New morphological and molecular data reveal an important underestimation of species diversity and indicate evolutionary patterns in European Lepidocyrtus

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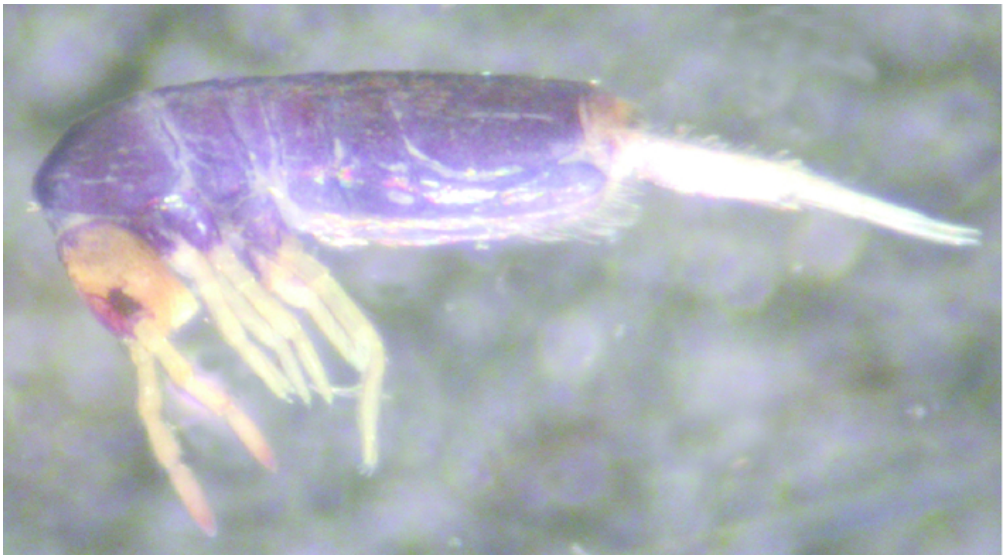

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New morphological and molecular data reveal an important underestimation of species diversity and indicate evolutionary patterns in European *Lepidocyrtus*

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Running title: European *Lepidocyrtus* under scrutiny.

Abstract

The proper identification of morphological species is a key task for species richness estimation of any ecosystem. Although the body colour is a widely used character identifying European *Lepidocyrtus* species, recent investigations using molecular data have revealed that species delineation using body color can result in an underestimation of the real species diversity because of the presence of cryptic species. *Lepidocyrtus violaceus* is a European species characterised by its dark violet body colour. Its wide worldwide distribution leads us to suspect that under this morphospecies several cryptic species can be hidden. Since traditional morphological characters have revealed insufficient for real diversity identification in *Lepidocyrtus*, new morphological characters are needed in order to describe the cryptic diversity detected by molecular data in this genus. Pseudopores are integumentary structures present in all *Lepidocyrtus* species, but the distribution of these structures has not been properly described in the genus. In the present work we aim to analyse whether morphopecies *L. violaceus* is a monophyletic entity in Europe. Moreover, we aim to determine if the position and number of pseudopores in the different parts of the body and appendages is a character with phylogenetic signal useful in the identification of the species or superspecific entities. 14 populations of *L. violaceus* from 5 European countries, and another 25 *Lepidocyrtus* species from 9 European countries have been studied. In total 208 specimens have been analysed morphologycally and half of them were studied molecularly using sequences of genes COXII and EF-1α. Molecular data reveals that the worldwide distributed *Lepidocyrtus violaceus* morphospecies is a polyphiletic entity in Europe. Between six and twelve diferent cryptic species have been detected under the appeareance of this European morphospecies, and only the presence of pseudopores on the basal plate of the fourth abdominal segment has been detected as a promising diagnostic character between them. A common basic-pattern of pseudopores distribution has been recognized in the European membres of the genus, and also a diferential pattern within each European species group. As a general trend, an increase in pseudopores number is detected in the evolutive line of the genus *Lepidocyrtus* in Europe.

Additional keywords

Chaetotaxy, COXII, EF-1α, cryptic species, DNA, phylogeny, taxonomy, pseudopores.

Short summary

Species richness estimation of European Collembola using morphology is challenged by molecular detection of cryptic species. The monophyly of old described *Lepidocyrtus* species and the search for new morphological characters useful for diagnose the detected molecular diversity in this genus is under scrutiny. 26 European *Lepidocyrtus* species, including 14 populations of L. violaceus, have been morphologically and molecularly studied using sequences of the COXII and EF-1α genes. Molecular data reveals that the worldwide distributed *Lepidocyrtus violaceus* morphospecies is a polyphiletic entity in Europe. The number and distribution of pseudopores on body and appendages is a promising morphological character with clear phylogenetic signal. As a general trend, an increase in pseudopores number is detected in the evolutive line of the genus *Lepidocyrtus* in Europe.

Introduction

Species richness is usually considered as a proxy of the ecological status of the ecosystems, and "morphological species" is the standard unit of measure for biodiversity purposes. In this context the proper identification of morphological species is a key task for species richness estimation. In soil environments, Collembola is one of the most diverse animal groups, and the estimation of Collembola species richness is important for the assessment of global soil biodiversity.

The genus *Lepidocyrtus* Bourlet, 1839 comprises ~250 species (Bellinger et al. 1996–2019). Based on chaetotaxy and molecular phylogenetic analysis, European species have been ascribed to five monophyletic groups clearly diferentated by the chaetotaxy (Mateos et al. 2018a), and each group contains unpigmented and pigmented species. Pigment is always dark blue or violet and could be present covering all body (fully-pigmented species) or forming dots or bands in several parts of the body. In both unpigmented and pigmented species pigment is usually present on several parts of the appendages. In all five European species groups at least one

fully-pigmented species (or subspecies) is present (see Mateos 2008b, 2011, 2012, Mateos and Petersen 2012, Winkler and Traser 2012). In the present work we deal with *L. violaceus* (Geoffroy, 1762) Lubbock, 1873, the oldest described *Lepidocyrtus* species.

Lepidocytus violaceus*, a brief history of the oldest species of the genus*

Lepidocyrtus violaceus (Geoffroy, 1762) Lubbock, 1873 was originally described as *Podura violacea* Geoffroy, 1762 from specimens from Paris (France). This original description only described the (in French in the original) "*purple color a little leaded*" as characteristic of the species, without any other morphological description. More than a century later, Lubbock (1873) moved the species to the genus *Lepidocyrtus* and made a brief description accompanied by a picture of a specimen in dorsal view. In the same publication, Beck (1873) described the scales of *L. violaceus* and drew a picture of one of them. Some years later, Parfitt (1891), based on specimens from Exeter (Devonshire, UK), described the pigmentation variability of the species as: "*Some are blue, purple, yellowish, bronze-green. Some specimens I collected … had the head and the last two segments of the body golden-yellow, the rest of the body a splendid purple. Antennae yellow, with the apex of each joint purple. Legs pale yellow*." Sixty five years later, Gisin (1944), based on specimens from Sweden, made another diagnose of the species and described the pigmentation as: (in German in the original) "*Blue-violet granulated body. Manubrium and femora (especially femur III)* ± *purple, without differentiating from body color. Intersegmental bands and dentes clearer. Antennae of uniform violet color, with the antennae bases barely lighter than the head and distal portion of the antennae joints".* Years later Gisin (1964a, 1964b) described the dorsal macrochaetae formula of the head and body (as A0A2A3Pa5/00/0101+3), the basal labium chaetotaxy (as MMRELL, with chaeta R half in length as M chaetae) and the accessory chaetae of anterior trichobothrium of Abd.IV (with chaetae D1, a and m pointed and strongly ciliated). Szeptycki (1967) described the branched morphology of the labral apical chaetae. Based on North American specimens, Snider (1967) described the Abd.III chaetotaxy of dorsal trichobothrial area (were chaeta d3 is absent); Snider also stated that, for years, taxonomists have confused the species *L. violaceus* and *L. cyaneus* Tullberg. Based on specimens from Indiana (USA), Christiansen & Bellinger (1980) described the dorsomedial chaetotaxy of Abd.II–III (Abd.II without chaetae ml and p5p; Abd.III

without chaeta d3), and fore foot unguis complex (unguis with basal tooth and two unpaired teeth, empodium lanceolate and with few serrations on the outer edge, tenent hair spatulate); the authors stated that *"it is not certain that Neartic populations are conspecific with European violaceus. … The general similarity is sufficient to justify combinig them for the moment*". Berg & Heijerman (2002) drew a picture of *L. violaceus* from The Netherlands where mesothorax appears not protruding over the head. Mateos (2008a) summarized the available information about the published descriptions of *L. violaceus* (and other European species of the genus), and several authors in recent years have elaborated identification keys where *L. violaceus* is included (Fjellberg 2007, Hopkin 2007, Mateos 2008a, Mateos 2011).

It is notorious that, since 1792 different authors have made (partial) descriptions of *L. violaceus* based on specimens from different countries and different continents. As a result, *L. violaceus* is nowadays considered a widely distributed species across Europe, Asia, Central and North America, and has also been cited from the Arctic and Antarctic regions (see Bellinger et al. 1996-2019). But, won't *L. violaceus* be a chimera?

About body colour and the need for new characters in Lepidocyrtus

For centuries, *L. violaceus* has been cited worldwide, and its dark blue pigmented body has always been a key character for its identification. However, DNA-based analyses suggest that the current body colour and morphology-based species delimitation is insufficient for delimiting species in genus *Lepidocyrtus* due to the presence of cryptic species (Zhang et al. 2019a). Thus, the search for new morphological characters, capable of describing the cryptic species that molecular studies are revealing is becoming increasingly necessary.

Pseudopores, despite being structures present in most Collembola groups, are usually overlooked in taxonomic descriptions. Only in a few Collembola species the complete distribution of pseudopores on the body has been properly described. Deharveng et al. (2018) presented an overview of pseudopore patterns across Collembola and underlined their interest at different taxonomic level.

Pseudopores were described for the first time in Collembola by Gisin (1963). Since this discovery, a pair of dorsal pseudopores from Th.II to Abd.IV and several pseudopores on coxae is known to be characteristic to all Entomobryioidea. One year later, Gisin (1964a) described the presence of pseudopores on each manubrial

plate (dorsodistal regions of manubrium) as a characteristic for all Entomobryidae. Since these two referenced Gisin's papers, all *Lepidocyrtus* species descriptions mention the 1+1 dorsal psedopores form Th.II to Abd.IV, and the 2+2 (rarely 3+3 or 4+4) pseudopores on the dorsal manubrial plate, while pseudopores on coxae have been remarked only in a few *Lepidocyrtus* species descriptions (see for example Mateos 2008a, Cipola et al. 2018c).

During the postembryonic development of *Lepidocyrtus* (and all Entomobryoidea) chaetotaxy is variable, but the number and position of pseudopores (and also bothriotricha and S-chaetae, see Zhang et al. 2019b) are stable, at least those pseudopores located on the dorsal region of Th.II to Abd.IV, coxae and dorsal manubrial plate. But there is little information about the pseudopores located on other regions of the body, revealing the need to map the complete distribution of pseudopores for taxonomic purposes.

The aim of the present work

As the base of the present work we have identified 14 European populations of *L. violaceus* based on the information currently available for the species, conducting morphological and molecular analysis of these populations and 25 more European *Lepidocyrtus* species (comprising the five groups of European species currently recognised). We are interested in determining whether *L. violaceus* morphospecies corresponds to a monophyletic entity. Taking into account the historical review carried out for this species, we hypothesize a non-monophyletic status for *L. violaceus*. For this purpose, we performed a phylogenetic analysis based on two molecular markers (COXII and EF-1 α).

With the aim of finding new useful characters for the delineation of the putative cryptic species within *L. violaceus* morphospecies, we have analized the distribution of pseudopores on the body of the 14 *L. violaceus* populations. We further have analised pseudopore number and distribution on the body of the other 25 studied species in order to find some pattern with clear phylogenetic signal at specific or supra-specific level.

