Evolution and diversification of the geckos of the Arabian Peninsula and Socotra Archipelago, compared to other mainland-island systems

Evolución y diversificación de los gecos de la Peninsula Arábiga y el archipiélago de Socotra, comparado con otros sistemas continente-isla

Joan Garcia Porta

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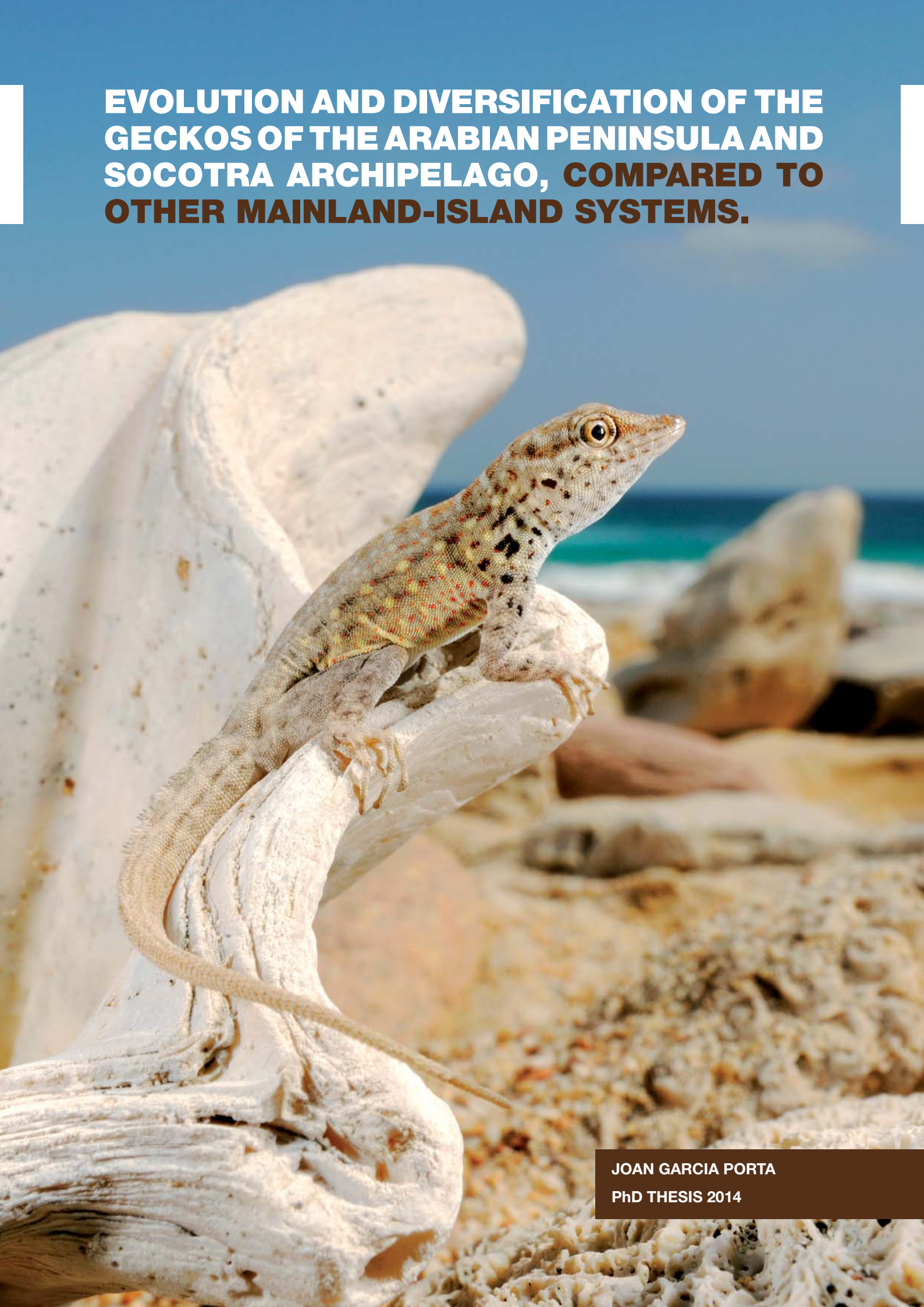

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PhD THESIS 2014

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Dedicat a la meva mare, la tieta i al Jaume.

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GENERAL INTRODUCTION

The diversity of Life is amazing, not only in terms of number of species, but also in terms of the different colors, sizes, shapes, life histories and even metabolisms. The contemplation of this beauty has inspired many generations of naturalists, philosophers and scientists since the dawn of our civilization, engaging their natural curiosity in the task of understanding how all this variety of forms and shapes came about. This endeavor experienced its greatest step forward the 24th of November of 1859 with the publication of "The Origin of Species". In this book, Charles Darwin proposed an evolutionary mechanism that revolutionized his time: the theory of evolution by means of natural selection. Darwin argued that the distributions of traits in the natural populations changed over time as those individuals with the combination of traits that better fit to the environment succeed in reproducing at a higher rate than those with traits less well fit (provided that the traits that increase fitness are heritable) (Darwin, 1859). From this idea derives a simple but powerful reasoning: if different populations within a species are exposed to different environments, the particular trait combinations that maximize fitness in each of the environments may not be the same ones. As a consequence, natural selection may act in radically different directions in different populations within the same species. These divergent selective pressures existing between environments can potentially drive phenotypic differentiation among the populations, at least in those combinations of traits used to exploit the different environments (Huxley et al.,1942; Mayr, 1942). George G. Simpson subsequently elaborated around this idea providing a simple concept in which the nature of these divergent selective pressures could be easily visualized: the adaptive landscape. In this concept, inspired by Wright's fitness landscapes for gene frequencies (Wright, 1984), fitness is visualized as the height of a surface that, in turn, varies as a function of the values of two or more phenotypic traits (Simpson, 1944; 1965). In this way, the combination of trait values that allow higher fitness are visualized as "peaks" in the surface while those combinations of trait values that determine low fitness are visualized as "valleys".

In such a “landscape” formed by peaks and valleys, populations diverge because they are “pulled” toward different adaptive peaks and away from the valleys of lower fitness. From an ecological perspective, each of these adaptive peaks can be seen as a particular combination of trait values that allow an efficient use of a particular niche (Schluter, 2000) (Fig. 1).

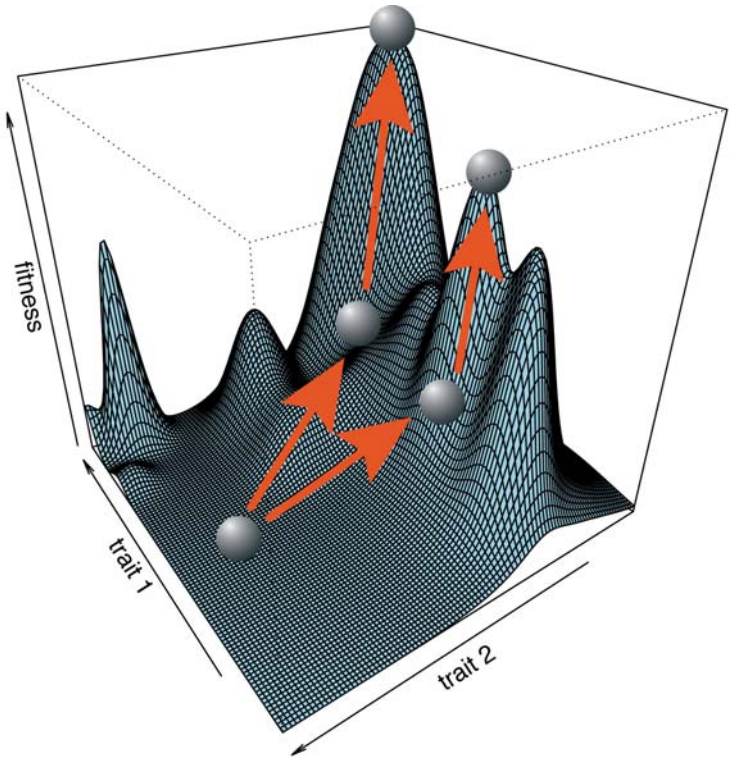

Figure 1. Example of an adaptive landscape where the height of the surface correlates with fitness and the two horizontal axes represent the variation existing along two traits. Groups can potentially experience a great expansion of the morphospace as they move across this imaginary surface.

Sometimes Nature suddenly exposes groups to a great variety of empty niches, which can be visualized as adaptive landscapes with many unoccupied fitness peaks (Martin & Wainwright, 2013). In a possible outcome derived from this situation, the divergent selective regimes that operate across the landscape may rapidly pull different populations into the different adaptive peaks producing phenotypic (and genetic) divergence among the populations. Under such scenario, speciation occurs as an incidental “by product” of adaptive divergence (Nosil, 2012).

In this way, new niche availability or, as coined by Simpson, “ecological opportunity”, can theoretically prompt great levels of phenotypic and species diversification. In fact, ecological opportunity has been invoked as one of the major drivers of diversification in many of the greatest examples of adaptive radiations, from the Darwin's finches to the great diversity of cetaceans (Grant & Grant, 2011; Schluter, 2000; Slater et al., 2010).

From a dynamic perspective, this process predicts different phases that differ in their pace of diversification. Rates of phenotypic and species diversification are predicted to be maximal at the beginning of the diversification when groups are in the process of rapidly filling all ecological space available. Then rates tend to slow down as the amount of available niches decreases progressively. This high acceleration in the evolutionary rates at the onset of the diversification is known as the “early burst” and is supposed to be very common in adaptive radiations (Schluter, 2000; but see Harmon et al., 2010).

Simpson proposed three different scenarios in which high levels of ecological opportunity could theoretically drive high amounts of evolutionary diversification (Simpson, 1944; 1965). These were the following:

EXTINCTION OF ANTAGONISTS

This occurs when a number of niches become available after the extinction of the species that occupied these niches. This is typically the case after a massive extinction event in which a large number of niches become suddenly empty. In these situations surviving groups often rapidly fill the new-formed ecological vacancies producing outstanding levels of phenotypic and species diversity. One of the notable examples of this is the great phenotypic and species diversification that experienced mammals after the mass extinction event in the Cretaceous-Tertiary boundary (around 65 Ma) (Meredith et al, 2011).

EXPOSURE TO NEW ENVIRONMENTS

This can either be the consequence of a group dispersing to a new area or the consequence of environmental changes within the distribution of a group. The most obvious examples of evolutionary diversification driven by the exposure to new environments is the great diversifications that follow island colonization. The astonishing variety of sizes and shapes found in many insular groups (e. g. Hawaiian honeycreepers) exemplify the role of new environments (as insular environments) at triggering diversification (Losos & Ricklefs, 2009).

ACQUISITION OF A KEY INNOVATION

In its earliest definition, a key innovation is a trait that allows a new interaction with the environment. Very often this grants the access to a number of new, previously inaccessible, niches which in turn may trigger high rates of phenotypic and species diversification. One of the most prominent examples of the sorts of outstanding diversifications that follow the acquisition of key innovations is the acquisition of feathers by theropod dinosaurs (which enabled the capacity of flying and ultimately led to the great diversity of birds existing nowadays; Hunter, 1998).

The main aim of this thesis is to examine the two last sources of ecological opportunity—specifically, the colonization of islands and the evolution of key innovations—and explore the extent in which these drive phenotypic and species diversification at different taxonomic and geographic contexts. In the following pages I elaborate about how theoretically these two particular sources of ecological opportunity can potentially prompt evolutionary diversification, exposing the strong and weak points of this idea.

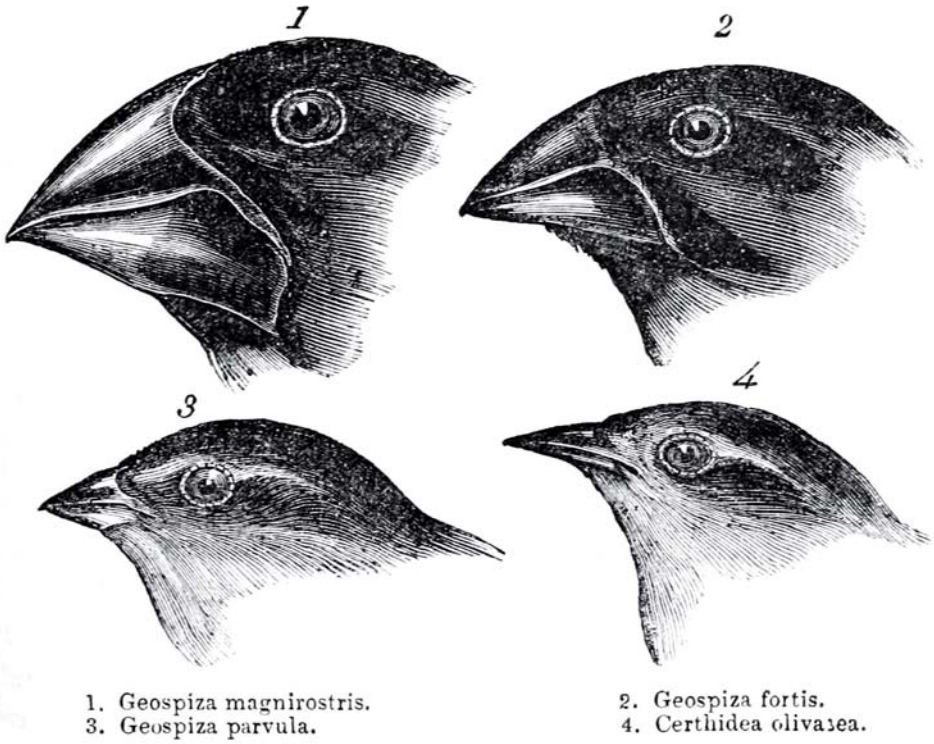

Figure 2. The Darwin's finches represent one of the most prominent radiations in islands. In this case, birds have evolved into different beak shapes (among other traits) to partition the resources existing in the islands (drawings extracted from the book "The voyage of the Beagle", published in 1845).

ISLANDS AS DRIVERS OF EVOLUTIONARY DIVERSIFICATION

Islands are widely known for their outstanding diversifications. The astonishing diversities in the Hawaiian honeycreepers or in the Darwin's finches are examples of the sorts of diversifications attained after island colonization (Losos & Ricklefs, 2009) (Fig. 2).

The notion of ecological opportunity lies at the very heart of the link between island colonization and evolutionary diversification. Ecological opportunity derives from the great number of ecological vacancies existing in the early stages of island evolution (Whittaker et al. 2008). In this context of many available resources with few competitors, colonizing groups tend to use a wider array of niches compared to continental groups, as island species are able to use resources that are normally used by other species elsewhere (phenomenon very often known as "ecological release") (Yoder et al., 2010). One of the consequences of this niche expansion is that species may also experience an increase in the heterogeneity of the selective regimes that act upon them. As exposed before, these different selective regimes acting on different populations within the same species can potentially induce phenotypic divergence ultimately leading to speciation (Schluter, 2000; Nosil, 2012).

Coupled with the absence of competitors, the absence of predators is also a key aspect mediating in island diversifications. Predators in the continent usually keep preys at low-density levels (Millien, 2011) and very often impose strong stabilizing selection on many of the traits of their preys (Yoder et al., 2010). For instance, prey species in the continent often tend to evolve towards an optimal body

size that maximizes the chances to hide or scape from predators. In islands, due to the low density and diversity of predators, preys are suddenly released from these constrains and therefore can diversify into a greater variety of phenotypes (for instance increasing or decreasing sizes beyond the limits of their relatives in mainland) (Yoder et al., 2010).

Also related to the absence of predators (and to the absence of competitors), species in islands normally present higher population densities compared to the continent. This is known as "density compensation" and is a phenomenon widely detected in many island species (Bennett & Gorman, 1979; Buckley & Jetz, 2007; Case, 1975; Rodda & Bradley, 2002). In such situations, disruptive selection may arise if phenotypically intermediate (and more common) phenotypes compete more strongly for resources than those at the tails of the distribution. This results in a lower fitness in the intermediate phenotypes and leads to an expansion towards new and less exploited resources. This process is also expected to drive great phenotypic divergence ultimately leading to speciation (Nosil, 2012).

All the processes explained above set a theoretical stage that helps to explain why islands present so many astonishing examples of diversification. However, empirical evidence shows that not all island taxa experience similar amounts of evolutionary diversification. There are instances in which island lineages even fail to undergo minimal amounts of diversification. An example of this is the Darwin's finch of the Cocos Island. Although the populations of these finches show a clearly expanded niche compared to continental species (Werner & Sherry, 1987), they failed to diversify into the variety of ecomorphs found in their closest relatives of Galapagos. Examples like this outline a more complex scenario in which islands might not necessarily induce great levels of evolutionary diversification.

Figure 3. Island area and island isolation are among the most important features that mediate in the ecological opportunity provided by islands (photo credit: Peter Porta).

There are a number of factors that can potentially play a role at determining whether insular groups will take or not the path of evolutionary diversification. In the following pages I introduce the most important ones separating them in two main groups: *extrinsic factors*, mostly modulated by the geography or geology of the island, and *intrinsic factors*, mostly determined by the biological characteristics of the groups that colonized islands.

EXTRINSIC FACTORS

ISLAND ISOLATION

The relationship between island isolation and species richness goes back to the seminal work of MacArthur and Wilson on island biogeography. According to their theory, the lower the isolation, the higher the expected rate of colonization from the continent and, as a consequence, the higher the species richness expected to occur at the island's equilibrium (MacArthur & Wilson, 1963). However by the same token, islands that are exposed to a higher influx of immigrants likely preclude opportunities of diversification (Losos & Parent, 2009). This is because, in such situations, ecological vacancies in the islands will be more likely occupied by continental immigrants, which already have all adaptations required to efficiently use the vacancies, rather than by species derived from intra-island speciation in which adaptations to use a particular niche will require time to evolve. We can thus say that, the more isolated an island is the greater the ecological opportunity it likely offers, as fewer potential competitors reach the island. Isolation itself can also join forces with ecological opportunity to impulse *in situ* diversification: the more isolated an island is, the easier for island species is to interrupt gene flow with the conspecifics inhabiting the source region (Losos & Parent, 2009). This allow island taxa the possibility to take their own evolutionary path as completely isolated gene pools and increase the chances that island populations speciate from their ancestral species and diversify *in situ*.

ISLAND AREA

This is another island feature classically investigated by biogeographers. According to MacArthur and Wilson, the area of an island is one of the best predictors of its species richness. This is because groups inhabiting bigger islands are likely exposed to lower rates of extinction, allowing higher number of species coexisting at the island equilibrium (MacArthur & Wilson, 1963). In recent times, many studies have also shown that area is, not only a good predictor of species richness, but is also a good predictor of the amount of *in situ* diversification that takes place in an island (Gillespie, 2004; Losos & Parent, 2009; Parent & Crespi, 2006; Steppan et al., 2003). This was first demonstrated for the *Anolis* in the Greater Antilles (Losos & Ricklefs, 2009) and indicated that the relationship between area and diversification resulted primarily from an increase in the rate of speciation with area, rather than from a decrease in the

rate of extinction. But how an increase in area may enhance diversification? There are different theories that explain this, although not all of them require an adaptive component. For instance, the potential for allopatric speciation may increase with island area (not necessarily implying adaptive diversification). But also, island area is often correlated with ecological heterogeneity (reviewed in Ricklefs & Lovette, 1999; Whittaker & Fernandez-Palacios, 2007), which could potentially increase the available niche space on larger islands, therefore also increasing the chances for adaptive diversification. We find an example of the previous in the bulimulid snails in the Galapagos Islands. In these, *in situ* speciation is correlated with area, but it is even more correlated with vegetational diversity, a good proxy to niche heterogeneity (Losos & Parent, 2009).

ISLAND AGE

The age of an island has been proposed as one of the most important modulators of *in situ* diversification (Losos & Parent, 2009). Age can be related to island diversification in two main (and opposite) ways. First, older islands may have greater chances to contain *in situ* diversifications simply because they likely possess groups that have been evolving *in situ* for longer and, as a consequence, have had more opportunities to diversify (Heaney, 2000). In line with this, a positive correlation between island age and *in situ* diversification has been detected for the bulimulid snails and other invertebrates in the Galapagos Islands (Losos & Parent, 2009; Sequeira et al., 2008). However island age can also play against diversification. This is because the ecological opportunity provided by an island likely decrease, as the forces of erosion tend to simplify its topography and reduce its area (Whitaker et al., 2008). Evidence of a negative relationship between age and diversification comes from the *Tarphius* beetles from the Canary Islands. In this group most of the diversification occurs in intermediate-aged islands and decrease in the oldest islands (Emerson et al., 2000).

GEOLOGIC ORIGIN OF ISLANDS

Regarding their geologic origins, islands can be categorized in two broad groups: oceanic islands and continental islands. Oceanic islands typically originate by volcanic processes (usually involving oceanic crust) and form landmasses isolated from the mainland source pools by ocean. On the contrary, continental islands typically originate from continental landmasses either by being part of the continent at past sea-level minima (land-bridge islands) or by being continental fragments that once detached from the continent by tectonic processes and drifted into the ocean (continental fragments) (Whittaker & Fernandez-Palacios, 2007). The differences in the processes of community assembly existing in both types of islands can greatly determine their chances to experience *in situ* diversification. In oceanic islands, diversity is built up *de novo* by the interaction of two processes: dispersal from the continent (or another island) and/or *in situ* diversification. By contrast, in continental islands, aside of these two

biogeographic processes, part of the diversity may have been passively inherited from the continental stage of these islands, when part of the continental stocks became isolated in the islands at the moment of mainland-island detachment (vicariance) (McDowall, 2004). If this is the case, continental islands would offer lesser amounts of ecological opportunity compared to oceanic islands, as many of the niches existing in continental islands would be already occupied by the vicariant components of their diversity.

ISLANDS VERSUS ARCHIPELAGOS

Islands can exist as isolated landmasses or in archipelagos, forming sets of landmasses in proximity of each other. Evidence shows that these different geographical settings can potentially influence the chances of adaptive diversification. We find one of the most remarkable examples of this in the famed Darwin's finches of the Galapagos Islands. Detailed analysis on the variation among populations show that the 13 species of the archipelago likely speciated in different islands, becoming secondarily sympatric as a result of dispersal events between islands. Once in sympatry, if coexisting species did not differ yet in their phenotypes and ecologies, they probably started to diverge as a consequence of character displacement (Grant & Grant, 2011; Losos & Ricklefs, 2009). Therefore, in this case, allopatric speciation occurring on different islands in an archipelago followed by secondary invasions can result in the build-up of diversity through adaptive processes.

INTRINSIC FACTORS

Will different groups arriving to the same island or archipelago experience similar amounts of evolutionary diversification? There are not many studies that explore in detail this question, however circumstantial evidence shows that this might not be the case. In the Galapagos Islands for example, Darwin's finches are the sole birds to have diversified to any extent (Jackson, 1993). Likewise, in the Greater Antilles, aside of the geckos of the genus *Sphaerodactylus*, not a single other reptilian group has diversified into the amounts of phenotypic and species diversification experienced by the iguanian lizards of the genus *Anolis* (Losos, 2009). These examples caution that the physical and geographic attributes of an island are probably not the whole story. There may be a number of factors intrinsic to the different colonizing groups that determine whether they will take or not the path of diversification.

GROUP-DEPENDENT MODULATORS OF ECOLOGICAL OPPORTUNITY

We have seen that the amounts of ecological opportunity offered by an island are greatly determined by its geographical, geological and physical attributes. However, these being equal, it is conceivable that not all groups experience similar levels of ecological opportunity. This is because the amount of

niches accessible by a species ultimately depends on its particular anatomy, physiology or behavior. As a consequence, appropriate resources may not be equally available for all groups of organisms. This could explain the lack of substantial diversification in the warbler finches in the Galapagos Islands. The absence of discrete resources to which different warbler-like species could adapt might potentially explain its failure to undergo extensive phenotypic and species diversification (Grant & Grant, 2011; Rundell & Price, 2009). Another important variable that accounts for the amounts of ecological opportunity that a group will likely experience is its timing of arrival to the island. Early colonizers will likely experience greater amounts of ecological opportunity while groups colonizing the island in a later stage, may encounter a situation with more competitors and fewer free niches to occupy (Losos, 2010).

DIFFERENT PREDISPOSITIONS TO SPECIATION

Not all colonizing groups may present the same proneness to speciate in an island even when exposed to high levels of ecological opportunity. For instance, groups with complex courtships, or relying on complex visual, kinetical or acoustic signals, may increase the likelihood that populations exposed to different environments (or using different resources) will become reproductively isolated (Losos, 2009). Also, in some groups, niche expansion may be mostly mediated by behavioral plasticity, not necessarily implying (at least in the short term) the specialization of different populations to different portions of the spectrum of resources offered by an island. In such a situation of individuals constantly moving among different environments and using alternatively different resources, the chances to attain reproductive isolation might be more limited.

DIFFERENT EVOLVABILITIES

Different evolvabilities, or different abilities to evolve into different forms (Schluter, 2000), could also be crucial to explain the different capabilities of different groups to diversify in islands. For instance, some groups might possess anatomical, physiological or biomechanical constrains that make them unable to undergo extensive evolutionary diversification even when exposed to great ecological opportunity. This notion has been invoked to explain why the *Anolis* in the Caribbean have produced such a great phenotypic diversity while another lizard group, inhabiting similar tropical islands, the genus *Phelsuma*, shows only moderate amounts of phenotypic diversification (Losos, 2010). In geckos, especially in arboreal species, limbs are laterally oriented with respect to the body and form a low angle with the substrate keeping their center of gravity close to it. This type of body design in arboreal geckos may have constrained the extent in which geckos have been able to adapt to different microhabitats (Losos, 2010). *Anolis*, however, are free from this anatomical constrain, which seem to have allowed them to diversity into the myriad of shapes and sizes attained in the Caribbean islands (Losos, 2009).

EVOLUTIONARY DIVERSIFICATION IN ISLANDS VERSUS CONTINENTS

Island colonizers normally shift between a context of high competence and predation to a context almost devoid of competitors and predators. This great ecologic asymmetry between the lineages that colonize an island (exposed to high levels of ecological opportunity) compared to their continental close relatives (exposed to low levels of ecological opportunity) likely has its reflection on how evolutionary diversification carries on in both domains. In mainland settings, communities are usually complex and composed by many species that typically share a long history of coexistence. In such scenario, most of the continental niches will likely be filled, leaving little free ecological space to newly formed species (Losos & Ricklefs, 2009). In this context, high levels of inter-specific competition are expected and, in turn, these will tend to limit an efficient niche (and morphospace) expansion in diversifying groups. In addition to this, predation is usually very intense in continental communities, which will likely contribute also to limit morphological diversification by inducing stabilizing selection on many traits (Yoder et al., 2010).

As a result of the combined effects of both inter-specific competition and predation, evolutionary diversification in continents is expected to produce small variations of already successful adaptive themes, enforcing a great morphologic conservatism (Moen et al., 2009; Losos & Ricklefs, 2009). This contrasts with the situation experienced by the early colonizers of islands; for all reasons previously exposed, insular groups in many cases will experience great diversifications as they move across the new adaptive landscape provided by islands (Losos & Ricklefs, 2009). This will likely produce two main differences compared to the diversification of continental groups: First, island taxa will experience higher rates of phenotypic and/or species diversification compared to continental taxa as a consequence of the predicted “early burst” taking place in islands (Schluter, 2000). Second, given that insular communities are necessarily formed by a subset of continental communities, groups that diversify in islands can use niches that in the continent are occupied by even distantly related groups.

In Darwin's own words (Darwin, 1859):

“*Oceanic islands are sometimes deficient in certain classes, and their places are apparently occupied by the other inhabitants; in the Galapagos Islands reptiles, and in New Zealand gigantic wingless birds, take the place of mammals*.”

As a consequence, evolutionary diversification in islands may open the door to an adaptive expansion of the morphospace, enabling the great phenotypic disparities so often found in insular groups (Carlquist, 1974). This mosphospace expansion may take island taxa well beyond the limits of the continental

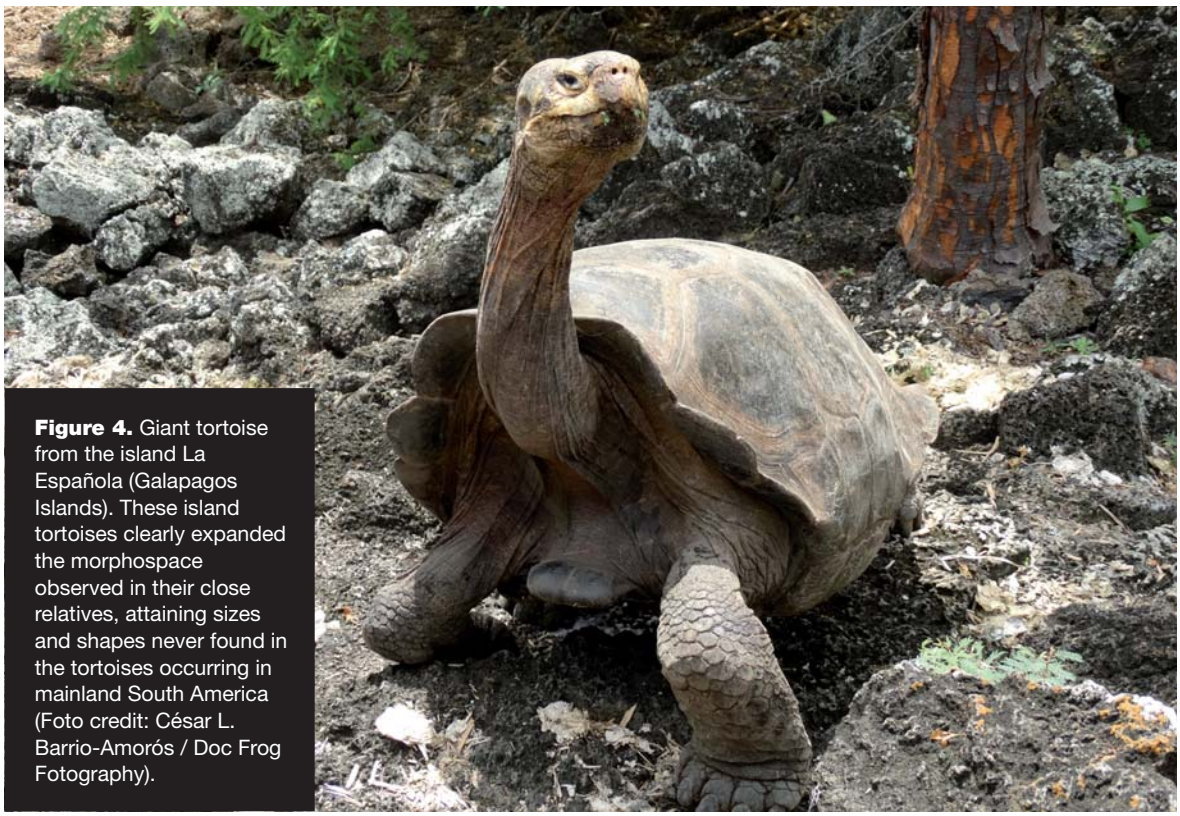

Figure 4. Giant tortoise from the island La Española (Galapagos Islands). These island tortoises clearly expanded the morphospace observed in their close relatives, attaining sizes and shapes never found in the tortoises occurring in mainland South America (Foto credit: César L. Barrio-Amorós / Doc Frog Fotography).

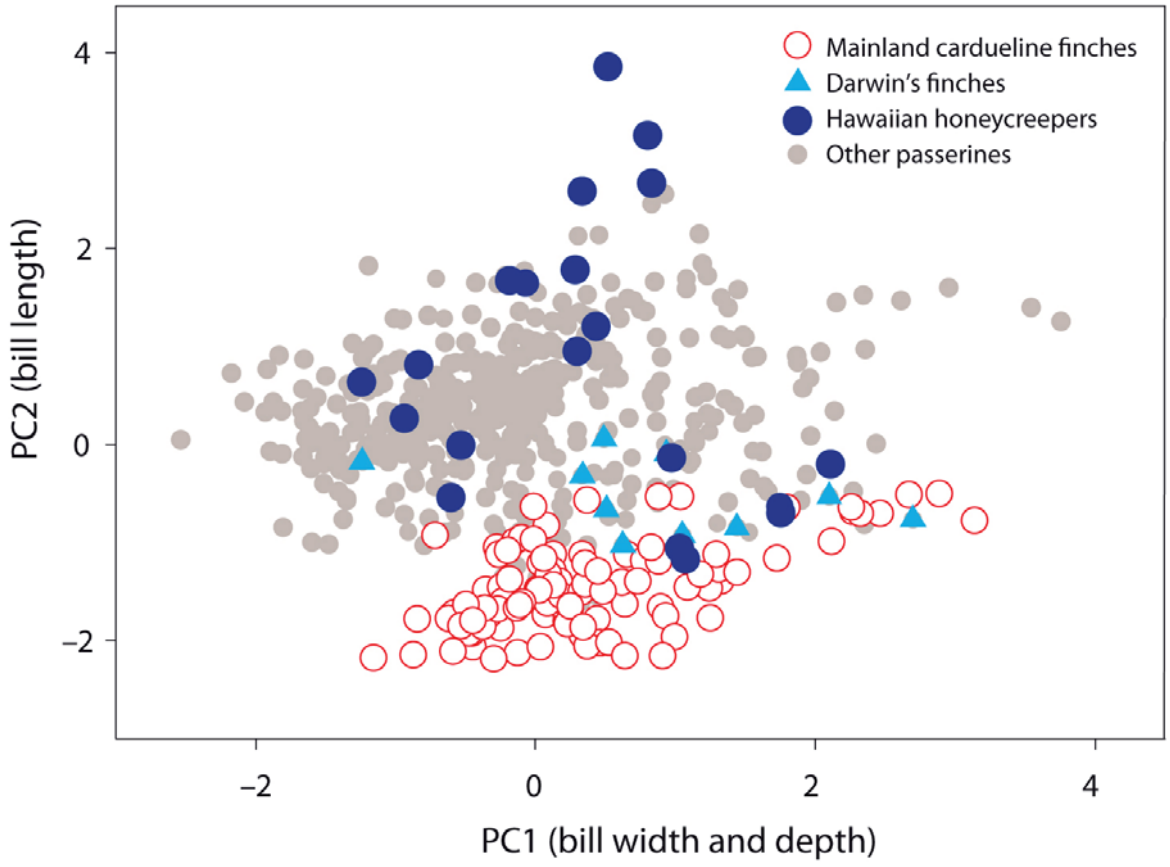

Figure 5. Plot showing variation in beak morphology existing in a sample of passerines worldwide, the continental cardueline finches, the Darwin's finches in the Galapagos Islands and the Hawaiian Honeycreepers in Hawaii (Modified from Losos & Ricklefs, 2009).

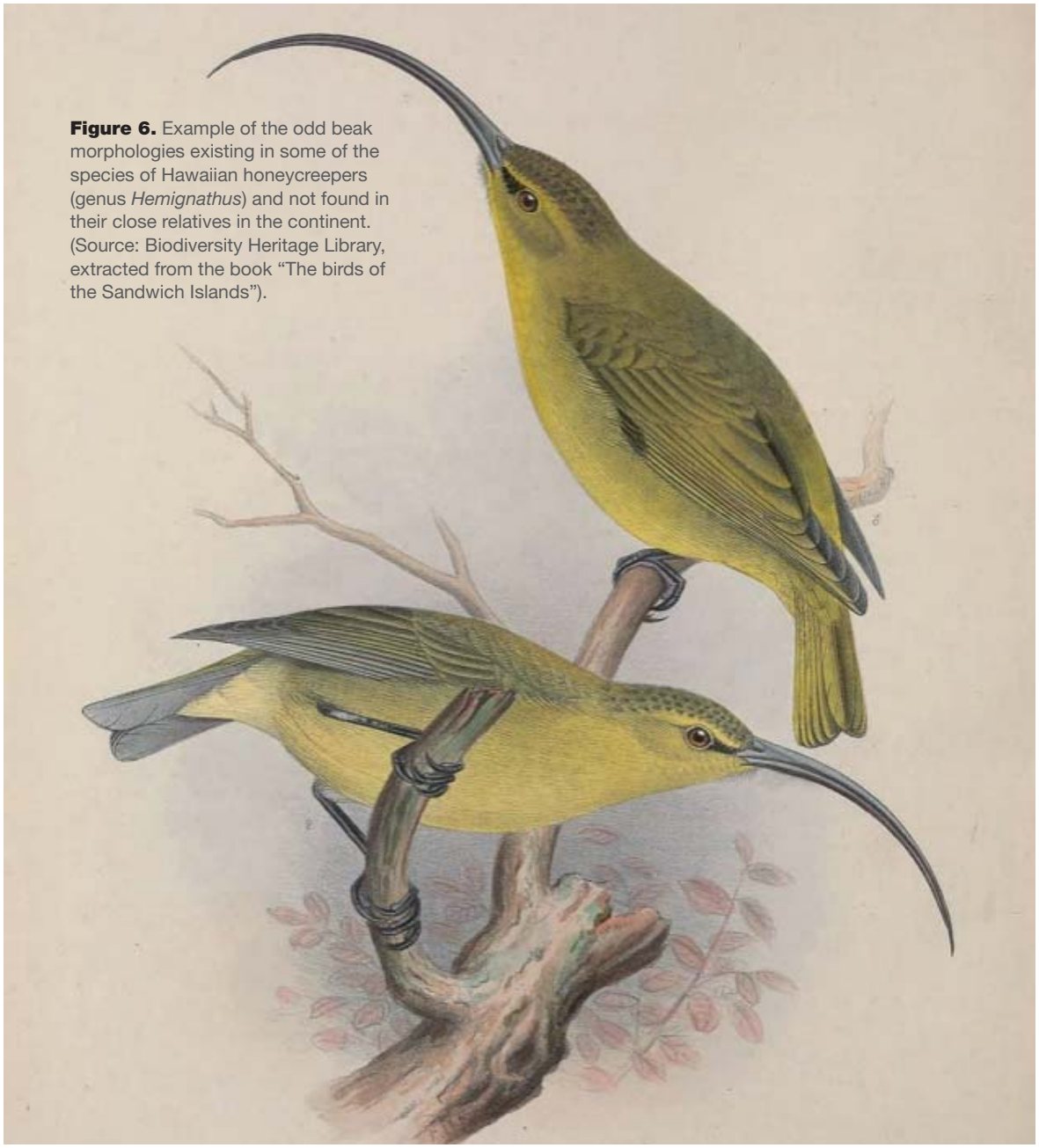

Figure 6. Example of the odd beak morphologies existing in some of the species of Hawaiian honeycreepers (genus *Hemignathus*) and not found in their close relatives in the continent. (Source: Biodiversity Heritage Library, extracted from the book "The birds of the Sandwich Islands").

morphospace, producing so many examples of sizes and shapes of island species radically divergent from those found in their continental close relatives (Fig. 4) (Losos & Ricklefs, 2009).

One of the most extreme examples of this is found in the comparison between the Hawaiian honeycreepers and their cardueline relatives in the continent. Continental carduelines have diversified into a very narrow space of bill shapes (essentially size variations of the standard finch-like morphology). However, the Hawaiian honeycreepers have greatly expanded this morphospace, replicating, only in Hawaii, most of the variation existing in the *entire* order of the passerines (Losos & Ricklefs, 2009; Lovette et al., 2002) (Fig. 5). Furthermore, the morphospace occupied by the honeycreepers contain morphologies that even go beyond the limits attained by any continental passerine (Fig. 6).

