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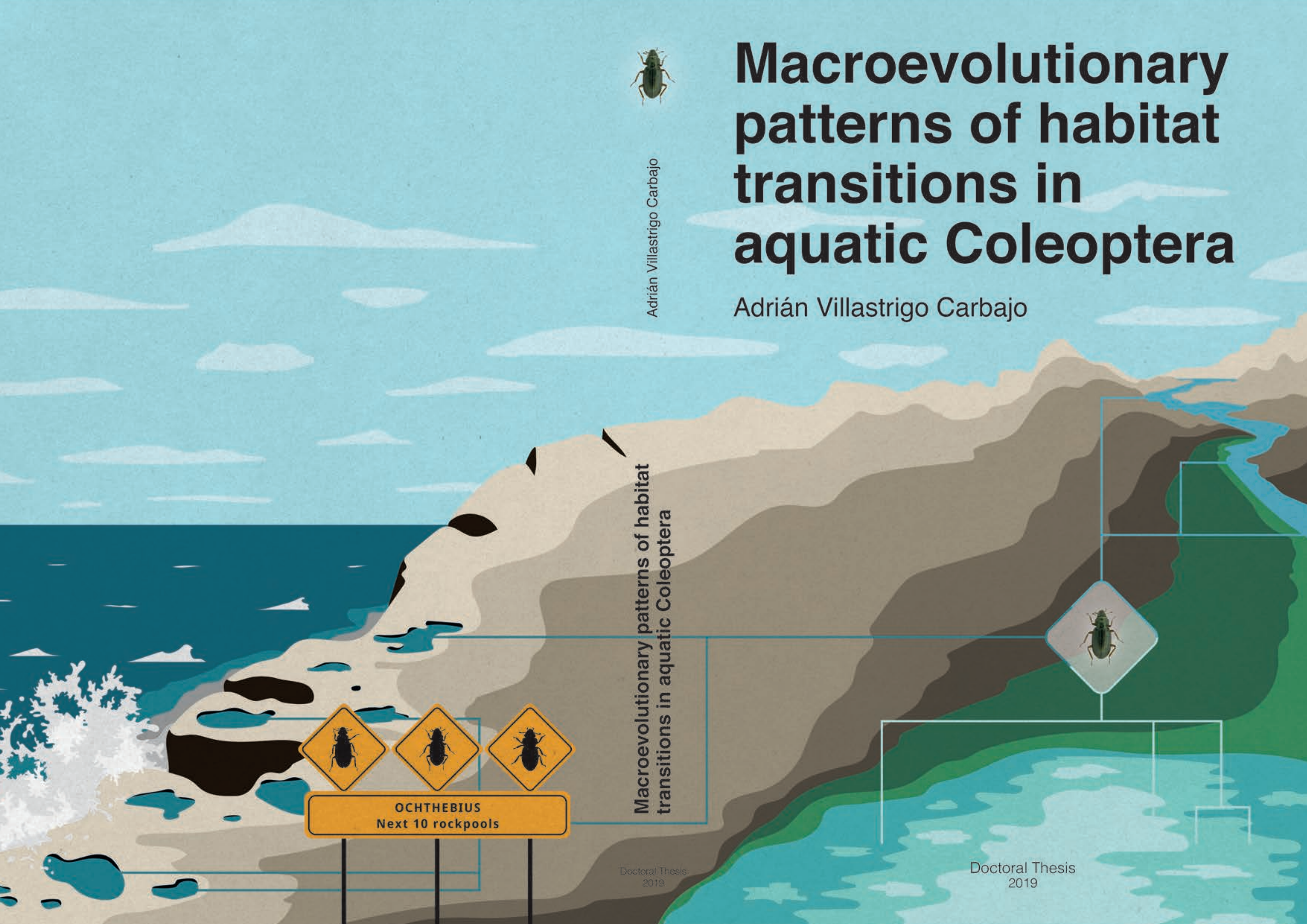
Adrián Villastrigo Carbajo



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Facultad de Biología

Departamento de Biología Evolutiva, Ecología y Ciencias Ambientales

Programa de Doctorado: Biodiversidad

Macroevolutionary patterns of habitat transitions in aquatic Coleoptera

Memoria presentada por

Adrián Villastrigo Carbajo

para optar al grado de doctor por la Universidad de Barcelona

Trabajo realizado en el Instituto de Biología Evolutiva (CSIC-UPF)

Barcelona, junio del 2019

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“*...ignorance more frequently begets confidence than does knowledge: it is those who know little, and not who know much, who so positively assert that this or that problem will never be solved by science*”

Darwin, C. (1871) *The descent of man, and selection in relation to sex*. John Murray.

Recommended citation:

Villastrigo, A. (2019). Macroevolutionary patterns of habitat transitions in aquatic Coleoptera (PhD thesis). Universitat de Barcelona, Barcelona, Spain.

Cover desing by PeloPantón (www.pelopanton.com)

Contents

Abstract

Understanding the evolutionary processes underlying extant biodiversity may help us to comprehend why species richness is not equally distributed between clades. One of the main causes to explain the disparity of species richness is the development of key innovations in particular clades, increasing or decreasing their capabilities to occupy new environments, to exploit novel resources or to cope with competition. The present thesis focuses on explaining the macroevolutionary consequences of two of the most common habitat transitions in aquatic environments: between environments with different degree of salinity, and between running and standing waters. Among inhabitants of aquatic environments aquatic Coleoptera are one of the most diverse, with representatives living in all kind of environments, including both running and standing waters and, not infrequently, saline environments. The most speciose aquatic Coleoptera families living in aquatic habitats are Hydrophilidae, Dytiscidae and Hydraenidae, but only the evolution of the ecological transitions of the former have been previously studied under a phylogenetic perspective. In this thesis, we assessed the evolutionary patterns of selected tribes belonging to the other two families. Despite aquatic Coleoptera being a relatively well-studied fauna, we needed to address several systematic modifications that revealed the true evolutionary history of the studied groups, describing a genus and three subgenera of Hygrotini, plus other modifications of both Hygrotini and Ochthebiini taxonomy (Chapters 1 and 3), setting the basis of this thesis.

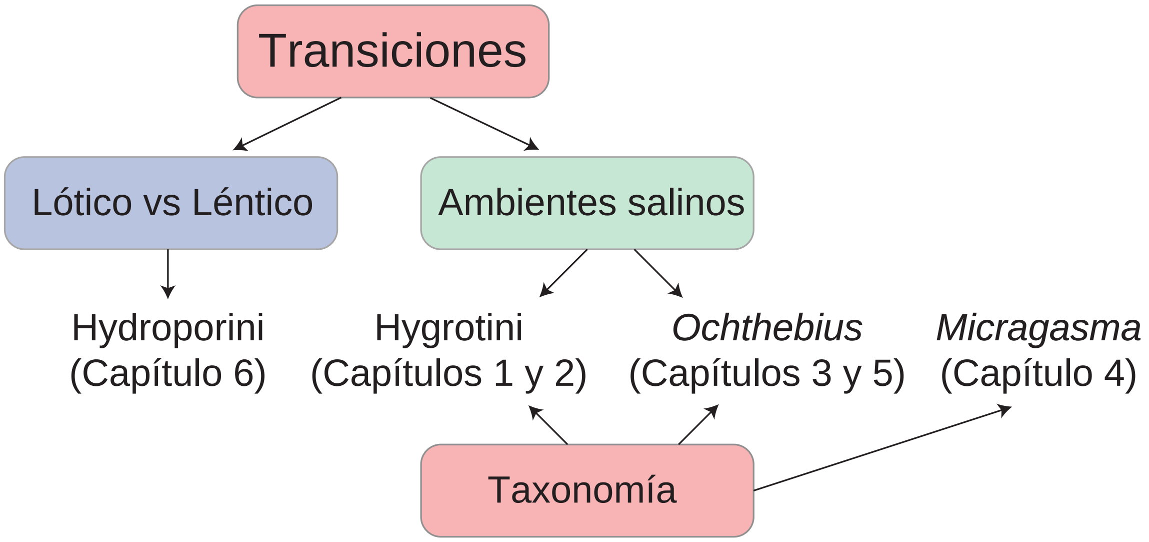
Transitions between environments with different degree of saline waters was addressed for Hygrotini (Dytiscidae family, Chapter 2) and Ochthebiini (Hydraenidae family, Chapter 5). We detected multiple origins of tolerance to saline waters, with a gradual acquisition in almost all cases and only direct transitions to hypersaline waters in fairly isolated clades, mostly associated with coastal rockpools. Moreover, tolerance to hypersaline waters was found to be irreversible, but it did not follow an evolutionary dead-end pattern as lineages exhibiting this trait still maintain their diversification capabilities (as seen in *Cobalius* subgenus, Chapter 5). Additionally, our results seem to link the origin of lineages exhibiting tolerance to saline waters to periods of global aridification, in accordance with previous studies in Hydrophilidae. Transitions between running and standing waters were studied in Hydroporini (Dytiscidae family, Chapter 6). We unveiled that species living in lotic and lentic habitats display similar diversification patterns, but the habitat preference is affecting the body size of the species, with reduced body sizes in specialized environments (i.e. smaller species in running waters). Finally, the field sampling in saline habitats led to the discovery of a new species for science, described as *Ochthebius* (*Micragasma*) *minoicus* (Chapter 4).

Resumen de la tesis

Estudiar los procesos evolutivos que ocasionan la biodiversidad actual puede ayudarnos a comprender mejor como la riqueza de especies está distribuida de forma desigual en diferentes clados. Una de las causas principales para explicar este fenómeno es el desarrollo de innovaciones clave que modifican las capacidades de las especies para sobrevivir en nuevos ambientes, acceder a nuevos recursos, o para lidiar la competencia con otras especies.

Esta tesis se focaliza en explicar las consecuencias macroevolutivas de dos de las transiciones de hábitat más comunes en ecosistemas acuáticos: aquellas entre ecosistemas con diferente nivel de salinidad, y aquellas entre ecosistemas de aguas corrientes y aguas estancas. Entre los habitantes de ambientes acuáticos, los escarabajos son uno de los grupos más diversos, con especies capaces de vivir en todo tipo de ambientes, incluyendo aguas corrientes y estancas, y en ocasiones, ambientes salinos. Las familias más diversas de escarabajos acuáticos son Hydrophilidae, Dytiscidae e Hydraenidae, aunque solo la primera ha sido estudiada desde una perspectiva evolutiva. En esta tesis, evaluamos los patrones evolutivos de varias tribus pertenecientes a las otras 2 familias.

Los objetivos principales de la tesis incluyen comprender el origen y la forma en la que se originan estas transiciones entre ambientes, así como comprender que patrones desencaden. Secundariamente, la tesis trata de ampliar el conocimiento ligado a los ecosistemas salinos, mucho menos estudiados que los ecosistemas de agua dulce. La tesis se ha elaborado según el siguiente esquema:



Taxonomía (Capítulos 1 y 3)

A pesar de que los escarabajos acuáticos son un grupo bien estudiado en general, ha sido necesario realizar modificaciones en la clasificación de algunos grupos para reflejar su verdadera historia evolutiva, describiendo un nuevo género y tres subgéneros para la tribu Hygrotini, dividiéndose actualmente en dos géneros: *Clemnius* e *Hygrotus*, divididos a su vez en 2 y 4 géneros respectivamente (*Clemnius* subg. y *Cyplopius* para *Clemnius*; *Coelambus*, *Hygrotus* subg., *Hyphoporus* y *Leptolambus* para *Hygrotus*). Adicionalmente, se han realizado pequeñas modificaciones tanto en Hygrotini como en Ochthebiini, siendo la base sobre la que se han desarrollado los posteriores estudios filogenéticos de esta tesis.

Transiciones entre ambientes salinos (Capítulos 2 y 5)

Las transiciones entre ambientes con diferente nivel de salinidad han sido estudiadas para Hygrotini (familia Dytiscidae, Capítulo 2) y Ochthebiini (familia Hydraenidae, Capítulo 5). Se han detectado múltiples orígenes de la tolerancia a la salinidad, adquiriéndose esta característica de forma gradual en la mayoría de los casos. Los casos en los que se han descubierto transiciones directas de ambientes de agua dulce hacia ambientes hipersalinos, son clados aislados en la filogenia y asociados con ambientes de charcas costeras.

Además, la tolerancia de aguas hipersalinas ha demostrado ser una característica irreversible, aunque no por ello se trata de un *dead-end* evolutivo (callejón sin salida), ya que las especies que viven en estos ambientes conservan su capacidad de diversificación, llegando incluso a ser superior a la de otras especies (por ejemplo, el subgénero *Cobalius*, Capítulo 5). A si mismo, nuestros resultados relacionan el origen de linajes tolerantes a la salinidad con periodos de aridificación a escala global, un patrón que confirma el encontrado en la familia Hydrophilidae.

Transiciones entre ambientes de agua corriente y agua estanca (Capítulo 6)

En cuanto a las transiciones entre aguas corrientes y estancas estudiada en Hydroporini (familia Dytiscidae, Capítulo 6), hemos encontrado patrones similares en las especies que viven en ambos ambientes, aunque los análisis sugieren la posibilidad de una mayor tasa de diversificación en ambientes de aguas estancadas. Nuestros resultados han destacado una correlación entre la preferencia del hábitat de las especies con su tamaño corporal, de manera que las especies que se encuentran en ambientes más especializados (en aguas corrientes) son aquellas con menor tamaño. Adicionalmente, se ha demostrado que la transición entre ambientes no es *per se* un factor que afecta al tamaño corporal de las tesis.

Biodiversidad en ambientes salinos (Capítulo 4)

Finalmente, gracias a los numeros trabajos de campo realizados enfocados en los ambientes salinos, se ha podido detectar la presencia de numerosos linages crípticos (Capítulo 5), además de la descripción de la especie *Ochthebius* (*Micragasma*) *minoicus* (Capítulo 4), perteneciente a un subgénero poco conocido con tan sólo otras 2 especies descritas.

General Introduction

Evolution can be understood as a natural process of change over a period of time, which Darwin associated with gradual modifications, but other authors put forward the idea of rapid events (e.g. Eldredge and Gould, 1972). In any case, evolution should be considered at different scales, from microevolution (evolution at short temporal scale) to macroevolution (evolution at large temporal scale). Whereas microevolution usually refers to evolution at level of the individual, e.g. mating selection of giraffe weevils (LeGrice *et al*., 2019), macroevolution works at the level of populations/species and over larger time periods, explaining the origin of new species and the hierarchy above them (Reznick & Ricklefs, 2009).

Research on macroevolution can enlighten us about the reasons for having such massive extant biodiversity, which Larsen *et al*. (2017) estimated from 1 to 6 billion species living on Earth, but of which only 1.5 million species are described – and more than 1 million are insects (Stork, 2018). But the species richness is not equally distributed between clades (groups of related animals). In fact, there is a huge variability in the number of species among them, e.g. within the described biodiversity, about 25% are represented by beetles, the most speciose group (Zhang *et al*., 2011). Such differences may be mainly explained by two hypotheses (McPeek & Brown, 2007) - see Figure 1:

- Different diversification rates. Clades with differences in their species richness may be explained by the rate at which a particular clade accumulates species. This hypothesis explains how young clades can be as speciose as older clades.
- Different diversification times. Besides diversification rates, it seems obvious that older clades had a greater time to accumulate species than younger clades. Therefore, clades with similar evolutionary patterns and diversification rates should yield the same diversity over time, leading to a higher number of species in older clades.

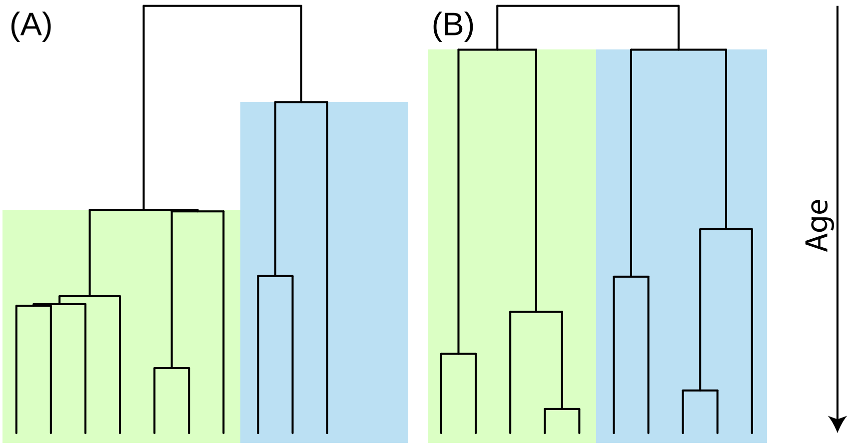


Figure 1. Summary of the main hypotheses for explain contrasting biodiversity across lineages. Different diversification rates may explain why younger clades are as speciose as older clades - or even more speciose (A), whilst time may explain similar number of species of equally older clades (B).

Although both hypotheses are valid and non-exclusive, diversification rates are believed to assume most of the variation among major clades (Wiens, 2017). Recent studies (Henao-Díaz *et al*., 2019; Li and Wiens, 2019) have demonstrated that clade age is the major explanation of extant species at regional scale, leaving open the answer to the question of *how species have diversified at global scale*. In the cases where clade age is not the main driver of diversification, other questions appear: What are the differences between clades that explain the disparity in extant species numbers? And how did those differences modify diversification rates? The most straightforward answer, but at the same time, not a simple one, is that organisms evolved key innovations (also called evolutionary novelties or traits) that reshaped their capabilities to diversify over time (Jablonski, 2008). Classic examples of key innovations include morphological novelties like the origin of flowers (Endress, 2011) and feathers (Prum & Brush, 2002), but other studies focus on functions like life-history strategy (Helmstetter *et al*., 2016).

Traits are one of the main topics in evolutionary studies (Wagner and Lynch, 2010), and some traits demonstrated to be associated with an alteration of either extinction rates, e.g. self-fertilization in plants (Wright *et al*., 2013), or speciation rates, e.g. immune system in teleost fishes (Malmstrøm *et al*., 2016), or, in some cases, both (Rolland *et al*., 2014). Also, other factors can influence imbalance diversity of species across clades, like colonization of new areas, e.g. adaptive radiation (see Gavrilets and Losos, 2009), competition between species (Meyer and Kassen, 2007; Silvestro *et al*., 2015), habitat filtering (Rodrigues and Diniz-Filho, 2016) or ecological niche differentiation (Moen and Morlon, 2014). Other traits may promote specialization to extreme conditions, opening ecological opportunities that alter speciation and extinction rates. In most cases, these extreme specializations are thought to be irreversible (Kelley & Farrel, 1998), and almost negligible alterations of environmental settings can increase extinction rates in what is called an evolutionary dead-end (Figure 2). Recent studies have tried to understand the implications of evolutionary dead-ends, but it is not clear whether extreme ecological specialization will lead to an increase in extinction rates (Cieslak *et al*., 2014; Day *et al*.,

Figure 2. Adaptations to extreme environments, such the ones of animal living in caves, are thought to affect negatively the diversification rate, but recent researches question this prior belief. Picture of the stygobitic *Iberoporus pluto* (Modified from Ribera and Reboleira, 2019).

2016; Stern *et al*., 2017; Cyriac *et al*., 2018).

Studying species diversity

To study novel traits and diversification across lineages, researchers use evolutionary trees or phylogenies. A phylogeny, a term firstly used by Ernst Haeckel (1866), is a branching diagram that represents the biological diversity in a hierarchical way. Phylogenies are built to reconstruct the evolutionary history of extant taxa, showing patterns of diversification that can be used to compare relationship between clades.

Building phylogenies is a complex task that can be achieved with different types of information whenever that information keeps a track of the evolutionary history, like morphological characters (e.g. Alarie and Michat, 2007), fossil remains (e.g. Brusatte and Carr, 2016) or DNA sequences (e.g. Carle *et al*., 2015). Each methodology has its own advantages and disadvantages, but this thesis was done using DNA sequences for several reasons:

- For comparing closely related organisms, morphology is likely a good choice. For comparing more distantly related organisms, it is likely that the chosen characters are not comparable or not informative between organisms. In the case of DNA, sequences rely in the same nucleotide language with homologous sequences, making comparison possible between all species.

- Some organisms show a high degree of morphological changes whilst others preserve almost the same morphology during long periods of time (e.g. morphological stasis in living fossils, like coelacanths or limulids). Also, morphological differenciation of cryptic species is not always possible (García-Porta *et al*., 2017). As DNA is continuously changing, it is possible to track the evolutionary history of all organisms whether morphological differences give or not clues about their relations.

- Fossil remains provide direct evidence of extinct taxa, and can be used during the inference of phylogenies, e.g. for calibrating clade ages, (McKenna *et al*., 2015). For lineages with a poor fossil record (Zhang *et al*., 2018) DNA is the only chance to study their history.

However, there are also inconveniences of working with DNA. Different sources of DNA may lead to diverse outcomes because they may have distinctive evolutionary histories (Salichos and Rokas, 2013). Selecting the appropriate sequences that reflect the real evolutionary history of life is complex. Animals present two main sources of DNA: nuclear and mitochondrial genomes. The nuclear genome contains molecular information about the species, and it is half paternally and maternally inherited. On the other side, mitochondrial genome is maternally inherited. These sources also have different evolutionary rates, with approximately 10-fold increased mutation rate in the mitochondrial genome compared to the nuclear one (Brown *et al*., 1979; Haag-Liautard *et al*., 2008). A higher mutation rate implies higher number of substitutions in the same nucleotide position of an alignment, saturating the DNA and erasing the track of evolution in the sequence (Philippe *et al*., 2011). Owing to DNA saturation, comparison of highly modified sequences may show conflicting phylogenetic signals. As the mitochondrial DNA is in general more easily saturated than nuclear DNA, is less ideal for studying deeply divergent taxa.

Once selected and obtained the appropriate genetic markers, the next step is aligning the sequences from each of the markers. Nucleotide alignment for extant taxa is the starting point for reconstructing evolutionary history, and alignments need to consider how those sequences evolved and which substitution of nucleotides may have experienced. For that purpose, the nucleotide substitution model that correlates better with the sequences should be used. These models estimate the genetic divergence (amount of substitutions) between DNA sequences considering that those sequences shared a common ancestor. There are a large number of models with different assumptions that reflect different probabilities of occurring a particular substitution, from the simplest Jukes-Cantor model (Jukes and Cantor, 1969) considering only one parameter (changes between nucleotides are identical), to the most complex Generalized Time-Reversible model (Tavaré, 1986) with no prior assumptions and establishing a different parameter for each kind of substitution plus considering unequal base composition. Another complex model is HKY (Hasegawa *et al*., 1985), which estimates different parameters for transversions (substitution of a purine for a pyrimidine or vice versa) and transitions (substitution of a purine for another purine or a pyrimidine for another pyrimidine) and unequal nucleotide composition. Model selection has been considered a critical step prior to reconstruct phylogenies (Hoff *et al*., 2016), but a recent study suggests that using a complex model with fewer prior assumptions will lead to similar inferences than the best fitted model (Abadi *et al*., 2019).

There are different approaches to make a molecular phylogeny, most of them starting with a multiple alignment of DNA sequences. During the last years, the two most common approaches have been Maximum Likelihood (developed by Felsenstein, 1981) and Bayesian Inference, both exploring the phylogenetic space in order to find the best tree or set of trees by changing parameter values and calculating likelihood of the hypothesis (in phylogenetic inference, the tree in consideration is the hypothesis). Besides, both approaches need to apply an evolutionary model to reconstruct the phylogeny, Maximum

Likelihood finds the parameter values that maximize the probability of the alignment giving the tree, whilst Bayesian Inference calculates the probability of a true tree based on the alignment. Bayesian Inference also considers prior information about the expected outcomes to reduce the computational time, but as the result is influenced by this information, it is better to use ample priors that will not compromise the result (Wang and Yang, 2014).

Although the approach used to reconstruct a phylogeny may be correct, the resolution obtained may not be enough to get a good support of the topology. The resolution of a tree depends on the support values calculated during the inference, and can be increased with the use of additional molecular markers that track the evolutionary history of the studied species. But increasing the number of markers usually involves a considerable increment of cost and time. Next Generation Sequencing (NGS) technologies are becoming essential to obtain massive amounts of data at a reasonable price, opening new fields like metabarcoding (Taberlet *et al*., 2012) or metagenomics (Quince *et al*., 2017). The latter has lead to a series of papers about a new methodology called Mitochondrial Metagenomics (e.g. Crampton-Platt *et al*., 2015; Andújar *et al*., 2015; Linard *et al*., 2018). It is used to build *de novo* mitochondrial genomes from a bulk DNA sample (Campton-Platt *et al*., 2016). As chapters 5 and 6 have been done using this methodology, a more detailed explanation it is presented below.

Mitochondrial Metagenomics

The main objective of this methodology is to increase the number of sequences available to boost the support of phylogenetic reconstruction (see Figure 3 for a summary of a mitochondrial metagenomics pipeline). This approach relies on the use of Sanger sequences as a baits database to be able to identify the expected mitochondrial genomes - in our case, we selected cytochrome c oxidase subunit I and the large ribosomal RNA as baits because they are located at both sides of the control region of the mitochondrial genome. Pooled DNA samples have to be equimolar to assure a similar number of reads during sequencing. However, terminals closely related (less than ca. 5 % differences in their mitochondrial bait sequences) should not be pooled together to avoid the assembly of chimeras. The last laboratory procedure is to built NGS libraries with insert-size as large as possible, as it has proved to improve the quality of the assemblies, obtaining higher coverage and more complete mitogenomes (Chen, Y.C. *et al*., 2018). As a general interpretation, each library can contain up to 60 terminals, and two libraries can share a low input MiSeq run.

Next procedures are performed with bioinformatic tools. Library processing starts by checking its integrity and quality, followed by the necessary steps to obtain high-quality data (i.e. remove adapters and sequences with poor quality values). The most useful programmes for this task are FastQC, Trimmomatic (Bolger *et al*., 2014), PrinSEQ (Schmieder and Edwards, 2011) and fastp (Chen, S. *et al*., 2018). For a better performance of the assembler software, short reads can be filtered based on their similarities with other mitochondrial genomes using BLAST (Madden *et al*., 2009). Assemblies have to be performed with different software with alternative assembling approaches to validate the outputs; in our case, we used IDBA-UD (Peng *et al*., 2012), RAY-meta (Boisvert *et al*., 2012), WGS-assembler (Myers *et al*., 2000) and SPAdes (Bankevich *et al*., 2012). Each assembler will

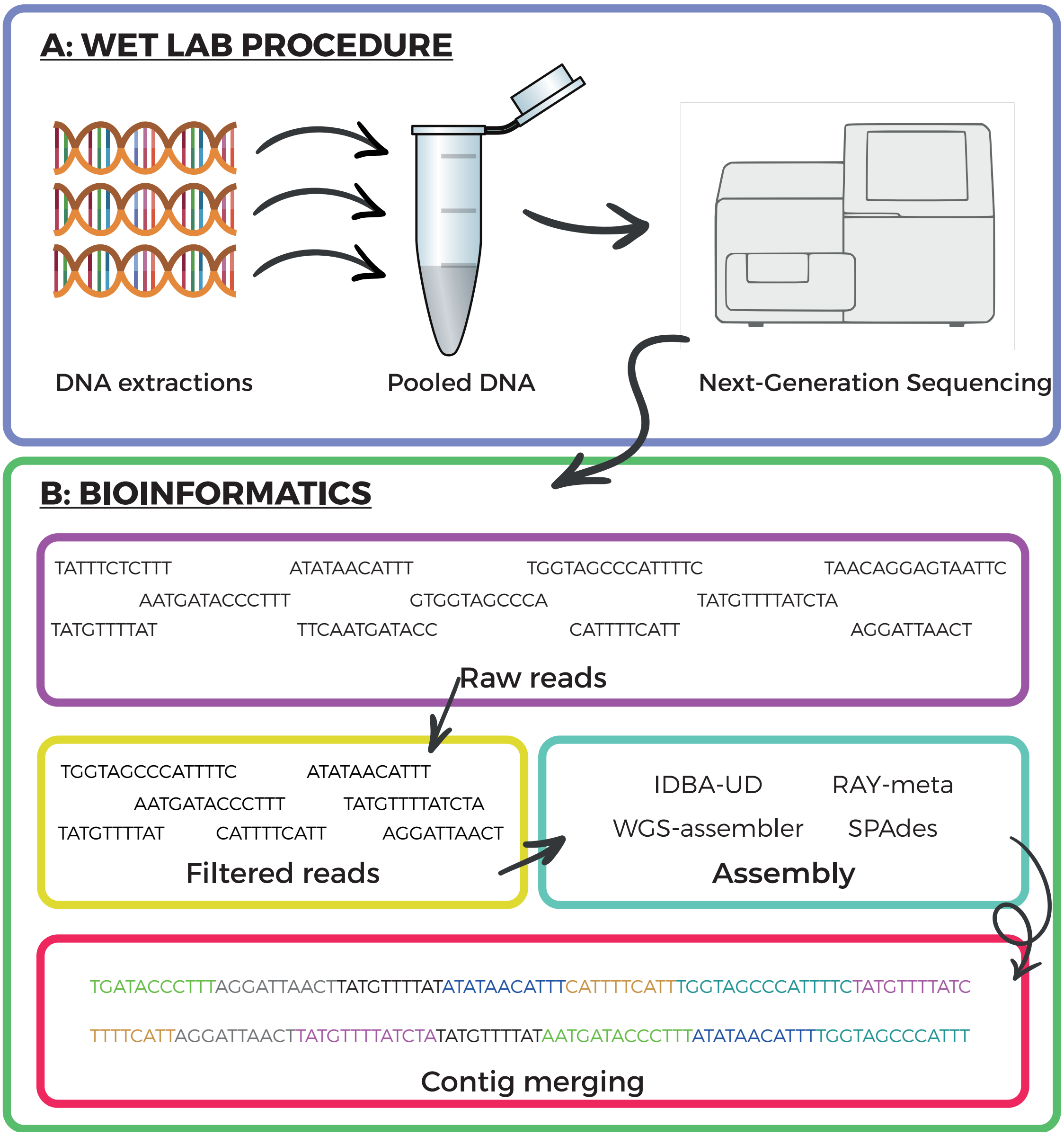


Figure 3. Mitochondrial Metagenomics pipeline is divided in two main procedures, wet lab (A) and bioinformatics (B). Wet lab consist in preparing an equimolar pool of DNA and sequencing with Next-Generation Sequencing technologies. Bioinformatics consists in a series of filters where raw reads are assembled into contigs, and a posterior identification.

provide a *fasta* file as output containing all the contigs found. For validating those *supercontigs* (contigs assembled with other contigs), assemblies of all outputs need to be done - to be considered a legitimate *supercontig* it needs the coincidence of at least 2 different assemblers. Identification of *supercontigs* is performed by blasting them against a custom baits database (obtained at the beginning of this methodology). The final step is the manual annotation of the mitochondrial genomes, using MITOS (Bernt *et al*., 2013) annotations as a template and multiple references of published mitochondrial genomes (see Figure 4).

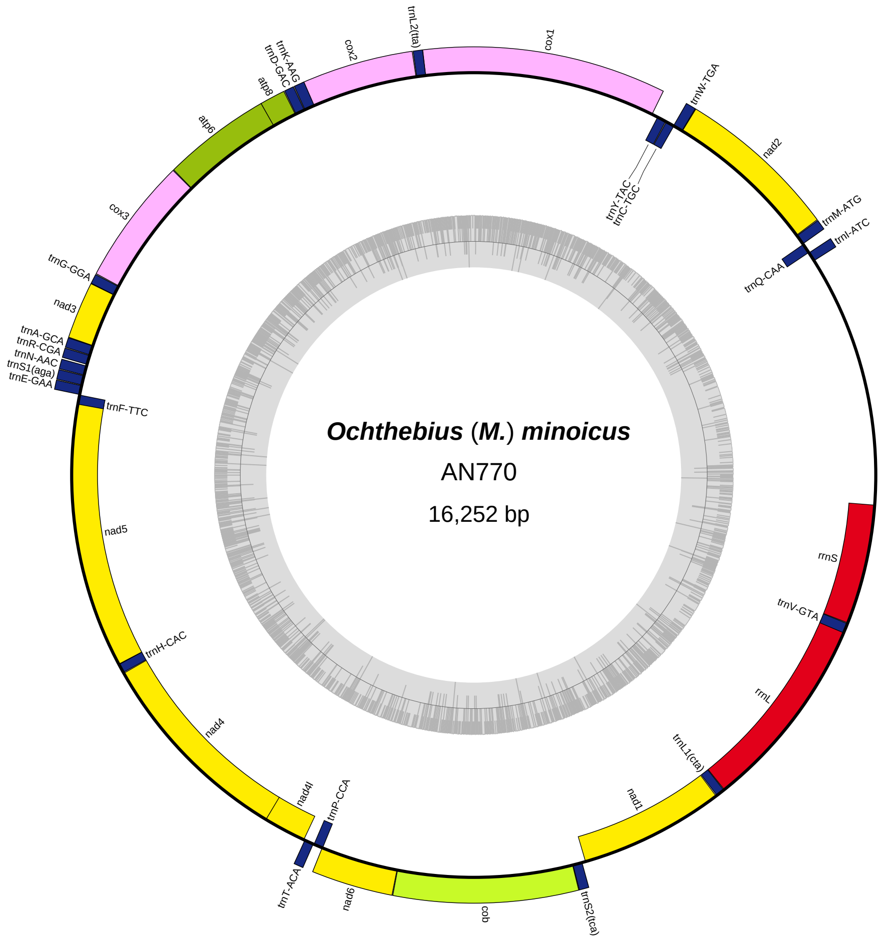


Figure 4. Mitochondrial genome of *Ochthebius* (*M*.) *minoicus*. A complete mitochondrial genome has 13 protein coding genes, 2 ribosomal genes and 22 tRNAs genes.

Calibrating trees

The relationship between the amount of molecular change across sequences and a measure of divergence time has become a key concept for reconstruction phylogenies and a crucial issue to understand the tempo and mode of evolutionary processes. The most basic approach relies on the assumption that the rate of molecular evolution is constant across lineages and time (strict molecular clock, Zuckerkandl and Pauling, 1965), but the rate of molecular evolution is influenced by several components, like the height of flowering plants (Lanfear *et al*., 2013), the body size of primates (Steiper and Seiffert, 2012) or climate-niche evolution of amphibians (Kozak and Wiens, 2010). Owing to the heterogeneous rate of molecular evolution, more complex assumptions are necessary to quantify its pace, like that different rates are present across a phylogeny, but some branches may share the rate based on a common history (local clocks, Yoder and Yang, 2000) or even reduce the expected uniformity of rates (relaxed clock, Drummond, *et al*., 2006). It is also possible to calibrate the rate of molecular substitution based on rates estimated by closely related lineages (e.g. the rates estimated for *Carabus* genus are a good approximation to measure the rate in the same family, Carabidae or in closely related ones, e.g. Dytiscidae, Andújar *et al*., 2012).

Estimates of divergence times have usually broad confident intervals and imprecise dates, but a more complex approach can be use incorporating other sources of information that constrain the confident interval of particular nodes, such as fossil record or biogeographic events (minimum and maximum age constrains respectively). For example, fossil dating relies on the idea of the superposition of rock strata, enabling the dating of the occurrence of extinct taxa (Benton *et al*., 2009). The quality of the fossil record is inversely correlated with age, and effective information can be recovered from fossils for at least the last 540 million years (Benton *et al*., 2000). Morphological resemblance of fossils with extant taxa allow researchers to constrain node age on phylogenetic analyses, using the guidelines of Hennig (1965) to place a fossil as stem or crown information.

Calculating diversification rates

Diversification rates allow researchers to address questions about macroevolutionary patterns (Rabosky *et al*., 2014). Diversification rate is modelled as the difference between two parameters: speciation rate and extinction rate. Unlike the estimates of extinction rates using phylogenies of extant taxa, that seem to be sensitive and error prone (May and Moore, 2016; Rabosky, 2009; Title and Rabosky, 2018), speciation rates can be inferred more precisely. Inferring diversification rates using phylogenies of extant taxa is intricate, but the patterns of internal nodes in time-calibrated phylogenies contain appropriate information about the pattern of diversification events (Ricklefs 2007). For that reason, comprehensive phylogenies are a requirement to not underestimate the number of speciation events (Barraclough and Nee, 2001). Underestimate the number of evolutionary lineages (i.e. cryptic species and undiscovered species) can mislead the outcome, thus, a sampling containing the whole number of described species does not imply a complete phylogeny. Also, phylogenetic reconstruction will lead to the most likely set of trees among the sampling space, but not necessary displaying the true evolutionary history, affecting the estimates of speciation and extinction rates (Kubo and Iwasa, 2006).

During the last decades, different methodologies made possible to estimate diversification rates. Simpler models just calculate diversification rate using clade age and species richness under different extinction scenarios (Magallón and Sanderson, 2001). More complex models link diversification rates with traits, using a SSE model (State-dependent Speciation and Extinction model). These models test diversification shifts against a binary trait (BiSSE, Maddison *et al*., 2007) or a multiple state trait (MuSSE, FitzJohn, 2012). Recently, SSE models have proved to produce false positives (see Caetano *et al*., 2018), so the inclusion of a hidden state reports a reliable state-dependent diversification and legitimate the models; HiSSE (Beaulieu and O'Meara, 2016) or the more recent SecSSE (Herrera-Alsina *et al*., 2019) are the most updated methods to account for rate heterogeneity using hidden states (applicable to binary traits or multiple state traits respectively).

In contrast with state-dependent models, state-independent diversification models can identify the position of a diversification shift (or shifts) in the phylogeny. This approach has become popular with the use of Bayesian Analysis of Macroevolutionary Mixtures (BAMM, Rabosky, 2014), which assumes rate heterogeneity through time and lineages. BAMM explores the number of shifts and their position using jump-reversible Markov Chain Monte Carlo methods, estimating those parameters from the posterior distribution. During the last years, a discussion about the use of BAMM arose (Moore *et al*., 2016; Rabosky *et al*., 2017; Meyer and Wiens, 2018; Rabosky, 2018; Meyer *et al*., 2018; Rabosky, 2019), showing that BAMM estimates are moderately accurate even with incomplete taxon sampling (around 25 %) and clade size is a critical factor to accurate estimates (extreme variance - inverse function of clade size - mislead to estimate rates). The latter was also found by Kodandaramaiah and Murali (2018) using simulated data, being BAMM the less accurate methodology under small clade sizes.

Trait reconstruction

Understanding the origin of novel traits and how they evolved may shed light about macroevolutionary processes and the mechanisms underlying diversification. Traits can be classified based on the type of data they provide: some traits focus on the presence of a particular quality whilst others target a continuous degree of change. The former are called discrete characters and can display multiple states or categories for the same trait (e.g. reconstruction the ancestral habitat of a genus with species living in both epigean and subterranean waters, Toussaint *et al*., 2016), whereas the latter are called continuous characters (e.g. body size evolution, Désamoré *et al*., 2018). Both types of traits use different evolutionary models to reconstruct the evolution of the character.

- Discrete traits: The simplest methodology to reconstruct the evolution of a discrete trait relies on the assumption that character evolution is relatively slow and species sharing the same character are likely to inherit it from a common ancestor. This idea that minimizing the number of changes across states will lead to the most likely reconstruction is called Parsimony (Hennig, 1965). However, more complex assumptions can be modelled, taking into account branch lengths: a slow rate of character evolution force closely related species to share the same character, whilst faster rates do not imply the same outcome. A Markov process is frequently used to estimate transition rates between characters using several assumptions and comparing their statistical power: (i) transitions between states have the same evolutionary rate; (ii) transition rates are symmetrical between states, but multiple states have different rates; and (iii) all transition rates are different.

  Advanced methodologies incorporate cost matrixes denoting asymmetrical character evolution. An extreme example of these asymmetrical traits can be the habitat preference for insect clades with species living in caves: insects adapted to live inside caves, e.g. eyeless animals with thin cuticles (see Howarth, 2009), are not likely able to survive in epigean environments, so transition from cave to epigean environments can be blocked in these matrixes whereas epigean to cave can still be estimated.

- Continuous traits: Traits related with morphological measurements are continuous variables, such as the body size (LeGrice *et al*., 2019). Continuous variables can be modelled using a simple Brownian motion evolutionary model (also called Random walk model), which accounts for an increase of variation in a trait over a period of time considering the pace of evolutionary change (Kaliontzopoulou and Adams, 2016). Again, more complex situations can be modelled based on our prior beliefs, like a Brownian Motion model assuming that the direction of trait evolution is not random, and optimum trait values influence evolutionary trends (known as Ornstein-Uhlenbeck model, Lande, 1976), or differential rate heterogeneity through time associated with fastest rates at the clade origin (Early-burst model, Harmon *et al*., 2010).

Aquatic beetles and their habitats

Beetles are the most successful order of animals on Earth, with more species than any other (Bilton *et al*., 2019). It is not surprising that such diversity results in a broad ecological and geographical distribution, with species capabilities allowing them to live in extreme habitats, such as caves (Polak, 2005) or arid environments (Parker and Lawrence, 2001). Beetles that live during some phase of their life in aquatic environments are called water beetles, and multiple independent transitions have led to radiations with more than 13.000 species in 30 beetle families (Short, 2018) - see Figure 5. The most remarkable trait that grant access to inhabit aquatic environments as adults is the capability to create a gas store under their elytra (Calosi *et al*., 2007) to breath beneath the water. However, water beetles exhibit other impressive adaptations that enable them to live in all kind of aquatic environments, like stygobitic *Paroster* living in groundwater (Leys & Watts, 2008) or

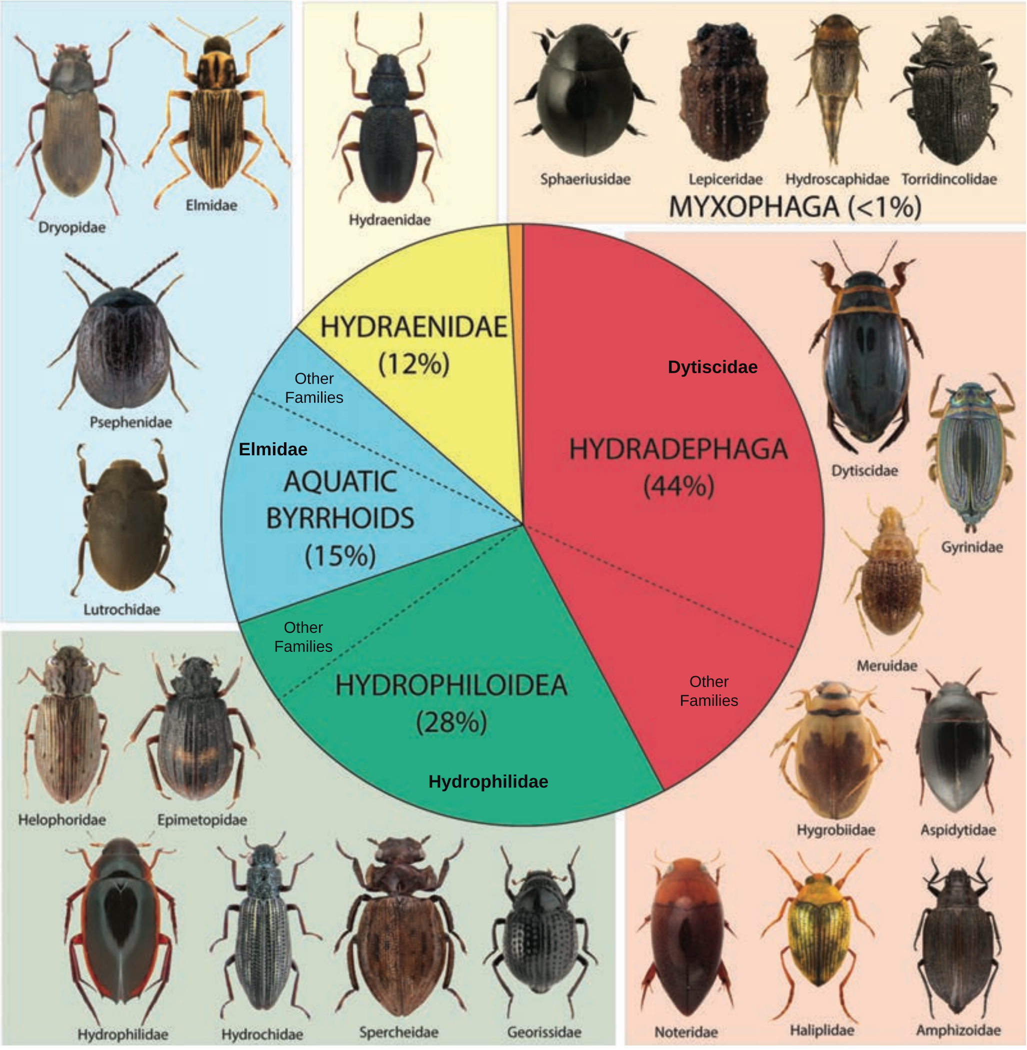


Figure 5. Relative abundance of described water beetles by groups (edited from Short, 2018). The core diversity of water beetles can be found in Dytiscidae, Hydrophilidae and Hydraenidae families.

Ochthebius living in hypersaline waters (Abellán *et al*., 2007; 2009). Extreme adaptations to those environments are found, for example, in *Paroster macrosturtensis* and *Ochthebius glaber*. The former retains negative phototactic behaviour from an ancestral interstitial species (Langille *et al*., 2018), and the latter displays an extreme tolerance to hypersaline waters, reaching nearly 250 g/l - more than 7 times the ocean mean concentration (see Millán *et al*., 2011).

Water beetles are distributed in all continents except Antarctica, with higher number of species in the Palaearctic region, followed by Neotropical, Afrotropical and Oriental regions, all of them outnumbering the Australian and Nearctic regions (Jäch & Balke, 2008). Species have different distribution ranges, being some species cosmopolitans whereas others are endemic for a particular region. That disparity is due to their distinct dispersal capabilities (for a detailed revision of fly capability see Jackson, 1952; also see Bilton *et al*., 2001), but those species linked to unstable habitats (e.g. temporary standing waters) are more likely to disperse to new environments than others (e.g. those that live in permanent streams) - see below for detailed information about habitat transitions.

Habitats

Water beetles are found in all kind of aquatic environments, from standing waters to running waters, including groundwaters (Cooper *et al*., 2002), intertidal rock crevices (Perkins, 2007), hygropetric and humicolous habitats (Perkins, 2006) - see Figure 6. This variety of habitats does not entail that species can live everywhere, because most of them are habitat specialists, like the species of *Ochthebius* subgenus *Calobius*, only found in intertidal rockpools (Villastrigo *et al*., 2019, Chapter 3) or *Paroster* living in Western Australia groundwaters (Watts and Humphreys, 2006). Studying why species are able to live in one habitat but not in others, and why different related species have different habitat preferences, can shed light of the evolutionary patterns driving water beetle history. To understand these patterns, transitions between contrasting aquatic habitats should be compared. Focusing on habitat preference, we can highlight two main transitions: 1) freshwater vs saline waters, 2) standing waters vs running waters.

- Freshwater and saline waters: Inland saline water habitats are both temporary or permanent environments with salinities higher than 3 g/L and are found around the world (Williams, 2002; Millán *et al*., 2011). Saline waters have a specific fauna, as their particular physiochemical characteristics may be a barrier for non-adapted species (Herbst, 2001). Although species deal with an energetic cost linked with

Figure 6. Water beetles can live in a broad range of habitats. Some examples are, from left to right and from top to bottom: salt lakes (Larnaca salt lake, Cyprus), mountain streams (Talassemtane National Park, Morocco), roadside pools (Plathiani Lagkada, Crete), rockpools (west from Qolla L-Bajda, Gozo) and salt pans (south from El Jadida, Morocoo).

stressful conditions, species may also benefit with lower costs due to avoiding competition and predation from other species (see Southwood, 1988 and Arribas *et al*., 2018 for more details). All the most diverse families of water beetles - Dytiscidae, Hydrophilidae and Hydraenidae - have species living in saline waters (Millán *et al*., 2011), but only Hydrophilidae (*Enochrus* genus) had been previously studied in an evolutionary framework (Arribas *et al*., 2014; Pallarés *et al*., 2017a). It has been shown that tolerance to saline waters has appeared multiple times independently during aridification periods (Arribas *et al*., 2014), and those tolerant species diversified retaining that trait. However, saline water inhabitants have been demonstrated to be generalist in their fundamental niches, with a similar fitness in freshwater habitats to closely related freshwater species (Arribas *et al*., 2018), implying a preference for saline waters but not the impossibility to live in freshwater.

- Standing waters and running waters: Aquatic beetles are primary divided based on the habitats where they live, with a main division between species living in standing water and species living in running waters (Gioria *et al*., 2014). Both habitat types present differences based on their physical and chemical characteristics - in essential long-term habitat stability - leading to different ecological dynamics at geological scale (Ribera and Vogler, 2000). Even though running waters can be physically altered (e.g. modification of the river bed location or drying up temporarily), they are more stable at geological scale and remain linked to other aquatic habitats within a drainage network. Small standing water bodies - the ones where aquatic beetles are usually found - are generally disconnected from other aquatic habitats in time and space (Hutchinson, 1957), and are likely to be filled by sediments over small periods of time (Ribera *et al*., 2001). A much richer classification includes multiple habitats, but they can be linked to one of these two main types (e.g. rivers, streams, creeks or waterfalls are running waters whilst ponds, pools, salt-pans or rockpools are standing waters), although some of them may be not as straightforward as others (e.g. pools associated with river sides or lake margins with wave action - Nilsson and Holmen, 1995). In general, most of the species are associated with one habitat type, but some widespread generalist can live in both types (Ribera, 2008).

Some studies about the consequences of living in different habitats have been made. Ribera and Vogler (2000) tested whether species living in running or standing waters have different range sizes, and they found a correlation. Monaghan *et al*. (2005) and Hof *et al*. (2006) also found the same correlation for other aquatic insects (mayflies and dragonflies respectively): species living in standing waters are forced to disperse when their habitat disappears, so those species have greater dispersal abilities that species living in running waters. Same result has been found for water beetles by Abellán and Ribera (2011), Millán *et al*. (2011) and Sánchez-Fernandez *et al*. (2012). Some of the hypothesized consequences of their different range sizes are an increased interpopulation gene flow in standing waters species, in contrast with a higher level of endemism (Ribera *et al*., 2003) and higher turnover in running water environments (Ribera *et al*., 2001; Abellán *et al*., 2009).

This thesis is focused on two water beetle families, dytiscids (Dytiscidae, also called diving beetles) and hydraenids (Hydraenidae, also called minute moss beetles) - Figure 7. In addition to Hydrophilidae, they are the most speciose families among water

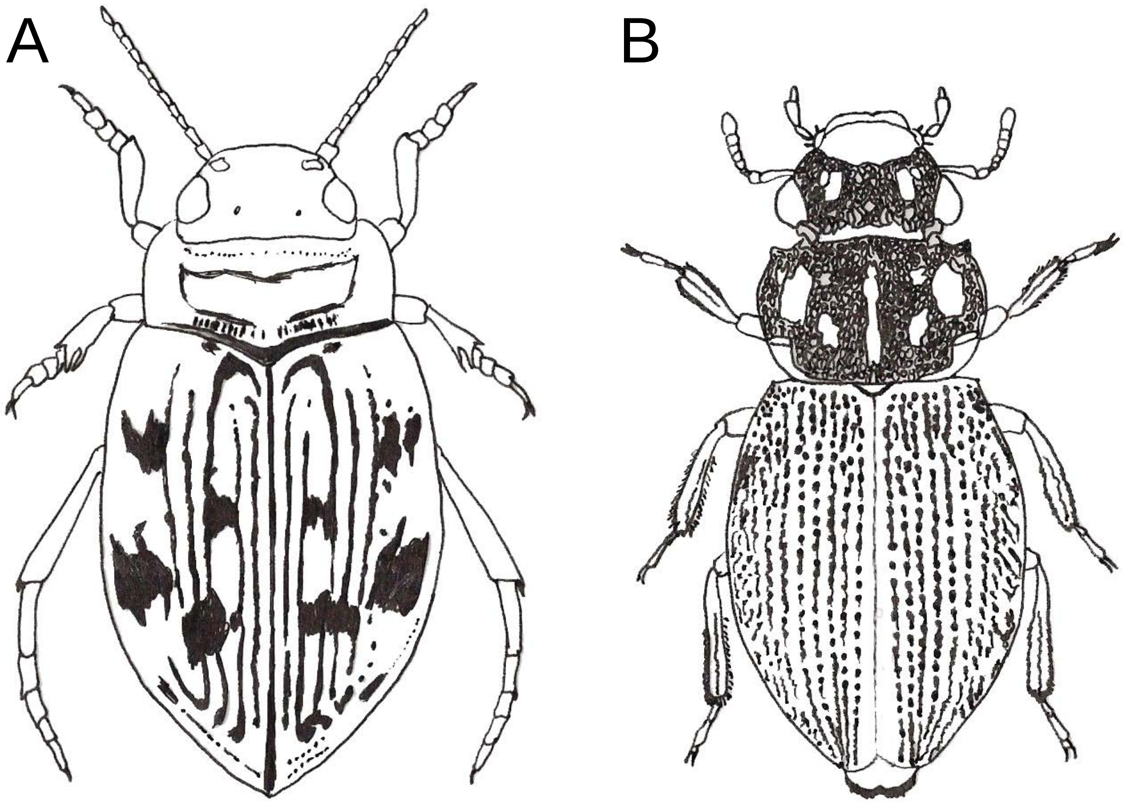


Figure 7. Draws of a diving beetle (A, *Nebrioporus canaliculatus*) and a hydraenid (B, *Ochthebius heydeni*). Draws by Carmen Victoria Romo Lanchas.

beetles (Jäch & Balke, 2008). They have species all around the world, including some species living in extreme latitude - e.g. some hydraenids occurring in subantarctic islands, like *Meropathus chuni* in Kerguelen (Bameul, 1989) or some diving beetles found in Greenland, like *Hydroporus morio* (Larson *et al*., 2000). Each family belongs to different suborders of Coleoptera, displaying contrasting morphologies: diving beetles get their name for the capabilities to dive, that is the reason of presenting a streamlined body with long legs (Miller and Bergsten, 2016); on the other hand, hydraenids are not great diving animals and most walk on the bottom of their habitats (Valladares *et al*., 2018). They also exhibit different body sizes, with hydraenids being small beetles between 0.8 to 3.4 mm long whilst diving beetles have a broad range of sizes, from the smallest *Limbodessus* around 0.9 mm long to the biggest *Megadytes* of 47 mm long. Both families have species able to live in all kind of environments, and due to their broad geographical distribution and an easy sampling, make them ideal models to study evolutionary questions.

General Objectives

Objectives and thesis outline

The main focus of this thesis is to obtain answers to some of the most frequent questions on evolutionary biology: what is the origin of novel traits and what are the evolutionary consequences of acquiring those traits; considering habitat transitions as novel traits. For answering these questions, new approaches needed to be developed combining different areas of knowledge. The two main objectives are:

- Determine the tempo and mode of evolution of the most common habitat transitions in aquatic Coleoptera: between environments with different degree of salinity, and from running water to standing waters and vice versa.
- Examine if habitat transitions have determined the evolutionary patterns of aquatic Coleoptera, more specifically on diversification rates.

More specific questions addressing the previous two objectives are:

- How supported are the current systematic status of Hygrotini, Ochthebiini and Hydroporini tribes? Is it necessary to perform any change? Is there any contradiction with the current classification, based largely on morphological characters?
- How many times did tolerance to salinity evolve and how it originated?
- Is the origin of tolerance to salinity linked to periods of aridification?
- Does extreme ecological specialization (i.e. tolerance to hypersaline waters) act as an evolutionary dead-end?
- Do diversification rates differ between species living in contrasting environments (i.e. between running and standing waters), and if so, do rates change during habitat transitions or along the evolution within each habitat?
- Does habitat specialization promote morphological specialization?, do specific habitats constrain the body size of species?

During the progress of this thesis, we needed to establish a framework to address both objectives and specific questions. The covered aspects were:

- Compilation of ecological information of habitats for each of the species in the studied tribes (Chapters 2, 5 and 6).

- Increase the knowledge of biodiversity associated with saline environments by effective field work, exploring undescribed taxa using molecular tools (Chapters 2, 4 and 5).
- Obtain robust phylogenies of Hydroporini, Hygrotini and Ochthebiini tribes, rearranging current taxonomy whenever was needed (Chapters 1, 3 and 6).
- Use a combination of classical plus Next Generation Sequencing tools to unveil the relationships of divergence taxa (Chapters 5 and 6).

This thesis is composed by six chapters, the first four chapters were published on international peer-review journals indexed in SCI while the last two chapters are in preparation to be submitted soon. Chapters are formatted as articles, presenting exactly the same content as those already published. Slight differences between chapters and articles refer only to reference style, abbreviations, the inclusion of references to chapters when self-citation was done and more readable figures of supplementary material. These six chapters are:

- Chapter 1. Villastrigo, A., Ribera, I., Manuel, M., Millán, A., and Fery, H. (2017) A new classification of the tribe Hygrotini Portevin, 1929 (Coleoptera: Dytiscidae: Hydraenidae). *Zootaxa*, **4317(3)**, 499-529.
- Chapter 2. Villastrigo, A., Fery, H., Manuel, M., Millán, A., and Ribera, I. (2018) Evolution of salinity tolerance in the diving beetle tribe Hygrotini (Coleoptera: Dytiscidae). *Zoologica Scripta*, **47**, 63-71.
- Chapter 3. Villastrigo, A., Jäch, M.A., Cardoso, A., Valladares, L.F., and Ribera I. (2019) A molecular phylogeny of the tribe Ochthebiini (Coleoptera, Hydraenidae, Ochthebiinae). *Systematic Entomology*, **44(2)**, 273-288.
- Chapter 4. Hernando, C., Villastrigo, A., and Ribera, I. (2017) A new species of *Micragasma* J. Sahlberg, 1900 (Coleoptera: Hydraenidae) from Crete. *Aquatic Insects*, **38**, 185-196.
- Chapter 5. Villastrigo, A., Arribas, P., Cardoso, A., and Ribera, I. (2019) Irreversible habitat specialisation does not constraint diversification in hypersaline water beetles. *Manuscript in preparation*.
- Chapter 6. Villastrigo, A., Abellán, P., Cardoso, A., and Ribera, I. (2019) Habitat preferences, body size and diversification in a speciose lineage of diving beetles. *Manuscript in preparation*.

Advisors’ report

Dr. Ignacio Ribera Galán and Andrés Millán Sánchez, as advisors of the PhD Thesis of Adrián Villastrigo Carbajo entitled “Macroevolutionary patterns of habitat transitions in aquatic Coleoptera”, report that the PhD Thesis is formed by six chapters consisting in 4 published papers and two complete manuscripts in preparation to be submitted.

Chapter 1. Villastrigo, A., Ribera, I., Manuel, M., Millán, A., and Fery, H. (2017) A new classification of the tribe Hygrotini Portevin, 1929 (Coleoptera: Dytiscidae: Hydraenidae). *Zootaxa*, **4317(3)**, 499-529.

Zootaxa had an impact factor in the lasted edition of the Journal of Citation Reports (2017) of 0.931. This journal is in the third quartile in the category “Zoology” (94th of 166).

Doctorate contributions: A. Villastrigo participated in the field sampling, laboratory work and data analyses, and contributed in the writing, discussion and editing of manuscript.

Co-authors contributions: I. Ribera, A. Millán and M. Manuel participated in the field sampling. I. Ribera contributed to the analyses of the data. H. Fery leadered the morphological analyses. I. Ribera and A. Millán led the experimental design and supervised the writing. All co-authors contributed in the discussion and edition of the manuscript.

Chapter 2. Villastrigo, A., Fery, H., Manuel, M., Millán, A., and Ribera, I. (2018) Evolution of salinity tolerance in the diving beetle tribe Hygrotini (Coleoptera: Dytiscidae). *Zoologica Scripta*, **47**, 63-71.

Zoologica Scripta had an impact factor in the latest edition of the Journal of Citation Reports (2017) of 3.057. This journal is in the first quartile in the category “Zoology” (10th of 166) and in the second quartile in the category “Evolutionary Biology” (21th of 48).

Doctorate contributions: A. Villastrigo participated in the field sampling, laboratory work and data analyses, and contributed in the writing, discussion and editing of manuscript.

Co-authors contributions: I. Ribera, A. Millán and M. Manuel participated in the field sampling. I. Ribera contributed to the analyses of the data. I. Ribera and A. Millán led the experimental design and supervised the writing. All co-authors contributed in the discussion and edition of the manuscript.

Chapter 3. Villastrigo, A., Jäch, M.A., Cardoso, A., Valladares, L.F., and Ribera I. (2019) A molecular phylogeny of the tribe Ochthebiini (Coleoptera, Hydraenidae, Ochthebiinae). *Systematic Entomology*, **44(2)**, 273-288.

Systematic Entomology had an impact factor in the latest edition of the Journal of Citation Reports (2017) of 4.237. This journal is in the first quartile in the categories "Entomology" (3th of 96) and "Evolutionary Biology" (12th of 49).

Doctorate contributions: A. Villastrigo participated in the field sampling, laboratory work and data analyses, and contributed in the writing, discussion and editing of manuscript.

Co-authors contributions: A. Cardoso led the laboratory work. M. Jäch, I. Ribera and L.F. Valladares participated in the morphological analyses. M. Jäch, I. Ribera and L.F. Valladares contributed in the discussion and writing of the manuscript.

Chapter 4. Hernando, C., Villastrigo, A., and Ribera, I. (2017) A new species of *Micragasma* J. Sahlberg, 1900 (Coleoptera: Hydraenidae) from Crete. *Aquatic Insects*, **38**, 185-196.

Aquatic Insects had an impact factor in the latest edition of the Journal of Citation Reports (2017) of 0.583. This journal is in the fourth quartile in the categories "Entomology" (76th of 96).

Doctorate contributions: A. Villastrigo participated in the field sampling and contributed in the writing of manuscript.

Co-authors contributions: All co-authors participated in the field sampling and in the discussion and writing of the manuscript.

Chapter 5. Villastrigo, A., Arribas, P., Cardoso, A., and Ribera, I. (2019) Irreversible habitat specialisation does not constraint diversification in hypersaline water beetles. *Manuscript in preparation for submission*.

Doctorate contributions: A. Villastrigo participated in the experimental design, field sampling, laboratory work and data analyses, and contributed in the writing, discussion and editing of manuscript.

Co-authors contributions: I. Ribera and P. Arribas led the experimental design. A. Cardoso led the laboratory work. I. Ribera and P. Arribas contributed in the discussion and writing of the manuscript.

Chapter 6. Villastrigo, A., Abellán, P., Cardoso, A., and Ribera, I. (2019) Habitat preferences, body size and diversification in a speciose lineage of diving beetles. *Manuscript in preparation for submission*.

Doctorate contributions: A. Villastrigo participated in the experimental design, field sampling, laboratory work and data analyses, and contributed in the discussion and writing of the manuscript.

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Barcelona, 19th June 2019

Ignacio Ribera Galán

Andrés Millán Sánchez

Chapters

Chapter 1

A new classification of the tribe Hygrotini Portevin, 1929 (Coleoptera: Dytiscidae: Hydroporinae)

Edited version of: Adrián Villastrigo, Ignacio Ribera,
Michaël Manuel, Andrés Millán and Hans Fery.
Published on: *Zootaxa* (2017) **4317 (3)**: 499-529.

Abstract

The tribe Hygrotini Portevin, 1929 is currently composed of four genera, *Heroceras* Guignot, 1950, *Herophydrus* Sharp, 1880, *Hygrotus* Stephens, 1828 (with two subgenera, *Coelambus* Thomson, 1860, and *Hygrotus* s. str.), and *Hyphoporus* Sharp, 1880. A recent molecular phylogeny of the tribe with ca. 45% of the 137 described species of Hygrotini, including the type species of all genus-level taxa, revealed extended para- or polyphyly of the current genera and subgenera (Villastrigo *et al*., 2018, Chapter 2), for which reason a new classification of the tribe Hygrotini is proposed. Within Hygrotini only two genera are recognised: *Clemnius* **n. gen.** (with two subgenera: *Clemnius* s. str. with type species *Hyphydrus decoratus* Gyllenhal, 1810, and *Cyclopius* **n. subgen.** with type species *Hydroporus acaroides* LeConte, 1855) and *Hygrotus* (with four subgenera: *Coelambus*, *Hygrotus* s. str., *Hyphoporus* **n. stat.** and *Leptolambus* **n. subgen.** with type species *Dytiscus impressopunctatus* Schaller, 1783). Two genera are synonymised under *Hygrotus* s. str., *Herophydrus* **n. syn.** and *Heroceras* **n. syn**. The following 67 new combinations, for species thus far treated under the genera *Heroceras*, *Herophydrus*, *Hygrotus* and *Hyphoporus*, result from the new classification: *Clemnius* (s. str.) *berneri* (Young & Wolfe, 1984) **n. comb.**, *Clemnius* (s. str.) *decoratus* (Gyllenhal, 1810) **n. comb.**, *Clemnius* (s. str.) *hydropicus* (LeConte, 1852) **n. comb.**, *Clemnius* (s. str.) *laccophilinus* (LeConte, 1878) **n. comb.**, *Clemnius* (s. str.) *sylvanus* (Fall, 1917) **n. comb.**, *Clemnius* (Cyclopius) *acaroides* (LeConte, 1855) **n. comb.**, *Clemnius* (Cyclopius) *farctus* (LeConte, 1855) **n. comb.**, *Clemnius* (Cyclopius) *marginipennis* (Blatchley, 1912) **n. comb.**, *Hygrotus* (s. str.) *assimilis* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *bilardoi* (Biström & Nilsson, 2002) **n. comb.**, *H.* (s. str.) *capensis* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *confusus* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *descarpentriesi* (Peschet, 1923) **n. comb.**, *H.* (s. str.) *discrepatus* (Guignot, 1954) **n. comb.**, *H.* (s. str.) *endroedyi* (Biström & Nilsson, 2002) **n. comb.**, *H.* (s. str.) *gigantoides* (Biström & Nilsson, 2002) **n. comb.**, *H.* (s. str.) *gigas* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *goldschmidti* (Pederzani & Rocchi, 2009) **n. comb.**, *H.* (s. str.) *gschwendtneri* (Omer-Cooper, 1957) **n. comb.**, *H.* (s. str.) *hyphoporoides* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *ignoratus* (Gschwendtner, 1933) **n. comb.**, *H.* (s. str.) *inquinatus* (Boheman, 1848) **n. comb.**, *H.* (s. str.) *janssensi* (Guignot, 1952) **n. comb.**, *H.* (s. str.) *kalaharii* (Gschwendtner, 1935) **n. comb.**, *H.* (s. str.) *morandi* (Guignot, 1952) **n. comb.**, *H.* (s. str.) *muticus* (Sharp, 1882) **n. comb.**, *H.* (s. str.) *natator* (Biström & Nilsson, 2002) **n. comb.**, *H.* (s. str.) *nigrescens* (Biström & Nilsson, 2002) **n. comb.**, *H.* (s. str.) *nodieri* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *obscurus* (Sharp, 1882) **n. comb.**, *H.* (s. str.) *obsoletus* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *ovalis* (Gschwendtner, 1932) **n. comb.**, *H.* (s. str.) *pallidus* (Omer-Cooper, 1931) **n. comb.**, *H.* (s. str.) *pauliani* (Guignot, 1950) **n. comb.**, *H.* (s. str.) quadrilineatus (Régimbart, 1895) **n. comb.**, *H.* (s. str.)

reticulatus (Pederzani & Rocchi, 2009) **n. comb.**, *H.* (s. str.) *ritsemae* (Régimbart, 1889) **n. comb.**, *H.* (s. str.) *rohani* (Peschet, 1924) **n. comb.**, *H.* (s. str.) *rufus* (Clark, 1863) **n. comb.**, *H.* (s. str.) *sjostedti* (Régimbart, 1908) **n. comb.**, *H.* (s. str.) *spadiceus* (Sharp, 1882) **n. comb.**, *H.* (s. str.) *sudanensis* (Guignot, 1952) **n. comb.**, *H.* (s. str.) *travniceki* (Šťastný, 2012) **n. comb.**, *H.* (s. str.) *tribolus* (Guignot, 1953) **n. comb.**, *H.* (s. str.) *variabilis secundus* (Régimbart, 1906) **n. comb.**, *H.* (s. str.) *variabilis variabilis* (Guignot, 1954) n. comb., *H.* (s. str.) *verticalis* (Sharp, 1882) **n. comb.**, *H.* (s. str.) *vittatus* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *wewalkai* (Biström & Nilsson, 2002) **n. comb.**, *Hygrotus* (*Hyphoporus*) *anitae* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *aper* (Sharp, 1882) **n. comb.**, *H.* (*Hyphoporus*) *bengalensis* (Severin, 1890) **n. comb.**, *H.* (*Hyphoporus*) *bertrandi* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *caliginosus* (Régimbart, 1899) **n. comb.**, *H.* (*Hyphoporus*) *dehraduni* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *elevatus* (Sharp, 1882) **n. comb.**, *H.* (*Hyphoporus*) *geetae* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *josephi* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *kempi* (Gschwendtner, 1936) **n. comb.**, *H.* (*Hyphoporus*) *montanus* (Régimbart, 1899) **n. comb.**, *H.* (*Hyphoporus*) *nilghiricus* (Régimbart, 1903) **n. comb.**, *H.* (*Hyphoporus*) *oudomxai* (Brancucci & Biström, 2013) **n. comb.**, *H.* (*Hyphoporus*) *pacistanus* (Guignot, 1959) **n. comb.**, *H.* (*Hyphoporus*) *pugnator* (Sharp, 1890) **n. comb.**, *H.* (*Hyphoporus*) *severini* (Régimbart, 1892) **n. comb.**, *H.* (*Hyphoporus*) *subaequalis* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *tonkinensis* (Régimbart, 1899) **n. comb**.

Introduction

The diving beetle tribe Hygrotini (Dytiscidae: Hydroporinae) is a relatively small and homogeneous lineage, with 137 species currently included in four genera: *Heroceras* Guignot, 1950, *Herophydrus* Sharp, 1880, *Hyphoporus* Sharp, 1880, and *Hygrotus* Stephens, 1828, the latter with two subgenera, *Hygrotus* s. str. and *Coelambus* Thomson, 1860 (Nilsson & Hájek, 2017a). They have a predominantly Holarctic and Ethiopian distribution, with some species reaching the Oriental and the north of the Neotropical regions (Nilsson & Hájek, 2017a).

The taxonomic history of Hygrotini has experienced several modifications since the original description of the tribe by Portevin (1929). Previous to this author, Sharp (1882: 389) listed *Coelambus* (in which he included species currently in *Hygrotus* and *Herophydrus*) as well as *Herophydrus* and *Hyphoporus* (both erected two years earlier) as the three first genera in his “Group Hydroporini”. Portevin (1929: 180) excluded from Hydroporini the genera *Oxynoptilus* Schaum, 1867 (junior objective synonym of *Hydrovatus* Motschulsky, 1853), *Hyphydrus* Illiger, 1802, and *Hygrotus*, and erected for these three genera the tribe Hygrotini, based on the common presence of an oblique epipleural carina near the elytral shoulders which separates the smaller anterior part (the genicular area or fossa, which receives the front- and midleg knees when the legs are folded; cf. Sharp, 1882: 242) from the longer posterior part of the elytral epipleura. However, most authors continued to include *Hygrotus* and allied genera within the wider tribe Hydroporini as defined by Sharp (1882). Nilsson & Holmen (1995: 30) reinstated the tribe Hygrotini, excluding the genera *Hyphydrus* and *Hydrovatus* (which were already in their own tribes Hyphydrini and Hydrovatini respectively), but including the genus *Pseudhydrovatus* Peschet, 1924, which subsequently was shown to be a junior synonym of *Hydrovatus* (see Biström, 2002).

The generic concepts within Hygrotini also suffered multiple changes. Thomson (1860) realised that *Hygrotus* sensu Stephens (1828) comprised two different morphological groups according to the aspect of the anterior margin of clypeus, establishing the genus *Coelambus* for the species without clypeal rim. The status of both *Coelambus* and *Hygrotus* has generated controversy among different authors, as the character of the clypeal rim in fact shows considerable variation (see Falkenström, 1933; Balfour-Browne, 1934; Anderson, 1971; or Biström & Nilsson, 2002; and the Appendix for a detailed discussion). Sharp (1882) considered *Coelambus* as a genus divided into different groups according to the morphology of the anterior part of the head and the clypeus, with *Dytiscus inaequalis* Fabricius, 1777 (the type species of the genus *Hygrotus*) included in his Group I. Other authors followed his criterion (e.g. Fall, 1919), but Balfour-Browne (1940) and Hatch (1953) (among others) considered again *Coelambus* as a subgenus of *Hygrotus*, a treatment that has prevailed since then (although Miller & Bergsten, 2016 recently re-established *Coelambus* as a valid genus, but without detailed discussion).

As for the other genera currently included in Hygrotini, Sharp (1880: cxlviii) erected within Hydroporini the new genus *Herophydrus* for what was known as *Hydroporus hyphydroides* Perris, 1864 (= *Hyphydrus guineensis* Aubé, 1838), plus five undescribed species from Africa and Madagascar, and the new genus *Hyphoporus* for *Hydroporus solieri* Aubé, 1838, and two other undescribed species. Two years later, Sharp (1882: 997; nec Branden 1885: 39) synonymised *H. hyphydroides* with *H. guineensis* and described the species mentioned in Sharp (1880) as new, five in *Herophydrus* and two in *Hyphoporus*. It is notable that in Sharp (1882) the current *Herophydrus musicus* (Klug, 1834) was maintained in the genus *Coelambus*.

The identity of the genera of Hygrotini has been problematic ever since. Guignot (1950) was the first author who characterised *Hyphoporus* and *Herophydrus* by using as main character the morphology of the male genitalia, asymmetric in the first and symmetric in the second. Finally, *Heroceras* was erected by Guignot (1950) for a species from Madagascar (*Herophydrus descarpentriesi* Peschet, 1923) with some peculiar characters (such as e.g. dilated antennae, see below).

Until recently the internal phylogeny of Hygrotini has only been addressed as part of wider studies on the phylogeny of Dytiscidae, or the revision of particular genera. Remarkably, all published studies failed to recover the respective monophyly of *Hygrotus* and *Herophydrus*, either using molecular (Ribera *et al.,* 2002, 2008; Abellán *et al.,* 2013; Miller & Bergsten, 2014) or morphological data, both of larvae (Alarie & Michat, 2007) and adults (Miller, 2001; Biström & Nilsson, 2002). The phylogenetic position of the genera *Hyphoporus* and *Heroceras* has only been addressed using morphological data by Biström & Nilsson (2002), who found *Hyphoporus* as sister to the studied species of *Coelambus*, and *Heroceras* as sister to the species of *Herophydrus* plus *Hygrotus*.

In a previous work by the same authors (Villastrigo *et al*., 2018, Chapter 2) we reconstructed the evolution of the tolerance to salinity within tribe Hygrotini, which includes some of the few diving beetles able to live at salt concentrations more than double that of seawater (e.g. *Hygrotus salinarius* (Wallis, 1924) or *H. pallidulus* (Aubé, 1850); Timms & Hammer, 1988;, Picazo *et al*., 2010). With that purpose, a molecular phylogeny of the tribe was estimated, including ca. 45% of the described species. Results revealed that two of the four currently recognised genera of Hygrotini and one subgenus were para- or polyphyletic

(*Hygrotus*, *Herophydrus* and subgenus *Coelambus*), and the monotypic *Heroceras* was deeply nested within a clade of Madagascan *Herophydrus*. The need of a new classification of Hygrotini in order to reconcile the taxonomic ordination of the tribe with its phylogeny was clear, but it was considered more appropriate to present the corresponding taxonomic changes in a separate work.

Material and Methods

Phylogenetic data: We used the phylogeny of Hygrotini obtained in Villastrigo *et al.* (2018, Chapter 2), which included sequence data from 99 specimens of 61 species representing all four currently recognised genera of Hygrotini (Table 1). Most importantly, the phylogeny included the type species of all nine genus-group names within Hygrotini (Nilsson & Hájek , 2017a). Outgroups included a selection of species of Hydroporini, and trees were rooted on *Laccornis* Gozis, 1914, considered to be outside Hydroporini and Hygrotini and in a basal position within Hydroporinae (Ribera *et al.,* 2008; Miller & Bergsten, 2014).

For a detailed explanation of the methods used to obtain the molecular data and the phylogeny see Villastrigo *et al.* (2018, Chapter 2). In summary, the phylogeny was built using fragments of seven genes in six sequencing reactions, three mitochondrial: (1) 5' end of cytochrome c oxidase subunit 1 (COI-5, the "barcode" fragment, Hebert *et al.,* 2003), (2) 3' end of cytochrome c oxidase subunit 1 (COI-3), (3) 5' end of 16S RNA plus the Leucine tRNA plus 5' end of NADH dehydrogenase subunit I (16S); and three nuclear: (4) an internal fragment of the large ribosomal unit 28S RNA (28S), (5) an internal fragment of the small ribosomal unit, 18S RNA (18S) and (6) an internal fragment of Histone 3 (H3). Vouchers and DNA samples of all specimens used in the phylogeny are kept in the collections of the Institute of Evolutionary Biology (IBE, Barcelona) and Museo Nacional de Ciencias Naturales (MNCN, Madrid).

To reconstruct the phylogeny, sequences were aligned using the online version of MAFFT 7 with the G-INS-I algorithm (Katoh *et al.,* 2009) and a fast Maximum Likelihood (ML) heuristic algorithm in RAxML-HPC2 (Stamatakis, 2006) in the CIPRES Science Gateway (Miller *et al.,* 2010), using a partition by genes with a GTR+G evolutionary model independently estimated for each partition and assessing node support with 100 pseudoreplicas with a rapid bootstrapping algorithm (Stamatakis *et al.,* 2008).

Morphological data: Specimens were studied with an Olympus SZX16 stereomicroscope. For the figures, stacks of micrographs were made with a Canon EOS 650D camera attached to the stereomicroscope. These stacks were subsequently treated with the image stacking software Helicon Focus Pro version 6.4.1. For the SEM-micrographs, specimens were placed on stubs and coated with gold (Sputter Coater, Quorum Technologies Ltd., Ashford, England). Micrographs were taken with an ESEM XL30 (Philips, Amsterdam, The Netherlands) and Scandium FIVE software (Olympus, Münster, Germany) in the Phyletisches Museum (Jena, Germany). Adobe Photoshop CS5 software was used to retouch micrographs and ink drawings. Most ink drawings are reproduced from Fery (2003) with the permission of M.A. Jäch (Vienna, Austria); this is not mentioned in the legends of the figures.

To estimate the likely phylogenetic relationships of the species for which no molecular data could be obtained, and to complete the taxonomic rearrangement of the tribe Hygrotini (see below), we studied all described species of the subgenera *Hygrotus* and *Coelambus* with the only exceptions of *H*. (*Coelambus*) *artus* (Fall, 1919), known only from the holotype and considered to be possibly extinct (see Anderson, 1983), and *H*. (*Coelambus*) *femoratus* (Fall, 1901), which is likely a junior synonym of *H*. (*Coelambus*) *nubilus* (LeConte, 1855) (see Anderson, 1983). Additionally, we have studied a selection of species of *Herophydrus* and *Hyphoporus* (see Table 1 for the studied material).

Species for which no molecular data were available were considered to be closely related to those showing a high morphological similarity, based both on external characters and on the female and male genitalia. For species without obvious close relatives we identified diagnostic characters or character combinations for the main clades in the phylogeny, and placed these species according to the presence or absence of these characters. We used Mesquite v3.20 (Maddison & Maddison, 2017) to manually place all species in their estimated position in the phylogenetic tree (used as a backbone tree), and collapsed uncertain nodes to create polytomies.

Throughout the text of the present work, we follow the classification and nomenclature of Nilsson & Hájek (2017a, b) until we introduce our new classification. The following abbreviations are used in the text: TL (total length) and MW (maximum width); MNHN is used for “Muséum National d’Histoire Naturelle, Paris, France” and FSCA for “Florida State Collection of Arthropods, Gainesville, Florida, USA”.

A new classification of the tribe Hygrotini

According to the phylogenetic results of Villastrigo *et al*. (2018, Chapter 2) the monophyly of Hygrotini is recovered with strong support, as well as the division of Hygrotini into two clades (see Fig. 1; here the former generic and subgeneric names are still used): (A) three Nearctic and one Palaearctic species of subgenera *Hygrotus* and *Coelambus* and (B) the remaining species of the tribe. The latter was in turn divided into four further clades: (B1) a group of Palaearctic species of *Coelambus* including *Hygrotus* (*Coelambus*) *confluens* (Fabricius, 1787) (the type species of *Coelambus*), (B2) the two sampled species of *Hyphoporus* (including its type species, *H. solieri*), (B3) a large group of species including *Heroceras*, all sampled *Herophydrus* (including its type species *H. guineensis*) and most species of *Hygrotus* s. str. (including its type species *H. inaequalis*) and (B4) the remaining species of *Coelambus*, in turn divided into two sister clades, one with mostly Palaearctic species and a second with mostly Nearctic species. The internal phylogeny of the main clades was in general in good agreement with the recognised species groups among *Hygrotus* and *Coelambus* based on morphology (see e.g. Anderson, 1971, 1976, 1983; Fery 1992, 1995, 2003).

Given the para- or polyphyly of the genera *Hygrotus* and *Herophydrus* and of the subgenus *Coelambus* in their current concepts, we provide here a new classification of the tribe with the aim to avoid para- or polyphyletic genera and subgenera. This section includes brief descriptions of the principal diagnostic characters of the newly classified taxa, which were delimited according to the main clades of the phylogeny (see Table 1 for a complete checklist of the species of the tribe, and Fig. 2 for a dendrogram representing graphically the new classification).

Tribe Hygrotini Portevin, 1929: 180, as tribe of subfamily Hydroporinae.

Type genus: *Hygrotus* Stephens, 1828: 38.

Diagnosis: Within Hydroporinae, species of Hygrotini are usually characterised by the following combination of characters:

- metepisternum (metepiventrite in Miller & Bergsten, 2016: 139) reaching mesocoxal cavities, not separated by mesepimeron (in contrast to members of Vatellini);
- apices of elytra and last abdominal ventrite not acuminate (in contrast to members of Methlini);
- prosternal process elongate with apex narrowly pointed or rounded (in contrast to members of Hydrovatini);
- dorsal (anterior) margin of metafemur separated from metacoxal lobe by metatrochanter (in contrast to members of Laccornellini and Laccornini);

- humeral portion of epipleuron with oblique carina delimiting genicular fossa (character shared with members of Hydrovatini, Hyphydrini and genus *Rhithrodytes* Bameul, 1989 in Hydroporini; cf. Fery, 2013, 2016; see Fig. 53 for *Rhithrodytes agnus* Foster, 1992, and Figs 49–52 for some *Hygrotus* species).
- metatarsal claws equal in length, with exception of members of *C. saginatus*-group (see Fery, 1992, 1995, 2003) and in contrast to members of Hyphydrini and Pachydrini (see e.g. Pederzani, 1995; Miller & Bergsten, 2016).

None of these characters is, however, an unambiguous synapomorphy of the group. We introduce here a potentially unambiguous synapomorphy of tribe Hygrotini, recognised by one of us (H.F.). In many members of Hydroporinae the antennal cavities in the fronto-lateral part of the head are rather deep and more or less conical (much flatter e.g. in Hyphydrini), allowing the movement of the first antennomere (the scape). These cavities are delimited dorsally by the anterior border of the clypeus ("b" in Figs 3–6). Inside each cavity there is a capsule in which the base of the scape (the condyle) is articulated. This capsule is delimited by a distinct more or less circular carina ("a" in Figs 3–6). In all studied species of Hygrotini there is an additional - more or less semicircular - carina ("c" in Figs 4–6) which is closer to the border of the clypeus and surrounds in part the other carina. In the species of Hydroporini this additional carina is not present (see Fig. 3 for *Hydroporus dorsalis* (Fabricius, 1787) or fig. 33 in Fery & Bouzid 2016 for *Tassilodytes parisii* (Gridelli, 1939)). In a few species of Hygrotini the second carina is difficult to observe or is very narrow (as in e.g. *Heroceras descarpentriesi* and *Hygrotus* (*Coelambus*) *salinarius*), but is nevertheless clearly perceptible when the specimens are properly illuminated and orientated. However, in most specimens studied of *Hygrotus* (*Coelambus*) *masculinus* (Crotch, 1874), the second carina is reduced to a short piece near the mandible.

According to the phylogeny reconstructed in Villastrigo *et al.* (2018, Chapter 2) the tribe Hygrotini is divided into two well supported monophyletic lineages, which are considered here with generic rank. Each of these two lineages is in turn divided into generally well supported clades, which are treated as subgenera. In some cases these clades have lower support in the molecular phylogeny, and are also not well defined morphologically (see below and Appendix for a discussion on the clypeal bead, the main character used so far to differentiate genera and subgenera within Hygrotini), so we opted for a subgeneric rather than a generic rank.

Due to the new classification 67 species are for the first time included in the genus *Clemnius* **n. gen**. or in *Hygrotus*, thus their names becoming new combinations. We have listed all these species in Table 1 marked with "**n. comb.**" These changes have also generated some homonymies which will be resolved in a separate work (H. Fery, manuscript in preparation).

The genera and subgenera are treated below in the same order as in Fig. 2, starting from the lower part of the figure.

Genus *Clemnius* n. gen.

Type species: *Hyphydrus decoratus* Gyllenhal, 1810: XVI, by present designation

The new genus *Clemnius* **n. gen.** includes eight described species distributed in the Nearctic (including the north of Mexico, sometimes treated as Neotropical) and in the Palaearctic zoogeographical region (Fig. 2 and Table 1).

Diagnosis: In the new classification *Clemnius* **n. gen**. is one of two genera of the tribe Hygrotini. All species of this and the second genus *Hygrotus* have two carinae in each antennal cavity (see Figs 4–6), a unique character among the entire subfamily Hydroporinae. The new genus—which corresponds to clade A in Fig. 1—is a heterogeneous assembly of relatively small species with different morphological characters. It is subdivided into two sister-clades each treated as a subgenus: *Cyclopius* **n. subgen.** and *Clemnius* s. str. **n. subgen.** (see Fig. 2).

Subgenus *Cyclopius* n. subgen.

Type species: *Hydroporus acaroides* LeConte, 1855: 294, by present designation.

The subgenus *Cyclopius* **n. subgen.** contains three species of the former subgenus *Hygrotus* (see Fig. 2 and Table 1).

Diagnosis: Body shape subglobose (TL/MW ca. 1.35–1.6); body size small (TL 2.1–2.6 mm) (cf. Fig. 7 for *Clemnius* (*Cyclopius*) *acaroides*). Head with anterior clypeal margin truncate and slightly emarginated, border not produced forwards; bead broadly interrupted medially (*C*. (*Cyclopius*) *acaroides* and *C*. (*Cyclopius*) *marginipennis* (Blatchley, 1912)) or absent (*C*. (*Cyclopius*) *farctus* (LeConte, 1855)); antennomeres simple, not broadened. Elytra with margin in lateral view rather strongly ascending to shoulder (similar to Fig. 54); epipleuron comparably broad, broader than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably small angle (< ca. 135°; similar to Fig. 49). Colour pattern of elytra variable, from yellowish with dark vittae to more or less uniformly dark brownish or blackish; venter brownish to dark brownish, partly paler.

Males with last abdominal ventrite with deep medial depression, its anterior margin with two long spines projecting backwards and more posteriorly with two rather short ones directed perpendicular to surface (see fig. 24B in Larson *et al*., 2000), a unique character in Hygrotini and also in Dytiscidae. Median lobe symmetric, robust in *C*. (*Cyclopius*) *acaroides* (Fig. 19) and *C*. (*Cyclopius*) *marginipennis*, but very thin in *C*. (*Cyclopius*) *farctus*. Parameres with condylar process short, forming an obtuse angle with distal part (see Fig. 29; cf. figs. 10–12 in Anderson 1971). Male metatarsal claws of equal length.

Etymology: From the Greek χύκλος (kyklos = circle), referring to the semicircular shape of the clypeus of the species of the subgenus. The gender of the name is masculine.

Distribution: Nearctic: Canada and USA.

Main habitat types: Species of this subgenus are typically known from lentic freshwater habitats; Blatchley (1912: 330) reported *C*. (*Cyclopius*) *marginipennis* from “shallow brackish ponds”.

Subgenus *Clemnius* s. str. n. subgen.

Type species: *Hyphydrus decoratus* Gyllenhal, 1810: XVI, by present designation.

The subgenus *Clemnius* **n. subgen.** contains five species of the former genus *Hygrotus*, subgenera *Hygrotus* and *Coelambus* (see Fig. 2 and Table 1).

Diagnosis: Body shape oval, either almost globose (TL/MW ca. 1.6) (see Fig. 10 for *C.* (s. str.) *decoratus*) or more elongated, “navicular” (TL/MW 1.7–1.8) (see Fig. 11 for *C.* (s. str.) *laccophilinus* (LeConte, 1878)), with MW short behind pronotum and distinctly before mid-length (still somewhat more elongated in *C*. (s. str.) *berneri* Young & Wolfe, 1984, with TL/MW ca. 2.05; see Fig. 12 for holotype and its labels; FSCA); body size small (TL 2.1–3.3 mm). *Clemnius* (s. str.) *decoratus* and *C*. (s. str.) *hydropicus* (LeConte, 1852) with anterior clypeal margin evenly and semicircularly rounded, with border produced forwards and bead complete as in subgenus *Hygrotus* s. str. In Contrast *Clemnius* (s. str.) *laccophilinus*, *C.* (s. str.) *berneri* and *C*. (s. str.) *sylvanus* (Fall, 1917) with anterior clypeal margin truncate and slightly emarginated, with border not produced forwards and bead absent. Antennomeres simple, not broadened. Elytra with margin in lateral view moderately ascending to shoulder (similar to Fig. 55); epipleuron comparably broad, broader than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably small angle (< ca. 135°; similar to Fig. 49). Elytra either dark, uniformly testaceous or with yellowish-brown dots, but not vittate; venter brown or testaceous. Last abdominal ventrite without deep depression.

Aedeagus with median lobe symmetric, robust in *C*. (s. str.) *decoratus* (Fig. 21) and *C*. (s. str.) *hydropicus* (cf. fig. 13 in Anderson 1971) or distally very narrow in *C*. (S. str.) *berneri* (Fig. 20), *C*. (s. str.) *laccophilinus* and *C.* (s. str.) *sylvanus* (cf. also figs 1A and 2A in Anderson 1976). Parameres with condylar process rather short and forming an obtuse angle with distal part (Figs 30 and 31; cf. also figs 1B and 2B in Anderson 1976). Male metatarsal claws of equal length.

Etymology: From the Greek χλεμμύς (klemmys = tortoise); referring to the almost hemispherical body shape of most species. The gender of the generic name is masculine.

Distribution: Palaearctic (Europe, Russia and Kazakhstan), Nearctic and Neotropical (northern part of Mexico).

Main habitat types: The subgenus includes species typical of lentic freshwater environments.

Notes: Within the subgenus there are two clear groups of species according to body shape and genital morphology, one formed by *C*. (s. str.) *decoratus* and *C*. (s. str.) *hydropicus* (body shape more globular, aedeagus robust with short medial lobe), and the other by *C*. (s. str.) *berneri*, *C*. (s. str.) *laccophilinus* and *C*. (s. str.) *sylvanus* (body shape more elongated, “navicular”, aedeagus slender with elongated median lobe). The phylogenetic relationships of the species for which molecular data were available are poorly supported (Fig. 1), but in any case we never recovered these two groups as respectively monophyletic. We thus refrain from splitting *Clemnius* **n. subgen.** into two taxa corresponding to these two groups, at least until more evidence becomes available.

Genus *Hygrotus* Stephens, 1828

Type species: *Dytiscus inaequalis* Fabricius, 1777: 239, by subsequent designation of Curtis (1835: pl. 531).

Diagnosis: In the new classification *Hygrotus* is the second of two genera of the tribe Hygrotini. As in *Clemnius* **n. gen**. all species of the genus have two carinae in each antennal cavity (see Figs 4–6).

Table 1. Checklist of all 137 species of Hygrotini with original, new and former (Nilsson & Hájek, 2017a) generic and subgemeric names; last three columns indicating new combinations, distribution and methods applied to material studied. See Villastrigo *et al*. (2018) for details on the specimens used in the molecular phylogeny and accession numbers of the sequences.

n	species	authors	original genus	genus in this work	subgenus in this work	genus in Nilsson & Hájek 2017a	subgenus in Nilsson & Hájek 2017a	new combination	distribution	methods applied
1	*acaroides*	(LeConte, 1855)	*Hydroporus*	*Clemnius*	*Cyclopius*	*Hygrotus*	*Hygrotus*	n. comb	NA	morphological & molecular
2	*aequalis*	Falkenström, 1932	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	-	PL	morphological
3	*ahmeti*	Hájek, Fery & Erman, 2005	*Hygrotus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
4	*anitae*	(Vazirani, 1969)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb	PL	only literature
5	*aper*	(Sharp, 1882)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb	OR, PL	only literature
6	*armeniacus*	(Zaitzev, 1927)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
7	*artus*	(Fall, 1919)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	only literature
8	*assimilis*	(Régimbart, 1895)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb	AF	morphological & molecular
9	*bengalensis*	(Severin, 1890)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb	OR	morphological
10	*berneri*	Young & Wolfe, 1984	*Hygrotus*	*Clemnius*	*Clemnius*	*Hygrotus*	*Coelambus*	n. comb	NA	morphological
11	*bertrandi*	(Vazirani, 1969)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	.	n. comb	PL	only literature
12	*bilardoi*	(Biström & Nilsson, 2002)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb	AF	only literature
13	*bruesi*	(Fall, 1928)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
14	*caliginosus*	(Régimbart, 1899)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb	OR	only literature
15	*capensis*	(Régimbart, 1895)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb	AF	only literature
16	*caspius*	(Wehncke, 1875)	*Hydroporus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
17	*chinensis*	(Sharp, 1882)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological
18	*cleopatrae*	(Peyron, 1858)	*Hydroporus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	-	PL	morphological
19	*collatus*	(Fall, 1919)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological
20	*compar*	(Fall, 1919)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
21	*confluens*	(Fabricius, 1787)	*Dytiscus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
22	*confusus*	(Régimbart, 1895)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb	AF	only literature
23	*corpulentus*	(Schaum, 1864)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
24	*curvilobus*	Fery, Sadegui & Hosseinie, 2005	*Hygrotus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological

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Table 1. (Continued)

n	species	authors	original genus	genus in this work	subgenus in this work	genus in Nilsson & Hájek 2017a	subgenus in Nilsson & Hájek 2017a	new combination	distribution	methods applied
25	*curvipes*	(Leech, 1938)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological
26	*decoratus*	(Gyllenhal, 1810)	*Hyphydrus*	*Clemnius*	*Clemnius*	*Hygrotus*	*Hygrotus*	n. comb.	PL	morphological & molecular
27	*dehraduni*	(Vazirani, 1969)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	PL	only literature
28	*descarpentriesi*	(Peschet, 1923)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Heroceras*	-	n. comb.	AF	morphological & molecular
29	*discrepatus*	(Guignot, 1954)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
30	*dissimilis*	(Geminger & Harold, 1868)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological
31	*diversipes*	Leech, 1966	*Hygrotus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
32	*elevatus*	(Sharp, 1882)	*Hydroporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR, PL	morphological
33	*endroedyi*	(Biström & Nilsson, 2002)	*Herophydurs*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
34	*enneagrammus*	(Ahrens, 1833)	*Hydroporus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*	-	PL	morphological
35	*falli*	(Wallis, 1924)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological
36	*farctus*	(LeConte, 1855)	*Hydroporus*	*Clemnius*	*Cyclopius*	*Hygrotus*	*Hygrotus*	n. comb.	NA	morphological
37	*femoratus*	(Fall, 1901)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	only literature
38	*flaviventris*	(Motschulsky, 1860)	*Hydroporus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
39	*fontinalis*	Leech, 1966	*Hygrotus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
40	*fraternus*	(LeConte, 1852)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA, NT	morphological
41	*fresnedai*	(Fery, 1992)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
42	*fumatus*	(Sharp, 1882)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
43	*geetae*	(Vazirani, 1969)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR	only literature
44	*gigantoides*	(Biström & Nilsson, 2002)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
45	*gigas*	(Régimbart, 1895)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	morphological
46	*goldschmidti*	(Pederzani & Rocchi, 2009)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
47	*gschwendtneri*	(Omer-Cooper, 1957)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
48	*guineensis*	(Aubé, 1838)	*Hyphydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	-	AF, PL	morphological & molecular
49	*heros*	(Sharp, 1882)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	-	AF	morphological

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Table 1. (Continued)

n	species	authors	original genus	genus in this work	subgenus in this work	genus in Nilsson & Hájek 2017a	subgenus in Nilsson & Hájek 2017a	new combination	distribution	methods applied
50	*hydropicus*	(LeConte, 1852)	*Hydroporus*	*Clemnius*	*Clemnius*	*Hygrotus*	*Hygrotus*	n. comb.	NA, NT	morphological & molecular
51	*hyphoporoides*	(Régimbart, 1895)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
52	*ignoratus*	(Gschwendtner, 1933)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
53	*impressopunctatus*	(Schaller, 1783)	*Dytiscus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	H	morphological & molecular
54	*inaequalis*	(Fabricius, 1777)	*Dytiscus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	-	PL	morphological & molecular
55	*infuscatus*	(Sharp, 1882)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological
56	*inquinatus*	(Boheman, 1848)	*Hydroporus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	morphological & molecular
57	*inscriptus*	(Sharp, 1882)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological
58	*intermedius*	(Fall, 1919)	*Coelambus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	-	NA	morphological
59	*janssensi*	(Guignot, 1952)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
60	*josephi*	(Vazirani, 1969)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR	only literature
61	*kalaharii*	(Gschwendtner, 1935)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
62	*kempi*	(Gschwendtner, 1936)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR, PL	only literature
63	*laccophilinus*	(LeConte, 1878)	*Hydroporus*	*Clemnius*	*Clemnius*	*Hygrotus*	*Coelambus*	n. comb.	NA	morphological & molecular
64	*lagari*	(Fery, 1992)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
65	*lernaeus*	(Schaum, 1857)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
66	*lutescens*	(LeConte, 1852)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA, NT	morphological & molecular
67	*marginipennis*	(Blatchley, 1912)	*Coelambus*	*Clemnius*	*Cyclopius*	*Hygrotus*	*Hygrotus*	n. comb.	NA	morphological
68	*marklini*	(Gyllenhal, 1813)	*Hyphydrus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	H	morphological & molecular
69	*masculinus*	(Crotch, 1874)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
70	*montanus*	(Régimbart, 1899)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR	only literature
71	*morandi*	(Guignot, 1952)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	OR	only literature
72	*musicus*	(Klug, 1834)	*Hydroporus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	-	AF, OR, PL	morphological & molecular
73	*muticus*	(Sharp, 1882)	*Coelambus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	-	AF	morphological & molecular
74	*natator*	(Biström & Nilsson, 2002)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature

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Table 1. (Continued)

n	species	authors	original genus	genus in this work	subgenus in this work	genus in Nilsson & Hájek 2017a	subgenus in Nilsson & Hájek 2017a	new combination	distribution	methods applied
75	*nigrescens*	(Biström & Nilsson, 2002)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	morphological & molecular
76	*nigrescens*	(Fall, 1919)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
77	*nigrolineatus*	(Steven, 1808)	*Hyphydrus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
78	*nilghiricus*	(Régimbart, 1903)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR, PL	only literature
79	*nodieri*	(Régimbart, 1895)	*Coelambus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	-	AF	morphological & molecular
80	*novemlineatus*	(Steven, 1829)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	H	morphological & molecular
81	*nubilus*	(LeConte, 1855)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA, NT [PC]	morphological
82	*obscureplagiatus*	(Fall, 1919)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological
83	*obscurus*	(Sharp, 1882)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	morphological & molecular
84	*obsoletus*	(Régimbart, 1895)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
85	*orthogrammus*	(Sharp, 1882)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
86	*oudomxai*	(Brancucci & Biström, 2013)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR	only literature
87	*ovalis*	(Gschwendtner, 1932)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
88	*pacistanus*	(Guignot, 1959)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	PL	only literature
89	*pallidulus*	(Aubé, 1850)	*Hydroporus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
90	*pallidus*	(Omer-Cooper, 1931)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
91	*parallellogrammus*	(Ahrens, 1812)	*Dytiscus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
92	*patruelis*	(LeConte, 1855)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
93	*pauliani*	(Guignot, 1950)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
94	*pectoralis*	(Motschulsky, 1860)	*Hydroporus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
95	*pedalis*	(Fall, 1901)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
96	*picatus*	(Kirby, 1837)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
97a	*polonicus polonicus*	(Aubé, 1842)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological
97b	*polonicus sahlbergi*	(Sharp, 1882)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological
98	*pugnator*	(Sharp, 1890)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR	only literature

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Table 1. (Continued)

n	species	authors	original genus	genus in this work	subgenus in this work	genus in Nilsson & Hájek 2017a	subgenus in Nilsson & Hájek 2017a	new combination	distribution	methods applied
99	*punctilineatus*	(Fall, 1919)	*Coelambus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*	-	NA	morphological
100	*quadrilineatus*	(Régimbart, 1895)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	morphological
101	*quinquelineatus*	(Zetterstedt, 1828)	*Hyphydrus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	-	PL	morphological & molecular
102	*reticulatus*	(Pedernazi & Rocchi, 2009)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	morphological & molecular
103	*ritsemae*	(Régimbart, 1889)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
104	*rohani*	(Peschet, 1924)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	molecular
105	*rufus*	(Clark, 1863)	*Hyphydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	morphological
106	*saginatus*	(Schaum. 1857)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
107	*salinarius*	(Wallis, 1924)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
108	*sanfilippoi*	(Fery, 1992)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
109	*sayi*	J. Balfour-Browne, 1944	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	-	NA	morphological & molecular
110	*sellatus*	(LeConte, 1866)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological
111	*semenowi*	(Jakovlev, 1899)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological
112	*semivittatus*	(Fall, 1919)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
113	*severini*	(Régimbart, 1892)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR, PL	morphological
114	*sjostedti*	(Régimbart, 1908)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
115	*solieri*	(Aubé, 1838)	*Hydroporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	-	PL	morphological & molecular
116	*spadiceus*	(Sharp, 1882)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	molecular
117	*stefanschoedli*	Fery, Sadegui & Hosseinie, 2005	*Hygrotus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological
118	*subaequalis*	(Vazirani, 1969)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR	only literature
119	*sudanensis*	(Guignot, 1952)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
120	*suturalis*	(LeConte, 1850)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
121	*sylvanus*	(Fall, 1917)	*Coelambus*	*Clemnius*	*Clemnius*	*Hygrotus*	*Coelambus*	n. comb.	NA	morphological
122	*thermarum*	(Darlington, 1928)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological
123	*tonkinensis*	(Régimbart, 1899)	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	-	n. comb.	OR	morphological & molecular

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Table 1. (Continued)

n	species	authors	original genus	genus in this work	subgenus in this work	genus in Nilsson & Hájek 2017a	subgenus in Nilsson & Hájek 2017a	new combination	distribution	methods applied
124	*travniceki*	(Šťastný, 2012)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	morphological
125	*tribolus*	(Guignot, 1953)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	morphological & molecular
126	*tumidiventris*	(Fall, 1919)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
127	*turbidus*	(LeConte, 1855)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA	morphological & molecular
128	*unguicularis*	(Crotch, 1874)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	H	morphological & molecular
129	*urgensis*	(Jakovlev, 1899)	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological & molecular
130a	*variabilis secundus*	(Régimbart, 1906)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
130b	*variabilis variabilis*	(Guignot, 1954)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
131	*vaziranii*	(Nilsson, 1999)	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	-	PL	only literature
132	*versicolor*	(Schaller, 1783)	*Dytiscus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	-	PL	morphological & molecular
133	*verticalis*	(Sharp, 1882)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	morphological & molecular
134	*vittatus*	(Régimbart, 1895)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
135	*wardii*	(Clar,¡k, 1862)	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	NA, NT	morphological
136	*wewalkai*	(Biström & Nilsson, 2002)	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	-	n. comb.	AF	only literature
137	*zigetangco*	Fery, 2003	*Hygrotus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*	-	PL	morphological

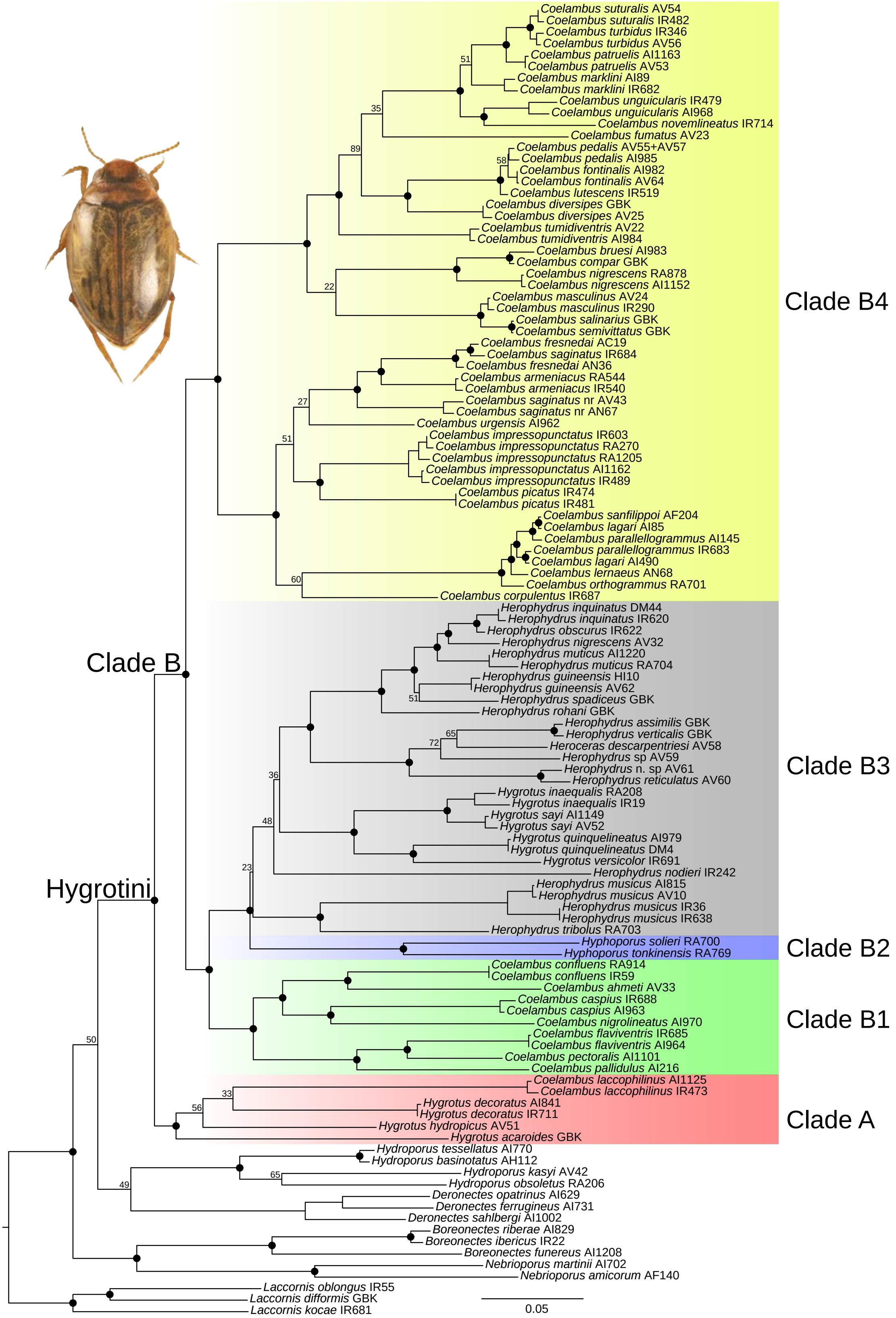


Figure 1. Phylogeny of Hygrotini, modified from Villastrigo *et al*. (2018, Chapter 2). Numbers above nodes are bootstrap support values. Note that here the former generic and subgeneric names are still used.

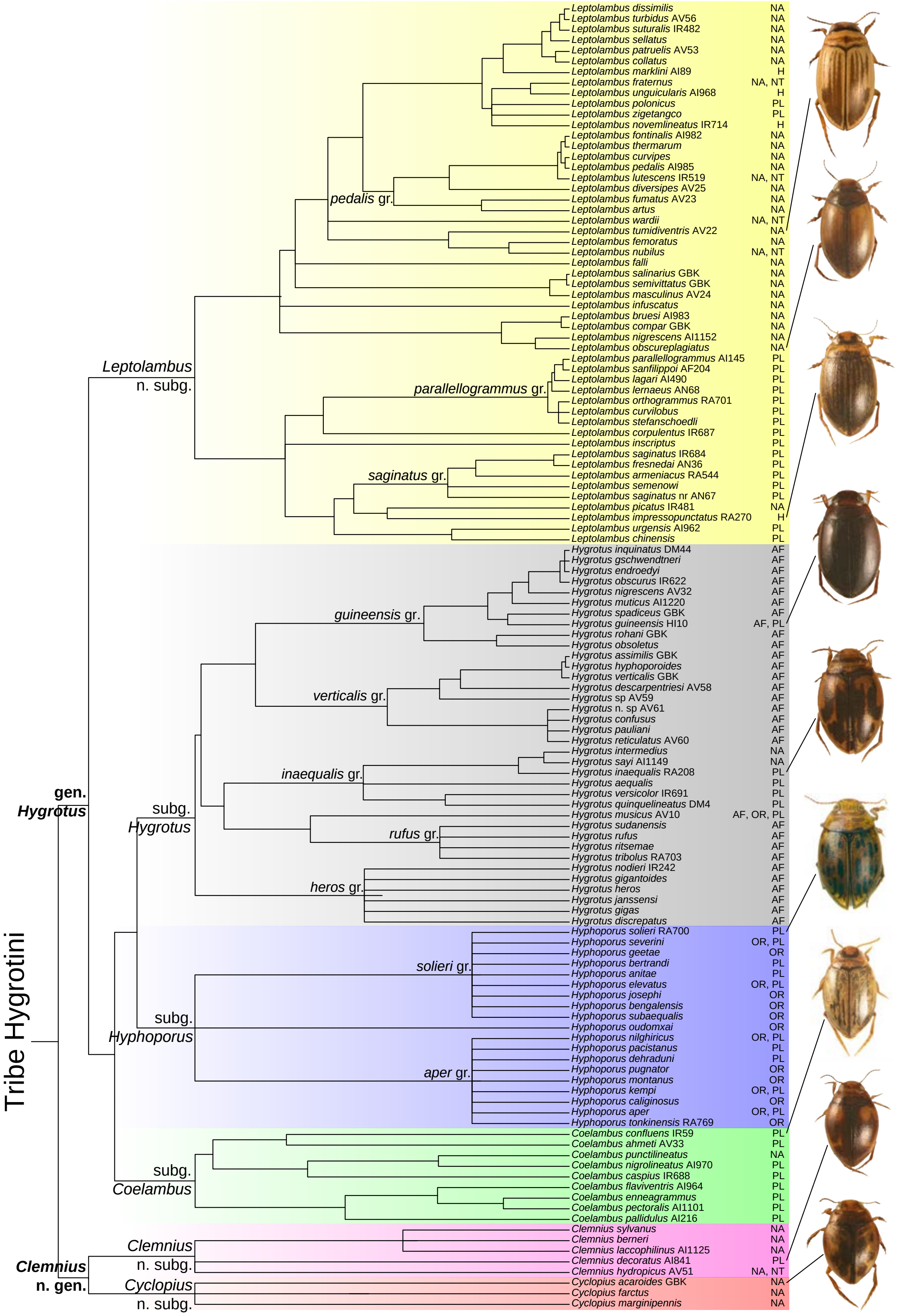
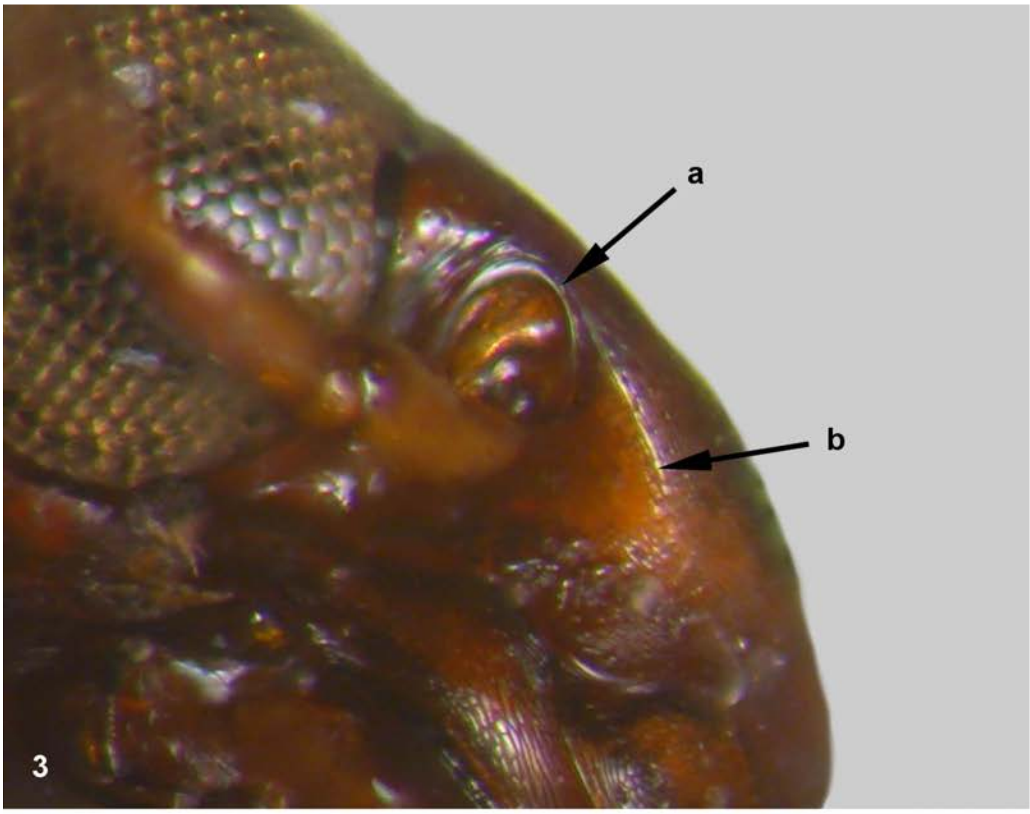
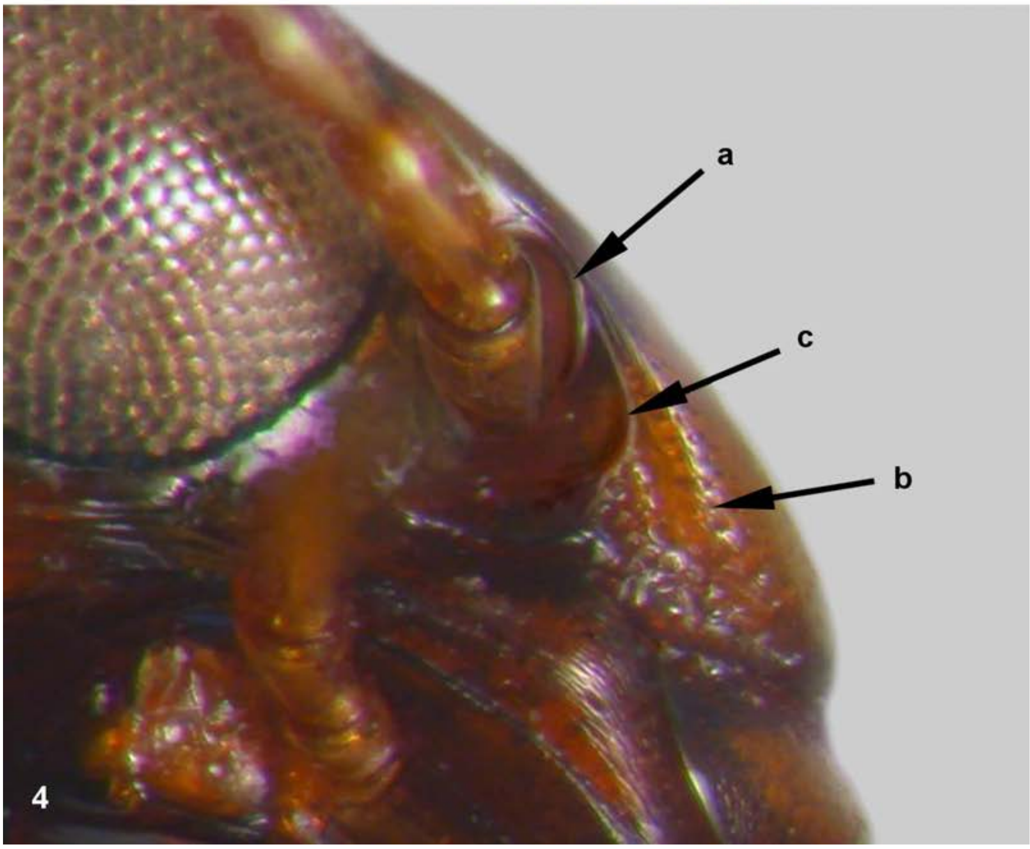
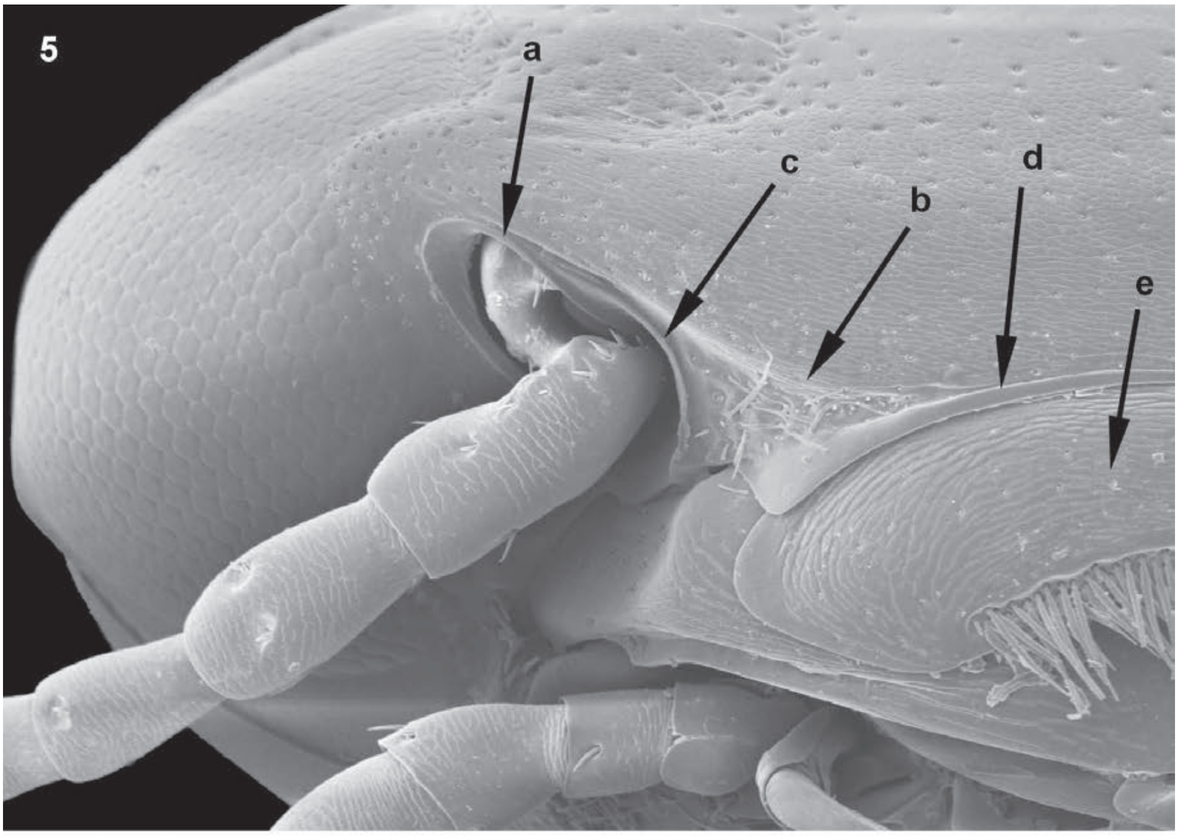


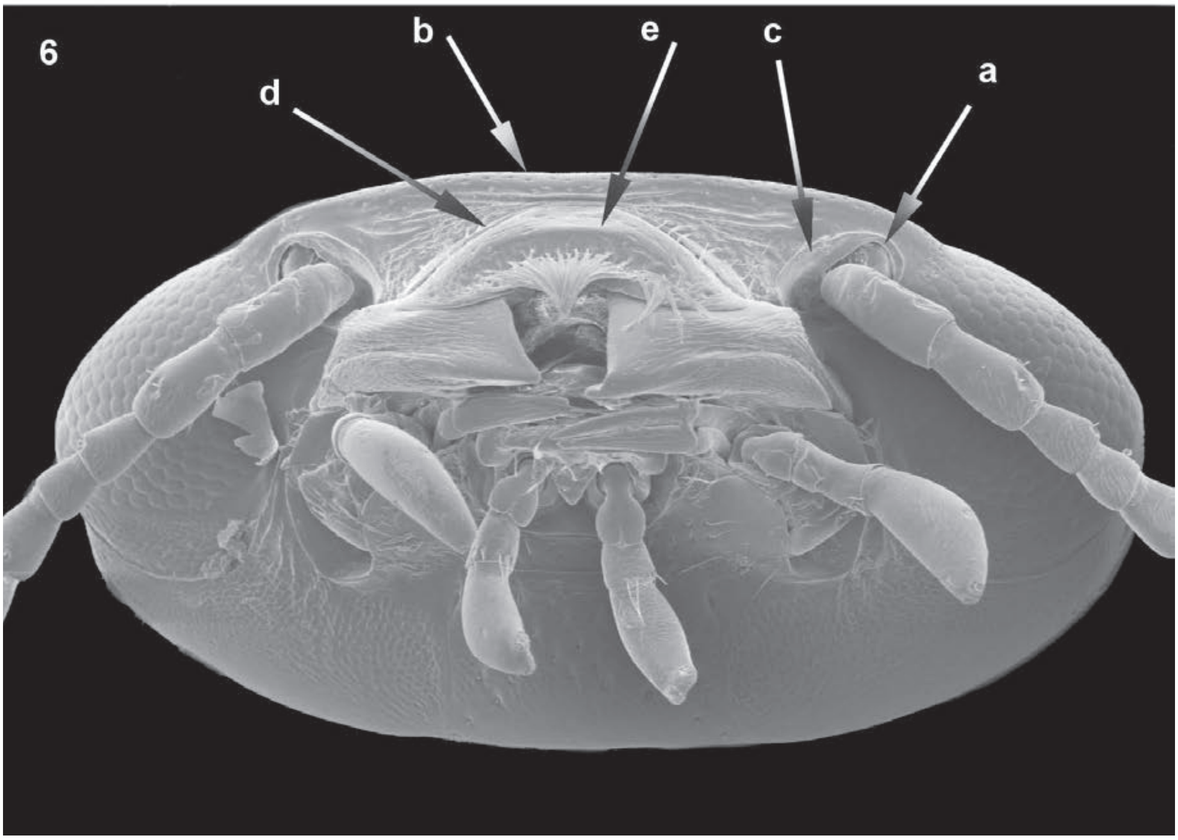
Figure 2. Dendrogram showing the new classification of Hygrotini. A number of species without molecular data (no voucher number) have been inserted near morphologically similar species with molecular data. Acronyms refer to biogeographical regions.





Figures 3-4. Antennal cavities of: (3) *Hydroporus dorsalis* (Hydroporini) and (4) *Hygrotus* (*Leptolambus*) *lagari* (Fery, 1992) (a: carina delimiting capsule for scape; b: fronto-lateral border of clypeus; c: second carina).





Figures 5-6. SEM micrographs with details of head and antennal cavities of: (5) *Hygrotus* (*Leptolambus*) *impressopunctatus* (frontal view) and (6) *Hygrotus* (s. str.) *inaequalis* (oblique ventral view) (a, b and c as in Figs 3-4: d: clypeal stripe; e: labrum).

According to the two main clades in the phylogeny of Hygrotini and several morphological characters, the genus (which corresponds to Clade B in Fig. 1) is subdivided into four clades corresponding to the following four subgenera: clade B1 = subgenus *Coelambus*, clade B2 = subgenus *Hyphoporus*, clade B3 = subgenus *Hygrotus* s. str. and clade B4 = *Leptolambus* **n. subgen.**

Subgenus *Coelambus* Thomson, 1860

Type species: *Dytiscus confluens* Fabricius, 1787: 193, by subsequent designation of Zaitzev (1953: 129).

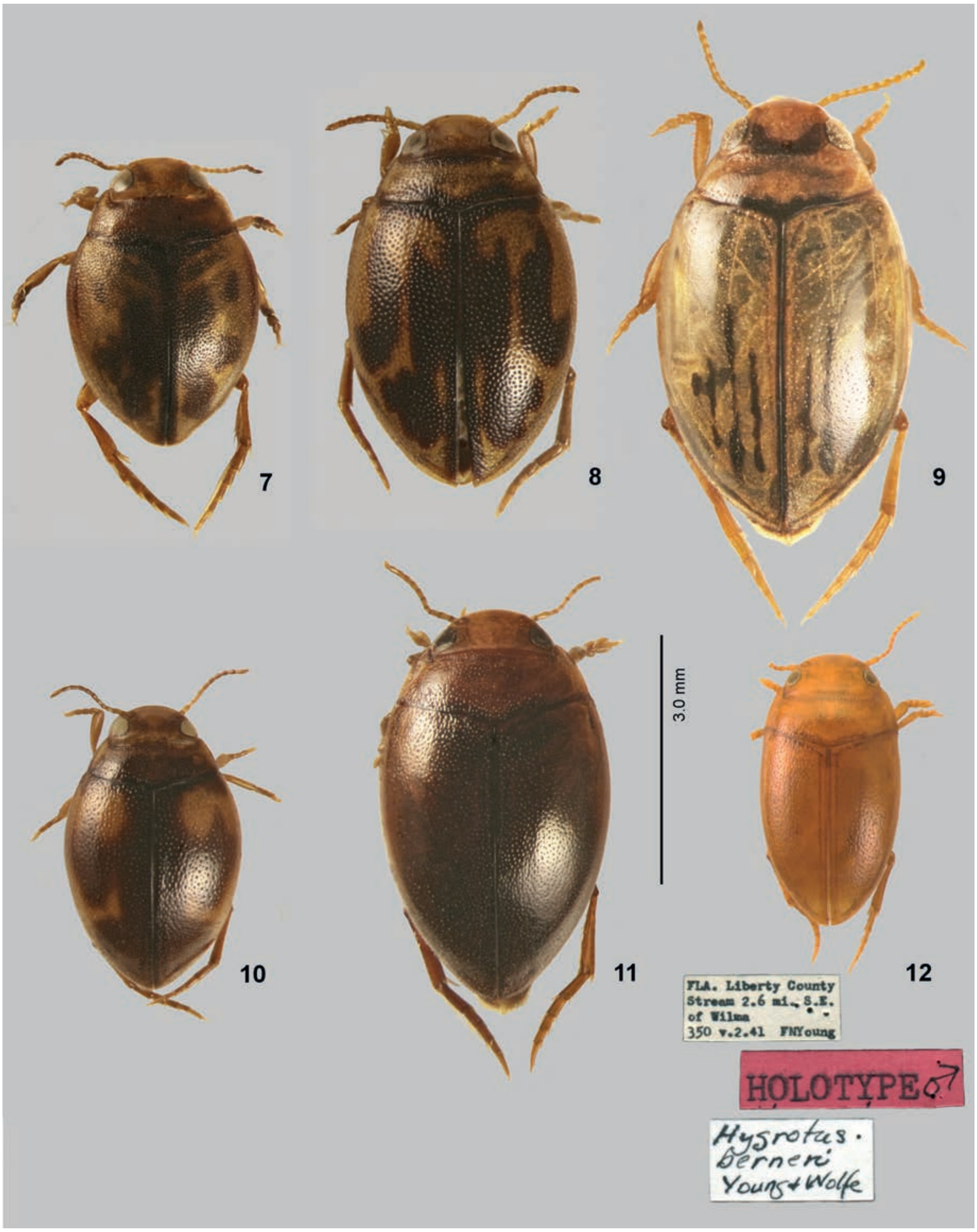
The subgenus *Coelambus* contains eight Palaearctic and one Nearctic species (*Hygrotus* (*C.*) *punctilineatus* (Fall, 1919)).

Diagnosis: Body shape moderately to elongate oval (TL/MW ca. 1.7–2.0); small to medium sized species (TL 3.0–4.5 mm) (see Fig. 9 for *H.* (*Coelambus*) *confluens*). Head with anterior clypeal margin truncate and slightly emarginated, border not produced forwards; bead absent. Antennomeres simple, not broadened. Elytra with margin in lateral view strongly ascending to shoulder (see Fig. 54 for *H*. (*Coelambus*) *caspius* (Wehncke, 1875)); epipleuron comparably narrow, narrower than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably wide angle (< ca. 150°; similar to Fig. 51). Elytra light yellowish (more yellowish brown in *H.* (*Coelambus*) *ahmeti* Hájek, Fery & Erman, 2005), distinctly vittate (in some species/individuals vittae strongly reduced in anterior half). Venter usually black, but females of some species (e.g. *H.* (*Coelambus*) *pallidulus*) with abdomen at least in part yellow or brownish. Last abdominal ventrite without deep depression.

Aedeagus with median lobe elongate, very slender (distal half very thin in lateral view), symmetric or almost symmetric (e.g. in *H.* (*Coelambus*) *enneagrammus* (Ahrens, 1833), and *H.* (*Coelambus*) *confluens*; see Figs 23 and 24). Parameres with condylar process rather long and forming an almost right angle with distal part (Figs. 37 and 38 for *H.* (*Coelambus*) *enneagrammus* and *H.* (*Coelambus*) *confluens*, respectively). Male metatarsal claws of equal length.

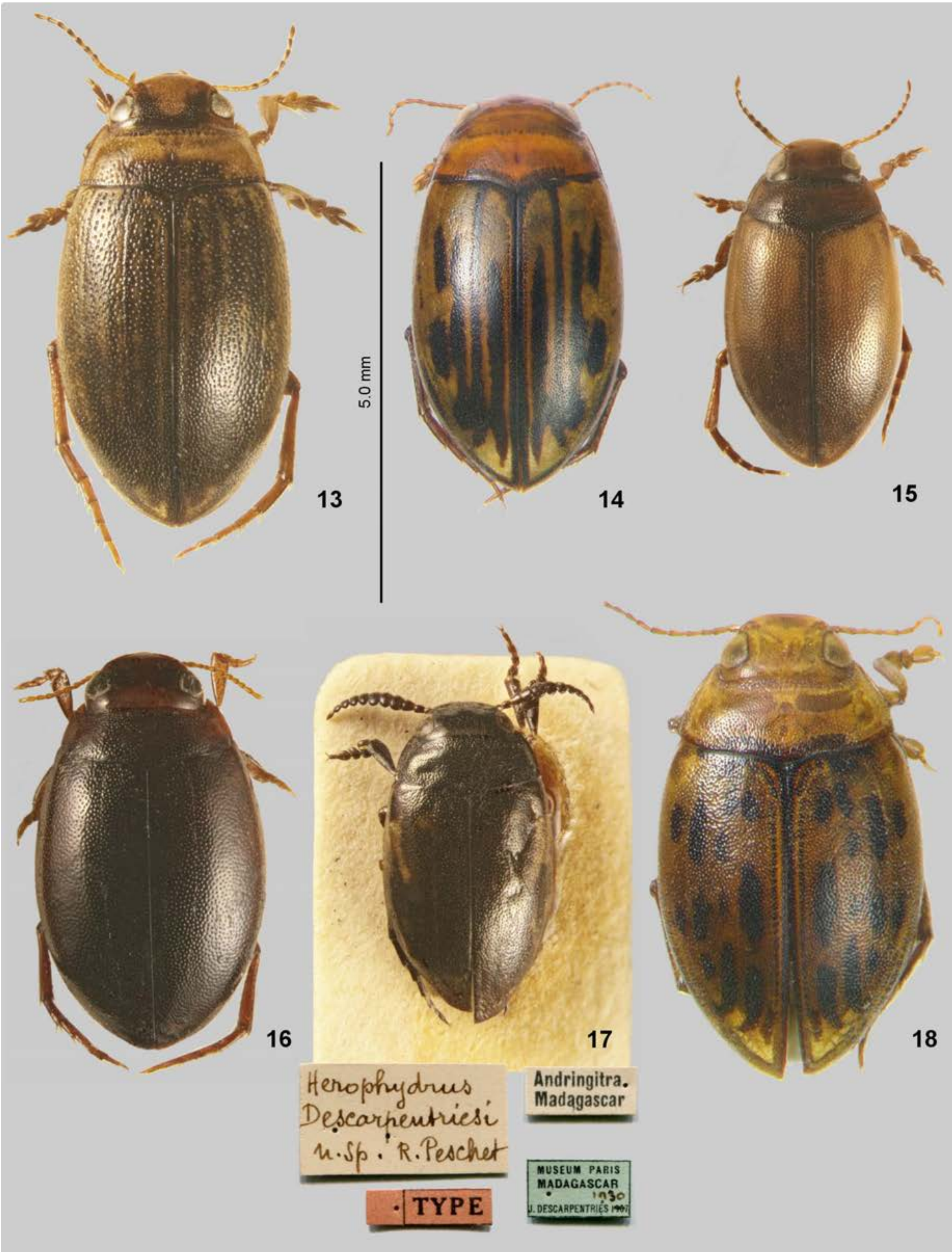
Distribution: Mainly Palaearctic, with one species in Canada and northern USA (*Hygrotus* (*Coelambus*) *punctilineatus*).

Main habitat types: The subgenus includes species typical of both lentic and lotic habitats, with varied salinity tolerance going from freshwater to hypersaline.



Figures 7-12. Habitus of: (7) *Clemnius* (*Cyclopius*) *acaroides*, (8) *Hygrotus* (s. str.) *inaequalis*, (9) *Hygrotus* (*Coelambus*) *confluens*, (10) *Clemnius* (s. str.) *decoratus*, (11) *Clemnius* (s. str.) *laccophilinus* and (12) *Clemnius* (s. str.) *berneri* (male holotype and labels).

The genus *Hygrotus* as here defined includes 129 described species (two of them bitypic) distributed in the Nearctic (including the north of Mexico), Palaearctic, Afrotropical and Oriental zoogeographical regions (Fig. 2 and Table 1). One Nearctic species was introduced to Hawaii (see below), which belongs to the Pacific region.



Figures 13-18. Habitus of: (13) *Hygrotus* (*Leptolambus*) *impresopunctatus*, (14) *H.* (*Leptolambus*) *orthogrammus*, (15) *H.* (*Leptolambus*) *obscureplagiatus*, (16) *H.* (s. str.) *guineensis*, (17) *H.* (s. str.) *descarpentriesi* (male syntype and labels) and (18) *H.* (*Hyphoporus*) *solieri* (Figs 14 and 18 are reproduced from Fery *et al*., 2012 with the permission of F. Gusenleitner, Linz, Austria).

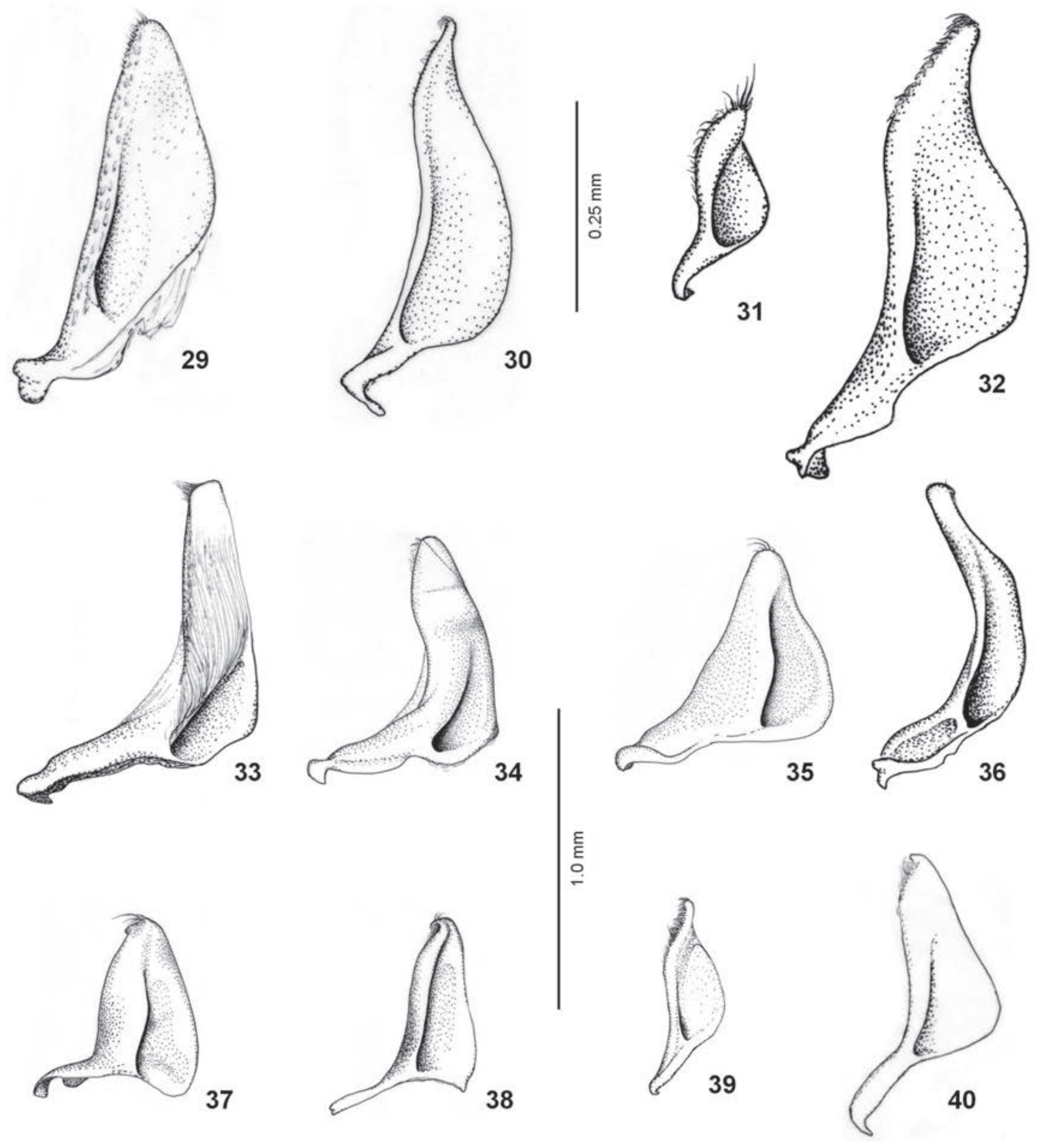
Figures 19-28. Median lbe in ventral and lateral view of: (19) *Clemnius* (*Cyclopius*) *acaroides*, (20) *Clemnius* (s. str.) *berneri*, (21) *Clemnius* (s. str.) *decoratus*, (22) *Hygrotus* (s. str.) *inaequalis*, (23) *H.* (*Coelambus*) *enneagrammus*, (24) *H.* (*Coelambus*) *confluens*, (25) *H.* (*Leptolambus*) *parallellogrammus*, (26) *H.* (*Leptolambus*) *impressopunctatus*, (27) *H.* (s. str.) *guineensis* and (28) *H.* (*Hyphoporus*) *tonkinensis* (Fig. 28 is a modified reproduction of figs 3 and 4 in Brancucci & Biström, 2013).

Subgenus *Hyphoporus* Sharp, 1880 n. stat.

Type species: *Hydroporus solieri* Aubé, 1838: 554, by monotypy.

The subgenus *Hyphoporus* contains 19 species distributed in the Oriental and Palaearctic regions (see Fig. 2 and Table 1).

Diagnosis: Body shape short oval (TL/MW ca. 1.6–1.7), rather globose; small to medium



Figures 29-40. Left paramere of: (29) *Clemnius* (*Cyclopius*) *acaroides*, (30) *Clemnius* (s. str.) *berneri*, (31) *Clemnius* (s. str.) *decoratus*, (32) *Hygrotus* (s. str.) *inaequalis*, (33) *H.* (*Leptolambus*) *fresnedai*, (34) *H.* (*Leptolambus*) *impressopunctatus*, (35) *H.* (*Leptolambus*) *parallellogrammus*, (36) *H.* (*Leptolambus*) *nubilus*, (37) *H.* (*Coelambus*) *enneagrammu,* (38) *H.* (*Coelambus*) *confluens*, (39) *H.* (s. str.) *guineensis* and (40) *H*. (*Leptolambus*) *tonkinensis* (Fig. 40 is a modified reproduction of fig 5 in Brancucci & Biström, 2013).

sized species (TL 3.5–5.6 mm) (see Fig. 18 for *H.* (*Hyphoporus*) *solieri*). Head with anterior clypeal margin truncate, straight or slightly emarginated, border not produced forwards; bead continuous, middle part narrowed in most species (see Fig. 42 for *H*. (*Hyphoporus*) *solieri*); (except *H.* (*Hyphoporus*) *bengalensis* (Severin, 1890) with continuous bead). Antennomeres simple, not broadened. Elytra with margin in lateral view moderately ascending to shoulder (similar to Fig. 55); epipleuron comparably broad, broader than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably small angle (< ca. 135°;

Figures 41-48. Head with details of clypeal rim of: (41) *Hygrotus* (*Leptolambus*) *impressopunctatus*, (42) *H.* (*Hyphoporus*) *solieri*, (43) *H.* (s. str.) *guineensis*, (44) *H.* (s. str.) *inaequalis*, (45) idem in lateral view, (46) *H.* (*Coelambus*) *confluens* (the lighter area before the anterior border of the clypeus is the "clypeal stripe"; see Appendix), (47) *H.* (*Leptolambus*) *masculinus* and (48) idem in lateral view.

similar to Fig. 50). Elytra with interrupted vittae or dotted (in some species very darkened); venter black or brown. Last abdominal ventrite without deep depression.

Aedeagus with median lobe robust, asymmetric (see Fig. 28 for *Hygrotus* (*Hyphoporus*) *tonkinensis* (Régimbart, 1899); in *H. bengalensis* median lobe more or less symmetric; see Vazirani 1969 for several further figures); parameres with condylar process of diverse length, forming an obtuse angle with distal part (see Fig. 40 for *H.* (*Hyphoporus*) *tonkinensis*). Male metatarsal claws of equal length.

Distribution: Palaearctic and Oriental, from Iran to India and south-east Asia; one species - *H.* (*Hyphoporus*) *solieri* - from Iran to the Arabian Peninsula and Egypt.

Main habitat types: There is no information on the habitat of most of the species of the subgenus, although they are likely to be mostly associated with freshwater environments. However, it shall be mentioned that Hájek (2006: 48) illustrated a rest-pool in a wadi (Pir Sohrab, Iran) where he has collected *H*. (*Hyphoporus*) *aper* (Sharp, 1882) together with *Neptosternus circumductus* Régimbart, 1899.

Subgenus *Hygrotus* Stephens, 1828

Type species: *Dytiscus inaequalis* Fabricius, 1777: 239, by subsequent designation of Curtis (1835: pl. 531).

Herophydrus Sharp, 1880: cxlviii; type species: *Hydroporus hyphydroides* Perris, 1864: 277 (= *Herophydrus guineensis* (Aubé 1838: 455)), by monotypy. **n. syn.**

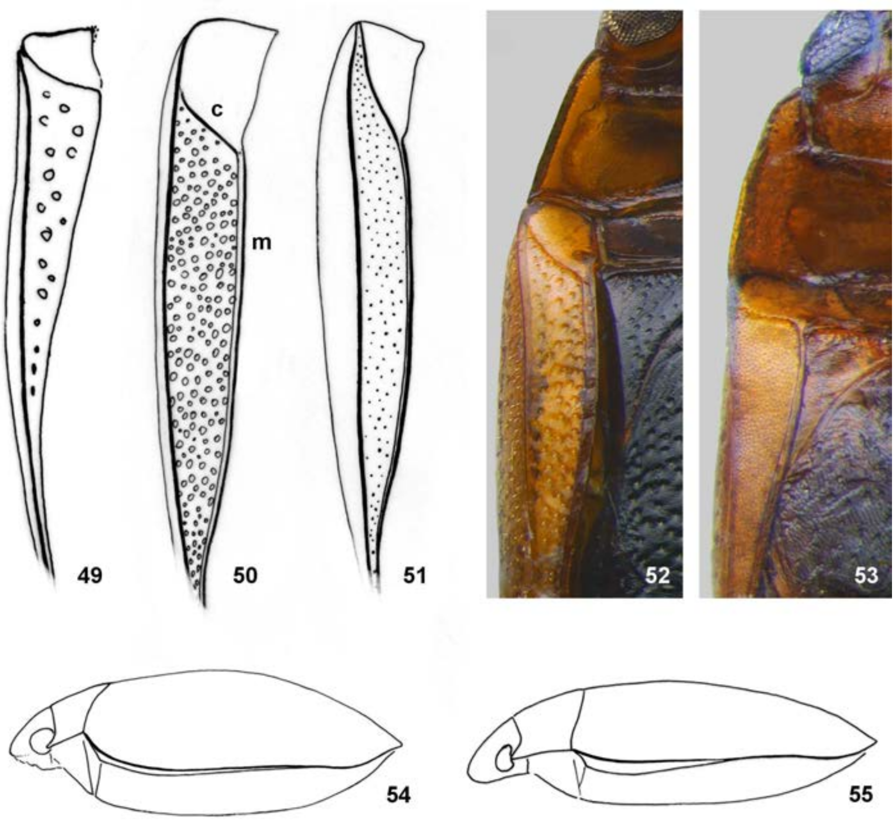
Dryephorus Guignot, 1950: 150; type species: *Coelambus nodieri* Régimbart, 1895: 37, by original designation of Guignot (1950: 150). **n. syn.**

Heroceras Guignot, 1950: 150; type species: *Herophydrus descarpentriesi* Peschet, 1923: 176, by original designation of Guignot (1950: 150). **n. syn.**

The newly defined subgenus *Hygrotus* s. str. includes six species previously included in the former subgenus *Hygrotus* s. str., the single species of former genus *Heroceras* (*H. descarpentriesi*) and all 44 species (one of them bitypic) of the former genus *Herophydrus* (see Fig. 2 and Table 1).

Diagnosis: Body shape short oval to moderately elongate oval (TL/MW ca. 1.7–1.8); species of former *Hygrotus* s. str. and *Heroceras* small (TL 2.8–3.6 mm) (see Fig. 8 for *H.* (s.

str.) *inaequalis* and Fig. 17 for *H*. (s. str.) *descarpentriesi*) and species of former *Herophydrus* small to large (TL 2.6–7.4 mm) (see Fig. 16 for *H.* (s. str.) *guineensis*). Head of species of former *Hygrotus* with anterior clypeal margin evenly and semicircularly rounded, border produced forwards and with complete bead (see Figs 44 and 45 for *H.* (s. str.) *inaequalis*); species of former *Herophydrus* and *Heroceras* with anterior clypeal margin truncate, straight or slightly emarginated, border not produced forwards; bead present, but in many species narrowed in medial part (see Fig. 43 for *H.* (s. str.) *guineensis*), in others medially obsolete or widely reduced except before eyes (cf. Appendix). Antennomeres simple, not broadened except in *H.* (s. str.) *descarpentriesi*, with antennomeres of both sexes, but especially males, strongly dilated (Fig. 17). Elytra with margin in lateral view rather strongly ascending to shoulder (similar to Fig. 54); epipleuron comparably broad, broader than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably small angle (< ca. 135°; similar to Fig. 49). Elytral pattern diverse (vittate, dotted or uniform); venter black or brown. Last abdominal ventrite without deep depression.



Figures 49-55. (49-53): Epipleuron with oblique epipleural carina and genicular fossa of: (49) *Clemnius* (s. str.) *berneri*, (50) *Hygrotus* (*Leptolambus*) *polonicus polonicus*, (51) *H.* (*Coelambus*) *caspius*, (52) *H.* (*Leptolambus*) *impressopunctatus* and (53) *Rhithrodytes agnus*; epipleural carina (c) and inner margin of epipleuron (m) including angle mentioned in diagnoses of subgenera. **(54-55)**: Elytral margin in lateral view of: (54) *H.* (*Coelambus*) *caspius* (strongly ascending to shoulder) and (55) *Clemnius* (s. str.) *berneri* (moderately ascending to shoulder) (Figs 52 and 53 are reproduced from Fery, 2013 and from Fery, 2016 with the permissions of F. Gusenleitner, Linz, Austria, and G. Foster, Ayr, UK, respectively.

Aedeagus with median lobe robust, distal part very diverse in ventral view, more or less symmetric (see Figs 22 and 27 for *H.* (s. str.) *inaequalis* and *H.* (s. str.) *guineensis*) or at most slightly asymmetric in some former *Herophydrus* (see figures in Biström & Nilsson 2002); parameres with condylar process generally short, forming an obtuse angle with distal part (see Fig. 32 and 39 for *H.* (s. str.) *inaequalis* and *H.* (s. str.) *guineensis*). Male metatarsal claws of equal length.

Distribution: Palaearctic, Nearctic, Ethiopian and Oriental. Species of the former subgenus *Hygrotus* (the *H.* (*Hygrotus*) *inaequalis*-group in Fig. 2) are distributed in Europe, northern Africa, Asia and northern America (reaching northern Mexico); species of former *Herophydrus* occur mainly in Africa, with five species in the Palaearctic, one (*H.* (*Hygrotus*) *musicus*) reaching the Oriental region and *H*. (*Hygrotus*) *morandi* (Guignot, 1952) known from Cambodia only; *H.* (*Hygrotus*) *descarpentriesi* is endemic to mountainous regions in south-eastern Madagascar.

Main habitat types: The subgenus includes species typical of lentic freshwater environments; some species can be found in inland mineralised or coastal brackish waters, such as for example *H*. (*Hygrotus*) *musicus* (Millán *et al*., 2006). The latter species can also be found in mineral and thermal spring-pools (pers. communication by J. Hájek, Prague, Czech Republic). The habitat of most African species of the subgenus is poorly known.

Subgenus *Leptolambus* n. subgen.

Type species: *Dytiscus impressopunctatus* Schaller, 1783: 312, by present designation.

The subgenus includes 51 species (one of them bitypic), all previously included in the former subgenus *Coelambus* (see Fig. 2 and Table 1). Notes: *Coelambus hudsonicus* Fall, 1919 is treated by us as junior subjective synonym of *Hygrotus* (*Leptolambus*) *novemlineatus* (Stephens, 1829) (according to Nilsson & Hájek, 2017a). We are aware that some authors (e.g. Foster *et al.,* 2016) accepted subspecific rank for this taxon and others (e.g. Alarie *et al.,* 1999) specific rank, but all without giving any justification for their proceeding.

Diagnosis: Body shape moderately to elongate oval (TL/MW ca. 1.75–2.05); small to medium sized species (TL 2.7–5.8 mm) (see Figs 13–15 for *H.* (*Leptolambus*) *impressopunctatus*, *H.* (*Leptolambus*) *orthogrammus* (Sharp, 1882) and *H.* (*Leptolambus*) *obscureplagiatus* (Fall, 1919)). Head with anterior clypeal margin truncate and emarginated (see Fig. 41 for *H*. (*Leptolambus*) *impressopunctatus*); in some species medially at least less curved than near eyes (see Fig. 47 for *H*. (*Leptolambus*) *masculinus*); border not produced forwards and bead absent (except in *H*. (*Leptolambus*) *masculinus* and *H*. (*Leptolambus*) *salinarius*; cf. Appendix). Antennomeres simple, not broadened. In most species elytral margin in lateral view somewhat less ascending to shoulder than in Fig. 54; in species of Anderson's (1976) group III (*H*. (*Leptolambus*) *bruesi* (Fall, 1928), *H.* (*Leptolambus*) *compar* (Fall, 1919), *H.* (*Leptolambus*) *nigrescens* (Fall, 1919), *H.* (*Leptolambus*) *dissimilis* (Gemminger & Harold, 1868), and *H*. (*Leptolambus*) *turbidus* (LeConte, 1855)) margin only moderately ascending (similar to Fig. 55); in most species epipleuron comparably broad, broader than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably small angle (ca. 135°; similar to Fig. 50 or Fig. 52). In four species of Anderson's (1983) group IV (*H*. (*Leptolambus*) *diversipes* (Leech, 1966), *H.* (*Leptolambus*) *fontinalis* Leech, 1966, *H*. (*Leptolambus*) *pedalis* (Fall, 1901), and *H*. (*Leptolambus*) *thermarum* (Darlington, 1928)) epipleuron narrower and carina meeting inner margin of epipleuron forming a comparably wide angle (> ca. 150°; similar to Fig. 51). In *H*. (*Leptolambus*) *curvipes* (Leech, 1938) (belonging also to group IV in Anderson, 1983) epipleuron slightly broader and carina meeting inner margin of epipleuron forming an angle of ca. 140°; however, in these five species of Anderson's (1983) group IV elytral margin not as strongly ascending as in species of newly defined subgenus *Coelambus* (see Fig. 54 for *H*. (*Coelambus*) *caspius*). Elytra pattern diverse (vittate, diffuse-vittate or "cloudlike") (see Anderson, 1983; see also Fig. 15 of *H.* (*Leptolambus*) *obscureplagiatus* as an example for diffuse-vittate or "cloudlike" elytral pattern); venter black. Last abdominal ventrite without deep depression.

Aedeagus with shape of median lobe diverse, from robust to very slender; symmetric in ventral view (see Figs 25 and 26 for *H*. (*Leptolambus*) *parallellogrammus* (Ahrens, 1812), and *H*. (*Leptolambus*) *impressopunctatus*). Species of *H*. (*Leptolambus*) *saginatus*-group (see Fig. 2) with distal part of parameres strap-like and condylar process also forming an almost right angle with distal part (see Fig. 33 for *H*. (*Leptolambus*) *fresnedai* (Fery, 1992)). Species of *H*. (*Leptolambus*) *parallellogrammus*-group (see Fig. 2) with parameres more or less triangular and condylar process indistinct and not forming an angle with distal part (see Fig. 35 for *H*. (*Leptolambus*) *parallellogrammus*). Other species with distal part of parameres triangular or broadly strap-like and condylar process forming an almost right angle with distal part (see Fig 34 for *H*. (*Leptolambus*) *impressopunctatus*) or with very unusually shaped parameres (see Fig. 36 for *H*. (*Leptolambus*) *nubilus*; cf. also fig. 7B in Anderson 1976 for *H*. (*Leptolambus*) *dissimilis*). Male metatarsal claws of equal length except in species of *H*. (*Leptolambus*) *saginatus*-group (only four species of all Hygrotini with metatarsal claws of unequal length).

Etymology: From Greek λεπτός (= leptos = narrow) and "lambus" in reference to *Coelambus*. The gender of the generic name is masculine.

Distribution: The subgenus is divided into two main clades, one with mostly Palaearctic distribution except for the Holarctic *H*. (*Leptolambus*) *impressopunctatus* and the Nearctic *H*. (*Leptolambus*) *picatus* (Kirby, 1837), and a second with mostly Nearctic species, except for *H*. (*Leptolambus*) *marklini* (Gyllenhal, 1813), *H.* (*Leptolambus*) *novemlineatus*, and *H*. (*Leptolambus*) *unguicularis* (Crotch, 1874) which are Holarctic. Some species of the Nearctic clade reach northern Mexico: *H*. (*Leptolambus*) *fraternus* (LeConte, 1852), *H.* (*Leptolambus*) *lutescens* (LeConte, 1852), *H.* (*Leptolambus*) *nubilus*, and *H*. (*Leptolambus*) *wardii* (Clark, 1862). *Hygrotus* (*Leptolambus*) *nubilus* was recently recorded from Hawaii, where it has likely been introduced (see Fery & Challet, 2015).

Main habitat types: Many species of this subgenus are found in mostly lentic freshwaters, but several in the Nearctic clade, as well as the species of the *H*. (*Leptolambus*) *parallellogrammus*-group, can tolerate from slightly saline to hypersaline waters (Villastrigo *et al*., 2018, Chapter 2). The subgenus includes the most salt-tolerant species of the tribe, *H*. (*Leptolambus*) *salinarius* and *H*. (*Leptolambus*) *masculinus*.

Discussion

The phylogenetic results of Villastrigo *et al*. (2018, Chapter 2) revealed the need of a thorough systematic rearrangement of the tribe Hygrotini. The close relationships between the former *Hygrotus*, *Herophydrus* and *Heroceras* had already been previously noted by several authors (see the Introduction and the Appendix), and suggested by the incomplete molecular and morphological phylogenies available prior to our study (Miller, 2001; Biström & Nilsson, 2002; Ribera *et al*., 2002, 2008; Alarie & Michat, 2007; Abellán *et al*., 2013; Miller & Bergsten, 2014).

Species of *Hyphoporus* have always been considered to be related to species of *Herophydrus*, from which they differ mainly by the male genital shape (e.g. Biström & Nilsson, 2002; Miller & Bergsten, 2016). We opted for maintaining *Hyphoporus* as a valid subgenus, not only based on our phylogenetic results, but also due to the distinctiveness of their male genitalia. It is, however, possible that in further analyses with a more complete sampling *Hyphoporus* proves to be a derived clade within the wider subgenus *Hygrotus* s. str.

Hygrotus descarpentriesi, formerly considered in its own genus *Heroceras*, is a morphologically very deviating species when compared with its closest relatives from Madagascar as resolved in the molecular phylogeny (Fig. 1). The habitus is more elongated, the elytral surface is smooth in the male and reticulated (although not matt) in the female (a character not found in any other Malagasy species of *Hygrotus*), the clypeal bead is almost absent (see the Appendix), and - the most apparent character - both sexes, but especially the males, have strongly dilated antennae. The latter character is present occasionally in other groups of Dytiscidae (e.g. in the genera *Agabus* Leach, 1817, *Limbodessus* Guignot, 1939, *Hydrovatus*, *Rhithrodytes*, *Exocelina* Broun, 1886, *Lioporeus* Guignot, 1950, and others; see e.g. Miller & Bergsten, 2016 for some examples and Fig. 17), but still exceptional within Hygrotini. Despite all these peculiarities, *H*. (s. str.) *descarpentriesi* was originally described in *Herophydrus*, and found at least to be related to this genus by the morphological phylogenetic analysis of Biström & Nilsson (2002).

The most unexpected result of the phylogeny was the division of Hygrotini into two clades (A and B in Fig. 1), the former (our *Clemnius* **n. gen.**) including a small number of species previously considered belonging in part to former subgenus *Hygrotus* s. str. and in part to former subgenus *Coelambus*. The species of *Clemnius* **n. gen**. had never been suggested to be closely related to each other within Hygrotini. They all share some likely plesiomorphic characters of body shape and colouration. Most of them are also similar in terms of male genitalia shape, which is in general simpler and more similar to those of other related tribes of Hydroporinae.

Of the two subgenera recognised within *Clemnius* **n. gen**., *Cyclopius* **n. subgen.** is well characterised by the special morphology of the male last abdominal ventrite, but we could not recognise any clear unambiguous synapomorphy for *Clemnius* **n. subgen.** The relationships within the latter are still poorly defined, and need further morphological and molecular studies to be clarified.

Although a formal biogeographic analysis is out of the scope of our paper, it is interesting to note that most of the recognised clades have a well-defined distribution within one of the main biogeographic regions:

- *Clemnius* **n. gen.** is Nearctic with the only exception of one species, the widespread Palaearctic *Clemnius* (s. str.) *decoratus* (see Nilsson & Hájek, 2017a, b).
- Subgenus *Coelambus* is almost exclusively Palaearctic, with only one exception, the Nearctic *C. punctilineatus*. However, this species is extremely similar to the Palaearctic species *C. nigrolineatus* (Steven, 1808) (this has not been recognised before), including the shape of the male protarsal claws. Their male and female genitalia show only some slight differences and their general shape could be considered virtually identical. Additionally, females of both species have the upper

side dull. It is thus most likely that *C. punctilineatus* has very recently colonised the Nearctic region from a Palaearctic ancestor.

- Subgenus *Hygrotus* s. str., as here defined, is largely of Afrotropical distribution (and likely origin), with only some species reaching the southern Palaearctic region and a small clade–the *H*. (s. str.) *inaequalis*-group–in the Palaearctic and Nearctic regions (Fig. 2).

- Subgenus *Hyphoporus* is mostly Oriental, with some species reaching the Palaearctic region in China (Yunnan), Afghanistan, northern India (Uttar Pradesh), Pakistan and in the west until Iran and Egypt.

- Subgenus *Leptolambus* **n. subgen.** is divided into two clades, one mostly Palaearctic and one mostly Nearctic (see above and Fig. 2). Only some northern species of *Leptolambus* **n. subgen.** have likely recently expanded their geographic range to the whole Holarctic, and three species have a discordant distribution: *H.* (*Leptolambus*) *picatus* is a Nearctic species in the Palaearctic clade, and *H.* (*Leptolambus*) *polonicus* (Aubé, 1842) and *H.* (*Leptolambus*) *zigetangco* Fery, 2003 are Palaearctic species likely to be in the Nearctic clade (see Fig. 2), although for the last two species there are no molecular data and thus their phylogenetic position is uncertain.

Acknowledgments

We cordially thank Rolf Beutel (Jena, Germany) for giving us access to the SEM microscope of the “Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum, Friedrich-Schiller-Universität Jena”. We thank also M.A. Jäch (Vienna, Austria), F. Gusenleitner (Vienna, Austria) and G.N. Foster (Ayr, UK) for permission to use numerous figures published previously in other works. Antoine Mantilleri (MNHN) and P. E. Skelley (FSCA) are warmly thanked for loan of material. We thank also J. Hájek (Prague, Czech Republic) anvd an anonymous reviewer for comments to previous versions of the manuscript. This work was partly funded by an FPI grant to A.V., and projects CGL2013-48950-C2-1-P and CGL2013-48950-C2-2-P (AEI/FEDER, UE) to I.R. and A.M. respectively.

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Appendix

Note: The generic and subgeneric names cited in the Appendix are those of the new classification unless stated otherwise.

Detailed description of the clypeus in Hygrotini

The presence and the shape of the clypeal bead (often also called “clypeal rim” or “raised clypeal margin”) are used by many authors to separate genera or subgenera within the tribe Hygrotini, mainly the former subgenus *Hygrotus* from former subgenus *Coelambus* and both from the former genera *Herophydrus* and *Hyphoporus* (see Table 1 for the authors and dates of all taxa mentioned here, as well as their past and new classification). Unfortunately, a determination is not always reliable or even impossible because the respective character states are not well recognisable in several species or are present in species which due to other characters, both morphological and molecular, should better be included in another genus or subgenus. Thus, the usefulness of the “clypeal bead” has been debated since long time. This clypeal bead is present in more than half of all members of Hygrotini, and varies considerably in shape. Although in the light of the results of the molecular phylogeny it is clear that the clypeal bead is a highly labile character, without much phylogenetic significance, a detailed description of its structure is necessary to understand the historical classification of Hygrotini, and it can nevertheless have some usefulness to identify some groups of species.

Former subgenera *Hygrotus* and *Coelambus*

The more common character state in members of the former subgenus *Coelambus* as recognised prior to this study (Nilsson & Hájek, 2017a, b) is the absence of a clypeal bead (as in e.g. *H.* (*Coelambus*) *confluens,* Fig. 46, and *H.* (*Leptolambus*) *impressopunctatus*, Fig. 41). The anterior part of the clypeus descends anteriad more or less evenly or in a slightly convex shape to the labrum (see Figs 5e and 6e). In almost all species of former *Coelambus* the anterior margin of the clypeus is bordered (directly before reaching/contacting the labrum) with a very narrow rim, which we here call the “clypeal line” (see Fig. 5d for *H.* (*Leptolambus*) *impressopunctatus* and Fig. 6d for *H*. (s. str.) *inaequalis*; see also below for more details). A rather broad transverse band posterior to this line (reaching more or less until the clypeal grooves) is often somewhat less reticulate (and thus more shiny) and more sparsely and finely punctured than the rest of the clypeus posterior to this band (e.g. recognisable “under the arrows” in Fig. 5 for *H*. (*Leptolambus*) *impressopunctatus*). Thus, this band might be interpreted as a separate part of the clypeus and - also due to the depressed clypeal grooves posterior to the band - can appear as if it was somewhat vaulted (although it is not), which might be interpreted as a (flat) bead. The structure of this band can vary considerably even among specimens of the same population of a species - in one specimen the band can appear as a flat bead, in another one found at the same locality the illusion of a bead is lacking. In all these typical members of the former subgenus *Coelambus* the anterior border of the clypeus (where it reaches/contacts the labrum) and that "clypeal line" (if present) can be recognised when the clypeus is viewed perpendicularly. Additionally, in this view it can be recognised that the anterior border of the clypeus of all these species of former subgenus *Coelambus* is not evenly rounded, but truncate and somewhat emarginated.

In members of the former subgenus *Hygrotus* the anterior border of the clypeus is evenly rounded in perpendicular view (see Fig. 44 for *H*. (s. str.) *inaequalis*). Additionally, the clypeus does not descend directly to the labrum, but is strongly and almost sharply produced forwards (see Figs. 6 and 45 for *H*. (s. str.) *inaequalis*), so that it reaches so far over the labrum that the real anterior border of the clypeus (where it reaches/contacts the labrum) cannot be recognised when the clypeus is viewed perpendicularly, as the anteriorly produced part of the clypeus covers most of the labrum and in particular the "clypeal line" (if that line is present at all). This is the reason why in species of the former subgenus *Hygrotus* it is necessary to differentiate between the anterior border of the clypeus and its anterior end, the latter being situated posterior (!) to the former. Short behind and parallel to its evenly curved anterior border the clypeus is depressed over the entire distance between the eyes; this depression is anteriorly delimited by a more or less sharp line (see Fig. 44 for *H*. (s. str.) *inaequalis*). That is why between this line and the anterior border an evenly curved "clypeal" bead is formed which has more or less the same width over its entire length.

There are two species of the former subgenus *Coelambus* that have a clypeus with almost exactly the same structure as the species of former subgenus *Hygrotus*, something that has confused generations of dytiscid specialists: *Hygrotus* (*Leptolambus*) *salinarius* and *H.* (*L.*) *masculinus* (see Figs 47 and 48 for *H*. (*L.*) *masculinus*). However, in contrast to members of the former subgenus *Hygrotus*, these two species have the anterior border of the clypeus truncate (in several specimens of *H.* (*L.*) *masculinus* at least less curved centrally than near the eyes) and also somewhat emarginated in *H.* (*L.*) *salinarius* - being in this respect typical *Hygrotus* (*Coelambus*) and not *Hygrotus* s. str.

Former genera *Hyphoporus* and *Herophydrus*

Members of former genera *Herophydrus* and *Hyphoporus* have a different structure of the anterior part of the clypeus. Whilst all former *Hyphoporus* have a complete clypeal bead which is mostly narrowed medially (see Fig. 42 for *Hygrotus* (*Hyphoporus*) *solieri*), in former *Herophydrus* some species have a complete bead, some a medially shortly interrupted bead (see Fig. 43 for *Hygrotus* (s. str.) *guineensis*) as well as others with a medially broadly interrupted bead and *Hygrotus* (s. str.) *rohani* (Peschet, 1924) with a hardly delimited bead (cf. Biström & Nilsson, 2002). In those species with incomplete bead, the situation in the middle of the clypeal border is similar to that in typical *Hygrotus* (*Leptolambus*) - the anterior part of the clypeus descends anteriad more or less evenly or in a slightly convex shape to the labrum and the labrum as well as an eventual "clypeal line" are not hidden by the anterior part of the clypeus. Left and right of the bead interruption, the clypeus is widely vaulted and sometimes slightly produced forwards, however never as strongly produced as in former subgenus *Hygrotus*. The two parts of the bead are backwards either delimited by a more or less distinct line or by an anteriorly rather sharply delimited depression (as in the *inaequalis*-group of *Hygrotus* s. str.).

In members of subgenus *Hyphoporus* and those of former genus *Herophydrus* with complete bead, the anterior border of the clypeus is medially also widely vaulted and thus a little produced anteriorly. This is why in perpendicular view an eventually existing "clypeal line" and a very small posterior part of the labrum are covered by the vaulted anterior border of the clypeus. It must, however, be emphasised that these structures are totally different from those in members of former subgenus *Hygrotus* - in all these species the clypeus is by far not as strongly and not as sharply produced forwards as in the latter. Additionally, the bead or its two lateral parts are considerably broader left and right of the middle, whilst it is of more or less of equal width over its entire length in members of the former subgenus *Hygrotus*.

Species of former *Herophydrus* and *Hyphoporus* have the anterior margin of the clypeus truncate and emarginated in perpendicular view (see Fig. 43 for *guineensis*; in some species, however, this emargination is only rather indistinct), but we must concede that we have not been able to study all species and must rely in part on the figures given in Biström & Nilsson (2002).

The separation of former *Herophydrus* from *Hyphoporus* by morphological characters has been debated since long time (see e.g. Guignot, 1950: 149; Vazirani, 1969: 203–205; Biström & Nilsson, 2002: 20–21). We did not find any satisfying solution for this problem and believe that a final classification can only be given once the molecular data of all species of these two genera are known. So far we must refer on what was given by Vazirani (1969: 204) in his key to these two genera, but add some exceptions.

According to Guignot (1950: 149; more or less repeated by Vazirani, 1969: 204) in former genus *Herophydrus* the punctation of the head reaches an imaginary line connecting the hind margins of the eyes, but on a small band posterior to this line (on the vertex) this punctation is absent or at most replaced by a few much smaller punctures. In former genus *Hyphoporus* the punctation of the head reaches distinctly beyond this imaginary line and is not replaced by finer punctation. Additionally, both authors note that in *Herophydrus* the median lobe is (more or less) symmetric and in *Hyphoporus* it is not. On the other hand, Guignot (1959: 339) himself conceded that "lack of punctation in that band on the vertex" [our translation from French] does not really hold in *H.* (s. str.) *musicus* and *H.* (s. str.) *rufus*, and we must concede that we were not able to use this character for reliable identifications.

In what refers to the symmetry of the median lobe we want to underline that *Hygrotus* (*Hyphoporus*) *bengalensis* has a more or less symmetric median lobe, and on the other hand that many former *Herophydrus* have a median lobe which is at least not strictly symmetric (as can be appreciated e.g. in some figures in Biström & Nilsson, 2002).

Former genus *Heroceras*

Hygrotus (s. str.) *descarpentriesi* is a species not well represented in collections; thus, some of the very few descriptions in the literature may not rely on careful studies of specimens. It is more or less known as a "*Herophydrus* with widened antennae" (cf. Guignot, 1950: 150 and Pederzani, 1995: 35). Guignot (1959: 372) provided for this species: "Tête bourrelet clypéal ininterrompu, ..." [= head with clypeal bead not interrupted ...] and "... bourrelet clypéal peu marqué ..." [= ... clypeal bead not well marked ...]. Miller & Bergsten (2016: 204) gave "... having the anterior clypeal margin broadly bordered."

We have studied one male and one female syntype of this species (coll. Peschet, MNHN; see Fig. 17), one additional female from the coll. Guignot (MNHN), and several further specimens collected recently by one of us (M.M.). To our great surprise, at first glance we were not able to detect any distinct clypeal bead. Only when adequately illuminated it was possible to see that in the female syntype the clypeus is very slightly vaulted before the anterior margin. In the male syntype a few transversely stretched punctures indicate a posterior margin of a bead - however, only on the right side of the clypeus. In particular, there are absolutely no traces of a bead recognisable before the eyes. The latter observation is surprising, because even *H.* (s. str.) *nodieri*, with a very broadly interrupted clypeal bead, shows laterally distinct rests of a bead next to the eyes. Similar observations were made with the recently collected material. Additionally, we want to state that only a small posterior part of the labrum is covered by the vaulted anterior border of the clypeus - this being in contrast to Biström & Nilsson (2002: 18, 19) who stated labrum “not visible from above”. On the other hand, our observation that the clypeal bead is practically lacking in former *Heroceras* is supported by Biström & Nilsson (2002: 18, 19, table 1) who gave the anterior margin of the clypeus (character 1 on p. 18) with state 0 (= without bead). These authors, however, did not comment this feature.

It shall be mentioned here that the almost total lack of a medial clypeal bead was the reason for Guignot (1950) to create his subgenus *Dryephorus* of genus *Herophydrus*. Similarly, other species of former *Herophydrus* show only rests of a bead before each eye, such as *Hygrotus* (s. str.) *heros* (Sharp, 1882) (cf. Zimmermann, 1919: 150). Although in a different subgenus, *Hygrotus* (*Leptolambus*) *polonicus polonicus* (Aubé, 1842) and its subspecies *sahlbergi* (Sharp, 1882) have the clypeus anteriorly somewhat vaulted (cf. Zimmermann, 1930: 96). Zimmermann (1919: 150) claimed that also *Hygrotus* (*Leptolambus*) *unguicularis* might be ranged under *Hygrotus* s. str. „... wegen der feinen, aber wenigstens in der Mitte deutlichen Clypeusrandung ...“ [= because of the fine, but at least medially distinct beading]. We have studied numerous specimens of *H. unguicularis* and can state that Zimmermann (1919) must have been misguided: most probably he mixed up what in Hygrotini is usually called clypeal “bead” (and which is rather broad) with what we call here “clypeal line”.

Falkenström (1933: 12) noted that on the one hand *Hygrotus* (s. str.) *versicolor* (Schaller, 1783) should be ranged in *Herophydrus* due to the shape of the clypeal border (meaning a medially reduced width of the clypeal bead), but on the other hand refused this classification.

The “clypeal stripe”

As mentioned above, in many species of Hygrotini, and in particular in species of former subgenus *Coelambus*, the anterior border of the clypeus is provided with a “clypeal line”. This line is in some species rather distinct, in others irregularly interrupted and in some not recognisable or absent. The distinctness of this line seems to show also some individual variation within a species. Balfour-Browne (1934: 150) pointed to a special feature of *Hygrotus* (*Coelambus*) *confluens*: at the base of the labrum, before the anterior border of the clypeus there is a flat, transverse stripe which is rather shiny, not reticulate, provided with only a very few punctures, broadest in middle and evenly tapering to the sides (Fig. 46). We interpret this “clypeal stripe” as a broadened “clypeal line”. Such stripe can be also found in *H*. (*C.*) *pallidulus*, *H*. (*C.*) *caspius*, *H.* (*C.*) *pectoralis* (Motschulsky, 1860) (stripe very thin), *H.* (*C.*) *nigrolineatus*, *H*. (*C.*) *punctilineatus*, *H*. (*C.*) *enneagrammus* and in *H*. (*C.*)

flaviventris (Motschulsky, 1860), but in all latter species this stripe is by far not as broad as in *H*. (*C.*) *confluens* and often difficult to observe, as it is strongly reduced to almost a simple line slightly broader medially than laterally. In *H*. (*C.*) *ahmeti* this “clypeal stripe” is in fact reduced to a line of more or less even width. We have found such medially very slightly broadened stripe/line also in a few other species, such as *H.* (*Leptolambus*) *marklini*, *H.* (*L.*). *fraternus*, *H.* (*L.*). *patruelis* (LeConte, 1855), and *H.* (*L.*). *pedalis*.

Finally, we want to emphasise, that this “clypeal stripe” is by no means homologous to the “clypeal bead” found in species of former *Herophydrus*, *Hyphoporus* and subgenus *Hygrotus*.