Material and methods

Specimens

Specimens of 14 populations of *L. violaceus* from 5 European countries and another 22 described and 3 undescribed *Lepidocyrtus* species from 9 European countries have been analysed morphologically and molecularly (two species were studied only morphologically, see Table 1). For molecular analyses 46 specimens of *L. violaceus* and 61 specimens of the other species were studied (Table 2). For morphologycal analyses the skin of the sequenced specimens plus additional 40 specimens of *L. violaceus* and 65 specimens of the other species were studied.

DNA sequencing, datasets, alignment and phylogenetic tree reconstruction

The DNA was obtained with the Speedtools tissue DNA extraction kit (Biotools, Madrid, Spain), following the manufacturer's protocol. The bodies were degraded with proteinase K and the exoskeleton was preserved in 70% ethanol for morphological studies. Polymerase Chain Reaction (PCR) amplification (25 µL) was performed to obtain a ~800 bp fragment of the mitochondrial cytochrome c oxidase subunit II (COXII) gene and fragments of the first and second exon (~ 500 bp), including an intron of the nuclear EF1-α gene, using the specific primers tRNA-K-LcuJ and tRNA-L-LcuN for COXII, and EFLcuJ and EFLcuN for EF-1 α (Cicconardi et al. 2010). Annealing temperatures of 48 °C and 55 °C for COXII and EF were applied, respectively. Finally, the PCR products were purified using a vacuum manifold (Millipore, SA) and sequenced, using the same primers as in amplification, in Macrogen Europe (Amsterdam, The Netherlands). Chromatograms were revised and contigs constructed in Geneious v 10.2.6. software (Biomatters; available from http://www.geneious.com).

Three different data sets were used to infer phylogenies, 1) COXII, 2) EF-1α and 3) Concatenated dataset (with the information of the two genes concatenated). The COXII gene and the coding region of EF-1α (first and second exons) were aligned following the amino acid pattern with ClustalW tool in BioEdit 7.2.5. (Hall 1999), using the genetic code in table 5 (mitochondrial invertebrate) for the COXII gene and the standard table for EF-1α gene. The intron of EF-1α was aligned using MAFFT online version 7 (Katoh et al. 2019), and Gblocks 0.91b (Talavera & Castresana 2007) was used to eliminate ambiguously aligned regions or with too many gaps.

Version 2.1.1 of PartitionFinder2 (Lanfear et al., 2017) was run on CIPRES Science Gateway (Miller et al. 2010) to identify an appropriate partition scheme and model for

each dataset. The data were divided by gene, with unlinked branch lengths, the 'raxml' models for selection and the selection criteria under the AICc model with the 'greedy' search algorithm. The evolutionary model GTR + G was applied with 8 categories for the Gamma distribution, as indicated by the PartitionFinder results. To infer the phylogenies, two different methods were used, Maximum Likelihood (ML) and Bayesian Inference (BI). ML trees were obtained by means of the IQtree software v1.6.1 (Nguyen et al., 2015). IQtree searches were carried out using the program's default configuration, with an initial random tree (option -t RANDOM) and evaluating branch support using 10000 ultrafast bootstrap replicates (Minh et al. 2013). Following the results obtained by PartitionFinder, for the analysis of the COXII gene, the first and second position of the codon were separated from the third, however, in EF-1α all positions were left in the same partition. Each partition was allowed to have its own set of branch lengths (-sp option). To infer phylogenies with the BI method, MrBayes v3.2.6 software was used (Ronquist et al. 2012), establishing the evolutionary model and appropriate partitions according to the results of PartitionFinder with unlinked parameters. Two independent runs of four chains each produced 5 million generations and, for each of them, 5,000 trees were stored. The predetermined 25% value of burnin was used, and it was verified that the probability values (logarithm) of the cold chains reached the stationarity and convergence of the two runs, checking that the average standard deviation of the divided frequencies was lower than 0.1. A consensus tree was obtained from the remaining trees.

The FigTree v1.4.4 program (Rambaut 2009) was used to visualize the trees and the iTOL v4 web server (Letunic & Bork 2007) to prepare and generate the tree figures.

Species delimitation analysis

To confirm the specific hypotheses formulated through the morphological data, a molecular method of species delimitation, ABGD (Puillandre et al. 2012) was applied. The method was implemented through the website http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. The COXII dataset was used for this analysis, with the default values of Pmin = 0.001 and Pmax = 0.10, steps = 10 and number of intervals = 20. The value of relative gap width value (X) was set at 1.0 and was used the correction of K80 Kimura with a MinSlope of 2.

Morphological analyses

For morphological studies the specimens were mounted on slides with the head separated from the body. The specimens were cleared using Nesbitt fluid and then slide-mounted in Hoyer medium. The slides were studied under a phase contrast microscope with a digital camera attached.

Morphological and chaetotaxic characters of the dorsal and ventral regions of the antennae, head, body and appendages were analysed. As a novelty, we studied the basal plate of the fourth abdominal segment (BP4 sensu Hopkin 1997, p. 71), a region of the body that has never been explored in *Lepidocyrtus* until now.

Species identification was done consulting the published original descriptions and also the descriptions and identification keys provided by several authors (Fjellberg 2007, Hopkin 2007, Mateos 2008a, Mateos 2011).

Each species was assigned to a *Lepidocyrtus* group using the diagnostic characters of the European species-groups proposed by Mateos (2008b, 2011, 2012), Mateos and Petersen (2012), and Winkler and Traser (2012).

Nomenclature used

For the taxonomic descriptions the following terms and codes were used: For dorsal cephalic chaetotaxy the “AMS” nomenclature system (see Soto-Adames 2010). For labial palp Fjellberg (1999). For labial chaetotaxy Gisin (1964b). For interocular chaetotaxy MariMutt (1986). For dorsal chaetotaxy schemes of thoracic and abdominal segments Gisin (1967), Szeptycki (1972, 1979), Wang et al. 2003 and Mateos (2008). For C1–B4/B4–B6 relation on abd. IV Mateos (2008).

Abbreviations used

The following abbreviations have been used in morphological descriptions: Ant.—antennal segment, Th.—thoracic segment, Abd.—abdominal segment, I–VI—segment numbers, pse—pseudopore, BP4—basal plate of fourth abdominal segment, VT—ventral tube, ret—retinaculum.

Results

Molecular analyses

1 - Datasets

The COXII dataset, with a final length of 688 bp, comprised 111 sequences from which three corresponded to the selected outgroup (genera *Cyphoderus* and *Orchesella,* Table 2). The EF-1α dataset, with a final length of 679 bp, included 93 sequences (comprising the three outgroups). Finally, the concatenated dataset was composed of 110 specimens (including outgroups) with representation of both genes in almost all of them (see Table 2) with a final length of 1367 bp.

2 - European groups

In the phylogeny inferred using the concatenated dataset (Figs. 1 and 2), the five European *Lepidocyrtus* species groups were recovered with both methods used (ML and BI) as monophyletic groups with high support (only *pallidus–serbicus*-group does not have high support in either method, although it is monophyletic in both, Fig. 1). The most basal clade (Fig. 1) was *lusitanicus*-group (sensu Mateos 2008b), including *L. bilobatus* Mateos, 2008, *L. lusitanicus* Gama, 1964 and *L. selvaticus* Arbea & Ariza, 2007 specimens. The *lanuginosus*-group (sensu Mateos 2011) clade included *L. bicoloris* Mateos, 2012, *L. cyaneus* Tullberg, 1871 and *L. lanuginosus* (Gmelin, 1788) specimens. The clade *pallidus–serbicus*-group (sensu Gisin 1965; Winkler and Traser 2012) included *L. arrabonicus* Traser, 2000, *L. pallidus* Reuter, 1890 and *L. serbicus* Denis, 1933 specimens. The *curvicollis*-group (sensu Mateos and Petersen 2012) clade consisted of the *L. flexicollis* Gisin, 1965, *L. montseniensis* Mateos, 1985 and *L. paradoxus* Uzel, 1890 specimens, and formed the sister group to the clade comprising the *lignorum*-group (sensu Mateos 2011) (Fig. 2), that included *L. barbulus* Mateos, 2011, *L. chorus* Mateos & Lukić, 2019, *L. intermedius* Mateos et al. Mateos, Escuer & Álvarez-Presas, 2018, *L. juliae* Mateos, 2011, *L. lignorum* (Fabricius, 1793), *L. pulchellus* Denis, 1926, *L.* spJ, *L.* spK, *L.* spL, *L. tellecheae* Arbea & Jordana, 1990, *L. traseri* Winkler, 2016, and *L. violaceus*. The species *Lepidocyrtus* spJ, spK, and spL are still under study, but their morphological characters perfectly comply with the definition of the lignorum-group. Within this *lignorum*-group clade, the 14 *L. violaceus* populations were grouped into six main lineages (Fig. 2). These main *violaceus* lineages have been coded with capital letters

and numerical subindexes from A1 to G, each capital letter grouping specimens from nearest geographical locations.

ABGD molecular species delimitation analysis defines 39 putative entities (=molecular entities) (Figs. 1 and 2), from which 12 correspond to the *violaceus* lineages inferred in the tree (Fig. 2). The specimens morphologically identified as *L. arrabonicus* (within the *pallidus–serbicus* group, Fig. 1), *L. lignorum*, *L*. spK and *L. violaceus* (within *lignorum*-group, Fig. 2) did not form a monophyletic entity, indicating the presence of cryptic species. The monophyletic subgroup within the *lignorum*-group formed by the molecular entities *violaceus* G, *juliae*, *lignorum*-1, and spL share the synapomorphy of the presence of pseuropores on the BP4. No other studied specimens have pseudopores on this region of the body.

3 - Lepidocyrtus violaceus

Geographycal proximity appears as a key element grouping the 12 molecular entities within the six main violaceus lineages (see Fig. 3). All populations in cluster A (including molecular entities *violaceus* A1, *violaceus* A2 and *violaceus* A3) were distributed in Pyrenean localities, in Northeastern Spain. Populations in cluster B (with molecular entities *violaceus* B1, *violaceus* B2 and *violaceus* B3) were located in Western Iberian Peninsula, near the border between Spain and Portugal. Population in cluster C (including molecular entities *violaceus* C) was located also in Western Iberian Peninsula, in the vicinity of Coimbra city (Portugal). The two populations in cluster D (including molecular entities *violaceus* D) have a close distribution (a few kilometres away) in the “Serra de Prades” mountains (Spain). Population in cluster E (including molecular entities *violaceus* E) was located in Italy. Populations in cluster F (including molecular entities *violaceus* F1 and *violaceus* F2) were placed in Hungary. Population in cluster G (including molecular entities *violaceus* G) was located in Moldova.

Systematics

Family ***Entomobryidae*** Schott

Genus ***Lepidocyrtus*** Bourlet

European *Lepidocyrtus* species groups

(Figs. 1, 4 – 11, Tables 1 – 3)

We have revised the number and position of pseudopores on all *Lepidocyrtus* species included in the present work. Pseudopores were present in 33 positions including coxae, dorsal head, dorsal and ventral body, ventral antennae, dorsal manubrium and dorsal dentes. We have detected the presence of a basic common scheme of pseudopores distribution for all species, and also a diferential scheme for several European species groups. Table 3 summarizes the number of pseudopores present on each position for all studied species. In the following paragraphs the diferent positions where pseudopores have been detected are described.

On the legs, pseudopores are located on the external face of the three coxae (positions 1 – 3). On Cx.I 2 pseudopores is the common number in all species except for *L. selvaticus* (with only one). On CX.II the number of pseudopores varies between 2 and 8 depending on species and species groups, and when more than three pseudopores are present the rest have smaller size. On CX.III always 2 pseudopores are present in all studied species.

On the head, pseudopores are located in two positions of the periantennal area on the anterior end (positions 4 and 5, Fig. 4, and always in the same number: 1+1 externally to antennal basis, and 2+2 internally to the antennal basis. These 2+2 pseudopores located between antennal bases are very dificult to observe because of the stacking of chaetae that occurs in microscopic slides.