This is the case of the akiapoolau (*Hemignathus munroi*) that exhibits a very bizarre bill with a strong

asymmetry in the lengths of the upper and lower mandibles. This species occupies the niche of the absent woodpeckers in Hawaii, eating the insect larvae that live in the wood. However it uses a completely different feeding strategy: akiapoolaus extract larvae from woody surfaces by excavating holes using their short lower mandible and then extracting them using their long and curved upper mandible (Losos & Ricklefs, 2009).

Other examples in Hawaii are the *Tetragnata* spiders and the aglycyderid weevils that have produced diversifications greatly surpassing the amounts of diversification existing in similar groups anywhere in the World (Gillespie et al., 1994; Paulay, 1994).

But do insular groups necessarily diversify at higher rates and always expand their morphospaces compared to their continental closest relatives?

Comparing insular and continental groups is not an easy task and the problem is normally to obtain a substantial sampling of the actual diversity existing in the continent. But when such a study has been conducted on one of the greatest examples of island diversifications, the *Anolis* of the Caribbean, the results were quite unexpected. Despite of constituting one of the most prominent examples of island diversification, the disparities and the rates of phenotypic diversification in the *Anolis* of the Caribbean failed to be substantially different from those found in the continental *Anolis* (Pinto et al., 2008). Cases like this caution that a pattern of unequal extent of morphological diversification between islands and the continent might not be a general rule.

In fact, it is conceivable that “island-like” patterns could also appear in continental groups although they might be more difficult to detect due to the great geographic and taxonomic scales involved (Claramunt et al., 2012). For instance, extrinsic factors as climate change, orogenic processes and episodic massive extinction events can provide novel niches that can potentially spur high levels of species and phenotypic diversification also in the continent (Simpson, 1944). Moreover, intrinsic factors as the appearance of a key innovation can facilitate the access to a wider range of niches in continental groups producing patterns of phenotypic diversification similar to those expected in islands (Claramunt et al., 2012; Simpson, 1944). More empirical studies relying on well-sampled continental-island systems will be crucial to shed light on this question.

KEY INNOVATIONS AS DRIVERS OF EVOLUTIONARY DIVERSIFICATION

Unfortunately the notion of key innovation is one of the most ambiguous concepts in evolutionary biology. In the most traditional sense, key innovations are features that allow groups the possibility to interact with their environments in novel ways. One of the important aspects of key innovations is that this new interaction with the environment may allow the access to completely new types of resources (de Queiroz, 2002; Hunter, 1998; Losos, 2009). In a similar way as described for islands, key innovations

can shift groups into contexts of great ecological opportunity which, in turn, can prompt great amounts of phenotypic and species diversification (Galis, 2001). Classic examples of key innovations that drive evolutionary diversification are the evolution of feathers and wings in dinosaurs (which allowed flight; Hunter, 1998) and the appearance of flowers in plants (which allowed animal pollination; Vamosi & Vamosi, 2010). The concepts of key innovation and adaptive radiation are tightly linked in the literature (see Losos, 2009; 2010 and references therein). However, the failure of a key innovation in driving evolutionary diversification has been reported in a number of groups (Claramunt et al., 2012; Hodges, 1997; Price et al, 2010). Prominent examples of this are taxa like the aardvarks (burrowing nocturnal mammals of the family Orycteropodidae native to Africa) or even ourselves, humans. Both groups possess a great variety of key innovations and exhibit only low morphological and species diversity (Hunter, 1998; Wood & Collard, 1999). Such examples caution that the evolution of key innovations not necessarily always open the door to greater evolutionary diversification (Fürsich & Jablonski, 1984).

The failure of a key innovation in driving evolutionary diversification can be explained by a variety of reasons. One of them may be the particular ecological setting in which a key innovation originates (de Queiroz, 2002; Hodges, 1997). For example, the evolution of the pharyngeal jaw in African cichlids is a key innovation that has led to an adaptive radiation only in recently formed, competitor-free rift lakes (Liem, 1973). Also, intrinsic morphological or genetic constrains (lack of evolvability) have been proposed to explain instances of low evolutionary diversification following the acquisition of a key innovation (Price et al., 2010; Schluter, 2000). Such constraints, for example, have been invoked to explain why innovations in the jaw design of parrotfishes have not been followed by a great morphological diversification in this group (Price et al., 2010), or why geckos possessing toepads (another key innovation) have not experienced levels of phenotypic diversification comparable to padded *Anolis* (which are free of constrains) (Losos, 2010).

METHODOLOGICAL APPROACHES TO STUDY PHENOTYPIC AND SPECIES DIVERSIFICATION

Do island colonization or the acquisition of key innovations induce greater evolutionary diversification? To answer this question we need methods to quantify phenotypic and species diversity and compare these across different clades. From a naïve perspective we could just compare the amounts of phenotypic disparity and species richness between island and continental clades or between clades possessing a key innovation and clades lacking it. However, these comparisons would be flawed as they fail to incorporate a crucial component: the evolutionary relationships between the species or, in other words, the phylogeny. Fortunately, we nowadays live in a golden age in the development of methodological approaches that integrate phenotypic or ecologic data with phylogenetic information. In the following

pages I highlight some of the approaches widely used in the chapters of this thesis particularly focusing on the estimation of rates of phenotypic and species diversification.

RATES OF PHENOTYPIC DIVERSIFICATION

One of the possible consequences that follow island colonization or the acquisition of a key innovation is an increment in the rates of phenotypic evolution as groups rapidly fill the available ecological space in their early stages of diversification (Schluter, 2000). As already said, in a naïve approximation we could just compare the phenotypic disparity (e. g. the variance of the trait of interest) between, for example, island and continental clades. If we compute a greater phenotypic disparity in island clades would this allow us to conclude that these experienced higher rates of phenotypic diversification?

The answer is, not necessarily, as this comparison fails to take into account some key components that might obscure the outcome of such a comparison. These are the effects of time and shared history. The effect of time is very intuitive: the phenotypes in each of the clades have been diversifying since the time of their most recent common ancestor (MRCA). If the age of the MRCA of the island clade (the clade presenting the greatest phenotypic variance) is considerably older than the age of the MRCA of the continental clade, then this difference in the time of evolution in both clades may be enough to

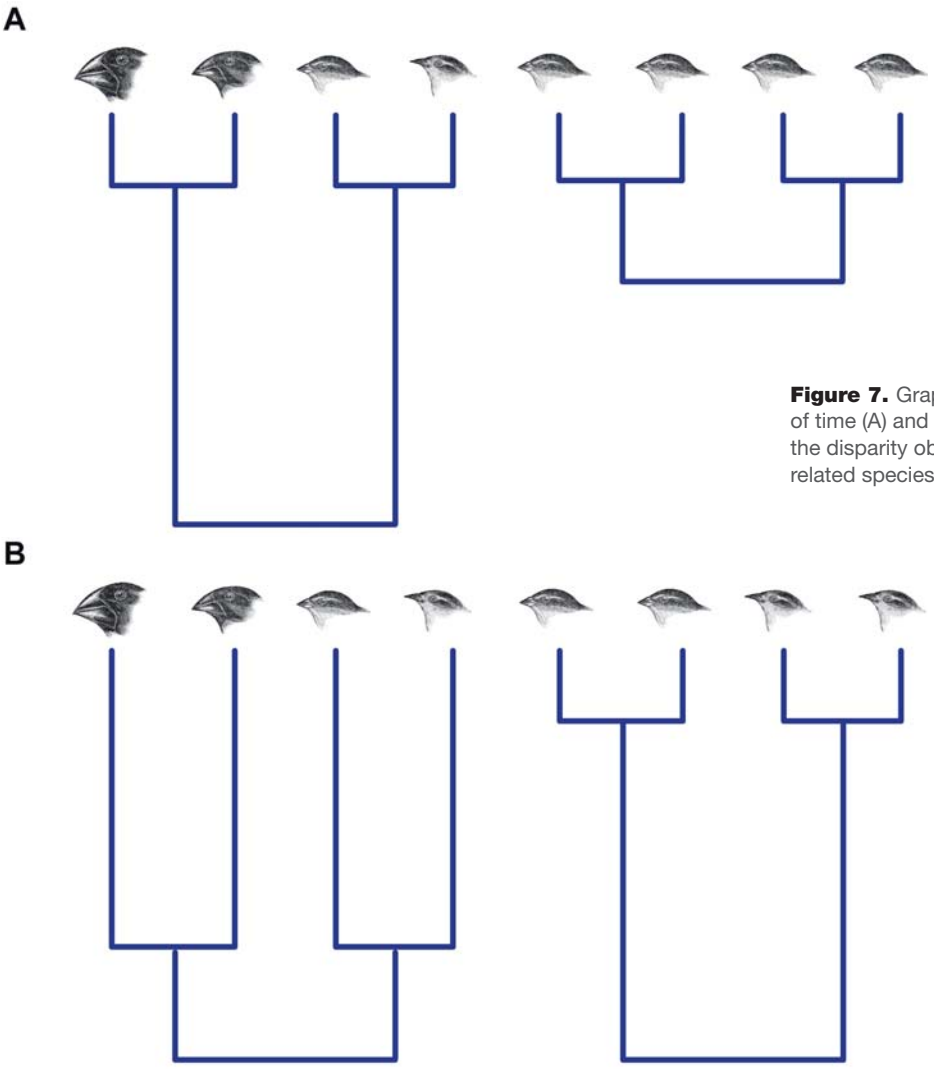

Figure 7. Graphs illustrating the effects of time (A) and shared ancestry (B) on the disparity observed between closely related species.

explain their differences in trait disparities (Wainwright, 2007). Simply, the longer a clade has to diversify, the greater the level of differentiation it can attain (Fig. 7A). But given the same ages of the MRCAs in both clades, we might also have another potential confounding effect: the amount of shared history between the species in each clade. The shorter the shared history between the species in a clade, the greater the expected phenotypic disparity in that clade (O'Meara et al., 2006; Wainwright, 2007). The reason is that the shorter the shared history between the species in a clade, the longer is their history as independent evolutionary units (each species has been evolving with its own independent evolutionary path for longer) (Fig. 7B).

Is therefore conceivable that the disparity measured in a clade depends not only on the rates of phenotypic evolution, but also is a function of the depth of the clade (the age of the MRCA) and the amount of shared history between lineages (Ackerly & Nyffeler, 2004; Garland et al., 1992; Mooers et al., 1999; O'Meara et al., 2006; Thomas et al., 2006). These three intuitive components are integrated in a very simple model of phenotypic evolution: the Brownian motion model (BM model).

According to this model, the amount of change of a trait over time can be described in the following simple equation (Butler & King, 2004):

$$dX(t) = \sigma dB(t)$$

Where $dX(t)$ is the change in the trait X over time, $dB(t)$ refers to "white noise" that is, independent and identically distributed random variables with mean 0 and variance dt, and finally, the parameter σ is the scaling parameter for the random distribution.

As specified in this equation, at any point in time:

- The character X can increase, decrease or stay the same.
- The direction and magnitude of change is independent of the current or past character states.
- The variance of change is constant and equals the variance of the random distribution (constant rate).

These basic points produce an interesting property when multiple Brownian processes are simulated from a common starting point (equivalent to multiple lineages evolving independently from a single ancestor): just as stated before, the expected variance (or disparity) will increase with time (Fig. 8) but moreover, the rate at which variance increases with time will be modulated by the parameter σ (the higher σ, the faster variance increases through time) (Fig. 9).

In other words, given the same amount of time, the clade presenting the greatest disparity will be the one presenting the highest σ. It is evident that the particular value of the parameter σ (also known as rate parameter) can be seen as a good proxy to the rates of phenotypic evolution. However, in the case

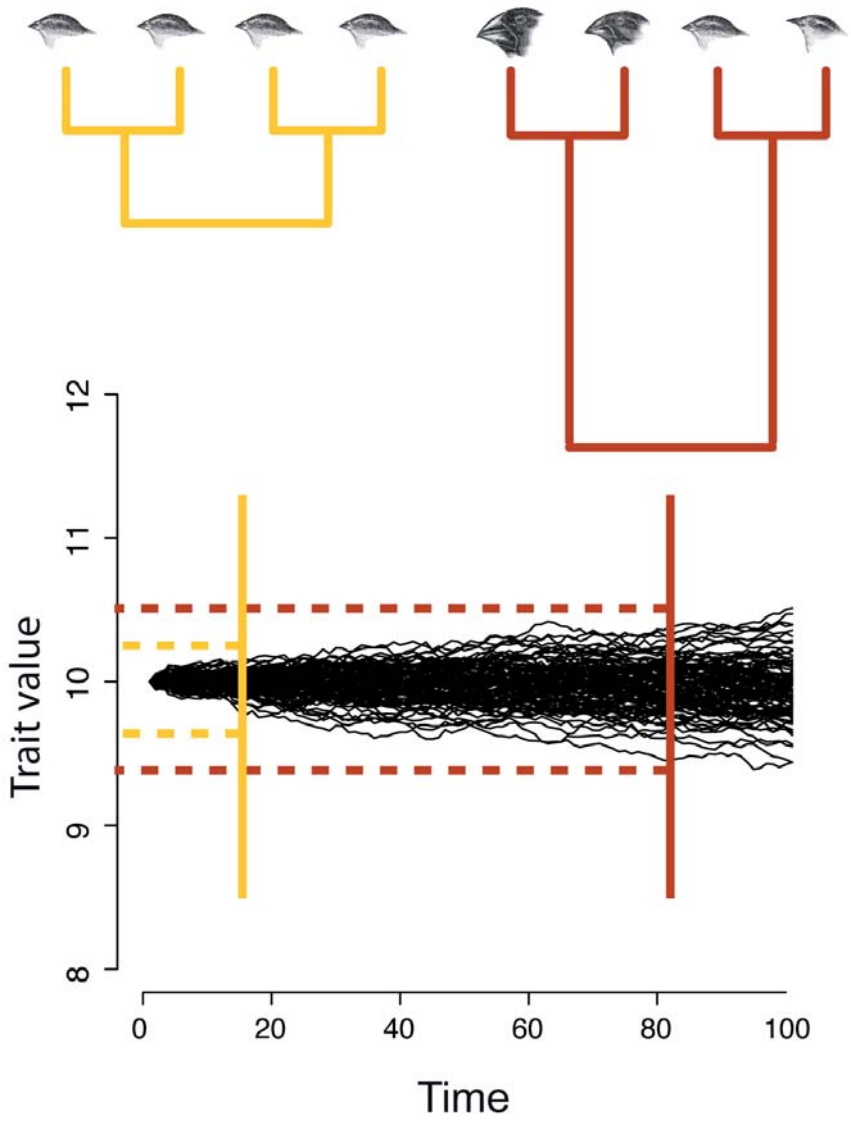

Figure 8. Visualization of a Browian motion process simulated 1,000 times from a common starting point. As it can be seen, one of the properties of this model is that, the longer the time, the greater is the variance observed in the trait values.

of having multiple lineages evolving along a phylogeny, their disparity at a given time will not only be a function of time and the rate parameter, but it will also be a function of the shared ancestry among taxa. This can be easily adapted into the BM model by means of the following equation (derived in O'Meara et al., 2006):

$$E(disparity) = \sigma^2[(1/N)tr(C) - (1/N^2)1'C1]$$

Where σ is the rate parameter, N the number of tips in the tree (number of species) and C is the phylogenetic variance-covariance matrix. This matrix is essential in many phylogenetic comparative methods as describes numerically the pattern of shared ancestry in a tree.

As seen in the above equation, the expected disparity is proportional to the rate parameter and to the difference between the time of the most recent common ancestor of the tree (equivalent to $(1/N)tr(C)$) and the amount of shared ancestry in the tree (the average entry of C, equivalent to $(1/N^2)1'C1$). In this way, we can see how the greater the shared ancestry, the lower the expected disparity.

It is important to mention that, despite of the random nature of BM, this model is not only valid for

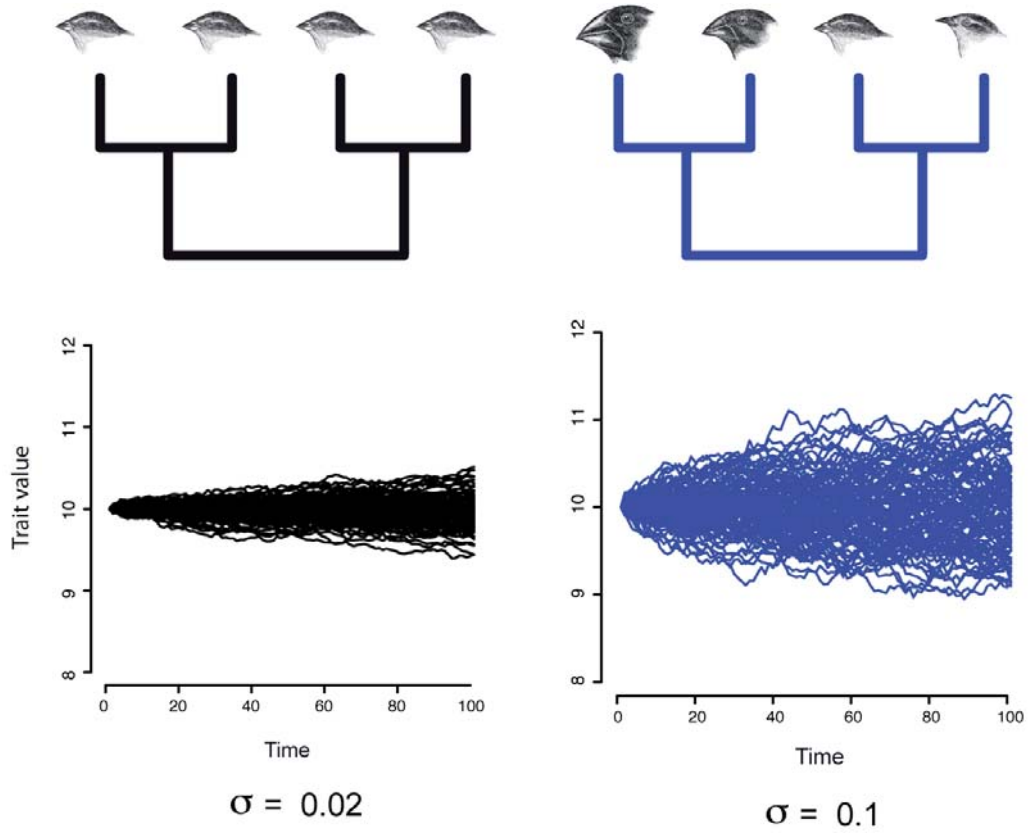

Figure 9. Plots of two independent Brownian motion processes that differ in the value of their rate parameter (σ). As observed, at any given time, the greatest variance will always be computed in the process presenting the higher σ.

describing purely random evolutionary process (e. g. genetic drift). It turns out to be also a reasonable good model to describe adaptive diversification with fluctuating optima (O'Meara et al., 2006).

RATES OF SPECIES DIVERSIFICATION

As in the case of phenotypic evolution, species richness might not be the whole story when it comes to compare rates of species diversification between groups (as for disparity, the older a clade is, the longer it has had to produce species). Therefore, we need methods to estimate rates of species diversification and, fortunately, the branching pattern in a phylogenetic tree contains information that can be used for this purpose (Ricklefs, 2007).

The estimation of rates of diversification from phylogenies strongly depends on the assumed diversification model. For instance, according to the simplest model of diversification, the Yule process, diversification rates are equivalent to a constant speciation rate without extinction. In this case, clade size will increase exponentially through time according to the following simple equation (Nee, 2006):

$$E(n) = \exp(\lambda t)$$

Where E(n) is the expected number of lineages in a clade, t is time and λ is a possible proxy for a diversification rate (as this parameter modulates the increment in species number through time).

However, if we add in the picture the component of extinction, the situation becomes more complicated. In this case the differences in species richness between two clades can result from differences in the rates of speciation, extinction or both (in addition to random fluctuations).

According to the simplest model of diversification involving extinction, the birth and death model, constant rates of speciation (λ) and extinction (μ) quantify the probabilities that a speciation or extinction event will occur within a particular interval of time (t) and the expected clade size varies following (Ricklefs, 2007):

$$E(n) = \exp[(\lambda - \mu)t]$$

In this case the rate of diversification corresponds to the difference between the speciation rate and the extinction rate (λ - μ). However, this model assumes that rates of speciation and extinction are the same for all lineages and do not vary through time and violations of this assumption can drastically bias our estimates in the diversification rates (Ricklefs, 2007). This issue is particularly problematic in the frame of the evolutionary questions explored in this thesis, as after island colonization or after the acquisition of a key innovation we expect a pattern that explicitly implies non-constant rates of diversification: high rates are expected at the onset of the diversification with a slow down towards the present (an "early burst" pattern) (Schluter, 2000). Therefore, in order to calculate and compare rates of diversification first we need to determine the right model of diversification in each particular case (assuming either constant and non-constant rates).

There are different approaches designed to test whether rates are constant or vary through time. One of the most classic ones is the Gamma statistic (Pybus & Harvey, 2000). This summary statistic quantifies the position of the nodes in a tree compared to the situation of constant-rates defined by the Yule process. Phylogenies with negative gamma values indicate that most of the nodes are situated close to the root of the tree which is interpreted as a signature of a slowdown in speciation rates. However, one of the problems of this approach (among others) is that it fails to detect decays in speciation rates in situations of non-zero extinction rates (Rabosky & Lovette, 2008). A second approach is based on the comparison of the likelihoods of the observed internode distances of a tree when fitted to different models assuming constant or non-constant diversification (Hey, 1992; Nee, 2006; Nee et al., 1994; Nee et al., 1992; Rabosky, 2006; Rabosky & Lovette, 2008). Nonetheless, a problem with this approach is that it tends to produce unrealistically low estimates of extinction rates (Rabosky & Lovette, 2008). Finally, in recent times, coalescence-based methods have been developed to distinguish between alternative models of diversification (Morlon et al., 2010). These methods model the internode distances of a phylogeny assuming that they are distributed according to a standard coalescent approximation (Griffiths & Tavare, 1994). This has the advantage of modeling species diversity from the present to the past assuming that it can take any value at any point in time (including a situation of constant diversity through time). It can also easily accommodate incomplete-sampled phylogenies (another major source

of bias in diversification analyses) since the coalescence theory stems from the theory of samples (Morlon et al., 2010).

INTRODUCTION TO OUR MODEL ORGANISMS: THE GECKOS OF ARABIA AND AUSTRALASIA.

Geckos (infraorder: Gekkota) with more than 1,500 species and 118 genera constitute one of the most diverse groups of reptiles (comprising around 25% of all lizard species) (Gamble et al., 2012). According to most of the molecular phylogenies spanning all Squamata (lizards and snakes), geckos appear as the sister group to all other squamates with the exception of dibamids (Hedges & Vidal, 2009). Their crown age varies depending on the different dating estimates but most of them coincide in placing the oldest split of all extant Gekkota somewhere during the Cretaceous (145 to 66 Ma) (Vidal & Hedges, 2005; Wiens et al., 2006; Hugall et al., 2007; Gamble et al., 2008). The oldest fossils attributable to gekkotans also come from this age although their phylogenetic placement as crowngroup or stemgroup gekkotans is still a matter of debate (Daza et al., 2014).

One of the most remarkable features of geckos was already noted by Aristotle more than 2,000 years ago. He wrote referring to the gecko-lizard: “It can run up and down a tree in any way, even with the head downwards” (Aristotle/Thompson, 1918). Aristotle refereed to the famous ability of geckos to defy

Figure 10. Detailed view of the toepads in the genus *Hemidactylus* (Foto credit: Salvador Carranza)

gravity as they run even on smooth vertical surfaces. We now know that the secret of such capabilities lies on a very special structure existing under their digits: the adhesive toepads (Fig. 10). These consist of a series of modified lamellae (also known as scansors), each one covered with millions of microscopic hair-like bristles called setae. First, it was hypothesized that these setae produced adhesion acting like micro-hooks, catching on surface irregularities (microinterlocking) (Dellit, 1933). However, the true mechanism of adhesion was far more amazing: setae are so thin and small that the atoms at the tip

of each seta are able to establish weak chemical bonds (Van der Waals forces) with the atoms of the substrate. Is then the sum of all these weak forces over the total surface of each of the toes what produces the extraordinary adhesive capabilities of geckos (Autumn & Peattie, 2002; Hiller, 1968).

Adhesive toepads are present in 60% of the species of geckos and have been acquired several independent times through their evolutionary history (Gamble et al., 2012). It is obvious that such an extraordinary mechanism grant padded geckos the possibility of interacting with the environment in a completely different way compared to pad-lacking species, allowing a more efficient use of highly tridimensional habitats (as arboreal habitats). For this reason, toepads are a paradigmatic example of key innovation and its acquisition in several lineages of geckos has been proposed as a crucial factor that explains the great diversification experienced in this group (Losos, 2009; 2010).

Another remarkable aspect of geckos is its great capacity of dispersal. This is reflected in its worldwide distribution, inhabiting all continents except Antarctica. Among the great dispersal abilities of geckos, we highlight their capacity to engage long distance dispersal events (Gamble et al., 2011), which make them good colonizers of remote islands (Austin et al., 2004; Bauer, 1994; Carranza et al., 2000; Rocha et al., 2007) and therefore also make them a good model to study evolutionary processes taking place in islands.

In this thesis we explored the effects of island colonization and other sources of ecological opportunity (as the acquisition of adhesive toepads) using three genera of Arabian geckos and all diplodactyloid geckos of Australasia as models. In the following pages I provide a brief introduction to these groups of geckos from a taxonomic, ecologic and biogeographic perspective.

Figure 11. *Pristurus carteri* from Oman (Foto credit: Salvador Carranza).

THE GECKOS OF ARABIA AND THE SOCOTRA ARCHIPELAGO

We explored the effects of island colonization and key innovations in three different genera belonging the mainland-island system of Arabia-Socotra: *Pristurus*, *Hemidactylus* and *Haemodracon*.

The genus *Pristurus* also known as semaphore geckos, belongs to the family Sphaerodactylidae although its phylogenetic position within this family is uncertain. It contains at least 23–26 species (Arnold, 2009; Sindaco & Jeremčenko 2008; Uetz 2014) and unlike most of geckos, they are diurnal and heliothermic. A remarkable particularity of this genus is that most of the species present a very conspicuous and elaborate signaling consisting in body and tail movements. These features are not very usual among geckos, as most of them are nocturnal and communicate predominantly by means of vocalizations or chemical cues. In fact, most of the species in *Pristurus* behave more like desert agamid lizards than typical geckoes (Arnold, 2009). They lack adhesive toepads, are sit and wait predators and occupy a great variety of habitats, from sandy grounds to rocky areas. Most of the species of semaphore geckos are found in northeast Africa (7 species with 4 endemics), the Arabian Peninsula (14 species with 12 endemics) and the Socotra Archipelago (7 endemic species), with one of the Arabian

Figure 12. *Hemidactylus pumilio* from the island of Socotra (Foto credit: Roberto Sindaco).

species *P. rupestris*, extending into the lowland coastal regions of Iran. As a striking biogeographic oddity, an isolated species of *Pristurus*, *P. adrarensis*, is found in a very small area in Mauritania 4,700 km away from its main distribution area in the East.

The genus *Hemidactylus* currently consists of 124 named species belonging to the family Gekkonidae distributed across all tropical and subtropical continental landmasses, including hundreds of intervening oceanic and continental islands (Sindaco & Jeremčenko, 2008; Uetz & Hosec, 2014). According to the latest molecular phylogenies, *Hemidactylus* is sister to the genus *Cyrtodactylus* (Gamble et al. 2012). Although a complete phylogeny of *Hemidactylus* is still lacking, partial molecular phylogenies indicate that all the species analyzed to date can be assigned to four phylogenetically divergent clades: 1) the

African-Atlantic clade; 2) the *H. angulatus* clade; 3) the tropical clade; and 4) the arid clade (Bansal & Karanth 2010; Bauer et al., 2010; Carranza & Arnold 2006, 2012; Šmíd et al., 2013a,b). In this thesis we focus in the last of the clades, by far the best known of the clades in this genus (Busais & Joger 2011a,b; Carranza & Arnold 2012; Moravec et al., 2011; Šmíd et al., 2013b). With more than a third of all species, the arid clade is currently the most speciose of the four main *Hemidactylus* clades.

All the species of the Arid clade of *Hemidactylus* are strictly nocturnal and occupy a great variety of habitats in Arabia, from extremely arid gravel plains and rocky outcrops to subtropical forest and high mountain areas. Across these varied landscapes the different species have adapted to exploit different

Figure 13. *Haemodracon riebecki* from the Socotra Archipelago (Foto credit: Roberto Sindaco).

spatial niches like the ground, rocks, cliffs, trees and some of the species can occupy man made constructions like walls and houses.

They possess highly developed toepads, although their relative area under the toes varies from one species to another.

Finally, the genus *Haemodracon* belongs to the family *Phyllodactylidae* and is formed by two species endemic to the Archipelago of Socotra. Phylogenies clearly indicate that the genus *Haemodracon* is sister to the Arabian and Asian radiation of geckos of the genus *Asaccus* (Gamble et al., 2012). The two species present very different sizes, with *Ha. riebeckii* being much larger than *Ha. trachyrhinus*. Both species have well-developed toepads of the phylodactylid type and present highly distinct habitats. *Ha. riebecki* normally is found inhabiting cliffs, rocks, large boulders, caves and tree trunks, while *Ha. trachyrhinus* is more ground-dweller or is found on tree branches and bushes.

Figure 14. *Strophurus sp.* from Western Australia. A representative of the Australasian diplodactyloid geckos (Foto credit: Joan Garcia-Porta).

THE GECKOS OF AUSTRALASIA

For comparative purposes, aside of studying the geckos from Arabia and the Socotra Archipelago, we also worked on another interesting mainland-island system: the diplodactyloid geckos from Australasia. These form a radiation of more than 200 species distributed across Australia, New Caledonia and New Zealand (Uetz & Hosec, 2014). This group contains three independent families: Diplodactylidae, Carphodactylidae and Pygopodidae and are phylogenetically placed as sister group of the rest of the geckos (Gamble et al., 2012). Aside of having colonized independently two island archipelagoes, this group is also remarkable for its great ecological and morphological diversity (Oliver & Sanders, 2009). Most—but not all—species in this group possess one of two putative key innovations in the form of adhesive toepads or in the form of another astonishing innovation: an elongated, near limbless snake-like phenotype (Fig. 15) (Cogger, 2014; Wilson & Swan, 2013; Hitchmough, 1997). This phenotype consists of an elongated body with no forelimbs and only small scaly flaps as hindlimbs and is found in all species belonging to the family Pygopodidae (Shine, 1986). There are a number of advantages for snake-like species, among them: 1) more efficient locomotion; 2) the ability to use narrow spaces

Figure 15. The snake-like phenotype in the Australasian diplodactyloid geckos *(Delma pax*) (Foto credit: Brian Bush).

like crevices for obtaining food, thermoregulation, or shelter, 3) the ability to burrow in soil or sand; and often, 4) the ability to ingest prey bigger than themselves (Gans, 1975; Shine, 1986). The presence in this group of two independent key innovations and two instances of island colonization offers a great opportunity of study the contribution of these alternative sources of ecological opportunity at driving evolutionary diversification.

OBJECTIVES

GENERAL OBJECTIVES

The main aim of this thesis is to examine the extent in which ecological opportunity—specifically, the colonization of islands (in oceanic or continental settings) and the evolution of key innovations—has driven phenotypic and species diversification in the geckos of Arabia and the Socotra Archipelago and compare it with another continental-island system: the diplodactyloid geckos in Australasia.

There are eight general questions that this thesis aims to shed light on:

1. Do insular groups experience an expansion of the morphospace compared to continental groups?
2. Do insular groups experience accelerated rates of phenotypic and species diversification compared to their continental close relatives?
3. Which are the relative contributions of key innovations and island colonization at driving evolutionary diversification?
4. Do different traits respond in the same way to the ecological opportunity provided by islands?
5. By which processes (dispersal, intra-island diversification or vicariance) continental islands build up their diversity?
6. Which is the relative contribution of dispersal, intra-island diversification and vicariance at producing the niche structure observed in islands?
7. Do different groups, diversifying in the same island, follow the same diversification paths?
8. How diversification proceeds in continental "island-like" environments?

SPECIFIC OBJECTIVES

1. Explore the effects of the colonization of Socotra and Abd al Kuri in the phenotypic diversification experienced by the *Hemidactylus* geckos belonging to the Arid clade. Particularly focusing on whether island species expand the morphospaces occupied by their close relatives in the continent and whether island lineages experience accelerations in the rates of phenotypic diversification.

2. Compare the effects of two key innovations (toepads and a snake-like phenotype) and island colonization (the colonization of New Caledonia and New Zealand) at producing the great diversification experienced in the Australasian diplodactyloid geckos. Particularly study whether insular groups or groups with a key innovation accelerate their rates of phenotypic and species diversification in comparison to continental groups or groups lacking key innovations.
3. Assess the relative contributions of vicariance, dispersal and *in situ* diversification in the species assembly of the geckos of the Socotra Archipelago, also study their relative role at producing the niche structure shown by geckos at different scales.
4. Examine whether all independent instances of *in situ* diversification in the geckos of the Socotra Archipelago follow equivalent paths of macroecologic and microecologic diversification.
5. Increase the sampling and advance in the understanding of the genetic diversity existing within *Pristurus rupestris*.
6. Understand the effects of the Arabian mountain ranges at producing the phenotypic and species diversity existing within the "subspecies" *P. rupestris rupestris*.

RESUMEN DE LAS PUBLICACIONES

CAPITULO 1:

Efectos de colonización de islas en la diversificación evolutiva: Evidencia en los gecos Hemidactylus del Archipiélago de Socotra

Las características únicas que tan a menudo presentan las especies insulares normalmente se explican por las grandes diferencias existentes entre ambientes insulares y continentales. En islas, niveles menores de competencia interespecífica y depredación a menudo permiten a los grupos insulares evolucionar en nuevas direcciones, expandiendo el espacio fenotípico que usan sus parientes cercanos en el continente. En este estudio exploramos si esta hipótesis puede ser validada en los

patrones de diversificación encontrados en un sistema continente-isla muestreado casi por completo: los *Hemidactylus* pertenecientes al clado Árido. Este clado se encuentra ampliamente distribuido a través de Africa Oriental y Medio Oriente, llegando hasta el Archipiélago de Socotra tres veces de manera independiente. Esto permite estudiar la relación entre la colonización de islas y la diversificación morfológica en tres eventos de colonización independientes. Nuestros resultados muestran que características diferentes (tamaño del cuerpo y proporciones de la cabeza) e islas diferentes (Abd al Kuri y Socotra) difieren en sus patrones y procesos de diversificación. El tamaño del cuerpo experimentó el nivel mas elevado de disparidad después de la colonización de islas, produciendo el tamaño más grande y más pequeño de toda la radiación. Estos niveles elevados de disparidad son la consecuencia del único evento de especiación intra-isla que tuvo lugar en Abd al Kuri y del inicio de la diversificación en la isla de Socotra. Es remarcable el caso de las dos especies de Abd al Kuri que presentan la disparidad máxima detectada en toda la radiación. Tanto en Abd al Kuri como en Socotra los niveles elevados de disparidad fueron consecuencia de tasas aceleradas de evolución fenotípica, también posiblemente involucrando la existencia de distintos óptimos de tamaño. Esto contrasta con nuestros resultados para las proporciones de la cabeza que muestran tasas de evolución equivalentes entre linajes insulares y continentales. Sin embargo, en este caso, la existencia de regímenes selectivos divergentes operando sobre las proporciones de la cabeza generaron niveles elevados de disparidad en Abd al Kuri. A pesar de las diferencias mencionadas entre diferentes caracteres e islas, los resultados de este estudio son consistentes con la existencia de un “efecto isla” sobre la diversificación fenotípica de las especies insulares de este grupo.

CAPITULO 2:

Innovaciones clave y colonización de islas como motores de diversificación evolutiva: un test comparativo con los gecos diplodactiloides australasiáticos

La adquisición de innovaciones clave y la invasión de nuevas áreas constituye dos de los mayores procesos facilitadores de oportunidad ecológica y la subsecuente diversificación evolutiva. En este estudio, utilizando una gran radiación de lagartos como modelo, los gecos diplodactiloides australasiáticos, exploramos los efectos de dos innovaciones clave (los lamelas adhesivas y el fenotipo en forma de serpiente) y la invasión de dos ambientes (colonización de islas) sobre la diversificación fenotípica y de especies en este grupo. No encontramos evidencia de que las lamelas adhesivas produjeran niveles significativamente grandes de diversificación evolutiva, lo cual pone en cuestión la hipótesis de que estas estructuras son las responsables de la radiación extensiva que caracteriza los gecos. Sin embargo, el impacto más claro sobre la diversificación fenotípica fue la colonización de Nueva Zelanda y Nueva Caledonia, dado que estuvieron asociadas con importantes aceleraciones en la

tasa de evolución del tamaño del cuerpo y diversificación de especies. Esto muestra que la colonización de nuevos ambientes puede generar elevados niveles de diversificación evolutiva, con o sin la existencia de innovaciones clave en un linaje. Por esta razón, futuros estudios orientados a estudiar el nexo entre las innovaciones clave y la diversificación evolutiva posterior deben asegurarse de que es la innovación clave en particular y no la colonización de nuevas áreas (actuando como factor de confusión) la que ha impulsado la diversificación.

CAPITULO 3:

Ensamblaje de especies y estructura de nicho en el Archipiélago de Socotra: los gecos no se rigen por un único patrón

Las biotas insulares son notorias por sus ejemplos de grandes diversificaciones, normalmente impulsadas por la gran oportunidad ecológica que las islas proveen. Pero es importante tener en cuenta también que las diversificaciones en islas pueden producirse a lo largo de gradientes ambientales (nichos beta) o involucrando una repartición de recursos locales ocupando distintos micro-hábitats (nichos alfa). Además, al margen de la diversificación *in situ*, otros procesos como dispersión o vicarianza pueden contribuir, no sólo a generar la diversidad en las islas, sino que también pueden contribuir a estructurar las especies de una isla a distintas escalas. En este estudio, nuestro objetivo fue explorar cómo toda la diversidad de gecos en el Archipiélago de Socotra (consistiendo en tres géneros diferentes: *Pristurus*, *Haemodracon* y *Hemidactylus*) se ha generado a partir de los procesos anteriormente mencionados. Además, también hemos investigado cómo estos procesos juegan un papel en la estructuración ecológica dentro de las islas. Nuestros resultados muestran como la mayor parte de la diversidad de gecos del archipiélago es la consecuencia de diversificación *in situ* y tuvo lugar cuando la isla ya se encontraba completamente separada del continente. No obstante, diferentes grupos presentaron patrones sustancialmente diferentes de estructuración morfológica y climática. Mientras que en *Haemodracon* y en *Hemidactylus* las especies tendían a diversificar en tamaños de cuerpo y a presentar un gran conservatismo climático, en *Pristurus* emergía un patrón completamente opuesto, en el que la gran mayoría de diversificación entre especies cercanas tuvo lugar a lo largo de ejes climáticos y no involucrando niveles apreciables de diversificación en tamaños. Esto es consistente con las tasas de diversificación del tamaño del cuerpo estimadas para los distintos géneros, que mostraron como *Pristurus* presentaba los niveles mas bajos de evolución fenotípica de los tres géneros. Estos resultados muestran como distintos grupos diversificando en las mismas islas pueden diferir extensivamente en sus patrones de estructuración de nicho y ponen en cuestión la existencia de una teoría general que pueda ser aplicada para una gran variedad de grupos.