Chapter 2

Evolution of salinity tolerance in the diving beetle tribe Hygrotini (Coleoptera: Dytiscidae)

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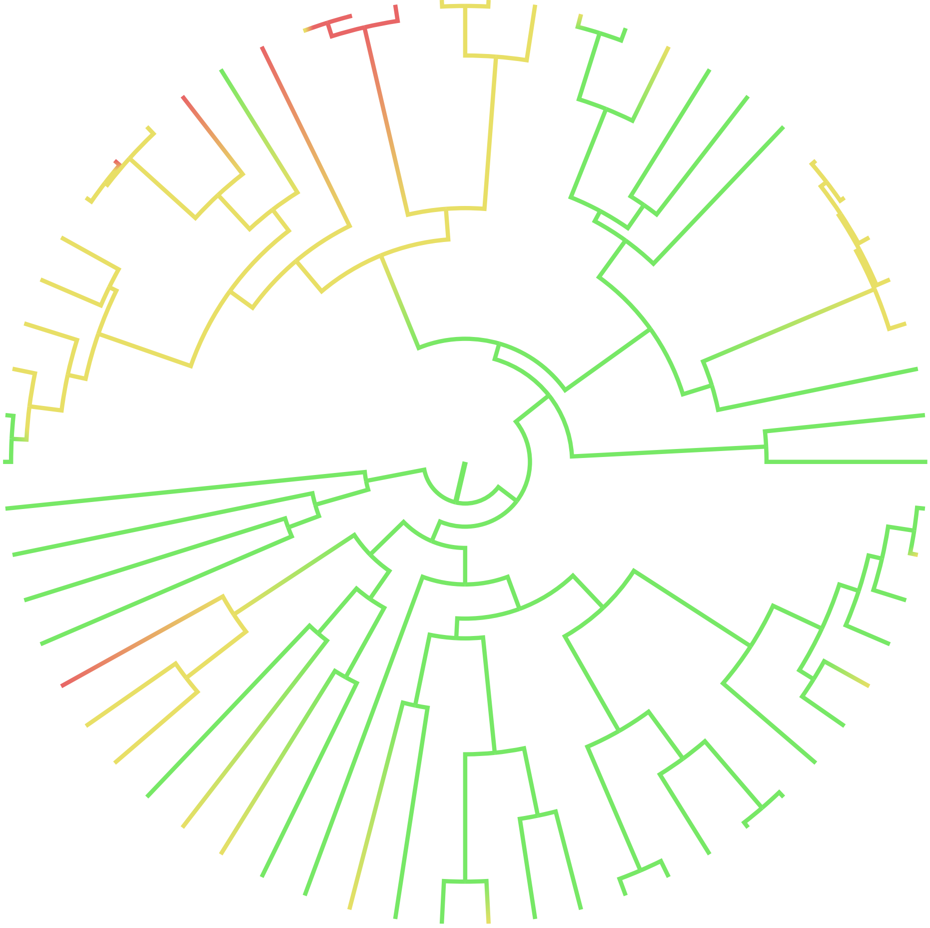
Published on: *Zoologica Scripta* (2018) **47**: 63-71



Abstract

Some species of the diving beetle tribe Hygrotini (subfamily Hydroporinae) are among the few insects able to tolerate saline concentrations more than twice that of seawater. However, the phylogenetic relationships of the species of Hygrotini, and the origin and evolution of tolerance to salinity in this lineage, are unknown. In this work, we aim to reconstruct how many times salinity tolerance did evolve in Hygrotini, whether this evolution was gradual or if tolerance to hypersalinity could evolve directly from strictly freshwater (FW) species, and to estimate the probabilities of transition between habitats. We build a phylogeny with ca. 45% of the 137 species of Hygrotini, including all major lineages and almost all of the known halophile or tolerant species. We used sequence data of four mitochondrial (COI-5', COI-3', 16S + tRNA and NADH1) and three nuclear (28S, 18S and H3) gene fragments, plus ecological data to reconstruct the history of the salinity tolerance using Bayesian inference. Our results demonstrate multiple origins of the tolerance to salinity, although most saline and hypersaline species were concentrated in two lineages. The evolution of salinity was gradual, with no direct transitions from FW to hypersaline habitats, but with some reversals from tolerant to FW species. The oldest transition to saline tolerance, at the base of the clade with the highest number of saline species, was dated in the late Eocene-early Oligocene, a period with decreasing temperature and precipitation. This temporal coincidence suggests a link between increased aridity and the development of tolerance to saline waters, in agreement with recent research in other groups of aquatic Coleoptera.

Introduction

Hydroporinae is the most diverse of the subfamilies of diving beetles (Dytiscidae) (Nilsson & Hájek, 2017a) and its species also display a large variety of ecologies and life habits (Miller & Bergsten, 2016). Many species of Hydroporinae live in extreme or unusual environments for diving beetles, such as subterranean aquifers, forest litter or hypersaline waters. While the origin and evolution of subterranean and terrestrial lifestyles has received recent attention (e.g. Leys & Watts, 2008; Tierney *et al.,* 2015; Toussaint *et al*., 2016), the origin of the species of diving beetles able to sustain extreme salt concentration has never been addressed in a phylogenetic context, whereas for other families of aquatic Coleoptera, such as Hydrophilidae or Hydraenidae, comparative studies on the evolution of saline tolerance are already available (Arribas *et al.,* 2014; Sabatelli *et al.,* 2016).

Within Hydroporinae, species which are exclusively halophile or which can tolerate saline or hypersaline waters have independently evolved in three tribes, Bidessini, Hydroporini and Hygrotini (Miller & Bergsten, 2016). The latter includes some of the most extreme examples of saline tolerance, with some species able to sustain concentrations above 70 g/L, twice that of seawater (Picazo, *et al*., 2010; Timms & Hammer, 1988). The physiological mechanism of salt tolerance of *Hygrotus* (*Coelambus*) *salinarius* (Wallis, 1924) has been studied (Tones, 1978). However, the species' phylogenetic relationships are unknown, and in consequence whether it may be related to other saline tolerant species of *Hygrotus* Stephens, 1828 or what could have been the origin of its saline tolerance.

With this work we aim to investigate the phylogenetic relationships among the salt tolerant species of Hygrotini, and their relationships with the rest of the species of the tribe. This will allow to answer some basic questions such as: (i) how many times did salinity tolerance evolve in Hygrotini?; (ii) was this evolution gradual (i.e. from FW to intermediate salinities to hypersaline), or could tolerance to hypersalinity have evolved directly from strictly FW species?; (iii) what were the probabilities of transitions between habitats in the evolution of Hygrotini? and (iv) are there any general patterns in the geographic and temporal origin of salinity tolerance in Hygrotini?

To answer these questions we built a molecular phylogeny with almost half of the 137 known species of the tribe, including all genera and recognised main species groups (Nilsson & Hájek, 2017a,b), and compiled data on the ecological tolerances of all described species from the literature and our own observations. In addition, our results demonstrated that two of the four currently recognised genera of Hygrotini and one subgenus are para- or polyphyletic, revealing the need of a new classification of the tribe, which will be presented in a separate paper (Villastrigo, *et al*., 2017, Chapter 1).

Material and Methods

Molecular data

We obtained molecular data from 101 specimens of 64 species, including all four currently recognised genera of Hygrotini: *Heroceras* Guignot, 1950, *Herophydrus* Sharp, 1880, *Hygrotus* (with two subgenera, *Hygrotus* and *Coelambus* Thompson, 1860) and *Hyphoporus* Sharp, 1880 (Nilsson & Hájek, 2017a,b; see Table S1). We used as outgroups a selection of 12 species from four genera belonging to Hydroporini, shown to be related to Hygrotini (Ribera, *et al*., 2008). Trees were rooted on *Laccornis* Gozis, 1914, considered to be outside Hydroporini and Hygrotini and in a basal position within Hydroporinae (Miller & Bergsten, 2014; Ribera *et al*., 2008).

DNA extraction and sequencing

Specimens were collected in the field and preserved in absolute ethanol. DNA was extracted using commercial kits (mostly DNeasy Tissue Kit, Qiagen, Hilden, Germany) following the instructions of the manufacturers. Vouchers and DNA samples are kept in the collections of the Institute of Evolutionary Biology (IBE, Barcelona) and Museo Nacional de Ciencias Naturales (MNCN, Madrid). We sequenced fragments of seven genes in six sequencing reactions, three mitochondrial: (i) 5' end of cytochrome c oxidase subunit 1 (COI-5, "barcode" fragment of Hebert, Ratnasingham, & De Waard, 2003), (ii) 3' end of cytochrome c oxidase subunit 1 (COI-3), (iii) 5' end of 16S RNA plus the Leucine tRNA plus 5' end of NADH dehydrogenase subunit I (16S); three nuclear fragments: (iv) an internal fragment of the large ribosomal unit 28S RNA (28S), (v) an internal fragment of the small ribosomal unit, 18S RNA (18S) and (vi) an internal fragment of Histone 3 (H3). Details on primers used and typical polymerase chain reaction (PCR) conditions are provided in Table S2. Sequences were assembled and edited with Geneious v6.0.6 (Kearse *et al*., 2012); new sequences have been submitted to the EMBL database with accession numbers LT882773-LT883126 (Table S1).

Phylogenetic analyses

Edited sequences were aligned using the online version of MAFFT 7 with the G-INS-I algorithm (Katoh *et al*., 2009). For one species (*Hygrotus* (*Coelambus*) *pedalis* (Fall, 1901)) we pooled sequences of two specimens in a chimera to complete the data set (Table

S1), after testing their monophyly with COI-3. We used PartitionFinder v1.1.1 (Lanfear *et al*., 2012) to estimate the best fitting evolutionary model initially using one partition for each gene fragment except for COI (split in COI-5 and COI-3) and 16S and tRNA (pooled in a single partition), and applied Akaike Information Criterion (AIC) scores as selected Criteria.

We used BEAST 1.8 (Drummond & Rambaut, 2007) for Bayesian phylogenetic analyses, using the partition and evolutionary models selected by PartitionFinder and a molecular-clock approach for estimating divergence times. We applied an uncorrelated lognormal relaxed clock to estimate substitution rates and a Yule speciation process as the tree prior. We calibrated the tree using rates estimated in Andújar *et al*., (2012) for a genus of Carabidae (*Carabus*), in the same suborder Adephaga (rate of 0.0113 [95% confidence interval 0.0081–0.0147] substitutions per site per million years (subst/s/Ma) for COI- 5; 0.0145 [0.01–0.0198] subst/s/Ma for COI-3 and 0.0016 [0.001–0.0022] subst/s/Ma for 16S + tRNA). Analyses were run for 100 million generations, assessing that convergence was correct and estimating the burn-in fraction with Tracer v1.6 (Drummond & Rambaut, 2007). We also used a fast maximum likelihood (ML) heuristic algorithm in RAxMLHPC2 (Stamatakis, 2006) in the CIPRES Science Gateway (Miller *et al*., 2010), using the same partition scheme as in BEAST with a GTR + G evolutionary model independently estimated for each partition and assessing node support with 100 pseudoreplicas with a rapid bootstrapping algorithm (Stamatakis *et al*., 2008).

Morphological data

To estimate the likely phylogenetic relationships of the species for which no molecular data could be obtained, we studied all described species of subgenera *Hygrotus* and *Coelambus* with the only exceptions of *H*. (*C*.) *artus* (Fall, 1919), known only from the holotype and considered to be possibly extinct (see Anderson, 1983), and *H*. (*C*.) *femoratus* (Fall, 1901), which is likely a junior synonym of *H*. (*C*.) *nubilus* (LeConte, 1855) (Anderson, 1983). We have also studied a large selection of species of *Herophydrus* and *Hyphoporus* (see Table S3 for the studied material).

The taxonomic classification of Hygrotini has suffered multiple changes, and some of the characters used to define genera (such as e.g., the morphology of the clypeus) are difficult to interpret and characterise (see e.g., Anderson, 1971; Balfour-Browne, 1934; Biström & Nilsson, 2002; Falkenström, 1933; Villastrigo *et al*., 2017, Chapter 1). Species for which no molecular data were available were thus considered to be closely related to those showing a high morphological similarity, based both on external characters and on the female and male genitalia. We also recognised diagnostic characters or character combinations of the different clades of the molecular phylogeny and tested their presence in the species without molecular data (see Villastrigo *et al*., 2017, Chapter 1, for more details). We used Mesquite v3.20 (Maddison & Maddison, 2017) to manually place all species in their most likely position in the phylogenetic tree, and collapsed uncertain nodes to create polytomies.

Salinity tolerance data

We compiled ecological data on habitat preferences of all species of Hygrotini from bibliography and from our own observations (Table S3). Recent work on salinity tolerance in aquatic Coleoptera has recognised six different categories of habitat preferences: (i) FW (<0.5 g/L); (ii) mineralized (0.5–5 g/L); (iii) hyposaline (≥5–20 g/L); (iv) mesosaline (≥20–40 g/L); (v) hypersaline (≥40–80 g/L); (vi) extreme hypersaline (>80 g/L) (Arribas *et al*., 2014). As quantitative observations in species of Hygrotini were very scarce we reduced these categories to three: (i) species strictly bounded to FW environments (approximately <0.5 g/L), corresponding to category (i) above; (ii) species that can tolerate a wide range of salinities (approximately 0.5–40 g/L), corresponding to categories (ii–iv) above; and (iii) hypersaline species (approximately >40 g/L), corresponding to categories (v) and (vi) above. To reconstruct the evolution of saline tolerance we pruned the data set to one specimen per species and deleted the outgroups, using salinity tolerance as a qualitative trait. This reduced matrix was analysed in BEAST using the same settings as for the phylogenetic reconstruction, with an asymmetric substitution model for the trait reconstruction and dating the ancestral node according to the results of the previous analysis (with a Gamma distribution with shape 30 and scale 2.227). We also reconstructed the evolution of saline tolerance in the extended phylogeny, including species for which no molecular data were available, using parsimony in Mesquite.

Results

Molecular data

The best partition schemes selected by PartitionFinder pooled the two fragments of COI and several of the nuclear genes, and favoured the most complex evolutionary models for most partitions (Table 1). The topological differences between the Bayesian analysis and the ML searches were minimal and always affected poorly supported nodes (Figures 1, S1), associated with the position of three species: *Hygrotus* (*Coelambus*) *fumatus* (Sharp, 1882), *Hygrotus* (*Coelambus*) *urgensis* (Jakovlev, 1899) and *Hygrotus* (*Hygrotus*) *hydrop-*

icus (LeConte, 1852). In all cases, the monophyly of Hygrotini was recovered with strong support, as were most of the internal nodes (Figures 1, S1).

Table 1. Best partition schemes and optimal evolutionary models as estimated with PartitionFinder.

Complete data set			**Reduced data set**		
P	**Genes**	**Model**	**P**	**Genes**	**Model**
#1	COI-5’ + COI-3’	GTR + I + G	#1	COI-5’ + COI-3’	GTR + I + G
#2	16S + NAD1	GTR + I + G	#2	16S + NAD1	GTR + I + G
#3	18S + 28S + H3	GTR + I + G	#3	18S + 28S	GTR + I
			#4	H3	GTR + I + G

P, partition

In all analyses, Hygrotini was divided into two lineages (posterior probability [pp] = 1; bootstrap support [BS] = 98): (A) three Nearctic and one Palaearctic species of subgenera *Hygrotus* and *Coelambus* and (B) the remaining species of the tribe (Fig. 1). The latter was in turn divided into four clades: (B1) a group of Palaearctic species of subgenus *Coelambus*, (B2) the two sampled species of *Hyphoporus*, (B3) a large group of species including *Heroceras*, all sampled *Herophydrus* and most species of *Hygrotus* s. str., and (B4) the remaining species of subgenus *Coelambus*, in turn divided into two sister clades, one with mostly *Palaearctic* species and a second with mostly Nearctic species.

The monophyly of all clades had strong support in both Bayesian and ML analyses except for clade B3 (Fig. 1). This clade included the genus *Herophydrus* as paraphyletic with respect to *Heroceras* and most species of the subgenus *Hygrotus*, the latter grouped in a monophyletic lineage. *Heroceras* and the sampled species of *Herophydrus* from Madagascar with the exception of *H. spadiceus* Sharp, 1882 formed a strongly supported clade (pp = 1, BS = 100), in turn placed (with lower support) inside a lineage with most of the remaining sampled African *Herophydrus* (Fig. 1).

The internal phylogeny of the main clades was generally in good agreement with the recognised species groups among subgenera *Hygrotus* and *Coelambus* based on morphology (see e.g., Anderson, 1971, 1976, 1983; Fery, 1992, 1995, 2003), although not with the phylogeny of *Herophydrus* obtained by Biström and Nilsson (2002).

Evolution of tolerance to salinity

Differences between the analyses of the complete and reduced data set referred mostly to the position of *Hyphoporus* (clade B2). In the complete data set it was placed as sister to clade B3, and both sister to clade B1, with very strong support (Fig. 1). In the re-

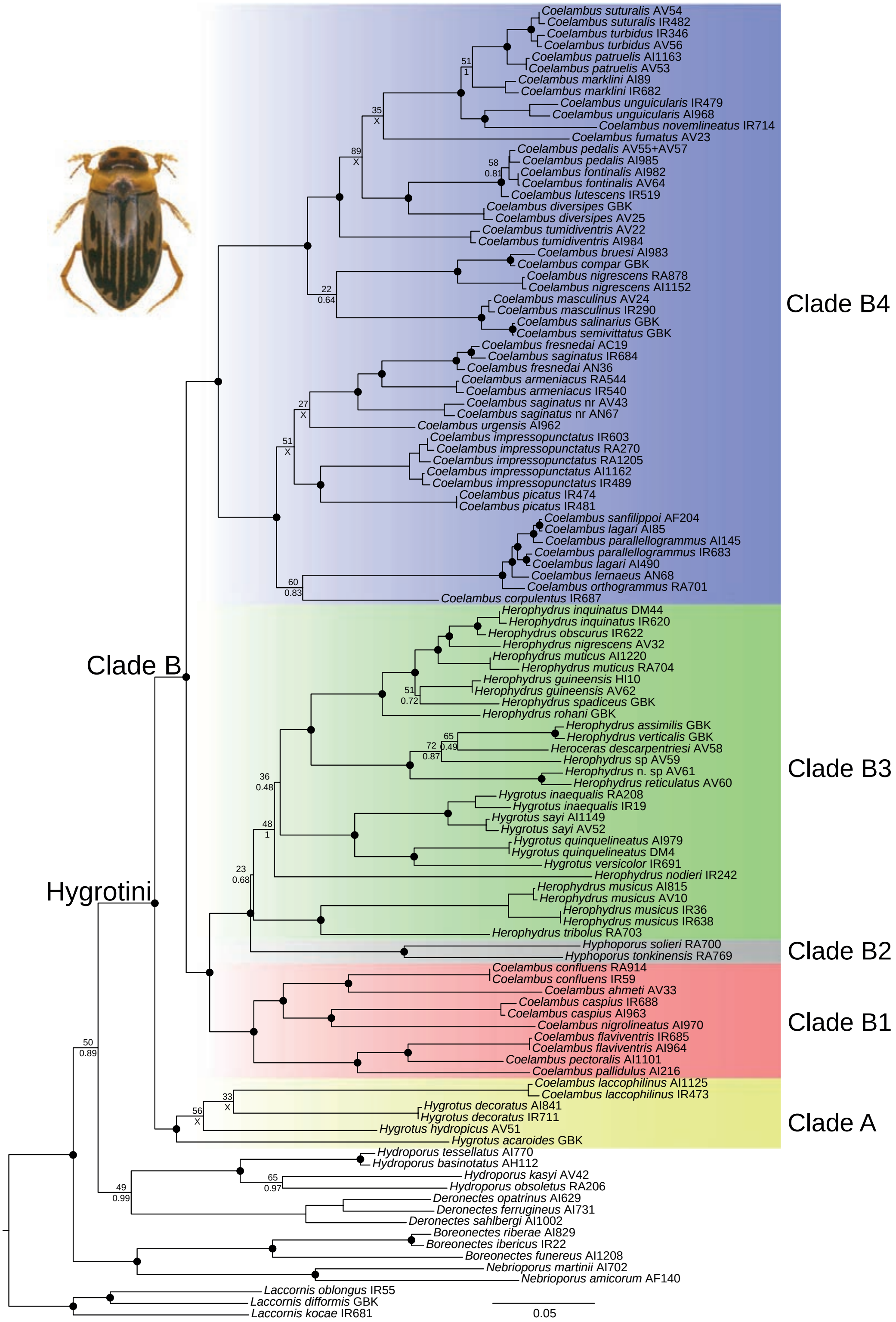


Figure 1. Best maximum ikelihood (ML) phylogram obtained in RAxML with the extended dataset of Hygrotini (including outgroups and multiple terminals per species). Black circles, nodes with bootstrap support in RAxML (BS) > 70 and posterior probability (pp) in BEAST > 0.95; when support values were lower: number above nodes, BS; number below nodes, pp. X, nodes not recovered in the BEAST analysis (Fig. S1). See Table S1 for details on the specimens. For clarity, only the subgenus name is given in *Hygrotus* s. str. and *Coelambus*. Habitus photograph: *Hygrotus* (*Coelambus*) *lagari* (Fery, 1992) (from Millán et al., 2014).

duced data set, without outgroups and with only one terminal per species, it was placed as sister to clade B4, but with low support (Fig. 2). However, the uncertainty in the phylogenetic position of *Hyphoporus* (clade B2) did not have any effect on the reconstruction, as the two possible sister clades, B3 (Fig. 1) or B4 (Figures 2, S2), were reconstructed to have a FW ancestor, and thus, their common ancestor was also reconstructed as living in FW habitats.

According to the Bayesian reconstruction in the reduced data set, tolerance to salinity emerged independently from a FW ancestor at least ten times within three of the main lineages of Hygrotini: B1, B3 and B4 (Fig. 2). There were five subsequent independent

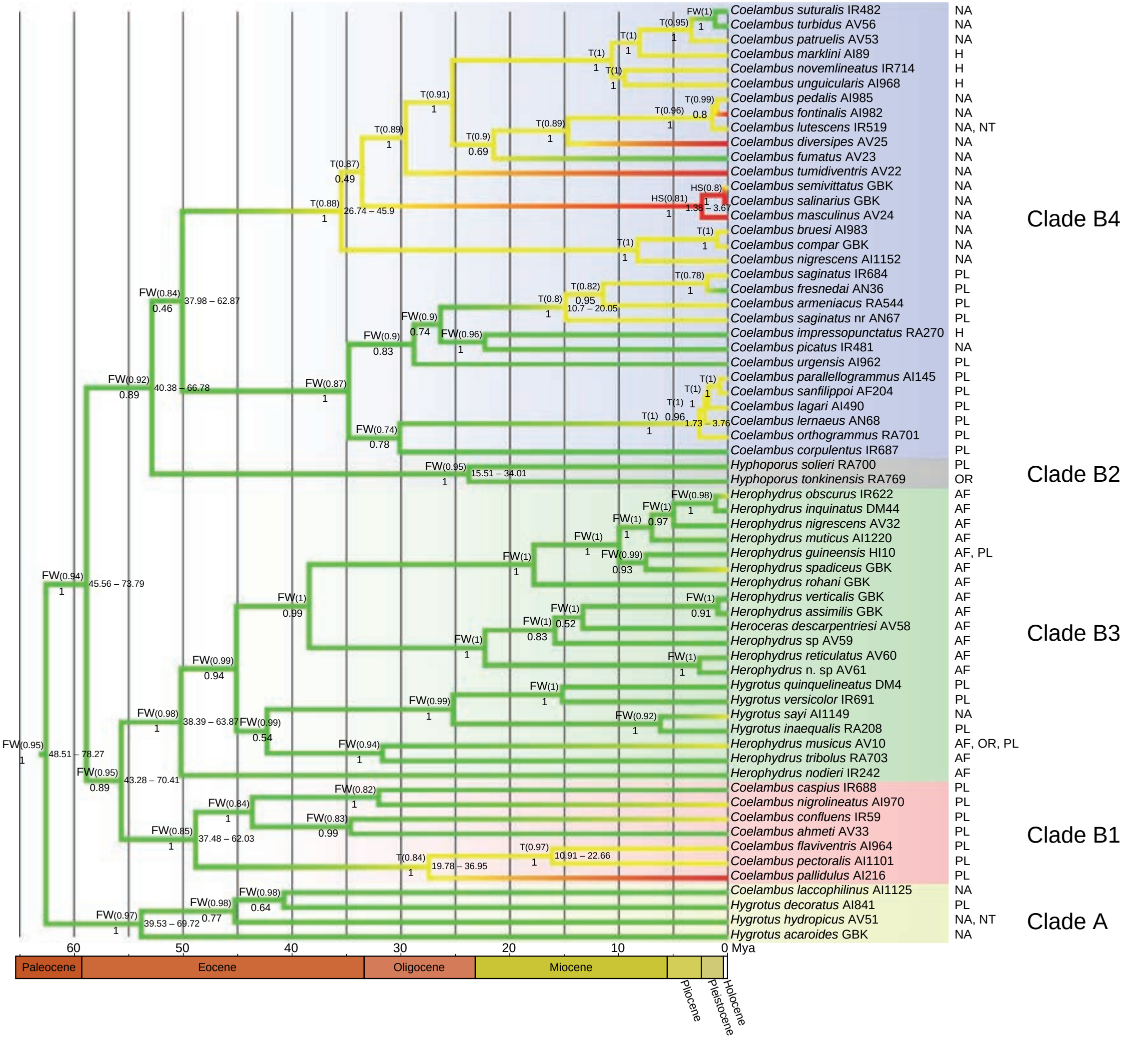


Figure 2. Reconstruction of the evolution of tolerance to salinity in the reduced data set of Hygrotini (excluding outgroups and with only one terminal per species) in BEAST. Letters above nodes: reconstructed state of the trait (FW, freshwater, green; T, tolerant, yellow; HS, hypersaline, red); numbers inside parentheses: posterior probability of the reconstructed discrete state; number below nodes: node support (pp); numbers inside nodes: 95% interval of the reconstructed age of selected nodes. For clarity, only the subgenus name is given in *Hygrotus* s. str. and *Coelambus*. See Table S1 and S3 for details on the specimens and the ecological typification of the species, respectively. Geographic distribution: AF: Afrotropical; H: Holarctic; NA: Nearctic; NT: Neotropical (northern Mexico); OR: Oriental; PL: Palaearctic.

transitions from tolerant to hypersaline (mainly in the Nearctic lineage of clade B4), although in some cases these transitions affected only the terminal branches in our phylogeny. Four of the transitions from FW to salinity tolerant and one of the transitions to hypersalinity led to clades with more than one species, and in only one clade (B4) there have been three reversal transitions from tolerant to FW. No direct transitions from FW to hypersaline habitats were found in the reconstruction (Fig. 2).

The oldest transition to salinity tolerance, at the base of the clade with the highest number of saline species, was dated to the late Eocene-early Oligocene. Three of the transitions to hypersalinity tolerance were estimated to have occurred during the Oligocene, one of them with a further diversification in the early Pleistocene, including *H*. (*C*.) *salinarius* and *Hygrotus* (*Coelambus*) *masculinus* (Crotch, 1874; Fig. 2). Other transitions to hypersalinity tolerance affecting single species occurred in the middle Miocene (*Hygrotus* (*Coelambus*) *diversipes* Leech, 1966) and the Pleistocene (*Hygrotus* (*Coelambus*) *fontinalis* Leech, 1966; see Fig. 2).

Table 2. Estimated transition rates in BEAST between the discrete states of tolerance to salinity.

	Freshwater	Tolerant	Hypersaline
Freshwater	-	1.97 (0.33-3.99)	0.23 (<0.001-0.72)
Tolerant	1.23 (0.09-2.83)	-	1.4 (0.03-3.02)
Hypersaline	0.37 (<0.001-1.17)	0.73(<0.001-1.9)	-

In brackets, 95% confidence interval.

For most species without molecular data a close relative included in the phylogeny could be identified based on similarities in the genitalia or the external morphology, although in a few cases no obvious relatives could be identified, and the species were placed in an unresolved polytomy in the less inclusive clade to which they could be ascribed (Fig. S2). The parsimony reconstruction of salinity tolerance in this extended phylogeny gave similar results to the reconstruction using only the molecular data. The last common ancestor of Hygrotini was a FW species, as was the reconstructed ancestors of clades A, B2 and B3. For nodes including species with the three states of the trait, such as in clades B1 and B4, the ancestral reconstruction was ambiguous in the parsimony analysis (Fig. S2). On the contrary, in the Bayesian analysis of the reduced data set they were reconstructed as FW with a high probability (Fig. 2). Most of the tolerant or hypersaline species were included within clades with at least some other tolerant species, in agreement with the results obtained with the phylogeny using only species with molecular data (Figures 2, S2).

Discussion

Molecular phylogeny

We obtained a robust phylogeny of Hygrotini, with good support for most internal nodes except for the monophyly of clade B3 and its relationship with *Hyphoporus*. Our species sampling was also very dense except for *Herophydrus* and for *Hyphoporus*, of which we could obtain fresh material of only two species. These two species, however, belong to the two different morphological groups recognised in the revision of the genus by Vazirani (1969).

The most unexpected result of our phylogeny was the deep division of Hygrotini in two clades, one of them formed by a small number of species. Despite the lack of clear synapomorphies, these species share some presumably plesiomorphic characters of body shape, colouration and male genitalia, the latter being in general simpler and more similar to those of other related tribes of Hydroporinae. The internal relationships within clade A are still poorly defined, and would need further morphological and molecular studies to be clarified. Within clade B3, the close relationships of *Hygrotus* s. str., *Herophydrus* and *Heroceras* had been previously noted by many authors (Abellán *et al*., 2013; Alarie & Michat, 2007; Biström & Nilsson, 2002; Miller & Bergsten, 2014; Ribera *et al*., 2002; Ribera *et al*., 2008), and suggested by the (incomplete) molecular and morphological phylogenies available prior to our study. Species of *Hyphoporus* have always been considered to be related to the species of *Herophydrus*, of which they differ mostly in the male genital shape (e.g., Biström & Nilsson, 2002; Miller & Bergsten, 2016). The non-monophyly of genera *Hygrotus* and *Herophydrus* and subgenera *Hygrotus* and *Coelambus* requires a revised classification of the tribe, which will be formalised in a separate paper (Villastrigo *et al*., 2017, Chapter 1).

Evolution of the tolerance to salinity in Hygrotini

We found several independent origins for the salinity tolerance in the tribe Hygrotini, with at least 10 transitions from FW to saline ecosystems both in the Palaearctic and the Nearctic. The multiple origin of saline species was expected, as they were included in different morphologically well characterised subgenera or species groups, but the number of transitions was lower than anticipated, as some of the saline species in clade B4 that were not thought to be related (as e.g., *Hygrotus* (*Coelambus*) *marklini* (Gyllenhall, 1813) and *H*. (*C*.) *salinarius*) were grouped in the same monophyletic radiation. In other families of aquatic Coleoptera, species that were apparently not closely related were also found to have a common origin of their tolerance to salinity, as for example in the genera *Ochthebius*

Leach, 1815 (Hydraenidae; see Sabatelli *et al*., 2016) and *Enochrus* Thomson, 1859 (Hydrophilidae; see Arribas *et al*., 2014).

We did not find any direct transition from FW to hypersaline habitats, a transition that had the lowest estimated probability, suggesting that in Hygrotini the adaptation to salinity has been a gradual process. The only possible exception was *Hygrotus* (*Coelambus*) *pallidulus* (Aubé, 1850), sister to a group of tolerant species, but the clade was reconstructed as having a FW ancestor with high probability. However, *H*. (*C*.) *pallidulus* together with its tolerant sisters are included in a wider lineage with several other tolerant species of which no molecular data could be obtained, rendering the condition of their common ancestor ambiguous (as can be seen in Fig. S2). This gradual evolution is in contrast to the direct transitions from FW to hypersaline tolerance found in a group of Mediterranean species of *Enochrus* (Arribas *et al*., 2014). These transitions were associated with periods of aridification of the climate, leading to the hypothesis that saline tolerance may have been a by-product (an exaptation) of adaptation to desiccation (Arribas *et al*., 2014; Pallarés *et al*., 2016). The capability to produce hyperosmotic excreta is a plesiomorphic character in insects, likely linked to the necessary adaptations to a terrestrial environment (Bradley, 2008; Bradley *et al*., 2009; Cloudsley-Thompson, 2001). In the only species for which the salinity tolerance mechanism is known, *H*. (*C*.) *salinarius*, adults maintain a hyposmotic haemolymph also with hyperosmotic excreta (Tones, 1978), with no evidence of any additional mechanism particular to this species. However, larvae of *H*. (*C*.) *salinarius* maintain a hyperosmotic haemolymph even at high saline concentrations (Tones, 1978), suggesting a different mechanism to that of adults. Although there is an increasing knowledge of the physiological basis of salinity tolerance in adult Coleoptera (e.g., Céspedes *et al*., 2013; Pallarés *et al*., 2015; Pallarés *et al*., 2012), the physiology of larval tolerance to salinity is still unknown. However, both the different characteristics of the cuticle (less sclerotised and without the protection of the elytra) and the biology (usually more strictly linked to the aquatic environment) suggest that the existence of different mechanisms to salinity tolerance in adults and larvae may be frequent.

In any case, it must be noted that we have data only on the ecological preferences of the species of Hygrotini, not on their physiological tolerances. If tolerance to salinity is an exaptation derived from a plesiomorphic adaptation to terrestrial environments (as hypothesised by Arribas *et al*., 2014; see Pallarés *et al*., 2017 for an experimental confirmation of the link between salinity and desiccation tolerance), tolerance to at least certain degree of salinity may be widespread even in species commonly found in FW habitats, as has been demonstrated to be the case in other groups of aquatic Coleoptera (Céspedes *et al*., 2013; Pallarés *et al*., 2015). Our ecological typification was also in most cases based on qualitative descriptions, without quantitative data. For some species there are few, if any, reports on their habitat, and in some cases we have assumed a FW habitat when the information was not very precise, as when species are found in saline or hypersaline habitats this is usually reported. In some cases qualitative reports can be ambiguous, such as for example when species are considered “halophile” or “halobiont” when they occur in slightly mineralised waters in a landscape otherwise lacking any true saline or hypersaline habitat, such as for example *Hygrotus* (*Hygrotus*) *inaequalis* (Fabricius, 1777) or *H*. (*Coelambus*) *impressopunctatus* (Schaller, 1783) in some areas in central and northern Europe (e.g., Bellstedt, 2008).

In Hygrotini, the transitions to saline habitats were estimated to have occurred in different periods, from the late Eocene to the Plio- and Pleistocene, without a clear pattern of associations to arid periods, in contrast to what happened in the genus *Enochrus* in the Mediterranean region (Arribas *et al*., 2014). However, the oldest transition to saline habitats in Hygrotini, and the one leading to the higher number of saline species, occurred at the end of the Eocene in clade B4 in the Nearctic region, coincident with a global decrease in temperatures and the onset of the first Oligocene glaciations (Liu *et al*., 2009; Zachos *et al*., 2001). In North America, this decrease in temperature was associated with a decrease in precipitation (Retallack, 2007), leaving open the possibility that this transition to saline habitats was also a response to an increased aridification in this lineage.

Acknowledgements

We would like to thank all colleagues mentioned in Table S1 for sending valuable material for study, as well as Ana Izquierdo (MNCN), Rocio Alonso and Anabela Cardoso (IBE) for laboratory work. M. Manuel is grateful to Andriamirado T. Ramahandrison for help during field work in Madagascar, in the context of the RicciaTeam expedition led by Catherine Reeb. We also thank Josefa Velasco and Susana Pallares (Universidad de Murcia) and Paula Arribas (Instituto de Productos Naturales y Agrobiologia-CSIC) For comments on the origin and evolution of salinity tolerance, and three anonymous referees for their comments and suggestions. This work was partly funded by an FPI grant to A.V., and projects CGL2013-48950- C2-1-P and CGL2013-48950-C2-2-P (AEI/FEDER, UE) to I.R. and A.M., respectively.

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Supporting Information

Table S1. Material studied in the molecular study, with voucher numbers, locality data and accession numbers of the sequences.

Table S2. A) Primers used in the amplifying and sequencing reactions and B) standard PCR conditions for the amplification of the studied fragments.

Table S3. Checklist of the species of Hygrotini (following Nilsson & Hàjek, 2017a, b) with the typical habitat and the species for which material could be studied. (AF, Afrotropical; H, Holarctic; NA, Nearctic; NT, Neotropical; OR, Oriental; PL, Palaearctic)

Fig. S1. Time-calibrated majority rule consensus tree obtained in BEAST with the complete dataset. Number in nodes: posterior probability values.

Fig. S2. Reconstructed evolution of salinity tolerance in the tribe Hygrotini, using parsimony with the estimated relationships of species for which no molecular data were available.

Table S1. Material studied in the molecular study, with voucher numbers, locality data and accesion numbers of the sequences. In Bold, newly obtained sequences.

ID	Genus	Subgenus	Species	Voucher	Red.	country	Loc.	leg	BAR	COI	16S	18S	H3	28S
1	*Heroceras*		*descarpentriesi*	IBE-AV58	Yes	Madagascar	Prov. Fianarantsoa / ca 4.5 km WSW Antanifotsy / Andringitra Massif. / S22°08'11.9" E46°51'55.5" / 2,127 m.	M. Manuel & Rahamandrison	**LT882848**	**LT882791**	**LT883018**	**LT882897**	**LT883058**	**LT882958**
2	*Herophydrus*		n. sp.	IBE-AV51	Yes	Madagascar	Prov. Fianarantsoa / ca 4.5 km WSW Antanifotsy / Andringitra Massif. / S22°08'11.9" E46°51'55.5" / 2,127 m.	M. Manuel & Rahamandrison	**LT882851**	**LT882793**	**LT883020**	**LT882904**	**LT883068**	
3	*Herophydrus*		*assimilis*	GENBANK	Yes	Madagascar	Ref (1)			HQ382803	HQ381465			
4	*Herophydrus*		*guineensis*	MNCN-HI10	Yes	Algeria	Raml-Souk (puit) / N 36° 47.494' E 008° 31.326' / 101.19 m	S. Bouzid		**LT882823**	**LT883047**	**LT882898**	**LT883059**	**LT882959**
5	*Herophydrus*		*guineensis*	IBE-AV62	No	Namibia		D.J. Mann		**LT882794**				
6	*Herophydrus*		*inquinatus*	MNCN-DM44	Yes	South Africa	W Cape / Prince Albert Road / pond in junction R407 with N1	I. Ribera & A. Cieslak	**LT882884**	**LT882822**	**LT883046**	**LT882899**	**LT883060**	**LT882960**
7	*Herophydrus*		*inquinatus*	NHM-IR620	No	South Africa	W Cape / Wilderness NP, Swartvlei / Montmere, ditch by the lake	I. Ribera & A. Cieslak		**LT882828**	**LT883053**			
8	*Herophydrus*		*musicus*	MNCN-AI815	No	Turkey	SINOP / rd. 785 btw Saraydüzü & Boyabat / slow stream 5 km S of Yesilyurt / 440m / N41º23'42.5" E34º49'51"	I. Ribera		**LT882818**	**LT883043**		**LT883061**	
9	*Herophydrus*		*musicus*	NHM-IR36	No	Spain	Albacete, Hellin, Charca de los Patos	I. Ribera		AJ850634	AJ850384	AJ318731	EF670206	
10	*Herophydrus*		*musicus*	NHM-IR638	No	Gran Canaria (ES)	Moya, Bco. de Azuaje	I. Ribera & A. Cieslak		**LT882829**	**LT883054**	**LT882900**	**LT883062**	**LT882961**
11	*Herophydrus*		*musicus*	IBE-AV10	Yes	Crete (GR)	Crete Potamies	R.B. Angus	**LT882838**	**LT882776**	**LT883007**	**LT882901**	**LT883063**	**LT882962**
12	*Herophydrus*		*muticus*	MNCN-AI1220	Yes	Ethiopia	Shewa Prov. / 25 km N Addis Ababa, Solutta / 2800m	G. Wewalka	**LT882866**	**LT882811**	**LT883036**	**LT882902**	**LT883064**	**LT882963**
13	*Herophydrus*		*muticus*	IBE-RA704	No	Ethiopia	Shewa Prov. / 40-60 km N Addis Ababa / 2500m	G. Wewalka		**LT882804**	**LT883030**			
14	*Herophydrus*		*nigrescens*	IBE-AV32	Yes	South Africa	Kwazulu-Natal / iSimangalis Wetland Park / False Bay site 3 / Woodland pool	D.T. Bilton		**LT882781**				
15	*Herophydrus*		*nodieri*	NHM-IR242	Yes	Namibia		D.J. Mann	**LT882886**	AJ850632	AJ850382	AJ850520	EF670204	
16	*Herophydrus*		*obscurus*	NHM-IR622	Yes	South Africa	W Cape / Du Toits Kloof, rd. N1 / pond and river Wit in resort	I. Ribera & A. Cieslak	**LT882891**	AJ850633	AJ850383	AJ850521	EF670205	**LT882964**
17	*Herophydrus*		*reticulatus*	IBE-AV60	Yes	Madagascar	Prov. Antananarivo / ca. 6 km WSW Manandona / Ibity Massif. / S20°04'18.4" E47°00'01" / Alt. 1,423 m.	M. Manuel & Rahamandrison	**LT882850**	**LT882792**	**LT883019**			**LT882965**
18	*Herophydrus*		*rohani*	GENBANK	Yes	Zambia	Ref (2)		KJ548554	KJ548554	KJ548382		KJ548799	
19	*Herophydrus*		sp	IBE-AV59	Yes	Madagascar	Prov. Antananarivo / ca. 13 km W Ambatolampy / Ankaratra Massif / S19°21'42.8" E47°18'53.8" / Alt. 1,737 m.	M. Manuel & Rahamandrison	**LT882849**				**LT883067**	
20	*Herophydrus*		*spadiceus*	GENBANK	Yes	Madagascar	Ref (1)			HQ383502				
21	*Herophydrus*		*tribolus*	IBE-RA703	Yes	Zambia	Northern Province / Kapisha / S011°10'09.4 E031°36'01.6 / 1440 m	R. Vila	**LT882856**	**LT882803**	**LT883029**	**LT882903**	**LT883066**	**LT882966**
22	*Herophydrus*		*verticalis*	GENBANK	Yes	Madagascar	Ref (1)			HQ382802	HQ381464			
23	*Hygrotus*	*Coelambus*	*ahmeti*	IBE-AV33	Yes	Turkey	Erzurum, Çat	Ö.K. Erman		**LT882782**	**LT883012**	**LT882905**	**LT883069**	
24	*Hygrotus*	*Coelambus*	*armeniacus*	IBE-RA544	Yes	Turkey	Erzurum / Toprakkaleköyü / slow stream in grassland / 2174 m / N40 14 22.9 E40 59 16.7	I. Ribera	**LT882855**	**LT882800**	**LT883026**	**LT882906**	**LT883071**	**LT882967**
25	*Hygrotus*	*Coelambus*	*armeniacus*	NHM-IR540	No	Turkey	Erzurum / ca. 14 Km S Tortum ca. 2 km S Guzelyayla pass / ponds on meadow	H. Fery		HF931283	HF931520		**LT883070**	
26	*Hygrotus*	*Coelambus*	*bruesi*	MNCN-AI983	Yes	US (CA)	Mono Co. / Long Valley, Owens river Rd. / tributary r. Owen	I. Ribera & A. Cieslak	**LT882881**	HF931259	HF931495	LT882907	**LT883072**	
27	*Hygrotus*	*Coelambus*	*caspius*	NHM-IR688	Yes	Russia		A.N. Nilsson	**LT882894**	AJ850635	AJ850385	AJ850522	EF670207	**LT882968**
28	*Hygrotus*	*Coelambus*	*caspius*	MNCN-AI963	No	Mongolia	Tov / Ondorshireet / Tuul River ~1.5 km W of Tuul Ovoot bridge, downstream of road to Arvayheer / N47.31096 E105.27119 / 1042 m	A.E.Z. Short		**LT882819**	**LT883044**			
29	*Hygrotus*	*Coelambus*	*compar*	GENBANK	Yes	US (WY)	Ref (2)		KJ548527	KJ548527	KJ548332		KJ548765	

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Table S1. (Continued)

ID	Genus	Subgenus	Species	Voucher	Red.	country	Loc.	leg	BAR	COI	16S	18S	H3	28S
30	*Hygrotus*	*Coelambus*	*confluens*	NHM-IR59	Yes	Portugal	Sa. Da Estrela, Torre / lagoon	I. Ribera	**LT882890**	AY250964	AJ850386	AJ318735	EF670208	**LT882969**
31	*Hygrotus*	*Coelambus*	*confluens*	IBE-RA914	No	Azores (Pot.)	Terceira / Guinjal, euthrophic lagoon / 38°41’44.6”N 27° 9’27.2”W / 390m	I. Ribera & A. Cieslak		**LT882807**	**LT883032**			
32	*Hygrotus*	*Coelambus*	*corpulentus*	NHM-IR687	Yes	Russia	Astrakhan oblast / Pirogovka / steppe lake NW of village	A.N. Nilsson	**LT882893**	AJ850637	AJ850387	AJ850523	EF670209	**LT882970**
33	*Hygrotus*	*Coelambus*	*diversipes*	IBE-AV25	Yes	US (CA)	Soda Lake (near Baker) / 35º9’19’’N,116º6’22’’W	P. Abellán	**LT882842**	**LT882780**	**LT883011**	**LT882908**	**LT883073**	**LT882971**
34	*Hygrotus*	*Coelambus*	*diversipes*	GENBANK	No	US (WY)	Ref (2)			KJ548528	KJ548333		KJ548766	
35	*Hygrotus*	*Coelambus*	*flaviventris*	MNCN-AI964	Yes	Mongolia	Tov / Ondorshireet / Tuul River ~1.5 km W of Tuul Ovoot bridge, downstream of road to Arvayheer / N47.31096 E105.27119 / 1042 m	A.E.Z. Short	**LT882877**	HF931251	HF931487	**LT882909**	**LT883074**	
36	*Hygrotus*	*Coelambus*	*flaviventris*	NHM-IR685	No	Russia		A.N. Nilsson		**LT882831**	**LT883056**			
37	*Hygrotus*	*Coelambus*	*fontinalis*	MNCN-AI982	Yes	US (CA)	Mono Co. / Long Valley, Owens river Rd. / tributary r. Owen	I. Ribera & A. Cieslak	**LT882880**	HF931258	HF931494	**LT882910**	**LT883075**	
38	*Hygrotus*	*Coelambus*	*fontinalis*	IBE-AV64	No	US (CA)	Mono Co. / big alkali lake at Benton crossing rd	G. Challet		**LT882795**	**LT883021**			
39	*Hygrotus*	*Coelambus*	*fresnedai*	MNCN-AC19	No	Spain	Segovia / ponds beside cross rd between Abades-Otero de los Herreros SG723 and Segovia-Villacastin N110	R.B. Angus	HF947982	HF931108	HF931325			
40	*Hygrotus*	*Coelambus*	*fresnedai*	IBE-AN36	Yes	Spain	Guadalajara / El Pobo de Dueñas / pond / N40º47’5.0” W1º38’30.3”	I. Ribera & A. Cieslak	**LT882835**	**LT882773**	**LT883004**	**LT882911**	**LT883076**	**LT882972**
41	*Hygrotus*	*Coelambus*	*fumatus*	IBE-AV23	Yes	US (CA)	Mendocino Co. / Rd. 1 Manchester / pond S City	I. Ribera & A. Cieslak	**LT882840**	**LT882778**	**LT883009**	**LT882912**	**LT883077**	**LT882973**
42	*Hygrotus*	*Coelambus*	*impressopunctatus*	MNCN-AI1162	No	Canada (AL)		T. Berendonk		**LT882810**	**LT883035**		**LT883078**	
43	*Hygrotus*	*Coelambus*	*impressopunctatus*	NHM-IR489	No	Canada (AL)	Rd. 22, 5 km S Longview	I. Ribera & A. Cieslak		**LT882827**	**LT883052**			
44	*Hygrotus*	*Coelambus*	*impressopunctatus*	NHM-IR603	No	Corsica (FR)	Cassamozza: r. l’Abatescu	I. Ribera & A. Cieslak		AJ850653	AJ850405	AJ850537 EF670302	EF670210	
45	*Hygrotus*	*Coelambus*	*impressopunctatus*	IBE-RA270	Yes	Ireland	Gortlecka / Lough Gealain, turlough / N52º59’52.2” W9º01’28.3”	I. Ribera	**LT882854**	**LT882799**	**LT883025**	**LT882913**	**LT883079**	**LT882974**
46	*Hygrotus*	*Coelambus*	*impressopunctatus*	IBE-RA1205	No	Kyrgyzstan	Son Kol Lake area / rheohelocrenic spring 2 / 3069 m / 41º55.720’ N, 75º12.057’ E	Pešić		**LT882796**	**LT883022**			**LT882975**
47	*Hygrotus*	*Coelambus*	*laccophilinus*	NHM-IR473	No	Canada (ON)	Manitoulin island, Ck. At Hwy 540, 20 km E Little Curreny	Y. Alarie		**LT882825**	**LT883049**	**LT882915**	**LT883081**	
48	*Hygrotus*	*Coelambus*	*laccophilinus*	MNCN-AI1125	Yes	Canada (ON)	Manitoulin island, Ck. At Hwy 540, 20 km E Little Curreny	Y. Alarie	**LT882862**	HF931163	HF931382	**LT882914**	**LT883080**	**LT882976**
49	*Hygrotus*	*Coelambus*	*lagari*	MNCN-AI85	No	Spain	Zaragoza /Chiprana pond rd. S. Marcos / 41°14’30“N 0°09‘27“ W / 120m	I. Ribera & A. Cieslak		HF931236	HF931465		**LT883082**	
50	*Hygrotus*	*Coelambus*	*lagari*	MNCN-AI490	Yes	Spain	Guadalajara /El Pobo de Dueñas / pond in cross N211-CM2112 / N40º47’05.6” W1º38’30.1”	I. Ribera & A. Cieslak	**LT882869**	**LT882813**	**LT883038**	**LT882916**	**LT883083**	**LT882977**
51	*Hygrotus*	*Coelambus*	*lernaeus*	IBE-AN68	Yes	Azerbaijan	Qobustan / ponds in beach / 40°5’38.9”N 49°25’19.5”E /26m	I. Ribera & A. Rudoy	**LT882837**	**LT882775**	**LT883006**	**LT882917**	**LT883084**	**LT882978**
52	*Hygrotus*	*Coelambus*	*lutescens*	NHM-IR519	Yes	Canada (BC)	B.C. / Rd. 5A, Stump Lake / ponds by road	I. Ribera & A. Cieslak	**LT882889**	HF931281	HF931518	**LT882918**	**LT883085**	**LT882979**
53	*Hygrotus*	*Coelambus*	*marklini*	MNCN-AI89	Yes	Mongolia	Zavkhan Aimag, Telmen Soum,Ideriin Gol ~15 km SSW of Telmen / Ovogdii / N48.53255,E97.52093 / 1823 m	A.E.Z. Short	**LT882876**	HF931237	HF931467	**LT882919**	**LT883086**	
54	*Hygrotus*	*Coelambus*	*marklini*	NHM-IR682	No	Russia	Volgograd oblast / SW Lake of Elton / steppe pond with much vegetation	A.N. Nilsson		**LT882830**	**LT883055**			
55	*Hygrotus*	*Coelambus*	*masculinus*	NHM-IR290	No	US (CA)	Mono Co. / Long Valley, Owens river Rd. / saline lagoons	I. Ribera & A. Cieslak		HF931273	HF931510			
56	*Hygrotus*	*Coelambus*	*masculinus*	IBE-AV24	Yes	US (CA)	Mono Co. / stream outlet of big alkali lake at Benton crossing road	G. Challet	**LT882841**	**LT882779**	**LT883010**	**LT882920**	**LT883087**	**LT882980**
57	*Hygrotus*	*Coelambus*	*nigrescens*	MNCN-AI1152	Yes	Canada (AL)		T. Berendonk	**LT882864**	HF931167	HF931386	**LT882921**	**LT883088**	**LT882981**
58	*Hygrotus*	*Coelambus*	*nigrescens*	IBE-RA878	No	Canada (AL)	N53º39.346 W110º58.922	T. Berendonk	**LT882858**	**LT882806**				

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Table S1. (Continued)

ID	Genus	Subgenus	Species	Voucher	Red.	country	Loc.	leg	BAR	COI	16S	18S	H3	28S
59	*Hygrotus*	*Coelambus*	*nigrolineatus*	MNCN-AI970	Yes	Mongolia	Arkhangay / Bulgan / Urd Tamir Gol braid upstream of bridge, ~63 km SW of Tsetserleg / N47.11192 E101.01048 / 2066 m	A.E.Z. Short	**LT882879**	HF931254	HF931490	**LT882922**	**LT883089**	
60	*Hygrotus*	*Coelambus*	*novemlineatus*	NHM-IR714	Yes	UK (SC)	E. Sutherland, Loch Brora	I. Ribera	**LT882896**	HF931286	HF931523	**LT882923**	**LT883090**	**LT882982**
61	*Hygrotus*	*Coelambus*	*orthogrammus*	IBE-RA701	Yes	Iran	Khuzestan / 5 km SW Bandare Emam, -21 m / 30 5714N 49 0524E	Darvishzadej		**LT882802**	**LT883028**	**LT882924**	**LT883091**	**LT882983**
62	*Hygrotus*	*Coelambus*	*pallidulus*	MNCN-AI216	Yes	Spain	Alcolea de las Peñas / arroyo salino / 30TWL 17757/62735 / 992 m / Sal: 37,1 gr/l	A. Millán & col.	**LT882868**	HF931184	HF931407	**LT882925**	**LT883092**	
63	*Hygrotus*	*Coelambus*	*parallellogrammus*	MNCN-AI145	Yes	Germany	Mecklenburg-Vorpom. Hiddensee, N Witte St. 4 013º06'26"E 54º34'4"N	L. Hendrich	**LT882867**	**LT882812**	**LT883037**	**LT882926**	**LT883093**	
64	*Hygrotus*	*Coelambus*	*parallellogrammus*	NHM-IR683	No	Russia	Astrakhan oblast / Nishniy Baskuntyak / small stream from small lake at entrance to Bogdo Zapovednik	A.N. Nilsson		HF931284	HF931521			
65	*Hygrotus*	*Coelambus*	*patruelis*	MNCN-AI1163	No	Canada (AL)		T. Berendonk		HF931169	HF931388		**LT883094**	
66	*Hygrotus*	*Coelambus*	*patruelis*	IBE-AV53	Yes	Canada		T. Berendonk	**LT882845**	**LT882787**	**LT883015**	**LT882927**	**LT883095**	
67	*Hygrotus*	*Coelambus*	*pectoralis*	MNCN-AI1101	Yes	Mongolia	Zavkhan / Tosontsengel / Ideriin Gol ~15 km SSW of Telmen/Ovogdii / N48.53255 E97.52093 / 1823 m	A.E.Z. Short	**LT882861**	HF931157	HF931376	**LT882928**	**LT883096**	
68	*Hygrotus*	*Coelambus*	*pedalis*	MNCN-AI985	Yes	US (CA)	Mono Co. / Long Valley, Owens river Rd. / tributary r. Owen	I. Ribera & A. Cieslak	**LT882882**	**LT882821**	**LT883045**	**LT882929**	**LT883097**	**LT882984**
69	*Hygrotus*	*Coelambus*	*pedalis*	IBE-AV55	No	US (CA)	Amargosa River in Dumont Dunes / 35º41'45''N,116º15'5''W	P. Abellán	**LT882846**	**LT882789**			**LT883098**	
70	*Hygrotus*	*Coelambus*	*pedalis*	IBE-AV57	No	US (CA)	Coal Oil Point Reserve (Slough road) / 34º24'47''N,119º52'31''W	P. Abellán			**LT883016**			
71	*Hygrotus*	*Coelambus*	*picatus*	NHM-IR474	No	Canada (ON)	Manitoulin island, Ck. At Hwy 540, 20 km E Little Curreny	Y. Alarie			**LT883050**			
72	*Hygrotus*	*Coelambus*	*picatus*	NHM-IR481	Yes	Canada (AL)	Waterton lakes Nat. Park / junc. Rd. 5&6	I. Ribera & A. Cieslak	**LT882887**	HF931280	HF931517	**LT882930**	**LT883099**	**LT882985**
73	*Hygrotus*	*Coelambus*	*saginatus*	NHM-IR684	Yes	Russia	Astrakhan oblast / Kharabali / flooded margin of Volga just S of city	A.N. Nilsson	**LT882892**	AJ850639	AJ850389	AJ850524	EF670211	**LT882986**
74	*Hygrotus*	*Coelambus*	*saginatus nr*	IBE-AV43	No	Cyprus	Paralimni / ditch with reed / 35º02'22.7"N 33º57'38.6"E / 81m	A. Millán, I. Ribera, J. Velasco & A. Villastrigo		**LT882784**	**LT883014**	**LT882931**	**LT883100**	
75	*Hygrotus*	*Coelambus*	*saginatus nr*	IBE-AN67	Yes	Azerbaijan	Qobustan / ponds in beach / 40°5'38.9"N 49°25'19.5"E /26m	I. Ribera & A. Rudoy	**LT882836**	**LT882774**	**LT883005**	**LT882932**	**LT883101**	**LT882987**
76	*Hygrotus*	*Coelambus*	*salinarius*	GENBANK	Yes	US (WY)	Ref (2)		KJ548531	KJ548531	KJ548335		KJ548769	
77	*Hygrotus*	*Coelambus*	*sanfilippoi*	IBE-AF204	Yes	Sardinia (IT)	Sassari prov. / S Stintino (NW Porto Torres) / Stagno di Casaraccio (brakish pond, 0 m) / 40.91403N 8.23021E	H. Fery & M. Toledo	**LT882834**	HF931123	HF931342	**LT882933**	**LT883102**	**LT882988**
78	*Hygrotus*	*Coelambus*	*semivittatus*	GENBANK	Yes	US (WY)	Ref (3)			KF575509	KF575573		KF575392	
79	*Hygrotus*	*Coelambus*	*suturalis*	NHM-IR482	Yes	Canada (AL)	Waterton lakes Nat. Park / junc. Rd. 5&6	I. Ribera & A. Cieslak	**LT882888**	AJ850640	AJ850390	AJ850525	EF670212	**LT882991**
80	*Hygrotus*	*Coelambus*	*suturalis*	IBE-AV54	No	Canada		T. Berendonk		**LT882788**				
81	*Hygrotus*	*Coelambus*	*tumidiventris*	MNCN-AI984	No	US (CA)	Mono Co. / Long Valley, Owens river Rd. / tributary r. Owen	I. Ribera & A. Cieslak		HF931260	HF931496	**LT882934**	**LT883103**	
82	*Hygrotus*	*Coelambus*	*tumidiventris*	IBE-AV22	Yes	US (CA)	Mono Co. / Long Valley, Owens river Rd. / saline lagoons	I. Ribera & A. Cieslak	**LT882839**	**LT882777**	**LT883008**	**LT882935**	**LT883104**	**LT882989**
83	*Hygrotus*	*Coelambus*	*turbidus*	NHM-IR346	No	US (CA)	Mono Co. / Yosemite Ntal. Park / Rd. 120 Dana Meadows	I. Ribera & A. Cieslak		**LT882824**	**LT883048**			
84	*Hygrotus*	*Coelambus*	*turbidus*	IBE-AV56	Yes	US (CA)	Amargosa River in Dumont Dunes / 35º41'45''N,116º15'5''W	P. Abellán	**LT882847**	**LT882790**	**LT883017**	**LT882936**	**LT883105**	**LT882990**
85	*Hygrotus*	*Coelambus*	*unguicularis*	MNCN-AI968	Yes	Mongolia	Arkhangay / Bulgan / Urd Tamir Gol braid upstream of bridge, ~63 km SW of Tsetserleg / N47.11192 E101.01048 / 2066 m	A.E.Z. Short	**LT882878**	HF931253	HF931489	**LT882937**	**LT883106**	**LT882992**
86	*Hygrotus*	*Coelambus*	*unguicularis*	NHM-IR479	No	Canada (AL)	Waterton lakes Nat. Park / junc. Rd. 5&6	I. Ribera & A. Cieslak		**LT882826**	**LT883051**			
87	*Hygrotus*	*Coelambus*	*urgensis*	MNCN-AI962	Yes	Mongolia	Tov / Ondorshireet / Tuul River ~1.5 km W of Tuul Ovoot bridge, downstream of road to Arvayheer / N47.31096 E105.27119 / 1042 m	A.E.Z. Short		HF931250	HF931486	**LT882938**	**LT883107**	**LT882993**

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Table S1. (Continued)

ID	Genus	Subgenus	Species	Voucher	Red.	country	Loc.	leg	BAR	COI	16S	18S	H3	28S
88	*Hygrotus*	*Hygrotus*	*acaroides*	GENBANK	Yes	US (AL)	Ref (3)		KF575482	KF575482	KF575542		KF575361	
89	*Hygrotus*	*Hygrotus*	*decoratus*	MNCN-AI841	Yes	UK (EN)	Norfolk / Ludham / How Hill Marsh	I. Ribera	**LT882875**	HF931235	HF931464	**LT882939**	**LT883108**	**LT882994**
90	*Hygrotus*	*Hygrotus*	*decoratus*	NHM-IR711	No	Austria	Voralberg / Rheintal, Bodensee. Hard Rheinvorland / 400 m / N47º29'48" E 9º39'59"	I. Ribera		**LT882832**	**LT883057**			
91	*Hygrotus*	*Hygrotus*	*hydropicus*	IBE-AV51	Yes	US (CA)	Monterrey Co., 35.9518ºN 121.3017ºW / Nacimiento-Ferguson rd.	M. Caterino & A.E.Z. Short	**LT882844**	**LT882785**		**LT882940**	**LT883109**	**LT882995**
92	*Hygrotus*	*Hygrotus*	*inaequalis*	NHM-IR19	No	Spain	Girona, Capmany, Estanys inferior	I. Ribera		EF056611	EF056681	AJ318737		**LT882996**
93	*Hygrotus*	*Hygrotus*	*inaequalis*	IBE-RA208	Yes	Greece	NE Peloponnese, Stymfalia Lake / open swamps, ca. 6 km ENE Lafka, net & bottle traps / 37.84411N 22.45478E / 610 m	H. Fery & L. Hendrich	**LT882853**	**LT882798**	**LT883024**	**LT882941**	**LT883110**	**LT882997**
94	*Hygrotus*	*Hygrotus*	*quinquelineatus*	MNCN-AI979	No	Mongolia	Zavkhan / Tosontsengel / Ideriin Gol ~15 km SSW of Telmen/Ovogdii / N48.53255 E97.52093 / 1823 m	A.E.Z. Short		**LT882820**				
95	*Hygrotus*	*Hygrotus*	*quinquelineatus*	MNCN-DM4	Yes	Sweden	Västerbotten prov. / Åmsele / Vindelälven river lagoon / 64º31'04"N, 19º20'52"E	A.N. Nilsson	**LT882883**	HF931268	HF931506	**LT882942**	**LT883111**	**LT882998**
97	*Hygrotus*	*Hygrotus*	*sayi*	MNCN-AI1149	Yes	US (NY)	Tompkins Co. / Marsh on Elllis Hollow Road, 4 mi. E of Pine Tree Road	A.E.Z. Short	**LT882863**	HF931164	HF931383	**LT882943**	**LT883112**	**LT882999**
98	*Hygrotus*	*Hygrotus*	*sayi*	IBE-AV52	No	Canada		T. Berendonk		**LT882786**				
99	*Hygrotus*	*Hygrotus*	*versicolor*	NHM-IR691	Yes	Austria	Voralberg, Pfänderstock, Bodensee	I. Ribera	**LT882895**	HF931285	HF931522	**LT882944**	**LT883113**	**LT883000**
100	*Hyphoporus*		*solieri*	IBE-RA700	Yes	Iran	Khuzestan / 8 km SW Abadan, 8 m / 30 3541N 48 2267E / pond	Darvishzadej		**LT882801**	**LT883027**	**LT882945**	**LT883114**	
101	*Hyphoporus*		*tonkinenesis*	IBE-RA769	Yes	China	Yunnan Xin ping Mamushu / cun 1127m 2011.v.20 / N24.02121 E102.12882	S. Keqing	**LT882857**	**LT882805**	**LT883031**	**LT882946**	**LT883115**	**LT883001**
102	*Hydroporus*		*basinotatus*	MNCN-AH112	No	Spain	Cádiz, Tarifa / stream 2K N Na. Sra de la Luz / 10m / N36º05'53.4" W5º37'35.0"	I. Ribera & P. Aguilera & C. Hernando	**LT882859**	HE610196	**LT883033**	**LT882955**	**LT883124**	
103	*Hydroporus*		*kasyi*	IBE-AV42	No	Cyprus	Akrotiri / Fassouri reedbeds / 34º37'57.2"N 32º56'01.5"E / 28m	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT882843**	**LT882783**	**LT883013**	**LT882957**	**LT883126**	
104	*Hydroporus*		*obsoletus*	IBE-RA206	No	Greece	Peloponnese / Afrodisio Mts / ca. 5 km NE Kondovazena / small spring / ca. 37.82044N 21.93570E / ca. 1098 m	H. Fery & L. Hendrich	**LT882852**	**LT882797**	**LT883023**	**LT882954**	**LT883123**	
105	*Hydroporus*		*tessellatus*	MNCN-AI770	No	Turkey	DÜZCE / Rd. to Kartalkaya from Çaydurt / small stream with vegetation / 1191m / N40º42'29" E31º45'56"	I. Ribera	**LT882873**	**LT882817**	**LT883042**	**LT882956**	**LT883125**	
106	*Deronectes*		*ferrugineus*	MNCN-AI731	No	Portugal	Guarda district / Sabugueiro (Serra da Estrela)	I. Ribera	LN995103	LN995072	LN995176	**LT882949**	LN995142	
107	*Deronectes*		opatrinus	MNCN-AI629	No	Spain	Córdova province / Sierra Morena	A. Castro	LN995112	HE610188	LN995185	**LT882948**	LN995151	
108	*Deronectes*		*sahlbergi*	MNCN-AI1002	No	Bulgaria	Haskovo province / Madjarovo (Eastern Rhodope mountains)	Pešić	**LT882860**	HE610191	HF931361	**LT882947**	**LT883116**	
109	*Boreonectes*		*funereus*	MNCN-AI1208	No	US (CA)		Y. Alarie	**LT882865**	HF931173	HF931393	**LT882950**	**LT883119**	
110	*Boreonectes*		*ibericus*	NHM-IR22	No	Portugal	Sa. Da Estrela, Torre, lagoon	I. Ribera	**LT882885**	EF670064	EF670030	EF670271 EF670298	EF670157	
111	*Boreonectes*		*riberae*	MNCN-AI829	No	Turkey	DÜZCE / Rd. to Kartalkaya from Çaydurt / pools in mountain pass / 1700m / N40º40'20" E31º47'05"	I. Ribera	**LT882874**	HF931232	HF931461	**LT882951**	LT796550	
112	*Nebrioporus*		*amicorum*	IBE-AF140	No	Crete (GR)	Heraklion, Arkalohori, Tsoutsouros / stream 34°59'05" 25°17'07'	Z. Csabai et al.	**LT882833**	HF931118	HF931335	**LT882953**	**LT883122**	**LT883003**
113	*Nebrioporus*		*martinii*	MNCN-AI702	No	Corsica (FR)	Corte, river Restonica, 2	I. Ribera & A. Cieslak	**LT882871**	**LT882815**	**LT883040**	**LT882952**	**LT883121**	**LT883002**
114	*Laccornis*		*difformis*	GENBANK	No	US (NY)	Ref (3)			KF575484	KF575544		KF575363	
115	*Laccornis*		*kocae*	NHM-IR681	No	Russia	Volgograd oblast / SW Lake of Elton / steppe pond with much vegetation	A.N. Nilsson		AJ850666	AJ850419	AJ850546	EF670226	
116	*Laccornis*		*oblongus*	NHM-IR55	No	Scotland (UK)	Mount Bog / Peebles	D.T. Bilton		AF309298	AF309241	AJ318715	EF056579	

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Table S2.

A) Primers used in the amplifying and sequencing reactions.

gene	primer	sequence	ref.
COI-3’	Jerry (5’)	CAACATTTATTTTGATTTTTTGG	6
	Pat (3’)	TCCAATGCACTAATCTGCCATATTA	6
	Chy (5’)	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	4
	Tom (3’)	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	4
COI-5’	Uni LepF1b	TAATACGACTCACTATAGGGATTCAACCAATCATAAAGATATTGGAAC	2
	Uni LepR1	ATTAACCCTCACTAAAGTAAACTTCTGGATGTCCAAAAAATCA	2
16S+trnL+nad1	16SaR (5’)	CGCCTGTTTAACAAAAACAT	6
	ND1 (3’)	GGTCCCTTACGAATTTGAATATATCCT	6
16S	16Sb	CCGGTCTGAACTCAGATCATGT	6
18S	18S 5’	GACAACCTGGTTGATCCTGCCAGT(1)	5
	18s b5.0	TAACCGCAACAACTTTAAT(1)	5
H3	H3aF (5’)	ATGGCTCGTACCAAGCAGACRCG	1
	H3aR (3’)	ATATCCTTRGGCATRATRGTGAC	1
28S	ka	ACACGGACCAAGGAGTCTAGCATG	3
	kb	CGTCCTGCTGTCTTAAGTTAC	3

B) Standard PCR conditions for the amplification of the studied fragments.

step	time	temperature
1	30’	96
2	30”	94
3	30” - 1’	47-50 *
4	1’	72
5	Go to setp 2 and repeat 34 - 40 x	
6	10’	72

* Depending on the annealing temperatures of the primers pair used

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Table S3. Checklist of the species of Hygrotini (Nilsson & Hájek, 2017a,b) with the typical habitat and the species for which material could be studied. AF, Afrotropical; H, Holarctic; NA, Nearctic; NT, Neotropical; OR, Oriental; PL, Palaearctic.

ID	Genus	Subgenus	Species	Authors	Distribution	habitat	data type studied
1	*Heroceras*		*descarpentriesi*	(Peschet, 1923)	AF	?	morphological & molecular
2	*Herophydrus*		*assimilis*	Régimbart, 1895	AF	?	morphological & molecular
3	*Herophydrus*		*bilardoi*	Biström & Nilsson, 2002	AF	?	only literature
4	*Herophydrus*		*capensis*	Régimbart, 1895	AF	?	only literature
5	*Herophydrus*		*cleopatrae*	(Peyron, 1858)	PL	?	morphological
6	*Herophydrus*		*confusus*	Régimbart, 1895	AF	?	only literature
7	*Herophydrus*		*discrepatus*	Guignot, 1954	AF	Tolerant	only literature
8	*Herophydrus*		*endroedyi*	Biström & Nilsson, 2002	AF	Freshwater	only literature
9	*Herophydrus*		*gigantoides*	Biström & Nilsson, 2002	AF	?	only literature
10	*Herophydrus*		*gigas*	Régimbart, 1895	AF	Freshwater	morphological
11	*Herophydrus*		*goldschmidti*	Pedernazi & Rocchi, 2009	AF	?	only literature
12	*Herophydrus*		*gschwendtneri*	Omer-Cooper, 1957	AF	?	only literature
13	*Herophydrus*		*guineensis*	(Aubé, 1838)	AF, PL	Freshwater	morphological & molecular
14	*Herophydrus*		*heros*	Sharp, 1882	AF	Freshwater	morphological
15	*Herophydrus*		*hyphoporoides*	Régimbart, 1895	AF	?	only literature
16	*Herophydrus*		*ignoratus*	Gschwendtner, 1935	AF	?	only literature
17	*Herophydrus*		*inquinatus*	(Boheman, 1848)	AF	Freshwater	morphological & molecular
18	*Herophydrus*		*janssensi*	Guignot, 1952	AF	?	only literature
19	*Herophydrus*		*kalaharii*	Gschwendtner, 1935	AF	?	only literature
20	*Herophydrus*		*morandi*	Guignot, 1952	OR	?	only literature
21	*Herophydrus*		*musicus*	(Klug, 1834)	AF, OR, PL	Tolerant	morphological & molecular
22	*Herophydrus*		*muticus*	(Sharp, 1882)	AF	Freshwater	morphological & molecular
23	*Herophydrus*		*natator*	Biström & Nilsson, 2002	AF	?	only literature
24	*Herophydrus*		*nigrescens*	Biström & Nilsson, 2002	AF	Tolerant	morphological & molecular
25	*Herophydrus*		*nodieri*	(Régimbart, 1895)	AF	Freshwater	morphological & molecular
26	*Herophydrus*		*obscurus*	Sharp, 1882	AF	Tolerant	morphological & molecular
27	*Herophydrus*		*obsoletus*	Régimbart, 1895	AF	?	only literature
28	*Herophydrus*		*ovalis*	Gschwendtner, 1932	AF	?	only literature
29	*Herophydrus*		*pallidus*	Omer-Cooper, 1931	AF	?	only literature
30	*Herophydrus*		*pauliani*	Guignot, 1950	AF	?	only literature
31	*Herophydrus*		*quadrilineatus*	Régimbart, 1895	AF	?	morphological
32	*Herophydrus*		*reticulatus*	Pedernazi & Rocchi, 2009	AF	Freshwater	morphological & molecular
33	*Herophydrus*		*ritsemae*	Régimbart, 1889	AF	Freshwater	only literature
34	*Herophydrus*		*rohani*	Peschet, 1924	AF	Freshwater	molecular
35	*Herophydrus*		*rufus*	(Clark, 1863)	AF	?	morphological
36	*Herophydrus*		*sjostedti*	Régimbart, 1908	AF	?	only literature
37	*Herophydrus*		*spadiceus*	Sharp, 1882	AF	Tolerant	molecular
38	*Herophydrus*		*sudanensis*	Guignot, 1952	AF	Freshwater	only literature
39	*Herophydrus*		*travniceki*	Šťastný, 2012	AF	?	morphological
40	*Herophydrus*		*tribolus*	Guignot, 1953	AF	Freshwater	morphological & molecular
41	*Herophydrus*		*variabilis secundus*	Régimbart, 1906	AF	?	only literature
42	*Herophydrus*		*variabilis variabilis*	Guignot, 1954	AF	?	only literature
43	*Herophydrus*		*vazirani*	(Nilsson, 1999)	PL	?	only literature
44	*Herophydrus*		*verticalis*	Sharp, 1882	AF	Freshwater	morphological & molecular
45	*Herophydrus*		*vittatus*	Régimbart, 1895	AF	?	only literature
46	*Herophydrus*		*wewalkai*	Biström & Nilsson, 2002	AF	?	only literature
47	*Hygrotus*	*Coelambus*	*ahmeti*	Hájek, Fery & Erman, 2005	PL	Freshwater	morphological & molecular
48	*Hygrotus*	*Coelambus*	*armeniacus*	(Zaitzev, 1927)	PL	Tolerant	morphological & molecular
49	*Hygrotus*	*Coelambus*	*artus*	(Fall, 1919)	NA	Tolerant[2]	only literature
50	*Hygrotus*	*Coelambus*	*berneri*	Young & Wolfe, 1984	NA	?	morphological
51	*Hygrotus*	*Coelambus*	*bruesi*	(Fall, 1928)	NA	Tolerant	morphological & molecular
52	*Hygrotus*	*Coelambus*	*caspius*	(Wehncke, 1875)	PL	Freshwater	morphological & molecular

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Table S3. (Continued)

ID	Genus	Subgenus	Species	Authors	Distribution	habitat	data type studied
53	*Hygrotus*	*Coelambus*	*chinensis*	(Sharp, 1882)	PL	?	morphological
54	*Hygrotus*	*Coelambus*	*collatus*	(Fall, 1919)	NA	?	morphological
55	*Hygrotus*	*Coelambus*	*compar*	(Fall, 1919)	NA	Tolerant	morphological & molecular
56	*Hygrotus*	*Coelambus*	*confluens*	(Fabricius, 1787)	PL	Tolerant	morphological & molecular
57	*Hygrotus*	*Coelambus*	*corpulentus*	(Schaum, 1864)	PL	?	morphological & molecular
58	*Hygrotus*	*Coelambus*	*curvilobus*	Fery, Sadegui & Hosseinie, 2005	PL	?	morphological
59	*Hygrotus*	*Coelambus*	*curvipes*	(Leech, 1938)	NA	?	morphological
60	*Hygrotus*	*Coelambus*	*dissimilis*	(Geminger & Harold, 1868)	NA	?	morphological
61	*Hygrotus*	*Coelambus*	*diversipes*	Leech, 1966	NA	Hypersaline	morphological & molecular
62	*Hygrotus*	*Coelambus*	*enneagrammus*	(Ahrens, 1833)	PL	Tolerant	morphological
63	*Hygrotus*	*Coelambus*	*falli*	(Wallis, 1924)	NA	?	morphological
64	*Hygrotus*	*Coelambus*	*femoratus*	(Fall, 1901)	NA	?	only literature
65	*Hygrotus*	*Coelambus*	*flaviventris*	(Motschulsky, 1860)	PL	Tolerant	morphological & molecular
66	*Hygrotus*	*Coelambus*	*fontinalis*	Leech, 1966	NA	Hypersaline	morphological & molecular
67	*Hygrotus*	*Coelambus*	*fraternus*	(LeConte, 1852)	NA	?	morphological
68	*Hygrotus*	*Coelambus*	*fresnedai*	(Fery, 1992)	PL	Freshwater[3]	morphological & molecular
69	*Hygrotus*	*Coelambus*	*fumatus*	(Sharp, 1882)	NA	Freshwater	morphological & molecular
70	*Hygrotus*	*Coelambus*	*impressopunctatus*	(Schaller, 1783)	H	Freshwater[3]	morphological & molecular
71	*Hygrotus*	*Coelambus*	*infuscatus*	(Sharp, 1882)	NA	Tolerant	morphological
72	*Hygrotus*	*Coelambus*	*inscriptus*	(Sharp, 1882)	PL	Hypersaline	morphological
73	*Hygrotus*	*Coelambus*	*laccophilinus*	(LeConte, 1878)	NA	Freshwater	morphological & molecular
74	*Hygrotus*	*Coelambus*	*lagari*	(Fery, 1992)	PL	Tolerant	morphological & molecular
75	*Hygrotus*	*Coelambus*	*lernaeus*	(Schaum, 1857)	PL	Tolerant	morphological & molecular
76	*Hygrotus*	*Coelambus*	*lutescens*	(LeConte, 1852)	NA	Tolerant	morphological & molecular
77	*Hygrotus*	*Coelambus*	*marklini*	(Gyllenhal, 1813)	H	Tolerant[4]	morphological & molecular
78	*Hygrotus*	*Coelambus*	*masculinus*	(Crotch, 1874)	NA	Hypersaline	morphological & molecular
79	*Hygrotus*	*Coelambus*	*nigrescens*	(Fall, 1919)	NA	Freshwater	morphological & molecular
80	*Hygrotus*	*Coelambus*	*nigrolineatus*	(Steven, 1808)	PL	Tolerant	morphological & molecular
81	*Hygrotus*	*Coelambus*	*novemlineatus*	(Stephens, 1829)	H	Tolerant	morphological & molecular
82	*Hygrotus*	*Coelambus*	*nubilus*	(LeConte, 1855)	NA, NT	?	morphological
83	*Hygrotus*	*Coelambus*	*obscureplagiatus*	(Fall, 1919)	NA	?	morphological
84	*Hygrotus*	*Coelambus*	*orthogrammus*	(Sharp, 1882)	PL	Tolerant	morphological & molecular
85	*Hygrotus*	*Coelambus*	*pallidulus*	(Aubé, 1850)	PL	Hypersaline[1]	morphological & molecular
86	*Hygrotus*	*Coelambus*	*parallellogrammus*	(Ahrens, 1812)	PL	Tolerant	morphological & molecular
87	*Hygrotus*	*Coelambus*	*patruelis*	(LeConte, 1855)	NA	Tolerant	morphological & molecular
88	*Hygrotus*	*Coelambus*	*pectoralis*	(Motschulsky, 1860)	PL	Tolerant	morphological & molecular
89	*Hygrotus*	*Coelambus*	*pedalis*	(Fall, 1901)	NA	Tolerant	morphological & molecular
90	*Hygrotus*	*Coelambus*	*picatus*	(Kirby, 1837)	NA	Freshwater	morphological & molecular
91	*Hygrotus*	*Coelambus*	*polonicus polonicus*	(Aubé, 1842)	PL	Tolerant	morphological
92	*Hygrotus*	*Coelambus*	*polonicus sahlbergi*	(Sharp, 1882)	PL	Tolerant	morphological
93	*Hygrotus*	*Coelambus*	*punctilineatus*	(Fall, 1919)	NA	Tolerant	morphological
94	*Hygrotus*	*Coelambus*	*saginatus*	(Schaum, 1857)	PL	Tolerant	morphological & molecular
95	*Hygrotus*	*Coelambus*	*salinarius*	(Wallis, 1924)	NA	Hypersaline	morphological & molecular
96	*Hygrotus*	*Coelambus*	*sanfilippoi*	(Fery, 1992)	PL	Tolerant	morphological & molecular
97	*Hygrotus*	*Coelambus*	*sellatus*	(LeConte, 1866)	NA	Tolerant	morphological
98	*Hygrotus*	*Coelambus*	*semenowi*	(Jakovlev, 1899)	PL	Freshwater	morphological
99	*Hygrotus*	*Coelambus*	*semivittatus*	(Fall, 1919)	NA	Tolerant	morphological & molecular
100	*Hygrotus*	*Coelambus*	*stefanschoedli*	Fery, Sadegui & Hosseinie, 2005	PL	?	morphological
101	*Hygrotus*	*Coelambus*	*suturalis*	(LeConte, 1850)	NA	Freshwater	morphological & molecular
102	*Hygrotus*	*Coelambus*	*sylvanus*	(Fall, 1917)	NA	Freshwater	morphological
103	*Hygrotus*	*Coelambus*	*thermarum*	(Darlington, 1928)	NA	?	morphological
104	*Hygrotus*	*Coelambus*	*tumidiventris*	(Fall, 1919)	NA	Hypersaline	morphological & molecular
105	*Hygrotus*	*Coelambus*	*turbidus*	(LeConte, 1855)	NA	Freshwater	morphological & molecular
106	*Hygrotus*	*Coelambus*	*unguicularis*	(Crotch, 1874)	H	Tolerant	morphological & molecular
107	*Hygrotus*	*Coelambus*	*urgensis*	(Jakovlev, 1899)	PL	Freshwater	morphological & molecular
108	*Hygrotus*	*Coelambus*	*wardii*	(Clark, 1862)	NA, NT	?	morphological
109	*Hygrotus*	*Coelambus*	*zigetangco*	Fery, 2003	PL	?	morphological
110	*Hygrotus*	*Hygrotus*	*acaroides*	(LeConte, 1855)	NA	Freshwater	morphological & molecular

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Table S3. (Continued)

ID	Genus	Subgenus	Species	Authors	Distribution	habitat	data type studied
111	*Hygrotus*	*Hygrotus*	*aequalis*	Falkenström, 1932	PL	Freshwater	morphological
112	*Hygrotus*	*Hygrotus*	*decoratus*	(Gyllenhal, 1810)	PL	Freshwater	morphological & molecular
113	*Hygrotus*	*Hygrotus*	*farctus*	(LeConte, 1855)	NA	?	morphological
114	*Hygrotus*	*Hygrotus*	*hydropicus*	(LeConte, 1852)	NA	?	morphological & molecular
115	*Hygrotus*	*Hygrotus*	*inaequalis*	(Fabricius, 1777)	PL	Freshwater	morphological & molecular
116	*Hygrotus*	*Hygrotus*	*intermedius*	(Fall, 1919)	NA	Freshwater	morphological
117	*Hygrotus*	*Hygrotus*	*marginipennis*	(Blatchley, 1912)	NA	Tolerant	morphological
118	*Hygrotus*	*Hygrotus*	*quinquelineatus*	(Zetterstedt, 1828)	PL	Freshwater	morphological & molecular
119	*Hygrotus*	*Hygrotus*	*sayi*	J. Balfour-Browne, 1944	NA	Tolerant	morphological & molecular
120	*Hygrotus*	*Hygrotus*	*versicolor*	(Schaller, 1783)	PL	Freshwater	morphological & molecular
121	*Hyphoporus*		*anitae*	Vazirani, 1969	PL	Freshwater	only literature
122	*Hyphoporus*		*aper*	Sharp, 1882	OR, PL	?	only literature
123	*Hyphoporus*		*bengalensis*	Severini, 1890	OR	?	morphological
124	*Hyphoporus*		*bertrandi*	Vazirani, 1969	PL	Freshwater	only literature
125	*Hyphoporus*		*caliginosus*	Régimbart, 1899	OR	?	only literature
126	*Hyphoporus*		*dehraduni*	Vazirani, 1969	PL	?	only literature
127	*Hyphoporus*		*elevatus*	Sharp, 1882	OR, PL	?	morphological
128	*Hyphoporus*		*geetae*	Vazirani, 1969	OR	?	only literature
129	*Hyphoporus*		*josephi*	Vazirani, 1969	OR	?	only literature
130	*Hyphoporus*		*kempi*	Gschwendtner, 1936	OR, PL	Tolerant	only literature
131	*Hyphoporus*		*montanus*	Régimbart, 1899	OR	?	only literature
132	*Hyphoporus*		*nilghiricus*	Régimbart, 1903	OR, PL	Freshwater	only literature
133	*Hyphoporus*		*oudomxai*	Biström & Bilsson, 2013	OR	?	only literature
134	*Hyphoporus*		*pacistanus*	Guignot, 1959	PL	?	only literature
135	*Hyphoporus*		*pugnator*	Sharp, 1890	OR	?	only literature
136	*Hyphoporus*		*severini*	Régimbart, 1892	OR, PL	Tolerant	morphological
137	*Hyphoporus*		*solieri*	(Aubé, 1838)	PL	Freshwater	morphological & molecular
138	*Hyphoporus*		*subaequalis*	Vazirani, 1969	OR	?	only literature
139	*Hyphoporus*		*tonkinensis*	Régimbart, 1899	OR	?	morphological & molecular

Fig. S1. Time-calibrated majority rule consensus tree obtained in BEAST with the complete dataset. Number in nodes: posterior probability values.

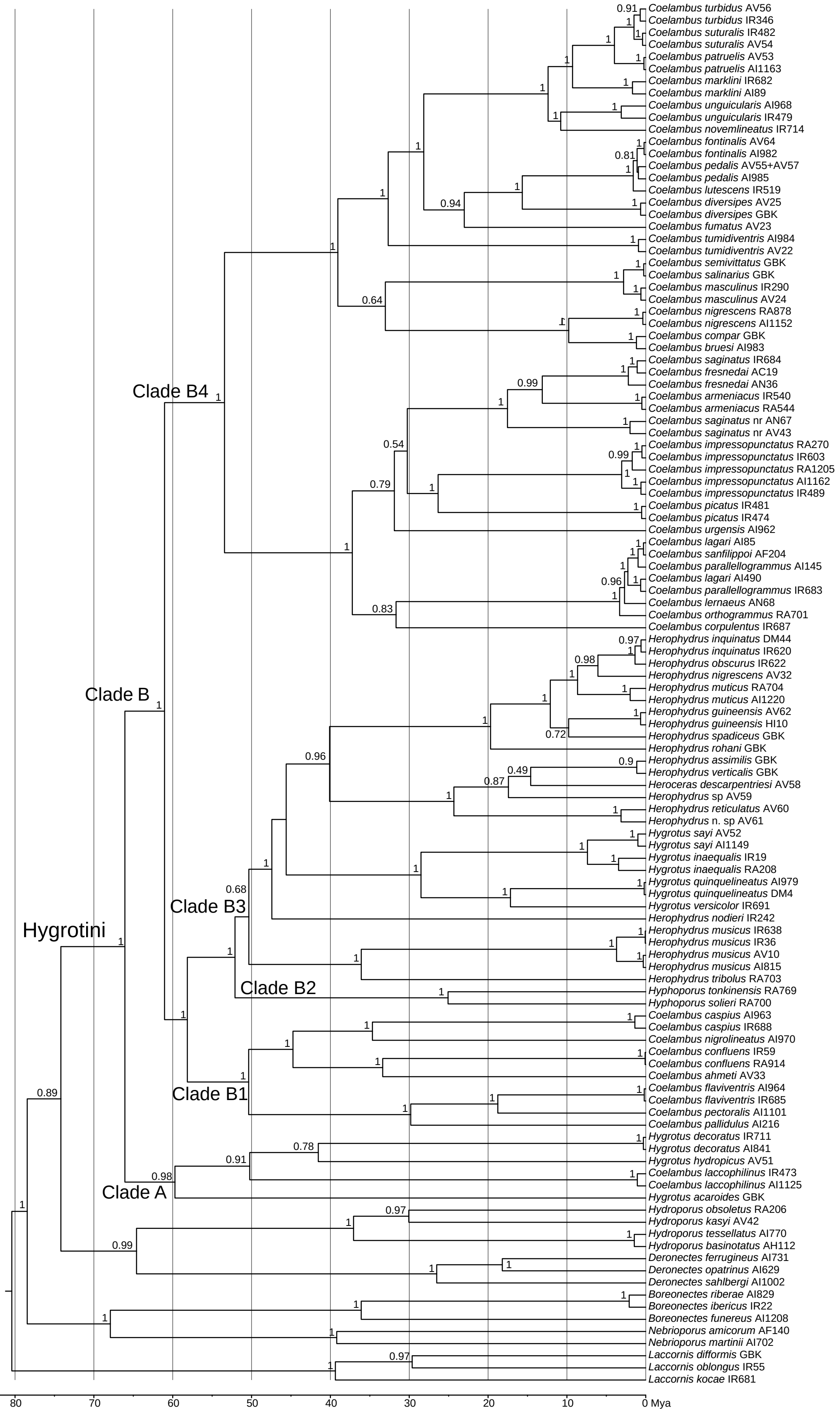
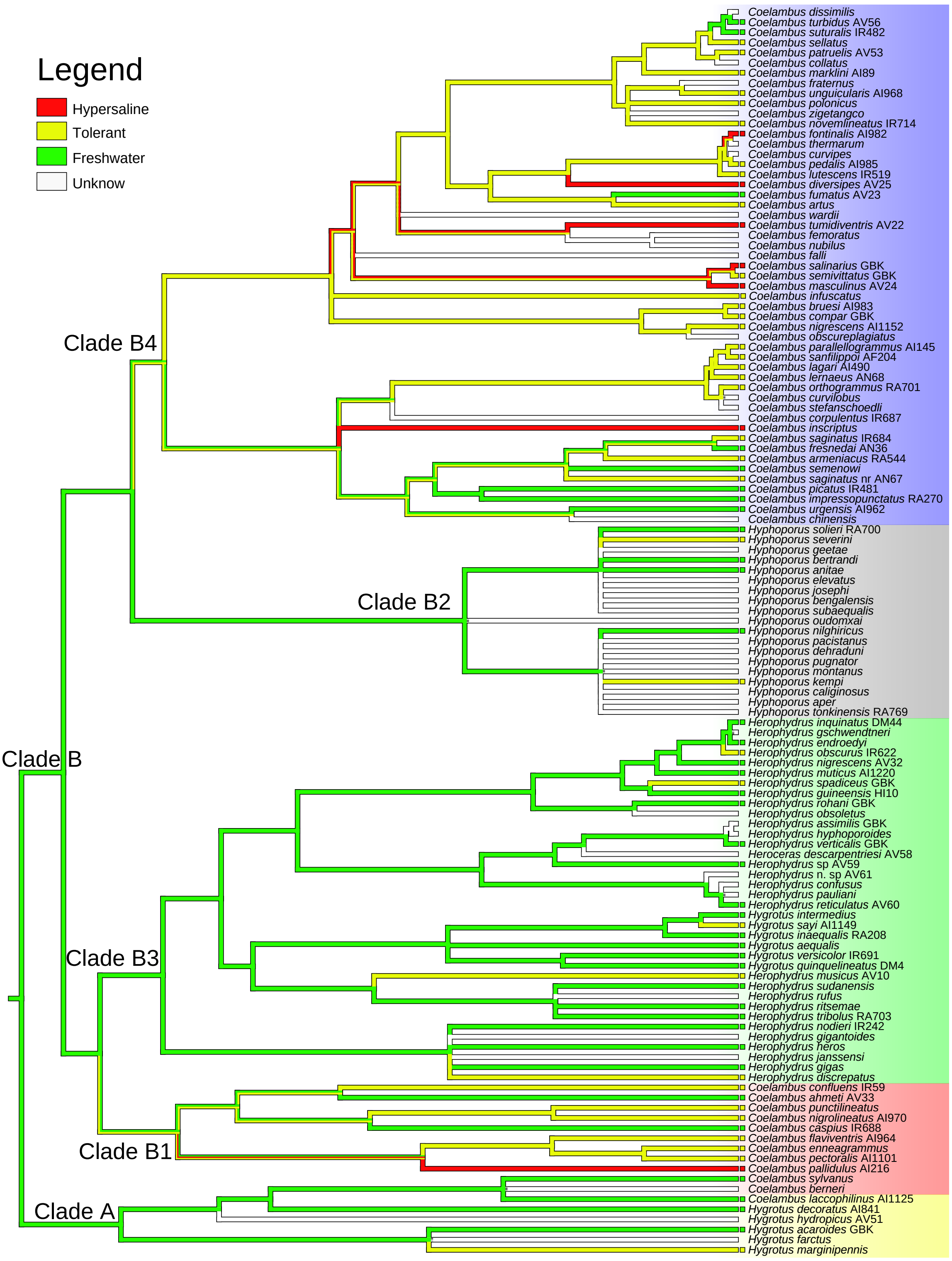


Fig. S2. Reconstructed evolution of salinity tolerance in the tribe Hygrotini, using parsimony with the estimated relationships of species for which no molecular data were available.



Chapter 3

A molecular phylogeny of the tribe Ochthebiini (Coleoptera, Hydraenidae, Ochthebiinae)

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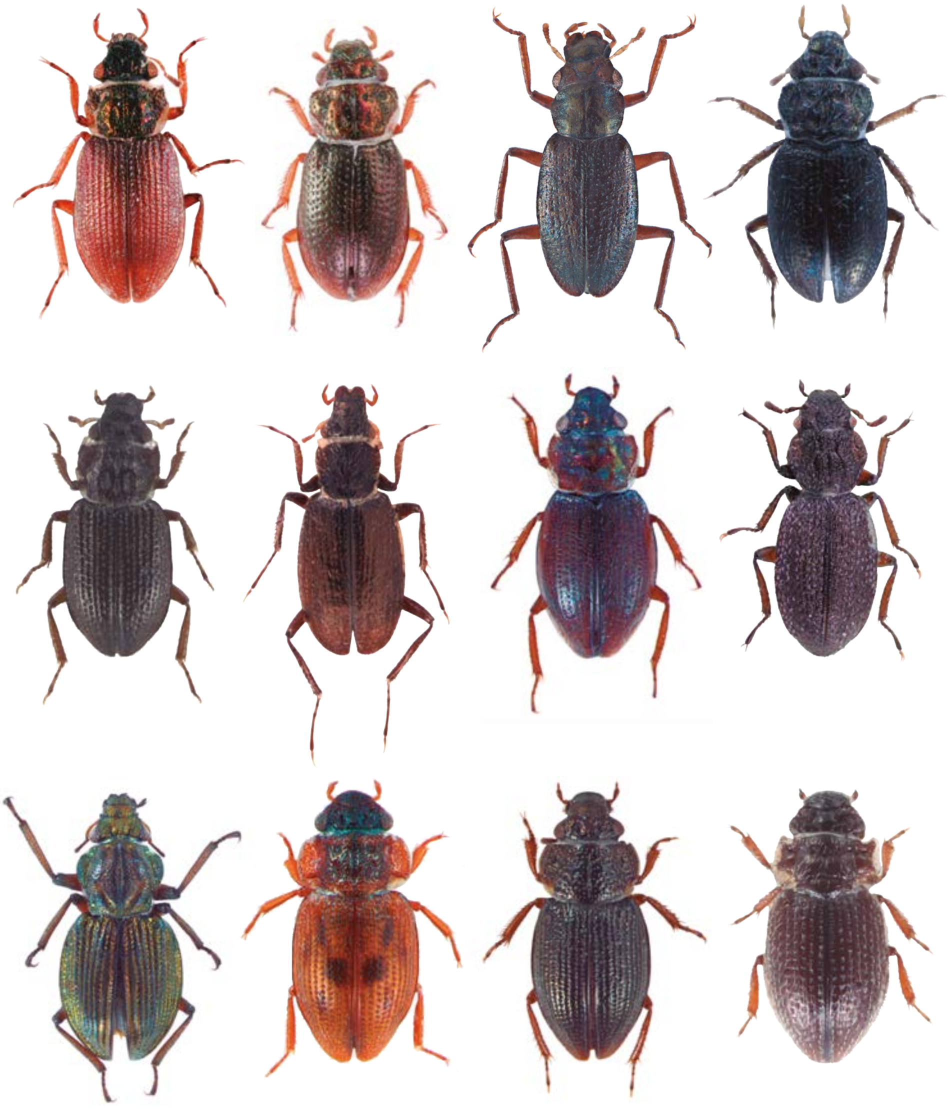
Published on: *Systematic Entomology* (2019) **44(2)**: 273-288



Abstract

Ochthebiinae, with c. 650 species distributed worldwide, are the second most speciose subfamily of the aquatic beetle family Hydraenidae. They are the ecologically most diverse subfamily, with terrestrial species as well as species in almost all types of aquatic habitats, including hypersaline waters. Ochthebiinae include the tribes Ochtheosini (four species in three genera) and Ochthebiini. We provide here the first comprehensive phylogeny of the tribe Ochthebiini, based on 188 species and four subspecies from most genera, subgenera and species groups. We obtained sequence data for a combination of mitochondrial and nuclear gene fragments including the 5' and 3' ends of the cytochrome *c* oxidase subunit 1, the 5' end of 16S RNA plus the leucine tRNA transfer plus 5' end of NADH dehydrogenase subunit I, and internal fragments of the large and small ribosomal units. The analyses with maximum likelihood and Bayesian probabilities consistently recovered a generally well supported phylogeny, with most currently accepted taxa and species groups as monophyletic. We provide a new classification of the tribe based on our phylogenetic results, with six genera: *Meropathus* Enderlein, *Ochthebius* Leach, *Protochthebius* Perkins, *Prototympanogaster* Perkins, *Tympallopatrum* Perkins, and *Tympanogaster* Janssens. The genus *Ochthebius* is here divided into nine subgenera in addition to *Ochthebius* s.str.: (1) *O*. (*Angiochthebius*) Jäch & Ribera; (2) *O*. (*Asiobates*) Thomson; (3) *O*. (*Aulacochthebius*) Kuwert; (4) *O*. (*Cobalius*) Rey; (5) *O*. (*Enicocerus*) Stephens; (6) *O*. (*Gymnanthelius*) Perkins **comb.n.**; (7) *O*. (*Gymnochthebius*) Orchymont comb.n.; (8) *O*. (*Hughleechia*) Perkins **comb.n.**; and (9) *O*. (*Micragasma*) Sahlberg. Within *Ochthebius* s.str., 17 species groups are proposed, five of them newly established (3, 9, 11, 13 and 16): (1) *andraei*; (2) *atriceps*; (3) *corrugatus*; (4) *foveolatus*; (5) *kosiensis*; (6) *lobicollis*; (7) *marinus*; (8) *metallescens*; (9) *nitidipennis*; (10) *notabilis*; (11) *peisonis*; (12) *punctatus*; (13) *quadricollis*; (14) *rivalis*; (15) *strigosus*; (16) *sumatrensis*; and (17) *vandykei*. We elevated to species rank two subspecies of *Ochthebius*: *O. fallaciosus* Ganglbauer **stat.n.** (former subspecies of *O. viridis* Peyron) and *O. deletus* Rey **stat.rest.** (former subspecies of *O. subpictus* Wollaston).

Introduction

Ochthebiinae, with c. 650 species and 11 subspecies described, are the second most diverse subfamily of the water beetle family Hydraenidae (Hansen, 1998; Jäch & Balke, 2008; Tables 1, S1). They occur in all biogeographic regions, including the Antarctic islands of Kerguelen and Heard, where they are the only Hydraenidae present (Hansen, 1998). Ochthebiinae are the ecologically most diverse hydraenid subfamily, with terrestrial species, species living in the interface between land and water, as well as in most types of aquatic environments (Jäch *et al*., 2016). A large number of species are tolerant to hypersaline waters, living in coastal rockpools (e.g. *Cobalius* Rey or *Calobius* Wollaston, Antonini *et al*., 2010; Sabatelli *et al*., 2016), coastal or inland saltpans or inland hypersaline streams (especially the O. *notabilis* group, but also many other species in different groups, Abellán *et al*., 2009; Millán *et al*., 2011).

The external morphology of the species of Ochthebiinae is more heterogeneous than in other Holarctic lineages of Hydraenidae [e.g. *Hydraena* Kugelann (Trizzino *et al*., 2013) or *Limnebius* Leach (Rudoy *et al*., 2016)]. This has resulted in a more complex taxonomy, with mostly all of the described genera or subgenera with uncertain relationships. Thus, the genus *Ochthebius* Leach was divided in 16 subgenera by Kuwert (1887), but all of them with the exception of *Aulacochthebius* Kuwert, were later synonymised (see Tables 1, S2 for a synopsis of the classification and Table S1 for a complete checklist of Ochthebiini).

Ochthebiinae were divided by Perkins (1980) into two tribes, Ochthebiini and Ochtheosini, the latter for the single terrestrial genus *Ochtheosus* Perkins, with two species. The monotypic genera *Edaphobates* Jäch & Díaz and *Ginkgoscia* Jäch & Díaz, for which we could not obtain fresh material for DNA extraction, were tentatively hypothesized to be related to *Ochtheosus* by Jäch & Díaz (2003, 2004) and thus we consider them within Ochtheosini. Perkins (1997) divided Ochthebiini in five newly defined subtribes: Enicocerina (for the single genus *Enicocerus* Stephens), Meropathina (*Meropathus* Enderlein, *Tympallopatrum* Perkins, *Tympanogaster* Perkins and the recently described *Prototympanogaster* Perkins; Perkins, 2018), Neochthebiina (*Neochthebius* Orchymont), Ochthebiina (*Ochthebius*, *Gymnochthebius* Orchymont, *Hughleechia* Perkins, *Gymnanthelius* Perkins, *Aulacochthebius* and *Micragasma* Sahlberg) and Protochthebiina (*Protochthebius* Perkins). *Enicocerus* was treated as a subgenus by several subsequent authors (e.g. Jäch, 1998; Ribera *et al*., 2010; Jäch & Skale, 2015), and *Neochthebius* was treated as a synonym of *Ochthebius* s.str. by Jäch & Delgado (2014b), leaving ten genera in Ochthebiini, most of them described in the 20th century (Table 1). *Ochthebius* is the oldest available generic name (Leach, 1815; Hansen, 1998; Table 1), grouped into four recognised subgenera with mostly Palaearctic distribution: *Asiobates* Thomson, *Calobius*, *Enicocerus* and *Ochthebius* (Jäch & Skale, 2015; Tables 1, S1; see the detailed taxonomic history of subgenera and species groups in the Discussion). Within *Ochthebius* s.str., the most diverse subgenus, several informal species groups have been defined, which have undergone important modifications through their taxonomic history (Tables S1, S2).

The classification and proposed relationships within Ochthebiini have also experienced many modifications during the last four decades. Perkins (1980) revised the by then known American species, and proposed a phylogeny derived from the examination of some morphological characters. *Gymnochthebius* was placed as sister to the remaining taxa, which were divided in two lineages: (i) *Meropathus* plus *Neochthebius* (currently a synonym of *Ochthebius*, Table S2) and (ii) *Ochthebius* plus *Asiobates*. Subsequently, Perkins (1997) synonymized four subgenera with *Ochthebius* (*Calobius*, *Cobalius*, *Liochthebius* Sahlberg and *Notochthebius* Orchymont), and described three additional genera (Tables 1, S2). Based mostly on the exocrine secretion delivery system (ESDS), he divided the subfamily in two tribes, Ochtheosini for the newly described *Ochtheosus* and Ochthebiini, divided in turn into subtribes, with unresolved relationship among them. *Ochtheosus* was considered to have some plesiomorphic characters similar to some southern African genera (e.g. antennae with 11 antennomeres, as in many Prosthetopinae, Perkins, 1997; see also Beutel *et al*., 2003), and did not share several of the most characteristic synapomorphies with the remaining Ochthebiinae, in particular the structure of the tentorial arms, galea and lacinia.

The first formal cladistic analysis of the family Hydraenidae was published by Beutel *et al*. (2003), but sampling was too incomplete to resolve internal relationships within Ochthebiinae other than the sister relationship of *Meropathus* with *Ochthebius* + *Gymnochthebius*. There is no published global molecular phylogeny of the entire family Hydraenidae or subfamily Ochthebiinae, but in recent years some detailed molecular phylogenies for some lineages have been published, such as the *Ochthebius notabilis* group (Abellán *et al.*, 2009) and *Enicocerus* (Ribera *et al*., 2010). In Abellán *et al.* (2013) an extensive phylogeny of *Ochthebius* and some related genera using only mitochondrial markers was used to estimate the phylogenetic diversity of the Iberian fauna. The sampling of some geographical areas was, however, very incomplete, as the intention was not to produce a phylogenetic study. Still, most Palaearctic lineages were represented, which allowed us to establish the monophyly of most of the included genera/subgenera and of the recognized species groups, although internal groups had poor relationships between them. Sabatelli *et al.* (2016) used these data to study the origin of species typical of rockpools, recovering basically the same relationships and establishing a new species group for the South African *O. capicola* Péringuey. In the same paper, the subgenus *Cobalius* was found to be outside

Ochthebius s.str., but *Calobius* nested within it, referring to it as the '*Calobius*' lineage.

In this study we provide a comprehensive phylogeny of Ochthebiini, based on mitochondrial and nuclear sequence data, including representatives of most lineages. We introduce several changes in the taxonomic classification to accommodate our phylogenetic results, and provide a complete checklist based on our new classification (Table S1).

Material and Methods

Taxon sampling

We studied 186 species and four subspecies of the 641 described species and 11 described subspecies of Ochthebiini, plus 29 specimens corresponding to undetermined or still undescribed species (Table S3). For two species with an isolated or unsupported placement (*O. plesiotypus* Perkins and *O. peisonis* Ganglbauer) we sequenced two specimens to test for possible sequencing mistakes. We included examples of eight of the 11 genera currently recognised in the tribe, all subgenera but two (within genus *Tympanogaster*), and all recognised species groups within the genus *Ochthebius* but one (*O. kosiensis* group, Tables 1, S1, S3). The three missing genera, *Tympallopatrum* (Australia) and *Protochthebius* (Asia) and *Prototympanogaster* (Lord Howe Island), have four, seven and a single species, respectively (Table S1).

We used as outgroups 31 species of other Hydraenidae genera (*Hydraena*, *Laeliaena* Sahlberg and *Limnebius*) and of Ptiliidae. Trees were rooted in the split between Hydraenidae and Ptiliidae, considered to be sister groups both based on molecular (e.g. Hunt *et al*., 2007; McKenna *et al.*, 2015; Zhang *et al*., 2018) and morphological evidence (Hansen, 1997; Lawrence *et al*., 2011).

DNA extraction and sequencing

Specimens were killed and preserved in absolute ethanol. DNA was extracted with a standard phenol-chloroform extraction or by commercial extraction kits (mostly Quiagen DNeasy Tissue Kit, Hildesheim, Germany) following the manufacturers' instructions. DNA samples and voucher specimens are kept in the collections of the Institute of Evolutionary Biology (IBE, Barcelona, Spain), Museo Nacional de Ciencias Naturales (MNCN, Madrid, Spain) and Naturhistorisches Museum Wien (NMW, Vienna, Austria). We sequenced fragments of six genes in five sequencing reactions, three mitochondrial: [(i) 5' end of the cytochrome *c* oxidase subunit 1 (the standard barcode, Hebert *et al*., 2003) (COI-5'), (ii)

3’ end of cytochrome *c* oxidase subunit 1 (COI-3’), (iii) 5’ end of 16S RNA (16S) plus the leucine tRNA transfer (tRNA-Leu) plus 5’ end of NADH dehydrogenase subunit I (NAD1)]; and two nuclear [(iv) an internal fragment of the large ribosomal unit, 28S RNA (28S) and (v) an internal fragment of the small ribosomal unit, 18S RNA (18S)] (see Table S4 for details on primers used and typical PCR conditions). Sequences were assembled and edited with GENEIOUS v10.1 (Kearse *et al*., 2012); new sequences (a total of 897) were deposited in the ENA database with accession numbers LT990690-LT991586.

Phylogenetic analyses

Edited sequences were aligned using the online version of MAFFT v.7 with the G-INS-I algorithm (Katoh *et al*., 2009). We used PartitionFinder v1.1.1 (Lanfear *et al*., 2012) to estimate the evolutionary model that best fitted the data, using one partition for each gene fragment (six partitions in total), and using Akaike Information Criterion (AIC) scores as selection criteria. Phylogenetic analyses were made using Bayesian probabilities in BEAST 1.8 (Drummond & Rambaut, 2007), using the partition and evolutionary models selected by PartitionFinder, with a Yule speciation process as tree prior. There are few fossils usable for calibrating a phylogeny of Hydraenidae. The oldest recognised members of the family are *Ochthebiites* Ponomarenko, from the Jurassic (Arnol’di *et al.*, 1991; Ponomarenko & Prokin, 2015; Yamamoto *et al*., 2017), but they cannot confidently be placed in any extant lineage. One of the best preserved fossils is *Archaeodraena cretacea* Jäch & Yamamoto from Upper Cretaceous Burmese amber (c. 99 Ma, Yamamoto et al., 2017), which probably belongs to the crown Hydraenidae. Both fossils are compatible with an estimate of c. 170 Ma for the split between Hydraenidae and Ptiliidae obtained in recent molecular phylogenies calibrated with a range of fossils (Hunt *et al*., 2007; McKenna *et al*., 2015). We thus used this estimation to calibrate our tree, with a normal distribution with a standard deviation of 1 Ma and an uncorrelated lognormal relaxed clock. A Middle Jurassic separation between Hydraenidae and Ptiliidae is considerably younger than the estimation of Toussaint *et al*. (2016) (Middle Triassic, 243 Ma), but older than the more recent of Zhang *et al*. (2018) (Upper Jurassic, c. 150 Ma), both of which we consider to be less plausible. In any case, it must be noted that the main objectives of our study do not require an absolute calibration of the phylogeny of Ochthebiini, which is done only as a preliminary exploration.

We ran the analyses for 100 million generations, logging results for every 5,000, and checked convergence to estimate the burn-in fraction with Tracer v1.6 (Drummond & Rambaut, 2007). We ran an additional ML phylogenetic reconstruction with RAxML-HPC2 (Stamatakis, 2006) in the CIPRES portal (Miller *et al*., 2010), using the same partition scheme as in BEAST with a GTR+G model estimated independently for each partition.

Node supports values were estimated with 100 pseudoreplicas using a rapid bootstrapping algorithm (Stamatakis *et al*., 2008). The same ML analysis was repeated only with the nuclear sequence (18S and 28S).

Table 1. Synopsis of the genus-level classification of Ochthebiini, with notes of the former status of the taxa (following Jäch & Skale, 2015, Jäch *et al.* 2016 and Perkins, 2018), total number of species and species included in the phylogeny (see Table S1 for a complete checklist and Table S1 for synonyms and type species). In brackets, number of subspecies. Species of uncertain identification included in the phylogeny noted after '+'.

New status	Former status	No. sp. (spp.)	DNA sp. (spp.)
Genus *Meropathus* Enderlein, 1901	Genes	8	1
Genus *Ochthebius* Leach, 1815	Genus	540[a] (9)	186 + 27 (2)
Subgenus *Angiochthebius* Jäch & Ribera, 2018	Subgenus of *Ochthebius*	3	1
Subgenus *Asiobates* Thomson, 1859	Subgenus of *Ochthebius*	105 (3)	34 + 6
Subgenus *Aulacochthebius* Kuwert, 1887	Genus	13	4 + 6
Subgenus *Cobalius* Rey, 1886	Subgenus of *Ochthebius* s.str.[b]	9 (2)	6
Subgenus *Enicocerus* Stephens, 1829	Subgenus of *Ochthebius*	16	9 + 1
Subgenus *Gymnanthelius* Perkins, 1997	Genus	8	2
Subgenus *Gymnochthebius* Orchymont, 1943	Genus	58	7 + 1
Subgenus *Hughleechia* Perkins, 1981	Genus	2	1
Subgenus *Micragasma* Sahlberg, 1900	Genus[c]	3	1
Subgenus *Ochthebius* Leach, 1815	Subgenus of *Ochthebius*	322 (4)	121 + 13 (2)
Genus *Protochthebius* Perkins, 1997	Genus	7	0
Genus *Prototympanogaster* Perkins, 2018	Genus	1	0
Genus *Tympallopatrum* Perkins, 1977	Genus	4	0
Genus *Tympanogaster* Perkins, 1967	Genus	84	3
Subgenus *Hygrotympanogaster* Perkins, 2006	Subgenus of *Tympanogaster*	36	1
Subgenus *Plesiotympanogaster* Perkins, 2006	Subgenus of *Tympanogaster*	2	0
Subgenus *Topotympanogaster* Perkins, 2006	Subgenus of *Tympanogaster*	8	0
Subgenus *Tympanogaster* Janssens, 1967	Subgenus of *Tympanogaster*	38	2

[a] Includes one species 'incertae sedis'.
[b] Considered as a subgenus of *Ochthebius* by Sabatelli *et al*. (2016) and Jäch & Delgado (2017a).
[c] Considered as a subgenus of *Ochthebius* by Hernando *et al*. (2017), based on the results of this study.

Results

The final matrix included 252 terminals with 3,656 aligned characters. Protein-coding regions had no indels except for the 3' end of COI-3, where some species had an additional codon. The best partitioning scheme obtained with PartitionFinder had six partitions corresponding to (i) COI-5, (ii) COI-3, (iii) 16S+tRNA-Leu, (iv) NAD1, (v) 18S and (vi) 28S. The optimal evolutionary model was GTR+I+G for all partitions except for NAD1 (best model TMV) and 28S (best model SYM). The BEAST run implementing the best models did not converge properly, however, mostly due to the parameters related to the estimation of the branch lengths, especially for the genes NAD1, 18S and 28S. We thus did a second run with simpler models for these genes (HKY+G+I), which converged adequately. The topologies of the two Bayesian analyses were, however, almost identical (Figs 1, S1), and unless specified we report only the results of the analyses with the better parameter convergence (i.e. with the simpler evolutionary models).

Molecular phylogeny

The topologies obtained in the ML and the two Bayesian analyses were very similar, differing only in some poorly supported nodes (Figs 1, S1, S2), most notably in the position of *Hughleechia* (see below). The ML tree with the nuclear sequence only had a topology very similar to that obtained with the combined data, although with a generally lower resolution and support. Main difference was the recovery of Ochthebiini as paraphyletic, with the genus *Hydraena* as sister to *Tympanogaster* plus *Meropathus*, although with very low support (bootstrap support, BS = 53%; Fig. S3). Genera, subgenera and most species groups were, however, recovered as monophyletic with strong support, with internal topologies very similar to that of the combined ML tree (Figs S2, S3).

In the ML and Bayesian trees with the combined nuclear and mitochondrial data, the monophyly of Ochthebiini was strongly supported, as well as their separation into two clades, (i) *Meropathus* plus *Tympanogaster* and (ii) *Ochthebius* s.l. *Meropathus* was nested within a paraphyletic *Tympanogaster* in the ML tree (combined and nuclear only) and in the Bayesian tree with the best models, and sister to *Tympanogaster* with low support in the Bayesian tree with simpler models (posterior probability, PP = 0.63; Figs 1a, S1, S2).

Within *Ochthebius* s.l. *Asiobates* and *Aulacochthebius* were sister groups in the Bayesian tree with low support (PP = 0.85), and both sisters to the rest of Ochthebiini. In the ML analysis, *Asiobates* and *Aulacochthebius* were paraphyletic with respect to the rest of Ochthebiini, also with low support (BS < 50%) (Figs 1a, S2). In both analyses Ochthebiini minus *Asiobates* and *Aulacochthebius* were monophyletic with strong support (BS = 80%; PP = 1; Fig. 1a).

The remaining Ochthebiini were divided in a series of well supported clades corresponding to traditionally recognised genera or subgenera, but with poorly resolved relationships among them: (i) *Enicocerus*, strongly supported and with well-resolved internal relationships, sister to the Australian *Hughleechia* in ML and the Bayesian analysis with the simpler models (BS = 81%, PP = 0.76; Figs 1b, S2); in the Bayesian analysis with the best models *Hughleechia* was sister to the clade formed by *Micragasma* and *Cobalius*, with low support (PP = 0.88; Fig. S1); (ii) a clade including *Gymnochthebius* and *Gymnanthelius*, the latter as sister to *Angiochthebius* Jäch & Ribera (*Gymnochthebius plesiotypus*

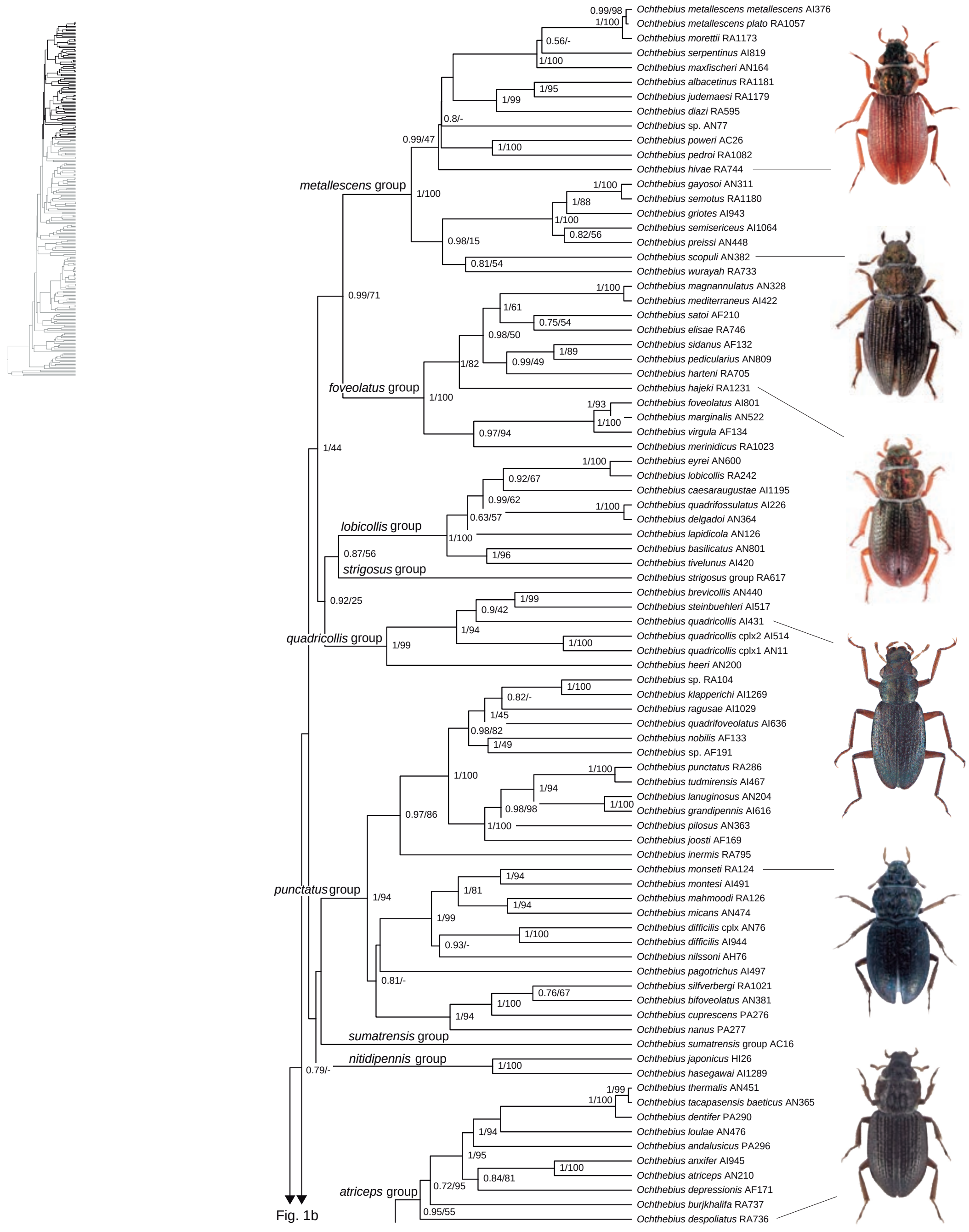


Figure 1. Majority rule consensus tree obtained with BEAST for the phylogeny of Ochthebiini, with the simple evolutionary models (see text). Numbers in nodes, posterior probabilities/bootstrap support values in RAxML. Names in nodes refer to the new classification. Habitus photographs correspond to species used in the analyses, with the addition of *Limnebius papposus* Mulsant, *Hydraena riparia* Kugelann (a), *Ochthebius* (s.s.) *bernhardi* Jäch & Delgado and *O*. (*Micragasma*) *minoicus* Hernando, Villastrigo & Ribera (b).

group of Perkins, 1980; see Jäch & Ribera, 2018) (BS = 94%, PP = 0.96); within *Gymnochthebius*, the Australian and American species were respectively monophyletic and sisters, with very strong support both in the ML and Bayesian trees (Figs 1b, S2); (iii) *Cobalius*,

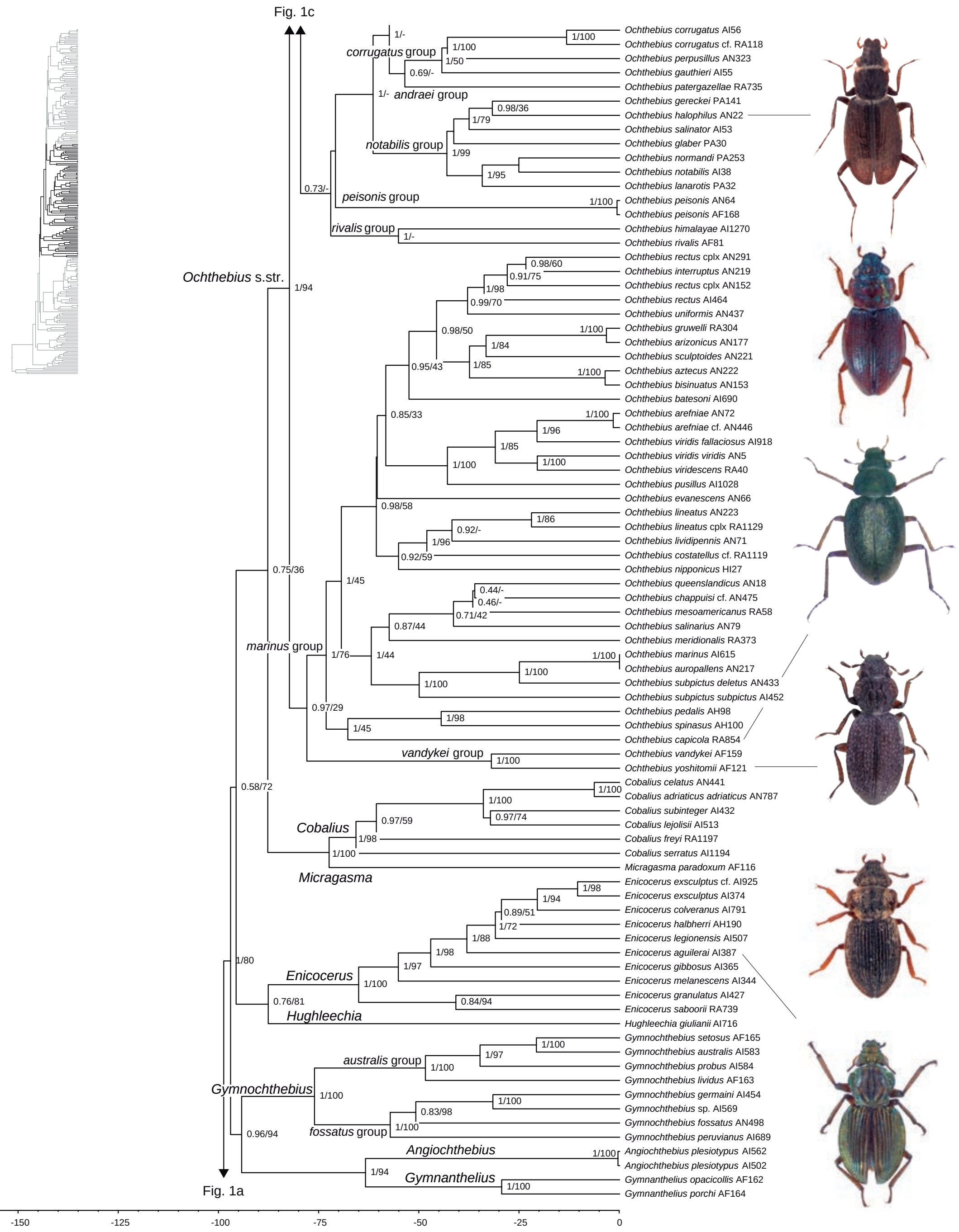


Figure 1. Continued.

with a strongly supported monophyly (BS= 98%, PP = 1) and sister to the only sequenced species of *Micragasma*, also with strong support (BS = 100%, PP = 1) (Figs 1b, S2); (iv) *Ochthebius* s.str., including *Calobius*, strongly supported both in the ML (BS = 94%) and Bayesian (PP = 1) trees (Figs 1b, S2).

Within *Ochthebius* s.str. the most established Palaearctic species groups were recovered as monophyletic (see Discussion, Figs 1b, 1c, S2). Their monophyly was strongly

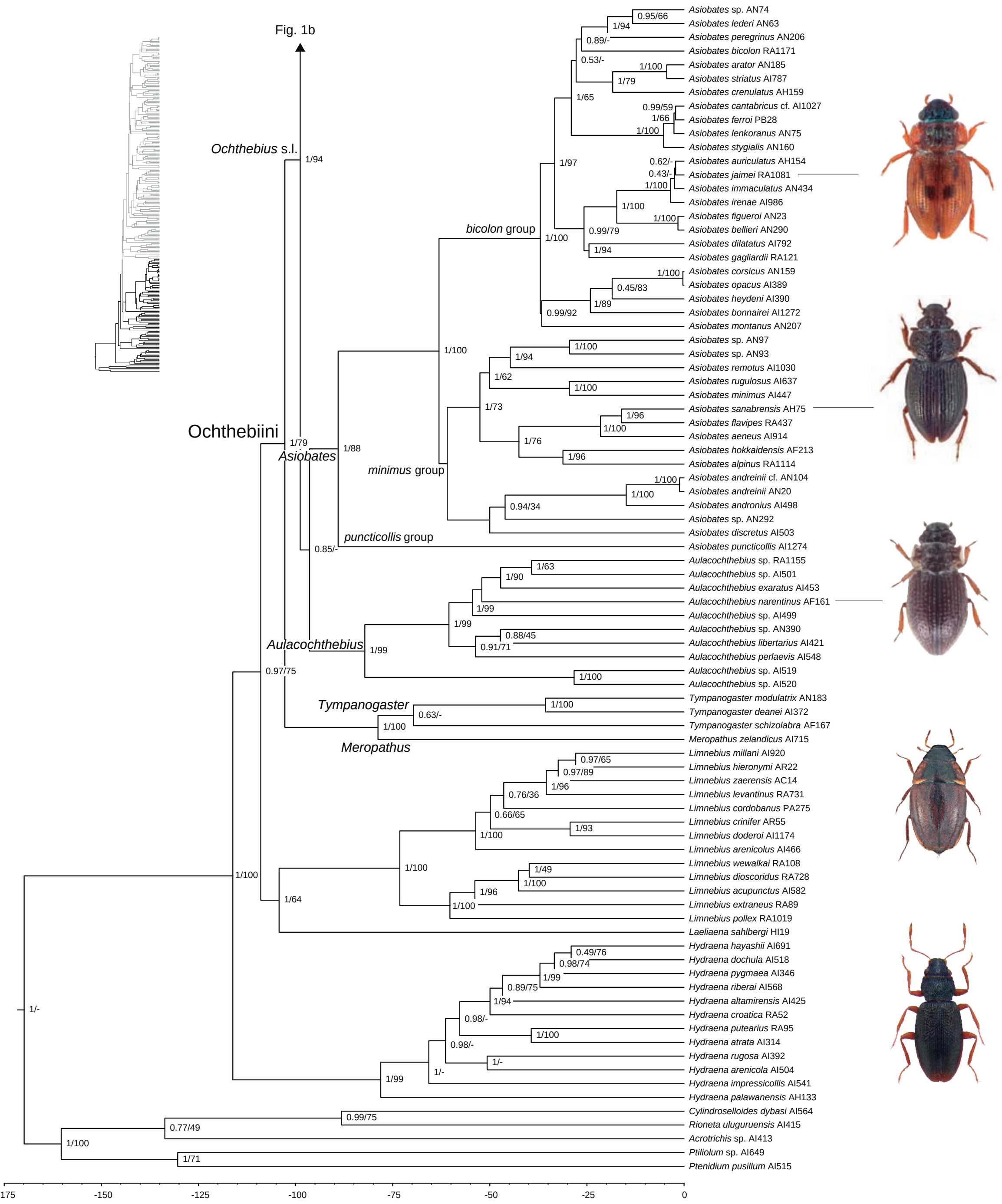


Figure 1. Continued.

supported in the ML and Bayesian trees, with the only exception of the group of species related to the *O. atriceps* and *O. notabilis* groups in the ML analyses. The main difference with established groups was the expansion of the *O. marinus* group to include the South African *O. capicola* and the American *O. biincisus*, *bisinuatus* and *interruptus* groups of Perkins (1980). The *O. foveolatus* group of Jäch (1991) was split in three clades: (i) *O. foveolatus* group, sister to the *O. metallescens* group with strong support in both ML and Bayesian trees (BS = 71%, PP = 0.99); (ii) *O. atriceps* group and (iii) *O. corrugatus* group.

The latter two formed a clade with the species of the *O. notabilis* and *O. andraei* groups, strongly supported in the Bayesian tree (PP = 1) but not in the ML tree (BS < 50%), in which the group also included one of the two sampled species of the *O. rivalis* group (Figs 1c, S2). Two coastal lineages, *Calobius* and the *O. vandykei* group (formerly genus *Neochthebius*), were nested within *Ochthebius* s.str., the former as sister to the *O. lobicollis* + *O. strigosus* groups (BS < 50%, PP = 0.92) and the latter as sister to the *O. marinus* group (BS < 50%, PP = 0.97) (Figs 1b, 1c, S2).

Table 2. Estimated evolutionary rates of the different partitions (substitutions per site per Ma per lineage) and 95% high posterior density interval (HPD) in the Bayesian analysis with simple evolutionary models (see text).

Partition	Mean rate	95% HPD interval
COI-5	0.0085	[0.0070, 0.0102]
COI-3	0.0111	[0.0091, 0.0133]
16S + tRNA	0.0024	[0.0020, 0.0029]
NAD1	0.0039	[0.0030, 0.0048]
18S	0.00014	[0.00010, 0.00017]
28S	0.00052	[0.00040, 0.00064]

According to our calibration scheme, with a separation between Hydraenidae and Ptiliidae at 170 Ma, the estimated age of crown Hydraenidae was 106 Ma [highest posterior density (HPD): 122.8–90.2 Ma], and that of the crown Ochthebiini 93 Ma (HPD: 109.7–80.8 Ma). The basal diversification of Ochthebiini was reconstructed as having occurred in a relatively short temporal window, with genera, subgenera and most species groups with an origin between c. 87–60 Ma (Fig. 1; see Table 2 for the estimated evolutionary rates of all partitions).

Discussion

Our results strongly support the monophyly of Ochthebiini, but our sampling did not allow us to test for the monophyly of Ochthebiinae, or its position within Hydraenidae. Within Ochthebiini our results recover two well-supported clades: *Meropathus* plus *Tympanogaster*, and *Ochthebius* s.l. (Fig. 2; see below for a detailed discussion of the taxonomic classification of Ochthebiini). We did not find evidence to support the five subtribes proposed by Perkins (1997), which are therefore not considered here.

We did not find evidence for a clear separation between the studied species of *Tympanogaster* and *Meropathus*, in agreement with previous studies (Hansen, 1991). However, we could not obtain material of the genera *Prototympanogaster* and *Tympallopatrum*, considered to be closely related to *Tympanogaster* by Perkins (1997, 2018), and two of the subgenera of *Tympanogaster* (*Plesiotympanogaster* Perkins and *Topotympanogaster* Perkins), so until more data become available we refrain from any taxonomic change and consider *Prototympanogaster*, *Tympallopatrum* and *Tympanogaster* as valid genera (Tables 1, S1; Fig. 2) (see Perkins, 2006 for a discussion on the subgeneric classification of *Tympanogaster*). The species of *Meropathus*, *Prototympanogaster*, *Tympallopatrum* and *Tympanogaster* are found in the Australian Region, on two Antarctic islands (Kerguelen and Heard) and on several Subantarctic islands, such as Campbell Island, Crozet Islands, Prince Edward Island and Falkland Islands.

The second lineage, genus *Ochthebius* s.l., included all the non-Australian Ochthebiini, as well as several Australian species. Our results agree remarkably well with the currently recognised subgenera and many of the established species groups, which were recovered as monophyletic with general strong support (Fig. 2). The relationships between these lineages, however, do not confirm some previous hypotheses on their relationships. Thus, *Aulacochthebius* was not found to be closely related to *Gymnochthebius*, as proposed in Hansen (1991), but to *Asiobates*; *Micragasma* and *Hughleechia* were not among the basal lineages and *Cobalius* and *Calobius* were not closely related, as hypothesised in Perkins (1997). Novel relationships found here are the possible sister relationship between *Hughleechia* and *Enicocerus*, and the close relationship between *Gymnochthebius* and *Gymnanthelius*. Interestingly, within the clade *Gymnochthebius* + *Gymnanthelius* + *Angiochthebius* there are two cladogenetic events separating American from Australian species: one within *Gymnochthebius*, dated at 73 Ma (95% HPD 87–60 Ma), and another separating the Australian *Gymnanthelius* and the Chilean *Angiochthebius*, dated at 60 Ma (95% HPD 78–51 Ma). Although a detailed biogeographic analysis is outside the scope of this paper, it is interesting to note that these estimations are too recent for a tectonic split between Australia and South America (i.e. west and east Gondwana), dated at c. 130 Ma (McIntyre *et al*., 2017). Our calibration would thus require a different scenario, probably through the colonization of some southern islands or the Antarctica. An older age for these nodes is unlikely, given that our rate estimations are already slower than most recent estimations for the same genes in other groups of Coleoptera (Table 2; see e.g. Papadopoulou *et al*., 2010; Andújar *et al*., 2012; Cieslak *et al*., 2014).

Taxonomic classification of Ochthebiini Thomson, 1859

Genus *Meropathus* Enderlein, 1910

Type species: *Meropathus chuni* Enderlein, 1910, by monotypy.

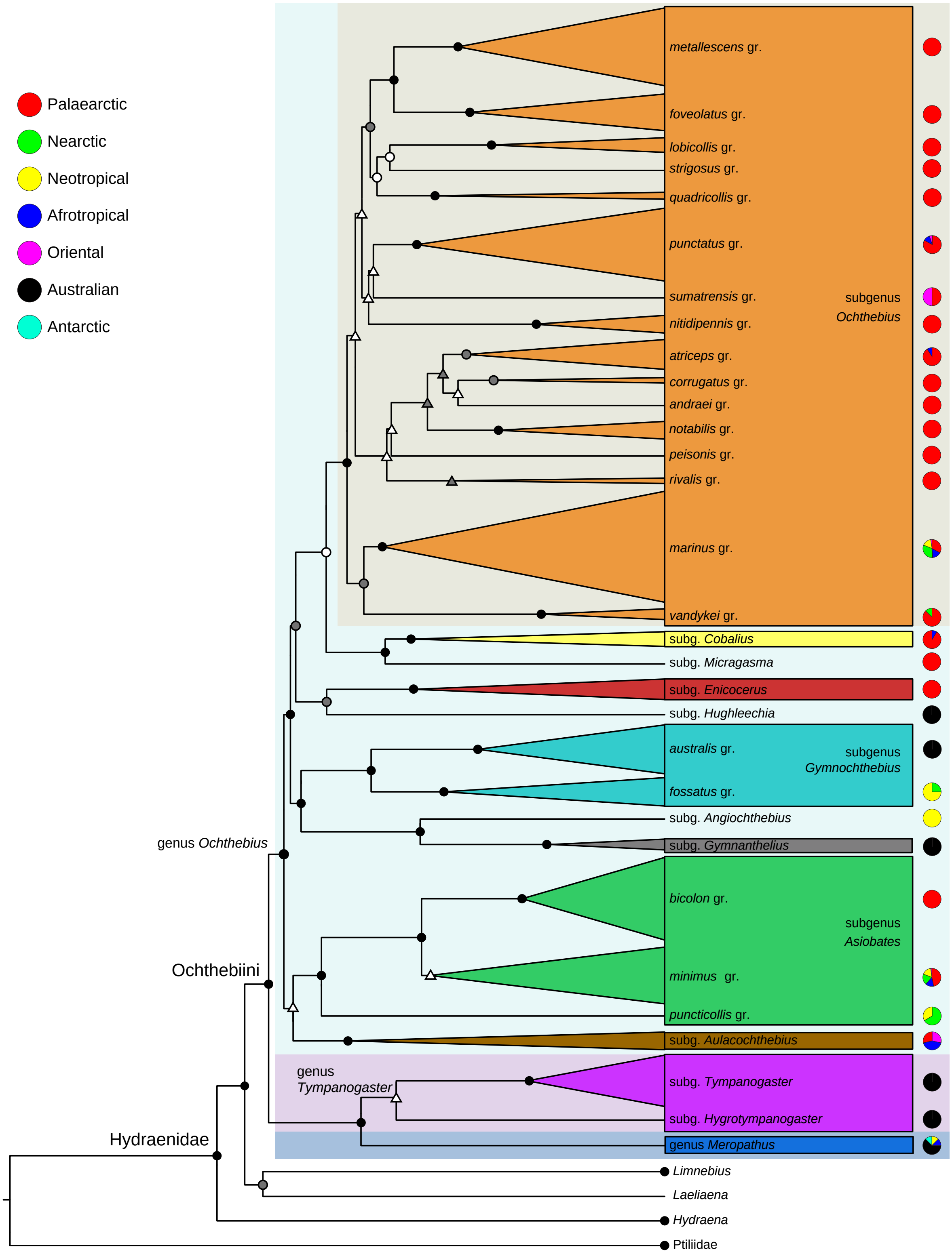


Figure 2. Summary tree of the phylogenetic relationships of the Ochthebiini main lineages. The width of the triangles reflects the number of species of the respective clade in the tree. Symbols in nodes: circles, congruent topology in the maximum likelihood and Bayesian analyses; triangles, incongruent topologies; in black, nodes with good support (Bayesian posterior probability > 95% and maximum likelihood bootstrap support > 70%) in both analyses; in grey, in one analysis only; in white, not supported nodes. Pie charts reflect the geographical distribution of the species of the respective clades.

Meropathus was described as genus, considered as subgenus of *Ochthebius* by Orchymont (1938) and reinstated again as genus by Jeannel (1940). Bameul (1989) redescribed the genus and recognised 12 species (in two species groups), transferring *Ochthebius schizolabrus* Deane to *Meropathus*. Hansen (1991) noted the difficulty in establishing clear distinctions within the *Meropathus*-*Tympanogaster* complex. *Meropathus* includes seven New Zealand, Antarctic and Subantarctic species plus *M. labratus* Deane from Queensland (Table S1). They are all found in coastal habitats, usually among debris and algae (Bameul, 1989).

Genus *Prototympanogaster* Perkins, 2018

Type species: *Prototympanogaster lordhowensis* Perkins, 2018, by original designation.

Prototympanogaster was described by Perkins (2018) as a monotypic genus based on two males collected in 2003 in Lord Howe Island (Australia). This genus seems to be closely related to *Tympanogaster*, but without its characteristic glabrous tabella in the metaventrite (Perkins, 2018).

Genus *Tympallopatrum* Perkins, 1997

Type species: *Tympallopatrum longitudum* Perkins, 1997, by original designation.

Tympallopatrum was described by Perkins (1997) as a monotypic genus within Meropathina. Subsequently, Perkins (2004a) revised the genus and described three additional species, all of them from western Australia (Table S1). We could not obtain any representative of this genus for our study, and thus its phylogenetic placement remains untested.

Genus *Tympanogaster* Janssens, 1967

Type species: *Tympanogaster deanei* Perkins, 1979 (replacement name for *Ochthebius longipes* Deane, 1931), by monotypy.

Described by Janssens (1967) as a monotypic genus for *O. longipes* (= *T. deanei* Perkins), Perkins (1997) redescribed *Tympanogaster* and transferred some species from *Meropathus*. Perkins (2006) revised the genus and described three subgenera and 76 new species, raising the total number of the species in the genus to 84 (Tables 1, S1), all distributed in Australia and Tasmania.

Subgenus *Hygrotympanogaster* Perkins, 2006. Type species: *Tympanogaster maureenae* Perkins, 2006, by original designation.

Hygrotympanogaster Perkins was described by Perkins (2006) as a subgenus of *Tympanogaster*, to include mostly hygropetric species in southwestern Australia. Currently it includes 36 species (Perkins, 2006) (Table S1).

Subgenus *Plesiotympanogaster* Perkins, 2006. Type species: *Tympanogaster thayerae* Perkins, 2006, by original designation.

Plesiotympanogaster was described by Perkins (2006) as a subgenus of *Tympanogaster* to include the type species plus *Ochthebius costatus* Deane (Table S1). Both species were considered to have plesiomorphic characters within the genus.

Subgenus *Topotympanogaster* Perkins, 2006. Type species: *Tympanogaster crista* Perkins, 2006, by original designation.

Topotympanogaster was described by Perkins (2006) as a subgenus of *Tympanogaster* to include eight Australian species, all described in Perkins (2006) (Table S1). We could not obtain any representative of this and the previous subgenus for our study, and thus their phylogenetic placement remain untested.

Subgenus *Tympanogaster* Janssens, 1967. Type species: *Tympanogaster deanei* Perkins, 1979 (replacement name for *Ochthebius longipes* Deane, 1931), by monotypy.

Tympanogaster s.str. was revised by Perkins (2006), raising the total number of the species to 38 (Tables 1, S1), all distributed in Australia and Tasmania.

Genus *Ochthebius* Leach, 1815

Type species: *Helophorus marinus* Paykull, 1798, fixed by Orchymont (1942).

The second well-supported lineage within Ochthebiini includes the remaining genera/subgenera with, in some cases, uncertain relationships among them. We consider *Ochthebius* a single genus with 540 species and nine subspecies in ten well-supported subgenera, corresponding in most cases to currently recognised taxa (Fig. 2). One additional species, *O. eremita* Knisch from Fiji, cannot be confidently placed in any of the described subgenera, and it is left as incertae sedis within the genus *Ochthebius* (Hansen, 1998; Table S1).

Subgenus *Angiochthebius* Jäch & Ribera, 2018. Type species: *Gymnochthebius plesiotypus* Perkins, 1980, by original designation.

The subgenus *Angiochthebius* was created for the *Gymnochthebius plesiotypus* species group (sensu Perkins, 1980), which now includes three South American species (Jäch & Ribera, 2018; Table S1). The species of the *G. plesiotypus* group were included within *Gymnochthebius* by Perkins (1980) as they share a bifid apex of the aedeagus, but external characters (e.g. the pubescent fifth abdominal ventrite) and some aedeagal characters (Jäch & Ribera, 2018) as well as molecular data (Figs 1b, S2) warrant their removal from *Gymnochthebius* and their status as a distinct subgenus of *Ochthebius.*

Subgenus *Asiobates* Thomson, 1859. Type species: *Ochthebius rufimarginatus* Stephens, 1829 (= *O. bicolon* Germar, 1824), by monotypy.

Originally described as a genus, but downgraded to subgenus by Seidlitz (1875), and treated as such by most authors (e.g. Jäch, 1990a; Hansen, 1991; Perkins, 1997). Jäch (1990a) divided the Palaearctic species in the *bicolon* and *minimus* groups, which were recovered as respectively monophyletic with strong support. The sampled American species were divided in the *puncticollis* group of Perkins (1980), with only one sampled species being sister to the rest of the subgenus with strong support (BS = 88%, PP = 1; Figs 1a, S2), plus the *discretus* group of Perkins (1980). The placement of the studied Nearctic species of the *A. discretus* group and two of the Afrotropical species (*O. andreiini* Régimbart and *O. andronius* Orchymont) was uncertain both in the ML and the Bayesian trees (Figs 1a, S2). We provisionally consider them within the *A. minimus* group due to the similarities in their aedeagi and the morphology of the pronotum (Orchymont, 1948; Perkins, 1980; Jäch, 1990a). The subgenus *Asiobates* currently includes 105 described species and three subspecies occurring in all biogeographical regions, except the Oriental and Australian Realms. While the *puncticollis* group is restricted to the Nearctic Region, the *bicolon* and *minimus* groups are more widespread. The former occurs in the Palaearctic and (with several undescribed species) Afrotropical regions, and the latter occurs in the Nearctic, Neotropical, Palaearctic, and Afrotropical Regions. Many additional species of *Asiobates* await description, several of them included in our phylogeny (Table S3).

Subgenus *Aulacochthebius* Kuwert, 1887. Type species: *Ochthebius exaratus* Mulsant, 1844, by monotypy.

Considered as a subgenus until Perkins (1997) raised it to genus level. There are no species groups defined within this subgenus, and our sampling is too incomplete to draw firm conclusions. Currently the subgenus includes 13 Palaearctic, Oriental and Afrotropical species (Table S1), although the taxonomy of the subgenus is in clear need of revision and it is expected that the number of species will increase considerably (Table S3).

Subgenus *Cobalius* Rey, 1886. Type species: *Ochthebius lejolisii* Mulsant & Rey, 1861, fixed by Jäch (1989b).

Described as a subgenus of *Ochthebius* by Rey (1886), synonymised by Perkins (1997) with *Ochthebius* s.str. and considered again as subgenus by Sabatelli *et al*. (2016). We recovered it here as a strongly supported monophyletic lineage clearly outside *Ochthebius* s.str., confirming its status as subgenus. Its nine recognised species and two subspecies occur along the coasts of the Mediterranean Sea, the Black Sea and the eastern Atlantic Ocean from Cape Verde to Scotland (Jäch, 1989b; Jäch & Skale, 2015; Jäch & Delgado, 2017a). Its taxonomy is in need of revision (Sabatelli *et al*., 2016; Jäch & Delgado, 2017a; I. Ribera *et al*., unpublished results).

Subgenus *Enicocerus* Stephens, 1829. Type species: *Enicocerus viridiaeneus* Stephens, 1829 (= *Ochthebius exsculptus* Germar, 1824), by monotypy.

Enicocerus was originally described as a genus, downgraded to subgenus of *Ochthebius* by Chenu (1851), reinstated again as genus by Perkins (1997) (within its own subtribe, Enicocerina), but treated as a subgenus by subsequent authors (e.g. Jäch, 1998; Ribera *et al*., 2010; Jäch & Skale, 2015). Our results support the exclusion of the East Palaearctic and Oriental species, confirming Jäch (1998) and Skale & Jäch (2009), and are in agreement with the phylogeny of Ribera *et al*. (2010). *Enicocerus* in its current sense includes 16 species with a mostly Mediterranean distribution, with some species reaching the British Isles, Central Europe, the Middle East and the Caucasus. One species from eastern North America, *O. benefossus* LeConte, not included in our phylogeny, is here tentatively placed in *Enicocerus* (following Perkins, 1980); it might, however, instead belong to the *O*. (s.str.) *nitidipennis* group, or to a species group of its own.

Subgenus *Gymnanthelius* Perkins, 1997 **comb.n**. Type species: *Ochthebius hieroglyphicus* Deane, 1933, by original designation.

The genus *Gymnanthelius* was introduced by Perkins (1997) for *O*. *hieroglyphicus*. Subsequently, Perkins (2004b) revised the genus and transferred to *Gymnanthelius* two other Australian species described by Deane (1931, 1937) within *Ochthebius* (Table S1). The eight described species are distributed mostly in southeastern Australia, with some reaching as far north as Queensland (Perkins, 2004b).

Subgenus *Gymnochthebius* Orchymont, 1943. Type species: *Ochthebius nitidus* LeConte, 1850 by original designation.

Gymnochthebius was originally described as a subgenus of *Ochthebius* (Orchymont, 1943) to place several American species described under *Ochthebius* that could not be placed in any of the described subgenera, which had been established mostly for Palaearctic species. Orchymont (1943) also included three Australian species for which he could examine the aedeagus and confirmed that they had the same general structure as the American species. Perkins (1980) revised the American species and Perkins (2005) the Australian and Papuan species, recognizing another four species in addition to the three previously noted by Orchymont (1943) and raising the total number of species in the subgenus to 58 (Table S1). The Australian and the American species of the subgenus form two well-supported clades, the *O*. *australis* and *O*. *fossatus* groups, with 36 and 22 species respectively (Table S1).

Subgenus *Hughleechia* Perkins, 1981 **comb.n**. Type species: *Hughleechia giulianii* Perkins, 1981, by original designation.

Originally described as a monotypic genus (Perkins, 1981), a second species was described by Perkins (2007a). Both species inhabit coastal rockpools in southern Australia and Tasmania, in the intertidal zone and (most frequently) above the tide (Perkins, 2007a).

Subgenus *Micragasma* Sahlberg, 1900 comb.n. Type species: *Micragasma paradoxum* Sahlberg, 1900, by monotypy.

Described as a monotypic genus for *M. paradoxum* (Sahlberg, 1900). Jäch (1997a) redescribed the genus and transferred *O. substrigosus* Reitter to *Micragasma*. A third species was recently described from Crete (Hernando *et al*., 2017), and there are two additional undescribed species from Central Asia (M.A. Jäch *et al*., unpublished data). Our results clearly show that *Micragasma* is nested within *Ochthebius* s.l., and thus we consider it a subgenus of *Ochthebius*.

Subgenus *Ochthebius* Leach, 1815. Type species: *Helophorus marinus* Paykull, 1798, fixed by Orchymont (1942).

Within *Ochthebius* s.str. we recovered, with strong support, most of the currently recognised species groups as monophyletic. Most species groups are entirely Palaearctic, or with mostly Palaearctic species, and thus the basis for the taxonomy of the subgenus is the revisionary work of Jäch (e.g. 1989a, 1990a, 1991, 1992a), who distinguished 13 groups and subgroups. With only one exception (*O. jengi* group), they were all, with some modifications, recovered as monophyletic. According to our results, the 322 described species and four subspecies of *Ochthebius* s.str. are separated in 17 species groups, five of them newly established herein (Fig. 2). A few species still have an uncertain phylogenetic placement. This is particularly the case for *O. belucistanicus* Ferro, *O. caudatus* Frivaldszky, *O. fissicollis* Janssens and *O. pierottii* Ferro, which presently cannot be confidently included in any of the recognised species groups, mainly because their original descriptions lack information about relevant characters (Table S1).

(1) *O. andraei* group: Defined and revised in Jäch (1992a), with additional species described in Jäch (2002) and Jäch & Delgado (2010). Currently this group includes six species and one subspecies of Palaearctic distribution (Table S1), typical of saline or hypersaline habitats. We could study a single species (*O. patergazellae* Jäch & Delgado, Table S3), included in a clade together with the species of the *O. notabilis*, *corrugatus* and *atriceps* groups (Fig. 1). The close relationship between the species of the *O. andraei*, *corrugatus*, *notabilis* and *atriceps* groups were already suggested in Jäch (1991, 1992a).

(2) *O. atriceps* group: In Jäch (1991) the species of the *O. foveolatus* group were divided in two subgroups, (A) *foveolatus* subgroup, sharing some characters with the species of the *O. metallescens* group, and (B) *atriceps* subgroup, sharing some characters with the species of the *O. notabilis* group. We recovered both subgroups as respectively monophyletic, and confirm the suspected relationships proposed by Jäch (1991) (see below). *Ochthebius burjkhalifa* Jäch & Delgado and *O. despoliatus* Jäch & Delgado, both from the UAE and of uncertain affinities, although hypothesised to be related to the *O. atriceps* group (Jäch & Delgado, 2014a), were found to be sister to the rest of the species of the group, with strong support in the Bayesian analysis (PP = 0.95) but weaker in the ML (BS = 55%) (Figs 1, S2). With the inclusion of these two species the *O. atriceps* group includes 20 species and one subspecies (Table S1). They have a mostly Palaearctic distribution but extending to East Africa (Djibouti) (Jäch & Delgado, 2017b).

(3) *O. corrugatus* group: Jäch (1992a) suggested that *O. corrugatus* Rosenhauer, despite being related to the species of the *O. andraei* and *notabilis* groups, could not be included in either of them. Our results confirm this hypothesis, but extend the *O. corrugatus* group to include two additional Mediterranean species previously included in the *O. atriceps* subgroup (Jäch, 1991; Table S1).

(4) *O. foveolatus* group: The *O. foveolatus* group as here defined corresponds to the *O. foveolatus* subgroup of Jäch (1991), recovered as sister of the *O. metallescens* group with strong support (Fig. 1). After the additions and corrections of Delgado & Jäch (2009) and Jäch & Delgado (2010, 2014b) it currently includes 27 species, all Palaearctic (Fig. 1; Table S1).

(5) *O. kosiensis* group: Jäch (1997b) established this group for *O. kosiensis* Champion, described within *Ochthebius* but originally not placed in any subgenus (Champion, 1920). Knisch (1924) placed it in *Asiobates* due to the resemblance of the general habitus, although the male genitalia do not correspond to those of the species of *Asiobates* (Jäch, 1997b). Jäch (2003) recognised the similarity between *O. kosiensis*, *O. strigosus* and related species, and included both species in the *strigosus* subgroup of the *metallescens* group. The study of some undescribed species deposited in the NMW (M.A. Jäch, unpublished data) suggests that the *strigosus* subgroup as defined in Jäch (2003) should be divided in the *kosiensis* and *strigosus* groups, with two and 16 described species respectively (see below; Table S1). We could not obtain any species of the *kosiensis* group suitable for DNA extraction, and thus their phylogenetic relationships (and composition) remain untested. Based on described and undescribed species the group is so far known from the Himalaya and Myanmar.

(6) *O. lobicollis* group: Jäch (1990b) revised the *lobicollis* group, with subsequent additions by Jäch (1994) and Jäch *et al*. (1998). It currently includes 11 species with a West Palaearctic distribution (Table S1; Fig. 1b).

(7) *O. marinus* group: The Palaearctic species of the *O. marinus* group, the most speciose within *Ochthebius* s.str., were revised by Jäch (1992b). According to our results it includes the species of the *O. jengi* group sensu Jäch (1998) and also species from the Nearctic and Neotropical Regions (*O. biincisus*, *bisinuatus* and *interruptus* groups of Perkins, 1980); the Afrotropical Region (*O. extremus* and *salinarius* groups of Perkins & Balfour-Browne, 1994; Perkins, 2011; *O. capicola* group of Sabatelli *et al*., 2016), including Madagascar (*O. alluaudi* Régimbart; Perkins, 2017); the Oriental Region (*O. masatakasatoi* Jäch; Jäch, 1992b; Jäch & Delgado, 2017a); and the Australian Region (*O. queenslandicus* Hansen; Jäch, 2001a; Perkins, 2007b).

Two of the studied subspecies were not recovered as sisters to the nominal subspecies in any of the analyses: *O. viridis fallaciosus* Ganglbauer (sister to *O. arefniae* Jäch & Delgado and another specimen probably representing an undescribed species), and *O. subpictus deletus* Rey (sister to *O. marinus* plus *O. auropallens* Fairmaire), in both cases with strong support (Figs 1, S1, S2; Table S1). We thus upgrade the two subspecies to species, *O. fallaciosus* Ganglbauer, 1901 **stat.n.** and *O. deletus* Rey, 1885 **stat.rest.** (see Jäch, 1992b and Jäch & Delgado, 2008 for a detailed description of the species). The *O. marinus* group as here defined includes 78 species, plus two species of uncertain adscription (Table S1). Most of these species seem to be associated to lentic habitats, frequently saline and, especially those outside the Palaearctic, coastal.

(8) *O. metallescens* group: The *O. metallescens* group was revised by Jäch (1989a). It is well defined morphologically, but many species have variable isolated populations, making species recognition and diagnosis difficult. This difficulty is reflected in the complex taxonomic history of the group, with multiple changes in the status of some species (e.g. Jäch, 1989a, 1999, 2001b). A number of species are typical of hygropetric habitats covered by a thin film of water, such as seepages or the marginal areas of stony surfaces in streams. The species group has currently 56 Palaearctic species and one subspecies (Table S1). Due to the somewhat cryptic habits and restricted geographic ranges of many species it is expected that this number will increase considerably.

(9) *O. nitidipennis* group: We include in the *O. nitidipennis* group the Asian species formerly included in the subgenus *Enicocerus*. As suggested by previous authors (Jäch, 1989b; Skale & Jäch, 2009; Yoshitomi & Satô, 2011), morphological similarities between these species and those of *Enicocerus* are the result of evolutionary convergence, probably

due to occupying similar microhabitats on the surface of rocks and stones partially submerged in streams. The group currently includes 12 species in the Himalaya Region and East Asia (Table S1).

(10) *O. notabilis* group: Jäch (1992a) recognised the *O. notabilis* group for species formerly included in the subgenus *Doryochthebius* Kuwert, establishing its synonymy with *Ochthebius* s.str. and differentiating the members of this group from the species of *Calobius* (see below). The group includes 13 Palaearctic species, all typical of saline or hypersaline habitats.

(11) *O. peisonis* group: *Ochthebius peisonis* was included in the *O. marinus* group by Jäch (1992b). Our results, however, place the species in a very isolated and uncertain position within *Ochthebius* s.str. We provisionally consider it in its own group, until additional evidence clarifies its phylogenetic relationships.

(12) *O. punctatus* group: The *punctatus* group was defined by Jäch (1992c) to include the species formerly considered under subgenus *Bothochius* Rey, with irregular elytral punctation (Jäch, 1989c), and a series of species with similar morphological characters but with regular elytral striae. The *Ochthebius punctatus* group includes 53 species and one subspecies, mostly Palaearctic (reaching the Oriental Region) but with some Afrotropical species, among them the *namibiensis* group of Perkins & Balfour-Browne (1994) (Jäch, 1992c; Hansen, 1998; Perkins, 2011; Jäch & Delgado, 2017b; Table S1).

(13) *O. quadricollis* group: The *O. quadricollis* group corresponds to the genus *Calobius*, described for *C. heeri* Wollaston from Madeira. The concept of *Calobius* was expanded by subsequent authors to include species now in different species groups (e.g. Reitter, 1886 included among them *O. notabilis* Rosenhauer and *O. quadrifoveolatus* Wollaston), and was usually treated as a subgenus. It was revised by Jäch (1993), who still considered it a subgenus, but was synonymised with *Ochthebius* s.str. by Perkins (1997), who considered it to be closely related to *Cobalius*. Its status remained uncertain, however, with some authors treating it as a genus (e.g. Audisio *et al*., 2010) or subgenus (e.g. Jäch & Skale, 2015). Finally, Sabatelli *et al*. (2016) provided evidence of the phylogenetic position of *Calobius*, demonstrating its derived status within *Ochthebius* s.str. and considering it as the “*Calobius*” lineage, named here the *O. quadricollis* group for consistency with other species groups within *Ochthebius* s.str. Sabatelli *et al*. (2016) also found that the group includes more than the five species currently recognised (Table S1), in agreement with previous results from the Italian species (e.g. Urbanelli & Porretta, 2008; Audisio *et al*., 2010). Our results support this impression, as happens with the subgenus *Cobalius*, which is also

in need of taxonomic revision. All species of the *O. quadricollis* group are found in coastal rockpools in the Mediterranean basin and the islands of Madeira and the Canaries.

(14) *O. rivalis* group: *Ochthebius rivalis* Champion and two similar species were originally considered to be a subgroup of the *O. metallescens* group (Jäch, 2003). Our results, however, do not support a close relationship with the species of the *O. metallescens* group, but with *O. peisonis* and the *O. notabilis*, *corrugatus* and *andraei* groups (Figs 1, S2), with low support. In the Bayesian analysis the two sampled species of the group were sister with strong support (PP = 1), but in the ML analysis they were not placed together, although with low support (BS < 50%) (Fig. S2). Currently the group includes four Asian species (including *O. himalayae* Jäch, originally described within the *O. metallescens* group, Jäch, 1989a), distributed from the Himalaya to Hainan Island (Table S1).

(15) *Ochthebius strigosus* group: *Ochthebius strigosus* Champion, described as *Ochthebius* s.str., was included in the subgenus *Asiobates* by Jäch (1989b) based on the study of female specimens only. After the discovery of males of a related species (*O. strigoides* Jäch) they were placed in their own subgroup within the *O. metallescens* group (Jäch, 1998). We found the only sequenced species of the group to be sister of the *O. lobicollis* group with low support (BS = 56%, PP = 0.87), and we consider it here as a distinct species group within *Ochthebius* s.str. The *O. strigosus* group currently includes 16 described plus some undescribed species, one of them included here (voucher IBE-RA617). The group is distributed in the eastern Palaearctic, including Taiwan (Jäch, 2003; Table S1).

(16) *Ochthebius sumatrensis* group: In the original description, *O. sumatrensis* Jäch could not be placed in any of the by then described groups, although some similarities with *O. jengi* Jäch (currently in the *marinus* group) were noted (Jäch, 2001a). Several undescribed species similar to *O. sumatrensis* have been collected in recent years (M.A. Jäch, unpublished data), among them the one from Hong Kong included here (specimen voucher MNCN-AC16; Table S1), recovered as sister to the rest of the species of *Ochthebius* s.str. with low support in the ML analysis (BS < 50%; Fig. S2) and as sister to the *punctatus* group in the Bayesian analysis, also with low support (PP < 0.5; Fig. 1). They live in hygropetric surfaces, which makes them prone to evolutionary convergence with non-related species sharing the same habitat, thus obscuring their relationships. The group is distributed from the Himalaya to eastern China and southward to Sumatra, where it is the only known species of *Ochthebius* s.str.

(17) *O. vandykei* group: The species of the *O. vandykei* group correspond to the former *Neochthebius*, originally described as subgenus but raised to genus (within its own

subtribe, Neochthebiina) by Perkins (1997) based on peculiarities of their antennae and the lack of ESDS. Jäch & Delgado (2014b), based on unpublished molecular data and on aedeagal characters, synonymised *Neochthebius* and considered it as a species group within *Ochthebius* s.str. The group currently includes eight species from the northern Pacific coast, seven in Asia and one in North America (Jäch & Delgado, 2014b; Table S1). They are all typical of rocky seashores or other coastal microhabitats.

Genus *Protochthebius* Perkins, 1997

Type species: *Protochthebius satoi* Perkins, 1997, by original designation.

The genus *Protochthebius* was described by Perkins (1997) for *P. satoi* and *O. jagthanae* Champion, who erected also the subtribe Protochthebiina based on peculiarities of the antennae and the ESDS. Subsequently, Jäch (1997b) and Perkins (1998) described another two and three species respectively. All seven known species of *Protochthebius* are found in the Himalaya Region, Meghalaya and Laos (Table S1). Some of them have been found by sifting forest litter or moss (Jäch, 1997b; Perkins, 1998).

We could not obtain molecular data of any of the species of *Protochthebius*, and thus the phylogenetic placement of the genus remains uncertain. Perkins (1997) noted some presumably plesiomorphic characters of the pronotum and postocular area of the head. The species of the genus have also a reduced ESDS system (Perkins, 1997), but this might be a secondary loss due to their microhabitat preferences. Their male genitalia are, however, typical of *Ochthebius* s.str., and when molecular data become available the taxonomic status of *Protochthebius* may have to be changed to a subgenus of *Ochthebius* or a species group within *Ochthebius* s.str., in which case *P. satoi* would become a junior homonym.

Acknowledgements

We thank all collectors mentioned in Table S3 for allowing us to study their material, Ana Izquierdo (MNCN) and Rocío Alonso (IBE) for laboratory work and three anonymous referees for comments to previous versions of the manuscript. A.V. has a FPI-MINECO PhD grant from the Spanish Government. This work has been partly funded by project CGL2013-48950-C2 (AEI/FEDER, UE) to I.R.

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Supporting Information

Table S1. Checklist of the species of Ochthebiini, with the current (following Jäch & Skale, 2015 and Jäch *et al*., 2016) and new classifications. In bold, type species. phyl, species included in the phylogeny (in brackets, species for which the sequenced specimen was a female). Distribution: PAL, Palaearctic; AFR, Afrotropical; AUR, Australia; NAR, Nearctic; NTR, Neotropical; ORR, Oriental; ANR, Antarctic. In brackets, specimens considered to have a Palaearctic distribution in Jäch & Skale (2015), but including the Oriental or Afrotropical Regions in Hansen (1998).

Table S2. Current classification of Ochthebiini, with synonymies and type species (following Jäch & Skale, 2015 and Jäch *et al*., 2016). In bold, taxa included in the phylogeny.

Table S3. List of material used in the molecular phylogeny, including voucher numbers, accession numbers of the sequences and locality data. In bold, newly obtained sequences.

Table S4. (A) primers used for DNA amplification and sequencing reactions; (B) Typical conditions for the polymerase chain reaction.

Fig. S1. Majority rule consensus tree obtained with BEAST for the phylogeny of Ochthebiini with the best partition models. Numbers in nodes, posterior probabilities.

Fig. S2. Phylogeny obtained with RAxML, including current Ochthebiini classification. Numbers in nodes, bootstrap support values.

Fig. S3. Phylogeny obtained with RAxML with the nuclear genes only. Numbers in nodes, bootstrap support values.

Table S1: Checklist of the species of Ochthebiini, with the current (following Jäch & Skale, 2015 and Jäch *et al*., 2016) and new classifications. In bold, type species. phyl, species included in the phylogeny (in brackets, species for which the sequenced specimen was a female).
Distribution: PAL, Palaearctic; AFR, Afrotropical; AUR, Australia; NAR, Nearctic; NTR, Neotropical; ORR, Oriental; ANR, Antarctic. In brackets, specimens considered to have a Palaearctic distribution in Jäch & Skale (2015), but including the Oriental or Afrotropical Regions in Hansen (1998).