On the dorsal region of the body 1+1 pseudopores in mid dorsal position from Th.II to Abd.IV are always present (positions 6 – 11); other pseudopores on dorsal Abd.IV (position 12) were present only in one species (*L. curvicollis*).

On the furca, pseudopores are located on the dorsal region of manubrium and dentes. On the manubrium, 1+1 pseudopores in dorsobasal region (position 13, Fig. 5) and 2-3+2-3 in dorsodistal region (2-3 on each manubrial plate, position 14) are always present. On the dentes, when present, pseudopores are disposed on dorsobasal region (position 15) and, when there is more than one, they are disposed forming a row one behind the other from the base towards the apical end.

On the antennal joints I – III (positions 16 – 18), pseudopores, when present, are always located ventrally on the apical membranous area (Fig. 6), between the apical row of chaetae and the tip of the joint.

On the ventral region of the thorax 1+1 pseudopores from Th.I to Th.III (near coxae) are always present (positions 19 – 21). On the ventral region of the abdomen 1+1 pseudopores anterior to the ventral tube (position 22) and 1+1 posterior to the ventral tube (position 23) (on Abd.I) are always present; also at least 2 pseudopores on Abd.II (position 24, between ventral tube and retinaculum, Fig. 7), 2 on Abd.III (position 25, in a linear disposition posterior to retinaculum, Fig. 8), and 1 on Abd.V (position 27, anterior to genital plate, Fig. 5). On the ventral region of Abd.IV, pseudopores are only present on several species of *curvicollis*-group, located internally to BP4 (position 26).

On the lateral region of the body, pseudopores are only present on *L. mariani*, with many small (compared to dorsomedial ones) pseudopores from Th.III to Abd.IV (positions 28 – 32). On four molecular entities of *lignorum*-group pseudopores are located on the BP4 (position 33), these species are *L. juliae* with 10 pseudopores on BP4, *L. lignorum-1* with 6 pseudopores, *L.* spL with 4 – 6 pseudopores, and *L. violaceus* G with 7 pseudopores. All four mentioned species form a monophyletic group in the concatenated tree (Fig. 2).

The outgroup *Cyphoderus* cf. *bidenticulati* has pseudopores located in the same positions as *Lepidocyrtus* species (Table 3), with only two main differences: presence of 3 pseudopores located behind the posterior row of chaetae on the dorsum of Abd.IV (position not coded, see Deharveng et al. 2018), and absence of pseudopores on the manubrial base (position 13). No data available on the distribution of pseudopores in the genus *Orchesella*.

Remarks

As is shown in Table 3, several positions of the body and apendages have a fixed number of pseudopores on all studied species (positions marked with asterisk in Table 3), while other regions show within-groups and betwen-groups variability. In Figs 9 and 10 pseudopores on dorsal, lateral and ventral positions are mapped for the five European species groups of *Lepidocyrtus*, indicating the variability observed on the diferent positions.

Lusitanicus-group and *lanugiosus*-group are characterised by the absence of pseudopores on dorsal dentes and ventral Ant.II (positions 15 and 17). *curvicollis*-group is characterised by the presence of pseudopores on ventral Ant.I (position 18).

Abd.IV is a region where pseudopores can be present in different positions depending on the species or species groups. In this segment, all species have the 1+1 dorsomedial pseudopores in position 11; in dorsolateral position 12 only species *L. curvicollis* (from the *curvicollis*-group) has a group of 5 – 7 pseudopores; on ventral position 26 only the species *L. flexicollis*, *L. mariani* and *L. montseniensis* (from the *curvicollis*-group) have several pseudopores; on lateral position 32 only the species *L. mariani* (from *curvicollis*-group) has pseudopores; and on lateral position 33 the four molecular entities *L. lignorum*-1, *L. juliae*, *L. violaceus* G, and *L.* spL (from *lignorum*-group) have several pseudopores on BP4 (Fig. 11).

Lepidocyrtus violaceus (Geoffroy 1762) Lubbock 1873

(Figs. 2, 3, 12 – 15, Tables 1, 2, 4, 5)

Diagnosis

Uniform violet body color on dorsal and ventral surfaces from Th.II to Abd.IV, Abd.V–VI, head and manubrium with or without pigment, legs with pigment on coxae, hind legs also with pigment on trocanter and femur (see pictures on Fig. 2). Mesothorax slightly projected over the head. Ant.I–II, legs and dorsal side of manubrium scaled. Labium chaetotaxy M1M2REL1L2 (R half in length than M chaetae). Labral chaetae formula 4/554; prelabral chaetae ciliated; apical row of labral chaetae branched. Dorsal macrochaetae formula A0A2A3Pa5/00/0101+3. On dorsal head the short macrochaeta A2a is present in front of macrochaeta A2. On Abd.II the dorsal macrochaeta is m3, on Abd.IV dorsal macrochaetae are C1+B4, B5, B6. Ungues with paired basal teeth, one sub-equal median tooth, and a tiny apical tooth. Unguiculi lanceolate, with denticles along outer edge.

The pseudopores number and position in all the specimens studied are as follows (Table 4, Fig. 12):

Legs: 2 on Cx.I; 4 on Cx.II; 2 on Cx.III.

Head: 1+1 located anteriorly to each eye patch (dorsoexternally to Ant.I), and 2+2 located dorsointernally to each Ant.I.

Dorsal body: 1+1 on each segment form Th.II to Abd.IV (near the central axis of the segments).

Furca: 1+1 on dorsal manubrial base; 2 on each dorsal manubrial plate; 3 on each dorsal dentes base.

Antenna: 1 on ventral side of Ant.II and Ant.III, located distally on the membranous area between the chaetae of the apical row and the tip of the antennal joints.
Ventral body: 1+1 on each thoracic segment (near the base of coxae), 1+1 anteriorly to the base of the ventral tube and 1+1 posteriorly to the base of the ventral tube, 3 – 4 forming a triangle or a rectengle between ventral tube and retinaculum, 2 in a line posterior to retinaculum, and 1 anterior to genital plate on Abd.V.
Lateral body: Lateral pseudopores only presents in population *L. violaceus*_392, with 7 pseudopores located on BP4.

Remarks: Intrapopulational and intraindividual variability

Although all the specimens labelled as *L. violaceus* match the species diagnosis, variability in the presence/absence of some chaetae or pseudopores has been detected in some populations. Also, bilateral asymmetries in several specimens have been observed. This variability affects the interocular chaeta q, Abd.II chaetae ml and p5p, Abd.III chaetae mi and d3, Abd.IV ratio B4-B5/B5-B6, development of macrochaeta B6 on Abd.IV, and presence/absence of pseudopores on BP4. This variability is summarized in Table 5 and described in the following paragraphs.
- Interocular chaeta q (Fig. 13) is absent in all specimens, but in the population *L.violaceus*_539 two specimens have asymmetric interocular chaetotaxy, with chaeta q present only on one ocular area.
- Abd.II chaeta ml (associated with trichobothrium m2, Fig. 14-a) is absent in ten studied populations, present in two populations, and present or absent in two populations. On population *L.violaceus*_457 one specimen has asymmetric chaetotaxy with ml present only on one side of the body.
- Abd.II chaeta p5p (Fig. 14-b) is present in four populations, absent in eight populations, and present or absent in two populations. In population *L.violaceus*_539 three specimens have asymmetric chaetotaxy with p5p present only on one side of the body.
- Abd.III chaeta mi (associated with trichobothrium m2) is present in two populations, absent in nine populations, and present or absent in three populations. In population *L.violaceus*_539 one specimen has asymmetric chaetotaxy with chaeta mi present only on one side of the body.
- Abd.III chaeta d3 (Fig. 15) is present in ten populations and absent in four populations.

- On Abd.IV the ratio B4-B5/B5-B6 is <1.6 in six populations (inner rectangle in Fig. 15) and >2 in eight populations (outer rectangle in Fig. 15). In the specimens with ratio <1.6 Abd.IV macrochaetae B4, B5 and B6 are of the same shape (broad ciliated macrochaetae), while in specimens with ratio >2 Abd.IV chaeta B6 is a thin ciliated macrochaeta, with a reduced socket respect to macrochaetae B4 and B5. Gisin (1964a) described the species *L. violaceus* indicating that mactrochaetae B5 and B6 on Abd.IV are close to each other, and B6 is thinner and acumuninate.
- BP4 pseudopores are only present in population *L. violaceus*_392, with seven pseudopores on each side of the body (Fig. 12).

With this variability observed in the 14 populations of *L. violaceus* it is not possible to diagnose the 12 molecular entities detected by the ABGD analysis. Only the presence of pseudopores on BP4 in the speciemen *L. violaceus*_392, defined as molecular entities *violaceus* G, can be considered a diagnostic character defining this species.

General discussion

Subgenera and species groups in European Lepidocyrtus

The species analysed in the present study cover all the five European species groups of *Lepidocyrtus* currently recognized. Also, the two subgenera of *Lepidocyrtus* present in Europe are represented, *Lanocyrtus* Yoshii & Suhardjono, 1989 and *Lepidocyrtus* s. str. Yosii, 1959. As in Mateos et al. (2018a), our results suggest that subgenus *Lanocyrtus* (including species groups *lusitanicus*, *lanuginosus* and *pallidus–serbicus*) is a non-monophyletic group, whereas *Lepidocyrtus* s. str. subgenus (including species groups *lignorum* and *curvicollis* as sister groups) is a monophyletic group. The presence of scales on antennae and legs beyond coxae are the main morphologycal characters diagnosing the species groups included in *Lepidocyrtus* s. str. subgenus, and within this subgenus, *curvicollis*-group is characterizaed by the presence of chaeta “s” on Abd.IV (Mateos et al. 2018a). Our results suggest that the presence of pseudopores on the ventral apical region of Ant.I is another diagnostic character of the species included in the *curvicollis*-group.

About pseudopores in Lepidocyrtus *and other Entomobryoidea*

We strongly agree with the comment of Deharveng et al. (2018) stating that "*Evidence is therefore increasing that these tiny pseudopores have diversified distribution patterns that provide (or may provide) powerful characters at all taxonomic levels*." But published information dealing with pseudopores number and position in Entomobryoidea are scarce, and in no case the complete mapping of these structures has been described for any species.

With the current published information, it is not possible to know the stability of the number and position of pseudopores in *Lepidocyrtus* (and in Collembola in general) during postembrionic development. For example, in *Lepidocyrtus mariani* Mateos & Winkler (2018) found that the number of small dorsolateral pseudopores located on Th.III to Abd.IV is variable depending on the size of the specimen. The information currently available about pseudopores in *Lepidocyrtus* and other Entomobryoidea is presented below.

Legs: Coxal pseudopores are known in Collembola since Gisin (1963), who described the presence of this structure on Cx.I – II in several genera of Entomobryidae, including *Lepidocyrtus*. Subsequently, pseudopores on Cx.I – III have been described in many Entomobryoidea genera: *Acanthocyrtus* (Cipola et al. 2018a), *Amazhomidia* (Cipola et al. 2016a), *Campylothorax* (Santos et al. 2016, Bellini & Cipola 2017), *Coecobrya* (Cipola & Bellini 2016, Jantarit et al. 2019), *Cyphoderus* (Nunes & Bellini 2018), Entomobrya (Ma & Shi 2018), *Heteromurus* (Lukić et al. 2015), *Homidia* (Pan et al. 2015), *Lepidocyrtoides* (Cipola et al. 2017), *Lepidonella* (Deharveng et al. 2018), *Troglobius* (Cipola et al. 2016b), *Trogolaphysa* (Bellini & Cipola 2017, Nunes & Bellini 2018), *Seira* (Cipola et al. 2018b), and *Tyrannoseira* (Cipola et al. 2019). In all cited genera Cx.II is where the number of pseudopores is more varible, as is the case described in the present work for position 2.

Head: Hüther (1986) described in *Lepidocyrtus* the presence of the "antennobasal-organ" as a "*small deep grove seeing with the light microscope*" and located on the external face of each Ant.I base. In our opinion this "antennobasal-organ" corresponds to the pseudopore described for *Lepidonella doveri* by Deharveng et al. (2018) as those "*located externally to antennal basis on head in the periantennal area*". This structure represents the 1+1 dorsal head pseudopores, located externally

to antennal basis on the periantennal area, described in the present work in position 4.