CAPÍTULO 4.1:

Relaciones filogenéticas en el grupo de gecos semáforo (Squamata: Sphaerodactylidae: Pristurus) evaluando la taxonomia en Pristurus rupestris.

Se ha inferido una filogenia molecular de los gecos del género *Pristurus*, pertenecientes a la familia de los sphaerodactílidos. Esta filogenia se basó en un alineamiento de 1,845 pares de bases consistente en cinco genes concatenados: uno mitocondrial (*12S*) y cuatro nucleares (*acm4, cmos, rag1, rag2*), los cuales fueron amplificados para 80 especímenes pertenecientes a 18 especies (de las 23-26 especies existentes en este género). Igualmente se incluyeron tres subespecies de *P. rupestris*. Los resultados indican que *P. rupestris* es polifilético y que esta formado por dos linajes extremadamente divergentes: el clado oriental, distribuido desde Irán hasta las montañas Hajar de Oman y Emiratos Arabes. Y un clado occidental distribuido desde la costa central de Oman hasta Jordania, pasando por Arabia Saudita y Yemen. Las redes de haplotipos inferidas para los cuatro genes nucleares muestran que los clados oriental y occidental están genéticamente muy diferenciados, no compartiendo ningún alelo. Además los dos clados se encuentran diferenciados morfológicamente, aunque ningún conjunto particular de caracteres pudo ser caracterizado como diagnóstico. Basado en los análisis moleculares de los especímenes de la localidad tipo de *P. rupestris rupestris*, el nombre de *P. rupestris* debería ser aplicado solamente para el clado oriental. El nombre que debería darse al clado occidental no puede clarificarse hasta que datos morfológicos y genéticos de los "*P. rupestris*" de la localidad de Bosaso (Somalia) estén disponibles para comparar. Mientras tanto preferimos denominar esta especie como *Pristurus sp 1* a la espera de nuevos datos y análisis. El árbol filogenético de *Pristurus* apoya la hipótesis de que *P. celerrimus* es especie hermana de todas las otras especies de *Pristurus*. Además, según nuestros resultados, la colonización del Archipiélago de Socotra por *Pristurus*, tuvo lugar como mínimo dos veces de manera independiente.

CAPITULO 4.2:

Diversificando en las "sky islands" de Arabia: el caso de la diversidad críptica dentro de la subespecie Pristurus rupestris rupestris

La biodiversidad del planeta se encuentra importantemente subestimada. Esto se aplica tradicionalmente sobretodo a les zonas tropicales, pero también podría ser el caso de zonas áridas, no tradicionalmente consideradas como reservorios de diversidad. En estas regiones, la existencia de regiones montañosas puede tener un papel muy importante como generadores de diversidad, probablemente escondiendo una gran cantidad de diversidad desconocida. Este trabajo se centra en uno de estos casos de diversidad desconocida: el complejo de especies existente dentro de la subespecie *Pristurus rupestris rupestris*,

distribuido en las montañas Hajar en el suroeste de Arabia. De acuerdo con nuestros resultados, esta subespecie en realidad esta formada por 14 especies, muchas de ellas presentando grandes divergencias, las cuales se solapan ampliamente con las divergencias encontradas para otras especies de gecos. Se trata probablemente de la diversificación de vertebrado más grande de toda Arabia. Nuestros análisis revelaron que la mayor parte de los eventos de diversificación, mayoritariamente, se encuentran después de la fase orogénica de las montañas. El modelo de diversificación que se ajusta más a nuestros resultados es un modelo consistente con pulsos de expansiones y retracciones de las distribuciones de las especies. Estos pulsos dándose entre los dos grandes bloques aislados entre montañas explica, por lo menos, parte de la diversidad observada. Encontramos niveles bajos de diversificación fenotípica lo cual concuerda con la baja simpatría existente entre las especies. Sin embargo, la variación de la forma del cuerpo parece estar asociada a un gradiente altitudinal, encontrándose en las especies habitantes de hábitats elevados morfotipos más robustos. Por último, la dinámica de diversificación de especies aparece como claramente expansiva, indicando que la diversificación en este grupo aun se encuentra en una fase activa.

DIRECTOR'S REPORT

ADVISOR'S REPORT ON THE PUBLICATION STATUS OF THE RESULTS AND THE IMPACT FACTOR OF THE PUBLISHED PAPERS

Dr. Salvador Carranza, supervisor of the doctoral thesis prepared by Joan Garcia-Porta, entitled "Evolution and diversification of the geckos of the Arabian Peninsula and Socotra Archipelago, compared to other mainland-island systems", reports that the thesis is made as a compendium of five publications presented in Chapters 1 – 4 of the thesis.

CHAPTER 1

Garcia-Porta, J.; Šmíd, J.; Fasola, M. & Carranza , S.

Testing the effects of island colonization on morphological diversification: insights from the *Hemidactylus* geckos from the Socotra Archipelago

The manuscript corresponding to Chapter 1 has been finished and is now in the phase of friendly review and it will be submitted to the journal *Evolution*. This journal has in the latest available edition of the Journal Citation Reports 2012 an impact factor of 4.864 and is in the first quartile (9 of 47) of the area "Evolutionary Biology". *Evolution* is a referent in the field of evolutionary biology.

CHAPTER 2

Garcia-Porta, J. & Ord, T. (2013). Key innovations and island colonization as engines of evolutionary diversification: a comparative test with the Australasian diplodactyloid geckos. *Journal of Evolutionary Biology*, 26: 2662-2680.

Journal of Evolutionary Biology has in the latest available edition of the Journal Citation Reports 2012 an impact factor of 3.479. This journal is in the second quartile (17 of 47) of the area "Evolutionary Biology" and in the first quartile (30 of 136) of the area "Ecology". *Journal of Evolutionary Biology* is a referent in the use of phylogenies for evolutionary studies.

CHAPTER 3

Garcia-Porta, J.; Morales, H.; Gómez-Díaz, E.; Sindaco, R. & Carranza, S. (in preparation)
Species assembly and niche structure in the Socotra Archipelago: not a single pattern to rule all geckos.

The manuscript corresponding to chapter 3 is currently at the last stage of preparation and therefore it is still too early to indicate to which journal is going to be sent. However, it is planned that it will be submitted to a journal of high impact factor belonging to the first quartile.

CHAPTER 4.1

Badiane, A., Garcia-Porta, J., Červenka, J., Kratochvíl, L., Sindaco, R., Robinson, M.D., Morales, H., Mazuch, T., Price, T., Amat, F., Shobrak, M.Y., Wilms, T., Simó-Riudalbas, M., Ahmadzadeh, F., Papenfuss, T.J., Cluchier, A., Viglione, J. & Carranza, S. (2014).
Phylogenetic relationships of Semaphore geckos (Squamata: Sphaerodactylidae: *Pristurus*) with an assessment of the taxonomy of *Pristurus rupestris*. *Zootaxa*, 3835(1), 33-58.

Zootaxa has in the latest available edition of the Journal Citation Reports 2012 an impact factor of 0.974. This journal is in the third quartile (83 of 151) of the area "Zoology". *Zootaxa* is mega-journal referent for zoological Taxonomy and Systematics. With 2135 papers published in 2013 (108 monographs of more than 60 pages) and 23,373 new taxa published till the end of 2012 (410 of Reptilia), it is the journal that is contributing most to a better understanding and cataloguing of the world's animal biodiversity.

CHAPTER 4.2

Garcia-Porta, J., Simó-Riudalbes, M., Robinson, M. & Carranza, S. (in preparation).

Diversiying in the sky islands of Arabia: The case of the hidden diversity within the subspecies *Pristurus rupestris rupestris*

The manuscript corresponding to chapter 4.2 is currently at the last stage of preparation and therefore it is still too early to indicate to which journal is going to be sent. However, it is planned that it will be submitted to a journal of high impact factor belonging to the first quartile.

Barcelona, July 2014

Salvador Carranza Gil-Dolz del Castellar

ADVISOR'S REPORT ON THE CONTRIBUTION OF THE PhD CANDIDATE IN THE PAPERS

Dr. Salvador Carranza, as advisor of the PhD thesis of Joan Garcia-Porta entitled "Evolution and diversification of the geckos of the Arabian Peninsula and Socotra Archipelago, compared to other mainland-island systems", I hereby present the following report regarding the contribution of the PhD candidate in each one of the five presented chapters:

CHAPTER 1

JGP and SC conceived the study. SC, JS and MF collected samples in the field and/or provided tissue samples. JS and SC obtained the sequences. JGP, JS and SC obtained the morphological data. JGP carried out all the analyses and drafted the manuscript. All authors read and approved the final manuscript.

CHAPTER 2

JGP and TO conceived the study. JGP assembled the dataset, carried out all the analyses and drafted the manuscript. Both authors approved the final manuscript.

CHAPTER 3

JGP and SC conceived the study. RS and SC collected samples in the field and/or provided tissue samples. HM, EGD and SC obtained the sequences. JGP obtained the morphological data. JGP carried out all the molecular and morphological analyses and drafted the manuscript. SC revised the manuscript.

CHAPTER 4.1

SC and JGP conceived the study. All authors collected samples in the field and/or provided tissue samples. AB, HM and SC obtained the sequences. SC, JGP and AB carried out the analyses. SC and JGP drafted the manuscript. All authors read and approved the final manuscript.

CHAPTER 4.2

JGP and SC conceived the study. JGP, SC and MR collected samples in the field and/or provided tissue samples. MSR and SC obtained the sequences. JGP and MSR obtained the morphological data. JGP carried out all the analyses and drafted the manuscript. SC revised the manuscript.

Barcelona, July 2014

Salvador Carranza Gil-Dolz del Castellar

CHAPTER 1

TESTING THE EFFECTS OF ISLAND COLONIZATION ON MORPHOLOGICAL DIVERSIFICATION: INSIGHTS FROM THE HEMIDACTYLUS GECKOS FROM THE SOCOTRA ARCHIPELAGO

Photo credit: Fabio Pupin

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ABSTRACT

The distinctiveness of many insular species assemblages is usually explained by the prominent ecological differences existing between island and mainland environments. In islands, the lower levels of inter-specific competition and predation often enable groups the possibility to evolve into novel directions, expanding the phenotypic space used by their continental relatives. Our study explored whether this scenario could be detected in the patterns of phenotypic diversification experienced in a nearly completely sampled mainland-island system, the geckos *Hemidactylus* of the Arid clade. This clade, widely distributed across Eastern Africa and Middle East, reached the Archipelago of Socotra three times independently, providing the opportunity to explore the effects of these island colonizations on the phenotypic diversification experienced by the group. Our results show that different traits (size and head proportions) and different islands (Abd al Kuri and Socotra) differed in their patterns and processes of phenotypic diversification. Body size experienced the highest levels of trait disparification after island colonization, producing the biggest and the smallest size in the radiation. This size differentiation was the consequence of the unique intra-island speciation occurring in Abd al Kuri and, although with lesser magnitude, the first speciation event occurring in Socotra. In both cases size diversification was mediated by high rates of phenotypic evolution possibly also involving different size optima. This contrasts with our results for head proportions that show equal rates of diversification in islands and in the continent. However, in this case, the existence of divergent selective regimes operating on head dimensions produced high disparities in Abd al Kuri. Despite these differences among traits and islands, our results are consistent with an "island effect" on phenotypic diversification that led to an increased trait variation after island colonization.

Keywords: island colonization; body size; head proportions; morphospace; disparity; evolutionary rate; gecko.

INTRODUCTION

Islands are usually the home of singular faunas and floras. These singularities exist both at a species level, with many instances of island species with morphologies greatly departing from those found in their continental relatives (e. g. the dwarf elephants of the Mediterranean islands) (Raia & Meiri, 2006), and at a community level, with many examples of island biotas occupying a wider (or different) array of niches compared to their relatives in the continent (e.g. the Hawaiian Honeycreepers) (Losos & Ricklefs, 2009). This distinctiveness of many insular species and species assemblages is thought to reflect the prominent differences existing between island and mainland environments (Harmon et al., 2008a; Losos & Ricklefs, 2009). Continental communities are complex and composed by many species that typically

share a long history of coexistence. In such scenario, most of the continental niches will likely be filled, leaving little free ecological space to newly formed species. In this context, high levels of inter-specific competition will tend to limit an efficient niche (and morphospace) expansion in diversifying groups. In addition to this, predation is usually very intense in continental communities, which will also contribute to limit morphological diversification by inducing stabilizing selection on many traits (Yoder et al., 2010). As a result of the combined effects of inter-specific competition and predation, evolutionary diversification in continents is expected to produce small variations of already successful adaptive themes, enforcing a great morphologic conservatism (Moen & Wiens, 2009; Losos & Ricklefs, 2009).

This contrast with the situation in islands, usually presenting impoverished communities compared to continental ones (Carliquist, 1974; Leigh et al., 2007; Losos & Ricklefs, 2009; Losos & Mahler, 2010). Predators in islands are likely less diverse and abundant compared to the continent and moreover, especially at the early stages of island colonization, groups diversifying in islands will likely encounter a wide array of niche vacancies (Harmon & Gibson, 2006; Losos & Mahler, 2010; Millien, 2011). This is considered to trigger an "ecological release", producing an expanded habitat or resource use by island species. In this context of great ecological opportunity, evolutionary diversification in islands may open the door to an adaptive expansion of the morphospace. This mosphospace expansion, usually coupled with accelerated rates of trait evolution, can potentially take island taxa well beyond the limits of the continental morphospace, producing so many examples of sizes and shapes highly divergent from those found in their continental close relatives (Losos & Ricklefs 2009).

However, although this conceptual frame helps to explain so many instances of dissimilarities between mainland and island biotas, there are other plausible scenarios that not necessarily lead to greater diversification in islands compared to the continent.

For instance, the ecological opportunity that islands provide, might not always necessarily induce increased morphological diversification in island groups. This would be the case if the expanded resource use in islands were mediated by phenotypic aspects other than morphology (e. g. behavior) or by one or few generalist phenotypes (not implying diversification into different specialized phenotypes).

Furthermore, it is conceivable that "island-like" patterns can also appear in continental groups although they may be more difficult to detect due to the great geographic and taxonomic scales involved (Claramunt et al., 2012 and references therein). Extrinsic factors as climate change, orogenic processes and episodic massive extinction events can provide novel niches that can potentially trigger high levels of species and phenotypic diversification also in the continent (Simpson, 1944). Also, intrinsic factors as the appearance of a key innovation can facilitate the access to a wider range of niches in continental groups producing patterns of phenotypic diversification similar to those found in islands (Simpson 1944, Claramunt et al. 2012, Garcia-Porta & Ord, 2013).

In this study, our aim was to explore the role of these different scenarios at producing the morphological diversification in a nearly completely sampled mainland-island system: the *Hemidactylus* geckos of

the so-called Arid clade (Carranza & Arnold, 2006). This clade represents a well studied monophyletic radiation with more than 40 species distributed across the arid regions of northeast Africa, the Levant, Arabia, adjoining areas of southwest Asia and, interestingly for our purposes, also in the Archipelago of Socotra (Carranza & Arnold, 2006; Moravec et al., 2011; Carranza & Arnold, 2012; Gómez-Diaz et al., 2012; Šmíd et al., 2013) (Fig.1).

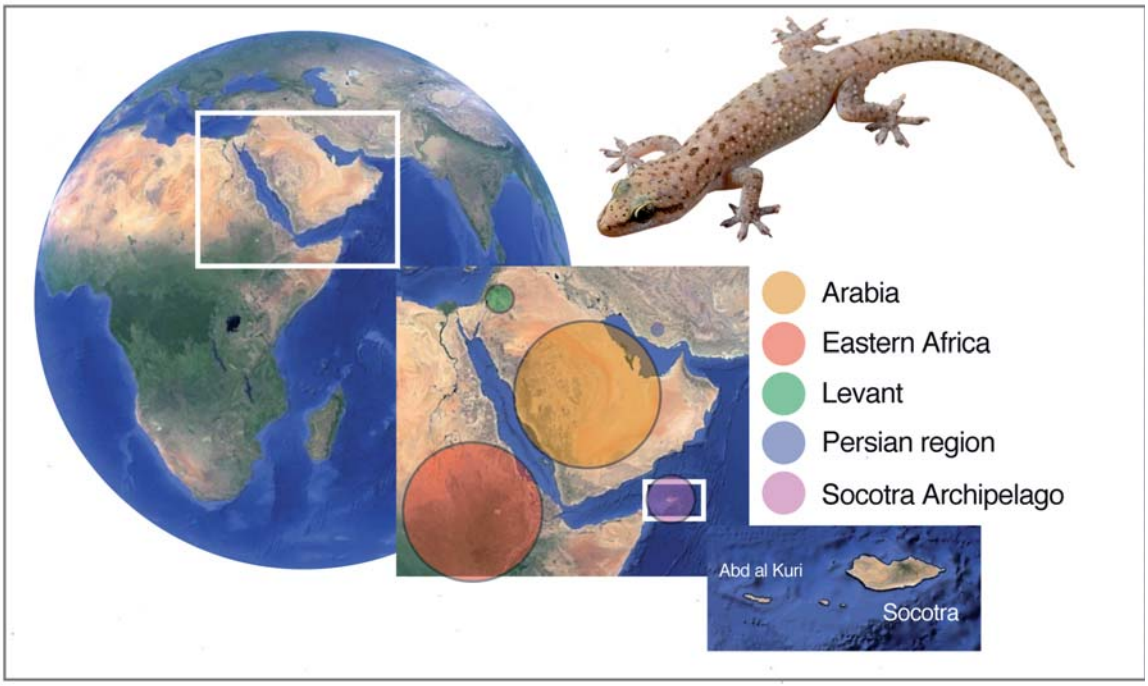

Figure 1. Map showing the geographic limits of this study. The diameters of the circles are proportional to the species richness of the Arid clade of *Hemidactylus* within each geographic area.

This archipelago, until now one of the least known archipelagos in the World, is located in the northwestern Indian Ocean and originated as a continental fragment that detached from Arabia around 30 Ma (Davison et al., 1994; Ghebreab, 1998; Bosworth et al., 2005; Autin et al., 2010). It comprises two main islands, Socotra and Abd al Kuri (3,625 km^2 and 133 km^2 respectively), which are situated 380 km southeast from the coast of Arabia (Yemen) and approximately 100 km east off the Horn of Africa (Somalia) (Fig. 1). It is known that the Arid clade has have arrived to the archipelago three times independently, two of them producing subsequent intra-island diversification events (Gómez-Díaz et al. 2012; Smid et al. 2013). The existence of multiple instances of colonization and diversification in the same archipelago provided the opportunity to explore the existence of an "island effect" on different and independent replicates. Moreover, the great sampling existing in the Arid clade allowed us the possibility to compare the patterns and processes of morphological diversification observed in the islands with those observed in the continent.

We used two proxies to study morphological evolution across the radiation: body size and head proportions. Body size is one of the most general proxies to reflect, not only morphology, but also general phenotype. This is because body size is correlated to many other phenotypic and ecologic

attributes, from behavior to metabolic rates (Peters, 1986; Brown et al., 2004). Furthermore, body size diversification is a common outcome of island colonization in many groups (Diamond, 1973; Williams, 1995; Price et al., 1997; Raia & Meiri, 2006), which makes size a trait of utmost importance to explore a possible link between island colonization and morphologic diversification. Regarding head proportions, their variation is known to be involved in multiple and highly relevant ecological activities in lizards, from feeding and refuge use to mating and aggressive interactions (Vitt & Cooper, 1985; Arnold ,1998; Herrel et al., 1999; Vanhooydonck & Van Damme, 1999; Kaliontzopoulou et al., 2008), and very often reflect differences in resource use within lizard communities (Losos, 2009). This makes head dimensions an interesting complement to body size to explore the effects of island colonization on phenotypic diversification.

To assess the existence of an “island effect” on the morphological diversification experienced by the *Hemidactylus* geckos of the Socotra Archipelago, we address three main questions in this study: 1) Do island and continental species share the same morphospace? 2) Do insular species assemblages present higher phenotypic disparities compared to continental species assemblages? And in this case, 3) which is the role of different tempos and modes of phenotypic diversification at producing these higher disparities? To answer these questions, we began by developing a robust phylogeny for the whole Arid clade on which we mapped body size and head proportions. We then applied a variety of comparative methods to detect whether an "island effect" could be detected on the patterns of phenotypic diversification observed in the island species.

METHODS

PHYLOGENETIC ANALYSIS

Sequences of two mitochondrial (12S rRNA (*12S*) and cytochrome *b* (*cytb*)) and four nuclear genes (oocyte maturation factor MOS (c-*mos*), the melano-cortin 1 receptor (*mc1r*) and the recombination activating genes 1 (*rag1*) and 2 (*rag2*)) were assembled from previous phylogenetic studies that focused on the *Hemidactylus* geckos of the Arid clade (Carranza & Arnold, 2012; Moravec et al., 2011; Gómez-Díaz et al., 2012; Smid et al., 2013). In addition, we sequenced all six gene fragments listed above for two *Hemidactylus* species not included in previous studies on the Arid clade: *H. barbierii* from Kenya and *H. macropholis* from Ethiopia and Kenya. Primers and conditions used for the amplification and sequencing of the different fragments followed methods described elsewhere (Smid et al. 2013).

Our final dataset included the sequences for all 37 species known to occur in Arabia, the Socotra Archipelago, Levant, and also incorporated two species distributed in adjoining areas of the Persian region and 10 species present in the Horn of Africa. This dataset is the most complete to date comprising all species, subspecies and several yet undescribed species (work in progress) of the Arid clade of *Hemidactylus* (Carranza & Arnold, 2006, 2012; Smid et al., 2013) (Fig. 1). Only a total of 16 species in

the Horn of Africa, one in Arabia and one in Asia, could not be included in this study. In the worst-case scenario, provided that all these missing species actually belonged to the Arid clade, our sampling would represent a 74% of the actual diversity.

We complemented the molecular dataset formed by these 48 species of *Hemidactylus* of the Arid clade with the sequences of the same genes for *H. mabouia*, *H. platycephalus*, *H. smithi*, *H. flavividiris*, *H. angulatus* and *H. ruspolii*. All these are known to be outside the Arid clade (Smid et al., 2013) and were used as outgroups to confirm the monophyletic nature of the clade. These were afterwards removed from the phylogeny.

DNA sequences were aligned using MAFFT v.6 (Katoh & Toh, 2008) with the options “maxiterate 1000" and “localpair”. Poorly aligned positions of the non-transcribed *12S* mtDNA region were eliminated by means of G-blocks (Castresana, 2000), using low stringency options (Talavera & Castresana, 2007). The final alignment consisted of 4,016 bp and included: 379 bp of *12S*, 1,137 bp of *cytb*, 402 bp of c-*mos,* 666 bp of *mc1r*, 1,024 bp of *rag1* and 408 bp of *rag2*. Best fitting nucleotide substitution models were selected for each partition under the Akaike information criterion (AIC) (Akaike, 1973) using jModeltest v.0.1.1 (Posada, 2008). The best models consisted in GTR+I+G for *12s*, *cytb* and *mc1r*, and TrN+G for c-*mos*, *rag1* and *rag2*. Alignment gaps were treated as missing data and the nuclear gene sequences were not phased.

The phylogenetic analyses were conducted by means of the package BEAST v1.6.2 (Drummond & Rambaut, 2007). The prior for the distribution of branching times was based on a birth-death process. The variation of nucleotide substitution rates across the tree was assumed to be non-autocorrelated and log-normally distributed. The nucleotide substitution models (see above) were applied to the six partitions and the global substitution rate was set to one. This produced branch lengths expressed in units of substitutions per site (relative time).

We ran two independent Markov Chain Monte Carlo (MCMC) analyses for $50*10^6$ generations, with parameters and trees sampled every 5,000 generations. These two independent runs converged on very similar posterior estimates and were combined using LogCombiner v1.6.2 (included in the package BEAST) after excluding the first 10% generations in each MCMC chain. Tracer v.1.5 (Drummond & Rambaut, 2007) was used to confirm convergence and good mixing of each of the MCMC chains.

We calculated the summary tree as the maximum clade credibility tree with median node heights using TreeAnnotator v1.6.2 (also included in BEAST package), setting the posterior probability limit at 0.5. Finally, in order to incorporate the phylogenetic uncertainty into our comparative analyses (see below), we resampled the posterior distribution of trees resulting from our BEAST analysis to obtain a sample of 1,500 trees that varied in topology and branch lengths.

PHENOTYPIC DATA COLLECTION

We obtained body size, measured as the snout-vent length (SVL), for all 48 taxa known to belong to

the Arid clade of *Hemidactylus* or confirmed to be part of it herein. For most of the species, SVL was taken with a caliper to the nearest 0.1 mm, with the exception of the rare *Hemidactylus modestus* and *H. funaiolii*, for which SVL was obtained from the original species descriptions (Gunter, 1894; Lanza, 1978). The head measurements consisted in its length, measured from the tip of the snout to the retroarticular process of the jaw; the head width, measured at the widest part of the head; and the head depth, taken as the maximum depth of the head (Carranza & Arnold, 2012; Moravec et al., 2011). These measures were obtained for 42 species with a caliper to the nearest 0.1 mm. All measurements were $\log_{10}$ transformed to improve normality and homoscedasticity. The sample sizes consisted in 715 specimens for body size and 694 specimens for head proportions, with a mean of 14 and 16 specimens per species, respectively. Only adult specimens were measured and, given that preliminary analyses showed no significant differences between males and females in any of the measurements taken (data not shown; see also Carranza & Arnold, 2012), both sexes were pooled together. To remove the effect of body size on the head variables, we computed the residuals by regressing each variable against SVL. This was done using the species means and correcting by the expected phylogenetic covariances among species (Revell, 2010). This process was repeated for each tree in the set of 1,500 trees when these were used in comparative analyses. Given the low dimensionality of our head data (with only three variables) we kept the analyses in the original data space to allow an easy interpretation of the head morphospace.

ANCESTRAL STATE RECONSTRUCTIONS OF ISLAND-CONTINENT TRANSITIONS

Lineage assignation to Socotra, Abd al Kuri or mainland was informed by 1,000 possible stochastic ancestral reconstructions computed on the summary tree and one reconstruction computed on each of the 1,500 trees sampled from the posterior distribution. This was conducted using the function "make.simmap" from the R package "phytools" (Revell, 2012). This function essentially fits a continuous-time reversible Markov model (in our case allowing all transition rates to be different) and simulates plausible stochastic character histories along the tree using the most likely model in combination with the states assigned to the tips of the tree.

EXPLORING MORPHOLOGIC VARIATION IN ISLAND AND MAINLAND GECKOS

We used the function "phenogram" in the R package "phytools" (Revell, 2012) to visualize size variation across the summary tree. This function essentially projects the phylogeny into a space defined by the phenotype (on the y axis, including the values at the tips and the values reconstructed at the nodes) and time (on the x axis). Given the multivariate nature of the head proportions, we visualized their variation by means of the "phylomorphospace". In this case the tree is projected into a bivariate space represented by the species values and the reconstructed states at the nodes for each combination of two head variables (Sidlauskas, 2008). Both representations were very useful to visualize size and head

morphospace in a phylogenetic context, allowing us to identify the major trends of change across the tree, as well as the different magnitudes variation (proportional to the vertical component of each branch in the "phenogram", and proportional to the branch lengths in the "phylomorphospace"; Sidlauskas, 2008). For head proportions, we also used the function "contMap" in the R package "phytools" to visualize the variation of each independent head dimension across the phylogeny. This method essentially reconstructs the ancestral states of continuous characters at the nodes and interpolates the values along the branches ("Method 2" in Revell, 2013). Finally, only for head proportions, we defined the limits of the morphospace occupied by continental lineages by a minimum convex hull enclosing all species from the continent. This allowed a clear visualization of the island morphospace in relation to the morphospace occupied by continental species. The R packages "phytools" (Revell, 2012) and "cluster" (Maechler et al. 2012) were used for all analyses conducted in this section.

To formally assess whether mainland and island species differed in morphology, we performed a phylogenetic ANOVA and MANOVA on size and head proportions respectively. Significance of the empirical F statistic (for ANOVA) and the Wilk's lambda (for MANOVA) was assessed by means of null distributions of these statistics based on 10,000 Brownian motion simulations. For size, these simulations were based on a maximum likelihood (ML) estimate of the empirical rate parameter. For head proportions, simulations were based on the ML estimate of the evolutionary variance-covariance (vcv) matrix. Both analyses were performed in the R package "geiger" (Harmon et al., 2008b) and were conducted on the summary tree and on the set of 1,500 trees.

TESTING FOR DIFFERENCES IN PHENOTYPIC DISPARITIES BETWEEN ISLANDS AND MAINLAND

Disparity was defined as the average squared euclidean distance computed between the sizes and head proportions of all pairs of species coexisting in a given area (Harmon et al., 2008a). In this way we calculated the disparity in size and head proportions in Socotra, Abd al Kuri and in the continent. We posteriorly measured the overlap between continental and island disparities by calculating the ratios between the disparities of Socotra and the continent, and between Abd al Kuri and the continent. These ratios were then compared to a null model consisting in 10,000 simulations in which size and head proportions were stochastically simulated according to a Brownian motion model. Simulations were based on an empirical estimate of the rate parameter for body size, and on the estimated evolutionary vcv matrix for head proportions.

By comparing the empirical ratios to the simulated ratios according to the stochastic model, we assessed whether island disparities significantly departed from the mean continental disparity. This analysis was performed on the summary tree but it was also replicated for each of the 1,500 trees in order to incorporate phylogenetic uncertainty into our empirical and simulated disparity ratios.

All the analyses of this section were performed using the "ape" (Paradis et al., 2004) and "geiger" (Harmon et al., 2008b) packages in R.

TESTING FOR DIFFERENCES IN TEMPOS OF PHENOTYPIC DIVERSIFICATION

To test whether insular and continental groups differed in their tempos of phenotypic evolution, we used Brownian motion to model rates of phenotypic evolution. We implemented this model with three different approaches.

Brownie

We first calculated the rates of phenotypic evolution of islands compared to the continent, by means of the “non censored” approach described in O’Meara et al. (2006). This approach is essentially based on the fitting of two alternative Brownian motion models; one assumed that the lineages in the continent, Abd al Kuri and Socotra evolved according to different Brownian rate parameters (σ^2) (model 2), while the other—the null model—assumed a single rate across all the lineages in the phylogeny (model 1). For the summary tree, we fitted model 1 and model 2 for each trait on each of the 1,000 stochastic reconstructions described before (which reflected the uncertainty of the assignation of categories across the tree). For the set of 1,500 trees, the two models were fitted on each of the stochastic reconstructions conducted on each of the trees (which reflected the uncertainty in both the assignation of categories across the tree plus phylogenetic uncertainty). In all cases, models 1 and 2 were evaluated by comparing their computed AICc values in each of the different trees and reconstructions.

For body size, which was the only trait for which we detected rate heterogeneity (see results), we also compared these two models to three additional ones in order to discern between three possible scenarios consistent with rate heterogeneity, namely: (1) a significant rate acceleration only in Abd al Kuri, with Socotra and the continent presenting similar rates, (2) an acceleration solely involving Socotra, with Abd al Kuri and the continent presenting even rates and finally (3) a situation in which Abd al Kuri and Socotra present equal rates but both are accelerated with respect to the continent. These three different scenarios were tested by three models enforcing different patterns of rate heterogeneity across the tree: In model 3 Socotra and Abd al Kuri were constrained to evolve at the same rate, but this could differ from the rate existing in the continent. In model 4, Socotran and continental groups were forced to evolve at the same rate, but this could be different from the rate in Abd al Kuri and finally, in model 5, lineages in Abd al Kuri and in the continent were forced to share the same rate while Socotran lineages could differ. As previously described, the AICc values computed for each these models were compared in the summary tree and on each of the 1,500 trees. The analyses described in this section were conducted by the function "brownie.lite" in the R package "phytools" (Revell, 2012).

Auteur

Secondly, we estimated rates of phenotypic evolution along branches of the phylogeny without *a priori* specifying which lineages of the tree corresponded with island or continental domains. Within the R

package "auteur" (Eastman et al., 2011) we conducted a reversible-jump Markov Chain Monte Carlo sampling to estimate the rates of evolution of all traits examined in this study (body size and each of the axis of head variation). In this approach, rates were estimated along the branches of our summary tree with no prior assumption that evolutionary rates had changed at specific points in the phylogeny (Eastman et al., 2011). The analysis consisted in three independent chains that ran for $20*10^6$ generations each one, with a sampling interval of 3,000 generations. The posterior estimates of these three runs were subsequently pooled with the first 10% of generations excluded as "burnin". These analyses allowed us to estimate (and visualize) the posterior rates of evolution for each trait along branches. For each trait we also compared the support of multiple versus single rate models by means of Bayes factors (BF). For body size (the only trait that presented rate heterogeneity, see results), we replicated the analysis for each of the 1,500 trees, to ensure that this rate heterogeneity could still be detected when topological and branch length uncertainties were incorporated into the analysis. In this case, for each of the 1,500 trees, we ran a single chain of $2*10^6$ generations with a sample interval set at 1,000 generations. For each tree we compared the support of multiple versus single rate models by means of BF and we also detected the clade (or lineages) associated with the rate shift that presented the highest computed posterior probability.

Independent contrasts

Finally in order to investigate how the heterogeneity in the rates of body size evolution was structured along time, we computed the absolute values of the standardized independent contrasts (another proxy to the Brownian rate parameters; Felsenstein, 1985; McPeek, 1995) and plotted them against the height of the node that produced them. In order to test whether the computed contrasts were in the range of the expected values assuming a single Brownian rate operating across the tree, we computed 10,000 simulated datasets generated by Brownian motion using an empirical estimate of the rate parameter. We then computed all independent contrasts for each simulated dataset and we plotted the 95% of the contrast variation.

TESTING FOR DIFFERENCES IN THE MODES OF PHENOTYPIC DIVERSIFICATION

Aside of studying whether island and continental clades differed in their tempos (rates) of phenotypic evolution, we also examined whether they differed in their modes of phenotypic evolution (Sidlauskas, 2008). We explored the existence of different phenotypic optima in islands and in the continent by means of an Ornstein-Uhlenbeck process (OU). This model can be seen as an extension of the Brownian motion model in which a deterministic tendency toward adaptive optimum is incorporated (Hansen, 2007; Butler & King, 2004). Four parameters regulate an OU model, namely: a Brownian rate parameter (σ^2), a rate of adaptation towards an optimum (α), and the optimum trait value (θ).

We used the package "surface" (Ingram & Mahler, 2013) to explore how many different OU models (or selective regimes) could be fitted across the Arid clade. The fitting of the OU models by "surface" is conducted in two phases. First, in a "forward" stepwise phase, the analysis starts with a single-peak OU model (OU1) to which new OU models are added keeping the combination of models that produces the lowest AICc values. This is followed by a "backward" phase in which all lineages sharing the same selective regime are collapsed in the same regime if this produces an additional decrease of the AICc values. This procedure allowed the detection, not only of different selective regimes, but also of convergent regimes across the phylogeny (Ingram & Mahler, 2013).

For all analyses, in both forward and backward phases, we accepted all improvements of AICc values and allowed multiple compatible regimes to collapse during each step in the backward phase.

Given that for an optimal implementation of this method multivariate datasets are recommended (Ingram & Mahler, 2013), we ran two analyses, one solely including the three head dimensions and another including these plus body size (which required pruning from the tree all species for which only size data was available). It is important to note that in the particular implementation of "surface", both σ^2 and α are constant for all selective regimes detected for each trait (although they are allowed to vary between traits). In the summary tree, once the different selective regimes were defined, these were painted on the phylogeny and the different optima were visualized by means of bivariate plots. In order to evaluate the effects of phylogenetic uncertainty on the delimitation of selective regimes, we replicated the analysis for each of the trees in the set of 1,500 trees obtained from the Bayesian posterior distribution.

RESULTS

PHYLOGENY AND ANCESTRAL STATE RECONSTRUCTIONS

We recovered 86% of nodes of the summary tree with a posterior probability (pp) higher than 0.90 (high to very high support; Fig. 2A). The phylogenetic relationships depicted by our summary tree (Fig. 2A) were generally consistent with the most recent and complete phylogenies of the Arid clade of the genus *Hemidactylus* (Carranza & Arnold, 2012; Gómez-Díaz et al., 2012; Smid et al., 2013).

The stochastic state reconstructions over the summary tree revealed negligible rates of transition from islands to the continent ($q < 10^{-5}$), and high rates of transitions from the continent to Socotra and Abd al Kuri ($q = 9.00$ and 4.26, respectively). Similar values were computed in those reconstructions involving each of the 1,500 trees. In both summary tree and in the set of 1,500 trees, most of the stochastic reconstructions placed all nodes splitting island lineages within island categories, indicating that they probably reflected intra-island speciation events (Fig. 2B). This is congruent with the dating estimates of Gómez-Díaz et al., (2012) and Smid et al., (2013), who dated all intra-island nodes well after the detachment between Arabia and the Socotra Archipelago.