	New classification								**current classification**										
No	**genus**	**subgenus**	**species group**	**species**	**subspecies**	**author**	**taxonomic act**	**original genus**	**genus**	**subgenus**	**obs.**	**phyl.**	**PAL**	**AFR**	**AUR**	**NAR**	**NTR**	**ORR**	**ANR**
1	*Meropathus*			*aucklandicus*		Ordish, 1971		*Meropathus*	*Meropathus*						X				
2	*Meropathus*			*campbellensis*		Brookes, 1951		*Meropathus*	*Meropathus*						X				
3	*Meropathus*			***chuni***		Enderlein, 1901		*Meropathus*	*Meropathus*										X
4	*Meropathus*			*johnsi*		Ordish, 1971		*Meropathus*	*Meropathus*						X				
5	*Meropathus*			*labratus*		Deane, 1933		*Ochthebius*	*Meropathus*						X				
6	*Meropathus*			*randi*		Jeannel, 1953		*Meropathus*	*Meropathus*					X					
7	*Meropathus*			*vectis*		Perkins, 1980		*Meropathus*	*Meropathus*								X		
8	*Meropathus*			*zelandicus*		Ordish, 1984		*Meropathus*	*Meropathus*			1			X				
9	*Ochthebius*	*Angiochthebius*		*jensenhaarupi*		Knisch, 1924		*Ochthebius*	*Gymnochthebius*								X		
10	*Ochthebius*	*Angiochthebius*		*octonarius*		Perkins, 1980		*Gymnochthebius*	*Gymnochthebius*								X		
11	*Ochthebius*	*Angiochthebius*		***plesiotypus***		Perkins, 1980		*Gymnochthebius*	*Gymnochthebius*			1					X		
12	*Ochthebius*	*Asiobates*	*bicolon*	*abeillei*		Guillebeae, 1896		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
13	*Ochthebius*	*Asiobates*	*bicolon*	*amplicollis*		Champion, 1925		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
14	*Ochthebius*	*Asiobates*	*bicolon*	*annae*		Ferro, 1979		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
15	*Ochthebius*	*Asiobates*	*bicolon*	*arator*		Ertorun & Jäch, 2014		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
16	*Ochthebius*	*Asiobates*	*bicolon*	*atricapillus*		Reitter, 1901		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
17	*Ochthebius*	*Asiobates*	*bicolon*	*auriculatus*		Rey, 1886		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
18	*Ochthebius*	*Asiobates*	*bicolon*	*avdati*		Delgado & Jäch, 2007		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
19	*Ochthebius*	*Asiobates*	*bicolon*	*belleiri*		Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
20	*Ochthebius*	*Asiobates*	*bicolon*	***bicolon***		Germar, 1824		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
21	*Ochthebius*	*Asiobates*	*bicolon*	*bonnairei*		Guillebeau, 1896		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
22	*Ochthebius*	*Asiobates*	*bicolon*	*cantabricus*		Balfour-Browne, 1979		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
23	*Ochthebius*	*Asiobates*	*bicolon*	*corcyraeus*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
24	*Ochthebius*	*Asiobates*	*bicolon*	*corsicus*		Saint-Claire Deville, 1908		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
25	*Ochthebius*	*Asiobates*	*bicolon*	*crenulatus*		Mulsant & Rey, 1850		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
26	*Ochthebius*	*Asiobates*	*bicolon*	*cyprensis*		Kuwert, 1890		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
27	*Ochthebius*	*Asiobates*	*bicolon*	*cyrenaeus*		Ferro, 1985		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
28	*Ochthebius*	*Asiobates*	*bicolon*	*czwalinae*		Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
29	*Ochthebius*	*Asiobates*	*bicolon*	*depressus*		Sahlberg, 1900		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
30	*Ochthebius*	*Asiobates*	*bicolon*	*dilatatus*		Stephens, 1829		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
31	*Ochthebius*	*Asiobates*	*bicolon*	*dilucidus*		Orchymont, 1940		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
32	*Ochthebius*	*Asiobates*	*bicolon*	*ferganensis*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
33	*Ochthebius*	*Asiobates*	*bicolon*	*ferroi*		Fresneda, Lagar & Hernando, 1993		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
34	*Ochthebius*	*Asiobates*	*bicolon*	*figueroi*		Garrido, Valladares & Régil, 1992		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
35	*Ochthebius*	*Asiobates*	*bicolon*	*flagellifer*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
36	*Ochthebius*	*Asiobates*	*bicolon*	*gagliardii*		Orchymont, 1940		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
37	*Ochthebius*	*Asiobates*	*bicolon*	*haberfelneri*		Reitter, 1890		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
38	*Ochthebius*	*Asiobates*	*bicolon*	*haelii*		Ferro, 1983		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
39	*Ochthebius*	*Asiobates*	*bicolon*	*heydeni*		Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
40	*Ochthebius*	*Asiobates*	*bicolon*	*hyblaemajoris*		Ferro, 1986		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
41	*Ochthebius*	*Asiobates*	*bicolon*	*immaculatus*		Breit, 1908		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
42	*Ochthebius*	*Asiobates*	*bicolon*	*impressipennis*		Rey, 1886		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
43	*Ochthebius*	*Asiobates*	*bicolon*	*irenae*		Ribera & Millán, 1999		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
44	*Ochthebius*	*Asiobates*	*bicolon*	*italicus*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
45	*Ochthebius*	*Asiobates*	*bicolon*	*jaimei*		Delgado & Jäch, 2007		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
46	*Ochthebius*	*Asiobates*	*bicolon*	*kaninensis*		Poppius, 1909		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
47	*Ochthebius*	*Asiobates*	*bicolon*	*khnzoriani*		Janssens, 1974		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
48	*Ochthebius*	*Asiobates*	*bicolon*	*kiesenwetteri*		Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
49	*Ochthebius*	*Asiobates*	*bicolon*	*laevisculptus*		Reitter, 1901		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
50	*Ochthebius*	*Asiobates*	*bicolon*	*laticullos*		Pankow, 1986		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
51	*Ochthebius*	*Asiobates*	*bicolon*	*lederi*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
52	*Ochthebius*	*Asiobates*	*bicolon*	*lenkoranus*		Reitter, 1885		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
53	*Ochthebius*	*Asiobates*	*bicolon*	*limbicollis*		Reitter, 1885		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
54	*Ochthebius*	*Asiobates*	*bicolon*	*maculatus*		Reiche, 1872		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
55	*Ochthebius*	*Asiobates*	*bicolon*	*montanus*		Frivaldszky, 1881		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						

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Table S1: (Continued)

	New classification								current classification										
No	**genus**	**subgenus**	**species group**	**species**	**subspecies**	**author**	**taxonomic act**	**original genus**	**genus**	**subgenus**	**obs.**	**phyl.**	**PAL**	**AFR**	**AUR**	**NAR**	**NTR**	**ORR**	**ANR**
56	*Ochthebius*	*Asiobates*	*bicolon*	*monychus*		Orchymont, 1941		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
57	*Ochthebius*	*Asiobates*	*biclon*	*naxianus*		Ieniştea, 1988		*Asiobates*	*Ochthebius*	*Asiobates*			X						
58	*Ochthebius*	*Asiobates*	*biclon*	*oezkani*		Jäch, Kasapoğlu & Erman, 2003		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
59	*Ochthebius*	*Asiobates*	*biclon*	*olicinium*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
60	*Ochthebius*	*Asiobates*	*biclon*	*opacus*		Baudi, 1882		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
61	*Ochthebius*	*Asiobates*	*biclon*	*peregrinus*		Orchymont, 1941		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
62	*Ochthebius*	*Asiobates*	*biclon*	*perkinsi*		Pankow, 1986		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
63	*Ochthebius*	*Asiobates*	*biclon*	*pui*		Perkins, 1979		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
64	*Ochthebius*	*Asiobates*	*biclon*	*rivibelli*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
65	*Ochthebius*	*Asiobates*	*biclon*	*sardus*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
66	*Ochthebius*	*Asiobates*	*biclon*	*striatus*		Castelnau, 1840		*Hydraena*	*Ochthebius*	*Asiobates*		1	X						
67	*Ochthebius*	*Asiobates*	*biclon*	*stygialis*		Orchymont, 1937		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
68	*Ochthebius*	*Asiobates*	*biclon*	*subopacus*		Reitter, 1885		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
69	*Ochthebius*	*Asiobates*	*biclon*	*tadilatus*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
70	*Ochthebius*	*Asiobates*	*biclon*	*thraciae*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
71	*Ochthebius*	*Asiobates*	*biclon*	*turcmeniae*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
72	*Ochthebius*	*Asiobates*	*minimus*	*adventicius*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
73	*Ochthebius*	*Asiobates*	*minimus*	*aeneus*		Stephens, 1835		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
74	*Ochthebius*	*Asiobates*	*minimus*	*akbuluti*		Jäch, Kasapoğlu & Erman, 2003		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
75	*Ochthebius*	*Asiobates*	*minimus*	*alpinus*		Ienişte, 1988		*Homalochthebius*	*Ochthebius*	*Asiobates*		1	X						
76	*Ochthebius*	*Asiobates*	*minimus*	*andreinii*	*andreinii*	Régimbart, 1905		*Ochthebius*	*Ochthebius*	*Asiobates*		1		X					
77	*Ochthebius*	*Asiobates*	*minimus*	*andreinii*	*andriscus*	Orchymont, 1948		*Ochthebius*	*Ochthebius*	*Asiobates*				X					
78	*Ochthebius*	*Asiobates*	*minimus*	*andreinii*	*androsthenus*	Orchymont, 1948		*Ochthebius*	*Ochthebius*	*Asiobates*				X					
79	*Ochthebius*	*Asiobates*	*minimus*	*andronius*		Orchymont, 1948		*Ochthebius*	*Ochthebius*	*Asiobates*		1		X					
80	*Ochthebius*	*Asiobates*	*minimus*	*apache*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Asiobates*						X	X		
81	*Ochthebius*	*Asiobates*	*minimus*	*apicalis*		Sharp, 1882		*Ochthebius*	*Ochthebius*	*Asiobates*							X		
82	*Ochthebius*	*Asiobates*	*minimus*	*brevipennis*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Asiobates*						X			
83	*Ochthebius*	*Asiobates*	*minimus*	*browni*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Asiobates*							X		
84	*Ochthebius*	*Asiobates*	*minimus*	*bupunctus*		Perkins, 2011		*Ochthebius*	*Ochthebius*	*Asiobates*				X					
85	*Ochthebius*	*Asiobates*	*minimus*	*coomani*		Orchymont, 1925		*Ochthebius*	*Ochthebius*	*Asiobates*								X	
86	*Ochthebius*	*Asiobates*	*minimus*	*cribricollis*		LeConte, 1850		*Ochthebius*	*Ochthebius*	*Asiobates*						X			
87	*Ochthebius*	*Asiobates*	*minimus*	*discretus*		LeConte, 1878		*Ochthebius*	*Ochthebius*	*Asiobates*		1				X	X		
88	*Ochthebius*	*Asiobates*	*minimus*	*flavipes*		Dalla Torre, 1877		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
89	*Ochthebius*	*Asiobates*	*minimus*	*formosanus*		Jäch, 1998		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
90	*Ochthebius*	*Asiobates*	*minimus*	*furcatus*		Pu, 1958		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
91	*Ochthebius*	*Asiobates*	*minimus*	*hebaueri*		Jäch, 1983		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
92	*Ochthebius*	*Asiobates*	*minimus*	*hibernus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Asiobates*						X			
93	*Ochthebius*	*Asiobates*	*minimus*	*hokkaidensis*		Jäch, 1998		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
94	*Ochthebius*	*Asiobates*	*minimus*	*hungaricus*		Endrödy-Younga, 1967		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
95	*Ochthebius*	*Asiobates*	*minimus*	*lurugosus*		Jäch, 1998		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
96	*Ochthebius*	*Asiobates*	*minimus*	*mexicanus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Asiobates*							X		
97	*Ochthebius*	*Asiobates*	*minimus*	*mimicus*		Brown, 1933		*Ochthebius*	*Ochthebius*	*Asiobates*						X			
98	*Ochthebius*	*Asiobates*	*minimus*	*minervus*	*minervus*	Orchymont, 1940		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
99	*Ochthebius*	*Asiobates*	*minimus*	*minervus*	*semechonitis*	Jäch, 1998		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
100	*Ochthebius*	*Asiobates*	*minimus*	*minimus*		Fabricius, 1792		*Helophorus*	*Ochthebius*	*Asiobates*		1	X						
101	*Ochthebius*	*Asiobates*	*minimus*	*monticola*		Orchymont, 1948		*Ochthebius*	*Ochthebius*	*Asiobates*				X					
102	*Ochthebius*	*Asiobates*	*minimus*	*nonaginta*		Jäch, 1998		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
103	*Ochthebius*	*Asiobates*	*minimus*	*orbus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Asiobates*						X			
104	*Ochthebius*	*Asiobates*	*minimus*	*otavalensis*		Anderson, 1983		*Ochthebius*	*Ochthebius*	*Asiobates*							X		
105	*Ochthebius*	*Asiobates*	*minimus*	*perdurus*		Reitter, 1899		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
106	*Ochthebius*	*Asiobates*	*minimus*	*pliginskiyi*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
107	*Ochthebius*	*Asiobates*	*minimus*	*putnamensis*		Blatchley, 1910		*Ochthebius*	*Ochthebius*	*Asiobates*						X			
108	*Ochthebius*	*Asiobates*	*minimus*	*regimbarti*		Knisch, 1924		*Ochthebius*	*Ochthebius*	*Asiobates*				X					
109	*Ochthebius*	*Asiobates*	*minimus*	*remotus*		Reitter, 1885		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
110	*Ochthebius*	*Asiobates*	*minimus*	*reticulocostus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Asiobates*							X		
111	*Ochthebius*	*Asiobates*	*minimus*	*rugulosus*		Wollaston, 1857		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						

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Table S1: (Continued)

	New classification								current classification										
No	**genus**	**subgenus**	**species group**	**species**	**subspecies**	**author**	**taxonomic act**	**original genus**	**genus**	**subgenus**	**obs.**	**phyl.**	**PAL**	**AFR**	**AUR**	**NAR**	**NTR**	**ORR**	**ANR**
112	*Ochthebius*	*Asiobates*	*minimus*	*sanabrensis*		Valladares & Jäch, 2008		*Ochthebius*	*Ochthebius*	*Asiobates*		1	X						
113	*Ochthebius*	*Asiobates*	*minimus*	*similis*		Sharp, 1882		*Ochthebius*	*Ochthebius*	*Asiobates*						X	X		
114	*Ochthebius*	*Asiobates*	*minimus*	*unimaculatus*		Pu, 1958		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
115	*Ochthebius*	*Asiobates*	*minimus*	*yunnanensis*		Orchymont, 1925		*Ochthebius*	*Ochthebius*	*Asiobates*			X						
116	*Ochthebius*	*Asiobates*	*puncticollis*	*angularidus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Asiobates*						X	X		
117	*Ochthebius*	*Asiobates*	*puncticollis*	*leechi*		Wood & Perkins, 1978		*Ochthebius*	*Ochthebius*	*Asiobates*						X			
118	*Ochthebius*	*Asiobates*	*puncticollis*	*martini*		Fall, 1919		*Ochthebius*	*Ochthebius*	*Asiobates*						X			
119	*Ochthebius*	*Asiobates*	*puncticollis*	*puncticollis*		LeConte, 1852		*Ochthebius*	*Ochthebius*	*Asiobates*		1				X	X		
120	*Ochthebius*	*Aulacochthebius*		*alienus*		Orchymont, 1929		*Ochthebius*	*Aulacochthebius*					X					
121	*Ochthebius*	*Aulacochthebius*		*amrishi*		Makhan, 2004		*Ochthebius*	*Ochthebius*	*Ochthebius*	possibly a synonym of *O. asiaticus*							X	
122	*Ochthebius*	*Aulacochthebius*		*asiaticus*		Orchymont, 1929		*Ochthebius*	*Aulacochthebius*									X	
123	*Ochthebius*	*Aulacochthebius*		*continentalis*		Orchymont, 1929		*Ochthebius*	*Aulacochthebius*					X					
124	*Ochthebius*	*Aulacochthebius*		*densus*		Orchymont, 1929		*Ochthebius*	*Aulacochthebius*									X	
125	*Ochthebius*	*Aulacochthebius*		***exaratus***		Mulsant, 1844		*Ochthebius*	*Aulacochthebius*			1	X	X					
126	*Ochthebius*	*Aulacochthebius*		*hunanensis*		Pu, 1958		*Ochthebius*	*Aulacochthebius*				X						
127	*Ochthebius*	*Aulacochthebius*		*libertarius*		Aguilera, Ribera & Hernando, 1998	comb.n.	*Aulacochthebius*	*Aulacochthebius*			1	X						
128	*Ochthebius*	*Aulacochthebius*		*manipurensis*		Orchymont, 1929		*Ochthebius*	*Aulacochthebius*									X	
129	*Ochthebius*	*Aulacochthebius*		*narentinus*		Reitter, 1885		*Ochthebius*	*Aulacochthebius*			1	X						
130	*Ochthebius*	*Aulacochthebius*		*perlaevis*		Perkins, 2017	comb.n.	*Aulacochthebius*	*Aulacochthebius*			1		X					
131	*Ochthebius*	*Aulacochthebius*		*plicicollis*		Fairmaire, 1898		*Ochthebius*	*Aulacochthebius*					X					
132	*Ochthebius*	*Aulacochthebius*		*tenuipunctus*		Régimbart, 1906		*Ochthebius*	*Aulacochthebius*					X					
133	*Ochthebius*	*Cobalius*		*adriaticus*	*adriaticus*	Reitter, 1886		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
134	*Ochthebius*	*Cobalius*		*adriaticus*	*moreanus*	Pretner, 1929		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
135	*Ochthebius*	*Cobalius*		*adriaticus*	*pleuralis*	Reitter, 1886		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
136	*Ochthebius*	*Cobalius*		*algicola*		Wollaston, 1871		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
137	*Ochthebius*	*Cobalius*		*balfourbrownei*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
138	*Ochthebius*	*Cobalius*		*biltoni*		Jäch & Delgado, 2017		*Ochthebius*	*Ochthebius*	*Cobalius*			X						
139	*Ochthebius*	*Cobalius*		*celatus*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
140	*Ochthebius*	*Cobalius*		*freyi*		Orchymont, 1940		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
141	*Ochthebius*	*Cobalius*		***lejolisii***		Mulsant & Rey, 1861		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
142	*Ochthebius*	*Cobalius*		*serratus*		Rosenhauer, 1856		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
143	*Ochthebius*	*Cobalius*		*subinteger*		Mulsant & Rey, 1861		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
144	*Ochthebius*	*Enicocerus*		*aguilerai*		Ribera, Castro & Hernando, 2010		*Ochthebius*	*Ochthebius*	*Enicocerus*		1	X						
145	*Ochthebius*	*Enicocerus*		*albanicus*		Orchymont, 1941		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
146	*Ochthebius*	*Enicocerus*		*anatolicus*		Janssens, 1963		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
147	*Ochthebius*	*Enicocerus*		*angelinii*		Ferro, 2008		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
148	*Ochthebius*	*Enicocerus*		*colveranus*		Ferro, 1979		*Ochthebius*	*Ochthebius*	*Enicocerus*		1	X						
149	*Ochthebius*	*Enicocerus*		*delyi*		Hebauer, 1990		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
150	*Ochthebius*	*Enicocerus*		***exsculptus***		Germar, 1824		*Ochthebius*	*Ochthebius*	*Enicocerus*		1	X						
151	*Ochthebius*	*Enicocerus*		*gibbosus*		Germar, 1824		*Ochthebius*	*Ochthebius*	*Enicocerus*		1	X						
152	*Ochthebius*	*Enicocerus*		*granulatus*		Mulsant, 1844		*Ochthebius*	*Ochthebius*	*Enicocerus*		1	X						
153	*Ochthebius*	*Enicocerus*		*halbherri*		Reitter, 1890		*Henicocerus*	*Ochthebius*	*Enicocerus*		1	X						
154	*Ochthebius*	*Enicocerus*		*legionensis*		Hebauer & Valladares Díez, 1985		*Ochthebius*	*Ochthebius*	*Enicocerus*		1	X						
155	*Ochthebius*	*Enicocerus*		*melanescens*		Dalla Torre, 1877		*Ochthebius*	*Ochthebius*	*Enicocerus*		1	X						
156	*Ochthebius*	*Enicocerus*		*montenegrinus*		Ganglbauer, 1901		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
157	*Ochthebius*	*Enicocerus*		*saboorii*		Skale & Jäch, 2009		*Ochthebius*	*Ochthebius*	*Enicocerus*		1	X						
158	*Ochthebius*	*Enicocerus*		*sattmanni*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
159	*Ochthebius*	*Enicocerus?*		*benefossus*		LeConte, 1878		*Ochthebius*	*Ochthebius*	*Enicocerus*						X			
160	*Ochthebius*	*Gymnanthelius*		*clypeatus*		Deane, 1931		*Ochthebius*	*Gymnanthelius*						X				
161	*Ochthebius*	*Gymnanthelius*		*cupreus*		Deane, 1937		*Ochthebius*	*Gymnanthelius*						X				
162	*Ochthebius*	*Gymnanthelius*		***hieroglyphicus***		Deane, 1933		*Ochthebius*	*Gymnanthelius*						X				
163	*Ochthebius*	*Gymnanthelius*		*lamingtonensis*		Perkins, 2004	comb.n.	*Gymnanthelius*	*Gymnanthelius*						X				
164	*Ochthebius*	*Gymnanthelius*		*maxipunctus*		Perkins, 2004	comb.n.	*Gymnanthelius*	*Gymnanthelius*						X				
165	*Ochthebius*	*Gymnanthelius*		*opacicollis*		Perkins, 2004	comb.n.	*Gymnanthelius*	*Gymnanthelius*			1			X				
166	*Ochthebius*	*Gymnanthelius*		*porchi*		Perkins, 2004	comb.n.	*Gymnanthelius*	*Gymnanthelius*			1			X				
167	*Ochthebius*	*Gymnanthelius*		*tunicus*		Perkins, 2004	comb.n.	*Gymnanthelius*	*Gymnanthelius*						X				

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Table S1: (Continued)

	New classification								current classification										
No	genus	subgenus	species group	species	subspecies	author	taxonomic act	original genus	genus	subgenus	obs.	phyl.	PAL	AFR	AUR	NAR	NTR	ORR	ANR
168	*Ochthebius*	*Gymnochthebius*	*australis*	*angulonotus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
169	*Ochthebius*	*Gymnochthebius*	*australis*	*australis*		Blackburn, 1888		*Ochthebius*	*Gymnochthebius*			1			X				
170	*Ochthebius*	*Gymnochthebius*	*australis*	*bacchusi*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
171	*Ochthebius*	*Gymnochthebius*	*australis*	*benesculptus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
172	*Ochthebius*	*Gymnochthebius*	*australis*	*brisbanensis*		Blackburn, 1898		*Ochthebius*	*Gymnochthebius*						X				
173	*Ochthebius*	*Gymnochthebius*	*australis*	*clarki*		Deane, 1931		*Ochthebius*	*Gymnochthebius*						X				
174	*Ochthebius*	*Gymnochthebius*	*australis*	*coruscus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
175	*Ochthebius*	*Gymnochthebius*	*australis*	*fontinalis*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
176	*Ochthebius*	*Gymnochthebius*	*australis*	*fumosus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
177	*Ochthebius*	*Gymnochthebius*	*australis*	*hesperius*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
178	*Ochthebius*	*Gymnochthebius*	*australis*	*inlineatus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
179	*Ochthebius*	*Gymnochthebius*	*australis*	*levis*		Deane, 1933		*Ochthebius*	*Gymnochthebius*						X				
180	*Ochthebius*	*Gymnochthebius*	*australis*	*lividus*		Deane, 1933		*Ochthebius*	*Gymnochthebius*			1			X				
181	*Ochthebius*	*Gymnochthebius*	*australis*	*lustrosulcus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
182	*Ochthebius*	*Gymnochthebius*	*australis*	*minipunctus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
183	*Ochthebius*	*Gymnochthebius*	*australis*	*nanosetus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
184	*Ochthebius*	*Gymnochthebius*	*australis*	*nicki*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
185	*Ochthebius*	*Gymnochthebius*	*australis*	*nigriceps*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
186	*Ochthebius*	*Gymnochthebius*	*australis*	*notalis*		Deane, 1933		*Ochthebius*	*Gymnochthebius*						X				
187	*Ochthebius*	*Gymnochthebius*	*australis*	*papua*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
188	*Ochthebius*	*Gymnochthebius*	*australis*	*perpunctus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
189	*Ochthebius*	*Gymnochthebius*	*australis*	*pluvipennis*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
190	*Ochthebius*	*Gymnochthebius*	*australis*	*probus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*			1			X				
191	*Ochthebius*	*Gymnochthebius*	*australis*	*radiatus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
192	*Ochthebius*	*Gymnochthebius*	*australis*	*resplendens*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
193	*Ochthebius*	*Gymnochthebius*	*australis*	*rhombus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
194	*Ochthebius*	*Gymnochthebius*	*australis*	*semicylindrus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
195	*Ochthebius*	*Gymnochthebius*	*australis*	*setosus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*			1			X				
196	*Ochthebius*	*Gymnochthebius*	*australis*	*sexplanatus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
197	*Ochthebius*	*Gymnochthebius*	*australis*	*squamifer*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
198	*Ochthebius*	*Gymnochthebius*	*australis*	*subsulcatus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
199	*Ochthebius*	*Gymnochthebius*	*australis*	*tenebricosus*		Deane, 1931		*Ochthebius*	*Gymnochthebius*						X				
200	*Ochthebius*	*Gymnochthebius*	*australis*	*trilineatus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
201	*Ochthebius*	*Gymnochthebius*	*australis*	*truncatus*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
202	*Ochthebius*	*Gymnochthebius*	*australis*	*wattsi*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
203	*Ochthebius*	*Gymnochthebius*	*australis*	*weiri*		Perkins, 2005	comb.n.	*Gymnochthebius*	*Gymnochthebius*						X				
204	*Ochthebius*	*Gymnochthebius*	*fossatus*	*bartyrae*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*								X		
205	*Ochthebius*	*Gymnochthebius*	*fossatus*	*bisagittatus*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*								X		
206	*Ochthebius*	*Gymnochthebius*	*fossatus*	*chilenus*		Balfour-Browne, 1971		*Ochthebius*	*Gymnochthebius*								X		
207	*Ochthebius*	*Gymnochthebius*	*fossatus*	*clandestinus*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*								X		
208	*Ochthebius*	*Gymnochthebius*	*fossatus*	*compactus*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*								X		
209	*Ochthebius*	*Gymnochthebius*	*fossatus*	*crassipes*		Sharp, 1882		*Ochthebius*	*Gymnochthebius*								X		
210	*Ochthebius*	*Gymnochthebius*	*fossatus*	*curvus*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*								X		
211	*Ochthebius*	*Gymnochthebius*	*fossatus*	*fossatus*		LeConte, 1855		*Ochthebius*	*Gymnochthebius*			1				X	X		
212	*Ochthebius*	*Gymnochthebius*	*fossatus*	*francki*		Bruch, 1915		*Ochthebius*	*Gymnochthebius*								X		
213	*Ochthebius*	*Gymnochthebius*	*fossatus*	*germaini*		Zaitzev, 1908		*Ochthebius*	*Gymnochthebius*			1					X		
214	*Ochthebius*	*Gymnochthebius*	*fossatus*	*ischigualasto*		Perkins & Archangelsky, 2002	comb.n.	*Gymnochthebius*	*Gymnochthebius*								X		
215	*Ochthebius*	*Gymnochthebius*	*fossatus*	*laevipennis*		LeConte, 1878		*Ochthebius*	*Gymnochthebius*							X	X		
216	*Ochthebius*	*Gymnochthebius*	*fossatus*	*maureenae*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*							X			
217	*Ochthebius*	*Gymnochthebius*	*fossatus*	***nitidus***		LeConte, 1850		*Ochthebius*	*Gymnochthebius*							X			
218	*Ochthebius*	*Gymnochthebius*	*fossatus*	*oppositus*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*							X	X		
219	*Ochthebius*	*Gymnochthebius*	*fossatus*	*perlabidus*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*								X		
220	*Ochthebius*	*Gymnochthebius*	*fossatus*	*peruvianus*		Balfour-Browne, 1971		*Ochthebius*	*Gymnochthebius*			1					X		
221	*Ochthebius*	*Gymnochthebius*	*fossatus*	*reticulatissimus*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*								X		
222	*Ochthebius*	*Gymnochthebius*	*fossatus*	*reticulatus*		Orchymont, 1943		*Ochthebius*	*Gymnochthebius*								X		
223	*Ochthebius*	*Gymnochthebius*	*fossatus*	*seminole*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*							X			

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Table S1: (Continued)

	New classification								current classification										
No	genus	subgenus	species group	species	subspecies	author	taxonomic act	original genus	genus	subgenus	obs.	phyl.	PAL	AFR	AUR	NAR	NTR	ORR	ANR
224	*Ochthebius*	*Gymnochthebius*	*fossatus*	*tectus*		Perkins, 1980	comb.n.	*Gymnochthebius*	*Gymnochthebius*								X		
225	*Ochthebius*	*Gymnochthebius*	*fossatus*	*topali*		Balfour-Browne, 1971		*Ochthebius*	*Gymnochthebius*								X		
226	*Ochthebius*	*Hughleechia*		*giulianii*		Perkins, 1981	comb.n.	*Hughleechia*	*Hughleechia*			1			X				
227	*Ochthebius*	*Hughleechia*		*gracilis*		Perkins, 2007	comb.n.	*Hughleechia*	*Hughleechia*						X				
228	*Ochthebius*	*Micragasma*		*minoicus*		Hernando, Villastrigo & Ribera, 2017		*Ochthebius*	*Ochthebius*				X						
229	*Ochthebius*	*Micragasma*		*paradoxus*		Sahlberg, 1900		*Micragasma*	*Micragasma*			1	X						
230	*Ochthebius*	*Micragasma*		*substrigosus*		Reitter, 1897		*Ochthebius*	*Micragasma*				X						
231	*Ochthebius*	*Ochthebius*	*andraei*	*andraei*	*andraei*	Breit, 1920		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
232	*Ochthebius*	*Ochthebius*	*andraei*	*andraei*	*explanatus*	Orchymont, 1937		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
233	*Ochthebius*	*Ochthebius*	*andraei*	*caspius*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
234	*Ochthebius*	*Ochthebius*	*andraei*	*cupricollis*		Sahlberg, 1903		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
235	*Ochthebius*	*Ochthebius*	*andraei*	*inelegans*		Jäch, 2002		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
236	*Ochthebius*	*Ochthebius*	*andraei*	*madli*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
237	*Ochthebius*	*Ochthebius*	*andraei*	*patergazellae*		Jäch & Delgado, 2010		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
238	*Ochthebius*	*Ochthebius*	*atriceps*	*andalusicus*		Jäch & Castro, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
239	*Ochthebius*	*Ochthebius*	*atriceps*	*anxifer*		Balfour-Browne, 1979		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
240	*Ochthebius*	*Ochthebius*	*atriceps*	*atriceps*		Fairmaire, 1879		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
241	*Ochthebius*	*Ochthebius*	*atriceps*	*burjkhalifa*		Jäch & Delgado, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
242	*Ochthebius*	*Ochthebius*	*atriceps*	*cameroni*		Balfour-Browne, 1951		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
243	*Ochthebius*	*Ochthebius*	*atriceps*	*dentifer*		Rey, 1885		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
244	*Ochthebius*	*Ochthebius*	*atriceps*	*depressionis*		Jäch, 1991		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
245	*Ochthebius*	*Ochthebius*	*atriceps*	*despoliatus*		Jäch & Delgado, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
246	*Ochthebius*	*Ochthebius*	*atriceps*	*falcatus*		Jäch, 1991		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
247	*Ochthebius*	*Ochthebius*	*atriceps*	*fausti*		Sharp, 1887		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
248	*Ochthebius*	*Ochthebius*	*atriceps*	*faustinus*		Orchymont, 1940		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
249	*Ochthebius*	*Ochthebius*	*atriceps*	*hesperides*		Balfour-Browne, 1976		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
250	*Ochthebius*	*Ochthebius*	*atriceps*	*karasui*		Ferro, 1986		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
251	*Ochthebius*	*Ochthebius*	*atriceps*	*lacustatta*		Jäch, 1991		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
252	*Ochthebius*	*Ochthebius*	*atriceps*	*loulae*		Jäch & Delgado, 2017		*Ochthebius*	*Ochthebius*	*Ochthebius*		1		X					
253	*Ochthebius*	*Ochthebius*	*atriceps*	*praetermissus*		Jäch, 1991		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
254	*Ochthebius*	*Ochthebius*	*atriceps*	*recurvatus*		Jäch, 1991		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
255	*Ochthebius*	*Ochthebius*	*atriceps*	*sculpturatus*		Sahlberg, 1900		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
256	*Ochthebius*	*Ochthebius*	*atriceps*	*tacapasensis*	*baeticus*	Ferro, 1984		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
257	*Ochthebius*	*Ochthebius*	*atriceps*	*tacapasensis*	*tacapasensis*	Ferro, 1983		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
258	*Ochthebius*	*Ochthebius*	*atriceps*	*thermalis*		Janssens, 1965		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
259	*Ochthebius*	*Ochthebius*	*corrugatus*	*corrugatus*		Rosenhauer, 1856		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
260	*Ochthebius*	*Ochthebius*	*corrugatus*	*gauthieri*		Peyerimhoff, 1924		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
261	*Ochthebius*	*Ochthebius*	*corrugatus*	*perpusillus*		Ferro, 1985		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
262	*Ochthebius*	*Ochthebius*	*foveolatus*	*afghanicus*		Jäch, 1991		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
263	*Ochthebius*	*Ochthebius*	*foveolatus*	*alpheius*		Janssens, 1959		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
264	*Ochthebius*	*Ochthebius*	*foveolatus*	*elisae*		Sahlberg, 1900		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
265	*Ochthebius*	*Ochthebius*	*foveolatus*	*foveolatus*		Germar, 1824		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
266	*Ochthebius*	*Ochthebius*	*foveolatus*	*hajeki*		Jäch & Delgado, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
267	*Ochthebius*	*Ochthebius*	*foveolatus*	*harteni*		Jäch & Delgado, 2010		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
268	*Ochthebius*	*Ochthebius*	*foveolatus*	*hellenicus*		Ieniştea, 1988		*Hymenodes*	*Ochthebius*	*Ochthebius*			X						
269	*Ochthebius*	*Ochthebius*	*foveolatus*	*inconspicuus*		Jäch, 1991		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
270	*Ochthebius*	*Ochthebius*	*foveolatus*	*indicus*		Ieniştea, 1988		*Hymenodes*	*Ochthebius*	*Ochthebius*			X						
271	*Ochthebius*	*Ochthebius*	*foveolatus*	*innexus*		Balfour-Browne, 1951		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
272	*Ochthebius*	*Ochthebius*	*foveolatus*	*magnannulatus*		Delgado & Jäch, 2009		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
273	*Ochthebius*	*Ochthebius*	*foveolatus*	*marginalis*		Rey, 1886		*Ochthobius*	*Ochthebius*	*Ochthebius*		1	X						
274	*Ochthebius*	*Ochthebius*	*foveolatus*	*mediterraneus*		Ieniştea, 1988		*Hymenodes*	*Ochthebius*	*Ochthebius*		1	X						
275	*Ochthebius*	*Ochthebius*	*foveolatus*	*medius*		Jäch, 1991		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
276	*Ochthebius*	*Ochthebius*	*foveolatus*	*merinidicus*		Ferro, 1985		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
277	*Ochthebius*	*Ochthebius*	*foveolatus*	*mutatus*		Jäch, 1991		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
278	*Ochthebius*	*Ochthebius*	*foveolatus*	*pakistanicus*		Delgado & Jäch, 2009		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
279	*Ochthebius*	*Ochthebius*	*foveolatus*	*pallidulus*		Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						

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Table S1: (Continued)

	New classification								current classification										
No	genus	subgenus	species group	species	subspecies	author	taxonomic act	original genus	genus	subgenus	obs.	phyl.	PAL	AFR	AUR	NAR	NTR	ORR	ANR
280	*Ochthebius*	*Ochthebius*	*foveolatus*	*parvannulatus*		Delgado & Jäch, 2009		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
281	*Ochthebius*	*Ochthebius*	*foveolatus*	*pedicularius*		Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
282	*Ochthebius*	*Ochthebius*	*foveolatus*	*pudilaceris*		Ferro, 1982		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
283	*Ochthebius*	*Ochthebius*	*foveolatus*	*satoi*		Nakane, 1965		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
284	*Ochthebius*	*Ochthebius*	*foveolatus*	*siculus*		Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
285	*Ochthebius*	*Ochthebius*	*foveolatus*	*sidanus*		Orchymont, 1942		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
286	*Ochthebius*	*Ochthebius*	*foveolatus*	*speculator*		Jäch, 1991		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
287	*Ochthebius*	*Ochthebius*	*foveolatus*	*uskubensis*		Hebauer, 1986		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
288	*Ochthebius*	*Ochthebius*	*foveolatus*	*virgula*		Ferro, 1986		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
289	*Ochthebius*	*Ochthebius*	*kosiensis*	*asperatus*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
290	*Ochthebius*	*Ochthebius*	*kosiensis*	*kosiensis*		Champion, 1920		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
291	*Ochthebius*	*Ochthebius*	*lobicollis*	*basilicatus*		Fiori, 1915		*Ochthebius*	*Ochthebius*	*Ochthebius*		(1)	X						
292	*Ochthebius*	*Ochthebius*	*lobicollis*	*caesaraugustae*		Jäch, Ribera & Aguilera, 1998		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
293	*Ochthebius*	*Ochthebius*	*lobicollis*	*delgadoi*		Jäch, 1994		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
294	*Ochthebius*	*Ochthebius*	*lobicollis*	*eyrei*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
295	*Ochthebius*	*Ochthebius*	*lobicollis*	*fossulatus*		Mulsant, 1844		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
296	*Ochthebius*	*Ochthebius*	*lobicollis*	*lapidicola*		Wollaston, 1864		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
297	*Ochthebius*	*Ochthebius*	*lobicollis*	*lobicollis*		Rey, 1885		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
298	*Ochthebius*	*Ochthebius*	*lobicollis*	*mauretanicus*		Jäch, 1990		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
299	*Ochthebius*	*Ochthebius*	*lobicollis*	*quadrifossulatus*		Waltl, 1835		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
300	*Ochthebius*	*Ochthebius*	*lobicollis*	*tivelunus*		Ferro, 1984		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
301	*Ochthebius*	*Ochthebius*	*lobicollis*	*velutinus*		Fairmaire, 1883		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
302	*Ochthebius*	*Ochthebius*	*marinus*	*alluaudi*		Régimbart, 1903		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
303	*Ochthebius*	*Ochthebius*	*marinus*	*alpinopetrus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
304	*Ochthebius*	*Ochthebius*	*marinus*	*anchorus*		Perkins, 2011		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
305	*Ochthebius*	*Ochthebius*	*marinus*	*angusi*		Jäch, 1994		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
306	*Ochthebius*	*Ochthebius*	*marinus*	*arabicus*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
307	*Ochthebius*	*Ochthebius*	*marinus*	*arefniae*		Jäch & Delgado, 2008		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
308	*Ochthebius*	*Ochthebius*	*marinus*	*arenicolus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
309	*Ochthebius*	*Ochthebius*	*marinus*	*arizonicus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*		(1)				X			
310	*Ochthebius*	*Ochthebius*	*marinus*	*attritus*		LeConte, 1878		*Ochthebius*	*Ochthebius*	*Ochthebius*						X	X		
311	*Ochthebius*	*Ochthebius*	*marinus*	*auropallens*		Fairmaire, 1879		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
312	*Ochthebius*	*Ochthebius*	*marinus*	*aztecus*		Sharp, 1887		*Ochthebius*	*Ochthebius*	*Ochthebius*		1				X	X		
313	*Ochthebius*	*Ochthebius*	*marinus*	*bactrianus*		Janssens, 1962		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
314	*Ochthebius*	*Ochthebius*	*marinus*	*batesoni*		Blair, 1933		*Ochthebius*	*Ochthebius*	*Ochthebius*		1					X		
315	*Ochthebius*	*Ochthebius*	*marinus*	*bellstedti*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
316	*Ochthebius*	*Ochthebius*	*marinus*	*bernhardi*		Jäch & Delgado, 2008		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
317	*Ochthebius*	*Ochthebius*	*marinus*	*bicomicus*		Perkins, 2011		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
318	*Ochthebius*	*Ochthebius*	*marinus*	*biincisus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
319	*Ochthebius*	*Ochthebius*	*marinus*	*bisinuatus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*		(1)				X			
320	*Ochthebius*	*Ochthebius*	*marinus*	*borealis*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X	X		
321	*Ochthebius*	*Ochthebius*	*marinus*	*californicus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
322	*Ochthebius*	*Ochthebius*	*marinus*	*capicola*		Péringuey, 1892		*Hydraena*	*Ochthebius*	*Ochthebius*		1		X					
323	*Ochthebius*	*Ochthebius*	*marinus*	*chappuisi*		Orchymont, 1948		*Ochthebius*	*Ochthebius*	*Ochthebius*		(1)		X					
324	*Ochthebius*	*Ochthebius*	*marinus*	*costatellus*		Reitter, 1897		*Ochthebius*	*Ochthebius*	*Ochthebius*			X			X			
325	*Ochthebius*	*Ochthebius*	*marinus*	*costipennis*		Fall, 1901		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
326	*Ochthebius*	*Ochthebius*	*marinus*	*crassalus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
327	*Ochthebius*	*Ochthebius*	*marinus*	*crenatus*		Hatch, 1965		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
328	*Ochthebius*	*Ochthebius*	*marinus*	*deletus*		Rey, 1885	stat.rest.	*Ochthebius*	*Ochthebius*	*Ochthebius*	currently a subspecies of *O. subpictus*	1	X						
329	*Ochthebius*	*Ochthebius*	*marinus*	*delhiensis*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*			X					X	
330	*Ochthebius*	*Ochthebius*	*marinus*	*endroedyi*		Perkins, 2011		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
331	*Ochthebius*	*Ochthebius*	*marinus*	*erzerumi*		Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
332	*Ochthebius*	*Ochthebius*	*marinus*	*evanescens*		Sahlberg, 1875		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
333	*Ochthebius*	*Ochthebius*	*marinus*	*extremus*		Péringuey, 1892		*Hydraena*	*Ochthebius*	*Ochthebius*				X					
334	*Ochthebius*	*Ochthebius*	*marinus*	*fallaciosus*		Ganglbauer, 1901	stat.n.	*Ochthebius*	*Ochthebius*	*Ochthebius*	currently a subspecies of *O. viridis*	1	X						
335	*Ochthebius*	*Ochthebius*	*marinus*	*granulinus*		Perkins, 2011		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					

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Table S1: (Continued)

	New classification								current classification										
No	**genus**	**subgenus**	**species group**	**species**	**subspecies**	**author**	**taxonomic act**	**original genus**	**genus**	**subgenus**	**obs.**	**phyl.**	**PAL**	**AFR**	**AUR**	**NAR**	**NTR**	**ORR**	**ANR**
336	*Ochthebius*	*Ochthebius*	*marinus*	*gruwelli*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*		1				X	X		
337	*Ochthebius*	*Ochthebius*	*marinus*	*hainanensis*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
338	*Ochthebius*	*Ochthebius*	*marinus*	*interruptus*		LeConte, 1852		*Ochthebius*	*Ochthebius*	*Ochthebius*		1				X	X		
339	*Ochthebius*	*Ochthebius*	*marinus*	*involatus*		Perkins, 2011		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
340	*Ochthebius*	*Ochthebius*	*marinus*	*janssensi*		Ferro, 1983		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
341	*Ochthebius*	*Ochthebius*	*marinus*	*jengi*		Jäch, 1998		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
342	*Ochthebius*	*Ochthebius*	*marinus*	*kuwerti*		Reitter, 1897		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
343	*Ochthebius*	*Ochthebius*	*marinus*	*lecontei*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
344	*Ochthebius*	*Ochthebius*	*marinus*	*lenensis*		Poppius, 1907		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
345	*Ochthebius*	*Ochthebius*	*marinus*	*lineatus*		LeConte, 1852		*Ochthebius*	*Ochthebius*	*Ochthebius*		1				X	X		
346	*Ochthebius*	*Ochthebius*	*marinus*	*lividipennis*		Peyron, 1858		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
347	*Ochthebius*	*Ochthebius*	*marinus*	*madrensis*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X	X		
348	*Ochthebius*	*Ochthebius*	*marinus*	*marinus*		Paykull, 1798		*Elophorus*	*Ochthebius*	*Ochthebius*		1	X			X			
349	*Ochthebius*	*Ochthebius*	*marinus*	*masatakasatoi*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*								X	
350	*Ochthebius*	*Ochthebius*	*marinus*	*meridionalis*		Rey, 1885		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
351	*Ochthebius*	*Ochthebius*	*marinus*	*mesoamericanus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*		1					X		
352	*Ochthebius*	*Ochthebius*	*marinus*	*mexcavatus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*							X		
353	*Ochthebius*	*Ochthebius*	*marinus*	*minabensis*		Ferro, 1983		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
354	*Ochthebius*	*Ochthebius*	*marinus*	*mongolensis*		Janssens, 1967		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
355	*Ochthebius*	*Ochthebius*	*marinus*	*nipponicus*		Jäch, 1998		*Ochthebius*	*Ochthebius*	*Ochthebius*	Ryukyu	1	X						
356	*Ochthebius*	*Ochthebius*	*marinus*	*pacificus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
357	*Ochthebius*	*Ochthebius*	*marinus*	*pauli*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*							X		
358	*Ochthebius*	*Ochthebius*	*marinus*	*pedalis*		Balfour-Browne, 1954		*Ochthebius*	*Ochthebius*	*Ochthebius*		1		X					
359	*Ochthebius*	*Ochthebius*	*marinus*	*pusillus*		Stephens, 1835		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
360	*Ochthebius*	*Ochthebius*	*marinus*	*queenslandicus*		Hansen, 1998		*Ochthebius*	*Ochthebius*	*Ochthebius*		1			X				
361	*Ochthebius*	*Ochthebius*	*marinus*	*recticulus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
362	*Ochthebius*	*Ochthebius*	*marinus*	*rectus*		LeConte, 1878		*Ochthebius*	*Ochthebius*	*Ochthebius*		1				X			
363	*Ochthebius*	*Ochthebius*	*marinus*	*rectusalsus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X	X		
364	*Ochthebius*	*Ochthebius*	*marinus*	*richmondi*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
365	*Ochthebius*	*Ochthebius*	*marinus*	*rubripes*		Boheman, 1860		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
366	*Ochthebius*	*Ochthebius*	*marinus*	*salinarius*		Balfour-Browne, 1954		*Ochthebius*	*Ochthebius*	*Ochthebius*		1		X					
367	*Ochthebius*	*Ochthebius*	*marinus*	*sculptoides*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*		1					X		
368	*Ochthebius*	*Ochthebius*	*marinus*	*sculptus*		LeConte, 1878		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
369	*Ochthebius*	*Ochthebius*	*marinus*	*sharpi*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*							X		
370	*Ochthebius*	*Ochthebius*	*marinus*	*sierrensis*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
371	*Ochthebius*	*Ochthebius*	*marinus*	*sitiensis*		Perkins, 2011		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
372	*Ochthebius*	*Ochthebius*	*marinus*	*spanglerorum*		Wood & Perkins, 1978		*Ochthebius*	*Ochthebius*	*Ochthebius*						X			
373	*Ochthebius*	*Ochthebius*	*marinus*	*spinasus*		Perkins & Balfour-Browne, 1994		*Ochthebius*	*Ochthebius*	*Ochthebius*		1		X					
374	*Ochthebius*	*Ochthebius*	*marinus*	*subaeneus*		Janssens, 1967		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
375	*Ochthebius*	*Ochthebius*	*marinus*	*subpictus*		Wollaston, 1857		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
376	*Ochthebius*	*Ochthebius*	*marinus*	*tubus*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*						X	X		
377	*Ochthebius*	*Ochthebius*	*marinus*	*uniformis*		Perkins, 1980		*Ochthebius*	*Ochthebius*	*Ochthebius*		(1)				X			
378	*Ochthebius*	*Ochthebius*	*marinus*	*virens*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
379	*Ochthebius*	*Ochthebius*	*marinus*	*viridescens*		Ieniştea, 1988		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
380	*Ochthebius*	*Ochthebius*	*marinus*	*viridis*		Peyron, 1858		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
381	*Ochthebius*	*Ochthebius*	*marinus?*	*romanicus*		Ieniştea, 1968		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
382	*Ochthebius*	*Ochthebius*	*metallescens*	*albacetinus*		Ferro, 1984		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
383	*Ochthebius*	*Ochthebius*	*metallescens*	*anaxagoras*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
384	*Ochthebius*	*Ochthebius*	*metallescens*	*aristoteles*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
385	*Ochthebius*	*Ochthebius*	*metallescens*	*ciliciae*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
386	*Ochthebius*	*Ochthebius*	*metallescens*	*colchicus*		Janssens, 1963		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
387	*Ochthebius*	*Ochthebius*	*metallescens*	*dalmatinus*		Ganglbauer, 1904		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
388	*Ochthebius*	*Ochthebius*	*metallescens*	*decianus*		Orchymont, 1942		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
389	*Ochthebius*	*Ochthebius*	*metallescens*	*diazi*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
390	*Ochthebius*	*Ochthebius*	*metallescens*	*empedocles*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
391	*Ochthebius*	*Ochthebius*	*metallescens*	*gayosoi*		Jäch, 2001		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						

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Table S1: (Continued)

	New classification								current classification										
No	**genus**	**subgenus**	**species group**	**species**	**subspecies**	**author**	**taxonomic act**	**original genus**	**genus**	**subgenus**	**obs.**	**phyl.**	**PAL**	**AFR**	**AUR**	**NAR**	**NTR**	**ORR**	**ANR**
392	*Ochthebius*	*Ochthebius*	*metallescens*	*gestroi*		Gridelli, 1926		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
393	*Ochthebius*	*Ochthebius*	*metallescens*	*griotes*		Ferro, 1985		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
394	*Ochthebius*	*Ochthebius*	*metallescens*	*hanshebaueri*		Jäch, 1994		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
395	*Ochthebius*	*Ochthebius*	*metallescens*	*hatayensis*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
396	*Ochthebius*	*Ochthebius*	*metallescens*	*hivae*		Jäch, Irani & Delgado, 2013		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
397	*Ochthebius*	*Ochthebius*	*metallescens*	*hofratvukovitsi*		Jäch, 1994		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
398	*Ochthebius*	*Ochthebius*	*metallescens*	*huberti*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
399	*Ochthebius*	*Ochthebius*	*metallescens*	*insidiosus*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
400	*Ochthebius*	*Ochthebius*	*metallescens*	*iternuptialis*		Jäch, 2001		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
401	*Ochthebius*	*Ochthebius*	*metallescens*	*javieri*		Jäch, 2000		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
402	*Ochthebius*	*Ochthebius*	*metallescens*	*judemaesi*		Delgado & Jäch, 2007		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
403	*Ochthebius*	*Ochthebius*	*metallescens*	*khuzestanicus*		Ferro, 1982		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
404	*Ochthebius*	*Ochthebius*	*metallescens*	*kieneri*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
405	*Ochthebius*	*Ochthebius*	*metallescens*	*kirschenhoferi*		Jäch, 1994		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
406	*Ochthebius*	*Ochthebius*	*metallescens*	*kurdistanicus*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
407	*Ochthebius*	*Ochthebius*	*metallescens*	*latinorum*		Ieniştea, 1988		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
408	*Ochthebius*	*Ochthebius*	*metallescens*	*levantinus*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
409	*Ochthebius*	*Ochthebius*	*metallescens*	*libanus*		Jäch & Dia, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
410	*Ochthebius*	*Ochthebius*	*metallescens*	*marijanmatoki*		Jäch & Delgado, 2015		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
411	*Ochthebius*	*Ochthebius*	*metallescens*	*maxfischeri*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*		(1)	X						
412	*Ochthebius*	*Ochthebius*	*metallescens*	*metallescens*	*metallescens*	Rosenhauer, 1847		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
413	*Ochthebius*	*Ochthebius*	*metallescens*	*metallescens*	*plato*	Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
414	*Ochthebius*	*Ochthebius*	*metallescens*	*metallicus*		Orchymont, 1942		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
415	*Ochthebius*	*Ochthebius*	*metallescens*	*metarius*		Orchymont, 1942		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
416	*Ochthebius*	*Ochthebius*	*metallescens*	*metellus*		Orchymont, 1942		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
417	*Ochthebius*	*Ochthebius*	*metallescens*	*morettii*		Pirisinu, 1974		*Ochthebius*	*Ochthebius*	*Ochthebius*		(1)	X						
418	*Ochthebius*	*Ochthebius*	*metallescens*	*pedroi*		Jäch, 2000		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
419	*Ochthebius*	*Ochthebius*	*metallescens*	*poweri*		Rye, 1869		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
420	*Ochthebius*	*Ochthebius*	*metallescens*	*preissi*		Jäch, 2001		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
421	*Ochthebius*	*Ochthebius*	*metallescens*	*pretneri*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
422	*Ochthebius*	*Ochthebius*	*metallescens*	*puberulus*		Reitter, 1885		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
423	*Ochthebius*	*Ochthebius*	*metallescens*	*rectilobus*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
424	*Ochthebius*	*Ochthebius*	*metallescens*	*schoedli*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
425	*Ochthebius*	*Ochthebius*	*metallescens*	*schuberti*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
426	*Ochthebius*	*Ochthebius*	*metallescens*	*scitulus*		Ferro, 1982		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
427	*Ochthebius*	*Ochthebius*	*metallescens*	*scopuli*		Köhler, Jäch & Delgado, 2016		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
428	*Ochthebius*	*Ochthebius*	*metallescens*	*semisericeus*		Sainte-Claire Deville, 1914		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
429	*Ochthebius*	*Ochthebius*	*metallescens*	*semotus*		Orchymont, 1942		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
430	*Ochthebius*	*Ochthebius*	*metallescens*	*sempronius*		Orchymont, 1942		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
431	*Ochthebius*	*Ochthebius*	*metallescens*	*sennius*		Orchymont, 1942		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
432	*Ochthebius*	*Ochthebius*	*metallescens*	*serpentinus*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
433	*Ochthebius*	*Ochthebius*	*metallescens*	*smyrnensis*		Sahlberg, 1908		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
434	*Ochthebius*	*Ochthebius*	*metallescens*	*trapezuntinus*		Jäch, 1999		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
435	*Ochthebius*	*Ochthebius*	*metallescens*	*ustaoglui*		Topkara, Jäch & Kasapoğlu, 2011		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
436	*Ochthebius*	*Ochthebius*	*metallescens*	*vedovai*		Ferro, 1987		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
437	*Ochthebius*	*Ochthebius*	*metallescens*	*viganoi*		Pirisinu, 1974		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
438	*Ochthebius*	*Ochthebius*	*metallescens*	*wurayah*		Jäch & Delgado, 2010		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
439	*Ochthebius*	*Ochthebius*	*nitidipennis*	*amami*		Yoshitomi & Satô, 2001		*Ochthebius*	*Ochthebius*	*Enicocerus*	Ryukyu		X						
440	*Ochthebius*	*Ochthebius*	*nitidipennis*	*championi*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
441	*Ochthebius*	*Ochthebius*	*nitidipennis*	*exiguus*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
442	*Ochthebius*	*Ochthebius*	*nitidipennis*	*hasegawai*		Nakane & Matsui, 1986		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
443	*Ochthebius*	*Ochthebius*	*nitidipennis*	*ilanensis*		Jäch, 1998		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
444	*Ochthebius*	*Ochthebius*	*nitidipennis*	*japonicus*		Jäch, 1998		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
445	*Ochthebius*	*Ochthebius*	*nitidipennis*	*nakanei*		Matsui, 1986		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
446	*Ochthebius*	*Ochthebius*	*nitidipennis*	*nigrasperulus*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
447	*Ochthebius*	*Ochthebius*	*nitidipennis*	*nitidipennis*		Champion, 1920		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						

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Table S1: (Continued)

	New classification								current classification										
No	genus	subgenus	species group	species	subspecies	author	taxonomic act	original genus	genus	subgenus	obs.	phyl.	PAL	AFR	AUR	NAR	NTR	ORR	ANR
448	*Ochthebius*	*Ochthebius*	*nitidipennis*	*obesus*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
449	*Ochthebius*	*Ochthebius*	*nitidipennis*	*rotundatus*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
450	*Ochthebius*	*Ochthebius*	*nitidipennis*	*scintillans*		Champion, 1920		*Ochthebius*	*Ochthebius*	*Enicocerus*			X						
451	*Ochthebius*	*Ochthebius*	*notabilis*	*eburneus*		Sahlberg, 1900		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
452	*Ochthebius*	*Ochthebius*	*notabilis*	*elburzi*		Ferro, 1987		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
453	*Ochthebius*	*Ochthebius*	*notabilis*	*gereckei*		Jäch, 1993		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
454	*Ochthebius*	*Ochthebius*	*notabilis*	*glaber*		Montes & Soler, 1988		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
455	*Ochthebius*	*Ochthebius*	*notabilis*	*halophilus*		Ertorun & Jäch, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
456	*Ochthebius*	*Ochthebius*	*notabilis*	*jermakovi*		Orchymont, 1933		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
457	*Ochthebius*	*Ochthebius*	*notabilis*	*lanarotis*		Ferro, 1985		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
458	*Ochthebius*	*Ochthebius*	*notabilis*	*maroccanus*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
459	*Ochthebius*	*Ochthebius*	*notabilis*	*normandi*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
460	*Ochthebius*	*Ochthebius*	*notabilis*	*notabilis*		Rosenhauer, 1856		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
461	*Ochthebius*	*Ochthebius*	*notabilis*	*ponticus*		Ieniştea, 1956		*Ochthebius*	*Ochthebius*	*Ochthebius*	possibly a synonym of *O. jermakovi*		X						
462	*Ochthebius*	*Ochthebius*	*notabilis*	*salinator*		Peyerimhoff, 1924		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
463	*Ochthebius*	*Ochthebius*	*notabilis*	*zugmayeri*		Kniž, 1909		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
464	*Ochthebius*	*Ochthebius*	*peisonis*	*peisonis*		Ganglbauer, 1901		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
465	*Ochthebius*	*Ochthebius*	*punctatus*	*almorensis*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
466	*Ochthebius*	*Ochthebius*	*punctatus*	*argentatus*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
467	*Ochthebius*	*Ochthebius*	*punctatus*	*atratulus*		Régimbart, 1905		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
468	*Ochthebius*	*Ochthebius*	*punctatus*	*bifoveolatus*		Waltl, 1835		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
469	*Ochthebius*	*Ochthebius*	*punctatus*	*caucasicus*		Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
470	*Ochthebius*	*Ochthebius*	*punctatus*	*ciffidilis*		Ferro, 1984		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
471	*Ochthebius*	*Ochthebius*	*punctatus*	*cuprescens*		Guillebeau, 1893		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
472	*Ochthebius*	*Ochthebius*	*punctatus*	*danjo*		Nakane, 1990		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
473	*Ochthebius*	*Ochthebius*	*punctatus*	*darius*		Balfour-Browne, 1979		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
474	*Ochthebius*	*Ochthebius*	*punctatus*	*difficilis*		Mulsant, 1844		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
475	*Ochthebius*	*Ochthebius*	*punctatus*	*flexus*		Pu, 1958		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
476	*Ochthebius*	*Ochthebius*	*punctatus*	*flumineus*		Orchymont, 1937		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
477	*Ochthebius*	*Ochthebius*	*punctatus*	*gonggashanensis*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
478	*Ochthebius*	*Ochthebius*	*punctatus*	*grandipennis*		Fairmaire, 1879		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
479	*Ochthebius*	*Ochthebius*	*punctatus*	*hauseri*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
480	*Ochthebius*	*Ochthebius*	*punctatus*	*imbensimbi*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
481	*Ochthebius*	*Ochthebius*	*punctatus*	*inermis*		Sharp, 1884		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
482	*Ochthebius*	*Ochthebius*	*punctatus*	*iranicus*		Balfour-Browne, 1979		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
483	*Ochthebius*	*Ochthebius*	*punctatus*	*joosti*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
484	*Ochthebius*	*Ochthebius*	*punctatus*	*klapperichi*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X					X	
485	*Ochthebius*	*Ochthebius*	*punctatus*	*lanuginosus*		Reiche & Saulcy, 1856		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
486	*Ochthebius*	*Ochthebius*	*punctatus*	*lobatus*		Pu, 1958		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
487	*Ochthebius*	*Ochthebius*	*punctatus*	*mahmoodi*		Jäch & Delgado, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
488	*Ochthebius*	*Ochthebius*	*punctatus*	*micans*		Balfour-Browne, 1951		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X	(X)					
489	*Ochthebius*	*Ochthebius*	*punctatus*	*mongolicus*		Janssens, 1967		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
490	*Ochthebius*	*Ochthebius*	*punctatus*	*monseti*		Jäch & Delgado, 2010		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
491	*Ochthebius*	*Ochthebius*	*punctatus*	*montesi*		Ferro, 1984		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
492	*Ochthebius*	*Ochthebius*	*punctatus*	*namibiensis*		Perkins & Balfour-Browne, 1994		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
493	*Ochthebius*	*Ochthebius*	*punctatus*	*nanus*		Stephens, 1829		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
494	*Ochthebius*	*Ochthebius*	*punctatus*	*nepalensis*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
495	*Ochthebius*	*Ochthebius*	*punctatus*	*nilssoni*		Hebauer, 1986		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
496	*Ochthebius*	*Ochthebius*	*punctatus*	*nobilis*		Villa & Villa, 1835		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
497	*Ochthebius*	*Ochthebius*	*punctatus*	*opacipennis*		Champion, 1920		*Ochthebius*	*Ochthebius*	*Ochthebius*			X					X	
498	*Ochthebius*	*Ochthebius*	*punctatus*	*orientalis*		Janssens, 1962		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
499	*Ochthebius*	*Ochthebius*	*punctatus*	*ovatus*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X					(X)	
500	*Ochthebius*	*Ochthebius*	*punctatus*	*pagotrichus*		Perkins & Balfour-Browne, 1994		*Ochthebius*	*Ochthebius*	*Ochthebius*		1		X					
501	*Ochthebius*	*Ochthebius*	*punctatus*	*pilosus*		Waltl, 1835		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
502	*Ochthebius*	*Ochthebius*	*punctatus*	*punctatoides*		Jäch, 1994		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
503	*Ochthebius*	*Ochthebius*	*punctatus*	*punctatus*		Stephens, 1829		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						

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Table S1: (Continued)

	New classification								current classification											
No	genus	subgenus	species group	species	subspecies	author	taxonomic act	original genus	genus	subgenus	obs.	phyl.	PAL	AFR	AUR	NAR	NTR	ORR	ANR
504	*Ochthebius*	*Ochthebius*	*punctatus*	*quadrifoveolatus*		Wollaston, 1854		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X	X					
505	*Ochthebius*	*Ochthebius*	*punctatus*	*ragusae*		Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
506	*Ochthebius*	*Ochthebius*	*punctatus*	*schneideri*	*orchymonti*	Jäch, 1984		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
507	*Ochthebius*	*Ochthebius*	*punctatus*	*schneideri*	*schneideri*	Kuwert, 1887		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
508	*Ochthebius*	*Ochthebius*	*punctatus*	*sexfoveatus*		Champion, 1920		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
509	*Ochthebius*	*Ochthebius*	*punctatus*	*silfverbergi*		Jäch, 1992		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
510	*Ochthebius*	*Ochthebius*	*punctatus*	*spatulus*		Balfour-Browne, 1954		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
511	*Ochthebius*	*Ochthebius*	*punctatus*	*sulpuris*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
512	*Ochthebius*	*Ochthebius*	*punctatus*	*tudmirensis*		Jäch, 1997		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
513	*Ochthebius*	*Ochthebius*	*punctatus*	*tunisicus*		Jäch, 1997		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
514	*Ochthebius*	*Ochthebius*	*punctatus*	*turcicus*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
515	*Ochthebius*	*Ochthebius*	*punctatus*	*turkestanus*		Kuwert, 1892		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
516	*Ochthebius*	*Ochthebius*	*punctatus*	*verrucosus*		Pu, 1942		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
517	*Ochthebius*	*Ochthebius*	*punctatus*	*wewalkai*		Jäch, 1984		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
518	*Ochthebius*	*Ochthebius*	*punctatus*	*zulu*		Perkins, 2011		*Ochthebius*	*Ochthebius*	*Ochthebius*				X					
519	*Ochthebius*	*Ochthebius*	*quadricollis*	*brevicollis*		Baudi a Selve, 1864		*Calobius*	*Ochthebius*	*Calobius*		1	X						
520	*Ochthebius*	*Ochthebius*	*quadricollis*	*heeri*		Wollaston, 1854		*Calobius*	*Ochthebius*	*Calobius*		1	X						
521	*Ochthebius*	*Ochthebius*	*quadricollis*	*quadricollis*		Mulsant, 1844		*Ochthebius*	*Ochthebius*	*Calobius*		1	X						
522	*Ochthebius*	*Ochthebius*	*quadricollis*	*steinbuehleri*		Reitter, 1886		*Calobius*	*Ochthebius*	*Calobius*		1	X						
523	*Ochthebius*	*Ochthebius*	*quadricollis*	*urbanelliae*		Audisio, Trizzino & De Biase, 2010		*Calobius*	*Ochthebius*	*Calobius*			X						
524	*Ochthebius*	*Ochthebius*	*rivalis*	*himalayae*		Jäch, 1989		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
525	*Ochthebius*	*Ochthebius*	*rivalis*	*octofoveatus*		Pu, 1958		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
526	*Ochthebius*	*Ochthebius*	*rivalis*	*rivalis*		Champion, 1920		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
527	*Ochthebius*	*Ochthebius*	*rivalis*	*wuzhishanensis*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
528	*Ochthebius*	*Ochthebius*	*strigosus*	*andreasi*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
529	*Ochthebius*	*Ochthebius*	*strigosus*	*andreasoides*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
530	*Ochthebius*	*Ochthebius*	*strigosus*	*asiobatoides*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
531	*Ochthebius*	*Ochthebius*	*strigosus*	*caligatus*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
532	*Ochthebius*	*Ochthebius*	*strigosus*	*castellanus*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
533	*Ochthebius*	*Ochthebius*	*strigosus*	*enicoceroides*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
534	*Ochthebius*	*Ochthebius*	*strigosus*	*fujianensis*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
535	*Ochthebius*	*Ochthebius*	*strigosus*	*guangdongensis*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
536	*Ochthebius*	*Ochthebius*	*strigosus*	IBE-RA617		undescribed		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
537	*Ochthebius*	*Ochthebius*	*strigosus*	*jilanzhui*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
538	*Ochthebius*	*Ochthebius*	*strigosus*	*salebrosus*		Pu, 1958		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
539	*Ochthebius*	*Ochthebius*	*strigosus*	*sichuanensis*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
540	*Ochthebius*	*Ochthebius*	*strigosus*	*stastnyi*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
541	*Ochthebius*	*Ochthebius*	*strigosus*	*strigoides*		Jäch, 1998		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
542	*Ochthebius*	*Ochthebius*	*strigosus*	*strigosus*		Champion, 1921		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
543	*Ochthebius*	*Ochthebius*	*strigosus*	*wangmiaoi*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
544	*Ochthebius*	*Ochthebius*	*strigosus*	*yaanensis*		Jäch, 2003		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
545	*Ochthebius*	*Ochthebius*	*sumatrensis*	MNCN-AC16		undescribed		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
546	*Ochthebius*	*Ochthebius*	*sumatrensis*	*sumatrensis*		Jäch, 2001		*Ochthebius*	*Ochthebius*	*Ochthebius*								X	
547	*Ochthebius*	*Ochthebius*	*vandykei*	*ahni*		Jäch & Delgado, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
548	*Ochthebius*	*Ochthebius*	*vandykei*	*asanoae*		Jäch & Delgado, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
549	*Ochthebius*	*Ochthebius*	*vandykei*	*granulosus*		Satô, 1963		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
550	*Ochthebius*	*Ochthebius*	*vandykei*	*hayashii*		Jäch & Delgado, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
551	*Ochthebius*	*Ochthebius*	*vandykei*	*matsudae*		Jäch & Delgado, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
552	*Ochthebius*	*Ochthebius*	*vandykei*	*parki*		Jäch & Delgado, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
553	*Ochthebius*	*Ochthebius*	*vandykei*	*vandykei*		Knisch, 1924		*Ochthebius*	*Ochthebius*	*Ochthebius*		1				X			
554	*Ochthebius*	*Ochthebius*	*vandykei*	*yoshitomii*		Jäch & Delgado, 2014		*Ochthebius*	*Ochthebius*	*Ochthebius*		1	X						
555	*Ochthebius*	*Ochthebius*	incertae sedis	*belucistanicus*		Ferro, 1948		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
556	*Ochthebius*	*Ochthebius*	incertae sedis	*caudatus*		Frivaldszky, 1883		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
557	*Ochthebius*	*Ochthebius*	incertae sedis	*fissicollis*		Janssens, 1970		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
558	*Ochthebius*	*Ochthebius*	incertae sedis	*pierottii*		Ferro, 1979		*Ochthebius*	*Ochthebius*	*Ochthebius*			X						
559	*Ochthebius*	incertae sedis		*eremita*		Knisch, 1922		*Ochthebius*	*Ochthebius*	incertae sedis								X	

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Table S1: (Continued)

	New classification								current classification										
No	genus	subgenus	species group	species	subspecies	author	taxonomic act	original genus	genus	subgenus	obs.	phyl.	PAL	AFR	AUR	NAR	NTR	ORR	ANR
560	*Protochthebius*			*convexus*		Perkins, 1998		*Protochthebius*	*Protochthebius*				X						
561	*Protochthebius*			*jagthanae*		Champion, 1921		*Ochthebius*	*Protochthebius*				X						
562	*Protochthebius*			*jendeki*		Jäch, 1997		*Protochthebius*	*Protochthebius*									X	
563	*Protochthebius*			*loebli*		Perkins, 1998		*Protochthebius*	*Protochthebius*				X						
564	*Protochthebius*			***satoi***		Perkins, 1997		*Protochthebius*	*Protochthebius*				X					(X)	
565	*Protochthebius*			*schillhammeri*		Jäch, 1997		*Protochthebius*	*Protochthebius*									X	
566	*Protochthebius*			*smetanai*		Perkins, 1998		*Protochthebius*	*Protochthebius*				X						
567	*Prototympanogaster*			*lordhowensis*		Perkins, 2018		*Prototympanogaster*	*Prototympanogaster*						X				
568	*Tympallopatrum*			*aureolum*		Perkins, 2004		*Tympanopatrum*	*Tympallopatrum*						X				
569	*Tympallopatrum*			*callosum*		Perkins, 2004		*Tympanopatrum*	*Tympallopatrum*						X				
570	*Tympallopatrum*			*curvicostum*		Perkins, 2004		*Tympanopatrum*	*Tympallopatrum*						X				
571	*Tympallopatrum*			*longitudum*		Perkins, 1997		*Tympallopatrum*	*Tympallopatrum*						X				
572	*Tympanogaster*	*Hygrotympanogaster*		*arcuata*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
573	*Tympanogaster*	*Hygrotympanogaster*		*atroargenta*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
574	*Tympanogaster*	*Hygrotympanogaster*		*barronensis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
575	*Tympanogaster*	*Hygrotympanogaster*		*bondi*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
576	*Tympanogaster*	*Hygrotympanogaster*		*bryosa*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
577	*Tympanogaster*	*Hygrotympanogaster*		*buffalo*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
578	*Tympanogaster*	*Hygrotympanogaster*		*canobolas*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
579	*Tympanogaster*	*Hygrotympanogaster*		*cascadensis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
580	*Tympanogaster*	*Hygrotympanogaster*		*clandestina*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
581	*Tympanogaster*	*Hygrotympanogaster*		*clypeata*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
582	*Tympanogaster*	*Hygrotympanogaster*		*coopracambra*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
583	*Tympanogaster*	*Hygrotympanogaster*		*cornutus*		Janssens, 1967		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
584	*Tympanogaster*	*Hygrotympanogaster*		*cudgee*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
585	*Tympanogaster*	*Hygrotympanogaster*		*decepta*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
586	*Tympanogaster*	*Hygrotympanogaster*		*duobifida*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
587	*Tympanogaster*	*Hygrotympanogaster*		*grampians*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
588	*Tympanogaster*	*Hygrotympanogaster*		*gushi*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
589	*Tympanogaster*	*Hygrotympanogaster*		*kuranda*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
590	*Tympanogaster*	*Hygrotympanogaster*		*macrognatha*		Lea, 1926		*Ochthebius*	*Tympanogaster*	*Hygrotympanogaster*					X				
591	*Tympanogaster*	*Hygrotympanogaster*		***maureenae***		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
592	*Tympanogaster*	*Hygrotympanogaster*		*megamorpha*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
593	*Tympanogaster*	*Hygrotympanogaster*		*merrijig*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
594	*Tympanogaster*	*Hygrotympanogaster*		*nargun*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
595	*Tympanogaster*	*Hygrotympanogaster*		*newtoni*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
596	*Tympanogaster*	*Hygrotympanogaster*		*novicia*		Blackburn, 1896		*Ochthebius*	*Tympanogaster*	*Hygrotympanogaster*					X				
597	*Tympanogaster*	*Hygrotympanogaster*		*porchi*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
598	*Tympanogaster*	*Hygrotympanogaster*		*punctata*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
599	*Tympanogaster*	*Hygrotympanogaster*		*schizolabra*		Deane, 1933		*Ochthebius*	*Tympanogaster*	*Hygrotympanogaster*		1			X				
600	*Tympanogaster*	*Hygrotympanogaster*		*spicerensis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
601	*Tympanogaster*	*Hygrotympanogaster*		*storeyi*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
602	*Tympanogaster*	*Hygrotympanogaster*		*subcostata*		Deane, 1933		*Ochthebius*	*Tympanogaster*	*Hygrotympanogaster*					X				
603	*Tympanogaster*	*Hygrotympanogaster*		*tabula*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
604	*Tympanogaster*	*Hygrotympanogaster*		*tallawarra*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
605	*Tympanogaster*	*Hygrotympanogaster*		*trilineata*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
606	*Tympanogaster*	*Hygrotympanogaster*		*truncata*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
607	*Tympanogaster*	*Hygrotympanogaster*		*wahroonga*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Hygrotympanogaster*					X				
608	*Tympanogaster*	*Plesiotympanogaster*		*costata*		Deane, 1933		*Ochthebius*	*Tympanogaster*	*Plesiotympanogaster*					X				
609	*Tympanogaster*	*Plesiotympanogaster*		*thayerae*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Plesiotympanogaster*					X				
610	*Tympanogaster*	*Topotympanogaster*		*crista*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Topotympanogaster*					X				
611	*Tympanogaster*	*Topotympanogaster*		*dorsa*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Topotympanogaster*					X				
612	*Tympanogaster*	*Topotympanogaster*		*finniganensis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Topotympanogaster*					X				
613	*Tympanogaster*	*Topotympanogaster*		*intricata*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Topotympanogaster*					X				
614	*Tympanogaster*	*Topotympanogaster*		*juga*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Topotympanogaster*					X				
615	*Tympanogaster*	*Topotympanogaster*		*monteithi*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Topotympanogaster*					X				

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Table S1: (Continued)

	New classification								current classification										
No	**genus**	**subgenus**	**species group**	**species**	**subspecies**	**author**	**taxonomic act**	**original genus**	**genus**	**subgenus**	**obs.**	**phyl.**	**PAL**	**AFR**	**AUR**	**NAR**	**NTR**	**ORR**	**ANR**
616	*Tympanogaster*	*Topotympanogaster*		*parallela*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Topotympanogaster*					X				
617	*Tympanogaster*	*Topotympanogaster*		*summa*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Topotympanogaster*					X				
618	*Tympanogaster*	*Tympanogaster*		*aldinga*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
619	*Tympanogaster*	*Tympanogaster*		*amaroo*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
620	*Tympanogaster*	*Tympanogaster*		*ambigua*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
621	*Tympanogaster*	*Tympanogaster*		*bluensis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
622	*Tympanogaster*	*Tympanogaster*		*cardwellensis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
623	*Tympanogaster*	*Tympanogaster*		*cooloogatta*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
624	*Tympanogaster*	*Tympanogaster*		*cunninghamensis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
625	*Tympanogaster*	*Tympanogaster*		*darlingtoni*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
626	*Tympanogaster*	*Tympanogaster*		*deanei*		Perkins, 1979		*Ochthebius*	*Tympanogaster*	*Tympanogaster*		1			X				
627	*Tympanogaster*	*Tympanogaster*		*dingabledinga*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
628	*Tympanogaster*	*Tympanogaster*		*dorrigoensis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
629	*Tympanogaster*	*Tympanogaster*		*eungella*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
630	*Tympanogaster*	*Tympanogaster*		*foveova*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
631	*Tympanogaster*	*Tympanogaster*		*hypipamee*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
632	*Tympanogaster*	*Tympanogaster*		*illawarra*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
633	*Tympanogaster*	*Tympanogaster*		*jaechi*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
634	*Tympanogaster*	*Tympanogaster*		*lamingtonensis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
635	*Tympanogaster*	*Tympanogaster*		*magarra*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
636	*Tympanogaster*	*Tympanogaster*		*millaamillaa*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
637	*Tympanogaster*	*Tympanogaster*		*modulatrix*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*		1			X				
638	*Tympanogaster*	*Tympanogaster*		*moondarra*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
639	*Tympanogaster*	*Tympanogaster*		*mysteriosa*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
640	*Tympanogaster*	*Tympanogaster*		*obcordata*		Deane, 1931		*Ochthebius*	*Tympanogaster*	*Tympanogaster*					X				
641	*Tympanogaster*	*Tympanogaster*		*ovipennis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
642	*Tympanogaster*	*Tympanogaster*		*pagetae*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
643	*Tympanogaster*	*Tympanogaster*		*perpendicula*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
644	*Tympanogaster*	*Tympanogaster*		*plana*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
645	*Tympanogaster*	*Tympanogaster*		*precariosa*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
646	*Tympanogaster*	*Tympanogaster*		*protecta*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
647	*Tympanogaster*	*Tympanogaster*		*ravenshoensis*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
648	*Tympanogaster*	*Tympanogaster*		*robinae*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
649	*Tympanogaster*	*Tympanogaster*		*serrata*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
650	*Tympanogaster*	*Tympanogaster*		*tenax*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
651	*Tympanogaster*	*Tympanogaster*		*tora*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
652	*Tympanogaster*	*Tympanogaster*		*volata*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
653	*Tympanogaster*	*Tympanogaster*		*wattsi*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
654	*Tympanogaster*	*Tympanogaster*		*weiri*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				
655	*Tympanogaster*	*Tympanogaster*		*wooloomgabba*		Perkins, 2006		*Tympanogaster*	*Tympanogaster*	*Tympanogaster*					X				

Table S2: Current classification of Ochthebiini, with synonymies and type species (following Jäch & Skale, 2015 and Jäch *et al*., 2016). In bold, taxa included in the phylogeny.

genus	subgenus	synonyms	type species
***Aulacochthebius* Kuwert, 1887: 376**			***Ochthebius exaratus* Mulsant, 1844**
		Chirochthebius Kuwert, 1887: 383	***Ochthebius narentinus* Reitter, 1885**
		Eochirochthebius Ieniştea 1988: 220	***Ochthebius narentinus* Reitter, 1885**
***Gymnanthelius* Perkins, 1997: 139**			*Ochthebius hieroglyphicus* Deane, 1931
Gymnochthebius Orchymont, 1943: 38			*Ochthebius nittidus* LeConte 1850
***Hughleechia* Perkins, 1981: 300**			***Hughleechia giulianii Perkins, 1981***
***Meropathus* Enderlein, 1901: 121**			*Meropathus chuni* Enderlein, 1901
***Micragasma* Sahlberg, 1900: 199**			***Micragasma paradoxum*** Sahlberg, 1900
***Ochthebius* Leach, 1815: 95**	***Asiobates* Thomson, 1859: 15**		***Ochthebius rufimarginatus* Stephens, 1829 (=*bicolon* Germar, 1824)**
		Trymochthebius Kuwert, 1887: 380	***Ochthebius bicolon* German, 1824**
		Homalochthebius Kuwert, 1887: 383	***Helophorus minimus* Fabricius, 1792**
		Lunzochthebius Ieniştea, 1988: 220	*Ochthebius haberfelneri* Reitter, 1890
		Mimasiobates Ieniştea, 1988: 220	***Ochthebius montanus* Frivaldszky, 1881**
	***Calobius* Wollaston, 1854: 92[1]**		***Calobius heeri* Wollaston, 1854**
		Calochthebius Kuwert, 1887: 372	***Ochthebius quadricollis* Mulsant, 1844**
	***Enicocerus* Stephens, 1829: 196**		***Enicocerus viridiaeneus* Stephens, 1829 (=*exsculptus* Germar, 1824)**
		Henicocerus Agassiz, 1846: 178	***Enicocerus viridiaeneus* Stephens, 1829 (=*exsculptus* Germar, 1824)**
		Cyrtochthebius Kuwert, 1887: 371	***Ochthebius exsculptus* Germar, 1824**
		Sphaerochthebius Kuwert, 1887: 371	***Ochthebius gibbosus* Germar, 1824**
		Eosphaerochthebius Ieniştea, 1988: 218	***Ochthebius gibbosus* Germar, 1824**
	***Ochthebius* Leach, 1815: 95[2]**		***Helophorus marinus* Paykull, 1798**
		Hymenodes Mulsant, 1844: 68	***Ochthebius punctatus* Stephens, 1829**
		Cobalius Rey, 1886: 24[3]	***Ochthebius lejolisii* Mulsant & Rey, 1861**
		Bothochius Rey, 1886: 53	***Ochthebius nobilis*, Villa & Villa, 1835**
		Ochthobius Rey, 1886: 14	***Helophorus marinus* Paykull, 1798**
		Doryochthebius Kuwert, 1887: 373	***Ochthebius notabilis* Rosenhauer, 1856**
		Prionochthebius Kuwert, 1887: 373	***Ochthebius lejolisii* Mulsant & Rey, 1861**
		Cheilochthebius Kuwert, 1887: 374	***Ochthebius metallescens* Rosenhauer, 1856**
		Camptochthebius Kuwert, 1887: 377	***Ochthebius bifoveolatus* Waltl, 1835**
		Odontochthebius Kuwert, 1887: 377	***Ochthebius bifoveolatus* Waltl, 1835**
		Colpochthebius Kuwert, 1887: 379	***Ochthebius punctatus* Stephens, 1829**
		Eccoptochthebius Kuwert, 1887: 379	***Ochthebius difficilis* Mulsant, 1844**
		Acanthochthebius Kuwert, 1887: 383	***Ochthebius serratus* Rosenhauer, 1856**
		Pseudhydraena Acloque, 1896: 96	***Helophorus marinus* Paykull, 1798**
		Liochthebius Sahlberg, 1900: 198	*Ochthebius eburneus* Sahlberg, 1900
		Neochthebius Orchymont, 1932: 43	***Hydraena vandykei* Knisch, 1924**
		Notochthebius Orchymont, 1933: 408	***Hydraena capicola* Péringuey, 1892**

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Table S2: (Continued)

genus	subgenus	synonyms	type species
		Nyxochthebius Orchymont, 1933: 408	*Ochthebius rubripes* Boheman, 1860
		Neohymenodes Ieniştea, 1988: 218	***Ochthebius pedicularius* Kuwert, 1887**
		Parahymenodes Ieniştea, 1988: 218	***Ochthebius metallescens* Rosenhauer, 1856**
		Eocolpochthebius Ieniştea, 1988: 219	*Ochthebius punctatus* Stephens, 1829
		Metahymenodes Ieniştea, 1988: 219	*Ochthebius semisericeus* Sainte-Claire Deville, 1914
		Chaetochthebius Ieniştea, 1988: 220	*Ochthebius quadrifoveolatus* Wollaston, 1854
		Balfourochthebius Ieniştea, 1988: 220	*Ochthebius lindbergi* Balfour-Browne, 1976 (=*balfourbrownei* Jäch, 1989)
Protochthebius Perkins, 1997: 154			*Protochthebius satoi* Perkins, 1997
Prototympanogaster Perkins, 2018			*Prototympanogaster lordhowensis* Perkins, 2018
Tympallopatrum Perkins, 1977: 147			*Tympallopatrum longitudum* Perkins, 1997
***Tympanogaster* Janssens, 1967: 8**	***Hygrotympanogaster* Perkins, 2006: 11**		*Tympanogaster maureenae* Perkins, 2006
	Plesiotympanogaster Perkins, 2006: 12		*Tympanogaster thayerae* Perkins, 2006
	Topotympanogaster Perkins, 2006: 11		*Tympanogaster crista* Perkins, 2006
	***Tympanogaster* Janssens, 1967: 8**		***Ochthebius longipes* Deane, 1931 (=*deanei* Perkins, 1979)**

1. Considered as the "*Calobius* lineage" of *Ochthebius* s.s. by Sabatelli *et al*. (2016).
2. The subgenus *Angiochthebius* was described by Jäch & Ribera (2018).
3. Considered as a subgenus of *Ochthebius* by Sabatelli *et al*. (2016).

Table S3: List of material used in the molecular phylogeny, including voucher numbers, accesion numbers of the sequences and locality data. In bold, newly obtained sequences.

id	family	subfamily	genus	subgenus	sp. group	species	subspecies	voucher	locality	leg	COI-5	COI-3	16S	18S	28S
1	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Holcohydraena*		*arenicola*		MNCN-AI504	California (USA); Trinity Co., Forest Glen, Rattlesnake Creek 22.6.2000	I. Ribera & A. Cieslak	**LT991251**	HM588381	HM588525	HM588584	HM588441
2	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Holcohydraena*		*rugosa*		MNCN-AI392	Spain; Palencia, Castil de Vela 5.2005	L.F. Valladares	HE970908	HM588366	HM588510	HM588575	HM588432
3	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Hydraena*		*altamirensis*		MNCN-AI425	Spain; Ciudad Real, Navas de Estena, Río Estena 18.8.2005	A. Castro	HF947944	HM588371	HM588515	HM588578	HM588435
4	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Hydraena*		*croatica*		IBE-RA52	Corfu (Greece); South Corfu, 2 km S Lefkimi, stream 20.7.2009	M.A. Jäch	**LT991252**	HE970794	HE971027	HE970936	HE970982
5	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Hydraena*		*dochula*		MNCN-AI518	Bhutan; Timphu, 12 km east Timphu Town, stream 24.11.2005	M.A. Jäch	**LT991253**	FM946170	FM946171	FM946174	FM946175
6	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Hydraena*		*hayashii*		MNCN-AI691	Japan; Honshu, Shimane Pref., Mitarai-taki, Jinji river 19.2.2006	M. Hayashi	**LT991254**	HM588389	HM588533	HM588590	HM588447
7	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Hydraena*		*pygmaea*		MNCN-AI346	Austria; Niederösterreich, Schwarzenbach, Sankt Veit an der Gölsen 6.8.2005	I. Ribera & A. Cieslak	HE970901	HM588353	HM588497	HM588572	HM588429
8	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Hydraena*		*riberai*		MNCN-AI568	Morocco; Assif-Oumarhouz, Oued Massa 25.4.2000	I. Ribera	**LT991255**	HM588388	HM588532	HM588589	HM588446
9	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Hydraenopsis*		*palawanensis*		MNCN-AH133	Philippines; North Palawan, Taytay, Manguao Stream 2007	H. Freitag	**LT991256**	HM588309	HM588454	HM588546	HM588403
10	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Monomadraena*		*impressicollis*		MNCN-AI541	Madagascar; Montagne d'Ambre, Petit Cascade 12.2004	M. Balke	**LT991257**	HE970850	HE971081	HE970961	HE970998
11	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Phothydraena*		*atrata*		MNCN-AI314	Spain; Albacete, Robledo, Laguna del Arquillo 2.6.2002	I. Ribera & A. Cieslak	HE970895	HM588344	HM588488	HM588568	HM588425
12	*Hydraenidae*	*Hydraeninae*	*Hydraena*	*Phothydraena*		*putearius*		IBE-RA95	Oman; Al-Akhdar, Ghul 5.4.2010	I. Ribera, C. Hernando & A. Cieslak	**LT991258**	**LT991448**	HE971029	HE610241	HE610244
13	*Hydraenidae*	*Limnebiinae*	*Laeliaena*			*sahlbergi*		MNCN-HI19	India; Uttarakhand, 15 km north Bageshwar, River Falda 14.11.2006	M.A. Jäch	LN995192	LN995232	LN995260	LN995286	**LT991039**
14	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Bilimneus*		*acupunctus*		MNCN-AI582	Australia; South Australia, Cudlee Creek, River Torrens 14.1.2006	C.H.S. Watts	LN995196	LN995238	LN995266	LN995292	LN995353
15	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Bilimneus*		*dioscoridus*		IBE-RA728	Socotra (Yemen); Wadi Ayhaft 8.11.2010	J. Hájek	LN995198	LN995239	**LT990690**	LN995310	LN995355
16	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Bilimneus*		*extraneus*		IBE-RA89	Spain; Huelva, Encinasola, Río Barranco Frío 26.7.1998	I. Ribera	LN995199	HF931321	HF931544	LN995312	LN995357
17	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Bilimneus*		*pollex*		IBE-RA1019	Mauritius; Le Pouce, S Port Louis 29.11.2012	M.A. Jäch	LN995201	**LT991449**	LN995268	LN995294	LN995364
18	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Bilimneus*		*wewalkai*		IBE-RA108	Oman; Al-Akhdar, wadi Bani Awf 6.4.2010	I. Ribera, C. Hernando & A. Cieslak	LN995202	LN995241	LN995269	LN995295	LN995365
19	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Limnebius*		*arenicolus*		MNCN-AI466	California (USA); Trinity Co., Forest Glen, Rattlesnake Creek 22.6.2000	I. Ribera & A. Cieslak	LN995229	HF931204	HF931429	LN995344	LN995407
20	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Limnebius*		*cordobanus*		IBE-PA275	Spain; Teruel, Beceite, river Matarranya, El Parrisal 3.6.2006	I. Ribera	**LT991259**	HF931292	HF931527	LN995319	LN995368
21	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Limnebius*		*crinifer*		IBE-AR55	Sweden; Öland, Möckelmossen, ponds 22.5.2011	I. Ribera	LN995221	LN995255	LN995281	LN995305	LN995400
22	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Limnebius*		*doderoi*		MNCN-AI1174	Corsica (France); Cap Corse, Bettolacce 21.9.1999	I. Ribera & A. Cieslak	LN995222	HF931170	HF931389	LN995341	LN995401
23	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Limnebius*		*hieronymi*		IBE-AR22	Italy; Abruzzo National Park, Pescasseroli, Fiume Sangro 26.7.2010	I. Ribera & A. Cieslak	LN995212	LN995249	LN995276	LN995301	LN995381
24	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Limnebius*		*levantinus*		IBE-RA731	Iran; Fars, Arzhan oak nature reserve 29.4.2010	A. Skale	LN995206	**LT991450**	LN995273	LN995298	LN995376
25	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Limnebius*		*millani*		MNCN-AI920	Spain; Albacete, Paterna del Madera, Arroyo de la Fuenfría 30.7.1998	I. Ribera	**LT991260**	HE610235	HF931476	**LT990834**	LN995389
26	*Hydraenidae*	*Limnebiinae*	*Limnebius*	*Limnebius*		*zaerensis*		MNCN-AC14	Morocco; Rommani, tributary Oued Korifla 10.4.2007	I. Ribera, P. Aguilera & C. Hernando	**LT991261**	EU365868	EU365865	HE970937	HE970984
27	*Hydraenidae*	*Ochthebiinae*	*Meropathus*			*zelandicus*		MNCN-AI715	New Zealand; South Island, Papatowai, beach 7.12.2005	M. Thayer, A. Newton & J. Nunn	**LT991262**	**LT991451**	**LT990691**	**LT990835**	**LT991040**
28	*Hydraenidae*	*Ochthebiinae*	*Tympanogaster*	*Hygrotympanogaster*		*schizolabra*		IBE-AF167	Australia; Victoria, Beech Forest below Hopetoun Falls 21.7.2009	I. Ribera & A. Cieslak	**LT991263**	**LT991452**	**LT990692**	**LT990836**	**LT991041**
29	*Hydraenidae*	*Ochthebiinae*	*Tympanogaster*	*Tympanogaster*		*deanei*		MNCN-AI372	Australia; Queensland, 40 km SSE Brisbane, Cedar Creek Road, above Cedar Creek Falls 22.8.2004	M.A. Jäch	**LT991264**	**LT991453**	**LT990693**	**LT990837**	**LT991042**
30	*Hydraenidae*	*Ochthebiinae*	*Tympanogaster*	*Tympanogaster*		*modulatrix*		IBE-AN183	Australia; Queensland, Lamington NP, Morans falls 26.4.2014	J. Maté	**LT991265**	**LT991454**	**LT990694**	**LT990838**	**LT991043**
31	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Angiochthebius*		*plesiotypus*		MNCN-AI502	Chile; IX Región, Termas de Río Blanco 8.2.2001	M. Guerrero	**LT991266**	**LT991455**	**LT990695**	**LT990839**	**LT991044**
32	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Angiochthebius*		*plesiotypus*		MNCN-AI562	Chile; Región Metropolitana, Alto Cantillana 8.1.2001	M. Guerrero	**LT991267**	**LT991456**	**LT990696**	**LT990840**	**LT991045**
33	*Hydraenidae*	*Ochthebius*	*Ochthebius*	*Asiobates*	*bicolon*	*arator*		IBE-AN185	Turkey; Uşak, Çatalbayır village 19.6.2012	N. Ertorun	**LT991268**	**LT991457**		**LT990841**	**LT991046**

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Table S3: (continued)

id	family	subfamily	genus	subgenus	sp. group	species	subspecies	voucher	locality	leg	COI-5	COI-3	16S	18S	28S
34	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*auriculatus*		MNCN-AH154	England (UK); S Devon, Dawlish Warren Saltmarsh 29.9.2007	D.T. Bilton	**LT991269**	HF931134	HF931352	**LT990842**	**LT991047**
35	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*bellieri*		IBE-AN290	Spain; Albacete, Paterna del Madera, Arroyo de la Fuenfría 30.7.1998	I. Ribera	**LT991270**	**LT991458**	**LT990697**	**LT990843**	**LT991048**
36	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*bicolon*		IBE-RA1171	England (UK); N Devon, 1 km S Hartland Quay, stream 28.7.2013	D.T. Bilton	**LT991271**	**LT991459**	**LT990698**	**LT990844**	**LT991049**
37	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*bonnairei*		MNCN-AI1272	Spain; Jaén, Santiago de la Espada, Río Zumeta 16.6.2006	A. Millán & collaborators	**LT991272**	HF931177	HF931398	**LT990845**	**LT991050**
38	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*cantabricus* cf.		MNCN-AI1027	Turkey; Kastamonu, Aşağı Kayı 27.4.2006	A. Castro	**LT991273**	HF931145	HF931364	**LT990846**	**LT991051**
39	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*corsicus*		IBE-AN159	Corsica (France); Col de Bavella 14.5.2014	R. Vila	**LT991274**	**LT991460**	**LT990699**	**LT990847**	**LT991052**
40	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*crenulatus*		MNCN-AH159	Sicily (Italy); Parco dei Nebrodi, trail Lago Urio - Portella dell'Obolo 13.6.2007	P. Abellán & F. Picazo	**LT991275**	HF931136	HF931353	**LT990848**	**LT991053**
41	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*dilatatus*		MNCN-AI792	Turkey; Bartın, between Topallar & Çakraz, calcareous stream 25.4.2006	I. Ribera	**LT991276**	HF931227	HF931456	**LT990849**	**LT991054**
42	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*ferroi*		IBE-PB28	Spain; Huesca, Sta. Cruz de Serós, Barranco Carbonera 3.8.2013	I. Esteban	HG915303	**LT991461**	**LT990700**	**LT990850**	**LT991055**
43	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*figueroi*		IBE-AN23	Spain; Cantabria, Río Soto 15.7.2014	D.T. Bilton	**LT991277**	**LT991462**	**LT990701**	**LT990851**	**LT991056**
44	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*gagliardii*		IBE-RA121	Italy; Toscana, Lorenzana, Gello Mattaccino, pond 26.5.2008	I. Ribera	**LT991278**	HF931301	**LT990702**	**LT990852**	**LT991057**
45	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*heydeni*		MNCN-AI390	Spain; Ourense, Sierra de Queixa, river San Lázaro 9.7.2005	I. Ribera & A. Cieslak	**LT991279**	HF931194	HF931419	**LT990853**	**LT991058**
46	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*immaculatus*		IBE-AN434	Mallorca (Spain); Mallorca, Salines de Llevant 28.3.2016	I. Ribera & A. Cieslak	**LT991280**	**LT991463**	**LT990703**	**LT990854**	**LT991059**
47	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*irenae*		MNCN-AI986	Spain; Navarra, Tudela, ditch near Balsa de Purguer 20.7.2004	I. Ribera & A. Cieslak	HG915302	HF931261	HF931497	**LT990855**	**LT991060**
48	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*jaimei*		IBE-RA1081	Spain; Alicante, Albatera 12.5.2013	I. Esteban	**LT991281**	HG915307	**LT990704**	**LT990856**	**LT991061**
49	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*lederi*		IBE-AN63	Azerbaijan; Alishanli, 7 km NE Masally 8.5.2014	I. Ribera & A. Rudoy	**LT991282**	**LT991464**	**LT990705**	**LT990857**	**LT991062**
50	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*lenkoranus*		IBE-AN75	Azerbaijan; Gosmalijion, stream 7.5.2014	I. Ribera & A. Rudoy	**LT991283**	**LT991465**	**LT990706**	**LT990858**	**LT991063**
51	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*montanus*		IBE-AN207	Greece; Evvoia, Seta, mountain stream 8.4.2013	I. Ribera & A. Cieslak	**LT991284**	**LT991466**	**LT990707**	**LT990859**	**LT991064**
52	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*opacus*		MNCN-AI389	Italy; Liguria, 2 km E Cosio di Arroscia 31.7.2005	I. Ribera & A. Cieslak	**LT991285**	HF931193	HF931418	**LT990860**	**LT991065**
53	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*peregrinus*		IBE-AN206	Greece; Sterea Ellada, Fokida, Itea, stream E of Kirra 7.4.2013	I. Ribera & A. Cieslak	**LT991286**	**LT991467**	**LT990708**	**LT990861**	**LT991066**
54	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*sp.*		IBE-AN74	Azerbaijan; Elabad, road to Garaybayli, stream 4.5.2014	I. Ribera & A. Rudoy	**LT991287**	**LT991468**	**LT990709**	**LT990862**	**LT991067**
55	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*striatus*		MNCN-AI787	Turkey; Bartın, between Amasra & Inpiri, spring & stream 25.4.2006	I. Ribera	**LT991288**	HF931226	HF931455	**LT990863**	**LT991068**
56	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*bicolon*	*stygialis*		IBE-AN160	Turkey; Antalya, 1 km N Hacıyusuflar 24.6.2014	D.T. Bilton	**LT991289**	**LT991469**	**LT990710**	**LT990864**	**LT991069**
57	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*minimus*	*aeneus*		MNCN-AI914	Morocco; Ou-Maghous, Amaghouz 24.4.2000	I. Ribera, P. Aguilera, C. Hernando & A. Millán	**LT991290**	HF931240	HF931472	**LT990865**	**LT991070**
58	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*minimus*	*alpinus*		IBE-RA1114	Belarus; Berezinsky Biosphere Reserve 24.6.2013	I. Ribera	**LT991291**	**LT991470**	**LT990711**	**LT990866**	**LT991071**
59	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*minimus*	*andreiini*		IBE-AN20	Ethiopia; Amhara, 30 km NE Gashena 13.6.2014	R. Vila & G. Talavera	**LT991292**	**LT991471**	**LT990712**	**LT990867**	**LT991072**
60	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*minimus*	*andreinii* cf.		IBE-AN104	Ethiopia; Oromia, Bale Mountains, Goba Forest, Togona River 22.2.2014	M.A. Jäch	**LT991293**	**LT991472**	**LT990713**	**LT990868**	**LT991073**
61	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*minimus*	*andronius*		MNCN-AI498	South Africa; West Cape, Prince Albert Road, pond 23.3.2001	I. Ribera & A. Cieslak	**LT991294**	**LT991473**	**LT990714**	**LT990869**	**LT991074**
62	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*minimus*	*discretus*		MNCN-AI503	California (USA); Trinity Co., Forest Glen, Rattlesnake Creek 22.6.2000	I. Ribera & A. Cieslak	**LT991295**	**LT991474**	**LT990715**	**LT990870**	**LT991075**
63	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*minimus*	*flavipes*		IBE-RA437	Slovakia; Hámske tŕstie, near Číčov, ditch 7.6.2009	I. Ribera	**LT991296**	HF931315	**LT990716**	**LT990871**	**LT991076**
64	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*minimus*	*hokkaidensis*		IBE-AF213	Japan; Hokkaido, Togeshita, Rumoi-shi 18.7.2007	H. Yoshitomi		HF931125	HF931344	**LT990872**	**LT991077**
65	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*minimus*	*minimus*		MNCN-AI447	Slovakia; Banská Bystrica Region, Cerovo 2005	via R.G. Beutel	HE970917	HE970842	HE971074	HE970955	HE970995
66	*Hydraenidae*	*Ochthebiinae*	*Ochthebius*	*Asiobates*	*minimus*	*remotus*		MNCN-AI1030	Turkey; Karabük, stream in Çayörengüney 28.4.2006	A. Castro	**LT991297**	HF931148	HF931367	**LT990873**	**LT991078**

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Table S3: (continued)

id	family	subfamily	genus	subgenus	sp. group	species	subspecies	voucher	locality	leg	COI-5	COI-3	16S	18S	28S
67	Hydraenidae	Ochthebiinae	*Ochthebius*	*Asiobates*	*minimus*	*rugulosus*		MNCN-AI637	Gran Canaria (Spain); Moya, Barranco de Azuaje 15.4.2001	I. Ribera & A. Cieslak	**LT991298**	HF931219	HF931447	**LT990874**	**LT991079**
68	Hydraenidae	Ochthebiinae	*Ochthebius*	*Asiobates*	*minimus*	*sanabrensis*		MNCN-AH75	Spain; Zamora, Parque Natural Lago Sanabria, Laguna de la Yegua 4.7.2007	L.F. Valladares	**LT991299**	EU660055	HF931357		**LT991080**
69	Hydraenidae	Ochthebiinae	*Ochthebius*	*Asiobates*	*minimus*	sp.		IBE-AN292	Alberta (Canada); 2 km W Lundbreck 27.6.2000	I. Ribera & A. Cieslak	**LT991300**	**LT991475**	**LT990717**	**LT990875**	**LT991081**
70	Hydraenidae	Ochthebiinae	*Ochthebius*	*Asiobates*	*minimus*	sp.		IBE-AN97	Ethiopia; Oromia, Jemjem Forest, small stream 25.2.2014	M.A. Jäch	**LT991301**	**LT991476**	**LT990718**	**LT990876**	**LT991082**
71	Hydraenidae	Ochthebiinae	*Ochthebius*	*Asiobates*	*minimus*	sp.		IBE-AN93	Ethiopia; Amhara, Debark, Simien Mountains 18.6.2014	R. Vila & G. Talavera	**LT991302**	**LT991477**	**LT990719**	**LT990877**	**LT991083**
72	Hydraenidae	Ochthebiinae	*Ochthebius*	*Asiobates*	*puncticollis*	*puncticollis*		MNCN-AI1274	California (USA); Santa Barbara Co., Sedgewick Reserve 6.7.2006	A.E.Z. Short	**LT991303**	**LT991478**	**LT990720**		**LT991084**
73	Hydraenidae	Ochthebiinae	*Ochthebius*	*Aulacochthebius*		*exaratus*		MNCN-AI453	Tunisia; road Jendouba-Makthar, 1 km NW crossroad to Mt. Kebbouch 24.10.2001	I. Ribera & A. Cieslak	**LT991304**	HF931202	HF931427	**LT990878**	**LT991085**
74	Hydraenidae	Ochthebiinae	*Ochthebius*	*Aulacochthebius*		*libertarius*		MNCN-AI421	Morocco; Ou-Maghous, Amaghouz 24.4.2000	I. Ribera, P. Aguilera, C. Hernando & A. Millán	**LT991305**	HF931196	HF931421	**LT990879**	**LT991086**
75	Hydraenidae	Ochthebiinae	*Ochthebius*	*Aulacochthebius*		*narentinus*		IBE-AF161	Slovakia; Studienka, Rudava river 5.6.2009	I. Ribera	**LT991306**	HF931120	HF931338	**LT990880**	**LT991087**
76	Hydraenidae	Ochthebiinae	*Ochthebius*	*Aulacochthebius*		*perlaevis*		MNCN-AI548	Madagascar; Ankarana, 1er canyon, river 12.2004	M. Balke	**LT991307**	HF931211	HF931437	**LT990881**	**LT991088**
77	Hydraenidae	Ochthebiinae	*Ochthebius*	*Aulacochthebius*		sp.		IBE-AN390	South Africa; West Cape, Baardskeerdersbos, Boesmans River 10.5.2010	J. Den Heijer & A. Hidalgo-Galiana	**LT991308**	**LT991479**		**LT990882**	**LT991089**
78	Hydraenidae	Ochthebiinae	*Ochthebius*	*Aulacochthebius*		sp.		MNCN-AI499	South Africa; West Cape, 3 km SE Franschhoek 25.3.2001	I. Ribera & A. Cieslak	**LT991309**	**LT991480**	**LT990721**	**LT990883**	**LT991090**
79	Hydraenidae	Ochthebiinae	*Ochthebius*	*Aulacochthebius*		sp.		MNCN-AI501	South Africa; Eastern Cape, Kareedouwberg, river in Skoop Drif 23.3.2001	I. Ribera & A. Cieslak	**LT991310**	HF931207	HF931432	**LT990884**	**LT991091**
80	Hydraenidae	Ochthebiinae	*Ochthebius*	*Aulacochthebius*		sp.		MNCN-AI519	Bhutan; Sarpang, 11 km NW Sarpang, Bhur Khola river 27.11.2005	M.A. Jäch	**LT991311**	**LT991481**	**LT990722**	**LT990885**	**LT991092**
81	Hydraenidae	Ochthebiinae	*Ochthebius*	*Aulacochthebius*		sp.		MNCN-AI520	Bhutan; Punakha, 16 km NW Punakha, Mo Chhu river 28.11.2005	M.A. Jäch	**LT991312**	**LT991482**	**LT990723**	**LT990886**	**LT991093**
82	Hydraenidae	Ochthebiinae	*Ochthebius*	*Aulacochthebius*		sp.		IBE-RA1155	Tanzania; Mwanza Region, near Kashishi, ponds 24.7.2010	R. Sites & A. Mbogho	**LT991313**	**LT991483**	**LT990724**	**LT990887**	**LT991094**
83	Hydraenidae	Ochthebiinae	*Ochthebius*	*Cobalius*		*adriaticus*	*adriaticus*	IBE-AN787	Croatia; Trsteno, rockpools 5.5.2017	A. Faille, J. Fresneda & I. Ribera	**LT991314**	**LT991484**	**LT990725**	**LT990888**	**LT991095**
84	Hydraenidae	Ochthebiinae	*Ochthebius*	*Cobalius*		*celatus*		IBE-AN441	Cyprus; Akamas peninsula, Lara beach 29.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT991315**	**LT991485**	**LT990726**	**LT990889**	**LT991096**
85	Hydraenidae	Ochthebiinae	*Ochthebius*	*Cobalius*		*freyi*		IBE-RA1197	Azores (Portugal); Terceira, São Pedro, rockpools 4.9.2012	I. Ribera	**LT991316**	**LT991486**	**LT990727**	**LT990890**	**LT991097**
86	Hydraenidae	Ochthebiinae	*Ochthebius*	*Cobalius*		*lejolisii*		MNCN-AI513	Spain; Pontevedra, Nigran, Playa Patos 3.10.2005	J. Garrido	**LT991317**	HF931208	HF931433	**LT990891**	**LT991098**
87	Hydraenidae	Ochthebiinae	*Ochthebius*	*Cobalius*		*serratus*		MNCN-AI1194	Morocco; El Ouedaya, Oued Sebt 18.4.2006	A. Millán & collaborators	**LT991318**	HF931171	HF931391	**LT990892**	**LT991099**
88	Hydraenidae	Ochthebiinae	*Ochthebius*	*Cobalius*		*subinteger*		MNCN-AI432	Corsica (France); Cap Corse, Port de Centuri 20.9.2000	I. Ribera & A. Cieslak	**LT991319**	HF931200	HF931425	**LT990893**	**LT991100**
89	Hydraenidae	Ochthebiinae	*Ochthebius*	*Enicocerus*		*aguilerai*		MNCN-AI387	Spain; Ávila, Arenas de San Pedro, río Pelayo 15.5.2005	I. Ribera	HF947939	GU143761	GU143728	GU143770	GU143778
90	Hydraenidae	Ochthebiinae	*Ochthebius*	*Enicocerus*		*colveranus*		MNCN-AI791	Turkey; Bartın, between Topallar & Çakraz, calcareous stream 25.4.2006	I. Ribera	**LT991320**	GU143744	GU143733	GU143768	GU143776
91	Hydraenidae	Ochthebiinae	*Ochthebius*	*Enicocerus*		*exsculptus*		MNCN-AI374	Spain; Barcelona, Guardiola del Berguedà, torrent Gavarrós 25.7.2005	I. Ribera	**LT991321**	GU143763	GU143741	GU143772	GU143780
92	Hydraenidae	Ochthebiinae	*Ochthebius*	*Enicocerus*		*exsculptus* cf.		MNCN-AI925	Spain; Albacete, Sierra de Segura, 10 km N Yeste, Rio Tús 10.4.2003	V. Assing & P. Wunderle	**LT991322**	GU143750	GU143737	**LT990894**	**LT991101**
93	Hydraenidae	Ochthebiinae	*Ochthebius*	*Enicocerus*		*gibbosus*		MNCN-AI365	Italy; Piemonte, Móngia, torrente Móngia 31.7.2005	I. Ribera & A. Cieslak	**LT991323**	GU143755	GU143727	GU143769	GU143777
94	Hydraenidae	Ochthebiinae	*Ochthebius*	*Enicocerus*		*granulatus*		MNCN-AI427	Austria; Niederösterreich, Kleiner Ötscherbach, Langau 25.9.2005	M.A. Jäch	**LT991324**	GU143765	GU143725	GU143774	GU143782
95	Hydraenidae	Ochthebiinae	*Ochthebius*	*Enicocerus*		*halbherri*		MNCN-AH190	Italy; Emilia Romagna, Bosco, Torrente Parma 24.5.2008	I. Ribera	**LT991325**	GU143764	GU143736	GU143773	GU143781
96	Hydraenidae	Ochthebiinae	*Ochthebius*	*Enicocerus*		*legionensis*		MNCN-AI507	Spain; León, Puerto de Panderrueda 12.7.2005	L.F. Valladares	HF947941	GU143762	GU143735	GU143771	GU143779
97	Hydraenidae	Ochthebiinae	*Ochthebius*	*Enicocerus*		*melanescens*		MNCN-AI344	Austria; Niederösterreich, Schwarzenbach, Sankt Veit an der Gölsen 6.8.2005	I. Ribera & A. Cieslak	HE970900	GU143766	GU143743	GU143775	GU143783
98	Hydraenidae	Ochthebiinae	*Ochthebius*	*Enicocerus*		*saboorii*		IBE-RA739	Iran; Mazandaran, near Nowshar, Kheiroud Kenar Forest 2.5.2010	A. Skale	**LT991326**		**LT990728**	**LT990895**	**LT991102**
99	Hydraenidae	Ochthebiinae	*Ochthebius*	*Gymnanthelius*		*opacicollis*		IBE-AF162	Australia; Victoria, Allambee, 19 km N Leongatha 8.7.2009	I. Ribera & A. Cieslak	**LT991327**	**LT991487**	**LT990729**	**LT990896**	**LT991103**

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Table S3: (continued)

id	family	subfamily	genus	subgenus	sp. group	species	subspecies	voucher	locality	leg	COI-5	COI-3	16S	18S	28S
100	Hydraenidae	Ochthebiinae	*Ochthebius*	*Gymnanthelius*		*porchi*		IBE-AF164	Australia; Victoria, Tarra-Bulga NP, River Tarra 9.7.2009	I. Ribera & A. Cieslak	**LT991328**	**LT991488**	**LT990730**	**LT990897**	**LT991104**
101	Hydraenidae	Ochthebiinae	*Ochthebius*	*Gymnochthebius*	*australis*	*australis*		MNCN-AI583	Australia; South Australia, 1 km W Cudlee Creek, River Torrens 14.1.2006	C.H.S. Watts	**LT991329**	**LT991489**	**LT990731**	**LT990898**	**LT991105**
102	Hydraenidae	Ochthebiinae	*Ochthebius*	*Gymnochthebius*	*australis*	*lividus*		IBE-AF163	Australia; Victoria, Tarra-Bulga NP, River Tarra 9.7.2009	I. Ribera & A. Cieslak	**LT991330**	**LT991490**	**LT990732**	**LT990899**	**LT991106**
103	Hydraenidae	Ochthebiinae	*Ochthebius*	*Gymnochthebius*	*australis*	*probus*		MNCN-AI584	Australia; South Australia, 1 km W Cudlee Creek, River Torrens 14.1.2006	C.H.S. Watts	**LT991331**	**LT991491**	**LT990733**	**LT990900**	**LT991107**
104	Hydraenidae	Ochthebiinae	*Ochthebius*	*Gymnochthebius*	*australis*	*setosus*		IBE-AF165	Australia; Victoria, Tarra-Bulga NP, River Tarra 9.7.2009	I. Ribera & A. Cieslak	**LT991332**	**LT991492**	**LT990734**	**LT990901**	**LT991108**
105	Hydraenidae	Ochthebiinae	*Ochthebius*	*Gymnochthebius*	*fossatus*	*fossatus*		IBE-AN498	Dominican Republic; Independencia, La Colonia 15.8.2014	A. Deler-Hernández, M. Fikácek & M. Gimmel	**LT991333**	**LT991493**	**LT990735**	**LT990902**	**LT991109**
106	Hydraenidae	Ochthebiinae	*Ochthebius*	*Gymnochthebius*	*fossatus*	*germaini*		MNCN-AI454	Chile; IX Región, 5 km E Lonquimay, road to Lolén 26.1.1999	I. Ribera & M. Guerrero	**LT991334**	**LT991494**	**LT990736**	**LT990903**	**LT991110**
107	Hydraenidae	Ochthebiinae	*Ochthebius*	*Gymnochthebius*	*fossatus*	*peruvianus*		MNCN-AI689	Peru; San Clemente, km 222 Panamericana Sur 31.8.2005	P. Aguilera	**LT991335**	**LT991495**	**LT990737**	**LT990904**	**LT991111**
108	Hydraenidae	Ochthebiinae	*Ochthebius*	*Gymnochthebius*	*fossatus*	sp.		MNCN-AI569	Chile; Chiloé, 6 km E Huillinco, river in Notuco 3.2.1999	I. Ribera & M. Guerrero	**LT991336**	**LT991496**	**LT990738**	**LT990905**	**LT991112**
109	Hydraenidae	Ochthebiinae	*Ochthebius*	*Hughleechia*		*giuliani*		MNCN-AI716	Australia; West Australia, Peron Point 2.10.2003	C.H.S. & G.A. Watts	**LT991337**	**LT991497**	**LT990739**	**LT990906**	**LT991113**
110	Hydraenidae	Ochthebiinae	*Ochthebius*	*Micragasma*		*paradoxum*		IBE-AF116	Russia; Volgogradskaya Oblast, Lake Elton, residual pools 17.4.2008	A. Prokin	**LT991338**	HF931114	HF931331	**LT990907**	**LT991114**
111	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*andraei*	*patergazellae*		IBE-RA735	United Arab Emirates; Ajman, salt water pools NE Ajman City 25.1.2010	M.A. Jäch	**LT991339**	**LT991498**	**LT990740**	**LT990908**	**LT991115**
112	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*atriceps*	*andalusicus*		IBE-PA296	Spain; Cádiz, Salinas de Hortales 10.11.2003	A. Millán & collaborators	**LT991340**	HF931297	**LT990741**	**LT990909**	**LT991116**
113	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*atriceps*	*anxifer*		MNCN-AI945	Morocco; Immouzzer Ida Ou Tanane, Assif Tanit 21.4.2001	I. Ribera & A. Cieslak	**LT991341**	HF931247	HF931482	**LT990910**	**LT991117**
114	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*atriceps*	*atriceps*		IBE-AN210	Tunisia; road Jendouba-Makthar, 1 km NW crossroad to Mt. Kebbouch 24.10.2001	I. Ribera & A. Cieslak	**LT991342**	**LT991499**	**LT990742**	**LT990911**	**LT991118**
115	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*atriceps*	*burjkhalifa*		IBE-RA737	United Arab Emirates; Ajman, salt water pools NE Ajman City 25.1.2010	M.A. Jäch	**LT991343**	**LT991500**	**LT990743**	**LT990912**	**LT991119**
116	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*atriceps*	*dentifer*		IBE-PA290	Spain; Navarra, Barranco Salado de Mendavia 30.10.2008	A. Millán & collaborators	**LT991344**	**HF931296**	**LT990744**	**LT990913**	**LT991120**
117	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*atriceps*	*depressionis*		IBE-AF171	Russia; Volgogradskaya Oblast, Lake Elton, nr Lantsug River 14.4.2008	A. Prokin	**LT991345**	HF931122	HF931340	**LT990914**	**LT991121**
118	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*atriceps*	*loulae*		IBE-AN476	Djibouti; 50 km W Dikhil, Lac Abbé, thermal springs 2.2.2016	M.A. Jäch	**LT837919**	**LT991501**	**LT990745**	**LT990915**	**LT991122**
119	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*atriceps*	*tacapasensis*	*baeticus*	IBE-AN365	Spain; Málaga, Laguna de Fuente de Piedra 3.5.2011	A. Millán & collaborators	**LT991346**	**LT991502**	**LT990746**	**LT990916**	**LT991123**
120	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*atriceps*	*thermalis*		IBE-AN451	Cyprus; Larnaka, saline coastal pond 25.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT991347**	**LT991503**	**LT990747**		**LT991124**
121	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*atriceps?*	*despoliatus*		IBE-RA736	United Arab Emirates; Ajman, salt water pools NE Ajman City 25.1.2010	M.A. Jäch		**LT991504**		**LT990917**	**LT991125**
122	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*corrugatus*	*corrugatus*		MNCN-AI56	Mallorca (Spain); Mallorca, Salines de Llevant 15.10.2004	I. Ribera & A. Cieslak		HF931213	HF931438	**LT990918**	**LT991126**
123	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*corrugatus*	*gauthieri*		MNCN-AI55	Tunisia; road Kebili-Tozeur, 37 km SE Tozeur, salines 26.10.2001	I. Ribera & A. Cieslak		HF931212		**LT990919**	**LT991127**
124	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*corrugatus*	*perpusillus*		IBE-AN323	Morocco; Ouezzane, Sidi Kacem, Oued Khendek 18.4.2006	A. Millán & collaborators		**LT991505**		**LT990920**	**LT991128**
125	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*elisae*		IBE-RA746	Iran; Khuzestan, Behbahan, Morvarid spring 4.9.2010	E. Irani	**LT991348**	**LT991506**		**LT990921**	**LT991129**
126	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*foveolatus*		MNCN-AI801	Turkey; Kastamonu, Çocukören 26.4.2006	I. Ribera	**LT991349**	HF931228	HF931457	**LT990922**	**LT991130**
127	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*hajeki*		IBE-RA1231	Socotra (Yemen); Dixam plateau, Firmihin, Dracaena woodland 15.6.2012	J. Hájek	**LT991350**	**LT991507**	**LT990748**	**LT990923**	**LT991131**
128	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*harteni*		IBE-RA705	Oman; Al-Akhdar, Bahla, wadi in city 5.4.2010	I. Ribera, C. Hernando & A. Cieslak	**LT991351**	**LT991508**	**LT990749**	**LT990924**	**LT991132**
129	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*magnannulatus*		IBE-AN328	Bulgaria; Kotlari, river Arda 27.5.2015	I. Ribera	**LT991352**	**LT991509**	**LT990750**	**LT990925**	**LT991133**
130	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*marginalis*		IBE-AN522	France; Drôme, river Méouge 2016	A. Faille	**LT991353**	**LT991510**	**LT990751**	**LT990926**	**LT991134**
131	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*mediterraneus*		MNCN-AI422	Morocco; Ou-Maghous, Amaghouz 24.4.2000	I. Ribera, P. Aguilera, C. Hernando & A. Millán	**LT991354**	HF931197	HF931422	**LT990927**	**LT991135**
132	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*merinidicus*		IBE-RA1023	Spain; Albacete, River Mundo, Azud de Liétor 26.7.2012	A. Millán & collaborators	**LT991355**	**LT991511**	**LT990752**	**LT990928**	**LT991136**

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Table S3: (continued)

id	family	subfamily	genus	subgenus	sp. group	species	subspecies	voucher	locality	leg	COI-5	COI-3	16S	18S	28S
133	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*pedicularius*		IBE-AN809	Italy; Udine, Flagogna 9.4.2017	A. Eckelt	**LT991356**	**LT991512**	**LT990753**	**LT990929**	**LT991137**
134	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*satoi*		IBE-AF210	Japan; Hokkaido, Taiki 11.7.2009	H. Yoshitomi	**LT991357**	HF931124	HF931343		**LT991138**
135	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*sidanus*		IBE-AF132	Italy; Emilia Romagna, Bosco, Torrente Parma 24.5.2008	I. Ribera	**LT991358**	HF931115	HF931332	**LT990930**	**LT991139**
136	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*foveolatus*	*virgula*		IBE-AF134	Italy; Emilia Romagna, Vigoleno, Parco dello Stirone, Torrente Stirone 25.5.2008	I. Ribera	**LT991359**	HF931116	HF931333	**LT990931**	**LT991140**
137	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*lobicollis*	*basilicatus*		IBE-AN801	Sardinia (Italy); Cabras, Stagno di Cabras 10.4.2017	I. Ribera & A. Cieslak	**LT991360**	**LT991513**	**LT990754**	**LT990932**	**LT991141**
138	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*lobicollis*	*caesaraugustae*		MNCN-AI1195	Spain; Zaragoza, Medianan de Aragón, saline river 20.7.2006	A. Millán & collaborators	**LT991361**	HF931172	HF931392	**LT990933**	**LT991142**
139	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*lobicollis*	*delgadoi*		IBE-AN364	Spain; Murcia, Rambla del Reventón 9.3.2012	A. Millán & collaborators	**LT991362**	**LT991514**	**LT990755**	**LT990934**	**LT991143**
140	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*lobicollis*	*eyrei*		IBE-AN600	Sicily (Italy); Palermo, Fiume Salso 28.7.2009	C. Gutiérrez-Cánovas	**LT991363**	**LT991515**	**LT990756**	**LT990935**	**LT991144**
141	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*lobicollis*	*lapidicola*		IBE-AN126	Tenerife (Spain); Puerto de la Cruz, Roque Grande, 9.4.2015	R. Lyszkowski		**LT991516**	**LT990757**	**LT990936**	**LT991145**
142	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*lobicollis*	*lobicollis*		IBE-RA242	Menorca (Spain); Cap de Favàritx 26.2.2010	I. Ribera & A. Cieslak	**LT991364**	HF931308	HF931534	**LT990937**	**LT991146**
143	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*lobicollis*	*quadrifossulatus*		MNCN-AI226	Spain; Guadalajara, Alcolea de las Peñas 21.5.2005	A. Millán & collaborators		**LT991517**	**LT990758**	**LT990938**	**LT991147**
144	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*lobicollis*	*tivelunus*		MNCN-AI420	Morocco; Agarzane, tributary of Oued Drâa 17.4.2001	I. Ribera & A. Cieslak	**LT991365**	HF931195	HF931420	**LT990939**	**LT991148**
145	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*arefniae*		IBE-AN72	Azerbaijan; road Budzhakah-Bash-Dashagyi, ponds 5.5.2014	I. Ribera & A. Rudoy	**LT991366**	**LT991518**	**LT990759**	**LT990940**	**LT991149**
146	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*arefniae* cf.		IBE-AN446	Cyprus; Loukrounou, upstream of Evretou reservoir 29.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT991367**	**LT991519**	**LT990760**		**LT991150**
147	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*arizonicus*		IBE-AN177	Arizona (USA); Yavapai Co., Agua Fria River 22.6.2007	W.D. Shepard	**LT991368**	**LT991520**	**LT990761**	**LT990941**	**LT991151**
148	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*auropallens*		IBE-AN217	Tunisia; road Kairouan-Enfida, 13 km N Kairouan, lagoon 27.10.2001	I. Ribera & A. Cieslak		**LT991521**	**LT990762**	**LT990942**	**LT991152**
149	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*aztecus*		IBE-AN222	California (USA); Inyo Co., Shoshone, Amargosa River 8.4.2008	P. Abellán	**LT991369**	**LT991522**	**LT990763**	**LT990943**	**LT991153**
150	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*batesoni*		MNCN-AI690	Peru; San Clemente, km 222 Panamericana Sur 31.8.2005	P. Aguilera	**LT991370**	**LT991523**	**LT990764**	**LT990944**	**LT991154**
151	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*bisinuatus*		IBE-AN153	California (USA); Mendocino Co., circa 5 mi W Davis Creek 9.6.2007	W.D. Shepard	**LT991371**	**LT991524**	**LT990765**	**LT990945**	**LT991155**
152	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*capicola*		IBE-RA854	South Africa; West Cape, West Cape NP, Tsaarsbank, rookpools 2.10.2011	D.T. Bilton	**LT991372**	**LT991525**	**LT990766**	**LT990946**	**LT991156**
153	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*chappuisi* cf.		IBE-AN475	Djibouti; 50 km W Dikhil, Lac Abbé, thermal springs 2.2.2016	M.A. Jäch	**LT837918**	**LT991526**	**LT990767**	**LT990947**	**LT991157**
154	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*costatellus* cf.		IBE-RA1119	Alberta (Canada); Alberta, Derwent, circa Lac Coté 2000	T. Berendonk	**LT991373**	**LT991527**	**LT990768**	**LT990948**	**LT991158**
155	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*evanescens*		IBE-AN66	Azerbaijan; Alishanli, 7 km NE Masally 8.5.2014	I. Ribera & A. Rudoy	**LT991374**	**LT991528**	**LT990769**	**LT990949**	**LT991159**
156	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*gruwelli*		IBE-RA304	California (USA); Riverside Co., Morongo Valley, Whitewater river 12.12.2010	I. Ribera & J. Bergsten	**LT991375**	HF931311	HF931535	**LT990950**	**LT991160**
157	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*interruptus*		IBE-AN219	California (USA); Santa Barbara Co., Coal Oil Point Reserve (Slough road) 26.3.2008	P. Abellán	**LT991376**	**LT991529**	**LT990770**	**LT990951**	**LT991161**
158	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*lineatus*		IBE-AN223	California (USA); Inyo Co., Ballarant, Post Office Spring 10.4.2008	P. Abellán	**LT991377**	**LT991530**			**LT991162**
159	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*lineatus* cplx		IBE-RA1129	Venezuela; Guárico state, Río San Antonio 4.2.2010	A.E.Z. Short, M. García & L. Joly		**LT991531**	**LT990771**	**LT990952**	**LT991163**
160	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*lividipennis*		IBE-AN71	Azerbaijan; road Budzhakah-Bash-Dashagyi, ponds 7.5.2014	I. Ribera & A. Rudoy	**LT991378**	**LT991532**	**LT990772**	**LT990953**	**LT991164**
161	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*marinus*		MNCN-AI615	Spain; Madrid, Aranjuez, salinas 11.2.2006	I. Ribera & A. Cieslak	**LT991379**	HF931215	HF931441	**LT990954**	**LT991165**
162	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*meridionalis*		IBE-RA373	Morocco; Moulay Bousselham, Oued Drader 12.4.2007	I. Ribera, P. Aguilera & C. Hernando	**LT991380**	HF931313	HF931538	**LT990955**	**LT991166**
163	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*mesoamericanus*		IBE-RA58	Costa Rica; Cartago, 7.8 km SE Turrialba, Río Tuis 15.3.2009	M. Brojer		**LT991533**			
164	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*nipponicus*		MNCN-HI27	Japan; Honshu, Ibaraki Pref., Kobodihana, Ohmika-machi, Hitachi-shi 22.7.2006	N. Hikida	**LT991381**	HF931270	LT990773	**LT990956**	**LT991167**
165	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*pedalis*		MNCN-AH98	South Africa; West Cape, Papendorp, brackish pond 23.8.2006	G. Challet	**LT991382**	**LT991534**	**LT990774**	**LT990957**	**LT991168**

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Table S3: (continued)

id	family	subfamily	genus	subgenus	sp. group	species	subspecies	voucher	locality	leg	COI-5	COI-3	16S	18S	28S
166	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*pusillus*		MNCN-AI1028	Turkey; Sinop, road Saraydüzü-Boyabat, stream 5 km S Yeşilyurt 27.4.2006	A. Castro	**LT991383**	HF931146	HF931365	**LT990958**	**LT991169**
167	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*queenslandicus*		IBE-AN18	Australia; Queensland, Brisbane, Shorncliffe 2.5.2014	J. Maté	**LT991384**	**LT991535**	**LT990775**		**LT991170**
168	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*rectus*		MNCN-AI464	California (USA); Mono Co., Long Valley, Owens River road 19.6.2000	I. Ribera & A. Cieslak	**LT991385**	**LT991536**	**LT990776**	**LT990959**	**LT991171**
169	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*rectus* cplx		IBE-AN152	California (USA); Ventura Co., Los Padres National Forest, Upper Sespe Creek 1.7.2006	A.E.Z. Short & M. Caterino	**LT991386**	**LT991537**	**LT990777**	**LT990960**	**LT991172**
170	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*rectus* cplx		IBE-AN291	Alberta (Canada); 2 km W Lundbreck 27.6.2000	I. Ribera & A. Cieslak	**LT991387**	**LT991538**	**LT990778**	**LT990961**	**LT991173**
171	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*salinarius*		IBE-AN79	South Africa; West Cape, Wilderness NP, road to Swartvlei, stream 22.3.2001	I. Ribera & A. Cieslak	**LT991388**	**LT991539**	**LT990779**	**LT990962**	**LT991174**
172	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*sculptoides*		IBE-AN221	California (USA); Inyo Co., Tecopa, Amargosa River 8.4.2008	P. Abellán	**LT991389**	**LT991540**	**LT990780**	**LT990963**	**LT991175**
173	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*spinasus*		MNCN-AH100	South Africa; West Cape, Berg River, saline stream 3.9.2006	G. Challet	**LT991390**	**LT991541**	**LT990781**	**LT990964**	**LT991176**
174	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*subpictus*	*deletus*	IBE-AN433	Mallorca (Spain); Mallorca, Salines de Llevant 28.3.2016	I. Ribera & A. Cieslak	**LT991391**	**LT991542**	**LT990782**	**LT990965**	**LT991177**
175	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*subpictus*	*subpictus*	MNCN-AI452	France; Bouches du Rhône, Salin-de-Girau 29.7.2005	I. Ribera & A. Cieslak	HE970918	HE970844	HE971076	HE970957	HE970996
176	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*uniformis*		IBE-AN437	California (USA); Mendocino Co., Manchester 30.6.2000	I. Ribera & A. Cieslak	**LT991392**	**LT991543**	**LT990783**	**LT990966**	**LT991178**
177	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*viridescens*		IBE-RA40	Spain; Zaragoza, Cinco Villas, Estanca de Castiliscar 1.11.2009	I. Ribera & A. Cieslak	**LT991393**	HF931314	HF931539	**LT990967**	**LT991179**
178	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*viridis*	*fallaciosus*	MNCN-AI918	Morocco; Azrou, Lac Afenourir 29.4.2000	I. Ribera	**LT991394**	HF931242	HF931474	**LT990968**	**LT991180**
179	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*marinus*	*viridis*	*viridis*	IBE-AN5	Turkey; Izmir, 6 km E Foça, head of reservoir 26.7.2014	I. Ribera & A. Cieslak	**LT991395**	**LT991544**	**LT990784**	**LT990969**	**LT991181**
180	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*albacetinus*		IBE-RA1181	Spain; Jaén, Río Madera 7.9.2013	D.T. Bilton	**LT991396**	HG915306	**LT990785**	**LT990970**	**LT991182**
181	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*diazi*		IBE-RA595	Spain; Cádiz, 15 km W Los Barrios, Arroyo del Tiradero 22.6.2011	D.T. Bilton	HF948001	**LT991545**	**LT990786**	**LT990971**	**LT991183**
182	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*gayosoi*		IBE-AN311	Portugal; Algarve, Caldas de Monchique 11.6.2015	D.T. Bilton	**LT991397**	**LT991546**	**LT990787**	**LT990972**	**LT991184**
183	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*griotes*		MNCN-AI943	Morocco; Immouzzer Ida Ou Tanane, Assif Tanit 21.4.2001	I. Ribera & A. Cieslak	**LT991398**	HF931245	HF931480	**LT990973**	**LT991185**
184	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*hivae*		IBE-RA744	Iran; Khuzestan, Behbahan, Morvarid spring 4.9.2010	E. Irani	**LT991399**	**LT991547**	**LT990788**	**LT990974**	**LT991186**
185	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*judemaesi*		IBE-RA1179	Spain; Albacete, Chorros del Río Mundo 7.9.2013	D.T. Bilton	**LT991400**	**LT991548**	**LT990789**	**LT990975**	**LT991187**
186	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*maxfischeri*		IBE-AN164	Turkey; Antalya, 1 km N Hacıyusuflar 24.6.2014	D.T. Bilton	**LT991401**	**LT991549**	**LT990790**	**LT990976**	**LT991188**
187	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*metallescens*	*metallescens*	MNCN-AI376	Austria; Niederösterreich, Lunz 2002	M.A. Jäch	**LT991402**	HF931191	HF931414	**LT990977**	**LT991189**
188	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*metallescens*	*plato*	IBE-RA1057	Greece; Peloponnese, Achaea, 1.5 km NE Kleitor 3.4.2013	I. Ribera & A. Cieslak	**LT991403**	**LT991550**	**LT990791**		**LT991190**
189	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*morettii*		IBE-RA1173	Italy; Liguria, Imperia, 1 km S Pigna 7.7.2013	D.T. Bilton	**LT991404**	**LT991551**	**LT990792**	**LT990978**	**LT991191**
190	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*pedroi*		IBE-RA1082	Mallorca (Spain); Sa Calobra, Font de sa Mata 8.5.2013	I. Ribera	**LT991405**	**LT991552**	**LT990793**	**LT990979**	**LT991192**
191	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*poweri*		MNCN-AC26	England (UK); S Devon, Ladram Bay, seepage 4.7.2007	D.T. Bilton	**LT991406**	**LT991553**	**LT990794**	**LT990980**	**LT991193**
192	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*preissi*		IBE-AN448	Cyprus; Loukrounou, river Kaboura 28.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT991407**	**LT991554**	**LT990795**	**LT990981**	**LT991194**
193	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*scopuli*		IBE-AN382	Sardinia (Italy); Golf of Orosei, Cala Mariolu 12.10.2015	J. Köhler	**LT602656**	**LT602657**	**LT990796**	**LT990982**	**LT991195**
194	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*semisericeus*		MNCN-AI1064	Spain; Teruel, Beceite, river Matarranya, El Parrisal 7.8.2006	I. Ribera & A. Cieslak		HF931151	HF931370	**LT990983**	**LT991196**
195	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*semotus*		IBE-RA1180	Spain; Albacete, Chorros del Río Mundo 7.9.2013	D.T. Bilton		HG915305	**LT990797**	**LT990984**	**LT991197**
196	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*serpentinus*		MNCN-AI819	Turkey; Kastamonu, road Ağlı-Azdavay 28.4.2006	I. Ribera	**LT991408**	HF931229	HF931458	**LT990985**	**LT991198**
197	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	sp.		IBE-AN77	Azerbaijan; Gosmalijion, stream 7.5.2014	I. Ribera & A. Rudoy	**LT991409**	**LT991555**	**LT990798**	**LT990986**	**LT991199**
198	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*metallescens*	*wurayah*		IBE-RA733	United Arab Emirates; Fujairah (north), Hajar Mountains, Wadi Wurayah 24.1.2010	M.A. Jäch		**LT991556**	**LT990799**	**LT990987**	**LT991200**

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Table S3: (continued)

id	family	subfamily	genus	subgenus	sp. group	species	subspecies	voucher	locality	leg	COI-5	COI-3	16S	18S	28S
199	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*nitidipennis*	*hasegawai*		MNCN-AI1289	Japan; Honshu, Shimane Pref., Mt. Sentsu, Okuizumo 15.7.2006	N. Hayashi	**LT991410**	**LT991557**	**LT990800**	**LT990988**	**LT991201**
200	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*nitidipennis*	*japonicus*		MNCN-HI26	Japan; Shikoku, Ehime Pref., Oda-gawa, Mizumoto, Uchiko-chō 30.9.2006	Y. Kamite	**LT991411**	**LT991558**	**LT990801**	**LT990989**	**LT991202**
201	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*notabilis*	*corrugatus* cf.		IBE-RA118	Tunisia; road Safax-Gabes, Wadi 7 km N Hachichina 25.10.2001	I. Ribera & A. Cieslak		HF931300	**LT990802**	**LT990990**	**LT991203**
202	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*notabilis*	*gereckei*		MNCN-PA141	Sicily (Italy); Caltanissetta, Torto Valley 11.6.2007	P. Abellán & F. Picazo	**LT991412**	FJ944176	**LT990803**	**LT990991**	**LT991204**
203	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*notabilis*	*glaber*		MNCN-PA30	Spain; Córdoba, Priego de Córdoba, Río Salado de Priego 21.7.2006	A. Millán & collaborators	HF948002	FJ944214	**LT990804**	**LT990992**	**LT991205**
204	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*notabilis*	*halophilus*		IBE-AN22	Turkey; Kırıkkale, Delice Çoğul salt pond 14.6.2012	T. Söylemez	**LT991413**	**LT991559**	**LT990805**	**LT990993**	**LT991206**
205	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*notabilis*	*lanarotis*		MNCN-PA32	Morocco; Ouezzane, Sidi Kacem, Oued Khendek 18.4.2006	A. Millán & collaborators	**LT991414**	FJ944251	**LT990806**	**LT990994**	**LT991207**
206	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*notabilis*	*normandi*		MNCN-PA253	Algeria; Blida, Mellaha 23.8.2007	S. Bouzid	**LT991415**	FJ944275	**LT990807**	**LT990995**	**LT991208**
207	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*notabilis*	*notabilis*		MNCN-AI38	Spain; Albacete, Pinilla, Salinas de Pinilla 2.6.2002	I. Ribera & A. Cieslak	**LT991416**	FJ944107	HF931416	**LT990996**	**LT991209**
208	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*notabilis*	*salinator*		MNCN-AI53	Tunisia; road Kebili-Tozeur, 37 km SE Tozeur, salines 26.10.2001	I. Ribera & A. Cieslak	**LT991417**	FJ944271	HF931436	**LT990997**	**LT991210**
209	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*peisonis*	*peisonis*		IBE-AF168	Russia; Volgogradskaya Oblast, Lake Elton, residual pools 17.4.2008	A. Prokin	**LT991418**	**LT991560**	**LT990808**		**LT991211**
210	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*peisonis*	*peisonis*		IBE-AN64	Azerbaijan; Alishanli, 7 km NE Masally 8.5.2014	I. Ribera & A. Rudoy	**LT991419**	**LT991561**	**LT990809**	**LT990998**	**LT991212**
211	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*bifoveolatus*		IBE-AN381	Spain; Cádiz, Bahía de Cádiz, marshes 27.4.2011	A. Millán & collaborators		**LT991562**	**LT990810**	**LT990999**	**LT991213**
212	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*cuprescens*		IBE-PA276	Tunisia; road Tozeur-Gafsa, 24 km SW Gafsa, Oued El Melah 16.10.2001	I. Ribera & A. Cieslak	**LT991420**	HF931293	HF931528	**LT991000**	**LT991214**
213	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*difficilis*		MNCN-AI944	Morocco; Immouzzer Ida Ou Tanane, Assif Tanit 21.4.2001	I. Ribera & A. Cieslak	**LT991421**	HF931246	HF931481	**LT991001**	**LT991215**
214	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*difficilis* cplx		IBE-AN76	Azerbaijan; Gosmalijion, stream 7.5.2014	I. Ribera & A. Rudoy	**LT991422**	**LT991563**	**LT990811**	**LT991002**	**LT991216**
215	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*grandipennis*		MNCN-AI616	Spain; Madrid, Aranjuez, salinas 11.2.2006	I. Ribera & A. Cieslak	**LT991423**	HF931216	HF931442	**LT991003**	**LT991217**
216	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*inermis*		IBE-RA795	Japan; Honshu, Ibaraki Pref., Kobodihana, Ohmika-machi, Hitachi-shi 22.7.2006	N. Hikida		**LT991564**	**LT990812**	**LT991004**	**LT991218**
217	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*joosti*		IBE-AF169	Russia; Volgogradskaya Oblast, Lake Elton, residual pools 17.4.2008	A. Prokin	**LT991424**	HF931121	HF931339	**LT991005**	**LT991219**
218	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*klapperichi*		MNCN-AI1269	Bhutan; Timphu, Taba, Wang Chhu river 23.11.2005	M.A. Jäch	**LT991425**	**LT991565**	**LT990813**	**LT991006**	**LT991220**
219	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*lanuginosus*		IBE-AN204	Greece; Arkadia, Astros, Lake Moustou 6.4.2013	I. Ribera & A. Cieslak	**LT991426**	**LT991566**	**LT990814**	**LT991007**	**LT991221**
220	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*mahmoodi*		IBE-RA126	Oman; 1 km W Qalhat, residual pools in wadi 9.4.2010	I. Ribera, C. Hernando & A. Cieslak	**LT991427**	HF931304	HF931532	**LT991008**	**LT991222**
221	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*micans*		IBE-AN474	Djibouti; Arta, wadi Alloulli (oasis), Oued Kalou 31.1.2016	M.A. Jäch	**LT837917**	**LT991567**	**LT990815**	**LT991009**	**LT991223**
222	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*monseti*		IBE-RA124	Oman; 15 km SW Sur, residual pools in wadi 9.4.2010	I. Ribera, C. Hernando & A. Cieslak	**LT991428**	HF931302	**LT990816**	**LT991010**	**LT991224**
223	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*montesi*		MNCN-AI491	Spain; Murcia, Caravaca, Rambla Pozo Enmedio 2.6.2005	A. Millán & collaborators		HF931206	HF931431	**LT991011**	**LT991225**
224	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*nanus*		IBE-PA277	Morocco; Âït-Rahhal, Oued Akka 17.2.2001	I. Ribera & A. Cieslak		HF931294	HF931529	**LT991012**	**LT991226**
225	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*nilssoni*		MNCN-AH76	Ireland; Clare, Lough Gealáin 31.7.2007	D.T. Bilton	**LT991429**	**LT991568**	**LT990817**	**LT991013**	**LT991227**
226	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*nobilis*		IBE-AF133	Italy; Emilia Romagna, Bosco, Torrente Parma 24.5.2008	I. Ribera	**LT991430**	**LT991569**	**LT990818**	**LT991014**	**LT991228**
227	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*pagotrichus*		MNCN-AI497	South Africa; West Cape, Prince Albert Road, pond 23.3.2001	I. Ribera & A. Cieslak	**LT991431**	**LT991570**	**LT990819**	**LT991015**	**LT991229**
228	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*pilosus*		IBE-AN363	Spain; Cádiz, Bahía de Cádiz 27.4.2011	A. Millán & collaborators	**LT991432**	**LT991571**	**LT990820**	**LT991016**	**LT991230**
229	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*punctatus*		IBE-RA286	Ireland; Clare, Finavarra, Lough Muree 23.5.2010	I. Ribera	**LT991433**	HF931310	**LT990821**	**LT991017**	**LT991231**
230	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*quadrifoveolatus*		MNCN-AI636	Gran Canaria (Spain); Moya, Barranco de Azuaje 15.4.2001	I. Ribera & A. Cieslak	**LT991434**	HF931218	HF931446	**LT991018**	**LT991232**
231	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*ragusae*		MNCN-AI1029	Turkey; Sinop, road Saraydüzü-Boyabat, stream 5 km S Yeşilyurt 27.4.2006	A. Castro	**LT991435**	HF931147	HF931366	**LT991019**	**LT991233**

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Table S3: (continued)

id	family	subfamily	genus	subgenus	sp. group	species	subspecies	voucher	locality	leg	COI-5	COI-3	16S	18S	28S
232	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*silfverbergi*		IBE-RA1021	Morocco; Tan-Tan, Oued Drâa 3.4.2007	A. Millán & collaborators	**LT991436**	**LT991572**	**LT990822**	**LT991020**	**LT991234**
233	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		IBE-RA104	Oman; Al-Akhdar, source of wadi Bani Awf 6.4.2010	I. Ribera, C. Hernando & A. Cieslak	**LT991437**	**LT991573**	**LT990823**	**LT991021**	**LT991235**
234	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		IBE-AF191	Bhutan; Sarpang, 11 km NW Sarpang, Bhur Khola river 27.11.2005	M.A. Jäch	**LT991438**	**LT991574**	**LT990824**	**LT991022**	**LT991236**
235	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*punctatus*	*tudmirensis*		MNCN-AI467	Spain; Guadalajara, Salinas de Imón 22.5.2005	I. Ribera & A. Cieslak	HF948004	HF931205	HF931430	**LT991023**	**LT991237**
236	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*quadricollis*	*brevicollis*		IBE-AN440	Cyprus; Akamas peninsula, Lara beach 29.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT991439**	**LT991575**	**LT990825**	**LT991024**	**LT991238**
237	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*quadricollis*	*heeri*		IBE-AN200	Tenerife (Spain); Tenerife, La Orotava, rookpools in El Barco 4.4.2015	R. Lyszkowski	**LT991440**	**LT991576**	**LT990826**	**LT991025**	**LT991239**
238	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*quadricollis*	*quadricollis*		MNCN-AI431	Corsica (France); Cap Corse, Port de Centuri 20.9.2000	I. Ribera & A. Cieslak	**LT991441**	HF931199	HF931424	**LT991026**	**LT991240**
239	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*quadricollis*	*quadricollis* cplx1		IBE-AN11	Spain; Murcia, La Manga del Mar Menor, Punta del Cocedor 10.10.2009	J. Sánchez-Meca	**LT991442**	**LT991577**	**LT990827**	**LT991027**	**LT991241**
240	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*quadricollis*	*quadricollis* cplx2		MNCN-AI514	Spain; Pontevedra, Nigran, Playa Patos 3.10.2005	J. Garrido	**LT991443**	HF931209	HF931434	**LT991028**	**LT991242**
241	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*quadricollis*	*steinbuehleri*		MNCN-AI517	Greece; Halkidiki, Kassandra 29.6.2002	M.A. Jäch	**LT991444**	HF931210	HF931435	**LT991029**	**LT991243**
242	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*rivalis*	*himalayae*		MNCN-AI1270	Bhutan; Timphu, Taba, Wang Chhu river 23.11.2005	M.A. Jäch		**LT991578**	**LT990828**	**LT991030**	**LT991244**
243	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*rivalis*	*rivalis*		IBE-AF81	India; Uttarakhand, 10 km SW Rudraprayag, River Alaknanda 11.11.2006	M.A. Jäch		**LT991579**	**LT990829**	**LT991031**	**LT991245**
244	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*strigosus*	sp.		IBE-RA617	China; Shaanxi, 110 km ENE Xian, Huayin vill., Hija Mt. 10.5.2011	M. Balke & J. Hájek		**LT991580**	**LT990830**	**LT991032**	**LT991246**
245	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*sumatrensis*	sp.		MNCN-AC16	Hong-Kong (China); Hong Kong Island, hygropetric 19.2.2007	J. Maté	**LT991445**	**LT991581**	**LT990831**	**LT991033**	**LT991247**
246	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*vandykei*	*vandykei*		IBE-AF159	California (USA); San Luis Obispo Co., Rancho Marino Res. 28.6.2008	M. Caterino		**LT991582**		**LT991034**	**LT991248**
247	Hydraenidae	Ochthebiinae	*Ochthebius*	*Ochthebius*	*vandykei*	*yoshitomii*		IBE-AF121	Japan; Hokkaido, Shirakami, Matsumae-chô 14.7.2006	H. Yoshitomi		**LT991583**		**LT991035**	**LT991249**
248	Ptiliidae		*Acrotrichis*			sp.		MNCN-AI413	Germany; Thuringia, Jena, Pennickental 19.7.2005	R.G. Beutel		**LT991584**	**LT990832**	**LT991036**	
249	Ptiliidae		*Cylindroselloides*			*dybasi*		MNCN-AI564	Ontario (Canada); Eastern Ontario, circa Westport 25.7.2005	V.V. Grebennikov		**LT991585**	**LT990833**	**LT991037**	**LT991250**
250	Ptiliidae		*Ptenidium*			*pusillum*		MNCN-AI515	Morocco; Immouzzer Ida Ou Tanane, Assif Tanit 21.4.2001	I. Ribera & A. Cieslak	**LT991446**	HE970847	HE971079	HE970959	HE970997
251	Ptiliidae		*Ptiliolum*			sp.		MNCN-AI649	Spain; Córdoba, Cabra, La Nava 22.1.2006	A. Castro	**LT991447**	HE970857	HE971087	HE970967	HE971004
252	Ptiliidae		*Rioneta*			*uluguruensis*		MNCN-AI415	Tanzania; Uluguru Mts., between Tchenzema vil. and Lukvangule Plateau 19-21.10.2002	V.V. Grebennikov		**LT991586**		**LT991038**	

Table S4. (A) primers used for DNA amplification and sequencing reactions; (B) Typical conditions for the polymerase chain reaction.

(A)

gene	primer	F/R	sequence	ref.
COI-5’	Uni LepF1b	F	TAATACGACTCACTATAGGGATTCAACCAATCATAAAGATATTGGAAC	1
	Uni LepR1	R	ATTAACCCTCACTAAAGTAAACTTCTGGATGTCCAAAAAATCA	1
COI-3’	Jerry	F	CAACATTTATTTTGATTTTTTGG	5
	Pat	R	TCCAATGCACTAATCTGCCATATTA	5
	Chy	F	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	3
	Tom	R	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	3
16S	16sAr	R	CGCCTGTTTAACAAAAACAT	5
	ND1A	F	GGTCCCTTACGAATTTGAATATATCCT	5
	16Sb	R	CCGGTCTGAACTCAGATCATGT	5
28S	ka	F	ACACGGACCAAGGAGTCTAGCATG	2
	kb	R	CGTCCTGCTGTCTTAAGTTAC	2
18S	18S 5’	F	GACAACCTGGTTGATCCTGCCAGT(1)	4
	18s b5.0	R	TAACCGCAACAACTTTAAT(1)	4

(B)

COI-3’ / 18S

step	time	temperature
1	3’	96°
2	30”	94°
3	30”	50°
4	1’	72°
5	Go to setp 2 and repeat 39 x	
6	10’	72°

16S / 28S

step	time	temperature
1	3’	96°
2	30”	94°
3	1’	48°
4	1’	72°
5	Go to setp 2 and repeat 34 x	
6	10’	72°

COI-5’

step	time	temperature
1	3’	96°
2	40”	94°
3	40”	48°
4	1’	72°
5	Go to setp 2 and repeat 4 x	
6	40”	94°
7	40”	51°
8	1’	72°
9	Go to setp 6 and repeat 29 x	
10	10’	72°

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3. Ribera, I., Fresneda, J., Bucur, R., Izquierdo, A., Vogler, A.P., Salgado, J.M. & Cieslak, A. (2010). Ancient origin of a Western Mediterranean radiation of subterranean beetles. *BMC Evolutionary Biology,* **10**, 29.
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Figure S1. Majority rule consensus tree obtained with BEAST for the phylogeny of Ochthebiini with the best partition models. Numbers in nodes, posterior probabilities.

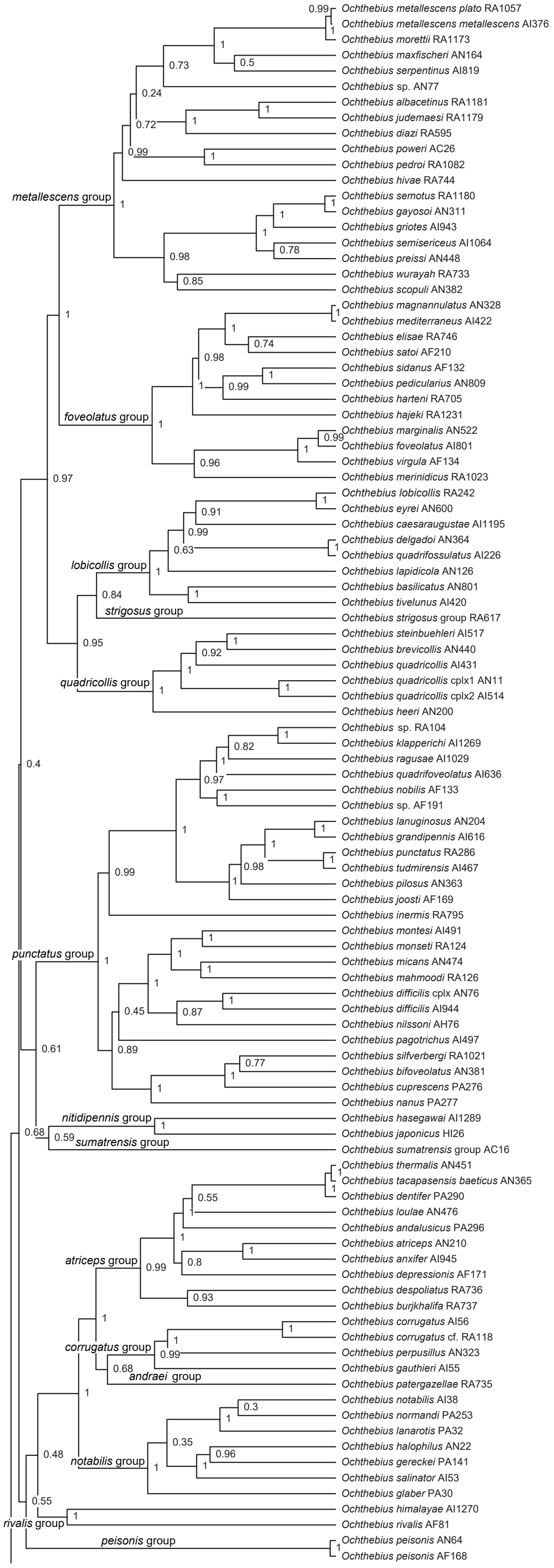


Figure S1. (Continued)

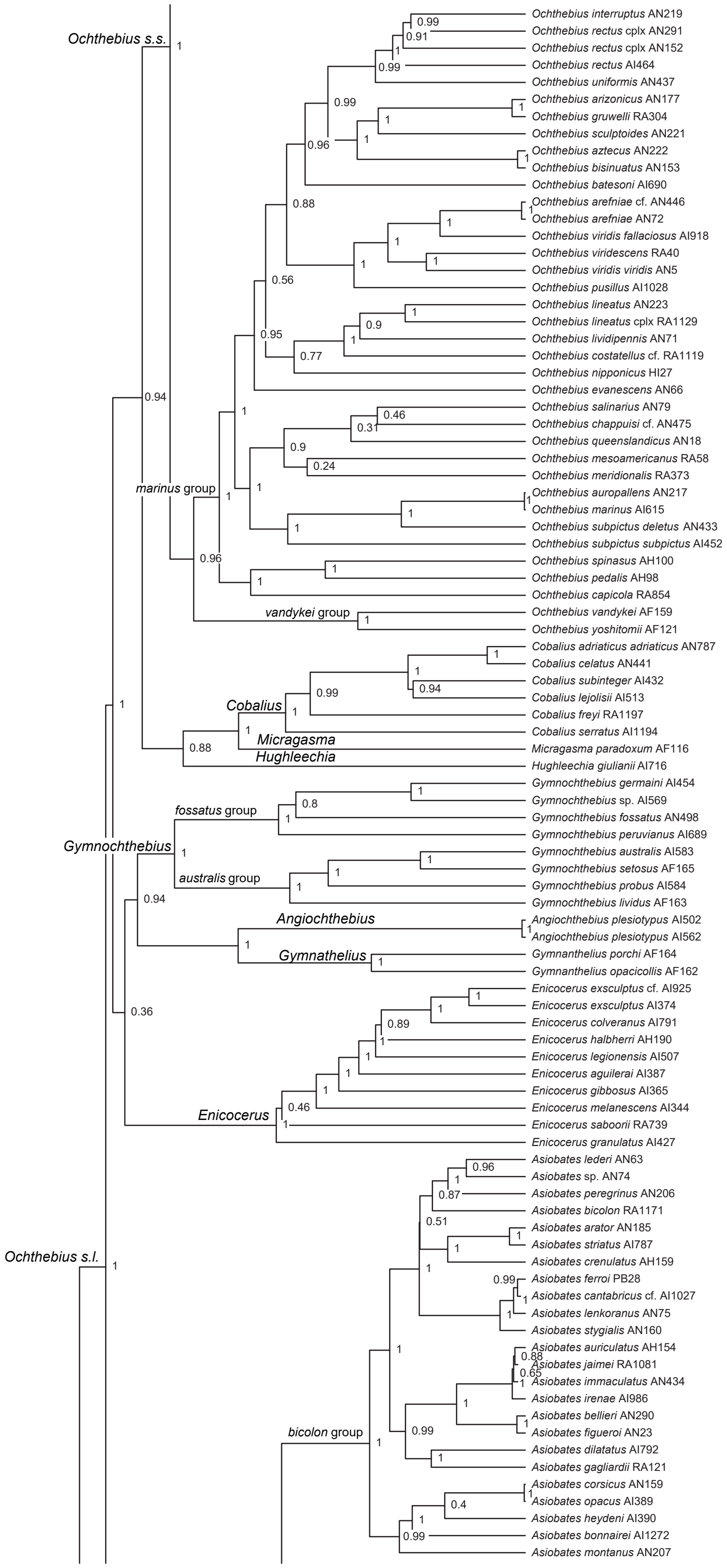


Figure S1. (Continued)

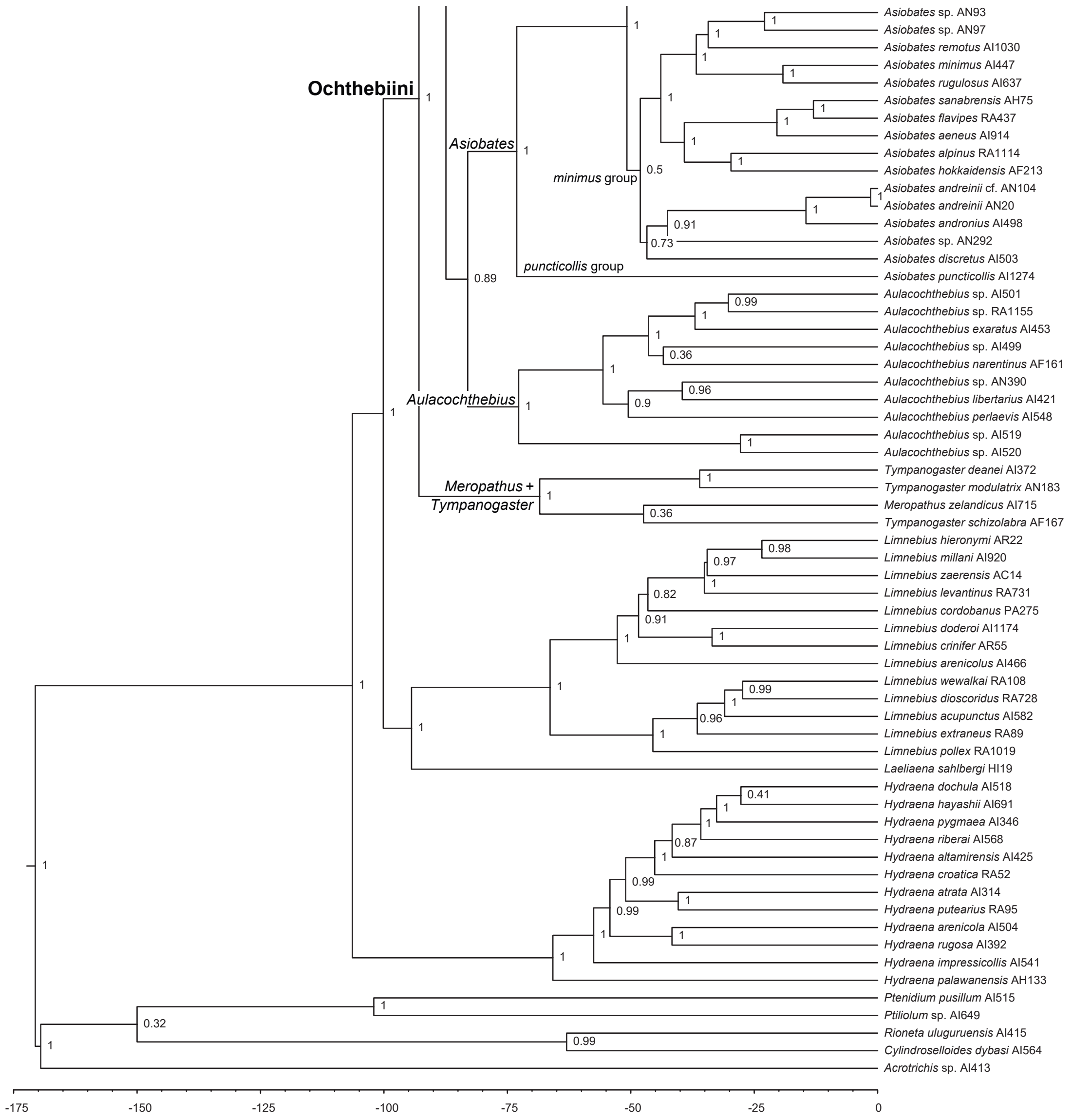


Figure S2. Phylogeny obtained with RAxML, including current Ochthebiini classification. Numbers in nodes, bootstrap support values.

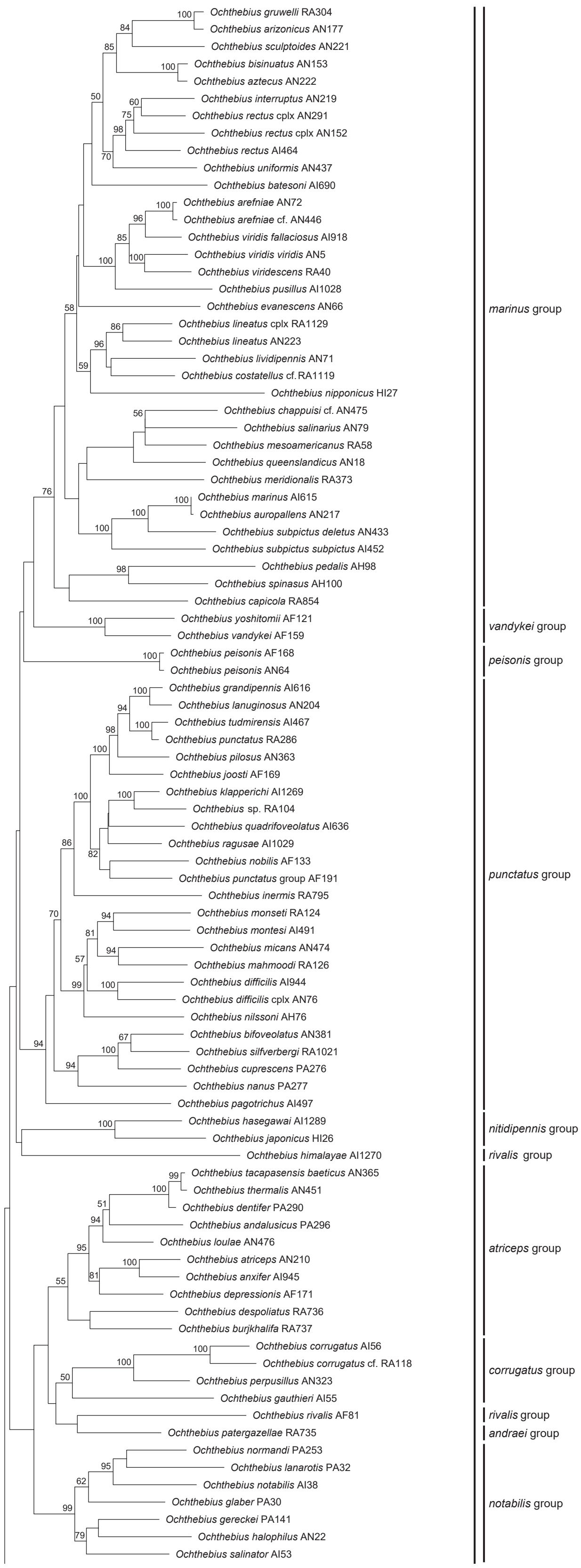


Figure S2. (Continued)

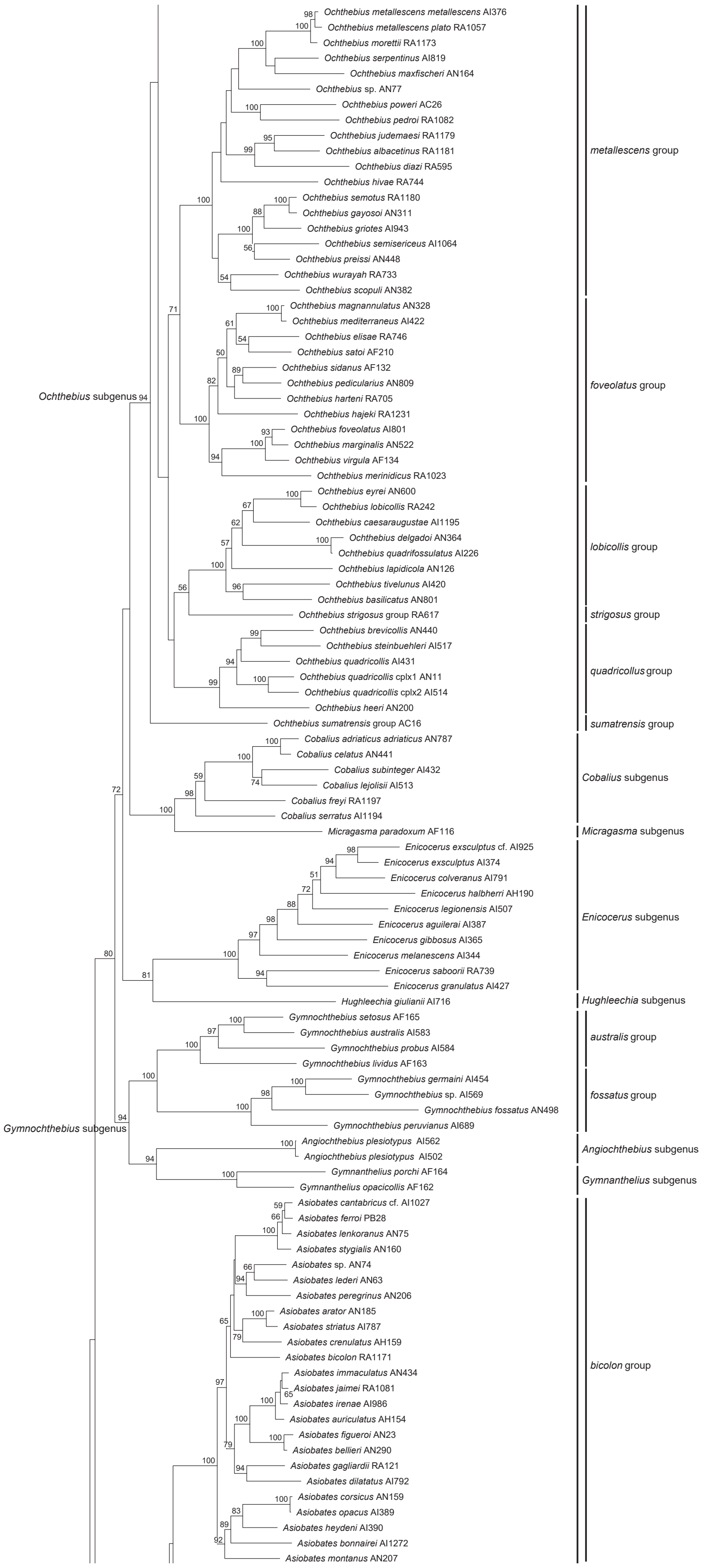


Figure S2. (Continued)

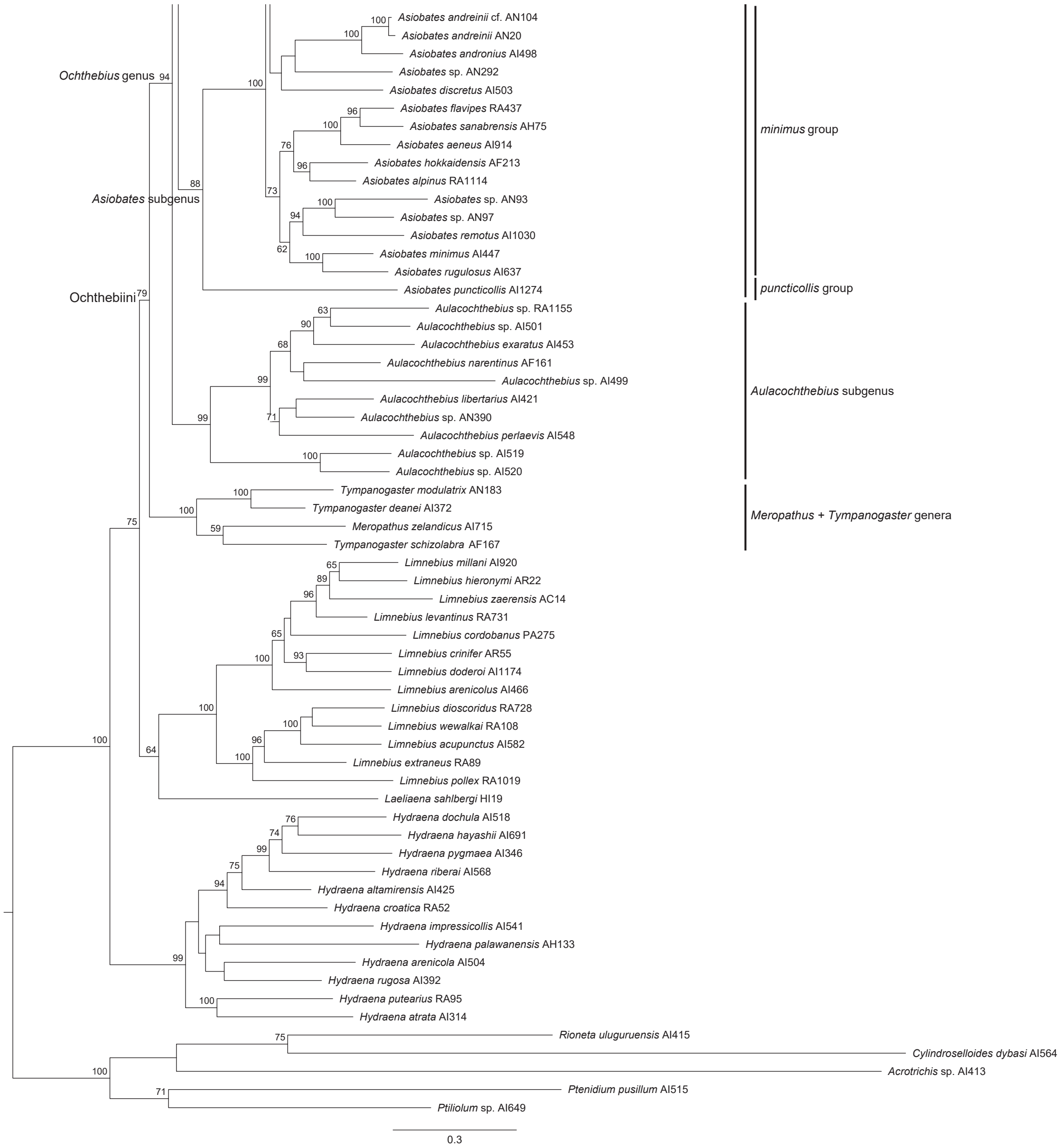


Figure S3. Phylogeny obtained with RAxML with the nuclear genes only. Numbers in nodes, bootstrap support values. Upper part of the figure.

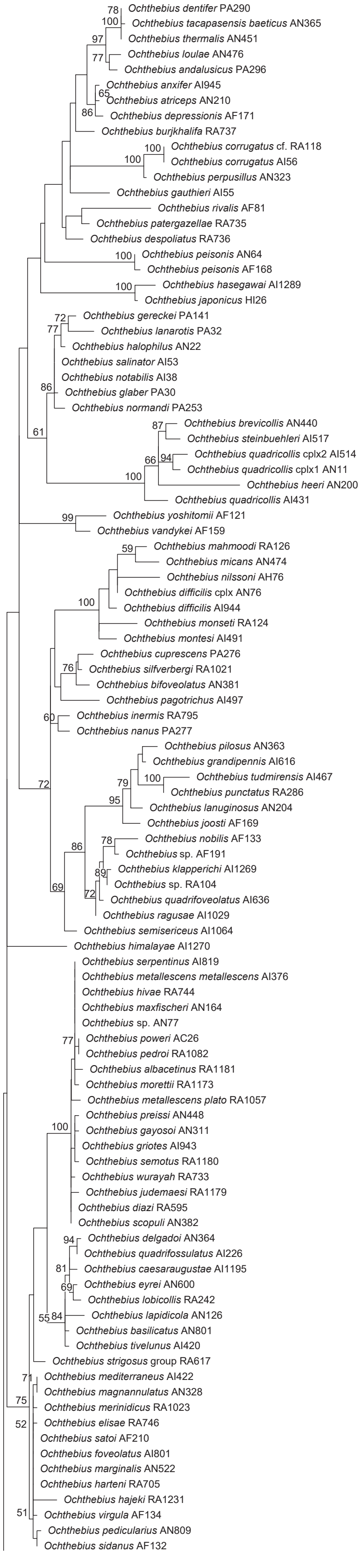


Figure S3. (Continued)

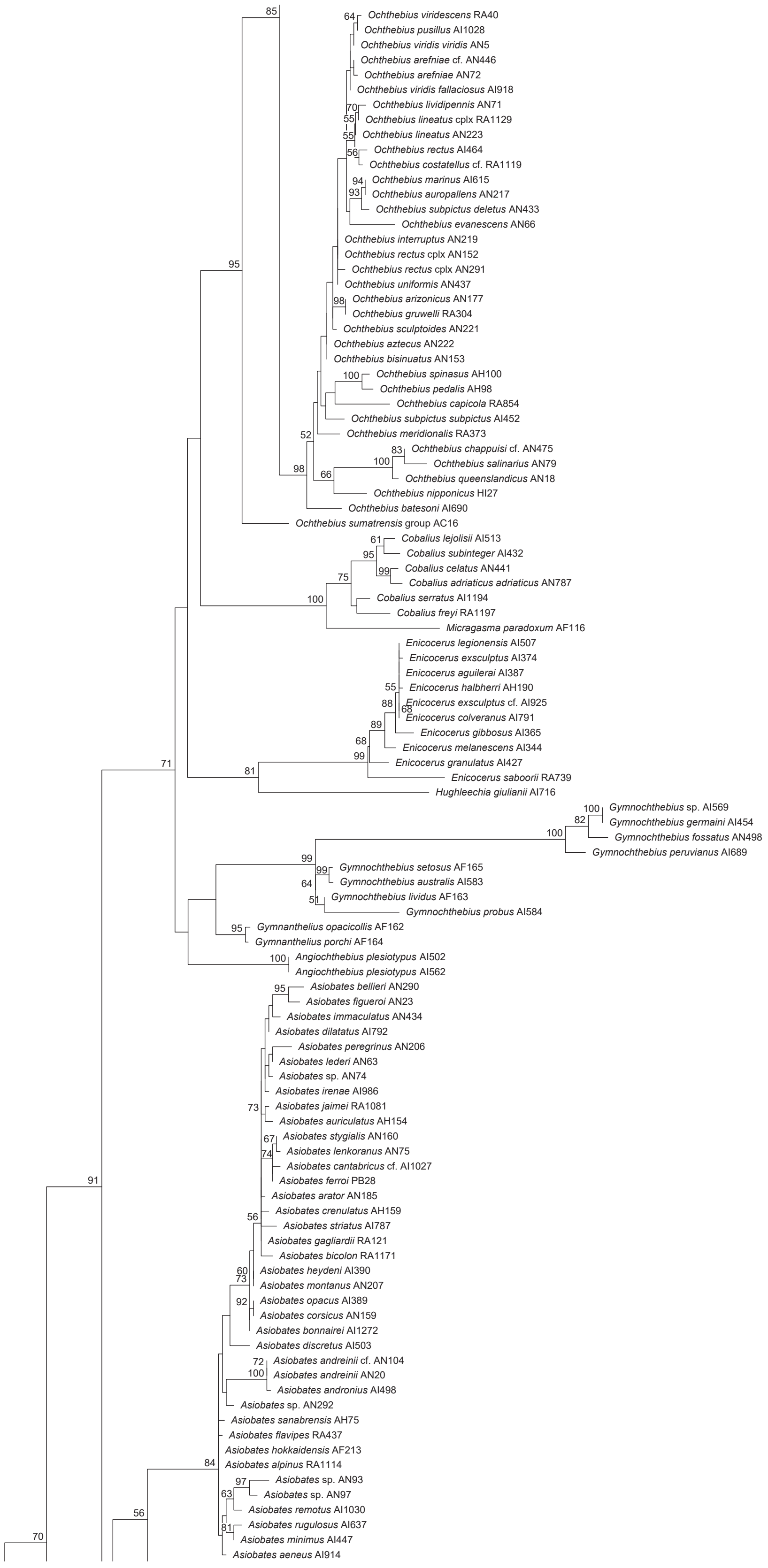
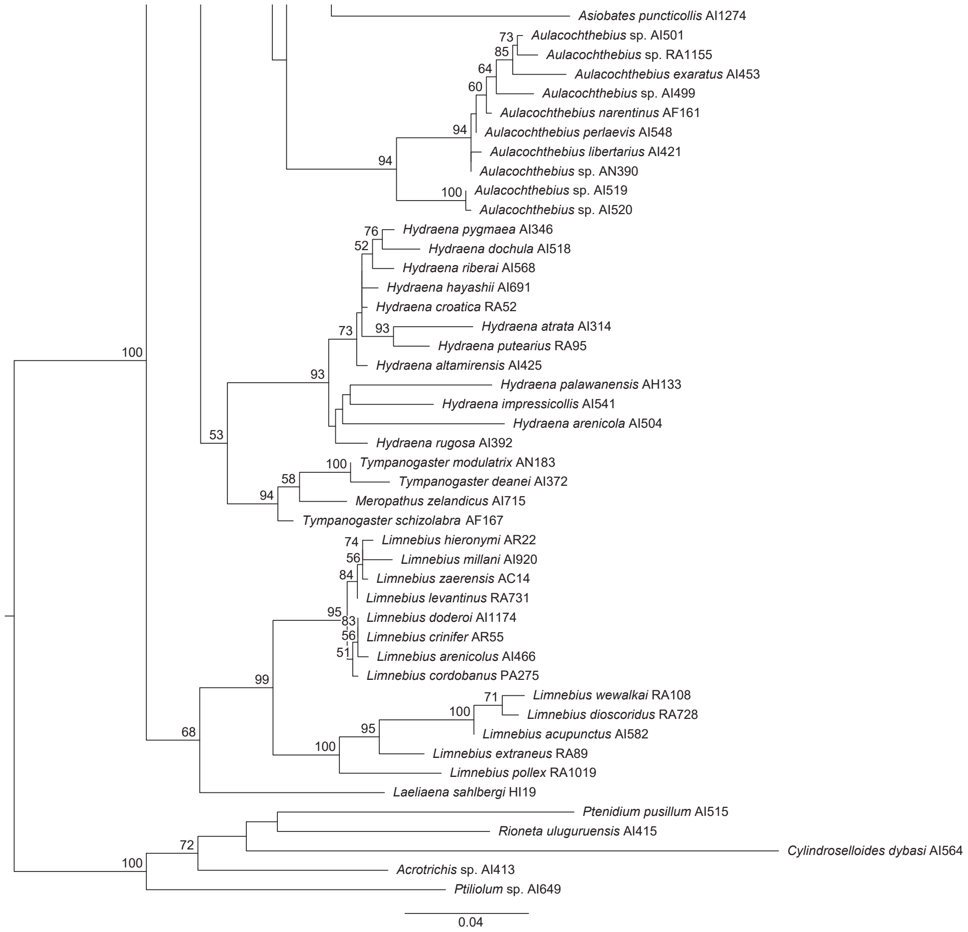


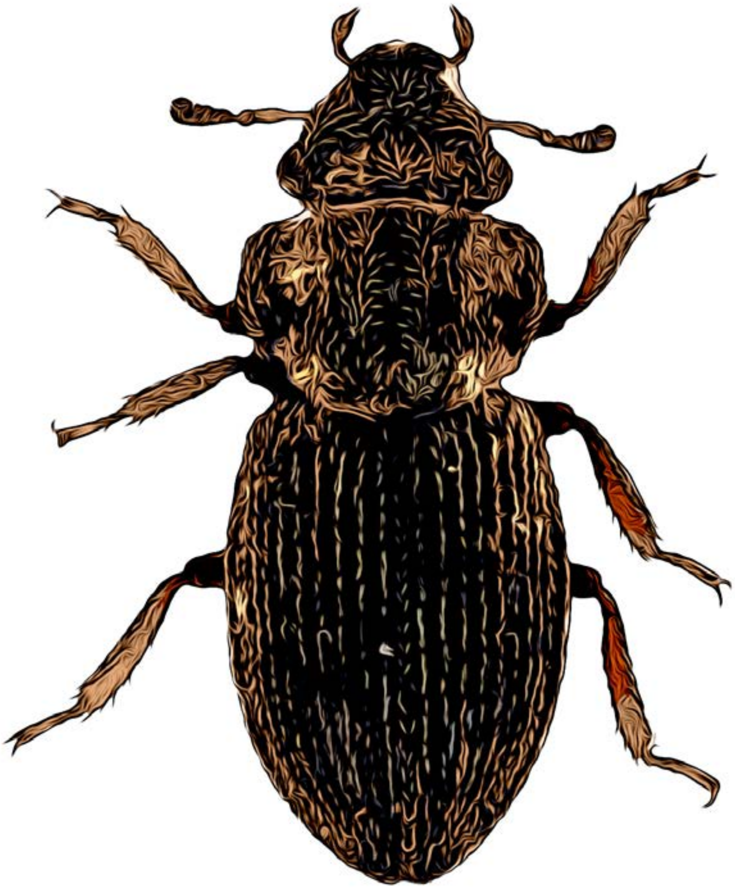
Figure S3. (Continued)



Chapter 4

A new species of *Micragasma* J. Sahlberg, 1900 (Coleoptera: Hydraenidae) from Crete

Edited version of: Carles Hernando, Adrián Villastrigo,
and Ignacio Ribera
Published on: *Aquatic Insects* (2017) **38**: 185-196



Abstract

We describe a new species in of *Micragasma* J. Sahlberg, 1900 (Coleoptera, Hydraenidae), which is here treated as a subgenus of *Ochthebius* Leach, 1815. The new species, *O*. (*Micragasma*) *minoicus* sp. n., was found at the margins of a coastal rockpool in the island of Crete.

The species differs from the other two known species of the genus in both external and genital characters, but shares with them the presence of small setiferous tubercles on the surface of the head, pronotum and elytra, and a strong medial gibbosity on the head. In some characters, such as the structure and shape of the aedeagus, *O*. (*Micragasma*) *minoicus* sp. n. is more similar to other species of the genus *Ochthebius*, in particular of the subgenus *Cobalius* Rey, 1886, typical of coastal rockpools.

Introduction

Micragasma J. Sahlberg, 1900, one of the most elusive and lesser known genera of European aquatic Coleoptera, was described for a single species, *M. paradoxum* J. Sahlberg, 1900 from the island of Corfu. In the original description, Sahlberg (1900) reports collecting several specimens in December, 1895 amongst flooded vegetation in the mouth of the Potamos River and some additional specimen in November, 1898 again amongst flooded grasses in the Kalichiopulo [=Calichiopulo] Lagoon. The species had not been collected again until 1993 when a small series (three specimens) was collected in Puglia, southern Italy (Figure 1), amongst plant debris not far from the sea. Subsequently, a larger series was collected in the same area by sifting sand among *Salicornia* L. next to the coast (Ferro *et al*.,1996). Jäch (1997) redescribed the genus and transferred *Ochthebius substrigosus* Reitter, 1897 to *Micragasma*. This species was described from a small series collected in the mouth of the Kura River, in Azerbaijan (Figure 1), although no details on the habitat were given in the original description (Reitter, 1897). The species has never been reported again. More recently, *M. paradoxum* has been found in the surrounding area of Lake Elton, in Volgograd Province, Russia (Makarov *et al*., 2009; Prokin *et al*., 2016), in saline ditches and pools, and i two localities on the north coast of the Black Sea, in detritus or in overgrown grass puddles (Shatrovskiy, 2015) (Figure 1). There are no other published records of *Micragasma*.