Winkler & Traser (2012) described the presence of two pseudopores on the inner side of each antennal base in *Lepidocyrtus tomosvaryi*; the same number and position of pseudopores have been described by Winkler (2016, 2017) for *L. traseri* and *L. isabellae* respectively, and by Mateos & Winkler (2018) for *L. florae*. These pseudopores correspond to the 2+2 anterointernal pseudopores located on the periantennal area described in the present work in position 5 for all studied *Lepidocyrtus* species. Jantarit et al. (2014) described in *Cyphoderus* (and they say "*for the first time in Collembola*") the presence of 1 or 2+2 pseudopores on head anteriorly to the antenno-basal line (position 5), and Jantarit et al. (2019) described the presence of 1+1 pseudopores on this position in *Coecobrya sirindhornae*.

Dorsal body: In *Lepidocyrtus* (and all Entomobryoidea) 1+1 dorsal pseudopores from Th.II to Abd.IV are always present, and represent those described from position 6 to position 11 in the present work. Pseudopores located on other positions of dorsal body in the genus have been only described for *L. curvicollis* (see Mateos 2008a), with its characteristic 5–7+5–7 pseudopores on dorsal Abd.IV in position 12.

Cipola et al. (2018b) described the presence of 10 pseudopores on the anterior region of dorsal Abd.IV in *Seira baetica*, and several pseudopores on the dorsolateral region of Th.III – Abd.III (with 10, 11, 3 and 12 pseudopores on each segment respectively) in *Seira burgersi*; the authors stated that the presence of pseudopores on anterior and dorsolateral regions of thoracic and abdominal segments is an exclusive feature registered for the first time in *Seira*. Deharveng et al. (2018) indicated that the presence of several pseudopores behind the posterior row of chaetae on the dorsum of Abd.IV is a synapomorphy of the group formed by Troglopedetinae + Cyphoderidae, separating them from other Paronellidae and Entomobryidae.

Furca: The presence of pseudopores on dorsobasal position of manubrium in the genus *Lepidocyrtus* has been only described by Winkler & Traser (2012), who noted the presence of 3 pseudopores (forming a triangle) on this position in species *L. tomosvaryi*. Likewise, Deharveng et al. (2018) described the same number and position of pseudopores in dorsobasal region on manubrium (3, forming a triangle near the genital plate) in *Lepidonella doveri*. We consider that these 3 pseudopores

forming a triangle described for *L. tomosvaryi* and *L. doveri* are actually 1+1 on dorsobasal manubrium (position 13) and 1 (odd) on ventral Abd.V located anterior to genital plate (position 27, see Fig. 5). Also in *Coecobrya xui* the presence of 2 pseudopores has been described on dorsobasal position of manubrium on position 13 (Zhang & Dong 2014).

The presence of pseudopores on the manubrial plate (dorsodistal region of manubrium, position 14) in *Lepidocyrtus* is largelly described for all species since its detection by Gisin (1964a). In all Entomobryidae species the usual number of pseudopores in this position is 2–3+2–3, but in *Verhoeffiella* 4–14 has been described as a usual number (Lukić et al. 2015). This high number of pseudopores on the apical dorsal región of manubrium is considered taxonomically important by Lukić et al. (2018).

In *Tomocerus* the presence of a large number of dorsal pseudopores (up to 27+27) on each side of the manubrium seems to be a general rule (see Yu et al. 2014). Pseudopores on this area of the manubrium have not been detected so far in other Entomobryiomorpha.

In *Lepidocyrtus* the presence of pseudopores on the basal region of dorsal dentes (position 15) has been only described by Winkler & Mateos (2018) in the especies *L. mariani*. Deharveng et al. (2018) also cited the presence of pseudopores on this position, saying verbatim "*new location, observed in many Entomobryidae*", but without indicating any specific case.

Ventral body: In *Lepidocyrtus* the presence of pseudopores on the ventral region of the body has been described only in a few species. Deharveng et al. (2018) cited that the presence of pseudopores between tenaculum and ventral tube has been observed in *Lepidocyrtus* sp. and *Cyphoderus* sp., but without indicating any specific case. For *Lepidocyrtus mateosi* Cipola et al. (2018c) described the presence of 1+1 basal pseudopores on the anterior región of the corpus of the VT. On other Entomobryoidea pseudopores on the corpus of VT have been described for *Tyrannoseira* as 1+1 on the base of the anterior region (Cipola et al. 2019), and for *Sinella abietis* as 1+1 on the middle of the anterior part (Ding & Zhang 2015). On other ventral regions of the body pseudopores have never been formally described for any Entomobryoidea. So the presence in *Lepidocyrtus* of 1+1 pseudopores on the ventral region of Th.I to Th.III on positions 19 to 21 (near the coxae bases), 1+1 on positions 22 and 23 (anterior and posterior base of VT), 3 – 5 on postition 24

(between the base of VT and retinaculum), a pair (forming a line) on position 25 (posterior to retinaculum), and several on position 26 (ventral Abd.IV) on several species of the *curvicollis*-group, are formally described for the first time in Collembola in the present work.

Antenna: In *Lepidocyrtus* the presence of pseudopores on the ventral region of Ant.I – III joints has only been described for the species *L. mariani* (Winkler & Mateos 2018). These pseudopores are located distally on the membranous area between the tip of the antennal joints and the chaetae of the apical row (positions 16, 17 and 18), and their presence on all *Lepidocyrtus* species is stated for the first time in the present work.

Also the presence of ventral antennal pseudopores has been described on Ant.I – III for the genera *Coecobrya* (Jantarit et al. 2019), *Dicranocentrus* (Ren et al. 2018) and *Verhoeffiella* (Lukić et al. 2015, 2018), on Ant.II – III for genera *Cyphoderus* (Jantarit et al. 2014) and *Lepidonella* (Deharveng et al. 2018), and on Ant.III for the genus *Paronella* (Barra 1969). The highest number of pseudopores on antennal joints is found in *Verhoeffiella* (Lukićet al. 2018), specially on Ant.II with a maximum of 15.

Lateral boby: In genus *Lepidocyrtus* the presence of pseudopores on the lateral regions of the body has been described in a few ocasions. Mateos & Greenslade (2015) described the presence of two pseudopores on lateral Abd.IV (along the suture tergum-BP4, position 32) for several species of *Setogaster* subgenus. Cipola et al. (2018c) described the presence of dorsolateral pseudopores on Th.II–III (2 pseudopores on each segment) and Abd.III (5 pseudopores) in *Lepidocyrtus amazonicus*. Winkler & Mateos (2018) described the presence of numerous small pseudopores on dorsolateral regions of Th.III to Abd.IV (positions 28 to 32) in *Lepidocyrtus mariani*. For other Entomobryoidea only Deharveng et al. (2018) described the presence of pseudopores on lateral Abd.IV along the suture tergum-BP4 in *Lepidonella doveri*. The presence of lateral pseudopores on the BP4 (position 33), found in four *Lepidocyrtus* populations from the lignorum-group in the present work, is here described for the first time for Entomobryoidea. Interestingly, these populations with pseudopores on BP4 (*L. violaceus* G, *L. juliae*, *L.* spL and *L. lignorum*-1) are grouped forming a monophyletic group in the concatenated phylogeny (Fig. 2), suggesting a phylogenetic signal for the presence of these pseudopores.

Cryptic species

The 14 populations of *L. violaceus* studied perfectly comply with the currently morphologycal diagnosis proposed for this species. But within these morphospecies, phylogenetic analyses showed six (non-sister groups) main clusters, and ABGD analysis showed twelve putative molecular (cryptic) entities. The morphological study has not detected any character that allows a differential diagnosis for each of these main clusters nor for the putative molecular entities. Only the presence of pseudopores on BP4 in specimen L. violaceus_392_2 is a diferential character for the molecular entities *L. violaceus* G (Figure 2). This scenario is congruent with the presence of 6 to 12 cryptic species within the European morphospecies *L. violaceus*. The existence of distinct lineages/cryptic species in the genus *Lepidocyrtus* has been detected in several studies using molecular data (Cicconardi et al. 2010; 2013; Mateos et al. 2018; Zhang et al. 2018a; 2019a), and also other studies suggested their existence in several groups of Collembola (Katz et al. 2015; Anslan and Tedersoo 2015; Nilsai et al. 2017; Zhang et al. 2018b). Results of all these studies (and the present study) suggest that sampling additional locations and/or habitats would reveal even more lineages/cryptic species in Collembola. With the data presented in this work, it is not possible to diagnose any of the molecular entities as new species, which would have to be analysed more deeply at the morphological level. We can consider, then, such molecular entities as Unconfirmed Candidate Species (UCS).

Conclusions

Molecular data reveal that the worldwide distributed *Lepidocyrtus violaceus* morphospecies is a polyphiletic entity. Between six and twelve diferent cryptic species have been detected in Europe under the appeareance of this morphospecies. This indicates that the true diversity of Collembola can be strongly underestimated if only traditional morphological characters (including body colour) are used for the identification of the species. The results obtained in the present paper suggest that the number and position of pseudopores on the body and appendages are promising useful diagnostic characters for species or species-groups identification in European *Lepidocyrtus*. Species of the *lusitanicus*-group and

lanuginosus-group, in a phylogenetic basal position within the European *Lepidocyrtus*, have the most basic scheme of pseudopores distribution, indicating an increase in pseudopores number in the evolutive line of the genus *Lepidocyrtus* in Europe. Our results suggest that the current richness of Collembola species in Europe is underestimated.

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Conflicts of Interest

The authors declare no conflicts of interest.

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965

966 **Tables**

967

968 Table 1. Localities of the studied species. Abb: abbreviations of species names used
969 on other sections of the manuscript. * species studied only morphologically.

970

971 Table 2. List of sequences used for the molecular phylogenetic inferences and the
972 molecular species delimitation analyses with GenBank Accession numbers.

973

974 Table 3. Number of pseudopores on each position for all species studied. For each
975 position the pseudopores number on one side of the body is indicated, except
976 numbers between parentheses which correspond to odd groups of pseudopores
977 located at the centre of the segments involved. Pos, position number. lu, lusitanicus-
978 group; la, lanuginosus-group; pa, pallidus–serbicus-group; li, lignorum-group; cu,
979 curvicollis-group. *, same pseudopores number for all species in this position. #1,
980 including species: bic+cya+lan. #2, including species: cho+lig1+pul+spJ+spK+tra.
981 See Table 1 for explanation of abbreviatures used for each species.

982

983 Table 4. *Lepidocyrtus violaceus*. Number of pseudopores on each position for each
984 population studied. Pos, position number. #: including populations A1 + A3 + B1 +
985 B2 + B3 + C + D + F1 + F2. See Table 1 for explanation of abbreviatures used for
986 each species.

987

988 Table 5. *Lepidocyrtus violaceus*. Differential characters between populations. ABGD,
989 molecular entities detected by the ABGD delimitation analysis. N, number of
990 specimens morphologically studied. Size, body size (without head) in mm. Ocular-q,
991 presence or absence of interocular chaeta q. Abd.II-ml, presence or absence of
992 chaeta ml on Abd.II. Abd.II-p5p, presence or absence of chaeta p5p on Abd.II.
993 Abd.III-mi, presence or absence of chaeta mi on Abd.III. Abd.III-d3, presence or
994 absence of chaeta d3 on Abd.III. B4-B5/B5-B6, ratio between distances of
995 macrochaetae B4 to B5 and B5 to B6 on Abd.IV. Abd.IV-B6, shape of macrochaeta
996 B6 on Abd.IV, bcm = broad ciliated macrochaeta, tcm = thin ciliated
997 macrochaeta.BP4-pse, presence (+) or absence (-) of pseudopores on BP4. In all
998 cases: +=present, -=absent, -/+=absent or present depending on the specimen, (-
999 /+)=absent or present as intraindividual asymmetry.