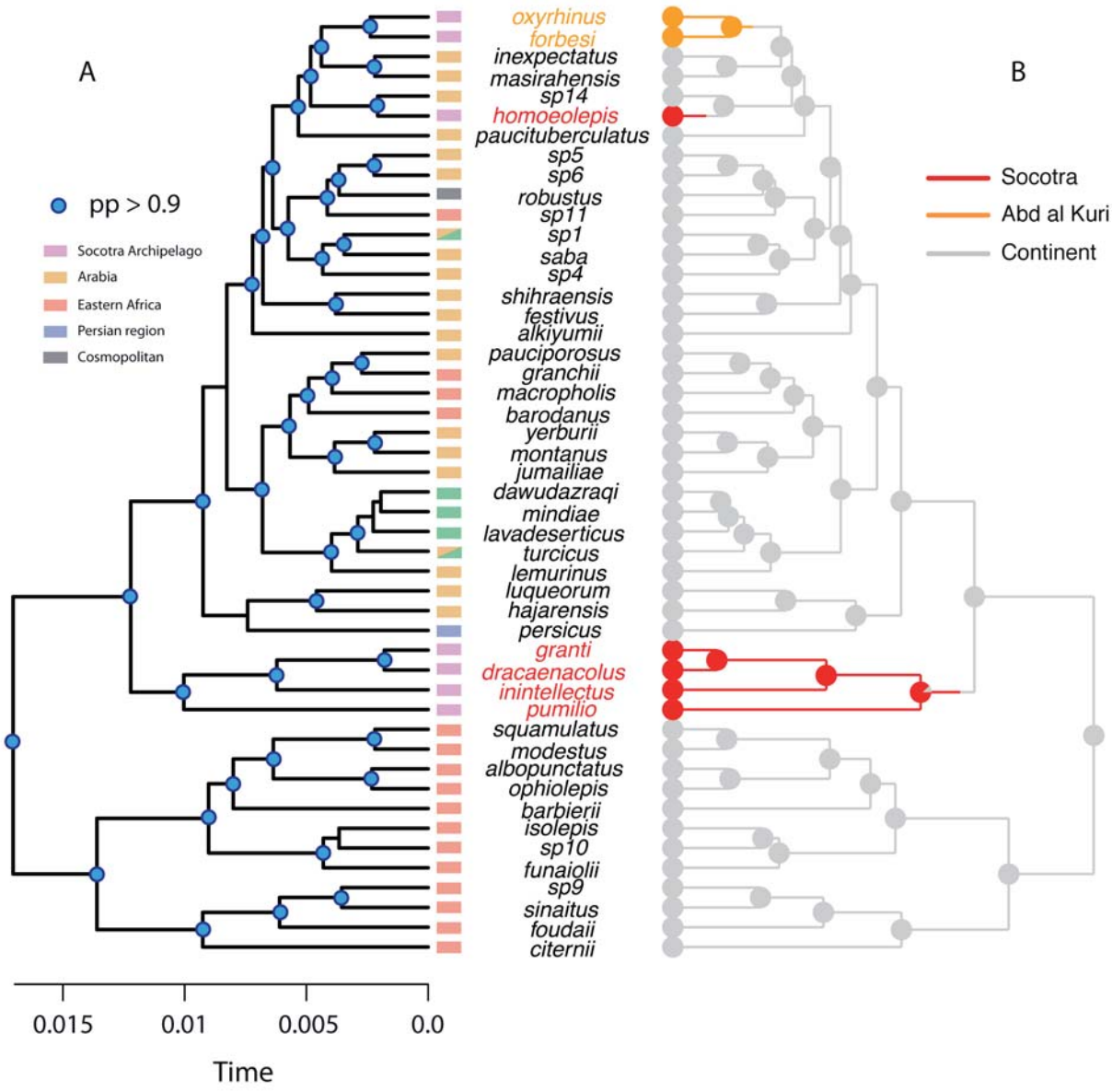

Figure 2. (A) Ultrametric tree of the Arid clade of *Hemidactylus* geckos derived from the BEAST analysis (summary tree). The blue circles indicate nodes with a posterior probability higher than 0.90. The colored rectangles correspond to the different geographic origins of the species covered in the phylogeny (consistent with colors in Fig. 1). (B) Summary tree with the transitions among "continental", "Abd al Kuri" and "Socotra" (grey, orange and red, respectively) reconstructed according to one possible stochastic character history. The circles at the nodes provide a visualization of the uncertainty of the ancestral state reconstructions.

TESTING FOR DIFFERENCES IN MORPHOLOGY AND DISPARITY BETWEEN ISLAND AND CONTINENTAL GROUPS

For body size, the phenogram showed how island species attained the most extreme sizes in the Arid clade, with *H. forbesii* (from Abd al Kuri) being the largest and *H. pumilio* (from Socotra) being the smallest (Fig. 3). These two size extremes where the consequence of substantial amounts of size change associated with two of the intra-island splits: the intra-Abd al Kuri speciation event (separating

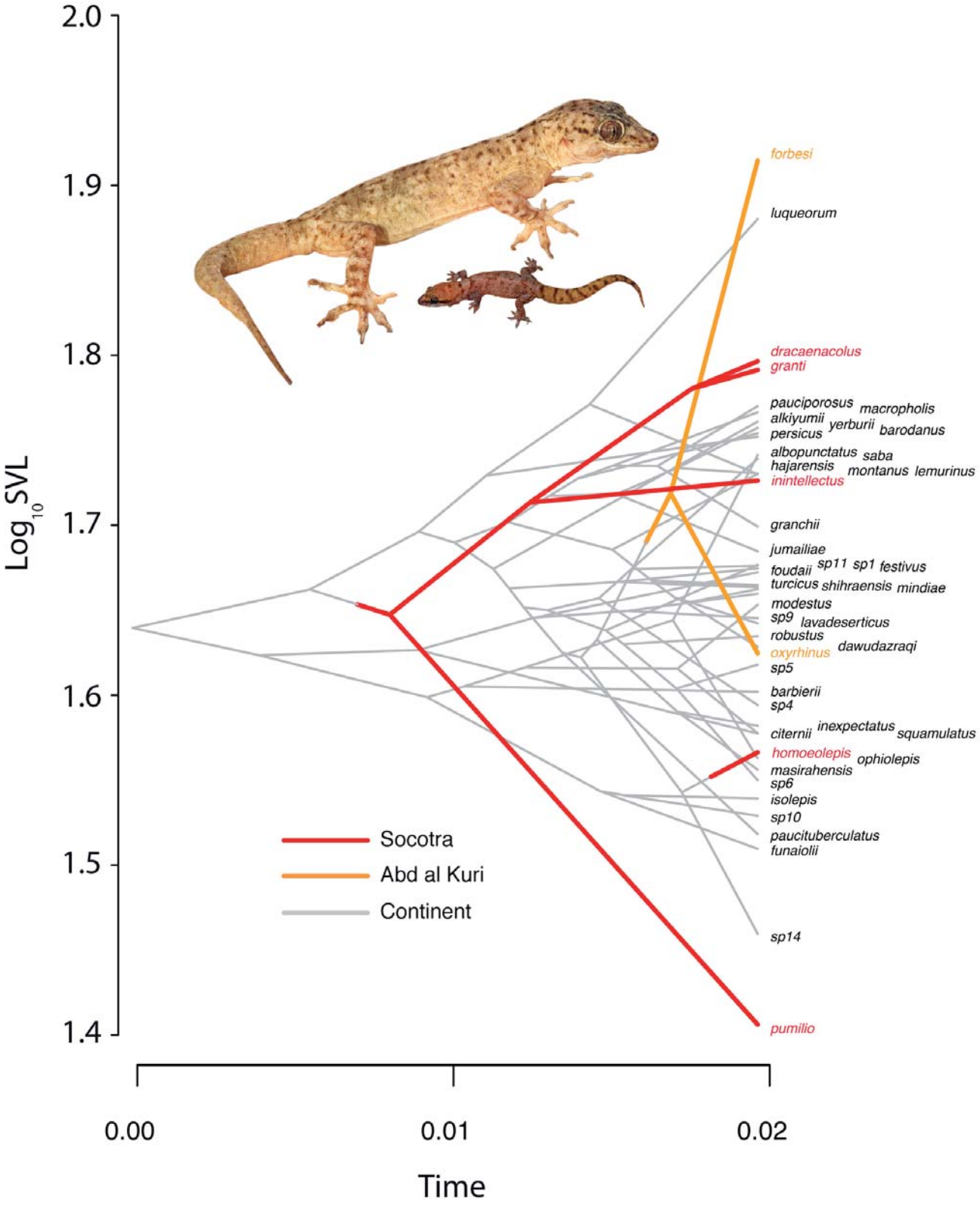

Figure 3. Phenogram showing the body size variation across the 48 taxa that form the Arid clade of *Hemidactylus* geckos. The vertical position of nodes and tips represent the known or estimated (log_{10}-transformed) body sizes, while the horizontal position reflects relative time. Island and continental lineages are highlighted in different colors: grey (for the continent), orange (for Abd al Kuri) and red (for Socotra). The pictures show the biggest and the smallest species in the radiation shown at the same scale (*Hemidactylus forbesii* from Abd al Kuri and *H. pumilio* from Socotra). In the figure SVL refers to snout-vent length.

H. forbesii and *H. oxyrhinus*) and the basal-most split within Socotra (separating *H. pumilio* from the remaining Socotran species). Also, with the exception of the *H. dracaneocolus* and *H. granti,* the sizes of the different species coexisting within the same island tended to be widely separated across the spectrum of size variation. This pattern contrasts with the observed for continental clades in which most of the species tend to converge on similar, intermediate sizes (Fig. 3).

The variation in head proportions was roughly distributed along a "short-narrow-low" to "long-wide-high" continuum of head shapes, with most of the intra-island splits segregating along this continuum (Fig. 4). Regarding the magnitude of phenotypic change (proportional the branch lengths in the phylomorphospace), the two species from Abd al Kuri experienced one of the greatest amounts of

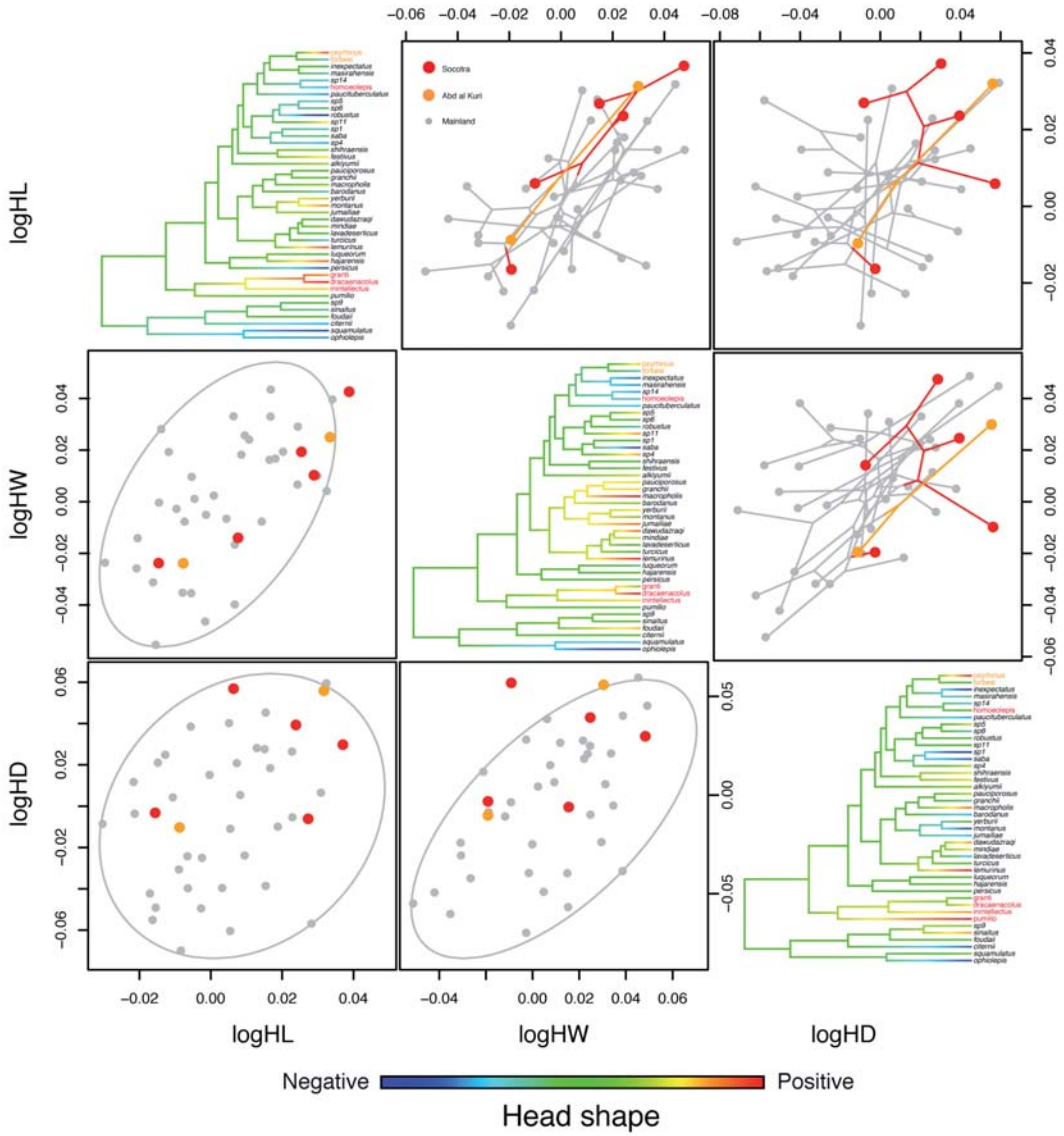

Figure 4. Visualization of the variation in head proportions for the 46 species for which head dimensions were obtained. The upper-right portion of the panel represents the phylomorphospace occupied by the residuals of head dimensions. The lower-left portion provides a visualization of the insular morphospace in the context of the region of the head morphospace occupied by continental species. The grey ovals represent the limits of the continental morphospace (minimum convex hull). The trees in the diagonal show the residuals of each head dimension reconstructed along the nodes and branches of the summary tree using the Method 2 described in Revell (2013). Island and continental species are highlighted in different colors: grey (continental), orange (Abd al Kuri), red (Socotra). In the figure HD stands for "head depth", HW refers to "head width" and HL refers to "head length".

head differentiation in the group, being the amount of change in Socotra comparatively smaller in extent (Fig. 4). With the exception of the mentioned size extremes detected for *H. forbesii* and *H. pumilio,* the visual comparison between island and continental morphospaces (in both size and head proportions) revealed great overlap (Fig. 3-4).

In agreement with this observed overlap, the results of the phylogenetic ANOVA and MANOVA showed no significant differences in size or in head proportions between island and mainland clades, with independence of whether islands were grouped together or not in the same category and whether the analyses were performed on the summary tree or in each of the 1,500 trees (with all *p*-values > 0.05, Table S1).

However the ratios of island versus mainland disparities revealed an extreme body size differentiation in islands. Disparities in islands ranged from 3.58 to 5.30 times the mean disparity of the continent (for Socotra and Abd al Kuri respectively). Both values were significantly higher than the null distribution produced on the summary tree (with *p*-values computed at 0.01 and 10^{-4}, for Socotra and Abd al Kuri respectively) and on the set of 1,500 trees (with *p*-values ranging from 0.004 to 0.0077 and from 0.0014 to 10^{-4}, for Socotra and Abd al Kuri respectively) (Fig. 5). Regarding head proportions, only Abd al Kuri produced an island/mainland ratio significantly higher than the random expectation (*p*-value on the

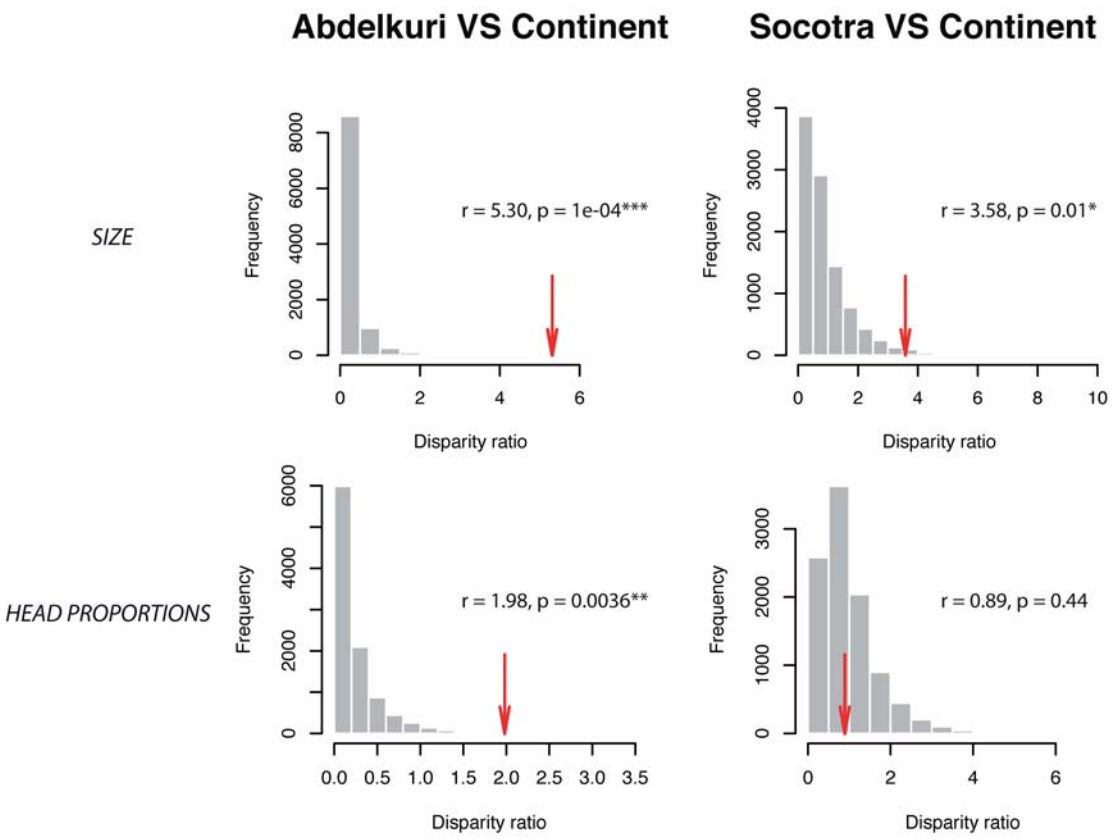

Figure 5. Ratios of island versus continent in body size (upper) and head shape (lower) disparities. Empirical values (arrows) are given with the *p*-values calculated by means of 10,000 Brownian motion simulations computed on the summary tree. The distributions of values according to the simulations in each case are also represented (grey bars).

summary tree computed at 0.0036 and *p*-value on the 1,500 trees ranging from 0.01 to 0.001). These disparities were approximately twice the observed disparity in the continent when the summary tree was used (Fig. 5) and from 1.10 to 2.8 times the continental disparity when the analysis was conducted on the 1,500 trees.

TESTING FOR DIFFERENCES IN TEMPOS OF PHENOTYPIC DIVERSIFICATION

Brownie

Our results on body size, comparing model 1 (assuming no rate heterogeneity across the tree) and model 2 (assuming differences among Socotra, Abd al Kuri and the continent), revealed a compelling support for the second (with the model 1 presenting a mean of 13 AICc units over the model 2 in the summary tree and a mean at 12.80 units above the model 2 in the analyses conducted on the 1,500 trees, Fig. S1). This implies that rate heterogeneity between island and continental categories is the scenario that better depicts body size evolution across the radiation.

However the results of fitting the additional models (models 3, 4 and 5) in both the summary tree and 1,500 trees, highlighted model 4, the one enforcing the same rate of body size evolution in Socotra and in the continent, as highly supported (either by being the best model or by being at less than 4 ΔAICc units from the best model, Fig. S2). Model 5, the one that forced Abd al Kuri and the continent to evolve at the same rate never received any support (with a mean of 14 ΔAICc units away from the best or the set of best supported models). This suggests that the rate heterogeneity detected for body size was mainly the consequence accelerated rates in the lineages in Abd al Kuri, but not necessarily the consequence of accelerated rates in Socotra.

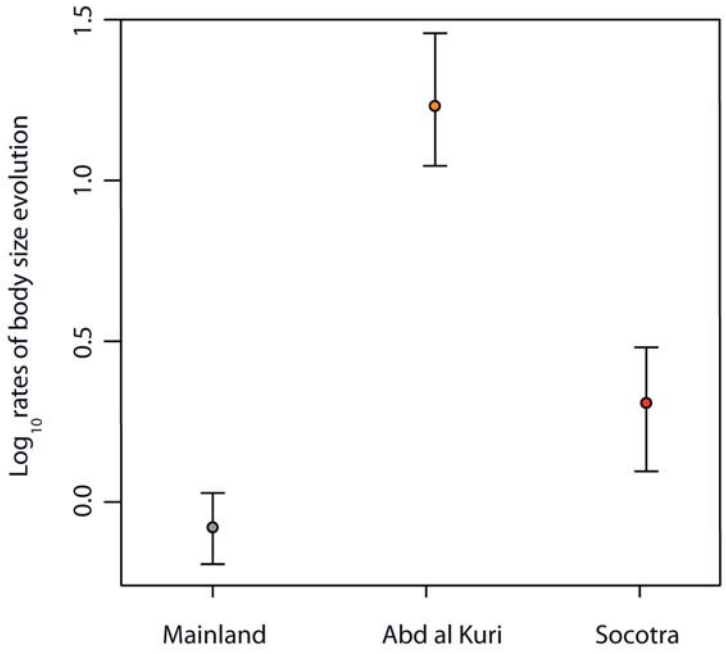

Figure 6. Plot of the relative rates (in a $\log_{10}$-scale) of body size evolution and their associated 95% confidence intervals for each category, estimated according to the model 2 (allowing Socotra, Abd al Kuri and the continent to evolve at different rates) on the set of 1,500 trees (results in the summary tree were identical).

The visualization of the rate estimates according to model 2 with their confidence intervals was consistent with this scenario: the clade of Abd al Kuri presented the highest rates of body size evolution with mean rates more than 20 times the mean rate observed in the continent and more than eight times the rates computed for Socotra. However in Socotra, rates, although high, were only around two times the mean rates of the continent (Fig. 6).

The results for body size greatly differed from the pattern depicted by head proportions. In this case model 1 and model 2 appeared equally supported (in all cases showing only slight differences in their AICc values, Fig. S1). This suggests that a situation of rate heterogeneity among categories failed to be the most likely scenario depicting the evolution of head proportions.

Auteur

The results of "auteur" produced congruent results with "brownie.lite" with the BF analyses comparing multiple *vs* single rates highlighting a multi-rate scenario for body size (BF = 14.62) and a single rate scenario of each of the head proportions (all BF < 1) (Fig. 7B). The multi-rate scenario for body size was also confirmed by the set of 1,500 trees, which produced similarly high BF values for most of the trees (mean BF comparing multiple *vs* single rates = 30.24). In the summary tree and in most of the 1,500 trees, the comparison between the prior and posterior rate distributions revealed a two-rates situation as the pattern of rate heterogeneity with the highest posterior probability (Fig. 7B). In the summary tree and in 99% of the 1,500 trees, the clade formed by *H. oxyrhinus* and *H. forbesii* was the one associated with the rate shift presenting the highest posterior probability, always in the direction of increasing rates of body size evolution. When the posterior rates were visualized on the branches of the summary tree, Abd al Kuri appeared to be nested within a continental clade with high rates of size evolution, although it appeared as highly accelerated compared to its phylogenetic immediate surroundings (with more than seven units of difference detected between the highest continental rates and the rates computed for Abd al Kuri). In Socotra, however, only *H. homoeolepis* and *H. pumilio* presented high rates of body size evolution, with only the later reflecting a true acceleration with respect to the continental background rate (although presenting rate values in the range of those found other clades in the continent) (Fig. 7A). All the evidence provided by "auteur" supports the existence of two rates of body size evolution: one in Abd al Kuri (higher) and another in the continent plus Socotra (lower). Head proportions, however, evolved under an single rate of evolution across the whole radiation.

Independent contrasts

Finally, the visualization of the size contrasts through time (another proxy to Brownian rates) showed how the intra-island split in Abd al Kuri presented the most extreme contrast value in the radiation (Fig.

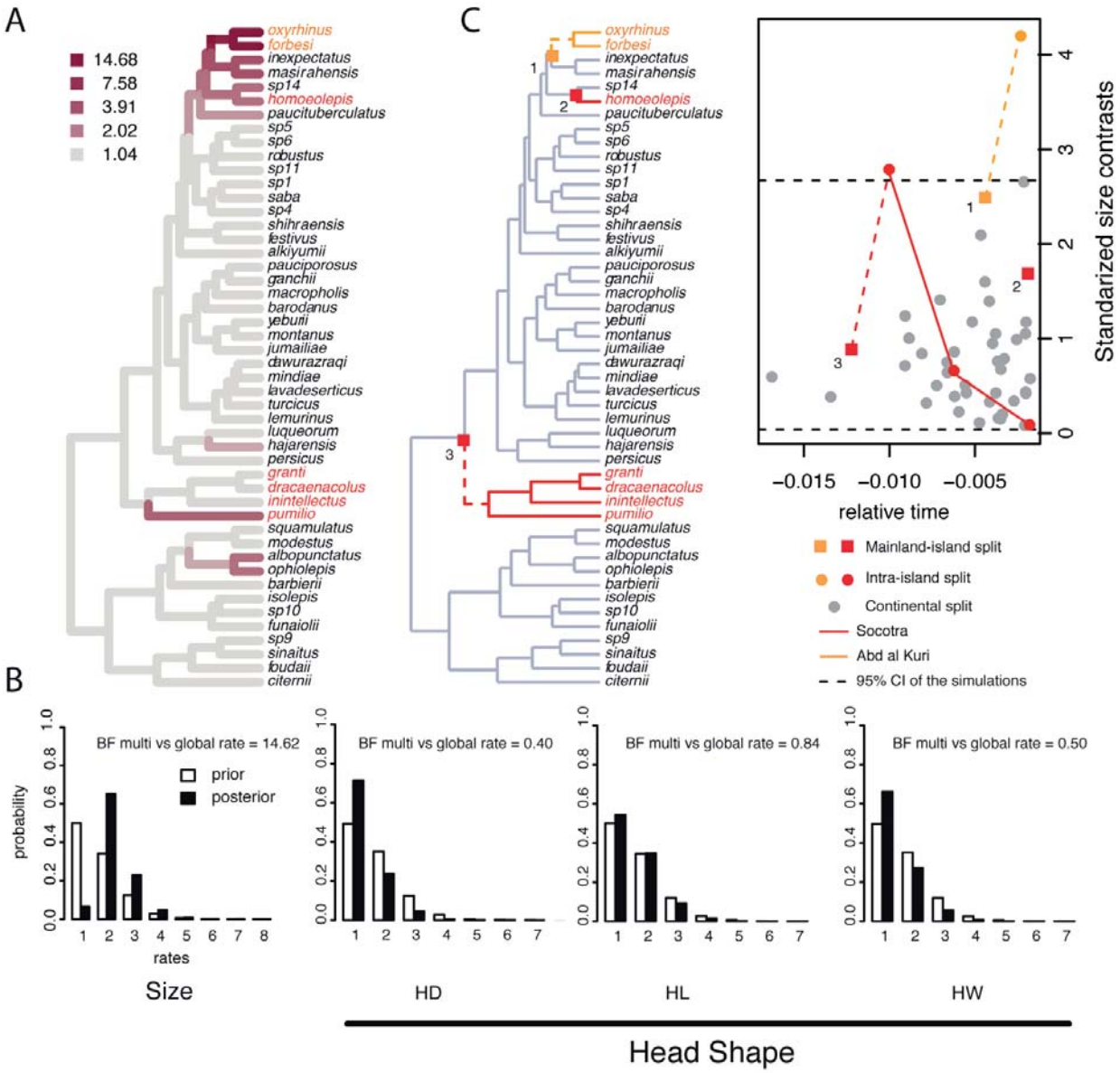

Figure 7. (A) Summary tree with branches colored to reflect the magnitude of shifts in the rates of body size evolution as computed by “auteur”. Background rates (those not deviating from the median rate across the tree) are colored light gray; those branches with rates higher than background rates are colored in red and their intensity varies in proportion to their rate value. (B) Distributions of the prior and posterior scenarios of rate heterogeneity along with the estimates of Bayes factors comparing a multi vs single rate pattern of phenotypic evolution. (C) Distribution of the standarized body size contrasts across time. The colors and numbers in the chart match with those in the tree on its left side. The dashed lines indicate the 95% CI of 10,000 simulations generated assuming a single rate parameter across the tree. In the figure HD stands for "head depth", HW refers to "head width" and HL refers to "head length".

7C), pattern entirely consistent with the evidence provided by the previous analyses. Interestingly, this analysis also revealed that although the innermost split within Socotra presented contrast values similar to those found in some of the most recent continental splits (therefore supporting a scenario of no rate differences between Socotra and the continent), at the same time it presented a very high contrast value compared to the continental splits occurring at similar times (Fig. 7C). Furthermore, this value was significantly higher than the null distribution of 10,000 simulations based on a single rate parameter across the tree (p-value = 0.01). The splits that followed the onset of diversification in Socotra, presented decreasing contrast values, pattern consistent with a slowing down in the rates of body size evolution as the intra-island diversification proceeds (Fig. 7C).

Our analyses also showed that although most of the continental lineages presented very low rates of body size evolution, some recently appeared continental clades presented high rates, some of them attaining values similar to those found in the innermost speciation event in Socotra (Fig. 7A and 7C). This indicates that rates in the continent are far from being saturated, with some groups actively increasing size variation also in the continent.

In summary, our results provide strong evidence for the existence of an acceleration in the rates of body size evolution after the colonization of Abd al Kuri and, although lesser in extent, also Socotra. The rate acceleration in Socotra was only realized when the rates involving the deep-most node in the island were compared to the rates computed for similarly aged nodes in the continent. Regarding head proportions, our analyses failed to detect any difference in the tempos of the evolution between islands and the continent.

TESTING FOR DIFFERENCES IN THE MODES OF PHENOTYPIC DIVERSIFICATION

The "surface" analysis on head proportions produced five distinct regimes in the forward phase, three of which were collapsed in two regimes at the end of the backward phase. This process involved a drop of 14.19 units in the forward phase with an additional drop of 13.09 units in the backward phase (Fig. S3). These two regimes involved two well-separated head shape optima: one consisting in short, narrow and low heads and the other consisting in long, wide and high heads. The values at these optima were found to be in the range of empirical values, being therefore realistic (Table S2). The rates of adaptation were similarly high in all three head dimensions presenting $t_{1/2}$ (computed as "ln(2)/α") ranging from 0.5% to 1% of the total length of the tree. The computed Brownian rate parameters were also very high and produced a great overdispersion around the optima (Fig. 8). The distribution of the selective regimes across the phylogeny, revealed the independent appearance of these two head shape optima in Africa, Arabia, Abd al Kuri and Socotra (Fig. 8), providing evidence for head shape convergence across these four different landmasses. This supports our results from the MANOVA, which showed not significant head shape differentiation between insular and continental groups. Remarkably, the split in Abd al Kuri was associated with the two optima, which indicates the existence of two selective regimes operating in the frame of an intra-island speciation event. For Socotra, however, these two regimes were allocated in the two independent island colonizations (Fig. 8). The analyses conducted on the 1,500 trees produced the same two selective regimes found in the summary tree in 67% of the trees or these two plus an additional one (in the "long-wide-high" region of the morphospace and always including *H. oxyrhinus*) in 33% of the trees (Fig. 8). In either case, the intra-island split in Abd al Kuri was always associated to two different selective regimes (always involving "short-narrow-low" and "long-wide-high" optima).

When we combined body size plus head shape in the summary tree, the forward phase produced six different selective regimes (with a drop of 22 units in AICc values), which were posteriorly pooled in

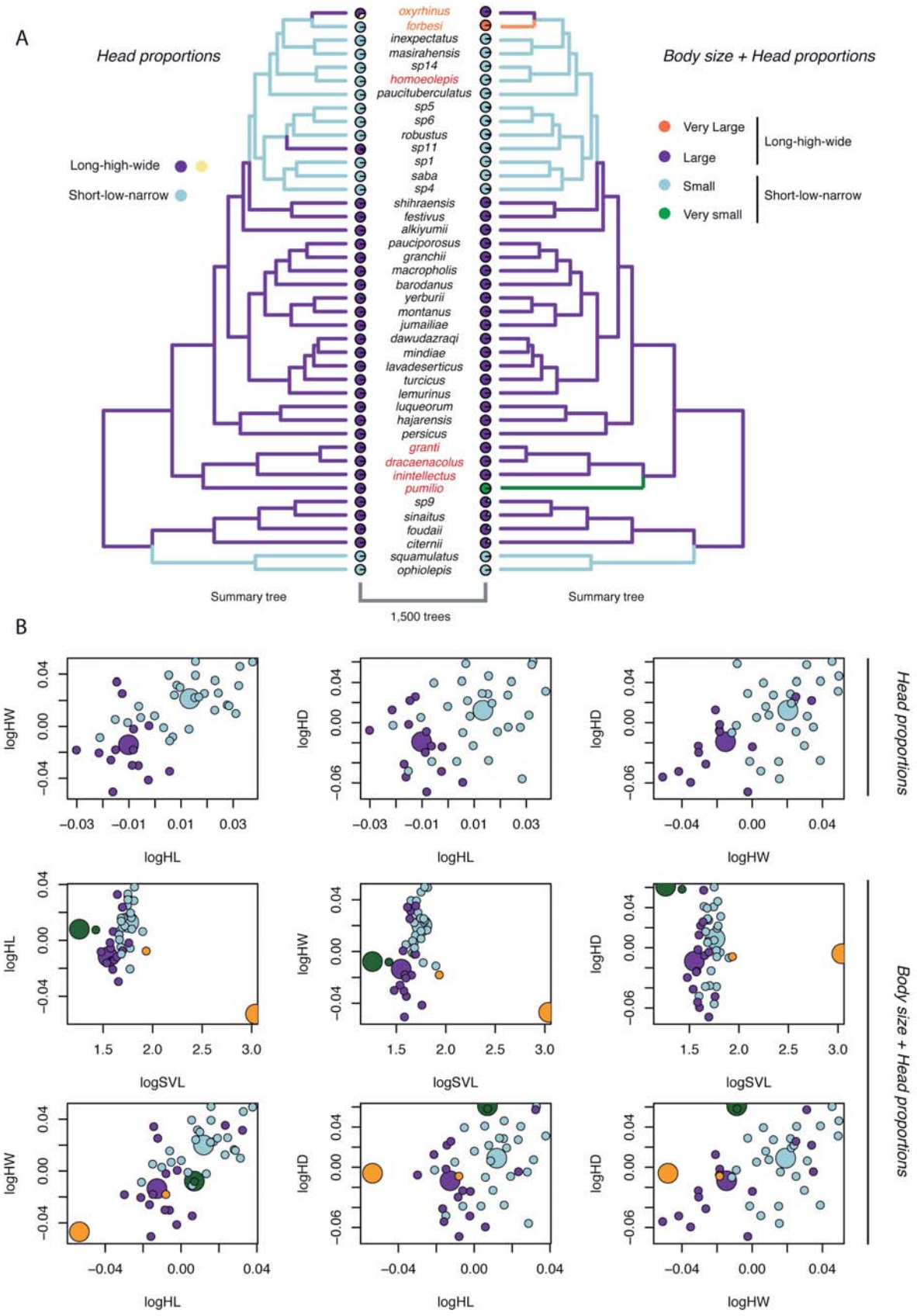

Figure 8. (A) Results of a surface analysis based on the head proportions (left) and based on the head proportions + body size (right) on the summary tree. In both cases a visualization of the distribution of the different selective regimes across the phylogeny is provided along with a qualitative description their optima. Pie charts summarize the distribution of selective regimes across the 100% of the 1,500 trees for head proportions and in 91% of them for head proportions + body size (the pie charts corresponding to the rest of the trees are shown in Figure S4). (B) Scatterplots showing the trait values for each species (small circles) and estimated optima (large circles), with colors matching those in the tree.

four regimes at the end of the backward phase (with an additional drop of 15.44 units in AICc values) (Fig. S3). The distribution of the four selective regimes across the tree was consistent with the pattern produced by the dataset solely based on head proportions, and remarkably, the two additional selective regimes that emerged in this analysis were associated with two intra-island splits: the lineage leading to *H. forbesii* in the island of Abd al Kuri (the biggest size of the radiation) and the lineage leading to *H. pumilio* in Socotra (the smallest size of the radiation), both being exclusive (non-convergent) optima (Fig. 8). However the values associated to the optima involving these two regimes fell well outside the limits of the empirical values (Table S2, Fig. 8). This probably reflects the low accuracy of "surface" at estimating parameters in selective regimes that involve few branches (due to lack of information). Alternatively, this might be due to the fact that the parameter heterogeneity conflicted with the assumption made by "surface" of constant parameters for each trait across the tree. The analyses conducted on the 1,500 trees detected the same four regimes found in the summary tree in 91% of the trees, five regimes in 6% of the trees and three regimes in 3% of the trees. Despite this variation in the number of regimes, two divergent size optima were always detected in the frame of the onset of intra-island diversification in Socotra and Abd al Kuri (Fig. 8 and Fig. S4).

DISCUSSION

It is often assumed that after island colonization groups are released from the high inter-specific competition and predation existing in the continent (Yoder et al., 2010). This release allows insular groups the possibility to expand their niches and phenotypes as they rapidly move across this context of great ecological opportunity (Schluter, 2000; Losos & Ricklefs 2009). In this study our aim was to test this hypothesis on the morphological diversification of a nearly completely mainland-island system: the *Hemidactylus* geckos of the Arid clade. Our results, although generally consistent with this notion, at the same time revealed a complex picture in which different island communities and different traits, may produce vastly different patterns and processes in the same mainland-island system.

BODY SIZE

Body size was highlighted as the trait that presented the greatest diversification after island colonization, with size disparities in islands always significantly higher than the mean disparities in the continent. This is consistent with the notion that after island colonization groups are able to expand their trait variation in response to the novel ecological opportunities they encounter (Losos & Ricklefs, 2009). In the Arid clade, this expansion was beyond the limits of size variation existing in the continent, with island clades producing the largest and the smallest sizes of the radiation. Similar patterns have been reported in many other mainland-island systems where the biggest and/or the smallest species are found in islands (Raia et al., 2010).

In both Socotra and Abd al Kuri, these great island disparities were mainly the consequence of accelerated rates of body size evolution that occurred at the onset of intra-island diversifications: in the unique intra-island split occurring in Abd al Kuri (separating *H. oxyrhinus* and *H. forbesi*), which produced the highest rate of size of evolution existing in the Arid clade and, although with lesser magnitude, also in the basal-most split in Socotra (separating *H. pumilio* and its sister lineage). However, in both cases, this size differentiation was also consistent with the existence of two different size optima. This suggests that the existence of long-lasting selective pressures separating sister species towards different size optima, could also have contributed to produce the observed size divergence. These results are consistent with the evidence provided by many other groups in which body size is the first trait to experience adaptive divergence after island colonization (Moen & Wiens, 2009; Losos, 2009).

It is remarkable the case of the little island of Abd al Kuri, in which the two sister species present extremely diverging body sizes in the frame of an *in situ* speciation event (Fig. S5). The small size of the island along with its low physiographic complexity makes conceivable the possibility of phenotypic differentiation (and speciation) in sympatry. In a plausible scenario, this extreme body size divergence could be the consequence of disruptive selection driven by intra-specific competition. In a context of great ecological opportunity (low competition and predation) species usually increase their densities in islands (phenomenon known as "density compensation", Macarthur et al., 1972; Buckley & Jetz, 2007; Yoder et al., 2010). In such situations disruptive selection may arise if phenotypically intermediate (and more common) phenotypes (e.g. intermediate sizes) compete more strongly for resources than those at the tails of the distribution (extreme sizes). This results in lower fitness of the intermediate phenotypes leading an expansion to new and less exploited resources. This is expected to drive great phenotypic divergence ultimately leading to speciation (Nosil, 2012). In Abd al Kuri, the absence of predators normally occurring in mainland (e. g. snakes) could have also contributed to this size divergence, releasing groups from stabilizing selection on optimal sizes to scape or hide and hence allowing the possibility to produce the observed size extremes (particularly the biggest size in the radiation, *H. forbesii*).

This is a very appealing hypothesis, as it would explain both the *in situ* speciation event and the large degree of size disparity occurring in the island. However, although this hypothesis is consistent with studies conducted on many other insular taxa, showing size differentiation based on resource use (e. g. different prey sizes) (Boback, 2003; Keogh et al., 2005; Harmon & Gibson, 2006; Losos, 2009), additional empirical evidence should be added to this picture to either confirm or deny this possibility. For instance, data shedding light on the diets of both species would be crucial in order to verify whether this observed size disparity allow them to effectively exploit different resources (e. g. different prey sizes). Similar processes could also explain the size divergence existing in the innermost split occurring in Socotra, however in this case given the greater area and topographic complexity of the island, other scenarios as allopatric speciation coupled with size divergence in the context of a secondary contact cannot be excluded.