The phylogenetic affinities of *Micragasma* are also not well understood. Reitter (1897) described *O. substrigosum* within subgenus *Chirochthebius* Kuwert, 1887, currently a synonymy of *Aulacochthebius* Kuwert, 1887 (Jäch and Skale, 2015), noting its resemblance to *A. narentinus* (Reitter, 1885). Sahlberg (1900) compared the newly described genus with species of *Ochthebius* Leach, 1815, and, in his revision of Hydraenidae genera, Hansen (1991) notes that it is a distinct an apparently well-justified genus. However, Jäch (1997), after including *O. substrigosum* in the genus *Micragasma*, noted the similarity between *Micragasma* and *Ochthebius*, questioning its status as a separate genus. In Beutel, Anton and Jäch (2003), some peculiarities of the head of *Micragasma* were noted in reference to other species of *Ochthebius* or the wider Hydraenidae (absence of ocelli in *M. paradoxum*, a shield-like clypeus with distinctly raised lateral margin), but the genus was not included in the phylogenetic analyses and nothing was said about its possible relationships. Unpublished molecular data place *Micragasma* as sister to the species of the Cobalius Rey, 1886, treated as a synonym of *Ochthebius* s. str. in the last reference catalogues (e.g., Jäch and Skale, 2015). Here we treat both *Micragasma* and *Cobalius* as subgenera of *Ochthebius*; a more detailed discussion on the phylogenetic relatioship of both taxa will be published in a forthcoming work.

The close relationship between *Micragasma* and *Cobalius* suggested by molecular data may apear suprising, given the strong differences in external morphology. This relationship seems more likely in the light of the species newly described here, found during a recent trip to Crete in the margins of a rock pool in the south western coast of the island.

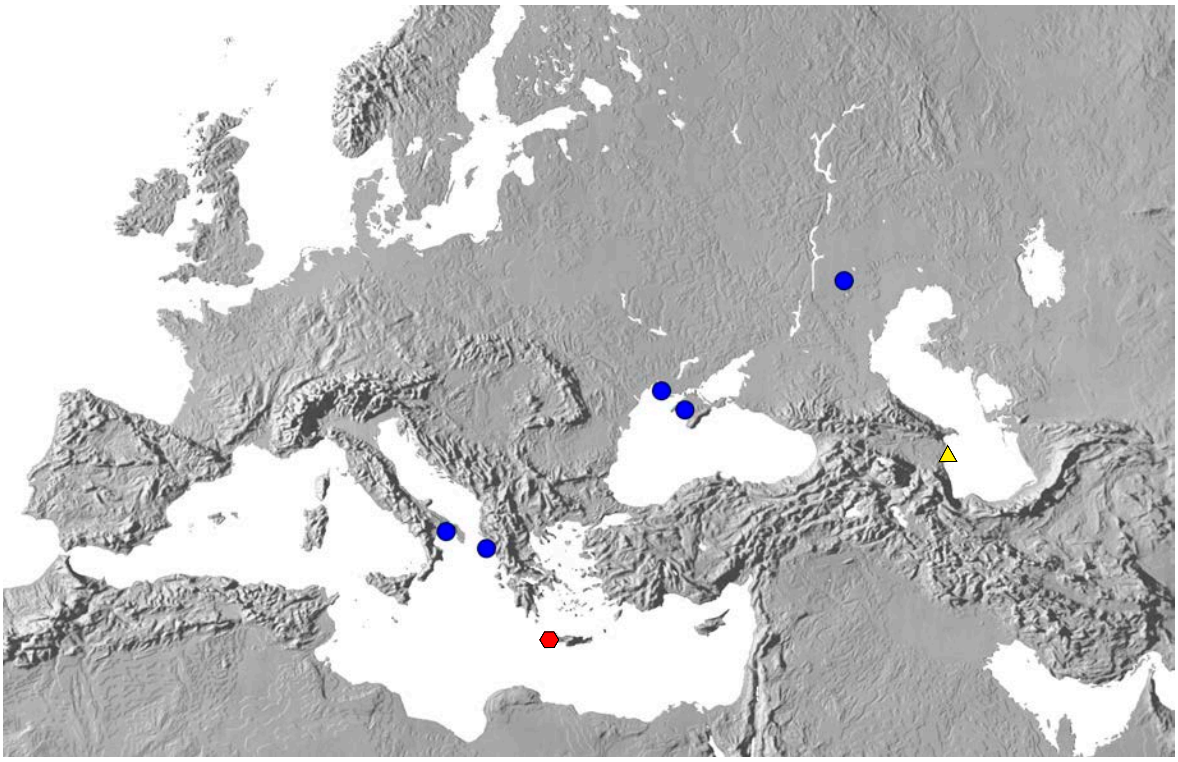


Figure 1. Distribution of the known species of *Micragasma* J. Sahlberg, 1900: circles, *Ochthebius* (*M.*) *paradoxus* (J. Sahlberg, 1900); triangle, *O.* (*M.*) *substrigosus* Reitter, 1897; hexagon, O. (M.) *minoicus* sp. n.

Material and methods

We studied one species of *O.* (*M.*) *paradoxus* labelled 'RUSSIA: 17.4.2008, Volgogradskaya Oblast, 49°07'43.6''N/ 46°47'39.5''E, Lake Elton, -18 m below sea level, residual pools of temp. water course, leg. A. Prokin', 'Voucher IBE-AF116' (one of the specimens reported in Prokin et al. 2016). The DNA of one paratype of the new species was extracted non-destructively (voucher IBE-AN770, see below) with commercial kits (DNeasy Tissue Kit, Qiagen, Hilden, Germany).

The specimen used for DNA extraction (voucher IBE-AN770) was subsequently used for SEM observation. After the extraction process, it was mounted on a stub with double-sided carbon conductive tab and coated with gold in a Quorum Q150R S sputter coater unit (Quorum Technologies Ltd., UK). The sample was observed using a Hitachi S3500N scanning electron microscope (Hitachi High-Technologies Co. Ltd., Japan) operated at 4 kV in the Electron Microscopy Service of the Institute of Marine Sciences (CSIC), in Barcelona (Spain).

Specimens are deposited in the following collections: CHB, Collection of C. Hernando, Badalona, Spain; IBE, Institute of Evolutionary Biology, Barcelona, Spain; MNCN, Museo Nacional de Ciencias Naturales, Madrid, Spain; NMW, Naturhistorisches Museum, Wien, Austria.

Taxonomy

Ochthebius (*M*.) *minoicus* sp. n.

Figures 2-10.

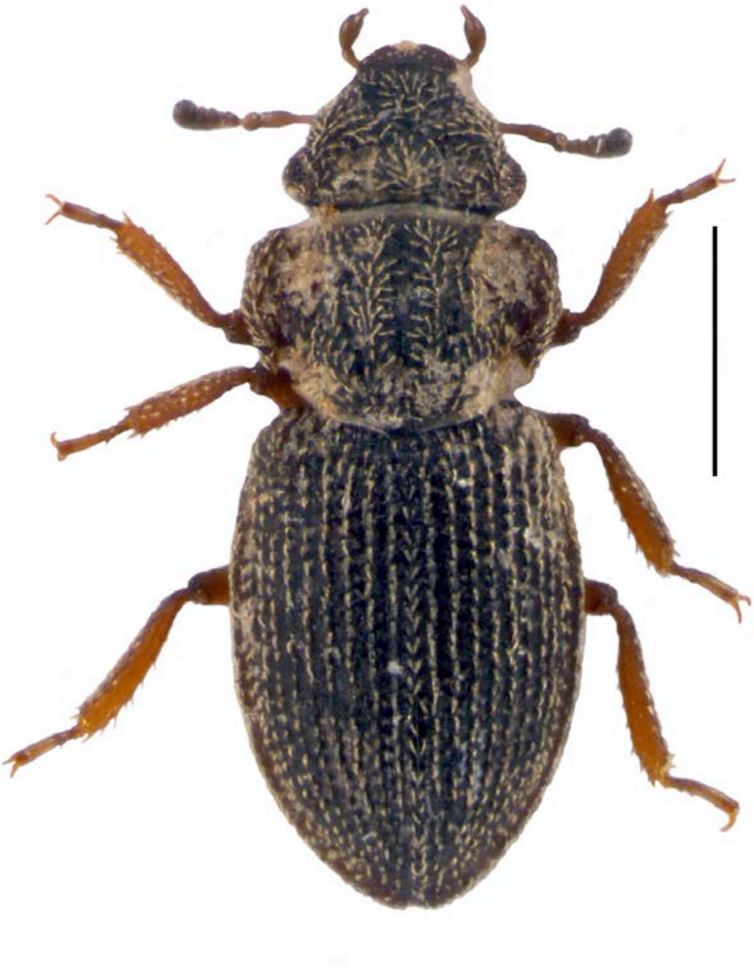


Figure 2. Habitus of the holotype of *Ochthebius* (*M*.) *minoicus* sp. n. Photo by M. Brojer, Scale bar 0.5 mm.

Figure 3. SEM habitus of a paratype of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770). Scale bar 0.5 mm.

Type locality

Greece, Crete, Elafonisi, 35°16’9.2”N, 23°32’42”E (Figure 1).

Type material

Holotype. ♂: ‘22 Crete, Elafonisi 20.iv.2017 / rockpools on calcareous bed / 35°16’9.2”N 23°32’42.”E 0m / Hernando, Ribera & Villastrigo leg.’ (NMW); aedeagus dissected and mounted in DMHF on a transparent card, pinned with the specimen; plus red holotype label. *Paratypes*. 6 ♂ 3 ♀, same data as holotype, with paratype labels (IBE, CHB, NMW, MNCN). DNA of one paratype extracted (voucher number IBE-AN770).

Description

Total length 1.68-1.73 mm, maximum width 0.65-0.70 mm, without apparent differences between males and females. Body shape oval (Figures 2 and 3). Body colour dark brown; head, disk of pronotum and elytra darker, appendages paler (Figure 2).

Head (Figures 4 and 5). Labrum deflexed, deeply emarginated; surface smooth, with a shallow reticulation; with sparse small setae. Clypeus trapezoidal, narrower distally; surface rugose, with small tubercles, with sparse setiferous punctures with small setae; finely bordered; fronto-clypeal suture well marked. Frons expanded laterally, with a medial gibbosity; two lateral elongated excavations bordering the gibbosity joining posteriorly, without pubescence or tubercles (in most specimens, these excavations are not visible due to salt concretions, but are clearly visible in the specimen used for DNA extraction). Margins of head only slightly elevated. Head covered with small tubercles with very robust, short, recurved silverish-whitish setae, almost squamiform; as observed with the SEM, setae are lanceolate, wider in the middle and with lateral expansions (specially on the elytra, see below), with surface rugose. Eyes widely separated, laterally very prominent. Ocelli absent.

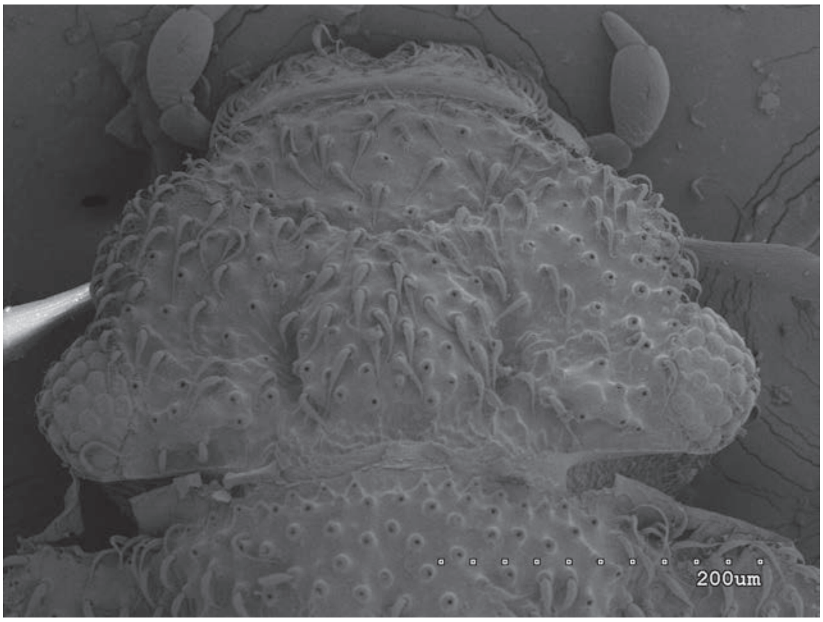


Figure 4. Head of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770), dorsal view.

Thorax. Pronotum transverse, with margins explanate (Figures 5 and 6); disc elevated, with a medial irregular longitudinal sulcus; central area covered with small, very dense setiferous tubercles; lateral area rugose; with two basal elevated areas forming a ‘V’. Lateral margins irregular, but uniformly arched, elevated, with a fringe of denser setae; anterior margin with a narrow hyaline expansion; posterior margin constricted, with postero-lateral hyaline expansion following the contour of the pronotum; postero-lateral hyaline expansions densely covered with setae; posterior margin of pronotum covering base of elytra, partly covering the scutellum.

Elytra elongated, oval (Figures 2 and 3); apparently fused; shoulders with a strong gibbosity; apex acuminate; margins slightly explanate, with a fringe of denser setae. With 10 very regular rows of punctures in each elytron, with smaller punctures distributed irregularly, some of them with smaller setae; main sculpture composed of a large puncture with a tubercle on the anterior margin, with a setae on top (Figure 7); surface rugose-chagrinated (not as rugose as pronotum and head); setae as on pronotum and head; apex of elytra and, to a lesser extend, marginal areas with an irregular surface, with tubercles united forming small carinnae (Figure 8). Apterous (observed in one paratype).

Figure 5. Head and pronotum of *Ochthebius* (*M*.) *minoicus* sp. n. (voucher IBE-AN770), frontal view.

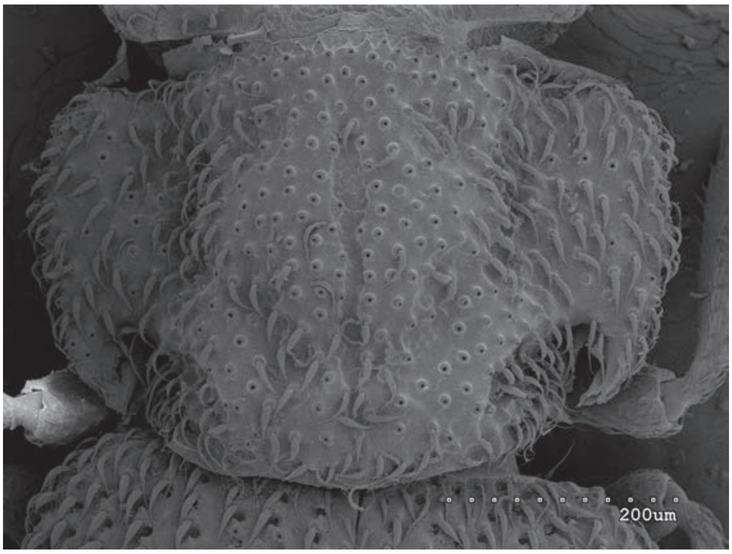


Figure 6. Pronotum of *Ochthebius* (*M*.) *minoicus* sp. n. (voucher IBE-AN770), dorsal view.

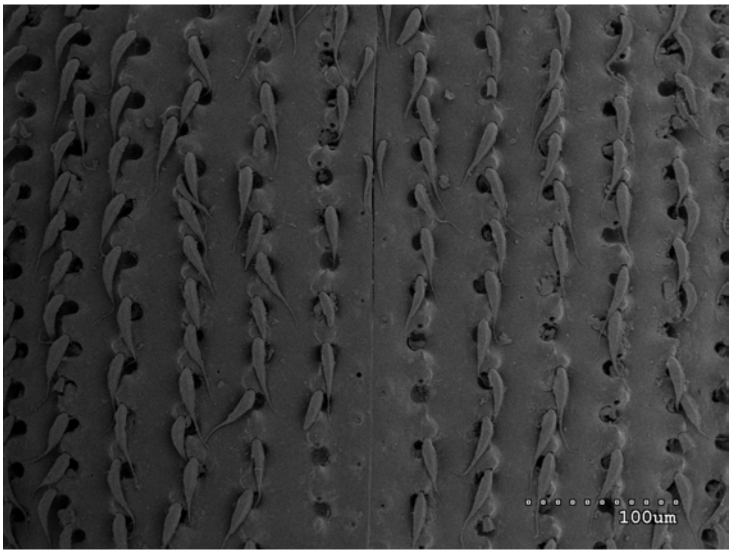


Figure 7. Details of the elytral disk of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770).

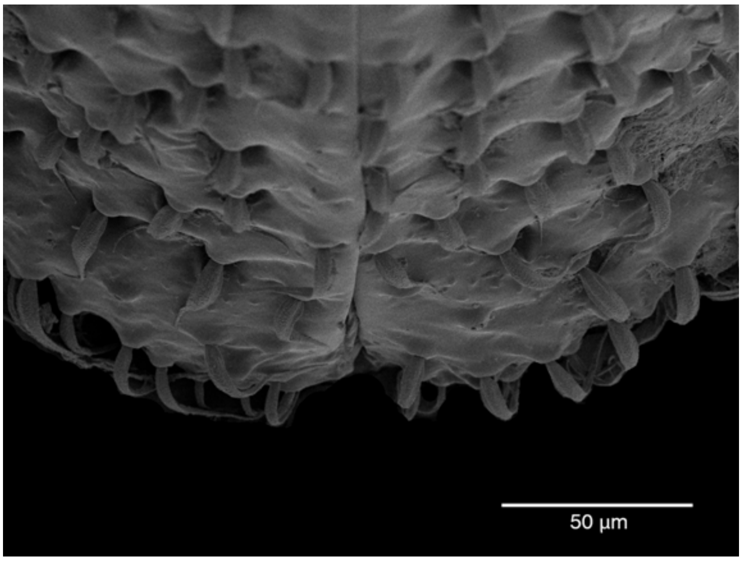


Figure 8. Apex of the elytral of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770), dorsal view.

Hypomera with deep antennal grooves, with a hyaline margin. Metaventrite short, covered with hydrophobe pubescence except on medial part, which is covered with a longer and more robust pubescence; prosternum and mesoventrite glabrous, with a rugose surface; mesoventrite with a small anterior longitudinal carina, with an acuminate apophysis.

Legs short, robust; tarsi short, specially protarsi, without natatory setae; with spiniform setae.

Abdomen. Ventrites 1-5 with short hydrophobe pubescence and longer and recurved sparse setae, denser in the medial part (Figure 9); last ventrites without hydrophobe pubescene, with short, straight spiniform setae. Surface irregular, with a chagrinated aspect. Last ventrite with a row of 7-9 long, explanate setae.

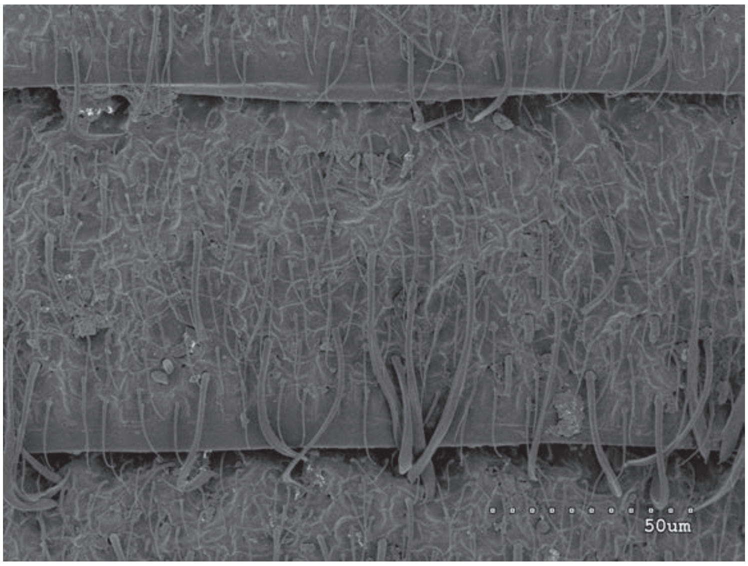


Figure 9. Surface of the abdominal ventrite 2 of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770).

Figure 10. Aedeagus of *Ochthebius* (*M.*) *minoicus* sp. n. (paratype), lateral view.

Aedeagus as in Figure 10, asymmetrical, medial lobe slightly arced; distal piece elongated, widest in the middle.

Females

Pronotum with anterior angles more rounded and lateral margins more arced than in males.

Etymology

Named in reference to the Minoan civilization of Crete. The name is an adjective in the nominative singular.

Distribution

So far only known from the type locality.

Habitat

Specimens were collected at the margins of a rock pool close to the seashore, with saline water over rocky substratum with gravel and sand on the margins, with a tuff of vegetation (Figure 11). They were dislodged from the shore and the tuff of vegetation by splashing water. Species were collected in association with *Bledius* sp. (Staphylinidae), *Ochthebius* (*Cobalius*) *adriaticus moreanus* Pretner, 1929, *O.* (*Cobalius*) *subinteger* Mulsant & Rey, 1861 and *O*. (*Calobius*) *steinbuehleri* Reitter, 1886.

Discussion

Of the characters listed by Jäch (1997) as diagnostic for the genus *Micragasma* based on the two only known species at the time (*O*. (*M.*) *paradoxus* and *O*. (*M.*) *substrigosus*), potential synapomorphies are the presence of small tubercles covering most of the surface of the body and the presence of a well-developed medial gibbosity on the vertex surrounded by more or less impressed foveae. As already noted by Jäch (1997), all other characters occur in various species of *Ochthebius*. In *O*. (*M.*) *minoicus* sp. n., the shape of the labrum is more similar to other species of *Ochthebius*, not as strongly deflexed as in *O*. (*M.*) *paradoxus* and less transverse. The head is also less transverse and explanate, without the strong lateral elevations present in *O*. (*M.*) *paradoxus*. The pronotum in *O*. (*M.*) *minoicus* sp. n. is also less explanate, with the marginal areas less flat and with a more rugose surface. Other differences refer to the general shape and size, more elongated in *O*. (*M.*) *minoicus* sp. n. (the studied specimen of *O*. (*M.*) *paradoxus*, a female, was 1.58 mm length and 0.75 mm wide), with a darker body colour, and a more dense and robust pubescence, with a more regular appearance on the elytra. The studied specimen of *O*. (*M.*) *paradoxus* was brachypterous, with a non-functional small stump only slightly longer than the metaventrite, but the elytra are not fused.

The aedeagus of *O*. (*M.*) *minoicus* sp. n. has the same general structure as that of other species of the subgenus, but without the strongly deviating features of *O*. (*M.*) *paradoxus*, and with a simpler distal piece than *O*. (*M.*) *substrigosus* (see Jäch 1997, Figures 2 and 3). This results in an aedeagus that is surprisingly similar to those of the species of the *Cobalius*, in particular to that of *O*. (*C.*)*celatus* Jäch, 1989 (see Jäch 1989, Figure 7).

Figure 11. Type locality of *Ochthebius* (*M*.) *minoicus* sp. n.: rockpool in Elafonisi, Crete. Note the edge of the pool with some vegetation, from where specimens were flushed.

Some species of the *Cobalius* have also very regular elytral striae with strong setae, and deep fossae on the head (but without gibbosity) (e.g. *O*. (*C.*) *subinteger*). Although the ecology of the species of *Micragasma* is still poorly known, they are undoubtedly associated to coastal saline habitats, with the only know exception of the population of *O*. (*M.*) *paradoxus* near Lake Elton (Prokin *et al*., 2016). They seem to be, however, less linked to water than the species of the *Cobalius*, as suggested also by some morphological features (shorter legs, more robust pubescence), and some specimens seem to have been found in a fully terrestrial environment (e.g., Shatrovskiy, 2015). When washed into the water, specimens of *O*. (*M.*) *minoicus* sp. n. floated upside down with a film of air attached to the ventrites, as happens with other species of *Ochthebius* (including those of the *Cobalius*), suggesting that the ventral pubescence may be hydrophobic. It is interesting to note, however, that the dorsal surface is extremely hydrophilic: when dry specimens were touched with a wet brush, the whole dorsal surface was immediately covered by a film of water, maybe through the capillary effect of the tubercles and setae, or perhaps due to a possible strong hydrophilic effect of the highly modified expanded setae. The potential function of a hydrophylic dorsal surface in combination with a hydrophobic ventral surface is unknown, but it may be related with respiration or to avoid dehydration in the sometimes extreme saline and dry environment in which the species may be found.

Acknowledgements

We thank Anabela Cardoso (IBE) for laboratory work, J.M. Fortuño (ICM) for help with the SEM observations, Michaela Brocher (NMW) for the habitus photograph and M.A. Jäch (NMW) and A. Prokin for making available the specimen of *O*. (*M.*) *paradoxus* for study and M.A. Jäch for useful comments to the manuscript.

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Chapter 5

Irreversible habitat specialisation does not constraint diversification in hypersaline water beetles

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Manuscript in preparation for submission.

Abstract

Specialization to extreme environments is often considered an evolutionary dead-end, leading to irreversible adaptations and reduced evolvability. There is, however, mixed evidence of this macroevolutionary pattern, and limited data from speciose lineages. Here, we tested the effect of habitat specialization to hypersaline waters in the diversification rates of aquatic beetles of the genus *Ohthebius* (Coleoptera, Hydraenidae), using a molecular phylogeny with 279 of the 541 recognised species. Phylogenies were built with three mitochondrial and two nuclear genes, with the addition of 42 mitochondrial genomes obtained through NGS. Using Bayesian methods of character reconstruction we show that hypersaline tolerance is an irreversible adaptation that arose multiple times independently. Two lineages had a significant increase in diversification rates, one of them inhabiting hypersaline waters, but there was no overall correlation with habitat or any significant decrease in diversification rates despite the irreversibility of the hypersaline habits.

Living in extreme environments often requires key innovations that allow organisms to cope with extreme conditions, like anti-freezing proteins in cold-tolerant organisms (Duman, 2015), modifications in the life cycle or metabolism in cave beetles (Deleurance, 1963; Delay, 1978), or the tolerance to hypersaline waters (Arribas *et al*., 2018). It has been hypothesised that these traits concede species the ability to live in new environments, representing an ecological opportunity that allow species to diversify swiftly until niches are saturated, but producing a decline on diversification rates later on the history of the lineage (Rabosky, 2009; Henao-Díaz *et al*., 2019). Some extreme traits are considered evolutionary dead ends: irreversible specializations (Kelley and Farrell, 1998) that affect negatively the diversification rate, normally due to an increased extinction (Wright *et al*., 2013). However, recent works have shown that adaptations assumed to be dead-ends, such as those of subterranean organisms, have some potential for diversification due to differentiation of niches (Fišer *et al*., 2012) or differential life-histories (Cieslak *et al*., 2014). More evidence seems thus clearly needed to understand the effect of habitat specialisation on diversification rates.

During the last years, tolerance to hypersaline water - an extreme ecological specialization on aquatic Coleoptera - has been the focus of some research (Picazo *et al*., 2010; Arribas *et al*., 2014; Sabatelli *et al*., 2016; Pallarés *et al*., 2017; Villastrigo *et al*., 2018, Chapter 2). Some species of water beetles can tolerate salt concentrations several fold higher than that of the oceans (Picazo *et al*., 2010; Timms and Hammer, 1988), and together with some Diptera and Crustacea are the only macrorganisms able to live in these environments. The study of the origin of salinity tolerance in *Enochrus* Thomson (family Hydrophilidae, Arribas *et al*., 2014; Pallarés *et al*., 2017) and Hygrotini (family Dytiscidae, Villastrigo *et al*., 2018, Chapter 2) gave similar results: the ecological adaptation to hypersaline waters appeared during periods of global aridification, possibly as an exaptation to aridity, and it is irreversible. However, the limited number of species in these studies did not allow to investigate the possible relationship between salinity tolerance and diversification rates.

The aim of this research is to explore the macroevolutionary consequences of the origin and evolution of hypersalinity in the genus *Ochthebius* Leach (family Hydraenidae), the most speciose group of water beetles able to live in these environments, with lineages living in inland hypersaline streams, coastal rockpools or saltpans (Millán *et al*., 2014; Villastrigo *et al*., 2019, Chapter 3). We hypothesize that this trait evolved independently several times as in other aquatic beetles (Arribas *et al*., 2014; Villastrigo *et al*., 2018, Chapter 2), following a pattern of a dead end due to its irreversibility. As the transition to hypersaline habitats requires the evolution of traits that allow to cope with very stressful conditions of these singular aquatic systems, it may also be expected to play an important role on diversification rates, with differences among freshwater and salt tolerant lineages.

Results

Molecular phylogeny

We used the dataset based of Villastrigo *et al*. (2019, Chapter 3) with the addition of 66 specimens, complementing the data with 42 new mitogenome sequences (see Methods). This represents ca. 50% of the recognised species of *Ochthebius* (Villastrigo *et al*., 2019, Chapter 3). The best convergence in the Bayesian analyses was obtained with the simplest evolutionary model, HKY+G+I under a strict clock. The consensus topology (Fig. 1) was similar to previous phylogenies of the group (e.g. Villastrigo *et al*., 2019 Chapter 3), but with higher support values in most nodes. Main differences were the fully supported relationships between subgenera (posterior probability [pp] > 0.98), with the clade *Aulacochthebius* plus *Asiobates* as sister to the rest of *Ochthebius*, followed by *Enicocerus* plus *Hughleechia* (Fig. 1). The addition of the newly described *Micragasma minoicus* (Hernando *et al*., 2017, Chapter 4) resulted in a non-monophyletic *Micragasma*, recovering *M. minoicus* as sister to *Ochthebius* s.str. (with low support, pp= 0.59; Fig. S1) whilst the type species of the subgenus, *M. paradoxum*, was sister to subgenus *Cobalius* with strong support (Figs 1, S1). The monophyly of all subgenera and species groups (as defined in Villastrigo *et al*., 2019, Chapter 3) were strongly supported (pp > 0.99) except for the notabilis group of *Ochthebius* s.str.

Ochthebius diversification

We used three different phylogenies to estimates diversification rates: A) the consensus tree; B) the consensus tree with the manual inclusion of the missing species of *Ochthebius*; and C) 100 randomly selected post-burnin trees plus the missing species (see Methods for details on how we placed missing species in the phylogeny). We ran BAMM and MM-estimator on phylogenies B and C.

The overall diversification rate through time showed a standard pattern of decelerated speciation with time (Fig. 2). According to the Bayes factors scores, phylogeny B showed evidence of 1 to 3 diversification shifts (Fig. 3; scores 34.26, 29.44 and 21.25 respectively). Shifts were most frequently in subgenus *Asiobates* (mainly in the *bicolon* group, but also on all *Asiobates* except the puncticollis group) and the eastern Mediterranean species of *Cobalius*. Phylogenies C had a similar pattern, with 0 to 4 diversification shifts (mean number of core shifts= 1.56, mode= 1). Additional diversification shifts detected in

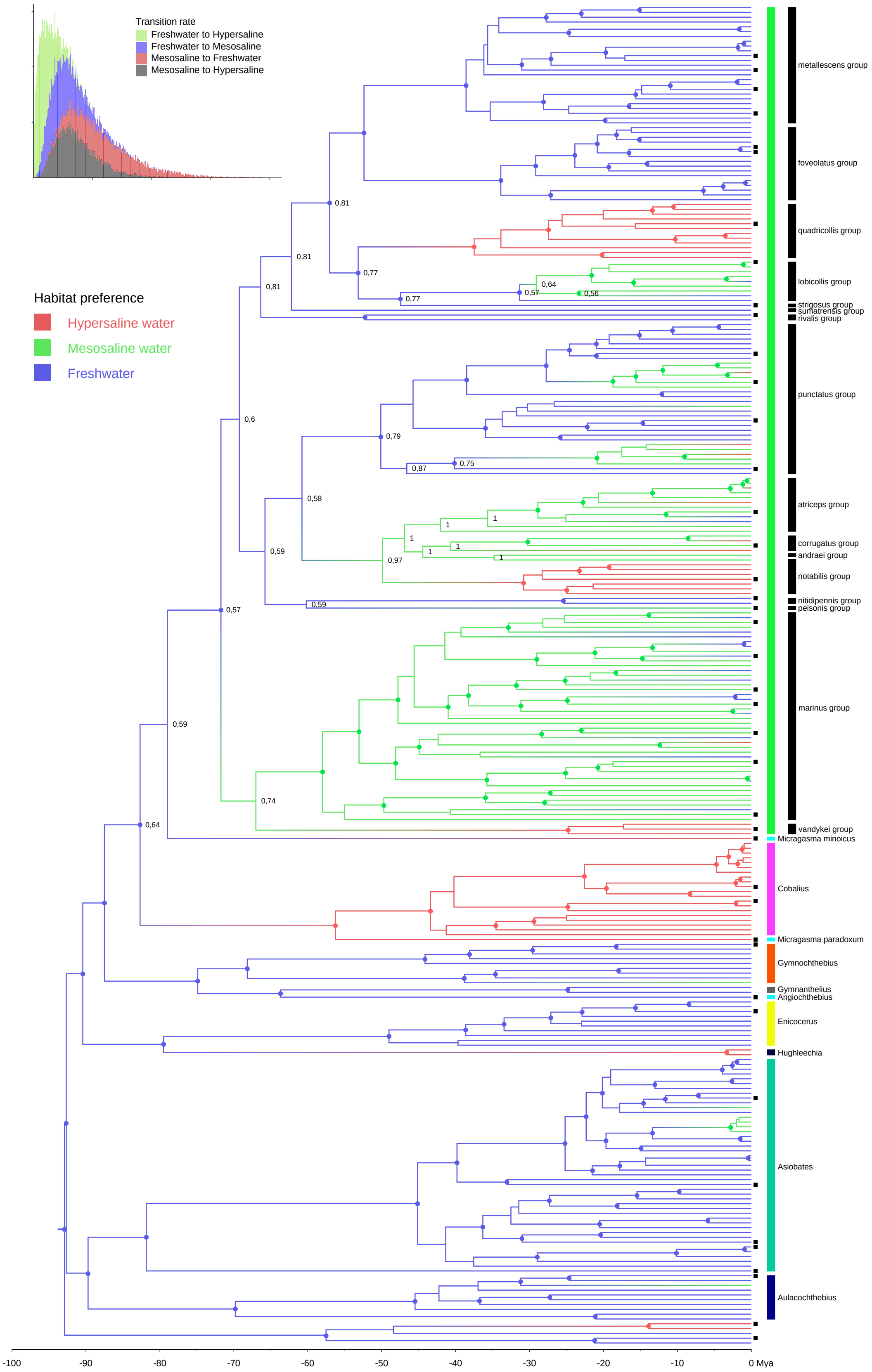

Figure 1. Time calibrated majority rule consensus tree obtained in BEAST, with habitat reconstruction. Number in nodes, posterior probability (circles when pp=1). Top-left corner, marginal frequency distribution of the estimated transitions between habitats.

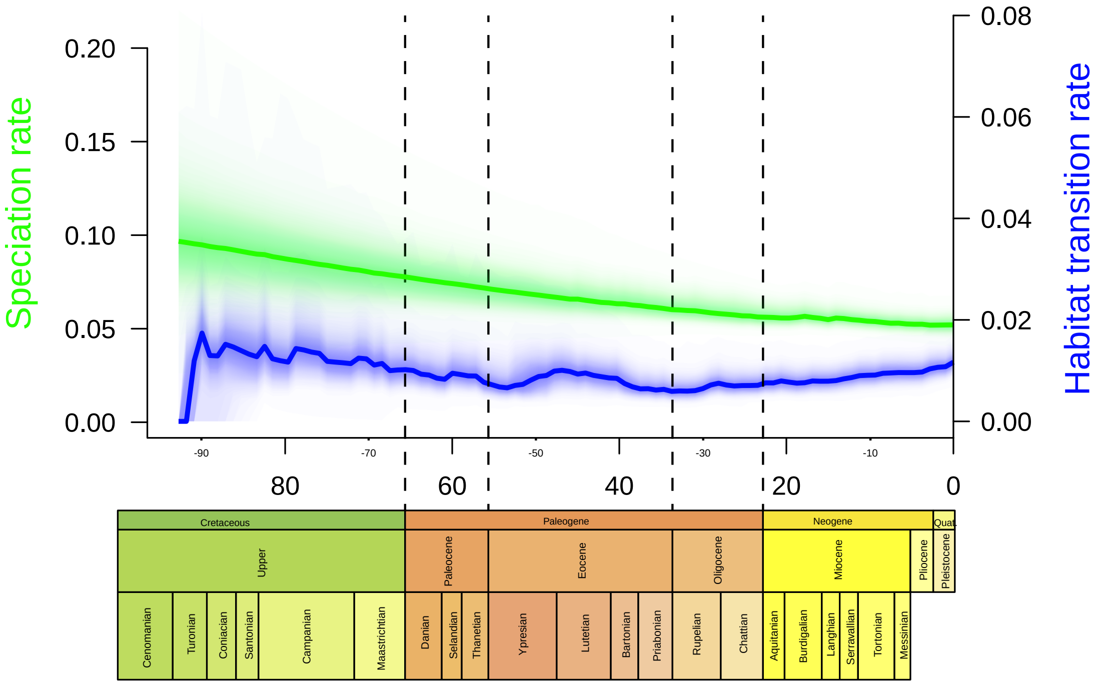

Figure 2. Speciation rate (green) and habitat transition rate (blue) of *Ochthebius* through time.

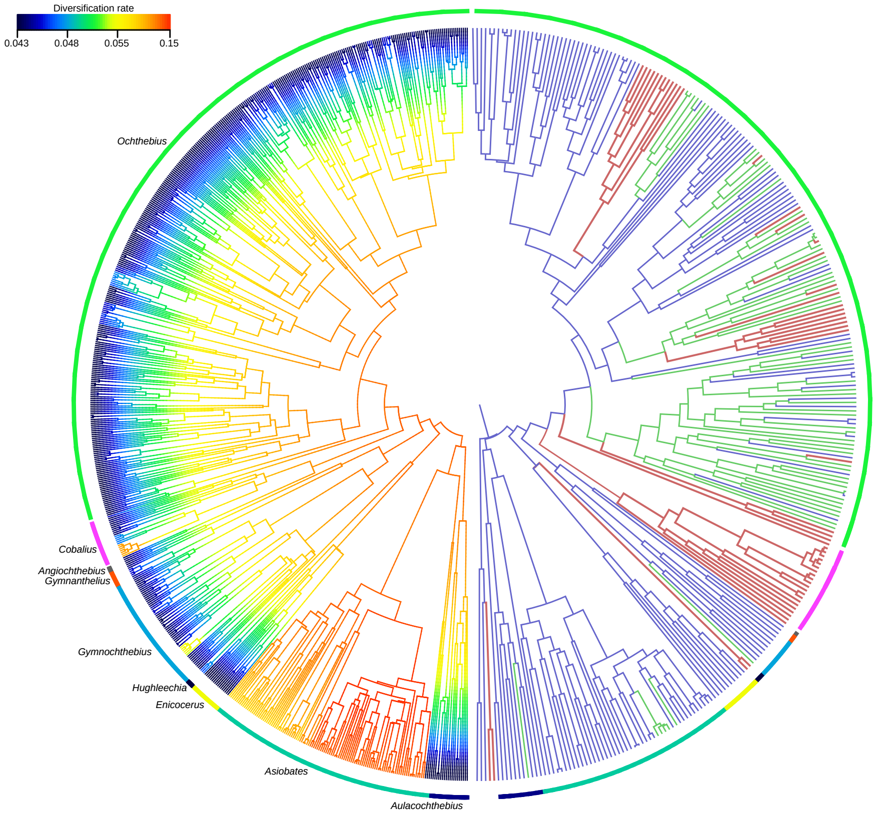

Figure 3. Left, diversification rate of *Ochthebius* obtained in Beast (warmer colours indicate a faster rate); right, habitat preference of *Ochthebius* reconstructed by Beast.

phylogenies B and C were in species groups with a low proportion of species included in the phylogeny (e.g. the *strigosus* group, Table S1). BAMM rates calculated for the main clades of phylogenies B and C separately (mean values) were basically the same, with a slightly lower rate in phylogeny B for the *bicolon* group of *Asiobates* and the eastern *Cobalius* (Table 1). The mean diversification rate for *Ochthebius* using the MM-estimator was between 0.044 and 0.062 (high extinction and no extinction fraction scenario respectively), while BAMM calculated a mean rate of 0.048, similar to the high extinction scenario (extinction fraction = 0.9). Rates of phylogeny B and the mean of diversification rates of Phylogenies C were similar, and rates calculated using MM-estimator were also similar to those with BAMM results for a mid-extinction scenario (Table 1). The only disagreements between BAMM and MM-estimator were for the rates in subgenus *Hughleechia* and the east Mediterranean *Cobalius*, with MM crown clade rates overestimated and stem clade rates underestimated in comparison with BAMM.

Table 1. Diversification rates for main groups (i.e. subgenera plus groups with higher rates) estimated by *method of moments* (stem and crown ages, ε = extinction fraction) and BAMM.

	MM estimator						**BAMM**	
	crown groups			**stem groups**				
	ε = 0	**ε = 0.5**	**ε = 0.9**	**ε = 0**	**ε = 0.5**	**ε = 0.9**	**Mean Phylogeny C**	**Consensus Phylogeny B**
Ochthebius genus	0.062	0.059	0.044	NA	NA	NA	0.048	0.048
Angiochthebius	0.040	0.035	0.012	0.006	0.006	0.002	0.051	0.049
Asiobates	0.050	0.046	0.030	0.045	0.042	0.028	0.062	0.059
Aulacochthebius	0.032	0.029	0.014	0.025	0.022	0.011	0.049	0.047
Cobalius	0.055	0.050	0.025	0.042	0.038	0.019	0.047	0.046
Enicocerus	0.044	0.039	0.018	0.027	0.024	0.011	0.047	0.046
Gymnanthelius	0.056	0.049	0.019	0.019	0.016	0.006	0.046	0.047
Gymnochthebius	0.050	0.046	0.027	0.045	0.042	0.025	0.044	0.046
Hughleechia	0.125	0.109	0.036	0.005	0.004	0.002	0.057	0.054
Ochthebius	0.073	0.069	0.050	0.066	0.063	0.045	0.045	0.045
bicolon group	0.136	0.125	0.076	0.086	0.080	0.048	0.083	0.072
East *Cobalius*	0.269	0.232	0.089	0.029	0.025	0.010	0.060	0.048

Evolution of salinity tolerance

We reconstructed multiple independent origins of salinity tolerance across the phylogeny of *Ochthebius* using a Bayesian approach in BEAST. Species were divided in three categories according to their ecological preferences: freshwater, mesohaline (i.e. tolerant) and hypersaline (see Methods). The ancestor of *Ochthebius* was estimated to have a freshwater preference, but the most common recent ancestor of subgenera *Ochthebius*, *Micragasma* and *Cobalius* was undefined, with similar probabilities for mesohaline and freshwater states (0.57/0.43, 0.59/0.38 and 0.64/0.33 for freshwater and mesohaline respectively).

Most transitions where found to be gradual, but there were some direct transitions from freshwater to hypersaline waters in old lineages with long branches (e.g. *quadricollis* group, *Micragasma minoicus*, *Cobalius* plus *Micragasma paradoxum* and *Hughleechia*). The most common estimated habitat transition was from mesohaline to freshwater, followed by mesohaline to hypersaline, freshwater to mesohaline and finally freshwater to hypersaline (Table 2). The estimated transitions from hypersaline water to mesohaline or freshwater were very low (Table 2).

Table 2. Habitat transitions rates obtained in Beast v1.10.1.

	Transition rate (transition / My)
Freshwater to hypersaline	0.438
Freshwater to mesohaline	1.116
Mesohaline to freshwater	2.634
Mesohaline to hypersaline	1.508
Hypersaline to mesohaline	0.165
Hypersaline to freshwater	0.159

The best evolutionary model for habitat preference under the Markov model estimated all transition rates independently (Table 3). All estimated transitions were gradual, and there was no reversal from the hypersaline condition. The most common transition was again from mesohaline to freshwater, followed by mesohaline to hypersaline and mesohaline to tolerant (Table 3).

Table 3. AIC weight values of three Mk models for discrete trait evolution.

	AIC weight
Equal rates model	0.00000
Symmetrical rates model	0.00005
All rates different model	0.99995

The rates calculated for habitat transitions on BAMM were not constant through time, with a tendency to increase over the last 30 million years (Fig. 2). An additional, smaller increase on rate was also identified between 55 to 45 million years ago (Fig. 2).

We further tested the influence of the trait in the diversification rates using Hidden State Speciation and Extinction (Herrera-Alsina, 2019). The best performing model using the salinity tolerance (the “examined-trait-diversification” or ETD, see methods) included four independent transition rates (Table 4), with lineages found exclusively on freshwater environments having the lowest speciation rates (λf = 0.045) compared with those that that can tolerate some range of salinity (mesohaline) (λt = 0.059) and those found in hypersaline waters (λh = 0.061, Table 4). However, when comparing this model with the constant rate (CR) and concealed trait diversification (CTD) models we found a significant effect of a concealed trait rather than the examined trait (CTD was most likely than CR and ETD models, Table 4), suggesting that despite the heterogeneity of speciation rates across the phylogeny there is no significant evidence for salinity being responsible for this speciation rate variation. Results were similar when the effect of the incomplete taxon sampling across trait states was considered (Table 4).

Table 4. Maximum Likelihood (ML) framework of SecSSE for different models of trait-dependent diversification. A) Model comparison for a set of nested examined trait-dependent diversification models (ETD) that included different numbers of transition rates (from 1 to 4) between the three examined trait states. B) Model comparison for different settings of the speciation rates: constant-rate (CR); concealed trait-dependent (CTD) and examined trait-dependent diversification (ETD) models using four transition rates between the three examined trait states. C) and D) are analogous to A) and B) but considering the potential effect of the incomplete taxon sampling across the three different trait states. Models are compared using Akaike weights (AICw), best model in bold.

A)	*model*	*k*	*ML*	*AICw*	C)	*model*	*k*	*ML*	*AICw*
	ETD_1	5	-1342.2	0.00006		ETD_1	5	-1324.8	0.00432
	ETD_2	6	-1342.1	0.00003		ETD_2	6	-1324.8	0.00160
	ETD_3	7	-1339.8	0.00010		ETD_3	7	-1322.3	0.00746
	ETD_4	**8**	**-1329.5**	**0.99981**		**ETD_4**	**8**	**-1316.4**	**0.98662**
B)	*model*	*k*	*ML*	*AICw*	D)	*model*	*k*	*ML*	*AICw*
	CR_4	6	-1330.2	0.00000		CR_4	6	-1319.2	0.00140
	CTD_4	**8**	**-1313.9**	**1.00000**		**CTD_4**	**8**	**-1310.7**	**0.99531**
	ETD_4	8	-1329.5	0.00000		ETD_4	8	-1316.4	0.00329

Discussion

Molecular phylogeny

The topology of consensus tree (A) was essentially the same as in Villastrigo *et al*. (2019, Chapter 3). The addition of mitogenome sequences into the core dataset has improved the support values of mostly all basal nodes except for a few fast speciation branches at the origin of *Ochthebius* subgenus. This unresolved fast diversification splits occurred between the end of the Cretaceous and the early Paleocene, a period of global changes linked to mass extinction (e.g. Longrich *et al*., 2012). The inclusion of *Micragasma minoicus* established *Micragasma* as polyphyletic, something unexpected given the morphological resemblance of the two sampled species, but not completely given the complex taxonomic history of the genus (Hernando *et al*., 2017, Chapter 4). The phylogenetic position of *Ochthebius zugmayeri* was also unexpected, raising questions about the taxonomical status of the *andraei*, *atriceps*, *corrugatus* and *notabilis* species groups (Jäch, 1991, 1992). Other minor unresolved relationships between species groups were likely due to an incomplete sampling (*andraei*, *nitidipennis* and *vandykei* groups) or for being part of isolated old lineages (*peisonis*, *rivalis* and *sumatrensis* groups).

Tempo and Mode of the evolution of tolerance to salinity

We found multiple origins of salinity tolerance, in accordance with previous studies (Arribas *et al*., 2014; Pallarés *et al*., 2017; Villastrigo *et al*., 2018, Chapter 2). The first appearance of tolerant ancestors was likely at the end of Campanian (Upper Cretaceous) as the most common recent ancestor of subgenera *Ochthebius* plus *Micragasma* plus *Cobalius*. The BEAST analysis was inconclusive with the reconstruction of that node, with almost the same state probability for freshwater and mesosaline but with the exclusion of a hypersaline state. However, BEAST and fitMk established mesosaline as the most common origin of habitat transitions, supporting the idea of an early origin of salinity tolerance. Based on our results, hypersalinity tolerance appeared during the Paleocene-Eocene thermal maximum (PETM), a period associated with a decreased precipitation and increased temperatures (Barrón *et al*., 2010; Zachos *et al*. 2001) which may be linked with a reduction of freshwater and the increase of saline habitats.

BAMM found non-constant rate for habitat transitions through time, starting with a general decline during Cretaceous, increasing fast at the beginning of PETM but decreasing again after 10 million years, and increasing during early Oligocene in a trend that it is currently maintained. The increase in diversification during the PETM may be related to the climatic changes of that period, and associated to the increase of the availability of saline habitats, as noted above. In agreement with this, the early Oligocene increase is again coincident with a period of abrupt climate change, and coincident also with the findings by Villastrigo *et al*. (2018, Chapter 2), who linked the appearance of hypersalinity tolerance in Dytiscidae with a global decrease in temperature in the Early Oligocene (Liu *et al*., 2009).

Tolerance to salinity evolved gradually except for some lineages linked with coastal rockpools. Two of these lineages are associated with the likely origin of salinity tolerance (see above), one without any extant close relatives (*Hughleechia*) and another (*quadricollis* group) related to mesosaline taxa. This gradual evolution is in agreement with the reconstructed evolution of Hygrotini (family Dytiscidae, Villastrigo *et al*., 2018, Chapter 2), but in contrast with the direct transition from freshwater to hypersaline species in the hydrophylid genus *Enochrus* (Arribas *et al*., 2014; Pallarés *et al*., 2017). In any case, the origin of tolerance to hypersaline in all studied groups seems to be associated with periods of increasing aridity, despite the unavoidable uncertainties in the calibration of the trees.

Diversification rate

Both BAMM and MM-estimator showed similar results when crown ages were used. There were some expected differences in clades with a small number of species (4 or less) such as *Angiochthebius* or *Hughleechia*, as rate estimates is an inverse function of clade size (Rabosky, 2019). As both analyses were coincident, only BAMM is considered below.

BAMM supported at least one shift in diversification rate. The analyses with 100 post-burnin trees showed 0 to 4 shifts, but some of them were due to artefacts on lineages with just a few species or with only one sampled species (e.g. *strigosus* group). Unsampled species were added into the consensus phylogeny randomly within the crown clade, but if there was only one species in that clade, the first species was included randomly on the stem branch, affecting the estimated diversification rate by altering the rate of speciation and detecting an artefactual shift. Without considering those artefacts, BAMM consistently detected shifts on subgenus *Asiobates* (specially on the *bicolon* group) and the eastern Mediterranean species of *Cobalius*. *Asiobates* has more than 100 species (Villastrigo *et al*., 2019, Chapter 3), almost all included in the *bicolon* and *minimus* groups. Both groups are mostly linked to freshwater environments, but many of the species are known only by the type locality or a few number of isolated records (see Jäch, 1990; Ribera, 2000 or Delgado & Jäch, 2007 for examples). The scarcity of records is probably due to their cryptic habitats, being likely that some species are linked to semi-aquatic or terrestrial lifestyles.

Most species of subgenus *Cobalius* live in coastal rockpools. The core shift in diversification was detected in a clade comprising species that live in the eastern Mediterranean islands. These islands (like Crete or Cyprus) arose early in the early Miocene - about 20 million years ago (Ring *et al*., 2001), opening a vast amount of new coastal areas to be colonized. The dispersal ability of species of *Cobalius* seems to be more limited than usually assumed, and largely determined by predominant wind currents (*personal observations*). The emergence of hundreds of islands in a reduced area represented an ecological opportunity that likely increased diversification.

Methods

Dataset and taxon sampling

We compiled ca. 200 *Ochthebius* species from Villastrigo *et al*. (2019, Chapter 3) plus four species as outgroups (genera *Meropathus* and *Tympanogaster*). To this dataset we added 66 new specimens representing 25 recognised species plus 41 unidentified species, for what we extracted their DNA using Qiagen DNeasy Tissue Kit (Hildesheim, Germany). New DNA samples and voucher specimens are stored in the collection of Institute of Evolutionary Biology (IBE, Barcelona, Spain). In total, we include 279 specimens of 209 recognised species and 6 subspecies plus 68 unidentified species of the known 541 species and 9 subspecies of *Ochthebius* sensu lato (s.l.), with examples of all subgenera and species groups except for *kosiensis* (see Villastrigo *et al*., 2019, Chapter 3) (Table S1). We amplified six genes in five sequencing reactions, including mitochondrial and nuclear genes – two cytochrome c oxidase subunit I fragments (COI-5' and COI-3'), 5' end of rrnL RNA plus leucine tRNA transfer (tRNA-L1) plus 5' end of NADH dehydrogenase subunit I (NAD1), and one internal fragment of both larger ribosomal unit (28S RNA) and small ribosomal unit (18S RNA) (see Table S4 in Villastrigo *et al*., 2019, Chapter 3, for details). Sequences were edited using Geneious v10.1 (Kearse *et al*., 2012) and were uploaded in the ENA database with accession numbers XXXXX-XXXXX (Table S1).

With the aim to increase the support of some poorly supported basal nodes at Villastrigo *et al*. (2019, Chapter 3) we obtained 42 complete or partial mitochondrial genomes (mt genomes) plus one outgroup mt genome obtained from Genbank (*Meropathus* sp., accession number KX035131). Mt genomes were obtained with a modified Mitochondrial metagenomics protocol based on Crampton-Platt *et al*. (2016): DNA extractions for species with less than 95% pairwise similarities based on the COI gene were equimolarly pooled, and libraries were prepared with TruSeq Nano DNA kit. In total, four libraries were run in two Illumina MiSeq runs (2 x 300 bp). Libraries were trimmed and filtered with Trimmomatic (Bolger *et al*., 2014) and PrinSEQ (Schmieder & Edwards, 2011) respectively. Non mitochondrial reads were excluded with BLAST (Madden *et al*., 2009) prior to assembly with four different approaches: (i) IDBA-UD (Peng *et al*., 2012), (ii) RAY-meta (Boisvert *et al*., 2012), (iii) WGS-assembler (Myers *et al*., 2000) and (iv) SPAdes (Bankevich *et al*., 2012). Contigs found in at least three assemblers were considered valid and were identified using Sanger sequences of COI and rrnL genes. Consensus contigs were annotated with MITOS WebServer (Bernt *et al*., 2013), checking every note by hand using reference sequences available of closely related aquatic beetles on Genbank (Clark et al., 2016). Newly obtained sequences have been deposited in Genbank (XXXXXXX-XXXXXXX; Table S1), and a list of samples per gene and their length can be found in Table S2.

Phylogenetic analyses

Genes and tRNAs sequences extracted from the mt genomes plus Sanger sequences were aligned with the MAFFT plugin (Katoh *et al*., 2009) in Geneious v10.1 with the G-INS-I algorithm. We established seven partitions according to the type of data: (i) nuclear genes (28S plus 18S), (ii) tRNA present in both the Sanger sequencing and the mt genomes (tRNA-L1), (iii) the remaining tRNAs, (iv) protein coding genes present in both the Sanger sequencing and the mt genomes (COI plus NAD1), (v) the remaining 11 protein coding genes, (vi) rRNAs present in both the Sanger sequencing and the mt genomes (16S), (vii) the remaining rRNAs (12S) (see S2 for more information). We tested four different scenarios, combining two evolutionary models (HKY+G+I and GTR+G+I) and two clocks models (uncorrelated relaxed lognormal and strict) with Beast v10.0.1 (Suchard *et al*., 2018) under a Yule speciation process as tree prior. For dating the phylogeny, we used the crown age of Ochthebiini estimated by Villastrigo *et al*. (2019, Chapter 3) (93 Ma), as a normal prior with SD 0.01. Convergence of all parameters were checked on Tracer v.1.7.1 (Rambaut *et al*., 2018) in order to select the best model.

The best model was used to reconstruct different phylogenies in BEAST, in all of them without outgroups and constraining the basal node to match the topology with outgroups: (A) consensus tree, (B) consensus tree plus unsampled species and (C) 100 randomly selected post-burnin trees plus unsampled species. We included the remaining 343 unsampled species and 5 subspecies of *Ochthebius* s.l. randomly using the *phytools* r-package (Revell, 2012) following current recognised species groups and subgenera (Villastrigo *et al*., 2019, Chapter 3). Two *incertae sedis* species were discarded (*Ochthebius belucistanicus* and *Ochthebius eremita*), and the species of the *kosiensis* group of *Ochthebius* s.l. were clustered together with that of the *strigosus* group based on their morphological similarities (Villastrigo *et al*., 2019, Chapter 3; Jäch, 2003).

Diversification analyses

We used Bayesian Analysis of Macroevolutionary Mixtures - BAMM (Rabosky, 2014) to detect shifts in diversification rates for all subgenera except *Micragasma*, as it was not reconstructed as monophyletic. Given the current controversy around BAMM (Moore *et al*., 2016; Rabosky *et al*., 2017; Meyer *et al*., 2018; Meyer & Wiens, 2018; Rabosky, 2018), we also calculate diversification rates based on the *method of moments* estimators (MM-estimator) (Magallón & Sanderson, 2001) using *geiger* r-package (Harmon et al., 2008). Both methodologies were assessed using the complete phylogenies (i.e. with the inclusion of the non-sampled species).

BAMM analyses were run on phylogenies B and C. Priors were calculate using setBAMMpriors from *BAMMtools* r-package (Rabosky *et al*., 2014) during 10 million generations unless the effective sample sizes where below 200, in which cases they were run for 20 million generations. MM-estimator were calculated under three extinction fractions, (i) low ($\varepsilon = 0.0$), (ii) intermediate ($\varepsilon = 0.5$), and (iii) high ($\varepsilon = 0.9$) for both crown and stem subgenera. Additionally, we calculated diversification rates for the lineages where BAMM detected diversification shifts.

Evolution of salinity tolerance

Ecological data on habitat preference were obtained for all the species with molecular data based on bibliography and direct observation on the field (Table S1). As quantitative data on environmental salinity were sparse, we reconstructed habitat preference using three qualitative states, as in similar previous study (Villastrigo *et al*., 2018, Chapter 2): (i) species found exclusively on freshwater environments, (ii) species that can tolerate some range of salinity (including freshwater) but never (or only very occasionally) found on hypersaline environments, and (iii) species that are mostly restricted to hypersaline waters, found regularly on them (> 40 g/L). Analyses were run on the consensus tree after deleting the outgroups.

Habitat reconstruction were performed by BEAST using an asymmetrical model of trait evolution, and AIC weights (Wagenmakers & Farrell, 2004) was used to compare different model of discrete trait evolution under a MK model (fitMK function on *phytools* r-package): model with equal rates, model with symmetrical rates and model with different rates among states. BAMM was used to detect shifts in transition rates associated with habitat preference.

Trait-dependent diversification was analysed using SecSSE (Herrera-Alsina *et al*., 2019), that allows to simultaneously infer state-dependent diversification across multiple (observed) trait states while accounting for the role of a possible concealed (hidden) trait. Firstly, we compared among a set of nested Examined-Trait-Dependent speciation models (ETD, see below) that included different numbers of transition rates between the examined trait states. Four models were tested from single to four transition rates that were sequentially constrained according with the results of fitMk (all different rates as best model, see details in Table 3). Second, we compared models with different settings for the speciation rates: (i) a Constant Rates model (CR) where all species have the same speciation rate λ regardless of their trait state; (ii) a Concealed-Trait-Dependent speciation model (CTD) where speciation rates are allowed to vary only between concealed (hidden) trait states and (iii) the Examined-Trait-Dependent speciation model (ETD) where the speciation rates are allowed to vary only between the examined trait states. For all the models the Maximum Likelihood search was performed with the simplex algorithm offered by the *DDD* package, using the best transition rates constraint as tested before and the rest of parameters as recommended1. Finally, model selection based on Akaike weights was carried to select the best-performing model in each comparison. Additionally, all the analyses were repeated as before but considering the potential effect of the incomplete taxon sampling across different trait states, i.e. including *sampling_fraction* = c (0.64, 0.39, 0.36) according with the number of species in the phylogeny by the total number of described species for freshwater, tolerant and hypersaline states.

Acknowledgements

We thank all collectors mentioned in Table S1 for their contribution to this paper. AV has a FPI-MINECO PhD grant (BES-2014-069398) from the Spanish Government and part of the analyses were done under a short-stay grant (EEBB-I-17-11993) from the Spanish Goverment. This work has been partially funded by project CGL2013-48950-C2 (AEI/FEDER EU) to IR.

Author contributions

PA and IR designed the study. AV, PA and IR prepared the methodology. AV and AC obtain sanger sequences. AV and PA obtain mitogenome sequences. AV and PA ran the analyses. AV, PA and IR prepared the manuscript with contributions of AC.

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Supporting Information

Table S1. List of material used in the molecular phylogeny, including voucher numbers, accession numbers of the sequences and locality data

Table S2. List of genes used in phylogenetic reconstruction, including samples per gene and length of each aligment.

Figure S1. Majority rule consensus tree obtained in Beast. Number in nodes: posterior probability values.

Table S1. List of material used in the molecular phylogeny, including voucher numbers, accession numbers of the sequences and locality data.

all	genus	subgenus	species group	species	subspecies	salinity	voucher	locality	leg	mtgenome	COI-5	COI-3	rrnL	18S	28S
1	*Meropathus*	*Hygrotympanogaster*		*schizolabra*			IBE-AF167	Australia; Victoria, Beech Forest below Hopetoun Falls 21.7.2009	I. Ribera & A. Cieslak		LT991263	LT991452	LT990692	LT990836	LT991041
2	*Meropathus*	*Tympanogaster*		*deanei*			MNCN-AI372	Australia; Queensland, 40 km SSE Brisbane, Cedar Creek Road, above Cedar Creek Falls 22.8.2004	M.A. Jäch		LT991264	LT991453	LT990693	LT990837	LT991042
3	*Meropathus*	*Tympanogaster*		*modulatrix*			IBE-AN183	Australia; Queensland, Lamington NP, Morans falls 26.4.2014	J. Maté	**XXXXXX**	LT991265	LT991454	LT990694	LT990838	LT991043
4	*Meropathus*	*Meropathus*		*zealandicus*			MNCN-AI715	New Zealand; South Island, Papatowai, beach 7.12.2005	M. Thayer, A. Newton & J. Nunn		LT991262	LT991451	LT990691	LT990835	LT991040
5	*Meropathus*	*Meropathus*		*vectis*			genbank	-	-	**XXXXXX**					
6	*Ochthebius*	*Angiochthebius*		*plesiotypus*		Freshwater	MNCN-AI562	Chile; Región Metropolitana, Alto Cantillana 8.1.2001	M. Guerrero	**XXXXXX**	LT991267	LT991456	LT990696	LT990840	LT991045
7	*Ochthebius*	*Asiobates*	*bicolon*	*arator*		Freshwater	IBE-AN185	Turkey; Uşak, Çatalbayır village 19.6.2012	N. Ertorun		LT991268	LT991457		LT990841	LT991046
8	*Ochthebius*	*Asiobates*	*bicolon*	*auriculatus*		Tolerant	MNCN-AH154	England (UK); S Devon, Dawlish Warren Saltmarsh 29.9.2007	D.T. Bilton		LT991269	HF931134	HF931352	LT990842	LT991047
9	*Ochthebius*	*Asiobates*	*bicolon*	*bellieri*		Freshwater	IBE-AN290	Spain; Albacete, Paterna del Madera, Arroyo de la Fuenfría 30.7.1998	I. Ribera		LT991270	LT991458	LT990697	LT990843	LT991048
10	*Ochthebius*	*Asiobates*	*bicolon*	*bicolon*		Freshwater	IBE-RA1171	England (UK); N Devon, 1 km S Hartland Quay, stream 28.7.2013	D.T. Bilton		LT991271	LT991459	LT990698	LT990844	LT991049
11	*Ochthebius*	*Asiobates*	*bicolon*	*bonnairei*		Freshwater	MNCN-AI1272	Spain; Jaén, Santiago de la Espada, Río Zumeta 16.6.2006	A. Millán & collaborators		LT991272	HF931177	HF931398	LT990845	LT991050
12	*Ochthebius*	*Asiobates*	*bicolon*	*cantabricus* cf.		Freshwater	MNCN-AI1027	Turkey; Kastamonu, Aşağı Kayı 27.4.2006	A. Castro		LT991273	HF931145	HF931364	LT990846	LT991051
13	*Ochthebius*	*Asiobates*	*bicolon*	*corsicus*		Freshwater	IBE-AN159	Corsica (France); Col de Bavella 14.5.2014	R. Vila		LT991274	LT991460	LT990699	LT990847	LT991052
14	*Ochthebius*	*Asiobates*	*bicolon*	*crenulatus*		Freshwater	MNCN-AH159	Sicily (Italy); Parco dei Nebrodi, trail Lago Urio - Portella dell'Obolo 13.6.2007	P. Abellán & F. Picazo		LT991275	HF931136	HF931353	LT990848	LT991053
15	*Ochthebius*	*Asiobates*	*bicolon*	*dilatatus*		Freshwater	MNCN-AI792	Turkey; Bartın, between Topallar & Çakraz, calcareous stream 25.4.2006	I. Ribera		LT991276	HF931227	HF931456	LT990849	LT991054
16	*Ochthebius*	*Asiobates*	*bicolon*	*ferroi*		Freshwater	IBE-PB28	Spain; Huesca, Sta. Cruz de Serós, Barranco Carbonera 3.8.2013	I. Esteban		HG915303	LT991461	LT990700	LT990850	LT991055
17	*Ochthebius*	*Asiobates*	*bicolon*	*figueroi*		Freshwater	IBE-AN23	Spain; Cantabria, Río Soto 15.7.2014	D.T. Bilton		LT991277	LT991462	LT990701	LT990851	LT991056
18	*Ochthebius*	*Asiobates*	*bicolon*	*gagliardii*		Freshwater	IBE-RA121	Italy; Toscana, Lorenzana, Gello Mattaccino, pond 26.5.2008	I. Ribera		LT991278	HF931301	LT990702	LT990852	LT991057
19	*Ochthebius*	*Asiobates*	*bicolon*	*heydeni*		Freshwater	MNCN-AI390	Spain; Ourense, Sierra de Queixa, river San Lázaro 9.7.2005	I. Ribera & A. Cieslak		LT991279	HF931194	HF931419	LT990853	LT991058
20	*Ochthebius*	*Asiobates*	*bicolon*	*immaculatus*		Tolerant	IBE-AN434	Mallorca (Spain); Mallorca, Salines de Llevant 28.3.2016	I. Ribera & A. Cieslak		LT991280	LT991463	LT990703	LT990854	LT991059
21	*Ochthebius*	*Asiobates*	*bicolon*	*irenae*		Tolerant	MNCN-AI986	Spain; Navarra, Tudela, ditch near Balsa de Purguer 20.7.2004	I. Ribera & A. Cieslak		HG915302	HF931261	HF931497	LT990855	LT991060
22	*Ochthebius*	*Asiobates*	*bicolon*	*jaimei*		Tolerant	IBE-RA1081	Spain; Alicante, Albatera 12.5.2013	I. Esteban		LT991281	HG915307	LT990704	LT990856	LT991061
23	*Ochthebius*	*Asiobates*	*bicolon*	*lederi*		Freshwater	IBE-AN63	Azerbaijan; Alishanli, 7 km NE Masally 8.5.2014	I. Ribera & A. Rudoy	**XXXXXX**	LT991282	LT991464	LT990705	LT990857	LT991062
24	*Ochthebius*	*Asiobates*	*bicolon*	*lenkoranus*		Freshwater	IBE-AN75	Azerbaijan; Gosmalijion, stream 7.5.2014	I. Ribera & A. Rudoy		LT991283	LT991465	LT990706	LT990858	LT991063
25	*Ochthebius*	*Asiobates*	*bicolon*	*montanus*		Freshwater	IBE-AN207	Greece; Evvoia, Seta, mountain stream 8.4.2013	I. Ribera & A. Cieslak		LT991284	LT991466	LT990707	LT990859	LT991064
26	*Ochthebius*	*Asiobates*	*bicolon*	*opacus*		Freshwater	MNCN-AI389	Italy; Liguria, 2 km E Cosio di Arroscia 31.7.2005	I. Ribera & A. Cieslak		LT991285	HF931193	HF931418	LT990860	LT991065
27	*Ochthebius*	*Asiobates*	*bicolon*	*peregrinus*		Tolerant	IBE-AN206	Greece; Sterea Ellada, Fokida, Itea, stream E of Kirra 7.4.2013	I. Ribera & A. Cieslak		LT991286	LT991467	LT990708	LT990861	LT991066
28	*Ochthebius*	*Asiobates*	*bicolon*	sp.		Freshwater	IBE-AN74	Azerbaijan; Elabad, road to Garaybayli, stream 4.5.2014	I. Ribera & A. Rudoy		LT991287	LT991468	LT990709	LT990862	LT991067
29	*Ochthebius*	*Asiobates*	*bicolon*	sp.		Freshwater	IBE-AV154	Kyrgyzstan; Kara-Darya, Uzgen 4.7.2017	D. Palatov		**XXXXXX**	**XXXXXX**	**XXXXXX**	**XXXXXX**	**XXXXXX**
30	*Ochthebius*	*Asiobates*	*bicolon*	*striatus*		Freshwater	MNCN-AI787	Turkey; Bartın, between Amasra & Inpiri, spring & stream 25.4.2006	I. Ribera		LT991288	HF931226	HF931455	LT990863	LT991068
31	*Ochthebius*	*Asiobates*	*bicolon*	*stygialis*		Freshwater	IBE-AN160	Turkey; Antalya, 1 km N Hacıyusuflar 24.6.2014	D.T. Bilton		LT991289	LT991469	LT990710	LT990864	LT991069
32	*Ochthebius*	*Asiobates*	*minimus*	*aeneus*		Freshwater	MNCN-AI914	Morocco; Ou-Maghous, Amaghouz 24.4.2000	I. Ribera, P. Aguilera, C. Hernando & A. Millán		LT991290	HF931240	HF931472	LT990865	LT991070
33	*Ochthebius*	*Asiobates*	*minimus*	*adventicius*		Freshwater	IBE-AV122	Russia; Adygea, Maikop Distr. 3.9.2014	Saprykin & Khiryanov		**XXXXXX**	**XXXXXX**	**XXXXXX**	**XXXXXX**	**XXXXXX**

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Table S1. (continued)

all	genus	subgenus	species group	species	subspecies	salinity	voucher	locality	leg	mtgenome	COI-5	COI-3	rrnL	18S	28S
34	*Ochthebius*	*Asiobates*	*minimus*	*alpinus*		Freshwater	IBE-RA1114	Belarus; Berezinsky Biosphere Reserve 24.6.2013	I. Ribera		LT991291	LT991470	LT990711	LT990866	LT991071
35	*Ochthebius*	*Asiobates*	*minimus*	*andreiini*	*andreiini*	Freshwater	IBE-AN20	Ethiopia; Amhara, 30 km NE Gashena 13.6.2014	R. Vila & G. Talavera		LT991292	LT991471	LT990712	LT990867	LT991072
36	*Ochthebius*	*Asiobates*	*minimus*	sp.		Freshwater	IBE-AN104	Ethiopia; Oromia, Bale Mountains, Goba Forest, Togona River 22.2.2014	M.A. Jäch	XXXXXX	LT991293	LT991472	LT990713	LT990868	LT991073
37	*Ochthebius*	*Asiobates*	*minimus*	*andronius*		Freshwater	MNCN-AI498	South Africa; West Cape, Prince Albert Road, pond 23.3.2001	I. Ribera & A. Cieslak		LT991294	LT991473	LT990714	LT990869	LT991074
38	*Ochthebius*	*Asiobates*	*minimus*	*discretus*		Freshwater	MNCN-AI503	California (USA); Trinity Co., Forest Glen, Rattlesnake Creek 22.6.2000	I. Ribera & A. Cieslak		LT991295	LT991474	LT990715	LT990870	LT991075
39	*Ochthebius*	*Asiobates*	*minimus*	*flavipes*		Freshwater	IBE-RA437	Slovakia; Hámske tŕstie, near Čičov, ditch 7.6.2009	I. Ribera		LT991296	HF931315	LT990716	LT990871	LT991076
40	*Ochthebius*	*Asiobates*	*minimus*	*hokkaidensis*		Freshwater	IBE-AF213	Japan; Hokkaido, Togeshita, Rumoi-shi 18.7.2007	H. Yoshitomi			HF931125	HF931344	LT990872	LT991077
41	*Ochthebius*	*Asiobates*	*minimus*	*minimus*		Freshwater	MNCN-AI447	Slovakia; Banská Bystrica Region, Cerovo 2005	via R.G. Beutel		HE970917	HE970842	HE971074	HE970955	HE970995
42	*Ochthebius*	*Asiobates*	*minimus*	*perdurus*		Freshwater	IBE-AV151	China; Xinjiang 24.7.2017	D. Palatov		XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
43	*Ochthebius*	*Asiobates*	*minimus*	*remotus*		Freshwater	MNCN-AI1030	Turkey; Karabük, stream in Çayörengüney 28.4.2006	A. Castro	XXXXXX	LT991297	HF931148	HF931367	LT990873	LT991078
44	*Ochthebius*	*Asiobates*	*minimus*	*rugulosus*		Freshwater	IBE-AN822	Greece; Crete, Latomia, rockpools 18.4.2017	C. Hernando, I. Ribera & A. Villastrigo		XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
45	*Ochthebius*	*Asiobates*	*minimus*	sp.		Freshwater	MNCN-AI637	Gran Canaria (Spain); Moya, Barranco de Azuaje 15.4.2001	I. Ribera & A. Cieslak		LT991298	HF931219	HF931447	LT990874	LT991079
46	*Ochthebius*	*Asiobates*	*minimus*	*sanabrensis*		Freshwater	MNCN-AH75	Spain; Zamora, Parque Natural Lago Sanabria, Laguna de la Yegua 4.7.2007	L.F. Valladares		LT991299	EU660055	HF931357		LT991080
47	*Ochthebius*	*Asiobates*	*minimus*	sp.		Freshwater	IBE-AN292	Alberta (Canada); 2 km W Lundbreck 27.6.2000	I. Ribera & A. Cieslak	XXXXXX	LT991300	LT991475	LT990717	LT990875	LT991081
48	*Ochthebius*	*Asiobates*	*minimus*	sp.		Freshwater	IBE-AN97	Ethiopia; Oromia, Jemjem Forest, small stream 25.2.2014	M.A. Jäch		LT991301	LT991476	LT990718	LT990876	LT991082
49	*Ochthebius*	*Asiobates*	*minimus*	sp.		Freshwater	IBE-AN93	Ethiopia; Amhara, Debark, Simien Mountains 18.6.2014	R. Vila & G. Talavera		LT991302	LT991477	LT990719	LT990877	LT991083
50	*Ochthebius*	*Asiobates*	*minimus*	sp.		Freshwater	IBE-AV167	Cameroon; 22.1.2018	R. Vila		XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
51	*Ochthebius*	*Asiobates*	*puncticollis*	*puncticollis*		Freshwater	MNCN-AI1274	California (USA); Santa Barbara Co., Sedgewick Reserve 6.7.2006	A.E.Z. Short	XXXXXX	LT991303	LT991478	LT990720		LT991084
52	*Ochthebius*	*Aulacochthebius*		*exaratus*		Tolerant	MNCN-AI453	Tunisia; road Jendouba-Makthar, 1 km NW crossroad to Mt. Kebbouch 24.10.2001	I. Ribera & A. Cieslak		LT991304	HF931202	HF931427	LT990878	LT991085
53	*Ochthebius*	*Aulacochthebius*		*libertarius*		Freshwater	MNCN-AI421	Morocco; Ou-Maghous, Amaghouz 24.4.2000	I. Ribera, P. Aguilera, C. Hernando & A. Millán		LT991305	HF931196	HF931421	LT990879	LT991086
54	*Ochthebius*	*Aulacochthebius*		*narentinus*		Freshwater	IBE-AF161	Slovakia; Studienka, Rudava river 5.6.2009	I. Ribera		LT991306	HF931120	HF931338	LT990880	LT991087
55	*Ochthebius*	*Aulacochthebius*		*perlaevis*		Freshwater	MNCN-AI548	Madagascar; Ankarana, 1er canyon, river 12.2004	M. Balke		LT991307	HF931211	HF931437	LT990881	LT991088
56	*Ochthebius*	*Aulacochthebius*		sp.		Freshwater	IBE-AN390	South Africa; West Cape, Baardskeerdersbos, Boesmans River 10.5.2010	J. Den Heijer & A. Hidalgo-Galiana		LT991308	LT991479	XXXXXX	LT990882	LT991089
57	*Ochthebius*	*Aulacochthebius*		sp.		Freshwater	MNCN-AI499	South Africa; West Cape, 3 km SE Franschhoek 25.3.2001	I. Ribera & A. Cieslak		LT991309	LT991480	LT990721	LT990883	LT991090
58	*Ochthebius*	*Aulacochthebius*		sp.		Freshwater	MNCN-AI501	South Africa; Eastern Cape, Kareedouwberg, river in Skoop Drif 23.3.2001	I. Ribera & A. Cieslak		LT991310	HF931207	HF931432	LT990884	LT991091
59	*Ochthebius*	*Aulacochthebius*		sp.		Freshwater	MNCN-AI519	Bhutan; Sarpang, 11 km NW Sarpang, Bhur Khola river 27.11.2005	M.A. Jäch		LT991311	LT991481	LT990722	LT990885	LT991092
60	*Ochthebius*	*Aulacochthebius*		sp.		Freshwater	MNCN-AI520	Bhutan; Punakha, 16 km NW Punakha, Mo Chhu river 28.11.2005	M.A. Jäch		LT991312	LT991482	LT990723	LT990886	LT991093
61	*Ochthebius*	*Aulacochthebius*		sp.		Freshwater	IBE-RA1155	Tanzania; Mwanza Region, near Kashishi, ponds 24.7.2010	R. Sites & A. Mbogho	XXXXXX	LT991313	LT991483	LT990724	LT990887	LT991094
62	*Ochthebius*	*Cobalius*		*adriaticus*	*adriaticus*	Hypersaline	IBE-AN787	Croatia; Trsteno, rockpools 5.5.2017	A. Faille, J. Fresneda & I. Ribera		LT991314	LT991484	LT990725	LT990888	LT991095
63	*Ochthebius*	*Cobalius*		*adriaticus*	*moreanus*	Hypersaline	IBE-AV80	Greece; Crete, Elafonisi, rockpools 20.4.2017	C. Hernando, I. Ribera & A. Villastrigo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
64	*Ochthebius*	*Cobalius*		sp.		Hypersaline	IBE-AN821	Greece; Crete, Latomia, rockpools 18.4.2017	C. Hernando, I. Ribera & A. Villastrigo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
65	*Ochthebius*	*Cobalius*		*algicola*		Hypersaline	IBE-AV117	Portugal; Madeira. Camara de Lobos 17.10.2017	I. Ribera & A. Cieslak		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
66	*Ochthebius*	*Cobalius*		*asper*		Hypersaline	IBE-AV217	Spain; Girona, Cala de Sant Francesc	A. Cardoso & A. Villastrigo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX

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Table S1. (continued)

all	genus	subgenus	species group	species	subspecies	salinity	voucher	locality	leg	mtgenome	COI-5	COI-3	rrnL	18S	28S
67	*Ochthebius*	*Cobalius*		*balfourbrownei*		Hypersaline	genbank	-	-			XXXXXXX	XXXXXXX		XXXXXXX
68	*Ochthebius*	*Cobalius*		*biltoni* gr1		Hypersaline	IBE-AV177	Morocco; Temara, rockpools 11.4.2018	Alfambra, C. Hernando, I. Ribera & A. Villastrigo	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
69	*Ochthebius*	*Cobalius*		*biltoni* gr2		Hypersaline	IBE-AV193	Morocco; Essaouira, rockpools 9.4.2018	Alfambra, C. Hernando, I. Ribera & A. Villastrigo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
70	*Ochthebius*	*Cobalius*		*celatus*		Hypersaline	IBE-AN441	Cyprus; Akamas peninsula, Lara beach 29.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo		LT991315	LT991485	LT990726	LT990889	LT991096
71	*Ochthebius*	*Cobalius*		sp.		Hypersaline	IBE-AN838	Italy; Civitavecchia, rockpools 12.5.2017	A. Faille, J. Fresneda & I. Ribera		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
72	*Ochthebius*	*Cobalius*		*freyi*		Hypersaline	IBE-RA1197	Azores (Portugal); Terceira, São Pedro, rockpools 4.9.2012	I. Ribera		LT991316	LT991486	LT990727	LT990890	LT991097
73	*Ochthebius*	*Cobalius*		*lanthanus*		Hypersaline	IBE-AV169	Spain; Gran Canaria, Taliarte, rockpools 14.3.2018	A. Millán, I. Ribera & A. Villastrigo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
74	*Ochthebius*	*Cobalius*		*lejolisii*		Hypersaline	MNCN-AI513	Spain; Pontevedra, Nigran, Playa Patos 3.10.2005	J. Garrido		LT991317	HF931208	HF931433	LT990891	LT991098
75	*Ochthebius*	*Cobalius*		sp.		Hypersaline	IBE-RA1232	Spain; Ceuta, Cala del Desnarigado, 16.11.2013	J.L. Ruiz & K. Bensusan	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX		
76	*Ochthebius*	*Cobalius*		sp.		Hypersaline	IBE-AV181	Morocoo; Sidi Ifni, rockpools 6.4.2018	Alfambra, C. Hernando, I. Ribera & A. Villastrigo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
77	*Ochthebius*	*Cobalius*		*serratus*		Hypersaline	MNCN-AI1194	Morocco; El Ouedaya, Oued Sebt 18.4.2006	A. Millán & collaborators		LT991318	HF931171	HF931391	LT990892	LT991099
78	*Ochthebius*	*Cobalius*		sp.		Hypersaline	IBE-AV180	Morocoo; Sidi Ifni, rockpools 6.4.2018	Alfambra, C. Hernando, I. Ribera & A. Villastrigo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
79	*Ochthebius*	*Cobalius*		*subinteger*		Hypersaline	MNCN-AI432	Corsica (France); Cap Corse, Port de Centuri 20.9.2000	I. Ribera & A. Cieslak		LT991319	HF931200	HF931425	LT990893	LT991100
80	*Ochthebius*	*Cobalius*		sp.		Hypersaline	IBE-AV100	Spain; Cabrera, Caló de ses Agulles 5.7.2017	I. Ribera & A. Faille		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
81	*Ochthebius*	*Cobalius*		sp.		Hypersaline	IBE-AV79	Greece; Crete, Pacheia Ammos, rockpools 17.4.2017	C. Hernando, I. Ribera & A. Villastrigo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
82	*Ochthebius*	*Enicocerus*		*aguilerai*		Freshwater	MNCN-AI387	Spain; Ávila, Arenas de San Pedro, río Pelayo 15.5.2005	I. Ribera		HF947939	GU143761	GU143728	GU143770	GU143778
83	*Ochthebius*	*Enicocerus*		*colveranus*		Freshwater	MNCN-AI818	Turkey; Kastamonu, fast stream in forest 28.4.2006	I. Ribera	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
84	*Ochthebius*	*Enicocerus*		*exsculptus*		Freshwater	MNCN-AI374	Spain; Barcelona, Guardiola del Berguedà, torrent Gavarrós 25.7.2005	I. Ribera		LT991321	GU143763	GU143741	GU143772	GU143780
85	*Ochthebius*	*Enicocerus*		sp.		Freshwater	MNCN-AI925	Spain; Albacete, Sierra de Segura, 10 km N Yeste, Rio Tús 10.4.2003	V. Assing & P. Wunderle		LT991322	GU143750	GU143737	LT990894	LT991101
86	*Ochthebius*	*Enicocerus*		*gibbosus*		Freshwater	MNCN-AI365	Italy; Piemonte, Móngia, torrente Móngia 31.7.2005	I. Ribera & A. Cieslak		LT991323	GU143755	GU143727	GU143769	GU143777
87	*Ochthebius*	*Enicocerus*		*granulatus*		Freshwater	MNCN-AI427	Austria; Niederösterreich, Kleiner Ötscherbach, Langau 25.9.2005	M.A. Jäch		LT991324	GU143765	GU143725	GU143774	GU143782
88	*Ochthebius*	*Enicocerus*		*halbherri*		Freshwater	MNCN-AH190	Italy; Emilia Romagna, Bosco, Torrente Parma 24.5.2008	I. Ribera		LT991325	GU143764	GU143736	GU143773	GU143781
89	*Ochthebius*	*Enicocerus*		*legionensis*		Freshwater	MNCN-AI507	Spain; León, Puerto de Panderrueda 12.7.2005	L.F. Valladares		HF947941	GU143762	GU143735	GU143771	GU143779
90	*Ochthebius*	*Enicocerus*		*melanescens*		Freshwater	MNCN-AI344	Austria; Niederösterreich, Schwarzenbach, Sankt Veit an der Gölsen 6.8.2005	I. Ribera & A. Cieslak		HE970900	GU143766	GU143743	GU143775	GU143783
91	*Ochthebius*	*Enicocerus*		*saboorii*		Freshwater	IBE-RA739	Iran; Mazandaran, near Nowshar, Kheiroud Kenar Forest 2.5.2010	A. Skale		LT991326		LT990728	LT990895	LT991102
92	*Ochthebius*	*Gymnanthelius*		*opacicollis*		Freshwater	IBE-AF162	Australia; Victoria, Allambee, 19 km N Leongatha 8.7.2009	I. Ribera & A. Cieslak		LT991327	LT991487	LT990729	LT990896	LT991103
93	*Ochthebius*	*Gymnanthelius*		*porchi*		Freshwater	IBE-AF164	Australia; Victoria, Tarra-Bulga NP, River Tarra 9.7.2009	I. Ribera & A. Cieslak		LT991328	LT991488	LT990730	LT990897	LT991104
94	*Ochthebius*	*Gymnochthebius*	*australis*	*australis*		Freshwater	MNCN-AI583	Australia; South Australia, 1 km W Cudlee Creek, River Torrens 14.1.2006	C.H.S. Watts		LT991329	LT991489	LT990731	LT990898	LT991105
95	*Ochthebius*	*Gymnochthebius*	*australis*	*lividus*		Freshwater	IBE-AF163	Australia; Victoria, Tarra-Bulga NP, River Tarra 9.7.2009	I. Ribera & A. Cieslak	XXXXXXX	LT991330	LT991490	LT990732	LT990899	LT991106
96	*Ochthebius*	*Gymnochthebius*	*australis*	*probus*		Freshwater	MNCN-AI584	Australia; South Australia, 1 km W Cudlee Creek, River Torrens 14.1.2006	C.H.S. Watts		LT991331	LT991491	LT990733	LT990900	LT991107
97	*Ochthebius*	*Gymnochthebius*	*australis*	*setosus*		Freshwater	IBE-AF165	Australia; Victoria, Tarra-Bulga NP, River Tarra 9.7.2009	I. Ribera & A. Cieslak		LT991332	LT991492	LT990734	LT990901	LT991108
98	*Ochthebius*	*Gymnochthebius*	*australis*	*semicylindrus*		Freshwater	IBE-AV228	Australia; Kangaroo Island, Waterfall Creek, stream 25.8.2018	A. Villastrigo		XXXXXXX	XXXXXXX		XXXXXXX	
99	*Ochthebius*	*Gymnochthebius*	*fossatus*	*fossatus*		Freshwater	IBE-AN498	Dominican Republic; Independencia, La Colonia 15.8.2014	A. Deler-Hernández, M. Fikácek & M. Gimmel		LT991333	LT991493	LT990735	LT990902	LT991109

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Table S1. (continued)

all	genus	subgenus	species group	species	subspecies	salinity	voucher	locality	leg	mtgenome	COI-5	COI-3	rrnL	18S	28S
100	*Ochthebius*	*Gymnochthebius*	*fossatus*	*germaini*		Freshwater	MNCN-AI454	Chile; IX Región, 5 km E Lonquimay, road to Lolén 26.1.1999	I. Ribera & M. Guerrero		LT991334	LT991494	LT990736	LT990903	LT991110
101	*Ochthebius*	*Gymnochthebius*	*fossatus*	*peruvianus*		Tolerant	MNCN-AI689	Peru; San Clemente, km 222 Panamericana Sur 31.8.2005	P. Aguilera		LT991335	LT991495	LT990737	LT990904	LT991111
102	*Ochthebius*	*Gymnochthebius*	*fossatus*	sp.		Freshwater	MNCN-AI569	Chile; Chiloé, 6 km E Huillinco, river in Notuco 3.2.1999	I. Ribera & M. Guerrero		LT991336	LT991496	LT990738	LT990905	LT991112
103	*Ochthebius*	*Hughleechia*		*giuliani*		Hypersaline	MNCN-AI716	Australia; West Australia, Peron Point 2.10.2003	C.H.S. & G.A. Watts		LT991337	LT991497	LT990739	LT990906	LT991113
104	*Ochthebius*	*Hughleechia*		sp.		Hypersaline	IBE-AV226	Australia; Kangaroo Island, Vivonne Bay, rockpools 24.8.2018	A. Villastrigo		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
105	*Ochthebius*	*Micragasma*		*minoicus*		Hypersaline	AN770	Greece; Crete, Elafonisi, rockpools 20.4.2017	C. Hernando, I. Ribera & A. Villastrigo	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
106	*Ochthebius*	*Micragasma*		*paradoxum*		Hypersaline	IBE-AF116	Russia; Volgogradskaya Oblast, Lake Elton, residual pools 17.4.2008	A. Prokin	**XXXXXXX**	LT991338	HF931114	HF931331	LT990907	LT991114
107	*Ochthebius*	*Ochthebius*	*andraei*	*patergazellae*		Tolerant	IBE-RA735	United Arab Emirates; Ajman, salt water pools NE Ajman City 25.1.2010	M.A. Jäch		LT991339	LT991498	LT990740	LT990908	LT991115
108	*Ochthebius*	*Ochthebius*	*atriceps*	*andalusicus*		Hypersaline	IBE-PA296	Spain; Cádiz, Salinas de Hortales 10.11.2003	A. Millán & collaborators		LT991340	HF931297	LT990741	LT990909	LT991116
109	*Ochthebius*	*Ochthebius*	*atriceps*	*anxifer*		Freshwater	MNCN-AI945	Morocco; Immouzzer Ida Ou Tanane, Assif Tanit 21.4.2001	I. Ribera & A. Cieslak		LT991341	HF931247	HF931482	LT990910	LT991117
110	*Ochthebius*	*Ochthebius*	*atriceps*	*atriceps*		Tolerant	IBE-AN210	Tunisia; road Jendouba-Makthar, 1 km NW crossroad to Mt. Kebbouch 24.10.2001	I. Ribera & A. Cieslak	**XXXXXXX**	LT991342	LT991499	LT990742	LT990911	LT991118
111	*Ochthebius*	*Ochthebius*	*atriceps*	*burjkhalifa*		Tolerant	IBE-RA737	United Arab Emirates; Ajman, salt water pools NE Ajman City 25.1.2010	M.A. Jäch		LT991343	LT991500	LT990743	LT990912	LT991119
112	*Ochthebius*	*Ochthebius*	*atriceps*	*dentifer*		Tolerant	IBE-PA290	Spain; Navarra, Barranco Salado de Mendavia 30.10.2008	A. Millán & collaborators		LT991344	HF931296	LT990744	LT990913	LT991120
113	*Ochthebius*	*Ochthebius*	*atriceps*	*depressionis*		Freshwater	IBE-AF171	Russia; Volgogradskaya Oblast, Lake Elton, nr Lantsug River 14.4.2008	A. Prokin		LT991345	HF931122	HF931340	LT990914	LT991121
114	*Ochthebius*	*Ochthebius*	*atriceps*	*loulae*		Tolerant	IBE-AN476	Djibouti; 50 km W Dikhil, Lac Abbé, thermal springs 2.2.2016	M.A. Jäch		LT837919	LT991501	LT990745	LT990915	LT991122
115	*Ochthebius*	*Ochthebius*	*atriceps*	*recurvatus*		Tolerant	IBE-AN595	Tunisia; Safax-Gabes, oued 25.10.2001	I. Ribera & A. Cieslak			**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
116	*Ochthebius*	*Ochthebius*	*atriceps*	sp.		Tolerant	IBE-AN436	Morocco; Our Draa	A. Millán & collaborators		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
117	*Ochthebius*	*Ochthebius*	*atriceps*	*tacapasensis*	*baeticus*	Tolerant	IBE-AN365	Spain; Málaga, Laguna de Fuente de Piedra 3.5.2011	A. Millán & collaborators		LT991346	LT991502	LT990746	LT990916	LT991123
118	*Ochthebius*	*Ochthebius*	*atriceps*	*thermalis*		Hypersaline	IBE-AN451	Cyprus; Larnaka, saline coastal pond 25.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo		LT991347	LT991503	LT990747	XXXXXXX	LT991124
119	*Ochthebius*	*Ochthebius*	*atriceps?*	*despoliatus*		Tolerant	IBE-RA736	United Arab Emirates; Ajman, salt water pools NE Ajman City 25.1.2010	M.A. Jäch			LT991504	XXXXXXX	LT990917	LT991125
120	*Ochthebius*	*Ochthebius*	*corrugatus*	*corrugatus*		Tolerant	MNCN-AI56	Mallorca (Spain); Mallorca, Salines de Llevant 15.10.2004	I. Ribera & A. Cieslak			HF931213	HF931438	LT990918	LT991126
121	*Ochthebius*	*Ochthebius*	*corrugatus*	*gauthieri*		Hypersaline	MNCN-AI55	Tunisia; road Kebili-Tozeur, 37 km SE Tozeur, salines 26.10.2001	I. Ribera & A. Cieslak			HF931212		LT990919	LT991127
122	*Ochthebius*	*Ochthebius*	*corrugatus*	*perpusillus*		Tolerant	IBE-AN323	Morocco; Ouezzane, Sidi Kacem, Oued Khendek 18.4.2006	A. Millán & collaborators	**XXXXXXX**		LT991505		LT990920	LT991128
123	*Ochthebius*	*Ochthebius*	*corrugatus*	sp.		Hypersaline	MNCN-AI54	Tunisia; Kebili-Tozeur, salines 26.10.2001	I. Ribera & A. Cieslak		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
124	*Ochthebius*	*Ochthebius*	*foveolatus*	*elisae*		Freshwater	IBE-RA746	Iran; Khuzestan, Behbahan, Morvarid spring 4.9.2010	E. Irani		LT991348	LT991506		LT990921	LT991129
125	*Ochthebius*	*Ochthebius*	*foveolatus*	*foveolatus*		Freshwater	MNCN-AI801	Turkey; Kastamonu, Çocukören 26.4.2006	I. Ribera		LT991349	HF931228	HF931457	LT990922	LT991130
126	*Ochthebius*	*Ochthebius*	*foveolatus*	*hajeki*		Freshwater	IBE-RA1231	Socotra (Yemen); Dixam plateau, Firmihin, Dracaena woodland 15.6.2012	J. Hájek		LT991350	LT991507	LT990748	LT990923	LT991131
127	*Ochthebius*	*Ochthebius*	*foveolatus*	*harteni*		Freshwater	IBE-RA705	Oman; Al-Akhdar, Bahla, wadi in city 5.4.2010	I. Ribera, C. Hernando & A. Cieslak		LT991351	LT991508	LT990749	LT990924	LT991132
128	*Ochthebius*	*Ochthebius*	*foveolatus*	*magnannulatus*		Freshwater	IBE-AN328	Bulgaria; Kotlari, river Arda 27.5.2015	I. Ribera	**XXXXXXX**	LT991352	LT991509	LT990750	LT990925	LT991133
129	*Ochthebius*	*Ochthebius*	*foveolatus*	*marginalis*		Freshwater	IBE-AN522	France; Drôme, river Méouge 2016	A. Faille		LT991353	LT991510	LT990751	LT990926	LT991134
130	*Ochthebius*	*Ochthebius*	*foveolatus*	*mediterraneus*		Freshwater	IBE-AN213	Tunisia; Ouessalatia, muddy pond 24.10.2001	I. Ribera & A. Cieslak	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
131	*Ochthebius*	*Ochthebius*	*foveolatus*	*merinidicus*		Freshwater	IBE-RA1023	Spain; Albacete, River Mundo, Azud de Liétor 26.7.2012	A. Millán & collaborators		LT991355	LT991511	LT990752	LT990928	LT991136
132	*Ochthebius*	*Ochthebius*	*foveolatus*	*pedicularius*		Freshwater	IBE-AN809	Italy; Udine, Flagogna 9.4.2017	A. Eckelt		LT991356	LT991512	LT990753	LT990929	LT991137

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Table S1. (continued)

all	genus	subgenus	species group	species	subspecies	salinity	voucher	locality	leg	mtgenome	COI-5	COI-3	rrnL	18S	28S
133	*Ochthebius*	*Ochthebius*	*foveolatus*	*satoi*		Freshwater	IBE-AF210	Japan; Hokkaido, Taiki 11.7.2009	H. Yoshitomi		LT991357	HF931124	HF931343		LT991138
134	*Ochthebius*	*Ochthebius*	*foveolatus*	*sidanus*		Freshwater	IBE-AF132	Italy; Emilia Romagna, Bosco, Torrente Parma 24.5.2008	I. Ribera		LT991358	HF931115	HF931332	LT990930	LT991139
135	*Ochthebius*	*Ochthebius*	*foveolatus*	sp.		Freshwater	IBE-AV114	Russia; Krasnodar, Pshekha River 16.8.2016	A. Prokin		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
136	*Ochthebius*	*Ochthebius*	*foveolatus*	sp.		Freshwater	IBE-AV127	Russia; Agoy River near Tuapse	A. Prokin		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
137	*Ochthebius*	*Ochthebius*	*foveolatus*	sp.		Freshwater	IBE-AV161	Azerbaijan; Devechi-chay River 27.5.2017	A. Prokin		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
138	*Ochthebius*	*Ochthebius*	*foveolatus*	sp.		Freshwater	IBE-AV163	Azerbaijan; Kyzyl-chay River 5.6.2017	A. Prokin		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
139	*Ochthebius*	*Ochthebius*	*foveolatus*	*virgula*		Freshwater	IBE-AF134	Italy; Emilia Romagna, Vigoleno, Parco dello Stirone, Torrente Stirone 25.5.2008	I. Ribera		LT991359	HF931116	HF931333	LT990931	LT991140
140	*Ochthebius*	*Ochthebius*	*lobicollis*	*basilicatus*		Freshwater	IBE-AN801	Sardinia (Italy); Cabras, Stagno di Cabras 10.4.2017	I. Ribera & A. Cieslak		LT991360	LT991513	LT990754	LT990932	LT991141
141	*Ochthebius*	*Ochthebius*	*lobicollis*	*caesaraugustae*		Tolerant	MNCN-AI1195	Spain; Zaragoza, Medianan de Aragón, saline river 20.7.2006	A. Millán & collaborators		LT991361	HF931172	HF931392	LT990933	LT991142
142	*Ochthebius*	*Ochthebius*	*lobicollis*	*delgadoi*		Tolerant	IBE-AN364	Spain; Murcia, Rambla del Reventón 9.3.2012	A. Millán & collaborators	XXXXXXX	LT991362	LT991514	LT990755	LT990934	LT991143
143	*Ochthebius*	*Ochthebius*	*lobicollis*	*eyrei*		Tolerant	IBE-AN600	Sicily (Italy); Palermo, Fiume Salso 28.7.2009	C. Gutiérrez-Cánovas		LT991363	LT991515	LT990756	LT990935	LT991144
144	*Ochthebius*	*Ochthebius*	*lobicollis*	*lapidicola*		Freshwater	IBE-AN126	Tenerife (Spain); Puerto de la Cruz, Roque Grande, 9.4.2015	R. Lyszkowski			LT991516	LT990757	LT990936	LT991145
145	*Ochthebius*	*Ochthebius*	*lobicollis*	*lobicollis*		Freshwater	IBE-RA242	Menorca (Spain); Cap de Favàritx 26.2.2010	I. Ribera & A. Cieslak		LT991364	HF931308	HF931534	LT990937	LT991146
146	*Ochthebius*	*Ochthebius*	*lobicollis*	*quadrifossulatus*		Tolerant	MNCN-AI226	Spain; Guadalajara, Alcolea de las Peñas 21.5.2005	A. Millán & collaborators			LT991517	LT990758	LT990938	LT991147
147	*Ochthebius*	*Ochthebius*	*lobicollis*	sp.		Tolerant	IBE-AH155	Italy; Sicilia, Agrigento 11.6.2007	P. Abellán & F. Picazo			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
148	*Ochthebius*	*Ochthebius*	*lobicollis*	*tivelunus*		Tolerant	MNCN-AI420	Morocco; Agarzane, tributary of Oued Drâa 17.4.2001	I. Ribera & A. Cieslak		LT991365	HF931195	HF931420	LT990939	LT991148
149	*Ochthebius*	*Ochthebius*	*marinus*	*arefniae*		Freshwater	IBE-AN72	Azerbaijan; road Budzhakah-Bash-Dashagyi, ponds 5.5.2014	I. Ribera & A. Rudoy		LT991366	LT991518	LT990759	LT990940	LT991149
150	*Ochthebius*	*Ochthebius*	*marinus*	*arizonicus*		Freshwater	IBE-AN177	Arizona (USA); Yavapai Co., Agua Fria River 22.6.2007	W.D. Shepard		LT991368	LT991520	LT990761	LT990941	LT991151
151	*Ochthebius*	*Ochthebius*	*marinus*	*auropallens*		Freshwater	IBE-AN217	Tunisia; road Kairouan-Enfida, 13 km N Kairouan, lagoon 27.10.2001	I. Ribera & A. Cieslak			LT991521	LT990762	LT990942	LT991152
152	*Ochthebius*	*Ochthebius*	*marinus*	*aztecus*		Tolerant	IBE-AN222	California (USA); Inyo Co., Shoshone, Amargosa River 8.4.2008	P. Abellán		LT991369	LT991522	LT990763	LT990943	LT991153
153	*Ochthebius*	*Ochthebius*	*marinus*	*batesoni*		Tolerant	MNCN-AI690	Peru; San Clemente, km 222 Panamericana Sur 31.8.2005	P. Aguilera		LT991370	LT991523	LT990764	LT990944	LT991154
154	*Ochthebius*	*Ochthebius*	*marinus*	*bisinuatus*		Freshwater	IBE-AN153	California (USA); Mendocino Co., circa 5 mi W Davis Creek 9.6.2007	W.D. Shepard		LT991371	LT991524	LT990765	LT990945	LT991155
155	*Ochthebius*	*Ochthebius*	*marinus*	*capicola*		Tolerant	IBE-RA854	South Africa; West Cape, West Cape NP, Tsaarsbank, rookpools 2.10.2011	D.T. Bilton	XXXXXXX	LT991372	LT991525	LT990766	LT990946	LT991156
156	*Ochthebius*	*Ochthebius*	*marinus*	*caudatus*		Tolerant	IBE-AV112	Poland; Sanocki District, Tyrawa Solna 19.5.2017	D. Twardy		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
157	*Ochthebius*	*Ochthebius*	*marinus*	*chappuisi*		Tolerant	IBE-AN475	Djibouti; 50 km W Dikhil, Lac Abbé, thermal springs 2.2.2016	M.A. Jäch		LT837918	LT991526	LT990767	LT990947	LT991157
158	*Ochthebius*	*Ochthebius*	*marinus*	*costatellus*		Tolerant	IBE-RA1119	Alberta (Canada); Alberta, Derwent, circa Lac Coté 2000	T. Berendonk		LT991373	LT991527	LT990768	LT990948	LT991158
159	*Ochthebius*	*Ochthebius*	*marinus*	*deletus*		Tolerant	IBE-AN433	Mallorca (Spain); Mallorca, Salines de Llevant 28.3.2016	I. Ribera & A. Cieslak	XXXXXXX	LT991391	LT991542	LT990782	LT990965	LT991177
160	*Ochthebius*	*Ochthebius*	*marinus*	*evanescens*		Tolerant	IBE-AN66	Azerbaijan; Alishanli, 7 km NE Masally 8.5.2014	I. Ribera & A. Rudoy		LT991374	LT991528	LT990769	LT990949	LT991159
161	*Ochthebius*	*Ochthebius*	*marinus*	*extremus*		Tolerant	IBE-AV140	South Africa; 6.11.2017	R. Vila		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
162	*Ochthebius*	*Ochthebius*	*marinus*	*fallaciosus*		Tolerant	MNCN-AI918	Morocco; Azrou, Lac Afenourir 29.4.2000	I. Ribera		LT991394	HF931242	HF931474	LT990968	LT991180
163	*Ochthebius*	*Ochthebius*	*marinus*	*gruwelli*		Freshwater	IBE-RA304	California (USA); Riverside Co., Morongo Valley, Whitewater river 12.12.2010	I. Ribera & J. Bergsten		LT991375	HF931311	HF931535	LT990950	LT991160
164	*Ochthebius*	*Ochthebius*	*marinus*	*interruptus*		Tolerant	IBE-AN219	California (USA); Santa Barbara Co., Coal Oil Point Reserve (Slough road) 26.3.2008	P. Abellán		LT991376	LT991529	LT990770	LT990951	LT991161
165	*Ochthebius*	*Ochthebius*	*marinus*	*involatus*		Freshwater	IBE-AV142	South Africa; 6.11.2017	R. Vila		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX

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Table S1. (continued)

all	genus	subgenus	species group	species	subspecies	salinity	voucher	locality	leg	mtgenome	COI-5	COI-3	rrnL	18S	28S
166	*Ochthebius*	*Ochthebius*	*marinus*	*lineatus*		Tolerant	IBE-AN223	California (USA); Inyo Co., Ballarant, Post Office Spring 10.4.2008	P. Abellán		LT991377	LT991530	XXXXXXX		LT991162
167	*Ochthebius*	*Ochthebius*	*marinus*	*lividipennis*		Tolerant	IBE-AN71	Azerbaijan; road Budzhakah-Bash-Dashagyi, ponds 7.5.2014	I. Ribera & A. Rudoy	XXXXXXX	LT991378	LT991532	LT990772	LT990953	LT991164
168	*Ochthebius*	*Ochthebius*	*marinus*	*marinus*		Tolerant	MNCN-AI615	Spain; Madrid, Aranjuez, salinas 11.2.2006	I. Ribera & A. Cieslak		LT991379	HF931215	HF931441	LT990954	LT991165
169	*Ochthebius*	*Ochthebius*	*marinus*	*meridionalis*		Tolerant	IBE-RA373	Morocco; Moulay Bousselham, Oued Drader 12.4.2007	I. Ribera, P. Aguilera & C. Hernando		LT991380	HF931313	HF931538	LT990955	LT991166
170	*Ochthebius*	*Ochthebius*	*marinus*	*nipponicus*		Freshwater	MNCN-HI27	Japan; Honshu, Ibaraki Pref., Kobodihana, Ohmika-machi, Hitachi-shi 22.7.2006	N. Hikida		LT991381	HF931270	LT990773	LT990956	LT991167
171	*Ochthebius*	*Ochthebius*	*marinus*	*pedalis*		Tolerant	MNCN-AH98	South Africa; West Cape, Papendorp, brackish pond 23.8.2006	G. Challet		LT991382	LT991534	LT990774	LT990957	LT991168
172	*Ochthebius*	*Ochthebius*	*marinus*	*pusillus*		Tolerant	MNCN-AI1028	Turkey; Sinop, road Saraydüzü-Boyabat, stream 5 km S Yeşilyurt 27.4.2006	A. Castro		LT991383	HF931146	HF931365	LT990958	LT991169
173	*Ochthebius*	*Ochthebius*	*marinus*	*queenslandicus*		Freshwater	IBE-AN18	Australia; Queensland, Brisbane, Shorncliffe 2.5.2014	J. Maté		LT991384	LT991535	LT990775		LT991170
174	*Ochthebius*	*Ochthebius*	*marinus*	*rectus*		Tolerant	MNCN-AI464	California (USA); Mono Co., Long Valley, Owens River road 19.6.2000	I. Ribera & A. Cieslak		LT991385	LT991536	LT990776	LT990959	LT991171
175	*Ochthebius*	*Ochthebius*	*marinus*	*salinarius*		Tolerant	IBE-AN79	South Africa; West Cape, Wilderness NP, road to Swartvlei, stream 22.3.2001	I. Ribera & A. Cieslak	XXXXXXX	LT991388	LT991539	LT990779	LT990962	LT991174
176	*Ochthebius*	*Ochthebius*	*marinus*	*sculptoides*		Tolerant	IBE-AN221	California (USA); Inyo Co., Tecopa, Amargosa River 8.4.2008	P. Abellán	XXXXXXX	LT991389	LT991540	LT990780	LT990963	LT991175
177	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Freshwater	IBE-AN446	Cyprus; Loukrounou, upstream of Evretou reservoir 29.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo		LT991367	LT991519	LT990760	XXXXXXX	LT991150
178	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Freshwater	IBE-RA1129	Venezuela; Guárico state, Río San Antonio 4.2.2010	A.E.Z. Short, M. García & L. Joly			LT991531	LT990771	LT990952	LT991163
179	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Freshwater	IBE-AN152	California (USA); Ventura Co., Los Padres National Forest, Upper Sespe Creek 1.7.2006	A.E.Z. Short & M. Caterino		LT991386	LT991537	LT990777	LT990960	LT991172
180	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Freshwater	IBE-AN291	Alberta (Canada); 2 km W Lundbreck 27.6.2000	I. Ribera & A. Cieslak		LT991387	LT991538	LT990778	LT990961	LT991173
181	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Hypersaline	IBE-AN452	Cyprus; Larnaka, saline wetland 25.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
182	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Tolerant	IBE-AV149	Mongolia; Tatsyn-Tsaagan-nuur Lake 14.8.2017	A. Prokin & A. Sazhnev		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
183	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Freshwater	IBE-AV150	China; Xinjiang 24.7.2017	D. Palatov		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
184	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Freshwater	IBE-AV165	Uzbekistan; Amu-Darya valley 24.6.2017	D. Palatov		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
185	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Tolerant	IBE-RA884	South Africa; 30.9.2011	D.T. Bilton		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
186	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Tolerant	MNCN-AI452	France; Bouches du Rhône, Salin-de-Girau 29.7.2005	I. Ribera & A. Cieslak		HE970918	HE970844	HE971076	HE970957	HE970996
187	*Ochthebius*	*Ochthebius*	*marinus*	sp.		Tolerant	IBE-AN216	Tunisia; Hachichina 25.10.2001	I. Ribera & A. Cieslak		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
188	*Ochthebius*	*Ochthebius*	*marinus*	*spinasus*		Tolerant	MNCN-AH100	South Africa; West Cape, Berg River, saline stream 3.9.2006	G. Challet		LT991390	LT991541	LT990781	LT990964	LT991176
189	*Ochthebius*	*Ochthebius*	*marinus*	*subpictus*		Tolerant	IBE-AN880	Portugal; Madeira, Praia da Maiata, river mouth 21.10.2017	I. Ribera & A. Cieslak		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
190	*Ochthebius*	*Ochthebius*	*marinus*	*uniformis*		Tolerant	IBE-AN437	California (USA); Mendocino Co., Manchester 30.6.2000	I. Ribera & A. Cieslak	XXXXXXX	LT991392	LT991543	LT990783	LT990966	LT991178
191	*Ochthebius*	*Ochthebius*	*marinus*	*viridescens*		Tolerant	IBE-RA40	Spain; Zaragoza, Cinco Villas, Estanca de Castiliscar 1.11.2009	I. Ribera & A. Cieslak		LT991393	HF931314	HF931539	LT990967	LT991179
192	*Ochthebius*	*Ochthebius*	*marinus*	*viridis*		Freshwater	IBE-AN5	Turkey; Izmir, 6 km E Foça, head of reservoir 26.7.2014	I. Ribera & A. Cieslak	XXXXXXX	LT991395	LT991544	LT990784	LT990969	LT991181
193	*Ochthebius*	*Ochthebius*	*metallescens*	*albacetinus*		Freshwater	IBE-RA1181	Spain; Jaén, Río Madera 7.9.2013	D.T. Bilton		LT991396	HG915306	LT990785	LT990970	LT991182
194	*Ochthebius*	*Ochthebius*	*metallescens*	*bernard*		Freshwater	IBE-RA96	Oman; Al-Akhdar, 5.4.2010	I. Ribera, A. Cieslak & C. Hernando			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
195	*Ochthebius*	*Ochthebius*	*metallescens*	*diazi*		Freshwater	IBE-RA595	Spain; Cádiz, 15 km W Los Barrios, Arroyo del Tiradero 22.6.2011	D.T. Bilton		HF948001	LT991545	LT990786	LT990971	LT991183
196	*Ochthebius*	*Ochthebius*	*metallescens*	*gayosoi*		Freshwater	IBE-AN311	Portugal; Algarve, Caldas de Monchique 11.6.2015	D.T. Bilton		LT991397	LT991546	LT990787	LT990972	LT991184
197	*Ochthebius*	*Ochthebius*	*metallescens*	*griotes*		Freshwater	IBE-AN130	Morocco; Taza, stream 22.3.2008	I. Ribera, C. Hernando & P. Aguilera	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
198	*Ochthebius*	*Ochthebius*	*metallescens*	*hivae*		Freshwater	IBE-RA744	Iran; Khuzestan, Behbahan, Morvarid spring 4.9.2010	E. Irani		LT991399	LT991547	LT990788	LT990974	LT991186

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Table S1. (continued)

all	genus	subgenus	species group	species	subspecies	salinity	voucher	locality	leg	mtgenome	COI-5	COI-3	rrnL	18S	28S
199	*Ochthebius*	*Ochthebius*	*metallescens*	*judemaesi*		Freshwater	IBE-RA1179	Spain; Albacete, Chorros del Río Mundo 7.9.2013	D.T. Bilton		LT991400	LT991548	LT990789	LT990975	LT991187
200	*Ochthebius*	*Ochthebius*	*metallescens*	*metallescens*	*metallescens*	Freshwater	MNCN-AI376	Austria; Niederösterreich, Lunz 2002	M.A. Jäch		LT991402	HF931191	HF931414	LT990977	LT991189
201	*Ochthebius*	*Ochthebius*	*metallescens*	*metallescens*	*plato*	Freshwater	IBE-RA1057	Greece; Peloponnese, Achaea, 1.5 km NE Kleitor 3.4.2013	I. Ribera & A. Cieslak		LT991403	LT991550	LT990791		LT991190
202	*Ochthebius*	*Ochthebius*	*metallescens*	*morettii*		Freshwater	IBE-RA1173	Italy; Liguria, Imperia, 1 km S Pigna 7.7.2013	D.T. Bilton		LT991404	LT991551	LT990792	LT990978	LT991191
203	*Ochthebius*	*Ochthebius*	*metallescens*	*pedroi*		Freshwater	IBE-RA1082	Mallorca (Spain); Sa Calobra, Font de sa Mata 8.5.2013	I. Ribera		LT991405	LT991552	LT990793	LT990979	LT991192
204	*Ochthebius*	*Ochthebius*	*metallescens*	*poweri*		Freshwater	MNCN-AC26	England (UK); S Devon, Ladram Bay, seepage 4.7.2007	D.T. Bilton		LT991406	LT991553	LT990794	LT990980	LT991193
205	*Ochthebius*	*Ochthebius*	*metallescens*	*preissi*		Freshwater	IBE-AN448	Cyprus; Loukrounou, river Kaboura 28.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo		LT991407	LT991554	LT990795	LT990981	LT991194
206	*Ochthebius*	*Ochthebius*	*metallescens*	*puberulus*		Freshwater	IBE-AV164	Azerbaijan; 2.6.2017	A. Prokin		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	
207	*Ochthebius*	*Ochthebius*	*metallescens*	*scopuli*		Freshwater	IBE-AN382	Sardinia (Italy); Golf of Orosei, Cala Mariolu 12.10.2015	J. Köhler	XXXXXXX	LT602656	LT602657	LT990796	LT990982	LT991195
208	*Ochthebius*	*Ochthebius*	*metallescens*	*semisericeus*		Freshwater	MNCN-AI1064	Spain; Teruel, Beceite, river Matarranya, El Parrisal 7.8.2006	I. Ribera & A. Cieslak			HF931151	HF931370	LT990983	LT991196
209	*Ochthebius*	*Ochthebius*	*metallescens*	*semotus*		Freshwater	IBE-RA1180	Spain; Albacete, Chorros del Río Mundo 7.9.2013	D.T. Bilton			HG915305	LT990797	LT990984	LT991197
210	*Ochthebius*	*Ochthebius*	*metallescens*	*serpentinus*		Freshwater	MNCN-AI819	Turkey; Kastamonu, road Ağlı-Azdavay 28.4.2006	I. Ribera		LT991408	HF931229	HF931458	LT990985	LT991198
211	*Ochthebius*	*Ochthebius*	*metallescens*	sp.		Freshwater	IBE-AN164	Turkey; Antalya, 1 km N Hacıyusuflar 24.6.2014	D.T. Bilton	XXXXXXX	LT991401	LT991549	LT990790	LT990976	LT991188
212	*Ochthebius*	*Ochthebius*	*metallescens*	sp.		Freshwater	IBE-RA584	Spain; Cádiz, Garganta de la Balsa 23.6.2011	D.T. Bilton		XXXXXXX	XXXXXXX	XXXXXXX		
213	*Ochthebius*	*Ochthebius*	*metallescens*	sp.		Freshwater	IBE-AN77	Azerbaijan; Gosmalijion, stream 7.5.2014	I. Ribera & A. Rudoy	XXXXXXX	LT991409	LT991555	LT990798	LT990986	LT991199
214	*Ochthebius*	*Ochthebius*	*metallescens*	sp.		Freshwater	IBE-AV130	Russia; Pshysh River upstream of Khodyzhensk	A. Prokin		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
215	*Ochthebius*	*Ochthebius*	*metallescens*	sp.		Freshwater	IBE-AV144	Kazakhstan; Karatau Mts. 2.6.2016	S. Litovkin		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
216	*Ochthebius*	*Ochthebius*	*metallescens*	sp.		Freshwater	IBE-AN1016	Morocco, Talassemtane National Park, mountain stream 3.4.2018	Alfambra, C. Hernando, I. Ribera & A. Villastrigo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
217	*Ochthebius*	*Ochthebius*	*metallescens*	*wurayah*		Freshwater	IBE-RA733	United Arab Emirates; Fujairah (north), Hajar Mountains, Wadi Wurayah 24.1.2010	M.A. Jäch			LT991556	LT990799	LT990987	LT991200
218	*Ochthebius*	*Ochthebius*	*nitidipennis*	*hasegawai*		Freshwater	MNCN-AI1289	Japan; Honshu, Shimane Pref., Mt. Sentsu, Okuizumo 15.7.2006	N. Hayashi	XXXXXXX	LT991410	LT991557	LT990800	LT990988	LT991201
219	*Ochthebius*	*Ochthebius*	*nitidipennis*	*japonicus*		Freshwater	MNCN-HI26	Japan; Shikoku, Ehime Pref., Oda-gawa, Mizumoto, Uchiko-chô 30.9.2006	Y. Kamite		LT991411	LT991558	LT990801	LT990989	LT991202
220	*Ochthebius*	*Ochthebius*	*notabilis*	*gereckei*		Hypersaline	MNCN-PA141	Sicily (Italy); Caltanissetta, Torto Valley 11.6.2007	P. Abellán & F. Picazo		LT991412	FJ944176	LT990803	LT990991	LT991204
221	*Ochthebius*	*Ochthebius*	*notabilis*	*glaber*		Hypersaline	MNCN-PA30	Spain; Córdoba, Priego de Córdoba, Río Salado de Priego 21.7.2006	A. Millán & collaborators	XXXXXXX	HF948002	FJ944214	LT990804	LT990992	LT991205
222	*Ochthebius*	*Ochthebius*	*notabilis*	*halophilus*		Hypersaline	IBE-AN22	Turkey; Kırıkkale, Delice Çoğul salt pond 14.6.2012	T. Söylemez		LT991413	LT991559	LT990805	LT990993	LT991206
223	*Ochthebius*	*Ochthebius*	*notabilis*	*lanarotis*		Hypersaline	MNCN-PA32	Morocco; Ouezzane, Sidi Kacem, Oued Khendek 18.4.2006	A. Millán & collaborators		LT991414	FJ944251	LT990806	LT990994	LT991207
224	*Ochthebius*	*Ochthebius*	*notabilis*	*normandi*		Hypersaline	MNCN-PA253	Algeria; Blida, Mellaha 23.8.2007	S. Bouzid		LT991415	FJ944275	LT990807	LT990995	LT991208
225	*Ochthebius*	*Ochthebius*	*notabilis*	*notabilis*		Hypersaline	MNCN-AI38	Spain; Albacete, Pinilla, Salinas de Pinilla 2.6.2002	I. Ribera & A. Cieslak		LT991416	FJ944107	HF931416	LT990996	LT991209
226	*Ochthebius*	*Ochthebius*	*notabilis*	*salinator*		Hypersaline	MNCN-AI53	Tunisia; road Kebili-Tozeur, 37 km SE Tozeur, salines 26.10.2001	I. Ribera & A. Cieslak		LT991417	FJ944271	HF931436	LT990997	LT991210
227	*Ochthebius*	*Ochthebius*	*notabilis*	*zugmayeri*		Tolerant	IBE-AV113	Russia; Astrakhan Oblast, Krasnoyarsk District 6.5.2016	P. Petrov			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
228	*Ochthebius*	*Ochthebius*	*peisonis*	*peisonis*		Tolerant	IBE-AN64	Azerbaijan; Alishanli, 7 km NE Masally 8.5.2014	I. Ribera & A. Rudoy	XXXXXXX	LT991419	LT991561	LT990809	LT990998	LT991212
229	*Ochthebius*	*Ochthebius*	*punctatus*	*bifoveolatus*		Tolerant	IBE-AN381	Spain; Cádiz, Bahía de Cádiz, marshes 27.4.2011	A. Millán & collaborators			LT991562	LT990810	LT990999	LT991213
230	*Ochthebius*	*Ochthebius*	*punctatus*	*cuprescens*		Tolerant	IBE-PA276	Tunisia; road Tozeur-Gafsa, 24 km SW Gafsa, Oued El Melah 16.10.2001	I. Ribera & A. Cieslak		LT991420	HF931293	HF931528	LT991000	LT991214
231	*Ochthebius*	*Ochthebius*	*punctatus*	*difficilis*		Freshwater	MNCN-AI944	Morocco; Immouzzer Ida Ou Tanane, Assif Tanit 21.4.2001	I. Ribera & A. Cieslak		LT991421	HF931246	HF931481	LT991001	LT991215

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Table S1. (continued)

all	genus	subgenus	species group	species	subspecies	salinity	voucher	locality	leg	mtgenome	COI-5	COI-3	rrnL	18S	28S
232	*Ochthebius*	*Ochthebius*	*punctatus*	*grandipennis*		Tolerant	MNCN-AI616	Spain; Madrid, Aranjuez, salinas 11.2.2006	I. Ribera & A. Cieslak		LT991423	HF931216	HF931442	LT991003	LT991217
233	*Ochthebius*	*Ochthebius*	*punctatus*	*inermis*		Freshwater	IBE-RA795	Japan; Honshu, Ibaraki Pref., Kobodihana, Ohmika-machi, Hitachi-shi 22.7.2006	N. Hikida			LT991564	LT990812	LT991004	LT991218
234	*Ochthebius*	*Ochthebius*	*punctatus*	*joosti*		Tolerant	IBE-AF169	Russia; Volgogradskaya Oblast, Lake Elton, residual pools 17.4.2008	A. Prokin		LT991424	HF931121	HF931339	LT991005	LT991219
235	*Ochthebius*	*Ochthebius*	*punctatus*	*klapperichi*		Freshwater	MNCN-AI1269	Bhutan; Timphu, Taba, Wang Chhu river 23.11.2005	M.A. Jäch		LT991425	LT991565	LT990813	LT991006	LT991220
236	*Ochthebius*	*Ochthebius*	*punctatus*	*lanuginosus*		Tolerant	IBE-AN204	Greece; Arkadia, Astros, Lake Moustou 6.4.2013	I. Ribera & A. Cieslak		LT991426	LT991566	LT990814	LT991007	LT991221
237	*Ochthebius*	*Ochthebius*	*punctatus*	*mahmoodi*		Freshwater	IBE-RA126	Oman; 1 km W Qalhat, residual pools in wadi 9.4.2010	I. Ribera, C. Hernando & A. Cieslak		LT991427	HF931304	HF931532	LT991008	LT991222
238	*Ochthebius*	*Ochthebius*	*punctatus*	*micans*		Freshwater	IBE-AN474	Djibouti; Arta, wadi Alloulli (oasis), Oued Kalou 31.1.2016	M.A. Jäch		LT837917	LT991567	LT990815	LT991009	LT991223
239	*Ochthebius*	*Ochthebius*	*punctatus*	*monseti*		Freshwater	IBE-RA124	Oman; 15 km SW Sur, residual pools in wadi 9.4.2010	I. Ribera, C. Hernando & A. Cieslak		LT991428	HF931302	LT990816	LT991010	LT991224
240	*Ochthebius*	*Ochthebius*	*punctatus*	*montesi*		Tolerant	MNCN-AI491	Spain; Murcia, Caravaca, Rambla Pozo Enmedio 2.6.2005	A. Millán & collaborators			HF931206	HF931431	LT991011	LT991225
241	*Ochthebius*	*Ochthebius*	*punctatus*	*nanus*		Freshwater	IBE-PA277	Morocco; Âït-Rahhal, Oued Akka 17.2.2001	I. Ribera & A. Cieslak	XXXXXXX		HF931294	HF931529	LT991012	LT991226
242	*Ochthebius*	*Ochthebius*	*punctatus*	*nilssoni*		Freshwater	MNCN-AH76	Ireland; Clare, Lough Gealáin 31.7.2007	D.T. Bilton		LT991429	LT991568	LT990817	LT991013	LT991227
243	*Ochthebius*	*Ochthebius*	*punctatus*	*nobilis*		Freshwater	IBE-AF133	Italy; Emilia Romagna, Bosco, Torrente Parma 24.5.2008	I. Ribera	XXXXXXX	LT991430	LT991569	LT990818	LT991014	LT991228
244	*Ochthebius*	*Ochthebius*	*punctatus*	*pagotrichus*		Freshwater	MNCN-AI497	South Africa; West Cape, Prince Albert Road, pond 23.3.2001	I. Ribera & A. Cieslak		LT991431	LT991570	LT990819	LT991015	LT991229
245	*Ochthebius*	*Ochthebius*	*punctatus*	*pilosus*		Tolerant	IBE-AN363	Spain; Cádiz, Bahía de Cádiz 27.4.2011	A. Millán & collaborators	XXXXXXX	LT991432	LT991571	LT990820	LT991016	LT991230
246	*Ochthebius*	*Ochthebius*	*punctatus*	*punctatus*		Tolerant	IBE-RA286	Ireland; Clare, Finavarra, Lough Muree 23.5.2010	I. Ribera		LT991433	HF931310	LT990821	LT991017	LT991231
247	*Ochthebius*	*Ochthebius*	*punctatus*	*quadrifoveolatus*		Freshwater	MNCN-AI636	Gran Canaria (Spain); Moya, Barranco de Azuaje 15.4.2001	I. Ribera & A. Cieslak		LT991434	HF931218	HF931446	LT991018	LT991232
248	*Ochthebius*	*Ochthebius*	*punctatus*	*ragusae*		Freshwater	MNCN-AI1029	Turkey; Sinop, road Saraydüzü-Boyabat, stream 5 km S Yeşilyurt 27.4.2006	A. Castro		LT991435	HF931147	HF931366	LT991019	LT991233
249	*Ochthebius*	*Ochthebius*	*punctatus*	*silfverbergi*		Tolerant	IBE-RA1021	Morocco; Tan-Tan, Oued Drâa 3.4.2007	A. Millán & collaborators		LT991436	LT991572	LT990822	LT991020	LT991234
250	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		Hypersaline	IBE-AH164	Italy; Sicily, Caltanissetta 12.6.2007	P. Abellán & F. Picazo		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
251	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		Hypersaline	IBE-PA288	Spain; Murcia, río Chícamo 23.1.2008	A. Millán & collaborators		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
252	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		Freshwater	IBE-AN76	Azerbaijan; Gosmalijion, stream 7.5.2014	I. Ribera & A. Rudoy	XXXXXXX	LT991422	LT991563	LT990811	LT991002	LT991216
253	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		Freshwater	IBE-AV159	Taiwan; Taichung city, Heping 6.1.2018 / Fikácek, Liang, Hsiao	M. Fikácek, Liang & Hsiao		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
254	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		Freshwater	IBE-RA247	China, Yunnan 18.5.2010	V.V. Grevennikov		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
255	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		Freshwater	IBE-RA104	Oman; Al-Akhdar, source of wadi Bani Awf 6.4.2010	I. Ribera, C. Hernando & A. Cieslak		LT991437	LT991573	LT990823	LT991021	LT991235
256	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		Freshwater	IBE-AF191	Bhutan; Sarpang, 11 km NW Sarpang, Bhur Khola river 27.11.2005	M.A. Jäch		LT991438	LT991574	LT990824	LT991022	LT991236
257	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		Freshwater	IBE-AV147	Kyrgyzstan; Jalal-Abad Reg. 19.7.2017	S. Litovkin		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
258	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		Freshwater	IBE-AV152	Tajikistan; 2.7.2016	D. Palatov		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
259	*Ochthebius*	*Ochthebius*	*punctatus*	sp.		Freshwater	IBE-RA745	Iran; Khuzestan, Begbahan, river 5.5.2011	E. Irani		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
260	*Ochthebius*	*Ochthebius*	*punctatus*	*tudmirensis*		Hypersaline	MNCN-AI467	Spain; Guadalajara, Salinas de Imón 22.5.2005	I. Ribera & A. Cieslak		HF948004	HF931205	HF931430	LT991023	LT991237
261	*Ochthebius*	*Ochthebius*	*quadricollis*	*brevicollis*		Hypersaline	IBE-AN440	Cyprus; Akamas peninsula, Lara beach 29.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo		LT991439	LT991575	LT990825	LT991024	LT991238
262	*Ochthebius*	*Ochthebius*	*quadricollis*	*heeri*		Hypersaline	IBE-AV116	Portugal; Madeira, Sao Martinho 17.10.2017	I. Ribera & A. Cieslak		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
263	*Ochthebius*	*Ochthebius*	*quadricollis*	*quadricollis*		Hypersaline	IBE-AN140	Italy; Sicily, Acicastello, rockpools 1.6.2015	A. Rudoy	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
264	*Ochthebius*	*Ochthebius*	*quadricollis*	sp.		Hypersaline	IBE-AN200	Tenerife (Spain); Tenerife, La Orotava, rookpools in El Barco 4.4.2015	R. Lyszkowski		LT991440	LT991576	LT990826	LT991025	LT991239

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Table S1. (continued)

all	genus	subgenus	species group	species	subspecies	salinity	voucher	locality	leg	mtgenome	COI-5	COI-3	rrnL	18S	28S
265	*Ochthebius*	*Ochthebius*	*quadricollis*	sp.		Hypersaline	IBE-AV179	Spain; Lanzarote, Caletón Blanco, rockpools 29.3.2018	C. Andújar		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
266	*Ochthebius*	*Ochthebius*	*quadricollis*	sp.		Hypersaline	IBE-AN11	Spain; Murcia, La Manga del Mar Menor, Punta del Cocedor 10.10.2009	J. Sánchez-Meca		LT991442	LT991577	LT990827	LT991027	LT991241
267	*Ochthebius*	*Ochthebius*	*quadricollis*	sp.		Hypersaline	MNCN-AI514	Spain; Pontevedra, Nigran, Playa Patos 3.10.2005	J. Garrido		LT991443	HF931209	HF931434	LT991028	LT991242
268	*Ochthebius*	*Ochthebius*	*quadricollis*	sp.		Hypersaline	IBE-AN609	Spain; Cartagena, Cabo de Palos, rockpools 23.10.2016	A. Millán & J. Velasco		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
269	*Ochthebius*	*Ochthebius*	*quadricollis*	sp.		Hypersaline	IBE-AV78	Greece; Crete, Pacheia Ammos, rockpools 17.4.2017	C. Hernando, I. Ribera & A. Villastrigo			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
270	*Ochthebius*	*Ochthebius*	*quadricollis*	sp.		Hypersaline	MNCN-AI517	Greece; Halkidiki, Kassandra 29.6.2002	M.A. Jäch		LT991444	HF931210	HF931435	LT991029	LT991243
271	*Ochthebius*	*Ochthebius*	*quadricollis*	*steinbuehleri*		Hypersaline	IBE-AN791	Croatia; Trsteno, rockpools 5.5.2017	A. Faille, J. Fresneda & I. Ribera		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
272	*Ochthebius*	*Ochthebius*	*quadricollis*	*urbanelliae*		Hypersaline	genbank	-	-				XXXXXXX		XXXXXXX
273	*Ochthebius*	*Ochthebius*	*rivalis*	*himalayae*		Freshwater	MNCN-AI1270	Bhutan; Timphu, Taba, Wang Chhu river 23.11.2005	M.A. Jäch	XXXXXXX		LT991578	LT990828	LT991030	LT991244
274	*Ochthebius*	*Ochthebius*	*rivalis*	*rivalis*		Freshwater	IBE-AF81	India; Uttarakhand, 10 km SW Rudraprayag, River Alaknanda 11.11.2006	M.A. Jäch			LT991579	LT990829	LT991031	LT991245
275	*Ochthebius*	*Ochthebius*	*strigosus*	sp.		Freshwater	IBE-RA617	China; Shaanxi, 110 km ENE Xian, Huayin vill., Hija Mt. 10.5.2011	M. Balke & J. Hájek	XXXXXXX		LT991580	LT990830	LT991032	LT991246
276	*Ochthebius*	*Ochthebius*	*sumatrensis*	sp.		Freshwater	MNCN-AC16	Hong-Kong (China); Hong Kong Island, hygropetric 19.2.2007	J. Maté		LT991445	LT991581	LT990831	LT991033	LT991247
277	*Ochthebius*	*Ochthebius*	*vandykei*	*vandykei*		Hypersaline	IBE-AF159	California (USA); San Luis Obispo Co., Rancho Marino Res. 28.6.2008	M. Caterino			LT991582		LT991034	LT991248
278	*Ochthebius*	*Ochthebius*	*vandykei*	sp.		Hypersaline	IBE-RA1018	Japan; Okinawa 2010	H. Yoshitomi						XXXXXXX
279	*Ochthebius*	*Ochthebius*	*vandykei*	*yoshitomii*		Hypersaline	IBE-AF121	Japan; Hokkaido, Shirakami, Matsumae-chô 14.7.2006	H. Yoshitomi	XXXXXXX		LT991583		LT991035	LT991249

Table S2. List of genes used in phylogenetic reconstruction, including samples per gene and length of each alignment.

gene	**partition**	**# terminals**	**# nucleotides**
18S	nuclear	271	603
28S	nuclear	279	644
atp6	CDS mitogenome	42	672
atp8	CDS mitogenome	42	165
cob	CDS mitogenome	41	1140
cox1	CDS all data	282	1506
cox2	CDS mitogenome	42	706
cox3	CDS mitogenome	42	789
nad1	CDS all data / CDS mitogenome	231 / 42	104 / 847
nad2	CDS mitogenome	42	1011
nad3	CDS mitogenome	41	357
nad4	CDS mitogenome	41	1336
nad4l	CDS mitogenome	41	282
nad5	CDS mitogenome	41	1740
nad6	CDS mitogenome	41	507
rrnL	ribosomal all data / ribosomal mitogenome	273 / 41	692 / 723
rrnS	ribosomal mitogenome	40	812
trnA	trn mitogenome	41	69
trnC	trn mitogenome	42	69
trnD	trn mitogenome	42	71
trnE	trn mitogenome	41	70
trnF	trn mitogenome	41	73
trnG	trn mitogenome	41	68
trnH	trn mitogenome	41	70
trnI	trn mitogenome	40	73
trnK	trn mitogenome	42	72
trnL1	trn all data	237	69
trnL2	trn mitogenome	42	67
trnM	trn mitogenome	40	69
trnN	trn mitogenome	41	67
trnP	trn mitogenome	41	71
trnQ	trn mitogenome	40	69
trnR	trn mitogenome	41	68
trnS1	trn mitogenome	41	68
trnS2	trn mitogenome	41	71
trnT	trn mitogenome	41	66
trnV	trn mitogenome	40	72
trnW	trn mitogenome	42	73
trnY	trn mitogenome	41	68

Figure S1. Majority rule consensus tree obtained in Beast. Number in nodes: posterior probability values.

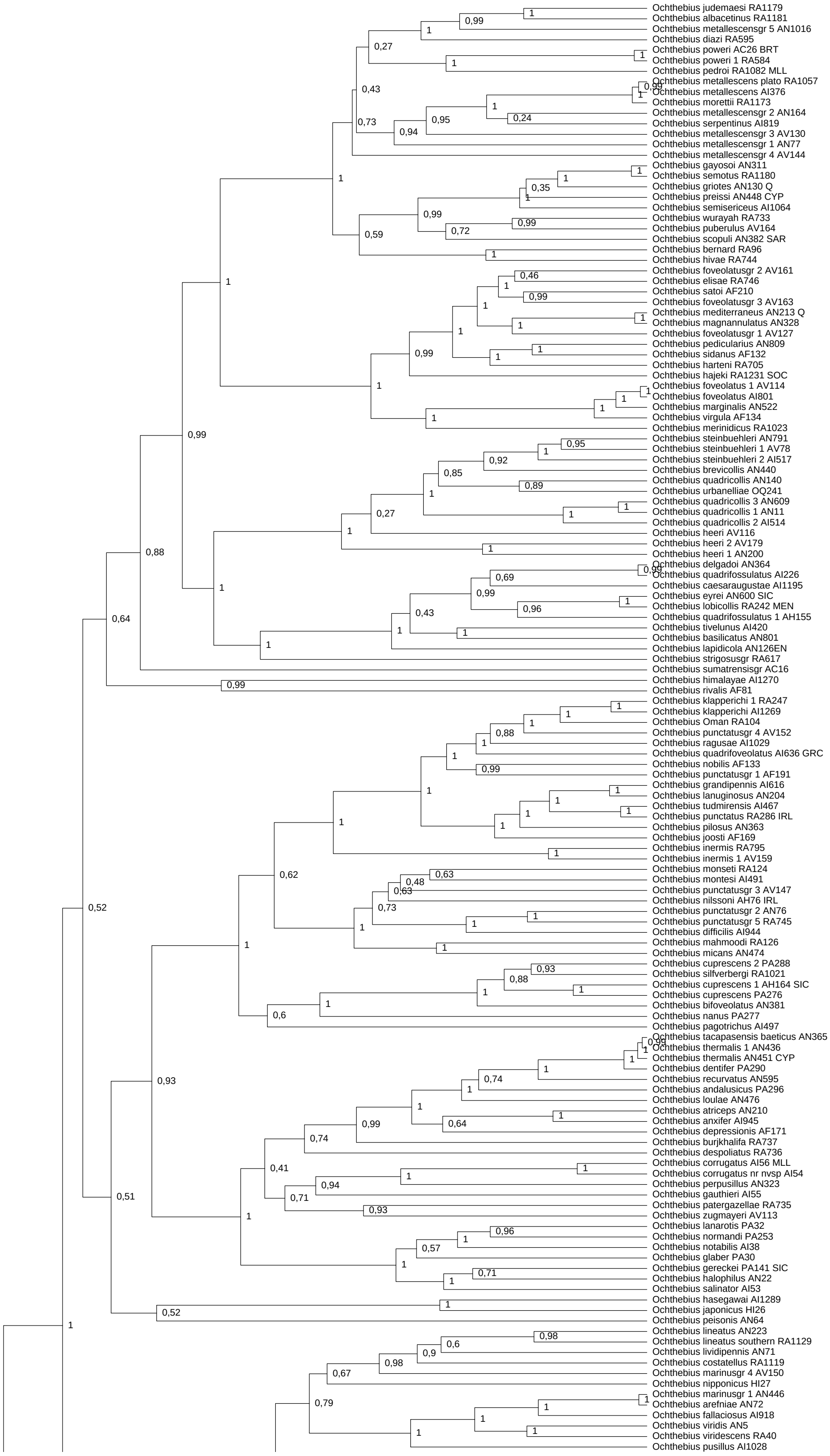
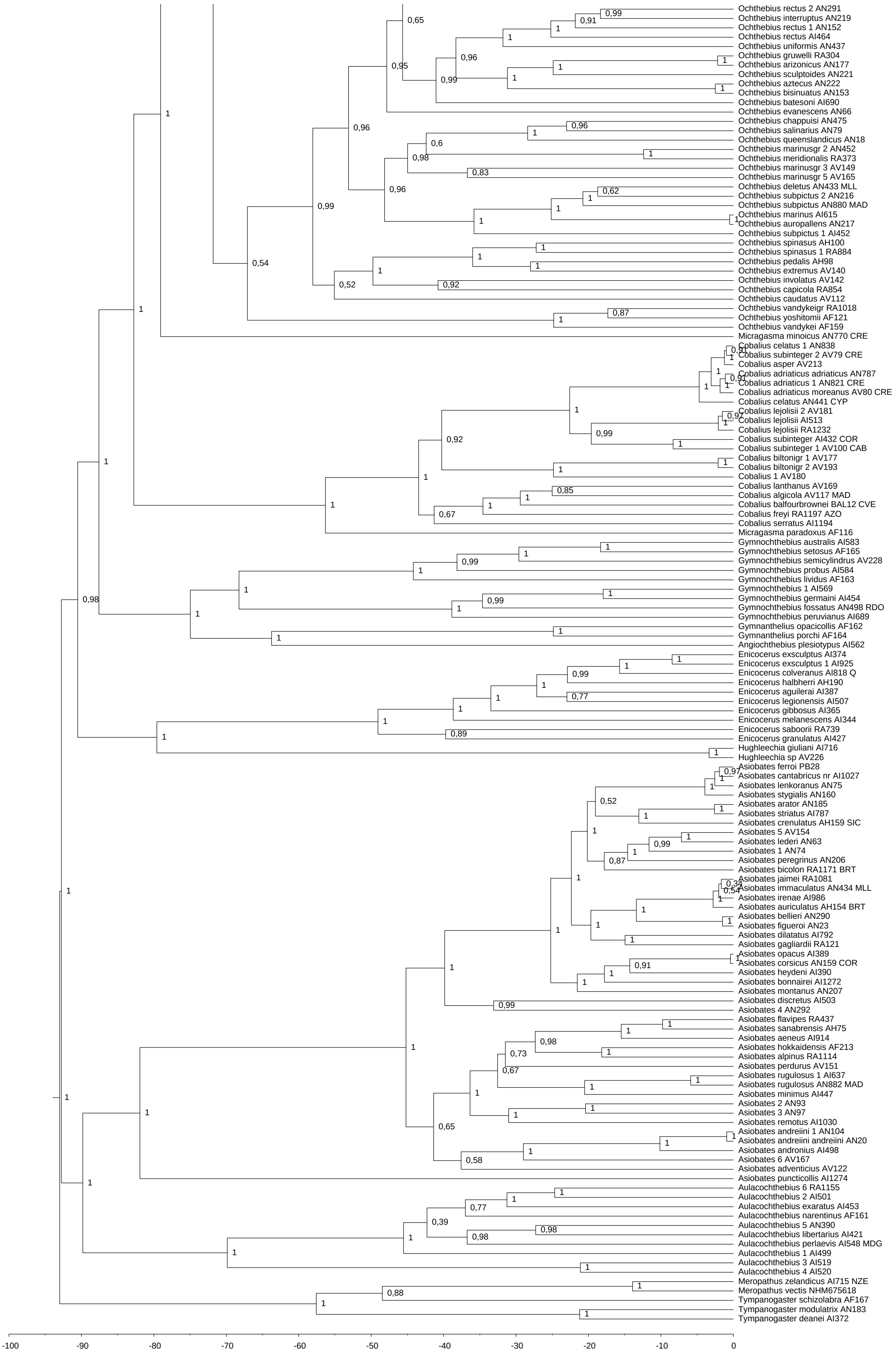


Figure S1. (Continued)

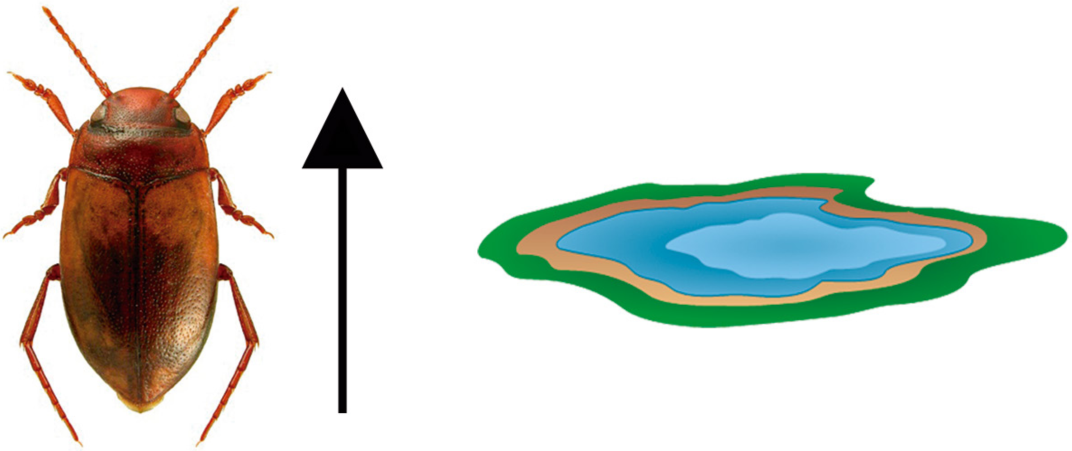


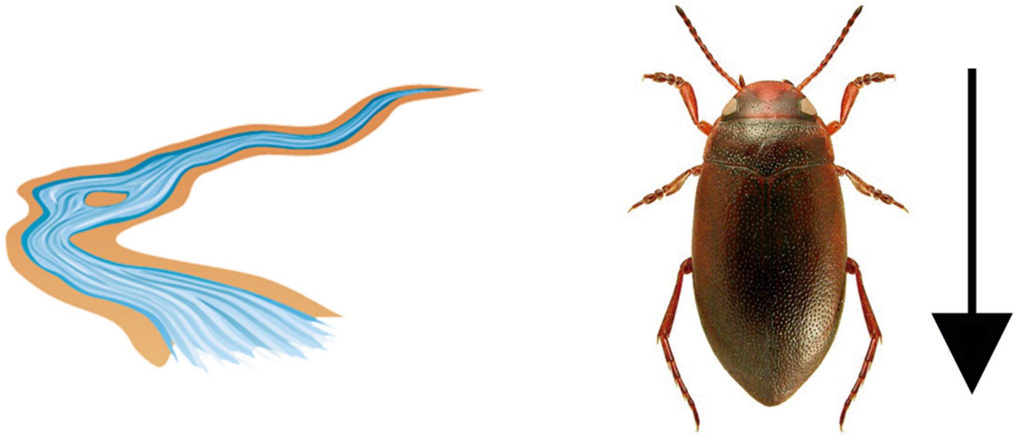
Chapter 6

Habitat preferences, body size and diversification in a speciose lineage of diving beetles

Adrián Villastrigo, Pedro Abellán, Anabela Cardoso
and Ignacio Ribera

Manuscript in preparation for submission.





Abstract

The long-term geological stability of aquatic habitats has demonstrated to be determinant in the evolution of their macroinvertebrate fauna, with species in running (lotic) waters having lower dispersal abilities, smaller ranges and higher gene flow between populations that species in standing (lentic) environments. Lotic species have been hypothesized to be more specialised, but the diversification dynamics of both habitat types, and the possible constraints in morphology, have not been studied in detail. Using a speciose lineage of water beetles we test here whether diversification rates and body size are related to the habitat preference of the species. We built a comprehensive molecular phylogeny with 473 terminals representing 421 of the 689 known species tribe Hydroporini (Coleoptera, Dytiscidae), using a combination of sequences from three mitochondrial and two nuclear genes plus 69 mitogenomes obtained with NGS. We found a general pattern of gradual acceleration of diversification rate with time, with 2 to 3 significant diversification shifts. Habitat diversification rates estimated by MuSSE were similar for both lotic and lentic environments, with overlapping posterior distribution but a slightly higher rate for lentic. The most recent common ancestor of Hydroporini was reconstructed as a lotic species, with multiple shifts to lentic environments. Most frequent transitions were estimated from lentic and lotic habitats to the category “both” followed by transitions from lotic to lentic and lentic to lotic respectively, although with very similar rates. Body size of species in category ‘Both’ were larger than the rest, followed by lentic and then lotic species. Model comparison for body size evolution selected an Ornstein-Uhlenbeck evolutionary model with preference over a Brownian Motion, but the variance and range of body size of the species of each of the defined clades were not correlated with their main habitat. Changes in body size were not related to habitat transitions but with evolution within each habitat. Contrary to expectations, we found little evidence for differences in diversification dynamics or body size evolution between habitats, with lotic environments clearly not acting as evolutionary dead-ends or strongly constraining the body size of the species of Hydroporini.

Introduction

The unevenness in species richness across the Tree of Life is one of the most striking features of biological diversity. Two major hypotheses have been proposed to explain differences in richness among clades (Wiens, 2017): time as the main diversification factor - clade age hypothesis (Li and Wiens, 2019) and / or differential diversification rates among clades - rates hypothesis (Hugall and Stuart-Fox, 2012; Baker *et al*., 2014; McGuire *et al*., 2014; Wiens *et al*., 2015a; Seeholzer *et al*., 2017). A possible cause for differential speciation or extinction rates between clades is the constraint posed by the habitats to the species (Southwood, 1977), tested multiple times in both terrestrial and aquatic environments, such as the shift from saline to freshwaters in amphibians (Hou *et al*., 2011), the type of nesting of some birds (Irestedt *et al*., 2009), or the habitat preference in lizards (Collar *et al*., 2010).

In aquatic environments, the main habitat constraint is the one set between running (lotic) and standing (lentic) waters (e.g. Ribera *et al*., 2001; 2008; Kalkman *et al*., 2018; Ye *et al*., 2019). The lower habitat stability in geological times of isolated lentic water bodies forces a higher dispersal capability of the species living in them, and in consequence broader geographical ranges for lentic than for lotic species (Ribera and Vogler, 2000, Marten *et al*., 2006; Hof *et al*., 2006; Sánchez-Fernández *et al*., 2012). Ribera (2008) hypothesized that habitat constraints determine a higher gene flow between populations of lentic species, which lead to lower speciation and extinction probabilities compared to lotic species. However, the net outcome of the balance between speciation and extinction is uncertain, and there is no strong evidence that lotic or lentic habitats endorse a higher biodiversity.

Limited efforts have been made to understand whether diversification rates are influenced by habitat preference in aquatic environments, perhaps due to the difficulties to estimate diversification rates (Rabosky, 2009) and the need of comprehensive phylogenies to account for precise rates (Barraclough and Nee, 2001). Letsch *et al*. (2016) demonstrated heterogeneous species rates across Odonata, with lentic environments linked to higher rates. However, an incomplete sampling effort (c. 17% of recognized anisopteran species) and the heterogeneous complex biological traits across species may have affected their results. More recently, Désamoré *et al*. (2018) suggested no differential diversification rates between lotic and lentic environments and an uncoupled evolution of morphology and diversification in aquatic Coleoptera, but again with an incomplete sampling (c. 4% of dytiscid species) that underestimated the number of habitat shifts within genera (e.g. *Hydroporus*, with 189 species, has an heterogeneous habitat, but in Désamoré *et al*. (2018) only five of them were included and it was considered as an homogeneous lentic genus).

Here we tested whether diversification rates and body size are related with habitat preference, under the main hypothesis that lotic environments are more specialized habitats than lentic ones (Ribera, 2008). We specifically tested if diversification rates differ between habitats and whether habitat transitions were associated to changes in diversification rates. In the case of lotic environments acting as an ecological specialization, we tested whether they are an evolutionary dead-end (i.e., what are the probabilities of habitat transitions between habitats, and what are their net diversification rates). Additionally, we compared the body size of the species in the two main habitats, and if differences were associated to transitions between habitats or to the evolution of lineages within a habitat. We also tested if lotic habitats show evidence of morphological specialization, as reflected in a reduced variance, or a directional evolution of body size.

To answer those questions, we studied the tribe Hydroporini of the diving beetle family Dytiscidae with a comprehensive dataset including c. 65% of the 688 known species (Nilsson and Hájek, 2019). Species of Hydroporini are more homogeneous in morphology and general life style than other tribes within Dytiscidae, but they are highly diverse and with numerous species both in lotic and lentic environments.

Methods

Molecular data

Molecular sequences were obtained from 473 Hydroporini specimens, representatives of all genera except *Amurodytes*, *Etruscodytes*, *Siamoporus* and *Tassilodytes*, all of them monotypic. We included in the analyses only species of Hydroporini as currently understood (Nilsson and Hájek, 2019), following the most recent phylogenies of Dytiscidae (Désamore *et al*., 2018). We compiled available published sequences plus 500 newly obtained sequences. DNA was extracted with a non-destructive protocol using commercial kits, mostly Qiagen DNeasy Tissue Kit (Hildesheim, Germany). Voucher specimens and DNA samples are kept in the collections of Institute of Evolutionary Biology (IBE, Barcelona, Spain) and Museo Nacional de Ciencias Naturales (MNCN, Madrid). DNA was amplified by PCR for six gene fragments in five reactions for a set of mitochondrial and nuclear genes: i) 5' end of cytochrome c oxidase subunit I (COI-5), ii) 3' end of cytochrome c oxidase subunit I (COI-3), iii) 5' end of rrnL RNA (16S) plus leucine tRNA (tRNA-L1) plus 5' end of NADH dehydrogenase subunit I (NAD1), iv) an internal fragment of small ribosomal unit (18S) and v) an internal fragment of large ribosomal subunit (28S). PCR primers and conditions can be found in Table S1.

Additionally, we sequenced 69 mitochondrial genomes from an equimolar bulk of DNA following a Mitochondrial Metagenomic protocol adapted from Crampton-Platt *et al*. (2016). Our pipeline includes preparation of DNA libraries with TruSeq Nano DNA kit, sequencing in an Illumina MiSeq (insert size 300 bp) and *de novo* assemblies with different softwares (IDBA-UD (Peng *et al*., 2012), RAY-meta (Boisvert *et al*., 2012), WGS-assembler (Myers *et al*., 2000), and SPAdes (Bankevich *et al*., 2012)) to validate each contigs. To assure correct identification of contigs, BLAST (Madden *et al*., 2009) searches were made against a custom database with Sanger sequences of COI and rrnL genes (see Villastrigo *et al*., Chapter 5, for detailed instructions). All sequences were trimmed using Geneious v10.2.5 (Kearse *et al*., 2012) and were uploaded to ENA database with accession numbers (XXXXXXX-XXXXXXX) (see Table S2 for a complete list of voucher specimens, mitochondrial genomes and accession numbers).

Phylogenetic reconstruction

Sequences from both mitochondrial genomes and sanger sequencing were aligned using MAFFT 7.409 (Katoh *et al*., 2009) with the G-INS-I algorithm. To avoid alignments with partitions with very unequal amount of missing data, the partition scheme was based on the type and source of the sequence: (i) nuclear genes (18S plus 28S), (ii) leucine tRNA, present in both Sanger and mitogenomes sequences, (iii) tRNAs present only in mitogenome sequences, (iv) coding sequences present in both Sanger and mitogenome sequences (COI plus partial NAD1), (v) coding sequences present only in mitogenome sequences, (vi) rRNA present in both Sanger and mitogenome sequences (rrnL) and (vii) rRNA present only in mitogenome sequences (rrnS).

We tested four scenarios under a Yule speciation process in Beast v1.10.4 (Suchard *et al*., 2018) for 100 million generations: two complex evolutionary models (HKY+G+I and GTR+G+I) in combination with two different clock model (strict and uncorrelated lognormal clocks). Phylogenies were calibrated with the crown age of Hydroporini (103.6 Ma) obtained by the most recent phylogeny of Dytiscidae, obtained using a combination of fossils (Désamoré *et al*. 2018). To reduce computation time, the monophyly of the subtribes was constrained based on the results of a separate analysis with RAxML-HPC2 (Stamatakis, 2006) without any topological constraint in the CIPRES Science Gateway (Miller *et al*., 2010) with the same partition scheme under a GTR+G model (Fig. S1). Convergence was assessed using Tracer v1.7 (Rambaut *et al*., 2018).

Pattern of diversification and its relationship with habitat

Diversification rates were calculated for all genera with more than two sampled terminals in BAMM v2.5.0 (Rabosky, 2014). In addition to the consensus tree obtained in Beast, we randomly selected a set of 100 trees from the posterior distribution to account for phylogenetic uncertainty, considering in both cases the sampled fraction of species following BAMM specifications. We also estimated diversification rates of the same clades using the *method of moments* estimator (MM-estimator, Magallón and Sanderson, 2001) using the *geiger* r-package (Harmon *et al*., 2008) for both crown and stem ages under different extinction scenarios (no extinction - $\varepsilon = 0$, intermediate - $\varepsilon = 0.5$, and high extinction - $\varepsilon = 0.9$).

To test whether habitat preference states had a different diversification pattern we used Multistate Speciation and Extinction model (MuSSE) under the most complex scenario (one speciation rate and one extinction rate for each state, allowing transitions from and to all habitats preferences), obtaining diversification rate per habitat association. Estimates were calculated in the *Diversitree* r-package (Fitzjohn, 2012) after running a Markov Chain MonteCarlo (MCMC) for 1000 generations.

We also tested the current diversification patterns in each habitat preference state. Mean diversification rates of extant species (tip rates) plus its variance were obtained from the set of 100 post-burnin trees, and the *slouch* r-package (Hansen *et al*., 2008) was used to obtain pairwise comparison of each habitat states trends. Tip rates and its variance were used and habitat preference reconstructed in Beast was included as a fixed factor. The *slouch* package can integrate a measure of variance within the response, minimizing errors attributed to an uneven number of measures across taxa.

Evolution of body size and habitat preference

We compiled average body size values of all species from de data set used by Nilsson-Örtman and Nilsson (2010). Beast was used to reconstruct the evolution of body size and habitat preference under a simple Brownian Motion evolutionary model and an asymmetric substitution model respectively. Alternative evolutionary models were tested for body size evolution using fitContinuous function from *phytools* r-package, selecting the best fitting evolutionary model based on AIC weights (Wagenmakers and Farrell, 2004). We tested the phylogenetic signal of body size using the *phylosignal* r-package (Keck *et al*., 2016) following three different statistics (Abouheif's Cmean, Blomberg's K and Pagel's λ). Phylogenetic signal of habitat was calculated using a likelihood ratio test that compared the maximized likelihood of a model with strong signal ($\lambda = 1$) and a model without signal ($\lambda =$

0), and transitions rates between habitat categories were obtained in Beast.

The relationship between body size and habitat was assessed using Phylogenetic Generalized Least Squares (PGLS) under a linear model. Analysis of variance (ANOVA) was used to test significance.

Specialized habitat as morphological evolutionary drivers

To explore whether habitat transitions affected body size evolution, we used the maximum clade credibility tree to calculate the rate at which body size evolved per node (i.e. the body size difference between each node and its most recent one divided by the branch length between both nodes) and whether a transition has occurred between a node and its most recent one (a variable with seven states, one for no transition between nodes, and six for different habitat preference transitions). We also extracted the rate of habitat evolution and the reconstructed state for habitat at each node. Relations between rate of body size evolution and rate of habitat transition, and for each of those variables against transitions and habitat at each node were tested with ANOVA.

To explore whether the habitat constrained variation in body size, we tested for differences in the variance and absolute range of body size within the most inclusive clades with a homogeneous habitat, allowing for isolated transitions of terminal species within the clade in some cases to increase the size of the clade (see Fig. S2 for the collapsed phylogeny and Table S2 for the delimitation of the clades). Differences in variance and absolute range of body size between clades were tested using PGLS.

Results

Molecular phylogeny and taxonomic status

The phylogenetic reconstruction included 473 terminals representing 423 species and 41 subspecies, plus unidentified lineages (Table S4), covering c. 65% of the recognized species of Hydroporini (688 species plus 27 subspecies, Nilsson and Hájek, 2019). Analyses under uncorrelated lognormal clock did not converge adequately, whilst strict clock analyses did, with essentially the same topology (results not shown). The HKY+G+I model obtained a higher effective sample size than the GTR+G+I and therefore was selected for subsequent analyses.

The consensus phylogeny (Fig. S3) fully resolved the relationships between major

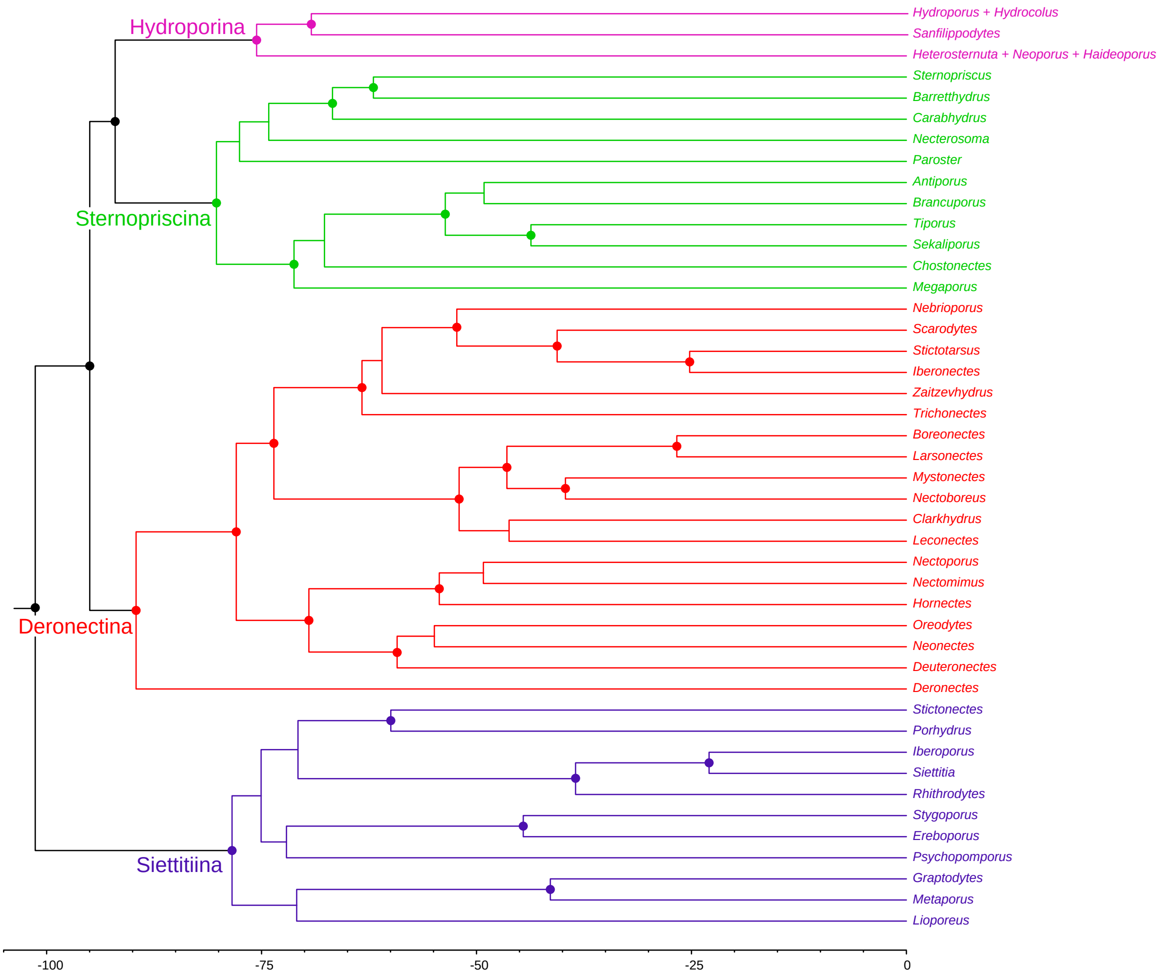


Figure 1. Collapsed molecular phylogeny of the genera of Hydroporini. Each colour represents one of the four subtribes. Circles in nodes when posterior probabilities are equal to 1.

clades (posterior probability [pp] > 1 for subtribe relationships) and considered mostly all genera as monophyletic, except for *Tiporus* (which included the monotypic genus *Sekaliporus*), a clade including the American genera *Neoporus* plus *Heterosternuta* plus *Haideoporus*, and a clade with *Hydrocolus* plus *Hydroporus*. The only non-expected result was the position of *Lioporeus* within Siettitiina (Fig. S1, Fig. S3), while it is currently considered within Hydroporina (Nilsson and Hájek, 2019). A collapsed phylogeny showing major clades is provided in Figure 1.

Pattern of diversification and its relationship with habitat

Estimated diversification rates were similar for most of the major clades analyzed in both analyses, specially comparing BAMM against MM-estimator under a scenario with low extinction for crown groups (Fig. 2, Table S3). Main differences between analyses occurred only in clades with a low number of species: *Barretthydrus*, *Carabhydrus*, *Chostonectes*, *Iberoporus*, *Megaporus*, *Mystonectes*, *Porhydrus* and *Stictotarsus* (Table S4).

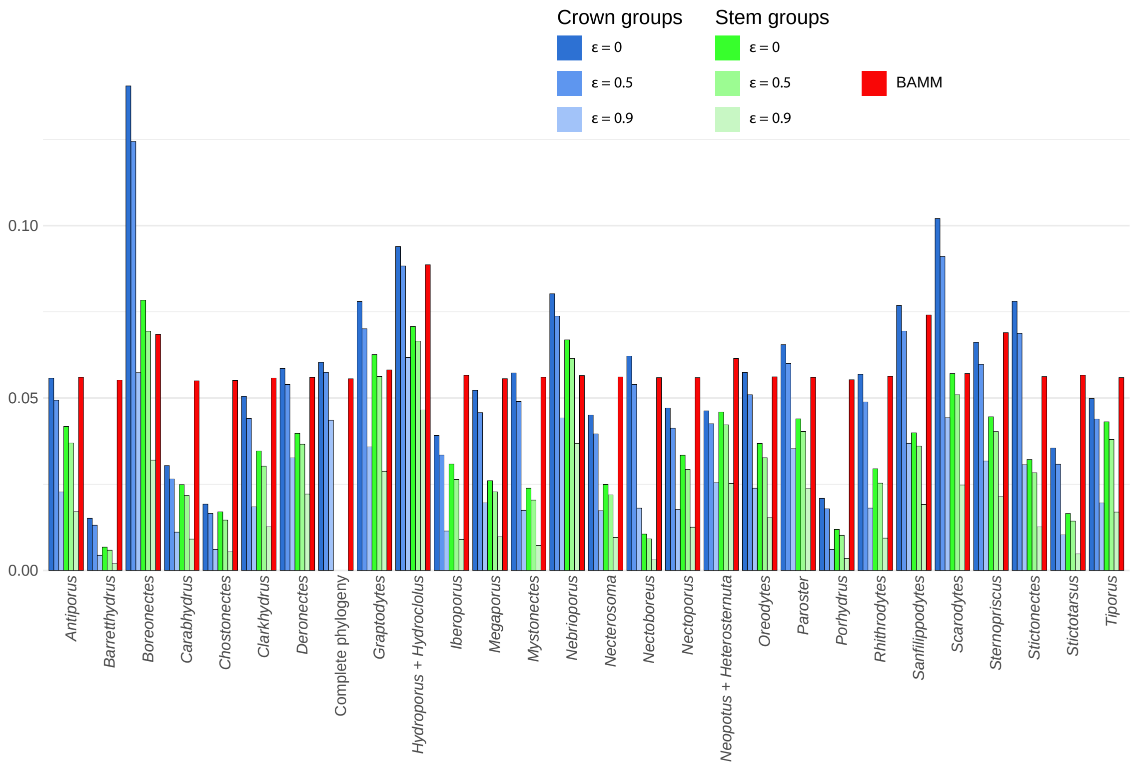


Figure 2. Mean diversification rate for major clades.

The net diversification rate of Hydroporini was estimated by BAMM as 0.056 species/lineage/My, in accordance with the range of estimates obtained by MM-estimator (0.044-0.060 species/lineage/My). The general pattern of diversification reflects a gradually acceleration of diversification rates (Figure 3). BAMM analyses detected 2 to 3 core diversification shifts, with a mean number of shifts in the 100 post-burnin trees of 2.19, and a mode of 2. Shifts were detected in clades within *Boreonectes* and *Sternopriscus*, but also in the clade formed by *Hydrocolus* plus *Hydroporus*, with higher diversification rates in *Sternopriscus* (Figure 3).

Habitat diversification rates estimated by MuSSE were similar for both lotic and lentic environments, with overlapping posterior distribution but a slightly higher rate for lentic (Figure 4). The posterior distribution of category ‘Both’ had a large variance, with a distribution overlapping that of the other two habitats, although the average was smaller than the other categories.

Table 1. Pairwise contrast of expected increase of diversification rate of species considering habitat categories (in diversification rate/My).

	Contrast	**Standard error**
Lentic - Both	0.0007	0.0006
Lotic - Both	0.0002	0.0005

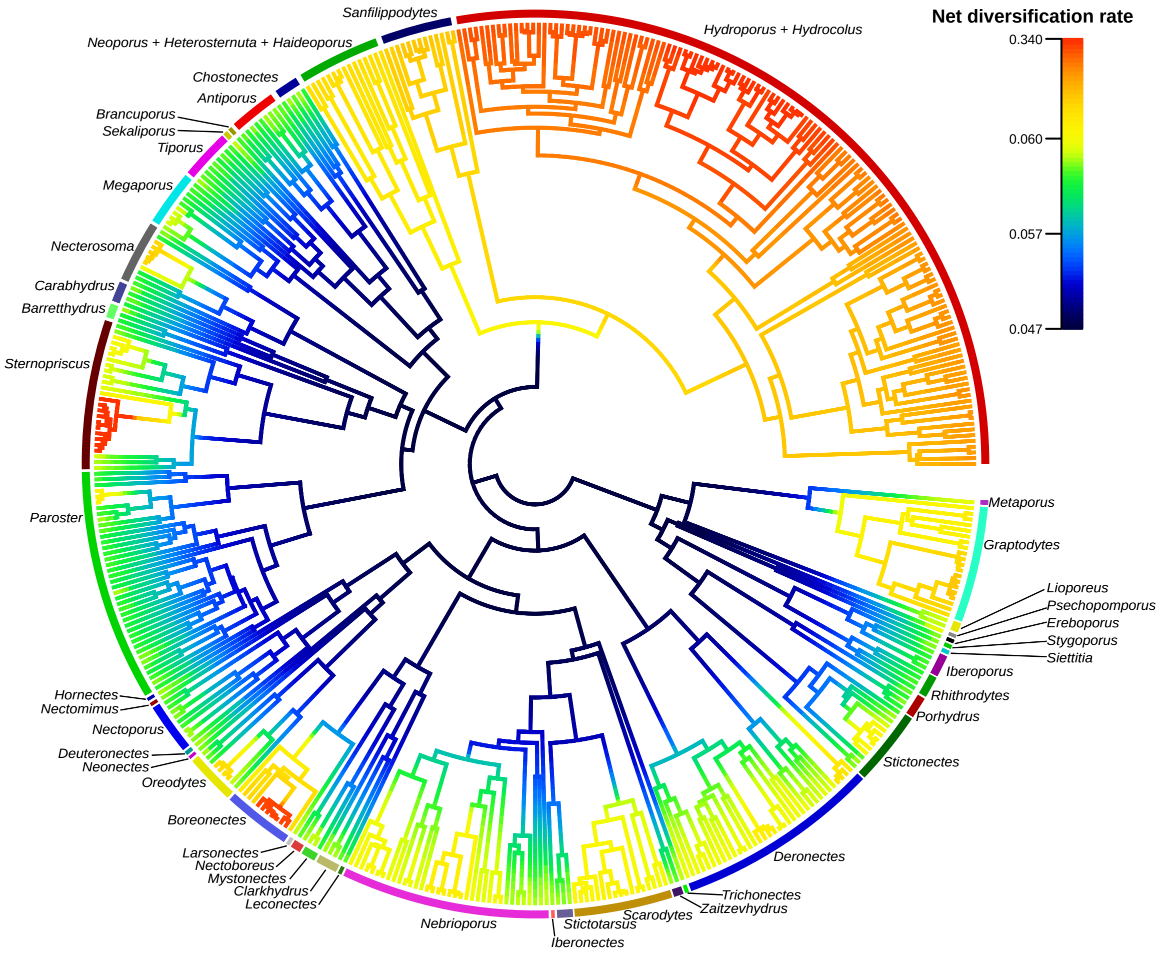


Figure 3. BAMM tree plot of the diversification rates in Hydroporini.

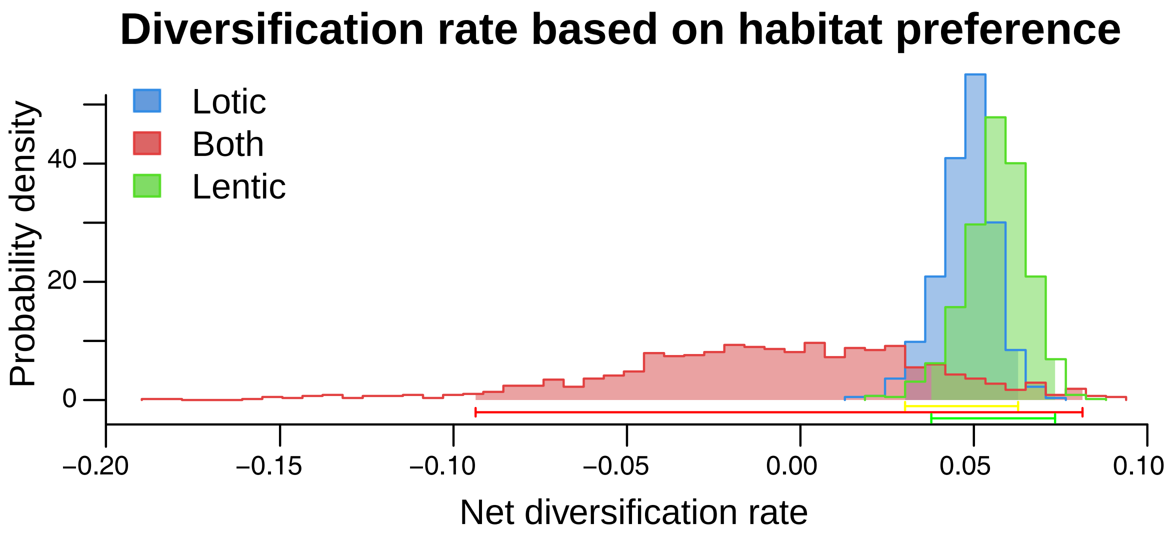


Figure 4. Marginal probabilities of the diversification rates estimated for each habitat preference states in MuSSE.

Lotic - Lentic	-0.0004	0.0002

The diversification trends estimated in *slouch* for the terminals (see tip diversification rates in Table S5) were similar to those found in MuSSE (Table 1, Figure 4): species living in lentic environments tend to increase their diversification rates in comparison with the other categories. Differences between lotic and both categories did not show an evident difference due to their broad standard error.