1000

Figures

Figure 1. Tree inferred with the Concatenated dataset using Bayesian Inference. Numbers at nodes correspond to Posterior Probability (PP) over the branch and bootstrap values obtained in the ML inference, under the branch. Clade "*lignorum* group" is detailed in Figure 2. Tree scale indicates number of substitutions per site. Outgroups correspond to the genera *Orchesella* and *Cyphoderus* gr. *bidenticulati*. To the right of the tree, the color bars and associated labels indicate the molecular entities inferred with the ABGD species delimitation analysis.

Figure 2. Clade "*lignorum* group". Tree inferred with the Concatenated dataset using Bayesian Inference. Numbers at nodes correspond to Posterior Probability (PP) over the branch and bootstrap values obtained in the ML inference, under the branch. Tree scale indicates number of substitutions per site. To the right of the tree, the color bars and associated labels indicate the molecular entities inferred with the ABGD species delimitation analysis. The pictures show the habitus of the diferent molecular entities of *Lepidocyrtus violaceus* predicted with the ABGD analysis.

Figure 3. *Lepidocyrtus violaceus*. Map of Europe showing the localities (black dots) where specimens were collected. See Table 1 for the explanation of LP### sample codes.

Figure 4. *Lepidocyrtus* sp. Dorsal view of the head. Black arrows point to pseudopores (black dots) on positions 4 and 5.

Figure 5. *Lepidocyrtus* sp. Basal portion of the anterior region of manubrium and ventral region of abdominal segments V – VI. (a) female, (b) male. The two superior arrows point to pseudopores located on manubrium (position 13). The inferior arrow points to a pseudopore located on Abd.V in front of the genital plate (position 27).

Figure 6. *Lepidocyrtus* sp. Apex of ventral region of second antennal joint. The white arrow points to a pseudopore on position 17.

Figure 7. *Lepidocyrtus* sp. Ventral view of abdominal segments I – III. Pseudopores on positions 23 and 24 are inside the white circles.

Figure 8. *Lepidocyrtus* sp. Ventral view of the third abdominal segment showing the retinaculum. The white arrows point to pseudopores on position 25.

Figure 9. *Lepidocyrtus* sp. Distribution of pseudopores on dorsal positions. Black dots are pseudopores common to all species. Arrows point to dorsal positions where pseudopores vary between species-groups *lusitanicus* (lu), *lanuginosus* (la), *pallidus* (pa), *lignorum* (li) and *curvicollis* (cu). Under each species-group abbreviation the number of pseudopores on each position is indicated.

Figure 10. *Lepidocyrtus* sp. Distribution of pseudopores on ventral+lateral positions. Black dots are pseudopores common to all species. Arrows point to ventral and lateral positions where pseudopores vary between species-groups *lusitanicus* (lu), *lanuginosus* (la), *pallidus* (pa), *lignorum* (li) and *curvicollis* (cu). Under each species-group abbreviation the number of pseudopores on each position is indicated.

Figure 11. *Lepidocyrtus* sp. lateral habitus. Shaded areas are the positions where pseudopores can be located on Abd.IV. Numbers refer to positions (see Table 3). Dashed arrow indicates that position 26 is located on the ventral region of the segment.

Figure 12. *Lepidocyrtus violaceus*. Distribution of pseudopores (black dots) on dorsal (left image) and ventral+lateral postions.

Figure 13. *Lepidocyrtus violaceus*. Right ocular area. A – G eyes. The arrow points to chaeta q, that is present or absent depending on the specimens.

Figure 14. *Lepidocyrtus violaceus*. Dorsal scheme of (a) Abd.II and (b) Abd. III chaetotaxy (left side). Arrows point to chaetae that are present or absent depending on the specimens.

Figure 15. *Lepidocyrtus violaceus*. Dorsal scheme of abd.IV chaetotaxy (left side). Dashed rectangle delimits chaetae B4, B5 and B6 on specimens with ratio B4-B5/B5-B6<2 (a) and ratio B4-B5/B5-B6>2 (b). Large black dots indicate broad ciliated macrochaetae, small black dots indicate thin ciliated macrochaetae.

Table 1.

Sample code	Genus	Species	Abb	Municipality	Province/County	Country	Position (WGS84)	Collecting date
LP325	*Lepidocyrtus*	*arrabonicus*	arr	Morasti	Brașov	Romania	N45.2245 E24.5019	01.iv.2013
LP239	*Lepidocyrtus*	*barbulus*	bar	Kakopetros	Creta	Greece	N35.408150 E23.755342	07.iv.2009
LP190	*Lepidocyrtus*	*bicoloris*	bic	Cabrils	Barcelona	Spain	N41.54661 E2.36532	28.xi.2007
LP175	*Lepidocyrtus*	*bilobatus*	bil	Sotoserrano	Salamanca	Spain	N40.4110 W6.0535	02.viii.2007
LP383	*Lepidocyrtus*	*chorus*	cho	Sibenick	Miljacka	Croatia	N44.001145 E16.018398	29.iv.2015
*LP580	*Lepidocyrtus*	*curvicollis*	cur	St Laurent de Minier	Gard	France	N43.93823 E3.67255	30.vii.1991
LP277	*Lepidocyrtus*	*cyaneus*	cya	Torla	Huesca	Spain	N42.6840719 W0.1118863	31.v.2009
LP223	*Lepidocyrtus*	*flexicollis*	fle	Alcalá de los Gazules	Cádiz	Spain	N36.5222285 W5.6501549	28.ii.2009
LP114	*Lepidocyrtus*	*intermedius*	int	Aiguafreda	Barcelona	Spain	N41.7999 E2.3007	10.ii.2007
LP229	*Lepidocyrtus*	*juliae*	jul	Georgioupoli	Creta	Greece	N35.360540 E24.251808	07.iv.2009
LP130	*Lepidocyrtus*	*lanuginosus*	lan	Vallgorguina	Barcelona	Spain	N41.6583 E2.5199	18.iv.2007
LP118	*Lepidocyrtus*	*lignorum*	lig1	Barcelona	Barcelona	Spain	N41.448950 E2.140780	18.ii.2007
LP266	*Lepidocyrtus*	*lignorum*	lig2	Drios	Paros	Greece	N37.000678 E25.197013	10.iv.2009
LP100	*Lepidocyrtus*	*lusitanicus*	lus	Poblet	Tarragona	Spain	N41.35677 E1.07869	03.i.2007
*LP455	*Lepidocyrtus*	*mariani*	mar	Nagykapornak	Zala	Hungary	N46°50'35" E16°58'26"	15-vii.2015
LP129	*Lepidocyrtus*	*montseniensis*	mon	Vallgorguina	Barcelona	Spain	N41.6583 E2.5199	18.iv.2007
LP362	*Lepidocyrtus*	*pallidus*	pal	Tjöme	Vestfold	Norway	N59.1521 E10.4321	06.iv.2014
LP328	*Lepidocyrtus*	*paradoxus*	par	Chisinau	Moldova	Moldova	N47.0354 E28.7981	20.iii.2013
LP389	*Lepidocyrtus*	*pulchellus*	pul	Varese Ligure	La Spezia	Italy	44°22'48.53"N, 9°30'40.63"E	04.v.2015
LP195	*Lepidocyrtus*	*selvaticus*	sel	Tossa de Mar	Girona	Spain	N41.71908 E2.90280	21.ii.2008
LP234	*Lepidocyrtus*	*serbicus*	ser	Kalamafka	Creta	Greece	N35.080339 E25.650831	06.iv.2009
LP125	*Lepidocyrtus*	*spJ*	spj	Sant Celoni	Barcelona	Spain	N41.66449 E2.56279	18.iv.2007
LP108	*Lepidocyrtus*	*spK*	spk	Aiguafreda	Barcelona	Spain	N41.7892 E2.3113	10.ii.2007
LP122	*Lepidocyrtus*	*spK*	spk	Sinarcas	Valencia	Spain	N39.765328 W1.226981	02.iv.2007
LP250	*Lepidocyrtus*	*spL*	spl	Laerma	Rodas	Greece	N36.146639 E27.920374	04.iv.2009

LP106	*Lepidocyrtus*	*tellecheae*	tel	Aiguafreda	Barcelona	Spain	N41.7705 E2.2722	10.ii.2007
LP546	*Lepidocyrtus*	*traseri*	tra	Nagybörzsöny	Pest	Hungary	N 47º53'54" E18º51'10"	05.10.2017
LP061	*Lepidocyrtus*	*violaceus*	vio	Huesca	Huesca	Spain	N42.599770 E0.756810	19.vii.2006
LP065	*Lepidocyrtus*	*violaceus*	vio	Vall d'Aran	Lleida	Spain	N42.777980 E0.834220	19.vii.2006
LP068	*Lepidocyrtus*	*violaceus*	vio	Caldes de Boi	Lleida	Spain	N42.553490 E0.832250	20.vii.2006
LP076	*Lepidocyrtus*	*violaceus*	vio	Vall d'Aran	Lleida	Spain	N42.628210 E0.762160	20.vii.2006
LP096	*Lepidocyrtus*	*violaceus*	vio	Pontedeume	A Coruña	Spain	N43.417185 W8.063563	11.xi.2006
LP101	*Lepidocyrtus*	*violaceus*	vio	Cogullons	Tarragona	Spain	N41.334020 E1.077590	03.i.2007
LP157	*Lepidocyrtus*	*violaceus*	vio	Gerês	Braga	Portugal	N41.7507 W8.1528	14.vii.2007
LP159	*Lepidocyrtus*	*violaceus*	vio	Coimbra	Coimbra	Portugal	N40.2092 W8.4191	19.vii./2007
LP392	*Lepidocyrtus*	*violaceus*	vio	Bugeac	Gagaucia	Moldova	N46.36606 E28.6757	20.iv.2013
LP457	*Lepidocyrtus*	*violaceus*	vio	Villaggio San Michelle	Trentino-Alto Adigio	Italy	45°42'15.73"N, 10°58'23.94"E	04.v.2015
LP539	*Lepidocyrtus*	*violaceus*	vio	Poblet	Tarragona	Spain	N41.35677 E1.07869	28.xii.2017
LP543	*Lepidocyrtus*	*violaceus*	vio	Csáfordjánosfa	Győr-Moson-Sopron	Hungary	N 47°24'45" E 16°57'52"	15.iv.2017
LP544	*Lepidocyrtus*	*violaceus*	vio	Nagymaros	Pest	Hungary	N 47º46'34" E18º55'42"	11.iv.2017
LP545	*Lepidocyrtus*	*violaceus*	vio	Sopron	Győr-Moson-Sopron	Hungary	N 47º40'39" E16º27'35"	10.xi.2018
LP440	*Cyphoderus*	*gr.bidenticulati*	Cyp	Cellers	Lleida	Spain	N42º03'41.4" E0º54'04.7"	01.xii.2015
Orches	*Orchsella*	*sp*	–	–	–	–	–	–

Table 2.