In the case of Socotra the comparison between the patterns of size variation and geographic distribution provide additional evidence consistent with size-based resource partitioning: all the species substantially differing in size are mostly found in sympatry while the species presenting similar sizes (e. g. the sister species, *H. dracaenacolus* and *H. granti*) occur in strict allopatry (Razzeti et al., 2011). Still in Socotra, rates of body size evolution are higher at the beginning of the diversification and then tend to decrease towards the present. This pattern is consistent with the "niche-filling" process found in many other insular groups (Mahler et al., 2010). All this combined evidence suggests that size-based resource partitioning in Socotra may be a plausible scenario that future studies will need to address (see conclusions).

Finally, regarding the patterns of size variation observed in the continent, our results provide a very complex picture. Most of the continental clades present low disparities and converge into similar intermediate sizes with low rates of evolution. However, towards the end of the diversification in the Arid clade, some lineages substantially increased their rates of body size diversification, attaining magnitudes comparable to the rates computed at the onset of the diversification in Socotra. This suggests that some clades in the continent are in an active process of phenotypic expansion. Nonetheless, in this case, body size differentiation does not involve different optima according to our "surface" analysis, calling into question the role of adaptive-mediated size divergence also operating in the continent.

HEAD PROPORTIONS

Our results failed to detect any acceleration in the evolution of head proportions after island colonization. This is consistent with the disparity pattern found for Socotra in which similar disparities in head proportions were computed for Socotra and Mainland. However, this contrasts with the situation found in Abd al Kuri where significantly greater disparities compared to the continent were detected. A possible explanation of this, comes from the realization that higher tempos of phenotypic evolution are not the only processes driving phenotypic diversification; long lasting selective pressures directing phenotypic change towards different optima (stable adaptive landscape, sensu Mahler et al., 2013), can also produce great levels of disparification (Sidlaukas, 2008).

When we tested this with "surface" we detected the existence of different selective regimes acting along a "short-narrow-low" and a "long-wide-high" continuum. These two optima appeared independently in Africa, Arabia, Socotra and Abd al Kuri, providing evidence of independent shape convergence across these landmasses.

Remarkably in Abd al Kuri, these two phenotypic optima appeared in the frame of an intra-island speciation event, likely being responsible for the great phenotypic disparities found in this island. Again, as in body size, such a pattern of strong differentiation in head proportions could obey to resource partitioning. In fact, different head proportions involve ecological segregation in a number of ways: head height can be related to refuge use, with species with lower heads being able to exploit narrower

spaces as refuges. Furthermore, head width and length are related to bite force, with species with wider and shorter heads being capable to bite harder, and therefore being able to feed on preys with stronger exoskeletons (Herrel et al. 2001; Losos, 2009). Although in Abd al Kuri, a scenario of resource partitioning based on head shape is a plausible one, as in the case of body size, further work is required to better understand how these differences in head shape can be translated in ecological differences.

In the island of Socotra, species failed to show higher head shape disparities compared to the continent. This may be explained by the fact that, in this case, the two selective regimes operating on head proportions were not associated to any intra-island split, but to two independent island invasions: one corresponding to *H. pumilio, H. inintellectus, H. granti* and *H. dracaenacolus* in the "short-narrow-low" end of the spectrum and the recently arrived *H. homoeolepis* at the "long-wide-high" extreme. The fact that in Socotra these two optima did not arise as a consequence of an *in situ* speciation event, coupled with the fact that most of the species share the same optimum of head proportions, likely explains the low disparities of head proportions in this island.

CONCLUSIONS AND FUTURE PERSPECTIVES

In this study we investigated the patterns and processes behind the phenotypic diversification experienced by the *Hemidactylus* geckos in the Socotra Archipelago, in the context of a nearly completely sampled mainland-island system. According to our results, the colonization of the little island of Abd al Kuri triggered high disparities in body size and head proportions, both being significantly higher than in continental clades. However the Socotran *Hemidactylus*, although also presented significantly higher size disparities compared to the species inhabiting mainland, disparities of head proportions were in the range of those found in the continent.

Size and head proportions also differed in their major drivers of phenotypic diversification. Size diversification was mediated by high rates of phenotypic diversification, possibly also coupled with the existence of different selective regimes toward different optima. This is consistent with size-based resource partitioning, scenario that needs to be contrasted with further evidence.

In the case of head proportions, great disparities when detected in islands were mainly the consequence of different selective regimes, not necessarily involving accelerated rates of evolution.

Finally, the head morphospaces of both island and continental communities were essentially overlapping. This shows that the morphospace expansion in Abd al Kuri mostly occurred within the limits of the continental morphospace, likely not implying a radical shift in the ecologies of continental and insular groups. This contrasts with our results on size that show how island species presented the most extreme sizes of the radiation, well beyond the limits of the continental range of variation and seemingly presenting exclusive, non-convergent optima. This highlights how different traits and different island communities within the same archipelago, can be decoupled in terms of the patterns

and processes of phenotypic diversification. More data on the Socotran *Hemidactylus* will be crucial to assemble a more complete picture of how diversification took place in the archipelago compared to the continent. Other aspects of the morphology of these geckos, as limb lengths, could provide additional axes of eco-morphological segregation not explored in this study. Also the extension of these studies to other Socotran and continental groups will be of utmost importance in order, not only to provide other replicates of island diversification, but also to integrate all these replicates together to acquire a community perspective.

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SUPPLEMENTARY FIGURES

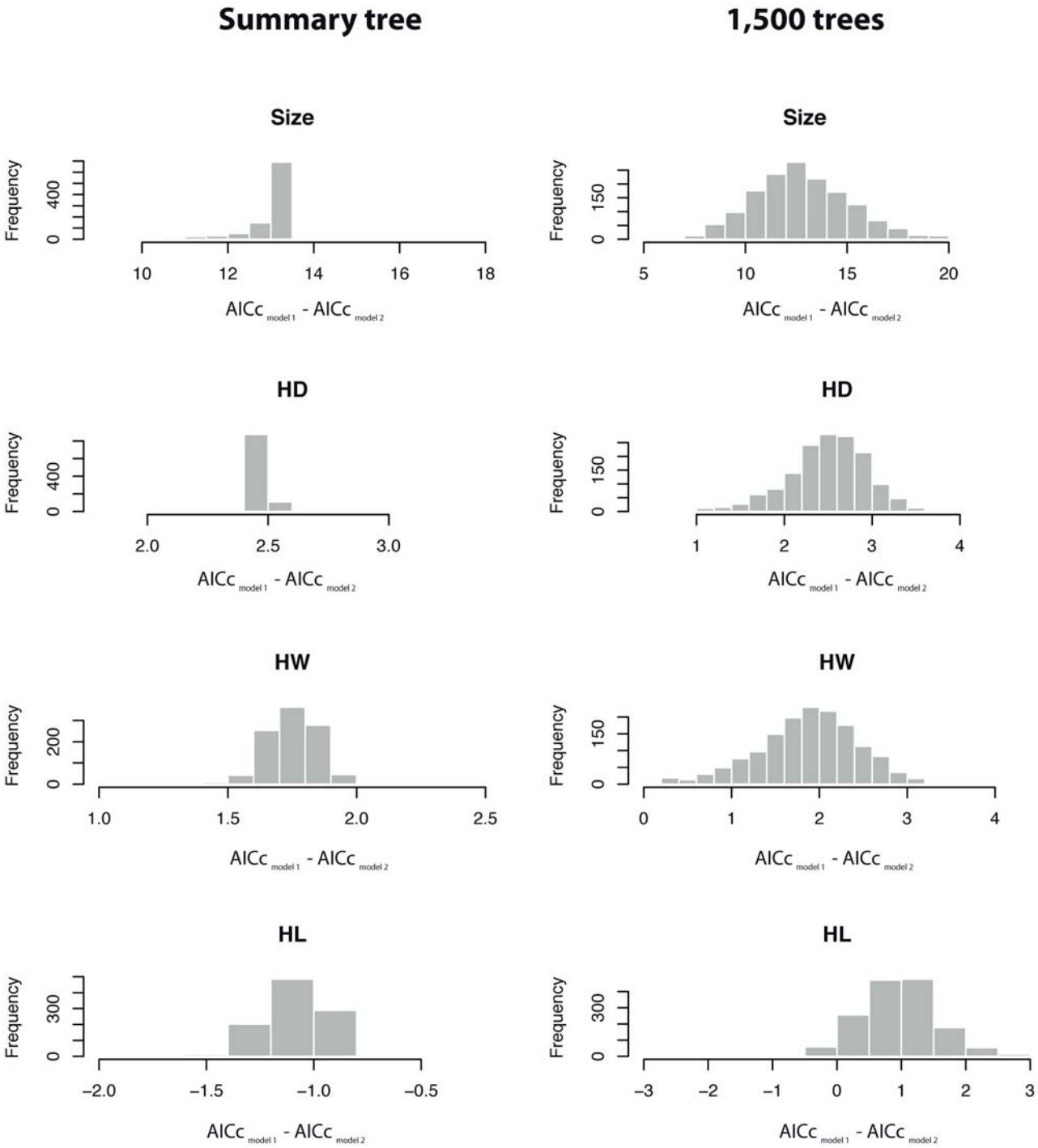

Figure S1. Distributions of the computed differences of Akaikes (ΔAICc) between model 2 (assuming rate heterogeneity) and model 1 (assuming a single rate across the tree) for each of the measured traits. This is shown for all the analyses conducted on the summary tree (left) and on the set of 1,500 trees (right). In the figure HD stands for "head depth", HW refers to "head width" and HL refers to "head length".

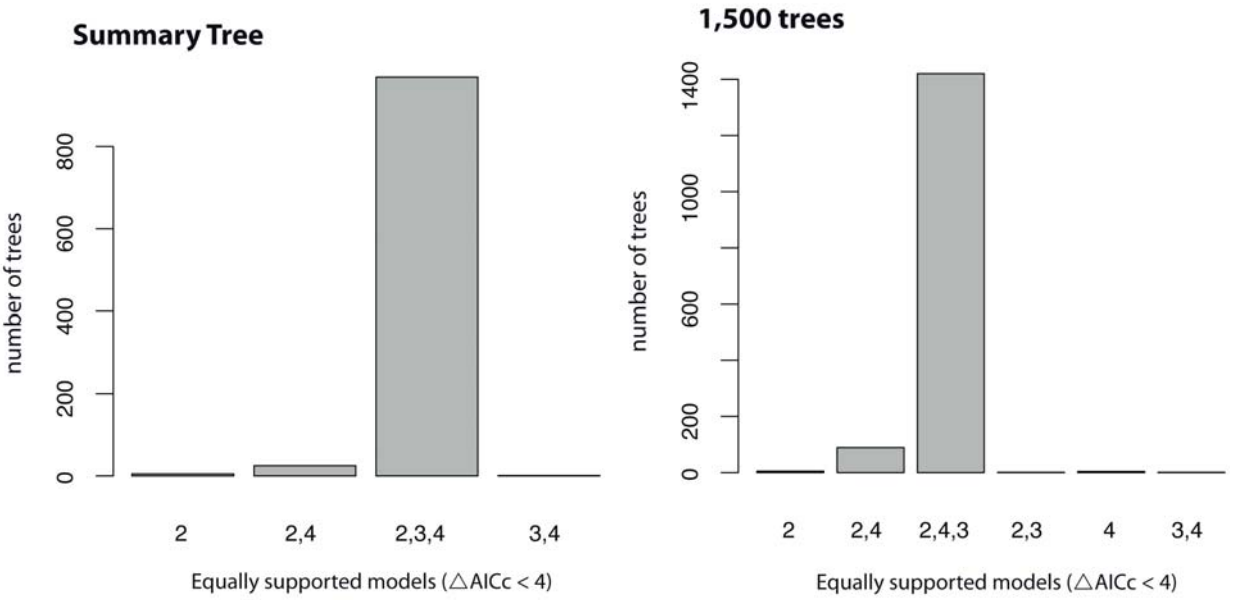

Figure S2. Barplots showing the frequency of similarly supported models (models at less than four ΔAICc from the model with the lowest AICc value) when models 1 and 2 are compared to models 3, 4 and 5. This is shown for the summary tree (left) and for the set of 1,500 trees (right).

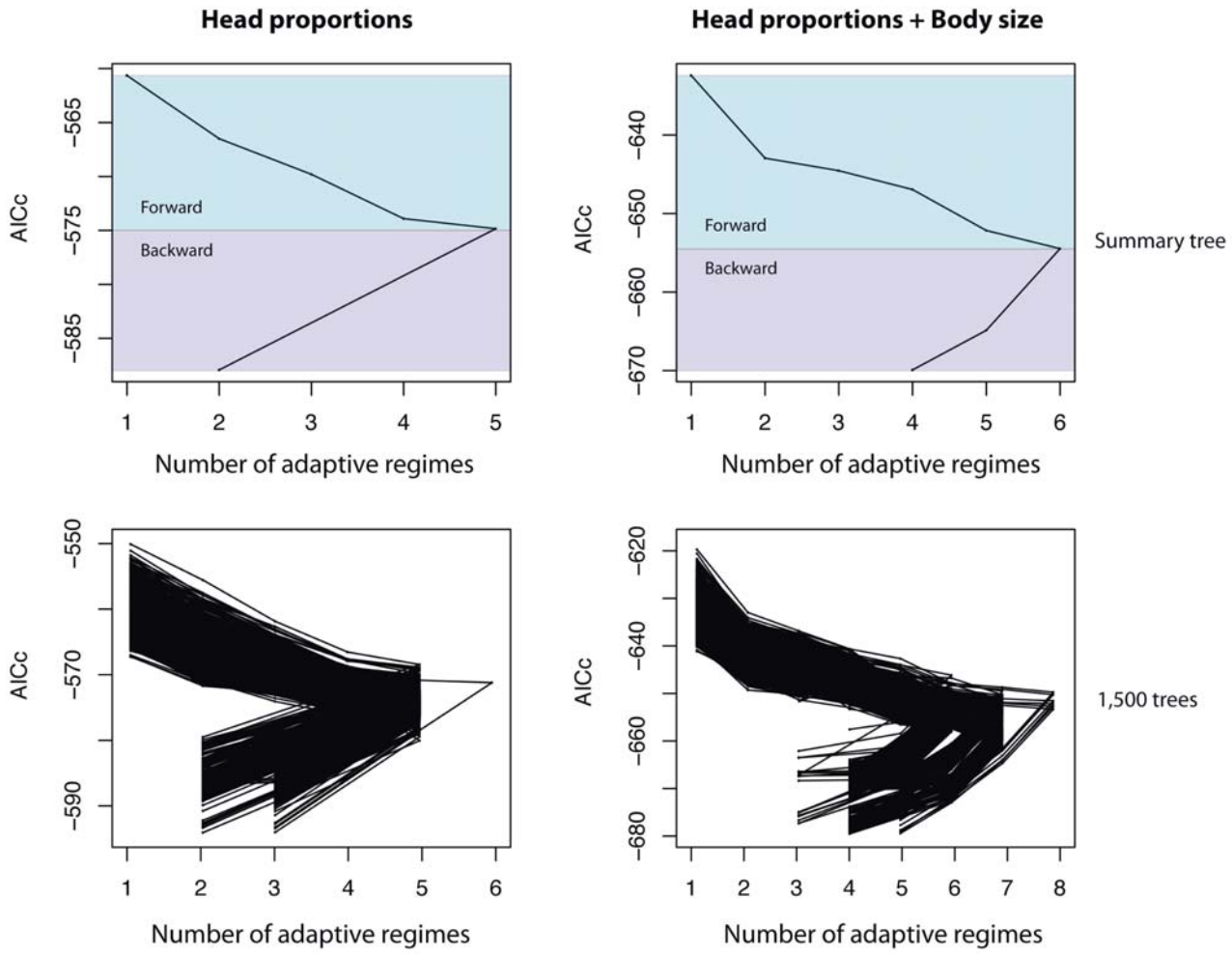

Figure S3. Sequence of OU model improvement for the "surface" analysis on head proportions (upper left) and on head proportions + body size (upper right) on the summary tree. In both cases, "forward" and "backward" phases are highlighted with different colors. Also shown in the figure are the lines tracing the model support in each of the 1,500 trees, using head proportions (lower left) and head proportions + body size (lower right).

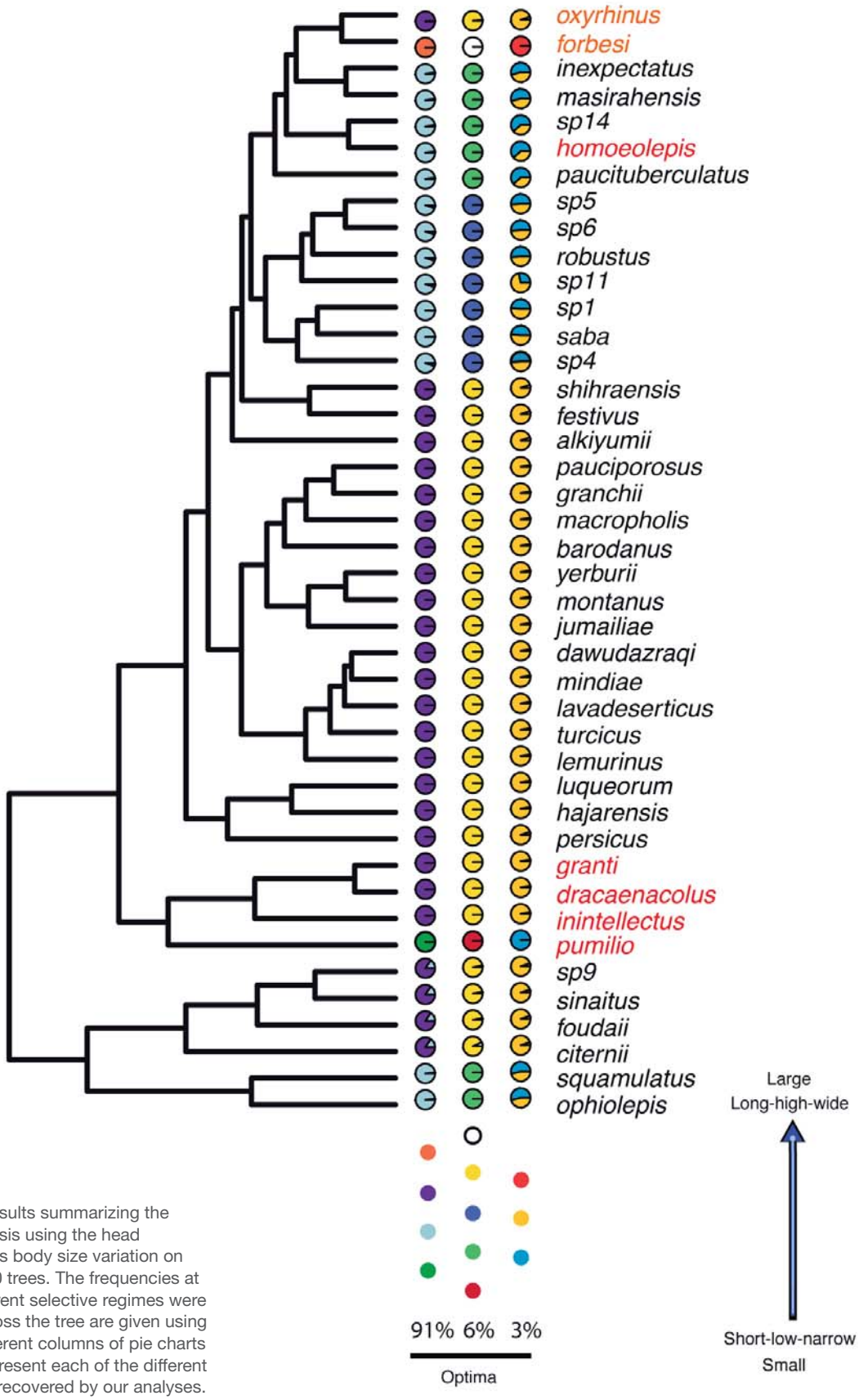

Figure S4. Results summarizing the "surface" analysis using the head proportions plus body size variation on the set of 1,500 trees. The frequencies at which the different selective regimes were distributed across the tree are given using pie charts. Different columns of pie charts are used to represent each of the different sets of optima recovered by our analyses. Also given are the frequencies of each of the sets of optima across the 1,500 trees.

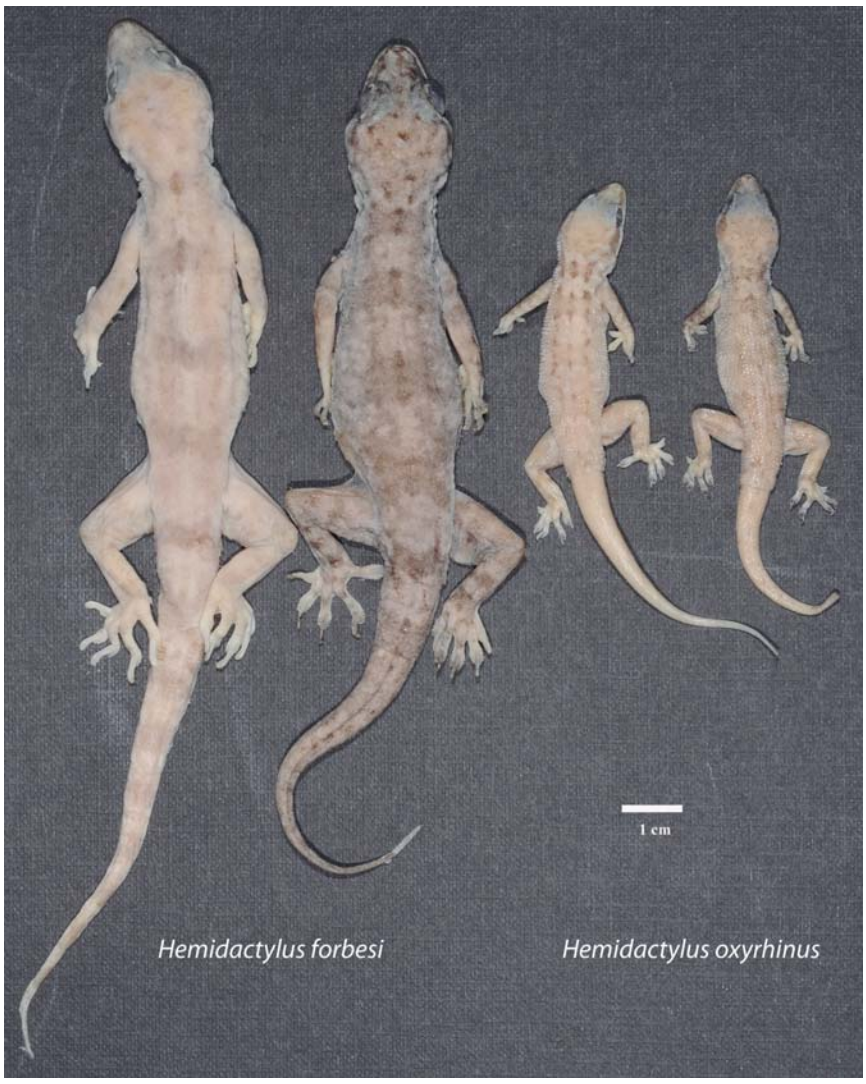

Figure S5. Picture showing the difference in size between the two species in Abd al Kuri: *Hemidactylus forbesii* (left) and *H. oxyrhinus* (right).

SUPPLEMENTARY TABLES

GROUPING	TRAIT	STATISTIC - SUMMARY TREE	P-VALUE - SUMMARY TREE	STATISTIC - 1500 TREES	P-VALUE - 1500 TREES
mainland vs islands	SVL (F - value)	1,20	0,53	0,69	0.54-0.65
mainland vs islands	SHAPE (Wilks lambda)	0,79	0,35	0.79-0.80	0.22-0.45
mainland vs Socotra vs Abd al Kuri	SVL (F - value)	0,69	0,60	1,20	0.48-0.62
mainland vs Socotra vs Abd al Kuri	SHAPE (Wilks lambda)	0,80	0,70	0.79-0.80	0.58-0.80

Table S1. Results of the phylogenetic ANOVA (on body size) and MANOVA (on head proportions) on the summary tree and the 1,500 trees, using different groupings for island and continental categories. The F-values for ANOVA and the Wilks lambda for MANOVA are given in each case with their associated *p*-values.

ANALYSIS	TRAIT	θ	α	σ2
HEAD SHAPE	HL	0,01	7754,39	2,68
		-0,01		
	HW	0,02	6128,15	4,61
		-0,02		
	HD	0,01	3775,39	7,57
		-0,02		
HEAD SHAPE + SIZE	SIZE	1,71	88,29	0,84
		3,17		
		1,17		
		1,48		
	HL	0,01	209,17	0,08
		-0,05		
		0,00		
		-0,02		
	HW	0,02	260,07	0,22
		-0,06		
		-0,01		
		-0,02		
	HD	0,01	3770,51	7,11
		-0,01		
		0,06		
		-0,02		

Table S2. Parameter estimates of the best OU models found by "surface" in two analyses: one only involving head proportions and another involving the combination of body size plus head proportions. In the table, σ^2 refers to the Brownian rate parameter, α to the rate of adaptation towards an optimum and θ refers to the trait values existing at the optima (one parameter per adaptive peak).

CHAPTER 2

KEY INNOVATIONS AND ISLAND COLONIZATION AS ENGINES OF EVOLUTIONARY DIVERSIFICATION: A COMPARATIVE TEST WITH THE AUSTRALASIAN DIPLODACTYLOID GECKOS

Photo credit: Joan Garcia Porta

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Key innovations and island colonization as engines of evolutionary diversification: a comparative test with the Australasian diplodactyloid geckos

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Keywords:

adaptive radiation;
body size disparity;
evolutionary rate;
lizard;
padless;
snakelike phenotype;
toepads.

Abstract

The acquisition of key innovations and the invasion of new areas constitute two major processes that facilitate ecological opportunity and subsequent evolutionary diversification. Using a major lizard radiation as a model, the Australasian diplodactyloid geckos, we explored the effects of two key innovations (adhesive toepads and a snake-like phenotype) and the invasion of new environments (island colonization) in promoting the evolution of phenotypic and species diversity. We found no evidence that toepads had significantly increased evolutionary diversification, which challenges the common assumption that the evolution of toepads has been responsible for the extensive radiation of geckos. In contrast, a snakelike phenotype was associated with increased rates of body size evolution and, to a lesser extent, species diversification. However, the clearest impact on evolutionary diversification has been the colonization of New Zealand and New Caledonia, which were associated with increased rates of both body size evolution and species diversification. This highlights that colonizing new environments can drive adaptive diversification in conjunction or independently of the evolution of a key innovation. Studies wishing to confirm the putative link between a key innovation and subsequent evolutionary diversification must therefore show that it has been the acquisition of an innovation specifically, not the colonization of new areas more generally, that has prompted diversification.

Introduction

A major challenge in evolutionary biology is understanding the main drivers that underlie morphological and species diversity (Wainwright, 2007). Ecological opportunity – access to new or previously inaccessible niches – has been identified as one of the most important drivers of both phenotypic and species diversification (Simpson, 1944; Losos & de Queiroz, 1997; Schluter, 2000; Nosil & Reimchen, 2005; Harmon *et al.*, 2008; Mahler *et al.*, 2010; Yoder *et al.*, 2010). This is because the exploitation of new ecological niches is often accompanied by phenotypic differentiation among closely related taxa. This can in turn facilitate species diversification if phenotypic differentiation is associated with the appearance of reproductive isolation (Gavrilets & Vose, 2009).

Ecological opportunity can arise from three main sources (Simpson, 1944, 1953): (i) the extinction of ecological competitors that open up previously 'filled' niches; (ii) exposure to new environments through dispersal (e. g. island colonization) or changes to existing environments through extrinsic forces that modify the environment (e.g. climate change); and (iii) the evolution of key innovations that allow taxa to use environments or resources in novel ways. These sources of ecological opportunity can appear in concert and interact in complex ways in diversifying groups. Our study examined the latter two sources of ecological opportunity – specifically, the colonization of islands and the

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evolution of two putative key innovations – and explored the extent that these have driven evolutionary diversification in a morphologically diverse and species-rich vertebrate group: the Australasian diplodactyloid geckos.

Island colonization and key innovations can affect evolutionary differentiation in a number of ways. First, we can expect the colonization of islands to result in ecological opportunity if colonizing taxa encounter new or unoccupied ecological niches. Adaptation to these newly available niches can trigger accelerated rates of phenotypic change and can be coupled with accelerated rates of speciation (Losos & Ricklefs, 2009). The Darwin finches of the Galapagos Islands (Grant & Grant, 2011), the explosive speciation of *Drosophila* in Hawaii (Zimmerman, 1970) or the numerous endemic *Anolis* lizard species found across the islands of the Caribbean (Losos, 2009) are classic examples of the sorts of adaptive radiations that can follow island colonization. Nevertheless, island colonization may not always lead to new ecological opportunities or result in accelerated evolutionary differentiation. In fact, reduced rates of evolutionary diversification might be expected if available ecological niches are filled by one or a few generalist species (Roughgarden, 1972), or if the composition of island communities reflects immigration rather than *in situ* island speciation (Whittaker *et al.*, 2010).

Second, key innovations are features that allow taxa to interact with their environment in novel ways and reach previously inaccessible regions of the adaptive landscape (Miller, 1949; Hunter, 1998; de Queiroz, 2002; Losos, 2009). The filling up of these newly accessed niches following the evolution of a key innovation may prompt increased rates of change in other phenotypic characteristics or high species diversification (Galis, 2001). Classic examples of key innovations are the evolution of feathers and wings in dinosaurs (which allowed flight; Hunter, 1998) and the appearance of flowers in plants (which allowed animal pollination; Vamosi & Vamosi, 2010). The concepts of key innovation and adaptive radiation are tightly linked in the literature (see Losos, 2009, 2010 and references therein). However, taxa such as the aardvarks (family Orycteropodidae) or even ourselves, humans, possess various key innovations and exhibit only low morphological and species diversity (Hunter, 1998; Wood & Collard, 1999). Such examples caution that the evolution of key innovations need not always open up the door to greater evolutionary diversification (Fürsich & Jablonski, 1984).

The Australasian diplodactyloid geckos (Vidal & Hedges 2009; Wilson & Swan, 2010) offer a wonderful opportunity to assess the contribution of island colonization and the evolution of key innovations in evolutionary diversification. The almost 200 species described so far in this group (Reptile Database: Uetz, 2010; accessed in February 2013) represent the greatest morphological diversity found in geckos (Oliver & Sanders, 2009). The group, containing three different families (Diplodactylidae, Carphodactylidae and Pygopodidae), forms an extensive radiation throughout Australia and New Guinea with, and of special relevance to our study, independent colonization of the island archipelagos of New Caledonia and New Zealand. Many – but not all – species possess one of two putative key innovations in the form of adhesive toepads or an elongated, near limbless snakelike phenotype (Hitchmough, 1997; Cogger, 2002; Wilson & Swan, 2010). Toepads are classically believed to have promoted ecological and species diversification in squamate lizards because they allow lizards to adhere to almost any surface (Autumn & Peattie, 2002; Hansen & Autumn, 2005; Huber *et al.*, 2005), greatly expanding the ecological niches available to species. Toepad evolution has consequently been inferred to have culminated in the extensive (and often adaptive) radiations of both the Caribbean *Anolis* lizards and geckos (Losos, 2009). In the case of the *Anolis*, these lizards subdivide more of their habitat than closely related padless genera (Warheit *et al.*, 1999). Such comparisons have led to the belief that the evolution of toepads was probably a critical step in the subsequent adaptive radiation of the *Anolis* lizards and presumably geckos as well (Losos, 2009).

Another candidate key innovation within geckos is a snakelike phenotype. Although most of the Australasian geckos have fully developed limbs, a subset of species (family Pygopodidae) possesses an elongated body with no forelimbs and only small scaly flaps as hindlimbs (hereafter referred as 'snakelike phenotype', Shine, 1986). This represents one of the most dramatic transformations in the tetrapod body plan and provides a new way to interact with the environment, enabling (i) more efficient locomotion; (ii) the ability to use narrow spaces like crevices for obtaining food, thermoregulation or shelter; (iii) the ability to burrow in soil or sand; and often, (iv) the ability to ingest prey bigger than themselves (Gans, 1975; Shine, 1986). This involves a combination of profound anatomical transformations that take place at different organismic levels, usually involving an extreme reduction in limbs and girdles, an increase in the vertebral number, visceral rearrangements and significant cranial transformations among others (Gans, 1975). The snakelike phenotype has appeared multiple times independently across the evolutionary history of squamates (Wiens *et al.*, 2006) and is associated with instances of high levels of species diversity, as in the case of the snakes or amphisbaenians.

In this study, we examined how the invasion of new environments associated with island colonization and the evolution of key innovations such as adhesive toepads and a snakelike phenotype have affected – independently or in synergy – the rates of phenotypic evolution and the diversity dynamics in a highly diverse group. We focused on changes in species body

size as a proxy for phenotypic evolution, as it is tightly correlated with a range of physiological and ecological characteristics, including metabolic rate, home range size and many life-history traits (Peters, 1986; Brown *et al.*, 2004). Furthermore, divergence in body size is a common outcome of evolutionary diversification with an adaptive component (Williams, 1972; Diamond, 1986; Richman & Price, 1992) because variance in body size among species tends to reflect the existence of resource partitioning (Moen & Wiens, 2009). In the particular case of Australasian geckos, body size varies extensively among species, from minute species of < 5 cm in snout-vent length to massive geckoes reaching well over 30 cm in snout-vent length (Bauer & Russell, 1986; Bauer *et al.*, 2006). Taken together, the Australasian geckos provide an ideal model to study the role of key innovations and island colonization in shaping the evolution of phenotypic and species diversity.

We began our investigation by developing a robust phylogeny of the whole radiation. Using this phylogeny, we then applied a variety of comparative methods to test whether key innovations and island colonization have been associated with accelerated rates of body size evolution and species diversification in the group.

Materials and methods

Phylogenetic analysis

The sequences of two mitochondrial (16S and ND2) and two nuclear genes (CMOS and RAG-1) were downloaded for all taxa assigned to Diplodactyloidea in GenBank (Benson *et al.*, 2011), plus 21 additional species of geckos outside of this group to calibrate the tree (GenBank was accessed in February 2013). The criterion to select genes was based on maximizing the number of species included in the phylogeny while minimizing the amount of missing species for each gene (with a minimum of 20% of representatives per gene). For each taxon, the longest sequence for each gene was retrieved with the additional requirement that all sequences had to be 200 bp or more for inclusion. After this procedure, our sequence data covered 82% of all currently described Australasian diplodactyloids (http://reptile-database.reptarium.cz; accessed February 2013), with an additional 35 undescribed species and nine highly divergent subspecies, resulting in a total of 202 taxa. Each gene was then trimmed and aligned using two procedures: the ribosomal coding 16S was aligned by means of MAFFT version 6 (http://www.ebi.ac.uk/Tools/msa/mafft/; Katoh *et al.*, 2002;) and the protein coding genes (ND2, CMOS and RAG-1) were aligned by means of the translation alignment algorithm implemented in the software Geneious (Drummond *et al.*, 2010). In both cases, the gap penalties and gap extension costs were left to default values. Finally, ambiguously aligned regions in the 16S alignment were excluded by means of Gblocks (Castresana, 2000). The final alignment consisted in a total of 3418 bp distributed in each gene as follows: 16S (227 bp), ND2 (939 bp), CMOS (372 bp) and RAG-1 (1880 bp).

The phylogenetic analysis was conducted by means of the package BEAST version 1.6.2 (Drummond & Rambaut, 2007). The prior for the distribution of branching times was based on a birth–death process. The nucleotide substitution model was set to GTR + G + I, and the variation of nucleotide substitution rates across the tree was assumed to be nonautocorrelated and log-normally distributed. The clock model and the nucleotide substitution models were applied independently to four partitions: 16S, ND2, CMOS and RAG-1, with every codon position considered separately in the protein coding genes.

Four calibrations were used to estimate branch lengths in units of time (Fig. S1):

1. The minimum age of the root node of Gekkota was set to 99.5 Ma based on the oldest fossil assigned to the crown group of Gekkota, *Hoburogekko suchanovi*, from the Early Cretaceous of Mongolia (Daza *et al.*, 2012) and a soft maximum of 180 Ma. This interval included the age of the oldest fossil of Gekkonomorpha (an undescribed fossil dated around 110 Ma) and the stem Squamatan *Parviraptor sp.*, dating back to 170 Ma (Conrad & Norell, 2006; Daza *et al.*, 2013). The prior was set by means of a gamma distribution ($\alpha = 3$, $\beta = 14$).
2. The minimum age for the radiation of *Sphaerodactylus* in the Caribbean was set to 20 Ma based on an amber fossil from the Dominican Republic (Daza & Bauer, 2012). The maximum age of this radiation was set conservatively to a soft maximum of 70 Ma. This was done by means of a gamma distribution ($\alpha = 2$, $\beta = 11$).
3. The age of the Tien Shan-Pamir uplift in western China, around 10 Ma, was used to calibrate the split between *Teratoscincus scincus* and the clade formed by *Teratoscincus przewalskii* and *Teratoscincus roborowskii* considering that this split originated via vicariance as a result of this geologic event (Macey *et al.*, 1999). A normal distribution with a mean positioned at 10 Ma and a standard deviation of 1 Ma were chosen to set the calibration prior of this node.
4. The minimum age of the clade represented by the *Pygopus* and *Paradelma* (including stem) was set at 20 Ma with a soft maximum of 50 Ma based on the oldest known fossil for this genus (*Pygopus hortulanus* – Hutchinson, 1997; Jennings *et al.*, 2003). A gamma distribution with an offset of 20 Ma was used to set the prior of this calibration point ($\alpha = 2$, $\beta = 6$).

The phylogenetic analysis consisted of two independent Markov Chain Monte Carlo (MCMC) analyses. Each chain was run for 50 000 000 generations with parameters, and trees sampled every 5000 generations. These two independent runs converged on very similar

posterior estimates and were combined using LogCombiner version 1.6.2 (http://beast.bio.ed.ac.uk/LogCombiner) after excluding the first 10% of generations in each one. Tracer version 1.5 (Rambaut & Drummond, 2007) was used to confirm convergence and good mixing of each MCMC chain.

To assess the effects of the interactions among the calibration priors, we ran one MCMC chain without sequences for 25 000 000 generations to estimate the distributions of the effective joint priors of our calibration points. We then compared these with the posterior distributions to assess congruence among calibration points (Sanders & Lee, 2007).

Finally, we calculated the summary tree as the maximum clade credibility tree with median node heights using TreeAnnotator version 1.6.2 (http://beast.bio.ed.ac.uk/TreeAnnotator), setting the posterior probability limit to 0.5. To incorporate uncertainty in both the topology and branch lengths of our recovered phylogeny in our comparative analyses, we resampled the posterior distribution of the trees generated by BEAST to obtain a set of 1000 trees. These 1000 trees were subsequently used for comparative tests of ancestor state reconstructions and diversification (see the following sections).