Evolution of body size and habitat preference

Beast reconstructed the most recent common ancestor of Hydroporini as a lotic species, with multiple shifts to lentic environments (Fig. 5). Transitions from lentic to lotic occurred multiple times and mostly on terminal branches (except in a clade within *Hydropo-*

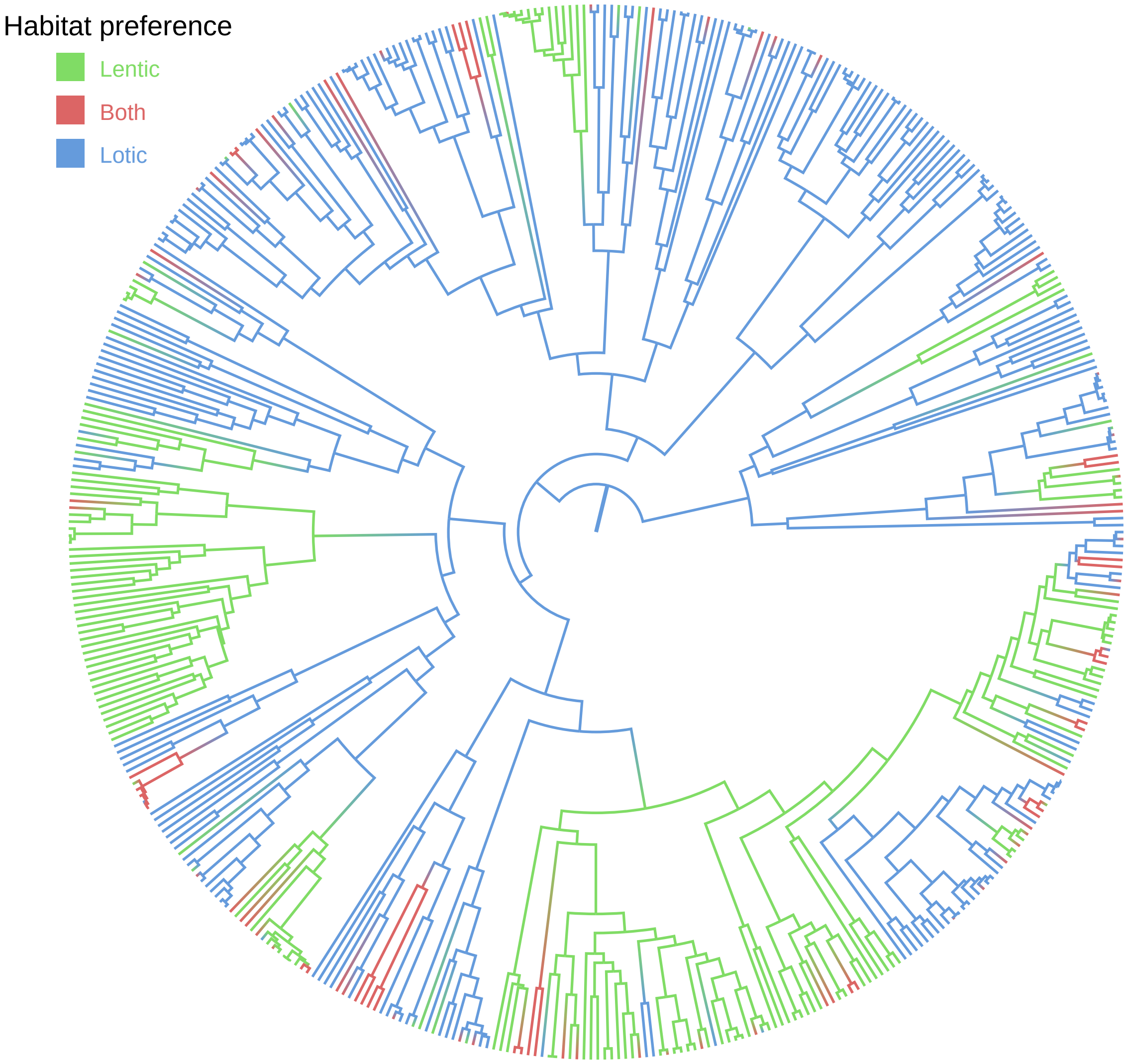


Figure 5. Reconstruction of the habitat preference in Hydroporini in BEAST using an asymmetrical model.

rus), with a similar pattern for species of category both. The reconstructed body size of the most recent common ancestor of Hydroporini was intermediate (3.59 mm), with the biggest species occurring in *Nebrioporus* (7.8 mm) and the smallest ones in the groundwater genus *Paroster* (1 mm).

Model comparison for body size evolution selected an Ornstein-Uhlenbeck evolutionary model with preference over a Brownian Motion based on AIC weights (Table 2). Body size also showed a strong support for Ornstein-Uhlenbeck process by all indexes (Pagel's $\lambda = 0.98$, $p = 0.001$; Cmean = 0.74, $p = 0.001$; Blomberg's K = 0.63, $p = 0.001$).

Table 2. AIC weight comparison for body size evolution

	Brownian Motion	Ornstein- Uhlenbeck	Early Bust
AIC weight	0.002	0.997	0.001

Habitat preference showed a solid phylogenetic signal based on the likelihood ratio test ($p = 1.6 \times 10^{-64}$). Most frequent transitions were estimated from Lentic to Both and from Lotic to Both (1.586 [95% HDP interval 0.42-3.06] and 1.536 [0.43-2.91] t [transitions]/My respectively), followed by transitions from Lotic to Lentic (1.130 [0.28-2.20] t/My) and Lentic to Lotic (0.835 [0.15-1.67] t/My), with transitions from category Both as the less frequent ones (0.490 [0.01-1.24] and 0.371 [0.01-0.90] t/My).

Phylogenetic Generalized Leas Squares provides evidence of body size response based on habitat preference ($p = 0.0005$). Model coefficients for each habitat state were obtained (Table 3), revealing that species for category 'Both' are larger than the rest, followed by lentic species and lotic species.

Table 3. Model coefficients obtained from the linear model applied in PGLS for each habitat state (absolute values)

	Both	Lentic	Lotic
Log(body size)	0.5469	0.5407	0.5288

<u>Specialized habitat as morphological evolutionary drivers</u>

The rate of body size evolution per branch was not correlated with the rate of habitat evolution (F value = 0.905, $p = 0.72$). Similarly, the rate of habitat evolution was not related to the reconstructed habitat at each node (F value = 0.015, $p = 0.99$). However, the rate of body size evolution was significantly correlated with the reconstructed habitat at each node (F value = 4.30, $p = 0.014$), with nodes reconstructed as lotic having accelerated rates of body size evolution (0.016 mm/My) than nodes reconstructed as lentic or both (-0.056 and -0.045 mm/My respectively).

The variance and range of body size of the species of each of the defined clades (Fig. S2, Table S2) were not correlated with their main habitat, as measured by PGLS (p = 0.5 and p = 0.9 respectively).

Discussion

Molecular phylogeny

We found some unexpected phylogenetic relationships, in disagreement with the current classification of Hydroporini. Previous authors noticed the morphological resemblance of *Neoporus* and *Heterosternuta* (e.g. Larson *et al.*, 2000; Miller and Bergsten, 2016), considering them as closely related genera - also, their distributions are overlapping (Larson *et al*. 2000) and they mostly share their habitat preference (Table S2). The main morphological character to distinguish between both genera is the apex of the aedeagus (bifid in *Heterosternuta*), and our samples were identified in base of that character. Our results shed light of a more complex scenario, with *Heterosternuta* as mononophyletic within a larger clade including both *Neoporus* and *Haideoporus*, suggesting the need of a revision of both genera. Miller *et al*. (2013) found a similar result, with a clade including *Haideoporus*, *Heterosternuta* and *Neoporus*. Other novel result was the ascription of *Lioporeus* to Siettitiina subtribe, formerly considered to be part of the *Hydroporus pulcher-undulatus* group by Fall (1923) (see also Miller and Bergsten, 2016). However, their morphological resemblance to other Siettitiina species, especially in the male genitalia, support this new relationship.

The paraphyly of *Hydroporus* is also not surprising, as *Hydrocolus* was erected by the former *Hydroporus oblitus* group (Larson *et al*., 2000). Recent studies considered *Hydrocolus* as closely related to *Hydroporus* (Ribera *et al*., 2008; Miller and Bergsten, 2014), but further studies with a more complete sampling are needed prior to rearrange their taxonomy.

Tiporus is a homogeneous group of species inhabiting lotic environments in northern Australia (Miller and Bergsten, 2016). The low support value of its monophyly (pp = 0.85) may be due to the inclusion of *Sekaliporus* in some of the topologies of the Bayesian analysis. Toussaint *et al*. (2015) found the same result, with a supported clade formed by both *Sekaliporus* and *Tiporus*.

Another interesting result is the relationship between subtribes. We identified Siettitiina as the sister to the rest of Hydroporini, followed by Deronectina and a clade formed by Hydroporina plus Sternopriscina, all of them with very strong support. Désamoré *et al*. (2018) found Hydroporina as sister to the rest, with Siettitiina sister to Deronectina, but with a more incomplete sampling and lower support.

Pattern of diversification and its relationship with the habitat

Despite the recent controversy on the accuracy of diversification rates estimated by BAMM (Moore *et al*., 2016; Rabosky *et al*., 2017; Meyer *et al*., 2018; Meyer and Wiens, 2018; Rabosky, 2018; Rabosky, 2019), our results provide useful insights of its power when using comprehensive phylogenies. Rabosky (2019) showed how rates were an inverse function of clade size and how variance should be taken into account when estimating diversification rates. The low number of species and associated higher variances may explain the differences between the estimates of BAMM and the method of moments in some genera (*Barretthydrus*, *Carabhydrus*, *Chostonectes*, *Iberoporus*, *Megaporus*, *Mystonectes*, *Porhydrus* and *Stictotarsus*).

The general trend of diversification through time is a gradual acceleration, contrary to the general trend of a slowdown reported in the literature (McPeek, 2008; Morlon *et al*., 2010; Moen and Morlon, 2014). A recent study (Henao-Díaz *et al*., 2019) suggested differential patterns within clades, with higher rates at their origin resulting in a slow-down with time. This patter was not observed in Hydroporini, that have undergone diversification events that have maintained or increased the rates of evolution in recent times. Cusimano and Renner (2010) considered that the general pattern of slow-down with time may be caused by non-random sampling, overestimating diversification events in deep nodes. However, some accelerated diversification rates can also be found linked to niche expansions (Nürk *et al*., 2015). BAMM analyses revealed a heterogeneous diversification rate across Hydroporini, with at least two main core shifts for clades within *Sternopriscus* and *Boreonectes*, and in some trees in the clade formed by *Hydrocolus* plus *Hydroporus*. Main shifts occurred in clades formed by species linked to lentic environments, although *Hydroporus* plus *Hydrocolus* has a more heterogeneous habitat preference. However, the amount of species of *Hydroporus* plus *Hydrocolus* that do not live in lotic environment is outnumbered by the others (40 lotic species against 94).

Additionally, the pattern of diversification based on habitat preference showed evidence of a slightly higher diversification rate in lentic environments, no matter whether MuSSE or tip rates were considered. The overlapping posterior distribution between lentic and lotic diversification rates revealed a similar diversification pattern, but slightly slower for species living in lotic environments. Other authors tried to find relationships between these variables: Letsch *et al*. (2016) discovered a similar pattern in dragonflies comparing speciation rates, whereas Désamoré *et al*. (2018) did not recovered any differential net diversification for diving beetles. However, our results can be considered as more robust due to the completeness of out sampling in comparison with these previous studies. Other studies have also tried to match differential diversification or speciation rates with the habitat of the species, such as fishes living in freshwater having higher diversification rates than those in marine habitats (Bloom *et al*., 2013), the coral reef-associated sharks displaying higher speciation rates (Sorenson *et al*., 2014), grassland and savannah as drivers of accelerated diversification rates for falcons (Fuchs *et al*., 2015), the humid forest in Madagascar increasing speciation rates for tenrecs (Everson *et al*., 2016) or the division between terrestrial and aquatic environments exhibit differential rates (Wiens, 2015a). Habitat has been found to explain between 30 to 37 % of the total variation in diversification rates among animal phyla (Wiens, 2015b).

Evolution of body size and habitat preference

Our analyses showed strong evidence of phylogenetic signal for both body size and habitat preference. All tests demonstrated signal for a body size evolution under an Ornstein-Uhlenbeck evolutionary model. Although indexes such as Blomberg’s K or Pagel’s λ assume a Brownian-Motion model, the estimated values for all indexes were compatible with an Ornstein-Uhlenbeck process with a low alpha value (Diniz-Filho *et al*., 2012; Münkemüller *et al*., 2012). The Ornstein-Uhlenbeck model is a random walk complex model based on Brownian Motion that assumes the existence of an optimum trait value than influences the direction of trait evolution (Lande, 1976)), Parameter-rich models like the Ornstein-Uhlenbeck may be favored by AIC comparison over Brownian Motion (Cooper *et al*., 2016), but our extensive sampling with more than 450 terminals is likely to not to be affected by this problem.

According to the habitat reconstruction in Beast, the most common recent ancestor of Hydroporini lived in lotic habitats, with most of the basal nodes being also lotic. The phylogenetic position of Hydroporini within the wider Hydroporinae is still uncertain (e.g. Ribera *et al*., 2008; Désamore *et al*., 2018), and in consequence it is not possible to consider the habitat preference of putative sister groups which may influence the reconstruction of the ancestral habitat of the tribe. The expected outcome of habitat transitions suggested by Ribera (2008) was that the more specialized habitats should have a lower frequency of transitions to other habitats, therefore, transitions from lotic habitats should be lower than those from lentic habitats. Contrary to this expectation, we found similar transition rates between lotic and lentic habitats, demonstrating that lotic habitats are not evolutionary dead-ends (Kelley and Farrel, 1998). The diversification of strictly lotic lineages of water beetles has been related to cycles of rapid expansion in temporal windows of favorable conditions followed by diversification by isolation when these conditions change, as for example during the interglaciars (Ribera *et al*., 2011; García-Vázquez *et al*., 2017). Other studies also found that habitat specialization does not imply an evolutionary dead-end, such as the adaptation to the subterranean environment in both beetles (Cieslak *et al*., 2014) and crayfishes (Stern *et al*., (2017), and in pollinator-specific plants and host-specific plants (Day *et al*., 2016).

Specialized habitat as morphological evolutionary drivers

Our analysis determined that body size evolution is linked to habitat preference, with a strong correlation as measured with PGLS. Mean body sizes were larger for species living in category “Both”, followed by lentic species and lotic species as the smallest ones. In agreement with our results, Gaston *et al*. (2012) found different body sizes in fishes depending on their microhabitats, with species living in pools being larger than species living in riffles. Haad *et al*. (2011) also found differential sizes in tadpoles, with larger species in lentic environments. Ribera and Nilsson (1995) hypothesized that the general absence of large-bodied Dytiscidae in lotic environments was due to the difficulties of overcoming the drag even for the best swimmers, but this referred to the largest species in subfamilies Dytiscinae and Cybistrinae, well over the size of the largest Hydroporini (*Nebrioporu kilimandjarensis*, with 7.8 mm, Table S5).

The mode of body size evolution showed a correlation with the habitat preference of the species but was not associated to the transitions between habitats. Changes in body size were thus related not to changes in main habitat type, but to the evolution of lineages within each type. Some authors have found previously the influence of habitat in morphological aspects, e.g. the relationship of the habitat of the agamid lizards and the evolution of their limbs and body form were found in Collar *et al*., (2010), or the body shapes in different habitats found in threespine stickleback (Aguirre, 2009) and *Mus musculus* (West and King, 2018). These evidences reflect that differential body shape and size are a consequence of evolution within the habitat, as it is usually found no correlation between body size and diversification rates (e.g. Thomas *et al*., 2006; Adams *et al*., 2009; Crouch and Ricklefs, 2019).

If specialized habitats constraint the body size of the species it would be expected that they had less interspecific variation than species living in habitat with less stringent constrains. However, we did not find any evidence of different variance or range of body size between habitats, supporting that body size has been evolving directionally to optimum values within each habitat, as expected under an Ornstein-Uhlenbeck evolutionary model (Lande, 1976).

In conclusion, we found differential diversification rates related to habitat preference in Hydroporini species, added to the influence of the habitats in species' body sizes. In addition, body sizes are not influenced by constrains posed by the habitats, but they are evolving towards optimum values, reflecting that more specialized habitats do not imply an irreversibility similar to an evolutionary dead-end.

Acknowledgements

We would like to thank all colleagues mentioned in Table S2 for sending valuable material for study, as well as Ana Izquierdo (MNCN) and Rocío Alonso (IBE) for laboratory work and A.N. Nilsson (Ümea) for making available the dataset of Dytiscidae body sizes. This work was partly funded by an FPI grant to A.V., and projects CGL2013-48950-C2-1-P and CGL2013-48950- C2-2-P (AEI/FEDER, UE) to I.R. and A.M., respectively.

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Supporting Information

Table S1. List of PCR primers (A) and PCR conditions (B).

Table S2. List of material used in the study, including voucher number, locatity data and accesion numbers of mitogenomes and sequences. In bold, newly obtained sequences.

Table S3. Mean diversification rates and standard deviations obtained in BAMM and MM-estimator (3 different ε scenarios).

Table S4. Number of species per genera currently recognized in Hydroporini and number of samples per genera.

Table S5. Rates estimated for extant taxa (speciation, extinction, diversification and its standard deviation - sd).

Figure S1. Phylogeny obtained with RAxML. Numbers in nodes, bootstrap support values.

Figure S2. Collapsed phylogeny for clades with homogeneous habitat.

Figure S3. Consensus topology obtained in Beast, including genera and tribes names. Numbers in nodes, posterior probability

Table S1. List of PCR primers (A) and PCR conditions (B)

(A) PCR primers

gene	primer	sequence	ref.
COI-3’	Jerry (5’)	CAACATTTATTTTGATTTTTTGG	5
	Pat (3’)	TCCAATGCACTAATCTGCCATATTA	5
	Chy (5’)	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	3
	Tom (3’)	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	3
COI-5’	Uni LepF1b	TAATACGACTCACTATAGGGATTCAACCAATCATAAAGATATTGGAAC	2
	Uni LepR1	ATTAACCCTCACTAAAGTAAACTTCTGGATGTCCAAAAAATCA	2
16S+trnL+nad1	16SaR (5’)	CGCCTGTTTAACAAAAACAT	5
	ND1 (3’)	GGTCCCTTACGAATTTGAATATATCCT	5
16S	16Sb	CCGGTCTGAACTCAGATCATGT	5
18S	18S 5’	GACAACCTGGTTGATCCTGCCAGT(1)	4
	18S b5.0	TAACCGCAACAACTTTAAT(1)	4
H3	H3aF (5’)	ATGGCTCGTACCAAGCAGACRCG	1
	H3aR (3’)	ATATCCTTRGGCATRATRGTGAC	1

(B) PCR conditions

COI-3’ / 18S

step	time	temperature
1	3’	96°
2	30”	94°
3	30”	50°
4	1’	72°
5	Go to setp 2 and repeat 39 x	
6	10’	72°

16S / 28S

step	time	temperature
1	3’	96°
2	30”	94°
3	1’	48°
4	1’	72°
5	Go to setp 2 and repeat 34 x	
6	10’	72°

COI-5’

step	time	temperature
1	3’	96°
2	40”	94°
3	40”	48°
4	1’	72°
5	Go to setp 2 and repeat 4 x	
6	40”	94°
7	40”	51°
8	1’	72°
9	Go to setp 6 and repeat 29 x	
10	10’	72°

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Table S2. List of material used in the study, including voucher number, locatity data, habitat and accesion numbers of mitogenomes and sequences. In bold, newly obtained sequences.

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
1	Deronectina	*Boreonectes*	*alpestris*		IBE-RA263	Italy; Piemonte; Gran Paradiso Nat. Park, Colle del Nivolet, roadside lake at ca 2500 m; 3.9.2010	R.B. Angus	Lentic	Boreonectes + Larsonectes		LS451062	LT796525	LT796551		LT796537
2	Deronectina	*Boreonectes*	*emmerichi*		IBE-RA891	Tibet; S Namtso lake 4750m, banks, 30º37’03”N 90º43’30”E; 21.vii.10	J. Schmidt	Lentic	Boreonectes + Larsonectes		LS451064	LT796532	LT796552	LS453502	LT796542
3	Deronectina	*Boreonectes*	*griseostriatus*	*griseostriatus*	MNCN-AI952	Sweden; Prov. Angermanland, Hörnefors parish, Norrbyskäv island, rock pool	A.N. Nilsson	Both	Boreonectes + Larsonectes		LS451110	LT796533	LT796553	LS453480	LT796544
4	Deronectina	*Boreonectes*	*griseostriatus*	*strandi*	MNCN-AI1082	Norway; Bugöynes; 29.7.2006	S. Ligaard & B. Andrén	Lentic	Boreonectes + Larsonectes			HF931153	HF931372		LT796548
5	Deronectina	*Boreonectes*	*griseostriatus* nr1		NHM-IR342	California (USA); Mono co., Long Valley, 7 km S Bodie, Cotton Wood Canyon Rd.; 19.6.2000	I. Ribera & A. Cieslak	Lentic	Boreonectes + Larsonectes			LS451162	LS452935		**XXXXXXX**
6	Deronectina	*Boreonectes*	*griseostriatus* nr2		MNCN-AI1160	California (USA); Napa Co., Knoxville Recreation Araea, N38º51.350 W122º24.401	T. Berendonk	Lentic	Boreonectes + Larsonectes		LS451065	HF931168	HF931387	LS453496	LT796547
7	Deronectina	*Boreonectes*	*griseostriatus* nr3		MNCN-AI1150	California (USA); Sacramento Co., Clay Station Rd.	W.D. Shepard & C.B. Barr	Lentic	Boreonectes + Larsonectes		LS451060	HF931166	HF931385	LS453497	LT796545
8	Deronectina	*Boreonectes*	*griseostriatus* nr4		IBE-RA483	California (USA); Marin Co., Seasonal Pools - Dillans Beach Dunes, N38.23877, W122.96545	D. Post	Lentic	Boreonectes + Larsonectes		LS451066	HF931317	HF931541	LS453503	LT796546
9	Deronectina	*Boreonectes*	*ibericus*		NHM-IR22	Portugal; Sa. Da Estrela, Torre, lagoon; 25.7.1998	I. Ribera	Lentic	Boreonectes + Larsonectes		LT882885 LS451061	EF670064	EF670030	EF670271 EF670298	EF670157
10	Deronectina	*Boreonectes*	*macedonicus*		MNCN-AI1120	Macedonia, Sar (Shar) Planina, Karanikolicko ezero, 6.9.2006	R.B. Angus	Lentic	Boreonectes + Larsonectes	**XXXXXXX**	LS451063	LT796534	LT796554	LS453505	LT796549
11	Deronectina	*Boreonectes*	*multilineatus*		IBE-RA255	France (Hautes-Pyrénées); Barèges, 2245m, Pond south of the Lac d’Oncet, 31T0266069 4756711, 11.9.2010	F.Bameul	Lentic	Boreonectes + Larsonectes		**XXXXXXX**	LS451148	LS452903	**XXXXXXX**	LS453111
12	Deronectina	*Boreonectes*	*riberae*		MNCN-AI829	Turkey; Düzce, Rd. to Kartalkaya from Çaydurt, pools in mountain pass, N40º40’20” E31º47’05”, 23.4.2006	I. Ribera	Lentic	Boreonectes + Larsonectes		LT882874 LS451113	HF931232	HF931461	LT882951 LS453506	LT796550
13	Deronectina	*Clarkhydrus*	*corvinus*		genbank			Lentic	Clarkhydrus + Leconectes					EU797415	
14	Deronectina	*Clarkhydrus*	*deceptus*		IBE-AV27	California (USA); San Luis Obispo Co., 35.5816ºN 121.0057ºW, Santa Rosa creek, 31.10.2007	M. Caterino & A.E.Z. Short	Lotic	Clarkhydrus + Leconectes		LS451031	LS451156	LS452883		LS453170
15	Deronectina	*Clarkhydrus*	*falli*		NHM-IR334	New Mexico (USA); 9.2000	Y. Alarie	Lentic	Clarkhydrus + Leconectes			EF670063	EF670029	EF670270	EF670155
16	Deronectina	*Clarkhydrus*	*roffii*		NHM-IR335	Texas (USA); 9.2000	Y. Alarie	Lotic	Clarkhydrus + Leconectes		LS451032	AJ850607	AJ850355	AJ850498	EF670158
17	Deronectina	*Deronectes*	*abnormicollis*		MNCN-AI120	Uzbekistan; Tashkent prov. Yakka-tut nr. Burchmulla, 41º38’N 70º03’E	L. Hendrich	Loric	Deronectes		LN995086	LN995059	LN995161		LN995126
18	Deronectina	*Deronectes*	*adanensis*		IBE-DV84	Turkey; Adana prov., Yakapinar, Kizildera env., rokle s potuckem, 36º56.430’N 35º 39.638’E, 30.3.2010	M. Boukal	Lotic	Deronectes			LN995060 LS451146			
19	Deronectina	*Deronectes*	*algibensis*		NHM-IR76	Spain; Cadiz, Puerto de Galis, rd., 27.7.1998	I. Ribera	Lotic	Deronectes			AF309318	AF309261		
20	Deronectina	*Deronectes*	*angelinii*		IBE-RA234	Italy, Quintodecimo, 21.7.09	M. Toledo	Lotic	Deronectes		LN995087	LN995061	LN995162	LS453465	LN995127
21	Deronectina	*Deronectes*	*angusi*		IBE-DV67	Spain; Galdos, Viveiro, Río Bravos, 08.11.2011	C. Benetti & A. Pérez	Lotic	Deronectes	**XXXXXXX**	LT602087	LT601819	LT602510	LS453466	LT602335

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
22	Deronectina	*Deronectes*	*aubei*	*aubei*	IBE-DV150	Italy; Brescia prov., Pisogne, val Palot, stream, in muddy spring, tributary of the stream, 16.6.2009		Lotic	Deronectes		LT602097	LT601829	LT602516	LS453485	LT602343
23	Deronectina	*Deronectes*	*aubei*	*sanfilippoi*	IBE-DV149	France; Gard, Le Vigan, Str. Coudoulous, Rd D48 pk11.5, N44º02'14.7" E3º34'56.8", 3.8.2008		Lotic	Deronectes		LT602141	LT601873	LT602532		LT602355
24	Deronectina	*Deronectes*	*bicostatus*		MNCN-AI639	Portugal; Serra Estrela, Manteigas , ponds in rd. to Torre, N40º19'57" W7º37'03"	I. Ribera	Lotic	Deronectes	**XXXXXXX**	HF947933 LN995091	HE610179	LN995166	LS453486	LN995130
25	Deronectina	*Deronectes*	*brannanii*		MNCN-AI178	Mallorca (Spain); Ternelles, Torrent de Ternelles, N 39º53'37.2" E3º00'14.9"	I. Ribera & A. Cieslak	Lotic	Deronectes		LN995094	HE610180	HF931404	LS453477	LN995133
26	Deronectina	*Deronectes*	*costipennis*	*costipennis*	MNCN-AI183	Portugal; Manteigas, river Zezere	I. Ribera	Lotic	Deronectes		HF947934 LN995095	HE610181	LN995169	LS453487	LN995134
27	Deronectina	*Deronectes*	*costipennis*	*gignouxi*	IBE-DV19	Spain; Valverdín, río Valverdín, 42º 56' 53'' N 5º 32' 10'' W, 25.9.2011	L.F. Valladares	Lotic	Deronectes	**XXXXXXX**	LN995096 HG793322	LN995068	LN995170		LN995135
28	Deronectina	*Deronectes*	*delarouzei*		IBE-DV1	Spain; Barcelona, Bagà, N42º16'05.3" E1º48"46.1", 25.7.05	I. Ribera, P. Aguilera & C. Hernando	Lotic	Deronectes		LT602166	LT601899	LT602538	LS453488	LT602361
29	Deronectina	*Deronectes*	*depressicollis*		MNCN-AI1023	Spain; Almeria, Abrucena, río Nacimiento	A. Castro	Lotic	Deronectes		HF947936 LN995098	HE610182	LN995172	LS453489	LN995137
30	Deronectina	*Deronectes*	*doriae*		MNCN-AI775	Turkey; Düzce., Rd. to Kartalkaya from Çaydurt, fast stream in coniferous forest, N40º39'20" E31º47'8.5", 23.4.2006	I. Ribera	Lotic	Deronectes	**XXXXXXX**	LN995099	HE610183	LN995173	LS453490	LN995138
31	Deronectina	*Deronectes*	*evelynae*		IBE-AV155	Turkey, Kulp distric.	via H. Fery	Lotic	Deronectes		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
32	Deronectina	*Deronectes*	*fairmairei*		MNCN-AI855	Spain; Teruel, Beceite, r. Matarranya, El Parrisal, N40º47'12.5" E0º12'13.5", 3.6.2006	I. Ribera	Lotic	Deronectes		LS451094	HE610184	LS452915	LS453479	LS453116
33	Deronectina	*Deronectes*	*ferrugineus*		MNCN-AI731	Portugal; Serra Estrela/Sabugueiro, N40º24'20" W7º37'43", 12.5.2005	I. Ribera	Lotic	Deronectes		LN995103 LT882872	LN995072 LT882816	LN995176 LT883041	LT882949 LS453491	LN995142 LT883118
34	Deronectina	*Deronectes*	*fosteri*		NHM-IR77	Spain; Barcelona, Saldes	P. Aguilera	Lotic	Deronectes			AF309317	AF309260		
35	Deronectina	*Deronectes*	*hispanicus*		IBE-DV49	Spain; Jaén, Sierra de Cazorla, Ayo. Km 43.3 cta. Del Tranco	A. Castro	Lotic	Deronectes	**XXXXXXX**	LS451102	LS451119	LS452920		LS453135
36	Deronectina	*Deronectes*	*lareynii*		NHM-IR165	Corsica; Vizzavona: Cascades des Anglais,16.9.1999	I. Ribera & A. Cieslak	Lotic	Deronectes		LN995105	AF309316	AF309259	LS453482	**XXXXXXX**
37	Deronectina	*Deronectes*	*latus*		IBE-DV80	Slovenia; Carniola, rv Cerkniščica, Topol pri Begunjah, N45º45'57.5" E14º21'40.0", 4.8.2007		Lotic	Deronectes		LT602190	LT601926	LT602553	LS453467	LT602372
38	Deronectina	*Deronectes*	*moestus*	*inconspectus*	MNCN-AI937	Morocco; 30º47'507"N 7º31'351"W, Tachokchte, Assif Siroua, 19.4.2001	I. Ribera & A. Cieslak	Lotic	Deronectes		LN995109	LN995077	LN995181	**XXXXXXX**	LN995147
39	Deronectina	*Deronectes*	*moestus*	*moestus*	IBE-DV69	Sardinia; Olbia-Tempio, Tempio Pausania, Monte Limbara, SW Cantoniera Curadureddu, 40.8585N 9.1226E 560m, brook, 29.5.2009		Lotic	Deronectes	**XXXXXXX**	LN995110	LN995078	LN995182	LS453478	LN995148
40	Deronectina	*Deronectes*	*nilssoni*		IBE-AF104	Iran; Khorasan Shamali prov., 15 km N Eshq Abad, (river valley; at light), 37º 48.2'N 56º55.5'E, 25-26.5.2006	J. Hájek & P. Chvojka	Lotic	Deronectes		LS451077	LN995080	LN995184	LS453508	LN995150
41	Deronectina	*Deronectes*	*opatrinus*		MNCN-AI629	Spain; Córdoba, Sierra Morena, Km 28.5 cta. Villaviciosa	A. Castro	Lotic	Deronectes		LN995112 LT882870	HE610188 LT882814	LN995185 LT883039	LT882948	LN995151 LT883117
42	Deronectina	*Deronectes*	*parvicollis*		MNCN-AI776	Turkey; Düzce, Rd. to Kartalkaya from Çaydurt, fast stream in coniferous forest N40º39'20" E31º47'8.5", 23.4.2006	I. Ribera	Lotic	Deronectes	**XXXXXXX**	LN995113	HF931225	HF931454	LS453509	LN995152

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
43	Deronectina	*Deronectes*	*persicus*		NHM-IR45	Iran; Fars, 18 km N Sepidan (=Ardakan) Sepidan-Yasuj rd. brook, 13.8.98	H. Fery	Lotic	Deronectes			AF309308	AF309251	AJ850479 EF670290	EF670140
44	Deronectina	*Deronectes*	*platynotus*		MNCN-AI1039	Bulgaria; Rila mts., stream above Rila village.	D.T. Bilton	Both	Deronectes		LN995115	HE610190	LN995186	LS453492	LN995154
45	Deronectina	*Deronectes*	*sahlbergi*		MNCN-AI1002	Bulgaria; Rhodopes, Madjarovo, stream Malkata Reka, 25.4.2006	V. Pesic	Lotic	Deronectes		LT882860 LS451103	HE610191	HF931361 LT883034	LT882947 LS453493	LT883116 LS453109
46	Deronectina	*Deronectes*	*semirufus*		IBE-DV59	Italy; Ascoli-Piceno prov., Acqvusanta, Rio della Volpara, nr. Umito, 2.6.2010	M. Toledo	Lotic	Deronectes		LT602305	LT602054	LT602599	LS453464	LT602410
47	Deronectina	*Deronectes*	*taron*		IBE-AV157	Turkey; Mus, ca. 13 km E Karhova, Karhova-Varto road, ca. 39.285N 41.160E, 18.5.2017	Aykut [via H. Fery]	Lotic	Deronectes		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
48	Deronectina	*Deronectes*	*theryi*		IBE-RA37	Morocco; Taza, stream in Tazzeka N.P., N34º08’56.8” W4º00’25.5”, 22.3.2008	I. Ribera, P. Aguilera & C. Hernando	Lotic	Deronectes		LN995119	LN995083	LN995189	LS453483	LN995158
49	Deronectina	*Deronectes*	*toledoi*		IBE-DV6	Turkey; Erzurum, Toprakkaleköyü,slow stream in grassland m, N40 14 22.9 E40 59 16.7, 11.6.2011	i. Ribera	Lotic	Deronectes		LN995120	LN995084	LN995190	LS453468	LN995159
50	Deronectina	*Deronectes*	*wewalkai*		MNCN-AI725	Spain; Guadalajara, r. Berbedillo, 7km E Cardoso de la S. 1159m, N41º05’34.3” W3º25’32.1”	I. Ribera & A. Cieslak	Lotic	Deronectes		HG793317 LN995121	LN995085	LN995191	LS453494	LN995160
51	Deronectina	*Deronectes*	*youngi*		NHM-IR182	Iran; Kohkiluyeh and Boyer Ahmad province, Gachsaran to Behbahan. road, 16 km N Gachsaran, stream, 24.3.99	H. Fery	Lotic	Deronectes		LS451079	AF309306	AF309249	AJ850480	EF670142
52	Deronectina	*Deuteronectes*	*picturatus*		NHM-IR369	California (USA); Trinity co., junc. Rd. 36 & 14, Forest Glen, Rattlesnake Creek, 22.6.2000	I. Ribera & A. Cieslak	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes	**XXXXXXX**		AJ850602	AJ850350	AJ850489	EF670150
53	Deronectina	*Hornectes*	*quadrimaculatus*		NHM-IR366	California (USA); Trinity co., junc. Rd. 36 & 14, Forest Glen, Rattlesnake Creek, 22.6.2000	I. Ribera & A. Cieslak	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		**XXXXXXX**	AJ850603	AJ850351	AJ850490	EF670151
54	Deronectina	*Iberonectes*	*bertrandi*		NHM-IR30	Spain; Leon, Puente del Rey, r. Burbia, 23.7.1998	I. Ribera	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451059	AY250984	AY250946	AJ850494 EF670296	EF670154
55	Deronectina	*Larsonectes*	*minipi*		IBE-AN861	Saskatchewan (Canada; Thomson Bay, Athabasca Sansa Park, 59º05’00”N 109º01’13”W, 2.7.2004	R. Hooper	Lentic	Boreonectes + Larsonectes		**XXXXXXX**	LS451131	**XXXXXXX**		LS453114
56	Deronectina	*Leconectes*	*striatellus*		IBE-RA327	California (USA); San Diego Co. rd. 78, Cuyamaca Rancho State Park, Lotic veg. rich cold small creek, N 32° 56.711’ W 116° 34.225’, 11.12.2010	I. Ribera & J. Bergsten	Both	Clarkhydrus + Leconectes	**XXXXXXX**	LS451114	LS451125	LS452886	LS453504	LS453112
57	Deronectina	*Mystonectes*	*coelamboides*		IBE-PB35	California (USA)		Both	Mystonectes + Nectoboreus	**XXXXXXX**	LS451058	LS451169	LS452924	LS453481	LS453142
58	Deronectina	*Mystonectes*	*neomexicanus*		IBE-RA775	Mexico; Chihuahua, Alvaro Obregon, 1,3 miles NW, 10.10.1975	J.R. Zimmermann	Lotic	Mystonectes + Nectoboreus						LS453158
59	Deronectina	*Mystonectes*	*panaminti*		IBE-RA1086	California (USA) Inyo Co., Salt Crek@Interprtive, Site. Death Valley Nat.Park	G. Challet	Lotic	Mystonectes + Nectoboreus		LS451112	LS451170	LS452923	**XXXXXXX**	LS453141
60	Deronectina	*Nebrioporus*	*abyssinicus*		MNCN-AI1227	Ethiopia; Welo Prov. 10 km N. Lalibela, 2200m	G. Wewalka	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451048	LS451124	LS452902	LS453516	LS453129
61	Deronectina	*Nebrioporus*	*airumlus*		MNCN-AI1179	Iran; Fars prov., road Sepidan-Yasuj, Vezek, 13 km S Yasuj, brook	H. Fery	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	**XXXXXXX**	**XXXXXXX**	LS451150	LS452907	**XXXXXXX**	LS453175
62	Deronectina	*Nebrioporus*	*amicorum*		IBE-AF140	Crete; Heraklion, Arkalohori, Tsoutsouros: stream 34°59’05’’ 25°17’07’’, 22.2.2008	Z. Csabai et al.	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	**XXXXXXX**	LT882833 LS451024	HF931118	HF931335	LT882953 LS453462	LT883122 LS453162
63	Deronectina	*Nebrioporus*	*assimilis*		MNCN-AI607	Sweden; Västerbotten prov., Åmsele, Vindelälven, river lagoon, 64º31’04’’N, 19º20’52’’E	A.N. Nilsson	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		**XXXXXXX**	LS451115	LS452910	LS453534	LS453117

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
64	Deronectina	*Nebrioporus*	*baeticus*		NHM-IR10	Spain; Sevilla, Osuna, Arroyo el Pintado, 27.7.1998	I. Ribera	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		**XXXXXXX**	AF309302	AF309245	AJ850481 EF670292	EF670143
65	Deronectina	*Nebrioporus*	*bucheti*	*cazorlensis*	MNCN-AI926	Spain; Albacete, E-No. 10, N Sierra de Segura, 10 km N Yeste, Rio Tús, 38º25'N,02º19W	V. Assing & P. Wunderle	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		HF947997	LS451139	LS452918	LS453521	LS453122
66	Deronectina	*Nebrioporus*	*canaliculatus*		MNCN-AI1044	Spain; Bascara, Rio Fluvia, 10.9.2000	P. Aguilera	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	**XXXXXXX**	LS451073	HF931149	HF931368	LS453517	LS453157
67	Deronectina	*Nebrioporus*	*canariensis*		MNCN-AI1091	Tenerife (Spain); Chamorga, Bco. Roque Bermejo	A. Castro	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451051	HF931155	HF931374	LS453532	LS453133
68	Deronectina	*Nebrioporus*	*capensis*		IBE-RA850	South Africa; N. Cape, Oorlogshloof river, on R 6.7 caolen [?], E of Neeuwouedt ville, 25.9.2011	D.T. Bilton	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes			LS451123	LS452908	LS453547	LS453097
69	Deronectina	*Nebrioporus*	*carinatus*		NHM-IR17	Spain; Leon, Puente del Rey, r. Burbia, 23.7.1998	I. Ribera	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes			AF309303	AF309246	AJ850482 EF670293	EF670144
70	Deronectina	*Nebrioporus*	*ceresyi*		MNCN-AI57	Malta; Ghadira Nat. reserve, saline pool	N. Barbara	Lentic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	**XXXXXXX**	LS451027	FJ944417	LS452881	LS453522	LS453121
71	Deronectina	*Nebrioporus*	*clarkii*		NHM-IR46	Spain; Sevilla, Osuna, Arroyo el Pintado, 27.7.1998	I. Ribera	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes			EF056623	AY250924	EF056657 EF670294	EF056585
72	Deronectina	*Nebrioporus*	*cooperi*		IBE-AN21	Ethiopia; Amhara, Debark, Simien Mountains, 13.193N 37.891E, 18.6.2014	R. Vila & G. Talavera	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	**XXXXXXX**	**XXXXXXX**	LS451127	LS452900	LS453535	LS453127
73	Deronectina	*Nebrioporus*	*croceus*		MNCN-AI82	Spain; Soria, Calatañazor, R. Abion	P. Aguilera	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		HF947999	LS451149	LS452914	LS453523	LS453118
74	Deronectina	*Nebrioporus*	*depressus*		MNCN-AI331	Russia; Karelen	J. Damgaard	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451055	HF931189	HF931412	LS453524	LS453120
75	Deronectina	*Nebrioporus*	*dubius*		MNCN-AI83	Madeira (Portugal); Achada, Curral das Freiras	G.N. Foster	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451105	HF931233	HF931462	LS453533	LS453134
76	Deronectina	*Nebrioporus*	*elegans*		MNCN-AI606	Sweden; Västerbotten prov., Åmsele, Vindelälven, river lagoon, 64º31'04''N, 19º20'52''E	A.N. Nilsson	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	**XXXXXXX**	LS451054	HF931214	HF931440	LS453525	LS453119
77	Deronectina	*Nebrioporus*	*fabressei*		NHM-IR169	Spain; Navarra, Barindano, r. Itxaco, 2.11.1997	I. Ribera	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes			AY250966	AY250926		**XXXXXXX**
78	Deronectina	*Nebrioporus*	*fenestratus*		MNCN-AH175	Sicily (Italy); Parco dei Nebrodi, Fiume Sanbárbaro Fughetto, 33N 461042 E, 4206949 N, 13.6.07	P. Abellán & F. Picazo	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	**XXXXXXX**	LS451053	HF931138	HF931355	LS453526	LS453104
79	Deronectina	*Nebrioporus*	*kilimandjarensis*		IBE-AN690	Tanzania, Kilimandjaro, Alluaud	via H. Fery	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes						LS453130
80	Deronectina	*Nebrioporus*	*lanceolatus*		NHM-IR650	Iran; Fars, 38 Km NE Shiraz, road Shiraz-Band-e-Amir, ditch running water, 24.4.2001	K. Elmi & H. Fery	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451092	AY250967	AY250927	LS453518	LS453143
81	Deronectina	*Nebrioporus*	*luctuosus*		IBE-RA724	Spain; Zaragoza, 19.9.2009	I. Esteban	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451050	LS451151	LS452916	LS453527	LS453123
82	Deronectina	*Nebrioporus*	*macronychus*		genbank			Lentic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		KJ204116				
83	Deronectina	*Nebrioporus*	*martinii*		MNCN-AI702	Corsica; Corte, river Restonica	I. Ribera & A. Cieslak	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LT882871 LS451045	LT882815 LS451118	LT883040 LS452917	LT882952 LS453461	LT883121 LS453128
84	Deronectina	*Nebrioporus*	*mascatensis*		IBE-RA107	Oman; J. Al-Akhdar, wadi Bani Awf, village below source, N23 11 38.6 E57 23 41.7, 6.4.2010	I. Ribera, C. Hernando & A. Cieslak	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451091	HF931299	HF931531	LS453519	LS453163

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
85	Deronectina	*Nebrioporus*	*millingeni*		IBE-RA607	Yemen; Sana'a gov. 5 km SW of Matnah vill., 15º13.7'N, 43º59.7'E., 19.11.2010	J. Hájek	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	**XXXXXXX**	LS451109	LS451126	LS452909	LS453463	LS453159
86	Deronectina	*Nebrioporus*	*nemethi*		MNCN-AI307	Morocco; Laatamna, Afl. Oued Larbâa	P. Aguilera	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451026	FJ944375	LS452906	LS453528	LS453101
87	Deronectina	*Nebrioporus*	*nipponicus*		NHM-IR610	Japan; Honshu, Gifu Pref., Tokuyama, 29.8.2000	J. Bergsten	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		**XXXXXXX**	AY250971	AY250931	LS453520	LS453173
88	Deronectina	*Nebrioporus*	*rotundatus*		MNCN-AC41	Nova Scotia (Canada); Cumberland c., river next Rd. 2, Mapleton, N45º34'54.3" W64º08'30.3", 23.8.2007	I. Ribera & A. Cieslak	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451056	HF931111	HF931328	LS453529	LS453124
89	Deronectina	*Nebrioporus*	*sansii*		IBE-RA725	Spain; Teruel, Aliaga, 30.7.2010	I. Esteban	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451068	LS451164	LS452919	LS453530	LS453174
90	Deronectina	*Nebrioporus*	*sardus*		IBE-RA17	Sardinia; Ogliastra, W Villanova Strisaili, 39.96332N 9.45989E 834m, 27.5.2009	H. Fery & M. Toledo	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451108	HF931306	HF931533	LS453531	LS453131
91	Deronectina	*Nebrioporus*	*scotti*		MNCN-AI1225	Ethiopia; Shewa Prov., 95 km N Addis Ababa, Debre Libanos	G. Wewalka	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		**XXXXXXX**	LS451158	LS452901	LS453536	LS453132
92	Deronectina	*Nebrioporus*	*seriatus*		IBE-RA613	Yemen; Sana'a gov. 5 km SW of Matnah vill., 15º13.7'N, 43º59.7'E., 19.11.2010	J. Hájek	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451052	LS451120	LS452936	LS453548	LS453126
93	Deronectina	*Nebrioporus*	*stearinus*	*stearinus*	NHM-IR134	Iran; Fars prov., road Sepidan-Yasuj, 65 km N Sepidan, almost dry brook, 13.8.1998	H. Fery	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes			AY250972	AY250932	AJ850484	EF670145
94	Deronectina	*Nebrioporus*	*stearinus*	*suavis*	MNCN-AI109	Chios (Greece); Katasavi	G.N. Foster	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451047	HF931154	HF931373	**XXXXXXX**	LS453161
95	Deronectina	*Nebrioporus*	*vagrans*		IBE-RA588	South Africa; NC /Kamiesberg Stream on Witwater-/ Longkloof road ca. 1 km S. Of / junction, 19.9.2010	D.T. Bilton	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	**XXXXXXX**	LS451111	LS451137	LS452911	LS453549	LS453181
96	Deronectina	*Nectoboreus*	*dolerosus*		IBE-AN128	California (USA); San Bernardino Co., San Benito River at Clear, Creek Rd., 36'358176 120'785887	G. Challet	Lotic	Mystonectes + Nectoboreus	**XXXXXXX**	**XXXXXXX**	LS451132	LS452884	LS453484	LS453113
97	Deronectina	*Nectoboreus*	*funereus*		MNCN-AI1208	California (USA); 9.2000	Y. Alarie	Lentic	Mystonectes + Nectoboreus		LT882865 LS451106	HF931173	HF931393	LT882950 LS453507	LT796543 LT883119
98	Deronectina	*Nectomimus*	*okulovi*		IBE-RA1218	Russia; Tyva, Ungesh River, stream, roiled, 3.9.06	Zaika [via H. Fery]	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes			LT855780 LS451145			LT856005 LS453102
99	Deronectina	*Nectoporus*	*abbreviatus*		MNCN-AI932	California (USA); El Dorado Co., E Placerville at Park Creek	A.I. Cognato	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		LS451099	HF931243	HF931477	LS453512	LT855994 LS453099
100	Deronectina	*Nectoporus*	*congruus*		NHM-IR440	British Columbia (Canada); Rd. 3, Rock Creek , 26.6.2000	I. Ribera & A. Cieslak	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes			AJ850599	AJ850347	AJ850485	EF670146
101	Deronectina	*Nectoporus*	*crassulus*		NHM-IR451	Alberta (Canada); Rd. 3, 2 km W Lund Breck, Crownsnest r. campground, 27.6.2000	I. Ribera & A. Cieslak	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		**XXXXXXX**	AJ850600	AJ850348	AJ850486	EF670147
102	Deronectina	*Nectoporus*	*obesus*	*cordillerensis*	NHM-IR452	Alberta (Canada); Rd. 3, 2 km W Lund Breck, Crownsnest r. campground, 27.6.2000	I. Ribera & A. Cieslak	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes			HF931279	HF931516	LS453514	**XXXXXXX**
103	Deronectina	*Nectoporus*	*obesus*	*obesus*	NHM-IR399	California (USA); Marin co., Olema, Olema Creek, Olema campground, 24.6.2000	I. Ribera & A. Cieslak	Both	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes			LT855762	LT856139		
104	Deronectina	*Nectoporus*	*rhyacophilus*		NHM-IR367	California (USA); Trinity co., junc. Rd. 36 & 14, Forest Glen, Rattlesnake Creek, 22.6.2000	I. Ribera & A. Cieslak	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		**XXXXXXX**	AJ850604	AJ850352	AJ850491	EF670152
105	Deronectina	*Nectoporus*	*sanmarkii*	*alienus*	MNCN-AI1134	Portugal; Manteigas, r. Zezere	I. Ribera	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		LT855757 LS451075	LT855813 LS451173	LT856168 LS452897	**XXXXXXX**	LT856018 LS453098

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
106	Deronectina	*Nectoporus*	*sanmarkii*	*sanmarkii*	MNCN-AI973	Mongolia; Ovorhangay, Bat-Olziy, Orkhon's Waterfall on Ulaan Gol, ~300 m S of Orkhon Gol, ~84 km W of Khujirt, N46.78742 E101.96021, 7.7.2004	A.E.Z. Short	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		LT855753 LS451069	LT855801 LS451167	LT856161 LS452891	LS453510	LT856014 LS453100
107	Deronectina	*Nectoporus*	*subrotundus*		IBE-RA485	California (USA); Mendocino Co., Chadbourne Gulch at Mouth, N - 39.61321, W - 123.78116	D. Post	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes	**XXXXXXX**	LT855759 LS451044	LT855817 LS451157	LT856173 LS452888	LS453511	LT856022 LS453103
108	Deronectina	*Neonectes*	*natrix*		NHM-IR611	Japan	J. Bergsten	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes			AJ850601	AJ850349	AJ850488	EF670149
109	Deronectina	*Oreodytes*	*alpinus*		MNCN-AI977	Scotland (UK); E Sutherland, Loch Brora	I. Ribera & G.N. Foster	Lentic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		LT855735 LS451087	HF931257	HF931493	LS453498	LT855995 LS453139
110	Deronectina	*Oreodytes*	*davisii*	*davisii*	MNCN-AI1127	Spain; Granada, Río Trévelez, Sierra Nevada	F.M. Cabezas	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes	**XXXXXXX**	LT855743 LS451085	LT855774 LS451134	LT856148 LS452893	LS453499	LT856003 LS453137
111	Deronectina	*Oreodytes*	*davisii*	*rhianae*	NHM-ER33	Spain; Avila, Sa. De Gredos, Gredos, ca., Gebirgsbach, 20.7.99	H. Fery	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes			AF309301	AF309244	AJ850487 EF670295	EF670148
112	Deronectina	*Oreodytes*	*mongolicus*		MNCN-AI972	Mongolia; Ovorhangay, Bat-Olziy, Orkhon's Waterfall on Ulaan Gol, ~300 m S of Orkhon Gol, ~84 km W of Khujirt, N46.78742 E101.96021, 7.7.2004	A.E.Z. Short	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		LS451086	HF931255	HF931491	LS453500	LT856006 LS453138
113	Deronectina	*Oreodytes*	*scitulus*	*bisulcatus*	IBE-AN613	California (USA); Humbolt Co., Redwood Creek at Hwy 101 at Orick, 18.8.2016	G.L. Challet	Both	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		LS451025	LS451129	LS452892		LS453177
114	Deronectina	*Oreodytes*	*scitulus*	*scitulus*	IBE-AV1	Nova Scotia (Canada); Cape Breton, c. Inverness, stream in Big Marsh Rd., N45º53'36.8" W61º12'16.8", 16.8.2007	I. Ribera & A. Cieslak	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes	**XXXXXXX**	**XXXXXXX**	LS451168	LS452889	**XXXXXXX**	LS453178
115	Deronectina	*Oreodytes*	*septentrionalis*		MNCN-AI974	Mongolia; Ovorhangay, Bat-Olziy, Orkhon's Waterfall on Ulaan Gol, ~300 m S of Orkhon Gol, ~84 km W of Khujirt, N46.78742 E101.96021, 7.7.2004	A.E.Z. Short	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		LS451049	HF931256	HF931492	LS453513	LT856019 LS453136
116	Deronectina	*Oreodytes*	*shorti*		MNCN-AI965	Mongolia; Arkhangay, Bulgan, Urd Tamir Gol braid upstream of bridge, ~63 km SW of Tsetserleg / N47.11192 E101.01048, 13-15.7.2004	A.E.Z. Short	Lotic	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		LS451089	HF931252	HF931488	LS453495	LT856021 LS453140
117	Deronectina	*Oreodytes*	*snoqualmie*		NHM-IR447	Alberta (Canada); Rd. 3, 2 km W Lund Breck, Crownsnest r. campground, 27.6.2000	I. Ribera & A. Cieslak	Both	Nectomimus + Nectoporus + Neonectes + Hornectes + Oreodytes + Deuteronectes		LS451088	LT855816 LS451143	LT856172 LS452890	LS453501	
118	Deronectina	*Scarodytes*	*antoni*		MNCN-AC6	Croatia; Istria	H. Fery	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451041	LS451121	EU156492	LS453537	LS453151
119	Deronectina	*Scarodytes*	*fuscitarsis*		IBE-RA4	Sardinia; Nuoro prov. (Ogliastra historical region), river W Villanova Strisàili, 39.96332N 9.45989E, 27.5.2009	H. Fery & M. Toledo	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451071	LS451142	LS452921	LS453552	LS453156
120	Deronectina	*Scarodytes*	*halensis*		MNCN-AI773	Turkey; Düzce, Rd. to Kartalkaya from Çaydurt, pools in mountain pass, N40º40'20" E31º47'05", 23.4.2006	I. Ribera	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451040	LS451140	LS452928	LS453538	LS453149
121	Deronectina	*Scarodytes*	*halensis* nr1		MNCN-AH121	Rhodos (Greece); Municipality of Kamiros, Apollona, River Gadouras, upper north arm 2. 36º12'58"N 27º57'35"E. 22.2.2007	Z. Csabai et al.	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451043	LS451141	LS452929	LS453459	LS453164
122	Deronectina	*Scarodytes*	*halensis* nr2		NHM-ER35	Spain; Barcelona, Saldes, Bach, 2.8.1999	H. Fery	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451042	AF309305	AF309248	AJ850492	EF670153
123	Deronectina	*Scarodytes*	*malickyi*		IBE-AF139	Crete; Chania, Kolimbari, Minothiana: stream 35°32'09,9" 23°47'30,5", 21.2.2008	Z. Csabai et al.	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	**XXXXXXX**	LS451039	HF931117	HF931334	LS453539	LS453146
124	Deronectina	*Scarodytes*	*nigriventris*		NHM-IR157	Corsica; Cassamozza, r. l'Abatescu, 19.9.1999	I. Ribera & A. Cieslak	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451070	AY250977	AY250938		**XXXXXXX**
125	Deronectina	*Scarodytes*	*pederzanii*		MNCN-AH168	Sicily (Italy); Parco dei Nebrodi, Stream Trail Lago Urio-Plla. dell'Obolo 2, 33N 448676 E, 4194243 N, 13.06.07	P. Abellán & F. Picazo	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451029	HF931137	HF931354	LS453550	LS453147
126	Deronectina	*Scarodytes*	*roberti*		IBE-RA204	Greece; S Peloponnese, ca. 12 km NW Sparti, NE Kambos, small stream, 37.15319N 22.35353E, ca. 294 m, 19.5.2010	H. Fery & L. Hendrich	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451038	FR773506	LS452927	LS453540	LS453154

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
127	Deronectina	*Scarodytes*	*ruffoi*		IBE-AF62	Italy; Abruzzi, prov. L'Aquila, ca. 4 km, N Pescasséroli, Fiume	H. Fery	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451030	HF931128	HF931347	LS453551	LS453148
128	Deronectina	*Scarodytes*	*savinensis*	*cercyrae*	IBE-RA219	Corfou (Greece); Municip. of Lefkimeon, Vitalades, stream, 39º24'12.70"N 20º01'44.20"E	Z. Csabai et al.	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes	XXXXXXX	LS451035	LS451122	LS452926	XXXXXXX	LS453153
129	Deronectina	*Scarodytes*	*savinensis*	*savinensis*	MNCN-AI135	Montenegro; Virpazar, Orahovska river	V. Pesic	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451034	EU156490	EU156491	LS453541	LS453155
130	Deronectina	*Scarodytes*	sp1		MNCN-AI304	France; Alpes Maritimes, river Carai, 2 km N Monti, 19.10.2002	I. Ribera & A. Cieslak	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451036	LS451117	LS452925	LS453543	LS453107
131	Deronectina	*Scarodytes*	sp2		NHM-IR653	Iran; Fars, 17 km SE Sepidan (=Ardakan) Shesh Pir. Ditch with running water, 18.4.2001	K. Elmi & H. Fery	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451057	AY250978	AY250939	LS453544	LS453152
132	Deronectina	*Scarodytes*	sp3		MNCN-AH167	Sicily (Italy); Parco dei Nebrodi, Stream Trail Lago Urio-Plla. dell'Obolo 2, 33N 448676 E, 4194243 N, 13.06.07	P. Abellán & F. Picazo	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451037	LS451128	LS452930	LS453542	LS453150
133	Deronectina	*Scarodytes*	sp4		MNCN-AC15	Morocco; Zaër Zaïane, Tachenrhout, rv. crsrd. Aguelmoussa-Goaïda, N33º7'59.4" W5º58'46.7", 9.4.2007	I. Ribera & P. Aguilera & C. Hernando	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451033	HF931107	HF931324	LS453460	LS453145
134	Deronectina	*Scarodytes*	sp5		MNCN-AI893	Spain; Madrid Sieteiglesias, Ayo. Quiñón Jábalo, N40º54'17" W3º34'34"	I. Ribera & A. Cieslak	Lotic	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451028	LS451116	LS452905	LS453553	LS453144
135	Deronectina	*Stictotarsus*	*duodecimpustulatus*		NHM-IR42	Spain; Cadiz, Ventorillo de las Canillas, Afl. R. Hozgarganta 27.7.1998	I. Ribera	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes			AF309304	AF309247	AJ850496 EF670297	EF670156
136	Deronectina	*Stictotarsus*	*maghrebinus*		NHM-IR236	Morocco; Tioulit, Oued Ait-Baha, 24.4.2000	I. Ribera	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451072	AY250985	AY250947	LS453545	LS453182
137	Deronectina	*Stictotarsus*	*procerus*		MNCN-AI1116	Corsica; River Golo below Calacuccia	D.T. Bilton	Both	Nebrioporus + Stictotarsus + Scarodytes + Iberonectes		LS451080	HF931160	HF931379	LS453546	LS453160
138	Deronectina	*Trichonectes*	*otini*		NHM-IR29	Spain; Jaen, Porcuna, Arroyo de las Salinas, A305 pk 35.5, 28.7.1998	I. Ribera	Lotic	*deleted			AJ850608	AJ850356	AJ850499 EF670299	EF670159
139	Deronectina	*Zaitzevhydrus*	*formaster*	*formaster*	MNCN-AI1102	Mongolia; Zavkhan, Ider, ponds by Ideriin Gol at Darkhjan Uul Brigade, ~12 km SE of Zuunmod/Ider, N48.13246 E97.48425, 23.7.2004	A.E.Z. Short	Lentic	Zaitzevhydrus		LS451067	HF931158	HF931377	LS453515	LS453166
140	Deronectina	*Zaitzevhydrus*	*formaster*	*ulanulana*	IBE-RA1170	China; Qinghai, Golo, Huamuxia, Zuimatan. Roadside pool., 19'52"N 99°03'57"E, 7.6.2013	R.B. Angus, F.L. Jia & Y. Zhang	Lentic	Zaitzevhydrus	XXXXXXX	LS451023	LS451130	LS452933		LS453165
141	Hydroporina	*Haideoporus*	*texanus*		genbank			Lotic	Neoporus + Heterosternuta + Haideoporus				KF575564		KF575393
142	Hydroporina	*Heterosternuta*	*allegheniana*		IBE-AV2	Nova Scotia (Canada); Cape Breton, c. Inverness, stream in Big Marsh Rd., N45º53'36.8" W61º12'16.8", 16.8.2007	I. Ribera & A. Cieslak	Lotic	Neoporus + Heterosternuta + Haideoporus	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
143	Hydroporina	*Heterosternuta*	*pulchra*		IBE-AV4	Nova Scotia (Canada) Regional M. Cape Breton, pond next Sand Lake Rd., Glace Bay, N46º08'01.9" W59º55'53.7", 19.8.2007	I. Ribera & A. Cieslak	Both	Neoporus + Heterosternuta + Haideoporus		XXXXXXX	XXXXXXX	XXXXXXX		XXXXXXX
144	Hydroporina	*Heterosternuta*	*wickhami*		NHM-IR330	Ontario (Canada); 9.2000	Y. Alarie	Both	Neoporus + Heterosternuta + Haideoporus				XXXXXXX		
145	Hydroporina	*Hydrocolus*	*paugus*		MNCN-AC43	Nova Scotia (Canada); Cumberland c., river next Rd. 2, Mapleton, N45º34'54.3" W64º08'30.3", 23.8.2007	I. Ribera & A. Cieslak	Both	Hydroporus + Hydrocolus Lentic			HE599648	XXXXXXX	XXXXXXX	XXXXXXX
146	Hydroporina	*Hydrocolus*	*persimilis*		IBE-AV5	Nova Scotia (Canada); Cape Breton, c. Richmond, str. in Battery Prov. P., St. Peters, N45º38'50.8" W60º51'57.3", 20.8.2007	I. Ribera & A. Cieslak	Both	Hydroporus + Hydrocolus Lentic		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
147	Hydroporina	*Hydrocolus*	*rubyi*		genbank			Lentic	Hydroporus + Hydrocolus Lentic		KJ203675				

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
148	Hydroporina	*Hydrocolus*	*sahlbergi*		NHM-ER21	Sweden; Prov. Vasterbotten, Ansele, 3.8.99	A.N. Nilsson	Lentic	Hydroporus + Hydrocolus Lentic		XXXXXXX	AJ850629	AJ850379	AJ850518	EF670199
149	Hydroporina	*Hydrocolus*	*stagnalis*		IBE-AV6	Nova Scotia (Canada); Cape Breton, c. Richmond, str. in Battery Prov. P., St. Peters, N45º38'50.8" W60º51'57.3", 20.8.2007	I. Ribera & A. Cieslak	Lentic	Hydroporus + Hydrocolus Lentic	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
150	Hydroporina	*Hydroporus*	*acutangulus*		NHM-ER1	Sweden; Prov. Vasterbotten, Vindeln, Skatan, 2.6.2004	A.N. Nilsson	Lentic	Hydroporus Lentic 1		XXXXXXX	AF518286	AF518256	XXXXXXX	XXXXXXX
151	Hydroporina	*Hydroporus*	*analis*		IBE-AV9	Sardinia; Nuoro prov. (Ogliastra historical region), river W Villanova Strisàili, 39.96332N 9.45989E, 27.5.2009	H. Fery & M. Toledo	Lentic	*deleted	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
152	Hydroporina	*Hydroporus*	*angustatus*		NHM-GR16	England (UK); Eping Forest, Goldings hill outer pond, 17.5.1999	I. Ribera	Lentic	*deleted		XXXXXXX	AY365300	AY365266	XXXXXXX	XXXXXXX
153	Hydroporina	*Hydroporus*	*apenninus*		IBE-AF60	Italy; Abruzzi, prov. L'Aquila, ca. 4 km, N Pescasséroli, Fiume	H. Fery	Lotic	Hydroporus Lotic 1	XXXXXXX	XXXXXXX	HF931127	HF931346	XXXXXXX	XXXXXXX
154	Hydroporina	*Hydroporus*	*appalachius*		genbank			Both	Hydroporus + Hydrocolus Lentic			JX434807	JX434765	JX434786	JX434826
155	Hydroporina	*Hydroporus*	*axillaris*		MNCN-DM32	California (USA); Shasta co., Manzanita Creek drive, ca. Lake McCumber, 21.6.2000	I. Ribera & A. Cieslak	Lotic	Hydroporus + Hydrocolus Lentic	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
156	Hydroporina	*Hydroporus*	*basinotatus*		MNCN-AH112	Spain; Cádiz, Tarifa, stream 2K N Na. Sra de la Luz, N36º05'53.4" W5º37'35.0", 29.3.2008	I. Ribera & P. Aguilera & C. Hernando	Lotic	Hydroporus Lentic 4		LT882859	HE610196 LT882808	LT883033	LT882955	LT883124
157	Hydroporina	*Hydroporus*	*bithynicus*		MNCN-AI782	Turkey; Bolu, Rd. 750 btw Yeniçaga & Mengen, fast stream in mixed forest, N40º50'49" E32º03'47.5", 24.4.2006	I. Ribera	Lotic	Hydroporus Lotic 1		XXXXXXX	HE599650	HE599681	XXXXXXX	XXXXXXX
158	Hydroporina	*Hydroporus*	*bodemeyeri*	*bodemeyeri*	MNCN-AI110	Chios (Greece); seepage lines with tufa, roadside ditch below oleander, Kapouries	G.N. Foster	Lotic	Hydroporus Lotic 1		XXXXXXX	HE599651	HE599675	XXXXXXX	XXXXXXX
159	Hydroporina	*Hydroporus*	*bodemeyeri*	*guignoti*	IBE-RA216	Greece; S Peloponnese, ca. 12 km NW Sparti, NE Kambos, small stream, 37.15319N 22.35353E, ca. 294 m, 19.5.2010	H. Fery & L. Hendrich	Lotic	Hydroporus Lotic 1		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
160	Hydroporina	*Hydroporus*	*brancoi*	*brancoi*	MNCN-AI228	Portugal; Serra de Arga, Pools on summit, 9.5.2005	D.T. Bilton	Both	Hydroporus Lotic 1		HF947975	HE599652	HE599678	XXXXXXX	XXXXXXX
161	Hydroporina	*Hydroporus*	*brancoi*	*gredensis*	MNCN-AC9	Spain; Madrid, Rascafría, PN Peñalara, below Laguna Grande, N40º50'14.4" W3º57'18.7", 2.6.07	I. Ribera	Both	Hydroporus Lotic 1		HF947976	HF931112	HF931329		
162	Hydroporina	*Hydroporus*	*brancuccii*		MNCN-AI180	Portugal; Serra de Arga, Pools on summit, 9.5.2005	D.T. Bilton	Lotic	Hydroporus Lotic 3		HF947977	HE610197	HF931406	XXXXXXX	XXXXXXX
163	Hydroporina	*Hydroporus*	*brevis*		MNCN-DM23	Sweden; Vasterbotten province, Vindeln district, 13.5.2000	A.N. Nilsson	Lentic	Hydroporus Lentic 3	XXXXXXX	XXXXXXX	HE610198	HF931505	XXXXXXX	XXXXXXX
164	Hydroporina	*Hydroporus*	*brucki*		IBE-AN4	Turkey; Izmir prov., Selçuk, delta Küçükmenderes, 37°57'24.3"N 27°17'32.8"E, 25.7.2014	I. Ribera & A. Cieslak	Lentic	*deleted		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
165	Hydroporina	*Hydroporus*	*cagrankaya*		MNCN-AI62	Turkey	Ö.K. Erman	Lotic	Hydroporus Lotic 1		XXXXXXX	FM946166	FM946167 FM946168 FM946169		
166	Hydroporina	*Hydroporus*	*cantabricus*		MNCN-AI122	Spain; Puerto de las Estacas de Trueba	H. Fery	Lentic	Hydroporus Lotic 1		HF947978	HE599653	HE599676	XXXXXXX	XXXXXXX
167	Hydroporina	*Hydroporus*	*carri*		genbank			Lotic	Hydroporus + Hydrocolus Lentic			JX434806	JX434764	JX434785	JX434825
168	Hydroporina	*Hydroporus*	*columbianus*		genbank			Both	Hydroporus + Hydrocolus Lentic			KR491461			

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
169	Hydroporina	*Hydroporus*	*compunctus*		NHM-IR181	Gomera (Spain); Garajonay, 15.1.2000	D.T. Bilton	Lotic	Hydroporus Lotic 2		**XXXXXXX**	AF518289	HE599693	**XXXXXXX**	**XXXXXXX**
170	Hydroporina	*Hydroporus*	*constantini*		MNCN-AI285	Spain; Burgos, Cruz de la Demanda, spring, N 42º11'52.6" W 3º6'1.9", 23.10.04	I. Ribera	Lotic	Hydroporus Lotic 1		HF947979	HE599654	HE599679	**XXXXXXX**	**XXXXXXX**
171	Hydroporina	*Hydroporus*	*cuprescens*		NHM-IR647	Cyprus; Ayia, Paphos forest, 13.3.2001	K. Miller	Lotic	Hydroporus Lotic 1		**XXXXXXX**	HE599655	HE599699	**XXXXXXX**	**XXXXXXX**
172	Hydroporina	*Hydroporus*	*decipiens*		MNCN-DM31	Spain; Avila, Sa. De Gredos, Gredos, ca., Gebirgsbach, 20.7.99	H. Fery	Both	*deleted			HE610200	XXXXXXX	**XXXXXXX**	**XXXXXXX**
173	Hydroporina	*Hydroporus*	*dentellus*		NHM-IR575	Vermont (USA), Bennington, 15.7.2000	C. Hernando	Lentic	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	AY365297	AY365263	**XXXXXXX**	**XXXXXXX**
174	Hydroporina	*Hydroporus*	*despectus*		NHM-IR483	Alberta (Canada); Waterton lakes Nat. Park, junc. Rd. 5&6, 28.6.2000	I. Ribera & A. Cieslak	Lentic	Hydroporus + Hydrocolus Lentic	**XXXXXXX**	**XXXXXXX**	AY365302	HE599696		**XXXXXXX**
175	Hydroporina	*Hydroporus*	*discretus*		MNCN-AI641	Spain; Logroño, Posadas, spring, rd. by r. Oja, N 42º12'52.7" W 3º3'40.3", 23.10.04	I. Ribera	Both	Hydroporus Lotic 3	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
176	Hydroporina	*Hydroporus*	*discretus* cplx1		MNCN-AI651	Spain; Ciudad Real, Fuencaliente, arroyo Cereceda	A. Castro	Both	Hydroporus Lotic 3		**XXXXXXX**	HF931220	HF931449	**XXXXXXX**	**XXXXXXX**
177	Hydroporina	*Hydroporus*	*discretus* cplx2		IBE-AV45	Cyprus; Troodos mt., stream 2 Km S Prodromos, 34º55'53.2"N 32º50'19.4"E, 27.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	Both	Hydroporus Lotic 3	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
178	Hydroporina	*Hydroporus*	*discretus* cplx3		IBE-RA142	Italy; Abbeverat. Ca., Str. Pianogrande, Força Canaping; 20.7.2009	M. Toledo	Both	Hydroporus Lotic 3		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
179	Hydroporina	*Hydroporus*	*distinguendus*		MNCN-HI15	Algeria; Feid 3, N 36° 43.960' E 008° 01.767', 16.3.2006	S. Bouzid	Both	Hydroporus Lentic 2			**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
180	Hydroporina	*Hydroporus*	*dobrogeanus*		MNCN-DM85	Serbia; Canyon of River Pschinja, spring at road to Monastir Prohor-Pchinjski, 28.4.2006	V. Pesic	Lotic	Hydroporus Lotic 1		**XXXXXXX**	HE599656	HE599689	**XXXXXXX**	**XXXXXXX**
181	Hydroporina	*Hydroporus*	*dobrogeanus* cplx		IBE-AV49	Cyprus; Pafos forest, spring in Cedar Valley,34º59'28.0"N 32º41'18.5"E, 28.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	Lotic	Hydroporus Lotic 1	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
182	Hydroporina	*Hydroporus*	*dorsalis*		IBE-RA939	Ireland; Limerick, Lough at Ballyhibin, 1.8.2007	D.T. Bilton	Both	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
183	Hydroporina	*Hydroporus*	*elongatulus*		MNCN-DM18	Germany; Bavaria, Dietramszell, Reith, irrigation ditch on meadow, slightly running	M. Balke	Lentic	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	HF931267	HF931504	**XXXXXXX**	**XXXXXXX**
184	Hydroporina	*Hydroporus*	*errans*		MNCN-AI638	Gran Canaria (Spain); Moya: Bco. de Azuaje, stream and ponds w. veget., 15.4.2001	I. Ribera & A. Cieslak	Lotic	Hydroporus Lotic 3		**XXXXXXX**	HE610203	HF931448	**XXXXXXX**	**XXXXXXX**
185	Hydroporina	*Hydroporus*	*erythrocephalus*		IBE-RA710	Ireland; Co. Clare, Corrofin, Ballymacaugh Lough, fen, N52º58'08.0" W9º04'00.2", 23.5.2010	I. Ribera	Lentic	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
186	Hydroporina	*Hydroporus*	*ferrugineus*		MNCN-AI929	Germany; Bayern, Rehau, Rehauer First, Mähringe bach	A. Skale	Lotic	Hydroporus Lotic 1		**XXXXXXX**	HE599657	HE599683	**XXXXXXX**	**XXXXXXX**
187	Hydroporina	*Hydroporus*	*feryi*		MNCN-DM33	Tunisia	I. Ribera & A. Cieslak	Lotic	Hydroporus Lotic 3	**XXXXXXX**		**XXXXXXX**	**XXXXXXX**		**XXXXXXX**
188	Hydroporina	*Hydroporus*	*figuratus*		NHM-IR67	England (UK); Norfolk	D.T. Bilton	Both	Hydroporus + Hydrocolus Lentic		LS451093	AF309299	HE599700	AJ318745	EF670203
189	Hydroporina	*Hydroporus*	*fortis*		NHM-IR353	California (USA); Shasta co., Manzanita Creek drive, ca. Lake McCumber, 21.6.2000	I. Ribera & A. Cieslak	Lotic	Hydroporus + Hydrocolus Lentic				**XXXXXXX**		

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
190	Hydroporina	*Hydroporus*	*foveolatus*		MNCN-DM8	Switzerland; Berner Alpen, Grimselpass, Oberaars Stausee, Bach b. Berghaus, N46º32.932' E08º16.684'	T. Ekrem	Lentic	Hydroporus Lentic 4		XXXXXXX	HE610205	HF931502	XXXXXXX	XXXXXXX
191	Hydroporina	*Hydroporus*	*foveolatus* nr		MNCN-DM14	Spain; Avila, Sierra de Gredos, S Hoyos del Espino, nr Plataforma	H. Fery	Lentic	Hydroporus Lentic 4		XXXXXXX	HF931269	HF931507	XXXXXXX	XXXXXXX
192	Hydroporina	*Hydroporus*	*fuscipennis*		NHM-IR491	Alberta (Canada); Rd. 22, 5 km S Longview, 28.6.2000	I. Ribera & A. Cieslak	Lentic	*deleted		XXXXXXX	HE610206	XXXXXXX	XXXXXXX	XXXXXXX
193	Hydroporina	*Hydroporus*	*galloprovincialis*		IBE-RA289	France; ca. 1 km SEE Jouques (13), little spring on the edge of a grassy marshland,9.10.2010	M. Manuel	Lotic	Hydroporus Lotic 1		XXXXXXX	HE793376	XXXXXXX	XXXXXXX	XXXXXXX
194	Hydroporina	*Hydroporus*	*geniculatus*		NHM-ER3	Sweden; Prov. Vasterbotten, Vindeln, Skatan, 2.6.2001	A.N. Nilsson	Lentic	Hydroporus + Hydrocolus Lentic		XXXXXXX	AY365303	AY365269	XXXXXXX	XXXXXXX
195	Hydroporina	*Hydroporus*	*glabriusculus*		NHM-GR33	England (UK); Norfolk, East Walton Common 1, 6.6.1999	I. Ribera	Lentic	Hydroporus Lentic 3			AY365304	AY365270		
196	Hydroporina	*Hydroporus*	*glasunovi*	*dolini*	IBE-RA1204	Kyrgyzstan; Ala Archa NP, rheocrenic spring, 42º36.203'N, 074º28.959'E, 9.7.2013	Pešić	Lotic	*deleted			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
197	Hydroporina	*Hydroporus*	*goldschmidti*		MNCN-AI118	Uzbekistan; Tashkent prov., Yakka-tut nr. Burchmulla, 41º38'N 70º03'E	L. Hendrich	Lotic	*deleted		XXXXXXX	HE610207	HF931390	XXXXXXX	XXXXXXX
198	Hydroporina	*Hydroporus*	*gueorguievi*		MNCN-DM20	Bulgaria; Pirin Mts. Cottongrass bog, 1200m above Dobriniste	D.T. Bilton	Lotic	Hydroporus Lotic 1		XXXXXXX	HE599660	HE599685	XXXXXXX	XXXXXXX
199	Hydroporina	*Hydroporus*	*guernei*		IBE-RA911	Azores (Portugal); Terceira, Casas da Falca, ditch & alberca, 38°43'36.6"N 27°16'53.8"W, 3.9.2012	I. Ribera & A. Cieslak	Both	*deleted		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
200	Hydroporina	*Hydroporus*	*gyllenhalii*		NHM-GR12	Spain; Huelva, Almonte, poblado forestal 26.7.1998	I. Ribera	Lentic	Hydroporus Lentic 2			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
201	Hydroporina	*Hydroporus*	*hebaueri*		MNCN-DM86	Bulgaria; Rhodopes, Madjarovo, village Senoklas, spring, 25.4.2006	V. Pesic	Both	Hydroporus Lotic 1		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
202	Hydroporina	*Hydroporus*	*incognitus*		IBE-RA691	Turkey; Erzurum, stream, Senyurtköyu, N40 11 34.0 E41 28 03.6, 12.6.2011	I. Ribera, A. Faille, J. Fresneda	Lentic	Hydroporus Lentic 2		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
203	Hydroporina	*Hydroporus*	*incommodus*		MNCN-AI382	Slovakia, Laksárska Nová Ves, Cerveny rybnik	R. Cséfalvay	Lentic	Hydroporus Lentic 2		XXXXXXX	HF931192	HF931417	XXXXXXX	XXXXXXX
204	Hydroporina	*Hydroporus*	*jonicus*		MNCN-AI1233	Greece; Peloppones, Korinthia, 5 km E lake Stymphalia, 10.7.2005	G. Wewalka	Both	Hydroporus Lentic 2		LS451078	HF931176	HF931396	LS453476	LS453171
205	Hydroporina	*Hydroporus*	*kabakovi*		MNCN-AI91	MONGOLIA; Zavkhan Aimag, Telmen Soum,Ideriin Gol ~15 km SSW of Telmen, Ovogdii,N48.53255,E97.52093	A.E.Z. Short	Lotic	*deleted		XXXXXXX	HE610208	HF931469	XXXXXXX	XXXXXXX
206	Hydroporina	*Hydroporus*	*kasyi*		IBE-AV42	Cyprus; Akrotiri, Fassouri reedbeds, 34º37'57.2"N 32º56'01.5"E, 26.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	Lentic	Hydroporus Lentic 2	XXXXXXX	LT882843	LT882783	LT883013	LT882957	LT883126
207	Hydroporina	*Hydroporus*	*kozlovskii*		MNCN-AI1264	GreeceM Makedonía (Pella), Voras Ski Resort, N40°54'12.0", E021°49'30.8"	P. & V. Ponel	Lentic	Hydroporus Lentic 4		XXXXXXX	HE610209	HF931397	XXXXXXX	
208	Hydroporina	*Hydroporus*	*kraatzi*		MNCN-AI602	Germany; Harz, Kaltenborn Quellgebiet	Spitzenberg	Both	Hydroporus Lotic 1			XXXXXXX	XXXXXXX		
209	Hydroporina	*Hydroporus*	*lapponum*		genbank			Lentic	Hydroporus + Hydrocolus Lentic			JX434818	JX434776	JX434797	JX434837
210	Hydroporina	*Hydroporus*	*larsoni*		genbank			Lentic	Hydroporus Lentic 3		KC017013				

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
211	Hydroporina	*Hydroporus*	*limbatus*		MNCN-AI736	Spain; Aranjuez, salinas cta. N400, 8.4.2006	I. Ribera & A. Cieslak	Lentic	*deleted		XXXXXXX	HE610210	XXXXXXX	XXXXXXX	XXXXXXX
212	Hydroporina	*Hydroporus*	*lluci*		IBE-RA368	Mallorca (Spain); Bunyola, te. de Bunyola, Ma 2100 pk 5.5, N39º43'24.7" E2º43'15.7", 13.5.2007	I. Ribera & A. Cieslak	Lotic	Hydroporus Lotic 1		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
213	Hydroporina	*Hydroporus*	*longicornis*		MNCN-DM22	Sweden; Vasterbotten province, Vindeln district, 13.5.2000	A.N. Nilsson	Lotic	Hydroporus Lotic 1		XXXXXXX	HE599663	HE599686	XXXXXXX	XXXXXXX
214	Hydroporina	*Hydroporus*	*longiusculus*		NHM-IR345	California (USA); Mono co., Yosemite Ntal. Park, Rd. 120 Dana Meadows, 19.6.2000	I. Ribera & A. Cieslak	Lentic	Hydroporus + Hydrocolus Lentic		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
215	Hydroporina	*Hydroporus*	*longulus*		MNCN-AI300	Italy; Brescia, val Trompia, stream rd. to Guiogo di Bala, 17.10.2002	I. Ribera & A. Cieslak	Lotic	Hydroporus Lotic 1		XXXXXXX	HE599664	HE599680	XXXXXXX	XXXXXXX
216	Hydroporina	*Hydroporus*	*lucasi*		MNCN-AI630	Spain; Córdoba, Sierra Morena, Km 28.5 cta. Villaviciosa	A. Castro	Both	*deleted		XXXXXXX	HE610211	XXXXXXX	XXXXXXX	XXXXXXX
217	Hydroporina	*Hydroporus*	*lundbladi*		NHM-IR664	Madeira (Portugal); Wet rock wall and adjacent spring pools below Paul do Serra by the way, 8.3.2001	D.T. Bilton	Lotic	Hydroporus Lotic 2			AF518297			
218	Hydroporina	*Hydroporus*	*macedonicus*		MNCN-AI1265	GreeceM Makedonía (Pella), Voras Ski Resort, N40°54'12.0", E021°49'30.8"	P. & V. Ponel	Lotic	Hydroporus Lotic 3		XXXXXXX	HE610212	XXXXXXX	XXXXXXX	XXXXXXX
219	Hydroporina	*Hydroporus*	*mannerheimi*		NHM-IR485	Alberta (Canada); Waterton lakes Nat. Park, Rd. 5, park limit, 28.6.2000	I. Ribera & A. Cieslak	Lentic	Hydroporus + Hydrocolus Lentic			XXXXXXX			
220	Hydroporina	*Hydroporus*	*mannerheimi* nr		NHM-IR526	British Columbia (Canada); Rd. 5, 40 km N Hope, ponds by motorway, 30.6.2000	I. Ribera & A. Cieslak	Lentic	Hydroporus + Hydrocolus Lentic	XXXXXXX		AY365295	AY365261		
221	Hydroporina	*Hydroporus*	*marginatus*		MNCN-AI61	Turkey; Erzurum	Ö.K. Erman	Both	*deleted	XXXXXXX	XXXXXXX	HE610213	XXXXXXX	XXXXXXX	XXXXXXX
222	Hydroporina	*Hydroporus*	*melanarius*		IBE-RA636	Hungary; Körös Maros, Nemzeti Park, Szabadkígyós 13.4.2011	R.B. Angus	Both	Hydroporus Lotic 1	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
223	Hydroporina	*Hydroporus*	*memnonius*		NHM-IR315	Switzerland; St. Gottardo pass, Rd. 2, summit, 27.7.00	I. Ribera & A. Cieslak	Both	Hydroporus Lotic 1		LS451097	HE599667	HE599694	LS453470	LS453176
224	Hydroporina	*Hydroporus*	*memnonius* gr		MNCN-AI764	Turkey; Düzce, Rd. to Samandere from Kaynasli, small shallow stream & pools, N40º45'13.5" E31º19'54", 23.4.2006	I. Ribera	Both	Hydroporus Lotic 1		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
225	Hydroporina	*Hydroporus*	*morio*		NHM-ER22	Sweden; Prov. Vasterbotten, Ansele, 3.8.99	A.N. Nilsson	Lentic	Hydroporus + Hydrocolus Lentic		XXXXXXX	AY365308	AY365274	XXXXXXX	XXXXXXX
226	Hydroporina	*Hydroporus*	*necopinatus*	*necopinatus*	MNCN-AI1130	Spain; Burgos, 1km W Soncillo, 25 km E Reinosa, ponds UTM VN34412 58004	H. Fery	Lentic	Hydroporus Lotic 1			HG793318			
227	Hydroporina	*Hydroporus*	*necopinatus*	*robertorum*	genbank			Lentic	Hydroporus Lotic 1			JN790723			
228	Hydroporina	*Hydroporus*	*necopinatus*	*roni*	genbank			Lentic	Hydroporus Lotic 1			JN790760			
229	Hydroporina	*Hydroporus*	*neglectus*		NHM-GR15	England (UK); Norfolk, East Walton Common 1, 6.6.1999	I. Ribera	Lentic	Hydroporus Lentic 1			AY365310	AY365276	XXXXXXX	XXXXXXX
230	Hydroporina	*Hydroporus*	*nevadensis*		MNCN-AI640	Spain; Posadas, spring, rd. by r. Oja, N 42º12'52.7" W 3º3'40.3", 23.10.04	I. Ribera	Both	Hydroporus Lotic 1		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
231	Hydroporina	*Hydroporus*	*nigellus*		NHM-IR506	Sweden; S. Ha. Onsala, Kustgol, VSV Rorvik, 3.11.2000	B. Andren	Lentic	Hydroporus + Hydrocolus Lentic			AY365311	AY365277	AJ850515	EF670195

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
232	Hydroporina	*Hydroporus*	*niger*		NHM-IR124	New York (USA); Tompkins Co., Ithaca, 1.11.1998	K.B. Miller	Both	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	AF518301	AF518270	**XXXXXXX**	**XXXXXXX**
233	Hydroporina	*Hydroporus*	*niger* gr1		IBE-AV7	California (USA); Inyo co., Amargosa River in Tecopa 35º50'57''N,116º13'49''W, 8.4.2008	P. Abellán	Lentic	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
234	Hydroporina	*Hydroporus*	*nigrita*		IBE-RA334	Spain; Huesca, torrentera del ibon de Plan	I. Esteban	Lentic	Hydroporus Lentic 4		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
235	Hydroporina	*Hydroporus*	*normandi*	*alhambrae*	IBE-RA461	Spain; Granada prov., Sierra Nevada, ca. 2 km NEE Capileira, spring, ca. 36.967N 3.339W, 26.5.2011	H. Fery	Lotic	Hydroporus Lotic 1		HF947981	HF931316	HF931540		**XXXXXXX**
236	Hydroporina	*Hydroporus*	*normandi*	*ifranensis*	NHM-GR13	Morocco; Azrou, 8, Lac Afenourir, 9.4.1999	R. Gerecke	Lotic	Hydroporus Lotic 1		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**		
237	Hydroporina	*Hydroporus*	*normandi*	*normandi*	MNCN-AH105	Morocco; Tanger, well in rd. to Melloussa, N35º48'15.9" W5º41'39.1", 27.3.2008	I. Ribera, P. Aguilera & C. Hernando	Lotic	Hydroporus Lotic 1		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
238	Hydroporina	*Hydroporus*	*notabilis*		MNCN-AI92	MONGOLIA; Zavkhan Aimag, Telmen Soum,Ideriin Gol ~15 km SSW of Telmen, Ovogdii,N48.53255,E97.52093	A.E.Z. Short	Both	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
239	Hydroporina	*Hydroporus*	*obscurus*		NHM-ER4	Sweden; Prov. Vasterbotten, Vindeln, Skatan, 2.6.2002	A.N. Nilsson	Lentic	Hydroporus + Hydrocolus Lentic			AF518303	AF518272	**XXXXXXX**	**XXXXXXX**
240	Hydroporina	*Hydroporus*	*obsoletus*		IBE-RA206	Greece; Peloponnese, Afrodisio Mts, ca. 5 km NE Kondovazena, small spring, ca. 37.82044N 21.93570E, 16.5.2010	H. Fery & L. Hendrich	Lotic	Hydroporus Lotic 1	**XXXXXXX**	LT882852	LT882797	LT883023	LT882954	LT883123
241	Hydroporina	*Hydroporus*	*occidentalis*		NHM-IR517	British Columbia (Canada); Glacier Ntal. Park; Rd. 1, 4 km N limit of the park, 29.6.2000	I. Ribera & A. Cieslak	Lentic	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
242	Hydroporina	*Hydroporus*	*paganettianus*		NHM-IR145	Portugal; Guarda, Sa. Estrela, close summit, pools & stream, 7.8.99	D.T. Bilton	Both	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	AY365324	AY365290	**XXXXXXX**	**XXXXXXX**
243	Hydroporina	*Hydroporus*	*palustris*		MNCN-AI19	Faroes (Denmark)	G.N. Foster	Both	Hydroporus Lentic 2		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
244	Hydroporina	*Hydroporus*	*pervicinus*		NHM-IR359	California (USA); Shasta co., Manzanita Creek drive, ca. Lake McCumber, 21.6.2000	I. Ribera & A. Cieslak	Lentic	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	AY365316	HE599695		**XXXXXXX**
245	Hydroporina	*Hydroporus*	*pfefferi*		IBE-RA1060	Greece; Achaïa, 1.5Km NE Klitor, mountain stream+fen w. dead trees, 37°53'31.55"N 22°3'26.92"E, 3.4.2013	I. Ribera & A. Cieslak	Lotic	Hydroporus Lotic 1			**XXXXXXX**	**XXXXXXX**		
246	Hydroporina	*Hydroporus*	*pilosus*		NHM-IR37	Tenerife (Spain); Anaga, Roque Chinobre, 12.1997	D.T. Bilton	Lotic	Hydroporus Lotic 2			AF518305	AF518274	AJ318733	EF670196
247	Hydroporina	*Hydroporus*	*planus*		IBE-AN82	Morocco; Oukaimeden, flooded field&stream, 3km N; N31º11'11" W7º50'36.4", 10.7.2011	I. Ribera & A. Cieslak	Lentic	*deleted	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**		**XXXXXXX**
248	Hydroporina	*Hydroporus*	*puberulus*		NHM-ER2	Sweden; Prov. Vasterbotten, Vindeln, Skatan, 2.6.1999	A.N. Nilsson	Lentic	Hydroporus Lentic 3	**XXXXXXX**	**XXXXXXX**	AY365317	AY365283	**XXXXXXX**	**XXXXXXX**
249	Hydroporina	*Hydroporus*	*pubescens*		IBE-AN383	Corsica; Porto-Vecchio, l'Ospedale, 18.9.1999	I. Ribera & A. Cieslak	Lentic	*deleted		LS451101	LS451133	LS452932		LS453172
250	Hydroporina	*Hydroporus*	*regularis*		NHM-IR166	Corsica; Vizzavona: Cascades des Anglais,16.9.1999	I. Ribera & A. Cieslak	Lotic	Hydroporus Lotic 1		**XXXXXXX**	AY365318	AY365284	**XXXXXXX**	**XXXXXXX**
251	Hydroporina	*Hydroporus*	*rifensis*		IBE-AV218	Morocco; Anasar (Chefchaouen), 35°01'12.06''N 04°59'59.17''W, 2.4.2018	A. Millán et al.	Lentic	Hydroporus Lentic 2		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
252	Hydroporina	*Hydroporus*	*rufifrons*		NHM-ER19	Sweden; Prov. Vasterbotten, Ansele, 3.8.99	A.N. Nilsson	Lentic	Hydroporus + Hydrocolus Lentic		HG915299	HG915299	HG915299	HG915299	HG915299

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
253	Hydroporina	*Hydroporus*	*rufinasus*		genbank			Lotic	Hydroporus + Hydrocolus Lentic		KJ204058				
254	Hydroporina	*Hydroporus*	*sabaudus*	*sabaudus*	MNCN-DM16	Spain; Teruel, Sierra de Albarracin, ca. 1 km NW Noguera	H. Fery	Lentic	Hydroporus Lentic 4		**XXXXXXX**	HE610217	HF931503	**XXXXXXX**	**XXXXXXX**
255	Hydroporina	*Hydroporus*	*sabaudus*	*sierranevadensis*	IBE-PA262	Spain; Granada, Sierra Nevada, Borreguitos del S. Juan	A. Castro	Lentic	Hydroporus Lentic 4		HG915299	HF931290	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
256	Hydroporina	*Hydroporus*	*sanfilippoi*		MNCN-AH189	Italy; Emilia Romagna, creek 2km SW Berceto, N44º30'01.1" E9º58'00.7", 23.5.2008	I. Ribera	Lotic	Hydroporus Lotic 1	**XXXXXXX**	**XXXXXXX**	HE599672	HE599674	**XXXXXXX**	**XXXXXXX**
257	Hydroporina	*Hydroporus*	*sardomontanus*		MNCN-DM62	Sardinia; Nuoro, Gennargentu entre Rifugio Sa Arena et Bruncu Spina, N 40º01'08.4" E 9º16'41.8"	P. & V. Ponel	Lotic	Hydroporus Lotic 1		**XXXXXXX**	HE599673	HE599688	**XXXXXXX**	**XXXXXXX**
258	Hydroporina	*Hydroporus*	*scalesianus*		NHM-IR38	England (UK); Dorset, Wareham, Morden bog, 5.7.1998	I. Ribera	Lentic	Hydroporus Lentic 1			AF518309	AF518278	AJ850516/ EF670300	EF670197
259	Hydroporina	*Hydroporus*	*semenowi*		MNCN-DM24	Sweden; Vasterbotten province, Vindeln district, 13.5.2000	A.N. Nilsson	Lentic	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
260	Hydroporina	*Hydroporus*	*signatus*	*signatus*	genbank			Lentic	Hydroporus + Hydrocolus Lentic			KR914955			
261	Hydroporina	*Hydroporus*	*signatus*		IBE-RA682	Nova Scotia (Canada); c. Annapolis, Greywood, Liverpool Head Lake, N44º34'57.7" W65º24'21.7", 13.8.2007	I. Ribera & A. Cieslak	Lentic	Hydroporus + Hydrocolus Lentic	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
262	Hydroporina	*Hydroporus*	*sinuatipes*		genbank			Lentic	Hydroporus + Hydrocolus Lentic				JX434774	JX434795	JX434835
263	Hydroporina	*Hydroporus*	*springeri*		NHM-IR632	Italy; Lombardia, Prov. Mantova, bogs of Marcaria, 24.3.2001	M. Toledo	Lentic	Hydroporus Lentic 2		**XXXXXXX**	AY365319	AY365285	**XXXXXXX**	**XXXXXXX**
264	Hydroporina	*Hydroporus*	*striola*		NHM-IR484	Alberta (Canada); Waterton lakes Nat. Park, junc. Rd. 5&6, 28.6.2000	I. Ribera & A. Cieslak	Lentic	Hydroporus Lentic 2			**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
265	Hydroporina	*Hydroporus*	*submuticus*		NHM-ER23	Sweden; Prov. Vasterbotten, Strycksele, 3.8.99	A.N. Nilsson	Both	Hydroporus + Hydrocolus Lentic	**XXXXXXX**	**XXXXXXX**	AY365321	AY365287	**XXXXXXX**	**XXXXXXX**
266	Hydroporina	*Hydroporus*	*subpubescens*		MNCN-AC46	Nova Scotia (Canada); Cumberland c., river next Rd. 2, Mapleton, N45º34'54.3" W64º08'30.3", 23.8.2007	I. Ribera & A. Cieslak	Lentic	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
267	Hydroporina	*Hydroporus*	*tenebrosus*		genbank			Lentic	Hydroporus + Hydrocolus Lentic		GU679513				
268	Hydroporina	*Hydroporus*	*tessellatus*		MNCN-AI770	Turkey; Düzce, Rd. to Kartalkaya from Çaydurt, small stream with vegetation, N40º42'29" E31º45'56", 23.4.2006	I. Ribera	Both	Hydroporus Lentic 4		LT882873	LT882817	LT883042	LT882956	LT883125
269	Hydroporina	*Hydroporus*	*tessellatus* nr		IBE-AN585	Croatia; spring of Cetina river, N 43º58'36.1" E16º25'48.6", 07.8.2016	I. Stankovic	Both	Hydroporus Lentic 4	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
270	Hydroporina	*Hydroporus*	*thracicus*		MNCN-AI336	Bulgaria; Pirin mts. Pools, 2,000m. Below Vihren	D.T. Bilton	Lentic	Hydroporus Lentic 4		**XXXXXXX**	HF931190	HF931413	**XXXXXXX**	**XXXXXXX**
271	Hydroporina	*Hydroporus*	*transgrediens*		IBE-RA217	Greece; E Peloponnese, Lyrkio Mts, 1 km SE Kefaloryso, fountain & spring, 37.69516N 22.47982E, 20.5.2010	H. Fery & L. Hendrich	Lotic	Hydroporus Lotic 3		**XXXXXXX**	HF931307	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
272	Hydroporina	*Hydroporus*	*transpunctatus*		NHM-IR341	California (USA); Fresno co., Sequoia Ntal. Forest. Alder Creek, 18.6.2000	I. Ribera & A. Cieslak	Lotic	Hydroporus + Hydrocolus Lentic		**XXXXXXX**	HF931277	HF931514	**XXXXXXX**	**XXXXXXX**
273	Hydroporina	*Hydroporus*	*tristis*		NHM-ER5	Sweden; Prov. Vasterbotten, Vindeln, Skatan, 2.6.2006	A.N. Nilsson	Lentic	Hydroporus Lentic 2		**XXXXXXX**	AY365323	AY365289	**XXXXXXX**	**XXXXXXX**

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
274	Hydroporina	*Hydroporus*	*tristis* nr		NHM-IR521	British Columbia (Canada); Rd. 5A, Stump Lake, ponds by road, 30.6.2000	I. Ribera & A. Cieslak	Lentic	Hydroporus Lentic 2			AY365293	AY365259	XXXXXXX	XXXXXXX
275	Hydroporina	*Hydroporus*	*umbrosus*		NHM-IR152	Finland	T. Berendonk	Lentic	Hydroporus Lentic 3			AF518311	AF518280	XXXXXXX	XXXXXXX
276	Hydroporina	*Hydroporus*	*vagepictus*		NHM-IR28	Portugal; Sa. Da Estrela, Torre, lagoon, 25.7.1998	I. Ribera	Both	Hydroporus Lentic 2			AF518312	AF518281	AJ850517 EF670301	
277	Hydroporina	*Hydroporus*	*vespertinus*		NHM-IR144	Portugal; Guarda, Sa. Estrela, close summit, pools & stream, 7.8.99	D.T. Bilton	Lentic	Hydroporus + Hydrocolus Lentic		XXXXXXX	AY365325	AY365291	XXXXXXX	XXXXXXX
278	Hydroporina	*Hydroporus*	*zimmermanni*		MNCN-AH123	Slovenia; Pivka	H. Fery	Lentic	Hydroporus Lentic 4		XXXXXXX	HE610220	HF931351	XXXXXXX	XXXXXXX
279	Hydroporina	*Neoporus*	*arizonicus*		NHM-IR331	New Mexico (USA); 9.2000	Y. Alarie	Both	Neoporus + Heterosternuta + Haideoporus			AJ850630	AJ850380	AJ850519	EF670193
280	Hydroporina	*Neoporus*	*clypealis*		MNCN-AC48	Nova Scotia (Canada); Cumberland c., river next Rd. 2, Mapleton, N45º34'54.3" W64º08'30.3", 23.8.2007	I. Ribera & A. Cieslak	Both	Neoporus + Heterosternuta + Haideoporus		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
281	Hydroporina	*Neoporus*	*dilatatus*		MNCN-AC49	Nova Scotia (Canada); Cumberland c., river next Rd. 2, Mapleton, N45º34'54.3" W64º08'30.3", 23.8.2007	I. Ribera & A. Cieslak	Lotic	Neoporus + Heterosternuta + Haideoporus			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
282	Hydroporina	*Neoporus*	*dimidiatus*		NHM-IR486	Alberta (Canada); Waterton lakes Nat. Park, Rd. 5, park limit, 28.6.2000	I. Ribera & A. Cieslak	Both	Neoporus + Heterosternuta + Haideoporus		XXXXXXX	XXXXXXX	XXXXXXX		XXXXXXX
283	Hydroporina	*Neoporus*	*mellitus*		genbank			Lotic	Neoporus + Heterosternuta + Haideoporus			KF575501	KF575562		KF575370
284	Hydroporina	*Neoporus*	sp1		NHM-IR598	South Carolina (USA); Baker Creek s.p., Sunter N.F. 28.7.2000	C. Hernando	Lotic	Neoporus + Heterosternuta + Haideoporus			XXXXXXX	XXXXXXX		
285	Hydroporina	*Neoporus*	sp2		NHM-IR597	South Carolina (USA); Baker Creek s.p., Sunter N.F. 28.7.2000	C. Hernando	Lotic	Neoporus + Heterosternuta + Haideoporus			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
286	Hydroporina	*Neoporus*	sp3		MNCN-AC47	Nova Scotia (Canada); Cumberland c., river next Rd. 2, Mapleton, N45º34'54.3" W64º08'30.3", 23.8.2007	I. Ribera & A. Cieslak	Lotic	Neoporus + Heterosternuta + Haideoporus	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
287	Hydroporina	*Neoporus*	sp4		NHM-IR594	Pennsylvania (USA); Fowlers Hollow s.p., Tuscarora s.f., 20.7.2000	C. Hernando	Both	Neoporus + Heterosternuta + Haideoporus	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
288	Hydroporina	*Neoporus*	*superioris*		MNCN-DM28	Alberta (Canada); Waterton lakes Nat. Park, junc. Rd. 5&6, 28.6.2000	I. Ribera & A. Cieslak	Both	Neoporus + Heterosternuta + Haideoporus		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
289	Hydroporina	*Neoporus*	*undulatus*		NHM-IR75	Ontario (Canada)	Y. Alarie	Both	Neoporus + Heterosternuta + Haideoporus			AJ850631	AJ850381	AJ318741	EF670200
290	Hydroporina	*Sanfilippodytes*	*adelardi*		IBE-RA487	California (USA); Mendocino Co., Rain Pool - Albion Little River Rd. ~2.9 mi NE Hwy 1, N - 39.25949, W - 123.74311	D. Post	Lentic	Sanfilippodytes	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
291	Hydroporina	*Sanfilippodytes*	*bidessoides*		IBE-RA488	California (USA); Mendocino County, Rain Pools - Nat. Cons. Pygmy Forest Near Ltl. R. Airport, N - 39.25343, W - 123.74945	D. Post	Lentic	Sanfilippodytes			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
292	Hydroporina	*Sanfilippodytes*	*compertus*		genbank			Both	Sanfilippodytes			MG062181			
293	Hydroporina	*Sanfilippodytes*	*edwardsi*		MNCN-DM42	California (USA)	I. Ribera & A. Cieslak	Lotic	Sanfilippodytes			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
294	Hydroporina	*Sanfilippodytes*	*edwardsi* nr		IBE-AV20	California (USA); Big Morongo Canyon Preserve (Morongo Valley) 34º1'50''N,116º33'43''W, 3.4.2008	P. Abellán	Lotic	Sanfilippodytes		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
295	Hydroporina	*Sanfilippodytes*	*hardyi*		MNCN-AI1279	California (USA); Santa Barbara Co., UC Sedgewick Reserve, upper creek crossing, 6.7.2006	A.E.Z. Short	Lotic	Sanfilippodytes			XXXXXXX	XXXXXXX		XXXXXXX
296	Hydroporina	*Sanfilippodytes*	*malkini*		NHM-IR372	California (USA); Trinity co., junc. Rd. 36 & 14, Forest Glen, Rattlesnake Creek, 22.6.2000	I. Ribera & A. Cieslak	Lotic	Sanfilippodytes			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
297	Hydroporina	*Sanfilippodytes*	*setifer*		IBE-RA489	California (USA); Mendocino County, Rain Pools - Nat. Cons. Pygmy Forest Near Ltl. R. Airport, N - 39.25343, W - 123.74945	D. Post	Lentic	Sanfilippodytes		XXXXXXX		XXXXXXX	XXXXXXX	XXXXXXX
298	Hydroporina	*Sanfilippodytes*	sp1		NHM-IR375	California (USA); Humbolt co., Avenue of the Giants, Stream nr. Red Crest, 22.6.2000	I. Ribera & A. Cieslak	Lotic	Sanfilippodytes		XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
299	Hydroporina	*Sanfilippodytes*	sp2		MNCN-DM40	California (USA)	I. Ribera & A. Cieslak	Lotic	Sanfilippodytes			XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
300	Hydroporina	*Sanfilippodytes*	sp3		NHM-IR441	British Columbia (Canada); Rd. 3, 11 km W junct. 3B, Champion L. Prov. Park Castlegar, 27.6.2000	I. Ribera & A. Cieslak	Lentic	Sanfilippodytes		XXXXXXX	XXXXXXX	XXXXXXX		XXXXXXX
301	Hydroporina	*Sanfilippodytes*	*terminalis*		NHM-IR412	California (USA); Mendocino co., Rd. 1 Manchester, pond S City, 23.6.2000	I. Ribera & A. Cieslak	Lotic	Sanfilippodytes			AJ850673	AJ850426	AJ850552	EF670202
302	Hydroporina	*Sanfilippodytes*	*vilis*		MNCN-DM26	California (USA); 9.2000	Y. Alarie	Both	Sanfilippodytes	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
303	Siettitiina	*Ereboporus*	*naturaconservatus*		genbank			Lotic	*deleted			KF575502	KF575565		KF575357
304	Siettitiina	*Graptodytes*	*aequalis*		NHM-IR206	Morocco; Debdou, 50, Meson forestiere, 6.4.1999	I. Ribera, P. Aguilera, C. Hernando & A. Millán	Lentic	Graptodytes Lotic		LS999725	AY250953 HM588264	AY250910	AJ850509	EF670184
305	Siettitiina	*Graptodytes*	*atlantis*		MNCN-AI921	Morocco; lac Afenourir, Azrou, 33º17'12.4"N 5º15'09.8"W, 29.4.2000	I. Ribera	Lentic	Graptodytes Lotic		LS999726	HM588265	HM588602	LS999692	LS999771
306	Siettitiina	*Graptodytes*	*bilineatus*		MNCN-AI608	Sweden; Västerbotten prov., Åmsele, Vindelälven, river lagoon, 64º31'04''N, 19º20'52''E	A.N. Nilsson	Lentic	Graptodytes Lentic		LS999727	HM588267	HM588603	LS999693	LS999772
307	Siettitiina	*Graptodytes*	*castilianus*		MNCN-AI1316	Spain; Navarra, Pitillas, pond in crossroad, 42°25'29"N 1°38'35" W, 21.7.04	I. Ribera & A. Cieslak	Both	Graptodytes Lotic		HF947943	HM588268	HM588604	LS999694	LS999773
308	Siettitiina	*Graptodytes*	*delectus*		MNCN-AI1092	Tenerife (Spain); Chamorga, Bco. Roque Bermejo	A. Castro	Lotic	Graptodytes Lotic		LS999728	HM588269	HM588605	LS999695	LS999774
309	Siettitiina	*Graptodytes*	*eremitus*		IBE-AF33	Morocco; Tiqqi, cave Doussoulile N 30°44,529'W9°19,803', 28.7.2008	J.M. Bichain et al.	Lotic	Graptodytes Lotic	XXXXXXX	LS999729	HM588271	HM588606	LS999696	LS999775
310	Siettitiina	*Graptodytes*	*flavipes*		NHM-IR40	Spain; Huelva, Almonte, poblado forestal 26.7.1998	I. Ribera	Both	*deleted			EF056604 HM588273	AY250914	AJ318730	EF056561
311	Siettitiina	*Graptodytes*	*fractus*		MNCN-AI627	Spain; Córdoba, Sierra de Córdoba, Arroyo de los Arenales	A. Castro	Lotic	Graptodytes Lotic		LS451100	HM588274	HM588608	LS453474	LS453168
312	Siettitiina	*Graptodytes*	*granularis*		MNCN-AI609	Sweden; Västerbotten prov., Åmsele, Vindelälven, river lagoon, 64º31'04''N, 19º20'52''E	A.N. Nilsson	Lentic	Graptodytes Lentic		LS999730	HM588278	HM588611	LS999697	LS999776
313	Siettitiina	*Graptodytes*	*ignotus*		NHM-IR531	Spain; Girona, Estanys de Capmany	P. Aguilera	Lotic	Graptodytes Lotic		LS999731	AY250956 HM588287	AY250915	AJ850510	EF670185
314	Siettitiina	*Graptodytes*	*kuchtae*		MNCN-AI177	Mallorca (Spain); Ternelles, Torrent de Ternelles, N 39º53'37.2" E3º00'14.9"	I. Ribera & A. Cieslak	Lotic	Graptodytes Lotic		LS999732	HM588288	HM588614	LS999698	LS999777
315	Siettitiina	*Graptodytes*	*laeticulus*		MNCN-HI16	Algeria; Aïn Damous, N 36° 25.350' E 007° 51.367', 24.8.2006	S. Bouzid	Lotic	Graptodytes Lotic			HM588300	HM588621	LS999699	LS999778

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
316	Siettitiina	*Graptodytes*	*pictus*		MNCN-AI660	Poland; Zachodniopomorsky, Dygowo: garden pond, N54º07’59” E15º42’49”, 16.8.04	I. Ribera & A. Cieslak	Both	Graptodytes Lentic	**XXXXXXX**	LS999733	HM588290	HM588615	LS999700	LS999779
317	Siettitiina	*Graptodytes*	*pietrii*		MNCN-DM37	Tunisia; Rd. Beja-Teboursouk , Oued 3 km NW Teboursouk, 23.10.2001	I. Ribera & A. Cieslak	Lotic	Graptodytes Lotic	**XXXXXXX**	LS999734	HM588292	HM588616	LS999701	LS999780
318	Siettitiina	*Graptodytes*	*sedilloti*	*phrygius*	MNCN-AI111	Chios (Greece); Marmaro marsh	G.N. Foster	Lentic	Graptodytes Lentic		LS999735	HM588293	HM588618	LS999702	LS999781
319	Siettitiina	*Graptodytes*	*sedilloti*	*sedilloti*	NHM-IR585	Cyprus	K. Miller	Lentic	Graptodytes Lentic		LS451098	HM588294	HM588619	LS453473	LS453167
320	Siettitiina	*Graptodytes*	*siculus*		MNCN-AH162	Sicily (Italy); Parco dei Nebrodi, Stream Trail Lago Urio - Plla. dell’Obolo, 33N 448991 E, 4193859 N, 13.06.07	P. Abellán & F. Picazo	Lotic	Graptodytes Lotic		LS999736	HM588295	HM588620	LS999703	LS999782
321	Siettitiina	*Graptodytes*	*varius*		MNCN-AH160	Sicily (Italy); Parco dei Nebrodi, Stream Trail Lago Urio - Plla. dell’Obolo, 33N 448991 E, 4193859 N, 13.06.07	P. Abellán & F. Picazo	Both	Graptodytes Lotic	**XXXXXXX**	LS999737	HM588297	HM588622	LS999704	LS999783
322	Siettitiina	*Graptodytes*	*veterator*	*behningi*	MNCN-AI774	Turkey; Düzce, Rd. to Kartalkaya from Çaydurt, pools in mountain pass, N40º40’20” E31º47’05”, 23.4.2006	I. Ribera	Both	Graptodytes Lentic		LS999738	HM588303	HM588624	LS999705	LS999784
323	Siettitiina	*Graptodytes*	*veterator*	*veterator*	MNCN-AH161	Sicily (Italy); Parco dei Nebrodi, Stream Trail Lago Urio - Plla. dell’Obolo, 33N 448991 E, 4193859 N, 13.06.07	P. Abellán & F. Picazo	Both	Graptodytes Lentic		LS451095	HM588304	HM588625	LS453472	LS453105
324	Siettitiina	*Iberoporus*	*agnus*		MNCN-AI1007	Portugal; Viana do Castelo, ca. 6 km N Ponte de Lima, W Labruja, rest ponds of brook under bridge, 28.5.2006	H. Fery	Lotic	Iberoporus		LS999744	HF931143	HF931362	LS999710	LS999789
325	Siettitiina	*Iberoporus*	*argaensis*		MNCN-AI179	Portugal; Serra de Arga, Pools on summit, 9.5.2005	D.T. Bilton	Lotic	Iberoporus		HF948005	HF931183	HF931405	LS999711	LS999790
326	Siettitiina	*Iberoporus*	*cermenius*		NHM-IR276	Spain; Cordoba, Priego de Cordoba 29.4.2000	A. Castro	Lotic	Iberoporus		LS451107	AY250958	AY250918	AJ850511	EF670186
327	Siettitiina	*Iberoporus*	*pluto*		IBE-AN151	Portugal; G. Soprador do Carvalho, S. Reboleira	S. Reboleira	Lotic	Iberoporus	**XXXXXXX**	LS999739	LS999756	LS999763	LS999706	LS999785
328	Siettitiina	*Lioporeus*	*pilatei*		IBE-AN699	USA	N. Manuel & Baca	Lotic	Lioporeus	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**		**XXXXXXX**
329	Siettitiina	*Lioporeus*	*triangularis*		IBE-AN698	USA	N. Manuel & Baca	Lotic	Lioporeus			**XXXXXXX**	**XXXXXXX**	**XXXXXXX**	**XXXXXXX**
330	Siettitiina	*Metaporus*	*meridionalis*		NHM-IR34	Spain; Albacete, Robledo, Ojos de Villaverde	I. Ribera	Both	*deleted			AY250959 HM588307	AY250919	AJ318739	EF670187
331	Siettitiina	*Porhydrus*	*genei*		IBE-RA86	Algeria; Garaet Aïn Nechma, 36º 50.261’N 7º 16.907’E, nr Ben-Azzouz (Skikda), 29.6.2009	S. Bouzid	Lentic	Porhydrus		LS999740	HF931320	HF931543	LS999707	LS999786
332	Siettitiina	*Porhydrus*	*lineatus*		NHM-IR24	England (UK); Sommerset Levels, Chilton Trinity, 4.7.1998	I. Ribera	Lentic	Porhydrus		LS999741	AY250973	AY250933	AJ318743	EF670188
333	Siettitiina	*Porhydrus*	*obliquesignatus*		IBE-RA147	Italy; Piano Grande, Piano di Castelluccio, 20.7.2009	M. Toledo	Lentic	Porhydrus	**XXXXXXX**	LS999742	HF931305	LS999764	LS999708	LS999787
334	Siettitiina	*Porhydrus*	*vicinus*		MNCN-AH113	Portugal; Cercal, ephemeral pond, N37º47’29.1” W8º41’43.0”, Rd. btw. Cercal & Vilanova, 24.1.2008	I. Ribera & P. Aguilera & C. Hernando	Lentic	Porhydrus		LS999743	HF931132	HF931350	LS999709	LS999788
335	Siettitiina	*Psychopomporus*	*felipi*		genbank			Lotic	*deleted				KF575574		KF575363
336	Siettitiina	*Rhithrodytes*	*bimaculatus*		IBE-RA727	Spain; Huesca, Aragués del Puerto, 23.7.2011	I. Esteban	Lotic	Rhithrodytes	**XXXXXXX**	LS999745	LS999757	LS999765	LS999712	LS999791

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
337	Siettitiina	*Rhithrodytes*	*crux*		MNCN-AI302	Italy; prov. Alessandria, stream 2.5 km S Praglia, 18.10.2002	I. Ribera & A. Cieslak	Lotic	Rhithrodytes	**XXXXXXX**	LS451084	HF931187	HF931410	LS453475	LS453108
338	Siettitiina	*Rhithrodytes*	*numidicus*		MNCN-DM34	Tunisia; Rd. Tabarka-Aïn-Draham, stream 1 km N Aïn-Draham, 23.10.2001	I. Ribera & A. Cieslak	Lotic	Rhithrodytes			LS999758	LS999766	LS999713	LS999792
339	Siettitiina	*Rhithrodytes*	*sexguttatus*		NHM-IR183	Corsica; Porto-Vecchio, l’Ospedale, 18.9.1999	I. Ribera & A. Cieslak	Lotic	Rhithrodytes			AY250975	AY250936	AJ850513	EF670190
340	Siettitiina	*Siettitia*	*avenionensis*		MNCN-AI897	France; Barbentane, 12.1989	J. Dalmon	Lotic	*deleted			LS999759		LS999714	
341	Siettitiina	*Stictonectes*	*abellani*		IBE-PA312	Spain; Arroyo de los Pescados, PN Cabañeros, Ciudad Real 7.7.2008	A. Millán & col.	Lotic	Stictonectes		LS451083	HF931298	HF931530	LS453469	LS453169
342	Siettitiina	*Stictonectes*	*azruensis*		NHM-IR661	Morocco; Moyen Atlas, nr. Azrou, Col du Zad, 16.4.2001	Pellecchia & Pizzetti	Lotic	Stictonectes		LS999746	AY250979	AY250940	LS999715	LS999793
343	Siettitiina	*Stictonectes*	*canariensis*		IBE-AF114	Gran Canarias (Spain); ca. 2 km SSW El Risco, Barranco Güigüi grande, 1.4.2008	J. Hájek & K. Kaliková	Lotic	Stictonectes		LS999747	HF931113	HF931330	LS999716	LS999794
344	Siettitiina	*Stictonectes*	*epipleuricus*		MNCN-AH73	Portugal; Serra de Sâo Mameme, Portalegre: r. Caia	I. Ribera	Lotic	Stictonectes		LS999748	LS999760	LS999767	LS999717	
345	Siettitiina	*Stictonectes*	*escheri*		MNCN-AH107	Morocco; Asilah, 27.3.2008, rd. N1, stream ca. 4 km S Asilah, N35º25’10.5” W6º01’32.5”, 27.3.2008	I. Ribera, P. Aguilera & C. Hernando	Lotic	Stictonectes	**XXXXXXX**	LS999749	HF931130	HF931349	LS999718	LS999795
346	Siettitiina	*Stictonectes*	*formosus*		MNCN-AH108	Morocco; Asilah, 27.3.2008, rd. N1, stream ca. 4 km S Asilah, N35º25’10.5” W6º01’32.5”, 27.3.2008	I. Ribera, P. Aguilera & C. Hernando	Lotic	Stictonectes		LS999750	HF931131	LS999768	LS999719	LS999796
347	Siettitiina	*Stictonectes*	*lepidus*		MNCN-AI632	Spain; Córdoba, Sierra Morena, Km 28.5 cta. Villaviciosa	A. Castro	Both	Stictonectes		LS999751	LS999761	LS999769	LS999720	LS999797
348	Siettitiina	*Stictonectes*	*occidentalis*		NHM-IR529	Portugal; Algarve	P. Aguilera	Lotic	Stictonectes			AY250980	AY250942		LS999798
349	Siettitiina	*Stictonectes*	*optatus*		MNCN-AI1089	Spain; Jaén, Sierra de Cazorla, Ayo. Km 43.3 cta. Del Tranco	A. Castro	Lotic	Stictonectes	**XXXXXXX**	LS999752	LS999762	LS999770	LS999721	LS999799
350	Siettitiina	*Stictonectes*	*rebeccae*		MNCN-AH72	Portugal; Serra Estrela, Sabugueiro, r. above village, N40º24’20” W7º37’43”, 12.5.2005	I. Ribera	Lotic	Stictonectes		LS999753	FR851207	FR851208	LS999722	LS999800
351	Siettitiina	*Stictonectes*	*rufulus*		MNCN-AI1299	Sardinia; Road from Óschiri to Mount Limbara, 8 km from Tempio, 32T UTH 05103/45230	G.N. Foster	Lotic	Stictonectes		LS999754	HF931179	HF931400	LS999723	LS999801
352	Siettitiina	*Stictonectes*	*samai*		IBE-AF142	Algeria; Oued Bagrat, 24.3.2006	S. Bouzid	Lotic	Stictonectes	**XXXXXXX**	LS999755	HF931119	HF931336	LS999724	LS999802
353	Siettitiina	*Stictonectes*	sp		MsC00C	Corsica; Porto-Vecchio, l’Ospedale, 18.9.1999	I. Ribera & A. Cieslak	Lotic	Stictonectes			AY250981	AY250943	AJ850514	EF670192
354	Siettitiina	*Stygoporus*	*oregonensis*		genbank			Lentic	*deleted			KX882134	KX882132		KX882138
355	Sternopriscina	*Antiporus*	*bakewellii*		genbank			Lotic	Antiporus Lotic			FR732791	FR727255	FR727281	FR727311
356	Sternopriscina	*Antiporus*	*blakeii*		genbank			Lotic	Antiporus Lotic			FR733155	FR727258	FR727265	FR727315
357	Sternopriscina	*Antiporus*	*femoralis*		NHM-IR97	Australia; SA, 1 k S Nangwarry	C.H.S. Watts	Lentic	Antiporus Lentic			AJ850561	AJ850309	AJ850437 EF670309	EF670093

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
358	Sternopriscina	*Antiporus*	*gilbertii*		genbank			Lentic	Antiporus Lentic			FR733428	FR727262	FR727269	FR727320
359	Sternopriscina	*Antiporus*	*hollingsworthi*		genbank			Lentic	*deleted			FR732532	FR727247	FR727274	HG965673
360	Sternopriscina	*Antiporus*	*interrogationis*		genbank			Lentic	Antiporus Lentic			FR732678	FR727252	FR727278	FR727308
361	Sternopriscina	*Antiporus*	*jenniferae*		genbank			Lotic	Antiporus Lotic			FR732618		FR727276	FR727306
362	Sternopriscina	*Antiporus*	*uncifer*		NHM-IR633	New Zealand; Glenariffe, upper Rahaia river, 6.12.2000	R.M. McDownll	Lotic	Antiporus Lentic		**XXXXXXX**	AJ850562	AJ850310	AJ850438	EF670094
363	Sternopriscina	*Antiporus*	*wilsoni*		genbank			Lentic	Antiporus Lotic			FR732824	FR727256	FR727282	FR727312
364	Sternopriscina	*Barretthydrus*	*geminatus*		genbank			Lotic	Barretthydrus			FR733549	HG940428		
365	Sternopriscina	*Barretthydrus*	*stepheni*		genbank			Lotic	Barretthydrus			FR732757	HE818935	HE818972	HE819087
366	Sternopriscina	*Barretthydrus*	*tibialis*		NHM-IR555	Australia; New South Wales, 4k N Eccleston	C.H.S. Watts	Lotic	Barretthydrus			AJ850563	AJ850311	AJ850439	EF670095
367	Sternopriscina	*Brancuporus*	*gottwaldi*		genbank			Lentic	*deleted			FR732968		FR727264	FR727314
368	Sternopriscina	*Carabhydrus*	*andreas*		genbank			Lotic	Carabhydrus			HG965634	HG940413	HG965590	HG965690
369	Sternopriscina	*Carabhydrus*	*niger*		NHM-IR563	Tasmania (Australia); Dentention River 2km W Montumana Tasmania 17.11.00	C.H.S. Watts	Lotic	Carabhydrus			AJ850564	AJ850312	AJ850440	EF670096
370	Sternopriscina	*Carabhydrus*	*plicatus*		genbank			Lotic	Carabhydrus				DQ888640		
371	Sternopriscina	*Carabhydrus*	*stephanieae*		genbank			Lotic	Carabhydrus				DQ888641		
372	Sternopriscina	*Chostonectes*	*gigas*		genbank			Lotic	Chostonectes			FR733160	HG940418	HG965595	HG965695
373	Sternopriscina	*Chostonectes*	*johnsoni*		genbank			Lotic	Chostonectes			FR733199	HG940419	HG965596	HG965696
374	Sternopriscina	*Chostonectes*	*maai*		genbank			Lotic	Chostonectes			HG965629			
375	Sternopriscina	*Chostonectes*	*nebulosus*		genbank			Lentic	Chostonectes			FR733265	HG940422	HG965599	HG965699
376	Sternopriscina	*Chostonectes*	*sharpi*		NHM-IR582	Australia; Queensland, Coolongatta, 2.01.2001	F. Johansson	Lotic	Chostonectes			AJ850565	AJ850313	AJ850441	EF670097
377	Sternopriscina	*Megaporus*	*feryi*		genbank			Lentic	*deleted			HG965628			
378	Sternopriscina	*Megaporus*	*fischeri*		genbank			Lotic	*deleted			FR733464			

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
379	Sternopriscina	*Megaporus*	*gardneri*		genbank			Lentic	Megaporus			FR733470	HG940426	HG965603	HG965703
380	Sternopriscina	*Megaporus*	*hamatus*		genbank			Lentic	Megaporus			FR732843	HG940408	HG965585	HG965685
381	Sternopriscina	*Megaporus*	*howittii*		genbank			Lentic	Megaporus			FR732717	HG940405	HG965581	HG965682
382	Sternopriscina	*Megaporus*	*natvigi*		genbank			Both	*deleted			FR733142	HG940417	HG965594	HG965694
383	Sternopriscina	*Megaporus*	*rufipes*		NHM-IR64	Australia; Kakadu, Jabiru lake, 4.10.98	D. Norton	Both	*deleted	**XXXXXXX**	**XXXXXXX**	AJ850566	AJ850314	AJ850442	
384	Sternopriscina	*Megaporus*	*solidus*		genbank			Lentic	Megaporus			HG965630	HG940397	HG965576	HG965674
385	Sternopriscina	*Megaporus*	*tristis*		genbank			Lotic	*deleted			HG965627			
386	Sternopriscina	*Megaporus*	*wilsoni*		genbank			Lotic	*deleted			FR732711	HG940404	HG965580	HG965681
387	Sternopriscina	*Necterosoma*	*aphrodite*		genbank			Both	Necterosoma			FR732628	HG940401	HG965579	HG965679
388	Sternopriscina	*Necterosoma*	*darwini*		genbank			Lentic	Necterosoma			FR733381	HG940424	HG965601	HG965701
389	Sternopriscina	*Necterosoma*	*dispar*		genbank			Both	Necterosoma			FR732558	HG940399	HG965577	HG965676
390	Sternopriscina	*Necterosoma*	*novaecaledoniae*		genbank			Lotic	Necterosoma			HG965639			
391	Sternopriscina	*Necterosoma*	*penicillatum*		NHM-IR699	Australia; NSW, 3 km W Cooma, 36.16.39S 149.00.05E, 4.11.2000	K.B. Miller	Both	Necterosoma	**XXXXXXX**	**XXXXXXX**	AJ850567	AJ850315	**XXXXXXX**	EF670241
392	Sternopriscina	*Necterosoma*	*regulare*		genbank			Lotic	Necterosoma			FR733378	HG940423	HG965600	HG965700
393	Sternopriscina	*Necterosoma*	*schmeltzi*		genbank			Both	Necterosoma			FR732864	HG940409	HG965586	HG965686
394	Sternopriscina	*Necterosoma*	*schoelleri*		genbank			Lotic	Necterosoma			HG965631			
395	Sternopriscina	*Necterosoma*	*susanna*		genbank			Lotic	Necterosoma			FR733576	HG940429	HG965604	HG965705
396	Sternopriscina	*Necterosoma*	*theonathani*		genbank			Lotic	Necterosoma			HG965636			
397	Sternopriscina	*Necterosoma*	*undecimlineatum*		genbank			Lotic	Necterosoma			FR732783	HG940406	HG965583	HG965683
398	Sternopriscina	*Paroster*	*angustus*		genbank			Lentic	Paroster			KU549161			
399	Sternopriscina	*Paroster*	*arachnoides*		genbank			Lentic	Paroster			EU616972	EU616917		

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
400	Sternopriscina	*Paroster*	*baylyi*		genbank			Lentic	Paroster			HG965632	HG940400	HG965578	HG965678
401	Sternopriscina	*Paroster*	*bulbus*		genbank			Lentic	Paroster			EU616973			
402	Sternopriscina	*Paroster*	*byroensis*		genbank			Lentic	Paroster			EU616965	EU616910		
403	Sternopriscina	*Paroster*	*copidotibiae*		genbank			Lentic	Paroster			EU616967	EU616912		
404	Sternopriscina	*Paroster*	*couragei*		genbank			Lentic	Paroster			EU644497	EU644493		
405	Sternopriscina	*Paroster*	*darlotensis*		genbank			Lentic	Paroster			EU616976	EU616921		
406	Sternopriscina	*Paroster*	*dingbatensis*		genbank			Lentic	Paroster			EU616966	EU616911		
407	Sternopriscina	*Paroster*	*eurypleuron*		genbank			Lentic	Paroster			EU616964	EU616909		
408	Sternopriscina	*Paroster*	*extraordinarius*		genbank			Lentic	Paroster			GQ380574	GQ380572		
409	Sternopriscina	*Paroster*	*fortispina*		genbank			Lentic	Paroster			AY350900	AY353847		
410	Sternopriscina	*Paroster*	*gibbi*		NHM-IR92	Australia; SA, 10k E Mt Compass	C.H.S. Watts	Lentic	Paroster		**XXXXXXX**	AJ850568	AJ850316	AJ318742	EF670098
411	Sternopriscina	*Paroster*	*hamoni*		genbank			Lentic	Paroster			AY350878	AY353825		
412	Sternopriscina	*Paroster*	*hinzeae*		genbank			Lentic	Paroster			AY350885	AY353832		
413	Sternopriscina	*Paroster*	*innoendyensis*		genbank			Lentic	Paroster			EU616968	EU616913		
414	Sternopriscina	*Paroster*	*insculptilis*		genbank			Lentic	Paroster			AY350876	AY353823		
415	Sternopriscina	*Paroster*	*killaraensis*		genbank			Lentic	Paroster			EU616992	EU616937		
416	Sternopriscina	*Paroster*	*macrocephalus*		genbank			Lentic	Paroster			EU616960	EU616905		
417	Sternopriscina	*Paroster*	*macrosturtensis*		genbank			Lentic	Paroster	MG912995					
418	Sternopriscina	*Paroster*	*megamacrocephalus*		genbank			Lentic	Paroster			EU616990	EU616935		
419	Sternopriscina	*Paroster*	*melroseensis*		genbank			Lentic	Paroster			EU616977	EU616922		
420	Sternopriscina	*Paroster*	*mesosturtensis*		genbank			Lentic	Paroster			EU616997	EU616942		

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
421	Sternopriscina	*Paroster*	*michaelseni*		genbank			Lentic	Paroster			EU616974	EU616919		
422	Sternopriscina	*Paroster*	*microsturtensis*		genbank			Lentic	Paroster			EU616996	EU616941		
423	Sternopriscina	*Paroster*	*milgunensis*		genbank			Lentic	Paroster			AY350879	AY353826		
424	Sternopriscina	*Paroster*	*napperbyensis*		genbank			Lentic	Paroster			AY350896	EU616906		
425	Sternopriscina	*Paroster*	*newhavenensis*		genbank			Lentic	Paroster			EU616962	EU616907		
426	Sternopriscina	*Paroster*	*niger*		genbank			Both	Paroster			HG965635	HG940415	HG965592	HG965692
427	Sternopriscina	*Paroster*	*nigroadumbratus*		genbank			Both	Paroster			FR732970	HG940414	HG965591	HG965691
428	Sternopriscina	*Paroster*	*pallescens*		genbank			Lentic	Paroster			EU616975	EU616920		
429	Sternopriscina	*Paroster*	*peelensis*		genbank			Lentic	Paroster			EU371111	EU371112		
430	Sternopriscina	*Paroster*	*pentameres*		genbank			Lentic	Paroster			EU616991	EU616936		
431	Sternopriscina	*Paroster*	*plutonicensis*		genbank			Lentic	Paroster			AY350892	AY353839		
432	Sternopriscina	*Paroster*	*skaphites*		genbank			Lentic	Paroster			EU616971	EU616916		
433	Sternopriscina	*Paroster*	*stegastos*		genbank			Lentic	Paroster			EU616969	EU616914		
434	Sternopriscina	*Paroster*	*tetrameres*		genbank			Lentic	Paroster			EU616985	EU616930		
435	Sternopriscina	*Paroster*	*ursulae*		genbank			Lentic	Paroster			FR733465			
436	Sternopriscina	*Paroster*	*verrucosus*		genbank			Lentic	Paroster			EU616963	EU616908		
437	Sternopriscina	*Paroster*	*wedgeensis*		genbank			Lentic	Paroster			EU616993	EU616938		
438	Sternopriscina	*Sekaliporus*	*kriegi*		genbank			Lotic	Tiporus + Sekaliporus			FR732624			
439	Sternopriscina	*Sternopriscus*	*alligatorensis*		genbank			Lotic	Sternopriscus Lotic			FR732624		HE818974	HE819089
440	Sternopriscina	*Sternopriscus*	*alpinus*		genbank			Lentic	Sternopriscus Lentic			FR732706	FR727253	HE818975	HE819092
441	Sternopriscina	*Sternopriscus*	*aquilonaris*		genbank			Lotic	Sternopriscus Lotic			FR732908			HE819094

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
442	Sternopriscina	*Sternopriscus*	*balkei*		genbank			Lotic	Sternopriscus Lotic			FR732881	HE818939	HE818978	HE819097
443	Sternopriscina	*Sternopriscus*	*barbarae*		genbank			Lotic	Sternopriscus Lentic			FR732974	HE818943	HE818980	HE819101
444	Sternopriscina	*Sternopriscus*	*browni*		genbank			Both	Sternopriscus Lentic			FR732708	HE818944	HE818981	HE819102
445	Sternopriscina	*Sternopriscus*	*clavatus*		NHM-IR698	Australia; Victoria, 24 km W Hamilton, 37.34.49S 141.46.31E, 11.11.2000	K.B. Miller	Both	Sternopriscus Lotic	**XXXXXXX**	**XXXXXXX**	AJ850569	AJ850317	AJ850444	EF670099
446	Sternopriscina	*Sternopriscus*	*eikei*		genbank			Lentic	*deleted			FR733384	HE818947	HE818984	HE819107
447	Sternopriscina	*Sternopriscus*	*emmae*		genbank			Lotic	Sternopriscus Lotic			HG965637			
448	Sternopriscina	*Sternopriscus*	*goldbergi*		genbank			Lotic	Sternopriscus Lotic			FR733029		HE818985	HE819109
449	Sternopriscina	*Sternopriscus*	*hansardii*		genbank			Lotic	Sternopriscus Lotic			FR733319	HE818949	HE818987	HE819110
450	Sternopriscina	*Sternopriscus*	*marginatus*		genbank			Lotic	Sternopriscus Lotic			FR733436	HE818951	HE818988	HE819112
451	Sternopriscina	*Sternopriscus*	*meadfootii*		genbank			Both	Sternopriscus Lentic			FR732582	HE818952		HE819113
452	Sternopriscina	*Sternopriscus*	*minimus*		genbank			Lentic	Sternopriscus Lentic			FR733393		HE818989	HE819114
453	Sternopriscina	*Sternopriscus*	*montanus*		genbank			Lentic	Sternopriscus Lentic			FR733462	HE818955	HE818990	HE819115
454	Sternopriscina	*Sternopriscus*	*multimaculatus*		genbank			Both	Sternopriscus Lentic			FR732594	HE818956	HE818991	HE819116
455	Sternopriscina	*Sternopriscus*	*mundanus*		genbank			Both	Sternopriscus Lentic			FR732906	HE818945	HE818982	HE819103
456	Sternopriscina	*Sternopriscus*	*storeyi*		genbank			Lentic	Sternopriscus Lentic			FR732720	HE818959	HE818994	HE819119
457	Sternopriscina	*Sternopriscus*	*tarsalis*		genbank			Both	Sternopriscus Lentic			FR733186	HE818961	HE818997	HE819122
458	Sternopriscina	*Sternopriscus*	*tasmanicus*		genbank			Lentic	Sternopriscus Lentic			FR732710	HE818962	HE818998	HE819123
459	Sternopriscina	*Sternopriscus*	*wallumphilia*		genbank			Lotic	Sternopriscus Lotic			FR732926			HE819125
460	Sternopriscina	*Sternopriscus*	*wattsi*		genbank			Both	Sternopriscus Lentic			FR732982	HE818963	HE818999	HE819127
461	Sternopriscina	*Sternopriscus*	*weckwerthi*		genbank			Lentic	Sternopriscus Lentic			FR732888	HE818966	HE819000	HE819130
462	Sternopriscina	*Sternopriscus*	*wehnckei*		genbank			Both	Sternopriscus Lentic			FR732936	HE818967	HE819001	HE819131

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Table S2. (continued)

id	subtribe	genus	species	subspecies	voucher	Loc.	leg	Habitat	clade	mtgenome	BAR	COI	16S	18S	H3
463	Sternopriscina	*Sternopriscus*	*weiri*		genbank			Lentic	Sternopriscus Lotic			FR732762	HE818970	HE819002	HE819134
464	Sternopriscina	*Sternopriscus*	*williamsi*		genbank			Lentic	Sternopriscus Lentic			FR732694	HE818971	HE819003	HE819135
465	Sternopriscina	*Tiporus*	*alastairi*		genbank			Lotic	Tiporus + Sekaliporus			FR732513			
466	Sternopriscina	*Tiporus*	*centralis*		genbank			Lotic	Tiporus + Sekaliporus			FR732645	HG940402		HG965680
467	Sternopriscina	*Tiporus*	*collaris*		genbank			Lotic	Tiporus + Sekaliporus			FR732880	HG940412	HG965589	HG965689
468	Sternopriscina	*Tiporus*	*emmae*		genbank			Lotic	Tiporus + Sekaliporus			FR732990			
469	Sternopriscina	*Tiporus*	*giuliani*		genbank			Lotic	Tiporus + Sekaliporus			FR733050			
470	Sternopriscina	*Tiporus*	*josepheni*		NHM-IR550	Australia; Queensland, 8k N Blue Water	C.H.S. Watts	Lotic	Tiporus + Sekaliporus			AJ850570	AJ850318	AJ850445	EF670100
471	Sternopriscina	*Tiporus*	*lachlani*		genbank			Lotic	Tiporus + Sekaliporus			FR732817	HG940407	HG965584	HG965684
472	Sternopriscina	*Tiporus*	*tambreyi*		genbank			Lotic	Tiporus + Sekaliporus			FR733420	HG940425	HG965602	HG965702
473	Sternopriscina	*Tiporus*	*undecimmaculatus*		genbank			Lotic	Tiporus + Sekaliporus			FR733046	HG940416	HG965593	HG965693

Table S3. Mean diversification rates and standard deviations obtained in BAMM and MM-estimator (3 different ε scenarios).

	***Method of moments* estimator** (Magallón and Sanderson, 2001)												**BAMM**
	ε = 0		ε = 0.5		ε = 0.9		ε = 0		ε = 0.5		ε = 0.9		
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	
complete phylogeny	0.060	0.007	0.057	0.006	0.044	0.005	NA	NA	NA	NA	NA	NA	0.056
Boreonectes	0.141	0.018	0.124	0.016	0.057	0.008	0.078	0.011	0.069	0.009	0.032	0.004	0.068
Clarkhydrus	0.051	0.010	0.044	0.009	0.018	0.004	0.035	0.005	0.030	0.004	0.013	0.002	0.056
Deronectes	0.059	0.007	0.054	0.006	0.033	0.004	0.040	0.005	0.037	0.004	0.022	0.003	0.056
Mystonectes	0.057	0.019	0.049	0.016	0.017	0.006	0.024	0.003	0.020	0.002	0.007	0.001	0.056
Nebrioporus	0.080	0.009	0.074	0.008	0.044	0.005	0.067	0.007	0.061	0.007	0.037	0.004	0.057
Nectoboreus	0.062	0.010	0.054	0.009	0.018	0.003	0.011	0.001	0.009	0.001	0.003	0.000	0.056
Nectoporus	0.047	0.006	0.041	0.005	0.018	0.002	0.033	0.005	0.029	0.004	0.013	0.002	0.056
Oreodytes	0.057	0.007	0.051	0.006	0.024	0.003	0.037	0.004	0.033	0.004	0.015	0.002	0.056
Scarodytes	0.102	0.013	0.091	0.011	0.044	0.005	0.057	0.007	0.051	0.006	0.025	0.003	0.057
Stictotarsus	0.036	0.005	0.031	0.005	0.010	0.002	0.017	0.002	0.014	0.002	0.005	0.001	0.057
Sanfilippodytes	0.077	0.010	0.069	0.009	0.037	0.005	0.040	0.005	0.036	0.004	0.019	0.002	0.074
Graptodytes	0.078	0.010	0.070	0.009	0.036	0.005	0.063	0.009	0.056	0.008	0.029	0.004	0.058
Iberoporus	0.039	0.005	0.033	0.005	0.011	0.002	0.031	0.005	0.026	0.004	0.009	0.001	0.057
Hydroporus plus *Hydrocolus*	0.094	0.011	0.088	0.010	0.062	0.007	0.071	0.008	0.067	0.008	0.047	0.005	0.089
Heterosternuta plus *Neoporus* plus *Haideoporus*	0.046	0.006	0.043	0.005	0.025	0.003	0.046	0.005	0.042	0.005	0.025	0.003	0.061
Porhydrus	0.021	0.003	0.018	0.002	0.006	0.001	0.012	0.001	0.010	0.001	0.003	0.000	0.055
Rhithrodytes	0.057	0.007	0.049	0.006	0.018	0.002	0.029	0.003	0.025	0.003	0.009	0.001	0.056
Stictonectes	0.078	0.009	0.069	0.008	0.031	0.004	0.032	0.004	0.028	0.003	0.013	0.001	0.056
Antiporus	0.056	0.007	0.049	0.006	0.023	0.003	0.042	0.005	0.037	0.005	0.017	0.002	0.056
Barretthydrus	0.015	0.002	0.013	0.002	0.004	0.001	0.007	0.001	0.006	0.001	0.002	0.000	0.055
Carabhydrus	0.030	0.004	0.027	0.004	0.011	0.002	0.025	0.003	0.022	0.003	0.009	0.001	0.055
Chostonectes	0.019	0.002	0.017	0.002	0.006	0.001	0.017	0.002	0.015	0.002	0.005	0.001	0.055
Megaporus	0.052	0.007	0.046	0.006	0.020	0.003	0.026	0.003	0.023	0.003	0.010	0.001	0.056
Necterosoma	0.045	0.007	0.040	0.006	0.017	0.003	0.025	0.003	0.022	0.003	0.010	0.001	0.056
Paroster	0.065	0.008	0.060	0.007	0.035	0.004	0.044	0.005	0.040	0.005	0.024	0.003	0.056
Sternopriscus	0.066	0.008	0.060	0.007	0.032	0.004	0.045	0.005	0.040	0.005	0.021	0.003	0.069
Tiporus	0.050	0.007	0.044	0.006	0.020	0.003	0.043	0.006	0.038	0.005	0.017	0.002	0.056

Table S4. Number of species per genera currently recognized in Hydroporini and number of samples per genera.

id	subtribe	genus	Catalogue		Samples		
			sp	spp	sp	spp	unknown lineages
1	Deronectina	*Amurodytes*	1		0		
2	Deronectina	*Boreonectes*	10	1	7	1	4
3	Deronectina	*Clarkhydrus*	10		4		
4	Deronectina	*Deronectes*	59	4	32	3	
5	Deronectina	*Deuteronectes*	2		1		
6	Deronectina	*Hornectes*	1		1		
7	Deronectina	*Iberonectes*	1		1		
8	Deronectina	*Larsonectes*	1		1		
9	Deronectina	*Leconectes*	1		1		
10	Deronectina	*Mystonectes*	5		3		
11	Deronectina	*Nebrioporus*	57	2	34	2	
12	Deronectina	*Nectoboreus*	3		2		
13	Deronectina	*Nectomimus*	1		1		
14	Deronectina	*Nectoporus*	9	2	7	2	
15	Deronectina	*Neonectes*	3		1		
16	Deronectina	*Oreodytes*	14	2	7	2	
17	Deronectina	*Scarodytes*	11	1	9	1	7
18	Deronectina	*Stictotarsus*	3		3		
19	Deronectina	*Trichonectes*	1		1		
20	Deronectina	*Zaitzevhydrus*	1	1	1	1	
21	Hydroporina	*Haideoporus*	1		1		
22	Hydroporina	*Heterosternuta*	14		3		
23	Hydroporina	*Hydrocolus*	12		5		
24	Hydroporina	*Hydroporus*	189	12	111	7	11
25	Hydroporina	*Neoporus*	39		7		4
26	Hydroporina	*Sanfilippodytes*	25		9		4
27	Siettitiina	*Ereboporus*	1		1		
28	Siettitiina	*Etruscodytes*	1		0		
29	Siettitiina	*Graptodytes*	22	2	18	2	
30	Siettitiina	*Iberoporus*	4		4		
31	Siettitiina	*Lioporeus*	2		2		
32	Siettitiina	*Metaporus*	2		1		
33	Siettitiina	*Porhydrus*	4		4		
34	Siettitiina	*Psychopomporus*	1		1		
35	Siettitiina	*Rhithrodytes*	6		4		
36	Siettitiina	*Siettitia*	2		1		
37	Siettitiina	*Stictonectes*	12		12		1
38	Siettitiina	*Stygoporus*	1		1		
39	Sternopriscina	*Antiporus*	15		9		
40	Sternopriscina	*Barretthydrus*	3		3		
41	Sternopriscina	*Brancuporus*	2		1		
42	Sternopriscina	*Carabhydrus*	10		4		
43	Sternopriscina	*Chostonectes*	6		5		
44	Sternopriscina	*Megaporus*	11		10		
45	Sternopriscina	*Necterosoma*	12		11		
46	Sternopriscina	*Paroster*	52		40		
47	Sternopriscina	*Sekaliporus*	2		1		
48	Sternopriscina	*Sternopriscus*	29		26		
49	Sternopriscina	*Tiporus*	13		9		
50	Unknown	*Siamoporus*	1		0		
51	Unknown	*Tassilodytes*	1		0		

Table S5. Rates estimated for extant taxa (speciation, extinction, diversification and its standard deviation - sd).

	Speciation	Extinction	Diversification	sd
Antiporus bakewellii GBK	0.060	0.003	0.058	0.007
Antiporus blakeii GBK	0.060	0.003	0.058	0.007
Antiporus femoralis IR97	0.060	0.003	0.057	0.007
Antiporus gilbertii GBK	0.060	0.003	0.058	0.007
Antiporus hollingsworthi GBK	0.060	0.003	0.057	0.007
Antiporus interrogationis GBK	0.060	0.003	0.057	0.007
Antiporus jenniferae GBK	0.060	0.003	0.057	0.007
Antiporus uncifer IR633	0.060	0.003	0.058	0.007
Antiporus wilsoni GBK	0.060	0.003	0.058	0.007
Barretthydrus geminatus GBK	0.060	0.003	0.057	0.007
Barretthydrus stepheni GBK	0.060	0.003	0.057	0.007
Barretthydrus tibialis IR555	0.060	0.003	0.057	0.007
Boreonectes alpestris RA263	0.146	0.034	0.111	0.028
Boreonectes emmerichi RA891	0.073	0.011	0.062	0.007
Boreonectes griseostriatus griseostriatus AI952	0.146	0.034	0.111	0.028
Boreonectes griseostriatus nr1 IR342	0.082	0.015	0.067	0.010
Boreonectes griseostriatus nr2 AI1160	0.076	0.013	0.064	0.008
Boreonectes griseostriatus nr3 AI1150	0.068	0.009	0.060	0.007
Boreonectes griseostriatus nr4 RA483	0.076	0.013	0.064	0.008
Boreonectes griseostriatus strandi AI1082	0.146	0.034	0.111	0.028
Boreonectes ibericus IR22	0.146	0.034	0.111	0.028
Boreonectes macedonicus AI1120	0.144	0.034	0.110	0.028
Boreonectes multilineatus RA255	0.145	0.034	0.111	0.028
Boreonectes riberae AI829	0.144	0.034	0.110	0.028
Brancuporus gottwaldi GBK	0.060	0.004	0.057	0.007
Carabhydrus andreas GBK	0.060	0.003	0.057	0.007
Carabhydrus niger IR563	0.060	0.003	0.057	0.007
Carabhydrus plicatus GBK	0.060	0.003	0.057	0.007
Carabhydrus stephanieae GBK	0.060	0.004	0.057	0.006
Chostonectes gigas GBK	0.060	0.003	0.057	0.007
Chostonectes johnsoni GBK	0.060	0.003	0.057	0.007
Chostonectes maai GBK	0.060	0.003	0.057	0.007
Chostonectes nebulosus GBK	0.060	0.003	0.057	0.007
Chostonectes sharpi IR582	0.060	0.003	0.057	0.007
Clarkhydrus corvinus GBK	0.060	0.003	0.058	0.007
Clarkhydrus deceptus AV27	0.061	0.003	0.058	0.007
Clarkhydrus falli IR334	0.060	0.003	0.058	0.007
Clarkhydrus roffii IR335	0.061	0.003	0.058	0.007
Deronectes abnormicollis AI120	0.060	0.003	0.058	0.007
Deronectes adanensis DV84	0.060	0.003	0.058	0.007
Deronectes algibensis IR76	0.061	0.003	0.058	0.007
Deronectes angelinii RA234	0.073	0.014	0.060	0.007
Deronectes angusi DV67	0.074	0.014	0.060	0.007
Deronectes aubei aubei DV150	0.066	0.006	0.060	0.007
Deronectes aubei sanfilippoi DV149	0.066	0.006	0.060	0.007
Deronectes bicostatus AI639	0.061	0.003	0.058	0.007
Deronectes brannanii AI178	0.061	0.003	0.058	0.007
Deronectes costipennis costipennis AI183	0.062	0.004	0.058	0.007
Deronectes costipennis gignouxi DV19	0.062	0.004	0.058	0.007
Deronectes delarouzei DV1	0.066	0.006	0.060	0.007
Deronectes depressicollis AI1023	0.061	0.003	0.058	0.007
Deronectes doriae AI775	0.061	0.003	0.058	0.007
Deronectes evelynae AV155	0.061	0.003	0.058	0.007
Deronectes fairmairei AI855	0.061	0.003	0.058	0.007
Deronectes ferrugineus AI731	0.061	0.004	0.058	0.007
Deronectes fosteri IR77	0.061	0.003	0.058	0.007

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Table S5. (continued)

	Speciation	Extinction	Diversification	sd
Deronectes hispanicus DV49	0.061	0.003	0.058	0.007
Deronectes lareynii IR165	0.061	0.004	0.058	0.007
Deronectes latus DV80	0.074	0.014	0.060	0.007
Deronectes moestus insconspectus AI937	0.061	0.003	0.058	0.007
Deronectes moestus moestus DV69	0.061	0.003	0.058	0.007
Deronectes nilssoni AF104	0.060	0.003	0.058	0.007
Deronectes opatrinus AI629	0.061	0.003	0.058	0.007
Deronectes parvicollis AI776	0.060	0.003	0.058	0.007
Deronectes persicus IR45	0.060	0.003	0.058	0.007
Deronectes platynotus AI1039	0.061	0.004	0.058	0.007
Deronectes sahlbergi AI1002	0.061	0.004	0.058	0.007
Deronectes semirufus DV59	0.066	0.006	0.060	0.007
Deronectes taron AV157	0.060	0.003	0.058	0.007
Deronectes theryi RA37	0.061	0.004	0.058	0.007
Deronectes toledoi DV6	0.073	0.014	0.060	0.007
Deronectes wewalkai AI725	0.061	0.004	0.058	0.007
Deronectes youngi IR182	0.060	0.003	0.058	0.007
Deuteronectes picturatus IR369	0.060	0.004	0.057	0.007
Ereboporus naturaconservatus GBK	0.060	0.004	0.057	0.007
Graptodytes aequalis IR206	0.084	0.020	0.064	0.008
Graptodytes atlantis AI921	0.075	0.016	0.059	0.007
Graptodytes bilineatus AI608	0.066	0.009	0.057	0.007
Graptodytes castilianus AI1316	0.084	0.020	0.064	0.008
Graptodytes delectus AI1092	0.086	0.021	0.065	0.009
Graptodytes eremitus AF33	0.078	0.018	0.061	0.007
Graptodytes flavipes IR40	0.063	0.006	0.057	0.007
Graptodytes fractus AI627	0.099	0.025	0.074	0.011
Graptodytes granularis AI609	0.066	0.009	0.058	0.007
Graptodytes ignotus IR531	0.099	0.025	0.074	0.011
Graptodytes kuchtai AI177	0.099	0.025	0.074	0.011
Graptodytes laeticulus HI16	0.099	0.025	0.074	0.011
Graptodytes pictus AI660	0.066	0.009	0.058	0.007
Graptodytes pietrii DM37	0.084	0.020	0.064	0.008
Graptodytes sedilloti phrygius AI111	0.066	0.009	0.058	0.007
Graptodytes sedilloti sedilloti IR585	0.066	0.009	0.058	0.007
Graptodytes siculus AH162	0.084	0.020	0.064	0.008
Graptodytes varius AH160	0.099	0.025	0.074	0.011
Graptodytes veterator behningi AI774	0.066	0.009	0.058	0.007
Graptodytes veterator veterator AH161	0.066	0.009	0.058	0.007
Haideoporus texanus GBK2	0.072	0.009	0.063	0.007
Heterosternuta allegheniana AV2	0.072	0.008	0.065	0.008
Heterosternuta pulchra AV4	0.072	0.008	0.065	0.008
Heterosternuta wickhami IR330	0.072	0.008	0.065	0.008
Hornectes quadrimaculatus IR366	0.060	0.004	0.057	0.007
Hydrocolus paugus AC43	0.121	0.035	0.087	0.010
Hydrocolus persimilis AV5	0.121	0.035	0.087	0.010
Hydrocolus rubyi GBK	0.121	0.035	0.086	0.010
Hydrocolus sahlbergi ER21	0.121	0.035	0.086	0.010
Hydrocolus stagnalis AV6	0.121	0.035	0.086	0.010
Hydroporus acutangulus ER1	0.135	0.045	0.091	0.011
Hydroporus analis AV9	0.135	0.045	0.091	0.011
Hydroporus angustatus GR16	0.122	0.036	0.087	0.010
Hydroporus apenninus AF60	0.156	0.057	0.099	0.012
Hydroporus appalachius GBK	0.122	0.035	0.087	0.010
Hydroporus axillaris DM32	0.121	0.035	0.087	0.010
Hydroporus basinotatus AH112	0.136	0.045	0.091	0.011

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Table S5. (continued)

	Speciation	Extinction	Diversification	sd
Hydroporus bithynicus AI782	0.142	0.049	0.093	0.011
Hydroporus bodemeyeri bodemeyeri AI110	0.154	0.056	0.098	0.012
Hydroporus bodemeyeri guignoti RA216	0.154	0.056	0.098	0.012
Hydroporus brancoi brancoi AI228	0.154	0.056	0.098	0.012
Hydroporus brancoi gredensis AC9	0.154	0.056	0.098	0.012
Hydroporus brancuccii AI180	0.136	0.045	0.091	0.011
Hydroporus brevis DM23	0.131	0.042	0.089	0.010
Hydroporus brucki AN4	0.135	0.045	0.091	0.011
Hydroporus cagrankaya AI62	0.156	0.057	0.099	0.012
Hydroporus cantabricus AI122	0.154	0.056	0.098	0.012
Hydroporus carri GBK	0.122	0.035	0.087	0.010
Hydroporus columbianus GBK	0.122	0.035	0.087	0.010
Hydroporus compunctus IR181	0.135	0.045	0.091	0.011
Hydroporus constantini AI285	0.155	0.057	0.099	0.012
Hydroporus cuprescens IR647	0.154	0.056	0.098	0.012
Hydroporus decipiens DM31	0.135	0.045	0.091	0.011
Hydroporus dentellus IR575	0.121	0.035	0.087	0.010
Hydroporus despectus IR483	0.121	0.035	0.087	0.010
Hydroporus discretus cplx1 AI651	0.135	0.045	0.091	0.011
Hydroporus discretus cplx2 AV45	0.135	0.045	0.091	0.011
Hydroporus discretus cplx3 RA142	0.135	0.045	0.091	0.011
Hydroporus discretus AI641	0.136	0.045	0.091	0.011
Hydroporus distinguendus HI15	0.130	0.041	0.089	0.010
Hydroporus dobrogeanus cplx AV49	0.156	0.057	0.099	0.012
Hydroporus dobrogeanus DM85	0.156	0.057	0.099	0.012
Hydroporus dorsalis RA939	0.121	0.035	0.086	0.010
Hydroporus elongatulus DM18	0.121	0.035	0.087	0.010
Hydroporus errans AI638	0.135	0.045	0.091	0.011
Hydroporus erythrocephalus RA710	0.121	0.035	0.087	0.010
Hydroporus ferrugineus AI929	0.142	0.049	0.093	0.011
Hydroporus feryi DM33	0.135	0.045	0.091	0.011
Hydroporus figuratus IR67	0.121	0.035	0.086	0.010
Hydroporus fortis IR353	0.121	0.035	0.087	0.010
Hydroporus foveolatus DM8	0.136	0.045	0.091	0.011
Hydroporus foveolatus nr DM14	0.136	0.045	0.091	0.011
Hydroporus fuscipennis IR491	0.135	0.045	0.091	0.011
Hydroporus galloprovincialis RA289	0.155	0.056	0.099	0.012
Hydroporus geniculatus ER3	0.122	0.035	0.087	0.010
Hydroporus glabriusculus GR33	0.131	0.042	0.090	0.010
Hydroporus glasunovi dolini RA1204	0.135	0.045	0.091	0.011
Hydroporus goldschmidti AI118	0.135	0.045	0.091	0.010
Hydroporus gueorguievi DM20	0.156	0.057	0.099	0.012
Hydroporus guernei RA911	0.135	0.045	0.091	0.011
Hydroporus gyllenhalii GR12	0.130	0.041	0.090	0.010
Hydroporus hebaueri DM86	0.154	0.056	0.098	0.012
Hydroporus incognitus RA691	0.130	0.041	0.090	0.010
Hydroporus incommodus AI382	0.130	0.041	0.090	0.010
Hydroporus jonicus AI1233	0.130	0.041	0.089	0.010
Hydroporus kabakovi AI91	0.135	0.045	0.091	0.011
Hydroporus kasyi AV42	0.130	0.041	0.089	0.010
Hydroporus kozlovskii AI1264	0.137	0.045	0.092	0.011
Hydroporus kraatzi AI602	0.156	0.057	0.099	0.012
Hydroporus lapponum GBK	0.121	0.035	0.087	0.010
Hydroporus larsoni GBK	0.131	0.042	0.090	0.010
Hydroporus limbatus AI736	0.135	0.045	0.091	0.011
Hydroporus lluci RA368	0.155	0.056	0.099	0.012

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Table S5. (continued)

	Speciation	Extinction	Diversification	sd
Hydroporus longicornis DM22	0.153	0.055	0.098	0.012
Hydroporus longiusculus IR345	0.122	0.035	0.087	0.010
Hydroporus longulus AI300	0.155	0.057	0.099	0.012
Hydroporus lucasi AI630	0.135	0.045	0.091	0.011
Hydroporus lundbladi IR664	0.135	0.045	0.091	0.011
Hydroporus macedonicus AI1265	0.136	0.045	0.091	0.011
Hydroporus mannerheimi IR485	0.122	0.035	0.087	0.010
Hydroporus mannerheimi nr IR526	0.122	0.035	0.087	0.010
Hydroporus marginatus AI61	0.135	0.045	0.091	0.010
Hydroporus melanarius RA636	0.154	0.056	0.098	0.012
Hydroporus memnonius gr AI764	0.153	0.055	0.098	0.012
Hydroporus memnonius IR315	0.154	0.056	0.098	0.012
Hydroporus morio ER22	0.121	0.035	0.086	0.010
Hydroporus necopinatus robertorum GBK	0.154	0.056	0.098	0.012
Hydroporus necopinatus roni GBK	0.154	0.056	0.098	0.012
Hydroporus necopinatus necopinatus AI1130	0.154	0.056	0.098	0.012
Hydroporus neglectus GR15	0.122	0.036	0.087	0.010
Hydroporus nevadensis AI640	0.155	0.057	0.099	0.012
Hydroporus nigellus IR506	0.122	0.035	0.087	0.010
Hydroporus niger gr1 AV7	0.121	0.035	0.086	0.010
Hydroporus signatus RA682	0.122	0.035	0.087	0.010
Hydroporus niger IR124	0.121	0.035	0.087	0.010
Hydroporus nigrita RA334	0.137	0.045	0.092	0.011
Hydroporus normandi alhambrae RA461	0.155	0.056	0.099	0.012
Hydroporus normandi ifnii GR13	0.154	0.056	0.098	0.012
Hydroporus normandi normandi AH105	0.155	0.056	0.099	0.012
Hydroporus notabilis AI92	0.121	0.035	0.087	0.010
Hydroporus obscurus ER4	0.121	0.035	0.087	0.010
Hydroporus obsoletus RA206	0.135	0.045	0.090	0.010
Hydroporus occidentalis IR517	0.122	0.035	0.087	0.010
Hydroporus paganettianus IR145	0.121	0.035	0.087	0.010
Hydroporus palustris AI19	0.130	0.041	0.090	0.010
Hydroporus pervicinus IR359	0.122	0.035	0.087	0.010
Hydroporus pfefferi RA1060	0.153	0.055	0.098	0.012
Hydroporus pilosus IR37	0.135	0.045	0.091	0.011
Hydroporus planus AN82	0.135	0.045	0.091	0.011
Hydroporus puberulus ER2	0.131	0.042	0.090	0.010
Hydroporus pubescens AN383	0.135	0.045	0.091	0.011
Hydroporus regularis IR166	0.155	0.057	0.099	0.012
Hydroporus rifensis AV218	0.130	0.041	0.090	0.010
Hydroporus rufifrons ER19	0.121	0.035	0.087	0.010
Hydroporus rufinasus GBK	0.121	0.035	0.087	0.010
Hydroporus sabaudus sabaudus DM16	0.137	0.045	0.092	0.011
Hydroporus sabaudus sierranevadensis PA262	0.137	0.045	0.092	0.011
Hydroporus sanfilippoi AH189	0.142	0.049	0.093	0.011
Hydroporus sardomontanus DM62	0.156	0.057	0.099	0.012
Hydroporus scalesianus IR38	0.122	0.036	0.086	0.010
Hydroporus semenowi DM24	0.121	0.035	0.087	0.010
Hydroporus signatus signatus GBK	0.122	0.035	0.087	0.010
Hydroporus sinuatipes GBK	0.121	0.035	0.087	0.010
Hydroporus springeri IR632	0.130	0.041	0.090	0.010
Hydroporus striola IR484	0.130	0.041	0.090	0.010
Hydroporus submuticus ER23	0.121	0.035	0.087	0.010
Hydroporus subpubescens AC46	0.122	0.035	0.087	0.010
Hydroporus tenebrosus GBK	0.122	0.035	0.087	0.010
Hydroporus tessellatus AI770	0.136	0.045	0.091	0.011

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Table S5. (continued)

	Speciation	Extinction	Diversification	sd
Hydroporus tessellatus nr AN585	0.136	0.045	0.091	0.011
Hydroporus thracicus AI336	0.137	0.045	0.092	0.011
Hydroporus transgrediens RA217	0.135	0.045	0.091	0.011
Hydroporus transpunctatus IR341	0.121	0.035	0.087	0.010
Hydroporus tristis ER5	0.130	0.041	0.090	0.010
Hydroporus tristis nr IR521	0.130	0.041	0.090	0.010
Hydroporus umbrosus IR152	0.131	0.042	0.090	0.010
Hydroporus vagepictus IR28	0.130	0.041	0.090	0.010
Hydroporus vespertinus IR144	0.121	0.035	0.087	0.010
Hydroporus zimmermanni AH123	0.136	0.045	0.091	0.011
Iberonectes bertrandi IR30	0.061	0.003	0.057	0.007
Iberoporus agnus AI1007	0.060	0.003	0.058	0.007
Iberoporus argaensis AI179	0.060	0.003	0.058	0.007
Iberoporus cermenius IR276	0.060	0.003	0.058	0.007
Iberoporus pluto AN151	0.060	0.003	0.058	0.007
Larsonectes minipi AN861	0.061	0.004	0.057	0.007
Leconectes striatellus RA327	0.060	0.004	0.057	0.006
Lioporeus pilatei AN699	0.061	0.004	0.057	0.007
Lioporeus triangularis AN698	0.061	0.004	0.057	0.007
Megaporus feryi GBK	0.060	0.003	0.057	0.007
Megaporus fischeri GBK	0.060	0.003	0.058	0.007
Megaporus gardneri GBK	0.062	0.004	0.058	0.007
Megaporus hamatus GBK	0.062	0.004	0.058	0.007
Megaporus howittii GBK	0.061	0.004	0.058	0.007
Megaporus natvigi GBK	0.060	0.003	0.057	0.007
Megaporus rufipes IR64	0.060	0.003	0.057	0.007
Megaporus solidus GBK	0.061	0.003	0.058	0.007
Megaporus tristis GBK	0.060	0.003	0.057	0.007
Megaporus wilsoni GBK	0.060	0.003	0.057	0.007
Metaporus meridionalis IR34	0.062	0.005	0.057	0.007
Mystonectes coelamboides PB35	0.061	0.003	0.058	0.007
Mystonectes neomexicanus RA775	0.060	0.003	0.058	0.007
Mystonectes panaminti RA1086	0.061	0.003	0.058	0.007
Nebrioporus abyssinicus AI1227	0.061	0.004	0.058	0.007
Nebrioporus airumlus AI1179	0.061	0.004	0.058	0.007
Nebrioporus amicorum AF140	0.061	0.003	0.058	0.007
Nebrioporus assimilis AI607	0.063	0.006	0.058	0.007
Nebrioporus baeticus IR10	0.062	0.004	0.058	0.007
Nebrioporus bucheti cazorlensis AI926	0.063	0.005	0.058	0.007
Nebrioporus canaliculatus AI1044	0.061	0.004	0.057	0.007
Nebrioporus canariensis AI1091	0.061	0.004	0.058	0.007
Nebrioporus capensis RA850	0.061	0.004	0.057	0.007
Nebrioporus carinatus IR17	0.070	0.009	0.061	0.007
Nebrioporus ceresyi AI57	0.061	0.004	0.058	0.007
Nebrioporus clarkii IR46	0.063	0.005	0.058	0.007
Nebrioporus cooperi AN21	0.061	0.004	0.058	0.007
Nebrioporus croceus AI82	0.070	0.009	0.061	0.007
Nebrioporus depressus AI331	0.068	0.008	0.060	0.007
Nebrioporus dubius AI83	0.061	0.004	0.058	0.007
Nebrioporus elegans AI606	0.068	0.008	0.060	0.007
Nebrioporus fabressei IR169	0.070	0.009	0.061	0.007
Nebrioporus fenestratus AH175	0.062	0.005	0.058	0.007
Nebrioporus kilimandjarensis AN690	0.061	0.004	0.058	0.007
Nebrioporus lanceolatus IR650	0.061	0.003	0.058	0.007
Nebrioporus luctuosus RA724	0.062	0.004	0.058	0.007
Nebrioporus macronychus GBK	0.068	0.008	0.060	0.007

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Table S5. (continued)

	Speciation	Extinction	Diversification	sd
Nebrioporus martinii AI702	0.063	0.005	0.058	0.007
Nebrioporus mascatensis RA107	0.061	0.003	0.058	0.007
Nebrioporus millingeni RA607	0.061	0.004	0.057	0.007
Nebrioporus nemethi AI307	0.062	0.004	0.058	0.007
Nebrioporus nipponicus IR610	0.061	0.004	0.058	0.007
Nebrioporus rotundatus AC41	0.068	0.008	0.060	0.007
Nebrioporus sansii RA725	0.063	0.005	0.058	0.007
Nebrioporus sardus RA17	0.063	0.005	0.058	0.007
Nebrioporus scotti AI1225	0.061	0.004	0.058	0.007
Nebrioporus seriatus RA613	0.061	0.004	0.058	0.007
Nebrioporus stearinus stearinus IR134	0.061	0.003	0.058	0.007
Nebrioporus stearinus suavis AI109	0.061	0.003	0.058	0.007
Nebrioporus vagrans RA588	0.061	0.004	0.057	0.007
Necterosoma aphrodite GBK	0.100	0.019	0.081	0.017
Necterosoma darwini GBK	0.099	0.019	0.081	0.017
Necterosoma dispar GBK	0.100	0.019	0.081	0.017
Necterosoma novaecaledoniae GBK	0.060	0.003	0.057	0.007
Necterosoma penicillatum IR699	0.062	0.005	0.058	0.007
Necterosoma regulare GBK	0.060	0.003	0.057	0.007
Necterosoma schmeltzi GBK	0.100	0.019	0.081	0.017
Necterosoma schoelleri GBK	0.060	0.003	0.057	0.007
Necterosoma susanna GBK	0.060	0.003	0.057	0.007
Necterosoma theonathani GBK	0.060	0.003	0.057	0.007
Necterosoma undecimlineatum GBK	0.100	0.019	0.081	0.017
Nectoboreus dolerosus AN128	0.060	0.003	0.058	0.007
Nectoboreus funereus AI1208	0.060	0.003	0.058	0.007
Nectomimus okulovi RA1218	0.060	0.004	0.057	0.007
Nectoporus abbreviatus AI932	0.061	0.003	0.058	0.007
Nectoporus congruus IR440	0.060	0.003	0.058	0.007
Nectoporus crassulus IR451	0.060	0.003	0.058	0.007
Nectoporus obesus cordillerensis IR452	0.060	0.003	0.058	0.007
Nectoporus obesus obesus IR399	0.060	0.003	0.058	0.007
Nectoporus rhyacophilus IR367	0.061	0.003	0.058	0.007
Nectoporus sanmarkii alienus AI1134	0.060	0.003	0.058	0.007
Nectoporus sanmarkii sanmarkii AI973	0.060	0.003	0.058	0.007
Nectoporus subrotundus RA485	0.060	0.003	0.057	0.007
Neonectes natrix IR611	0.060	0.004	0.057	0.007
Neoporus arizonicus IR331	0.075	0.010	0.066	0.008
Neoporus clypealis AC48	0.072	0.008	0.065	0.008
Neoporus dilatatus AC49	0.075	0.010	0.066	0.008
Neoporus dimidiatus IR486	0.075	0.010	0.066	0.008
Neoporus mellitus GBK9	0.072	0.010	0.063	0.007
Neoporus sp1 IR598	0.072	0.008	0.064	0.008
Neoporus sp2 IR597	0.072	0.008	0.064	0.007
Neoporus sp3 AC47	0.072	0.008	0.065	0.008
Neoporus sp4 IR594	0.072	0.008	0.065	0.008
Neoporus superioris DM28	0.072	0.008	0.065	0.007
Neoporus undulatus IR75	0.072	0.008	0.065	0.008
Oreodytes alpinus AI977	0.073	0.008	0.065	0.008
Oreodytes davisii davisii AI1127	0.073	0.008	0.065	0.008
Oreodytes davisii rhianae ER33	0.073	0.008	0.065	0.008
Oreodytes mongolicus AI972	0.073	0.008	0.065	0.008
Oreodytes scitulus scitulus AV1	0.060	0.003	0.058	0.007
Oreodytes scitulus bisulcatus AN613	0.060	0.003	0.058	0.007
Oreodytes septentrionalis AI974	0.060	0.003	0.058	0.007
Oreodytes shorti AI965	0.060	0.003	0.058	0.007

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Table S5. (continued)

	Speciation	Extinction	Diversification	sd
Oreodytes snoqualmie IR447	0.061	0.003	0.058	0.007
Paroster angustus GBK	0.060	0.003	0.057	0.007
Paroster arachnoides GBK	0.060	0.003	0.057	0.007
Paroster baylyi GBK	0.062	0.004	0.059	0.007
Paroster bulbus GBK	0.060	0.003	0.057	0.007
Paroster byroensis GBK	0.060	0.003	0.057	0.007
Paroster copidotibiae GBK	0.060	0.003	0.057	0.007
Paroster couragei GBK	0.060	0.003	0.058	0.007
Paroster darlotensis GBK	0.060	0.003	0.057	0.007
Paroster dingbatensis GBK	0.060	0.003	0.058	0.007
Paroster eurypleuron GBK	0.060	0.003	0.057	0.007
Paroster extraordinarius GBK	0.060	0.003	0.057	0.007
Paroster fortispina GBK	0.060	0.003	0.057	0.007
Paroster gibbi IR92	0.060	0.003	0.057	0.007
Paroster hamoni GBK	0.060	0.003	0.057	0.007
Paroster hinzeae GBK	0.060	0.003	0.057	0.007
Paroster innoendyensis GBK	0.060	0.003	0.057	0.007
Paroster insculptilis GBK	0.060	0.003	0.057	0.007
Paroster killaraensis GBK	0.060	0.003	0.057	0.007
Paroster macrocephalus GBK	0.060	0.003	0.057	0.007
Paroster macrosturtensis MG912995	0.060	0.003	0.057	0.007
Paroster megamacrocephalus GBK	0.060	0.003	0.057	0.007
Paroster melroseensis GBK	0.060	0.003	0.057	0.007
Paroster mesosturtensis GBK	0.060	0.003	0.057	0.007
Paroster michaelseni GBK	0.060	0.003	0.058	0.007
Paroster microsturtensis GBK	0.060	0.003	0.057	0.007
Paroster milgunensis GBK	0.060	0.003	0.057	0.007
Paroster napperbyensis GBK	0.060	0.003	0.057	0.007
Paroster newhavenensis GBK	0.060	0.003	0.057	0.007
Paroster niger GBK	0.060	0.003	0.057	0.007
Paroster nigroadumbratus GBK	0.060	0.003	0.058	0.007
Paroster pallescens GBK	0.062	0.004	0.059	0.007
Paroster peelensis GBK	0.060	0.003	0.057	0.007
Paroster pentameres GBK	0.060	0.003	0.057	0.007
Paroster plutonicensis GBK	0.060	0.003	0.057	0.007
Paroster skaphites GBK	0.060	0.003	0.057	0.007
Paroster stegastos GBK	0.060	0.003	0.057	0.007
Paroster tetrameres GBK	0.060	0.003	0.057	0.007
Paroster ursulae GBK	0.062	0.004	0.059	0.007
Paroster verrucosus GBK	0.060	0.003	0.057	0.007
Paroster wedgeensis GBK	0.060	0.003	0.057	0.007
Porhydrus genei RA86	0.060	0.003	0.058	0.007
Porhydrus lineatus IR24	0.060	0.003	0.057	0.007
Porhydrus obliquesignatus RA147	0.060	0.003	0.058	0.007
Porhydrus vicinus AH113	0.060	0.003	0.058	0.007
Psychopomporus felipi GBK14	0.061	0.008	0.053	0.006
Rhithrodytes bimaculatus RA727	0.060	0.003	0.058	0.007
Rhithrodytes crux AI302	0.060	0.003	0.058	0.007
Rhithrodytes numidicus DM34	0.060	0.003	0.058	0.007
Rhithrodytes sexguttatus IR183	0.060	0.003	0.058	0.007
Sanfilippodytes adelardi RA487	0.100	0.023	0.077	0.009
Sanfilippodytes bidessoides RA488	0.100	0.023	0.077	0.009
Sanfilippodytes compertus GBK	0.103	0.024	0.079	0.009
Sanfilippodytes edwardsi DM42	0.103	0.024	0.079	0.009
Sanfilippodytes edwardsi nr AV20	0.103	0.024	0.079	0.009
Sanfilippodytes hardyi AI1279	0.101	0.023	0.078	0.009

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Table S5. (continued)

	Speciation	Extinction	Diversification	sd
Sanfilippodytes malkini IR372	0.101	0.023	0.078	0.009
Sanfilippodytes setifer RA489	0.100	0.023	0.078	0.009
Sanfilippodytes sp1 IR375	0.100	0.023	0.078	0.009
Sanfilippodytes sp2 DM40	0.101	0.023	0.078	0.009
Sanfilippodytes sp3 IR441	0.103	0.024	0.079	0.009
Sanfilippodytes terminalis IR412	0.100	0.023	0.077	0.009
Sanfilippodytes vilis DM26	0.103	0.024	0.079	0.009
Scarodytes antoni AC6	0.064	0.006	0.059	0.007
Scarodytes fuscitarsis RA4	0.062	0.005	0.058	0.007
Scarodytes halensis nr1 AH121	0.063	0.005	0.058	0.007
Scarodytes halensis nr2 ER35	0.063	0.005	0.059	0.007
Scarodytes sp3 AH167	0.063	0.005	0.058	0.007
Scarodytes halensis AI773	0.063	0.005	0.059	0.007
Scarodytes sp4 AC15	0.062	0.004	0.058	0.007
Scarodytes sp5 AI893	0.062	0.004	0.058	0.007
Scarodytes malickyi AF139	0.063	0.005	0.058	0.007
Scarodytes nigriventris IR157	0.062	0.005	0.058	0.007
Scarodytes pederzanii AH168	0.062	0.004	0.058	0.007
Scarodytes roberti RA204	0.065	0.006	0.059	0.007
Scarodytes ruffoi AF62	0.062	0.004	0.058	0.007
Scarodytes savinensis cercyrae RA219	0.065	0.006	0.060	0.007
Scarodytes savinensis savinensis AI135	0.065	0.006	0.060	0.007
Scarodytes sp1 AI304	0.063	0.005	0.059	0.007
Scarodytes sp2 IR653	0.063	0.005	0.058	0.007
Sekaliporus kriegi GBK	0.060	0.003	0.057	0.007
Siettitia avenionensis AI897	0.060	0.003	0.058	0.007
Sternopriscus alligatorensis GBK	0.061	0.004	0.058	0.007
Sternopriscus alpinus GBK	0.348	0.104	0.238	0.056
Sternopriscus aquilonaris GBK	0.061	0.004	0.058	0.007
Sternopriscus balkei GBK	0.061	0.004	0.058	0.007
Sternopriscus barbarae GBK	0.348	0.104	0.238	0.056
Sternopriscus browni GBK	0.060	0.003	0.057	0.007
Sternopriscus clavatus IR698	0.061	0.003	0.058	0.007
Sternopriscus eikei GBK	0.060	0.003	0.057	0.007
Sternopriscus emmae GBK	0.061	0.003	0.058	0.007
Sternopriscus goldbergi GBK	0.061	0.004	0.058	0.007
Sternopriscus hansardii IR698	0.061	0.003	0.058	0.007
Sternopriscus marginatus GBK	0.060	0.003	0.057	0.007
Sternopriscus meadfootii GBK	0.348	0.104	0.238	0.056
Sternopriscus minimus GBK	0.063	0.005	0.058	0.007
Sternopriscus montanus GBK	0.348	0.104	0.238	0.056
Sternopriscus multimaculatus GBK	0.061	0.004	0.057	0.007
Sternopriscus mundanus GBK	0.348	0.104	0.238	0.056
Sternopriscus storeyi GBK	0.060	0.003	0.057	0.007
Sternopriscus tarsalis GBK	0.348	0.104	0.238	0.056
Sternopriscus tasmanicus GBK	0.348	0.104	0.238	0.056
Sternopriscus wallumphilia GBK	0.061	0.003	0.058	0.007
Sternopriscus wattsi GBK	0.060	0.003	0.057	0.007
Sternopriscus weckwerthi GBK	0.348	0.104	0.238	0.056
Sternopriscus wehnckei GBK	0.305	0.093	0.205	0.050
Sternopriscus weiri GBK	0.061	0.003	0.058	0.007
Sternopriscus williamsi GBK	0.348	0.104	0.238	0.056
Stictonectes abellani PA312	0.060	0.003	0.058	0.007
Stictonectes azruensis IR661	0.062	0.004	0.059	0.007
Stictonectes canariensis AF114	0.061	0.003	0.058	0.007
Stictonectes epipleuricus AH73	0.063	0.004	0.059	0.007

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Table S5. (continued)

	Speciation	Extinction	Diversification	sd
Stictonectes escheri AH107	0.060	0.003	0.058	0.007
Stictonectes formosus AH108	0.060	0.003	0.058	0.007
Stictonectes lepidus AI632	0.060	0.003	0.058	0.007
Stictonectes occidentalis IR529	0.063	0.004	0.059	0.007
Stictonectes sp MsCC	0.062	0.004	0.059	0.007
Stictonectes optatus AI1089	0.063	0.004	0.059	0.007
Stictonectes rebeccae AH72	0.063	0.004	0.059	0.007
Stictonectes rufulus AI1299	0.060	0.003	0.058	0.007
Stictonectes samai AF142	0.060	0.003	0.058	0.007
Stictotarsus duodecimpustulatus IR42	0.060	0.003	0.058	0.007
Stictotarsus maghrebinus IR236	0.060	0.003	0.058	0.007
Stictotarsus procerus AI1116	0.060	0.003	0.058	0.007
Stygoporus oregonensis GBK	0.060	0.004	0.057	0.006
Tiporus alastairi GBK	0.060	0.003	0.057	0.007
Tiporus centralis GBK	0.060	0.003	0.057	0.007
Tiporus collaris GBK	0.060	0.003	0.057	0.007
Tiporus emmae GBK	0.060	0.003	0.057	0.007
Tiporus giuliani GBK	0.060	0.003	0.057	0.007
Tiporus josepheni IR550	0.060	0.003	0.057	0.007
Tiporus lachlani GBK	0.060	0.003	0.057	0.007
Tiporus tambreyi GBK	0.060	0.003	0.057	0.007
Tiporus undecimmaculatus GBK	0.060	0.003	0.057	0.007
Trichonectes otini IR29	0.061	0.005	0.055	0.006
Zaitzevhydrus formaster formaster AI1102	0.060	0.003	0.057	0.007
Zaitzevhydrus formaster ulanulana RA1170	0.060	0.003	0.057	0.007

Figure S1. Phylogeny obtained with RAxML. Numbers in nodes, bootstrap support values.