		GenBank Accession Number	
Species	**Code**	**COII**	**EF-1α**
Lepidocyrtus arrabonicus	LP325_1	MF095510	-
	LP325_3	MF095512	MF095603
Lepidocyrtus barbulus	LP239_1	MF095498	MF095594
	LP239_2	MF095499	MF095595
Lepidocyrtus bicoloris	LP190_3	MF095482	MF095584
	LP190_4	MF095483	MF095585
Lepidocyrtus bilobatus	LP175_1	MF095533	-
	LP175_2	MF095532	MF095615
Lepidocyrtus chorus	LP383_1	MF095522	MF095609
	LP383_2	MF095523	-
Lepidocyrtus cyaneus	LP277_1	MF095506	MF095600
	LP277_2	MF095507	MF095601
Lepidocyrtus flexicolis	LP223_1	MF095485	MF095587
	LP223_2	MF095486	MF095588
Lepidocyrtus intermedius	LP114_1	MF095458	-
	LP114_2	MF095459	MF095569
Lepidocyrtus juliae	LP229_1	MF095490	MF095590
	LP229_2	MF095491	-
Lepidocyrtus lanuginosus	LP130_1	MF095474	MF095579
	LP130_2	MF095475	MF095574
Lepidocyrtus lignorum	LP118_1	MF095465	MF095570
	LP118_2	MF095466	*
	LP266_1	MF095503	MF095597
Lepidocyrtus lusitanicus	LP100_1	MF095537	MF095614
	LP100_2	MF095540	-
Lepidocyrtus montseniensis	LP129_1	MF095470	MF095571
	LP129_2	MF095471	MF095572
Lepidocyrtus pallidus	LP362_3	MF095519	MF095608
	LP362_4	MF095520	-
Lepidocyrtus paradoxus	LP328_1	MF095514	MF095605
	LP328_2	MF095515	MF095606
Lepidocyrtus pulchellus	LP389_7	*	-
	LP389_8	*	*
Lepidocyrtus selvaticus	LP195_1	MF095542	MF095616
	LP195_2	MF095544	-
Lepidocyrtus serbicus	LP234_3	MF095495	MF095591
	LP234_4	MF095496	MF095592
Lepidocyrtus tellecheae	LP106_2	MF095529	MF095564
	LP106_3	MF095530	MF095565
Lepidocyrtus traseri	LP546_1	*	*
	LP546_2	*	*
	LP546_3	*	*
Lepidocyrtus violaceus	LP061_1	*	*
	LP061_2	*	*
	LP061_3	*	*
	LP061_4	*	*
	LP061_5	*	*
	LP065_1	MF095436	MF095558

	LP065_2	*	*
	LP068_1	*	-
	LP068_2	*	*
	LP068_3	*	*
	LP068_4	*	*
	LP076_4	*	-
	LP076_5	*	*
	LP076_6	*	-
	LP076_7	*	*
	LP096_1	*	*
	LP096_2	*	*
	LP096_3	*	*
	LP096_4	*	*
	LP096_5	*	*
	LP101_3	*	-
	LP101_4	*	*
	LP157_1	*	*
	LP157_2	*	*
	LP157_3	*	*
	LP157_4	*	*
	LP157_5	*	*
	LP159_1	*	*
	LP159_2	*	*
	LP159_3	*	*
	LP159_4	*	*
	LP392_2	*	-
	LP457_4	*	*
	LP457_5	*	*
	LP539_5	*	*
	LP539_6	*	*
	LP539_7	*	-
	LP539_8	*	*
	LP543_1	*	*
	LP543_3	*	*
	LP544_1	*	*
	LP544_2	*	*
	LP544_3	*	*
	LP545_1	*	*
	LP545_2	*	*
	LP545_3	*	*
Lepidocyrtus spJ	LP125_1	*	*
	LP125_2	*	*
	LP125_3	*	*
	LP125_4	*	*
	LP125_5	*	*
Lepidocyrtus spK	LP108_1	*	*
	LP108_2	*	*
	LP108_3	*	*
	LP108_4	*	*
	LP108_5	*	*
	LP122_1	*	*

	LP122_2	*	*
	LP122_3	*	*
	LP122_4	*	*
	LP122_5	*	*
Lepidocyrtus spL	LP250_4	*	-
	LP250_5	*	*
	LP250_6	*	-
	LP250_7	*	-
OUTGROUP			
Orchesella sp.	Orches	EU016195	AY305473
Cyphoderus gr bidenticulati	LP440_1	MF095526	MF095612
	LP440_2	MF095527	MF095613

Table 3.

Body region	Position	Pos	lu bil+lus	lu sel	la #1	pa arr+pal	pa ser	li bar	li #2	li int	li jul	li lig2	li spL	li tel	li vio	cu cur	cu fle	cu mar	cu mon	cu par	Cyp
Legs	Cx.I	1	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Cx.II	2	2	2	2	2	2	3-5 [a]	4	2	3	3	4	6	4	5	4	5-8 [a]	3	4	2
	*Cx.III	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Head dorsal	*Cephalic - exterior	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Cephalic - interior	5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1-2
Body dorsal	*Th.II (mid dorsal)	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Th.III (mid dorsal)	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Abd.I (mid dorsal)	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Abd.II (mid dorsal)	9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Abd.III (mid dorsal)	10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Abd.IV (mid dorsal)	11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Abd.IV (dorsal)	12	-	-	-	-	-	-	-	-	-	-	-	-	-	5-7	-	-	-	-	3[c]
Furca dorsal	*Manubrial base	13	2	2	2	2	2	2	2	2	2	2	2	2	2	?	2	2	2	2	-
	Manubrial plate	14	2	2	2	2	2	2	2	2	2	2	2	2	2	?	2	3	2	2	2
	Dentes	15	-	-	-	1	1	2-4 [a]	2	2	1	1	2	3	2	11	3-5	11-17 [a]	2	5	-
Antenna ventral	Ant.III	16	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	5	1	1	1
	Ant.II	17	-	-	-	1	1	1	1	1	1	1	1	1	1	4	2	7	1-2	3	1
	Ant.I	18	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	5	1-2	2	-
Body ventral	*Th.I	19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Th.II	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Th.III	21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Abd.I - VT base anterior	22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Abd.I - VT base posterior	23	1	1	1	1	1	1	1	1	1	1	1	1	1	¿	1	1	1	1	1
	Abd.II	24	(3)	(3)	(2)	(3)	(3-5)	(5)	(3)	(3)	(3)	(3)	(3)	(3)	(3-4)	(?)	(3)	(3)	(3-4)	(3)	(4)
	*Abd.III - post ret	25	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
	Abd.IV	26	-	-	-	-		-	-	-	-	-	-	-	-	-	6-10 [a]	8	2	-	-
	*Abd.V – pre genital plate	27	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Body lateral	Th.III	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-
	Abd.I	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-
	Abd.II	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-
	Abd.III	31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-
	Abd.IV	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34	-	-	-
	BP4	33	-	-	-	-	-	-	-	-	10	6	6	-	0-7[b]	-	-	-	-	-	-

[a] the number of pseudopores on this position depends on the specimen size.

[b]only one population of morphological species *L. violaceus* has pseudopores on this position.

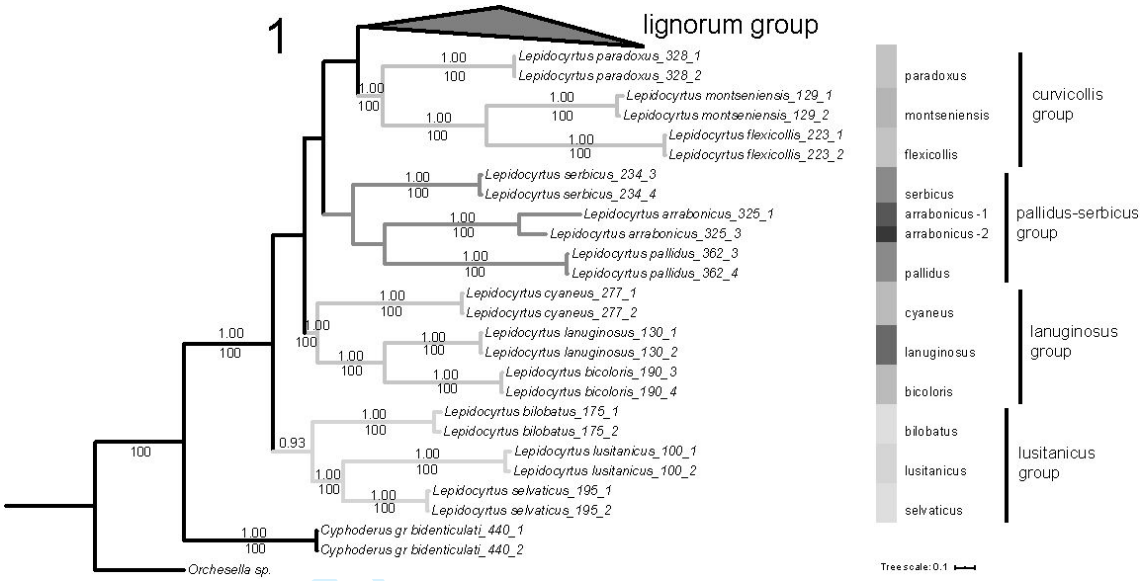
[c] in *Cyphoderus* these pseudopores are not on position 12 but on the basal part of dorsal Abd.IV.

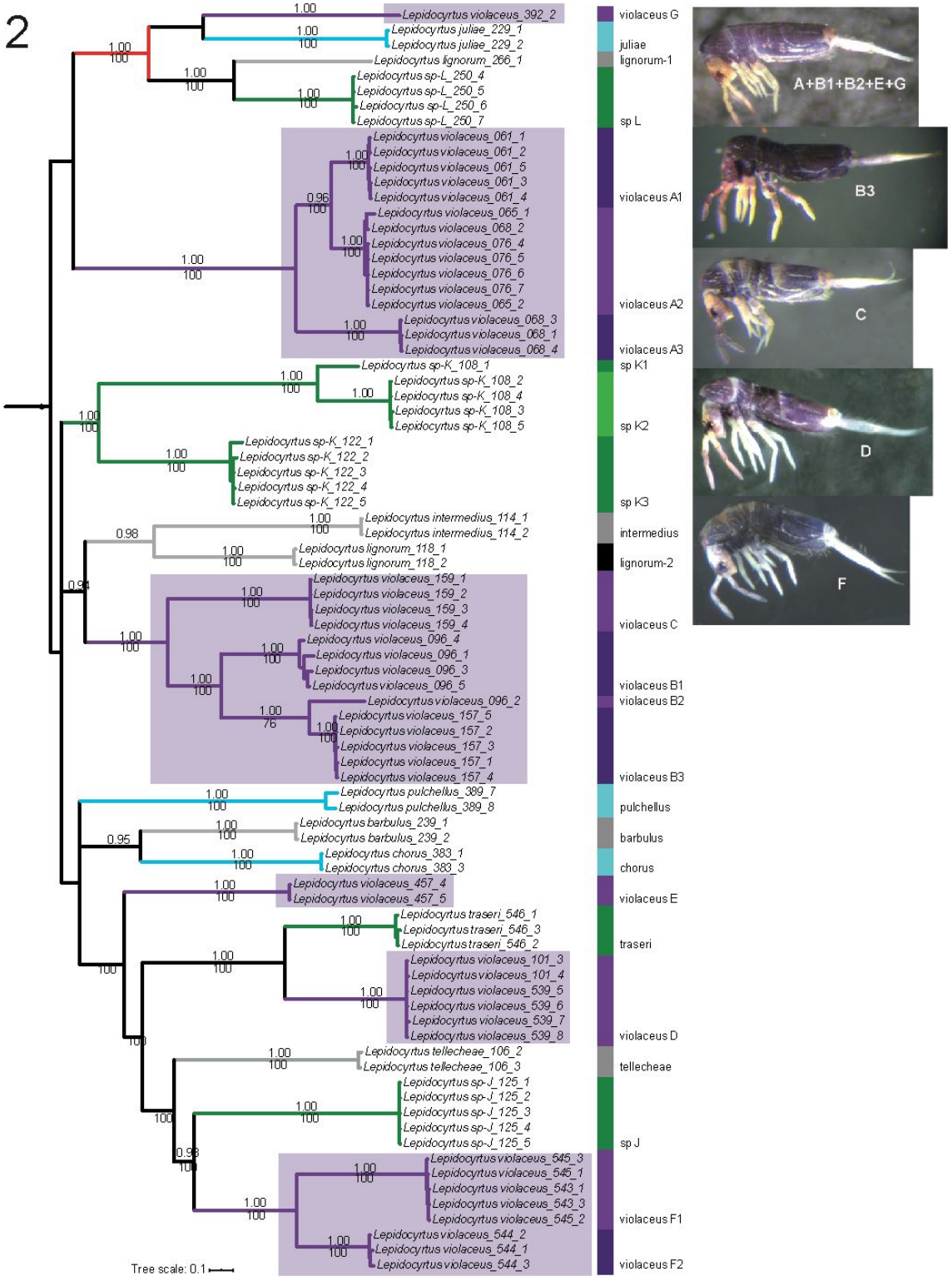
Table 4.