Species categories

We grouped species into one of five different categories: 'snakelike' (those taxa that with elongated body and lacking functional limbs; these occurred throughout Australia and New Guinea), 'padless' (those limbed taxa with no adhesive toepads; these were restricted to Australia), 'continental pad-bearing' (those taxa that possessed adhesive toepads and were found throughout the Australian continent), 'New Caledonian pad-bearing' (those taxa that possessed well-developed adhesive toepads and occurred in New Caledonia, abbreviated as NC) and 'New Zealand pad-bearing' (those taxa with well-developed toepads and occurred in New Zealand, abbreviated as NZ; Fig. 1). We distinguished the toepad-bearing categories for the continental and island species to single out the effects of toepads and island colonization (or island colonization plus toepads, in the case of a combined effect of both) on the rate of phenotypic and species diversification. We decided to split island species in New Caledonia and New Zealand given that the gecko radiations were monophyletic on each archipelago and large differences existed between these islands in terms of latitude (being 1700 km from one another), area and physiography (Bauer & Sadlier, 2000; Wallis & Trewick, 2009). New Guinea – represented by a single species (*Lialis jicari*) – was considered as a part of the radiation of Australia. Morphotype assignment of categories (snakelike, padless and pad-bearing) were based on the descriptions provided by Hitchmough (1997), Bauer & Sadlier (2000), Cogger (2002) and Wilson & Swan (2010).

Body size was measured as the maximum snout-vent length (SVL) reported for a given species. SVL data were compiled from Bauer & Russell (1986), Shea (1991), Bauer & Sadlier (2000), Bauer *et al.* (2006, 2009), Wilson & Swan (2010), Meiri *et al.* (2011), Bauer *et al.* (2012a,b) and the 'Electronic Atlas of the Amphibian & Reptiles of New Zealand' (EAARNZ, available at http://www.doc.govt.nz/conservation). All SVL data were log-10 transformed prior to analyses.

Analyses

Ancestral state reconstructions

We reconstructed the ancestral states of our categorical states by means of the function 'make.simmap' in the package 'phytools' version 0.2.80 (Revell, 2011). This function essentially fits a continuous-time reversible Markov model and simulates plausible stochastic character histories along the tree using the most likely model in combination with the states assigned to the tips of the tree (Revell, 2011).

For both the summary tree and the set of 1000 trees, we reconstructed the five categories described in the previous section (i.e. 'snakelike', 'padless', 'continental pad-bearing', 'NC pad-bearing' and 'NZ pad-bearing'). Reconstructions made on the summary tree relied on 100 stochastic character histories, whereas those made on the set of 1000 trees relied on a single stochastic history simulated on each tree. By implementing reconstructions on both the summary tree and the set of 1000 trees resampled from the posterior distribution used to estimate the summary tree, we effectively incorporated uncertainty in both the tree estimation and the character state reconstructions in subsequent comparative analyses.

In addition to these reconstructions, we created a second series using only the summary tree to reconstruct various groupings of these categories (Table S1) to assess whether rates of body size evolution differed or were similar among select categories in follow-up analyses (specifically those of MOTMOT; see next section). For example, all continental categories (snakelike, padless and continental pad-bearing) and island categories (NC pad-bearing and NZ pad-bearing) were grouped together to test whether evolutionary rates of body size evolution differed between continental and island lineages. Another set of reconstructions separated snakelike, padless and pad-bearing lineages (from the continent, NC and NZ) to assess whether evolutionary rates differed more between these lineage types. See Table S1 and the following section for other category groupings. All reconstructions followed the same protocol of simulating 100 stochastic character histories onto the summary tree.

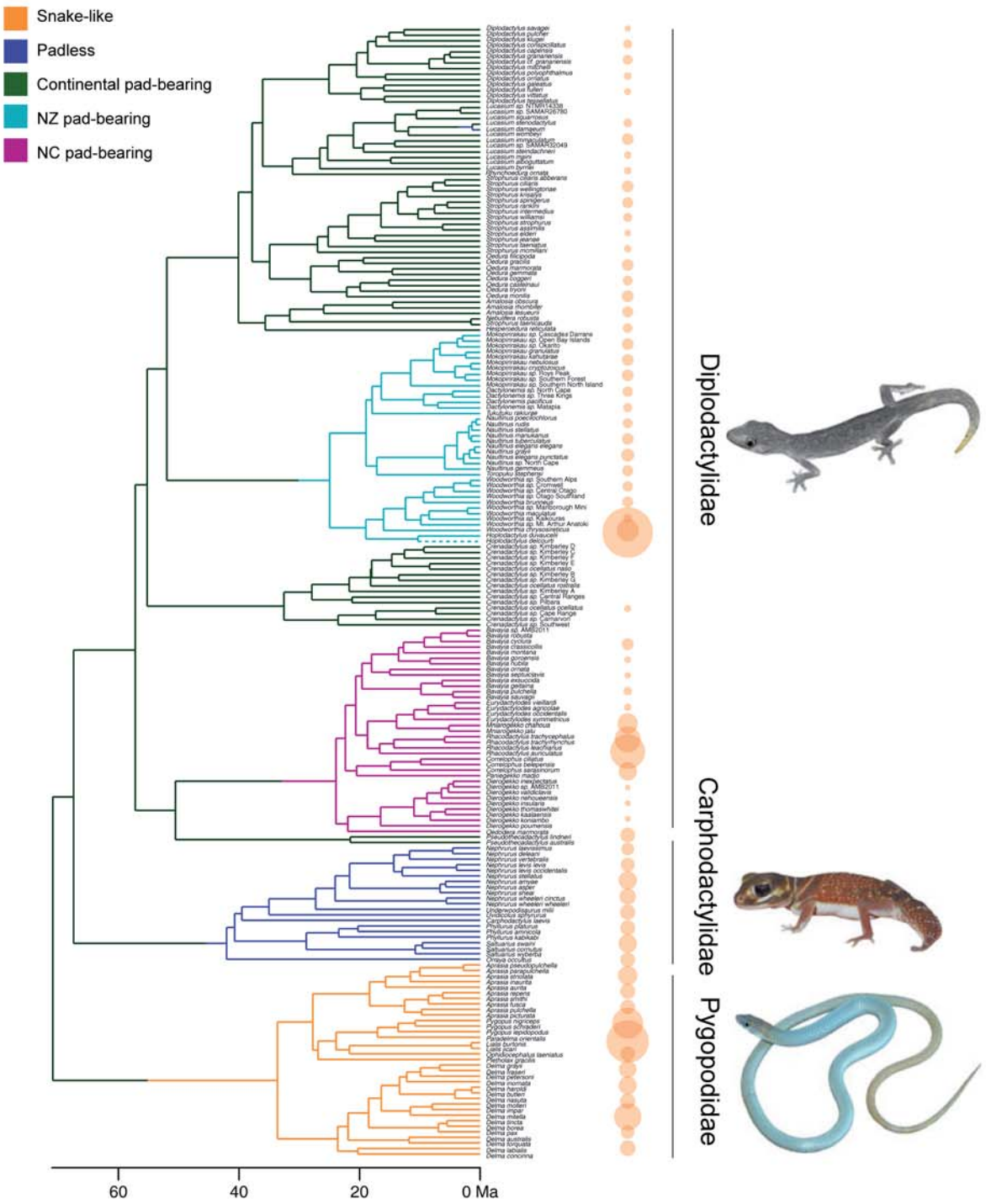

Fig. 1 Time-calibrated tree of the Australian diplodactyloid geckos with the evolutionary transitions among categories reconstructed according to one possible stochastic character history. The shading of the branches correspond to the following: 'snakelike' (elongated geckos that lack functional limbs), 'padless' species (limbed geckos with no adhesive toepads), 'continental pad-bearing' species (geckos that possessed adhesive toepads and occurred on continental Australia), 'NC pad-bearing' (geckos that possessed adhesive toepads and occurred in New Caledonia) and 'NZ pad-bearing' (geckos with toepads inhabiting New Zealand). The dashed line indicates the hypothetical phylogenetic position of the extinct gecko *Hoplodactylus delcourti*. The circles provide a visualization of the body size variation across the phylogeny with diameters proportional to the maximum snout-vent length (SVL) of a given species. Also shown are three representative species for each gecko family covered by the phylogeny.

Rates of body size evolution

We used two complementary approaches to estimate rates of body size evolution across the phylogeny. The first method was implemented by the R package MOTMOT version 1.0.1 (Thomas & Freckleton, 2012) and consisted of first specifying where on the phylogeny each categorical state had evolved. The relative rates of body size evolution among lineages assigned to a given category were then estimated via maximum likelihood (Thomas *et al.*, 2009). We fitted five alternative models each one based on a different category reconstruction (Table S1). Model 1 assumed that rates of body size evolution differed among all of our five categorical groups (snakelike, padless, continental pad-bearing, NC pad-bearing and NZ pad-bearing). Model 2 assumed that continental and island lineages differed in their rates. Model 3 assumed that evolutionary rates differed among the snakelike, continental and island lineages. Model 4 assumed that evolutionary rates differed among the snakelike, padless and pad-bearing lineages. Finally, Model 5 – the null model – assumed that rates of body size evolution were consistent across all lineages. Each model was run twice: once assuming that all categories shared a common phylogenetic mean (notated by 'a') and once assuming that categories did not share a common phylogenetic mean (notated by 'b'). This resulted in a total of ten models (Model 1a, Model 2a, Model 3a, Model 4a, Model 5a, Model 1b, Model 2b, Model 3b, Model 4b and Model 5b).

We evaluated the relative support for each model based on their computed mean second order Akaike's Information Criterion (AICc) across the 100 ancestor reconstructions on the summary tree (Burnham & Anderson, 2004). We also applied Model 1 (a and b; the most general model) to the set of 1000 trees in which each tree assumed a different stochastic history in the reconstruction of the snakelike, padless, continental pad-bearing, NC pad-bearing and NZ pad-bearing categories. This was done to assess the effect of uncertainties in both the phylogeny and ancestor reconstruction on the computed rates of body size evolution.

The recent extinction of what was the biggest gecko in the world, the New Zealand endemic *Hoplodactylus delcourti*, might have impacted our estimated rates of body size evolution for NZ pad-bearing category. To examine this, we refitted all models described before to the set same set of 100 trees in which *H. delcourti* had been positioned as a sister species of its probable closest relative, *H. duvaucelii* (based on morphological resemblance; Hitchmough, 1997) with a randomly set node height in each tree. We also applied Model 1 (a and b) to the set of 1000 trees in which each tree had *H. delcourti* positioned as sister of *H. duvaucelii* with a random height in each tree. As previously described, each tree incorporating *H. delcourti* assumed a different stochastic history in the reconstruction of the snakelike, padless, continental pad-bearing, NC pad-bearing and NZ pad-bearing categories.

The second method was implemented by the R package 'auteur' version 0.12 (Eastman *et al.*, 2011), which estimated rates of body size evolution along branches of the phylogeny without *a priori* specifying which regions of the tree corresponded with particular categories. That is, there was no prior assumption that evolutionary rates had changed at specific points in the phylogeny (e.g. those lineages reconstructed to have toepads). Within the 'auteur' package, we performed a reversible-jump Markov Chain Monte Carlo sampling to estimate the rates of body size evolution along the branches of our summary tree and the 1000 trees subset. Here, rates were computed as a weighted average of posterior rate estimates, where weighting was determined by branch lengths (Eastman *et al.*, 2011). To ensure an optimal mixing of the Markov chain, we first calibrated the proposal width with the summary tree by running three independent chains during 5 000 000 generations. We then ran three independent chains for 20 000 000 generations with a sampling interval of 3000 generations. The posterior estimates of these three runs were subsequently pooled with the first 50% of generations excluded. This analysis allowed us to estimate the posterior rates of body size evolution along branches as well as to localize rate shifts across the branches of the summary tree. To assess whether the results of 'auteur' were consistent with the scenario of rate heterogeneity depicted by MOTMOT, we extracted the posterior rate estimates of the branches belonging to each of the five categories (snakelike, padless, continental pad-bearing, NC pad-bearing and NZ pad-bearing). We then plotted their mean rates along with their 95% high posterior density (HPD) to visualize the rate variation among these 'a posteriori' defined groups. As described previously for the MOTMOT analyses, to assess the effect of the extinct *H. delcourti*, the analysis on the summary tree was conducted twice, once not including *H. delcourti* and once in which *H. delcourti* had been placed as sister clade to *H. duvaucelii*. For the 1000 trees (in which *H. delcourti* had positioned as sister of *H. duvaucelii* with a random height in each tree), we ran a single chain of 2 000 000 generations per tree with a sampling interval of 1000 generations. Posteriorly, for each tree, we localized which lineages were associated with shifts in rates of body size evolution (only shifts detected in more than 90% of the trees were considered as well supported).

To ensure good mixing and convergence of the Markov chains, all the traces of the summary tree analysis and a subset of the runs for the 1000 trees were analysed by means of the program Tracer version 1.5.

Diversity dynamics

To examine the effect of key innovations and island colonization on species diversification, we applied three approaches. First, we assessed whether snakelike, padless, continental pad-bearing (with the sole exception of *Lucasium damaeum*, which was a padless species in this otherwise toepad-bearing clade) NC pad-bearing and NZ pad-bearing clades differed in their diversity dynamics using the coalescent-based approach described by Morlon *et al.* (2010). This method models the internode distances of a phylogeny assuming that they are distributed according to a standard coalescent approximation (Griffiths & Tavaré, 1994). This has the advantage of modelling species diversity from the present to the past assuming that it can take any value at any point in time. It can also easily accommodate incomplete-sampled phylogenies as the coalescence theory stems from the theory of samples (Morlon *et al.*, 2010). We split our summary tree into five subtrees corresponding to each of the clades of interest. Six models of diversification that differed in their assumed diversity dynamics were then applied separately to each of the five subtrees: Models 1 and 2 assumed that speciation rates were constant through time (a constant birth–death and Yule process, respectively), and the rest of the models assumed that speciation rates varied exponentially through time and differed in the dynamics of the extinction rates: Model 3 assumed a constant extinction rate, Model 4 assumed a extinction rate that varied as a function of the speciation rate, Model 5 assumed an exponential change in extinction rate over time and finally Model 6 assumed no extinction rates (Table S2). The parameters and likelihood of each model were estimated using the R code provided in Morlon *et al.* (2010). The best-supported model was identified as the model with the highest computed Akaike weight (AICw) (see Morlon *et al.*, 2010). This model was then used to interpret the diversification dynamics for a given clade based on its computed parameters estimates.

Second, we compared the rates of diversification among the snakelike, padless, continental pad-bearing, NC pad-bearing and NZ pad-bearing using the 'Multiple State Speciation Extinction' (MuSSE) model in the R package Diversitree version 0.9.1 (FitzJohn, 2012). This method estimates the rates of change in a multistate character and the rates of speciation and extinction associated with each character state given the distribution of observed states along the tips of a tree. This is performed by combining the features of a Markov model of trait evolution (to estimate the rates of transition among characters) and a constant rates birth–death process (to estimate diversification rates in each state character) in the same evolutionary model. We estimated the rates of diversification across the subset of 1000 trees retained from the BEAST posterior, assuming an equal rates model of character evolution.

Finally, we also assessed the among-categories heterogeneity in diversification rates across the subset of 1000 trees by means of a diversity-dependent model (dd), in which the speciation rate was variable through time (varying according to the diversity in a given time) with constant extinction. This was implemented by splitting each of the 1000 trees into five subtrees corresponding to each category and applying the function 'dd_ML' (model 1) in the R package DDD version 1.11 (Etienne *et al.*, 2012). For both models, we assessed rate heterogeneity among categories by plotting mean diversification rates and associated 95% confidence intervals computed for each category.

Given that the number of nonsampled species in a phylogeny can produce a bias in the estimates of species diversification (Ricklefs, 2007), all analyses took into account an estimate of the number of species missing from the phylogeny. According to the Reptile Database and the EAARNZ (accessed in February 2013), our sampling coverage for each major group within the Australasian geckos was the following: 85% for the family Pygopodidae, 70% for the family Carphodactylidae, 80% for the continental Diplodactylidae, 88% for the New Zealand Diplodactylidae and the 100% of the described species of New Caledonian Diplodactylidae.

Another source of bias might also occur if the taxonomy within each of the categories was not equally known. For example, if New Caledonia and New Zealand were better taxonomically and phylogenetically studied than species on the Australian continent, this could lead to an underestimation of the real diversity on the continent and subsequently affect its estimated diversification rate. To assess this, we conducted a separate analysis by means of the dd model in which it was assumed that an additional 50% of the total number of currently known species of Pygopodidae, Carphodactylidae and continental Diplodactylidae would be discovered at some point in the future (that is, the current estimated number of species actually represents only two-thirds of the true diversity of the group).

Results

Phylogenetic analysis

We recovered 75% of nodes of the summary tree with a posterior probability (pp) > 0.90 (high to very high support; Fig. S1). The phylogenetic relationships depicted by our summary tree were generally consistent with previous published phylogenies of the Diplodactyloidea (Jennings *et al.*, 2003; Gamble *et al.*, 2008). The only major difference lay in the snakelike Pygopodidae not being recovered as the sister group to Carphodactylidae (see Gamble *et al.*, 2011; although the node in question had low support in our analysis). In addition, the positioning of *Strophurus taenicauda* was

unexpected because it was recovered as the sister species of *Nebulifera robusta*. Although this positioning was consistent with the results of Melville *et al.* (2004; with the same sequence we used in our study), we suspect that this may reflect a mislabelling of the sequence used for *S. taenicauda*. We repeated our analyses with this species removed and found that it had no impact on any of the comparative analyses performed (results not shown). For dating estimates, the medians of the posterior distributions of the calibrated nodes fell within the 95% HPD of the effective priors. This indicated that the priors of the calibration points were largely congruent with one another (Sanders & Lee, 2007).

According to our estimates, the diplodactyloid geckos started to radiate in Eastern Gondwana between 85 and 60 Ma, which is consistent with previous estimates (97–40 Ma – Gamble *et al.*, 2008; 91–53 Ma – Oliver & Sanders, 2009). The mainland–island splits were dated around 50 Ma for both archipelagos (95% HPD = 61–43 Ma for New Zealand and 62–40 Ma for New Caledonia). In both cases, the 95% HPD interval lies after the last contact (90–65 Ma; Neall & Trewick, 2008; Wallis & Trewick, 2009) between Zealandia (the continental fragment containing New Zealand and New Caledonia) and mainland (what was to become Australia, New Guinea and Antarctica; Wallis & Trewick, 2009). The beginning of the radiations in New Zealand and New Caledonia was estimated at 25 Ma (95% HPD = 31–20 Ma; congruent with Nielsen *et al.* 2011) and 24 Ma, respectively (95% HPD = 29–20 Ma; largely congruent with Oliver & Sanders, 2009). This agrees with several lines of evidence suggesting a complete (or almost complete) submersion of Zealandia between 65 and 37 Ma (according to geological evidence from New Caledonia; Espeland & Murienne, 2011) or even until 25 Ma (according to the geological evidence from New Zealand; Trewick *et al.*, 2007) and a subsequent recolonization of these islands by dispersal (Waters & Craw, 2006; Trewick *et al.*, 2007; Espeland & Murienne, 2011). The diversity of the geckos in New Zealand and New Caledonia therefore seem to have originated following at least one dispersal event from the continent to each archipelago and subsequently accumulated via within-island diversification (based on the fact that most of the lineage splits occur within the same island).

Ancestral reconstructions

The maximum likelihood ancestor state reconstructions of the five categories over the summary tree and the subset of 1000 trees generally assigned toepads as ancestral in the Australasian geckos. However, this assignment was not clear-cut with the relative support being low for toepads existing at the root of the phylogeny compared with some other morphotype (the mean scaled likelihood estimate for toepads existing at the root of the phylogeny was 0.55). Reconstructions across the 1000 trees also revealed that most of the major transitions among morphotypes (snakelike, padless and pad-bearing) occurred between 82 to 38 Ma. There was also an instance of toepad loss during the last 10 Ma in the lineage leading to *Lucasium damaeum* (Fig. 1).

Rate heterogeneity of body size evolution

MOTMOT

Models 3a, 3b and 1a were the best-supported models on the summary tree, with less than four AICc unit -difference between each model (i.e. all three were reasonably plausible scenarios; Table 1). These were the best-supported models regardless of whether the extinct giant gecko, *H. delcourti*, was or was not included in the analysis (see also next paragraph). Model 3 assumed homogeneous rates of body size evolution among padless and continental pad-bearing species, but different evolutionary rates for the snakelike phenotype and island species. The maximum likelihood estimates of the evolutionary rates of this model showed that the snakelike and island lineages had accelerated rates of body size evolution in respect to the padless and continental pad-bearing categories, which exhibit similar rates (Table 1). The other supported model, Model 1a, assumed rate heterogeneity among all categories. However, the estimated rates of body size evolution were consistent with Model 3 in that similar, low evolutionary rates were estimated for padless and continental pad-bearing species, whereas evolutionary rates were over three times higher for the snakelike and island lineages. Inspection of the mean evolutionary rates computed for Model 1a across the 1000 trees and their 95% CI (Fig. 2) again showed no difference between padless and continental pad-bearing lineages, but significant accelerations in body size evolution for the snakelike, New Caledonian and New Zealand clades. That is, the best-supported models based on the summary tree were consistent with the estimated differences in evolutionary rate computed across the set of 1000 trees that incorporated uncertainty in topology and branch lengths.

The effect of including the extinct giant gecko, *H. delcourti*, in the models and in the set of 1000 trees produced an increase in the estimated evolutionary rates for New Zealand species, which subsequently attained levels comparable with those computed for New Caledonia (Table 1, Fig 2).

Auteur

The analysis based on the summary tree revealed that virtually all lineages with accelerated rates of body size evolution (those with posterior rates above the median rate of evolution) were confined to New Caledonia, New Zealand and the snakelike radiation (Fig. 3a). By contrast, low rates of evolution were detected for most of the continental pad-bearing lineages (Fig. 3a). The

Table 1 Models summarizing alternative scenarios of body size diversification across the five defined categories, with their AICc values ranked relative to the best-supported model (ΔAICc). Also, given are parameter values corresponding to the mean relative rate of body size evolution for each category by model fitted. The results are based on 100 plausible ancestral state reconstructions of each model on the summary tree. The grey shades highlight similar supported models (those with ΔAICc < 4). (a) refers to a situation in which *Hoplodactylus delcourti* was excluded from the analysis, in (b) *H. delcourti* has been placed with randomized branch length as the sister species of *H. duvaucelii*.

(a)

			Relative rates of body size evolution (excluding *H. delcourti*)						
Model	Description	Common mean	Snakelike	Padless	Continental pad-bearing	NC pad-bearing	NZ pad-bearing	AICc	ΔAICc
Model 3a	Snakelike vs. Continental nonsnake-like vs. Islands	Yes	3.10	1	1	4.52	4.52	−308.72	0.00
Model 1a	Snakelike vs. Padless vs. Continental pad-bearer vs. NC pad-bearer vs. NZ pad-bearer	Yes	3.72	1	1.27	6.59	4.36	−306.38	2.34
Model 3b	Snakelike vs. Continental nonsnake-like vs. Islands	No	3.04	1	1	4.52	4.52	−305.29	3.43
Model 1b	Snakelike vs. Padless vs. Continental pad-bearer vs. NC pad-bearer vs. NZ pad-bearer	No	3.73	1	1.27	6.76	4.46	−300.18	8.53
Model 2a	Continental vs. island	Yes	1	1	1	2.69	2.69	−294.57	14.15
Model 2b	Continental vs. island	No	1	1	1	2.69	2.69	−292.49	16.23
Model 4a	Snakelike vs. Padless vs. Pad-bearer	Yes	3.85	1	3.84	3.84	3.84	−281.89	26.83
Model 4b	Snakelike vs. Padless vs. Pad-bearer	No	3.81	1	3.82	3.82	3.82	−278.80	29.92
Model 5a	Equal rates	Yes	1	1	1	1	1	−275.14	33.58
Model 5b	Equal rates	No	1	1	1	1	1	−268.16	40.56

(b)

			Relative rates of body size evolution (including *H. delcourti*)						
Model	Description	Common mean	Snake-like	Padless	Continental pad-bearing	NC pad-bearing	NZ pad-bearing	AICc	ΔAICc
Model 3a	Snakelike vs. Continental nonsnakelike vs. Islands	Yes	2.99	1	1	5.50	5.50	−291.91	0.00
Model 3b	Snakelike vs. Continental nonsnakelike vs. Islands	No	2.93	1	1	5.50	5.50	−288.47	3.44
Model 1a	Snakelike vs. Padless vs. Continental pad-bearer vs. NC pad-bearer vs. NZ pad-bearer	Yes	3.14	1	1.07	5.37	6.15	−288.23	3.68
Model 1b	Snakelike vs. Padless vs. Continental pad-bearer vs. NC pad-bearer vs. NZ pad-bearer	No	3.12	1	1.07	5.44	6.23	−281.75	10.16
Model 2a	Continental vs. island	Yes	1	1	1	3.35	3.35	−278.71	13.20
Model 2b	Continental vs. island	No	1	1	1	3.34	3.34	−276.76	15.15
Model 4a	Snakelike vs. Padless vs. Pad-bearer	Yes	3.29	1	4.04	4.04	4.04	−256.78	35.13
Model 4b	Snakelike vs. Padless vs. Pad-bearer	No	3.25	1	4.00	4.00	4.00	−253.46	38.45
Model 5a	Equal rates	Yes	1	1	1	1	1	−249.28	42.64
Model 5b	Equal rates	No	1	1	1	1	1	−242.24	49.67

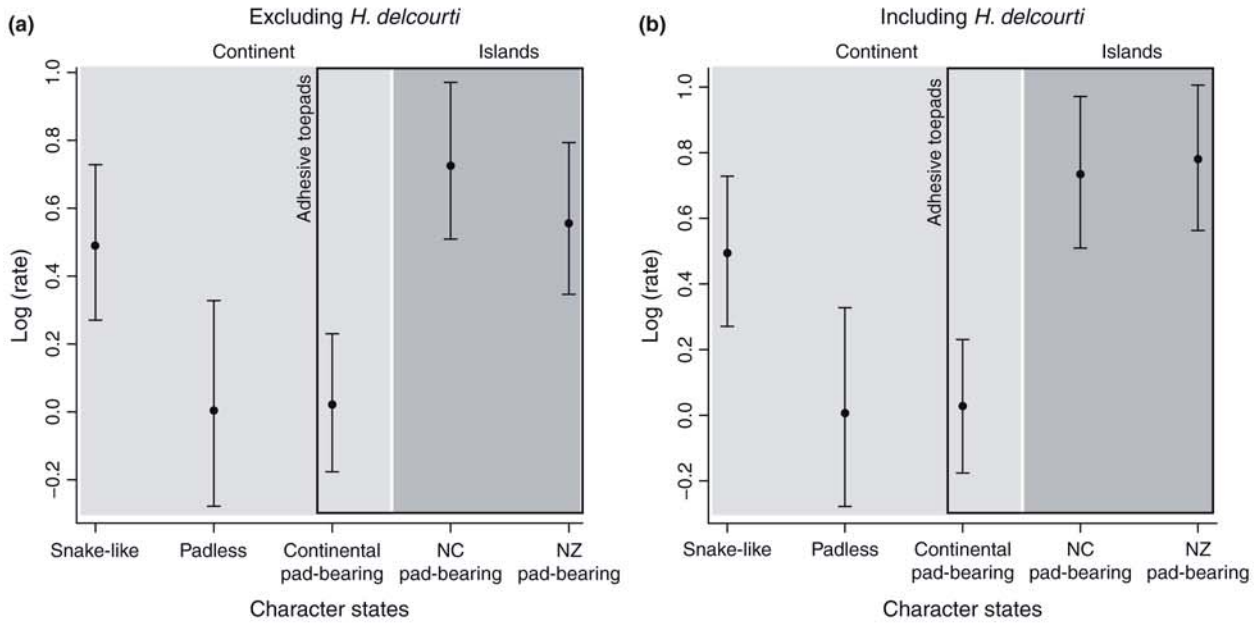

Fig. 2 Plot of the mean relative rates (in a $\log_{10}$.scale) of body size evolution and their associated 95% confidence intervals for each category estimated by Model 1a in which the extinct *Hoplodactylus delcourti* had been excluded (a) or included (b) in the analyses. The light grey rectangle and the darker grey rectangle represent continental and island lineages, respectively. The black frame groups together lineages possessing adhesive toepads. Results are based on a set of 1000 trees that varied in topology and branch lengths.

pattern of rate heterogeneity for those categories found to have accelerated rates of body size evolution was somewhat variable. In the New Caledonian radiation, with the exception of *Dierogekko*, most of the lineages experienced accelerated rates of body size evolution. In contrast, for the New Zealand radiation, high evolutionary rates were limited to select groups with other lineages not deviating from median (background) rates. In New Zealand, those lineages estimated to have experienced accelerated evolutionary rates basically involved all lineages leading to the *Naultinus* radiation (also involving *Toropuku stephensi*) and the lineage leading to *H. duvaucelii* (and to *H. delcourti* when this was included in the analysis, Fig. 3a). Within the snakelike category, high evolutionary rates were generally distributed across all genera.

For the analyses based on the 1000 trees, those shifts associated with high rates of body size evolution recovered in at least 90% of trees were found concentrated within the New Zealand (specifically lineages associated with *Naultinus* and the genus *Hoplodactylus*) and within the New Caledonian radiation, also affecting the split leading to the snakelike clade (Fig. 3a). The mean and 95% HDP intervals of evolutionary rates extracted from posterior rates defined by category depicted a scenario consistent with the MOTMOT results (Fig. 3b), with major increases in the rates of body size evolution found primarily in the snakelike, New Caledonian and New Zealand lineages. Also, consistent with the MOTMOT results was the effect of including the extinct *H. delcourti*, which resulted in an increase in the rates of body size evolution detected within the New Zealand clade (Fig. 3b).

Rate heterogeneity in species diversification

Diversity dynamics

The comparison of the six different diversification models fitted to the subtrees of each category extracted from the summary tree (Table 2) identified Model 6, which assumed a time-decaying speciation rate with no extinction, as the best model for the snakelike, continental pad-bearing and NC pad-bearing clades (in all cases with AICw > 0.5, Table 2, Fig. S2). For these three clades, speciation has generally slowed down through time ($\alpha > 0$), but this was estimated to have occurred at different rates within these clades: the New Caledonian radiation appears to have been associated with an early burst of speciation followed by a rapid decay in speciation rate ($\alpha = 0.1$); the continental pad-bearing radiation has experienced a slow decay in speciation ($\alpha = 0.02$); whereas the snakelike radiation has experienced an intermediate pattern of decay ($\alpha = 0.05$). In these clades, none of the two constant rate models (Models 1 and 2) received any substantial support (AICw < 0.01; Fig. S2). In padless and the NZ pad-bearing clades, Model 2, which assumed a pure-birth Yule process of diversification (implying diversification has been largely constant through time with no extinction), was highlighted as the best model. Although Model 6

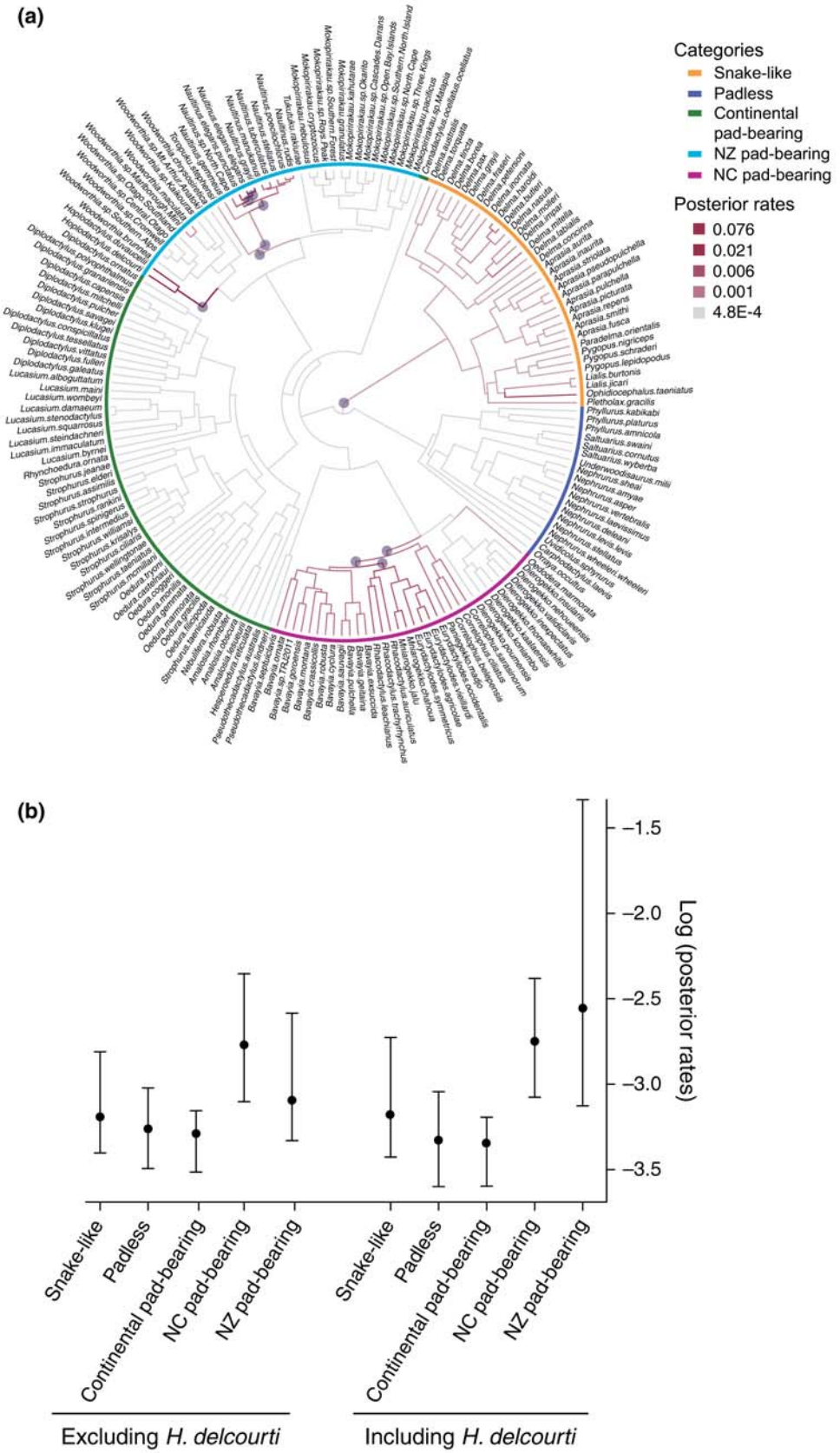

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Fig. 3 The top panel (a) shows the summary tree (including *Hoplodactylus delcourti*) with branches shaded to reflect how rates of body size evolution varied across the phylogeny. Background rates (those not deviating from the median rate across the tree) are shaded light grey; those rates greater than median rates are shaded in darker shading proportionally to their computed deviation from the median. Rates corresponding to each shade are indicated in the legend. The circles superimposed onto the phylogeny indicate rate shifts detected in more than 90% of the trees in the 1000 trees sample. The bottom panel (b) provides a plot comparing the posterior densities of the evolutionary rates estimated for the branches assigned to snakelike, padless, continental pad-bearing, NC pad-bearing and NZ pad-bearing. Two sets of analyses were conducted: one without the extinct giant gecko *H. delcourti* (left) and one including *H. delcourti* (posterior rate densities are plotted on a $\log_{10}$-scale).

Table 2 Parameter estimates and proportion of support (AICw) of the most likely models of diversity dynamics across the five categories. The results are based on the summary tree. Model 6 depicts a scenario of exponential variation in speciation rate and Model 2 corresponds to a Yule process in which speciation rate is constant through time. Here, λ_0 refers to the speciation rates at present day, and α refers to exponential variation in speciation rate.

Category	Best-fit model	AICw	Model properties			Parameter estimates
			Speciation	Extinction	Equation	
Snakelike	Model 6	0.57	Varying	–	$\lambda(t) = \lambda_0 e^{\alpha t}$	$\lambda_0 = 0.043$ $\alpha = 0.053$
Padless	Model 2	0.55	Constant	–	$\lambda(t) = \lambda_0$	$\lambda_0 = 0.062$
Continental pad-bearing	Model 6	0.54	Varying	–	$\lambda(t) = \lambda_0 e^{\alpha t}$	$\lambda_0 = 0.043$ $\alpha = 0.021$
NC pad-bearing	Model 6	0.66	Varying	–	$\lambda(t) = \lambda_0 e^{\alpha t}$	$\lambda_0 = 0.030$ $\alpha = 0.106$
NZ pad-bearing	Model 2	0.43	Constant	–	$\lambda(t) = \lambda_0$	$\lambda_0 = 0.137$

also received some support for the padless and the NZ pad-bearing clades (Fig. S2), the estimated rates of decay for the diversification rates in this model were very low ($\alpha = 0.02$ and $\alpha = 0.01$ for the padless and NZ pad-bearing clades respectively), implying little variation in diversification rates from the onset of the diversification to the present day (again, that diversification has been largely constant through time; see Model 2 above).

The MuSSE model applied to the subset of 1000 trees produced low mean transition rates among categories ($q = 4.2{\cdot}10^{-4}$) and generally lower diversification rates compared with the dd model. Extinction rates were also estimated very differently between the models, with a negligible effect in the MuSSE model but with high rates in some categories according to the dd model (Table S3). Despite these differences, both models produced consistent patterns of rate heterogeneity: both indicated high species diversification rates for the island radiations compared with continental pad-bearing lineages. The snakelike radiation also exhibited some rate acceleration, but the 95% confidence intervals overlapped those of the padless and continental pad-bearing lineages (particularly for the dd model; Fig. 4b). The padless clade showed similar diversification rates to the continental pad-bearing lineages (the MuSSE model; Fig. 4a), or perhaps even higher rates than the continental pad-bearing (the dd model; Fig. 4b), although the broad confidence intervals computed for the padless lineages made it difficult to interpret.

Finally, potential biases in the intensity of taxonomic sampling of islands versus continental communities were unlikely to have affected our results. When we applied the dd model with the assumption that only two-thirds of the true number of existing species have been described for the Pygopodidae, Carphodactylidae and continental Diplodactylidae, and our results were virtually unchanged (Fig. S3).

Discussion

We examined the effect of the invasion of new environments – island colonization – and the evolution of two key innovations – the acquisition of adhesive toepads or a snakelike phenotype – on evolutionary diversification in a morphologically diverse and species-rich group of lizards, the Australasian diplodactyloid geckos. Our results highlighted colonization of islands and the acquisition of a snakelike phenotype as the primary factors that have prompted accelerated rates of evolutionary diversification in geckos. The evolution of adhesive toepads seems to have had little impact on rates of body size evolution or species diversification, beyond its potential interaction with island colonization (see below).