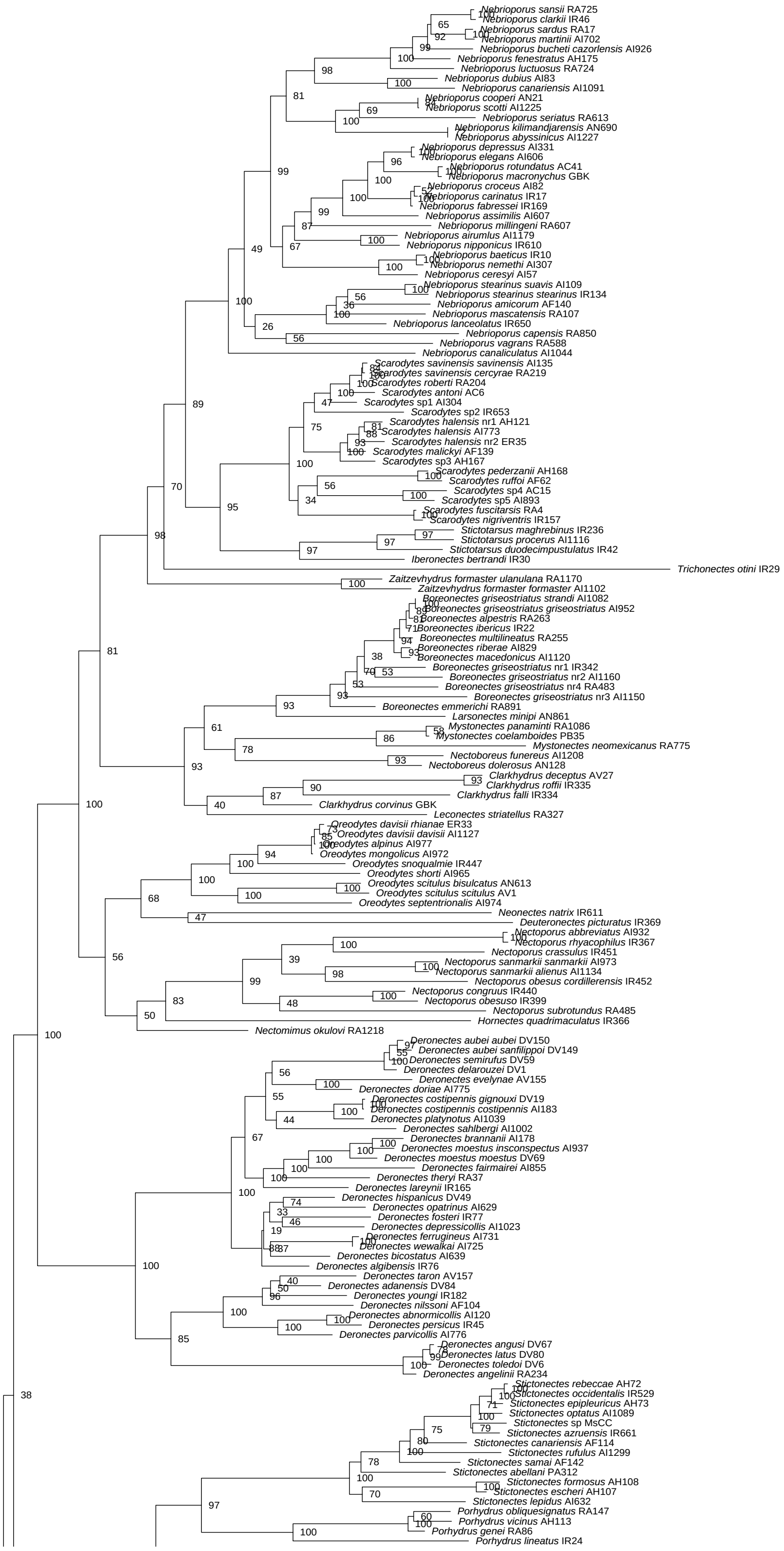


Figure S1. (continued)

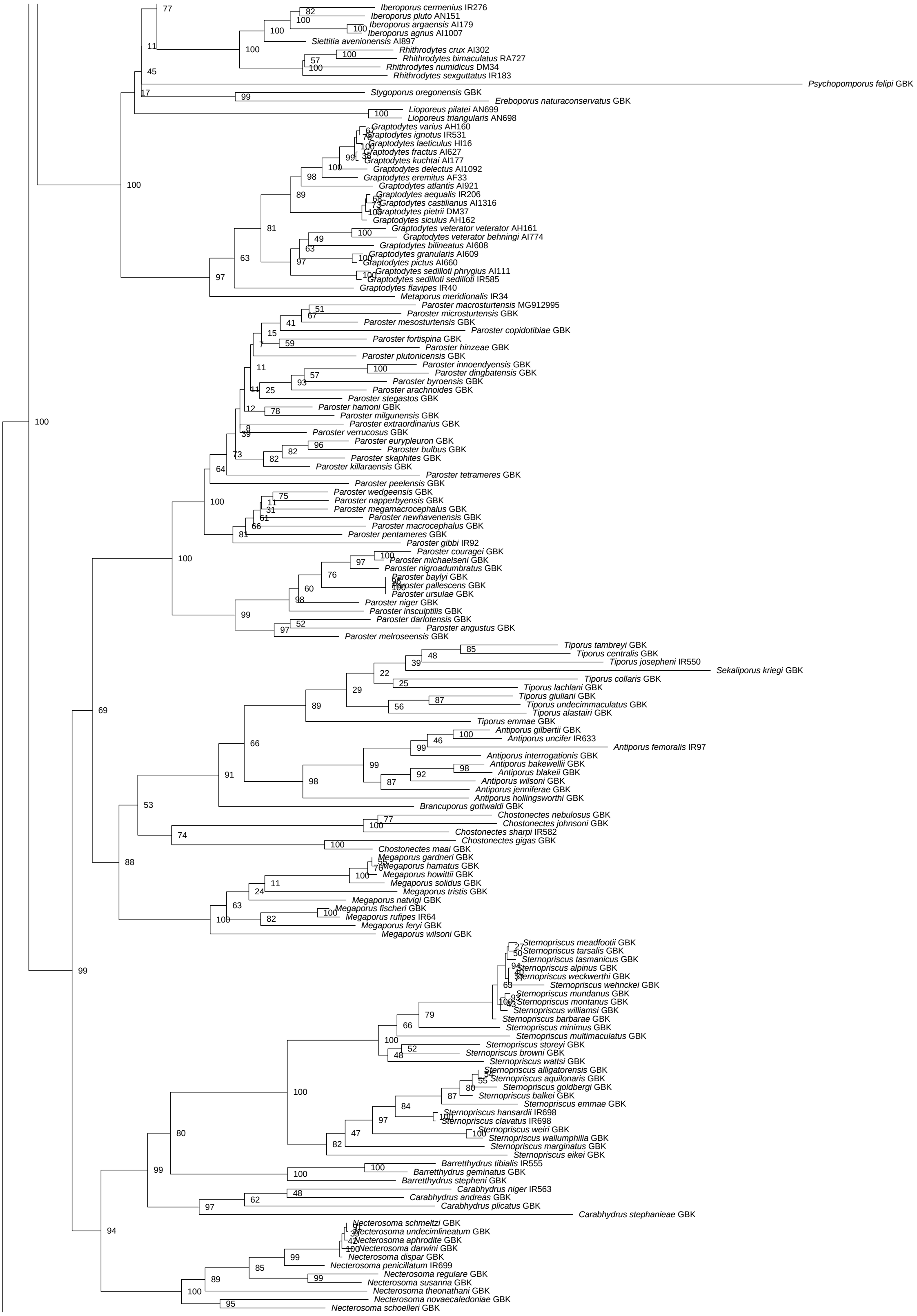


Figure S1. (continued)

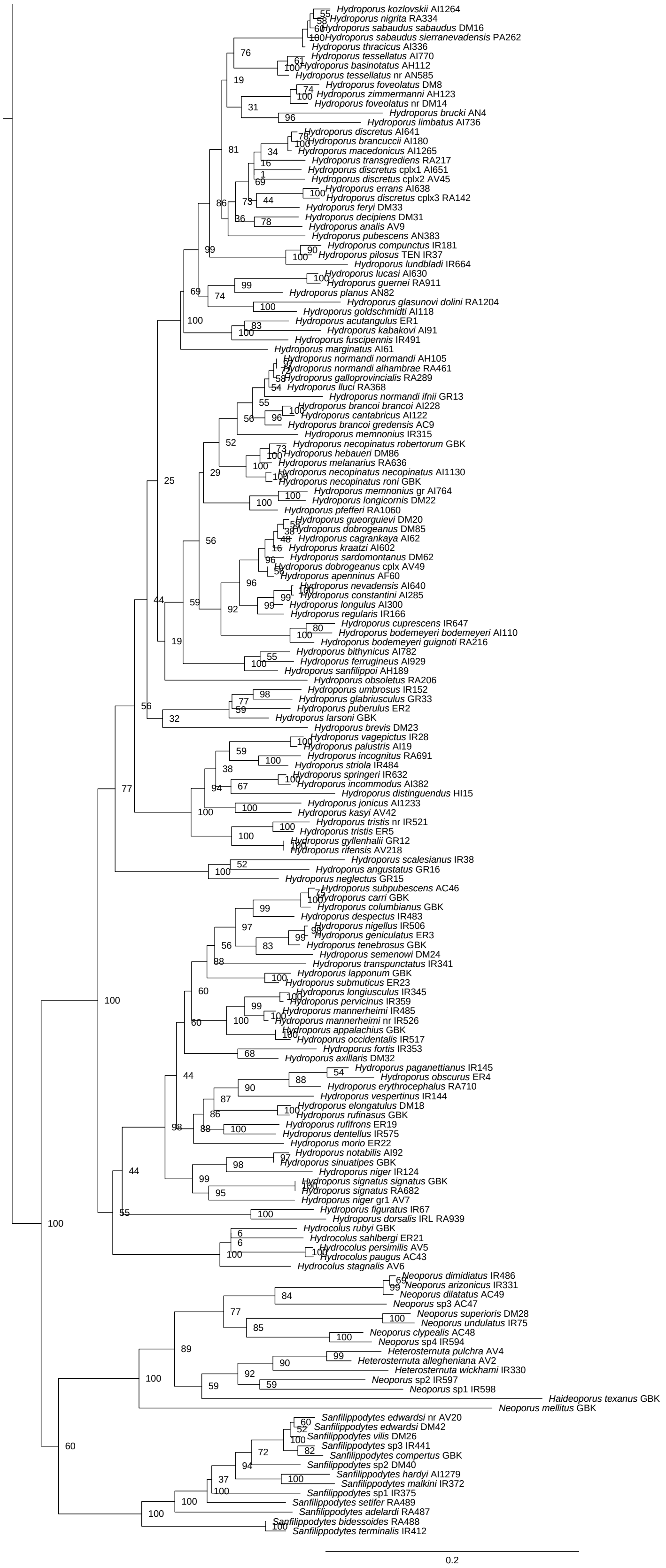


Figure S2. Collapsed phylogeny for clades with homogeneous habitat.

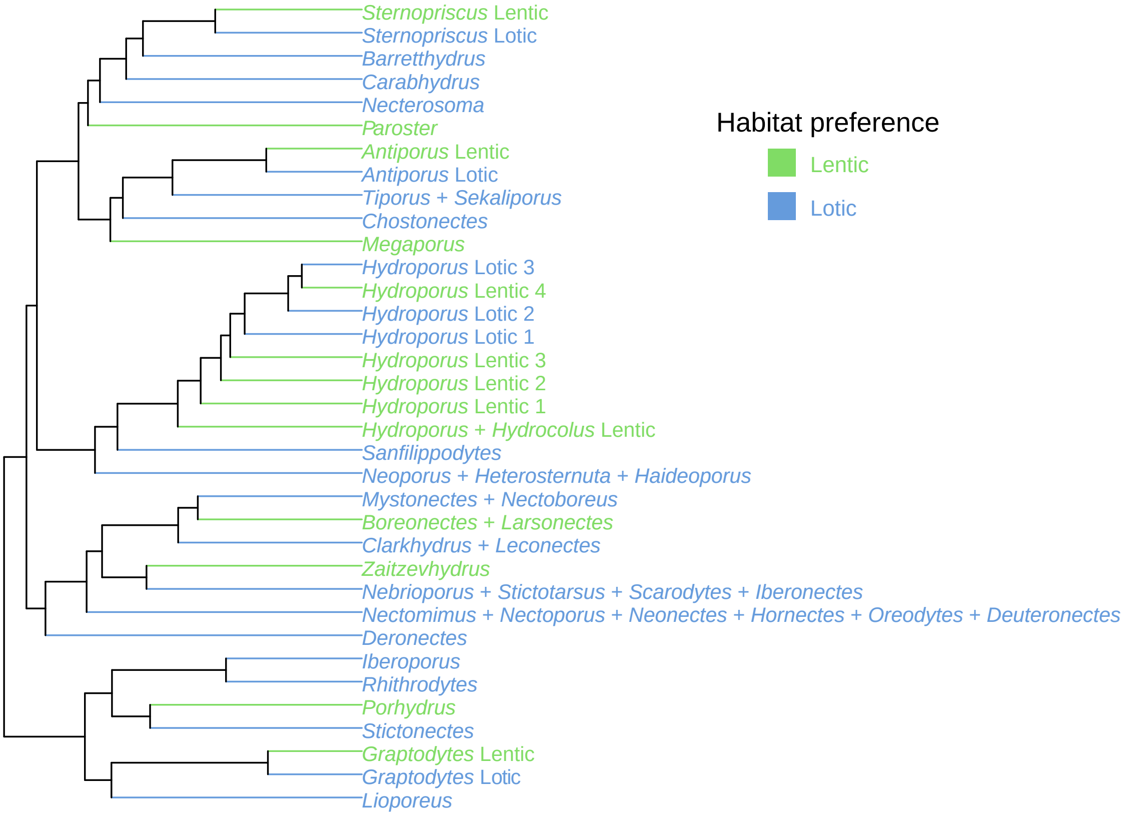


Figure S3. Consensus topology obtained in Beast, including genera and tribes names. Numbers in nodes, posterior probability.

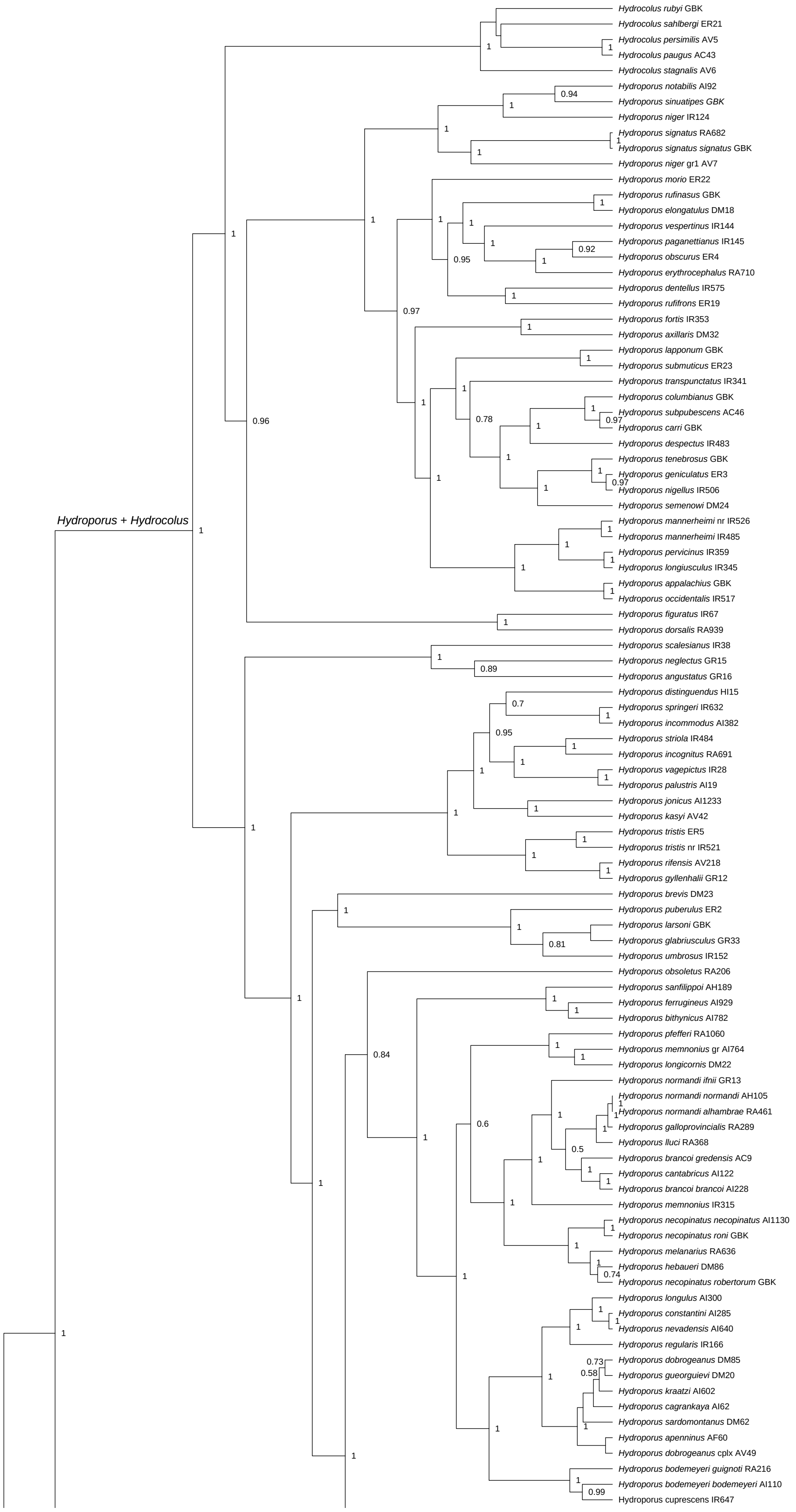


Figure S3. (Continued)

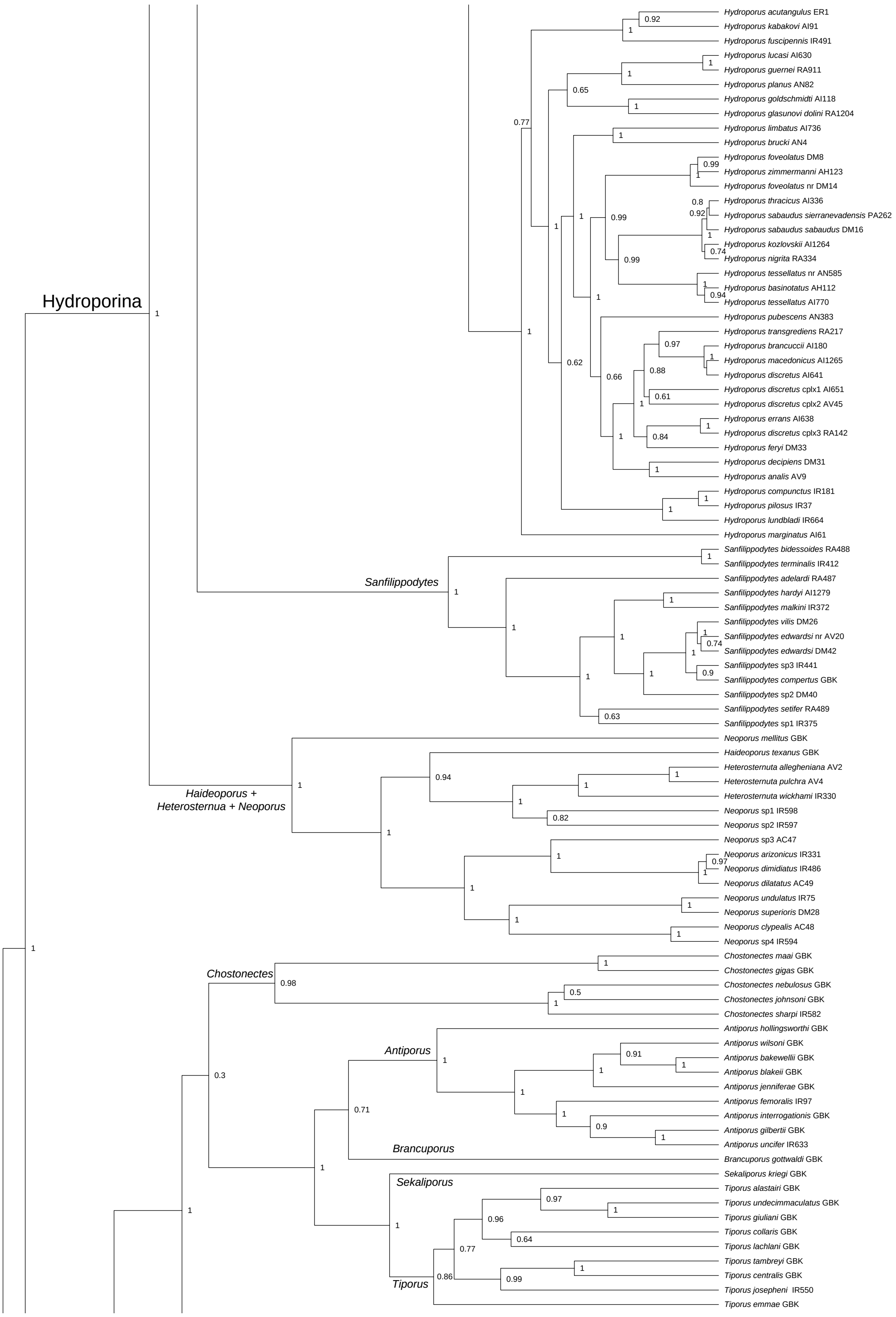


Figure S3. (Continued)

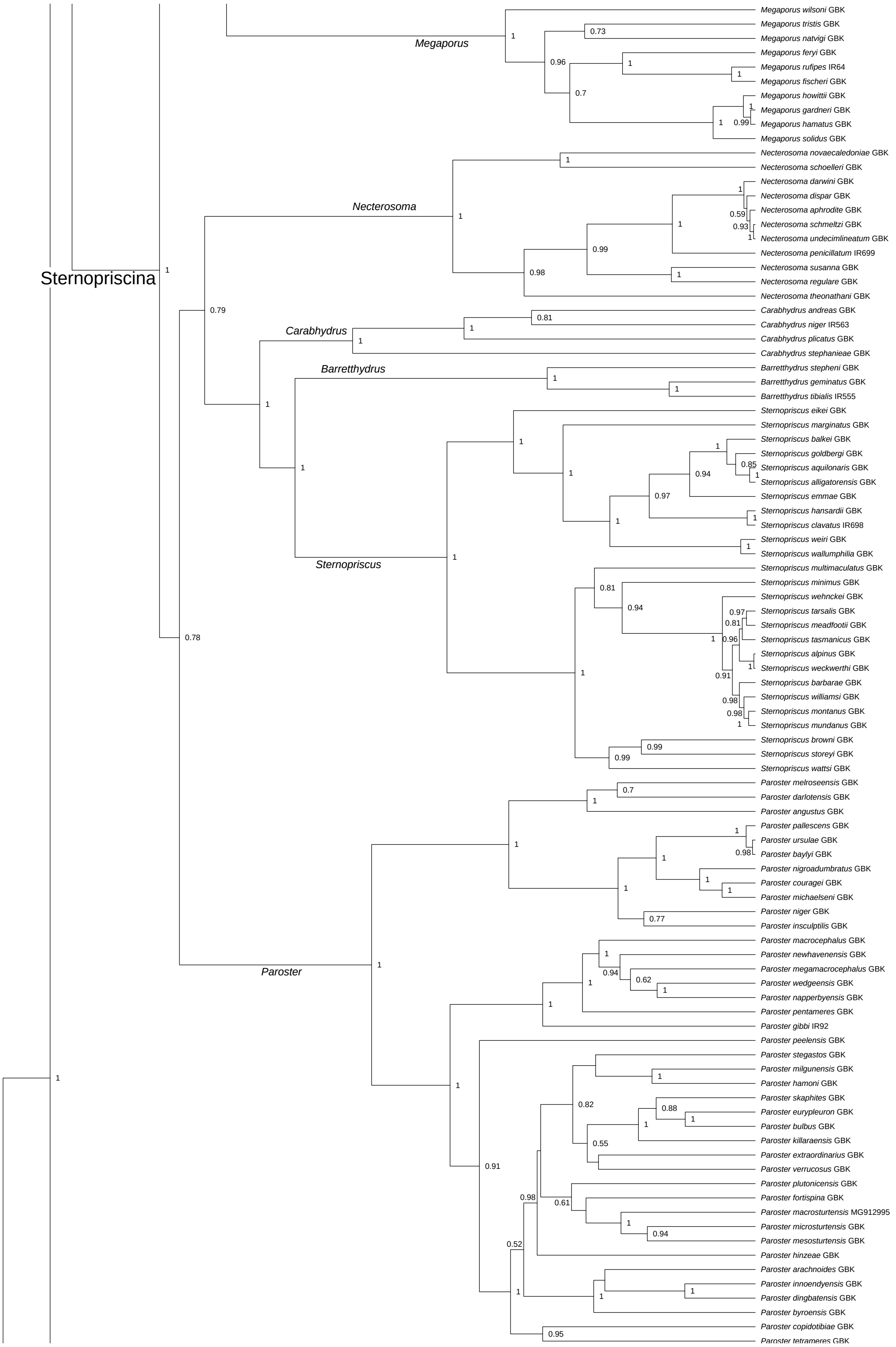


Figure S3. (Continued)

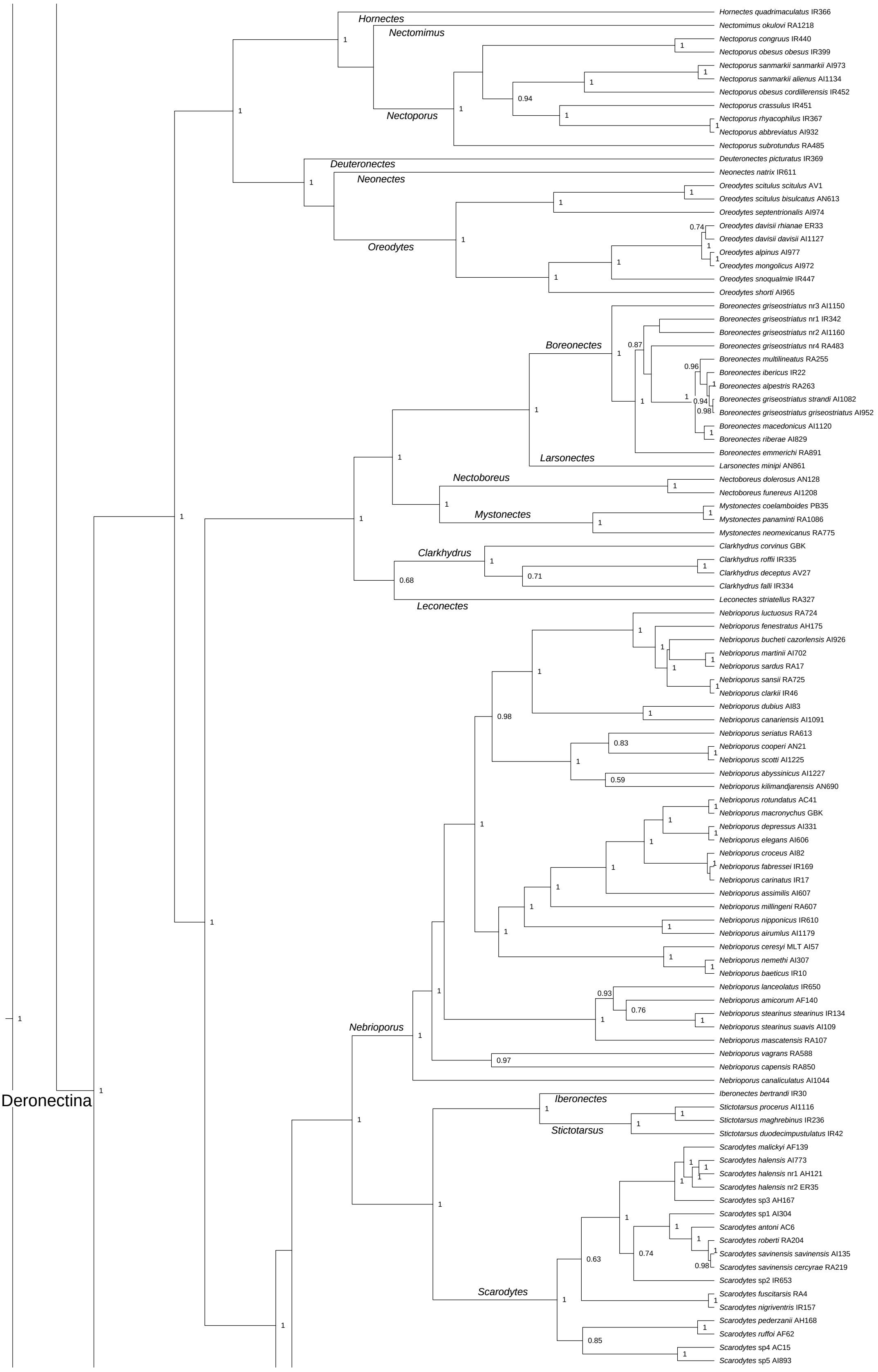
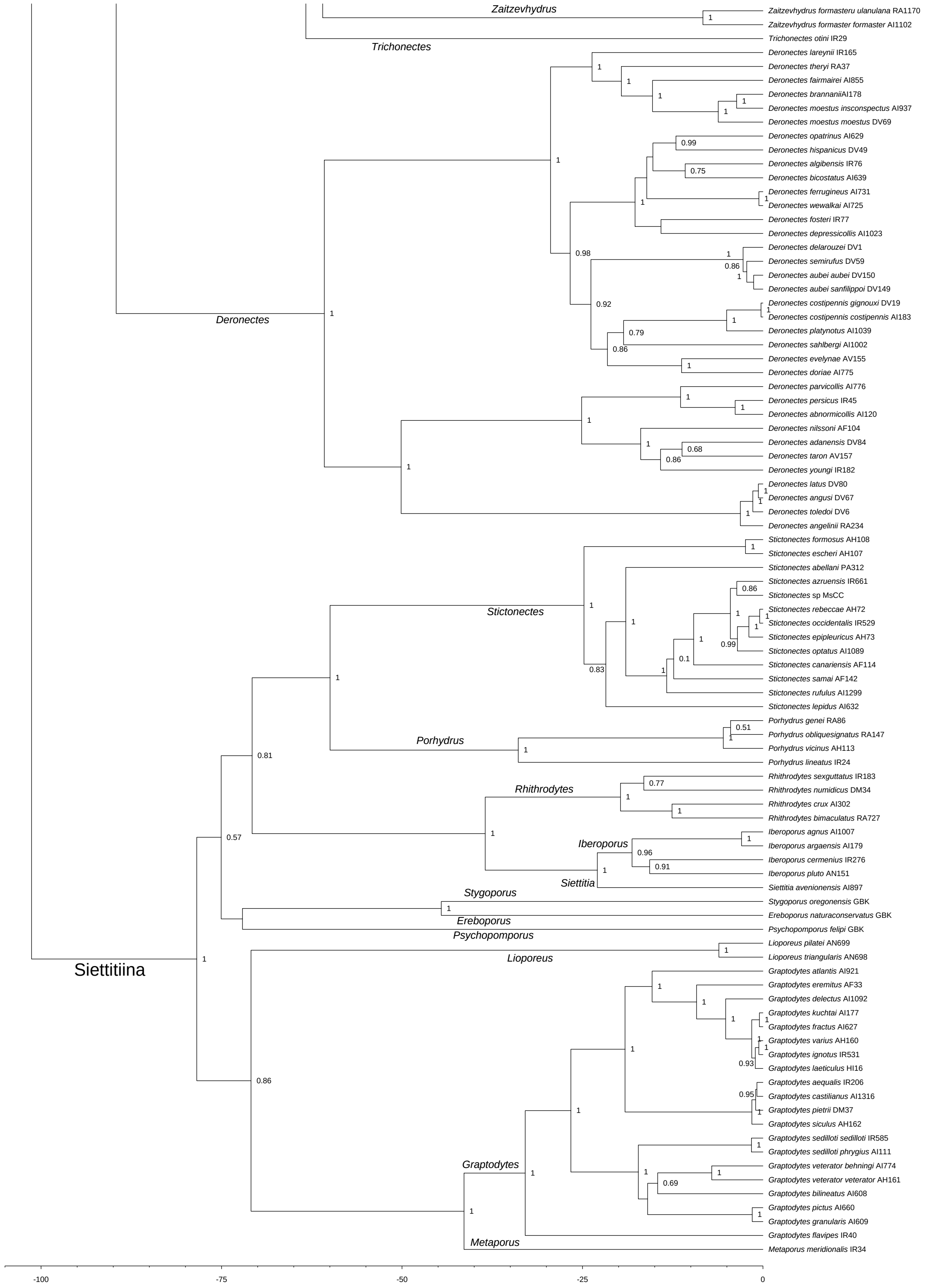


Figure S3. (Continued)



General Discussion

General discussion

Through the six chapters of this thesis we examined how habitats guided the history of some lineages of aquatic beetles, and how saline environments are more diverse than expected. Most of our results matched our prior hypotheses, but others shed light on novel findings that will be the basis of future research.

Molecular phylogenies to understand relationships between taxa

As noted in the general introduction, molecular phylogenies are useful tools to disentangling evolutionary histories and to provide an appropriate understanding of the relationships between taxa. The main example of the advantageous of these tools can be found on Chapter 1, where the choice of unreliable complex morphological characters has led to conflicts between the extant systematics of Hygrotini tribe and its true history. The new classification of Hygrotini agrees with some simple morphological aspects, as the general body shape, and recognised relationships previously ignored, as the highly modified *Hygrotus descarpentriesi* being considered as a member of the genus *Hygrotus* and not a monotypic genus (*Heroceras*), as in previous classifications. Other disagreements can be found in Chapter 6, where previously related genera were found to not being monophyletic, such as *Hydroporus* (paraphyletic with *Hydrocolus*) and *Neoporus* (paraphyletic with *Heterosternuta* and *Haideoporus*). Several examples can be found of disagreements between morphological and molecular classifications in the literature, not only within insects (Mugleston *et al*., 2013) but also in other lineages, like birds (Sweet *et al*., 2017).

But molecular phylogenies are also useful in combination with morphological analyses, as demonstrated in Chapter 3. Prior beliefs suggested more species groups within Ochthebiini based on morphological similarities, and the molecular analysis reinforces those ideas, leading to the establishment of new species groups. However, discordance between molecular and morphological information can also be found, for example, within *Micragasma* subgenus (Chapter 4 and 5). While two previously described species seem to form a monophyletic clade, and despite the morphological resemblance of *O*. (*M*.) *minoicus* with *O*. (*M*.) *paradoxum*, our results question the monophyly of the subgenus. One likely explanation of this issue is the morphological convergence due to similar habitats (Losos *et al*., 1998; Harmon *et al*., 2005), a common phenomenon affecting species under selection pressures - like the one for species associated with coastal environments affected by saline water or other still unknown ecological factors.

A novel approach addressed by this thesis was the combination of sequences obtained by classical Sanger sequencing with others obtained by Metagenomic tools. Metagenomic tools make use of Next Generation Sequencing technologies to produce high number of short sequences that can be easily assembled with bioinformatic tools at a reduced cost (Goodwin *et al*., 2016). A direct comparison of classical analysis and this new approach can be found in the Ochthebiini/*Ochthebius* phylogenies (Chapters 3 and 5 respectively), where the addition of 42 partial or complete mitogenomes increased the support of most of the basal nodes like the position of the clade formed by *Enicocerus* plus *Hughleechia* or *Aulacochthebius* as sister to *Asiobates*. This approach was also used for Chapter 6, leading again to a fully supported backbone of the tree.

Evolution of saline environments and their long-term consequences

Following with environments linked to saline waters, one of the main goals of this thesis was to increase the knowledge about these environments. Until last decades, ecosystems affected by saline waters did not receive the deserved attention, but this trend is gradually changing. More research related with specimens living in saline environments is being published, like the description of new species, e.g. *Ochthebius lanthanus* (Ribera and Foster, 2018), the physiological aspects that make possible to tolerate saline water (Pallarés *et al*., 2016; 2017b), the fundamental capabilities of saline water species (Arribas *et al*., 2018) and how diversification takes places in saline environments (Mitterboeck *et al*., 2016).

The research accomplished by this thesis for Dytiscidae and Hydraenidae families (Hygrotini and Ochthebiini, Chapters 2 and 5 respectively) complements the findings about the origin and evolution of tolerance to saline waters in family Hydrophilidae (Arribas *et al*., 2014; Pallarés *et al*., 2017a), and fulfils comprehensive understanding of salinity tolerance in the three most speciose families. In all cases, tolerance to saline waters had been acquired multiple times, but with slightly different evolutionary trends: Dytiscidae evolved the traits gradually, whilst Hydraenidae and specially Hydrophilidae (Arribas *et al*., 2014) present some direct transitions from freshwater to hypersaline waters. However, a more recent study on Hydrophilidae (Pallarés *et al*., 2017a) reconstructed tolerance to saline waters using a continuous variable under a Brownian Motion evolutionary model, finding a more gradual evolution of this trait in *Enochrus bicolor* group. Furthermore, direct transitions in Ochthebiini are always found in fairly isolated clades associated with coastal rockpools (Chapter 5).

Extreme specialization to saline waters (i.e. tolerance to hypersaline waters) was found to be irreversible in all cases. Results in chapter 5 shown that tolerance to slightly saline waters may be a synapomorphic trait in *Ochthebius*, which is in agreement with the most recent comprehensive review by Arribas *et al*. (2018). Arribas *et al*. discovered that tolerance to salinity does not act as a trade-off as was though by others authors (e.g. Herbst, 2001; Latta *et al*., 2012), so species inhabiting saline waters are generalist in their fundamental niches with similar fitness than freshwater species in freshwater environments, even if they only inhabit saline waters. These results may open a scenario where tolerance to some degree of salinity remained in most of the taxa within our study groups, so freshwater species may be able to persist in brackish waters (i.e. fundamental niches being larger than their realized niches, see Soberón and Arroyo-Peña, 2017 for a review on this hypothesis) in a similar mode than species currently living there.

Even if tolerance to hypersaline waters was irreversible - as expected for an evolutionary dead-end (Kelley and Farrel, 1998), we did not find evidence of differential diversification associated with freshwater, mesosaline or hypersaline species. Similar diversification patterns can be found for other expected dead-ends like cave adaptations, both in aquatic environments (Stern *et al*., 2017) or in terrestrial ones (Cieslak *et al*., 2014). These results confirm that extreme ecological adaptations may operate as diversification boost, such as the one found within *Cobalius* subgenus. This subgenus is well-adapted to hypersaline waters, and a core diversification shift (accelerating its diversification rate) was detected in Chapter 5 for species living in the eastern Mediterranean islands during the last five million years. We proposed that the increased habitat availability after the emergence of those islands may represent an ecological opportunity to diversification, even for this extremely adapted species (Stroud and Losos, 2016).

Moreover, all studied groups displayed a likely connection between periods of global aridification and the origin of tolerance to saline/hypersaline waters. The aridification events may accelerate diversification of lineages (Pepper *et al*., 2011; Deepak and Karanth, 2018; Gutiérrez-Ortega *et al*., 2018), but most of the lineages exhibit a pattern of gradual evolution through time with limited direct transitions to hypersaline waters.

Evolutionary patterns of habitat transitions between standing and running waters

Our prior hypotheses about diversification patterns across standing and running waters were eminently influenced by the habitat specialization hypothesis reviewed by Ribera (2008). In this work, several inferences were made about the expected evolutionary outcomes, but the ones linked to differential diversification between habitats were not well defined. This thesis tried to fill the gaps in that hypothesis, finding whether diversification rates are determined by species habitats or habitat transitions. Some of the consequences of habitat stability on diversification were studied by Letsch *et al*. (2016) and Désamoré *et al*. (2018) with Odonata and Dytiscidae respectively, but their conclusions are limited because of their very incomplete sampling (as detailed in Chapter 6). We found similar diversificarion patterns among habitats, with overlapping posterior distributions. These posterior distributions were found to be slightly higher for species living in standing waters, in agreement with the findginds in Letsch *et al*. (2016). We also recovered similar results in the state speciation and extinction analyses (SSE). Additionally, diversification core shifts were detected in clades living in lentic environments (*Sternopriscus* and *Boreonectes*) or in heterogeneous clades were lotic species were outnumbered by the other habitat preference categories (*Hydroporus* plus *Hydrocolus*). Based on the habitat specialization hypothesis, speciation and extinction rates should be higher for lotic species, but there was not prediction about net diversification rates. Based on these result, we did not find clear evidence of differential net diversification rate, but slight differencias may indicate a positive role of lentic environments in the diversification in aquatic Coleoptera (although not conclusive). The absence of reliable methodologies to decouple speciation and extinction rates did not allow us to test the hypothesized higher speciation and extinction rates of lotic species.

We also analysed the relationship between habitat preference and body size evolution, with smaller species in more specialized habitat (lotic) and larger species in the less specialized habitat (those of category ‘Both’). This gradient matched the habitat constraint on size predicted by Ribera and Nilsson (1995), and confirmed also by Pallarés *et al*. (2018) for *Hydroporus* and *Graptodytes* as well when they tested the Bergmann’s rule in water beetles. The novelty of our approach allowed us to identify that body size evolution was directional to optimum values (i.e. an Ornstein-Uhlenbeck model), and that it was mostly associated to the evolution of lineages in a particular habitat and not in the transitions to a different one.

The reconstruction of ancestral habitat preference estimated the most common recent ancestor of Hydroporini as lotic, but the absence of outgroups may compromise this finding. In any case, transitions from lotic environments had a similar probability than those from lentic environments, demonstrating that lotic environments, a more specialized habitat according to Ribera (2008), are not displaying the irreversibility expected if they were acting as an evolutionary dead-end (Kelley and Farrel, 1998).

General highlights of diversification patterns for water beetles

In addition to the results about the diversification estimates discussed previously, more general patterns can be extracted from this thesis. A recent review (Bilton *et al*., 2019) considered the research of diversification patterns a future issue to be address in water beetles due to the limiting sampling available to reconstruct comprehensive molecular phylogenies. Bloom *et al*. (2014) and Désamoré *et al*. (2018) tried to identify diversification patterns on Hydrophilidae and Dytiscidae respectively, but could not provide general conclusions due to their incomplete sampling (less than 4 % of the species were included in the phylogenies). As we showed during the introduction, the use of limited sampling during phylogenetic reconstruction affect the number of speciation events and the topology of phylogenies (Barraclough and Nee, 2001).

The use of comprehensive phylogenies in this thesis provide novel knowledge that shed light about how diversification rates have been evolving, and which methodologies are useful for that studies. One of the most criticized methodologies for estimate diversification rates is Bayesian Analysis of Macroevolutionary Mixtures (BAMM, Rabosky, 2014; see Chapter 6 for more information), but our results suggest similar rates estimates than another methodology widely used (Methods of Moments - Magallón and Sanderson, 2001). Most of the differences obtained between analyses were due to incompletely sampled clades or to isolated clades with small number of species, just as predicted by Rabosky (2019) in his defence of BAMM.

Conclusions

Conclusions

Chapter 1

- The tribe Hygrotini is monophyletic based on molecular data. The former five genera classification (sensu Miller & Bergsten, 2016) was not supported, revealing para- or polyphyly in all genera except for *Hyphoporus* Sharp.

- The definition of Hygrotini did not truly rely on unambiguous synapomorphic characters. A new character was presented with the intention to serve that role: the second carina at the base of the scape where it is articulated.

- Hygrotini is currently divided into two monophyletic genera: *Clemnius* Villastrigo et al. and *Hygrotus* Stephens, the former newly erected.

- *Clemnius* is a morphological heterogeneous genus distributed mainly in the Nearctic regions except for *Clemnius decoratus* Gyllenhal. *Clemnius* is divided into two subgenera: *Cyclopius* Villastrigo et al. and *Clemnius* s.str. *Cyclopius* is a well delimited subgenus whereas *Clemnius* s.str. is in turn divided into two groups based on morphological resemblance but not supported by molecular data.

- *Hygrotus* is widely distributed and comprise 4 subgenera: *Coelambus* Thomson, *Hygrotus* s.str., *Hyphoporus* and *Leptolambus* Villastrigo et al. *Hygrotus* s.str. is assembled by a combination of species from the former genera *Herophydrus* Guignot, *Heroceras* Guignot and *Hygrotus*.

Chapter 2

- The origin of Hygrotini is dated in the Paleocene, with primary splits that defined current taxonomic ranks during the first half of the Eocene.

- Multiple origins of tolerance to saline waters appeared in independent lineages in Hygrotini, whilst species able to tolerate hypersaline waters are concentrated in two clades, one in the Palaearctic and another in the Nearctic regions.

- The mode of evolution of tolerance to saline waters in Hygrotini was gradual, without direct transitions from freshwater to hypersaline habitats. There were reversal transitions from slightly saline waters to freshwater habitats, but hypersaline specialization is not reversible.

- There is not a clear link between aridification and tolerance to saline waters in

Hygrotini. However, the oldest transitions to saline waters that lead to the highest number of hypersaline species took place at the same period as a global decrease in temperature and precipitations, which can imply a connection between aridification and tolerance to saline waters.

Chapter 3

- The tribe Ochthebiini is monophyletic. It includes six genera: *Meropathus* Enderlein, *Ochthebius* Leach, *Protochthebius* Perkins, *Prototympanogaster* Perkins, *Tympallopatrum* Perkins and *Tympanogaster* Perkins. The genus *Ochthebius* comprise nine subgenera in addition to the nominal subgenus: *Angiochthebius* Jäch & Ribera, *Asiobates* Thomson, *Aulacochthebius* Kuwert, *Cobalius* Rey, *Enicocerus* Stephens, *Gymnanthelius* Perkins stat.n., *Gymnochthebius* Orchymont, *Hughleechia* Perkins stat.n., and *Micragasma* Sahlberg.

- New relationships between subgenera were discovered: *Aulacochthebius* as closely related to *Asiobates*, *Gymnochthebius* as closely related to *Gymnanthelius*, and a possible relationship between *Hughleechia* and *Enicocerus*. Surprisingly, *Micragasma* was not a basal lineage within *Ochthebius*, and the two rockpool lineages (*quadricollis* group and *Cobalius* subgenera) living mainly in the Mediterranean basin plus the Macaronesia were not related between them.

- We identified 17 species groups within *Ochthebius* s.str., four of them newly described (*corrugatus*, *nitidipennis*, *peisonis* and *sumatrensis* groups) and two of them (*atriceps* and *rivalis* groups) were created from the division of other species groups (*foveolatus* and *metallescens* groups respectively).

- Two subspecies of *Ochthebius* were elevated to species rank: *O*. *fallaciosus* Ganglbauer stat.n. and *O*. *deletus* Rey stat.rest.

Chapter 4

- *Ochthebius* (*Micragasma*) *minoicus* from Crete is described as a new species.

- *Micragasma* is a poorly known subgenus with only 3 species recorded in 7 localities.

- Although the habitat of *Micragasma* species is unknown, mostly all localities are placed in the coast. In the case of *O*. (*M*.) *minoicus*, it was found to be linked to coastal rockpools.

Chapter 5

- The most frequent transitions among habitat salinity in Ochthebiini are found from mesosaline species. Despite the reconstructed freshwater ancestor of Ochthebiini, the high frequency of transition may support an early origin of tolerance to saline waters.

- Tolerance to hypersaline waters is an irreversible trait in Ochthebiini and it was originated multiple independent times. Hypersalinity tolerance appeared during the Paleocene-Eocene thermal maximum (PETM), a period associated with increased temperatures and decreased precipitation likely linked with a reduction of freshwater habitat availability.

- Two core shifts were detected on diversification rates linked with *Asiobates* and the species of *Cobalius* inhabiting eastern Mediterranean islands that arose 20 million years ago. This increased habitat availability likely promote diversification in *Cobalius*.

- Hypersalinity tolerance was not correlated with a decrease in diversification rates, implying that extreme habitat specialization is not an evolutionary dead-end. Despite the likely influence of tolerance to saline waters on diversification rates, there is no evidence of it being the main factor promoting diversification.

Chapter 6

- Hydroporini exhibit heterogeneous diversification rates across lineages, with two to three core diversification shifts. Diversification shifts were detected within *Boreonectes*, *Sternopriscus* and the clade formed by *Hydrocolus* plus *Hydroporus*, all of them with a majority the species linked to lentic environments.

- We found similar diversification pattern between lotic and lentic habitats. The overlapping posterior distributions of both habitats showed a slightly higher rate for species living in lentic habitats. In any case, lotic environments did not follow an evolutionary dead-end pattern, as they showed similar transitions rates than lentic species.

- Body size evolution showed strong phylogenetic signal following an Ornstein-Uhlenbeck evolutionary model. Species living in lotic environments have the smallest body sizes, followed by lentic species and species in category ‘both’. This trend implies a negative correlation between body size and habitat specialization.

- Evolution of body size take place during the evolution of lineages within a habitat and it was not linked to habitat transitions.

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Supplements

Zootaxa 4317 (3): 499–529
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4317.3.4
http://zoobank.org/urn:lsid:zoobank.org:pub:5FD492A4-D41D-4F37-A121-FFA680E7E778

A new classification of the tribe Hygrotini Portevin, 1929 (Coleoptera: Dytiscidae: Hydroporinae)

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Abstract

The tribe Hygrotini Portevin, 1929 is currently composed of four genera, *Heroceras* Guignot, 1950, *Herophydrus* Sharp, 1880, *Hygrotus* Stephens, 1828 (with two subgenera, *Coelambus* Thomson, 1860, and *Hygrotus* s. str.), and *Hyphoporus* Sharp, 1880. A recent molecular phylogeny of the tribe with ca. 45% of the 137 described species of Hygrotini, including the type species of all genus-level taxa, revealed extended para- or polyphyly of the current genera and subgenera (Villastrigo *et al*., Zoologica Scripta, in press), for which reason a new classification of the tribe Hygrotini is proposed. Within Hygrotini only two genera are recognised: *Clemnius* **n. gen**. (with two subgenera: *Clemnius* s. str. with type species *Hyphydrus decoratus* Gyllenhal, 1810, and *Cyclopius* **n. subgen.** with type species *Hydroporus acaroides* LeConte, 1855) and *Hygrotus* (with four subgenera: *Coelambus*, *Hygrotus* s. str., *Hyphoporus* **n. stat.** and *Leptolambus* **n. subgen**. with type species *Dytiscus impressopunctatus* Schaller, 1783). Two genera are synonymised under *Hygrotus* s. str., *Herophydrus* **n. syn.** and *Heroceras* **n. syn.** The following 67 new combinations, for species thus far treated under the genera *Heroceras*, *Herophydrus*, *Hygrotus* and *Hyphoporus*, result from the new classification: *Clemnius* (s. str.) *berneri* (Young & Wolfe, 1984) **n. comb.**, *Clemnius* (s. str.) *decoratus* (Gyllenhal, 1810) **n. comb.**, *Clemnius* (s. str.) *hydropicus* (LeConte, 1852) **n. comb.**, *Clemnius* (s. str.) *laccophilinus* (LeConte, 1878) **n. comb.**, *Clemnius* (s. str.) *sylvanus* (Fall, 1917) **n. comb.**, *Clemnius* (*Cyclopius*) *acaroides* (LeConte, 1855) **n. comb.**, *Clemnius* (*Cyclopius*) *farctus* (LeConte, 1855) **n. comb.**, *Clemnius* (*Cyclopius*) *marginipennis* (Blatchley, 1912) **n. comb.**, *Hygrotus* (s. str.) *assimilis* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *bilardoi* (Biström & Nilsson, 2002) **n. comb.**, *H.* (s. str.) *capensis* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *confusus* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *descarpentriesi* (Peschet, 1923) **n. comb.**, *H.* (s. str.) *discrepatus* (Guignot, 1954) **n. comb.**, *H.* (s. str.) *endroedyi* (Biström & Nilsson, 2002) **n. comb.**, *H.* (s. str.) *gigantoides* (Biström & Nilsson, 2002) **n. comb.**, *H.* (s. str.) *gigas* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *goldschmidti* (Pederzani & Rocchi, 2009) **n. comb.**, *H.* (s. str.) *gschwendtneri* (Omer-Cooper, 1957) **n. comb.**, *H.* (s. str.) *hyphoporoides* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *ignoratus* (Gschwendtner, 1933) **n. comb.**, *H.* (s. str.) *inquinatus* (Boheman, 1848) **n. comb.**, *H.* (s. str.) *janssensi* (Guignot, 1952) **n. comb.**, *H.* (s. str.) *kalaharii* (Gschwendtner, 1935) **n. comb.**, *H.* (s. str.) *morandi* (Guignot, 1952) **n. comb.**, *H.* (s. str.) *muticus* (Sharp, 1882) **n. comb.**, *H.* (s. str.) *natator* (Biström & Nilsson, 2002) **n. comb.**, *H.* (s. str.) *nigrescens* (Biström & Nilsson, 2002) **n. comb.**, *H.* (s. str.) *nodieri* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *obscurus* (Sharp, 1882) **n. comb.**, *H.* (s. str.) *obsoletus* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *ovalis* (Gschwendtner, 1932) **n. comb.**, *H.* (s. str.) *pallidus* (Omer-Cooper, 1931) **n. comb.**, *H.* (s. str.) *pauliani* (Guignot, 1950) **n. comb.**, *H.* (s. str.) *quadrilineatus* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *reticulatus* (Pederzani & Rocchi, 2009) **n. comb.**, *H.* (s. str.) *ritsemae* (Régimbart, 1889) **n. comb.**, *H.* (s. str.) *rohani* (Peschet, 1924) **n. comb.**, *H.* (s. str.) *rufus* (Clark, 1863) **n. comb.**, *H.* (s. str.) *sjostedti* (Régimbart, 1908) **n. comb.**, *H.* (s. str.) *spadiceus* (Sharp, 1882) **n. comb.**, *H.* (s. str.) *sudanensis* (Guignot, 1952) **n. comb.**, *H.* (s. str.) *travniceki* (Šťastný, 2012) **n. comb.**, *H.* (s. str.) *tribolus* (Guignot, 1953) **n. comb.**, *H.* (s. str.) *variabilis secundus* (Régimbart, 1906) **n. comb.**, *H.* (s. str.) *variabilis variabilis* (Guignot, 1954) **n. comb.**, *H.* (s. str.) *verticalis* (Sharp, 1882) **n. comb.**, *H.* (s. str.) *vittatus* (Régimbart, 1895) **n. comb.**, *H.* (s. str.) *wewalkai* (Biström & Nilsson, 2002) **n. comb.**, *Hygrotus* (*Hyphoporus*) *anitae* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *aper* (Sharp, 1882) **n. comb.**, *H.* (*Hyphoporus*) *bengalensis* (Severin, 1890) **n. comb.**, *H.* (*Hyphoporus*) *bertrandi* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *caliginosus* (Régimbart, 1899) **n. comb.**, *H.* (*Hyphoporus*) *dehraduni* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *elevatus* (Sharp, 1882) **n. comb.**, *H.* (*Hyphoporus*) *geetae* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *josephi* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *kempi* (Gschwendtner, 1936) **n. comb.**, *H.* (*Hyphoporus*) *montanus* (Régim-

Accepted by M. Toledo: 7 Jul. 2017; published: 5 Sept. 2017

bart, 1899) **n. comb.**, *H.* (*Hyphoporus*) *nilghiricus* (Régimbart, 1903) **n. comb.**, *H.* (*Hyphoporus*) *oudomxai* (Brancucci & Biström, 2013) **n. comb.**, *H.* (*Hyphoporus*) *pacistanus* (Guignot, 1959) **n. comb.**, *H.* (*Hyphoporus*) *pugnator* (Sharp, 1890) **n. comb.**, *H.* (*Hyphoporus*) *severini* (Régimbart, 1892) **n. comb.**, *H.* (*Hyphoporus*) *subaequalis* (Vazirani, 1969) **n. comb.**, *H.* (*Hyphoporus*) *tonkinensis* (Régimbart, 1899) **n. comb**.

Key words: Coleoptera, Dytiscidae, Hydroporinae, Hygrotini, phylogeny, new classification, new genus, new subgenera, new combinations, new synonyms

Introduction

The diving beetle tribe Hygrotini (Dytiscidae: Hydroporinae) is a relatively small and homogeneous lineage, with 137 species currently included in four genera: *Heroceras* Guignot, 1950, *Herophydrus* Sharp, 1880, *Hyphoporus* Sharp, 1880, and *Hygrotus* Stephens, 1828, the latter with two subgenera, *Hygrotus* s. str. and *Coelambus* Thomson, 1860 (Nilsson & Hájek 2017a). They have a predominantly Holarctic and Ethiopian distribution, with some species reaching the Oriental and the north of the Neotropical regions (Nilsson & Hájek 2017a).

The taxonomic history of Hygrotini has experienced several modifications since the original description of the tribe by Portevin (1929). Previous to this author, Sharp (1882: 389) listed *Coelambus* (in which he included species currently in *Hygrotus* and *Herophydrus*) as well as *Herophydrus* and *Hyphoporus* (both erected two years earlier) as the three first genera in his "Group Hydroporini". Portevin (1929: 180) excluded from Hydroporini the genera *Oxynoptilus* Schaum, 1867 (junior ojective synonym of *Hydrovatus* Motschulsky, 1853), *Hyphydrus* Illiger, 1802, and *Hygrotus*, and erected for these three genera the tribe Hygrotini, based on the common presence of an oblique epipleural carina near the elytral shoulders which separates the smaller anterior part (the genicular area or fossa, which receives the front- and midleg knees when the legs are folded; cf. Sharp 1882: 242) from the longer posterior part of the elytral epipleura. However, most authors continued to include *Hygrotus* and allied genera within the wider tribe Hydroporini as defined by Sharp (1882). Nilsson & Holmen (1995: 30) reinstated the tribe Hygrotini, excluding the genera *Hyphydrus* and *Hydrovatus* (which were already in their own tribes Hyphydrini and Hydrovatini respectively), but including the genus *Pseudhydrovatus* Peschet, 1924, which subsequently was shown to be a junior synonym of *Hydrovatus* (see Biström 2002).

The generic concepts within Hygrotini also suffered multiple changes. Thomson (1860) realised that *Hygrotus* sensu Stephens (1828) comprised two different morphological groups according to the aspect of the anterior margin of clypeus, establishing the genus *Coelambus* for the species without clypeal rim. The status of both *Coelambus* and *Hygrotus* has generated controversy among different authors, as the character of the clypeal rim in fact shows considerable variation (see Falkenström 1933, Balfour-Browne 1934, Anderson 1971, or Biström & Nilsson 2002, and the Appendix for a detailed discussion). Sharp (1882) considered *Coelambus* as a genus divided into different groups according to the morphology of the anterior part of the head and the clypeus, with *Dytiscus inaequalis* Fabricius, 1777 (the type species of the genus *Hygrotus*) included in his Group I. Other authors followed his criterion (e.g. Fall 1919), but Balfour-Browne (1940) and Hatch (1953) (among others) considered again *Coelambus* as a subgenus of *Hygrotus*, a treatment that has prevailed since then (although Miller & Bergsten 2016 recently re-established *Coelambus* as a valid genus, but without detailed discussion).

As for the other genera currently included in Hygrotini, Sharp (1880: cxlviii) erected within Hydroporini the new genus *Herophydrus* for what was known as *Hydroporus hyphydroides* Perris, 1864 (= *Hyphydrus guineensis* Aubé, 1838), plus five undescribed species from Africa and Madagascar, and the new genus *Hyphoporus* for *Hydroporus solieri* Aubé, 1838, and two other undescribed species. Two years later, Sharp (1882: 997; nec Branden 1885: 39) synonymised *H. hyphydroides* with *H. guineensis* and described the species mentioned in Sharp (1880) as new, five in *Herophydrus* and two in *Hyphoporus*. It is notable that in Sharp (1882) the current *Herophydrus musicus* (Klug, 1834) was maintained in the genus *Coelambus*.

The identity of the genera of Hygrotini has been problematic ever since. Guignot (1950) was the first author who characterised *Hyphoporus* and *Herophydrus* by using as main character the morphology of the male genitalia, asymmetric in the first and symmetric in the second. Finally, *Heroceras* was erected by Guignot (1950) for a species from Madagascar (*Herophydrus descarpentriesi* Peschet, 1923) with some peculiar characters (such as e.g. dilated antennae, see below).

Until recently the internal phylogeny of *Hygrotini* has only been addressed as part of wider studies on the phylogeny of Dytiscidae, or the revision of particular genera. Remarkably, all published studies failed to recover the respective monophyly of *Hygrotus* and *Herophydrus*, either using molecular (Ribera *et al.* 2002, 2008; Abellán *et al.* 2013; Miller & Bergsten 2014) or morphological data, both of larvae (Alarie & Michat 2007) and adults (Miller 2001; Biström & Nilsson 2002). The phylogenetic position of the genera *Hyphoporus* and *Heroceras* has only been addressed using morphological data by Biström & Nilsson (2002), who found *Hyphoporus* as sister to the studied species of *Coelambus*, and *Heroceras* as sister to the species of *Herophydrus* plus *Hygrotus*.

In a previous work by the same authors (Villastrigo *et al*., in press) we reconstructed the evolution of the tolerance to salinity within tribe Hygrotini, which includes some of the few diving beetles able to live at salt concentrations more than double that of seawater (e.g. *Hygrotus salinarius* (Wallis, 1924) or *H. pallidulus* (Aubé, 1850); Timms & Hammer 1988, Picazo *et al*. 2010). With that purpose, a molecular phylogeny of the tribe was estimated, including ca. 45% of the described species. Results revealed that two of the four currently recognised genera of Hygrotini and one subgenus were para- or polyphyletic (*Hygrotus*, *Herophydrus* and subgenus *Coelambus*), and the monotypic *Heroceras* was deeply nested within a clade of Madagascan *Herophydrus*. The need of a new classification of Hygrotini in order to reconcile the taxonomic ordination of the tribe with its phylogeny was clear, but it was considered more appropriate to present the corresponding taxonomic changes in a separate work.

Material and methods

Phylogenetic data. We used the phylogeny of Hygrotini obtained in Villastrigo *et al.* (in press), which included sequence data from 99 specimens of 61 species representing all four currently recognised genera of Hygrotini (Table 1). Most importantly, the phylogeny included the type species of all nine genus-group names within Hygrotini (Nilsson & Hájek 2017a). Outgroups included a selection of species of Hydroporini, and trees were rooted on *Laccornis* Gozis, 1914, considered to be outside Hydroporini and Hygrotini and in a basal position within Hydroporinae (Ribera *et al.* 2008; Miller & Bergsten 2014).

For a detailed explanation of the methods used to obtain the molecular data and the phylogeny see Villastrigo *et al.* (in press). In summary, the phylogeny was built using fragments of seven genes in six sequencing reactions, three mitochondrial: (1) 5' end of cytochrome c oxidase subunit 1 (COI-5, the "barcode" fragment, Hebert *et al.* 2003), (2) 3' end of cytochrome c oxidase subunit 1 (COI-3), (3) 5' end of 16S RNA plus the Leucine tRNA plus 5' end of NADH dehydrogenase subunit I (16S); and three nuclear: (4) an internal fragment of the large ribosomal unit 28S RNA (28S), (5) an internal fragment of the small ribosomal unit, 18S RNA (18S) and (6) an internal fragment of Histone 3 (H3). Vouchers and DNA samples of all specimens used in the phylogeny are kept in the collections of the Institute of Evolutionary Biology (IBE, Barcelona) and Museo Nacional de Ciencias Naturales (MNCN, Madrid).

To reconstruct the phylogeny, sequences were aligned using the online version of MAFFT 7 with the G-INS-I algorithm (Katoh *et al.* 2009) and a fast Maximum Likelihood (ML) heuristic algorithm in RAxML-HPC2 (Stamatakis 2006) in the CIPRES Science Gateway (Miller *et al.* 2010), using a partition by genes with a GTR+G evolutionary model independently estimated for each partition and assessing node support with 100 pseudoreplicas with a rapid bootstrapping algorithm (Stamatakis *et al.* 2008).

Morphological data. Specimens were studied with an Olympus SZX16 stereomicroscope. For the figures, stacks of micrographs were made with a Canon EOS 650D camera attached to the stereomicroscope. These stacks were subsequently treated with the image stacking software Helicon Focus Pro version 6.4.1. For the SEM-micrographs, specimens were placed on stubs and coated with gold (Sputter Coater, Quorum Technologies Ltd., Ashford, England). Micrographs were taken with an ESEM XL30 (Philips, Amsterdam, The Netherlands) and Scandium FIVE software (Olympus, Münster, Germany) in the Phyletisches Museum (Jena, Germany). Adobe Photoshop CS5 software was used to retouch micrographs and ink drawings. Most ink drawings are reproduced from Fery (2003) with the permission of M.A. Jäch (Vienna, Austria); this is not mentioned in the legends of the figures.

To estimate the likely phylogenetic relationships of the species for which no molecular data could be obtained, and to complete the taxonomic rearrangement of the tribe Hygrotini (see below), we studied all described species of the subgenera *Hygrotus* and *Coelambus* with the only exceptions of *H.* (*Coelambus*) *artus* (Fall, 1919), known only from the holotype and considered to be possibly extinct (see Anderson 1983), and *H.* (*Coelambus*) *femoratus* (Fall, 1901), which is likely a junior synonym of *H.* (*Coelambus*) *nubilus* (LeConte, 1855) (see Anderson 1983). Additionally, we have studied a selection of species of *Herophydrus* and *Hyphoporus* (see Table 1 for the studied material).

Species for which no molecular data were available were considered to be closely related to those showing a high morphological similarity, based both on external characters and on the female and male genitalia. For species without obvious close relatives we identified diagnostic characters or character combinations for the main clades in the phylogeny, and placed these species according to the presence or absence of these characters. We used Mesquite v3.20 (Maddison & Maddison 2017) to manually place all species in their estimated position in the phylogenetic tree (used as a backbone tree), and collapsed uncertain nodes to create polytomies.

Throughout the text of the present work, we follow the classification and nomenclature of Nilsson & Hájek (2017a, b) until we introduce our new classification. The following abbreviations are used in the text: TL (total length) and MW (maximum width); MNHN is used for "Muséum National d'Histoire Naturelle, Paris, France" and FSCA for "Florida State Collection of Arthropods, Gainesville, Florida, USA".

A new classification of the tribe Hygrotini

According to the phylogenetic results of Villastrigo *et al*. (in press) the monophyly of Hygrotini is recovered with strong support, as well as the division of Hygrotini into two clades (see Fig. 1, in which the former generic and subgeneric names are still used): (A) three Nearctic and one Palaearctic species of subgenera *Hygrotus* and *Coelambus* and (B) the remaining species of the tribe. The latter was in turn divided into four further clades: (B1) a group of Palaearctic species of *Coelambus* including *Hygrotus* (*Coelambus*) *confluens* (Fabricius, 1787) (the type species of *Coelambus*), (B2) the two sampled species of *Hyphoporus* (including its type species, *H. solieri*), (B3) a large group of species including *Heroceras*, all sampled *Herophydrus* (including its type species *H. guineensis*) and most species of *Hygrotus* s. str. (including its type species *H. inaequalis*) and (B4) the remaining species of *Coelambus*, in turn divided into two sister clades, one with mostly Palaearctic species and a second with mostly Nearctic species. The internal phylogeny of the main clades was in general in good agreement with the recognised species groups among *Hygrotus* and *Coelambus* based on morphology (see e.g. Anderson 1971, 1976, 1983; Fery 1992, 1995, 2003).

Given the para- or polyphyly of the genera *Hygrotus* and *Herophydrus* and of the subgenus *Coelambus* in their current concepts, we provide here a new classification of the tribe with the aim to avoid para- or polyphyletic genera and subgenera. This section includes brief descriptions of the principal diagnostic characters of the newly classified taxa, which were delimited according to the main clades of the phylogeny (see Table 1 for a complete checklist of the species of the tribe, and Fig. 2 for a dendrogram representing graphically the new classification).

Tribe Hygrotini Portevin, 1929: 180, as tribe of subfamily Hydroporinae.

Type genus: *Hygrotus* Stephens, 1828: 38.

Diagnosis. Within Hydroporinae, species of Hygrotini are usually characterised by the following combination of characters:

- metepisternum (metepiventrite in Miller & Bergsten 2016: 139) reaching mesocoxal cavities, not separated by mesepimeron (in contrast to members of Vatellini);
- apices of elytra and last abdominal ventrite not acuminate (in contrast to members of Methlini);
- prosternal process elongate with apex narrowly pointed or rounded (in contrast to members of Hydrovatini);
- dorsal (anterior) margin of metafemur separated from metacoxal lobe by metatrochanter (in contrast to members of Laccornellini and Laccornini);
- humeral portion of epipleuron with oblique carina delimiting genicular fossa (character shared with members of Hydrovatini, Hyphydrini and genus *Rhithrodytes* Bameul, 1989 in Hydroporini; cf. Fery 2013, 2016; see Fig. 53 for *Rhithrodytes agnus* Foster, 1992, and Figs 49–52 for some *Hygrotus* species).

- metatarsal claws equal in length, with exception of members of *C. saginatus*-group (see Fery 1992, 1995, 2003) and in contrast to members of Hyphydrini and Pachydrini (see e.g. Pederzani 1995; Miller & Bergsten 2016).

None of these characters is, however, an unambiguous synapomorphy of the group. We introduce here a potentially unambiguous synapomorphy of tribe Hygrotini, recognised by one of us (H.F.). In many members of Hydroporinae the antennal cavities in the fronto-lateral part of the head are rather deep and more or less conical (much flatter e.g. in Hyphydrini), allowing the movement of the first antennomere (the scape). These cavities are delimited dorsally by the anterior border of the clypeus ("b" in Figs 3–6). Inside each cavity there is a capsule in which the base of the scape (the condyle) is articulated. This capsule is delimited by a distinct more or less circular carina ("a" in Figs 3–6). In all studied species of Hygrotini there is an additional–more or less semicircular–carina ("c" in Figs 4–6) which is closer to the border of the clypeus and surrounds in part the other carina. In the species of Hydroporini this additional carina is not present (see Fig. 3 for *Hydroporus dorsalis* (Fabricius, 1787) or fig. 33 in Fery & Bouzid 2016 for *Tassilodytes parisii* (Gridelli, 1939)). In a few species of Hygrotini the second carina is difficult to observe or is very narrow (as in e.g. *Heroceras descarpentriesi* and *Hygrotus* (*Coelambus*) *salinarius*), but is nevertheless clearly perceptible when the specimens are properly illuminated and orientated. However, in most specimens studied of *Hygrotus* (*Coelambus*) *masculinus* (Crotch, 1874), the second carina is reduced to a short piece near the mandible.

According to the phylogeny reconstructed in Villastrigo *et al.* (in press) the tribe Hygrotini is divided into two well supported monophyletic lineages, which are considered here with generic rank. Each of these two lineages is in turn divided into generally well supported clades, which are treated as subgenera. In some cases these clades have lower support in the molecular phylogeny, and are also not well defined morphologically (see below and Appendix for a discussion on the clypeal bead, the main character used so far to differentiate genera and subgenera within Hygrotini), so we opted for a subgeneric rather than a generic rank.

Due to the new classification 67 species are for the first time included in the genus *Clemnius* **n. gen**. or in *Hygrotus*, thus their names becoming new combinations. We have listed all these species in Table 1 marked with "**n. comb.**" These changes have also generated some homonymies which will be resolved in a separate work (H. Fery, manuscript in preparation).

The genera and subgenera are treated below in the same order as in Fig. 2, starting from the lower part of the figure.

Genus *Clemnius* n. gen.

Type species: *Hyphydrus decoratus* Gyllenhal, 1810: XVI, by present designation.

The new genus *Clemnius* **n. gen**. includes eight described species distributed in the Nearctic (including the north of Mexico, sometimes treated as Neotropical) and in the Palaearctic zoogeographical region (Fig. 2 and Table 1).

Diagnosis. In the new classification *Clemnius* **n. gen**. is one of two genera of the tribe Hygrotini. All species of this and the second genus *Hygrotus* have two carinae in each antennal cavity (see Figs 4–6), a unique character among the entire subfamily Hydroporinae. The new genus—which corresponds to clade A in Fig. 1—is a heterogeneous assembly of relatively small species with different morphological characters. It is subdivided into two sister-clades each treated as a subgenus: *Cyclopius* **n. subgen.** and *Clemnius* s. str. **n. subgen.** (see Fig. 2).

Subgenus *Cyclopius* n. subgen.

Type species: *Hydroporus acaroides* LeConte, 1855: 294, by present designation.

The subgenus *Cyclopius* **n. subgen.** contains three species of the former subgenus *Hygrotus* (see Fig. 2 and Table 1).

et al

acaroides	*Hydroporus*	*Clemnius*	*Cyclopius*	*Hygrotus*	*Hygrotus*
aequalis	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*
ahmeti	*Hygrotus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*
anitae	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
aper	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
armeniacus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
artus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
assimilis	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
bengalensis	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
berneri	*Hygrotus*	*Clemnius*	*Clemnius*	*Hygrotus*	*Coelambus*
bertrandi	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
bilardoi	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
bruesi	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
caliginosus	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
capensis	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
caspius	*Hydroporus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*
chinensis	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
cleopatrae	*Hydroporus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
collatus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
compar	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
confluens	*Dytiscus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*
confusus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
corpulentus	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
curvilobus	*Hygrotus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*

……continued on the next page

curvipes	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
decoratus	*Hyphydrus*	*Clemnius*	*Clemnius*	*Hygrotus*	*Hygrotus*
dehraduni	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
descarpentriesi	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Heroceras*	—
discrepatus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
dissimilis	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
diversipes	*Hygrotus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
elevatus	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
endroedyi	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
enneagrammus	*Hydroporus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*
falli	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
farctus	*Hydroporus*	*Clemnius*	*Cyclopius*	*Hygrotus*	*Hygrotus*
femoratus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
flaviventris	*Hydroporus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*
fontinalis	*Hygrotus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
fraternus	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
fresnedai	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
fumatus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
geetae	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
gigantoides	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
gigas	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
goldschmidti	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
gschwendtneri	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
guineensis	*Hyphydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
heros	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—

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hydropicus	*Hydroporus*	*Clemnius*	*Clemnius*	*Hygrotus*	*Hygrotus*
hyphoporoides	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
ignoratus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
impressopunctatus	*Dytiscus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
inaequalis	*Dytiscus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*
infuscatus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
inquinatus	*Hydroporus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
inscriptus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
intermedius	*Coelambus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*
janssensi	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
josephi	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
kalaharii	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
kempi	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
laccophilinus	*Hydroporus*	*Clemnius*	*Clemnius*	*Hygrotus*	*Coelambus*
lagari	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
lernaeus	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
lutescens	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
marginipennis	*Coelambus*	*Clemnius*	*Cyclopius*	*Hygrotus*	*Hygrotus*
marklini	*Hyphydrus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
masculinus	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
montanus	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
morandi	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
musicus	*Hydroporus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
muticus	*Coelambus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
natator	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—

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nigrescens	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
nigrescens	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
nigrolineatus	*Hyphydrus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*
nilghiricus	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
nodieri	*Coelambus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
novemlineatus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
nubilus	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
obscureplagiatus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
obscurus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
obsoletus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
orthogrammus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
oudomxai	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
ovalis	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
pacistanus	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
pallidulus	*Hydroporus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*
pallidus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
parallellogrammus	*Dytiscus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
patruelis	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
pauliani	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
pectoralis	*Hydroporus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*
pedalis	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
picatus	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
polonicus polonicus	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
polonicus sahlbergi	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
pugnator	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—

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punctilineatus	*Coelambus*	*Hygrotus*	*Coelambus*	*Hygrotus*	*Coelambus*
quadrilineatus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
quinquelineatus	*Hyphydrus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*
reticulatus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
ritsemae	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
rohani	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
rufus	*Hyphydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
saginatus	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
salinarius	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
sanfilippoi	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
sayi	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*
sellatus	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
semenowi	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
semivittatus	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
severini	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
sjostedti	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
solieri	*Hydroporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
spadiceus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
stefanschoedli	*Hygrotus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
subaequalis	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—
sudanensis	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
suturalis	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
sylvanus	*Coelambus*	*Clemnius*	*Clemnius*	*Hygrotus*	*Coelambus*
thermarum	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
tonkinensis	*Hyphoporus*	*Hygrotus*	*Hyphoporus*	*Hyphoporus*	—

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travniceki	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
tribolus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
tumidiventris	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
turbidus	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
unguicularis	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
urgensis	*Coelambus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
variabilis secundus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
variabilis variabilis	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
vaziranii	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
versicolor	*Dytiscus*	*Hygrotus*	*Hygrotus*	*Hygrotus*	*Hygrotus*
verticalis	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
vittatus	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
wardii	*Hydroporus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*
wewalkai	*Herophydrus*	*Hygrotus*	*Hygrotus*	*Herophydrus*	—
zigetangco	*Hygrotus*	*Hygrotus*	*Leptolambus*	*Hygrotus*	*Coelambus*

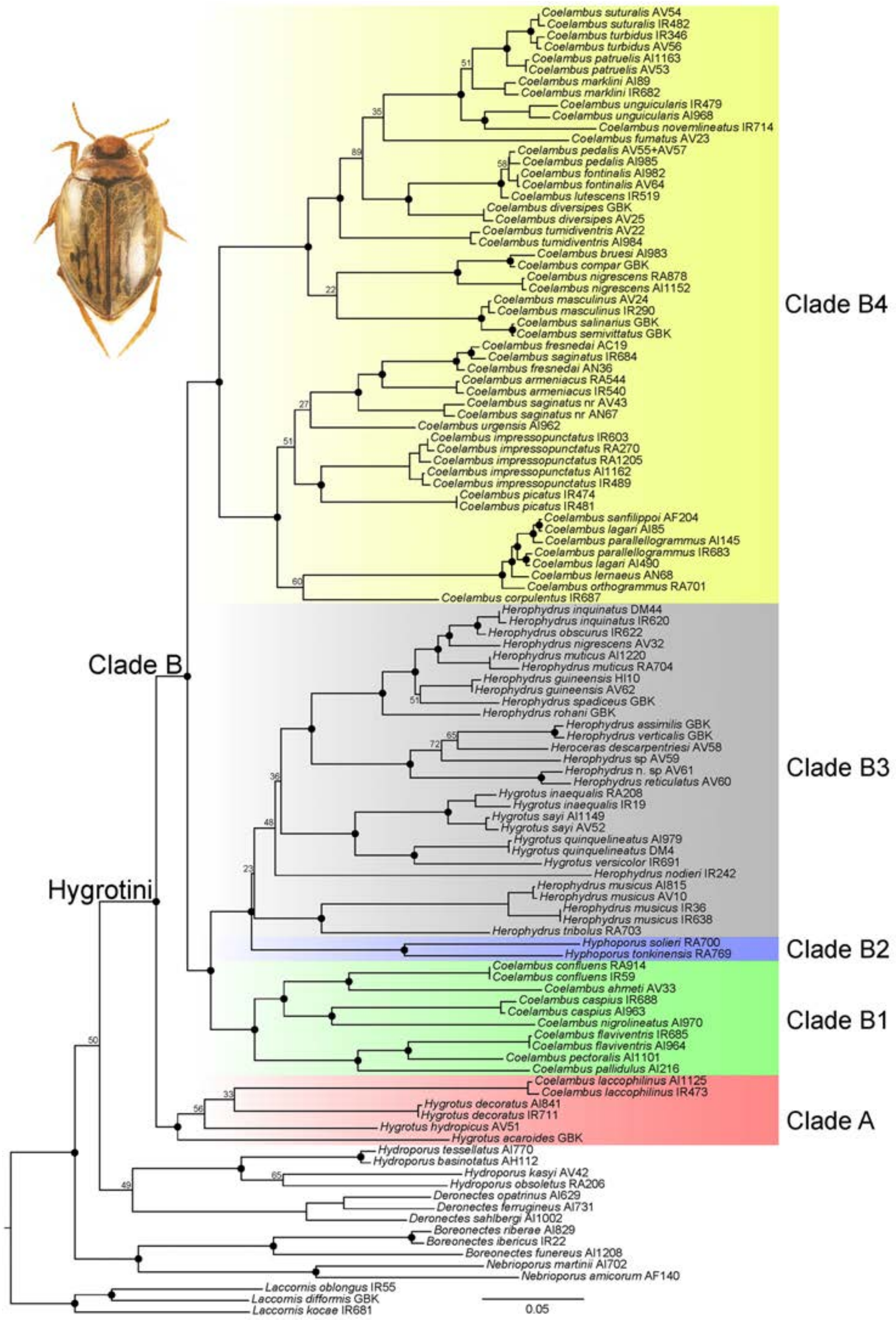


FIGURE 1. Phylogeny of Hygrotini, modified from Villastrigo *et al*. (in press). Numbers above nodes are bootstrap support values. Note that here the former generic and subgeneric names are still used.

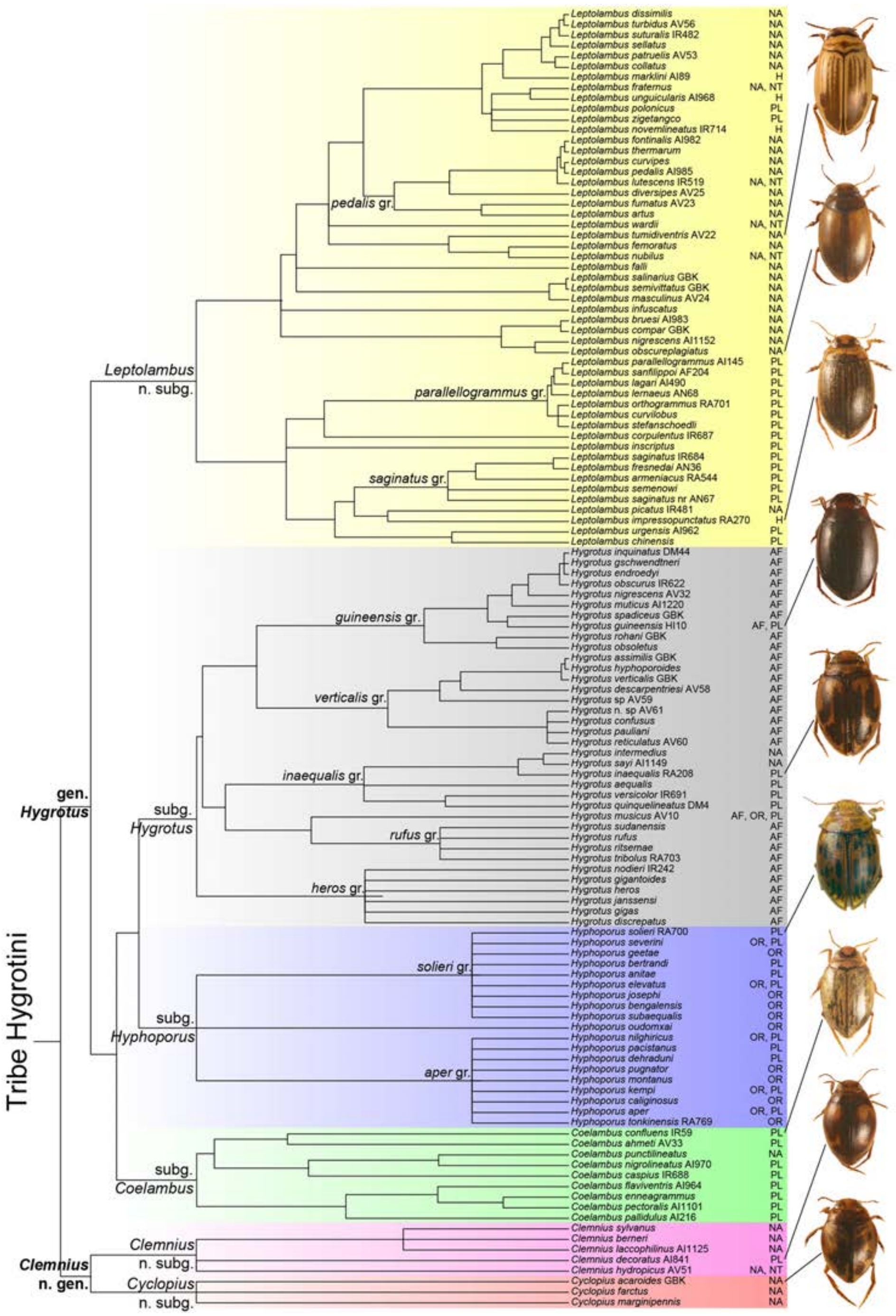
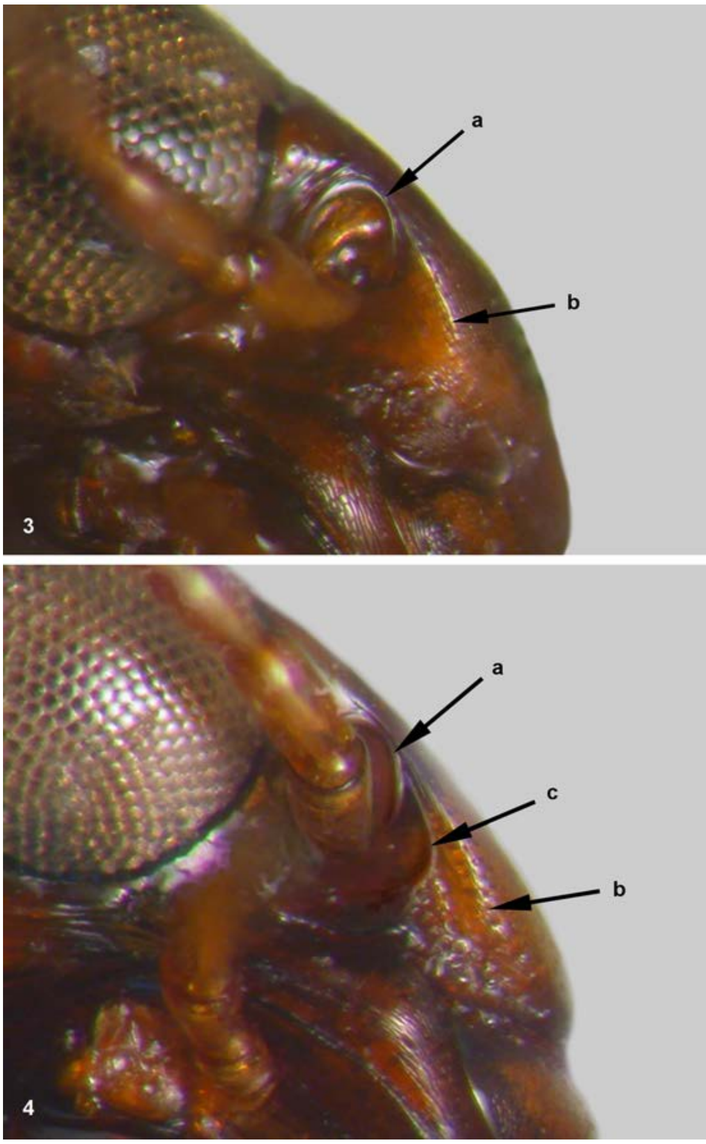


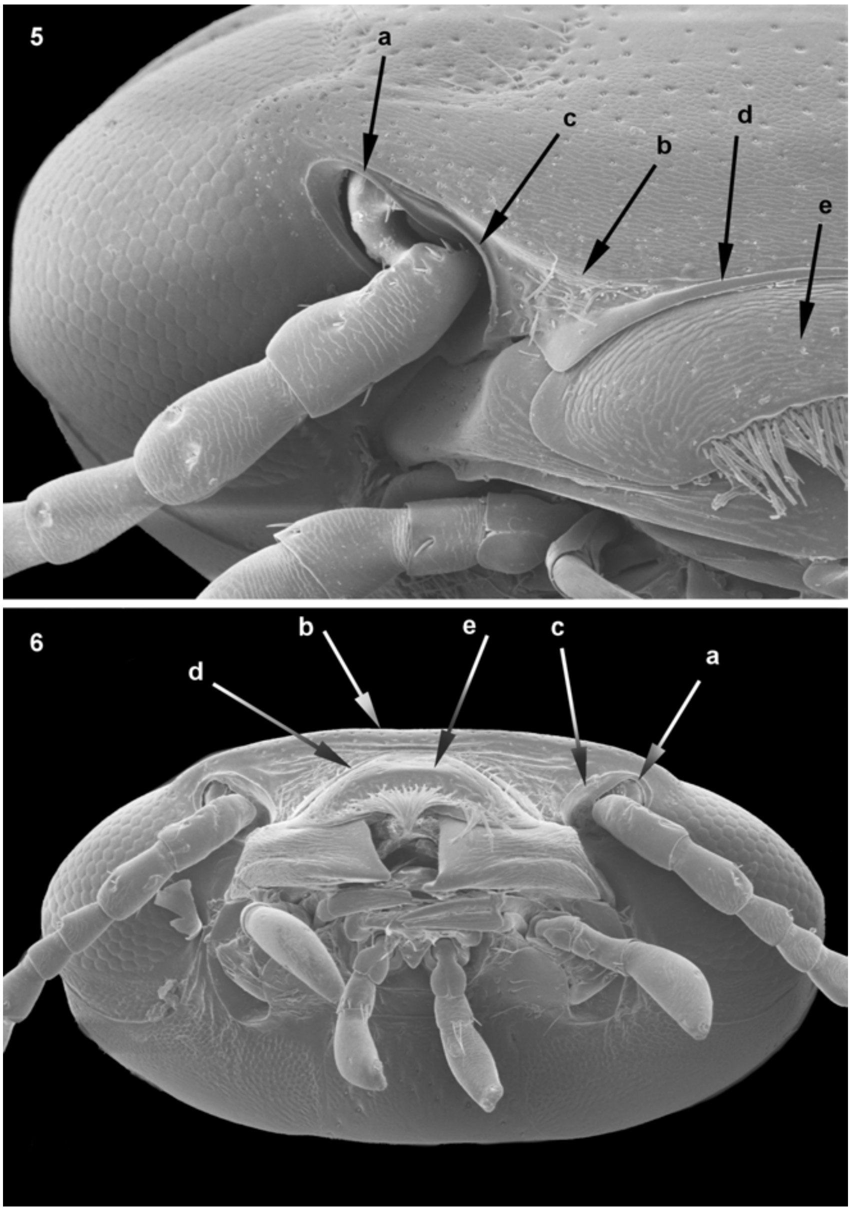
FIGURE 2. Dendrogram showing the new classification of Hygrotini. A number of species without molecular data (no voucher number) have been inserted near morphologically similar species with molecular data. Acronyms refer to biogeographical regions.



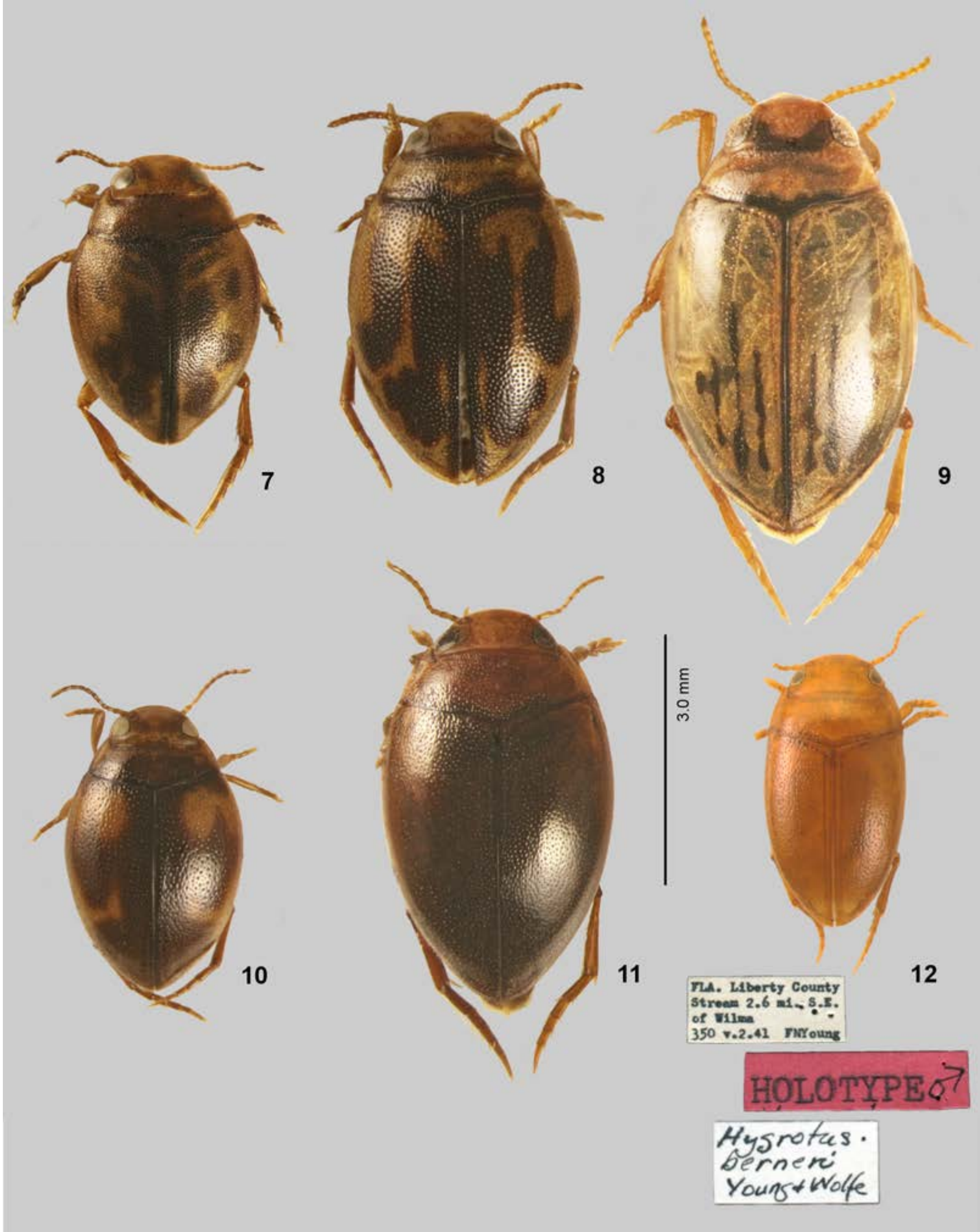
FIGURES 3–4. Antennal cavities of: (3) *Hydroporus dorsalis* (Hydroporini) and (4) *Hygrotus* (*Leptolambus*) *lagari* (Fery, 1992) (a: carina delimiting capsule for scape; b: fronto-lateral border of clypeus; c: second carina).

Diagnosis. Body shape subglobose (TL/MW ca. 1.35–1.6); body size small (TL 2.1–2.6 mm) (cf. Fig. 7 for *Clemnius* (*Cyclopius*) *acaroides*). Head with anterior clypeal margin truncate and slightly emarginated, border not produced forwards; bead broadly interrupted medially (*C*. (*Cyclopius*) *acaroides* and *C*. (*Cyclopius*) *marginipennis* (Blatchley, 1912)) or absent (*C*. (*Cyclopius*) *farctus* (LeConte, 1855)); antennomeres simple, not broadened. Elytra with margin in lateral view rather strongly ascending to shoulder (similar to Fig. 54); epipleuron comparably broad, broader than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably small angle (< ca. 135°; similar to Fig. 49). Colour pattern of elytra variable, from yellowish with dark vittae to more or less uniformly dark brownish or blackish; venter brownish to dark brownish, partly paler.

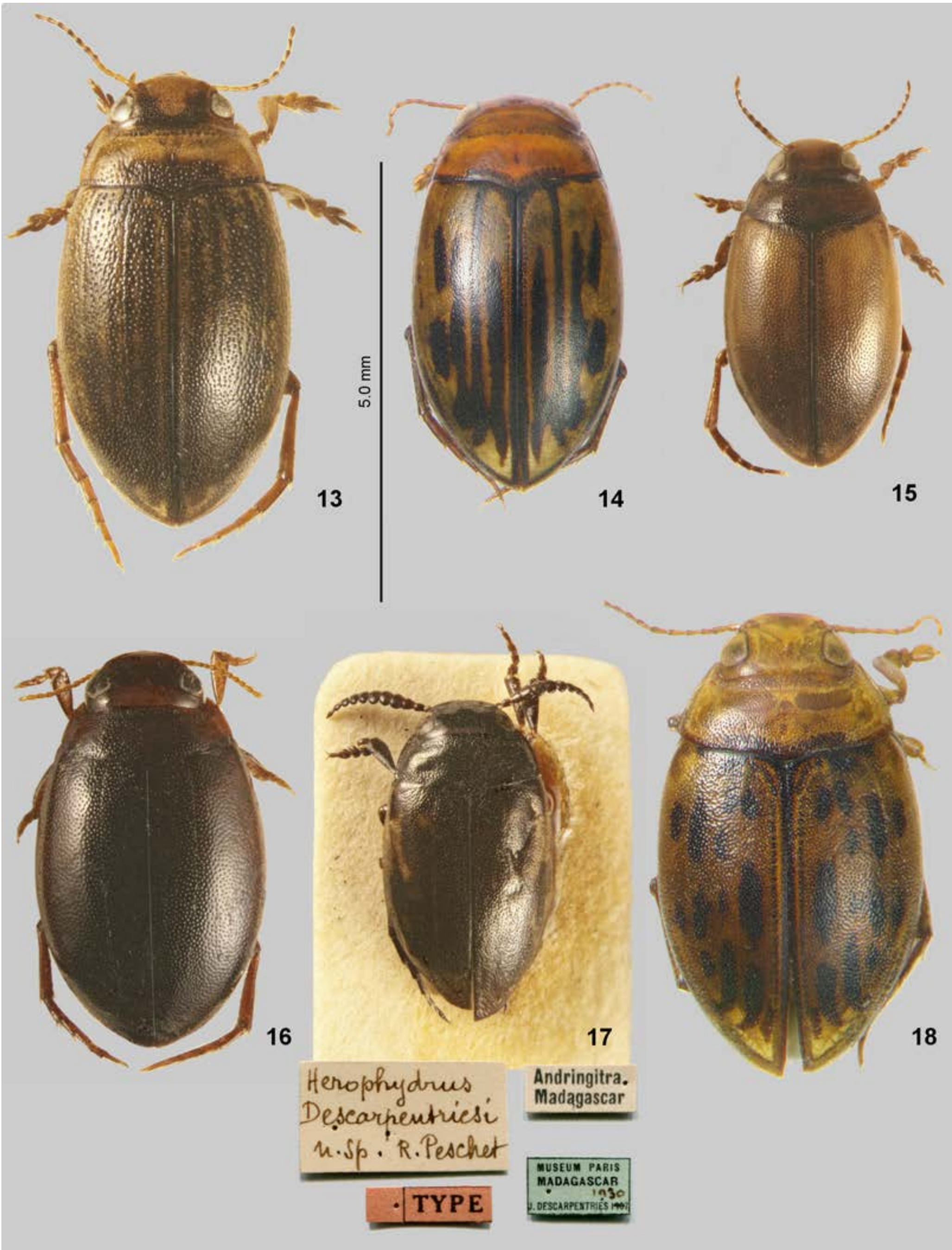
Males with last abdominal ventrite with deep medial depression, its anterior margin with two long spines projecting backwards and more posteriorly with two rather short ones directed perpendicular to surface (see fig. 24B in Larson *et al*. 2000), a unique character in Hygrotini and also in Dytiscidae. Median lobe symmetric, robust in *C*. (*Cyclopius*) *acaroides* (Fig. 19) and *C*. (*Cyclopius*) *marginipennis*, but very thin in *C*. (*Cyclopius*) *farctus*. Parameres with condylar process short, forming an obtuse angle with distal part (see Fig. 29; cf. figs. 10–12 in Anderson 1971). Male metatarsal claws of equal length.



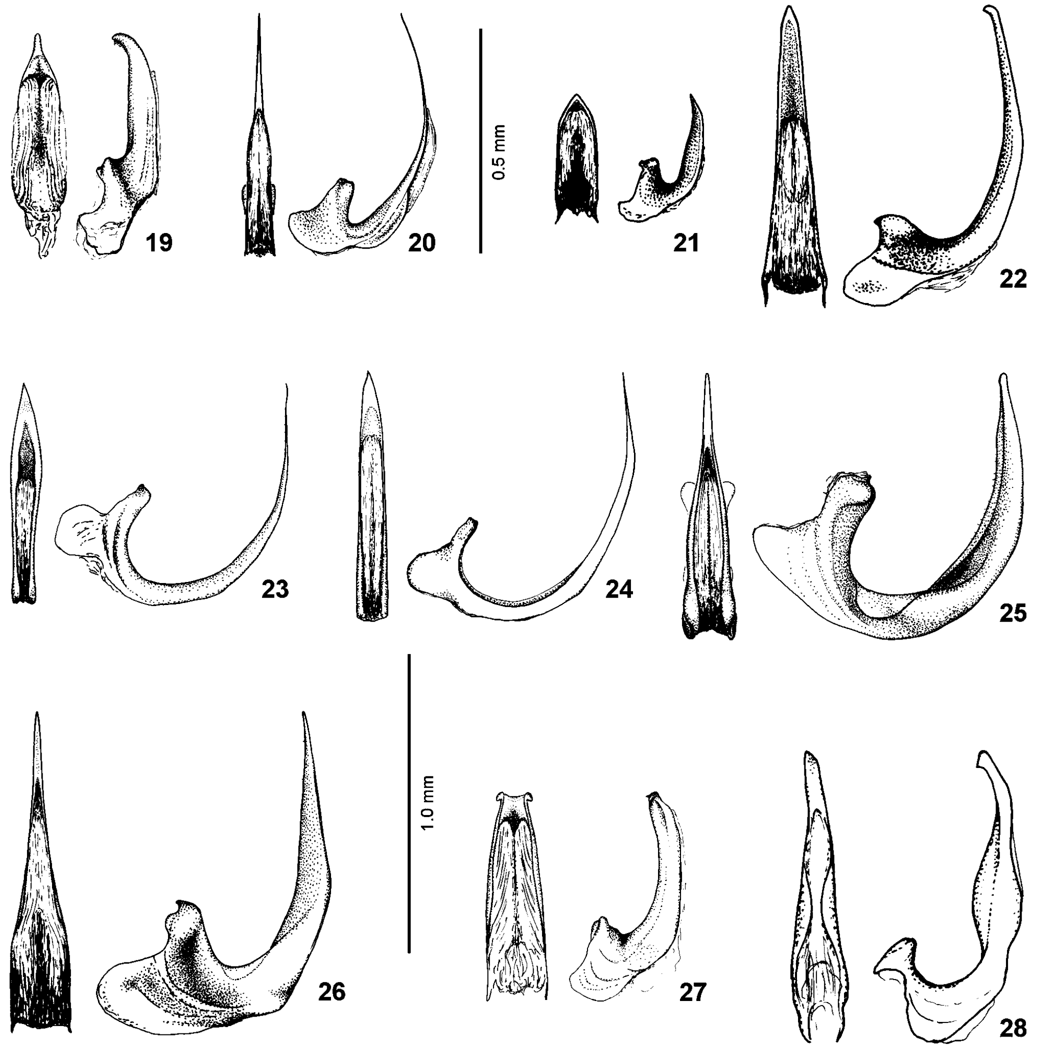
FIGURES 5–6. SEM micrographs with details of head and antennal cavities of: (5) *Hygrotus* (*Leptolambus*) *impressopunctatus* (frontal view) and (6) *Hygrotus* (s. str.) *inaequalis* (oblique ventral view) (a, b and c as in Figs 3–4; d: clypeal stripe; e: labrum).



FIGURES 7–12. Habitus of: (7) *Clemnius* (*Cyclopius*) *acaroides*, (8) *Hygrotus* (s. str.) *inaequalis*, (9) *Hygrotus* (*Coelambus*) *confluens*, (10) *Clemnius*. (s. str.) *decoratus*, (11) *Clemnius* (s. str.) *laccophilinus* and (12) *Clemnius* (s. str.) *berneri* (male holotype and labels).



FIGURES 13–18. Habitus of: (13) *Hygrotus* (*Leptolambus*) *impressopunctatus*, (14) *H.* (*Leptolambus*) *orthogrammus*, (15) *H.* (*Leptolambus*) *obscureplagiatus*, (16) *H.* (s. str.) *guineensis*, (17) *H.* (s. str.) *descarpentriesi* (male syntype and labels) and (18) *H.* (*Hyphoporus*) *solieri* (Figs 14 and 18 are reproduced from Fery *et al*. 2012 with the permission of F. Gusenleitner, Linz, Austria).

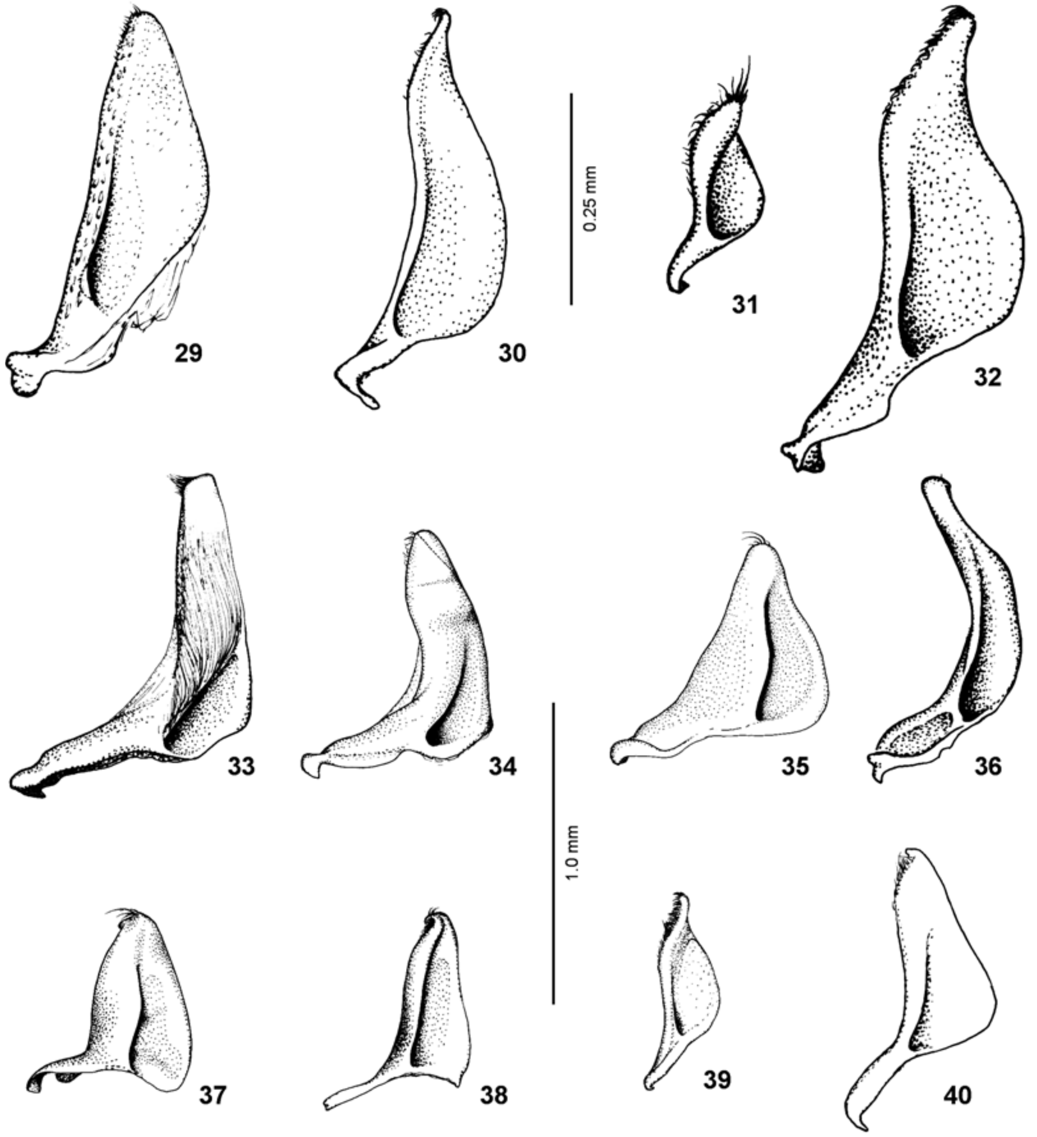


FIGURES 19–28. Median lobe in ventral and lateral view of: (19) *Clemnius* (*Cyclopius*) *acaroides*, (20) *Clemnius* (s. str.) *berneri*, (21) *Clemnius* (s. str.) *decoratus*, (22) *Hygrotus* (s. str.) *inaequalis*, (23) *H.* (*Coelambus*) *enneagrammus*, (24) *H.* (*Coelambus*) *confluens*, (25) *H.* (*Leptolambus*) *parallellogrammus*, (26) *H.* (*Leptolambus*) *impressopunctatus*, (27) *H.* (s. str.) *guineensis* and (28) *H.* (*Hyphoporus*) *tonkinensis* (Fig. 28 is a modified reproduction of figs 3 and 4 in Brancucci & Biström 2013).

Etymology. From the Greek χύκλος (kyklos = circle), referring to the semicircular shape of the clypeus of the species of the subgenus. The gender of the name is masculine.

Distribution. Nearctic: Canada and USA.

Main habitat types. Species of this subgenus are typically known from lentic freshwater habitats; Blatchley (1912: 330) reported *C.* (*Cyclopius*) *marginipennis* from "shallow brackish ponds".



FIGURES 29–40. Left paramere of: (29) *Clemnius* (*Cyclopius*) *acaroides*, (30) *Clemnius* (s. str.) *berneri*, (31) *Clemnius* (s. str.) *decoratus*, (32) *Hygrotus* (s. str.) *inaequalis*, (33) *H.* (*Leptolambus*) *fresnedai*, (34) *H.* (*Leptolambus*) *impressopunctatus*, (35) *H.* (*Leptolambus*) *parallellogrammus*, (36) *H.* (*Leptolambus*) *nubilus*, (37) *H.* (*Coelambus*) *enneagrammus*, (38) *H.* (*Coelambus*) *confluens*, (39) *H.* (s. str.) *guineensis* and (40) *H.* (*Hyphoporus*) *tonkinensis* (Fig. 40 is a modified reproduction of fig. 5 in Brancucci & Biström 2013).

Subgenus *Clemnius* s. str. n. subgen.

Type species: *Hyphydrus decoratus* Gyllenhal, 1810: XVI, by present designation.

The subgenus *Clemnius* **n. subgen.** contains five species of the former genus *Hygrotus*, subgenera *Hygrotus* and *Coelambus* (see Fig. 2 and Table 1).

Diagnosis. Body shape oval, either almost globose (TL/MW ca. 1.6) (see Fig. 10 for *C.* (s. str.) *decoratus*) or more elongated, "navicular" (TL/MW 1.7–1.8) (see Fig. 11 for *C.* (s. str.) *laccophilinus* (LeConte, 1878)), with MW short behind pronotum and distinctly before mid-length (still somewhat more elongated in *C.* (s. str.) *berneri* Young & Wolfe, 1984, with TL/MW ca. 2.05; see Fig. 12 for holotype and its labels; FSCA); body size small (TL 2.1–3.3 mm). *Clemnius* (s. str.) *decoratus* and *C.* (s. str.) *hydropicus* (LeConte, 1852) with anterior clypeal margin evenly and semicircularly rounded, with border produced forwards and bead complete as in subgenus *Hygrotus* s. str. In contrast *Clemnius* (s. str.) *laccophilinus*, *C.* (s. str.) *berneri* and *C.* (s. str.) *sylvanus* (Fall, 1917) with anterior clypeal margin truncate and slightly emarginated, with border not produced forwards and bead absent. Antennomeres simple, not broadened. Elytra with margin in lateral view moderately ascending to shoulder (similar to Fig. 55); epipleuron comparably broad, broader than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably small angle (< ca. 135°; similar to Fig. 49). Elytra either dark, uniformly testaceous or with yellowish-brown dots, but not vittate; venter brown or testaceous. Last abdominal ventrite without deep depression.

Aedeagus with median lobe symmetric, robust in *C.* (s. str.) *decoratus* (Fig. 21) and *C.* (s. str.) *hydropicus* (cf. fig. 13 in Anderson 1971) or distally very narrow in *C.* (s. str.) *berneri* (Fig. 20), *C.* (s. str.) *laccophilinus* and *C.* (s. str.) *sylvanus* (cf. also figs 1A and 2A in Anderson 1976). Parameres with condylar process rather short and forming an obtuse angle with distal part (Figs 30 and 31; cf. also figs 1B and 2B in Anderson 1976). Male metatarsal claws of equal length.

Etymology. From the Greek χλεμμύς (klemmys = tortoise); referring to the almost hemispherical body shape of most species. The gender of the generic name is masculine.

Distribution. Palaearctic (Europe, Russia and Kazakhstan), Nearctic and Neotropical (northern part of Mexico).

Main habitat types. The subgenus includes species typical of lentic freshwater environments.

Notes: Within the subgenus there are two clear groups of species according to body shape and genital morphology, one formed by *C.* (s. str.) *decoratus* and *C.* (s. str.) *hydropicus* (body shape more globular, aedeagus robust with short medial lobe), and the other by *C.* (s. str.) *berneri*, *C.* (s. str.) *laccophilinus* and *C.* (s. str.) *sylvanus* (body shape more elongated, "navicular", aedeagus slender with elongated median lobe). The phylogenetic relationships of the species for which molecular data were available are poorly supported (Fig. 1), but in any case we never recovered these two groups as respectively monophyletic. We thus refrain from splitting *Clemnius* **n. subgen.** into two taxa corresponding to these two groups, at least until more evidence becomes available.

Genus *Hygrotus* Stephens, 1828

Type species: *Dytiscus inaequalis* Fabricius, 1777: 239, by subsequent designation of Curtis (1835: pl. 531).

The genus *Hygrotus* as here defined includes 129 described species (two of them bitypic) distributed in the Nearctic (including the north of Mexico), Palaearctic, Afrotropical and Oriental zoogeographical regions (Fig. 2 and Table 1). One Nearctic species was introduced to Hawaii (see below), which belongs to the Pacific region.

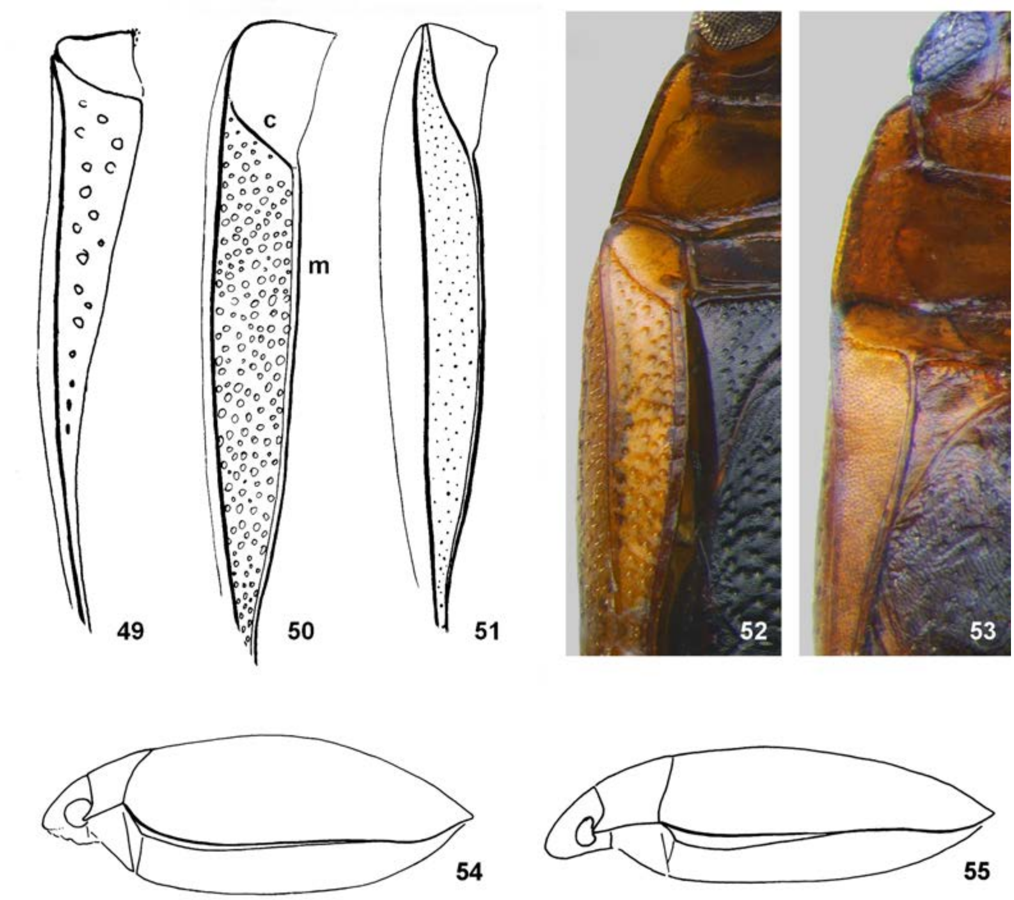
Diagnosis. In the new classification *Hygrotus* is the second of two genera of the tribe Hygrotini. As in *Clemnius* **n. gen**. all species of the genus have two carinae in each antennal cavity (see Figs 4–6).

According to the two main clades in the phylogeny of Hygrotini and several morphological characters, the genus (which corresponds to Clade B in Fig. 1) is subdivided into four clades corresponding to the following four subgenera: clade B1 = subgenus *Coelambus*, clade B2 = subgenus *Hyphoporus*, clade B3 = subgenus *Hygrotus* s. str. and clade B4 = *Leptolambus* **n. subgen.**

Subgenus *Coelambus* Thomson, 1860

Type species: *Dytiscus confluens* Fabricius, 1787: 193, by subsequent designation of Zaitzev (1953: 129).

FIGURES 41–48. Head with details of clypeal rim of (41) *Hygrotus* (*Leptolambus*) *impressopunctatus*, (42) *H.* (*Hyphoporus*) *solieri*, (43) *H.* (s. str.) *guineensis*, (44) *H.* (s. str.) *inaequalis*, (45) idem in lateral view, (46) *H.* (*Coelambus*) *confluens* (the lighter area before the anterior border of the clypeus is the "clypeal stripe"; see Appendix), (47) *H.* (*Leptolambus*) *masculinus* and (48) idem in lateral view.



FIGURES 49–55. (49–53): Epipleuron with oblique epipleural carina and genicular fossa of: (49) *Clemnius* (s. str.) *berneri*, (50) *Hygrotus* (*Leptolambus*) *polonicus polonicus*, (51) *H.* (*Coelambus*) *caspius*, (52) *H.* (*Leptolambus*) *impressopunctatus* and (53) *Rhithrodytes agnus*; epipleural carina (c) and inner margin of epipleuron (m) including angle mentioned in diagnoses of subgenera. **(54–55):** Elytral margin in lateral view of: (54) *H.* (*Coelambus*) *caspius* (strongly ascending to shoulder) and (55) *Clemnius* (s. str.) *berneri* (moderately ascending to shoulder) (Figs 52 and 53 are reproduced from Fery 2013 and from Fery 2016 with the permissions of F. Gusenleitner, Linz, Austria, and G. Foster, Ayr, UK, respectively).

The subgenus *Coelambus* contains eight Palaearctic and one Nearctic species (*Hygrotus* (*C.*) *punctilineatus* (Fall, 1919)).

Diagnosis. Body shape moderately to elongate oval (TL/MW ca. 1.7–2.0); small to medium sized species (TL 3.0–4.5 mm) (see Fig. 9 for *H.* (*Coelambus*) *confluens*). Head with anterior clypeal margin truncate and slightly emarginated, border not produced forwards; bead absent. Antennomeres simple, not broadened. Elytra with margin in lateral view strongly ascending to shoulder (see Fig. 54 for *H.* (*Coelambus*) *caspius* (Wehncke, 1875)); epipleuron comparably narrow, narrower than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably wide angle (< ca. 150°; similar to Fig. 51). Elytra light yellowish (more yellowish brown in *H.* (*Coelambus*) *ahmeti* Hájek, Fery & Erman, 2005), distinctly vittate (in some species/individuals vittae strongly reduced in anterior half). Venter usually black, but females of some species (e.g. *H.* (*Coelambus*) *pallidulus*) with abdomen at least in part yellow or brownish. Last abdominal ventrite without deep depression.

Aedeagus with median lobe elongate, very slender (distal half very thin in lateral view), symmetric or almost symmetric (e.g. in *H.* (*Coelambus*) *enneagrammus* (Ahrens, 1833), and *H.* (*Coelambus*) *confluens*; see Figs 23 and

24). Parameres with condylar process rather long and forming an almost right angle with distal part (Figs. 37 and 38 for *H.* (*Coelambus*) *enneagrammus* and *H.* (*Coelambus*) *confluens*, respectively). Male metatarsal claws of equal length.

Distribution. Mainly Palaearctic, with one species in Canada and northern USA (*Hygrotus* (*Coelambus*) *punctilineatus*).

Main habitat types. The subgenus includes species typical of both lentic and lotic habitats, with varied salinity tolerance going from freshwater to hypersaline.

Subgenus *Hyphoporus* Sharp, 1880 n. stat.

Type species: *Hydroporus solieri* Aubé, 1838: 554, by monotypy.

The subgenus *Hyphoporus* contains 19 species distributed in the Oriental and Palaearctic regions (see Fig. 2 and Table 1).

Diagnosis. Body shape short oval (TL/MW ca. 1.6–1.7), rather globose; small to medium sized species (TL 3.5–5.6 mm) (see Fig. 18 for *H.* (*Hyphoporus*) *solieri*). Head with anterior clypeal margin truncate, straight or slightly emarginated, border not produced forwards; bead continuous, middle part narrowed in most species (see Fig. 42 for *H.* (*Hyphoporus*) *solieri*); (except *H.* (*Hyphoporus*) *bengalensis* (Severin, 1890) with continuous bead). Antennomeres simple, not broadened. Elytra with margin in lateral view moderately ascending to shoulder (similar to Fig. 55); epipleuron comparably broad, broader than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably small angle (< ca. 135°; similar to Fig. 50). Elytra with interrupted vittae or dotted (in some species very darkened); venter black or brown. Last abdominal ventrite without deep depression. Aedeagus with median lobe robust, asymmetric (see Fig. 28 for *Hygrotus* (*Hyphoporus*) *tonkinensis* (Régimbart, 1899); in *H. bengalensis* median lobe more or less symmetric; see Vazirani 1969 for several further figures); parameres with condylar process of diverse length, forming an obtuse angle with distal part (see Fig. 40 for *H.* (*Hyphoporus*) *tonkinensis*). Male metatarsal claws of equal length.

Distribution. Palaearctic and Oriental, from Iran to India and south-east Asia; one species–*H.* (*Hyphoporus*) *solieri*–from Iran to the Arabian Peninsula and Egypt.

Main habitat types. There is no information on the habitat of most of the species of the subgenus, although they are likely to be mostly associated with freshwater environments. However, it shall be mentioned that Hájek (2006: 48) illustrated a rest-pool in a wadi (Pir Sohrab, Iran) where he has collected *H.* (*Hyphoporus*) *aper* (Sharp, 1882) together with *Neptosternus circumductus* Régimbart, 1899.

Subgenus *Hygrotus* Stephens, 1828

Type species: *Dytiscus inaequalis* Fabricius, 1777: 239, by subsequent designation of Curtis (1835: pl. 531).

Herophydrus Sharp, 1880: cxlviii; type species: *Hydroporus hyphydroides* Perris, 1864: 277 (= *Herophydrus guineensis* (Aubé 1838: 455)), by monotypy. **n. syn.**

Dryephorus Guignot, 1950: 150; type species: *Coelambus nodieri* Régimbart, 1895: 37, by original designation of Guignot (1950: 150). **n. syn.**

Heroceras Guignot, 1950: 150; type species: *Herophydrus descarpentriesi* Peschet, 1923: 176, by original designation of Guignot (1950: 150). **n. syn.**

The newly defined subgenus *Hygrotus* s. str. includes six species previously included in the former subgenus *Hygrotus* s. str., the single species of former genus *Heroceras* (*H. descarpentriesi*) and all 44 species (one of them bitypic) of the former genus *Herophydrus* (see Fig. 2 and Table 1).

Diagnosis. Body shape short oval to moderately elongate oval (TL/MW ca. 1.7–1.8); species of former *Hygrotus* s. str. and *Heroceras* small (TL 2.8–3.6 mm) (see Fig. 8 for *H.* (s. str.) *inaequalis* and Fig. 17 for *H.* (s. str.) *descarpentriesi*) and species of former *Herophydrus* small to large (TL 2.6–7.4 mm) (see Fig. 16 for *H.* (s. str.) *guineensis*). Head of species of former *Hygrotus* with anterior clypeal margin evenly and semicircularly rounded, border produced forwards and with complete bead (see Figs 44 and 45 for *H.* (s. str.) *inaequalis*); species of former

Herophydrus and *Heroceras* with anterior clypeal margin truncate, straight or slightly emarginated, border not produced forwards; bead present, but in many species narrowed in medial part (see Fig. 43 for *H.* (s. str.) *guineensis*), in others medially obsolete or widely reduced except before eyes (cf. Appendix). Antennomeres simple, not broadened except in *H.* (s. str.) *descarpentriesi*, with antennomeres of both sexes, but especially males, strongly dilated (Fig. 17). Elytra with margin in lateral view rather strongly ascending to shoulder (similar to Fig. 54); epipleuron comparably broad, broader than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably small angle (< ca. 135°; similar to Fig. 49). Elytral pattern diverse (vittate, dotted or uniform); venter black or brown. Last abdominal ventrite without deep depression.

Aedeagus with median lobe robust, distal part very diverse in ventral view, more or less symmetric (see Figs 22 and 27 for *H.* (s. str.) *inaequalis* and *H.* (s. str.) *guineensis*) or at most slightly asymmetric in some former *Herophydrus* (see figures in Biström & Nilsson 2002); parameres with condylar process generally short, forming an obtuse angle with distal part (see Fig. 32 and 39 for *H.* (s. str.) *inaequalis* and *H.* (s. str.) *guineensis*). Male metatarsal claws of equal length.

Distribution. Palaearctic, Nearctic, Ethiopian and Oriental. Species of the former subgenus *Hygrotus* (the *H.* (*Hygrotus*) *inaequalis*-group in Fig. 2) are distributed in Europe, northern Africa, Asia and northern America (reaching northern Mexico); species of former *Herophydrus* occur mainly in Africa, with five species in the Palaearctic, one (*H.* (s. str.) *musicus*) reaching the Oriental region and *H.* (s. str.) *morandi* (Guignot, 1952) known from Cambodia only; *H.* (s. str.) *descarpentriesi* is endemic to mountainous regions in south-eastern Madagascar.

Main habitat types. The subgenus includes species typical of lentic and lotic freshwater environments; some species can be found in inland mineralised or coastal brackish waters, such as for example *H.* (s. str.) *musicus* (Millán *et al*. 2006). The latter species can also be found in mineral and thermal spring-pools (pers. communication by J. Hájek, Prague, Czech Republic). The habitat of most African species of the subgenus is poorly known.

Subgenus *Leptolambus* n. subgen.

Type species: *Dytiscus impressopunctatus* Schaller, 1783: 312, by present designation.

The subgenus includes 51 species (one of them bitypic), all previously included in the former subgenus *Coelambus* (see Fig. 2 and Table 1). Notes: *Coelambus hudsonicus* Fall, 1919 is treated by us as junior subjective synonym of *Hygrotus* (*Leptolambus*) *novemlineatus* (Stephens, 1829) (according to Nilsson & Hájek 2017a). We are aware that some authors (e.g. Foster *et al.* 2016) accepted subspecific rank for this taxon and others (e.g. Alarie *et al.* 1999) specific rank, but all without giving any justification for their proceeding.

Diagnosis. Body shape moderately to elongate oval (TL/MW ca. 1.75–2.05); small to medium sized species (TL 2.7–5.8 mm) (see Figs 13–15 for *H.* (*Leptolambus*) *impressopunctatus*, *H.* (*Leptolambus*) *orthogrammus* (Sharp, 1882) and *H.* (*Leptolambus*) *obscureplagiatus* (Fall, 1919)). Head with anterior clypeal margin truncate and emarginated (see Fig. 41 for *H.* (*Leptolambus*) *impressopunctatus*); in some species medially at least less curved than near eyes (see Fig. 47 for *H.* (*Leptolambus*) *masculinus*); border not produced forwards and bead absent (except in *H.* (*Leptolambus*) *masculinus* and *H.* (*Leptolambus*) *salinarius*; cf. Appendix). Antennomeres simple, not broadened. In most species elytral margin in lateral view somewhat less ascending to shoulder than in Fig. 54; in species of Anderson's (1976) group III (*H.* (*Leptolambus*) *bruesi* (Fall, 1928), *H.* (*Leptolambus*) *compar* (Fall, 1919), *H.* (*Leptolambus*) *nigrescens* (Fall, 1919), *H.* (*Leptolambus*) *dissimilis* (Gemminger & Harold, 1868), and *H.* (*Leptolambus*) *turbidus* (LeConte, 1855)) margin only moderately ascending (similar to Fig. 55); in most species epipleuron comparably broad, broader than mesotibia distally; carina meeting inner margin of epipleuron forming a comparably small angle (ca. 135°; similar to Fig. 50 or Fig. 52). In four species of Anderson's (1983) group IV (*H.* (*Leptolambus*) *diversipes* (Leech, 1966), *H.* (*Leptolambus*) *fontinalis* Leech, 1966, *H.* (*Leptolambus*) *pedalis* (Fall, 1901), and *H.* (*Leptolambus*) *thermarum* (Darlington, 1928)) epipleuron narrower and carina meeting inner margin of epipleuron forming a comparably wide angle (> ca. 150°; similar to Fig. 51). In *H.* (*Leptolambus*) *curvipes* (Leech, 1938) (belonging also to group IV in Anderson 1983) epipleuron slightly broader and carina meeting inner margin of epipleuron forming an angle of ca. 140°; however, in these five species of Anderson's (1983) group IV elytral margin not as strongly ascending as in species of newly defined subgenus *Coelambus* (see Fig. 54 for *H.* (*Coelambus*) *caspius*). Elytra pattern diverse (vittate, diffuse-vittate or "cloudlike") (see Anderson

1983; see also Fig. 15 of *H.* (*Leptolambus*) *obscureplagiatus* as an example for diffuse-vittate or "cloudlike" elytral pattern); venter black. Last abdominal ventrite without deep depression.

Aedeagus with shape of median lobe diverse, from robust to very slender; symmetric in ventral view (see Figs 25 and 26 for *H.* (*Leptolambus*) *parallellogrammus* (Ahrens, 1812), and *H.* (*Leptolambus*) *impressopunctatus*). Species of *H.* (*Leptolambus*) *saginatus*-group (see Fig. 2) with distal part of parameres strap-like and condylar process also forming an almost right angle with distal part (see Fig. 33 for *H.* (*Leptolambus*) *fresnedai* (Fery, 1992)). Species of *H.* (*Leptolambus*) *parallellogrammus*-group (see Fig. 2) with parameres more or less triangular and condylar process indistinct and not forming an angle with distal part (see Fig. 35 for *H.* (*Leptolambus*) *parallellogrammus*). Other species with distal part of parameres triangular or broadly strap-like and condylar process forming an almost right angle with distal part (see Fig 34 for *H.* (*Leptolambus*) *impressopunctatus*) or with very unusually shaped parameres (see Fig. 36 for *H.* (*Leptolambus*) *nubilus*; cf. also fig. 7B in Anderson 1976 for *H.* (*Leptolambus*) *dissimilis*). Male metatarsal claws of equal length except in species of *H.* (*Leptolambus*) *saginatus*-group (only four species of all Hygrotini with metatarsal claws of unequal length).

Etymology. From Greek λεπτός (= leptos = narrow) and "lambus" in reference to *Coelambus*. The gender of the generic name is masculine.

Distribution. The subgenus is divided into two main clades, one with mostly Palaearctic distribution except for the Holarctic *H.* (*Leptolambus*) *impressopunctatus* and the Nearctic *H.* (*Leptolambus*) *picatus* (Kirby, 1837), and a second with mostly Nearctic species, except for *H.* (*Leptolambus*) *marklini* (Gyllenhal, 1813), *H.* (*Leptolambus*) *novemlineatus*, and *H.* (*Leptolambus*) *unguicularis* (Crotch, 1874) which are Holarctic. Some species of the Nearctic clade reach northern Mexico: *H.* (*Leptolambus*) *fraternus* (LeConte, 1852), *H.* (*Leptolambus*) *lutescens* (LeConte, 1852), *H.* (*Leptolambus*) *nubilus*, and *H.* (*Leptolambus*) *wardii* (Clark, 1862). *Hygrotus* (*Leptolambus*) *nubilus* was recently recorded from Hawaii, where it has likely been introduced (see Fery & Challet 2015).

Main habitat types. Many species of this subgenus are found in mostly lentic freshwaters, but several in the Nearctic clade, as well as the species of the *H.* (*Leptolambus*) *parallellogrammus*-group, can tolerate from slightly saline to hypersaline waters (Villastrigo *et al*., in press). The subgenus includes the most salt-tolerant species of the tribe, *H.* (*Leptolambus*) *salinarius* and *H.* (*Leptolambus*) *masculinus*.

Discussion

The phylogenetic results of Villastrigo *et al*. (in press) revealed the need of a thorough systematic rearrangement of the tribe Hygrotini. The close relationships between the former *Hygrotus*, *Herophydrus* and *Heroceras* had already been previously noted by several authors (see the Introduction and the Appendix), and suggested by the incomplete molecular and morphological phylogenies available prior to our study (Miller 2001; Biström & Nilsson 2002; Ribera *et al*. 2002, 2008; Alarie & Michat 2007; Abellán *et* al. 2013; Miller & Bergsten 2014).

Species of *Hyphoporus* have always been considered to be related to species of *Herophydrus*, from which they differ mainly by the male genital shape (e.g. Biström & Nilsson 2002; Miller & Bergsten 2016). We opted for maintaining *Hyphoporus* as a valid subgenus, not only based on our phylogenetic results, but also due to the distinctiveness of their male genitalia. It is, however, possible that in further analyses with a more complete sampling *Hyphoporus* proves to be a derived clade within the wider subgenus *Hygrotus* s. str.

Hygrotus descarpentriesi, formerly considered in its own genus *Heroceras*, is a morphologically very deviating species when compared with its closest relatives from Madagascar as resolved in the molecular phylogeny (Fig. 1). The habitus is more elongated, the elytral surface is smooth in the male and reticulated (although not matt) in the female (a character not found in any other Malagasy species of *Hygrotus*), the clypeal bead is almost absent (see the Appendix), and–the most apparent character–both sexes, but especially the males, have strongly dilated antennae. The latter character is present occasionally in other groups of Dytiscidae (e.g. in the genera *Agabus* Leach, 1817, *Limbodessus* Guignot, 1939, *Hydrovatus*, *Rhithrodytes*, *Exocelina* Broun, 1886, *Lioporeus* Guignot, 1950, and others; see e.g. Miller & Bergsten 2016 for some examples and Fig. 17), but still exceptional within Hygrotini. Despite all these peculiarities, *H.* (s. str.) *descarpentriesi* was originally described in *Herophydrus*, and found at least to be related to this genus by the morphological phylogenetic analysis of Biström & Nilsson (2002).

The most unexpected result of the phylogeny was the division of Hygrotini into two clades (A and B in Fig. 1), the former (our *Clemnius* **n. gen.**) including a small number of species previously considered belonging in part to former subgenus *Hygrotus* s. str. and in part to former subgenus *Coelambus*. The species of *Clemnius* **n. gen**. had never been suggested to be closely related to each other within Hygrotini. They all share some likely plesiomorphic characters of body shape and colouration. Most of them are also similar in terms of male genitalia shape, which is in general simpler and more similar to those of other related tribes of Hydroporinae.

Of the two subgenera recognised within *Clemnius* **n. gen**., *Cyclopius* **n. subgen.** is well characterised by the special morphology of the male last abdominal ventrite, but we could not recognise any clear unambiguous synapomorphy for *Clemnius* **n. subgen.** The relationships within the latter are still poorly defined, and need further morphological and molecular studies to be clarified.

Although a formal biogeographic analysis is out of the scope of our paper, it is interesting to note that most of the recognised clades have a well-defined distribution within one of the main biogeographic regions:

- *Clemnius* **n. gen.** is Nearctic with the only exception of one species, the widespread Palaearctic *Clemnius* (s. str.) *decoratus* (see Nilsson & Hájek 2017a, b).
- Subgenus *Coelambus* is almost exclusively Palaearctic, with only one exception, the Nearctic *C. punctilineatus*. However, this species is extremely similar to the Palaearctic species *C. nigrolineatus* (Steven, 1808) (this has not been recognised before), including the shape of the male protarsal claws. Their male and female genitalia show only some slight differences and their general shape could be considered virtually identical. Additionally, females of both species have the upper side dull. It is thus most likely that *C. punctilineatus* has very recently colonised the Nearctic region from a Palaearctic ancestor.
- Subgenus *Hygrotus* s. str., as here defined, is largely of Afrotropical distribution (and likely origin), with only some species reaching the southern Palaearctic region and a small clade–the *H.* (s. str.) *inaequalis*-group–in the Palaearctic and Nearctic regions (Fig. 2).
- Subgenus *Hyphoporus* is mostly Oriental, with some species reaching the Palaearctic region in China (Yunnan), Afghanistan, northern India (Uttar Pradesh), Pakistan and in the west until Iran and Egypt.
- Subgenus *Leptolambus* **n. subgen.** is divided into two clades, one mostly Palaearctic and one mostly Nearctic (see above and Fig. 2). Only some northern species of *Leptolambus* **n. subgen.** have likely recently expanded their geographic range to the whole Holarctic, and three species have a discordant distribution: *H.* (*Leptolambus*) *picatus* is a Nearctic species in the Palaearctic clade, and *H.* (*Leptolambus*) *polonicus* (Aubé, 1842) and *H.* (*Leptolambus*) *zigetangco* Fery, 2003 are Palaearctic species likely to be in the Nearctic clade (see Fig. 2), although for the last two species there are no molecular data and thus their phylogenetic position is uncertain.

Acknowledgments

We cordially thank Rolf Beutel (Jena, Germany) for giving us access to the SEM microscope of the "Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum, Friedrich-Schiller-Universität Jena". We thank also M.A. Jäch (Vienna, Austria), F. Gusenleitner (Vienna, Austria) and G.N. Foster (Ayr, UK) for permission to use numerous figures published previously in other works. Antoine Mantilleri (MNHN) and P. E. Skelley (FSCA) are warmly thanked for loan of material. We thank also J. Hájek (Prague, Czech Republic) and an anonymous reviewer for comments on previous versions of the manuscript. This work was partly funded by an FPI grant to A.V., and projects CGL2013-48950-C2-1-P and CGL2013-48950-C2-2-P (AEI/FEDER, UE) to I.R. and A.M. respectively.

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APPENDIX

Note: The generic and subgeneric names cited in the Appendix are those of the new classification unless stated otherwise.

Detailed description of the clypeus in Hygrotini.

The presence and the shape of the clypeal bead (often also called "clypeal rim" or "raised clypeal margin") are used by many authors to separate genera or subgenera within the tribe Hygrotini, mainly the former subgenus *Hygrotus* from former subgenus *Coelambus* and both from the former genera *Herophydrus* and *Hyphoporus* (see Table 1 for the authors and dates of all taxa mentioned here, as well as their past and new classification). Unfortunately, a determination is not always reliable or even impossible because the respective character states are not well recognisable in several species or are present in species which due to other characters, both morphological and molecular, should better be included in another genus or subgenus. Thus, the usefulness of the "clypeal bead" has been debated since long time. This clypeal bead is present in more than half of all members of Hygrotini, and varies considerably in shape. Although in the light of the results of the molecular phylogeny it is clear that the clypeal bead is a highly labile character, without much phylogenetic significance, a detailed description of its structure is necessary to understand the historical classification of Hygrotini, and it can nevertheless have some usefulness to identify some groups of species.

Former subgenera *Hygrotus* and *Coelambus*

The more common character state in members of the former subgenus *Coelambus* as recognised prior to this study (Nilsson & Hájek 2017a, b) is the absence of a clypeal bead (as in e.g. *H.* (*Coelambus*) *confluens,* Fig. 46, and *H.* (*Leptolambus*) *impressopunctatus*, Fig. 41). The anterior part of the clypeus descends anteriad more or less evenly or in a slightly convex shape to the labrum (see Figs 5e and 6e). In almost all species of former *Coelambus* the anterior margin of the clypeus is bordered (directly before reaching/contacting the labrum) with a very narrow rim, which we here call the "clypeal line" (see Fig. 5d for *H.* (*Leptolambus*) *impressopunctatus* and Fig. 6d for *H.* (s. str.) *inaequalis*; see also below for more details). A rather broad transverse band posterior to this line (reaching more or less until the clypeal grooves) is often somewhat less reticulate (and thus more shiny) and more sparsely and finely punctured than the rest of the clypeus posterior to this band (e.g. recognisable "under the arrows" in Fig. 5 for *H.* (*Leptolambus*) *impressopunctatus*). Thus, this band might be interpreted as a separate part of the clypeus and—also due to the depressed clypeal grooves posterior to the band—can appear as if it was somewhat vaulted (although it is not), which might be interpreted as a (flat) bead. The structure of this band can vary considerably even among specimens of the same population of a species—in one specimen the band can appear as a flat bead, in another one found at the same locality the illusion of a bead is lacking. In all these typical members of the former subgenus *Coelambus* the anterior border of the clypeus (where it reaches/contacts the labrum) and that "clypeal line" (if present) can be recognised when the clypeus is viewed perpendicularly. Additionally, in this view it can be recognised that the anterior border of the clypeus of all these species of former subgenus *Coelambus* is not evenly rounded, but truncate and somewhat emarginated.

In members of the former subgenus *Hygrotus* the anterior border of the clypeus is evenly rounded in perpendicular view (see Fig. 44 for *H.* (s. str.) *inaequalis*). Additionally, the clypeus does not descend directly to the labrum, but is strongly and almost sharply produced forwards (see Figs. 6 and 45 for *H.* (s. str.) *inaequalis*), so that it reaches so far over the labrum that the real anterior border of the clypeus (where it reaches/contacts the labrum) cannot be recognised when the clypeus is viewed perpendicularly, as the anteriorly produced part of the clypeus covers most of the labrum and in particular the "clypeal line" (if that line is present at all). This is the reason why in species of the former subgenus *Hygrotus* it is necessary to differentiate between the anterior border of the clypeus and its anterior end, the latter being situated posterior (!) to the former. Short behind and parallel to its evenly curved anterior border the clypeus is depressed over the entire distance between the eyes; this depression is anteriorly delimited by a more or less sharp line (see Fig. 44 for *H.* (s. str.) *inaequalis*). That is why between this line and the anterior border an evenly curved "clypeal" bead is formed which has more or less the same width over its entire length.

There are two species of the former subgenus *Coelambus* that have a clypeus with almost exactly the same structure as the species of former subgenus *Hygrotus*, something that has confused generations of dytiscid specialists: *Hygrotus* (*Leptolambus*) *salinarius* and *H.* (*L.*) *masculinus* (see Figs 47 and 48 for *H.* (*L.*) *masculinus*). However, in contrast to members of the former subgenus *Hygrotus*, these two species have the anterior border of the clypeus truncate (in several specimens of *H.* (*L.*) *masculinus* at least less curved centrally than near the eyes) and also somewhat emarginated in *H.* (*L.*) *salinarius*—being in this respect typical *Hygrotus* (*Coelambus*) and not *Hygrotus* s. str.

Former genera *Hyphoporus* and *Herophydrus*

Members of former genera *Herophydrus* and *Hyphoporus* have a different structure of the anterior part of the clypeus. Whilst all former *Hyphoporus* have a complete clypeal bead which is mostly narrowed medially (see Fig. 42 for *Hygrotus* (*Hyphoporus*) *solieri*), in former *Herophydrus* some species have a complete bead, some a medially shortly interrupted bead (see Fig. 43 for *Hygrotus* (s. str.) *guineensis*) as well as others with a medially broadly interrupted bead and *Hygrotus* (s. str.) *rohani* (Peschet, 1924) with a hardly delimited bead (cf. Biström & Nilsson 2002). In those species with incomplete bead, the situation in the middle of the clypeal border is similar to that in typical *Hygrotus* (*Leptolambus*)—the anterior part of the clypeus descends anteriad more or less evenly or in a slightly convex shape to the labrum and the labrum as well as an eventual "clypeal line" are not hidden by the anterior part of the clypeus. Left and right of the bead interruption, the clypeus is widely vaulted and sometimes slightly produced forwards, however never as strongly produced as in former subgenus *Hygrotus*. The two parts of the bead are backwards either delimited by a more or less distinct line or by an anteriorly rather sharply delimited depression (as in the *inaequalis*-group of *Hygrotus* s. str.).

In members of subgenus *Hyphoporus* and those of former genus *Herophydrus* with complete bead, the anterior border of the clypeus is medially also widely vaulted and thus a little produced anteriorly. This is why in perpendicular view an eventually existing "clypeal line" and a very small posterior part of the labrum are covered by the vaulted anterior border of the clypeus. It must, however, be emphasised that these structures are totally different from those in members of former subgenus *Hygrotus*—in all these species the clypeus is by far not as strongly and not as sharply produced forwards as in the latter. Additionally, the bead or its two lateral parts are considerably broader left and right of the middle, whilst it is of more or less of equal width over its entire length in members of the former subgenus *Hygrotus*.

Species of former *Herophydrus* and *Hyphoporus* have the anterior margin of the clypeus truncate and emarginated in perpendicular view (see Fig. 43 for *guineensis*; in some species, however, this emargination is only rather indistinct), but we must concede that we have not been able to study all species and must rely in part on the figures given in Biström & Nilsson (2002).

The separation of former *Herophydrus* from *Hyphoporus* by morphological characters has been debated since long time (see e.g. Guignot 1950: 149, Vazirani 1969: 203–205, Biström & Nilsson 2002: 20–21). We did not find any satisfying solution for this problem and believe that a final classification can only be given once the molecular data of all species of these two genera are known. So far we must refer on what was given by Vazirani (1969: 204) in his key to these two genera, but add some exceptions.

According to Guignot (1950: 149; more or less repeated by Vazirani 1969: 204) in former genus *Herophydrus* the punctation of the head reaches an imaginary line connecting the hind margins of the eyes, but on a small band posterior to this line (on the vertex) this punctation is absent or at most replaced by a few much smaller punctures. In former genus *Hyphoporus* the punctation of the head reaches distinctly beyond this imaginary line and is not replaced by finer punctation. Additionally, both authors note that in *Herophydrus* the median lobe is (more or less) symmetric and in *Hyphoporus* it is not. On the other hand, Guignot (1959: 339) himself conceded that "lack of punctation in that band on the vertex" [our translation from French] does not really hold in *H.* (s. str.) *musicus* and *H.* (s. str.) *rufus*, and we must concede that we were not able to use this character for reliable identifications.

In what refers to the symmetry of the median lobe we want to underline that *Hygrotus* (*Hyphoporus*) *bengalensis* has a more or less symmetric median lobe, and on the other hand that many former *Herophydrus* have a median lobe which is at least not strictly symmetric (as can be appreciated e.g. in some figures in Biström & Nilsson 2002).

Former genus *Heroceras*

Hygrotus (s. str.) *descarpentriesi* is a species not well represented in collections; thus, some of the very few descriptions in the literature may not rely on careful studies of specimens. It is more or less known as a "*Herophydrus* with widened antennae" (cf. Guignot 1950: 150 and Pederzani 1995: 35). Guignot (1959: 372) provided for this species: "Tête bourrelet clypéal ininterrompu, ..." [= head with clypeal bead not interrupted ...] and "... bourrelet clypéal peu marqué ..." [= ... clypeal bead not well marked ...]. Miller & Bergsten (2016: 204) gave "... having the anterior clypeal margin broadly bordered."

We have studied one male and one female syntype of this species (coll. Peschet, MNHN; see Fig. 17), one additional female from the coll. Guignot (MNHN), and several further specimens collected recently by one of us (M.M.). To our great surprise, at first glance we were not able to detect any distinct clypeal bead. Only when adequately illuminated it was possible to see that in the female syntype the clypeus is very slightly vaulted before the anterior margin. In the male syntype a few transversely stretched punctures indicate a posterior margin of a bead—however, only on the right side of the clypeus. In particular, there are absolutely no traces of a bead recognisable before the eyes. The latter observation is surprising, because even *H.* (s. str.) *nodieri*, with a very broadly interrupted clypeal bead, shows laterally distinct rests of a bead next to the eyes. Similar observations were made with the recently collected material. Additionally, we want to state that only a small posterior part of the labrum is covered by the vaulted anterior border of the clypeus—this being in contrast to Biström & Nilsson (2002: 18, 19) who stated labrum "not visible from above". On the other hand, our observation that the clypeal bead is practically lacking in former *Heroceras* is supported by Biström & Nilsson (2002: 18, 19, table 1) who gave the anterior margin of the clypeus (character 1 on p. 18) with state 0 (= without bead). These authors, however, did not comment this feature.

It shall be mentioned here that the almost total lack of a medial clypeal bead was the reason for Guignot (1950) to create his subgenus *Dryephorus* of genus *Herophydrus*. Similarly, other species of former *Herophydrus* show only rests of a bead before each eye, such as *Hygrotus* (s. str.) *heros* (Sharp, 1882) (cf. Zimmermann 1919: 150). Although in a different subgenus, *Hygrotus* (*Leptolambus*) *polonicus polonicus* (Aub, 1842) and its subspecies *sahlbergi* (Sharp, 1882) have the clypeus anteriorly somewhat vaulted (cf. Zimmermann 1930: 96). Zimmermann (1919: 150) claimed that also *Hygrotus* (*Leptolambus*) *unguicularis* might be ranged under *Hygrotus* s. str. "... wegen der feinen, aber wenigstens in der Mitte deutlichen Clypeusrandung ..." [= because of the fine, but at least medially distinct beading]. We have studied numerous specimens of *H. unguicularis* and can state that Zimmermann (1919) must have been misguided: most probably he mixed up what in Hygrotini is usually called clypeal "bead" (and which is rather broad) with what we call here "clypeal line".

Falkenström (1933: 12) noted that on the one hand *Hygrotus* (s. str.) *versicolor* (Schaller, 1783) should be ranged in *Herophydrus* due to the shape of the clypeal border (meaning a medially reduced width of the clypeal bead), but on the other hand refused this classification.

The "clypeal stripe"

As mentioned above, in many species of Hygrotini, and in particular in species of former subgenus *Coelambus*, the anterior border of the clypeus is provided with a "clypeal line". This line is in some species rather distinct, in others irregularly interrupted and in some not recognisable or absent. The distinctness of this line seems to show also some individual variation within a species. Balfour-Browne (1934: 150) pointed to a special feature of *Hygrotus* (*Coelambus*) *confluens*: at the base of the labrum, before the anterior border of the clypeus there is a flat, transverse stripe which is rather shiny, not reticulate, provided with only a very few punctures, broadest in middle and evenly tapering to the sides (Fig. 46). We interpret this "clypeal stripe" as a broadened "clypeal line". Such stripe can be also found in *H.* (*C.*) *pallidulus*, *H.* (*C.*) *caspius*, *H.* (*C.*) *pectoralis* (Motschulsky, 1860) (stripe very thin), *H.* (*C.*) *nigrolineatus*, *H.* (*C.*) *punctilineatus*, *H.* (*C.*) *enneagrammus* and in *H.* (*C.*) *flaviventris* (Motschulsky, 1860), but in all latter species this stripe is by far not as broad as in *H.* (*C.*) *confluens* and often difficult to observe, as it is strongly reduced to almost a simple line slightly broader medially than laterally. In *H.* (*C.*) *ahmeti* this "clypeal stripe" is in fact reduced to a line of more or less even width. We have found such medially very slightly broadened stripe/line also in a few other species, such as *H.* (*Leptolambus*) *marklini*, *H.* (*L.*). *fraternus*, *H.* (*L.*). *patruelis* (LeConte, 1855), and *H.* (*L.*). *pedalis*.

Finally, we want to emphasise, that this "clypeal stripe" is by no means homologous to the "clypeal bead" found in species of former *Herophydrus*, *Hyphoporus* and subgenus *Hygrotus*.

Received: 2 April 2017 | Accepted: 3 July 2017
DOI: 10.1111/zsc.12255

ORIGINAL ARTICLE

WILEY Zoologica Scripta

Evolution of salinity tolerance in the diving beetle tribe Hygrotini (Coleoptera, Dytiscidae)

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Funding information
Ministerio de Economía y Competitividad, Grant/Award Number: CGL2013-48950-C2-1-P and CGL2013-48950-C2-2-P; FPI Grant/Award Number: BES-2014-069398

Some species of the diving beetle tribe Hygrotini (subfamily Hydroporinae) are among the few insects able to tolerate saline concentrations more than twice that of seawater. However, the phylogenetic relationships of the species of Hygrotini, and the origin and evolution of tolerance to salinity in this lineage, are unknown. In this work, we aim to reconstruct how many times salinity tolerance did evolve in Hygrotini, whether this evolution was gradual or if tolerance to hypersalinity could evolve directly from strictly freshwater (FW) species, and to estimate the probabilities of transition between habitats. We build a phylogeny with ca. 45% of the 137 species of Hygrotini, including all major lineages and almost all of the known halophile or tolerant species. We used sequence data of four mitochondrial (COI-5′, COI-3′, 16S + tRNA and NADH1) and three nuclear (28S, 18S and H3) gene fragments, plus ecological data to reconstruct the history of the salinity tolerance using Bayesian inference. Our results demonstrate multiple origins of the tolerance to salinity, although most saline and hypersaline species were concentrated in two lineages. The evolution of salinity was gradual, with no direct transitions from FW to hypersaline habitats, but with some reversals from tolerant to FW species. The oldest transition to saline tolerance, at the base of the clade with the highest number of saline species, was dated in the late Eocene-early Oligocene, a period with decreasing temperature and precipitation. This temporal coincidence suggests a link between increased aridity and the development of tolerance to saline waters, in agreement with recent research in other groups of aquatic Coleoptera.

1 | INTRODUCTION

Hydroporinae is the most diverse of the subfamilies of diving beetles (Dytiscidae) (Nilsson & Hájek, 2017a) and its species also display a large variety of ecologies and life habits (Miller & Bergsten, 2016). Many species of Hydroporinae live in extreme or unusual environments for diving beetles, such as subterranean aquifers, forest litter or hypersaline waters. While the origin and evolution of subterranean and terrestrial lifestyles have received recent attention (e.g., Leys & Watts, 2008; Tierney et al., 2015; Toussaint, Hendrich, Escalona, Porch, & Balke, 2016), the origin of the species of diving beetles able to sustain extreme salt concentration has never been addressed in a phylogenetic context, whereas for other families of aquatic Coleoptera, such as Hydrophilidae or Hydraenidae, comparative studies on the evolution of saline tolerance are already available (Arribas et al., 2014; Sabatelli et al., 2016).

Within Hydroporinae, species which are exclusively halophile or which can tolerate saline or hypersaline waters have independently evolved in three tribes, Bidessini, Hydroporini and Hygrotini (Miller & Bergsten, 2016). The latter includes some of the most extreme examples of saline tolerance, with some species able to sustain concentrations above 70 g/L, twice that of seawater (Picazo, Moreno, & Millán, 2010; Timms & Hammer, 1988). The physiological mechanism of salt tolerance of *Hygrotus* (*Coelambus*) *salinarius* (Wallis, 1924) has been studied by Tones (1978). However, the species' phylogenetic relationships are unknown, and in consequence whether it may be related to other saline tolerant species of *Hygrotus* Stephens, 1828 or what could have been the origin of its saline tolerance.

With this work we aim to investigate the phylogenetic relationships among salt tolerant species of Hygrotini, and their relationships with the rest of the species of the tribe. This will allow to answer some basic questions such as: (i) how many times did salinity tolerance evolve in Hygrotini? (ii) was this evolution gradual (i.e., from FW to intermediate salinities to hypersaline), or could tolerance to hypersalinity have evolved directly from strictly FW species? (iii) which were the probabilities of transitions between habitats in the evolution of Hygrotini? and (iv) are there any general patterns in the geographic and temporal origin of salinity tolerance in Hygrotini?

To answer these questions we built a molecular phylogeny with almost half of the 137 known species of the tribe, including all genera and recognised main species groups (Nilsson & Hájek, 2017a,b), and compiled data on the ecological tolerances of all described species from the literature and our own observations. In addition, our results demonstrated that two of the four currently recognised genera of Hygrotini and one subgenus are para- or polyphyletic, revealing the need of a new classification of the tribe, which will be presented in a separate paper (Villastrigo, Ribera, Manuel, Millán, & Fery, 2017).

2 | MATERIALS AND METHODS

2.1 | Molecular data

We obtained molecular data from 101 specimens of 64 species, including all four currently recognised genera of Hygrotini: *Heroceras* Guignot, 1950, *Herophydrus* Sharp, 1880, *Hygrotus* (with two subgenera, *Hygrotus* and *Coelambus* Thompson, 1860) and *Hyphoporus* Sharp, 1880 (Nilsson & Hájek, 2017a,b; see Table S1). We used as outgroups a selection of 12 species from four genera belonging to Hydroporini, shown to be related to Hygrotini (Ribera, Vogler, & Balke, 2008). Trees were rooted on *Laccornis* Gozis, 1914, considered to be outside Hydroporini and Hygrotini and in a basal position within Hydroporinae (Miller & Bergsten, 2014; Ribera et al., 2008).

2.2 | DNA extraction and sequencing

Specimens were collected in the field and preserved in absolute ethanol. DNA was extracted using commercial kits (mostly DNeasy Tissue Kit, Qiagen, Hilden, Germany) following the instructions of the manufacturers. Vouchers and DNA samples are kept in the collections of the Institute of Evolutionary Biology (IBE, Barcelona) and Museo Nacional de Ciencias Naturales (MNCN, Madrid). We sequenced fragments of seven genes in six sequencing reactions, three mitochondrial: (i) 5′ end of cytochrome *c* oxidase subunit 1 (COI-5, “barcode” fragment of Hebert, Ratnasingham, & De Waard, 2003), (ii) 3′ end of cytochrome *c* oxidase subunit 1 (COI-3), (iii) 5′ end of 16S RNA plus the Leucine tRNA plus 5′ end of NADH dehydrogenase subunit I (16S); three nuclear fragments: (iv) an internal fragment of the large ribosomal unit 28S RNA (28S), (v) an internal fragment of the small ribosomal unit, 18S RNA (18S) and (vi) an internal fragment of Histone 3 (H3). Details on primers used and typical polymerase chain reaction (PCR) conditions are provided in Table S2. Sequences were assembled and edited with Geneious v6.0.6 (Kearse et al., 2012); new sequences have been submitted to the EMBL database with accession numbers LT882773-LT883126 (Table S1).

2.3 | Phylogenetic analyses

Edited sequences were aligned using the online version of MAFFT 7 with the G-INS-I algorithm (Katoh, Asimenos, & Toh, 2009). For one species (*Hygrotus* (*Coelambus*) *pedalis* (Fall, 1901)) we pooled sequences of two specimens in a chimera to complete the data set (Table S1), after testing their monophyly with COI-3. We used PartitionFinder v1.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012) to estimate the best fitting evolutionary model initially using one partition for each gene fragment except for COI (split in COI-5 and COI-3) and 16S and tRNA (pooled in a single partition), and applied Akaike Information Criterion (AIC) scores as selected criteria.

We used BEAST 1.8 (Drummond & Rambaut, 2007) for Bayesian phylogenetic analyses, using the partition and evolutionary models selected by PartitionFinder and a molecular-clock approach for estimating divergence times. We applied an uncorrelated lognormal relaxed clock to estimate substitution rates and a Yule speciation process as the tree prior. We calibrated the tree using rates estimated in Andújar, Serrano, and Gómez-Zurita (2012) for a genus of Carabidae (*Carabus*), in the same suborder Adephaga (rate of 0.0113 [95% confidence interval 0.0081–0.0147] substitutions per site per million years (subst/s/Ma) for COI-5; 0.0145 [0.01–0.0198] subst/s/Ma for COI-3 and 0.0016 [0.001–0.0022] subst/s/Ma for 16S + tRNA). Analyses were run for 100 million generations, assessing that convergence was correct and estimating the burn-in fraction with Tracer v1.6 (Drummond & Rambaut, 2007). We also used a fast maximum likelihood (ML) heuristic algorithm in RAxML-HPC2 (Stamatakis, 2006) in the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010), using the same partition scheme as in BEAST with a GTR + G evolutionary model independently estimated for each partition and assessing node support with 100 pseudoreplicas with a rapid bootstrapping algorithm (Stamatakis, Hoover, & Rougemont, 2008).

2.4 | Morphological data

To estimate the likely phylogenetic relationships of the species for which no molecular data could be obtained, we studied all described species of subgenera *Hygrotus* and *Coelambus* with the only exceptions of *H.* (*C.*) *artus* (Fall, 1919), known only from the holotype and considered to be possibly extinct (see Anderson, 1983), and *H.* (*C.*) *femoratus* (Fall, 1901), which is likely a junior synonym of *H.* (*C.*) *nubilus* (LeConte, 1855) (Anderson, 1983). We have also studied a large selection of species of *Herophydrus* and *Hyphoporus* (see Table S3 for the studied material).

The taxonomic classification of Hygrotini has suffered multiple changes, and some of the characters used to define genera (such as e.g., the morphology of the clypeus) are difficult to interpret and characterise (see e.g., Anderson, 1971; Balfour-Browne, 1934; Biström & Nilsson, 2002; Falkenström, 1933; Villastrigo et al., 2017). Species for which no molecular data were available were thus considered to be closely related to those showing a high morphological similarity, based both on external characters and on the female and male genitalia. We also recognised diagnostic characters or character combinations of the different clades of the molecular phylogeny and tested their presence in the species without molecular data (see Villastrigo et al., 2017 for more details). We used Mesquite v3.20 (Maddison & Maddison, 2017) to manually place all species in their most likely position in the phylogenetic tree, and collapsed uncertain nodes to create polytomies.

2.5 | Salinity tolerance data

We compiled ecological data on habitat preferences of all species of Hygrotini from bibliography and from our own observations (Table S3). Recent work on salinity tolerance in aquatic Coleoptera has recognised six different categories of habitat preferences: (i) FW (<0.5 g/L); (ii) mineralised (0.5–5 g/L); (iii) hyposaline (≥5–20 g/L); (iv) mesosaline (≥20–40 g/L); (v) hypersaline (≥40–80 g/L); (vi) extreme hypersaline (>80 g/L) (Arribas et al., 2014). As quantitative observations in species of Hygrotini were very scarce we reduced these categories to three: (i) species strictly bounded to FW environments (approximately <0.5 g/L), corresponding to category (i) above; (ii) species that can tolerate a wide range of salinities (approximately 0.5–40 g/L), corresponding to categories (ii–iv) above; and (iii) hypersaline species (approximately >40 g/L), corresponding to categories (v) and (vi) above. To reconstruct the evolution of saline tolerance we pruned the data set to one specimen per species and deleted the outgroups, using salinity tolerance as a qualitative trait. This reduced matrix was analysed in BEAST using the same settings as for the phylogenetic reconstruction, with an asymmetric substitution model for the trait reconstruction and dating the ancestral node according to the results of the previous analysis (with a Gamma distribution with shape 30 and scale 2.227). We also reconstructed the evolution of saline tolerance in the extended phylogeny, including species for which no molecular data were available, using parsimony in Mesquite.

3 | RESULTS

3.1 | Molecular phylogeny

The best partition schemes selected by PartitionFinder pooled the two fragments of COI and several of the nuclear genes, and favoured the most complex evolutionary models for most partitions (Table 1). The topological differences between the Bayesian analysis and the ML searches were minimal and always affected poorly supported nodes (Figures 1, S1), associated with the position of three species: *Hygrotus* (*Coelambus*) *fumatus* (Sharp, 1882), *Hygrotus* (*Coelambus*) *urgensis* (Jakovlev, 1899) and *Hygrotus* (*Hygrotus*) *hydropicus* (LeConte, 1852). In all cases, the monophyly of Hygrotini was recovered with strong support, as were most of the internal nodes (Figures 1, S1).

In all analyses, Hygrotini was divided into two lineages (posterior probability [pp] = 1; bootstrap support [BS] = 98): (A) three Nearctic and one Palaearctic species of subgenera *Hygrotus* and *Coelambus* and (B) the remaining species of the tribe (Fig. 1). The latter was in turn divided into four clades: (B1) a group of Palaearctic species of subgenus

TABLE 1 Best partition schemes and optimal evolutionary models as estimated with PartitionFinder

Complete data set			Reduced data set		
P	**Genes**	**Model**	**P**	**Genes**	**Model**
#1	COI-5 + COI-3	GTR + I + G	#1	COI-5 + COI-3	GTR + I + G
#2	16S + NAD1	GTR + I + G	#2	16S + NADH	GTR + I + G
#3	18S + 28S + H3	GTR + I + G	#3	18S + 28S	GTR + I
			#4	H3	GTR + I + G

P, partition.

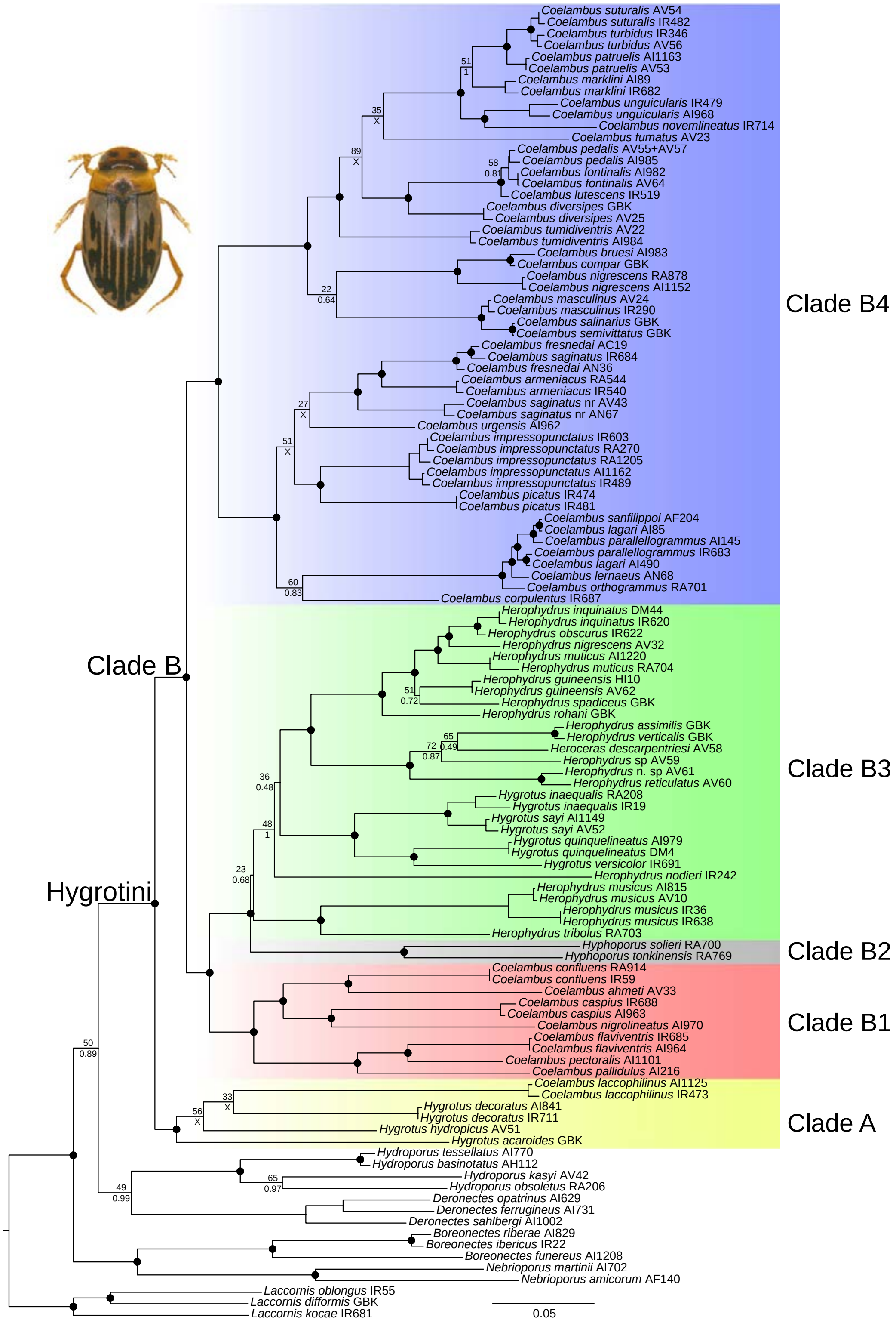
Coelambus suturalis AV54
Coelambus suturalis IR482
Coelambus turbidus IR346
Coelambus turbidus AV56
Coelambus patruelis AI1163
Coelambus patruelis AV53
Coelambus marklini AI89
Coelambus marklini IR682
Coelambus unguicularis IR479
Coelambus unguicularis AI968
Coelambus novemlineatus IR714
Coelambus fumatus AV23
Coelambus pedalis AV55+AV57
Coelambus pedalis AI985
Coelambus fontinalis AI982
Coelambus fontinalis AV64
Coelambus lutescens IR519
Coelambus diversipes GBK
Coelambus diversipes AV25
Coelambus tumidiventris AV22
Coelambus tumidiventris AI984
Coelambus bruesi AI983
Coelambus compar GBK
Coelambus nigrescens RA878
Coelambus nigrescens AI1152
Coelambus masculinus AV24
Coelambus masculinus IR290
Coelambus salinarius GBK
Coelambus semivittatus GBK
Coelambus fresnedai AC19
Coelambus saginatus IR684
Coelambus fresnedai AN36
Coelambus armeniacus RA544
Coelambus armeniacus IR540
Coelambus saginatus nr AV43
Coelambus saginatus nr AN67
Coelambus urgensis AI962
Coelambus impressopunctatus IR603
Coelambus impressopunctatus RA270
Coelambus impressopunctatus RA1205
Coelambus impressopunctatus AI1162
Coelambus impressopunctatus IR489
Coelambus picatus IR474
Coelambus picatus IR481
Coelambus sanfilippoi AF204
Coelambus lagari AI85
Coelambus parallelogrammus AI145
Coelambus parallelogrammus IR683
Coelambus lagari AI490
Coelambus lernaeus AN68
Coelambus orthogrammus RA701
Coelambus corpulentus IR687
Herophydrus inquinatus DM44
Herophydrus inquinatus IR620
Herophydrus obscurus IR622
Herophydrus nigrescens AV32
Herophydrus muticus AI1220
Herophydrus muticus RA704
Herophydrus guineensis HI10
Herophydrus guineensis AV62
Herophydrus spadiceus GBK
Herophydrus rohani GBK
Herophydrus assimilis GBK
Herophydrus verticalis GBK
Heroceras descarpentriesi AV58
Herophydrus sp AV59
Herophydrus n. sp AV61
Herophydrus reticulatus AV60
Hygrotus inaequalis RA208
Hygrotus inaequalis IR19
Hygrotus sayi AI1149
Hygrotus sayi AV52
Hygrotus quinquelineatus AI979
Hygrotus quinquelineatus DM4
Hygrotus versicolor IR691
Herophydrus nodieri IR242
Herophydrus musicus AI815
Herophydrus musicus AV10
Herophydrus musicus IR36
Herophydrus musicus IR638
Herophydrus tribolus RA703
Hyphoporus solieri RA700
Hyphoporus tonkinensis RA769
Coelambus confluens RA914
Coelambus confluens IR59
Coelambus ahmeti AV33
Coelambus caspius IR688
Coelambus caspius AI963
Coelambus nigrolineatus AI970
Coelambus flaviventris IR685
Coelambus flaviventris AI964
Coelambus pectoralis AI1101
Coelambus pallidulus AI216
Coelambus laccophilinus AI1125
Coelambus laccophilinus IR473
Hygrotus decoratus AI841
Hygrotus decoratus IR711
Hygrotus hydropicus AV51
Hygrotus acaroides GBK
Hydroporus tessellatus AI770
Hydroporus basinotatus AH112
Hydroporus kasyi AV42
Hydroporus obsoletus RA206
Deronectes opatrinus AI629
Deronectes ferrugineus AI731
Deronectes sahlbergi AI1002
Boreonectes riberae AI829
Boreonectes ibericus IR22
Boreonectes funereus AI1208
Nebrioporus martinii AI702
Nebrioporus amicorum AF140
Laccornis oblongus IR55
Laccornis difformis GBK
Laccornis kocae IR681
51
1
35
X
89
X
58
0.81
22
0.64
27
X
51
X
60
0.83
51
0.72
65
0.49
72
0.87
36
0.48
48
1
23
0.68
33
X
56
X
50
0.89
49
0.99
65
0.97
0.05
Clade B4
Clade B3
Clade B2
Clade B1
Clade A
Clade B
Hygrotini

FIGURE 1 Best maximum likelihood (ML) phylogram obtained in RAxML with the extended dataset of Hygrotini (including outgroups and multiple terminals per species). Black circles, nodes with bootstrap support in RAxML (BS) >70 and posterior probability (pp) in BEAST >0.95; when support values were lower: numbers above nodes, BS; numbers below nodes, pp. X, nodes not recovered in the BEAST analyses (Fig. S1). See Table S1 for details on the specimens. For clarity, only the subgenus name is given in *Hygrotus* s.str. and *Coelambus*. Habitus photograph: *Hygrotus* (*Coelambus*) *lagari* (Fery, 1992) (from Millán et al., 2014)

Coelambus, (B2) the two sampled species of *Hyphoporus*, (B3) a large group of species including *Heroceras*, all sampled *Herophydrus* and most species of *Hygrotus* s. str., and (B4) the remaining species of subgenus *Coelambus*, in turn divided into two sister clades, one with mostly Palaearctic species and a second with mostly Nearctic species.