Body region	Position	Pos	#	A2	E	G
Legs	Cx.I	1	2	2	2	2
	Cx.II	2	4	4	4	4
	Cx.III	3	2	2	2	2
Head dorsal	Cephalic - exterior	4	1	1	1	1
	Cephalic - interior	5	2	2	2	2
Body dorsal	Th.II (mid dorsal)	6	1	1	1	1
	Th.III (mid dorsal)	7	1	1	1	1
	Abd.I (mid dorsal)	8	1	1	1	1
	Abd.II (mid dorsal)	9	1	1	1	1
	Abd.III (mid dorsal)	10	1	1	1	1
	Abd.IV (mid dorsal)	11	1	1	1	1
	Abd.IV (dorsal)	12	-	-	-	-
Furca dorsal	Manubrial base	13	2	2	2	2
	Manubrial plate	14	2	2	2	2
	Dentes	15	2	2	2	2
Ant. ventral	Ant.III	16	1	1	1	1
	Ant.II	17	1	1	1	1
	Ant.I	18	-	-	-	-
Body ventral	Th.I	19	1	1	1	1
	Th.II	20	1	1	1	1
	Th.III	21	1	1	1	1
	Abd.I - VT base anterior	22	1	1	1	1
	Abd.I - VT base posterior	23	1	1	1	1
	Abd.II	24	(3)	(3-4)	(4)	(3)
	Abd.III - post retinaculum	25	(2)	(2)	(2)	(2)
	Abd.IV	26	-	-	-	-
	Abd.V – pre genital plate	27	(1)	(1)	(1)	(1)
Body lateral	Th.III	28	-	-	-	-
	Abd.I	29	-	-	-	-
	Abd.II	30	-	-	-	-
	Abd.III	31	-	-	-	-
	Abd.IV	32	-	-	-	-
	BP4	33	-	-	-	7

Table 5.

Sample Code	ABGD	N	Size	Ocular-q	Abd.II-ml	Abd.II-p5p	Abd.III-mi	Abd.III-d3	B4-B5/B5-B6	abd.IV-B6	BP4-pse
LP061	A1	6	1.3 –1.5	-	-	-	-	+	1.23	bcM	-
LP065	A2	5	1.1 –1.4	-	-	-	-	+	1.37	bcM	-
LP068	A2+A3	5	0.8 –1.2	-	-	-	-	+	1.26	bcM	-
LP076	A2	5	1.3 –1.6	-	-/+	-	-	+	1.18	bcM	-
LP096	B1+B2	2	1.0 –1.2	-	-	-	-	+	2.03	tcM	-
LP157	B3	2	1.4 –1.5	-	-	-	+	+	2.33	tcM	-
LP159	C	2	1.0 –1.1	-	-	-	-/+	+	2.06	tcM	-
LP101	D	2	2.0	-	+	+	+	+	2.49	tcM	-
LP539	D	4	1.6 –1.8	(-/+)	+	(-/+)	(-/+)	+	2.09	tcM	-
LP457	E	3	1.2 –1.4	-	(-/+)	-/+	-/+	+	0.93	bcM	-
LP543	F1	1	1.3	-	-	+	-	-	2.18	tcM	-
LP544	F2	1	1.5	-	-	+	-	-	2.26	tcM	-
LP545	F1	1	1.0	-	-	+	-	-	2.35	tcM	-
LP392	G	1	1.1	-	-	-	-	-	1.11	bcM	+

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Lepidocyrtus juliae_229_2
Lepidocyrtus lignorum_266_1
Lepidocyrtus sp-L_250_4
Lepidocyrtus sp-L_250_5
Lepidocyrtus sp-L_250_6
Lepidocyrtus sp-L_250_7
Lepidocyrtus violaceus_061_1
Lepidocyrtus violaceus_061_2
Lepidocyrtus violaceus_061_5
Lepidocyrtus violaceus_061_3
Lepidocyrtus violaceus_061_4
Lepidocyrtus violaceus_065_1
Lepidocyrtus violaceus_068_2
Lepidocyrtus violaceus_076_4
Lepidocyrtus violaceus_076_5
Lepidocyrtus violaceus_076_6
Lepidocyrtus violaceus_076_7
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Lepidocyrtus violaceus_068_3
Lepidocyrtus violaceus_068_1
Lepidocyrtus violaceus_068_4
Lepidocyrtus sp-K_108_1
Lepidocyrtus sp-K_108_2
Lepidocyrtus sp-K_108_4
Lepidocyrtus sp-K_108_3
Lepidocyrtus sp-K_108_5
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Lepidocyrtus sp-K_122_3
Lepidocyrtus sp-K_122_4
Lepidocyrtus sp-K_122_5
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Lepidocyrtus lignorum_118_1
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Lepidocyrtus violaceus_159_3
Lepidocyrtus violaceus_159_4
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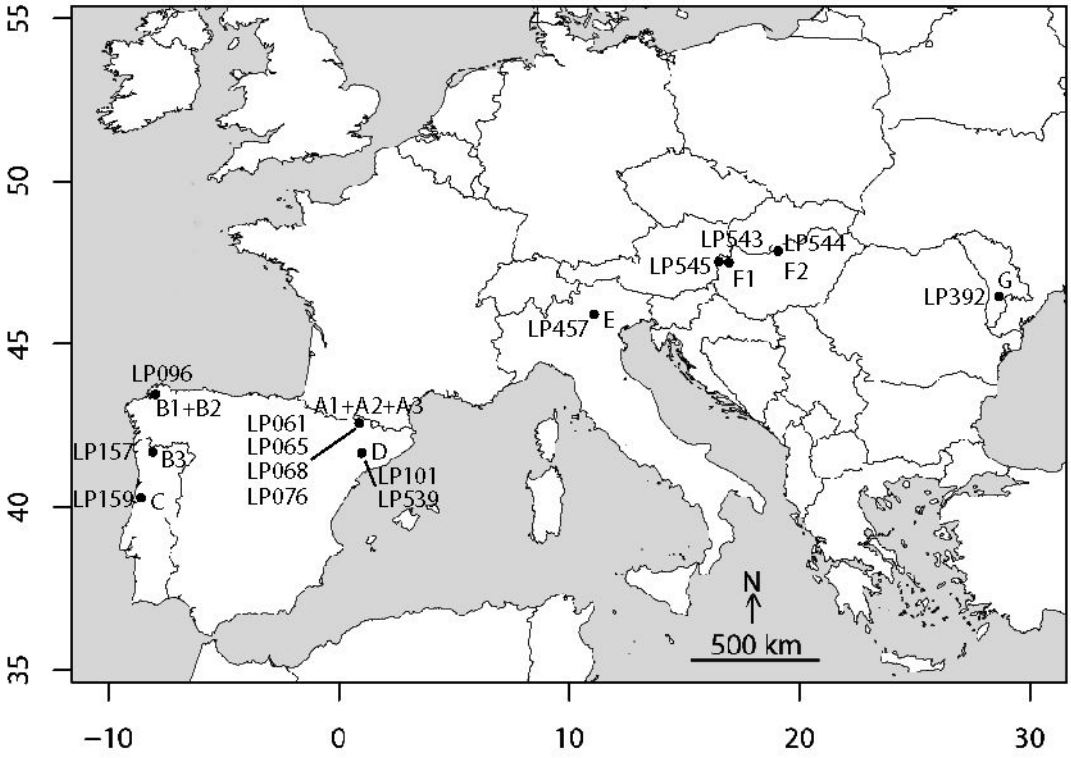
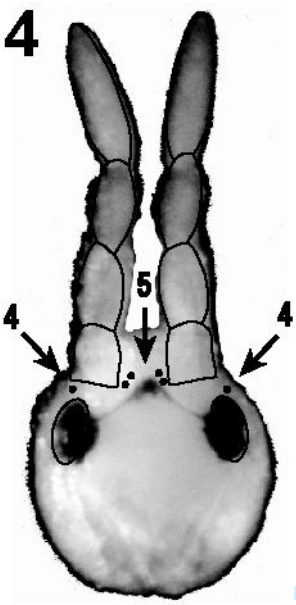
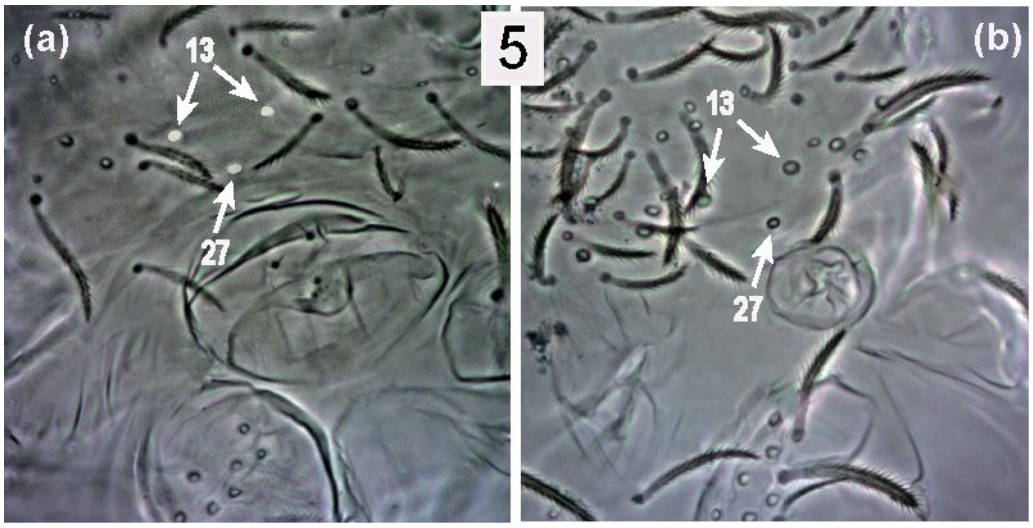
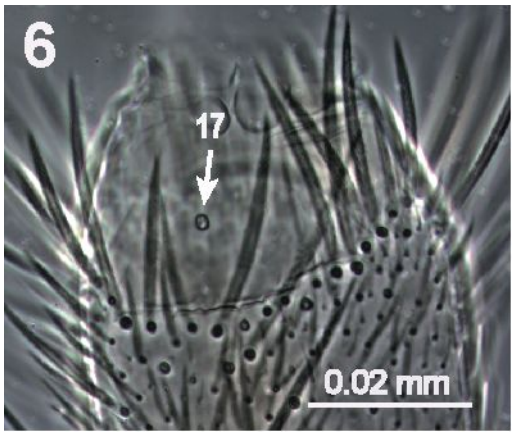
Lepidocyrtus violaceus_543_1
Lepidocyrtus violaceus_543_3
Lepidocyrtus violaceus_545_2
Lepidocyrtus violaceus_544_2
Lepidocyrtus violaceus_544_1
Lepidocyrtus violaceus_544_3
Tree scale: 0.1
violaceus G
juliae
lignorum-1
sp L
violaceus A1
violaceus A2
violaceus A3
sp K1
sp K2
sp K3
intermedius
lignorum-2
violaceus C
violaceus B1
violaceus B2
violaceus B3
pulchellus
barbulus
chorus
violaceus E
traseri
violaceus D
tellecheae
sp J
violaceus F1
violaceus F2
A+B1+B2+E+G
B3
C
D
F