Island colonization and diversification

Our dating estimates set the probable origin of the gekkotan radiations on New Caledonia and New Zealand soon after the emergence of the archipelagos from their submersion 37–25 Ma (Trewick *et al.*, 2007; Espeland & Murienne, 2011). Rapid colonization of a young

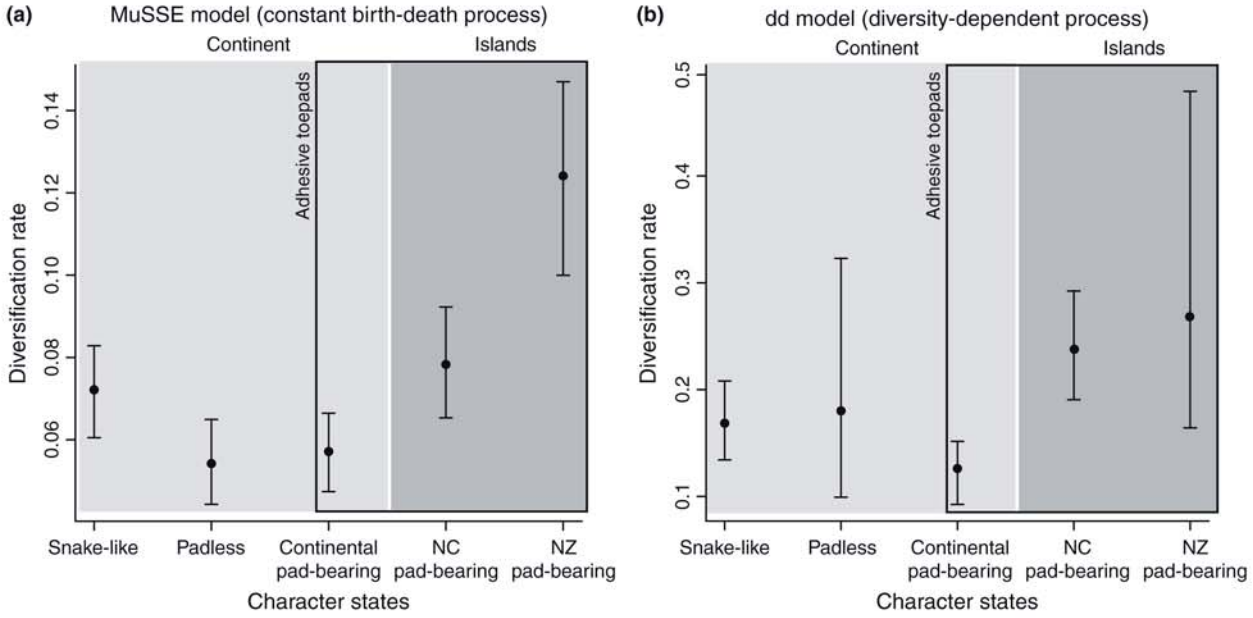

Fig. 4 Net diversification rates (speciation – extinction) and their 95% confidence intervals of each category across the 1000 trees. Results correspond to either data fit (a) with a constant rates birth and death model (bd) using the MuSSE model or (b) with a diversity-dependent model (dd).

island typically offers an array of vacant niches and sets the stage for subsequent elevated rates of cladogenesis (Whittaker *et al.*, 2010; Ortí *et al.*, 2012). In accordance with this, our results showed that the gekkotan radiations in New Caledonia and New Zealand have likely experienced accelerated rates of body size evolution and increased rates of species diversification relative to continental Australia. This is consistent with the classic notion that islands offer new ecological opportunities that can spur adaptive evolutionary diversification (Schluter, 2000). This is generally attributed to an ecological release in which species expand their resource or habitat use because of an absence of competitors (Thomas *et al.*, 2009). By contrast, in continental settings, most ecological niches have already been filled and provide fewer opportunities for niche expansion and subsequent adaptive diversification. An example of how island species expand their niches compared with their continental relatives can be found in the genus *Naultinus* in New Zealand. This genus has evolved a diurnal lifestyle (Nielsen *et al.* 2011), whereas all continental limbed geckos are nocturnal. In the case of New Caledonia, some evidence points towards a possible diurnal activity also in the genus *Eurydactyloides* (Bauer & Sadlier, 2000). Although diurnality could be considered an innovation in itself, the fact that in these geckos, it only appears after the colonization of New Zealand and New Caledonia likely reflects that both island archipelagos lack the diurnal competitors common in mainland environments (such as the large family of diurnal agamids). In the particular case of *Naultinus*, this shift to diurnality has also been associated with an accelerated rate of body size diversification (Fig. 3a) and provides a possible example of how low competitive environments on islands can spur evolutionary diversification.

Furthermore, predation is generally more severe in mainland habitats than on islands (where predators are often absent or less diverse; Millien, 2011), and any release from predation can allow phenotypic change in what were initially prey species. For example, less time is spent hiding from predators on islands, and this has allowed some lizards to expand their diets to include larger, more ellusive prey, and this has in turn facilitated extreme body size evolution (Case, 1978; Meiri, 2008). Gigantism has arisen independently after the colonization of both New Zealand and New Caledonia. New Zealand was home to the massive, now extinct gecko *H. delcourti*, which was more than 300% bigger than the mean size of current size of geckos on the island (Bauer & Russell, 1986), and is still home to *H. duvaucelii*, the biggest gecko in New Zealand, which is a 80% larger than the mean body size of all geckos in the archipelago. New Caledonia harbours the world's largest living gecko, *Rhacodacylus leachianus*, with a body size more than 200% bigger than the mean gecko size on the island. Not surprisingly, our analyses detected separate instances of accelerated rates of body size evolution associated with these lineages (Figs 2–3). Despite both island archipelagos exhibiting the highest rates of body size diversification in limbed species, not all within-island lineages were equal in rates of body size

evolution (Fig. 3a). In New Caledonia, although most of the lineages were associated with accelerated amounts of body size diversification, *Dierogekko* was an obvious exception. In New Zealand, virtually only the diurnal *Naultinus* and the giant genus *Hoplodactylus* (*H. duvaucelii* and *H. delcourti*) appeared to have experienced accelerated rates of body size diversification (Fig. 3a). This implies that not all lineages experienced the same degree of (or responded in the same way to) ecological opportunity on the same islands. Some groups for example might have diversified in traits other than body size. In line with this, other well-known lizard radiations on islands such as the *Anolis* have diversified not only in body size, but other phenotypic axes known to be involved, at least, in some stages of the adaptive diversification (Losos, 2009). For example, possible changes in diet could presumably have prompted morphological diversification in other functional characteristics independent of body size, such as head shape (e.g. larger prey items select for larger heads; e.g. Schoener, 1968) or perhaps limb length (more elusive prey might select for longer legs and faster sprint speeds; e.g. Irschick & Losos, 1999). This invites future comparative analyses of diversification in other morphological characteristics in these island clades.

It is also interesting to note how the clades in New Caledonia and New Zealand differed in the dynamics of diversification. In New Caledonia, we detected a strong diversity-dependent pattern of diversification (i.e. diversification that varied as a function of the number of species already in existence at a given time). This was characterized by an early burst of speciation following colonization, which subsequently slowed rapidly to much lower diversification rates towards the present day. This pattern has been detected for several other taxonomic groups on the same archipelago (e.g. flowering plants, diving beetles, spiders and reptiles) including skinks, which constitutes the other major radiation of lizards on the archipelago (Espeland & Murienne, 2011). This type of diversification pattern has often been interpreted as the signature of adaptive radiation following the colonization of new areas (Losos & Ricklefs, 2009). That is, speciation rates are high at the beginning of a radiation as taxa rapidly fill empty niche space, but diversification then slows as ecological opportunity decreases as niches are progressively filled (Rabosky & Lovette, 2008).

In New Zealand, on the other hand, we found that species diversification seems to have been relatively constant through time. This suggests that the New Zealand radiation of geckos might have yet to reach the saturation of its available niches (which should then result in a strong decline in diversification). In line with this, in the Caribbean *Anolis* lizards, species assemblages on big islands present proportionally slower declines in speciation compared with smaller islands. This reflects that big islands have greater carrying capacities than small islands and therefore take longer to reach niche saturation (Rabosky & Glor, 2010).

Key innovations and diversification

Snakelike phenotype

Our ancestral state reconstructions inferred that the snakelike phenotype evolved early in the gekkotan radiation in Australasia (before 35 Ma) and before the appearance of many of the other limbless reptiles that now inhabit Australia (typhlopids, colubrids, elapids and skinks which likely arrived to the continent in the last 30–25 Ma: Alfaro *et al.*, 2008; Kelly *et al.*, 2009; Skinner *et al.*, 2011; Marin *et al.*, 2012). Therefore, the onset of diversification in the snakelike geckos seems to have taken place in environments that were probably relatively free of other ecologically similar groups. Whereas in many groups the evolution of a snakelike phenotype seems to have been associated with the acquisition of a burrowing lifestyle (Wiens *et al.*, 2006), in Australian pygopodids, the acquisition of this phenotype seems to have predated the evolution of a burrowing lifestyle (Wiens *et al.*, 2006). This implies that the evolution of a snakelike phenotype is likely adaptive in a variety of ecological roles (and not simply a response to the restrictive use of the environment through burrowing). Indeed, the ecological diversity exhibited by Australian Pygopodidae is remarkable compared with the other continental geckos, including the diversity of habitats occupied, feeding strategies adopted and circadian activity (with many diurnal species; Shine, 1986). Consistent with this high ecological diversity, our analyses show that the snakelike geckos have likely experienced high rates of body size diversification and potentially high rates of species diversification as well.

Toepads

Despite being a classic example of a key innovation, and one that has been widely assumed to have facilitated the exemplarily diverse radiation of the geckos, the evolution of toepads appears not have had any impact on rates of body size and species diversification (Figs 2–4; see also Gamble *et al.*, 2012). The failure of a key innovation in driving evolutionary diversification has been reported in a number of other groups as well (Hodges, 1997; Price *et al.*, 2010; Claramunt *et al.*, 2012). This suggests that either the role of key innovations in spurring (potentially adaptive) radiations has been overplayed in the literature or the dependency of key innovations on the particular circumstances of a given taxonomic group has been underappreciated. Although a key innovation might provide the potential for a species to interact with the environment in new ways, this potential may nevertheless be limited by its particular ecological setting (Hodges, 1997; de Queiroz,

2002). For example, the evolution of the pharyngeal jaw in African cichlids is a key innovation that has only led to an adaptive radiation in recently formed, competitor-free rift lakes (Liem, 1973). In Australasian geckos, there are a number of context-related factors that could have similarly limited the impact of toepads on evolutionary diversification rates. One of these factors relates to the fact that most of the gekkotan diversity in continental Australia is associated to arid environments (Powney *et al.*, 2010). In these environments, selection may have constrained body sizes to minimize evaporative water loss (Vucko, 2008) and subsequently limited the potential for body size diversification to occur. This would be the case regardless of whether species occupied a structural-rich environment and possessed toepads that allowed them to exploit such an environment.

Furthermore, toepads might constitute a key innovation in some habitats but not others. Adhesive toepads are classically considered to be a key trait involved in the transition from terrestriality to arboreality (Kluge, 1967; Russell, 1979; King & Horner, 1993). The causal link between the presence of toepads and ecological opportunity has therefore usually been framed in terms of the evolution of arboreality (i.e. adhesive toepads enable animals to move more effectively in an arboreal environment and on a broader range of surfaces more generally – Irschick *et al.*, 2006; Losos, 2009). In a previous study comparing evolutionary diversification between lizard species with and without toepads, Warheit *et al.* (1999) documented significantly greater levels of body size disparity among species of the toepad-bearing *Anolis* genus compared with the closely related padless *Sceloporus* genus. This difference in body size diversity between the two genera was inferred to reflect the evolution of toepads providing greater ecological opportunity through a highly arboreal lifestyle of *Anolis* (Warheit *et al.*, 1999).

In Australia geckos occupy a range of terrestrial, saxicolous and arboreal habitats. Many of the species across this range of habitats possess toepads that appear similar in design (King & Horner, 1993). As an example, species within *Oedura* exhibit similar toepad designs and are arboreal, saxicolous or both (Wilson & Swan, 2010). Although there is a clear mechanistic link between toepads and greater ecological opportunity in arboreal environments (Irschick *et al.*, 2006; Losos, 2009), this link is less obvious in saxicolous or terrestrial environments. The possession of toepads in nonarboreal species might therefore have failed to translate into greater ecological opportunity compared with lineages that lack toepads more generally.

Finally, intrinsic morphological or genetic constrains on the evolution of phenotypic variation, or an inherent lack of 'evolvability' (*sensu* Losos, 2010), have been proposed to explain the low rates of evolutionary diversification following the acquisition of a key innovation in other taxa (Schluter, 2000; Price *et al.*, 2010). Such constraints, for example, have been invoked to explain why innovations in the jaw design of parrotfishes have not been followed by the evolution of greater morphological diversity among species (Price *et al.*, 2010). Although the toepads of anoles and geckos are functionally equivalent, the body designs of the two groups are quite different. In geckos, especially arboreal species, limbs are laterally oriented with respect to the body and form a low angle with the substrate. This keeps their centre of mass close to the substrate (Wang *et al.*, 2011 and references therein) and maximizes the pull-off force during the pad-to-substrate attachment process (Persson, 2007). This type of body design in arboreal geckos may have constrained the extent geckos have been able to adapt to different microhabitats (Losos, 2010). In line with this, the diurnal and arboreal geckos of the genus *Phelsuma* exhibit some habitat partitioning and associated body size segregation (Harmon *et al.*, 2007); however, this is relatively modest compared with the *Anolis* lizards (Losos, 2010). Alternatively, Bergmann & Irschick (2009) propose that the possession of toepads has been associated with constraints on evolutionary change in vertebral number, and these constraints may have subsequently limited evolutionary variation in SVL among species. However, it is clear that great evolutionary diversification has occurred within the Australasian geckos following island colonization. Therefore, a general lack of evolvability in these lizards is not a compelling argument for why the evolution of toepads in geckos has failed to promote accelerated body size evolution or speciation.

Concluding remarks

Our study shows how the influence of two key innovations and two independent island colonization can produce different outcomes in terms of body size evolution and species diversification. Island colonization has played the most prominent role in the evolutionary diversification of Australasian geckos, followed by the evolution of a snakelike phenotype. The evolution of adhesive toepads, however, appears not to have impacted diversification rates directly, although it is conceivable that island colonization promoted evolutionary diversification in geckos only because colonizing species possessed toepads. That is, although there was no evidence that toepads in themselves lead to changes in body size or species diversification, they might have facilitated the radiation of the groups that colonized islands. In this regard, untangling the interaction of toepad evolution and island colonization was not possible for the New Caledonia and New Zealand archipelagoes because both island radiations originated from toepad-bearing ancestors.

Nonetheless, for this very reason, our study offers an important cautionary note: the invasion of new environments (such as islands) needs to be incorporated in studies that explore the effects of key innovations on evolutionary diversification because it may not be the innovation specifically, but the invasion of a new environment more generally, that has driven diversification. Most studies usually address whether an innovation is coupled with shifts in the evolutionary diversification of a given group without considering other possible causal agents (Weber & Agrawal, 2012). In our case, had we pooled mainland and island species into the same category, 'geckos with toepads', the island effect would have inflated estimated rates of evolution and we might have inferred a link between the evolution of a key innovation and subsequent diversification (see for instance Model 4 in Table 1). Relaxing the assumption of where on the phylogeny evolutionary rate shifts are expected to have occurred can help circumvent this problem. This could be carried out using methods like 'auteur' that proved crucial in our study for detecting patterns of evolutionary diversification inconsistent with the key innovation hypothesis. These types of analyses can in turn prompt further investigation of the possible cause of unexpected evolutionary patterns and identify the more probable origin of evolutionary diversification.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Time-calibrated tree showing the radiation of the Australasian diplodactyloid geckos plus 20 species incorporated to place most of the calibration points.

Figure S2 Barplots representing the relative support for six models of diversity dynamics for each category.

Figure S3 Net diversification rates (speciation – extinction) and their 95% confidence intervals of each category across the 1000 trees corresponding to data fit with a diversity-dependent model (dd), assuming that an additional 50% of the currently known species of Pygopodidae, Carphodactylidae and continental Diplodactylidae ould be described at some point in the future.

Data deposited at Dryad: doi:10.5061/dryad.56vf1

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SUPPLEMENTARY FIGURES

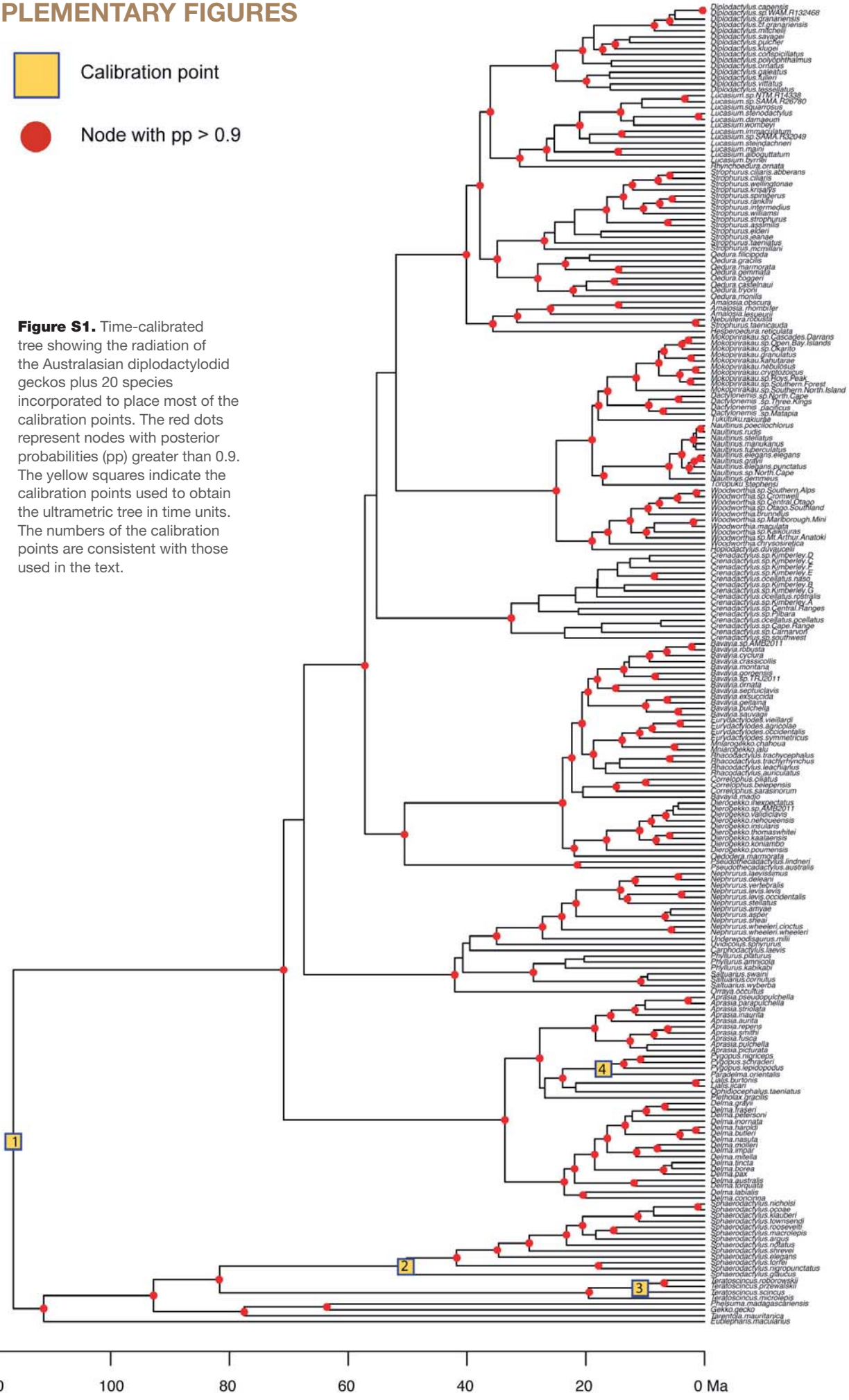

Figure S1. Time-calibrated tree showing the radiation of the Australasian diplodactylodid geckos plus 20 species incorporated to place most of the calibration points. The red dots represent nodes with posterior probabilities (pp) greater than 0.9. The yellow squares indicate the calibration points used to obtain the ultrametric tree in time units. The numbers of the calibration points are consistent with those used in the text.

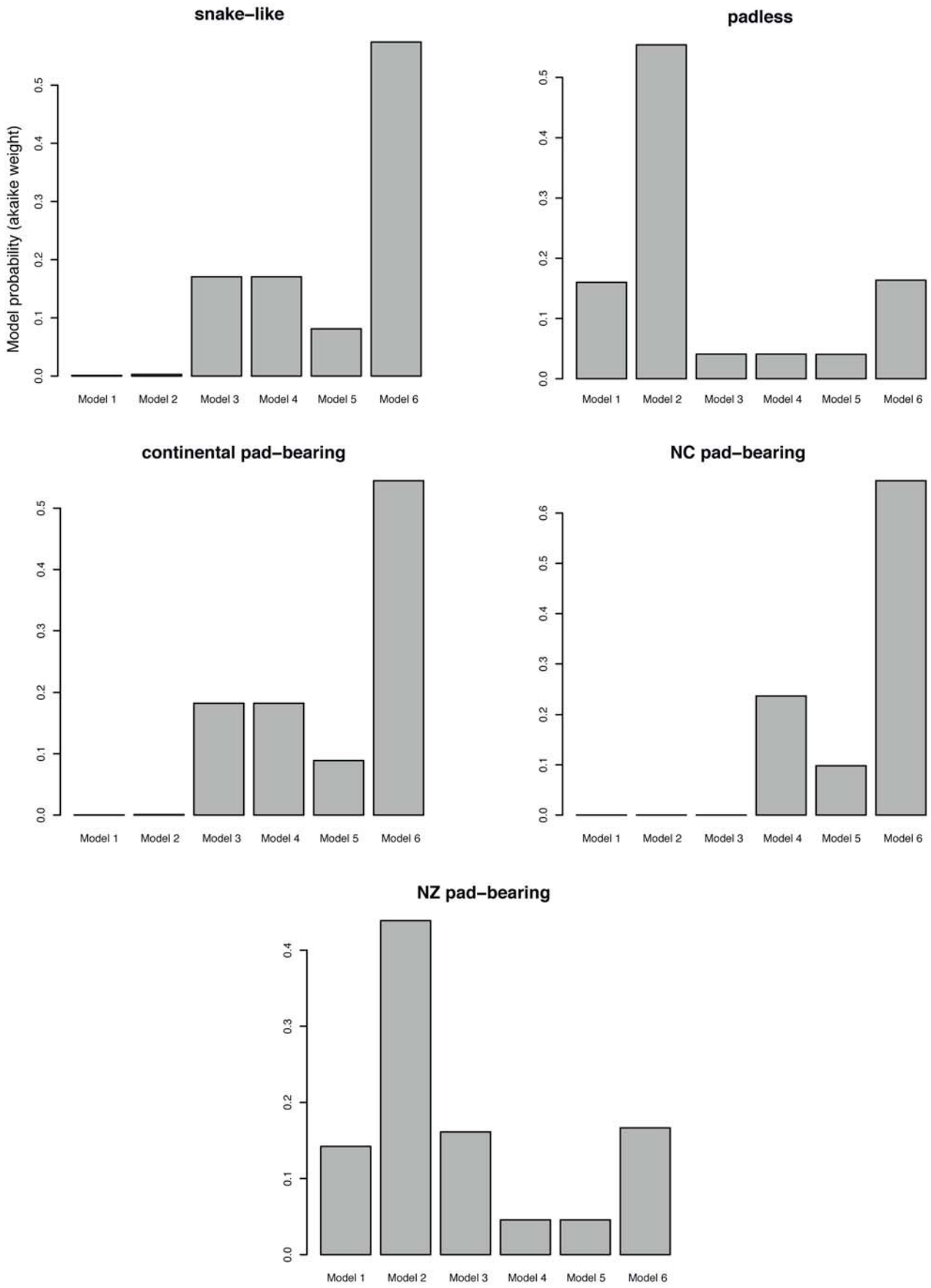

Figure S2. Barplots representing the relative support for six models of diversity dynamics for each categories. Each bar represents the relative level of support—measured as the Akaike weight (AICw) —that a given model predicts patterns of species diversification.

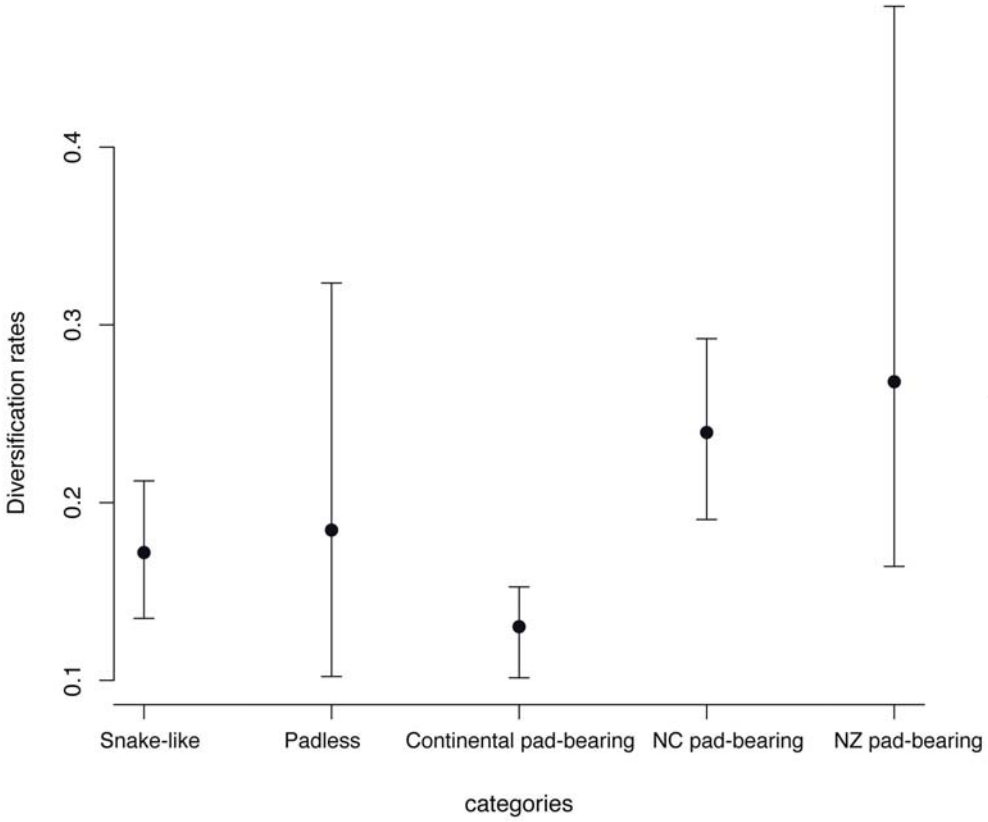

Figure S3. Net diversification rates (speciation – extinction) and their 95% confidence intervals of each category across the 1,000 trees corresponding to data fit with a diversity-dependent model (dd), assuming that an additional 50% of the currently known species of Pygopodidae, Carphodactylidae and continental Diplodactylidae would be described at some point in the future.

SUPPLEMENTARY TABLES

MODEL	SPECIATION	EXTINCTION	EQUATION
MODEL 1	constant	constant	$\lambda(t) = \lambda_0$
			$\mu(t) = \mu_0$
MODEL 2	constant	-	$\lambda(t) = \lambda_0$
MODEL 3	varying	constant	$\lambda(t) = \lambda_0 e^{\alpha t}$
			$\mu(t) = \mu_0$
MODEL 4	varying	varying	$\lambda(t) = \lambda_0 e^{\alpha t}$
			$\mu(t) = \varepsilon\lambda(t)$
MODEL 5	varying	varying	$\lambda(t) = \lambda_0 e^{\alpha t}$
			$\mu(t) = \mu_0 e^{\beta t}$
MODEL 6	varying	-	$\lambda(t) = \lambda_0 e^{\alpha t}$

Table S2. Summary of the six diversification models analyzed with a description of their properties and equations. Notations: λ = diversification rate; μ = extinction rate; α = exponential variation of speciation rate; ε = extinction fraction.

MODEL	DESCRIPTION	NUMBER OF CATEGORIES	SNAKE-LIKE	PADDLESS	CONTINENTAL PAD-BEARING	NC PAD-BEARING	NZ PAD-BEARING
MODEL 1	Snake-like (A) vs Padless (B) vs continental pad-bearing (C) vs NC pad-bearing (D) vs NZ pad-bearing (E)	5	A	B (1)	C	D	E
MODEL 2	Continental (snake-like, padless, pad-bearing:) (A) vs island (B)	2	A (1)	A (1)	A (1)	B	B
MODEL 3	Snake-like (A) vs Continental non-snake-like (B) vs Islands (NC + NZ) (C)	3	A	B (1)	B (1)	C	C
MODEL 4	Snake-like (A) vs Padless (B) vs Pad-bearing: (continental, NC, NZ) (C)	3	A	B (1)	C	C	C
MODEL 5	Snake-like (A) vs Padless (B) vs continental pad-bearing (C) vs NC pad-bearing (D) vs NZ pad-bearing (E), in this case all categories were set to evolve to equal rates	5	A (1)	B (1)	C (1)	D (1)	E (1)

Table S1. Summary of each of the mapping strategies implemented to produce the five models analyzed by MOTMOT. A notation of (1) indicates those categories fixed to the same relative evolutionary rate of 1.

		SNAKE-LIKE		PADLESS		CONTINENTAL PAD-BEARER		NC PAD-BEARER		NZ PAD-BEARER	
MODEL	*Transition rate*	*Speciation rate*	*Extinction rate*	*Speciation rate*	*Extinction rate*	*Speciation rate*	*Extinction rate*	*Speciation rate*	*Extinction rate*	*Speciation rate*	*Extinction rate*
MUSSE	4.20E-04	0.07	4.56E-06	0.05	5.01E-06	0.05	2.32E-06	0.07	4.13E-06	0.12	1.38E-05
DD	-	0.17	2.80E-03	0.22	0.046	0.12	7.20E-05	0.23	2.69E-04	0.4	0.13

Table S3. Table summarizing the parameter estimates of the MuSSE and dd models of diversification.

CHAPTER 3

SPECIES ASSEMBLY AND NICHE STRUCTURE IN THE SOCOTRA ARCHIPELAGO: NOT A SINGLE PATTERN TO RULE ALL GECKOS

Photo credit: Fabio Pupin

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ABSTRACT

Island biotas are notorious for their spectacular examples of diversifications, usually triggered by the great ecological opportunity provided by islands. Less appreciated is that these diversifications can occur at different scales, taking place along environmental gradients (beta niche) or involving the partition of local resources by occupying different microhabitats (alpha niche). Moreover, aside of *in situ* diversification, other processes as dispersal or vicariance can contribute, not only to build the diversity in the islands, but also to structure it in the different macro and microhabitats that islands provide. In this study our aim was to explore how the complete fauna of endemic geckos of the Socotra Archipelago (spanning three genera: *Pristurus*, *Hemidactylus* and *Haemodracon*) built up its diversity and structured it along axes of macro- and microniche variation. We found that most of the gekkotan diversity in the Socotra Archipelago was the consequence of intra-island speciation events that took place once the islands were completely detached from the continent. However different genera substantially differed in their patterns of climatic and morphological structure in the archipelago. While in *Hemidactylus* and *Haemodracon* species showed a strong tendency to differ in body size and presented a great conservatism in their climatic envelopes, in *Pristurus* an opposite pattern emerged with most of the diversification among closely related species taking place along climatic axes and involving almost no size differentiation. Consistently with this, our estimates of rates of body size evolution showed that *Pristurus* presented the lowest rates of the three genera. Overall this shows how different groups may substantially differ in their patterns of niche structuration in the same archipelago and questions the existence of a general theory applying to a wide range of groups.

KEY WORDS: gecko, macroniche, microniche, climatic envelope, body size, island, diversification

INTRODUCTION

Islands are known by their exceptional diversifications, usually interpreted as the consequence of niche differentiation in a context of great ecological opportunity (Losos & Ricklefs, 2009). Interestingly, this niche differentiation can operate at different scales. At large spatial scales, species can potentially diversify across different (and largely allopatric) macrohabitats or climatic envelopes (e.g. along an altitudinal gradient). At smaller scales, species can structure across the spectrum of existing microhabitats or resources to reduce overlap (e.g. species relying on different prey sizes or living on different tree heights) (Ackerly et al., 2006).

There are two main scenarios in which differentiation at these two different scales (often refereed as beta and alpha niche respectively; Ackerly et al., 2006) may be associated in diversifying groups. Diamond

(1986) based on his studies on New Guinean birds, proposed his "habitat-first" scenario, in which he argued that in the early stages of diversification, species tend to diversify into distinct (mostly allopatric) macrohabitats (e. g. along an altitudinal gradient) while maintaining similar microniches (e. g. presenting extensive overlap in their body sizes). It is only in the more mature stages of the diversification when species converge into similar macrohabitats and segregate into different microniches (e. g. diverging in body size). This model of diversification has been validated several times in posterior studies (Schluter, 2000) and has even been proposed as the most general pattern for vertebrate diversification (Streelman & Danley, 2003). An alternative scenario to the "habitat-first" model is exemplified by one of the greatest examples of island radiations, the *Anolis* of the Caribbean. In these lizards, compelling evidence shows that diversification initially takes place along a phenotypic axis (mostly involving size differentiation), reflecting different microhabitat uses, and later proceeds into differentiation along a physiological axis, reflecting different climatic niches (Williams, 1972; Losos, 2009; Hertz et al., 2013).

Intrinsic factors as different macro and microniche evolvabilities could explain these radical differences in the stages of diversification observed in different groups. For instance, if all traits reflecting alpha niche tend to be evolutionarily conserved, the "habitat first" pattern will be the most likely observed pattern. Alternatively, if these traits are labile enough, this would facilitate the use of different resources among closely related species in the same habitat, therefore setting the stage for microniche differentiation even before macroniche differentiation takes place (Ackerly et al., 2006).

The particular environment can also exert an important effect on the progress of diversification (Streelman & Danley, 2003). In the specific case of islands, these can provide different patterns of macro- and microniche availability, which in turn may determine the chances of macro- and microniche differentiation. For instance in oceanic islands, the length of an altitudinal gradient, and therefore the chances for altitudinal segregation, likely depends on the age of the island, as older islands normally present lower elevations than younger islands (Whittaker et al., 2008).

In spite of the great differences between the above-mentioned scenarios, both implicitly assume that niche structure between closely related species is mediated by evolutionary diversification. However, aside of diversification, islands build up their diversity also by dispersal and vicariance (McDowall, 2004), and both processes may also have roles at producing the niche structure observed in insular groups. For instance, in many of the Lesser Antilles, when two species of *Anolis* occur in the same island occupying different microniches (typically with different sizes), these mostly originated, not as a consequence of an *in situ* event of speciation, but as a consequence of two independent dispersal events (phenomenon known as "species assortment") (Losos, 2009). This outlines a complex picture in which niche structuration in insular groups may be the result of the combined effects of dispersal, vicariance or *in situ* diversification, which in turn might proceed according to above-presented different scenarios.

The aim of this study is to explore how all these processes interact for producing patterns of macro- and

microniche structuration using the complete fauna of endemic geckos of the Socotra Archipelago as a model.

The Socotra Archipelago comprises four islands of continental origin situated in the northwest Indian Ocean, 350 km from the Arabian coast, near the Gulf of Aden (Fig. 1). The easternmost and largest of these four islands is also called Socotra and, at just 3796 km^2, comprises about 95% of the total landmass of the archipelago. Abd Al Kuri, the second largest (133 km^2) and westernmost island of the archipelago, lies about 105 km to the west of Socotra and 100 km east of the Horn of Africa. Apart from the two main islands separated by great depths and likely never connected by emerged land (Fig. 1), the two small islands of Darsa (16 km^2) and Samha (40 km^2), situated 36 and 50 km respectively to the southwest of Socotra, are separated from it by shallow waters and were likely connected to Socotra during the last glacial event (Van Damme, 2009) (Fig. 1).

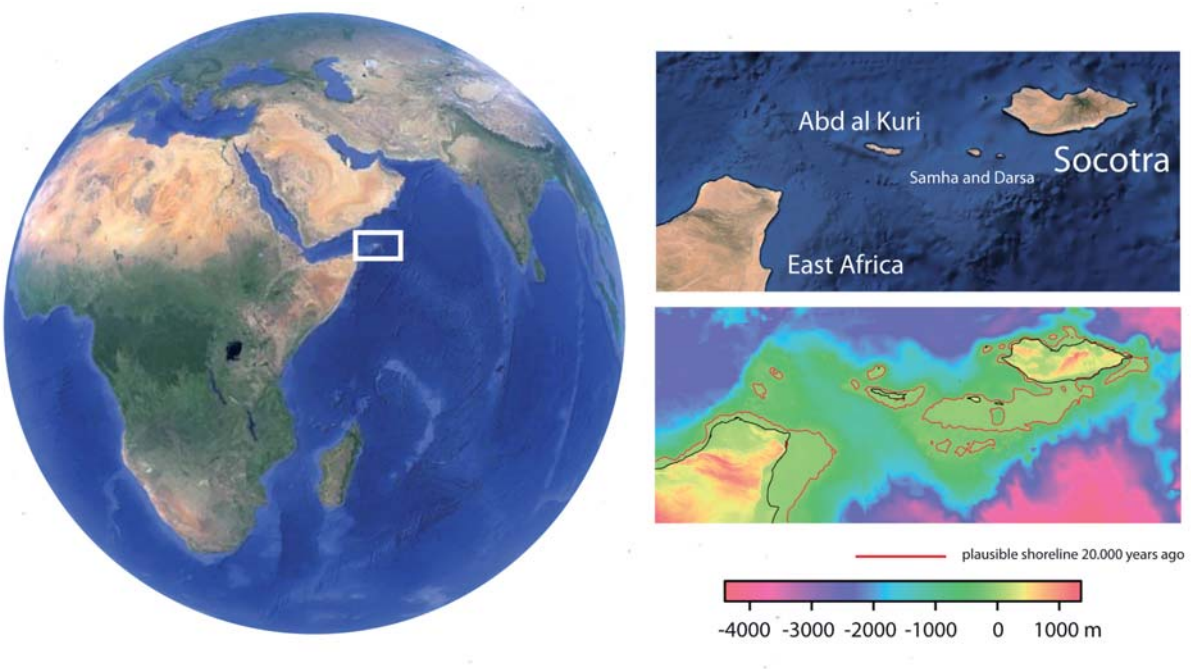

Figure 1. Map showing the geographic location of the Socotra Archipelago. The upper right map shows an enlarged view of the archipelago with the names of the main islands that constitute it. The lower right map provides a visualization of the ocean-floor bathymetry of the region on which we highlight (in red) the portion of topography lying above -120 m. This constitutes a plausible reconstruction of the paleo-shorelines 20,000 years ago, when sea levels dropped around 120 m from the present-day level (Siddall et al., 2003).