The monophyly of all clades had strong support in both Bayesian and ML analyses except for clade B3 (Fig. 1). This clade included the genus *Herophydrus* as paraphyletic with respect to *Heroceras* and most species of the subgenus *Hygrotus*, the latter grouped in a monophyletic lineage. *Heroceras* and the sampled species of *Herophydrus* from Madagascar with the exception of *H. spadiceus* Sharp, 1882 formed a strongly supported clade (pp = 1, BS = 100), in turn placed (with lower support) inside a lineage with most of the remaining sampled African *Herophydrus* (Fig. 1).

The internal phylogeny of the main clades was generally in good agreement with the recognised species groups among subgenera *Hygrotus* and *Coelambus* based on morphology (see e.g., Anderson, 1971, 1976, 1983; Fery, 1992, 1995, 2003), although not with the phylogeny of *Herophydrus* obtained by Biström and Nilsson (2002).

3.2 | Evolution of tolerance to salinity

Differences between the analyses of the complete and reduced data set referred mostly to the position of *Hyphoporus* (clade B2). In the complete data set it was placed as sister to clade B3, and both sister to clade B1, with very strong support (Fig. 1). In the reduced data set, without outgroups and with only one terminal per species, it was placed as sister to clade B4, but with low support (Fig. 2). However, the uncertainty in the phylogenetic position of *Hyphoporus* (clade B2) did not have any effect on the reconstruction, as the two possible sister clades, B3 (Fig. 1) or B4 (Figures 2, S2), were reconstructed to have a FW ancestor, and thus, their common ancestor was also reconstructed as living in FW habitats.

According to the Bayesian reconstruction in the reduced data set, tolerance to salinity emerged independently from a FW ancestor at least ten times within three of the main lineages of Hygrotini: B1, B3 and B4 (Fig. 2). There were five subsequent independent transitions from tolerant to hypersaline (mainly in the Nearctic lineage of clade B4), although in some cases these transitions affected only the terminal branches in our phylogeny. Four of the transitions from FW to salinity tolerant and one of the transitions to hypersalinity led to clades with more than one species, and in only one clade (B4) there have been three reversal transitions from tolerant to FW. No direct transitions from FW to hypersaline habitats were found in the reconstruction (Fig. 2).

The oldest transition to salinity tolerance, at the base of the clade with the highest number of saline species, was dated to the late Eocene-early Oligocene. Three of the transitions to hypersalinity tolerance were estimated to have occurred during the Oligocene, one of them with a further diversification in the early Pleistocene, including *H.* (*C.*) *salinarius* and *Hygrotus* (*Coelambus*) *masculinus* (Crotch, 1874; Fig. 2). Other transitions to hypersalinity tolerance affecting single species occurred in the middle Miocene (*Hygrotus* (*Coelambus*) *diversipes* Leech, 1966) and the Pleistocene (*Hygrotus* (*Coelambus*) *fontinalis* Leech, 1966; see Fig. 2).

The highest estimated transition rate was from FW to tolerant (1.97 transitions/branch/My [t/b/My]), followed by tolerant to hypersaline (1.45 t/b/My, Table 2). Direct transition from FW to hypersaline waters had the lowest rate (0.23 t/b/My), even lower than that estimated for reversal from hypersaline tolerance to FW (0.37 t/b/My, Table 2).

For most species without molecular data a close relative included in the phylogeny could be identified based on similarities in the genitalia or the external morphology, although in a few cases no obvious relatives could be identified, and the species were placed in an unresolved polytomy in the less inclusive clade to which they could be ascribed (Fig. S2). The parsimony reconstruction of salinity tolerance in this extended phylogeny gave similar results to the reconstruction using only the molecular data. The last common ancestor of Hygrotini was a FW species, as was the reconstructed ancestors of clades A, B2 and B3. For nodes including species with the three states of the trait, such as in clades B1 and B4, the ancestral reconstruction was ambiguous in the parsimony analysis (Fig. S2). On the contrary, in the Bayesian analysis of the reduced data set they were reconstructed as FW with a high probability (Fig. 2). Most of the tolerant or hypersaline species were included within clades with at least some other tolerant species, in agreement with the results obtained with the phylogeny using only species with molecular data (Figures 2, S2).

4 | DISCUSSION

4.1 | Molecular phylogeny

We obtained a robust phylogeny of *Hygrotini*, with good support for most internal nodes except for the monophyly of

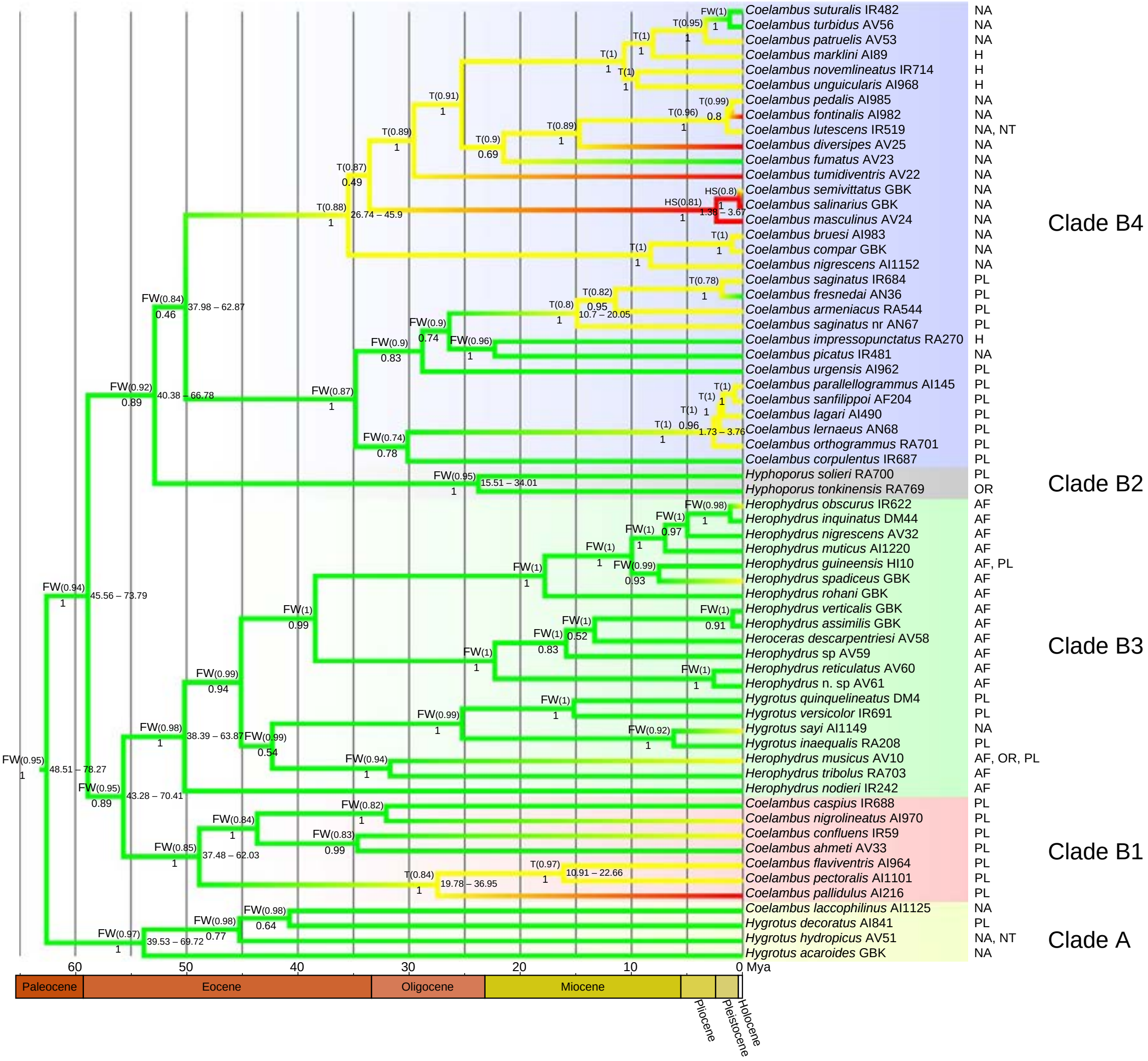

FIGURE 2 Reconstruction of the evolution of tolerance to salinity in the reduced data set of Hygrotini (excluding outgroups and with only one terminal per species) in BEAST. Letters above nodes: reconstructed state of the trait (FW, freshwater, green; T, tolerant, yellow; HS, hypersaline, red); numbers inside parentheses: posterior probability of the reconstructed discrete state; numbers below nodes: node support (pp); numbers inside nodes: 95% interval of the reconstructed age of selected nodes. For clarity, only the subgenus name is given in *Hygrotus* s.str. and *Coelambus*. See Tables S1 and S3 for details on the specimens and the ecological typification of the species, respectively. Geographic distribution: AF: Afrotropical; H: Holarctic; NA: Nearctic; NT: Neotropical (northern Mexico); OR: Oriental; PL: Palaearctic

TABLE 2 Estimated transition rates in BEAST between the discrete states of tolerance to salinity

	Freshwater	Tolerant	Hypersaline
Freshwater	–	1.97 (0.33–3.99)	0.23 (<0.001–0.72)
Tolerant	1.23 (0.09–2.83)	–	1.4 (0.03–3.02)
Hypersaline	0.37 (<0.001–1.17)	0.73 (<0.001–1.9)	–

In brackets, 95% confidence interval.

clade B3 and its relationship with *Hyphoporus*. Our species sampling was also very dense except for *Herophydrus* and for *Hyphoporus*, of which we could obtain fresh material of only two species. These two species, however, belong to the two different morphological groups recognised in the revision of the genus by Vazirani (1969).

The most unexpected result of our phylogeny was the deep division of Hygrotini in two clades, one of them formed by a small number of species. Despite the lack of clear synapomorphies, these species share some presumably plesiomorphic characters of body shape, colouration and male genitalia, the latter being in general simpler and more similar to those of other related tribes of Hydroporinae. The internal relationships within clade A are still poorly defined, and would need further morphological and molecular studies to be clarified. Within clade B3, the close relationships of *Hygrotus* s. str., *Herophydrus* and *Heroceras* had been previously noted by many authors (Abellán et al., 2013; Alarie & Michat, 2007; Biström & Nilsson, 2002; Miller & Bergsten, 2014; Ribera, Hogan, & Vogler, 2002; Ribera et al., 2008), and suggested by the (incomplete) molecular and morphological phylogenies available prior to our study. Species of *Hyphoporus* have always been considered to be related to the species of *Herophydrus*, of which they differ mostly in the male genital shape (e.g., Biström & Nilsson, 2002; Miller & Bergsten, 2016). The non-monophyly of genera *Hygrotus* and *Herophydrus* and subgenera *Hygrotus* and *Coelambus* requires a revised classification of the tribe, which will be formalised in a separate paper (Villastrigo et al., 2017).

4.2 | Evolution of the tolerance to salinity in Hygrotini

We found several independent origins for the salinity tolerance in the tribe Hygrotini, with at least 10 transitions from FW to saline ecosystems both in the Palaearctic and the Nearctic. The multiple origin of saline species was expected, as they were included in different morphologically well characterised subgenera or species groups, but the number of transitions was lower than anticipated, as some of the saline species in clade B4 that were not thought to be related (as e.g., *Hygrotus* (*Coelambus*) *marklini* (Gyllenhall, 1813) and *H.* (*C.*) *salinarius*) were grouped in the same monophyletic radiation. In other families of aquatic Coleoptera, species that were apparently not closely related were also found to have a common origin of their tolerance to salinity, as for example in the genera *Ochthebius* Leach, 1815 (Hydraenidae; see Sabatelli et al., 2016) and *Enochrus* Thomson, 1859 (Hydrophilidae; see Arribas et al., 2014).

We did not find any direct transition from FW to hypersaline habitats, a transition that had the lowest estimated probability, suggesting that in Hygrotini the adaptation to salinity has been a gradual process. The only possible exception was *Hygrotus* (*Coelambus*) *pallidulus* (Aubé, 1850), sister to a group of tolerant species, but the clade was reconstructed as having a FW ancestor with high probability. However, *H.* (*C.*) *pallidulus* together with its tolerant sisters are included in a wider lineage with several other tolerant species of which no molecular data could be obtained, rendering the condition of their common ancestor ambiguous (as can be seen in Fig. S2). This gradual evolution is in contrast to the direct transitions from FW to hypersaline tolerance found in a group of Mediterranean species of *Enochrus* (Arribas et al., 2014). These transitions were associated with periods of aridification of the climate, leading to the hypothesis that saline tolerance may have been a by-product (an exaptation) of adaptation to desiccation (Arribas et al., 2014; Pallarés, Velasco, Millán, Bilton, & Arribas, 2016). The capability to produce hyperosmotic excreta is a plesiomorphic character in insects, likely linked to the necessary adaptations to a terrestrial environment (Bradley, 2008; Bradley et al., 2009; Cloudsley-Thompson, 2001). In the only species for which the salinity tolerance mechanism is known, *H.* (*C.*) *salinarius*, adults maintain a hyposmotic haemolymph also with hyperosmotic excreta (Tones, 1978), with no evidence of any additional mechanism particular to this species. However, larvae of *H.* (*C.*) *salinarius* maintain a hyperosmotic haemolymph even at high saline concentrations (Tones, 1978), suggesting a different mechanism to that of adults. Although there is an increasing knowledge of the physiological basis of salinity tolerance in adult Coleoptera (e.g., Céspedes, Pallarés, Arribas, Millán, & Velasco, 2013; Pallarés, Arribas, Bilton, Millán, & Velasco, 2015; Pallarés, Arribas, Céspedes, Millán, & Velasco, 2012), the physiology of larval tolerance to salinity is still unknown. However, both the different characteristics of the cuticle (less sclerotised and without the protection of the elytra) and the biology (usually more strictly linked to the aquatic environment) suggest that the existence of different mechanisms to salinity tolerance in adults and larvae may be frequent.

In any case, it must be noted that we have data only on the ecological preferences of the species of Hygrotini, not on their physiological tolerances. If tolerance to salinity is an exaptation derived from a plesiomorphic adaptation to terrestrial environments (as hypothesised by Arribas et al., 2014; see Pallarés, Botella-Cruz, Arribas, Millán, & Velasco, 2017 for an experimental confirmation of the link between salinity and desiccation tolerance), tolerance to at least certain degree of salinity may be widespread even in species commonly found in FW habitats, as has been demonstrated to be the case in other groups of aquatic Coleoptera (Céspedes et al., 2013; Pallarés et al., 2015). Our ecological typification was also in most cases based on qualitative descriptions, without quantitative data. For some species there are few, if any, reports on their habitat, and in some cases we have assumed a FW habitat when the information was not very precise, as when species are found in saline or hypersaline habitats this is usually reported. In some cases qualitative reports can be ambiguous, such as for example when species are considered “halophile” or “halobiont” when they occur in slightly mineralised waters in a landscape otherwise lacking any true saline or hypersaline habitat, such as for example *Hygrotus*

(*Hygrotus*) *inaequalis* (Fabricius, 1777) or *H.* (*Coelambus*) *impressopunctatus* (Schaller, 1783) in some areas in central and northern Europe (e.g., Bellstedt, 2008).

In Hygrotini, the transitions to saline habitats were estimated to have occurred in different periods, from the late Eocene to the Plio- and Pleistocene, without a clear pattern of associations to arid periods, in contrast to what happened in the genus *Enochrus* in the Mediterranean region (Arribas et al., 2014). However, the oldest transition to saline habitats in Hygrotini, and the one leading to the higher number of saline species, occurred at the end of the Eocene in clade B4 in the Nearctic region, coincident with a global decrease in temperatures and the onset of the first Oligocene glaciations (Liu et al., 2009; Zachos, Pagani, Sloan, Thomas, & Billups, 2001). In North America, this decrease in temperature was associated with a decrease in precipitation (Retallack, 2007), leaving open the possibility that this transition to saline habitats was also a response to an increased aridification in this lineage.

ACKNOWLEDGEMENTS

We would like to thank all colleagues mentioned in Table S1 for sending valuable material for study, as well as Ana Izquierdo (MNCN), Rocío Alonso and Anabela Cardoso (IBE) for laboratory work. M. Manuel is grateful to Andriamirado T. Ramahandrison for help during field work in Madagascar, in the context of the RicciaTeam expedition led by Catherine Reeb. We also thank Josefa Velasco and Susana Pallarés (Universidad de Murcia) and Paula Arribas (Instituto de Productos Naturales y Agrobiología-CSIC) for comments on the origin and evolution of salinity tolerance, and three anonymous referees for their comments and suggestions. This work was partly funded by an FPI grant to A.V., and projects CGL2013-48950-C2-1-P and CGL2013-48950-C2-2-P (AEI/FEDER, UE) to I.R. and A.M., respectively.

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SUPPORTING INFORMATION

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How to cite this article: Villastrigo A, Fery H, Manuel M, Millán A, Ribera I. Evolution of salinity tolerance in the diving beetle tribe Hygrotini (Coleoptera, Dytiscidae). *Zool Scr*. 2018;47:63–71. https://doi.org/10.1111/zsc.12255

Evolution of salinity tolerance in the diving beetle tribe Hygrotini (Coleoptera, Dytiscidae)

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Material studied in the molecular study, with voucher numbers, locality data and accession numbers of the sequences.

Table S2. A) Primers used in the amplifying and sequencing reactions and B) standard PCR conditions for the amplification of the studied fragments.

Table S3. Checklist of the species of Hygrotini (following Nilsson & Hàjek 2017a, b) with the typical habitat and the species for which material could be studied. (AF, Afrotropical; H, Holarctic; NA, Nearctic; NT, Neotropical; OR, Oriental; PL, Palaearctic)

Fig. S1. Time-calibrated majority rule consensus tree obtained in BEAST with the complete dataset. Number in nodes: posterior probability values.

Fig. S2. Reconstructed evolution of salinity tolerance in the tribe Hygrotini, using parsimony with the estimated relationships of species for which no molecular data were available.

Evolution of salinity tolerance in the diving beetle tribe Hygrotini (Coleoptera, Dytiscidae)

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Table S1. Material studied in the molecular study, with voucher numbers, locality data and accession numbers of the sequences. In bold, newly obtained sequences.

ID	Genus	Subgenus	Species	voucher	Reduced	country	Loc.	leg	BAR	COI	16S	18S	H3	28S
1	*Heroceras*		*descarpentriesi*	IBE-AV58	Yes	Madagascar	Prov. Fianarantsoa / ca 4.5 km WSW Antanifotsy / Andringitra Massif. / S22°08'11.9" E46°51'55.5" / 2,127 m.	M. Manuel & Rahamandrison	**LT882848**	**LT882791**	**LT883018**	**LT882897**	**LT883058**	**LT882958**
2	*Herophydrus*		n. sp	IBE-AV61	Yes	Madagascar	Prov. Fianarantsoa / ca 4.5 km WSW Antanifotsy / Andringitra Massif. / S22°08'11.9" E46°51'55.5" / 2,127 m.	M. Manuel & Rahamandrison	**LT882851**	**LT882793**	**LT883020**	**LT882904**	**LT883068**	
3	*Herophydrus*		*assimilis*	GENBANK	Yes	Madagascar	Ref (1)			HQ382803	HQ381465			
4	*Herophydrus*		*guineensis*	MNCN-HI10	Yes	Algeria	Raml-Souk (puit) / N 36° 47.494' E 008° 31.326' / 101.19 m	S. Bouzid		**LT882823**	**LT883047**	**LT882898**	**LT883059**	**LT882959**
5	*Herophydrus*		*guineensis*	IBE-AV62	No	Namibia		D.J. Mann		**LT882794**				
6	*Herophydrus*		*inquinatus*	MNCN-DM44	Yes	South Africa	W Cape / Prince Albert Road / pond in junction R407 with N1	I. Ribera & A. Cieslak	**LT882884**	**LT882822**	**LT883046**	**LT882899**	**LT883060**	**LT882960**
7	*Herophydrus*		*inquinatus*	NHM-IR620	No	South Africa	W Cape / Wilderness NP, Swartvlei / Montmere, ditch by the lake	I. Ribera & A. Cieslak		**LT882828**	**LT883053**			
8	*Herophydrus*		*musicus*	MNCN-AI815	No	Turkey	SINOP / rd. 785 btw Saraydüzü & Boyabat / slow stream 5 km S of Yesilyurt / 440m / N41º23'42.5" E34º49'51"	I. Ribera		**LT882818**	**LT883043**		**LT883061**	
9	*Herophydrus*		*musicus*	NHM-IR36	No	Spain	Albacete, Hellin, Charca de los Patos	I. Ribera		AJ850634	AJ850384	AJ318731	EF670206	
10	*Herophydrus*		*musicus*	NHM-IR638	No	Gran Canaria (ES)	Moya, Bco. de Azuaje	I. Ribera & A. Cieslak		**LT882829**	**LT883054**	**LT882900**	**LT883062**	**LT882961**
11	*Herophydrus*		*musicus*	IBE-AV10	Yes	Crete (GR)	Crete Potamies	R.B. Angus	**LT882838**	**LT882776**	**LT883007**	**LT882901**	**LT883063**	**LT882962**
12	*Herophydrus*		*muticus*	MNCN-AI1220	Yes	Ethiopia	Shewa Prov. / 25 km N Addis Ababa, Solutta / 2800m	G. Wewalka	**LT882866**	**LT882811**	**LT883036**	**LT882902**	**LT883064**	**LT882963**
13	*Herophydrus*		*muticus*	IBE-RA704	No	Ethiopia	Shewa Prov. / 40-60 km N Addis Ababa / 2500m	G. Wewalka		**LT882804**	**LT883030**			
14	*Herophydrus*		*nigrescens*	IBE-AV32	Yes	South Africa	Kwazulu-Natal / iSimangalis Wetland Park / False Bay site 3 / Woodland pool	D.T. Bilton		**LT882781**			**LT883065**	
15	*Herophydrus*		*nodieri*	NHM-IR242	Yes	Namibia		D.J. Mann	**LT882886**	AJ850632	AJ850382	AJ850520	EF670204	
16	*Herophydrus*		*obscurus*	NHM-IR622	Yes	South Africa	W Cape / Du Toits Kloof, rd. N1 / pond and river Wit in resort	I. Ribera & A. Cieslak	**LT882891**	AJ850633	AJ850383	AJ850521	EF670205	**LT882964**
17	*Herophydrus*		*reticulatus*	IBE-AV60	Yes	Madagascar	Prov. Antananarivo / ca. 6 km WSW Manandona / Ibity Massif. / S20°04'18.4" E47°00'01" / Alt. 1,423 m.	M. Manuel & Rahamandrison	**LT882850**	**LT882792**	**LT883019**			**LT882965**
18	*Herophydrus*		*rohani*	GENBANK	Yes	Zambia	Ref (2)		KJ548554	KJ548554	KJ548382		KJ548799	
19	*Herophydrus*		sp	IBE-AV59	Yes	Madagascar	Prov. Antananarivo / ca. 13 km W Ambatolampy / Ankaratra Massif / S19°21'42.8" E47°18'53.8" / Alt. 1,737 m.	M. Manuel & Rahamandrison	**LT882849**				**LT883067**	
20	*Herophydrus*		*spadiceus*	GENBANK	Yes	Madagascar	Ref (1)			HQ383502				
21	*Herophydrus*		*tribolus*	IBE-RA703	Yes	Zambia	Northern Province / Kapisha / S011°10'09.4 E031°36'01.6 / 1440 m	R. Vila	**LT882856**	**LT882803**	**LT883029**	**LT882903**	**LT883066**	**LT882966**
22	*Herophydrus*		*verticalis*	GENBANK	Yes	Madagascar	Ref (1)			HQ382802	HQ381464			
23	*Hygrotus*	*Coelambus*	*ahmeti*	IBE-AV33	Yes	Turkey	Erzurum, Çat	Ö.K. Erman		**LT882782**	**LT883012**	**LT882905**	**LT883069**	
24	*Hygrotus*	*Coelambus*	*armeniacus*	IBE-RA544	Yes	Turkey	Erzurum / Toprakkaleköyü / slow stream in grassland / 2174 m / N40 14 22.9 E40 59 16.7	i. Ribera	**LT882855**	**LT882800**	**LT883026**	**LT882906**	**LT883071**	**LT882967**
25	*Hygrotus*	*Coelambus*	*armeniacus*	NHM-IR540	No	Turkey	Erzurum / ca. 14 Km S Tortum ca. 2 km S Guzelyayla pass / ponds on meadow	H. Fery		HF931283	HF931520		**LT883070**	
26	*Hygrotus*	*Coelambus*	*bruesi*	MNCN-AI983	Yes	US (CA)	Mono Co. / Long Valley, Owens river Rd. / tributary r. Owen	I. Ribera & A. Cieslak	**LT882881**	HF931259	HF931495	**LT882907**	**LT883072**	
27	*Hygrotus*	*Coelambus*	*caspius*	NHM-IR688	Yes	Russia		A.N. Nilsson	**LT882894**	AJ850635	AJ850385	AJ850522	EF670207	**LT882968**
28	*Hygrotus*	*Coelambus*	*caspius*	MNCN-AI963	No	Mongolia	Tov / Ondorshireet / Tuul River ~1.5 km W of Tuul Ovoot bridge, downstream of road to Arvayheer / N47.31096 E105.27119 / 1042 m	A.E.Z. Short		**LT882819**	**LT883044**			
29	*Hygrotus*	*Coelambus*	*compar*	GENBANK	Yes	US (WY)	Ref (2)		KJ548527	KJ548527	KJ548332		KJ548765	
30	*Hygrotus*	*Coelambus*	*confluens*	NHM-IR59	Yes	Portugal	Sa. Da Estrela, Torre / lagoon	I. Ribera	**LT882890**	AY250964	AJ850386	AJ318735	EF670208	**LT882969**
31	*Hygrotus*	*Coelambus*	*confluens*	IBE-RA914	No	Azores (Pot.)	Terceira / Guinjal, euthrophic lagoon / 38°41'44.6"N 27° 9'27.2"W / 390m	I. Ribera & A. Cieslak		**LT882807**	**LT883032**			
32	*Hygrotus*	*Coelambus*	*corpulentus*	NHM-IR687	Yes	Russia	Astrakhan oblast / Pirogovka / steppe lake NW of village	A.N. Nilsson	**LT882893**	AJ850637	AJ850387	AJ850523	EF670209	**LT882970**
33	*Hygrotus*	*Coelambus*	*diversipes*	IBE-AV25	Yes	US (CA)	Soda Lake (near Baker) / 35º9'19''N,116º6'22''W	P. Abellán	**LT882842**	**LT882780**	**LT883011**	**LT882908**	**LT883073**	**LT882971**
34	*Hygrotus*	*Coelambus*	*diversipes*	GENBANK	No	US (WY)	Ref (2)			KJ548528	KJ548333		KJ548766	
35	*Hygrotus*	*Coelambus*	*flaviventris*	MNCN-AI964	Yes	Mongolia	Tov / Ondorshireet / Tuul River ~1.5 km W of Tuul Ovoot bridge, downstream of road to Arvayheer / N47.31096 E105.27119 / 1042 m	A.E.Z. Short	**LT882877**	HF931251	HF931487	**LT882909**	**LT883074**	
36	*Hygrotus*	*Coelambus*	*flaviventris*	NHM-IR685	No	Russia		A.N. Nilsson		**LT882831**	**LT883056**			
37	*Hygrotus*	*Coelambus*	*fontinalis*	MNCN-AI982	Yes	US (CA)	Mono Co. / Long Valley, Owens river Rd. / tributary r. Owen	I. Ribera & A. Cieslak	**LT882880**	HF931258	HF931494	**LT882910**	**LT883075**	
38	*Hygrotus*	*Coelambus*	*fontinalis*	IBE-AV64	No	US (CA)	Mono Co. / big alkali lake at Benton crossing rd	G. Challet		**LT882795**	**LT883021**			
39	*Hygrotus*	*Coelambus*	*fresnedai*	MNCN-AC19	No	Spain	Segovia / ponds beside cross rd between Abades-Otero de los Herreros SG723 and Segovia-Villacastin N110.	R.B. Angus	HF947982	HF931108	HF931325			
40	*Hygrotus*	*Coelambus*	*fresnedai*	IBE-AN36	Yes	Spain	Guadalajara / El Pobo de Dueñas / pond / N40º47'5.0" W1º38'30.3"	I. Ribera & A. Cieslak	**LT882835**	**LT882773**	**LT883004**	**LT882911**	**LT883076**	**LT882972**
41	*Hygrotus*	*Coelambus*	*fumatus*	IBE-AV23	Yes	US (CA)	Mendocino Co. / Rd. 1 Manchester / pond S City	I. Ribera & A. Cieslak	**LT882840**	**LT882778**	**LT883009**	**LT882912**	**LT883077**	**LT882973**
42	*Hygrotus*	*Coelambus*	*impressopunctatus*	MNCN-AI1162	No	Canada (AL)		T. Berendonk		**LT882810**	**LT883035**		**LT883078**	
43	*Hygrotus*	*Coelambus*	*impressopunctatus*	NHM-IR489	No	Canada (AL)	Rd. 22, 5 km S Longview	I. Ribera & A. Cieslak		**LT882827**	**LT883052**			
44	*Hygrotus*	*Coelambus*	*impressopunctatus*	NHM-IR603	No	Corsica (FR)	Cassamozza: r. l'Abatescu	I. Ribera & A. Cieslak		AJ850653	AJ850405	AJ850537/EF670302	EF670210	
45	*Hygrotus*	*Coelambus*	*impressopunctatus*	IBE-RA270	Yes	Ireland	Gortlecka / Lough Gealain, turlough / N52º59'52.2" W9º01'28.3"	I. Ribera	**LT882854**	**LT882799**	**LT883025**	**LT882913**	**LT883079**	**LT882974**
46	*Hygrotus*	*Coelambus*	*impressopunctatus*	IBE-RA1205	No	Kyrgyzstan	Son Kol Lake area / rheohelocrenic spring 2 / 3069 m / 41º55.720' N, 75º12.057' E	Pešić		**LT882796**	**LT883022**			**LT882975**
47	*Hygrotus*	*Coelambus*	*laccophilinus*	NHM-IR473	No	Canada (ON)	Manitoulin island, Ck. At Hwy 540, 20 km E Little Curreny	Y. Alarie		**LT882825**	**LT883049**	**LT882915**	**LT883081**	
48	*Hygrotus*	*Coelambus*	*laccophilinus*	MNCN-AI1125	Yes	Canada (ON)	Manitoulin island, Ck. At Hwy 540, 20 km E Little Curreny	Y. Alarie	**LT882862**	HF931163	HF931382	**LT882914**	**LT883080**	**LT882976**
49	*Hygrotus*	*Coelambus*	*lagari*	MNCN-AI85	No	Spain	Zaragoza /Chiprana pond rd. S. Marcos / 41°14'30"N 0°09'27" W / 120m	I. Ribera & A. Cieslak		HF931236	HF931465		**LT883082**	
50	*Hygrotus*	*Coelambus*	*lagari*	MNCN-AI490	Yes	Spain	Guadalajara /El Pobo de Dueñas / pond in cross N211-CM2112 / N40º47'05.6" W1º38'30.1"	I. Ribera & A. Cieslak	**LT882869**	**LT882813**	**LT883038**	**LT882916**	**LT883083**	**LT882977**
51	*Hygrotus*	*Coelambus*	*lernaeus*	IBE-AN68	Yes	Azerbaijan	Qobustan / ponds in beach / 40°5'38.9"N 49°25'19.5"E /26m	I. Ribera & A. Rudoy	**LT882837**	**LT882775**	**LT883006**	**LT882917**	**LT883084**	**LT882978**
52	*Hygrotus*	*Coelambus*	*lutescens*	NHM-IR519	Yes	Canada (BC)	B.C. / Rd. 5A, Stump Lake / ponds by road	I. Ribera & A. Cieslak	**LT882889**	HF931281	HF931518	**LT882918**	**LT883085**	**LT882979**
53	*Hygrotus*	*Coelambus*	*marklini*	MNCN-AI89	Yes	Mongolia	Zavkhan Aimag, Telmen Soum,Ideriin Gol ~15 km SSW of Telmen / Ovogdii / N48.53255,E97.52093 / 1823 m	A.E.Z. Short	**LT882876**	HF931237	HF931467	**LT882919**	**LT883086**	
54	*Hygrotus*	*Coelambus*	*marklini*	NHM-IR682	No	Russia	Volgograd oblast / SW Lake of Elton / steppe pond with much vegetation	A.N. Nilsson		**LT882830**	**LT883055**			
55	*Hygrotus*	*Coelambus*	*masculinus*	NHM-IR290	No	US (CA)	Mono Co. / Long Valley, Owens river Rd. / saline lagoons	I. Ribera & A. Cieslak		HF931273	HF931510			
56	*Hygrotus*	*Coelambus*	*masculinus*	IBE-AV24	Yes	US (CA)	Mono Co. / stream outlet of big alkali lake at Benton crossing road	G. Challet	**LT882841**	**LT882779**	**LT883010**	**LT882920**	**LT883087**	**LT882980**
57	*Hygrotus*	*Coelambus*	*nigrescens*	MNCN-AI1152	Yes	Canada (AL)		T. Berendonk	**LT882864**	HF931167	HF931386	**LT882921**	**LT883088**	**LT882981**
58	*Hygrotus*	*Coelambus*	*nigrescens*	IBE-RA878	No	Canada (AL)	N53º39.346 W110º58.922	T. Berendonk	**LT882858**	**LT882806**				
59	*Hygrotus*	*Coelambus*	*nigrolineatus*	MNCN-AI970	Yes	Mongolia	Arkhangay / Bulgan / Urd Tamir Gol braid upstream of bridge, ~63 km SW of Tsetserleg / N47.11192 E101.01048 / 2066 m	A.E.Z. Short	**LT882879**	HF931254	HF931490	**LT882922**	**LT883089**	
60	*Hygrotus*	*Coelambus*	*novemlineatus*	NHM-IR714	Yes	UK (SC)	E. Sutherland, Loch Brora	I. Ribera	**LT882896**	HF931286	HF931523	**LT882923**	**LT883090**	**LT882982**
61	*Hygrotus*	*Coelambus*	*orthogrammus*	IBE-RA701	Yes	Iran	Khuzestan / 5 km SW Bandare Emam, -21 m / 30 5714N 49 0524E	Darvishzadej		**LT882802**	**LT883028**	**LT882924**	**LT883091**	**LT882983**
62	*Hygrotus*	*Coelambus*	*pallidulus*	MNCN-AI216	Yes	Spain	Alcolea de las Peñas / arroyo salino / 30TWL 17757/62735 / 992 m / Sal: 37,1 gr/l	A. Millán & col.	**LT882868**	HF931184	HF931407	**LT882925**	**LT883092**	
63	*Hygrotus*	*Coelambus*	*parallellogrammus*	MNCN-AI145	Yes	Germany	Mecklenburg-Vorpom. Hiddensee, N Witte St. 4 013º06'26"E 54º34'4"N	L. Hendrich	**LT882867**	**LT882812**	**LT883037**	**LT882926**	**LT883093**	
64	*Hygrotus*	*Coelambus*	*parallellogrammus*	NHM-IR683	No	Russia	Astrakhan oblast / Nishniy Baskuntyak / small stream from small lake at entrance to Bogdo Zapovednik	A.N. Nilsson		HF931284	HF931521			
65	*Hygrotus*	*Coelambus*	*patruelis*	MNCN-AI1163	No	Canada (AL)		T. Berendonk		HF931169	HF931388		**LT883094**	

66	*Hygrotus*	*Coelambus*	*patruelis*	IBE-AV53	Yes	Canada		T. Berendonk	**LT882845**	**LT882787**	**LT883015**	**LT882927**	**LT883095**	
67	*Hygrotus*	*Coelambus*	*pectoralis*	MNCN-AI1101	Yes	Mongolia	Zavkhan / Tosontsengel / Ideriin Gol ~15 km SSW of Telmen/Ovogdii / N48.53255 E97.52093 / 1823 m	A.E.Z. Short	**LT882861**	HF931157	HF931376	**LT882928**	**LT883096**	
68	*Hygrotus*	*Coelambus*	*pedalis*	MNCN-AI985	Yes	US (CA)	Mono Co. / Long Valley, Owens river Rd. / tributary r. Owen	I. Ribera & A. Cieslak	**LT882882**	**LT882821**	**LT883045**	**LT882929**	**LT883097**	**LT882984**
69	*Hygrotus*	*Coelambus*	*pedalis*	IBE-AV55	No	US (CA)	Amargosa River in Dumont Dunes / 35º41'45''N,116º15'5''W	P. Abellán	**LT882846**	**LT882789**			**LT883098**	
70	*Hygrotus*	*Coelambus*	*pedalis*	IBE-AV57	No	US (CA)	Coal Oil Point Reserve (Slough road) / 34º24'47''N,119º52'31''W	P. Abellán			**LT883016**			
71	*Hygrotus*	*Coelambus*	*picatus*	NHM-IR474	No	Canada (ON)	Manitoulin island, Ck. At Hwy 540, 20 km E Little Curreny	Y. Alarie			**LT883050**			
72	*Hygrotus*	*Coelambus*	*picatus*	NHM-IR481	Yes	Canada (AL)	Waterton lakes Nat. Park / junc. Rd. 5&6	I. Ribera & A. Cieslak	**LT882887**	HF931280	HF931517	**LT882930**	**LT883099**	**LT882985**
73	*Hygrotus*	*Coelambus*	*saginatus*	NHM-IR684	Yes	Russia	Astrakhan oblast / Kharabali / flooded margin of Volga just S of city	A.N. Nilsson	**LT882892**	AJ850639	AJ850389	AJ850524	EF670211	**LT882986**
74	*Hygrotus*	*Coelambus*	*saginatus* nr	IBE-AV43	No	Cyprus	Paralimni / ditch with reed / 35º02'22.7"N 33º57'38.6"E / 81m	A. Millán, I. Ribera, J. Velasco & A. Villastrigo		**LT882784**	**LT883014**	**LT882931**	**LT883100**	
75	*Hygrotus*	*Coelambus*	*saginatus* nr	IBE-AN67	Yes	Azerbaijan	Qobustan / ponds in beach / 40°5'38.9"N 49°25'19.5"E /26m	I. Ribera & A. Rudoy	**LT882836**	**LT882774**	**LT883005**	**LT882932**	**LT883101**	**LT882987**
76	*Hygrotus*	*Coelambus*	*salinarius*	GENBANK	Yes	US (WY)	Ref (2)		KJ548531	KJ548531	KJ548335		KJ548769	
77	*Hygrotus*	*Coelambus*	*sanfilippoi*	IBE-AF204	Yes	Sardinia (IT)	Sassari prov. / S Stintino (NW Porto Torres) / Stagno di Casaraccio (brakish pond, 0 m) / 40.91403N 8.23021E	H. Fery & M. Toledo	**LT882834**	HF931123	HF931342	**LT882933**	**LT883102**	**LT882988**
78	*Hygrotus*	*Coelambus*	*semivittatus*	GENBANK	Yes	US (WY)	Ref (3)			KF575509	KF575573		KF575392	
79	*Hygrotus*	*Coelambus*	*suturalis*	NHM-IR482	Yes	Canada (AL)	Waterton lakes Nat. Park / junc. Rd. 5&6	I. Ribera & A. Cieslak	**LT882888**	AJ850640	AJ850390	AJ850525	EF670212	**LT882991**
80	*Hygrotus*	*Coelambus*	*suturalis*	IBE-AV54	No	Canada		T. Berendonk		**LT882788**				
81	*Hygrotus*	*Coelambus*	*tumidiventris*	MNCN-AI984	No	US (CA)	Mono Co. / Long Valley, Owens river Rd. / tributary r. Owen	I. Ribera & A. Cieslak		HF931260	HF931496	**LT882934**	**LT883103**	
82	*Hygrotus*	*Coelambus*	*tumidiventris*	IBE-AV22	Yes	US (CA)	Mono Co. / Long Valley, Owens river Rd. / saline lagoons	I. Ribera & A. Cieslak	**LT882839**	**LT882777**	**LT883008**	**LT882935**	**LT883104**	**LT882989**
83	*Hygrotus*	*Coelambus*	*turbidus*	NHM-IR346	No	US (CA)	Mono Co. / Yosemite Ntal. Park / Rd. 120 Dana Meadows	I. Ribera & A. Cieslak		**LT882824**	**LT883048**			
84	*Hygrotus*	*Coelambus*	*turbidus*	IBE-AV56	Yes	US (CA)	Amargosa River in Dumont Dunes / 35º41'45''N,116º15'5''W	P. Abellán	**LT882847**	**LT882790**	**LT883017**	**LT882936**	**LT883105**	**LT882990**
85	*Hygrotus*	*Coelambus*	*unguicularis*	MNCN-AI968	Yes	Mongolia	Arkhangay / Bulgan / Urd Tamir Gol braid upstream of bridge, ~63 km SW of Tsetserleg / N47.11192 E101.01048 / 2066 m	A.E.Z. Short	**LT882878**	HF931253	HF931489	**LT882937**	**LT883106**	**LT882992**
86	*Hygrotus*	*Coelambus*	*unguicularis*	NHM-IR479	No	Canada (AL)	Waterton lakes Nat. Park / junc. Rd. 5&6	I. Ribera & A. Cieslak		**LT882826**	**LT883051**			
87	*Hygrotus*	*Coelambus*	*urgensis*	MNCN-AI962	Yes	Mongolia	Tov / Ondorshireet / Tuul River ~1.5 km W of Tuul Ovoot bridge, downstream of road to Arvayheer / N47.31096 E105.27119 / 1042 m	A.E.Z. Short		HF931250	HF931486	**LT882938**	**LT883107**	**LT882993**
88	*Hygrotus*	*Hygrotus*	*acaroides*	GENBANK	Yes	US (AL)	Ref (3)		KF575482	KF575482	KF575542		KF575361	
89	*Hygrotus*	*Hygrotus*	*decoratus*	MNCN-AI841	Yes	UK (EN)	Norfolk / Ludham / How Hill Marsh	I. Ribera	**LT882875**	HF931235	HF931464	**LT882939**	**LT883108**	**LT882994**
90	*Hygrotus*	*Hygrotus*	*decoratus*	NHM-IR711	No	Austria	Voralberg / Rheintal, Bodensee. Hard Rheinvorland / 400 m / N47º29'48" E 9º39'59"	I. Ribera		**LT882832**	**LT883057**			
91	*Hygrotus*	*Hygrotus*	*hydropicus*	IBE-AV51	Yes	US (CA)	Monterrey Co., 35.9518ºN 121.3017ºW / Nacimiento-Ferguson rd.	M. Caterino & A.E.Z. Short	**LT882844**	**LT882785**		**LT882940**	**LT883109**	**LT882995**
93	*Hygrotus*	*Hygrotus*	*inaequalis*	NHM-IR19	No	Spain	Girona, Capmany, Estanys inferior	I. Ribera		EF056611	EF056681	AJ318737		**LT882996**
94	*Hygrotus*	*Hygrotus*	*inaequalis*	IBE-RA208	Yes	Greece	NE Peloponnese, Stymfalia Lake / open swamps, ca. 6 km ENE Lafka, net & bottle traps / 37.84411N 22.45478E / 610 m	H. Fery & L. Hendrich	**LT882853**	**LT882798**	**LT883024**	**LT882941**	**LT883110**	**LT882997**
95	*Hygrotus*	*Hygrotus*	*quinquelineatus*	MNCN-AI979	No	Mongolia	Zavkhan / Tosontsengel / Ideriin Gol ~15 km SSW of Telmen/Ovogdii / N48.53255 E97.52093 / 1823 m	A.E.Z. Short		**LT882820**				
96	*Hygrotus*	*Hygrotus*	*quinquelineatus*	MNCN-DM4	Yes	Sweden	Västerbotten prov. / Åmsele / Vindelälven river lagoon / 64º31'04''N, 19º20'52''E	A.N. Nilsson	**LT882883**	HF931268	HF931506	**LT882942**	**LT883111**	**LT882998**
97	*Hygrotus*	*Hygrotus*	*sayi*	MNCN-AI1149	Yes	US (NY)	Tompkins Co. / Marsh on Elllis Hollow Road, 4 mi. E of Pine Tree Road	A.E.Z. Short	**LT882863**	HF931164	HF931383	**LT882943**	**LT883112**	**LT882999**
98	*Hygrotus*	*Hygrotus*	*sayi*	IBE-AV52	No	Canada		T. Berendonk		**LT882786**				
99	*Hygrotus*	*Hygrotus*	*versicolor*	NHM-IR691	Yes	Austria	Voralberg, Pfänderstock, Bodensee	I. Ribera	**LT882895**	HF931285	HF931522	**LT882944**	**LT883113**	**LT883000**
100	*Hyphoporus*		*solieri*	IBE-RA700	Yes	Iran	Khuzestan / 8 km SW Abadan, 8 m / 30 3541N 48 2267E / pond	Darvishzadej		**LT882801**	**LT883027**	**LT882945**	**LT883114**	
101	*Hyphoporus*		*tonkinensis*	IBE-RA769	Yes	China	Yunnan Xin ping Mamushu / cun 1127m 2011.v.20 / N24.02121 E102.12882	S. Keqing	**LT882857**	**LT882805**	**LT883031**	**LT882946**	**LT883115**	**LT883001**
102	*Hydroporus*		*basinotatus*	MNCN-AH112	No	Spain	Cádiz, Tarifa / stream 2K N Na. Sra de la Luz / 10m / N36º05'53.4" W5º37'35.0"	I. Ribera & P. Aguilera & C. Hernando	**LT882859**	HE610196	**LT883033**	**LT882955**	**LT883124**	
103	*Hydroporus*		*kasyi*	IBE-AV42	No	Cyprus	Akrotiri / Fassouri reedbeds / 34º37'57.2"N 32º56'01.5"E / 28m / Millán,Ribera,Velasco&Villastrigo leg.	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT882843**	**LT882783**	**LT883013**	**LT882957**	**LT883126**	
104	*Hydroporus*		*obsoletus*	IBE-RA206	No	Greece	Peloponnese / Afrodisio Mts / ca. 5 km NE Kondovazena / small spring / ca. 37.82044N 21.93570E / ca. 1098 m	H. Fery & L. Hendrich	**LT882852**	**LT882797**	**LT883023**	**LT882954**	**LT883123**	
105	*Hydroporus*		*tessellatus*	MNCN-AI770	No	Turkey	DÜZCE / Rd. to Kartalkaya from Çaydurt / small stream with vegetation / 1191m / N40º42'29" E31º45'56"	I. Ribera	**LT882873**	**LT882817**	**LT883042**	**LT882956**	**LT883125**	
106	*Deronectes*		*ferrugineus*	MNCN-AI731	No	Portugal	Guarda district / Sabugueiro (Serra da Estrela)	I. Ribera	LN995103	LN995072	LN995176	**LT882949**	LN995142	
107	*Deronectes*		*opatrinus*	MNCN-AI629	No	Spain	Córdova province / Sierra Morena	A. Castro	LN995112	HE610188	LN995185	**LT882948**	LN995151	
108	*Deronectes*		*sahlbergi*	MNCN-AI1002	No	Bulgaria	Haskovo province / Madjarovo (Eastern Rhodope mountains)	Pešić	**LT882860**	HE610191	HF931361	**LT882947**	**LT883116**	
109	*Boreonectes*		*funereus*	MNCN-AI1208	No	US (CA)		Y. Alarie	**LT882865**	HF931173	HF931393	**LT882950**	**LT883119**	
110	*Boreonectes*		*ibericus*	NHM-IR22	No	Portugal	Sa. Da Estrela, Torre, lagoon	I. Ribera	**LT882885**	EF670064	EF670030	EF670271/EF670298	EF670157	
111	*Boreonectes*		*riberae*	MNCN-AI829	No	Turkey	DÜZCE / Rd. to Kartalkaya from Çaydurt / pools in mountain pass / 1700m / N40º40'20" E31º47'05"	I. Ribera	**LT882874**	HF931232	HF931461	**LT882951**	LT796550	
112	*Nebrioporus*		*amicorum*	IBE-AF140	No	Crete (GR)	Heraklion, Arkalohori, Tsoutsouros / stream 34°59'05'' 25°17'07'	Z. Csabai et al.	**LT882833**	HF931118	HF931335	**LT882953**	**LT883122**	**LT883003**
113	*Nebrioporus*		*martinii*	MNCN-AI702	No	Corsica (FR)	Corte, river Restonica, 2	I. Ribera & A. Cieslak	**LT882871**	**LT882815**	**LT883040**	**LT882952**	**LT883121**	**LT883002**
114	*Lacconis*		*difformis*	GENBANK	No	US (NY)	Ref (3)			KF575484	KF575544		KF575363	
115	*Laccornis*		*kocae*	NHM-IR681	No	Russia	Volgograd oblast / SW Lake of Elton / steppe pond with much vegetation	A.N. Nilsson		AJ850666	AJ850419	AJ850546	EF670226	
116	*Lacconis*		*oblongus*	NHM-IR55	No	Scotland (UK)	Mount Bog / Peebles	D.T. Bilton		AF309298	AF309241	AJ318715	EF056579	

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2 Miller, K. B., & Bergsten, J. (2014). The phylogeny and classification of predaceous diving beetles. In Ecology, systematics, and the natural history of predaceous diving beetles (Coleoptera: Dytiscidae) (pp. 49-172). Springer Netherlands.

3 Miller, K., Jean, A., Alarie, Y., Hardy, N., & Gibson, R. (2013). Phylogenetic placement of North American subterranean diving beetles (Insecta: Coleoptera: Dytiscidae). Arthropod Systematics & Phylogeny, 71, 75-90.

Table S2:

A) Primers used in the amplifying and sequencing reactions.

gene	primer	sequence	ref.
COI-3’	Jerry (5')	CAACATTTATTTTGATTTTTTGG	6
	Pat (3')	TCCAATGCACTAATCTGCCATATTA	6
	Chy (5')	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	4
	Tom (3')	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	4
COI-5’	Uni LepF1b	TAATACGACTCACTATAGGGATTCAACCAATCATAAAGATATTGGAAC	2
	Uni LepR1	ATTAACCCTCACTAAAGTAAACTTCTGGATGTCCAAAAAATCA	2
16S+trnL+nad1 / 16S	16SaR (5')	CGCCTGTTTAACAAAAACAT	6
	ND1 (3')	GGTCCCTTACGAATTTGAATATATCCT	6
	16Sb	CCGGTCTGAACTCAGATCATGT	6
18S	18S 5'	GACAACCTGGTTGATCCTGCCAGT(1)	5
	18S b5.0	TAACCGCAACAACTTTAAT(1)	5
H3	H3aF (5')	ATGGCTCGTACCAAGCAGACRCG	1
	H3aR (3')	ATATCCTTRGGCATRATRGTGAC	1
28S	ka	ACACGGACCAAGGAGTCTAGCATG	3
	kb	CGTCCTGCTGTCTTAAGTTAC	3

B) Standard PCR conditions for the amplification of the studied fragments.

step	time	temperature
1	3’	96º
2	30”	94º
3	30”-1’	47-50º *
4	1’	72º
5	Go to step 2 and repeat 34-40 x	
6	10’	72º

* Depending on the annealing temperatures of the primers pair used

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Evolution of salinity tolerance in the diving beetle tribe Hygrotini (Coleoptera, Dytiscidae)

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Table S3

Checklist of the species of Hygrotini (following Nilsson & Hàjek 2017a,b) with the typical habitat and the species for which material could be studied.

AF, Afrotropical; H, Holarctic; NA, Nearctic; NT, Neotropical; OR, Oriental; PL, Palaearctic

ID	Genus	Subgenus	Species	Authors	Distribution	habitat	data type studied
1	*Heroceras*		*descarpentriesi*	(Peschet, 1923)	AF	?	morphological & molecular
2	*Herophydrus*		*assimilis*	Régimbart, 1895	AF	?	morphological & molecular
3	*Herophydrus*		*bilardoi*	Biström & Nilsson, 2002	AF	?	only literature
4	*Herophydrus*		*capensis*	Régimbart, 1895	AF	?	only literature
5	*Herophydrus*		*cleopatrae*	(Peyron, 1858)	PL	?	morphological
6	*Herophydrus*		*confusus*	Régimbart, 1895	AF	?	only literature
7	*Herophydrus*		*discrepatus*	Guignot, 1954	AF	Tolerant	only literature
8	*Herophydrus*		*endroedyi*	Biström & Nilsson, 2002	AF	Freshwater	only literature
9	*Herophydrus*		*gigantoides*	Biström & Nilsson, 2002	AF	?	only literature
10	*Herophydrus*		*gigas*	Régimbart, 1895	AF	Freshwater	morphological
11	*Herophydrus*		*goldschmidti*	Pedernazi & Rocchi, 2009	AF	?	only literature
12	*Herophydrus*		*gschwendtneri*	Omer-Cooper, 1957	AF	?	only literature
13	*Herophydrus*		*guineensis*	(Aubé, 1838)	AF, PL	Freshwater	morphological & molecular
14	*Herophydrus*		*heros*	Sharp, 1882	AF	Freshwater	morphological
15	*Herophydrus*		*hyphoporoides*	Régimbart, 1895	AF	?	only literature
16	*Herophydrus*		*ignoratus*	Gschwendtner, 1933	AF	?	only literature
17	*Herophydrus*		*inquinatus*	(Boheman, 1848)	AF	Freshwater	morphological & molecular
18	*Herophydrus*		*janssensi*	Guignot, 1952	AF	?	only literature
19	*Herophydrus*		*kalaharii*	Gschwendtner, 1935	AF	?	only literature
20	*Herophydrus*		*morandi*	Guignot, 1952	OR	?	only literature
21	*Herophydrus*		*musicus*	(Klug, 1834)	AF, OR, PL	Tolerant	morphological & molecular
22	*Herophydrus*		*muticus*	(Sharp, 1882)	AF	Freshwater	morphological & molecular
23	*Herophydrus*		*natator*	Biström & Nilsson, 2002	AF	?	only literature
24	*Herophydrus*		*nigrescens*	Biström & Nilsson, 2002	AF	Tolerant	morphological & molecular
25	*Herophydrus*		*nodieri*	(Régimbart, 1895)	AF	Freshwater	morphological & molecular
26	*Herophydrus*		*obscurus*	Sharp, 1882	AF	Tolerant	morphological & molecular
27	*Herophydrus*		*obsoletus*	Régimbart, 1895	AF	?	only literature
28	*Herophydrus*		*ovalis*	Gschwendtner, 1932	AF	?	only literature
29	*Herophydrus*		*pallidus*	Omer-Cooper, 1931	AF	?	only literature
30	*Herophydrus*		*pauliani*	Guignot, 1950	AF	?	only literature
31	*Herophydrus*		*quadrilineatus*	Régimbart, 1895	AF	?	morphological
32	*Herophydrus*		*reticulatus*	Pederzani & Rocchi, 2009	AF	Freshwater	morphological & molecular
33	*Herophydrus*		*ritsemae*	Régimbart, 1889	AF	Freshwater	only literature
34	*Herophydrus*		*rohani*	Peschet, 1924	AF	Freshwater	molecular
35	*Herophydrus*		*rufus*	(Clark, 1863)	AF	?	morphological
36	*Herophydrus*		*sjostedti*	Régimbart, 1908	AF	?	only literature
37	*Herophydrus*		*spadiceus*	Sharp, 1882	AF	Tolerant	molecular
38	*Herophydrus*		*sudanensis*	Guignot, 1952	AF	Freshwater	only literature
39	*Herophydrus*		*travniceki*	Šťastný, 2012	AF	?	morphological
40	*Herophydrus*		*tribolus*	Guignot, 1953	AF	Freshwater	morphological & molecular
41	*Herophydrus*		*variabilis secundus*	Régimbart, 1906	AF	?	only literature
42	*Herophydrus*		*variabilis variabilis*	Guignot, 1954	AF	?	only literature
43	*Herophydrus*		*vaziranii*	(Nilsson, 1999)	PL	?	only literature
44	*Herophydrus*		*verticalis*	Sharp, 1882	AF	Freshwater	morphological & molecular
45	*Herophydrus*		*vittatus*	Régimbart, 1895	AF	?	only literature
46	*Herophydrus*		*wewalkai*	Biström & Nilsson, 2002	AF	?	only literature
47	*Hygrotus*	*Coelambus*	*ahmeti*	Hájek, Fery & Erman, 2005	PL	Freshwater	morphological & molecular
48	*Hygrotus*	*Coelambus*	*armeniacus*	(Zaitzev, 1927)	PL	Tolerant	morphological & molecular
49	*Hygrotus*	*Coelambus*	*artus*	(Fall, 1919)	NA	Tolerant (2)	only literature
50	*Hygrotus*	*Coelambus*	*berneri*	Young & Wolfe, 1984	NA	?	morphological
51	*Hygrotus*	*Coelambus*	*bruesi*	(Fall, 1928)	NA	Tolerant	morphological & molecular
52	*Hygrotus*	*Coelambus*	*caspius*	(Wehncke, 1875)	PL	Freshwater	morphological & molecular
53	*Hygrotus*	*Coelambus*	*chinensis*	(Sharp, 1882)	PL	?	morphological
54	*Hygrotus*	*Coelambus*	*collatus*	(Fall, 1919)	NA	?	morphological
55	*Hygrotus*	*Coelambus*	*compar*	(Fall, 1919)	NA	Tolerant	morphological & molecular
56	*Hygrotus*	*Coelambus*	*confluens*	(Fabricius, 1787)	PL	Tolerant	morphological & molecular
57	*Hygrotus*	*Coelambus*	*corpulentus*	(Schaum, 1864)	PL	?	morphological & molecular
58	*Hygrotus*	*Coelambus*	*curvilobus*	Fery, Sadeghi & Hosseinie, 2005	PL	?	morphological
59	*Hygrotus*	*Coelambus*	*curvipes*	(Leech, 1938)	NA	?	morphological
60	*Hygrotus*	*Coelambus*	*dissimilis*	(Geminger & Harold, 1868)	NA	?	morphological
61	*Hygrotus*	*Coelambus*	*diversipes*	Leech, 1966	NA	Hypersaline	morphological & molecular
62	*Hygrotus*	*Coelambus*	*enneagrammus*	(Ahrens, 1833)	PL	Tolerant	morphological
63	*Hygrotus*	*Coelambus*	*falli*	(Wallis, 1924)	NA	?	morphological
64	*Hygrotus*	*Coelambus*	*femoratus*	(Fall, 1901)	NA	?	only literature
65	*Hygrotus*	*Coelambus*	*flaviventris*	(Motschulsky, 1860)	PL	Tolerant	morphological & molecular
66	*Hygrotus*	*Coelambus*	*fontinalis*	Leech, 1966	NA	Hypersaline	morphological & molecular
67	*Hygrotus*	*Coelambus*	*fraternus*	(LeConte, 1852)	NA	?	morphological
68	*Hygrotus*	*Coelambus*	*fresnedai*	(Fery, 1992)	PL	Freshwater (3)	morphological & molecular
69	*Hygrotus*	*Coelambus*	*fumatus*	(Sharp, 1882)	NA	Freshwater	morphological & molecular
70	*Hygrotus*	*Coelambus*	*impressopunctatus*	(Schaller, 1783)	H	Freshwater (3)	morphological & molecular
71	*Hygrotus*	*Coelambus*	*infuscatus*	(Sharp, 1882)	NA	Tolerant	morphological
72	*Hygrotus*	*Coelambus*	*inscriptus*	(Sharp, 1882)	PL	Hypersaline	morphological
73	*Hygrotus*	*Coelambus*	*laccophilinus*	(LeConte, 1878)	NA	Freshwater	morphological & molecular
74	*Hygrotus*	*Coelambus*	*lagari*	(Fery, 1992)	PL	Tolerant	morphological & molecular
75	*Hygrotus*	*Coelambus*	*lernaeus*	(Schaum, 1857)	PL	Tolerant	morphological & molecular
76	*Hygrotus*	*Coelambus*	*lutescens*	(LeConte, 1852)	NA	Tolerant	morphological & molecular

77	*Hygrotus*	*Coelambus*	*marklini*	(Gyllenhal, 1813)	H	Tolerant (4)	morphological & molecular
78	*Hygrotus*	*Coelambus*	*masculinus*	(Crotch, 1874)	NA	Hypersaline	morphological & molecular
79	*Hygrotus*	*Coelambus*	*nigrescens*	(Fall, 1919)	NA	Freshwater	morphological & molecular
80	*Hygrotus*	*Coelambus*	*nigrolineatus*	(Steven, 1808)	PL	Tolerant	morphological & molecular
81	*Hygrotus*	*Coelambus*	*novemlineatus*	(Stephens, 1829)	H	Tolerant	morphological & molecular
82	*Hygrotus*	*Coelambus*	*nubilus*	(LeConte, 1855)	NA, NT	?	morphological
83	*Hygrotus*	*Coelambus*	*obscureplagiatus*	(Fall, 1919)	NA	?	morphological
84	*Hygrotus*	*Coelambus*	*orthogrammus*	(Sharp, 1882)	PL	Tolerant	morphological & molecular
85	*Hygrotus*	*Coelambus*	*pallidulus*	(Aubé, 1850)	PL	Hypersaline (1)	morphological & molecular
86	*Hygrotus*	*Coelambus*	*parallellogrammus*	(Ahrens, 1812)	PL	Tolerant	morphological & molecular
87	*Hygrotus*	*Coelambus*	*patruelis*	(LeConte, 1855)	NA	Tolerant	morphological & molecular
88	*Hygrotus*	*Coelambus*	*pectoralis*	(Motschulsky, 1860)	PL	Tolerant	morphological & molecular
89	*Hygrotus*	*Coelambus*	*pedalis*	(Fall, 1901)	NA	Tolerant	morphological & molecular
90	*Hygrotus*	*Coelambus*	*picatus*	(Kirby, 1837)	NA	Freshwater	morphological & molecular
91	*Hygrotus*	*Coelambus*	*polonicus polonicus*	(Aubé, 1842)	PL	Tolerant	morphological
92	*Hygrotus*	*Coelambus*	*polonicus sahlbergi*	(Sharp, 1882)	PL	Tolerant	morphological
93	*Hygrotus*	*Coelambus*	*punctilineatus*	(Fall, 1919)	NA	Tolerant	morphological
94	*Hygrotus*	*Coelambus*	*saginatus*	(Schaum, 1857)	PL	Tolerant	morphological & molecular
95	*Hygrotus*	*Coelambus*	*salinarius*	(Wallis, 1924)	NA	Hypersaline	morphological & molecular
96	*Hygrotus*	*Coelambus*	*sanfilippoi*	(Fery, 1992)	PL	Tolerant	morphological & molecular
97	*Hygrotus*	*Coelambus*	*sellatus*	(LeConte, 1866)	NA	Tolerant	morphological
98	*Hygrotus*	*Coelambus*	*semenowi*	(Jakovlev, 1899)	PL	Freshwater	morphological
99	*Hygrotus*	*Coelambus*	*semivittatus*	(Fall, 1919)	NA	Tolerant	morphological & molecular
100	*Hygrotus*	*Coelambus*	*stefanschoedli*	Fery, Sadeghi & Hosseinie, 2005	PL	?	morphological
101	*Hygrotus*	*Coelambus*	*suturalis*	(LeConte, 1850)	NA	Freshwater	morphological & molecular
102	*Hygrotus*	*Coelambus*	*sylvanus*	(Fall, 1917)	NA	Freshwater	morphological
103	*Hygrotus*	*Coelambus*	*thermarum*	(Darlington, 1928)	NA	?	morphological
104	*Hygrotus*	*Coelambus*	*tumidiventris*	(Fall, 1919)	NA	Hypersaline	morphological & molecular
105	*Hygrotus*	*Coelambus*	*turbidus*	(LeConte, 1855)	NA	Freshwater	morphological & molecular
106	*Hygrotus*	*Coelambus*	*unguicularis*	(Crotch, 1874)	H	Tolerant	morphological & molecular
107	*Hygrotus*	*Coelambus*	*urgensis*	(Jakovlev, 1899)	PL	Freshwater	morphological & molecular
108	*Hygrotus*	*Coelambus*	*wardii*	(Clark, 1862)	NA, NT	?	morphological
109	*Hygrotus*	*Coelambus*	*zigetangco*	Fery, 2003	PL	?	morphological
110	*Hygrotus*	*Hygrotus*	*acaroides*	(LeConte, 1855)	NA	Freshwater	morphological & molecular
111	*Hygrotus*	*Hygrotus*	*aequalis*	Falkenström, 1932	PL	Freshwater	morphological
112	*Hygrotus*	*Hygrotus*	*decoratus*	(Gyllenhal, 1810)	PL	Freshwater	morphological & molecular
113	*Hygrotus*	*Hygrotus*	*farctus*	(LeConte, 1855)	NA	?	morphological
114	*Hygrotus*	*Hygrotus*	*hydropicus*	(LeConte, 1852)	NA	?	morphological & molecular
115	*Hygrotus*	*Hygrotus*	*inaequalis*	(Fabricius, 1777)	PL	Freshwater	morphological & molecular
116	*Hygrotus*	*Hygrotus*	*intermedius*	(Fall, 1919)	NA	Freshwater	morphological
117	*Hygrotus*	*Hygrotus*	*marginipennis*	(Blatchley, 1912)	NA	Tolerant	morphological
118	*Hygrotus*	*Hygrotus*	*quinquelineatus*	(Zetterstedt, 1828)	PL	Freshwater	morphological & molecular
119	*Hygrotus*	*Hygrotus*	*sayi*	J. Balfour-Browne, 1944	NA	Tolerant	morphological & molecular
120	*Hygrotus*	*Hygrotus*	*versicolor*	(Schaller, 1783)	PL	Freshwater	morphological & molecular
121	*Hyphoporus*		*anitae*	Vazirani, 1969	PL	Freshwater	only literature
122	*Hyphoporus*		*aper*	Sharp, 1882	OR, PL	?	only literature
123	*Hyphoporus*		*bengalensis*	Severin, 1890	OR	?	morphological
124	*Hyphoporus*		*bertrandi*	Vazirani, 1969	PL	Freshwater	only literature
125	*Hyphoporus*		*caliginosus*	Régimbart, 1899	OR	?	only literature
126	*Hyphoporus*		*dehraduni*	Vazirani, 1969	PL	?	only literature
127	*Hyphoporus*		*elevatus*	Sharp, 1882	OR, PL	?	morphological
128	*Hyphoporus*		*geetae*	Vazirani, 1969	OR	?	only literature
129	*Hyphoporus*		*josephi*	Vazirani, 1969	OR	?	only literature
130	*Hyphoporus*		*kempi*	Gschwendtnet, 1936	OR, PL	Tolerant	only literature
131	*Hyphoporus*		*montanus*	Régimbart, 1899	OR	?	only literature
132	*Hyphoporus*		*nilghiricus*	Régimbart, 1903	OR, PL	Freshwater	only literature
133	*Hyphoporus*		*oudomxai*	Brancucci & Biström, 2013	OR	?	only literature
134	*Hyphoporus*		*pacistanus*	Guignot, 1959	PL	?	only literature
135	*Hyphoporus*		*pugnator*	Sharp, 1890	OR	?	only literature
136	*Hyphoporus*		*severini*	Régimbart, 1892	OR, PL	Tolerant	morphological
137	*Hyphoporus*		*solieri*	(Aubé, 1838)	PL	Freshwater	morphological & molecular
138	*Hyphoporus*		*subaequalis*	Vazirani, 1969	OR	?	only literature
139	*Hyphoporus*		*tonkinensis*	Régimbart, 1899	OR	?	morphological & molecular

Notes

1 Although regularly found in low mineralized environments, it can tolerate hypersaline waters, in which it can be found in large numbers
2 Characterized as tolerant based on data of the type locality (the only known locality of the species)
3 Occasionally can be found in more mineralized environments, but their typical habitat is freshwater
4 In south Europe only found in freshwater habitats

Fig. S1. Time-calibrated majority rule consensus tree obtained in BEAST with the complete dataset. Number in nodes: posterior probability values.

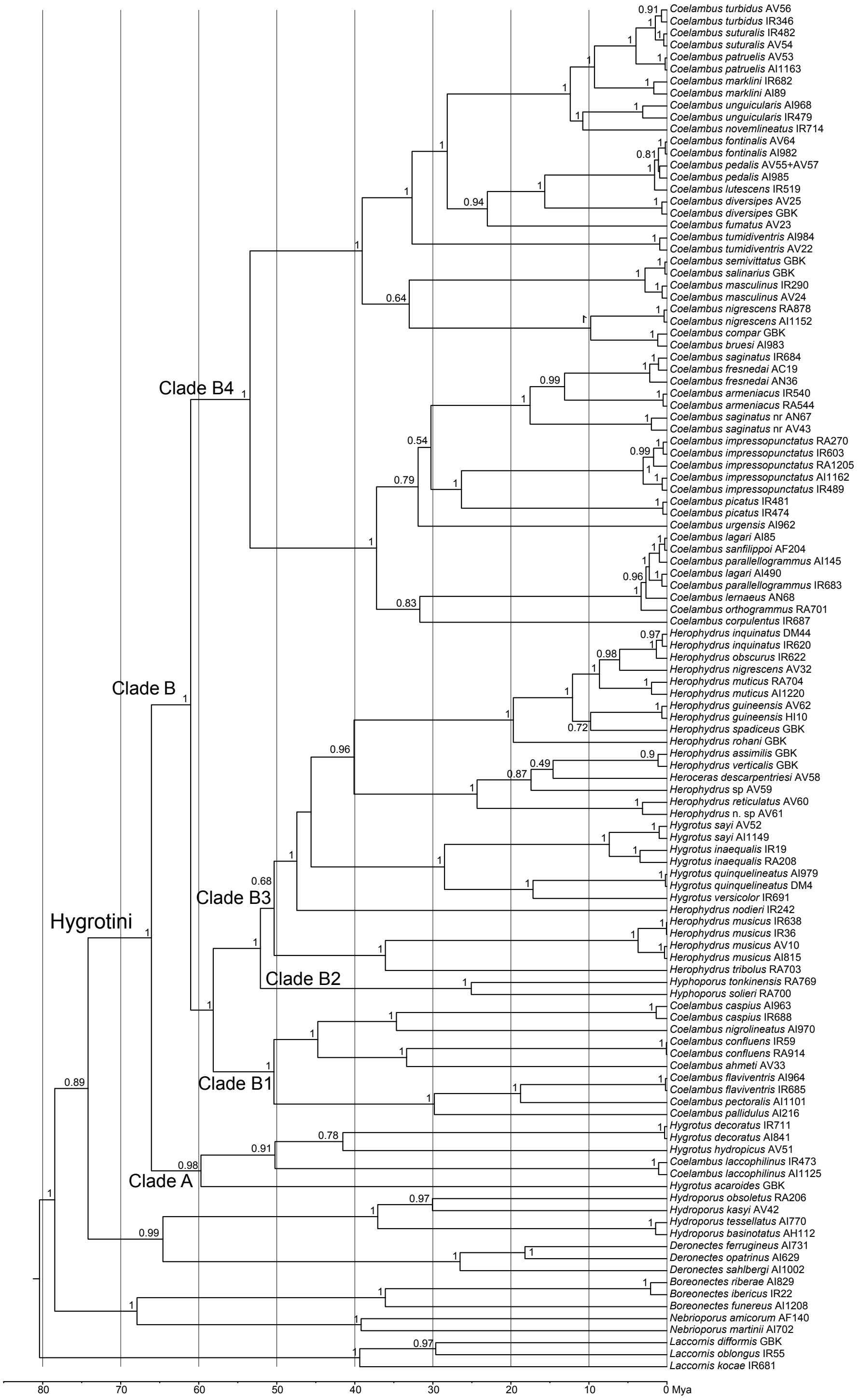
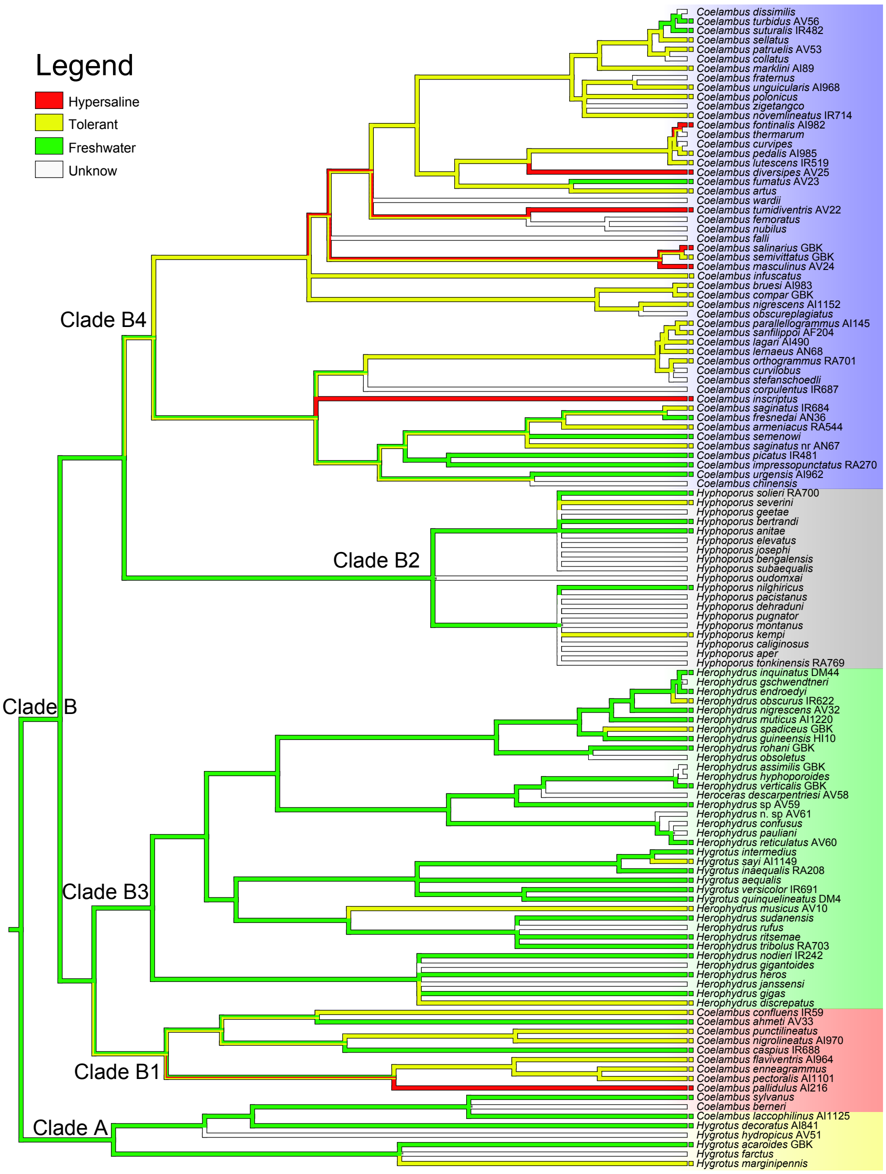


Fig. S2. Reconstructed evolution of salinity tolerance in the tribe Hygrotini, using parsimony with the estimated relationships of species for which no molecular data were available.



Systematic
Entomology

Systematic Entomology (2018), DOI: 10.1111/syen.12318

A molecular phylogeny of the tribe Ochthebiini (Coleoptera, Hydraenidae, Ochthebiinae)

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Abstract. Ochthebiinae, with *c*. 650 species distributed worldwide, are the second most speciose subfamily of the aquatic beetle family Hydraenidae. They are ecologically the most diverse hydraenid subfamily, with terrestrial species as well as species in almost all types of aquatic habitats, including hypersaline waters. Ochthebiinae include the tribes Ochtheosini (four species in three genera) and Ochthebiini. We provide here the first comprehensive phylogeny of the tribe Ochthebiini, based on 186 species and four subspecies from most genera, subgenera and species groups. We obtained sequence data for a combination of mitochondrial and nuclear gene fragments including the 5′ and 3′ ends of the cytochrome *c* oxidase subunit 1, the 5′ end of 16S RNA plus the leucine tRNA transfer plus 5′ end of NADH dehydrogenase subunit I, and internal fragments of the large and small ribosomal units. The analyses with maximum likelihood (ML) and Bayesian probabilities consistently recovered a generally well supported phylogeny, with most currently accepted taxa and species groups as monophyletic. We provide a new classification of the tribe based on our phylogenetic results, with six genera: *Meropathus* Enderlein, *Ochthebius* Leach, *Protochthebius* Perkins, *Prototympanogaster* Perkins, *Tympallopatrum* Perkins and *Tympanogaster* Janssens. The genus *Ochthebius* is here divided into nine subgenera in addition to *Ochthebius* s.s.: (1) *O*. (*Angiochthebius*) Jäch & Ribera; (2) *O*. (*Asiobates*) Thomson; (3) *O*. (*Aulacochthebius*) Kuwert; (4) *O*. (*Cobalius*) Rey; (5) *O*. (*Enicocerus*) Stephens; (6) *O*. (*Gymnanthelius*) Perkins **comb.n.**; (7) *O*. (*Gymnochthebius*) Orchymont; (8) *O*. (*Hughleechia*) Perkins **comb.n.**; and (9) *O*. (*Micragasma*) Sahlberg. Within *Ochthebius* s.s., 17 species groups are proposed, five of them newly established (3, 9, 11, 13 and 16): (1) *andraei*; (2) *atriceps*; (3) *corrugatus*; (4) *foveolatus*; (5) *kosiensis*; (6) *lobicollis*; (7) *marinus*; (8) *metallescens*; (9) *nitidipennis*; (10) *notabilis*; (11) *peisonis*; (12) *punctatus*; (13) *quadricollis*; (14) *rivalis*; (15) *strigosus*; (16) *sumatrensis*; and (17) *vandykei*. We elevated to species rank two subspecies of *Ochthebius*: *O. fallaciosus* Ganglbauer **stat.n.** (former subspecies of *O. viridis* Peyron) and *O. deletus* Rey **stat.rest.** (former subspecies of *O. subpictus* Wollaston).

Introduction

Ochthebiinae, with *c*. 650 species and 11 subspecies described, are the second most diverse subfamily of the water beetle family Hydraenidae (Hansen, 1998; Jäch & Balke, 2008; Tables 1, S1 in File S1). They occur in all biogeographic regions, including the Antarctic islands of Kerguelen and Heard, where they are the only Hydraenidae present (Hansen, 1998). Ochthebiinae are the ecologically most diverse hydraenid subfamily, with terrestrial species, species living in the interface between land and water, as

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well as in most types of aquatic environments (Jäch *et al.,* 2016). A large number of species are tolerant to hypersaline waters, living in coastal rockpools (e.g. *Cobalius* Rey or *Calobius* Wollaston; Antonini *et al.,* 2010; Sabatelli *et al.,* 2016), coastal or inland saltpans or inland hypersaline streams (especially the *O. notabilis* group, but also many other species in different groups; Abellán *et al.,* 2009; Millán *et al.,* 2011).

The external morphology of the species of Ochthebiinae is more heterogeneous than in other Holarctic lineages of Hydraenidae [e.g. *Hydraena* Kugelann (Trizzino *et al.* 2013) or *Limnebius* Leach (Rudoy *et al.*, 2016)]. This has resulted in a more complex taxonomy, with mostly all of the described genera or subgenera with uncertain relationships. Thus, the genus *Ochthebius* Leach was divided into 16 subgenera by Kuwert (1887), but all of them, with the exception of *Aulacochthebius* Kuwert, were later synonymized (see Tables 1, S2 in File S1 for a synopsis of the classification and Table S1 in File S1 for a complete checklist of Ochthebiini).

Ochthebiinae were divided by Perkins (1980) into two tribes, Ochthebiini and Ochtheosini, the latter for the single terrestrial genus *Ochtheosus* Perkins, with two species. The monotypic genera *Edaphobates* Jäch & Díaz and *Ginkgoscia* Jäch & Díaz, for which we could not obtain fresh material for DNA extraction, were tentatively hypothesized to be related to *Ochtheosus* by Jäch & Díaz (2003, 2004) and thus we consider them within Ochtheosini. Perkins (1997) divided Ochthebiini into five newly defined subtribes: Enicocerina (for the single genus *Enicocerus* Stephens), Meropathina (*Meropathus* Enderlein, *Tympallopatrum* Perkins, *Tympanogaster* Perkins and the recently described *Prototympanogaster* Perkins; Perkins, 2018), Neochthebiina (*Neochthebius* Orchymont), Ochthebiina (*Ochthebius*, *Gymnochthebius* Orchymont, *Hughleechia* Perkins, *Gymnanthelius* Perkins, *Aulacochthebius* and *Micragasma* Sahlberg) and Protochthebiina (*Protochthebius* Perkins). *Enicocerus* was treated as a subgenus by several subsequent authors (e.g. Jäch, 1998; Ribera *et al.*, 2010; Jäch & Skale, 2015), and *Neochthebius* was treated as a synonym of *Ochthebius* s.s. by Jäch & Delgado (2014b), leaving ten genera in Ochthebiini, most of them described in the 20th century (Table 1). *Ochthebius* is the oldest available generic name (Leach, 1815; Hansen, 1998; Table 1), grouped into four recognized subgenera with mostly Palaearctic distribution: *Asiobates* Thomson, *Calobius*, *Enicocerus* and *Ochthebius* (Jäch & Skale, 2015; Tables 1, S1 in File S1; see the detailed taxonomic history of subgenera and species groups in the Discussion). Within *Ochthebius* s.s., the most diverse subgenus, several informal species groups have been defined, which have undergone important modifications through their taxonomic history (Tables S1, S2 in File S1).

The classification and proposed relationships within Ochthebiini have also experienced many modifications during the last four decades. Perkins (1980) revised the by then known American species, and proposed a phylogeny derived from the examination of some morphological characters. *Gymnochthebius* was placed as sister to the remaining taxa, which were divided in two lineages: (i) *Meropathus* plus *Neochthebius* (currently a synonym of *Ochthebius*; Table S2 in File S1) and (ii) *Ochthebius* plus *Asiobates*. Subsequently, Perkins (1997) synonymized four subgenera with *Ochthebius* (*Calobius*, *Cobalius*, *Liochthebius* Sahlberg and *Notochthebius* Orchymont), and described three additional genera (Tables 1, S2 in File S1). Based mostly on the exocrine secretion delivery system (ESDS), he divided the subfamily in two tribes, Ochtheosini for the newly described *Ochtheosus* and Ochthebiini, divided in turn into subtribes, with unresolved relationshipa among them. *Ochtheosus* was considered to have some plesiomorphic characters similar to some southern African genera (e.g. antennae with 11 antennomeres, as in many Prosthetopinae; Perkins, 1997; see also Beutel *et al.*, 2003), and did not share several of the most characteristic synapomorphies with the remaining Ochthebiinae, in particular the structure of the tentorial arms, galea and lacinia.

The first formal cladistic analysis of the family Hydraenidae was published by Beutel *et al.* (2003), but sampling was too incomplete to resolve internal relationships within Ochthebiinae other than the sister relationship of *Meropathus* with *Ochthebius* + *Gymnochthebius*. There is no published global molecular phylogeny of the entire family Hydraenidae or subfamily Ochthebiinae, but in recent years some detailed molecular phylogenies for some lineages have been published, such as the *Ochthebius notabilis* group (Abellán *et al.*, 2009) and *Enicocerus* (Ribera *et al.*, 2010). In Abellán *et al.* (2013) an extensive phylogeny of *Ochthebius* and some related genera using only mitochondrial markers was used to estimate the phylogenetic diversity of the Iberian fauna. The sampling of some geographical areas was, however, very incomplete, as the intention was not to produce a phylogenetic study. Still, most Palaearctic lineages were represented, which allowed us to establish the monophyly of most of the included genera/subgenera and of the recognized species groups, although internal groups had poor relationships between them. Sabatelli *et al.* (2016) used these data to study the origin of species typical of rockpools, recovering basically the same relationships and establishing a new species group for the South African *O. capicola* Péringuey. In the same paper, the subgenus *Cobalius* was found to be outside *Ochthebius* s.s., but *Calobius* nested within it, referring to it as the '*Calobius*' lineage.

In this study we provide a comprehensive phylogeny of Ochthebiini, based on mitochondrial and nuclear sequence data, including representatives of most lineages. We introduce several changes in the taxonomic classification to accommodate our phylogenetic results, and provide a complete checklist based on our new classification (Table S1 in File S1).

Material and methods

Taxon sampling

We studied 186 species and four subspecies of the 641 described species and 11 described subspecies of Ochthebiini, plus 29 specimens corresponding to undetermined or still undescribed species (Tables S1, S3 in File S1). For two species with an isolated or unsupported placement (*O. plesiotypus* Perkins and *O. peisonis* Ganglbauer) we sequenced two specimens to test for possible sequencing mistakes. We included examples

Table 1. Synopsis of the genus-level classification of Ochthebiini, with notes of the former status of the taxa (following Jäch & Skale, 2015, Jäch *et al.*, 2016 and Perkins, 2018), total number of species and species included in the phylogeny (see Table S1 in File S1 for a complete checklist and Table S2 in File S1 for synonyms and type species). In brackets, number of subspecies. Species of uncertain identification included in the phylogeny noted after '+'.

New status	Former status	No. sp. (ssp.)	DNA sp. (ssp.)
Genus *Meropathus* Enderlein, 1901	Genus	8	1
Genus *Ochthebius* Leach, 1815	Genus	540[a] (9)	186 + 27(2)
Subgenus *Angiochthebius* Jäch & Ribera, 2018	Subgenus of *Ochthebius*	3	1
Subgenus *Asiobates* Thomson, 1859	Subgenus of *Ochthebius*	105 (3)	34 + 6
Subgenus *Aulacochthebius* Kuwert, 1887	Genus	13	4 + 6
Subgenus *Cobalius* Rey, 1886	Synonym of *Ochthebius* s.s.[b]	9 (2)	6
Subgenus *Enicocerus* Stephens, 1829	Subgenus of *Ochthebius*	16	9 + 1
Subgenus *Gymnanthelius* Perkins, 1997	Genus	8	2
Subgenus *Gymnochthebius* Orchymont, 1943	Genus	58	7 + 1
Subgenus *Hughleechia* Perkins, 1981	Genus	2	1
Subgenus *Micragasma* Sahlberg, 1900	Genus[c]	3	1
Subgenus *Ochthebius* Leach, 1815	Subgenus of *Ochthebius*	322 (4)	121 + 13 (2)
Genus *Protochthebius* Perkins, 1997	Genus	7	0
Genus *Prototympanogaster* Perkins, 2018	Genus	1	0
Genus *Tympallopatrum* Perkins, 1977	Genus	4	0
Genus *Tympanogaster* Janssens, 1967	Genus	84	3
Subgenus *Hygrotympanogaster* Perkins, 2006	Subgenus of *Tympanogaster*	36	1
Subgenus *Plesiotympanogaster* Perkins, 2006	Subgenus of *Tympanogaster*	2	0
Subgenus *Topotympanogaster* Perkins, 2006	Subgenus of *Tympanogaster*	8	0
Subgenus *Tympanogaster* Janssens, 1967	Subgenus of *Tympanogaster*	38	2

[a] Includes one species 'incertae sedis'.

[b] Considered as a subgenus of *Ochthebius* by Sabatelli *et al.* (2016) and Jäch & Delgado (2017a).

[c] Considered as a subgenus of *Ochthebius* by Hernando *et al.* (2017), based on the results of this study.

of eight of the 11 genera currently recognized in the tribe, all subgenera but two (within genus *Tympanogaster*), and all recognized species groups within the genus *Ochthebius* but one (*O. kosiensis* group, Tables 1, S1, S3 in File S1). The three missing genera, *Tympallopatrum* (Australia), *Protochthebius* (Asia) and *Prototympanogaster* (Lord Howe Island), have four, seven and a single species, respectively (Table S1 in File S1).

We used as outgroups 31 species of other Hydraenidae genera (*Hydraena*, *Laeliaena* Sahlberg and *Limnebius*) and of Ptiliidae. Trees were rooted in the split between Hydraenidae and Ptiliidae, considered to be sister groups both based on molecular (e.g. Hunt *et al.*, 2007; McKenna *et al.*, 2015; Zhang *et al.*, 2018) and morphological evidence (Hansen, 1997; Lawrence *et al.*, 2011).

DNA extraction and sequencing

Specimens were killed and preserved in absolute ethanol. DNA was extracted with a standard phenol-chloroform extraction or by commercial extraction kits (mostly Quiagen DNeasy Tissue Kit, Hildesheim, Germany) following the manufacturers' instructions. DNA samples and voucher specimens are kept in the collections of the Institute of Evolutionary Biology (IBE, Barcelona, Spain), Museo Nacional de Ciencias Naturales (MNCN, Madrid, Spain) and Naturhistorisches Museum Wien (NMW, Vienna, Austria). We sequenced fragments of six genes in five sequencing reactions, three mitochondrial [(i) 5′ end of the cytochrome *c* oxidase subunit I (the standard barcode, Hebert *et al.*, 2003) (COI-5′); (ii) 3′ end of cytochrome c oxidase subunit I (COI-3′); (iii) 5′ end of 16S RNA (16S) plus the leucine tRNA transfer (tRNA-Leu) plus 5′ end of NADH dehydrogenase subunit I (NAD1)]; and two nuclear [(iv) an internal fragment of the large ribosomal unit, 28S RNA (28S) and (v) an internal fragment of the small ribosomal unit, 18S RNA (18S)] (see Table S4 in File S1 for details on primers used and typical PCR conditions). Sequences were assembled and edited with GENEIOUS v10.1 (Kearse *et al.*, 2012); new sequences (a total of 897) were deposited in the ENA database with accession numbers LT990690–LT991586.

Phylogenetic analyses

Edited sequences were aligned using the online version of MAFFT v.7 with the G-INS-I algorithm (Katoh *et al.*, 2009). We used PARTITIONFINDER v1.1.1 (Lanfear *et al.*, 2012) to estimate the evolutionary model that best fitted the data, using one partition for each gene fragment (six partitions in total), and using Akaike information criterion (AIC) scores as selection criteria. Phylogenetic analyses were made using Bayesian probabilities in BEAST 1.8 (Drummond & Rambaut, 2007), using the partition and evolutionary models selected by PARTITIONFINDER, with a Yule speciation process as tree prior. There are few fossils usable for calibrating the phylogeny of Hydraenidae. The oldest recognized members of the family are *Ochthebiites*

Ponomarenko, from the Jurassic (Arnol'di *et al.*, 1991; Ponomarenko & Prokin, 2015; Yamamoto *et al.*, 2017), but they cannot confidently be placed in any extant lineage. One of the best-preserved hydraenid fossils is *Archaeodraena cretacea* Jäch & Yamamoto from Upper Cretaceous Burmese amber (*c.* 99 Ma, Yamamoto *et al.*, 2017), which probably belongs to the crown Hydraenidae. Both fossils are compatible with an estimate of *c.* 170 Ma for the split between Hydraenidae and Ptiliidae obtained in recent molecular phylogenies calibrated with a range of fossils (Hunt *et al.*, 2007; McKenna *et al.*, 2015). We thus used this estimation to calibrate our tree, with a normal distribution with a standard deviation of 1 Ma and an uncorrelated lognormal relaxed clock. A Middle Jurassic separation between Hydraenidae and Ptiliidae is considerably younger than the estimation of Toussaint *et al.* (2017) (Middle Triassic, 243 Ma), but older than the more recent estimation of Zhang *et al.* (2018) (Upper Jurassic, *c.* 150 Ma), both of which we consider to be less plausible. In any case, it must be noted that the main objectives of our study do not require an absolute calibration of the phylogeny of Ochthebiini, which is done only as a preliminary exploration.

We ran the analyses for 100 million generations, logging results for every 5000, and checked convergence to estimate the burn-in fraction with TRACER v1.6 (Drummond & Rambaut, 2007). We ran an additional ML phylogenetic reconstruction with RAXML-HPC2 (Stamatakis, 2006) in the CIPRES portal (Miller *et al.*, 2010), using the same partition scheme as in BEAST with a GTR+G model estimated independently for each partition. Node support values were estimated with 100 pseudoreplicas using a rapid bootstrapping algorithm (Stamatakis *et al.*, 2008). The same ML analysis was repeated only with the nuclear sequence (18S and 28S).

Results

The final matrix included 252 terminals with 3656 aligned characters. Protein-coding regions had no indels except for the $3'$ end of COI-3, where some species had an additional codon. The best partitioning scheme obtained with PARTITIONFINDER had six partitions corresponding to: (i) COI-5, (ii) COI-3, (iii) 16S+tRNA-Leu, (iv) NAD1, (v) 18S and (vi) 28S. The optimal evolutionary model was GTR+I+G for all partitions except for NAD1 (best model TMV) and 28S (best model SYM). The BEAST run implementing the best models did not converge properly, however, mostly due to the parameters related to the estimation of the branch lengths, especially for the genes NAD1, 18S and 28S. We therefore did a second run with simpler models for these genes (HKY+G+I), which converged adequately. The topologies of the two Bayesian analyses were, however, almost identical (Figs 1, S1 in File S1), and unless specified we report only the results of the analyses with the better parameter convergence (i.e. with the simpler evolutionary models).

Molecular phylogeny

The topologies obtained in the ML and the two Bayesian analyses were very similar, differing only in some poorly supported nodes (Figs 1, S1, S2 in File S1), most notably in the position of *Hughleechia* (see below). The ML tree with the nuclear sequence only had a topology very similar to that obtained with the combined data, although with a generally lower resolution and support. The main difference was the recovery of Ochthebiini as paraphyletic, with the genus *Hydraena* as sister to *Tympanogaster* plus *Meropathus*, although with very low support (bootstrap support, BS = 53%; Fig. S3 in File S1). Genera, subgenera and most species groups were, however, recovered as monophyletic with strong support, with internal topologies very similar to that of the combined ML tree (Figs S2, S3 in File S1).

In the ML and Bayesian trees with the combined nuclear and mitochondrial data, the monophyly of Ochthebiini was strongly supported, as well as their separation into two clades, (i) *Meropathus* plus *Tympanogaster* and (ii) *Ochthebius* s.l. *Meropathus* was nested within a paraphyletic *Tympanogaster* in the ML tree (combined and nuclear only) and in the Bayesian tree with the best models, and sister to *Tympanogaster* with low support in the Bayesian tree with simpler models (posterior probability, PP = 0.63; Figs 1a, S1, S2 in File S1).

Within *Ochthebius* s.l., *Asiobates* and *Aulacochthebius* were sister groups in the Bayesian tree with low support (PP = 0.85), and both sisters to the rest of Ochthebiini. In the ML analysis, *Asiobates* and *Aulacochthebius* were paraphyletic with respect to the rest of Ochthebiini, also with low support (BS < 50%) (Figs 1a, S2 in File S1). In both analyses Ochthebiini minus *Asiobates* and *Aulacochthebius* were monophyletic with strong support (BS = 80%; PP = 1; Fig. 1a).

The remaining Ochthebiini were divided into a series of well-supported clades corresponding to traditionally recognized genera or subgenera, but with poorly resolved relationships among them: (i) *Enicocerus*, strongly supported and with well-resolved internal relationships, sister to the Australian *Hughleechia* in ML and the Bayesian analysis with the simpler models (BS = 81%, PP = 0.76; Figs 1b, S2 in File S1); in the Bayesian analysis with the best models *Hughleechia* was sister to the clade formed by *Micragasma* and *Cobalius*, with low support (PP = 0.88; Fig. S1 in File S1); (ii) a clade including *Gymnochthebius* and *Gymnanthelius*, the latter as sister to *Angiochthebius* Jäch & Ribera (*Gymnochthebius plesiotypus* group of Perkins, 1980; see Jäch & Ribera, 2018) (BS = 94%, PP = 0.96); within *Gymnochthebius*, the Australian and American species were respectively monophyletic and sisters, with very strong support in both the ML and Bayesian trees (Figs 1b, S2 in File S1); (iii) *Cobalius*, with a strongly supported monophyly (BS = 98%, PP = 1) and sister to the only sequenced species of *Micragasma*, also with strong support (BS = 100%, PP = 1) (Figs 1b, S2 in File S1); (iv) *Ochthebius* s.s., including *Calobius*, strongly supported in both the ML (BS = 94%) and Bayesian (PP = 1) trees (Figs 1b, S2 in File S1).

Within *Ochthebius* s.s. the most established Palaearctic species groups were recovered as monophyletic (see Discussion; Figs 1b, c, S2 in File S1). Their monophyly was strongly supported in the ML and Bayesian trees, with the only exception of the group of species related to the *O. atriceps* and *O. notabilis* groups in the ML analyses. The main difference with

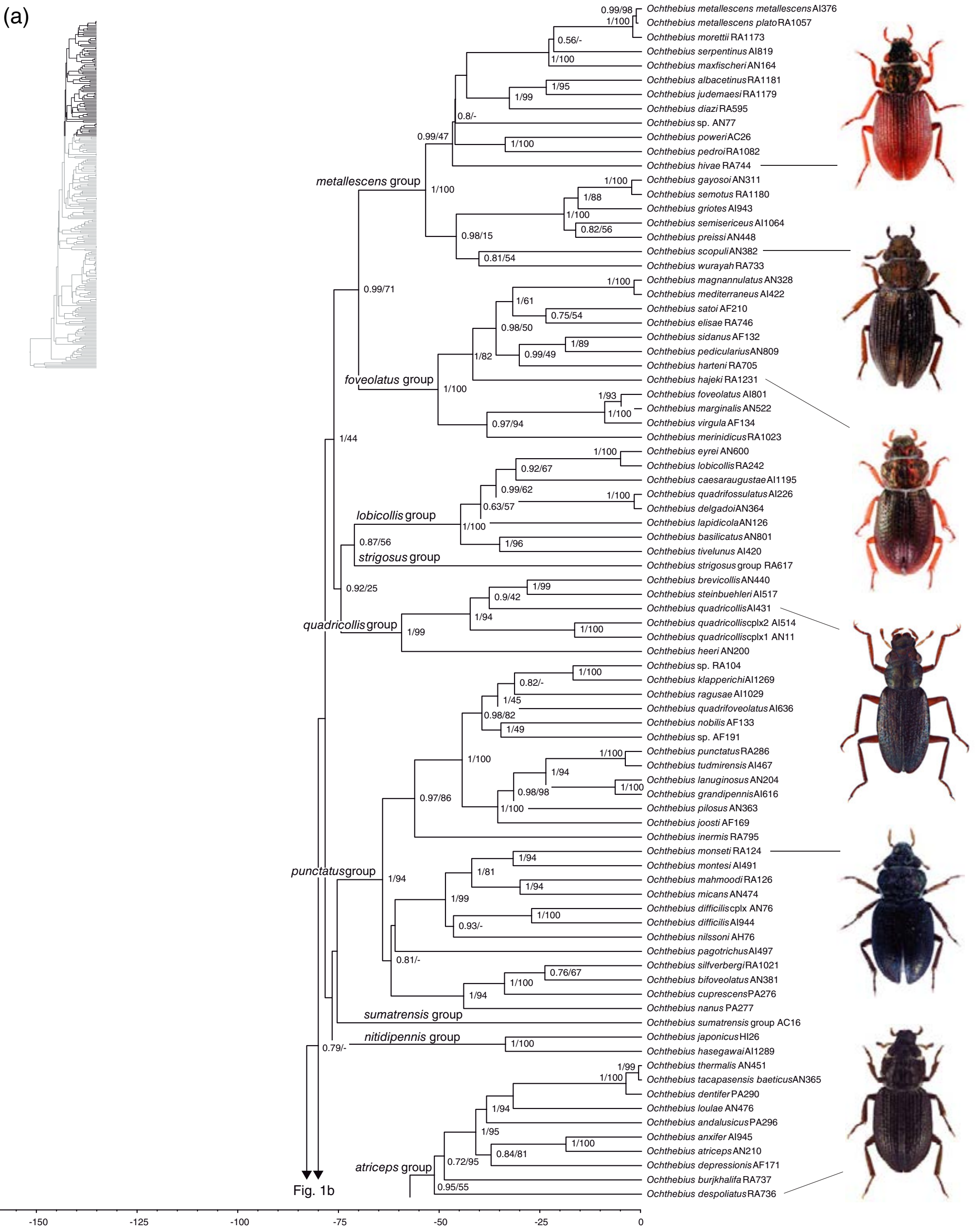

Fig. 1. Majority rule consensus tree obtained with BEAST for the phylogeny of Ochthebiini, with the simple evolutionary models (see text). Numbers in nodes, posterior probabilities/bootstrap support values in RAXML. Names in nodes refer to the new classification. Habitus photographs correspond to species used in the analyses, with the addition of *Limnebius papposus* Mulsant, *Hydraena riparia* Kugelann (a), *Ochthebius* (s.s.) *bernhardi* Jäch & Delgado and *O*. (*Micragasma*) *minoicus* Hernando, Villastrigo & Ribera (b).[Colour figure can be viewed at wileyonlinelibrary.com].

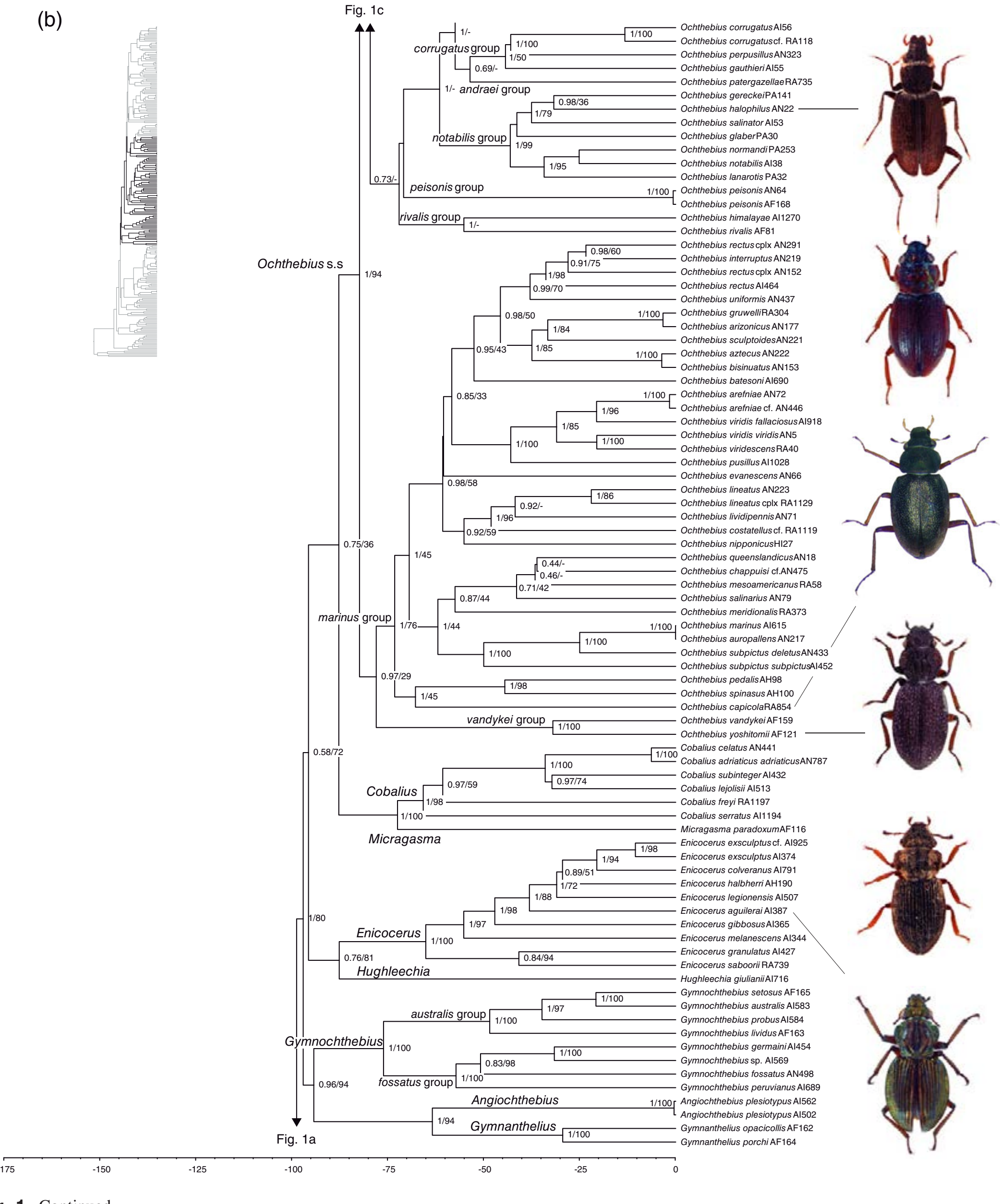

Fig. 1. Continued.

established groups was the expansion of the *O. marinus* group to include the South African *O. capicola* and the American *O. biincisus*, *bisinuatus* and *interruptus* groups of Perkins (1980). The *O. foveolatus* group of Jäch (1991) was split in three clades: (i) *O. foveolatus* group, sister to the *O. metallescens* group with strong support in both ML and Bayesian trees (BS = 71%, PP = 0.99); (ii) *O. atriceps* group; and (iii) *O. corrugatus* group. The latter two formed a clade with the species of the *O. notabilis* and *O. andraei* groups, strongly supported in the Bayesian tree (PP = 1) but not in the ML tree (BS < 50%), in which the group

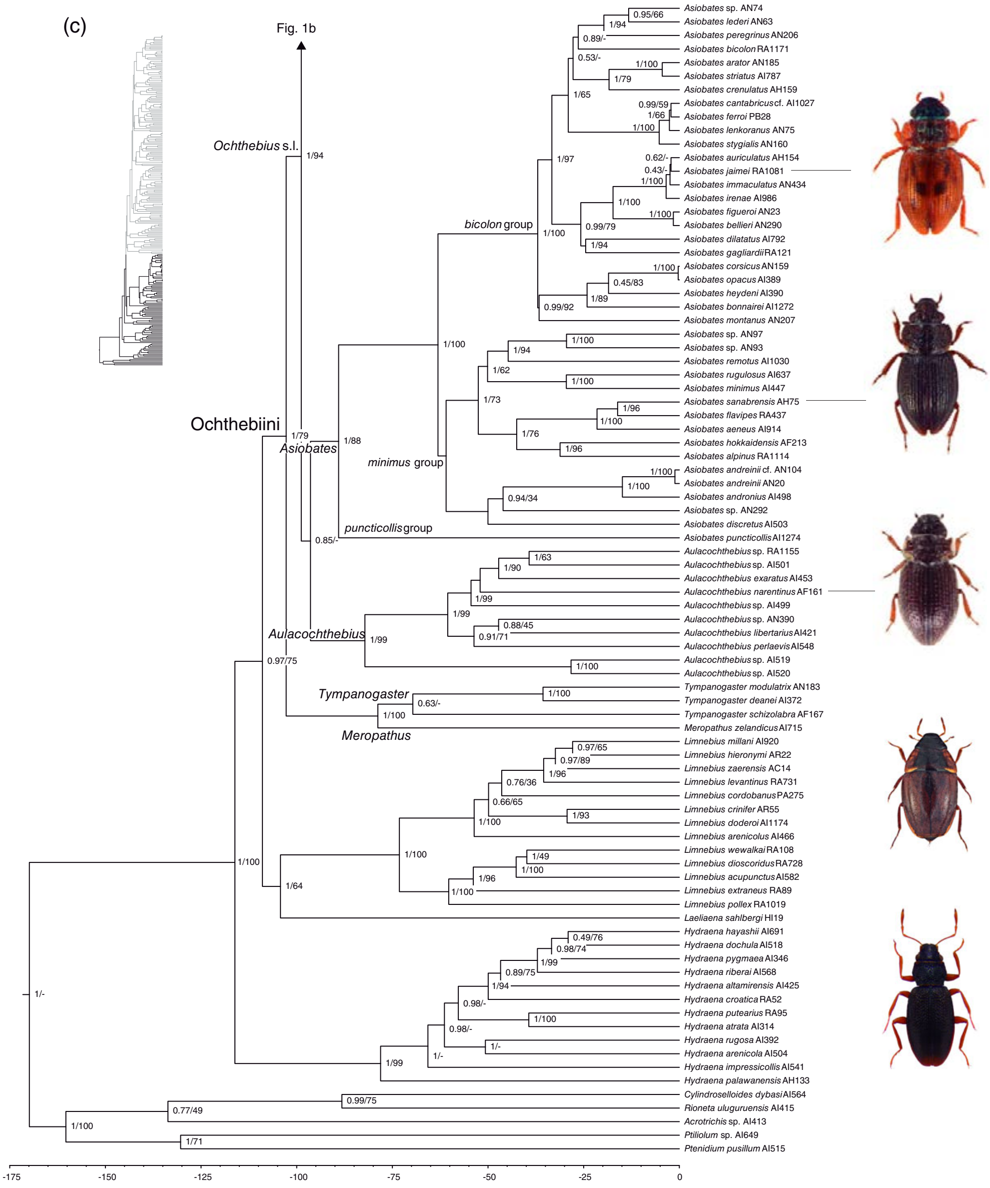

Fig. 1. Continued.

also included one of the two sampled species of the *O. rivalis* group (Figs 1c, S2 in File S1). Two coastal lineages, *Calobius* and the *O. vandykei* group (formerly genus *Neochthebius*), were nested within *Ochthebius* s.s., the former as sister to the *O. lobicollis* + *O. strigosus* groups (BS < 50%, PP = 0.92) and the latter as sister to the *O. marinus* group (BS < 50%, PP = 0.97) (Figs 1b, c, S2 in File S1).

According to our calibration scheme, with a separation between Hydraenidae and Ptiliidae at 170 Ma, the estimated age of crown Hydraenidae was 106 Ma [highest posterior density (HPD): 122.8–90.2 Ma], and that of the crown Ochthebiini was 93 Ma (HPD: 109.7–80.8 Ma). The basal diversification of Ochthebiini was reconstructed as having occurred in a relatively short temporal window, with genera, subgenera and most species

Table 2. Estimated evolutionary rates of the different partitions (substitutions per site per Ma per lineage) and 95% high posterior density interval (HPD) in the Bayesian analysis with simple evolutionary models (see text).

Partition	Mean rate	95% HPD interval
COI-5	0.0085	[0.0070, 0.0102]
COI-3	0.0111	[0.0091, 0.0133]
16S + tRNA	0.0024	[0.0020, 0.0029]
NAD1	0.0039	[0.0030, 0.0048]
18S	0.00014	[0.000 10, 0.000 17]
28S	0.00052	[0.00040, 0.00064]

groups with an origin in the range of *c*. 87–60 Ma (Fig. 1; see Table 2 for the estimated evolutionary rates of all partitions).

Discussion

Our results strongly support the monophyly of Ochthebiini, but our sampling did not allow us to test for the monophyly of Ochthebiinae, or its position within Hydraenidae. Within Ochthebiini our results recover two well-supported clades: *Meropathus* plus *Tympanogaster*, and *Ochthebius* s.l. (Fig. 2; see below for a detailed discussion of the taxonomic classification of Ochthebiini). We did not find evidence to support the five subtribes proposed by Perkins (1997), which are therefore not considered here.

We did not find evidence for a clear separation between the studied species of *Tympanogaster* and *Meropathus*, in agreement with previous studies (Hansen, 1991). However, we could not obtain material of the genera *Prototympanogaster* and *Tympallopatrum*, considered to be closely related to *Tympanogaster* by Perkins (1997, 2018), and two of the subgenera of *Tympanogaster* (*Plesiotympanogaster* Perkins and *Topotympanogaster* Perkins), so until more data become available we refrain from any taxonomic change and consider *Prototympanogaster*, *Tympallopatrum* and *Tympanogaster* as valid genera (Tables 1, S1 in File S1; Fig. 2) (see Perkins, 2006 for a discussion on the subgeneric classification of *Tympanogaster*). The species of *Meropathus*, *Prototympanogaster*, *Tympallopatrum* and *Tympanogaster* are found in the Australian Region, on two Antarctic islands (Kerguelen and Heard) and on several Subantarctic islands, such as Campbell Island, Crozet Islands, Prince Edward Island and Falkland Islands.

The second lineage, genus *Ochthebius* s.l., included all the non-Australian Ochthebiini, as well as several Australian species. Our results agree remarkably well with the currently recognized subgenera and many of the established species groups, which were recovered as monophyletic with general strong support (Fig. 2). The relationships between these lineages, however, do not confirm some previous hypotheses on their relationships. Thus, *Aulacochthebius* was not found to be closely related to *Gymnochthebius*, as proposed in Hansen (1991), but to *Asiobates*; *Micragasma* and *Hughleechia* were not among the basal lineages and *Cobalius* and *Calobius* were not closely related, as hypothesized in Perkins (1997). Novel relationships found here are the possible sister relationship between *Hughleechia* and *Enicocerus*, and the close relationship between *Gymnochthebius* and *Gymnanthelius*. Interestingly, within the clade *Gymnochthebius* + *Gymnanthelius* + *Angiochthebius* there are two cladogenetic events separating American from Australian species: one within *Gymnochthebius*, dated at 73 Ma (95% HPD: 87–60 Ma), and another separating the Australian *Gymnanthelius* and the Chilean *Angiochthebius*, dated at 60 Ma (95% HPD: 78–51 Ma). Although a detailed biogeographic analysis is outside the scope of this paper, it is interesting to note that these estimations are too recent for a tectonic split between Australia and South America (i.e. west and east Gondwana), dated at *c*. 130 Ma (McIntyre *et al.*, 2017). Our calibration would thus require a different scenario, probably through the colonization of some southern islands or the Antarctica. An older age for these nodes is unlikely, given that our rate estimations are already slower than most recent estimations for the same genes in other groups of Coleoptera (Table 2; see e.g., Papadopoulou *et al.*, 2010; Andújar *et al.*, 2012; Cieslak *et al.*, 2014).

Taxonomic classification of Ochthebiini Thomson, 1859

Genus *Meropathus* Enderlein, 1910

Type species: *Meropathus chuni* Enderlein, 1910, by monotypy.

Meropathus was described as genus, considered as subgenus of *Ochthebius* by Orchymont (1938) and reinstated again as genus by Jeannel (1940). Bameul (1989) redescribed the genus and recognized 12 species (in two species groups), transferring *Ochthebius schizolabrus* Deane to *Meropathus*. Hansen (1991) noted the difficulty in establishing clear distinctions within the *Meropathus–Tympanogaster* complex. *Meropathus* includes seven New Zealand, Antarctic and Subantarctic species plus *M. labratus* Deane from Queensland (Table S1 in File S1). They are all found in coastal habitats, usually among debris and algae (Bameul, 1989).

Genus *Prototympanogaster* Perkins, 2018

Type species: *Prototympanogaster lordhowensis* Perkins, 2018, by original designation.

Prototympanogaster was described by Perkins (2018) as a monotypic genus based on two males collected in 2003 in Lord Howe Island (Australia). This genus seems to be closely related to *Tympanogaster*, but without its characteristic glabrous tabella in the metaventrite (Perkins, 2018).

Genus *Tympallopatrum* Perkins, 1997

Type species: *Tympallopatrum longitudum* Perkins, 1997, by original designation.

Tympallopatrum was described by Perkins (1997) as a monotypic genus within Meropathina. Subsequently, Perkins (2004a) revised the genus and described three additional species, all of them from western Australia (Table S1 in File S1). We could not

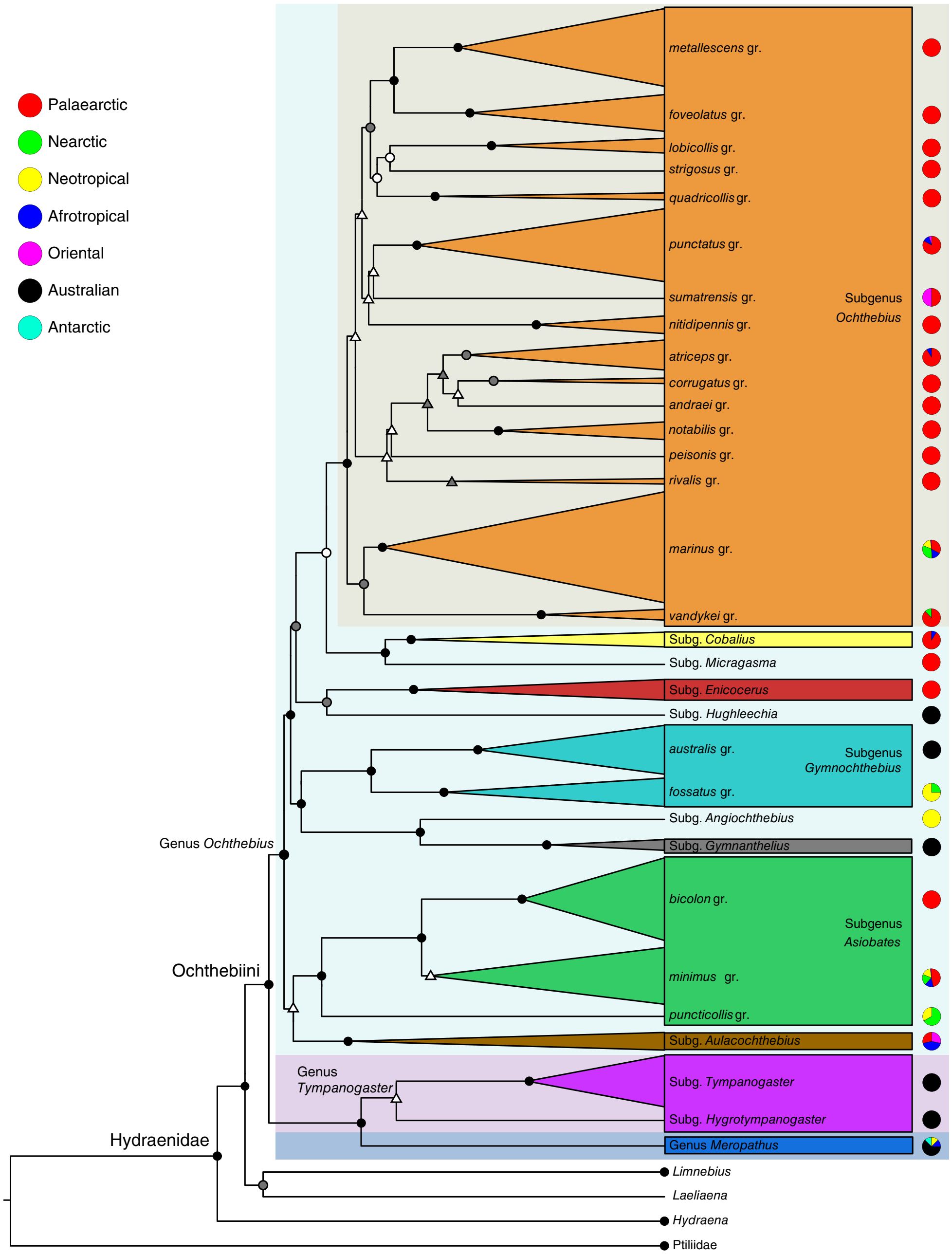

Fig. 2. Summary tree of the phylogenetic relationships of the Ochthebiini main lineages. The width of the triangles reflects the number of species of the respective clade in the tree. Symbols in nodes: circles, congruent topology in the maximum likelihood and Bayesian analyses; triangles, incongruent topologies; in black, nodes with good support (Bayesian posterior probability > 0.95 and maximum likelihood bootstrap support > 70%) in both analyses; in grey, in one analysis only; in white, not supported nodes. Pie charts reflect the geographical distribution of the species of the respective clades.

obtain any representative of this genus for our study, and thus its phylogenetic placement remains untested.

Genus *Tympanogaster* Janssens, 1967

Type species: *Tympanogaster deanei* Perkins, 1979 (replacement name for *Ochthebius longipes* Deane, 1931), by monotypy.

Described by Janssens (1967) as a monotypic genus for *O. longipes* (= *T. deanei* Perkins), Perkins (1997) redescribed *Tympanogaster* and transferred some species from *Meropathus*. Perkins (2006) revised the genus and described three subgenera and 76 new species, raising the total number of the species in the genus to 84 (Tables 1, S1 in File S1), all distributed in Australia and Tasmania.

Subgenus Hygrotympanogaster Perkins, 2006. Type species: *Tympanogaster maureenae* Perkins, 2006, by original designation.

Hygrotympanogaster Perkins was described by Perkins (2006) as a subgenus of *Tympanogaster*, to include mostly hygropetric species in southwestern Australia. Currently it includes 36 species (Perkins, 2006) (Table S1 in File S1).

Subgenus Plesiotympanogaster Perkins, 2006. Type species: *Tympanogaster thayerae* Perkins, 2006, by original designation.

Plesiotympanogaster was described by Perkins (2006) as a subgenus of *Tympanogaster* to include the type species plus *Ochthebius costatus* Deane (Table S1 in File S1). Both species were considered to have plesiomorphic characters within the genus.

Subgenus Topotympanogaster Perkins, 2006. Type species: *Tympanogaster crista* Perkins, 2006, by original designation.

Topotympanogaster was described by Perkins (2006) as a subgenus of *Tympanogaster* to include eight Australian species, all described in Perkins (2006) (Table S1 in File S1). We could not obtain any representative of this and the previous subgenus for our study, and thus their phylogenetic placement remain untested.

Subgenus Tympanogaster Janssens, 1967. Type species: *Tympanogaster deanei* Perkins, 1979 (replacement name for *Ochthebius longipes* Deane, 1931), by monotypy.

Tympanogaster s.s. was revised by Perkins (2006), raising the total number of the species to 38 (Tables 1, S1 in File S1), all distributed in Australia and Tasmania.

Genus *Ochthebius* Leach, 1815

Type species: *Helophorus marinus* Paykull, 1798, fixed by Orchymont (1942).

The second well-supported lineage within Ochthebiini includes the remaining genera/subgenera with, in some cases, uncertain relationships among them. We consider *Ochthebius* a single genus with 540 species and nine subspecies in ten well-supported subgenera, corresponding in most cases to currently recognized taxa (Fig. 2). One additional species, *O. eremita* Knisch from Fiji, cannot be confidently placed in any of the described subgenera, and it is left as incertae sedis within the genus *Ochthebius* (Hansen, 1998; Table S1 in File S1).

Subgenus Angiochthebius Jäch & Ribera, 2018. Type species: *Gymnochthebius plesiotypus* Perkins, 1980, by original designation.

The subgenus *Angiochthebius* was created for the *Gymnochthebius plesiotypus* species group (sensu Perkins, 1980), which now includes three South American species (Jäch & Ribera, 2018; Table S1 in File S1). The species of the *G. plesiotypus* group were included within *Gymnochthebius* by Perkins (1980) as they share a bifid apex of the aedeagus, but external characters (e.g. the pubescent fifth abdominal ventrite) and some aedeagal characters (Jäch & Ribera, 2018) as well as molecular data (Figs 1b, S2 in File S1) warrant their removal from *Gymnochthebius* and their status as a distinct subgenus of *Ochthebius*.

Subgenus Asiobates Thomson, 1859. Type species: *Ochthebius rufimarginatus* Stephens, 1829 (= *O. bicolon* Germar, 1824), by monotypy.

Originally described as a genus, but downgraded to subgenus by Seidlitz (1875), and treated as such by most authors (e.g. Jäch, 1990a; Hansen, 1991; Perkins, 1997). Jäch (1990a) divided the Palaearctic species in the *bicolon* and *minimus* groups, which were recovered as respectively monophyletic with strong support. The sampled American species were divided in the *puncticollis* group of Perkins (1980), with only one sampled species being sister to the rest of the subgenus with strong support (BS = 88%, PP = 1; Figs 1a, S2 in File S1), plus the *discretus* group of Perkins (1980). The placement of the studied Nearctic species of the *A. discretus* group and two of the Afrotropical species (*O. andreiini* Régimbart and *O. andronius* Orchymont) was uncertain in both the ML and the Bayesian trees (Figs 1a, S2 in File S1). We provisionally consider them within the *A. minimus* group due to the similarities in their aedeagi and the morphology of the pronotum (Orchymont, 1948; Perkins, 1980; Jäch, 1990a). The subgenus *Asiobates* currently includes 105 described species and three subspecies occurring in all biogeographical regions, except the Oriental and Australian Realms. While the *puncticollis* group is restricted to the Nearctic Region, the *bicolon* and *minimus* groups are more widespread. The former occurs in the Palaearctic and (with several undescribed species) Afrotropical regions, and the latter occurs in the Nearctic, Neotropical, Palaearctic, and Afrotropical regions. Many additional species of *Asiobates* await description, several of them included in our phylogeny (Table S3 in File S1).

Subgenus Aulacochthebius Kuwert, 1887. Type species: *Ochthebius exaratus* Mulsant, 1844, by monotypy.

Considered as a subgenus until Perkins (1997) raised it to genus level. There are no species groups defined within this subgenus, and our sampling is too incomplete to draw firm conclusions. Currently the subgenus includes 13 Palaearctic, Oriental and Afrotropical species (Table S1 in File S1), although the taxonomy of the subgenus is in clear need of revision and it is expected that the number of species will increase considerably (Table S3 in File S1).

Subgenus Cobalius Rey, 1886. Type species: *Ochthebius lejolisii* Mulsant & Rey, 1861, fixed by Jäch (1989b).

Described as a subgenus of *Ochthebius* by Rey (1886), synonymized by Perkins (1997) with *Ochthebius* s.s. and considered again as subgenus by Sabatelli *et al.* (2016). We recovered it here as a strongly supported monophyletic lineage clearly outside *Ochthebius* s.s., confirming its status as subgenus. Its nine recognized species and two subspecies occur along the coasts of the Mediterranean Sea, the Black Sea and the eastern Atlantic Ocean from Cape Verde to Scotland (Jäch, 1989b; Jäch & Skale, 2015; Jäch & Delgado, 2017a). Its taxonomy is in need of revision (Sabatelli *et al.*, 2016; Jäch & Delgado, 2017a; I. Ribera *et al.*, unpublished data).

Subgenus Enicocerus Stephens, 1829. Type species: *Enicocerus viridiaeneus* Stephens, 1829 (= *Ochthebius exsculptus* Germar, 1824), by monotypy.

Enicocerus was originally described as a genus, downgraded to subgenus of *Ochthebius* by Chenu (1851), reinstated again as genus by Perkins (1997) (within its own subtribe, Enicocerina), but treated as a subgenus by subsequent authors (e.g. Jäch, 1998; Ribera *et al.*, 2010; Jäch & Skale, 2015). Our results support the exclusion of the East Palaearctic and Oriental species, confirming Jäch (1998) and Skale & Jäch (2009), and are in agreement with the phylogeny of Ribera *et al.* (2010). *Enicocerus* in its current sense includes 16 species with a mostly Mediterranean distribution, with some species reaching the British Isles, Central Europe, the Middle East and the Caucasus. One species from eastern North America, *Ochthebius benefossus* LeConte, not included in our phylogeny, is here tentatively placed in *Enicocerus* (following Perkins, 1980); it might, however, instead belong to the *O.* (s.s.) *nitidipennis* group, or to a species group of its own.

Subgenus Gymnanthelius Perkins, 1997 **comb.n.** Type species: *Ochthebius hieroglyphicus* Deane, 1933, by original designation.

The genus *Gymnanthelius* was introduced by Perkins (1997) for *O. hieroglyphicus*. Subsequently, Perkins (2004b) revised the genus and transferred to *Gymnanthelius* two other Australian species described by Deane (1931, 1937) within *Ochthebius* (Table S1 in File S1). The eight described species are distributed mostly in southeastern Australia, with some reaching as far north as Queensland (Perkins, 2004b).

Subgenus Gymnochthebius Orchymont, 1943. Type species: *Ochthebius nitidus* LeConte, 1850 by original designation.

Gymnochthebius was originally described as a subgenus of *Ochthebius* (Orchymont, 1943) to place several American species described under *Ochthebius* that could not be placed in any of the described subgenera, which had been established mostly for Palaearctic species. Orchymont (1943) also included three Australian species for which he could examine the aedeagus and confirmed that they had the same general structure as the American species. Perkins (1980) revised the American species and Perkins (2005) the Australian and Papuan species, recognizing another four species in addition to the three previously noted by Orchymont (1943) and raising the total number of species in the subgenus to 58 (Table S1 in File S1). The Australian and the American species of the subgenus form two well-supported clades, the *O. australis* and *O. fossatus* groups, with 36 and 22 species, respectively (Table S1 in File S1).

Subgenus Hughleechia Perkins, 1981 **comb.n.** Type species: *Hughleechia giulianii* Perkins, 1981, by original designation.

Originally described as a monotypic genus (Perkins, 1981), a second species was described by Perkins (2007a). Both species inhabit coastal rockpools in southern Australia and Tasmania, in the intertidal zone and (most frequently) above the tide (Perkins, 2007a).

Subgenus Micragasma Sahlberg, 1900. Type species: *Micragasma paradoxum* Sahlberg, 1900, by monotypy.

Described as a monotypic genus for *M. paradoxum* (Sahlberg, 1900). Jäch (1997a) redescribed the genus and transferred *O. substrigosus* Reitter to *Micragasma*. A third species was recently described from Crete (Hernando *et al.*, 2017), and there are two additional undescribed species from Central Asia (M.A. Jäch *et al.*, unpublished data). Our results clearly show that *Micragasma* is nested within *Ochthebius* s.l., and thus we consider it a subgenus of *Ochthebius*.

Subgenus Ochthebius Leach, 1815. Type species: *Helophorus marinus* Paykull, 1798, fixed by Orchymont (1942).

Within *Ochthebius* s.s. we recovered, with strong support, most of the currently recognized species groups as monophyletic. Most species groups are entirely Palaearctic, or with mostly Palaearctic species, and thus the basis for the taxonomy of the subgenus is the revisionary work of Jäch (e.g. Jäch, 1989a, 1990a, 1991, 1992a), who distinguished 13 groups and subgroups. With only one exception (*O. jengi* group), they were all, with some modifications, recovered as monophyletic. According to our results, the 322 described species and four subspecies of *Ochthebius* s.s. are separated in 17 species groups, five of them newly established herein (Fig. 2). A few species still have an uncertain phylogenetic placement. This is particularly the case for *O. belucistanicus* Ferro, *O caudatus* Frivaldszky, *O. fissicollis* Janssens and *O. pierottii* Ferro, which presently cannot be confidently included in any of the recognized species groups, mainly because their original descriptions lack information about relevant characters (Table S1 in File S1).

(1) *O. andraei* group: Defined and revised in Jäch (1992a), with additional species described in Jäch (2002) and Jäch & Delgado (2010). Currently this group includes six species and one subspecies of Palaearctic distribution (Table S1 in File S1), typical of saline or hypersaline habitats. We could study a single species (*O. patergazellae* Jäch & Delgado, Table S3 in File S1), included in a clade together with the species of the *O. notabilis*, *corrugatus* and *atriceps* groups (Fig. 1). The close relationship between the species of the *O. andraei*, *corrugatus*, *notabilis* and *atriceps* groups were already suggested in Jäch (1991, 1992a).

(2) *O. atriceps* group: In Jäch (1991) the species of the *O. foveolatus* group were divided in two subgroups: (A) *foveolatus* subgroup, sharing some characters with the species of the *O. metallescens* group; and (B) *atriceps* subgroup, sharing some characters with the species of the *O. notabilis* group. We recovered both subgroups as respectively monophyletic, and confirmed the suspected relationships proposed by Jäch (1991) (see below). *Ochthebius burjkhalifa* Jäch & Delgado and *O. despoliatus* Jäch & Delgado, both from the UAE and of uncertain affinities, although hypothesized to be related to the *O. atriceps* group (Jäch & Delgado, 2014a), were found to be sister to the rest of the species of the group, with strong support in the Bayesian analysis ($PP = 0.95$) but weaker in the ML ($BS = 55\%$) (Figs 1, S2 in File S1). With the inclusion of these two species, the *O. atriceps* group includes 20 species and one subspecies (Table S1 in File S1). They have a mostly Palaearctic distribution but extending to East Africa (Djibouti) (Jäch & Delgado, 2017b).

(3) *O. corrugatus* group: Jäch (1992a) suggested that *O. corrugatus* Rosenhauer, despite being related to the species of the *O. andraei* and *notabilis* groups, could not be included in either of them. Our results confirm this hypothesis, but extend the *O. corrugatus* group to include two additional Mediterranean species previously included in the *O. atriceps* subgroup (Jäch, 1991; Table S1 in File S1).

(4) *O. foveolatus* group: the *O. foveolatus* group as here defined corresponds to the *O. foveolatus* subgroup of Jäch (1991), recovered as sister of the *O. metallescens* group with strong support (Fig. 1). After the additions and corrections of Delgado & Jäch (2009) and Jäch & Delgado (2010, 2014b) it currently includes 27 species, all Palaearctic (Fig. 1; Table S1 in File S1).

(5) *O. kosiensis* group: Jäch (1997b) established this group for *O. kosiensis* Champion, described within *Ochthebius* but originally not placed in any subgenus (Champion, 1920). Knisch (1924) placed it in *Asiobates* due to the resemblance of the general habitus, although the male genitalia do not correspond to those of the species of *Asiobates* (Jäch, 1997b). Jäch (2003) recognized the similarity between *O. kosiensis*, *O. strigosus* and related species, and included both species in the *strigosus* subgroup of the *metallescens* group. The study of some undescribed species deposited in the NMW (M.A. Jäch, unpublished data) suggests that the *strigosus* subgroup as defined in Jäch (2003) should be divided in the *kosiensis* and *strigosus* groups, with two and 16 described species respectively (see below; Table S1 in File S1). We could not obtain any species of the *kosiensis* group suitable for DNA extraction, and thus their phylogenetic relationships (and composition) remain untested. Based on described and undescribed species the group is so far known from the Himalaya and Myanmar.

(6) *O. lobicollis* group: Jäch (1990b) revised the *lobicollis* group, with subsequent additions by Jäch (1994) and Jäch *et al.* (1998). It currently includes 11 species with a West Palaearctic distribution (Table S1 in File S1; Fig. 1b).

(7) *O. marinus* group: the Palaearctic species of the *O. marinus* group, the most speciose within *Ochthebius* s.s., were revised by Jäch (1992b). According to our results it includes the species of the *O. jengi* group sensu Jäch (1998) and also species from the Nearctic and Neotropical regions (*O. biincisus*, *bisinuatus* and *interruptus* groups of Perkins, 1980); the Afrotropical Region (*O. extremus* and *salinarius* groups of Perkins & Balfour-Browne, 1994; Perkins, 2011; *O. capicola* group of Sabatelli *et al.*, 2016), including Madagascar (*O. alluaudi* Régimbart; Perkins, 2017); the Oriental Region (*O. masatakasatoi* Jäch; Jäch, 1992b; Jäch & Delgado, 2017a); and the Australian Region (*O. queenslandicus* Hansen; Jäch, 2001a; Perkins, 2007b).

Two of the studied subspecies were not recovered as sisters to the nominal subspecies in any of the analyses: *O. viridis fallaciosus* Ganglbauer (sister to *O. arefniae* Jäch & Delgado and another specimen probably representing an undescribed species), and *O. subpictus deletus* Rey (sister to *O. marinus* plus *O. auropallens* Fairmaire), in both cases with strong support (Figs 1, S1, S2; Table S1 in File S1). We thus upgrade the two subspecies to species, *O. fallaciosus* Ganglbauer, 1901 **stat.n.** and *O. deletus* Rey, 1885 **stat.rest.** (see Jäch, 1992b and Jäch & Delgado, 2008 for a detailed description of the species). The *O. marinus* group as here defined includes 78 species, plus one species of uncertain adscription (Table S1 in File S1). Most of these species seem to be associated to lentic habitats, frequently saline, and especially those outside the Palaearctic, coastal.

(8) *O. metallescens* group: the *O. metallescens* group was revised by Jäch (1989a). It is well defined morphologically, but many species have variable isolated populations, making species recognition and diagnosis difficult. This difficulty is reflected in the complex taxonomic history of the group, with multiple changes in the status of some species (e.g. Jäch, 1989a, 1999, 2001b). A number of species are typical of hygropetric habitats covered by a thin film of water, such as seepages or the marginal areas of stony surfaces in streams. The species group currently has 56 Palaearctic species and one subspecies (Table S1 in File S1). Due to the somewhat cryptic habits and restricted geographic ranges of many species, it is expected that this number will increase considerably.

(9) *O. nitidipennis* group: we include in the *O. nitidipennis* group the Asian species formerly included in the subgenus *Enicocerus*. As suggested by previous authors (Jäch, 1989b; Skale & Jäch, 2009; Yoshitomi & Satô, 2001), morphological similarities between these species and those of *Enicocerus* are the result of evolutionary convergence, probably due to occupying similar microhabitats on the surface of rocks and stones partially submerged in streams. The group currently includes 12 species in the Himalaya Region and East Asia (Table S1 in File S1).

(10) *O. notabilis* group: Jäch (1992a) recognized the *O. notabilis* group for species formerly included in the subgenus *Doryochthebius* Kuwert, establishing its synonymy with *Ochthebius* s.s. and differentiating the members of this group from the species of *Calobius* (see below). The group includes 13 Palaearctic species, all typical of saline or hypersaline habitats.

(11) *O. peisonis* group: *Ochthebius peisonis* was included in the *O. marinus* group by Jäch (1992b). Our results, however, place the species in a very isolated and uncertain position within *Ochthebius* s.s. We provisionally consider it in its

own group, until additional evidence clarifies its phylogenetic relationships.

(12) *O. punctatus* group: the *punctatus* group was defined by Jäch (1992c) to include the species formerly considered under subgenus *Bothochius* Rey, with irregular elytral punctation (Jäch, 1989c), and a series of species with similar morphological characters but with regular elytral striae. The *Ochthebius punctatus* group includes 53 species and one subspecies, mostly Palaearctic (reaching the Oriental Region) but with some Afrotropical species, among them the *namibiensis* group of Perkins & Balfour-Browne (1994) (Jäch, 1992c; Hansen, 1998; Perkins, 2011; Jäch & Delgado, 2017b; Table S1 in File S1).

(13) *O. quadricollis* group: the *O. quadricollis* group corresponds to the genus *Calobius*, described for *C. heeri* Wollaston from Madeira. The concept of *Calobius* was expanded by subsequent authors to include species now in different species groups (e.g. Reitter, 1886 included among them *O. notabilis* Rosenhauer and *O. quadrifoveolatus* Wollaston), and was usually treated as a subgenus. It was revised by Jäch (1993), who still considered it a subgenus, but was synonymized with *Ochthebius* s.s. by Perkins (1997), who considered it to be closely related to *Cobalius*. Its status remained uncertain, however, with some authors treating it as a genus (e.g. Audisio *et al.*, 2010) or subgenus (e.g. Jäch & Skale, 2015). Finally, Sabatelli *et al.* (2016) provided evidence of the phylogenetic position of *Calobius*, demonstrating its derived status within *Ochthebius* s.s. and considering it as the '*Calobius*' lineage, named here the *O. quadricollis* group for consistency with other species groups within *Ochthebius* s.s. Sabatelli *et al.* (2016) also found that the group includes more than the five species currently recognized (Table S1 in File S1), in agreement with previous results from the Italian species (e.g. Urbanelli & Porretta, 2008; Audisio *et al.*, 2010). Our results support this impression, as happens with the subgenus *Cobalius*, which is also in need of taxonomic revision. All species of the *O. quadricollis* group are found in coastal rockpools in the Mediterranean basin and the islands of Madeira and the Canaries.

(14) *O. rivalis* group: *Ochthebius rivalis* Champion and two similar species were originally considered to be a subgroup of the *O. metallescens* group (Jäch, 2003). Our results, however, do not support a close relationship with the species of the *O. metallescens* group, but with *O. peisonis* and the *O. notabilis*, *corrugatus* and *andraei* groups (Figs 1, S2 in File S1), with low support. In the Bayesian analysis, the two sampled species of the group were sister with strong support ($PP = 1$), but in the ML analysis they were not placed together, although with low support ($BS < 50\%$) (Fig. S2 in File S1). Currently the group includes four Asian species (including *O. himalayae* Jäch, originally described within the *O. metallescens* group, Jäch, 1989a), distributed from the Himalaya to Hainan Island (Table S1 in File S1).

(15) *Ochthebius strigosus* group: *Ochthebius strigosus* Champion, described as *Ochthebius* s.s., was included in the subgenus *Asiobates* by Jäch (1989b) based on the study of female specimens only. After the discovery of males of a related species (*O. strigoides* Jäch) they were placed in their own subgroup within the *O. metallescens* group (Jäch, 1998). We found the only sequenced species of the group to be sister of the *O. lobicollis* group with low support ($BS = 56\%$, $PP = 0.87$), and we consider it here as a distinct species group within *Ochthebius* s.s. The *O. strigosus* group currently includes 16 described plus some undescribed species, one of them included here (voucher IBE-RA617). The group is distributed in the eastern Palaearctic, including Taiwan (Jäch, 2003; Table S1 in File S1).

(16) *Ochthebius sumatrensis* group: in the original description, *O. sumatrensis* Jäch could not be placed in any of the by then described groups, although some similarities with *O. jengi* Jäch (currently in the *marinus* group) were noted (Jäch, 2001a). Several undescribed species similar to *O. sumatrensis* have been collected in recent years (M.A. Jäch *et al.*, unpublished data), among them the one from Hong Kong included here (specimen voucher MNCN-AC16; Table S1 in File S1), recovered as sister to the rest of the species of *Ochthebius* s.s. with low support in the ML analysis ($BS < 50\%$; Fig. S2 in File S1) and as sister to the *punctatus* group in the Bayesian analysis, also with low support ($PP < 0.5$; Fig. 1). They live in hygropetric surfaces, which makes them prone to evolutionary convergence with nonrelated species sharing the same habitat, thus obscuring their relationships. The group is distributed from the Himalayas to eastern China and southward to Sumatra, where it is the only known species of *Ochthebius* s.s.

(17) *O. vandykei* group: the species of the *O. vandykei* group correspond to the former *Neochthebius*, originally described as subgenus but raised to genus (within its own subtribe, Neochthebiina) by Perkins (1997) based on peculiarities of their antennae and the lack of ESDS. Jäch & Delgado (2014b), based on unpublished molecular data and on aedeagal characters, synonymized *Neochthebius* and considered it as a species group within *Ochthebius* s.s. The group currently includes eight species from the northern Pacific coast, seven in Asia and one in North America (Jäch & Delgado, 2014b; Table S1 in File S1). They are all typical of rocky seashores or other coastal microhabitats.

Genus *Protochthebius* Perkins, 1997

Type species: *Protochthebius satoi* Perkins, 1997, by original designation.

The genus *Protochthebius* was described by Perkins (1997) for *P. satoi* and *O. jagthanae* Champion, who erected also the subtribe Protochthebiina based on peculiarities of the antennae and the ESDS. Subsequently, Jäch (1997b) and Perkins (1998) described another two and three species, respectively. All seven known species of *Protochthebius* are found in the Himalaya Region, Meghalaya and Laos (Table S1 in File S1). Some of them have been found by sifting forest litter or moss (Jäch, 1997b; Perkins, 1998).

We could not obtain molecular data of any of the species of *Protochthebius*, and thus the phylogenetic placement of the genus remains uncertain. Perkins (1997) noted some presumably plesiomorphic characters of the pronotum and postocular area of the head. The species of the genus also have a reduced

ESDS system (Perkins, 1997), but this might be a secondary loss due to their microhabitat preferences. Their male genitalia are, however, typical of *Ochthebius* s.s., and when molecular data become available, the taxonomic status of *Protochthebius* may have to be changed to a subgenus of *Ochthebius* or a species group within *Ochthebius* s.s., in which case *P. satoi* would become a junior homonym.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1. Supporting Information file.

Acknowledgements

We thank all collectors mentioned in Table S3 in File S1 for allowing us to study their material, Ana Izquierdo (MNCN) and Rocío Alonso (IBE) for laboratory work and three anonymous referees for comments to previous versions of the manuscript. AV has a FPI-MINECO PhD grant from the Spanish Government. This work has been partly funded by project CGL2013-48950-C2 (AEI/FEDER, UE) to IR

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Accepted 20 June 2018

A molecular phylogeny of the tribe Ochthebiini (Coleoptera, Hydraenidae, Ochthebiinae)

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Supporting Information

Table S1: Checklist of the species of Ochthebiini, with the current (following Jäch & Skale, 2015 and Jäch *et al*., 2016) and new classifications. In bold, type species. phyl, species included in the phylogeny (in brackets, species for which the sequenced specimen was a female). Distribution: PAL, Palaearctic; AFR, Afrotropical; AUR, Australian; NAR, Nearctic; NTR, Neotropical; ORR, Oriental; ANR, Antarctic. In brackets, specimens considered to have a Palaearctic distribution in Jäch & Skale (2015), but including the Oriental or Afrotropical Regions in Hansen (1998).

Table S2: Current classification of Ochthebiini, with synonymies and type species (following Jäch & Skale, 2015 and Jäch *et al*., 2016). In bold, taxa included in the phylogeny.

Table S3: List of material used in the molecular phylogeny, including voucher numbers, accession numbers of the sequences and locality data. In bold, newly obtained sequences.

Table S4: (A) primers used for DNA amplification and sequencing reactions; (B) Typical conditions for the polymerase chain reaction.

Fig. S1: Majority rule consensus tree obtained with BEAST for the phylogeny of Ochthebiini with the best partition models. Numbers in nodes, posterior probabilities.

Fig. S2: Phylogeny obtained with RAxML, including current Ochthebiini classification. Numbers in nodes, bootstrap support values.

Fig. S3: Phylogeny obtained with RAxML with the nuclear genes only. Numbers in nodes, bootstrap support values.

Table S1: Checklist of the species of Ochthebiini, with the current (following Jäch & Skale, 2015 and Jäch *et al*., 2016) and new classifications. In bold, type species. phyl, species included in the phylogeny (in brackets, species for which the sequenced specimen was a female).
Distribution: PAL, Palaearctic; AFR, Afrotropical; AUR, Australian; NAR, Nearctic; NTR, Neotropical; ORR, Oriental; ANR, Antarctic. In brackets, specimens considered to have a Palaearctic distribution in Jäch & Skale (2015), but including the Oriental or Afrotropical Regions in Hansen (1998).

	new classification								**current classification**										
No	**genus**	**subgenus**	**species group**	**species**	**subspecies**	**author**	**taxonomic act**	**original genus**	**genus**	**subgenus**	**obs.**	**phyl.**	**PAL**	**AFR**	**AUR**	**NAR**	**NTR**	**ORR**	**ANR**
1	Meropathus			aucklandicus		Ordish, 1971		Meropathus	Meropathus						X				
2	Meropathus			campbellensis		Brookes, 1951		Meropathus	Meropathus						X				
3	Meropathus			**chuni**		Enderlein, 1901		Meropathus	Meropathus										X
4	Meropathus			johnsi		Ordish, 1971		Meropathus	Meropathus						X				
5	Meropathus			labratus		Deane, 1933		Ochthebius	Meropathus						X				
6	Meropathus			randi		Jeannel, 1953		Meropathus	Meropathus					X					
7	Meropathus			vectis		Perkins, 1980		Meropathus	Meropathus								X		
8	Meropathus			zelandicus		Ordish, 1984		Meropathus	Meropathus			1			X				
9	Ochthebius	Angiochthebius		jensenhaarupi		Knisch, 1924		Ochthebius	Gymnochthebius								X		
10	Ochthebius	Angiochthebius		octonarius		Perkins, 1980		Gymnochthebius	Gymnochthebius								X		
11	Ochthebius	Angiochthebius		**plesiotypus**		Perkins, 1980		Gymnochthebius	Gymnochthebius			1					X		
12	Ochthebius	Asiobates	bicolon	abeillei		Guillebeau, 1896		Ochthobius	Ochthebius	Asiobates			X						
13	Ochthebius	Asiobates	bicolon	amplicollis		Champion, 1925		Ochthebius	Ochthebius	Asiobates			X						
14	Ochthebius	Asiobates	bicolon	annae		Ferro, 1979		Ochthebius	Ochthebius	Asiobates			X						
15	Ochthebius	Asiobates	bicolon	arator		Ertorun & Jäch, 2014		Ochthebius	Ochthebius	Asiobates		1	X						
16	Ochthebius	Asiobates	bicolon	atricapillus		Reitter, 1901		Ochthebius	Ochthebius	Asiobates			X						
17	Ochthebius	Asiobates	bicolon	auriculatus		Rey, 1886		Ochthobius	Ochthebius	Asiobates		1	X						
18	Ochthebius	Asiobates	bicolon	avdati		Delgado & Jäch, 2007		Ochthebius	Ochthebius	Asiobates			X						
19	Ochthebius	Asiobates	bicolon	bellieri		Kuwert, 1887		Ochthebius	Ochthebius	Asiobates		1	X						
20	Ochthebius	Asiobates	bicolon	**bicolon**		Germar, 1824		Ochthebius	Ochthebius	Asiobates		1	X						
21	Ochthebius	Asiobates	bicolon	bonnairei		Guillebeau, 1896		Ochthobius	Ochthebius	Asiobates		1	X						
22	Ochthebius	Asiobates	bicolon	cantabricus		Balfour-Browne, 1979		Ochthebius	Ochthebius	Asiobates			X						
23	Ochthebius	Asiobates	bicolon	corcyraeus		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						
24	Ochthebius	Asiobates	bicolon	corsicus		Sainte-Claire Deville, 1908		Ochthebius	Ochthebius	Asiobates		1	X						
25	Ochthebius	Asiobates	bicolon	crenulatus		Mulsant & Rey, 1850		Ochthebius	Ochthebius	Asiobates		1	X						
26	Ochthebius	Asiobates	bicolon	cyprensis		Kuwert, 1890		Ochthebius	Ochthebius	Asiobates			X						
27	Ochthebius	Asiobates	bicolon	cyrenaeus		Ferro, 1985		Ochthebius	Ochthebius	Asiobates			X						
28	Ochthebius	Asiobates	bicolon	czwalinae		Kuwert, 1887		Ochthebius	Ochthebius	Asiobates			X						
29	Ochthebius	Asiobates	bicolon	depressus		Sahlberg, 1900		Ochthebius	Ochthebius	Asiobates			X						
30	Ochthebius	Asiobates	bicolon	dilatatus		Stephens, 1829		Ochthebius	Ochthebius	Asiobates		1	X						
31	Ochthebius	Asiobates	bicolon	dilucidus		Orchymont, 1940		Ochthebius	Ochthebius	Asiobates			X						
32	Ochthebius	Asiobates	bicolon	ferganensis		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						
33	Ochthebius	Asiobates	bicolon	ferroi		Fresneda, Lagar & Hernando, 1993		Ochthebius	Ochthebius	Asiobates		1	X						
34	Ochthebius	Asiobates	bicolon	figueroi		Garrido, Valladares & Régil, 1992		Ochthebius	Ochthebius	Asiobates		1	X						
35	Ochthebius	Asiobates	bicolon	flagellifer		Jäch, 2003		Ochthebius	Ochthebius	Asiobates			X						
36	Ochthebius	Asiobates	bicolon	gagliardii		Orchymont, 1940		Ochthebius	Ochthebius	Asiobates		1	X						
37	Ochthebius	Asiobates	bicolon	haberfelneri		Reitter, 1890		Ochthebius	Ochthebius	Asiobates			X						
38	Ochthebius	Asiobates	bicolon	haelii		Ferro, 1983		Ochthebius	Ochthebius	Asiobates			X						
39	Ochthebius	Asiobates	bicolon	heydeni		Kuwert, 1887		Ochthebius	Ochthebius	Asiobates		1	X						
40	Ochthebius	Asiobates	bicolon	hyblaemajoris		Ferro, 1986		Ochthebius	Ochthebius	Asiobates			X						
41	Ochthebius	Asiobates	bicolon	immaculatus		Breit, 1908		Ochthebius	Ochthebius	Asiobates		1	X						
42	Ochthebius	Asiobates	bicolon	impressipennis		Rey, 1886		Ochthobius	Ochthebius	Asiobates			X						
43	Ochthebius	Asiobates	bicolon	irenae		Ribera & Millán, 1999		Ochthebius	Ochthebius	Asiobates		1	X						
44	Ochthebius	Asiobates	bicolon	italicus		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						
45	Ochthebius	Asiobates	bicolon	jaimei		Delgado & Jäch, 2007		Ochthebius	Ochthebius	Asiobates		1	X						
46	Ochthebius	Asiobates	bicolon	kaninensis		Poppius, 1909		Ochthebius	Ochthebius	Asiobates			X						
47	Ochthebius	Asiobates	bicolon	khnzoriani		Janssens, 1974		Ochthebius	Ochthebius	Asiobates			X						
48	Ochthebius	Asiobates	bicolon	kiesenwetteri		Kuwert, 1887		Ochthebius	Ochthebius	Asiobates			X						
49	Ochthebius	Asiobates	bicolon	laevisculptus		Reitter, 1901		Ochthebius	Ochthebius	Asiobates			X						
50	Ochthebius	Asiobates	bicolon	laticollis		Pankow, 1986		Ochthebius	Ochthebius	Asiobates			X						
51	Ochthebius	Asiobates	bicolon	lederi		Jäch, 1990		Ochthebius	Ochthebius	Asiobates		1	X						
52	Ochthebius	Asiobates	bicolon	lenkoranus		Reitter, 1885		Ochthebius	Ochthebius	Asiobates		1	X						
53	Ochthebius	Asiobates	bicolon	limbicollis		Reitter, 1885		Ochthebius	Ochthebius	Asiobates			X						
54	Ochthebius	Asiobates	bicolon	maculatus		Reiche, 1872		Ochthebius	Ochthebius	Asiobates			X						
55	Ochthebius	Asiobates	bicolon	montanus		Frivaldszky, 1881		Ochthebius	Ochthebius	Asiobates		1	X						
56	Ochthebius	Asiobates	bicolon	monychus		Orchymont, 1941		Ochthebius	Ochthebius	Asiobates			X						
57	Ochthebius	Asiobates	bicolon	naxianus		Ieniştea, 1988		Asiobates	Ochthebius	Asiobates			X						
58	Ochthebius	Asiobates	bicolon	oezkani		Jäch, Kasapoğlu & Erman, 2003		Ochthebius	Ochthebius	Asiobates			X						
59	Ochthebius	Asiobates	bicolon	olicinium		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						
60	Ochthebius	Asiobates	bicolon	opacus		Baudi, 1882		Ochthebius	Ochthebius	Asiobates		1	X						
61	Ochthebius	Asiobates	bicolon	peregrinus		Orchymont, 1941		Ochthebius	Ochthebius	Asiobates		1	X						
62	Ochthebius	Asiobates	bicolon	perkinsi		Pankow, 1986		Ochthebius	Ochthebius	Asiobates			X						
63	Ochthebius	Asiobates	bicolon	pui		Perkins, 1979		Ochthebius	Ochthebius	Asiobates			X						
64	Ochthebius	Asiobates	bicolon	rivibelli		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						
65	Ochthebius	Asiobates	bicolon	sardus		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						
66	Ochthebius	Asiobates	bicolon	striatus		Castelnau, 1840		Hydraena	Ochthebius	Asiobates		1	X						
67	Ochthebius	Asiobates	bicolon	stygialis		Orchymont, 1937		Ochthebius	Ochthebius	Asiobates		1	X						
68	Ochthebius	Asiobates	bicolon	subopacus		Reitter, 1885		Ochthebius	Ochthebius	Asiobates			X						
69	Ochthebius	Asiobates	bicolon	tadilatus		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						
70	Ochthebius	Asiobates	bicolon	thraciae		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						
71	Ochthebius	Asiobates	bicolon	turcmeniae		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						
72	Ochthebius	Asiobates	minimus	adventicius		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						

73	Ochthebius	Asiobates	minimus	aeneus		Stephens, 1835		Ochthebius	Ochthebius	Asiobates		1	X						
74	Ochthebius	Asiobates	minimus	akbuluti		Jäch, Kasapoğlu & Erman, 2003		Ochthebius	Ochthebius	Asiobates			X						
75	Ochthebius	Asiobates	minimus	alpinus		Ieniştea, 1979		Homalochthebius	Ochthebius	Asiobates		1	X						
76	Ochthebius	Asiobates	minimus	andreinii	andreinii	Régimbart, 1905		Ochthebius	Ochthebius	Asiobates		1		X					
77	Ochthebius	Asiobates	minimus	andreinii	andriscus	Orchymont, 1948		Ochthebius	Ochthebius	Asiobates				X					
78	Ochthebius	Asiobates	minimus	andreinii	androsthenus	Orchymont, 1948		Ochthebius	Ochthebius	Asiobates				X					
79	Ochthebius	Asiobates	minimus	andronius		Orchymont, 1948		Ochthebius	Ochthebius	Asiobates		1		X					
80	Ochthebius	Asiobates	minimus	apache		Perkins, 1980		Ochthebius	Ochthebius	Asiobates						X	X		
81	Ochthebius	Asiobates	minimus	apicalis		Sharp, 1882		Ochthebius	Ochthebius	Asiobates							X		
82	Ochthebius	Asiobates	minimus	brevipennis		Perkins, 1980		Ochthebius	Ochthebius	Asiobates						X			
83	Ochthebius	Asiobates	minimus	browni		Perkins, 1980		Ochthebius	Ochthebius	Asiobates							X		
84	Ochthebius	Asiobates	minimus	bupunctus		Perkins, 2011		Ochthebius	Ochthebius	Asiobates				X					
85	Ochthebius	Asiobates	minimus	coomani		Orchymont, 1925		Ochthebius	Ochthebius	Asiobates								X	
86	Ochthebius	Asiobates	minimus	cribricollis		LeConte, 1850		Ochthebius	Ochthebius	Asiobates						X			
87	Ochthebius	Asiobates	minimus	discretus		LeConte, 1878		Ochthebius	Ochthebius	Asiobates		1				X	X		
88	Ochthebius	Asiobates	minimus	flavipes		Dalla Torre, 1877		Ochthebius	Ochthebius	Asiobates		1	X						
89	Ochthebius	Asiobates	minimus	formosanus		Jäch, 1998		Ochthebius	Ochthebius	Asiobates			X						
90	Ochthebius	Asiobates	minimus	furcatus		Pu, 1958		Ochthebius	Ochthebius	Asiobates			X						
91	Ochthebius	Asiobates	minimus	hebaueri		Jäch, 1983		Ochthebius	Ochthebius	Asiobates			X						
92	Ochthebius	Asiobates	minimus	hibernus		Perkins, 1980		Ochthebius	Ochthebius	Asiobates						X			
93	Ochthebius	Asiobates	minimus	hokkaidensis		Jäch, 1998		Ochthebius	Ochthebius	Asiobates		1	X						
94	Ochthebius	Asiobates	minimus	hungaricus		Endrödy-Younga, 1967		Ochthebius	Ochthebius	Asiobates			X						
95	Ochthebius	Asiobates	minimus	lurugosus		Jäch, 1998		Ochthebius	Ochthebius	Asiobates			X						
96	Ochthebius	Asiobates	minimus	mexicanus		Perkins, 1980		Ochthebius	Ochthebius	Asiobates							X		
97	Ochthebius	Asiobates	minimus	mimicus		Brown, 1933		Ochthebius	Ochthebius	Asiobates						X			
98	Ochthebius	Asiobates	minimus	minervius	minervius	Orchymont, 1940		Ochthebius	Ochthebius	Asiobates			X						
99	Ochthebius	Asiobates	minimus	minervius	semechonitis	Jäch, 1998		Ochthebius	Ochthebius	Asiobates			X						
100	Ochthebius	Asiobates	minimus	minimus		Fabricius, 1792		Helophorus	Ochthebius	Asiobates		1	X						
101	Ochthebius	Asiobates	minimus	monticola		Orchymont, 1948		Ochthebius	Ochthebius	Asiobates				X					
102	Ochthebius	Asiobates	minimus	nonaginta		Jäch, 1998		Ochthebius	Ochthebius	Asiobates			X						
103	Ochthebius	Asiobates	minimus	orbus		Perkins, 1980		Ochthebius	Ochthebius	Asiobates						X			
104	Ochthebius	Asiobates	minimus	otavalensis		Anderson, 1983		Ochthebius	Ochthebius	Asiobates							X		
105	Ochthebius	Asiobates	minimus	perdurus		Reitter, 1899		Ochthebius	Ochthebius	Asiobates			X						
106	Ochthebius	Asiobates	minimus	pliginskiyi		Jäch, 1990		Ochthebius	Ochthebius	Asiobates			X						
107	Ochthebius	Asiobates	minimus	putnamensis		Blatchley, 1910		Ochthebius	Ochthebius	Asiobates						X			
108	Ochthebius	Asiobates	minimus	regimbarti		Knisch, 1924		Ochthebius	Ochthebius	Asiobates				X					
109	Ochthebius	Asiobates	minimus	remotus		Reitter, 1885		Ochthebius	Ochthebius	Asiobates		1	X						
110	Ochthebius	Asiobates	minimus	reticulocostus		Perkins, 1980		Ochthebius	Ochthebius	Asiobates							X		
111	Ochthebius	Asiobates	minimus	rugulosus		Wollaston, 1857		Ochthebius	Ochthebius	Asiobates		1	X						
112	Ochthebius	Asiobates	minimus	sanabrensis		Valladares & Jäch, 2008		Ochthebius	Ochthebius	Asiobates		1	X						
113	Ochthebius	Asiobates	minimus	similis		Sharp, 1882		Ochthebius	Ochthebius	Asiobates						X	X		
114	Ochthebius	Asiobates	minimus	unimaculatus		Pu, 1958		Ochthebius	Ochthebius	Asiobates			X						
115	Ochthebius	Asiobates	minimus	yunnanensis		Orchymont, 1925		Ochthebius	Ochthebius	Asiobates			X						
116	Ochthebius	Asiobates	puncticollis	angularidus		Perkins, 1980		Ochthebius	Ochthebius	Asiobates						X	X		
117	Ochthebius	Asiobates	puncticollis	leechi		Wood & Perkins, 1978		Ochthebius	Ochthebius	Asiobates						X			
118	Ochthebius	Asiobates	puncticollis	martini		Fall, 1919		Ochthebius	Ochthebius	Asiobates						X			
119	Ochthebius	Asiobates	puncticollis	puncticollis		LeConte, 1852		Ochthebius	Ochthebius	Asiobates		1				X	X		
120	Ochthebius	Aulacochthebius		alienus		Orchymont, 1929		Ochthebius	Aulacochthebius					X					
121	Ochthebius	Aulacochthebius		amrishi		Makhan, 2004		Ochthebius	Ochthebius	Ochthebius	possibly a synonym of O. asiaticus							X	
122	Ochthebius	Aulacochthebius		asiaticus		Orchymont, 1929		Ochthebius	Aulacochthebius									X	
123	Ochthebius	Aulacochthebius		continentalis		Orchymont, 1929		Ochthebius	Aulacochthebius					X					
124	Ochthebius	Aulacochthebius		densus		Orchymont, 1929		Ochthebius	Aulacochthebius									X	
125	Ochthebius	Aulacochthebius		**exaratus**		Mulsant, 1844		Ochthebius	Aulacochthebius			1	X	X					
126	Ochthebius	Aulacochthebius		hunanensis		Pu, 1958		Ochthebius	Aulacochthebius				X						
127	Ochthebius	Aulacochthebius		libertarius		Aguilera, Ribera & Hernando, 1998	**comb.n.**	Aulacochthebius	Aulacochthebius			1	X						
128	Ochthebius	Aulacochthebius		manipurensis		Orchymont, 1929		Ochthebius	Aulacochthebius									X	
129	Ochthebius	Aulacochthebius		narentinus		Reitter, 1885		Ochthebius	Aulacochthebius			1	X						
130	Ochthebius	Aulacochthebius		perlaevis		Perkins, 2017	**comb.n.**	Aulacochthebius	Aulacochthebius			1		X					
131	Ochthebius	Aulacochthebius		plicicollis		Fairmaire, 1898		Ochthebius	Aulacochthebius					X					
132	Ochthebius	Aulacochthebius		tenuipunctus		Régimbart, 1906		Ochthebius	Aulacochthebius					X					
133	Ochthebius	Cobalius		adriaticus	adriaticus	Reitter, 1886		Ochthebius	Ochthebius	Ochthebius		1	X						
134	Ochthebius	Cobalius		adriaticus	moreanus	Pretner, 1929		Ochthebius	Ochthebius	Ochthebius			X						
135	Ochthebius	Cobalius		adriaticus	pleuralis	Reitter, 1886		Ochthebius	Ochthebius	Ochthebius			X						
136	Ochthebius	Cobalius		algicola		Wollaston, 1871		Ochthebius	Ochthebius	Ochthebius			X						
137	Ochthebius	Cobalius		balfourbrownei		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius				X					
138	Ochthebius	Cobalius		biltoni		Jäch & Delgado, 2017		Ochthebius	Ochthebius	Cobalius			X						
139	Ochthebius	Cobalius		celatus		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius		1	X						
140	Ochthebius	Cobalius		freyi		Orchymont, 1940		Ochthebius	Ochthebius	Ochthebius		1	X						
141	Ochthebius	Cobalius		**lejolisii**		Mulsant & Rey, 1861		Ochthebius	Ochthebius	Ochthebius		1	X						
142	Ochthebius	Cobalius		serratus		Rosenhauer, 1856		Ochthebius	Ochthebius	Ochthebius		1	X						
143	Ochthebius	Cobalius		subinteger		Mulsant & Rey, 1861		Ochthebius	Ochthebius	Ochthebius		1	X						
144	Ochthebius	Enicocerus		aguilerai		Ribera, Castro & Hernando, 2010		Ochthebius	Ochthebius	Enicocerus		1	X						
145	Ochthebius	Enicocerus		albanicus		Orchymont, 1941		Ochthebius	Ochthebius	Enicocerus			X						
146	Ochthebius	Enicocerus		anatolicus		Janssens, 1963		Ochthebius	Ochthebius	Enicocerus			X						
147	Ochthebius	Enicocerus		angelinii		Ferro, 2008		Ochthebius	Ochthebius	Enicocerus			X						
148	Ochthebius	Enicocerus		colveranus		Ferro, 1979		Ochthebius	Ochthebius	Enicocerus		1	X						
149	Ochthebius	Enicocerus		delyi		Hebauer, 1990		Ochthebius	Ochthebius	Enicocerus			X						
150	Ochthebius	Enicocerus		**exsculptus**		Germar, 1824		Ochthebius	Ochthebius	Enicocerus		1	X						

151	Ochthebius	Enicocerus		gibbosus		Germar, 1824		Ochthebius	Ochthebius	Enicocerus		1	X						
152	Ochthebius	Enicocerus		granulatus		Mulsant, 1844		Ochthebius	Ochthebius	Enicocerus		1	X						
153	Ochthebius	Enicocerus		halbherri		Reitter, 1890		Henicocerus	Ochthebius	Enicocerus		1	X						
154	Ochthebius	Enicocerus		legionensis		Hebauer & Valladares Díez, 1985		Ochthebius	Ochthebius	Enicocerus		1	X						
155	Ochthebius	Enicocerus		melanescens		Dalla Torre, 1877		Ochthebius	Ochthebius	Enicocerus		1	X						
156	Ochthebius	Enicocerus		montenegrinus		Ganglbauer, 1901		Ochthebius	Ochthebius	Enicocerus			X						
157	Ochthebius	Enicocerus		saboorii		Skale & Jäch, 2009		Ochthebius	Ochthebius	Enicocerus		1	X						
158	Ochthebius	Enicocerus		sattmanni		Jäch, 1992		Ochthebius	Ochthebius	Enicocerus			X						
159	Ochthebius	Enicocerus?		benefossus		LeConte, 1878		Ochthebius	Ochthebius	Enicocerus						X			
160	Ochthebius	Gymnanthelius		clypeatus		Deane, 1931		Ochthebius	Gymnanthelius						X				
161	Ochthebius	Gymnanthelius		cupreus		Deane, 1937		Ochthebius	Gymnanthelius						X				
162	Ochthebius	Gymnanthelius		**hieroglyphicus**		Deane, 1933		Ochthebius	Gymnanthelius						X				
163	Ochthebius	Gymnanthelius		lamingtonensis		Perkins, 2004	**comb.n.**	Gymnanthelius	Gymnanthelius						X				
164	Ochthebius	Gymnanthelius		maxipunctus		Perkins, 2004	**comb.n.**	Gymnanthelius	Gymnanthelius						X				
165	Ochthebius	Gymnanthelius		opacicollis		Perkins, 2004	**comb.n.**	Gymnanthelius	Gymnanthelius			1			X				
166	Ochthebius	Gymnanthelius		porchi		Perkins, 2004	**comb.n.**	Gymnanthelius	Gymnanthelius			1			X				
167	Ochthebius	Gymnanthelius		tunicus		Perkins, 2004	**comb.n.**	Gymnanthelius	Gymnanthelius						X				
168	Ochthebius	Gymnochthebius	australis	angulonotus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
169	Ochthebius	Gymnochthebius	australis	australis		Blackburn, 1888		Ochthebius	Gymnochthebius			1			X				
170	Ochthebius	Gymnochthebius	australis	bacchusi		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
171	Ochthebius	Gymnochthebius	australis	benesculptus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
172	Ochthebius	Gymnochthebius	australis	brisbanensis		Blackburn, 1898		Ochthebius	Gymnochthebius						X				
173	Ochthebius	Gymnochthebius	australis	clarki		Deane, 1931		Ochthebius	Gymnochthebius						X				
174	Ochthebius	Gymnochthebius	australis	coruscus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
175	Ochthebius	Gymnochthebius	australis	fontinalis		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
176	Ochthebius	Gymnochthebius	australis	fumosus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
177	Ochthebius	Gymnochthebius	australis	hesperius		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
178	Ochthebius	Gymnochthebius	australis	inlineatus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
179	Ochthebius	Gymnochthebius	australis	levis		Deane, 1933		Ochthebius	Gymnochthebius						X				
180	Ochthebius	Gymnochthebius	australis	lividus		Deane, 1933		Ochthebius	Gymnochthebius			1			X				
181	Ochthebius	Gymnochthebius	australis	lustrosulcus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
182	Ochthebius	Gymnochthebius	australis	minipunctus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
183	Ochthebius	Gymnochthebius	australis	nanosetus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
184	Ochthebius	Gymnochthebius	australis	nicki		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
185	Ochthebius	Gymnochthebius	australis	nigriceps		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
186	Ochthebius	Gymnochthebius	australis	notalis		Deane, 1933		Ochthebius	Gymnochthebius						X				
187	Ochthebius	Gymnochthebius	australis	papua		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
188	Ochthebius	Gymnochthebius	australis	perpunctus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
189	Ochthebius	Gymnochthebius	australis	pluvipennis		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
190	Ochthebius	Gymnochthebius	australis	probus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius			1			X				
191	Ochthebius	Gymnochthebius	australis	radiatus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
192	Ochthebius	Gymnochthebius	australis	resplendens		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
193	Ochthebius	Gymnochthebius	australis	rhombus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
194	Ochthebius	Gymnochthebius	australis	semicylindrus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
195	Ochthebius	Gymnochthebius	australis	setosus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius			1			X				
196	Ochthebius	Gymnochthebius	australis	sexplanatus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
197	Ochthebius	Gymnochthebius	australis	squamifer		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
198	Ochthebius	Gymnochthebius	australis	subsulcatus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
199	Ochthebius	Gymnochthebius	australis	tenebricosus		Deane, 1931		Ochthebius	Gymnochthebius						X				
200	Ochthebius	Gymnochthebius	australis	trilineatus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
201	Ochthebius	Gymnochthebius	australis	truncatus		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
202	Ochthebius	Gymnochthebius	australis	wattsi		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
203	Ochthebius	Gymnochthebius	australis	weiri		Perkins, 2005	**comb.n.**	Gymnochthebius	Gymnochthebius						X				
204	Ochthebius	Gymnochthebius	fossatus	bartyrae		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius								X		
205	Ochthebius	Gymnochthebius	fossatus	bisagittatus		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius								X		
206	Ochthebius	Gymnochthebius	fossatus	chilenus		Balfour-Browne, 1971		Ochthebius	Gymnochthebius								X		
207	Ochthebius	Gymnochthebius	fossatus	clandestinus		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius								X		
208	Ochthebius	Gymnochthebius	fossatus	compactus		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius								X		
209	Ochthebius	Gymnochthebius	fossatus	crassipes		Sharp, 1882		Ochthebius	Gymnochthebius								X		
210	Ochthebius	Gymnochthebius	fossatus	curvus		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius								X		
211	Ochthebius	Gymnochthebius	fossatus	fossatus		LeConte, 1855		Ochthebius	Gymnochthebius			1				X	X		
212	Ochthebius	Gymnochthebius	fossatus	francki		Bruch, 1915		Ochthebius	Gymnochthebius								X		
213	Ochthebius	Gymnochthebius	fossatus	germaini		Zaitzev, 1908		Ochthebius	Gymnochthebius			1					X		
214	Ochthebius	Gymnochthebius	fossatus	ischigualasto		Perkins & Archangelsky, 2002	**comb.n.**	Gymnochthebius	Gymnochthebius								X		
215	Ochthebius	Gymnochthebius	fossatus	laevipennis		LeConte, 1878		Ochthebius	Gymnochthebius							X	X		
216	Ochthebius	Gymnochthebius	fossatus	maureenae		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius							X			
217	Ochthebius	Gymnochthebius	fossatus	**nitidus**		LeConte, 1850		Ochthebius	Gymnochthebius							X			
218	Ochthebius	Gymnochthebius	fossatus	oppositus		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius							X	X		
219	Ochthebius	Gymnochthebius	fossatus	perlabidus		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius								X		
220	Ochthebius	Gymnochthebius	fossatus	peruvianus		Balfour-Browne, 1971		Ochthebius	Gymnochthebius			1					X		
221	Ochthebius	Gymnochthebius	fossatus	reticulatissimus		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius								X		
222	Ochthebius	Gymnochthebius	fossatus	reticulatus		Orchymont, 1943		Ochthebius	Gymnochthebius								X		
223	Ochthebius	Gymnochthebius	fossatus	seminole		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius							X			
224	Ochthebius	Gymnochthebius	fossatus	tectus		Perkins, 1980	**comb.n.**	Gymnochthebius	Gymnochthebius								X		
225	Ochthebius	Gymnochthebius	fossatus	topali		Balfour-Browne, 1971		Ochthebius	Gymnochthebius								X		
226	Ochthebius	Hughleechia		giulianii		Perkins, 1981	**comb.n.**	Hughleechia	Hughleechia			1			X				
227	Ochthebius	Hughleechia		gracilis		Perkins, 2007	**comb.n.**	Hughleechia	Hughleechia						X				
228	Ochthebius	Micragasma		minoicus		Hernando, Villastrigo & Ribera, 2017		Ochthebius	Ochthebius				X						