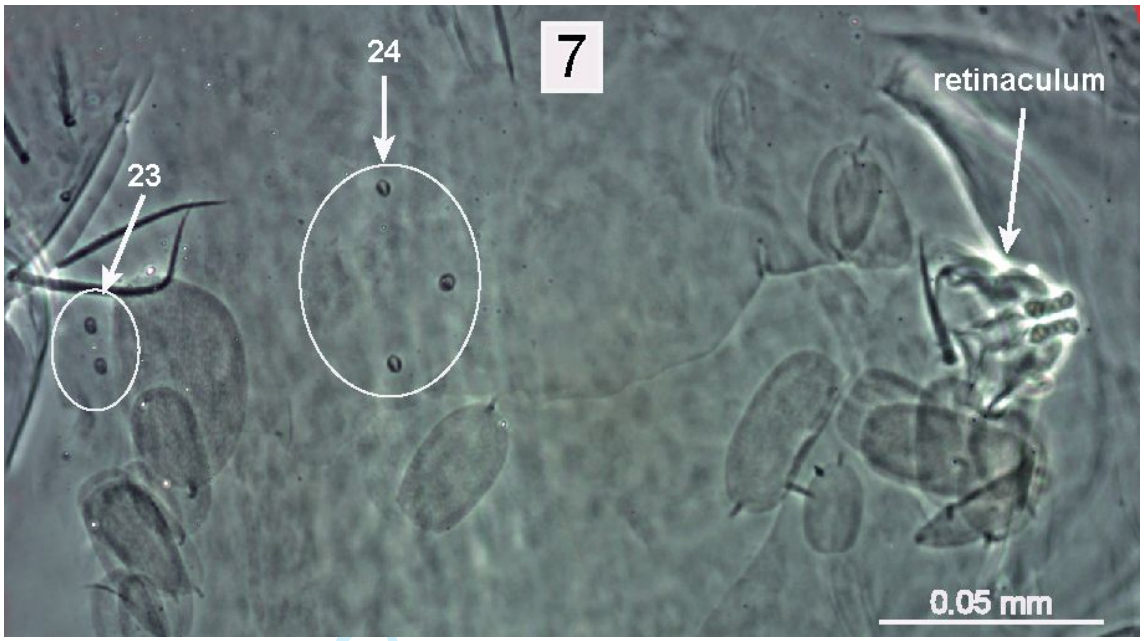
3

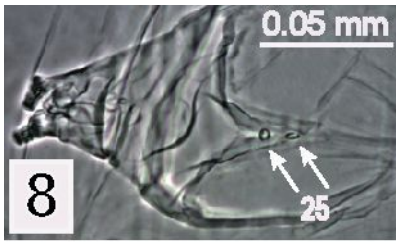


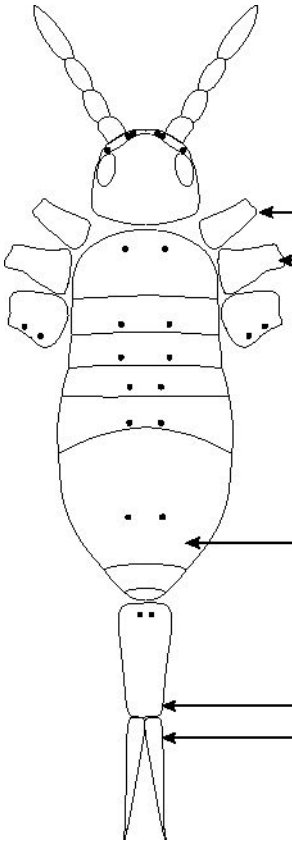












9

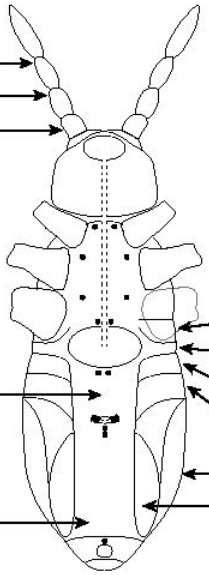
DORSAL

lu	la	pa	li	cu	Position
1-2	2	2	2	2	Cx.I (1)
2	2	2	2-6	3-8	Cx.II (2)
0	0	0	0	0-6	Abd.IV (12)
2	2	2	2	2-3	man. plate (14)
0	0	1	1-4	2-17	dentes (15)

10

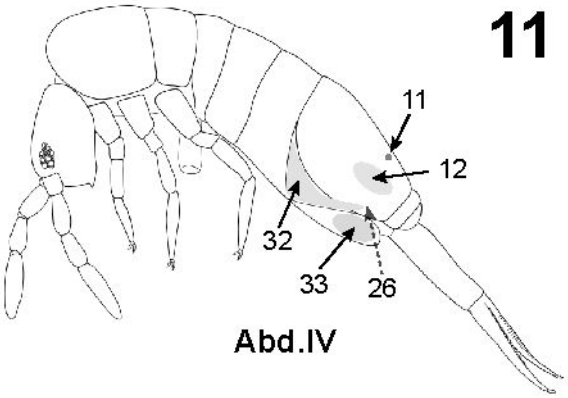
VENTRAL

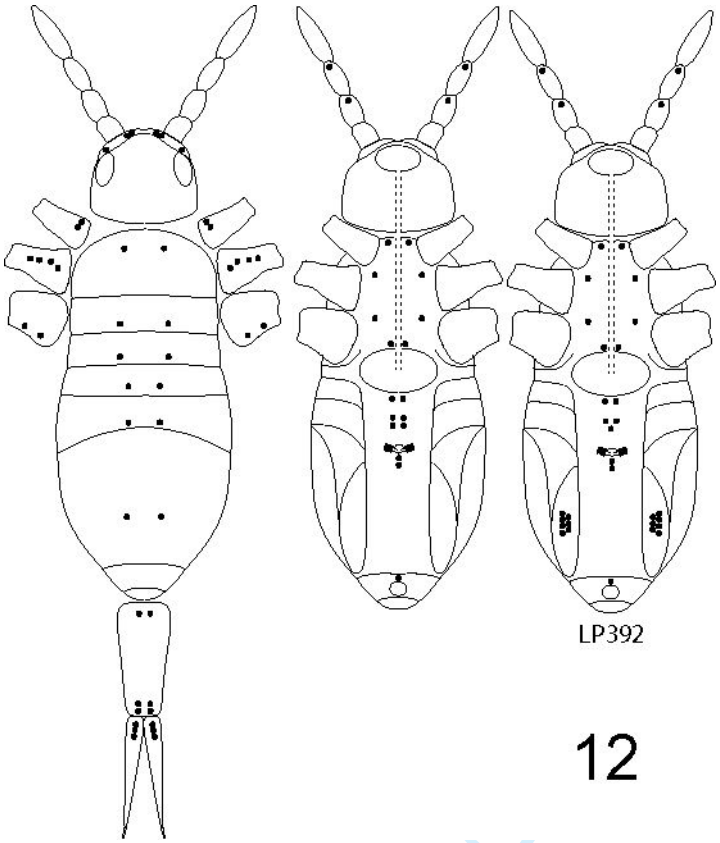
Position	lu	la	pa	li	cu
Ant.III (16)	1	1	1	1	1-5
Ant.II (17)	0	0	1	1	1-7
Ant.I (18)	0	0	0	0	1-5
Abd.II (24)	3	2	3-5	3-7	3-4
Abd.IV (26)	0	0	0	0	0-10



LATERAL

lu	la	pa	li	cu	Position
0	0	0	0	0-5	Th.III (28)
0	0	0	0	0-13	Abd.I (29)
0	0	0	0	0- 8	Abd.II (30)
0	0	0	0	0- 8	Abd.III (31)
0	0	0	0	0-24	Abd.IV (32)
0	0	0	0-10	0	BP4 (33)





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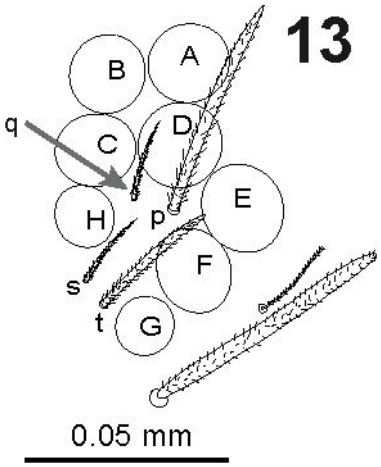

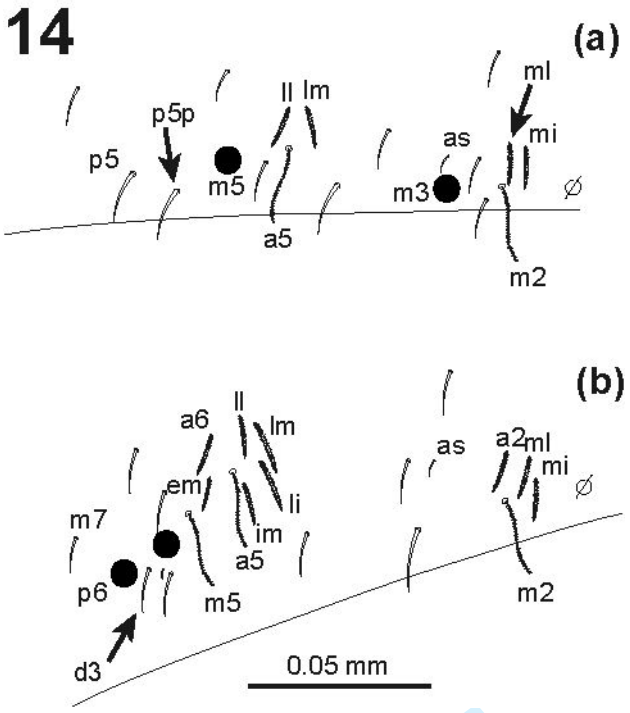
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(a)
ml
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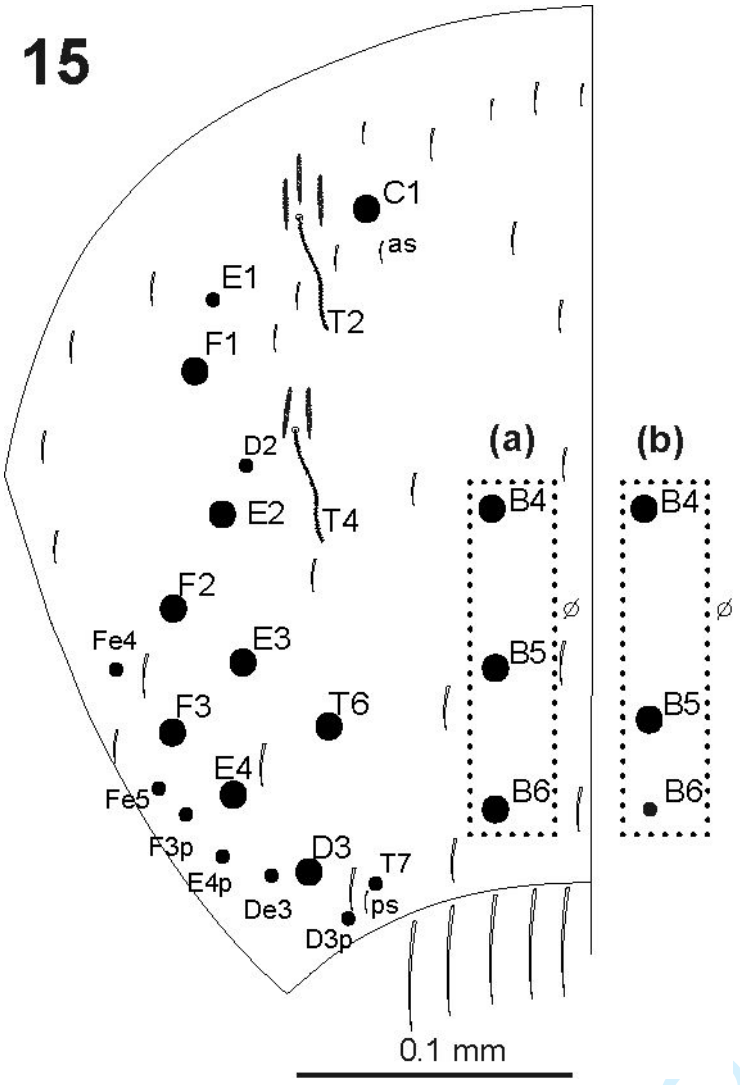
p5p
mi
as
p5
m5
m3
ø
a5
m2
(b)
ll
a6
lm
as
a2
ml
mi
em
li
ø
m7
im
a5
p6
m5
m2
d3
0.05 mm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T4
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E3
B5
B5
F3
T6
E4
B6
B6
Fe5
F3p
D3
T7
E4p
De3
ps
D3p
0.1 mm