229	Ochthebius	Micragasma		paradoxus		Sahlberg, 1900		Micragasma	Micragasma			1	X						
230	Ochthebius	Micragasma		substrigosus		Reitter, 1897		Ochthebius	Micragasma				X						
231	Ochthebius	Ochthebius	andraei	andraei	andraei	Breit, 1920		Ochthebius	Ochthebius	Ochthebius			X						
232	Ochthebius	Ochthebius	andraei	andraei	explanatus	Orchymont, 1937		Ochthebius	Ochthebius	Ochthebius			X						
233	Ochthebius	Ochthebius	andraei	caspius		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius			X						
234	Ochthebius	Ochthebius	andraei	cupricollis		Sahlberg, 1903		Ochthebius	Ochthebius	Ochthebius			X						
235	Ochthebius	Ochthebius	andraei	inelegans		Jäch, 2002		Ochthebius	Ochthebius	Ochthebius			X						
236	Ochthebius	Ochthebius	andraei	madli		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius			X						
237	Ochthebius	Ochthebius	andraei	patergazellae		Jäch & Delgado, 2010		Ochthebius	Ochthebius	Ochthebius		1	X						
238	Ochthebius	Ochthebius	atriceps	andalusicus		Jäch & Castro, 1999		Ochthebius	Ochthebius	Ochthebius		1	X						
239	Ochthebius	Ochthebius	atriceps	anxifer		Balfour-Browne, 1979		Ochthebius	Ochthebius	Ochthebius		1	X						
240	Ochthebius	Ochthebius	atriceps	atriceps		Fairmaire, 1879		Ochthebius	Ochthebius	Ochthebius		1	X						
241	Ochthebius	Ochthebius	atriceps	burjkhalifa		Jäch & Delgado, 2014		Ochthebius	Ochthebius	Ochthebius		1	X						
242	Ochthebius	Ochthebius	atriceps	cameroni		Balfour-Browne, 1951		Ochthebius	Ochthebius	Ochthebius			X						
243	Ochthebius	Ochthebius	atriceps	dentifer		Rey, 1885		Ochthebius	Ochthebius	Ochthebius		1	X						
244	Ochthebius	Ochthebius	atriceps	depressionis		Jäch, 1991		Ochthebius	Ochthebius	Ochthebius		1	X						
245	Ochthebius	Ochthebius	atriceps	despoliatus		Jäch & Delgado, 2014		Ochthebius	Ochthebius	Ochthebius		1	X						
246	Ochthebius	Ochthebius	atriceps	falcatus		Jäch, 1991		Ochthebius	Ochthebius	Ochthebius			X						
247	Ochthebius	Ochthebius	atriceps	fausti		Sharp, 1887		Ochthebius	Ochthebius	Ochthebius			X						
248	Ochthebius	Ochthebius	atriceps	faustinus		Orchymont, 1940		Ochthebius	Ochthebius	Ochthebius			X						
249	Ochthebius	Ochthebius	atriceps	hesperides		Balfour-Browne, 1976		Ochthebius	Ochthebius	Ochthebius				X					
250	Ochthebius	Ochthebius	atriceps	karasui		Ferro, 1986		Ochthebius	Ochthebius	Ochthebius			X						
251	Ochthebius	Ochthebius	atriceps	lacustatta		Jäch, 1991		Ochthebius	Ochthebius	Ochthebius			X						
252	Ochthebius	Ochthebius	atriceps	loulae		Jäch & Delgado, 2017		Ochthebius	Ochthebius	Ochthebius		1		X					
253	Ochthebius	Ochthebius	atriceps	praetermissus		Jäch, 1991		Ochthebius	Ochthebius	Ochthebius			X						
254	Ochthebius	Ochthebius	atriceps	recurvatus		Jäch, 1991		Ochthebius	Ochthebius	Ochthebius			X						
255	Ochthebius	Ochthebius	atriceps	sculpturatus		Sahlberg, 1900		Ochthebius	Ochthebius	Ochthebius			X						
256	Ochthebius	Ochthebius	atriceps	tacapasensis	baeticus	Ferro, 1984		Ochthebius	Ochthebius	Ochthebius		1	X						
257	Ochthebius	Ochthebius	atriceps	tacapasensis	tacapasensis	Ferro, 1983		Ochthebius	Ochthebius	Ochthebius			X						
258	Ochthebius	Ochthebius	atriceps	thermalis		Janssens, 1965		Ochthebius	Ochthebius	Ochthebius		1	X						
259	Ochthebius	Ochthebius	corrugatus	corrugatus		Rosenhauer, 1856		Ochthebius	Ochthebius	Ochthebius		1	X						
260	Ochthebius	Ochthebius	corrugatus	gauthieri		Peyerimhoff, 1924		Ochthebius	Ochthebius	Ochthebius		1	X						
261	Ochthebius	Ochthebius	corrugatus	perpusillus		Ferro, 1985		Ochthebius	Ochthebius	Ochthebius		1	X						
262	Ochthebius	Ochthebius	foveolatus	afghanicus		Jäch, 1991		Ochthebius	Ochthebius	Ochthebius			X						
263	Ochthebius	Ochthebius	foveolatus	alpheius		Janssens, 1959		Ochthebius	Ochthebius	Ochthebius			X						
264	Ochthebius	Ochthebius	foveolatus	elisae		Sahlberg, 1900		Ochthebius	Ochthebius	Ochthebius		1	X						
265	Ochthebius	Ochthebius	foveolatus	foveolatus		Germar, 1824		Ochthebius	Ochthebius	Ochthebius		1	X						
266	Ochthebius	Ochthebius	foveolatus	hajeki		Jäch & Delgado, 2014		Ochthebius	Ochthebius	Ochthebius		1	X						
267	Ochthebius	Ochthebius	foveolatus	harteni		Jäch & Delgado, 2010		Ochthebius	Ochthebius	Ochthebius		1	X						
268	Ochthebius	Ochthebius	foveolatus	hellenicus		Ieniştea, 1988		Hymenodes	Ochthebius	Ochthebius			X						
269	Ochthebius	Ochthebius	foveolatus	inconspicuus		Jäch, 1991		Ochthebius	Ochthebius	Ochthebius			X						
270	Ochthebius	Ochthebius	foveolatus	indicus		Ieniştea, 1988		Hymenodes	Ochthebius	Ochthebius			X						
271	Ochthebius	Ochthebius	foveolatus	innexus		Balfour-Browne, 1951		Ochthebius	Ochthebius	Ochthebius			X						
272	Ochthebius	Ochthebius	foveolatus	magnannulatus		Delgado & Jäch, 2009		Ochthebius	Ochthebius	Ochthebius		1	X						
273	Ochthebius	Ochthebius	foveolatus	marginalis		Rey, 1886		Ochthobius	Ochthebius	Ochthebius		1	X						
274	Ochthebius	Ochthebius	foveolatus	mediterraneus		Ieniştea, 1988		Hymenodes	Ochthebius	Ochthebius		1	X						
275	Ochthebius	Ochthebius	foveolatus	medius		Jäch, 1991		Ochthebius	Ochthebius	Ochthebius			X						
276	Ochthebius	Ochthebius	foveolatus	merinidicus		Ferro, 1985		Ochthebius	Ochthebius	Ochthebius		1	X						
277	Ochthebius	Ochthebius	foveolatus	mutatus		Jäch, 1991		Ochthebius	Ochthebius	Ochthebius			X						
278	Ochthebius	Ochthebius	foveolatus	pakistanicus		Delgado & Jäch, 2009		Ochthebius	Ochthebius	Ochthebius			X						
279	Ochthebius	Ochthebius	foveolatus	pallidulus		Kuwert, 1887		Ochthebius	Ochthebius	Ochthebius			X						
280	Ochthebius	Ochthebius	foveolatus	parvannulatus		Delgado & Jäch, 2009		Ochthebius	Ochthebius	Ochthebius			X						
281	Ochthebius	Ochthebius	foveolatus	pedicularius		Kuwert, 1887		Ochthebius	Ochthebius	Ochthebius		1	X						
282	Ochthebius	Ochthebius	foveolatus	pudilaceris		Ferro, 1982		Ochthebius	Ochthebius	Ochthebius			X						
283	Ochthebius	Ochthebius	foveolatus	satoi		Nakane, 1965		Ochthebius	Ochthebius	Ochthebius		1	X						
284	Ochthebius	Ochthebius	foveolatus	siculus		Kuwert, 1887		Ochthebius	Ochthebius	Ochthebius			X						
285	Ochthebius	Ochthebius	foveolatus	sidanus		Orchymont, 1942		Ochthebius	Ochthebius	Ochthebius		1	X						
286	Ochthebius	Ochthebius	foveolatus	speculator		Jäch, 1991		Ochthebius	Ochthebius	Ochthebius			X						
287	Ochthebius	Ochthebius	foveolatus	uskubensis		Hebauer, 1986		Ochthebius	Ochthebius	Ochthebius			X						
288	Ochthebius	Ochthebius	foveolatus	virgula		Ferro, 1986		Ochthebius	Ochthebius	Ochthebius		1	X						
289	Ochthebius	Ochthebius	kosiensis	asperatus		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
290	Ochthebius	Ochthebius	kosiensis	kosiensis		Champion, 1920		Ochthebius	Ochthebius	Ochthebius			X						
291	Ochthebius	Ochthebius	lobicollis	basilicatus		Fiori, 1915		Ochthebius	Ochthebius	Ochthebius		(1)	X						
292	Ochthebius	Ochthebius	lobicollis	caesaraugustae		Jäch, Ribera & Aguilera, 1998		Ochthebius	Ochthebius	Ochthebius		1	X						
293	Ochthebius	Ochthebius	lobicollis	delgadoi		Jäch, 1994		Ochthebius	Ochthebius	Ochthebius		1	X						
294	Ochthebius	Ochthebius	lobicollis	eyrei		Jäch, 1990		Ochthebius	Ochthebius	Ochthebius		1	X						
295	Ochthebius	Ochthebius	lobicollis	fossulatus		Mulsant, 1844		Ochthebius	Ochthebius	Ochthebius			X						
296	Ochthebius	Ochthebius	lobicollis	lapidicola		Wollaston, 1864		Ochthebius	Ochthebius	Ochthebius		1	X						
297	Ochthebius	Ochthebius	lobicollis	lobicollis		Rey, 1885		Ochthebius	Ochthebius	Ochthebius		1	X						
298	Ochthebius	Ochthebius	lobicollis	mauretanicus		Jäch, 1990		Ochthebius	Ochthebius	Ochthebius			X						
299	Ochthebius	Ochthebius	lobicollis	quadrifossulatus		Waltl, 1835		Ochthebius	Ochthebius	Ochthebius		1	X						
300	Ochthebius	Ochthebius	lobicollis	tivelunus		Ferro, 1984		Ochthebius	Ochthebius	Ochthebius		1	X						
301	Ochthebius	Ochthebius	lobicollis	velutinus		Fairmaire, 1883		Ochthebius	Ochthebius	Ochthebius			X						
302	Ochthebius	Ochthebius	marinus	alluaudi		Régimbart, 1903		Ochthebius	Ochthebius	Ochthebius				X					
303	Ochthebius	Ochthebius	marinus	alpinopetrus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X			
304	Ochthebius	Ochthebius	marinus	anchorus		Perkins, 2011		Ochthebius	Ochthebius	Ochthebius				X					
305	Ochthebius	Ochthebius	marinus	angusi		Jäch, 1994		Ochthebius	Ochthebius	Ochthebius			X						
306	Ochthebius	Ochthebius	marinus	arabicus		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius			X						

307	Ochthebius	Ochthebius	marinus	arefniae		Jäch & Delgado, 2008		Ochthebius	Ochthebius	Ochthebius		1	X						
308	Ochthebius	Ochthebius	marinus	arenicolus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X			
309	Ochthebius	Ochthebius	marinus	arizonicus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius		(1)				X			
310	Ochthebius	Ochthebius	marinus	attritus		LeConte, 1878		Ochthebius	Ochthebius	Ochthebius						X	X		
311	Ochthebius	Ochthebius	marinus	auropallens		Fairmaire, 1879		Ochthebius	Ochthebius	Ochthebius		1	X						
312	Ochthebius	Ochthebius	marinus	aztecus		Sharp, 1887		Ochthebius	Ochthebius	Ochthebius		1				X	X		
313	Ochthebius	Ochthebius	marinus	bactrianus		Janssens, 1962		Ochthebius	Ochthebius	Ochthebius			X						
314	Ochthebius	Ochthebius	marinus	batesoni		Blair, 1933		Ochthebius	Ochthebius	Ochthebius		1					X		
315	Ochthebius	Ochthebius	marinus	bellstedti		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius			X						
316	Ochthebius	Ochthebius	marinus	bernhardi		Jäch & Delgado, 2008		Ochthebius	Ochthebius	Ochthebius			X						
317	Ochthebius	Ochthebius	marinus	bicomicus		Perkins, 2011		Ochthebius	Ochthebius	Ochthebius				X					
318	Ochthebius	Ochthebius	marinus	biincisus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X			
319	Ochthebius	Ochthebius	marinus	bisinuatus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius		(1)				X			
320	Ochthebius	Ochthebius	marinus	borealis		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X	X		
321	Ochthebius	Ochthebius	marinus	californicus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X			
322	Ochthebius	Ochthebius	marinus	capicola		Péringuey, 1892		Hydraena	Ochthebius	Ochthebius		1		X					
323	Ochthebius	Ochthebius	marinus	chappuisi		Orchymont, 1948		Ochthebius	Ochthebius	Ochthebius		(1)		X					
324	Ochthebius	Ochthebius	marinus	costatellus		Reitter, 1897		Ochthebius	Ochthebius	Ochthebius			X			X			
325	Ochthebius	Ochthebius	marinus	costipennis		Fall, 1901		Ochthebius	Ochthebius	Ochthebius						X			
326	Ochthebius	Ochthebius	marinus	crassalus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X			
327	Ochthebius	Ochthebius	marinus	crenatus		Hatch, 1965		Ochthebius	Ochthebius	Ochthebius						X			
328	Ochthebius	Ochthebius	marinus	deletus		Rey, 1885	**stat.rest.**	Ochthebius	Ochthebius	Ochthebius	currently a subspecies of O. subpictus	1	X						
329	Ochthebius	Ochthebius	marinus	delhiensis		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius			X					X	
330	Ochthebius	Ochthebius	marinus	endroedyi		Perkins, 2011		Ochthebius	Ochthebius	Ochthebius				X					
331	Ochthebius	Ochthebius	marinus	erzerumi		Kuwert, 1887		Ochthebius	Ochthebius	Ochthebius			X						
332	Ochthebius	Ochthebius	marinus	evanescens		Sahlberg, 1875		Ochthebius	Ochthebius	Ochthebius		1	X						
333	Ochthebius	Ochthebius	marinus	extremus		Péringuey, 1892		Hydraena	Ochthebius	Ochthebius				X					
334	Ochthebius	Ochthebius	marinus	fallaciosus		Ganglbauer, 1901	**stat.n.**	Ochthebius	Ochthebius	Ochthebius	currently a subspecies of O. viridis	1	X						
335	Ochthebius	Ochthebius	marinus	granulinus		Perkins, 2011		Ochthebius	Ochthebius	Ochthebius				X					
336	Ochthebius	Ochthebius	marinus	gruwelli		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius		1				X	X		
337	Ochthebius	Ochthebius	marinus	hainanensis		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
338	Ochthebius	Ochthebius	marinus	interruptus		LeConte, 1852		Ochthebius	Ochthebius	Ochthebius		1				X	X		
339	Ochthebius	Ochthebius	marinus	involatus		Perkins, 2011		Ochthebius	Ochthebius	Ochthebius				X					
340	Ochthebius	Ochthebius	marinus	janssensi		Ferro, 1983		Ochthebius	Ochthebius	Ochthebius			X						
341	Ochthebius	Ochthebius	marinus	jengi		Jäch, 1998		Ochthebius	Ochthebius	Ochthebius			X						
342	Ochthebius	Ochthebius	marinus	kuwerti		Reitter, 1897		Ochthebius	Ochthebius	Ochthebius			X						
343	Ochthebius	Ochthebius	marinus	lecontei		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X			
344	Ochthebius	Ochthebius	marinus	lenensis		Poppius, 1907		Ochthebius	Ochthebius	Ochthebius			X						
345	Ochthebius	Ochthebius	marinus	lineatus		LeConte, 1852		Ochthebius	Ochthebius	Ochthebius		1				X	X		
346	Ochthebius	Ochthebius	marinus	lividipennis		Peyron, 1858		Ochthebius	Ochthebius	Ochthebius		1	X						
347	Ochthebius	Ochthebius	marinus	madrensis		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X	X		
348	Ochthebius	Ochthebius	marinus	**marinus**		Paykull, 1798		Elophorus	Ochthebius	Ochthebius		1	X			X			
349	Ochthebius	Ochthebius	marinus	masatakasatoi		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius								X	
350	Ochthebius	Ochthebius	marinus	meridionalis		Rey, 1885		Ochthebius	Ochthebius	Ochthebius		1	X						
351	Ochthebius	Ochthebius	marinus	mesoamericanus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius		1					X		
352	Ochthebius	Ochthebius	marinus	mexcavatus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius							X		
353	Ochthebius	Ochthebius	marinus	minabensis		Ferro, 1983		Ochthebius	Ochthebius	Ochthebius			X						
354	Ochthebius	Ochthebius	marinus	mongolensis		Janssens, 1967		Ochthebius	Ochthebius	Ochthebius			X						
355	Ochthebius	Ochthebius	marinus	nipponicus		Jäch, 1998		Ochthebius	Ochthebius	Ochthebius	Ryukyu	1	X						
356	Ochthebius	Ochthebius	marinus	pacificus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X			
357	Ochthebius	Ochthebius	marinus	pauli		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius							X		
358	Ochthebius	Ochthebius	marinus	pedalis		Balfour-Browne, 1954		Ochthebius	Ochthebius	Ochthebius		1		X					
359	Ochthebius	Ochthebius	marinus	pusillus		Stephens, 1835		Ochthebius	Ochthebius	Ochthebius		1	X						
360	Ochthebius	Ochthebius	marinus	queenslandicus		Hansen, 1998		Ochthebius	Ochthebius	Ochthebius		1			X				
361	Ochthebius	Ochthebius	marinus	recticulus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X			
362	Ochthebius	Ochthebius	marinus	rectus		LeConte, 1878		Ochthebius	Ochthebius	Ochthebius		1				X			
363	Ochthebius	Ochthebius	marinus	rectusalsus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X	X		
364	Ochthebius	Ochthebius	marinus	richmondi		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X			
365	Ochthebius	Ochthebius	marinus	rubripes		Boheman, 1860		Ochthebius	Ochthebius	Ochthebius				X					
366	Ochthebius	Ochthebius	marinus	salinarius		Balfour-Browne, 1954		Ochthebius	Ochthebius	Ochthebius		1		X					
367	Ochthebius	Ochthebius	marinus	sculptoides		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius		1					X		
368	Ochthebius	Ochthebius	marinus	sculptus		LeConte, 1878		Ochthebius	Ochthebius	Ochthebius						X			
369	Ochthebius	Ochthebius	marinus	sharpi		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius							X		
370	Ochthebius	Ochthebius	marinus	sierrensis		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X			
371	Ochthebius	Ochthebius	marinus	sitiensis		Perkins, 2011		Ochthebius	Ochthebius	Ochthebius				X					
372	Ochthebius	Ochthebius	marinus	spanglerorum		Wood & Perkins, 1978		Ochthebius	Ochthebius	Ochthebius						X			
373	Ochthebius	Ochthebius	marinus	spinasus		Perkins & Balfour-Browne, 1994		Ochthebius	Ochthebius	Ochthebius		1		X					
374	Ochthebius	Ochthebius	marinus	subaeneus		Janssens, 1967		Ochthebius	Ochthebius	Ochthebius			X						
375	Ochthebius	Ochthebius	marinus	subpictus		Wollaston, 1857		Ochthebius	Ochthebius	Ochthebius		1	X						
376	Ochthebius	Ochthebius	marinus	tubus		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius						X	X		
377	Ochthebius	Ochthebius	marinus	uniformis		Perkins, 1980		Ochthebius	Ochthebius	Ochthebius		(1)				X			
378	Ochthebius	Ochthebius	marinus	virens		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius			X						
379	Ochthebius	Ochthebius	marinus	viridescens		Ieniştea, 1988		Ochthebius	Ochthebius	Ochthebius		1	X						
380	Ochthebius	Ochthebius	marinus	viridis		Peyron, 1858		Ochthebius	Ochthebius	Ochthebius		1	X						
381	Ochthebius	Ochthebius	marinus?	romanicus		Ieniştea, 1968		Ochthebius	Ochthebius	Ochthebius			X						
382	Ochthebius	Ochthebius	metallescens	albacetinus		Ferro, 1984		Ochthebius	Ochthebius	Ochthebius		1	X						
383	Ochthebius	Ochthebius	metallescens	anaxagoras		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius			X						
384	Ochthebius	Ochthebius	metallescens	aristoteles		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius			X						

385	Ochthebius	Ochthebius	metallescens	ciliciae		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
386	Ochthebius	Ochthebius	metallescens	colchicus		Janssens, 1963		Ochthebius	Ochthebius	Ochthebius			X						
387	Ochthebius	Ochthebius	metallescens	dalmatinus		Ganglbauer, 1904		Ochthebius	Ochthebius	Ochthebius			X						
388	Ochthebius	Ochthebius	metallescens	decianus		Orchymont, 1942		Ochthebius	Ochthebius	Ochthebius			X						
389	Ochthebius	Ochthebius	metallescens	diazi		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius		1	X						
390	Ochthebius	Ochthebius	metallescens	empedocles		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius			X						
391	Ochthebius	Ochthebius	metallescens	gayosoi		Jäch, 2001		Ochthebius	Ochthebius	Ochthebius		1	X						
392	Ochthebius	Ochthebius	metallescens	gestroi		Gridelli, 1926		Ochthebius	Ochthebius	Ochthebius			X						
393	Ochthebius	Ochthebius	metallescens	griotes		Ferro, 1985		Ochthebius	Ochthebius	Ochthebius		1	X						
394	Ochthebius	Ochthebius	metallescens	hanshebaueri		Jäch, 1994		Ochthebius	Ochthebius	Ochthebius			X						
395	Ochthebius	Ochthebius	metallescens	hatayensis		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
396	Ochthebius	Ochthebius	metallescens	hivae		Jäch, Irani & Delgado, 2013		Ochthebius	Ochthebius	Ochthebius		1	X						
397	Ochthebius	Ochthebius	metallescens	hofratvukovitsi		Jäch, 1994		Ochthebius	Ochthebius	Ochthebius			X						
398	Ochthebius	Ochthebius	metallescens	huberti		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
399	Ochthebius	Ochthebius	metallescens	insidiosus		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius			X						
400	Ochthebius	Ochthebius	metallescens	iternuptialis		Jäch, 2001		Ochthebius	Ochthebius	Ochthebius			X						
401	Ochthebius	Ochthebius	metallescens	javieri		Jäch, 2000		Ochthebius	Ochthebius	Ochthebius			X						
402	Ochthebius	Ochthebius	metallescens	judemaesi		Delgado & Jäch, 2007		Ochthebius	Ochthebius	Ochthebius		1	X						
403	Ochthebius	Ochthebius	metallescens	khuzestanicus		Ferro, 1982		Ochthebius	Ochthebius	Ochthebius			X						
404	Ochthebius	Ochthebius	metallescens	kieneri		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius			X						
405	Ochthebius	Ochthebius	metallescens	kirschenhoferi		Jäch, 1994		Ochthebius	Ochthebius	Ochthebius			X						
406	Ochthebius	Ochthebius	metallescens	kurdistanicus		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
407	Ochthebius	Ochthebius	metallescens	latinorum		Ieniştea, 1988		Ochthebius	Ochthebius	Ochthebius			X						
408	Ochthebius	Ochthebius	metallescens	levantinus		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
409	Ochthebius	Ochthebius	metallescens	libanus		Jäch & Dia, 1992		Ochthebius	Ochthebius	Ochthebius			X						
410	Ochthebius	Ochthebius	metallescens	marijanmatoki		Jäch & Delgado, 2015		Ochthebius	Ochthebius	Ochthebius			X						
411	Ochthebius	Ochthebius	metallescens	maxfischeri		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius		(1)	X						
412	Ochthebius	Ochthebius	metallescens	metallescens	metallescens	Rosenhauer, 1847		Ochthebius	Ochthebius	Ochthebius		1	X						
413	Ochthebius	Ochthebius	metallescens	metallescens	plato	Jäch, 1999		Ochthebius	Ochthebius	Ochthebius		1	X						
414	Ochthebius	Ochthebius	metallescens	metallicus		Orchymont, 1942		Ochthebius	Ochthebius	Ochthebius			X						
415	Ochthebius	Ochthebius	metallescens	metarius		Orchymont, 1942		Ochthebius	Ochthebius	Ochthebius			X						
416	Ochthebius	Ochthebius	metallescens	metellus		Orchymont, 1942		Ochthebius	Ochthebius	Ochthebius			X						
417	Ochthebius	Ochthebius	metallescens	morettii		Pirisinu, 1974		Ochthebius	Ochthebius	Ochthebius		(1)	X						
418	Ochthebius	Ochthebius	metallescens	pedroi		Jäch, 2000		Ochthebius	Ochthebius	Ochthebius		1	X						
419	Ochthebius	Ochthebius	metallescens	poweri		Rye, 1869		Ochthebius	Ochthebius	Ochthebius		1	X						
420	Ochthebius	Ochthebius	metallescens	preissi		Jäch, 2001		Ochthebius	Ochthebius	Ochthebius		1	X						
421	Ochthebius	Ochthebius	metallescens	pretneri		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius			X						
422	Ochthebius	Ochthebius	metallescens	puberulus		Reitter, 1885		Ochthebius	Ochthebius	Ochthebius			X						
423	Ochthebius	Ochthebius	metallescens	rectilobus		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
424	Ochthebius	Ochthebius	metallescens	schoedli		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius			X						
425	Ochthebius	Ochthebius	metallescens	schuberti		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius			X						
426	Ochthebius	Ochthebius	metallescens	scitulus		Ferro, 1982		Ochthebius	Ochthebius	Ochthebius			X						
427	Ochthebius	Ochthebius	metallescens	scopuli		Köhler, Jäch & Delgado, 2016		Ochthebius	Ochthebius	Ochthebius		1	X						
428	Ochthebius	Ochthebius	metallescens	semisericeus		Sainte-Claire Deville, 1914		Ochthebius	Ochthebius	Ochthebius		1	X						
429	Ochthebius	Ochthebius	metallescens	semotus		Orchymont, 1942		Ochthebius	Ochthebius	Ochthebius		1	X						
430	Ochthebius	Ochthebius	metallescens	sempronius		Orchymont, 1942		Ochthebius	Ochthebius	Ochthebius			X						
431	Ochthebius	Ochthebius	metallescens	sennius		Orchymont, 1942		Ochthebius	Ochthebius	Ochthebius			X						
432	Ochthebius	Ochthebius	metallescens	serpentinus		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius		1	X						
433	Ochthebius	Ochthebius	metallescens	smyrnensis		Sahlberg, 1908		Ochthebius	Ochthebius	Ochthebius			X						
434	Ochthebius	Ochthebius	metallescens	trapezuntinus		Jäch, 1999		Ochthebius	Ochthebius	Ochthebius			X						
435	Ochthebius	Ochthebius	metallescens	ustaoglui		Topkara, Jäch & Kasapoğlu, 2011		Ochthebius	Ochthebius	Ochthebius			X						
436	Ochthebius	Ochthebius	metallescens	vedovai		Ferro, 1987		Ochthebius	Ochthebius	Ochthebius			X						
437	Ochthebius	Ochthebius	metallescens	viganoi		Pirisinu, 1974		Ochthebius	Ochthebius	Ochthebius			X						
438	Ochthebius	Ochthebius	metallescens	wurayah		Jäch & Delgado, 2010		Ochthebius	Ochthebius	Ochthebius		1	X						
439	Ochthebius	Ochthebius	nitidipennis	amami		Yoshitomi & Satô, 2001		Ochthebius	Ochthebius	Enicocerus	Ryukyu		X						
440	Ochthebius	Ochthebius	nitidipennis	championi		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
441	Ochthebius	Ochthebius	nitidipennis	exiguus		Jäch, 2003		Ochthebius	Ochthebius	Enicocerus			X						
442	Ochthebius	Ochthebius	nitidipennis	hasegawai		Nakane & Matsui, 1986		Ochthebius	Ochthebius	Ochthebius		1	X						
443	Ochthebius	Ochthebius	nitidipennis	ilanensis		Jäch, 1998		Ochthebius	Ochthebius	Ochthebius			X						
444	Ochthebius	Ochthebius	nitidipennis	japonicus		Jäch, 1998		Ochthebius	Ochthebius	Ochthebius		1	X						
445	Ochthebius	Ochthebius	nitidipennis	nakanei		Matsui, 1986		Ochthebius	Ochthebius	Enicocerus			X						
446	Ochthebius	Ochthebius	nitidipennis	nigrasperulus		Jäch, 2003		Ochthebius	Ochthebius	Enicocerus			X						
447	Ochthebius	Ochthebius	nitidipennis	nitidipennis		Champion, 1920		Ochthebius	Ochthebius	Enicocerus			X						
448	Ochthebius	Ochthebius	nitidipennis	obesus		Jäch, 2003		Ochthebius	Ochthebius	Enicocerus			X						
449	Ochthebius	Ochthebius	nitidipennis	rotundatus		Jäch, 2003		Ochthebius	Ochthebius	Enicocerus			X						
450	Ochthebius	Ochthebius	nitidipennis	scintillans		Champion, 1920		Ochthebius	Ochthebius	Enicocerus			X						
451	Ochthebius	Ochthebius	notabilis	eburneus		Sahlberg, 1900		Ochthebius	Ochthebius	Ochthebius			X						
452	Ochthebius	Ochthebius	notabilis	elburzi		Ferro, 1987		Ochthebius	Ochthebius	Ochthebius			X						
453	Ochthebius	Ochthebius	notabilis	gereckei		Jäch, 1993		Ochthebius	Ochthebius	Ochthebius		1	X						
454	Ochthebius	Ochthebius	notabilis	glaber		Montes & Soler, 1988		Ochthebius	Ochthebius	Ochthebius		1	X						
455	Ochthebius	Ochthebius	notabilis	halophilus		Ertorun & Jäch, 2014		Ochthebius	Ochthebius	Ochthebius		1	X						
456	Ochthebius	Ochthebius	notabilis	jermakovi		Orchymont, 1933		Ochthebius	Ochthebius	Ochthebius			X						
457	Ochthebius	Ochthebius	notabilis	lanarotis		Ferro, 1985		Ochthebius	Ochthebius	Ochthebius		1	X						
458	Ochthebius	Ochthebius	notabilis	maroccanus		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius			X						
459	Ochthebius	Ochthebius	notabilis	normandi		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius		1	X						
460	Ochthebius	Ochthebius	notabilis	notabilis		Rosenhauer, 1856		Ochthebius	Ochthebius	Ochthebius		1	X						
461	Ochthebius	Ochthebius	notabilis	ponticus		Ieniştea, 1956		Ochthebius	Ochthebius	Ochthebius	possibly a synonym of O. jermakovi (Jäch & Delgado 2014a)		X						
462	Ochthebius	Ochthebius	notabilis	salinator		Peyerimhoff, 1924		Ochthebius	Ochthebius	Ochthebius		1	X						

463	Ochthebius	Ochthebius	notabilis	zugmayeri		Kniž, 1909		Ochthebius	Ochthebius	Ochthebius			X						
464	Ochthebius	Ochthebius	peisonis	peisonis		Ganglbauer, 1901		Ochthebius	Ochthebius	Ochthebius		1	X						
465	Ochthebius	Ochthebius	punctatus	almorensis		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
466	Ochthebius	Ochthebius	punctatus	argentatus		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
467	Ochthebius	Ochthebius	punctatus	atratulus		Régimbart, 1905		Ochthebius	Ochthebius	Ochthebius				X					
468	Ochthebius	Ochthebius	punctatus	bifoveolatus		Waltl, 1835		Ochthebius	Ochthebius	Ochthebius		1	X						
469	Ochthebius	Ochthebius	punctatus	caucasicus		Kuwert, 1887		Ochthebius	Ochthebius	Ochthebius			X						
470	Ochthebius	Ochthebius	punctatus	ciffidilis		Ferro, 1984		Ochthebius	Ochthebius	Ochthebius			X						
471	Ochthebius	Ochthebius	punctatus	cuprescens		Guillebeau, 1893		Ochthebius	Ochthebius	Ochthebius		1	X						
472	Ochthebius	Ochthebius	punctatus	danjo		Nakane, 1990		Ochthebius	Ochthebius	Ochthebius			X						
473	Ochthebius	Ochthebius	punctatus	darius		Balfour-Browne, 1979		Ochthebius	Ochthebius	Ochthebius			X						
474	Ochthebius	Ochthebius	punctatus	difficilis		Mulsant, 1844		Ochthebius	Ochthebius	Ochthebius		1	X						
475	Ochthebius	Ochthebius	punctatus	flexus		Pu, 1958		Ochthebius	Ochthebius	Ochthebius			X						
476	Ochthebius	Ochthebius	punctatus	flumineus		Orchymont, 1937		Ochthebius	Ochthebius	Ochthebius			X						
477	Ochthebius	Ochthebius	punctatus	gonggashanensis		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
478	Ochthebius	Ochthebius	punctatus	grandipennis		Fairmaire, 1879		Ochthebius	Ochthebius	Ochthebius		1	X						
479	Ochthebius	Ochthebius	punctatus	hauseri		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius			X						
480	Ochthebius	Ochthebius	punctatus	imbensimbi		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
481	Ochthebius	Ochthebius	punctatus	inermis		Sharp, 1884		Ochthebius	Ochthebius	Ochthebius		1	X						
482	Ochthebius	Ochthebius	punctatus	iranicus		Balfour-Browne, 1979		Ochthebius	Ochthebius	Ochthebius			X						
483	Ochthebius	Ochthebius	punctatus	joosti		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius		1	X						
484	Ochthebius	Ochthebius	punctatus	klapperichi		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius		1	X					X	
485	Ochthebius	Ochthebius	punctatus	lanuginosus		Reiche & Saulcy, 1856		Ochthebius	Ochthebius	Ochthebius		1	X						
486	Ochthebius	Ochthebius	punctatus	lobatus		Pu, 1958		Ochthebius	Ochthebius	Ochthebius			X						
487	Ochthebius	Ochthebius	punctatus	mahmoodi		Jäch & Delgado, 2014		Ochthebius	Ochthebius	Ochthebius		1	X						
488	Ochthebius	Ochthebius	punctatus	micans		Balfour-Browne, 1951		Ochthebius	Ochthebius	Ochthebius		1	X	(X)					
489	Ochthebius	Ochthebius	punctatus	mongolicus		Janssens, 1967		Ochthebius	Ochthebius	Ochthebius			X						
490	Ochthebius	Ochthebius	punctatus	monseti		Jäch & Delgado, 2010		Ochthebius	Ochthebius	Ochthebius		1	X						
491	Ochthebius	Ochthebius	punctatus	montesi		Ferro, 1984		Ochthebius	Ochthebius	Ochthebius		1	X						
492	Ochthebius	Ochthebius	punctatus	namibiensis		Perkins & Balfour-Browne, 1994		Ochthebius	Ochthebius	Ochthebius				X					
493	Ochthebius	Ochthebius	punctatus	nanus		Stephens, 1829		Ochthebius	Ochthebius	Ochthebius		1	X						
494	Ochthebius	Ochthebius	punctatus	nepalensis		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
495	Ochthebius	Ochthebius	punctatus	nilssoni		Hebauer, 1986		Ochthebius	Ochthebius	Ochthebius		1	X						
496	Ochthebius	Ochthebius	punctatus	nobilis		Villa & Villa, 1835		Ochthebius	Ochthebius	Ochthebius		1	X						
497	Ochthebius	Ochthebius	punctatus	opacipennis		Champion, 1920		Ochthebius	Ochthebius	Ochthebius			X					X	
498	Ochthebius	Ochthebius	punctatus	orientalis		Janssens, 1962		Ochthebius	Ochthebius	Ochthebius			X						
499	Ochthebius	Ochthebius	punctatus	ovatus		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X					(X)	
500	Ochthebius	Ochthebius	punctatus	pagotrichus		Perkins & Balfour-Browne, 1994		Ochthebius	Ochthebius	Ochthebius		1		X					
501	Ochthebius	Ochthebius	punctatus	pilosus		Waltl, 1835		Ochthebius	Ochthebius	Ochthebius		1	X						
502	Ochthebius	Ochthebius	punctatus	punctatoides		Jäch, 1994		Ochthebius	Ochthebius	Ochthebius			X						
503	Ochthebius	Ochthebius	punctatus	punctatus		Stephens, 1829		Ochthebius	Ochthebius	Ochthebius		1	X						
504	Ochthebius	Ochthebius	punctatus	quadrifoveolatus		Wollaston, 1854		Ochthebius	Ochthebius	Ochthebius		1	X	X					
505	Ochthebius	Ochthebius	punctatus	ragusae		Kuwert, 1887		Ochthebius	Ochthebius	Ochthebius		1	X						
506	Ochthebius	Ochthebius	punctatus	schneideri	orchymonti	Jäch, 1984		Ochthebius	Ochthebius	Ochthebius			X						
507	Ochthebius	Ochthebius	punctatus	schneideri	schneideri	Kuwert, 1887		Ochthebius	Ochthebius	Ochthebius			X						
508	Ochthebius	Ochthebius	punctatus	sexfoveatus		Champion, 1920		Ochthebius	Ochthebius	Ochthebius			X						
509	Ochthebius	Ochthebius	punctatus	silfverbergi		Jäch, 1992		Ochthebius	Ochthebius	Ochthebius		1	X						
510	Ochthebius	Ochthebius	punctatus	spatulus		Balfour-Browne, 1954		Ochthebius	Ochthebius	Ochthebius				X					
511	Ochthebius	Ochthebius	punctatus	sulpuris		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
512	Ochthebius	Ochthebius	punctatus	tudmirensis		Jäch, 1997		Ochthebius	Ochthebius	Ochthebius		1	X						
513	Ochthebius	Ochthebius	punctatus	tunisicus		Jäch, 1997		Ochthebius	Ochthebius	Ochthebius			X						
514	Ochthebius	Ochthebius	punctatus	turcicus		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius			X						
515	Ochthebius	Ochthebius	punctatus	turkestanus		Kuwert, 1892		Ochthebius	Ochthebius	Ochthebius			X						
516	Ochthebius	Ochthebius	punctatus	verrucosus		Pu, 1942		Ochthebius	Ochthebius	Ochthebius			X						
517	Ochthebius	Ochthebius	punctatus	wewalkai		Jäch, 1984		Ochthebius	Ochthebius	Ochthebius			X						
518	Ochthebius	Ochthebius	punctatus	zulu		Perkins, 2011		Ochthebius	Ochthebius	Ochthebius				X					
519	Ochthebius	Ochthebius	quadricollis	brevicollis		Baudi a Selve, 1864		Calobius	Ochthebius	Calobius		1	X						
520	Ochthebius	Ochthebius	quadricollis	heeri		Wollaston, 1854		Calobius	Ochthebius	Calobius		1	X						
521	Ochthebius	Ochthebius	quadricollis	quadricollis		Mulsant, 1844		Ochthebius	Ochthebius	Calobius		1	X						
522	Ochthebius	Ochthebius	quadricollis	steinbuehleri		Reitter, 1886		Calobius	Ochthebius	Calobius		1	X						
523	Ochthebius	Ochthebius	quadricollis	urbanelliae		Audisio, Trizzino & De Biase, 2010		Calobius	Ochthebius	Calobius			X						
524	Ochthebius	Ochthebius	rivalis	himalayae		Jäch, 1989		Ochthebius	Ochthebius	Ochthebius		1	X						
525	Ochthebius	Ochthebius	rivalis	octofoveatus		Pu, 1958		Ochthebius	Ochthebius	Ochthebius			X						
526	Ochthebius	Ochthebius	rivalis	rivalis		Champion, 1920		Ochthebius	Ochthebius	Ochthebius		1	X						
527	Ochthebius	Ochthebius	rivalis	wuzhishanensis		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
528	Ochthebius	Ochthebius	strigosus	andreasi		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
529	Ochthebius	Ochthebius	strigosus	andreasoides		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
530	Ochthebius	Ochthebius	strigosus	asiobatoides		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
531	Ochthebius	Ochthebius	strigosus	caligatus		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
532	Ochthebius	Ochthebius	strigosus	castellanus		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
533	Ochthebius	Ochthebius	strigosus	enicoceroides		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
534	Ochthebius	Ochthebius	strigosus	fujianensis		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
535	Ochthebius	Ochthebius	strigosus	guangdongensis		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
536	Ochthebius	Ochthebius	strigosus	IBE-RA617		undescribed		Ochthebius	Ochthebius	Ochthebius		1	X						
537	Ochthebius	Ochthebius	strigosus	jilanzhui		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
538	Ochthebius	Ochthebius	strigosus	salebrosus		Pu, 1958		Ochthebius	Ochthebius	Ochthebius			X						
539	Ochthebius	Ochthebius	strigosus	sichuanensis		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
540	Ochthebius	Ochthebius	strigosus	stastnyi		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						

541	Ochthebius	Ochthebius	strigosus	strigoides		Jäch, 1998		Ochthebius	Ochthebius	Ochthebius			X						
542	Ochthebius	Ochthebius	strigosus	strigosus		Champion, 1921		Ochthebius	Ochthebius	Ochthebius			X						
543	Ochthebius	Ochthebius	strigosus	wangmiaoi		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
544	Ochthebius	Ochthebius	strigosus	yaanensis		Jäch, 2003		Ochthebius	Ochthebius	Ochthebius			X						
545	Ochthebius	Ochthebius	sumatrensis	MNCN-AC16		undescribed		Ochthebius	Ochthebius	Ochthebius		1	X						
546	Ochthebius	Ochthebius	sumatrensis	sumatrensis		Jäch, 2001		Ochthebius	Ochthebius	Ochthebius								X	
547	Ochthebius	Ochthebius	vandykei	ahni		Jäch & Delgado, 2014		Ochthebius	Ochthebius	Ochthebius			X						
548	Ochthebius	Ochthebius	vandykei	asanoae		Jäch & Delgado, 2014		Ochthebius	Ochthebius	Ochthebius			X						
549	Ochthebius	Ochthebius	vandykei	granulosus		Satô, 1963		Ochthebius	Ochthebius	Ochthebius			X						
550	Ochthebius	Ochthebius	vandykei	hayashii		Jäch & Delgado, 2014		Ochthebius	Ochthebius	Ochthebius			X						
551	Ochthebius	Ochthebius	vandykei	matsudae		Jäch & Delgado, 2014		Ochthebius	Ochthebius	Ochthebius			X						
552	Ochthebius	Ochthebius	vandykei	parki		Jäch & Delgado, 2014		Ochthebius	Ochthebius	Ochthebius			X						
553	Ochthebius	Ochthebius	vandykei	vandykei		Knisch, 1924		Ochthebius	Ochthebius	Ochthebius		1				X			
554	Ochthebius	Ochthebius	vandykei	yoshitomii		Jäch & Delgado, 2014		Ochthebius	Ochthebius	Ochthebius		1	X						
555	Ochthebius	Ochthebius	incertae sedis	belucistanicus		Ferro, 1948		Ochthebius	Ochthebius	Ochthebius			X						
556	Ochthebius	Ochthebius	incertae sedis	caudatus		Frivaldszky, 1883		Ochthebius	Ochthebius	Ochthebius			X						
557	Ochthebius	Ochthebius	incertae sedis	fissicollis		Janssens, 1970		Ochthebius	Ochthebius	Ochthebius			X						
558	Ochthebius	Ochthebius	incertae sedis	pierottii		Ferro, 1979		Ochthebius	Ochthebius	Ochthebius			X						
559	Ochthebius	incertae sedis		eremita		Knisch, 1922		Ochthebius	Ochthebius	incertae sedis								X	
560	Protochthebius			convexus		Perkins, 1998		Protochthebius	Protochthebius				X						
561	Protochthebius			jagthanae		Champion, 1921		Ochthebius	Protochthebius				X						
562	Protochthebius			jendeki		Jäch, 1997		Protochthebius	Protochthebius									X	
563	Protochthebius			loebli		Perkins, 1998		Protochthebius	Protochthebius				X						
564	Protochthebius			**satoi**		Perkins, 1997		Protochthebius	Protochthebius				X					(X)	
565	Protochthebius			schillhammeri		Jäch, 1997		Protochthebius	Protochthebius									X	
566	Protochthebius			smetanai		Perkins, 1998		Protochthebius	Protochthebius				X						
567	Prototympanogaster			lordhowensis		Perkins, 2018		Prototympanogaster	Prototympanogaster						X				
568	Tympallopatrum			aureolum		Perkins, 2004		Tympanopatrum	Tympallopatrum						X				
569	Tympallopatrum			callosum		Perkins, 2004		Tympanopatrum	Tympallopatrum						X				
570	Tympallopatrum			curvicostum		Perkins, 2004		Tympanopatrum	Tympallopatrum						X				
571	Tympallopatrum			longitudum		Perkins, 1997		Tympallopatrum	Tympallopatrum						X				
572	Tympanogaster	Hygrotympanogaster		arcuata		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
573	Tympanogaster	Hygrotympanogaster		atroargenta		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
574	Tympanogaster	Hygrotympanogaster		barronensis		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
575	Tympanogaster	Hygrotympanogaster		bondi		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
576	Tympanogaster	Hygrotympanogaster		bryosa		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
577	Tympanogaster	Hygrotympanogaster		buffalo		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
578	Tympanogaster	Hygrotympanogaster		canobolas		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
579	Tympanogaster	Hygrotympanogaster		cascadensis		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
580	Tympanogaster	Hygrotympanogaster		clandestina		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
581	Tympanogaster	Hygrotympanogaster		clypeata		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
582	Tympanogaster	Hygrotympanogaster		coopracambra		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
583	Tympanogaster	Hygrotympanogaster		cornutus		Janssens, 1967		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
584	Tympanogaster	Hygrotympanogaster		cudgee		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
585	Tympanogaster	Hygrotympanogaster		decepta		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
586	Tympanogaster	Hygrotympanogaster		duobifida		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
587	Tympanogaster	Hygrotympanogaster		grampians		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
588	Tympanogaster	Hygrotympanogaster		gushi		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
589	Tympanogaster	Hygrotympanogaster		kuranda		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
590	Tympanogaster	Hygrotympanogaster		macrognatha		Lea, 1926		Ochthebius	Tympanogaster	Hygrotympanogaster					X				
591	Tympanogaster	Hygrotympanogaster		**maureenae**		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
592	Tympanogaster	Hygrotympanogaster		megamorpha		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
593	Tympanogaster	Hygrotympanogaster		merrijig		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
594	Tympanogaster	Hygrotympanogaster		nargun		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
595	Tympanogaster	Hygrotympanogaster		newtoni		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
596	Tympanogaster	Hygrotympanogaster		novicia		Blackburn, 1896		Ochthebius	Tympanogaster	Hygrotympanogaster					X				
597	Tympanogaster	Hygrotympanogaster		porchi		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
598	Tympanogaster	Hygrotympanogaster		punctata		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
599	Tympanogaster	Hygrotympanogaster		schizolabra		Deane, 1933		Ochthebius	Tympanogaster	Hygrotympanogaster		1			X				
600	Tympanogaster	Hygrotympanogaster		spicerensis		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
601	Tympanogaster	Hygrotympanogaster		storeyi		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
602	Tympanogaster	Hygrotympanogaster		subcostata		Deane, 1933		Ochthebius	Tympanogaster	Hygrotympanogaster					X				
603	Tympanogaster	Hygrotympanogaster		tabula		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
604	Tympanogaster	Hygrotympanogaster		tallawarra		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
605	Tympanogaster	Hygrotympanogaster		trilineata		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
606	Tympanogaster	Hygrotympanogaster		truncata		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
607	Tympanogaster	Hygrotympanogaster		wahroonga		Perkins, 2006		Tympanogaster	Tympanogaster	Hygrotympanogaster					X				
608	Tympanogaster	Plesiotympanogaster		costata		Deane, 1933		Ochthebius	Tympanogaster	Plesiotympanogaster					X				
609	Tympanogaster	Plesiotympanogaster		thayerae		Perkins, 2006		Tympanogaster	Tympanogaster	Plesiotympanogaster					X				
610	Tympanogaster	Topotympanogaster		crista		Perkins, 2006		Tympanogaster	Tympanogaster	Topotympanogaster					X				
611	Tympanogaster	Topotympanogaster		dorsa		Perkins, 2006		Tympanogaster	Tympanogaster	Topotympanogaster					X				
612	Tympanogaster	Topotympanogaster		finniganensis		Perkins, 2006		Tympanogaster	Tympanogaster	Topotympanogaster					X				
613	Tympanogaster	Topotympanogaster		intricata		Perkins, 2006		Tympanogaster	Tympanogaster	Topotympanogaster					X				
614	Tympanogaster	Topotympanogaster		juga		Perkins, 2006		Tympanogaster	Tympanogaster	Topotympanogaster					X				
615	Tympanogaster	Topotympanogaster		monteithi		Perkins, 2006		Tympanogaster	Tympanogaster	Topotympanogaster					X				
616	Tympanogaster	Topotympanogaster		parallela		Perkins, 2006		Tympanogaster	Tympanogaster	Topotympanogaster					X				
617	Tympanogaster	Topotympanogaster		summa		Perkins, 2006		Tympanogaster	Tympanogaster	Topotympanogaster					X				
618	Tympanogaster	Tympanogaster		aldinga		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				

619	Tympanogaster	Tympanogaster		amaroo		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
620	Tympanogaster	Tympanogaster		ambigua		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
621	Tympanogaster	Tympanogaster		bluensis		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
622	Tympanogaster	Tympanogaster		cardwellensis		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
623	Tympanogaster	Tympanogaster		cooloogatta		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
624	Tympanogaster	Tympanogaster		cunninghamensis		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
625	Tympanogaster	Tympanogaster		darlingtoni		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
626	Tympanogaster	Tympanogaster		deanei		Perkins, 1979		Ochthebius	Tympanogaster	Tympanogaster		1			X				
627	Tympanogaster	Tympanogaster		dingabledinga		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
628	Tympanogaster	Tympanogaster		dorrigoensis		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
629	Tympanogaster	Tympanogaster		eungella		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
630	Tympanogaster	Tympanogaster		foveova		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
631	Tympanogaster	Tympanogaster		hypipamee		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
632	Tympanogaster	Tympanogaster		illawarra		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
633	Tympanogaster	Tympanogaster		jaechi		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
634	Tympanogaster	Tympanogaster		lamingtonensis		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
635	Tympanogaster	Tympanogaster		magarra		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
636	Tympanogaster	Tympanogaster		millaamillaa		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
637	Tympanogaster	Tympanogaster		modulatrix		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster		1			X				
638	Tympanogaster	Tympanogaster		moondarra		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
639	Tympanogaster	Tympanogaster		mysteriosa		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
640	Tympanogaster	Tympanogaster		obcordata		Deane, 1931		Ochthebius	Tympanogaster	Tympanogaster					X				
641	Tympanogaster	Tympanogaster		ovipennis		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
642	Tympanogaster	Tympanogaster		pagetae		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
643	Tympanogaster	Tympanogaster		perpendicula		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
644	Tympanogaster	Tympanogaster		plana		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
645	Tympanogaster	Tympanogaster		precariosa		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
646	Tympanogaster	Tympanogaster		protecta		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
647	Tympanogaster	Tympanogaster		ravenshoensis		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
648	Tympanogaster	Tympanogaster		robinae		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
649	Tympanogaster	Tympanogaster		serrata		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
650	Tympanogaster	Tympanogaster		tenax		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
651	Tympanogaster	Tympanogaster		tora		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
652	Tympanogaster	Tympanogaster		volata		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
653	Tympanogaster	Tympanogaster		wattsi		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
654	Tympanogaster	Tympanogaster		weiri		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				
655	Tympanogaster	Tympanogaster		wooloomgabba		Perkins, 2006		Tympanogaster	Tympanogaster	Tympanogaster					X				

Table S2: Current classification of Ochthebiini, with synonymies and type species (following Jäch & Skale, 2015 and Jäch *et al*., 2016). In bold, taxa included in the phylogeny.

genus	subgenus	synonyms	type species
***Aulacochthebius* Kuwert, 1887: 376**			***Ochthebius exaratus* Mulsant, 1844**
		Chirochthebius Kuwert, 1887: 383	***Ochthebius narentinus* Reitter, 1885**
		Eochirochthebius Ieniştea, 1988: 220	***Ochthebius narentinus* Reitter, 1885**
***Gymnanthelius* Perkins, 1997: 139**			*Ochthebius hieroglyphicus* Deane, 1931
***Gymnochthebius* Orchymont, 1943: 38**			*Ochthebius nitidus* LeConte, 1850
***Hughleechia* Perkins, 1981: 300**			***Hughleechia giulianii* Perkins, 1981**
***Meropathus* Enderlein, 1901: 121**			*Meropathus chuni* Enderlein, 1901
***Micragasma* Sahlberg, 1900: 199**			***Micragasma paradoxum* Sahlberg, 1900**
***Ochthebius* Leach, 1815: 95**	***Asiobates* Thomson, 1859: 15**		***Ochthebius rufimarginatus* Stephens, 1829 (=*bicolon* Germar, 1824)**
		Trymochthebius Kuwert, 1887: 380	***Ochthebius bicolon* Germar, 1824**
		Homalochthebius Kuwert, 1887: 383	***Helophorus minimus* Fabricius, 1792**
		Lunzochthebius Ieniştea, 1988: 220	*Ochthebius haberfelneri* Reitter, 1890
		Mimasiobates Ieniştea, 1988: 220	***Ochthebius montanus* Frivaldszky, 1881**
	***Calobius* Wollaston, 1854: 92**[1]		***Calobius heeri* Wollaston, 1854**
		Calochthebius Kuwert, 1887: 372	***Ochthebius quadricollis* Mulsant, 1844**
	***Enicocerus* Stephens, 1829: 196**		***Enicocerus viridiaeneus* Stephens, 1829 (=*exsculptus* Germar, 1824)**
		Henicocerus Agassiz, 1846: 178	***Enicocerus viridiaeneus* Stephens, 1829 (=*exsculptus* Germar, 1824)**
		Cyrtochthebius Kuwert, 1887: 371	***Ochthebius exsculptus* Germar, 1824**
		Sphaerochthebius Kuwert, 1887: 371	***Ochthebius gibbosus* Germar, 1824**
		Eosphaerochthebius Ieniştea, 1988: 218	***Ochthebius gibbosus* Germar, 1824**
	***Ochthebius* Leach, 1815: 95**[2]		***Helophorus marinus* Paykull, 1798**
		Hymenodes Mulsant, 1844: 68	***Ochthebius punctatus* Stephens, 1829**
		Cobalius Rey, 1886: 24[3]	***Ochthebius lejolisii* Mulsant & Rey, 1861**
		Bothochius Rey, 1886: 53	***Ochthebius nobilis* Villa & Villa, 1835**
		Ochthobius Rey, 1886: 14	***Helophorus marinus* Paykull, 1798**
		Doryochthebius Kuwert, 1887: 373	***Ochthebius notabilis* Rosenhauer, 1856**
		Prionochthebius Kuwert, 1887: 373	***Ochthebius lejolisii* Mulsant & Rey, 1861**
		Cheilochthebius Kuwert, 1887: 374	***Ochthebius metallescens* Rosenhauer, 1856**
		Camptochthebius Kuwert, 1887: 377	***Ochthebius nobilis* Villa & Villa, 1835**
		Odontochthebius Kuwert, 1887: 377	***Ochthebius bifoveolatus* Waltl, 1835**
		Colpochthebius Kuwert, 1887: 379	***Ochthebius punctatus* Stephens, 1829**
		Eccoptochthebius Kuwert, 1887: 379	***Ochthebius difficilis* Mulsant, 1844**
		Acanthochthebius Kuwert, 1887: 383	***Ochthebius serratus* Rosenhauer, 1856**
		Pseudhydraena Acloque, 1896: 96	***Helophorus marinus* Paykull, 1798**
		Liochthebius Sahlberg, 1900: 198	*Ochthebius eburneus* Sahlberg, 1900
		Neochthebius Orchymont, 1932: 43	***Hydraena vandykei* Knisch, 1924**
		Notochthebius Orchymont, 1933: 408	***Hydraena capicola* Péringuey, 1892**
		Nyxochthebius Orchymont, 1933: 408	*Ochthebius rubripes* Boheman, 1860
		Neohymenodes Ieniştea, 1988: 218	***Ochthebius pedicularius* Kuwert, 1887**
		Parahymenodes Ieniştea, 1988: 218	***Ochthebius metallescens* Rosenhauer, 1856**

		Eocolpochthebius Ienişteа, 1988: 219	***Ochthebius punctatus* Stephens, 1829**
		Metahymenodes Ienişteа, 1988: 219	***Ochthebius semisericeus* Sainte-Claire Deville, 1914**
		Chaetochthebius Ienişteа, 1988: 220	***Ochthebius quadrifoveolatus* Wollaston, 1854**
		Balfourochthebius Ienişteа, 1988: 220	*Ochthebius lindbergi* Balfour-Browne, 1976 (=*balfourbrownei* Jäch, 1989)
Protochthebius Perkins, 1997: 154			*Protochthebius satoi* Perkins, 1997
Prototympanogaster Perkins, 2018			*Prototympanogaster lordhowensis*, Perkins, 2018
Tympallopatrum Perkins, 1977: 147			*Tympallopatrum longitudum* Perkins, 1997
***Tympanogaster* Janssens, 1967: 8**	***Hygrotympanogaster* Perkins, 2006: 11**		*Tympanogaster maureenae* Perkins, 2006
	Plesiotympanogaster Perkins, 2006: 12		*Tympanogaster thayerae* Perkins, 2006
	Topotympanogaster Perkins, 2006: 11		*Tympanogaster crista* Perkins, 2006
	***Tympanogaster* Janssens, 1967: 8**		***Ochthebius longipes* Deane, 1931 (=*deanei* Perkins, 1979)**

1. Considered as the “*Calobius* lineage” of *Ochthebius* s.str. by Sabatelli *et al*. (2016).
2. The subgenus *Angiochthebius* was described by Jäch & Ribera (2018).
3. Considered as a subgenus of *Ochthebius* by Sabatelli *et al*. (2016).

Table S3: List of material used in the molecular phylogeny, including voucher numbers, accession numbers of the sequences and locality data. In bold, newly obtained sequences.

all	family	subfamily	genus	subgenus	species group	species	subspecies	voucher	locality	leg	COI-5	COI-3	16S	18S	28S
1	Hydraenidae	Hydraeninae	Hydraena	Holcohydraena		arenicola		MNCN-AI504	California (USA); Trinity Co., Forest Glen, Rattlesnake Creek 22.6.2000	I. Ribera & A. Cieslak	**LT991251**	HM588381	HM588525	HM588584	HM588441
2	Hydraenidae	Hydraeninae	Hydraena	Holcohydraena		rugosa		MNCN-AI392	Spain; Palencia, Castil de Vela 5.2005	L.F. Valladares	HE970908	HM588366	HM588510	HM588575	HM588432
3	Hydraenidae	Hydraeninae	Hydraena	Hydraena		altamirensis		MNCN-AI425	Spain; Ciudad Real, Navas de Estena, Río Estena 18.8.2005	A. Castro	HF947944	HM588371	HM588515	HM588578	HM588435
4	Hydraenidae	Hydraeninae	Hydraena	Hydraena		croatica		IBE-RA52	Corfu (Greece); South Corfu, 2 km S Lefkimi, stream 20.7.2009	M.A. Jäch	**LT991252**	HE970794	HE971027	HE970936	HE970982
5	Hydraenidae	Hydraeninae	Hydraena	Hydraena		dochula		MNCN-AI518	Bhutan; Timphu, 12 km east Timphu Town, stream 24.11.2005	M.A. Jäch	**LT991253**	FM946170	+FM946172+	FM946174	FM946175
6	Hydraenidae	Hydraeninae	Hydraena	Hydraena		hayashii		MNCN-AI691	Japan; Honshu, Shimane Pref., Mitarai-taki, Jinji river 19.2.2006	M. Hayashi	**LT991254**	HM588389	HM588533*	HM588590	HM588447
7	Hydraenidae	Hydraeninae	Hydraena	Hydraena		pygmaea		MNCN-AI346	Austria; Niederösterreich, Schwarzenbach, Sankt Veit an der Gölsen 6.8.2005	I. Ribera & A. Cieslak	HE970901	HM588353	HM588497	HM588572	HM588429
8	Hydraenidae	Hydraeninae	Hydraena	Hydraena		riberai		MNCN-AI568	Morocco; Assif-Oumarhouz, Oued Massa 25.4.2000	I. Ribera	**LT991255**	HM588388	HM588532	HM588589	HM588446
9	Hydraenidae	Hydraeninae	Hydraena	Hydraenopsis		palawanensis		MNCN-AH133	Philippines; North Palawan, Taytay, Manguao Stream 2007	H. Freitag	**LT991256**	HM588309	HM588454	HM588546	HM588403
10	Hydraenidae	Hydraeninae	Hydraena	Monomadraena		impressicollis		MNCN-AI541	Madagascar; Montagne d'Ambre, Petit Cascade 12.2004	M. Balke	**LT991257**	HE970850	HE971081	HE970961	HE970998
11	Hydraenidae	Hydraeninae	Hydraena	Phothydraena		atrata		MNCN-AI314	Spain; Albacete, Robledo, Laguna del Arquillo 2.6.2002	I. Ribera & A. Cieslak	HE970895	HM588344	HM588488	HM588568	HM588425
12	Hydraenidae	Hydraeninae	Hydraena	Phothydraena		putearius		IBE-RA95	Oman; Al-Akhdar, Ghul 5.4.2010	I. Ribera, C. Hernando & A. Cieslak	**LT991258**	**LT991448**	HE971029	HE610241	HE610244
13	Hydraenidae	Limnebiinae	Laeliaena			sahlbergi		MNCN-HI19	India; Uttarakhand, 15 km north Bageshwar, River Falda 14.11.2006	M.A. Jäch	LN995192	LN995232	LN995260	LN995286	**LT991039**
14	Hydraenidae	Limnebiinae	Limnebius	Bilimneus		acupunctus		MNCN-AI582	Australia; South Australia, Cudlee Creek, River Torrens 14.1.2006	C.H.S. Watts	LN995196	LN995238	LN995266	LN995292	LN995353
15	Hydraenidae	Limnebiinae	Limnebius	Bilimneus		dioscoridus		IBE-RA728	Socotra (Yemen); Wadi Ayhaft 8.11.2010	J. Hájek	LN995198	LN995239	**LT990690**	LN995310	LN995355
16	Hydraenidae	Limnebiinae	Limnebius	Bilimneus		extraneus		IBE-RA89	Spain; Huelva, Encinasola, Río Barranco Frío 26.7.1998	I. Ribera	LN995199	HF931321	HF931544	LN995312	LN995357
17	Hydraenidae	Limnebiinae	Limnebius	Bilimneus		pollex		IBE-RA1019	Mauritius; Le Pouce, S Port Louis 29.11.2012	M.A. Jäch	LN995201	**LT991449**	LN995268	LN995294	LN995364
18	Hydraenidae	Limnebiinae	Limnebius	Bilimneus		wewalkai		IBE-RA108	Oman; Al-Akhdar, wadi Bani Awf 6.4.2010	I. Ribera, C. Hernando & A. Cieslak	LN995202	LN995241	LN995269	LN995295	LN995365
19	Hydraenidae	Limnebiinae	Limnebius	Limnebius		arenicolus		MNCN-AI466	California (USA); Trinity Co., Forest Glen, Rattlesnake Creek 22.6.2000	I. Ribera & A. Cieslak	LN995229	HF931204	HF931429	LN995344	LN995407
20	Hydraenidae	Limnebiinae	Limnebius	Limnebius		cordobanus		IBE-PA275	Spain; Teruel, Beceite, river Matarranya, El Parrisal 3.6.2006	I. Ribera	**LT991259**	HF931292	HF931527	LN995319	LN995368
21	Hydraenidae	Limnebiinae	Limnebius	Limnebius		crinifer		IBE-AR55	Sweden; Öland, Möckelmossen, ponds 22.5.2011	I. Ribera	LN995221	LN995255	LN995281	LN995305	LN995400
22	Hydraenidae	Limnebiinae	Limnebius	Limnebius		doderoi		MNCN-AI1174	Corsica (France); Cap Corse, Bettolacce 21.9.1999	I. Ribera & A. Cieslak	LN995222	HF931170	HF931389	LN995341	LN995401
23	Hydraenidae	Limnebiinae	Limnebius	Limnebius		hieronymi		IBE-AR22	Italy; Abruzzo National Park, Pescasseroli, Fiume Sangro 26.7.2010	I. Ribera & A. Cieslak	LN995212	LN995249	LN995276	LN995301	LN995381
24	Hydraenidae	Limnebiinae	Limnebius	Limnebius		levantinus		IBE-RA731	Iran; Fars, Arzhan oak nature reserve 29.4.2010	A. Skale	LN995206	**LT991450**	LN995273	LN995298	LN995376
25	Hydraenidae	Limnebiinae	Limnebius	Limnebius		millani		MNCN-AI920	Spain; Albacete, Paterna del Madera, Arroyo de la Fuenfría 30.7.1998	I. Ribera	**LT991260**	HE610235	HF931476	**LT990834**	LN995389
26	Hydraenidae	Limnebiinae	Limnebius	Limnebius		zaerensis		MNCN-AC14	Morocco; Rommani, tributary Oued Korifla 10.4.2007	I. Ribera, P. Aguilera & C. Hernando	**LT991261**	EU365868	+EU365866+	HE970937	HE970984
27	Hydraenidae	Ochthebiinae	Meropathus			zelandicus		MNCN-AI715	New Zealand; South Island, Papatowai, beach 7.12.2005	M. Thayer, A. Newton & J. Nunn	**LT991262**	**LT991451**	**LT990691**	**LT990835**	**LT991040**
28	Hydraenidae	Ochthebiinae	Tympanogaster	Hygrotympanogaster		schizolabra		IBE-AF167	Australia; Victoria, Beech Forest below Hopetoun Falls 21.7.2009	I. Ribera & A. Cieslak	**LT991263**	**LT991452**	**LT990692**	**LT990836**	**LT991041**
29	Hydraenidae	Ochthebiinae	Tympanogaster	Tympanogaster		deanei		MNCN-AI372	Australia; Queensland, 40 km SSE Brisbane, Cedar Creek Road, above Cedar Creek Falls 22.8.2004	M.A. Jäch	**LT991264**	**LT991453**	**LT990693**	**LT990837**	**LT991042**
30	Hydraenidae	Ochthebiinae	Tympanogaster	Tympanogaster		modulatrix		IBE-AN183	Australia; Queensland, Lamington NP, Morans falls 26.4.2014	J. Maté	**LT991265**	**LT991454**	**LT990694**	**LT990838**	**LT991043**
31	Hydraenidae	Ochthebiinae	Ochthebius	Angiochthebius		plesiotypus		MNCN-AI502	Chile; IX Región, Termas de Río Blanco 8.2.2001	M. Guerrero	**LT991266**	**LT991455**	**LT990695**	**LT990839**	**LT991044**
32	Hydraenidae	Ochthebiinae	Ochthebius	Angiochthebius		plesiotypus		MNCN-AI562	Chile; Región Metropolitana, Alto Cantillana 8.1.2001	M. Guerrero	**LT991267**	**LT991456**	**LT990696**	**LT990840**	**LT991045**
33	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	arator		IBE-AN185	Turkey; Uşak, Çatalbayır village 19.6.2012	N. Ertorun	**LT991268**	**LT991457**		**LT990841**	**LT991046**
34	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	auriculatus		MNCN-AH154	England (UK); S Devon, Dawlish Warren Saltmarsh 29.9.2007	D.T. Bilton	**LT991269**	HF931134	HF931352	**LT990842**	**LT991047**
35	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	bellieri		IBE-AN290	Spain; Albacete, Paterna del Madera, Arroyo de la Fuenfría 30.7.1998	I. Ribera	**LT991270**	**LT991458**	**LT990697**	**LT990843**	**LT991048**
36	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	bicolon		IBE-RA1171	England (UK); N Devon, 1 km S Hartland Quay, stream 28.7.2013	D.T. Bilton	**LT991271**	**LT991459**	**LT990698**	**LT990844**	**LT991049**
37	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	bonnairei		MNCN-AI1272	Spain; Jaén, Santiago de la Espada, Río Zumeta 16.6.2006	A. Millán & collaborators	**LT991272**	HF931177	HF931398	**LT990845**	**LT991050**
38	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	cantabricus cf.		MNCN-AI1027	Turkey; Kastamonu, Aşağı Kayı 27.4.2006	A. Castro	**LT991273**	HF931145	HF931364	**LT990846**	**LT991051**
39	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	corsicus		IBE-AN159	Corsica (France); Col de Bavella 14.5.2014	R. Vila	**LT991274**	**LT991460**	**LT990699**	**LT990847**	**LT991052**
40	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	crenulatus		MNCN-AH159	Sicily (Italy); Parco dei Nebrodi, trail Lago Urio - Portella dell'Obolo 13.6.2007	P. Abellán & F. Picazo	**LT991275**	HF931136	HF931353	**LT990848**	**LT991053**
41	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	dilatatus		MNCN-AI792	Turkey; Bartın, between Topallar & Çakraz, calcareous stream 25.4.2006	I. Ribera	**LT991276**	HF931227	HF931456	**LT990849**	**LT991054**
42	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	ferroi		IBE-PB28	Spain; Huesca, Sta. Cruz de Serós, Barranco Carbonera 3.8.2013	I. Esteban	HG915303	**LT991461**	**LT990700**	**LT990850**	**LT991055**
43	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	figueroi		IBE-AN23	Spain; Cantabria, Río Soto 15.7.2014	D.T. Bilton	**LT991277**	**LT991462**	**LT990701**	**LT990851**	**LT991056**
44	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	gagliardii		IBE-RA121	Italy; Toscana, Lorenzana, Gello Mattaccino, pond 26.5.2008	I. Ribera	**LT991278**	HF931301	**LT990702**	**LT990852**	**LT991057**
45	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	heydeni		MNCN-AI390	Spain; Ourense, Sierra de Queixa, river San Lázaro 9.7.2005	I. Ribera & A. Cieslak	**LT991279**	HF931194	HF931419	**LT990853**	**LT991058**
46	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	immaculatus		IBE-AN434	Mallorca (Spain); Mallorca, Salines de Llevant 28.3.2016	I. Ribera & A. Cieslak	**LT991280**	**LT991463**	**LT990703**	**LT990854**	**LT991059**
47	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	irenae		MNCN-AI986	Spain; Navarra, Tudela, ditch near Balsa de Purguer 20.7.2004	I. Ribera & A. Cieslak	HG915302	HF931261	HF931497	**LT990855**	**LT991060**
48	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	jaimei		IBE-RA1081	Spain; Alicante, Albatera 12.5.2013	I. Esteban	**LT991281**	HG915307	**LT990704**	**LT990856**	**LT991061**
49	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	lederi		IBE-AN63	Azerbaijan; Alishanli, 7 km NE Masally 8.5.2014	I. Ribera & A. Rudoy	**LT991282**	**LT991464**	**LT990705**	**LT990857**	**LT991062**
50	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	lenkoranus		IBE-AN75	Azerbaijan; Gosmalijion, stream 7.5.2014	I. Ribera & A. Rudoy	**LT991283**	**LT991465**	**LT990706**	**LT990858**	**LT991063**
51	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	montanus		IBE-AN207	Greece; Evvoia, Seta, mountain stream 8.4.2013	I. Ribera & A. Cieslak	**LT991284**	**LT991466**	**LT990707**	**LT990859**	**LT991064**
52	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	opacus		MNCN-AI389	Italy; Liguria, 2 km E Cosio di Arroscia 31.7.2005	I. Ribera & A. Cieslak	**LT991285**	HF931193	HF931418	**LT990860**	**LT991065**
53	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	peregrinus		IBE-AN206	Greece; Sterea Ellada, Fokida, Itea, stream E of Kirra 7.4.2013	I. Ribera & A. Cieslak	**LT991286**	**LT991467**	**LT990708**	**LT990861**	**LT991066**
54	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	sp.		IBE-AN74	Azerbaijan; Elabad, road to Garaybayli, stream 4.5.2014	I. Ribera & A. Rudoy	**LT991287**	**LT991468**	**LT990709**	**LT990862**	**LT991067**
55	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	striatus		MNCN-AI787	Turkey; Bartın, between Amasra & Inpiri, spring & stream 25.4.2006	I. Ribera	**LT991288**	HF931226	HF931455	**LT990863**	**LT991068**
56	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	bicolon	stygialis		IBE-AN160	Turkey; Antalya, 1 km N Hacıyusuflar 24.6.2014	D.T. Bilton	**LT991289**	**LT991469**	**LT990710**	**LT990864**	**LT991069**
57	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	aeneus		MNCN-AI914	Morocco; Ou-Maghous, Amaghouz 24.4.2000	I. Ribera, P. Aguilera, C. Hernando & A. Millán	**LT991290**	HF931240	HF931472	**LT990865**	**LT991070**
58	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	alpinus		IBE-RA1114	Belarus; Berezinsky Biosphere Reserve 24.6.2013	I. Ribera	**LT991291**	**LT991470**	**LT990711**	**LT990866**	**LT991071**
59	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	andreiini		IBE-AN20	Ethiopia; Amhara, 30 km NE Gashena 13.6.2014	R. Vila & G. Talavera	**LT991292**	**LT991471**	**LT990712**	**LT990867**	**LT991072**
60	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	andreinii cf.		IBE-AN104	Ethiopia; Oromia, Bale Mountains, Goba Forest, Togona River 22.2.2014	M.A. Jäch	**LT991293**	**LT991472**	**LT990713**	**LT990868**	**LT991073**
61	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	andronius		MNCN-AI498	South Africa; West Cape, Prince Albert Road, pond 23.3.2001	I. Ribera & A. Cieslak	**LT991294**	**LT991473**	**LT990714**	**LT990869**	**LT991074**
62	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	discretus		MNCN-AI503	California (USA); Trinity Co., Forest Glen, Rattlesnake Creek 22.6.2000	I. Ribera & A. Cieslak	**LT991295**	**LT991474**	**LT990715**	**LT990870**	**LT991075**
63	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	flavipes		IBE-RA437	Slovakia; Hámske tŕstie, near Číčov, ditch 7.6.2009	I. Ribera	**LT991296**	HF931315	**LT990716**	**LT990871**	**LT991076**
64	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	hokkaidensis		IBE-AF213	Japan; Hokkaido, Togeshita, Rumoi-shi 18.7.2007	H. Yoshitomi		HF931125	HF931344	**LT990872**	**LT991077**
65	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	minimus		MNCN-AI447	Slovakia; Banská Bystrica Region, Cerovo 2005	via R.G. Beutel	HE970917	HE970842	HE971074	HE970955	HE970995
66	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	remotus		MNCN-AI1030	Turkey; Karabük, stream in Çayörengüney 28.4.2006	A. Castro	**LT991297**	HF931148	HF931367	**LT990873**	**LT991078**
67	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	rugulosus		MNCN-AI637	Gran Canaria (Spain); Moya, Barranco de Azuaje 15.4.2001	I. Ribera & A. Cieslak	**LT991298**	HF931219	HF931447	**LT990874**	**LT991079**

68	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	sanabrensis		MNCN-AH75	Spain; Zamora, Parque Natural Lago Sanabria, Laguna de la Yegua 4.7.2007	L.F. Valladares	**LT991299**	EU660055	HF931357		**LT991080**
69	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	sp.		IBE-AN292	Alberta (Canada); 2 km W Lundbreck 27.6.2000	I. Ribera & A. Cieslak	**LT991300**	**LT991475**	**LT990717**	**LT990875**	**LT991081**
70	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	sp.		IBE-AN97	Ethiopia; Oromia, Jemjem Forest, small stream 25.2.2014	M.A. Jäch	**LT991301**	**LT991476**	**LT990718**	**LT990876**	**LT991082**
71	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	minimus	sp.		IBE-AN93	Ethiopia; Amhara, Debark, Simien Mountains 18.6.2014	R. Vila & G. Talavera	**LT991302**	**LT991477**	**LT990719**	**LT990877**	**LT991083**
72	Hydraenidae	Ochthebiinae	Ochthebius	Asiobates	puncticollis	puncticollis		MNCN-AI1274	California (USA); Santa Barbara Co., Sedgewick Reserve 6.7.2006	A.E.Z. Short	**LT991303**	**LT991478**	**LT990720**		**LT991084**
73	Hydraenidae	Ochthebiinae	Ochthebius	Aulacochthebius		exaratus		MNCN-AI453	Tunisia; road Jendouba-Makthar, 1 km NW crossroad to Mt. Kebbouch 24.10.2001	I. Ribera & A. Cieslak	**LT991304**	HF931202	HF931427	**LT990878**	**LT991085**
74	Hydraenidae	Ochthebiinae	Ochthebius	Aulacochthebius		libertarius		MNCN-AI421	Morocco; Ou-Maghous, Amaghouz 24.4.2000	I. Ribera, P. Aguilera, C. Hernando & A. Millán	**LT991305**	HF931196	HF931421	**LT990879**	**LT991086**
75	Hydraenidae	Ochthebiinae	Ochthebius	Aulacochthebius		narentinus		IBE-AF161	Slovakia; Studienka, Rudava river 5.6.2009	I. Ribera	**LT991306**	HF931120	HF931338	**LT990880**	**LT991087**
76	Hydraenidae	Ochthebiinae	Ochthebius	Aulacochthebius		perlaevis		MNCN-AI548	Madagascar; Ankarana, 1er canyon, river 12.2004	M. Balke	**LT991307**	HF931211	HF931437	**LT990881**	**LT991088**
77	Hydraenidae	Ochthebiinae	Ochthebius	Aulacochthebius		sp.		IBE-AN390	South Africa; West Cape, Baardskeerdersbos, Boesmans River 10.5.2010	J. Den Heijer & A. Hidalgo-Galiana	**LT991308**	**LT991479**		**LT990882**	**LT991089**
78	Hydraenidae	Ochthebiinae	Ochthebius	Aulacochthebius		sp.		MNCN-AI499	South Africa; West Cape, 3 km SE Franschhoek 25.3.2001	I. Ribera & A. Cieslak	**LT991309**	**LT991480**	**LT990721**	**LT990883**	**LT991090**
79	Hydraenidae	Ochthebiinae	Ochthebius	Aulacochthebius		sp.		MNCN-AI501	South Africa; Eastern Cape, Kareedouwberg, river in Skoop Drif 23.3.2001	I. Ribera & A. Cieslak	**LT991310**	HF931207	HF931432	**LT990884**	**LT991091**
80	Hydraenidae	Ochthebiinae	Ochthebius	Aulacochthebius		sp.		MNCN-AI519	Bhutan; Sarpang, 11 km NW Sarpang, Bhur Khola river 27.11.2005	M.A. Jäch	**LT991311**	**LT991481**	**LT990722**	**LT990885**	**LT991092**
81	Hydraenidae	Ochthebiinae	Ochthebius	Aulacochthebius		sp.		MNCN-AI520	Bhutan; Punakha, 16 km NW Punakha, Mo Chhu river 28.11.2005	M.A. Jäch	**LT991312**	**LT991482**	**LT990723**	**LT990886**	**LT991093**
82	Hydraenidae	Ochthebiinae	Ochthebius	Aulacochthebius		sp.		IBE-RA1155	Tanzania; Mwanza Region, near Kashishi, ponds 24.7.2010	R. Sites & A. Mbogho	**LT991313**	**LT991483**	**LT990724**	**LT990887**	**LT991094**
83	Hydraenidae	Ochthebiinae	Ochthebius	Cobalius		adriaticus	adriaticus	IBE-AN787	Croatia; Trsteno, rockpools 5.5.2017	A. Faille, J. Fresneda & I. Ribera	**LT991314**	**LT991484**	**LT990725**	**LT990888**	**LT991095**
84	Hydraenidae	Ochthebiinae	Ochthebius	Cobalius		celatus		IBE-AN441	Cyprus; Akamas peninsula, Lara beach 29.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT991315**	**LT991485**	**LT990726**	**LT990889**	**LT991096**
85	Hydraenidae	Ochthebiinae	Ochthebius	Cobalius		freyi		IBE-RA1197	Azores (Portugal); Terceira, São Pedro, rockpools 4.9.2012	I. Ribera	**LT991316**	**LT991486**	**LT990727**	**LT990890**	**LT991097**
86	Hydraenidae	Ochthebiinae	Ochthebius	Cobalius		lejolisii		MNCN-AI513	Spain; Pontevedra, Nigran, Playa Patos 3.10.2005	J. Garrido	**LT991317**	HF931208	HF931433	**LT990891**	**LT991098**
87	Hydraenidae	Ochthebiinae	Ochthebius	Cobalius		serratus		MNCN-AI1194	Morocco; El Ouedaya, Oued Sebt 18.4.2006	A. Millán & collaborators	**LT991318**	HF931171	HF931391	**LT990892**	**LT991099**
88	Hydraenidae	Ochthebiinae	Ochthebius	Cobalius		subinteger		MNCN-AI432	Corsica (France); Cap Corse, Port de Centuri 20.9.2000	I. Ribera & A. Cieslak	**LT991319**	HF931200	HF931425	**LT990893**	**LT991100**
89	Hydraenidae	Ochthebiinae	Ochthebius	Enicocerus		aguilerai		MNCN-AI387	Spain; Ávila, Arenas de San Pedro, río Pelayo 15.5.2005	I. Ribera	HF947939	GU143761	GU143728	GU143770	GU143778
90	Hydraenidae	Ochthebiinae	Ochthebius	Enicocerus		colveranus		MNCN-AI791	Turkey; Bartın, between Topallar & Çakraz, calcareous stream 25.4.2006	I. Ribera	**LT991320**	GU143744	GU143733	GU143768	GU143776
91	Hydraenidae	Ochthebiinae	Ochthebius	Enicocerus		exsculptus		MNCN-AI374	Spain; Barcelona, Guardiola del Berguedà, torrent Gavarrós 25.7.2005	I. Ribera	**LT991321**	GU143763	GU143741	GU143772	GU143780
92	Hydraenidae	Ochthebiinae	Ochthebius	Enicocerus		exsculptus cf.		MNCN-AI925	Spain; Albacete, Sierra de Segura, 10 km N Yeste, Rio Tús 10.4.2003	V. Assing & P. Wunderle	**LT991322**	GU143750	GU143737	**LT990894**	**LT991101**
93	Hydraenidae	Ochthebiinae	Ochthebius	Enicocerus		gibbosus		MNCN-AI365	Italy; Piemonte, Móngia, torrente Móngia 31.7.2005	I. Ribera & A. Cieslak	**LT991323**	GU143755	GU143727	GU143769	GU143777
94	Hydraenidae	Ochthebiinae	Ochthebius	Enicocerus		granulatus		MNCN-AI427	Austria; Niederösterreich, Kleiner Ötscherbach, Langau 25.9.2005	M.A. Jäch	**LT991324**	GU143765	GU143725	GU143774	GU143782
95	Hydraenidae	Ochthebiinae	Ochthebius	Enicocerus		halbherri		MNCN-AH190	Italy; Emilia Romagna, Bosco, Torrente Parma 24.5.2008	I. Ribera	**LT991325**	GU143764	GU143736	GU143773	GU143781
96	Hydraenidae	Ochthebiinae	Ochthebius	Enicocerus		legionensis		MNCN-AI507	Spain; León, Puerto de Panderrueda 12.7.2005	L.F. Valladares	HF947941	GU143762	GU143735	GU143771	GU143779
97	Hydraenidae	Ochthebiinae	Ochthebius	Enicocerus		melanescens		MNCN-AI344	Austria; Niederösterreich, Schwarzenbach, Sankt Veit an der Gölsen 6.8.2005	I. Ribera & A. Cieslak	HE970900	GU143766	GU143743	GU143775	GU143783
98	Hydraenidae	Ochthebiinae	Ochthebius	Enicocerus		saboorii		IBE-RA739	Iran; Mazandaran, near Nowshar, Kheiroud Kenar Forest 2.5.2010	A. Skale	**LT991326**		**LT990728**	**LT990895**	**LT991102**
99	Hydraenidae	Ochthebiinae	Ochthebius	Gymnanthelius		opacicollis		IBE-AF162	Australia; Victoria, Allambee, 19 km N Leongatha 8.7.2009	I. Ribera & A. Cieslak	**LT991327**	**LT991487**	**LT990729**	**LT990896**	**LT991103**
100	Hydraenidae	Ochthebiinae	Ochthebius	Gymnanthelius		porchi		IBE-AF164	Australia; Victoria, Tarra-Bulga NP, River Tarra 9.7.2009	I. Ribera & A. Cieslak	**LT991328**	**LT991488**	**LT990730**	**LT990897**	**LT991104**
101	Hydraenidae	Ochthebiinae	Ochthebius	Gymnochthebius	australis	australis		MNCN-AI583	Australia; South Australia, 1 km W Cudlee Creek, River Torrens 14.1.2006	C.H.S. Watts	**LT991329**	**LT991489**	**LT990731**	**LT990898**	**LT991105**
102	Hydraenidae	Ochthebiinae	Ochthebius	Gymnochthebius	australis	lividus		IBE-AF163	Australia; Victoria, Tarra-Bulga NP, River Tarra 9.7.2009	I. Ribera & A. Cieslak	**LT991330**	**LT991490**	**LT990732**	**LT990899**	**LT991106**
103	Hydraenidae	Ochthebiinae	Ochthebius	Gymnochthebius	australis	probus		MNCN-AI584	Australia; South Australia, 1 km W Cudlee Creek, River Torrens 14.1.2006	C.H.S. Watts	**LT991331**	**LT991491**	**LT990733**	**LT990900**	**LT991107**
104	Hydraenidae	Ochthebiinae	Ochthebius	Gymnochthebius	australis	setosus		IBE-AF165	Australia; Victoria, Tarra-Bulga NP, River Tarra 9.7.2009	I. Ribera & A. Cieslak	**LT991332**	**LT991492**	**LT990734**	**LT990901**	**LT991108**
105	Hydraenidae	Ochthebiinae	Ochthebius	Gymnochthebius	fossatus	fossatus		IBE-AN498	Dominican Republic; Independencia, La Colonia 15.8.2014	A. Deler-Hernández, M. Fikácek & M. Gimmel	**LT991333**	**LT991493**	**LT990735**	**LT990902**	**LT991109**
106	Hydraenidae	Ochthebiinae	Ochthebius	Gymnochthebius	fossatus	germaini		MNCN-AI454	Chile; IX Región, 5 km E Lonquimay, road to Lolén 26.1.1999	I. Ribera & M. Guerrero	**LT991334**	**LT991494**	**LT990736**	**LT990903**	**LT991110**
107	Hydraenidae	Ochthebiinae	Ochthebius	Gymnochthebius	fossatus	peruvianus		MNCN-AI689	Peru; San Clemente, km 222 Panamericana Sur 31.8.2005	P. Aguilera	**LT991335**	**LT991495**	**LT990737**	**LT990904**	**LT991111**
108	Hydraenidae	Ochthebiinae	Ochthebius	Gymnochthebius	fossatus	sp.		MNCN-AI569	Chile; Chiloé, 6 km E Huillinco, river in Notuco 3.2.1999	I. Ribera & M. Guerrero	**LT991336**	**LT991496**	**LT990738**	**LT990905**	**LT991112**
109	Hydraenidae	Ochthebiinae	Ochthebius	Hughleechia		giuliani		MNCN-AI716	Australia; West Australia, Peron Point 2.10.2003	C.H.S. & G.A. Watts	**LT991337**	**LT991497**	**LT990739**	**LT990906**	**LT991113**
110	Hydraenidae	Ochthebiinae	Ochthebius	Micragasma		paradoxum		IBE-AF116	Russia; Volgogradskaya Oblast, Lake Elton, residual pools 17.4.2008	A. Prokin	**LT991338**	HF931114	HF931331	**LT990907**	**LT991114**
111	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	andraei	patergazellae		IBE-RA735	United Arab Emirates; Ajman, salt water pools NE Ajman City 25.1.2010	M.A. Jäch	**LT991339**	**LT991498**	**LT990740**	**LT990908**	**LT991115**
112	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	atriceps	andalusicus		IBE-PA296	Spain; Cádiz, Salinas de Hortales 10.11.2003	A. Millán & collaborators	**LT991340**	HF931297	**LT990741**	**LT990909**	**LT991116**
113	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	atriceps	anxifer		MNCN-AI945	Morocco; Immouzzer Ida Ou Tanane, Assif Tanit 21.4.2001	I. Ribera & A. Cieslak	**LT991341**	HF931247	HF931482	**LT990910**	**LT991117**
114	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	atriceps	atriceps		IBE-AN210	Tunisia; road Jendouba-Makthar, 1 km NW crossroad to Mt. Kebbouch 24.10.2001	I. Ribera & A. Cieslak	**LT991342**	**LT991499**	**LT990742**	**LT990911**	**LT991118**
115	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	atriceps	burjkhalifa		IBE-RA737	United Arab Emirates; Ajman, salt water pools NE Ajman City 25.1.2010	M.A. Jäch	**LT991343**	**LT991500**	**LT990743**	**LT990912**	**LT991119**
116	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	atriceps	dentifer		IBE-PA290	Spain; Navarra, Barranco Salado de Mendavia 30.10.2008	A. Millán & collaborators	**LT991344**	HF931296	**LT990744**	**LT990913**	**LT991120**
117	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	atriceps	depressionis		IBE-AF171	Russia; Volgogradskaya Oblast, Lake Elton, nr Lantsug River 14.4.2008	A. Prokin	**LT991345**	HF931122	HF931340	**LT990914**	**LT991121**
118	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	atriceps	loulae		IBE-AN476	Djibouti; 50 km W Dikhil, Lac Abbé, thermal springs 2.2.2016	M.A. Jäch	LT837919	**LT991501**	**LT990745**	**LT990915**	**LT991122**
119	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	atriceps	tacapasensis	baeticus	IBE-AN365	Spain; Málaga, Laguna de Fuente de Piedra 3.5.2011	A. Millán & collaborators	**LT991346**	**LT991502**	**LT990746**	**LT990916**	**LT991123**
120	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	atriceps	thermalis		IBE-AN451	Cyprus; Larnaka, saline coastal pond 25.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT991347**	**LT991503**	**LT990747**		**LT991124**
121	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	atriceps?	despoliatus		IBE-RA736	United Arab Emirates; Ajman, salt water pools NE Ajman City 25.1.2010	M.A. Jäch		**LT991504**		**LT990917**	**LT991125**
122	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	corrugatus	corrugatus		MNCN-AI56	Mallorca (Spain); Mallorca, Salines de Llevant 15.10.2004	I. Ribera & A. Cieslak		HF931213	HF931438	**LT990918**	**LT991126**
123	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	corrugatus	gauthieri		MNCN-AI55	Tunisia; road Kebili-Tozeur, 37 km SE Tozeur, salines 26.10.2001	I. Ribera & A. Cieslak		HF931212		**LT990919**	**LT991127**
124	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	corrugatus	perpusillus		IBE-AN323	Morocco; Ouezzane, Sidi Kacem, Oued Khendek 18.4.2006	A. Millán & collaborators		**LT991505**		**LT990920**	**LT991128**
125	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	elisae		IBE-RA746	Iran; Khuzestan, Behbahan, Morvarid spring 4.9.2010	E. Irani	**LT991348**	**LT991506**		**LT990921**	**LT991129**
126	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	foveolatus		MNCN-AI801	Turkey; Kastamonu, Çocukören 26.4.2006	I. Ribera	**LT991349**	HF931228	HF931457	**LT990922**	**LT991130**
127	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	hajeki		IBE-RA1231	Socotra (Yemen); Dixam plateau, Firmihin, Dracaena woodland 15.6.2012	J. Hájek	**LT991350**	**LT991507**	**LT990748**	**LT990923**	**LT991131**
128	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	harteni		IBE-RA705	Oman; Al-Akhdar, Bahla, wadi in city 5.4.2010	I. Ribera, C. Hernando & A. Cieslak	**LT991351**	**LT991508**	**LT990749**	**LT990924**	**LT991132**
129	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	magnannulatus		IBE-AN328	Bulgaria; Kotlari, river Arda 27.5.2015	I. Ribera	**LT991352**	**LT991509**	**LT990750**	**LT990925**	**LT991133**
130	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	marginalis		IBE-AN522	France; Drôme, river Méouge 2016	A. Faille	**LT991353**	**LT991510**	**LT990751**	**LT990926**	**LT991134**
131	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	mediterraneus		MNCN-AI422	Morocco; Ou-Maghous, Amaghouz 24.4.2000	I. Ribera, P. Aguilera, C. Hernando & A. Millán	**LT991354**	HF931197	HF931422	**LT990927**	**LT991135**
132	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	merinidicus		IBE-RA1023	Spain; Albacete, River Mundo, Azud de Liétor 26.7.2012	A. Millán & collaborators	**LT991355**	**LT991511**	**LT990752**	**LT990928**	**LT991136**
133	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	pedicularius		IBE-AN809	Italy; Udine, Flagogna 9.4.2017	A. Eckelt	**LT991356**	**LT991512**	**LT990753**	**LT990929**	**LT991137**
134	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	satoi		IBE-AF210	Japan; Hokkaido, Taiki 11.7.2009	H. Yoshitomi	**LT991357**	HF931124	HF931343		**LT991138**
135	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	sidanus		IBE-AF132	Italy; Emilia Romagna, Bosco, Torrente Parma 24.5.2008	I. Ribera	**LT991358**	HF931115	HF931332	**LT990930**	**LT991139**
136	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	foveolatus	virgula		IBE-AF134	Italy; Emilia Romagna, Vigoleno, Parco dello Stirone, Torrente Stirone 25.5.2008	I. Ribera	**LT991359**	HF931116	HF931333	**LT990931**	**LT991140**
137	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	lobicollis	basilicatus		IBE-AN801	Sardinia (Italy); Cabras, Stagno di Cabras 10.4.2017	I. Ribera & A. Cieslak	**LT991360**	**LT991513**	**LT990754**	**LT990932**	**LT991141**
138	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	lobicollis	caesaraugustae		MNCN-AI1195	Spain; Zaragoza, Medianan de Aragón, saline river 20.7.2006	A. Millán & collaborators	**LT991361**	HF931172	HF931392	**LT990933**	**LT991142**
139	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	lobicollis	delgadoi		IBE-AN364	Spain; Murcia, Rambla del Reventón 9.3.2012	A. Millán & collaborators	**LT991362**	**LT991514**	**LT990755**	**LT990934**	**LT991143**

140	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	lobicollis	eyrei		IBE-AN600	Sicily (Italy); Palermo, Fiume Salso 28.7.2009	C. Gutiérrez-Cánovas	**LT991363**	**LT991515**	**LT990756**	**LT990935**	**LT991144**
141	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	lobicollis	lapidicola		IBE-AN126	Tenerife (Spain); Puerto de la Cruz, Roque Grande, 9.4.2015	R. Lyszkowski		**LT991516**	**LT990757**	**LT990936**	**LT991145**
142	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	lobicollis	lobicollis		IBE-RA242	Menorca (Spain); Cap de Favàritx 26.2.2010	I. Ribera & A. Cieslak	**LT991364**	HF931308	HF931534	**LT990937**	**LT991146**
143	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	lobicollis	quadrifossulatus		MNCN-AI226	Spain; Guadalajara, Alcolea de las Peñas 21.5.2005	A. Millán & collaborators		**LT991517**	**LT990758**	**LT990938**	**LT991147**
144	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	lobicollis	tivelunus		MNCN-AI420	Morocco; Agarzane, tributary of Oued Drâa 17.4.2001	I. Ribera & A. Cieslak	**LT991365**	HF931195	HF931420	**LT990939**	**LT991148**
145	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	arefniae		IBE-AN72	Azerbaijan; road Budzhakah-Bash-Dashagyi, ponds 5.5.2014	I. Ribera & A. Rudoy	**LT991366**	**LT991518**	**LT990759**	**LT990940**	**LT991149**
146	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	arefniae cf.		IBE-AN446	Cyprus; Loukrounou, upstream of Evretou reservoir 29.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT991367**	**LT991519**	**LT990760**		**LT991150**
147	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	arizonicus		IBE-AN177	Arizona (USA); Yavapai Co., Agua Fria River 22.6.2007	W.D. Shepard	**LT991368**	**LT991520**	**LT990761**	**LT990941**	**LT991151**
148	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	auropallens		IBE-AN217	Tunisia; road Kairouan-Enfida, 13 km N Kairouan, lagoon 27.10.2001	I. Ribera & A. Cieslak		**LT991521**	**LT990762**	**LT990942**	**LT991152**
149	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	aztecus		IBE-AN222	California (USA); Inyo Co., Shoshone, Amargosa River 8.4.2008	P. Abellán	**LT991369**	**LT991522**	**LT990763**	**LT990943**	**LT991153**
150	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	batesoni		MNCN-AI690	Peru; San Clemente, km 222 Panamericana Sur 31.8.2005	P. Aguilera	**LT991370**	**LT991523**	**LT990764**	**LT990944**	**LT991154**
151	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	bisinuatus		IBE-AN153	California (USA); Mendocino Co., circa 5 mi W Davis Creek 9.6.2007	W.D. Shepard	**LT991371**	**LT991524**	**LT990765**	**LT990945**	**LT991155**
152	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	capicola		IBE-RA854	South Africa; West Cape, West Cape NP, Tsaarsbank, rookpools 2.10.2011	D.T. Bilton	**LT991372**	**LT991525**	**LT990766**	**LT990946**	**LT991156**
153	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	chappuisi cf.		IBE-AN475	Djibouti; 50 km W Dikhil, Lac Abbé, thermal springs 2.2.2016	M.A. Jäch	LT837918	**LT991526**	**LT990767**	**LT990947**	**LT991157**
154	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	costatellus cf.		IBE-RA1119	Alberta (Canada); Alberta, Derwent, circa Lac Coté 2000	T. Berendonk	**LT991373**	**LT991527**	**LT990768**	**LT990948**	**LT991158**
155	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	evanescens		IBE-AN66	Azerbaijan; Alishanli, 7 km NE Masally 8.5.2014	I. Ribera & A. Rudoy	**LT991374**	**LT991528**	**LT990769**	**LT990949**	**LT991159**
156	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	gruwelli		IBE-RA304	California (USA); Riverside Co., Morongo Valley, Whitewater river 12.12.2010	I. Ribera & J. Bergsten	**LT991375**	HF931311	HF931535	**LT990950**	**LT991160**
157	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	interruptus		IBE-AN219	California (USA); Santa Barbara Co., Coal Oil Point Reserve (Slough road) 26.3.2008	P. Abellán	**LT991376**	**LT991529**	**LT990770**	**LT990951**	**LT991161**
158	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	lineatus		IBE-AN223	California (USA); Inyo Co., Ballarant, Post Office Spring 10.4.2008	P. Abellán	**LT991377**	**LT991530**			**LT991162**
159	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	lineatus cplx		IBE-RA1129	Venezuela; Guárico state, Río San Antonio 4.2.2010	A.E.Z. Short, M. García & L. Joly		**LT991531**	**LT990771**	**LT990952**	**LT991163**
160	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	lividipennis		IBE-AN71	Azerbaijan; road Budzhakah-Bash-Dashagyi, ponds 7.5.2014	I. Ribera & A. Rudoy	**LT991378**	**LT991532**	**LT990772**	**LT990953**	**LT991164**
161	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	marinus		MNCN-AI615	Spain; Madrid, Aranjuez, salinas 11.2.2006	I. Ribera & A. Cieslak	**LT991379**	HF931215	HF931441	**LT990954**	**LT991165**
162	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	meridionalis		IBE-RA373	Morocco; Moulay Bousselham, Oued Drader 12.4.2007	I. Ribera, P. Aguilera & C. Hernando	**LT991380**	HF931313	HF931538	**LT990955**	**LT991166**
163	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	mesoamericanus		IBE-RA58	Costa Rica; Cartago, 7.8 km SE Turrialba, Río Tuis 15.3.2009	M. Brojer		**LT991533**			
164	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	nipponicus		MNCN-HI27	Japan; Honshu, Ibaraki Pref., Kobodihana, Ohmika-machi, Hitachi-shi 22.7.2006	N. Hikida	**LT991381**	HF931270	**LT990773**	**LT990956**	**LT991167**
165	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	pedalis		MNCN-AH98	South Africa; West Cape, Papendorp, brackish pond 23.8.2006	G. Challet	**LT991382**	**LT991534**	**LT990774**	**LT990957**	**LT991168**
166	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	pusillus		MNCN-AI1028	Turkey; Sinop, road Saraydüzü-Boyabat, stream 5 km S Yeşilyurt 27.4.2006	A. Castro	**LT991383**	HF931146	HF931365	**LT990958**	**LT991169**
167	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	queenslandicus		IBE-AN18	Australia; Queensland, Brisbane, Shorncliffe 2.5.2014	J. Maté	**LT991384**	**LT991535**	**LT990775**		**LT991170**
168	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	rectus		MNCN-AI464	California (USA); Mono Co., Long Valley, Owens River road 19.6.2000	I. Ribera & A. Cieslak	**LT991385**	**LT991536**	**LT990776**	**LT990959**	**LT991171**
169	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	rectus cplx		IBE-AN152	California (USA); Ventura Co., Los Padres National Forest, Upper Sespe Creek 1.7.2006	A.E.Z. Short & M. Caterino	**LT991386**	**LT991537**	**LT990777**	**LT990960**	**LT991172**
170	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	rectus cplx		IBE-AN291	Alberta (Canada); 2 km W Lundbreck 27.6.2000	I. Ribera & A. Cieslak	**LT991387**	**LT991538**	**LT990778**	**LT990961**	**LT991173**
171	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	salinarius		IBE-AN79	South Africa; West Cape, Wilderness NP, road to Swartvlei, stream 22.3.2001	I. Ribera & A. Cieslak	**LT991388**	**LT991539**	**LT990779**	**LT990962**	**LT991174**
172	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	sculptoides		IBE-AN221	California (USA); Inyo Co., Tecopa, Amargosa River 8.4.2008	P. Abellán	**LT991389**	**LT991540**	**LT990780**	**LT990963**	**LT991175**
173	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	spinasus		MNCN-AH100	South Africa; West Cape, Berg River, saline stream 3.9.2006	G. Challet	**LT991390**	**LT991541**	**LT990781**	**LT990964**	**LT991176**
174	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	subpictus	deletus	IBE-AN433	Mallorca (Spain); Mallorca, Salines de Llevant 28.3.2016	I. Ribera & A. Cieslak	**LT991391**	**LT991542**	**LT990782**	**LT990965**	**LT991177**
175	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	subpictus	subpictus	MNCN-AI452	France; Bouches du Rhône, Salin-de-Girau 29.7.2005	I. Ribera & A. Cieslak	HE970918	HE970844	HE971076	HE970957	HE970996
176	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	uniformis		IBE-AN437	California (USA); Mendocino Co., Manchester 30.6.2000	I. Ribera & A. Cieslak	**LT991392**	**LT991543**	**LT990783**	**LT990966**	**LT991178**
177	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	viridescens		IBE-RA40	Spain; Zaragoza, Cinco Villas, Estanca de Castiliscar 1.11.2009	I. Ribera & A. Cieslak	**LT991393**	HF931314	HF931539	**LT990967**	**LT991179**
178	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	viridis	fallaciosus	MNCN-AI918	Morocco; Azrou, Lac Afenourir 29.4.2000	I. Ribera	**LT991394**	HF931242	HF931474	**LT990968**	**LT991180**
179	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	marinus	viridis	viridis	IBE-AN5	Turkey; Izmir, 6 km E Foça, head of reservoir 26.7.2014	I. Ribera & A. Cieslak	**LT991395**	**LT991544**	**LT990784**	**LT990969**	**LT991181**
180	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	albacetinus		IBE-RA1181	Spain; Jaén, Río Madera 7.9.2013	D.T. Bilton	**LT991396**	HG915306	**LT990785**	**LT990970**	**LT991182**
181	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	diazi		IBE-RA595	Spain; Cádiz, 15 km W Los Barrios, Arroyo del Tiradero 22.6.2011	D.T. Bilton	HF948001	**LT991545**	**LT990786**	**LT990971**	**LT991183**
182	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	gayosoi		IBE-AN311	Portugal; Algarve, Caldas de Monchique 11.6.2015	D.T. Bilton	**LT991397**	**LT991546**	**LT990787**	**LT990972**	**LT991184**
183	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	griotes		MNCN-AI943	Morocco; Immouzzer Ida Ou Tanane, Assif Tanit 21.4.2001	I. Ribera & A. Cieslak	**LT991398**	HF931245	HF931480	**LT990973**	**LT991185**
184	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	hivae		IBE-RA744	Iran; Khuzestan, Behbahan, Morvarid spring 4.9.2010	E. Irani	**LT991399**	**LT991547**	**LT990788**	**LT990974**	**LT991186**
185	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	judemaesi		IBE-RA1179	Spain; Albacete, Chorros del Río Mundo 7.9.2013	D.T. Bilton	**LT991400**	**LT991548**	**LT990789**	**LT990975**	**LT991187**
186	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	maxfischeri		IBE-AN164	Turkey; Antalya, 1 km N Hacıyusuflar 24.6.2014	D.T. Bilton	**LT991401**	**LT991549**	**LT990790**	**LT990976**	**LT991188**
187	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	metallescens	metallescens	MNCN-AI376	Austria; Niederösterreich, Lunz 2002	M.A. Jäch	**LT991402**	HF931191	HF931414	**LT990977**	**LT991189**
188	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	metallescens	plato	IBE-RA1057	Greece; Peloponnese, Achaea, 1.5 km NE Kleitor 3.4.2013	I. Ribera & A. Cieslak	**LT991403**	**LT991550**	**LT990791**		**LT991190**
189	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	morettii		IBE-RA1173	Italy; Liguria, Imperia, 1 km S Pigna 7.7.2013	D.T. Bilton	**LT991404**	**LT991551**	**LT990792**	**LT990978**	**LT991191**
190	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	pedroi		IBE-RA1082	Mallorca (Spain); Sa Calobra, Font de sa Mata 8.5.2013	I. Ribera	**LT991405**	**LT991552**	**LT990793**	**LT990979**	**LT991192**
191	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	poweri		MNCN-AC26	England (UK); S Devon, Ladram Bay, seepage 4.7.2007	D.T. Bilton	**LT991406**	**LT991553**	**LT990794**	**LT990980**	**LT991193**
192	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	preissi		IBE-AN448	Cyprus; Loukrounou, river Kaboura 28.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT991407**	**LT991554**	**LT990795**	**LT990981**	**LT991194**
193	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	scopuli		IBE-AN382	Sardinia (Italy); Golf of Orosei, Cala Mariolu 12.10.2015	J. Köhler	LT602656	LT602657	**LT990796**	**LT990982**	**LT991195**
194	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	semisericeus		MNCN-AI1064	Spain; Teruel, Beceite, river Matarranya, El Parrisal 7.8.2006	I. Ribera & A. Cieslak		HF931151	HF931370	**LT990983**	**LT991196**
195	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	semotus		IBE-RA1180	Spain; Albacete, Chorros del Río Mundo 7.9.2013	D.T. Bilton		HG915305	**LT990797**	**LT990984**	**LT991197**
196	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	serpentinus		MNCN-AI819	Turkey; Kastamonu, road Ağlı-Azdavay 28.4.2006	I. Ribera	**LT991408**	HF931229	HF931458	**LT990985**	**LT991198**
197	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	sp.		IBE-AN77	Azerbaijan; Gosmalijion, stream 7.5.2014	I. Ribera & A. Rudoy	**LT991409**	**LT991555**	**LT990798**	**LT990986**	**LT991199**
198	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	metallescens	wurayah		IBE-RA733	United Arab Emirates; Fujairah (north), Hajar Mountains, Wadi Wurayah 24.1.2010	M.A. Jäch		**LT991556**	**LT990799**	**LT990987**	**LT991200**
199	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	nitidipennis	hasegawai		MNCN-AI1289	Japan; Honshu, Shimane Pref., Mt. Sentsu, Okuizumo 15.7.2006	N. Hayashi	**LT991410**	**LT991557**	**LT990800**	**LT990988**	**LT991201**
200	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	nitidipennis	japonicus		MNCN-HI26	Japan; Shikoku, Ehime Pref., Oda-gawa, Mizumoto, Uchiko-chô 30.9.2006	Y. Kamite	**LT991411**	**LT991558**	**LT990801**	**LT990989**	**LT991202**
201	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	notabilis	corrugatus cf.		IBE-RA118	Tunisia; road Safax-Gabes, Wadi 7 km N Hachichina 25.10.2001	I. Ribera & A. Cieslak		HF931300	**LT990802**	**LT990990**	**LT991203**
202	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	notabilis	gereckei		MNCN-PA141	Sicily (Italy); Caltanissetta, Torto Valley 11.6.2007	P. Abellán & F. Picazo	**LT991412**	FJ944176	**LT990803**	**LT990991**	**LT991204**
203	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	notabilis	glaber		MNCN-PA30	Spain; Córdoba, Priego de Córdoba, Río Salado de Priego 21.7.2006	A. Millán & collaborators	HF948002	FJ944214	**LT990804**	**LT990992**	**LT991205**
204	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	notabilis	halophilus		IBE-AN22	Turkey; Kırıkkale, Delice Çoğul salt pond 14.6.2012	T. Söylemez	**LT991413**	**LT991559**	**LT990805**	**LT990993**	**LT991206**
205	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	notabilis	lanarotis		MNCN-PA32	Morocco; Ouezzane, Sidi Kacem, Oued Khendek 18.4.2006	A. Millán & collaborators	**LT991414**	FJ944251	**LT990806**	**LT990994**	**LT991207**
206	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	notabilis	normandi		MNCN-PA253	Algeria; Blida, Mellaha 23.8.2007	S. Bouzid	**LT991415**	FJ944275	**LT990807**	**LT990995**	**LT991208**
207	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	notabilis	notabilis		MNCN-AI38	Spain; Albacete, Pinilla, Salinas de Pinilla 2.6.2002	I. Ribera & A. Cieslak	**LT991416**	FJ944107	HF931416	**LT990996**	**LT991209**
208	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	notabilis	salinator		MNCN-AI53	Tunisia; road Kebili-Tozeur, 37 km SE Tozeur, salines 26.10.2001	I. Ribera & A. Cieslak	**LT991417**	FJ944271	HF931436	**LT990997**	**LT991210**
209	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	peisonis	peisonis		IBE-AF168	Russia; Volgogradskaya Oblast, Lake Elton, residual pools 17.4.2008	A. Prokin	**LT991418**	**LT991560**	**LT990808**		**LT991211**
210	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	peisonis	peisonis		IBE-AN64	Azerbaijan; Alishanli, 7 km NE Masally 8.5.2014	I. Ribera & A. Rudoy	**LT991419**	**LT991561**	**LT990809**	**LT990998**	**LT991212**
211	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	bifoveolatus		IBE-AN381	Spain; Cádiz, Bahía de Cádiz, marshes 27.4.2011	A. Millán & collaborators		**LT991562**	**LT990810**	**LT990999**	**LT991213**

212	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	cuprescens		IBE-PA276	Tunisia; road Tozeur-Gafsa, 24 km SW Gafsa, Oued El Melah 16.10.2001	I. Ribera & A. Cieslak	**LT991420**	HF931293	HF931528	**LT991000**	**LT991214**
213	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	difficilis		MNCN-AI944	Morocco; Immouzzer Ida Ou Tanane, Assif Tanit 21.4.2001	I. Ribera & A. Cieslak	**LT991421**	HF931246	HF931481	**LT991001**	**LT991215**
214	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	difficilis cplx		IBE-AN76	Azerbaijan; Gosmalijion, stream 7.5.2014	I. Ribera & A. Rudoy	**LT991422**	**LT991563**	**LT990811**	**LT991002**	**LT991216**
215	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	grandipennis		MNCN-AI616	Spain; Madrid, Aranjuez, salinas 11.2.2006	I. Ribera & A. Cieslak	**LT991423**	HF931216	HF931442	**LT991003**	**LT991217**
216	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	inermis		IBE-RA795	Japan; Honshu, Ibaraki Pref., Kobodihana, Ohmika-machi, Hitachi-shi 22.7.2006	N. Hikida		**LT991564**	**LT990812**	**LT991004**	**LT991218**
217	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	joosti		IBE-AF169	Russia; Volgogradskaya Oblast, Lake Elton, residual pools 17.4.2008	A. Prokin	**LT991424**	HF931121	HF931339	**LT991005**	**LT991219**
218	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	klapperichi		MNCN-AI1269	Bhutan; Timphu, Taba, Wang Chhu river 23.11.2005	M.A. Jäch	**LT991425**	**LT991565**	**LT990813**	**LT991006**	**LT991220**
219	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	lanuginosus		IBE-AN204	Greece; Arkadia, Astros, Lake Moustou 6.4.2013	I. Ribera & A. Cieslak	**LT991426**	**LT991566**	**LT990814**	**LT991007**	**LT991221**
220	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	mahmoodi		IBE-RA126	Oman; 1 km W Qalhat, residual pools in wadi 9.4.2010	I. Ribera, C. Hernando & A. Cieslak	**LT991427**	HF931304	HF931532	**LT991008**	**LT991222**
221	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	micans		IBE-AN474	Djibouti; Arta, wadi Alloulli (oasis), Oued Kalou 31.1.2016	M.A. Jäch	LT837917	**LT991567**	**LT990815**	**LT991009**	**LT991223**
222	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	monseti		IBE-RA124	Oman; 15 km SW Sur, residual pools in wadi 9.4.2010	I. Ribera, C. Hernando & A. Cieslak	**LT991428**	HF931302	**LT990816**	**LT991010**	**LT991224**
223	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	montesi		MNCN-AI491	Spain; Murcia, Caravaca, Rambla Pozo Enmedio 2.6.2005	A. Millán & collaborators		HF931206	HF931431	**LT991011**	**LT991225**
224	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	nanus		IBE-PA277	Morocco; Âït-Rahhal, Oued Akka 17.2.2001	I. Ribera & A. Cieslak		HF931294	HF931529	**LT991012**	**LT991226**
225	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	nilssoni		MNCN-AH76	Ireland; Clare, Lough Gealáin 31.7.2007	D.T. Bilton	**LT991429**	**LT991568**	**LT990817**	**LT991013**	**LT991227**
226	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	nobilis		IBE-AF133	Italy; Emilia Romagna, Bosco, Torrente Parma 24.5.2008	I. Ribera	**LT991430**	**LT991569**	**LT990818**	**LT991014**	**LT991228**
227	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	pagotrichus		MNCN-AI497	South Africa; West Cape, Prince Albert Road, pond 23.3.2001	I. Ribera & A. Cieslak	**LT991431**	**LT991570**	**LT990819**	**LT991015**	**LT991229**
228	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	pilosus		IBE-AN363	Spain; Cádiz, Bahía de Cádiz 27.4.2011	A. Millán & collaborators	**LT991432**	**LT991571**	**LT990820**	**LT991016**	**LT991230**
229	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	punctatus		IBE-RA286	Ireland; Clare, Finavarra, Lough Muree 23.5.2010	I. Ribera	**LT991433**	HF931310	**LT990821**	**LT991017**	**LT991231**
230	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	quadrifoveolatus		MNCN-AI636	Gran Canaria (Spain); Moya, Barranco de Azuaje 15.4.2001	I. Ribera & A. Cieslak	**LT991434**	HF931218	HF931446	**LT991018**	**LT991232**
231	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	ragusae		MNCN-AI1029	Turkey; Sinop, road Saraydüzü-Boyabat, stream 5 km S Yeşilyurt 27.4.2006	A. Castro	**LT991435**	HF931147	HF931366	**LT991019**	**LT991233**
232	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	silfverbergi		IBE-RA1021	Morocco; Tan-Tan, Oued Drâa 3.4.2007	A. Millán & collaborators	**LT991436**	**LT991572**	**LT990822**	**LT991020**	**LT991234**
233	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	sp.		IBE-RA104	Oman; Al-Akhdar, source of wadi Bani Awf 6.4.2010	I. Ribera, C. Hernando & A. Cieslak	**LT991437**	**LT991573**	**LT990823**	**LT991021**	**LT991235**
234	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	sp.		IBE-AF191	Bhutan; Sarpang, 11 km NW Sarpang, Bhur Khola river 27.11.2005	M.A. Jäch	**LT991438**	**LT991574**	**LT990824**	**LT991022**	**LT991236**
235	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	punctatus	tudmirensis		MNCN-AI467	Spain; Guadalajara, Salinas de Imón 22.5.2005	I. Ribera & A. Cieslak	HF948004	HF931205	HF931430	**LT991023**	**LT991237**
236	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	quadricollis	brevicollis		IBE-AN440	Cyprus; Akamas peninsula, Lara beach 29.4.2016	A. Millán, I. Ribera, J. Velasco & A. Villastrigo	**LT991439**	**LT991575**	**LT990825**	**LT991024**	**LT991238**
237	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	quadricollis	heeri		IBE-AN200	Tenerife (Spain); Tenerife, La Orotava, rookpools in El Barco 4.4.2015	R. Lyszkowski	**LT991440**	**LT991576**	**LT990826**	**LT991025**	**LT991239**
238	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	quadricollis	quadricollis		MNCN-AI431	Corsica (France); Cap Corse, Port de Centuri 20.9.2000	I. Ribera & A. Cieslak	**LT991441**	HF931199	HF931424	**LT991026**	**LT991240**
239	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	quadricollis	quadricollis cplx1		IBE-AN11	Spain; Murcia, La Manga del Mar Menor, Punta del Cocedor 10.10.2009	J. Sánchez-Meca	**LT991442**	**LT991577**	**LT990827**	**LT991027**	**LT991241**
240	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	quadricollis	quadricollis cplx2		MNCN-AI514	Spain; Pontevedra, Nigran, Playa Patos 3.10.2005	J. Garrido	**LT991443**	HF931209	HF931434	**LT991028**	**LT991242**
241	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	quadricollis	steinbuehleri		MNCN-AI517	Greece; Halkidiki, Kassandra 29.6.2002	M.A. Jäch	**LT991444**	HF931210	HF931435	**LT991029**	**LT991243**
242	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	rivalis	himalayae		MNCN-AI1270	Bhutan; Timphu, Taba, Wang Chhu river 23.11.2005	M.A. Jäch		**LT991578**	**LT990828**	**LT991030**	**LT991244**
243	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	rivalis	rivalis		IBE-AF81	India; Uttarakhand, 10 km SW Rudraprayag, River Alaknanda 11.11.2006	M.A. Jäch		**LT991579**	**LT990829**	**LT991031**	**LT991245**
244	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	strigosus	sp.		IBE-RA617	China; Shaanxi, 110 km ENE Xian, Huayin vill., Hija Mt. 10.5.2011	M. Balke & J. Hájek		**LT991580**	**LT990830**	**LT991032**	**LT991246**
245	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	sumatrensis	sp.		MNCN-AC16	Hong-Kong (China); Hong Kong Island, hygropetric 19.2.2007	J. Maté	**LT991445**	**LT991581**	**LT990831**	**LT991033**	**LT991247**
246	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	vandykei	vandykei		IBE-AF159	California (USA); San Luis Obispo Co., Rancho Marino Res. 28.6.2008	M. Caterino		**LT991582**		**LT991034**	**LT991248**
247	Hydraenidae	Ochthebiinae	Ochthebius	Ochthebius	vandykei	yoshitomii		IBE-AF121	Japan; Hokkaido, Shirakami, Matsumae-chô 14.7.2006	H. Yoshitomi		**LT991583**		**LT991035**	**LT991249**
248	Ptiliidae		Acrotrichis			sp.		MNCN-AI413	Germany; Thuringia, Jena, Pennickental 19.7.2005	R.G. Beutel		**LT991584**	**LT990832**	**LT991036**	
249	Ptiliidae		Cylindroselloides			dybasi		MNCN-AI564	Ontario (Canada); Eastern Ontario, circa Westport 25.7.2005	V.V. Grebennikov		**LT991585**	**LT990833**	**LT991037**	**LT991250**
250	Ptiliidae		Ptenidium			pusillum		MNCN-AI515	Morocco; Immouzzer Ida Ou Tanane, Assif Tanit 21.4.2001	I. Ribera & A. Cieslak	**LT991446**	HE970847	HE971079	HE970959	HE970997
251	Ptiliidae		Ptiliolum			sp.		MNCN-AI649	Spain; Córdoba, Cabra, La Nava 22.1.2006	A. Castro	**LT991447**	HE970857	HE971087	HE970967	HE971004
252	Ptiliidae		Rioneta			uluguruensis		MNCN-AI415	Tanzania; Uluguru Mts., between Tchenzema vil. and Lukvangule Plateau 19-21.10.2002	V.V. Grebennikov		**LT991586**		**LT991038**	

Table S4: (A) primers used for DNA amplification and sequencing reactions; (B) Typical conditions for the polymerase chain reaction.

(A)

Gene	Primer	F/R	Sequence	Ref.
COI-5’	UniLep F1b	F	TAATACGACTCACTATAGGGATTCAACCAATCATAAAGATATTGGAAC	1
	UniLep R1	R	ATTAACCCTCACTAAAGTAAACTTCTGGATGTCCAAAAAATCA	1
COI-3’	Jerry	F	CAACATTTATTTTGATTTTTTGG	5
	Pat	R	TCCAATGCACTAATCTGCCATATTA	5
	Chy	F	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	3
	Tom	R	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	3
16S	16sAr	F	CGCCTGTTTAACAAAAACAT	5
	ND1 A	R	GGTCCCTTACGAATTTGAATATATCCT	5
	16Sb	R	CCGGTCTGAACTCAGATCATGT	5
28S	ka	F	ACACGGACCAAGGAGTCTAGCATG	2
	kb	R	CGTCCTGCTGTCTTAAGTTAC	2
18S	18S 5’	F	GACAACCTGGTTGATCCTGCCAGT(1)	4
	18S b5.0	R	TAACCGCAACAACTTTAAT(1)	4

(B)

COI-3’ / 18S

Step	Time	Temperature
1	3’	96º
2	30”	94º
3	30”	50º
4	1’	72º
5	Go to step 2 and repeat 39 x	
6	10’	72º

16S / 28S

Step	Time	Temperature
1	3’	96º
2	30”	94º
3	1”	48º
4	1’	72º
5	Go to step 2 and repeat 34 x	
6	10’	72º

COI-5’

Step	Time	Temperature
1	3’	96º
2	40”	94º
3	40”	48º
4	1’	72º
5	Go to step 2 and repeat 4x	
6	40”	94º
7	40”	51º
8	1’	72º
9	Go to step 6 and repeat 29x	
10	10’	72º

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Figure S1: Majority rule consensus tree obtained with BEAST for the phylogeny of Ochthebiini with the best partition models. Numbers in nodes, posterior probabilities. Part 1 of 3.

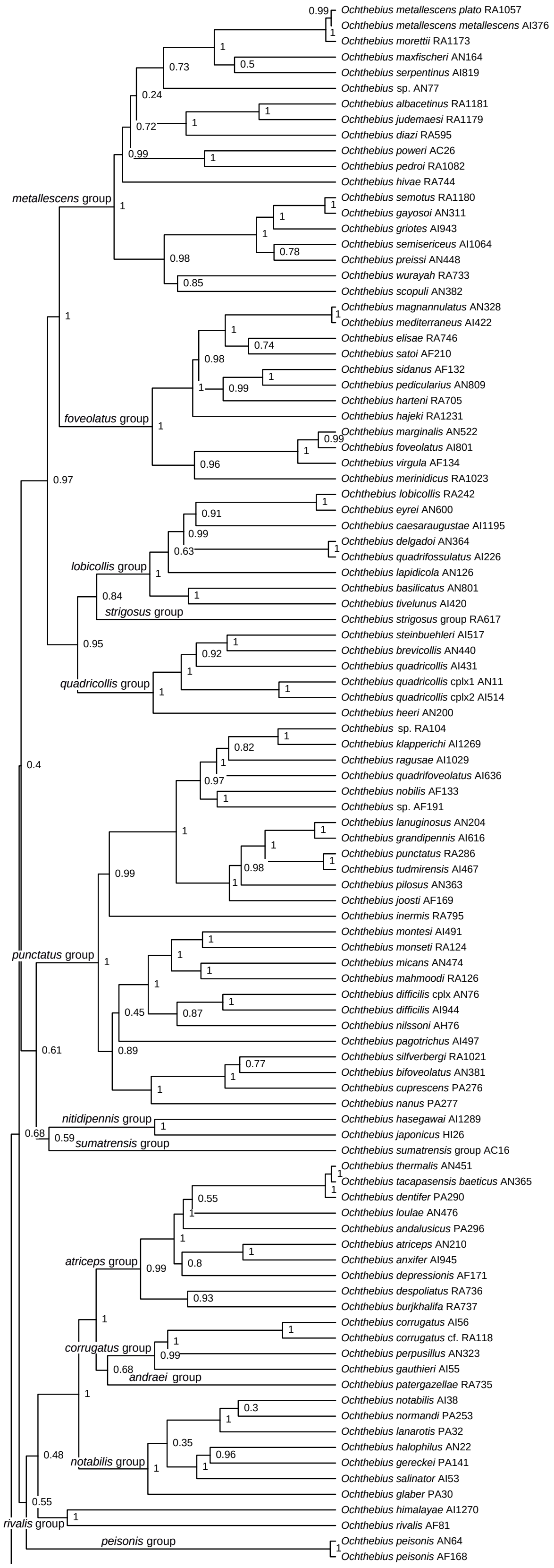


Figure S1: Majority rule consensus tree obtained with BEAST for the phylogeny of Ochthebiini with the best partition models. Numbers in nodes, posterior probabilities. Part 2 of 3.

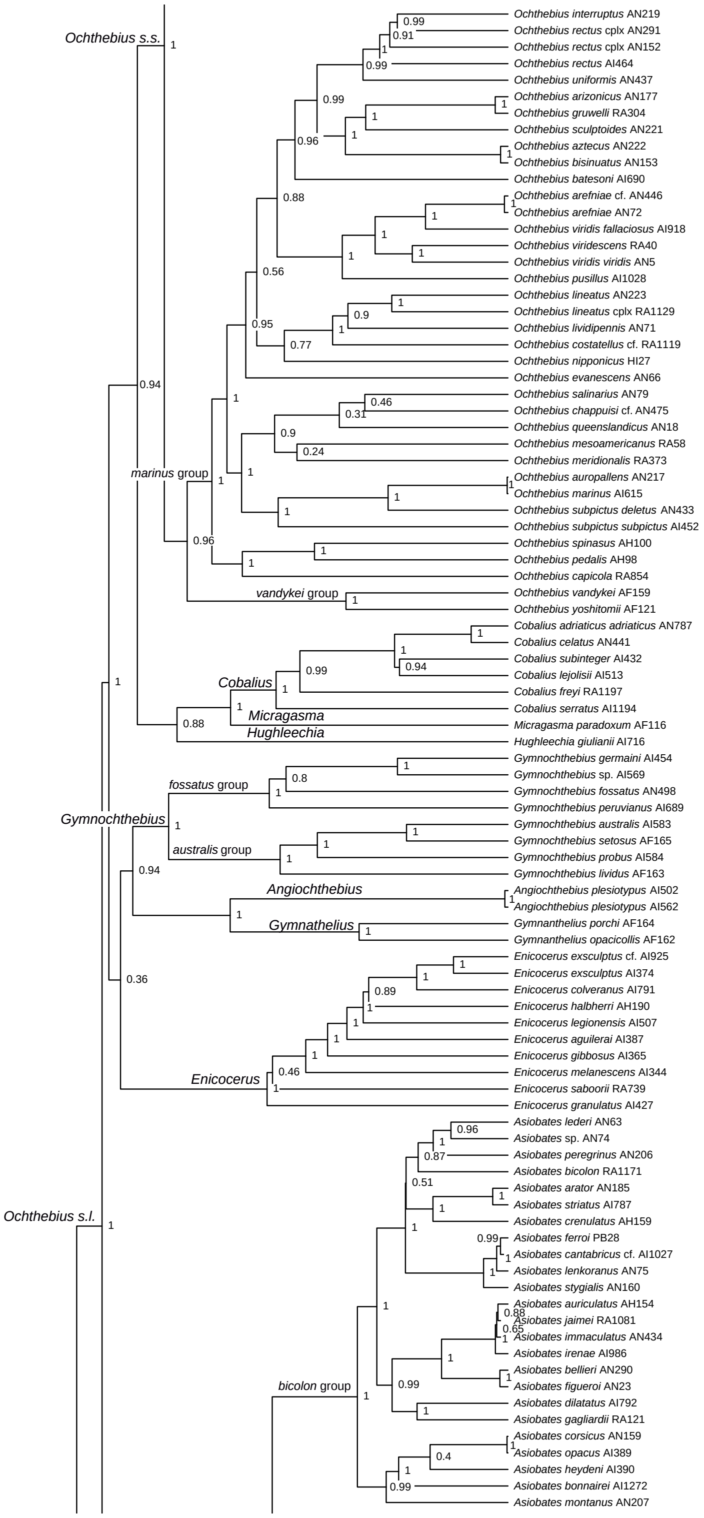


Figure S1: Majority rule consensus tree obtained with BEAST for the phylogeny of Ochthebiini with the best partition models. Numbers in nodes, posterior probabilities. Part 3 of 3.

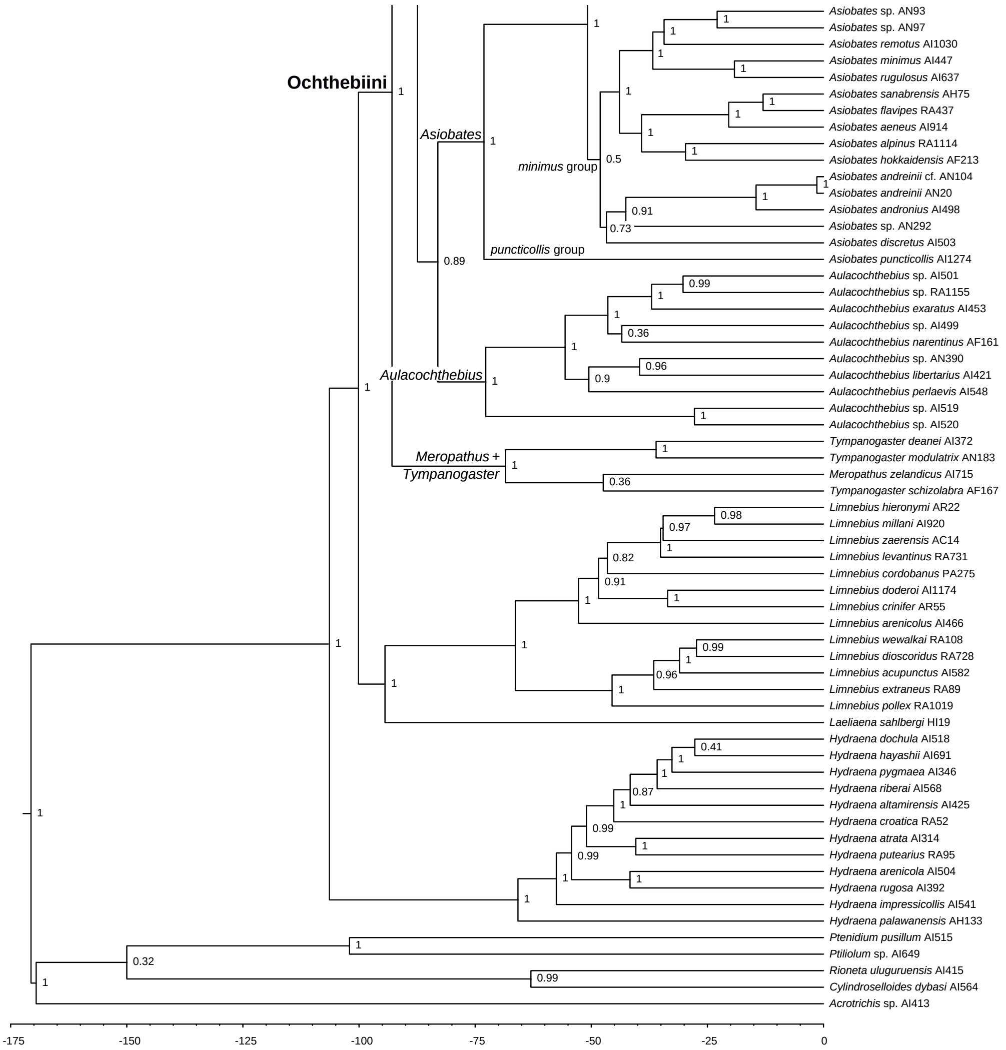


Figure S2: Phylogeny obtained with RAxML, including current Ochthebiini classification. Numbers in nodes, bootstrap support values. Part 1 of 3.

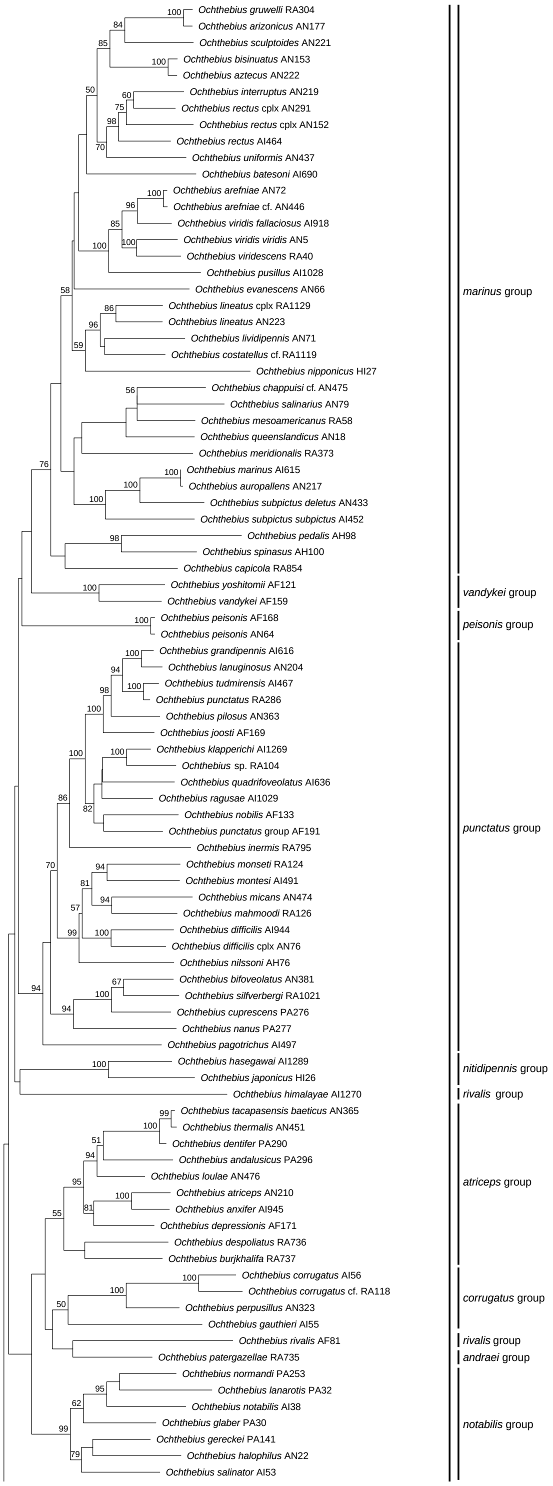


Figure S2: Phylogeny obtained with RAxML, including current Ochthebiini classification. Numbers in nodes, bootstrap support values. Part 2 of 3.

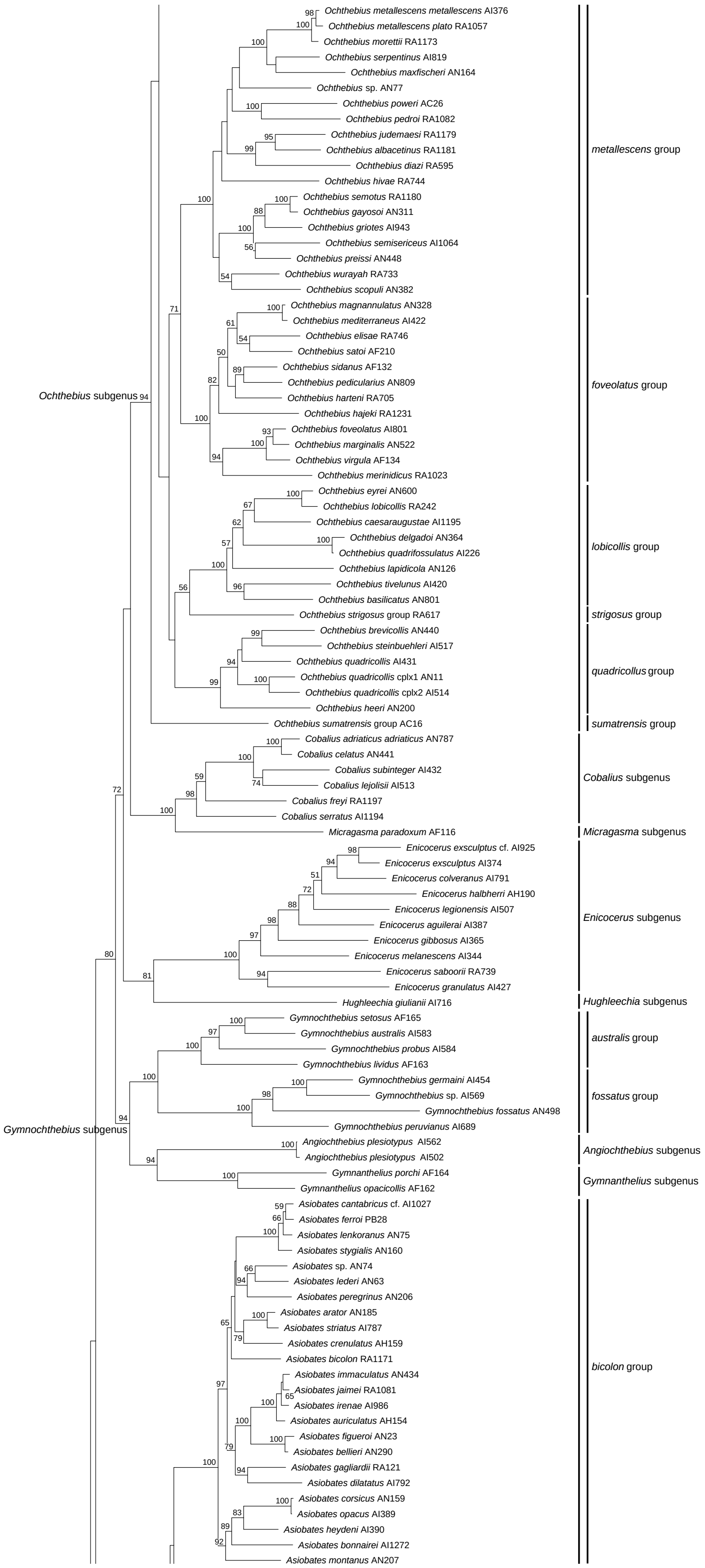


Figure S2: Phylogeny obtained with RAxML, including current Ochthebiini classification. Numbers in nodes, bootstrap support values. Part 3 of 3.

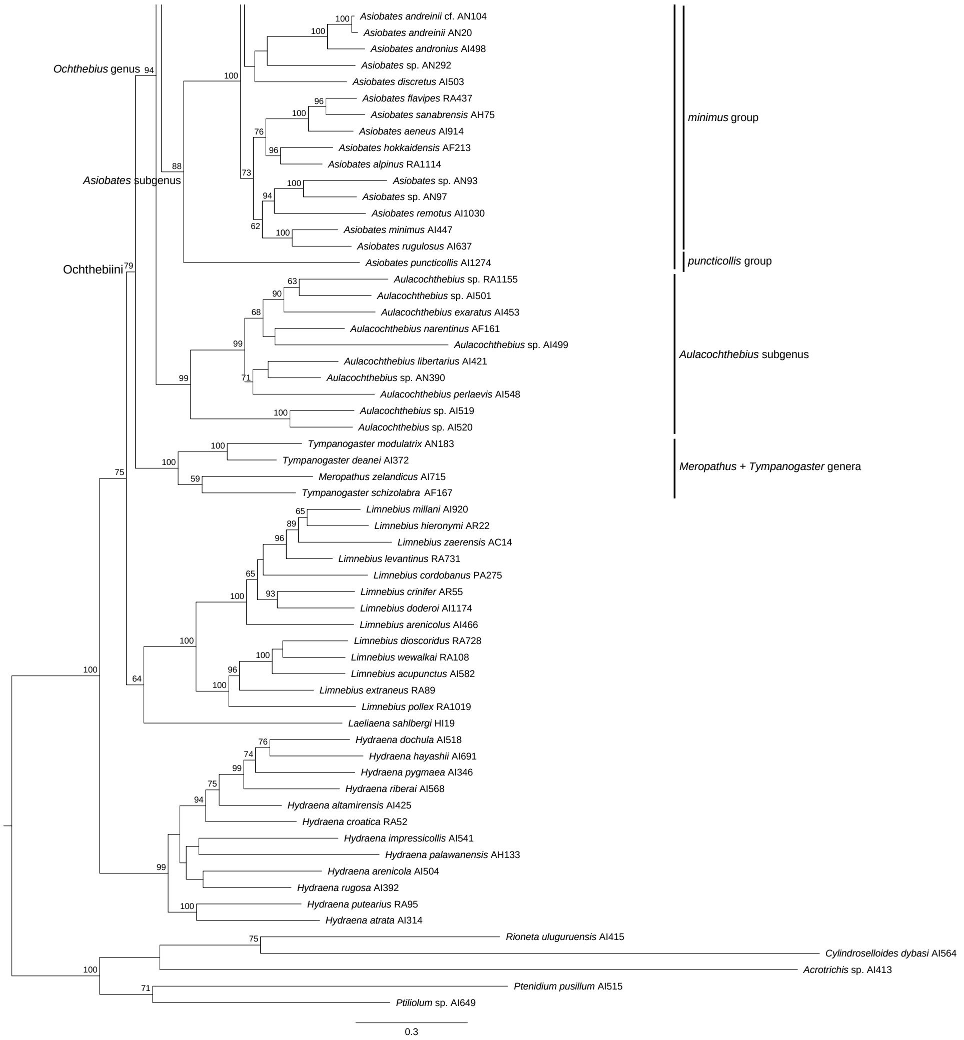


Figure S3: Phylogeny obtained with RAxML with the nuclear genes only. Numbers in nodes, bootstrap support values. Part 1 of 3.

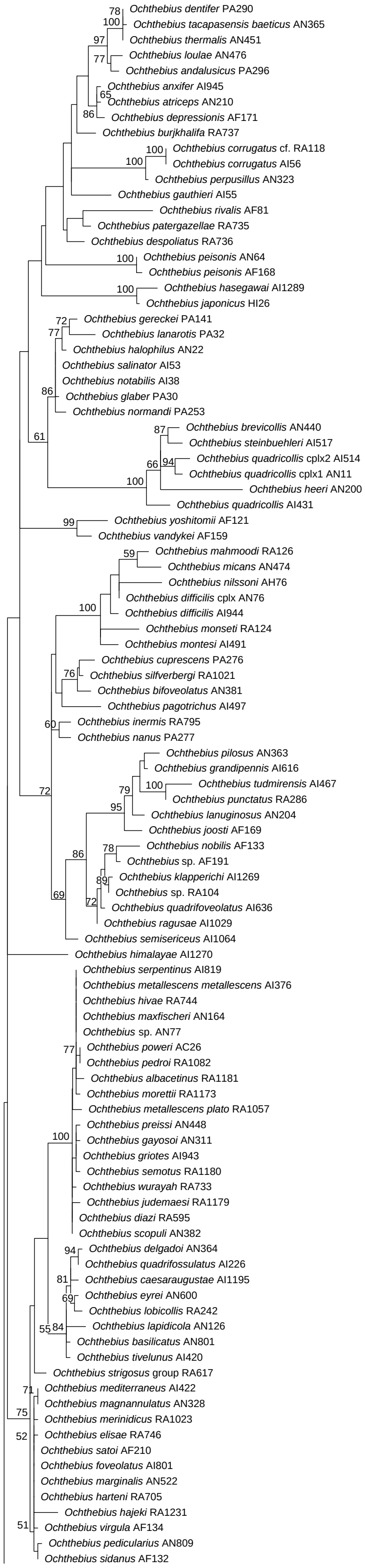


Figure S3: Phylogeny obtained with RAxML with the nuclear genes only. Numbers in nodes, bootstrap support values. Part 2 of 3.

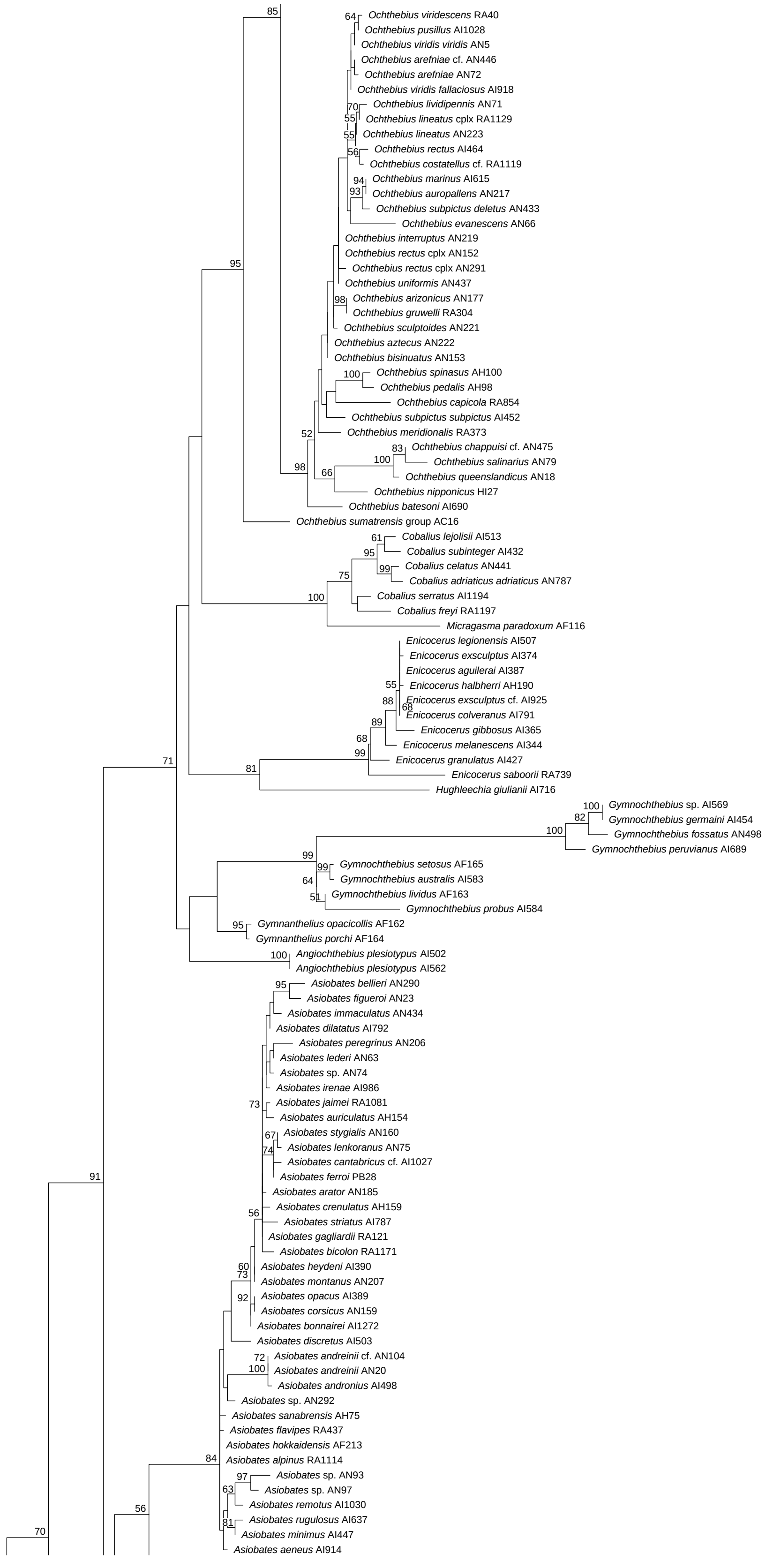
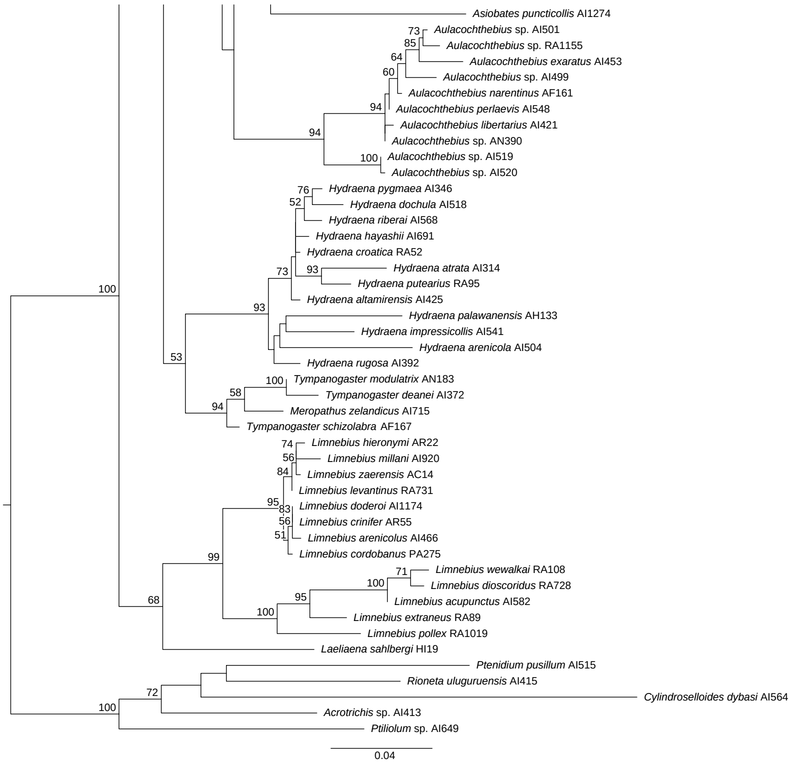


Figure S3: Phylogeny obtained with RAxML with the nuclear genes only. Numbers in nodes, bootstrap support values. Part 3 of 3.



AQUATIC INSECTS, 2017
VOL. 38, NO. 4, 185–196
https://doi.org/10.1080/01650424.2017.1384024

A new species of *Micragasma* J. Sahlberg, 1900 (Coleoptera: Hydraenidae) from Crete

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ABSTRACT

We describe a new species of *Micragasma* J. Sahlberg, 1900 (Coleoptera, Hydraenidae), which is here treated as a subgenus of *Ochthebius* Leach, 1815. The new species, *O.* (*Micragasma*) *minoicus* sp. n., was found at the margins of a coastal rockpool in the island of Crete. The species differs from the other two known species of *Micragasma* in both external and genital characters, but shares with them the presence of small setiferous tubercles on the surface of the head, pronotum and elytra, and a strong medial gibbosity on the head. In some characters, such as the structure and shape of the aedeagus, *O.* (*M.*) *minoicus* sp. n. is similar to other species of the genus *Ochthebius*, in particular of the subgenus *Cobalius* Rey, 1886, typical of coastal rockpools.

http://zoobank.org/urn:lsid:zoobank.org:act:BCEAE1EE-7C5E-4017-A753-559738221502

ARTICLE HISTORY
Received 26 July 2017
Accepted 14 September 2017
First published online
22 November 2017

KEYWORDS
Coleoptera; Hydraenidae; *Micragasma*; new species; rockpools; Crete

Introduction

Micragasma J. Sahlberg, 1900, one of the most elusive and lesser known genera of European aquatic Coleoptera, was described for a single species, *M. paradoxum* J. Sahlberg, 1900 from the island of Corfu. In the original description, Sahlberg (1900) reported collecting several specimens in December, 1895 amongst flooded vegetation in the mouth of the Potamos River and some additional specimens in November, 1898 again amongst flooded grasses in the Kalichiopulo [= Calichiopulo] Lagoon. The species had not been collected again until 1993 when a small series (three specimens) was collected in Puglia, southern Italy (Figure 1), amongst plant debris not far from the sea. Subsequently, a larger series was collected in the same area by sifting sand amongst *Salicornia* L. next to the coast (Ferro, Audisio, and De Biase 1996). Jäch (1997) redescribed the genus and transferred *Ochthebius substrigosus* Reitter, 1897 to *Micragasma*. This species was described from a small series collected in the upper part of the mouth of the Kura River, in Azerbaijan (Figure 1), although no details on the habitat were given in the original description (Reitter 1897). The species has never been reported again. More recently, *M. paradoxum* has been found in the surroundings of Lake Elton, in Volgograd Province, Russia (Makarov,

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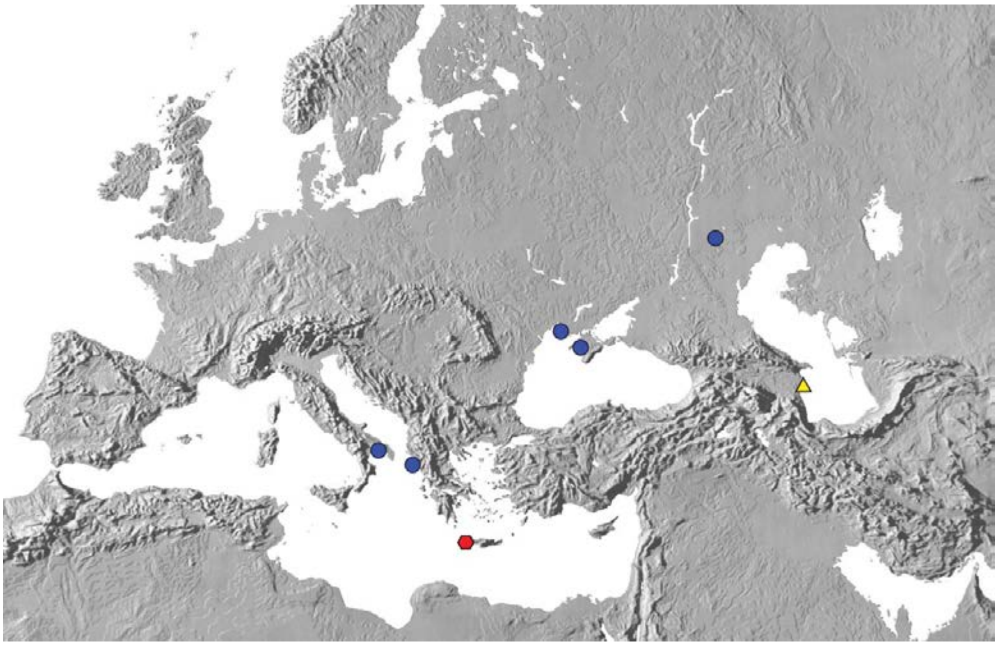

Figure 1. Distribution of the known species of *Micragasma* J. Sahlberg, 1900: circles, *Ochthebius* (*M.*) *paradoxus* (J. Sahlberg, 1900); triangle, *O.* (*M.*) *substrigosus* Reitter, 1897; hexagon, *O.* (*M.*) *minoicus* sp. n.

Matalin, and Komarov 2009; Prokin, Litovkin, and Jäch 2016), in saline ditches and pools, and in two localities on the north coast of the Black Sea, in detritus or in overgrown grass puddles (Shatrovskiy 2015) (Figure 1). There are no other published records of *Micragasma*.

The phylogenetic affinities of *Micragasma* are also not well understood. Reitter (1897) described *O. substrigosus* within subgenus *Chirochthebius* Kuwert, 1887, currently a synonym of *Aulacochthebius* Kuwert, 1887 (Jäch and Skale 2015), noting its resemblance to *A. narentinus* (Reitter, 1885). Sahlberg (1900) compared the newly described genus with species of *Ochthebius* Leach, 1815, and, in his revision of Hydraenidae genera, Hansen (1991) noted that it is a distinct and apparently well-justified genus. However, Jäch (1997), after including *O. substrigosus* in the genus *Micragasma*, noted the similarity between *Micragasma* and *Ochthebius*, questioning its status as a separate genus. In Beutel, Anton, and Jäch (2003), some peculiarities of the head of *Micragasma* were noted in reference to other species of *Ochthebius* or the wider Hydraenidae (absence of ocelli in *M. paradoxum*, a shield-like clypeus with distinctly raised lateral margin), but the genus was not included in the phylogenetic analysis and nothing was said about its possible relationships. Unpublished molecular data place *Micragasma* as sister to the species of *Cobalius* Rey, 1886, treated as a synonym of *Ochthebius* s.str. in the last reference catalogues (e.g., Jäch and Skale 2015). Here we treat both *Micragasma* and *Cobalius* as subgenera of *Ochthebius*; a more detailed discussion on the phylogenetic relationships of both taxa will be published in a forthcoming work.

The close relationship between *Micragasma* and *Cobalius* suggested by molecular data may appear surprising, given the strong differences in external morphology. This relationship seems to be more likely in the light of the species newly described here, found during a recent trip to Crete at the margins of a rock pool in the south western coast of the island.

Material and methods

We studied one specimen of *O.* (*M.*) *paradoxus* labelled 'RUSSIA: 17.4.2008, Volgogradskaya Oblast, 49°07′43.6″N/46°47′39.5″E, Lake Elton, −18 m below sea level, residual pools of temp. water course, leg. A. Prokin', 'Voucher IBE-AF116' (one of the specimens reported in Prokin et al. 2016). The DNA of one paratype of the new species was extracted non-destructively (voucher IBE-AN770, see below) with commercial kits (DNeasy Tissue Kit, Qiagen, Hilden, Germany).

The specimen used for DNA extraction (voucher IBE-AN770) was subsequently used for SEM observation. After the extraction process, it was mounted on a stub with double-sided carbon conductive tab and coated with gold in a Quorum Q150R S sputter coater unit (Quorum Technologies Ltd., UK). The sample was observed using a Hitachi S3500N scanning electron microscope (Hitachi High-Technologies Co. Ltd., Japan) operated at 4 kV in the Electron Microscopy Service of the Institute of Marine Sciences (CSIC), in Barcelona (Spain).

Specimens are deposited in the following collections: CHB, collection of C. Hernando, Badalona, Spain; IBE, Institute of Evolutionary Biology, Barcelona, Spain; MNCN, Museo Nacional de Ciencias Naturales, Madrid, Spain; NMW, Naturhistorisches Museum Wien, Austria.

Taxonomy

Ochthebius (Micragasma) minoicus sp. n.

Figures 2–10.

Type locality

Greece, Crete, Elafonisi, 35°16′9.2″N, 23°32′42″E (Figure 1).

Type material

Holotype. ♂ '22 Crete, Elafonisi 20.iv.2017/rockpools on calcareous bed/35°16′9.2″N 23°32′42″E 0m/Hernando, Ribera & Villastrigo leg' (NMW); aedeagus dissected and mounted in DMHF on a transparent card, pinned with the specimen; plus red holotype label. *Paratypes*. 6 ♂, 3 ♀, same data as holotype, with paratype labels (IBE, CHB, NMW, MNCN). DNA of one paratype female extracted (voucher number IBE-AN770).

Description

Total length 1.68–1.73 mm, maximum width 0.65–0.70 mm, without apparent differences between males and females. Body shape oval (Figures 2 and 3). Body colour dark brown; head, disk of pronotum and elytra darker, appendages paler (Figure 2).

Figure 2. Habitus of the holotype of *Ochthebius* (*M.*) *minoicus* sp. n. Photo by M. Brojer. Scale bar 0.5 mm.

Head (Figures 4 and 5). Labrum deflexed, deeply emarginated; surface smooth, with a shallow reticulation; with sparse small setae. Clypeus trapezoidal, narrower distally; surface rugose, with small tubercles, with sparse setiferous punctures with small setae; finely bordered; fronto-clypeal suture well marked. Frons expanded laterally, with a medial gibbosity; two lateral elongated excavations bordering the gibbosity joining posteriorly, without pubescence or tubercles (in most specimens, these excavations are not visible due to salt concretions, but are clearly visible in the specimen used for DNA extraction). Margins of head only slightly elevated. Head covered with small tubercles with very robust, short, recurved silverish-whitish setae, almost squamiform; as observed with the SEM, setae are lanceolate, wider in the middle and with lateral expansions (specially on the elytra, see below), with rugose surface. Eyes widely separated, laterally very prominent. Ocelli absent.

Thorax. Pronotum transverse, with margins explanate (Figures 5 and 6); disc elevated, with a medial irregular longitudinal sulcus; central area covered with small, very dense setiferous tubercles; lateral area rugose; with two basal elevated areas forming a 'V'. Lateral

Figure 3. SEM habitus of a paratype of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770). Scale bar 0.5 mm.

margins irregular, but uniformly arched, elevated, with a fringe of denser setae; anterior margin with a narrow hyaline expansion; posterior margin constricted, with postero-lateral hyaline expansion following the contour of the pronotum; postero-lateral hyaline expansions densely covered with setae; posterior margin of pronotum covering base of elytra, partly covering the scutellum.

Elytra elongated, oval (Figures 2 and 3); apparently fused; shoulders with a strong gibbosity; apex acuminate; margins slightly explanate, with a fringe of denser setae. With 10 very regular rows of punctures on each elytron, with smaller punctures distributed irregularly, some of them with smaller setae; main sculpture composed of a large puncture with a tubercle on the anterior margin, with a seta on top (Figure 7); surface rugose-

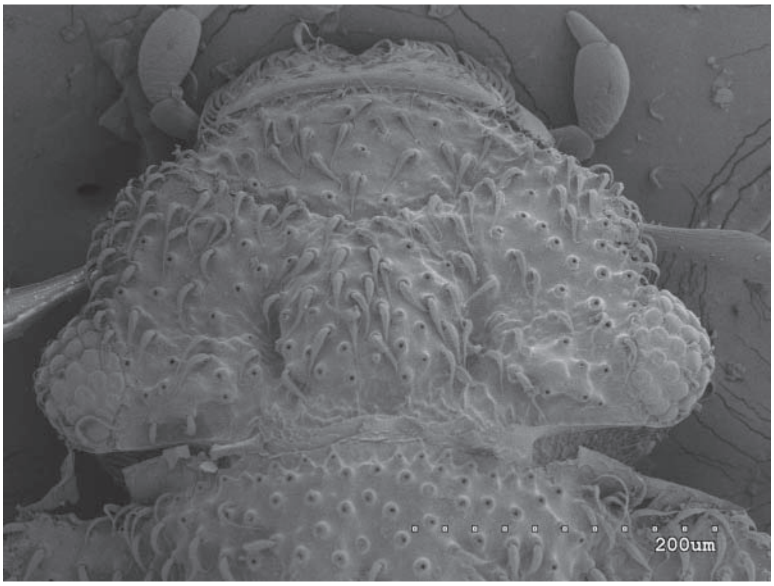

Figure 4. Head of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770), dorsal view.

chagrinated (not as rugose as pronotum and head); setae as on pronotum and head; apex of elytra and, to a lesser extent, marginal areas with an irregular surface, with tubercles united forming small carinae (Figure 8). Apterous (observed in one paratype).

Hypomera with deep antennal grooves, with a hyaline margin. Metaventrite short, covered with hydrophobe pubescence except on medial part, which is covered with a longer

Figure 5. Head and pronotum of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770), frontal view.

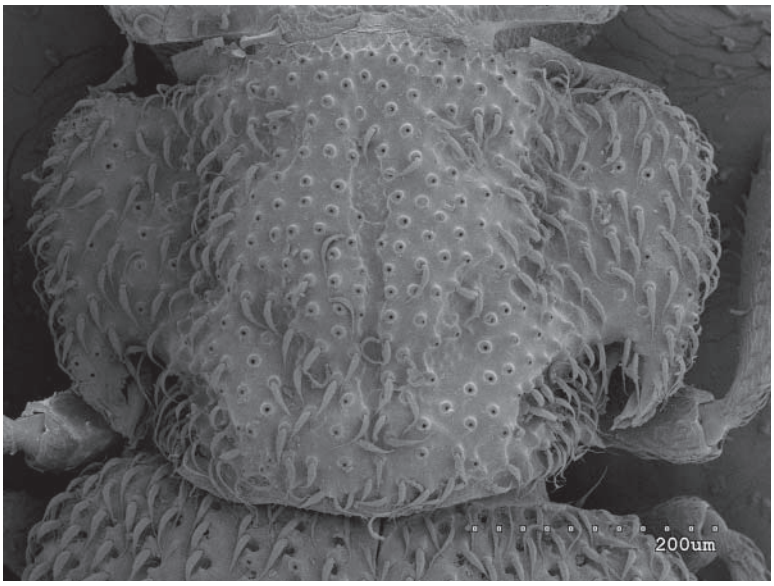

Figure 6. Pronotum of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770), dorsal view.

Figure 7. Detail of the elytral disk of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770).

and more robust pubescence; prosternum and mesoventrite glabrous, with a rugose surface; mesoventrite with a small anterior longitudinal carina, with an acuminate apophysis.

Legs short, robust; tarsi short, specially protarsi, without natatory setae; with spiniform setae.

Abdomen. Ventrites 1–5 with short hydrophobe pubescence and longer and recurved sparse setae, denser on the medial part (Figure 9); last ventrites without hydrophobe

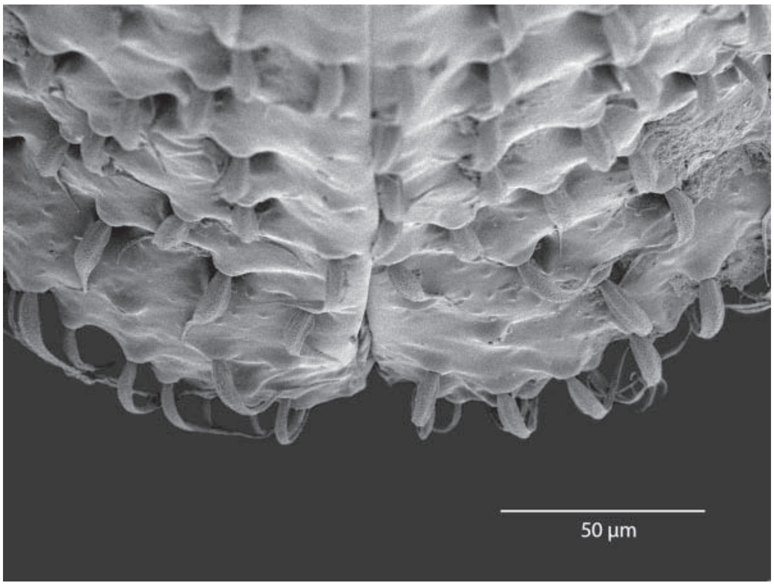

Figure 8. Apex of the elytra of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770), dorsal view.

pubescence, with short, straight spiniform setae. Surface irregular, with a chagrinated aspect. Last ventrite with a row of 7–9 long, explanate setae.

Aedeagus as in Figure 10, asymmetrical, medial lobe slightly arched; distal piece elongated, widest in the middle.

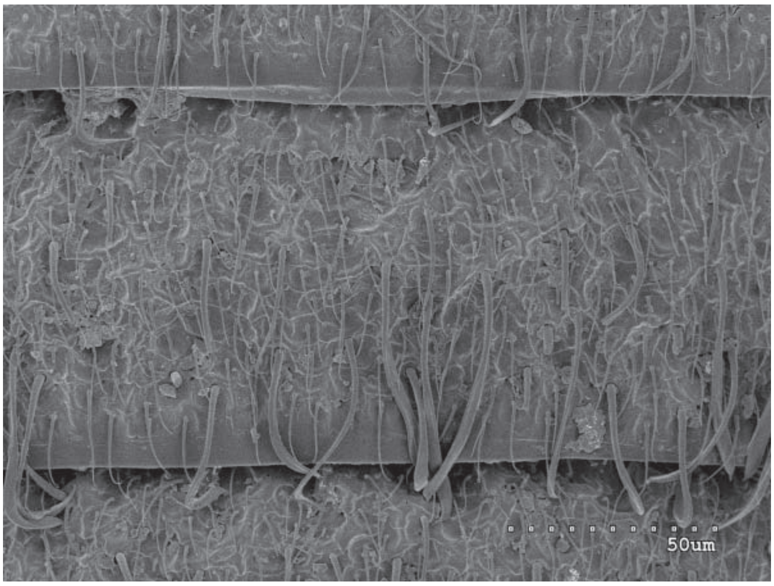

Figure 9. Surface of the abdominal ventrite 2 of *Ochthebius* (*M.*) *minoicus* sp. n. (voucher IBE-AN770).

Figure 10. Aedeagus of *Ochthebius* (*M.*) *minoicus* sp. n. (paratype), lateral view.

Females

Pronotum with anterior angles more rounded and lateral margins more arched than in males.

Etymology

Named in reference to the Minoan civilisation of Crete. The name is an adjective in the nominative singular.

Distribution

So far known only from the type locality.

Habitat

Specimens were collected at the margins of a rock pool close to the seashore, with saline water over rocky substratum with gravel and sand on the margins and with a tuft of vegetation (Figure 11). They were dislodged from the shore and the tuft of vegetation by splashing water. Specimens were collected in association with *Bledius* sp. (Staphylinidae), *Ochthebius* (*Cobalius*) *adriaticus moreanus* Pretner, 1929, *O.* (*Cobalius*) *subinteger* Mulsant and Rey, 1861 and *O.* (*Calobius*) *steinbuehleri* Reitter, 1886.

Discussion

Of the characters listed by Jäch (1997) as diagnostic for the genus *Micragasma* based on the two only known species at the time (*O.* (*M.*) *paradoxus* and *O.* (*M.*) *substrigosus*), potential synapomorphies are the presence of small tubercles covering most of the surface of the body and the presence of a well-developed medial gibbosity on the vertex surrounded by more or less impressed foveae. As already noted by Jäch (1997), all other characters occur in various species of *Ochthebius*. In *O.* (*M.*) *minoicus* sp. n., the shape of the labrum is more similar to other species of *Ochthebius*, not as strongly deflexed as in *O.* (*M.*) *paradoxus* and less transverse. The head is also less transverse and less explanate, without the strong lateral elevations present in *O.* (*M.*) *paradoxus*. The pronotum of *O.*

Figure 11. Type locality of *Ochthebius* (*M.*) *minoicus* sp. n.: rockpool in Elafonisi, Crete. Note the edge of the pool with some vegetation, from where specimens were flushed.

(*M.*) *minoicus* sp. n. is also less explanate, with the marginal areas less flat and with a more rugose surface. Other differences refer to the general shape and size, more elongated in *O.* (*M.*) *minoicus* sp. n. (the studied specimen of *O.* (*M.*) *paradoxus*, a female, is 1.58 mm long and 0.75 mm wide), with a darker body colour, and a more dense and robust pubescence, with a more regular appearance on the elytra. The studied specimen of *O.* (*M.*) *paradoxus* is brachypterous, with a non-functional small stump only slightly longer than the metaventrite, but the elytra are not fused.

The aedeagus of *O.* (*M.*) *minoicus* sp. n. has the same general structure as that of other species of the subgenus, but without the strongly deviating features of *O.* (*M.*) *paradoxus*, and with a simpler distal piece than *O.* (*M.*) *substrigosus* (see Jäch 1997; Figures 2 and 3). This results in an aedeagus that is surprisingly similar to those of the species of *Cobalius*, in particular to that of *O.* (*C.*) *celatus* Jäch, 1989 (see Jäch 1989; Figure 7). Some species of *Cobalius* have also very regular elytral striae with strong setae, and deep fossae on the head (but without gibbosity) (e.g., *O.* (*C.*) *subinteger*). Although the ecology of the species of *Micragasma* is still poorly known, they are undoubtedly associated with coastal saline habitats, with the only known exception of the population of *O.* (*M.*) *paradoxus* near Lake Elton (Prokin et al. 2016). They seem to be, however, less linked to water than the species of *Cobalius*, as suggested also by some morphological features (shorter legs, more robust pubescence), and some specimens seem to have been found in a fully terrestrial environment (e.g., Shatrovskiy 2015). When washed into the water, specimens of *O.* (*M.*) *minoicus* sp. n. floated upside down with a film of air attached to the ventrites, as happens with other species of *Ochthebius* (including those of *Cobalius*), suggesting that the ventral pubescence may be hydrophobic. It is interesting to note, however, that the dorsal surface is extremely hydrophilic: when dry specimens were touched with a wet brush, the whole dorsal surface was immediately covered by a film of water, maybe through the capillary effect of the tubercles and setae, or perhaps due to a possible strong hydrophilic effect of the highly modified expanded setae. The potential function of a hydrophilic dorsal surface in combination with a hydrophobic ventral surface is unknown, but it may be related with respiration or to avoid dehydration in the sometimes extreme saline and dry environment in which the species may be found.

Acknowledgements

We thank Anabela Cardoso (IBE) for laboratory work, J.M. Fortuño (ICM) for help with the SEM observations, Michaela Brojer (NMW) for the habitus photograph, M.A. Jäch (NMW) and A. Prokin for making available the specimen of *O.* (*M.*) *paradoxus* for study and M.A. Jäch for useful comments to the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The work was funded by project AEI/FEDER, UE [CGL2013-48950-C2-1-P] to Ignacio Ribera.

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Biosketch

My name is Adrián and I was born in León in 1987. I studied biology at University of León, where I acquired a love for nature. I was lucky to be supervised by Dr. Francisco García-Criado during Summer 2012 as part of an internship at Area of Ecology, learning for the first time about macroinvertebrate communities in aquatic environments. I finished the degree in Biology in 2013, with a final project focused on aquatic macroinvertebrates in streams. After that, I spent 3 months with a fellowship at Instituto de Medio Ambiente, Recursos Naturales y Biodiversidad (IMARENABIO), studying other water inhabitants: diatoms. My academic training continued with another project on aquatic communities, but this time on a mountain lake. That project lead to a publication entitled “Response of a littoral macroinvertebrate assemblage to a persistent hydrological drought in a permanent mountain shallow lake from northwestern Spain”, published during 2017 (as corresponding author).

In 2015, I obtained an FPI grant supervised by Ignacio Ribera and Andrés Millán under the project “The evolution of habitat transitions in aquatic Coleoptera” (CGL2013-48950-C2). The research during this PhD was undertaken mainly at Institute of Evolutionary Biology (CSIC-UPF), but also at University of Murcia. During this PhD I have been able to attend several European and International meetings and conferences: Balfour Browne Meeting (León, Spain – 2014; Chefchaouen, Morocco - 2018), European Meeting of PhD Student (Visby, Sweden – 2016), Iberian Congress of Biological Systematics (Palma de Mallorca, Spain – 2018) and Annual meeting of the Willi Hennig Society (Barcelona, Spain – 2018; Berkeley, CA, USA - 2019).

During the last years, I have done two short stays in recognised research institutions. I visited Brent Emerson’s lab (Instituto de Productos Naturales y Agrobiología, CSIC, La Laguna, Tenerife) in 2017, under the supervision of Paula Arribas and Carmelo Andújar. I obtained the knowledge required to perform mitochondrial metagenomics technique, essential in both chapters 5 and 6, and in my personal development as a scientist. In 2018, I visited The University of Adelaide (Australia) under the supervision of Dr. Steven Cooper, where I learnt about groundwater fauna in Western Australia and developed molecular phylogenies (part of chapter 6).

Additionally, a lot of fieldwork has been carried out to accomplish the objectives of this PhD, with major sampling in Cyprus (Spring 2016), Crete (Spring 2017), Morocco (Spring 2018) and Malta (Autumn 2018), but also minor fieldwork in south-eastern Spain (Murcia, Albacete and Cuenca, Autumn 2015), Tenerife (summer 2017), La Palma (summer 2017), Gran Canaria (spring 2018), Australia (Western Australia, Queensland and Kangaroo Island, Summer 2018), Girona (Spring 2019) and Lanzarote (Spring 2019).