The diversity of native geckos in the archipelago consists of three distinct genera and 16 species, all of them endemic. The sphaerodactylid geckos of the genus *Pristurus* is constituted by seven species and are characterized by lacking toepads and by being diurnal. The geckonid genus *Hemidactylus* and the phyllodactylid genus *Haemodracon* are composed by seven and two species respectively and both genera comprise strictly nocturnal and padded species (Razzetti et al. 2011). These three groups represent three independent replicates of the species assembly and niche structuration in the same archipelago, a fantastic opportunity to study these processes in a comparative framework.

Three are the main questions that this study intends to shed light on: which is the relative contribution of dispersal, vicariance or *in situ* diversification at producing the diversity of geckos existing on each island? Which is the relative contribution of these processes at producing the patterns of macro- and microniche structure shown by the species in the archipelago? And finally, when *in situ* diversification takes place, do all groups follow the same stages of intra-island diversification?

METHODS

TAXON SAMPLING, DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

We sampled and sequenced all native geckos of the Socotra Archipelago. Genomic DNA was extracted from ethanol-preserved tissue samples using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). Six genetic markers were PCR-amplified and sequenced: one mitochondrial fragment of the gene encoding the ribosomal 12S rRNA (*12S*; primers 12Sa and 12Sb – Kocher et al. 1989), and five nuclear fragments of the genes encoding the acetylcholinergic receptor M4 (*acm4*; primers tg-F and tg-R – Gamble et al. 2008), the oocyte maturation factor Mos (*cmos*; primers FUF and FUR – Gamble et al. 2008), a short fragment of the recombination-activating gene 1 (*rag1*; primers F700 and R700 – Bauer et al. 2007), the recombination-activating gene 2 (*rag2*; primers PyF1 and PyR – Gamble *et al*. 2008) and phosphoducin (*pdc*; primers PHOF2 and PHOR1 – Bauer et al. 2007). PCR conditions used for the amplification of the *12S* mitochondrial fragment as well as the nuclear genes *cmos*, *rag1* and *rag2* can be found in Šmíd et al. (2013), for the nuclear gene *acm4* in Barata et al. (2012) and for *pdc* in Greenbaum et al. (2007). These genes have been widely used in other large-scale phylogenetic studies of Gekkota (Gamble et al. 2008, 2011, 2012) and were therefore the ones preferred in this study. Having all the species in the archipelago sequenced allowed us to recover all intra-island speciation events. However, in order to detect cases of dispersal and vicariance we also required recovering all possible mainland-island speciation events (see below). To this end we sequenced the same genes for 28 species existing in mainland, which previous studies showed as close relatives to island groups (Gamble et al., 2012; Šmíd et al., 2013; Badiane et al., 2014).

PHYLOGENETIC ANALYSES

In order to discern between alternative processes of diversity assembly in the islands, we took a phylogenetic approach (see below), and produced a time-calibrated phylogeny that included all 44 species sequenced (island and continental). These were then placed in the phylogenetic context of a wide representation of all geckos with sequences available in GenBank.

Working at this phylogenetic scale allowed the possibility to use a variety of different calibration points placed in different parts of the phylogeny of geckos and calculate divergence times while limiting potential problems of biased and/or low sampling across the phylogeny (Venditti et al., 2006). To assemble this dataset we searched for all species of gecko existing in GenBank for which at least three of the genes

amplified for the Socotran species were available. We then retrieved the longest sequence for each species with the additional requirement that all sequences had to be 200 bp or more to be selected (search in GenBank conducted in September 2011). After this procedure, our sequence dataset included 346 species, 44 of which were sampled in this study and 302 were obtained from GenBank. Each gene was then aligned using two procedures: the ribosomal coding *12S* was aligned by means of MAFFT v6 (http://www.ebi.ac.uk/Tools/msa/mafft/; Katoh et al., 2002) and the protein coding genes (*acm4, cmos, rag1*, *rag2* and *pdc*) were aligned by means of the translation alignment algorithm implemented in the software Geneious (Drummond et al., 2010). The final alignment consisted of a total of 2,259 bp distributed in each gene as follows: *12S* (390 bp), *acm4* (453 bp), *cmos* (375 bp), *rag1* (303 bp), *rag2* (345 bp) and *pdc* (393 bp).

The phylogenetic analysis was conducted by means of the package BEAST v1.7.5 (Drummond & Rambaut, 2007). The prior for the distribution of branching times was based on a birth-death process. The nucleotide substitution model was set to GTR+G+I, and the variation of nucleotide substitution rates across the tree was assumed to be non-autocorrelated and log-normally distributed. The clock model and the nucleotide substitution models were applied independently to the six partitions: *12S*, *acm4*, *cmos*, *rag1*, *rag2* and *pdc*, with every codon position considered separately in the protein coding genes (as implemented in similar phylogenetic analysis encompassing all gekkota, Gamble et al., 2008, 2010). Five calibrations were used to estimate branch lengths in units of time (Fig. 2):

1. The minimum age for the radiation of *Sphaerodactylus* in the Caribbean was set to 20 Ma based on an amber fossil of this genus from the Dominican Republic (Daza & Bauer, 2012). The maximum age of this radiation was set conservatively to a soft maximum of 70 Ma. This was done by means of a gamma distribution (α=2, β=10).
2. The age of the Tien Shan-Pamir uplift in western China, around 10 Ma, was used to calibrate the split between *Teratoscincus scincus* and the clade formed by *T. przewalskii* and *T. roborowskii* considering that this split originated via vicariance as a result of this geologic event (Macey et al., 1999). A normal distribution with a mean positioned at 10 Ma and a standard deviation of 1 Ma was chosen to set the calibration prior of this node.
3. The age for the diplodactyloid radiation in New Caledonia was set to a soft maximum of 37 Ma. This is based on several lines of evidence (geological and biological) that show that the island was submerged until this approximate time (Nattier et al., 2011; Pillon, 2012; Papadopoulou et al., 2013 & Garcia-Porta & Ord, 2013). A normal distribution with a mean at 20 Ma and a standard deviation of 10 Ma was used to set the prior of this calibration point.
4. The split between *Phelsuma ornata* from the island of Mauritius and *P. inexpectata* from the island of Reunion was set to a soft maximum of 8.9 Ma based on the age of the oldest rocks of Mauritius (the oldest island in the Mascarenes, including both Mauritius and Reunion) (Moore et al., 2011). This prior was set by means of an exponential distribution with an offset of 0 and a mean at 3 Ma.

5. Finally, the deepest split in the diplodactyloid radiation of New Zealand was set to a minimum of 19 Ma based on the oldest fossils of geckos in the archipelago (Lee et al., 2009) with a conservative soft maximum of 65 Ma. This was set by means of a gamma distribution (α=3, β=7).

The phylogenetic analysis relied on four independent Markov Chain Monte Carlo (MCMC) that converged on similar posterior estimates. Each chain was run for 100,000,000 generations with parameters and trees sampled every 5,000 generations. These runs were combined using LogCombiner v1.7.5 (included in the package BEAST) after excluding, as burning, a suitable amount of generations in each one (from 10 to 30%). Tracer v.1.5 (Drummond & Rambaut, 2007) was used to confirm convergence and good mixing of each MCMC chain. We then calculated the summary tree as the maximum clade credibility tree with median node heights using the TreeAnnotator v1.7.5 program (also included in BEAST package), setting the posterior probability limit at 0.5. Moreover we randomly sampled 1,500 trees from the posterior distribution of trees generated by our BEAST analyses. This allowed us to incorporate the topological and branch lengths uncertainties in some of the phylogenetic comparative analysis performed (see below).

Finally, in order to investigate the reliability of our dating estimates, we took two complementary approaches. We first compared the age and confidence intervals estimated for the root of Gekkota with the estimates obtained from previous studies. We chose this node as its age is widely reported in many phylogenetic studies involving gekkotans. Complementarily, we also validated our analysis by comparing our empirical rate for *12S* with the rates for this gene obtained in other studies.

EXPLORING PROCESSES OF SPECIES ASSEMBLY

The dating estimates and the confidence intervals (95% HPD) of all recovered mainland-island splits and all intra-island splits were contrasted with the known phases of geological evolution of the Socotra Archipelago, namely: (1) a *continental stage*, moment at which the archipelago was still part of the continent, (2) a *transition stage* in which the archipelago was in the process of detachment from the continent (this ultimately reflects the uncertainty regarding the last contact between the archipelago and the continent) and finally (3) an *oceanic stage*, moment at which the archipelago was already surrounded and isolated by water (archipelago constituted by true islands). In the case of Socotra, the continental stage was defined as the time previous to the initiation of the rifting of the Gulf of Aden, the process that separated the Socotra Archipelago from Arabia. This process started around 30 Ma and was triggered by the emplacement of the Afar mantle plume in Eastern Africa (Burke, 1996; Baker et al., 1996; Hofmann et al., 1997; Rochette et al., 1998; Ebinger & Sleep, 1998; Ukstins et al., 2002). We therefore used the time of this event to set the end of the continental stage. The transition stage was defined as the period comprising the initiation of the rifting process and the upper-limit of the syn-rift deposits, which is constrained around 20 Ma (Watchorn et al., 1998; Fournier et al., 2010). During

this period of time the Socotra Archipelago was in the process of detachment from the continent or, if already separated, it was very close to it. And finally, in the oceanic stage, from 20 Ma to the present day, the archipelago was permanently isolated by ocean and well separated from mainland (Fournier et al., 2010).

Each node was assigned to the stage that presented the greatest overlap with the 95% high posterior density (HPD) interval of its dating estimates. A node separating two island species (intra-island split) with most of its HPD interval falling into the oceanic phase of the archipelago was interpreted as an "intra-island speciation event", any other HPD interval distribution in these nodes was considered as non-informative. Regarding the nodes separating mainland and island species (mainland-island splits), when their HPD intervals mostly fell in the oceanic stage of the archipelago, these were considered as "dispersal events" and when these mostly overlapped with the transition stage, were interpreted as "vicariant events". Finally when most of the HPD interval of a mainland-island split fell in the continental stage of the island, the situation was considered as non-informative.

EXPLORING MACRONICHE STRUCTURE

Macroniche differentiation among species was assessed by means of comparing their occurrence-based climatic envelopes. Species occurrence data was obtained from Razzeti et al. (2011), which are the result of 215 diurnal and nocturnal transects including mainly Socotra but also Abd al-Kuri, Samha, and Darsa, based on the Systematic Sampling Surveys (time-constrained) protocol (Heyer *et al.,* 1994). Only original data was used and included 834 localities. These were subsequently filtered by applying a grid of 1 x 1 km on the archipelago from which we randomly extracted a single locality per species and cell. This resulted in 555 localities with a mean and a minimum of 34.68 and 5 per species respectively. Climate in the archipelago was informed by the 19 Bioclim variables available in the WorldClim database (http://www.worldclim.org) at 30 arc-seconds of spatial resolution (Hijmans et al., 2005). These were subsequently reduced to six in order to minimize the amount of correlation between the climatic variables and to facilitate the interpretation of the environmental space. The ones selected were: Bio1, Bio 2, Bio 4, Bio 7, Bio 14 and Bio 16 and their values were obtained by retrieving all cell values at a resolution of 1 x 1 km in each of the six climatic rasters (Fig. S1). We used the "PCA-env" ordination technique (Broennimann et al., 2012) to characterize the climate existing in the archipelago and the climatic envelope of each species in it. This method essentially projects the selected climatic variables into the multivariate space defined by a principal component analysis (PCA). The environmental space defined by the two first components is then divided into r x r cells (100 x 100 cells in our case), each cell corresponding to a unique vector of environmental conditions present at one or more sites in the geographic space. After this, the smoothed density of available environments and the smoothed density of species occurrences are calculated across all cells by means of a kernel density function. These are subsequently combined in a metric to obtain the environmental occupancy of each species (derived in

Broennimann et al., 2012). We also obtained a point estimate of the niche position for each species by calculating the mean values on the first and the second PCA axes. These estimates allowed us to visualize macroniche variation in a phylogenetic context reconstructing these values onto the nodes of the phylogeny and then visualizing the values at the tips, the nodes and the phylogeny connecting them in a bivariate space (the "phyloclimatic space" by analogy with the "phylomorphospace"; Sidlauskas, 2008).

We calculated macroniche overlap among species of the same genus inhabiting the same island by means of the Schoener's D metric applied to the environmental occupancies of each of the species (Schoener 1970; Warren et al., 2008, Broennimann et al., 2012). This metric varies between 0 (no overlap between species) to 1 (complete overlap between species). To produce null distributions of the D values of each of the pairwise species comparisons we generated 1,000 sets of random occurrence points for each species in their respective islands, always maintaining the number of localities existing per species. We then recalculated the D metric for all pairwise comparisons using the random sets of species localities and we calculated the probability of our empirical value to be equal or smaller given the distribution of 1,000 simulated D values (one-tailed *p*-value). All calculations and data manipulations described in this section were conducted in R (R Core Team, 2014) using the packages "raster" (Hijmans, 2014), "dismo" (Hijmans et al., 2012), "ade4" (Dray & Dufour, 2007), "adehabitat" (Calenge, 2006), "sp" (Pebesma & Bivand, 2005; Roger et al., 2013), "phytools" (Revell, 2012) and a modified version of the R scripts provided by Broennimann et al., (2012) (available from http://www.unil.ch/ecospat/home/menuinst/tools--data/tools.html).

EXPLORING MICRONICHE STRUCTURE

Characterization of the morphological variation

To explore microniche differentiation we took an ecomorphological approach (Losos, 2009) and assumed that species morphology reflects microhabitat or resource specialization. This has been extensively demonstrated in many vertebrate groups. For instance, body size determines patterns of resource use in many organisms, strongly correlating with prey size preference (Fisher & Dickman 1993; Woodward & Hildrew, 2002; Duellman & Mendelson 2005; Moen & Wiens, 2009) and with microhabitat use (Losos, 2009). In lizards, limb lengths are often correlated with the partitioning of the habitat structure. For instance, species with shorter limbs (relative to SVL) normally use more vertical surfaces than species with longer limbs (Van Damme et al., 1997) and in arboreal species limb lengths are strongly correlated with perch diameter (Losos et al., 1997, 2001). Head proportions can also contribute to niche partitioning by enabling different bite forces: species with shorter, higher and broader heads are usually able to bite harder which can be useful to consume harder preys (Losos, 2009).

We characterized the morphology of each of the 16 species of endemic geckos in the archipelago by means of 18 different measurements. Body size was measured as the length between the snout and the opening of the cloaca (snout vent length = SVL), head shape was characterized by its length measured from the snout to the auricular opening (HL), its maximum width (HW), its maximum height (HD), the width at the level of the nasal openings (HWN), the head depth at the level of the nasal openings (HDN), the inter-nasal distance (IND), the distance from the anterior margin of the orbit to the nasal opening (END), the distance between the auricular opening to orbit (EED), the inter-orbital distance (IOD) and the orbital diameter (OD). Body proportions were measured as the axilla to groin distance (AGL) and the body amplitude at the level of the scapular and pelvic girdles (ASG and APG, respectively). Regarding limb proportions, forelimbs were measured as the length of the brachium (BL), length of the ante brachium (AL), and hindlimbs proportions were quantified as the thigh length (TL) and the crus length (CL). All measurements were taken by the same person (JGP) three times using a digital caliper (to the nearest 0.1 mm) with the average of the three replicates used as the final value. These were then $\log_{10}$-transformed to improve the normality and homoscedasticity of our data. A total of 201 specimens were measured, with a mean of 12.56 specimens per species and a minimum of four specimens per species. We characterized the morphospace occupied by all island species by means of a PCA conducted on the correlation matrix. We then visualized it in a phylogenetic context by projecting the tree of all island species into a bivariate space represented by the means of each species in the morphological space and the reconstructed values at the nodes (the “phylomorphospace”; Sidlauskas 2008). All analysis and data manipulations were performed in R, using the packages “stats” and “phytools” (Revell, 2012).

Defining sympatric species assemblages and exploring microniche overlap

To assess whether species coexisting in the same community differed in morphology we rasterized a shapefile containing all sampling localities in the archipelago to produce a grid of 1 x 1 km of cell size. We then extracted all assemblages of more than two con-generic species within each of the cells and we plotted their morphological variation along the PC1 (the component that explained 88% of the inter-specific variance; see results). We then compared the morphologies of all coexisting species in two different ways: we first conducted pairwise permutational ANOVAs on the scores of the PC1 to assess whether coexisting species occupied significantly different regions of the morphospace. Secondly we compared the amount of dissimilarity between coexisting species by computing their overlap along PC1. This was achieved by computing the density curve of the value distributions of each species by means of a Kernel density function, using Gaussian basis functions. We then computed the area overlap between the two density curves. Given that the area of the density curve distribution equals to one, we computed dissimilarity as (1- overlap)*100 and we calculated it for each pairwise combination of all species coexisting in the same community. Finally we repeated the same process in

all pairwise comparisons between non-coexisting species (species never found in the same community) to assess whether coexisting and non-coexisting species assemblages differed in their morphologies. The extraction of the species assemblages was performed using our own R scripts, which in turn were based on the packages "raster" (Hijmans, 2014), "sp" (Pebesma & Bivand, 2005; Roger et al., 2013) and "maptools" (Lewin-Koh et al., 2011). The computation of phenotypic overlap was performed with our scripts, all of them relying on the package "stats" (R Core Team, 2014) and permutational ANOVAS were conducted in R with the package RVAideMemoire.

Comparing rates of body size evolution across the three genera

In order to test whether differences in the amount of size differentiation within each genus (the trait that explained most of the variance in morphology; see results) could be explained by the existence of different rates of body size evolution among genera, we compiled the maximum SVL (the measure most widely available in literature) for most of the species of geckos of the families Sphaerodactylidae, Phyllodactylidae and Gekkonidae represented in our phylogeny. The data was obtained mainly from Meiri et al., (2011) and complemented from Arnold (1980), Moravec (2011) and Carranza & Arnold (2012), with the values for the Socotran species obtained from this study. We then calculated the rates of body size evolution by means of the function "BrownieREML" from the R package phytools (Revell, 2012). This function essentially fits a Brownian motion model that assumes that different parts of the tree may have evolved according to different Brownian rate parameters (our proxy to the rates of evolution) (O'Meara et al., 2006). We mapped the tree with the following species categories: (1) *Pristurus* from Socotra; (2) *Hemidactylus* from Socotra; (3) *Hemidactylus* from Abd al Kuri; (4) *Haemodracon*; and finally (5) the rest of the geckos. Given that the genus *Pristurus* in Abd al Kuri consisted of just a single species, we pooled it with the rest of the geckos. These species categories were then assigned to internal nodes and branches of the tree by means of a single stochastic reconstruction on each of the 1,500 trees obtained from the posterior distribution of the BEAST analysis.

This was done using the function "make.simmap" from the package "phytools" (Revell, 2012) assuming equal rates in the transitions between categories. Aside of fitting a model assuming independent rate parameter for each category we also fitted an alternative model that assumed that size evolution across the three families of geckos was governed by a single rate parameter. We then evaluated and compared these two models in each of the trees by means of their computed second order Akaike's Information criterion (AIC_c) (Akaike, 1973).

EXPLORING STAGES OF MACRO AND MICRONICHE DIVERSIFICATION

We studied the association between macro- and microniche differentiation in all instances of intra-island diversification events and, when possible (in the cases of more than one successive speciation

event), also exploring how this association evolved through time. Given the low number of diversification events that occurred in the archipelago, we relied on a qualitative approximation. This was based on the visualization of the climatic and morphological divergences associated to all diversification events occurring in the archipelago. Divergences were computed as the Euclidian distances separating two sister species in the climatic and morphologic space (using the first two PCA components in both cases). In the case of diversification events not involving extant species (nodes 1, 2 and 6 in Figure 9), we computed divergences using the climatic or morphological values estimated at the nodes by means of ancestral state reconstructions. These relied on a BM model and were computed using the function "fastAnc" in the package "phytools" (Revell, 2012).

RESULTS

PHYLOGENETIC ANALYSES AND SPECIES ASSEMBLY

We recovered 73% of nodes of the summary tree with a posterior probability (pp) greater than 0.90 (Fig. 2). The phylogenetic relationships depicted by our summary tree were generally consistent with previous published phylogenies of Gekkota (Gamble et al., 2008, 2011, 2012; Pyron et al., 2013). According to our dating estimates, the crown radiation of Gekkota dates from 80 to 150 Ma, which is in the range of most of the estimates provided by previous studies (90-133 Ma in Vidal & Hedges, 2009; 78-95 Ma in Wiens et al., 2006; 84-104 Ma in Hugall et al., 2007; 85-206 Ma in Gamble et al., 2008; 118-167 in Gamble et al. 2011; 52.4-101 Ma in Jones et al., 2013). On the other hand, the substitution rates for *12S* computed in our analyses (between 0.0052 and 0.0093, at a mean of 0.0071 substitutions per lineage per million year) are consistent with the rates estimated in other studies involving geckos (Carranza & Arnold 2012; Metallinou et al., 2012).

Our results show that while the island endemic *Haemodracon* is monophyletic, the Socotran species of *Pristurus* and *Hemidactylus* are polyphyletic implying more than a single colonization event of these genera into the archipelago (at least two in *Pristurus* and three in *Hemidactylus*). However, the precise number of independent colonization events can only be revealed by placing all intra-island splits and mainland-island splits in the context of the different stages of island evolution (Fig. 3). According to this, *Hemidactylus* arrived three times independently to the archipelago. The oldest mainland-island split in this genus fell within the transition stage of the island and therefore is consistent with vicariance or a very early dispersal event when the islands were still very close to mainland (consistently with Carranza & Arnold, 2012; Gómez-Díaz et al., 2012 and Šmíd et al., 2013). The two more recent mainland-island splits took place in the oceanic stage of the archipelago and therefore can unambiguously be interpreted as dispersal events (one to Socotra and one to Abd al Kuri). Still in *Hemidactylus*, all splits separating island species took place in the oceanic stage of the archipelago and are hence consistent with *in situ* (intra-island) speciation events. This is also the case of *Haemodracon*, in which the unique speciation

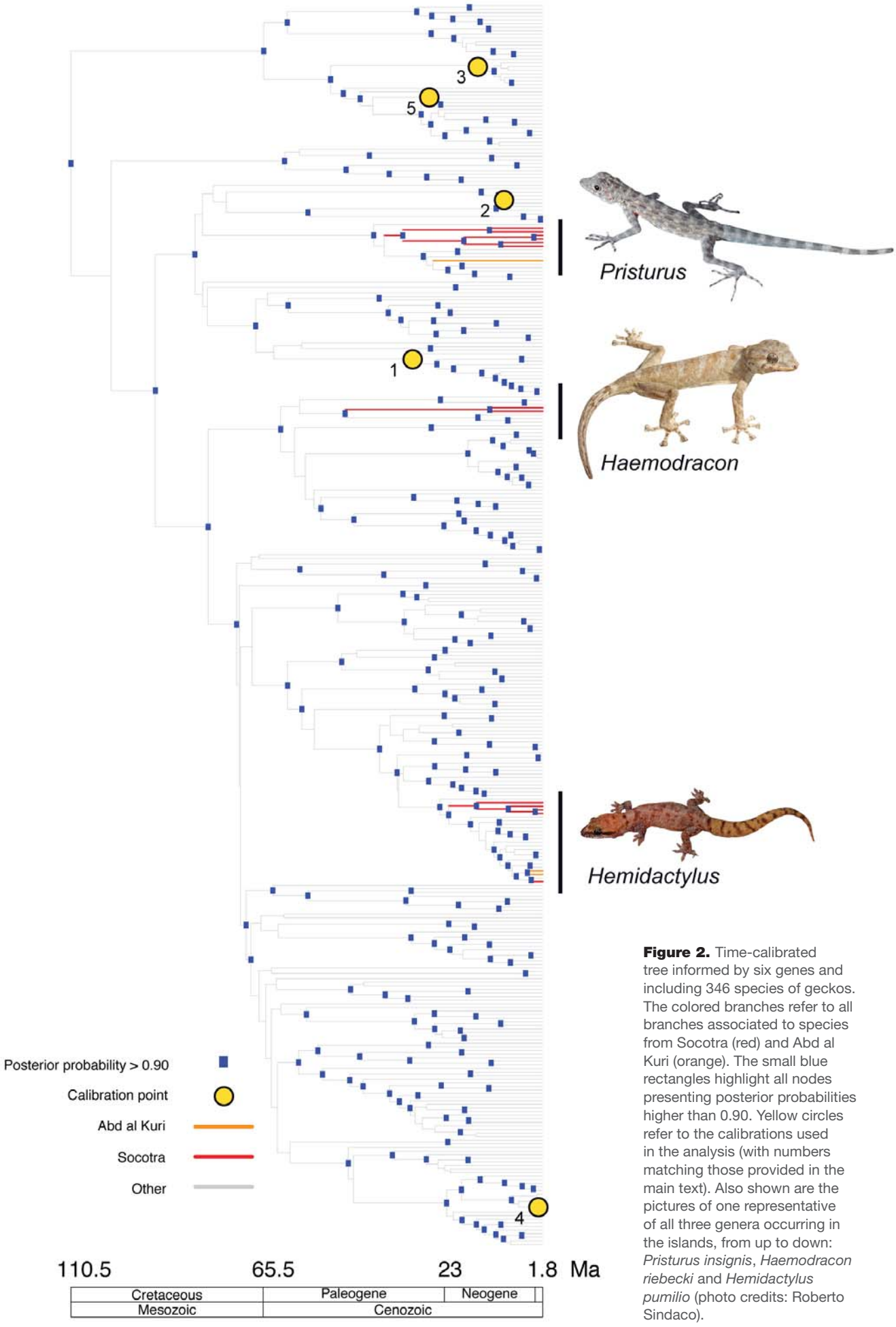

Figure 2. Time-calibrated tree informed by six genes and including 346 species of geckos. The colored branches refer to all branches associated to species from Socotra (red) and Abd al Kuri (orange). The small blue rectangles highlight all nodes presenting posterior probabilities higher than 0.90. Yellow circles refer to the calibrations used in the analysis (with numbers matching those provided in the main text). Also shown are the pictures of one representative of all three genera occurring in the islands, from up to down: *Pristurus insignis*, *Haemodracon riebecki* and *Hemidactylus pumilio* (photo credits: Roberto Sindaco).

event within this genus took place in the oceanic stage of the islands. However, in this case, our dating estimates place the most recent split of this genus with its closest relative in the continent (the genus *Assaccus*) during the continental stage, being therefore a non-informative mainland-island split (the true mainland-island split is not recovered in our phylogeny).

In *Pristurus*, our dating estimates place the split between the endemic species of Abd al Kuri (*Pristurus abdelkuri*) and its closest continental relatives in the transition stage, situation compatible with a possible case of vicariance (although the low support of this mainland-island node make us consider this with caution). Finally, the situation in *Pristurus* of the island of Socotra was the most unexpected, not only the most recent mainland-island split was placed in the continental stage, but also this was the case of the deep-most intra-island split (Fig. 3), both being therefore non-informative. According to this, the clade formed by *P. ignis* and *P. insignoides* (here and after "big *Pristurus*") and the clade formed by *P. sokotranus, P. guichardi, P. obsti* and *P. samhaensis* (here and after "small *Pristurus*") would be the consequence of two independent dispersal or vicariant events not recovered in our phylogeny. All remaining island-island splits are clearly in the oceanic stage of the island and are hence considered as *in situ* diversification events (Fig. 3).

MACRONICHE STRUCTURE

Characterization of macroniche differentiation across con-generic species

The first two components of the PCA conducted on the climatic variables of the archipelago explained a 61.32 and 34.28% of the total variance respectively (Table S1). PC1 essentially reflected an altitudinal gradient with lower values in this axis corresponding to lower annual mean temperatures, wider annual thermic ranges and higher precipitation. PC2 reflected variation along a longitudinal axis, with lower values in this component corresponding to lower values of isothermality and higher values in temperature seasonality (Table S1, Fig. S1). The visualization of the climatic space occupied by each of the islands shows how Socotra and Abd al Kuri are highly divergent in their climatic conditions (Fig. S2). Both are clearly separated along PC2 (with higher values in this component corresponding to Abd al Kuri) and show marked differences in their ranges along PC1, reflecting the differences in the altitudinal span of both islands. The islands of Samha and Darsa present an intermediate climatic position between Socotra and Abd al Kuri, but are substantially closer to the climatic conditions of Socotra (Fig. S2).

The visualization of the climatic envelopes of all gekkotan species across the archipelago reveal highly different climatic heterogeneities between islands (Fig. 4). While in Abd al Kuri all species present similar, highly overlapping climatic envelopes, species in Socotra show a substantially greater climatic heterogeneity: some species present wide climatic envelopes, occupying most of the climatic space offered by the island. This is the case of *Haemodracon riebeckii* and *Ha. trachyrhinus*, in *Hemidactylus*

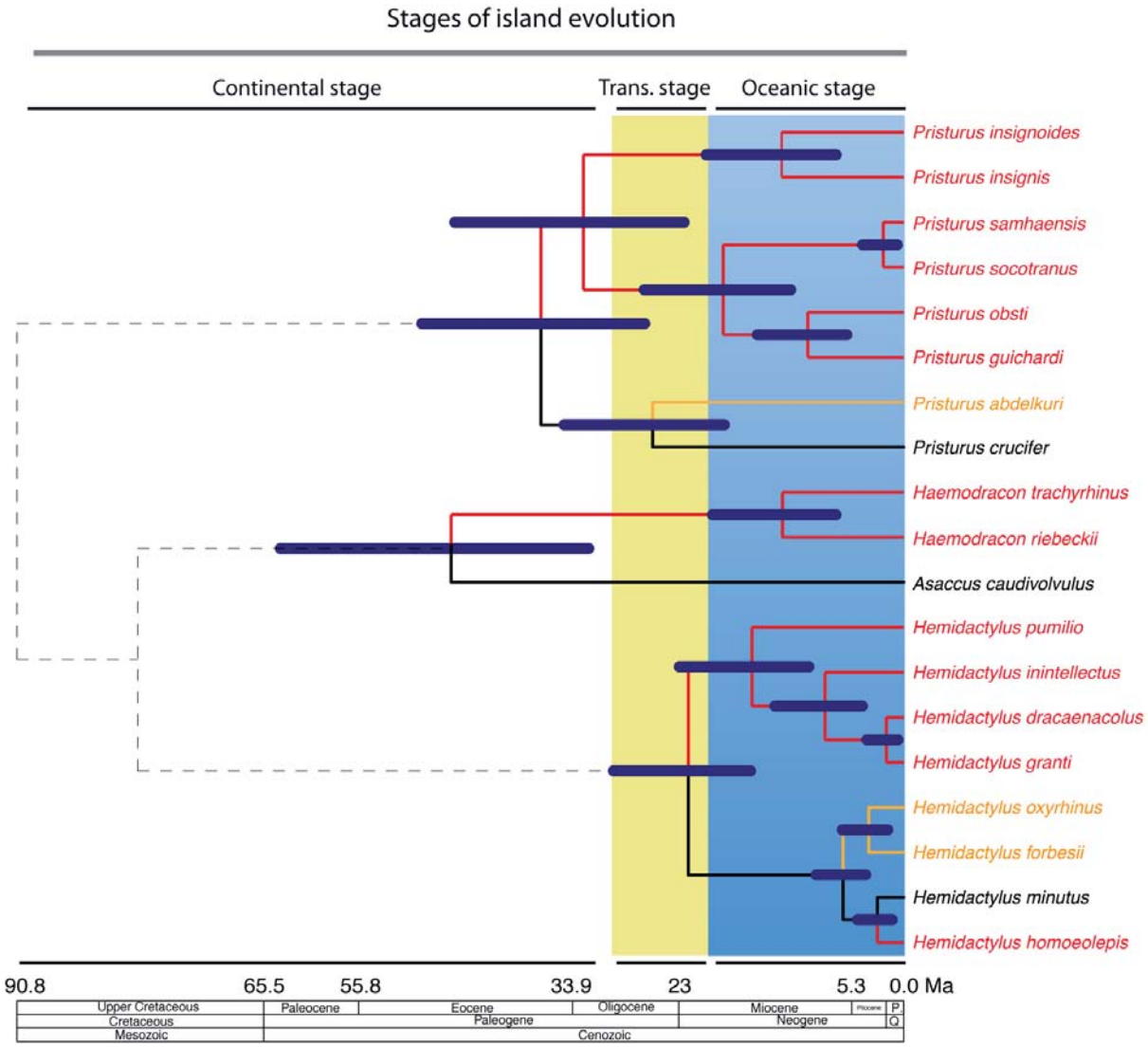

Figure 3. Time-calibrated tree showing all branches and nodes related to the Socotra Archipelago, including all nodes separating island lineages and the most recent mainland-island splits recovered in our tree. Blue bars depict the 95% posterior density intervals for all nodes. We also show, superimposed on the tree, the temporal span of the three stages of geologic evolution of the archipelago. From left to right: continental stage (until 30 Ma), transition stage (from 30 to 20 Ma) and the oceanic stage (from 20 Ma to present). Species from Socotra are shown in red and species from Abd al Kuri are shown in orange.

pumilio, *H. inintellectus*, *H. homoeolepis* and in *Pristurus insignis*, *P. sokotranus* and possibly also *P. guichardi*. Other species occupy discrete regions of the climatic space, as it is the case for *H. granti*, *H. dracaenacolus*, *P. insignoides*, *P. obsti* and *P. samhaensis*.

Exploring patterns of macroniche overlap between congeneric species

The patterns of niche overlap between congeneric species coexisting in the same island also present a great variability (Table 1, Figs. 4 and 5). Regarding the genus *Haemodracon*, the two sister species do not seem to have diverged into greatly different climatic niches (Fig. 5), both occupy large portions of the climatic space available (Fig. 4) and present an estimated niche overlap not significantly different from the random expectation (*p*-value = 0.33) (Table 1). This contrasts with the situation in the genus

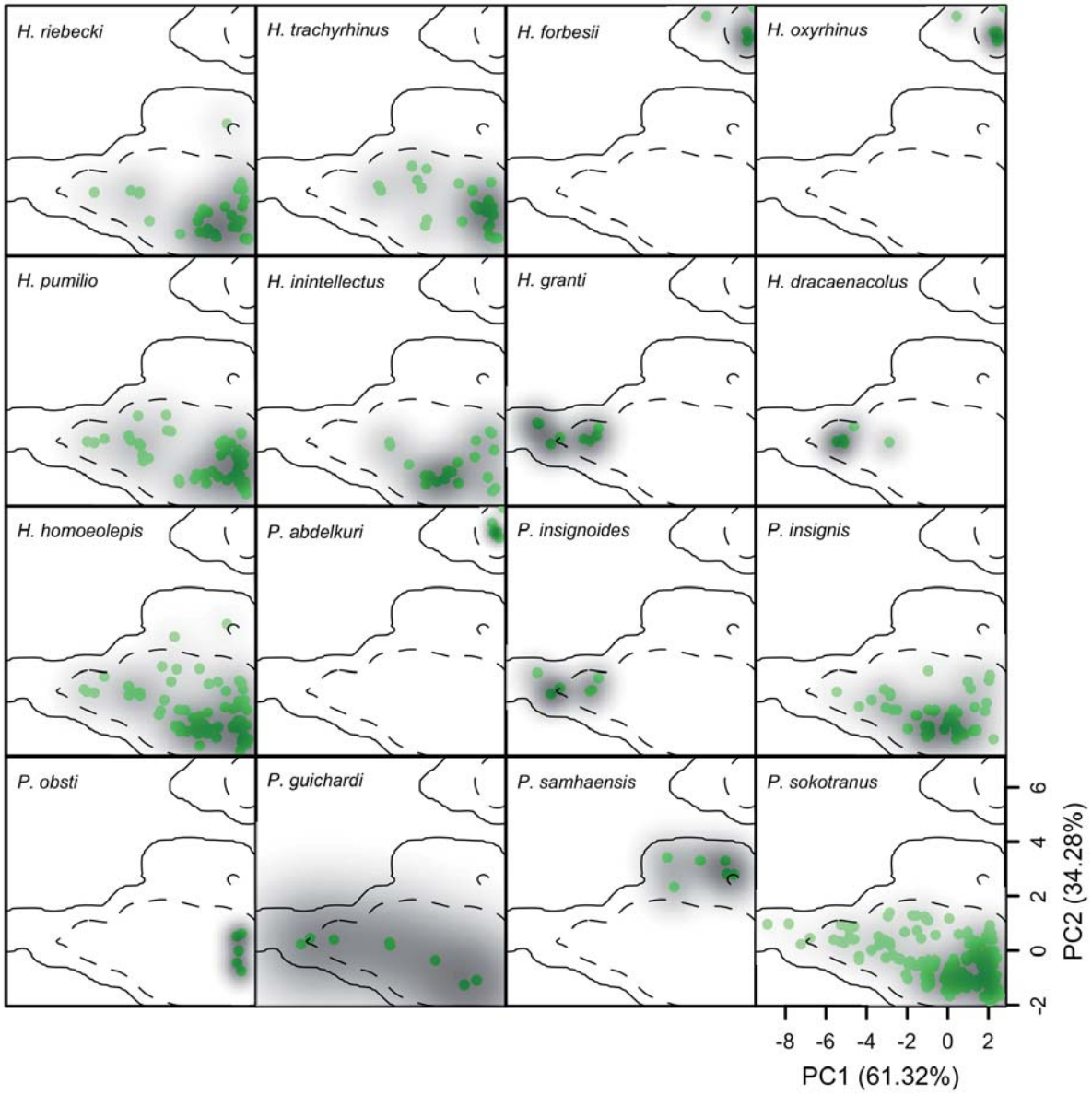

Figure 4. Visualization of the climatic space occupied by each of the 16 gecko species in the archipelago. The green points represent the actual (sampled) values of each species in the climatic space, the grey shading represent the climatic space as interpolated by means of a Kernel density function. The solid and dashed contour lines illustrate, respectively, 100% and 50% of the available (background) climatic space provided by the islands. This space is defined from a PCA performed on six bioclimatic variables (Bio 1, Bio 3, Bio 4, Bio 7, Bio 14 and Bio 16).

Pristurus in which all sister species present significantly different niches (*p*-values ranging from < 0.001 to 0.04) (Table 1), and are widely separated in the phyloclimatic space (Fig. 5). This is the case of the speciation event separating *P. insignis – P. insignoides*, in which macroniche divergence clearly occurs on an altitudinal gradient (PC1), with *P. insignis* being widely distributed from low to medium altitudes and *P. insignoides* restricted to high altitudes (Figs. 4 and 5). In the frame of the *in situ* speciation event separating *P. obsti* and *P. guichardi*, these sister species tend to segregate along both altitudinal and longitudinal axes of climatic variation, being *P. obsti* mostly restricted to lower altitudes on the western part of the island (presenting higher amounts of isothermality) and *P. guichardi* occurring across a larger range of altitudes (from medium to high altitudes) on the eastern side of the island (Fig. 4 and 5). In the

case of the split between *P. sokotranus* and *P. samhaensis*, macroniche divergence mostly occurs along the longitudinal axis (PC2), with *P. sokotranus* occurring in most of the climatic space existing in Socotra and *P. samhaensis* occupying the environmental space provided by the western islets of Samha and Darsa (Fig. 4 and 5). Despite these climatic differences between sister species, representatives of "big" and "small" *Pristurus* and instances of non-sister species in the "small" Pristurus (e.g. *P. sokotranus* and *P. guichardi*) seem to occupy similar climatic envelopes.

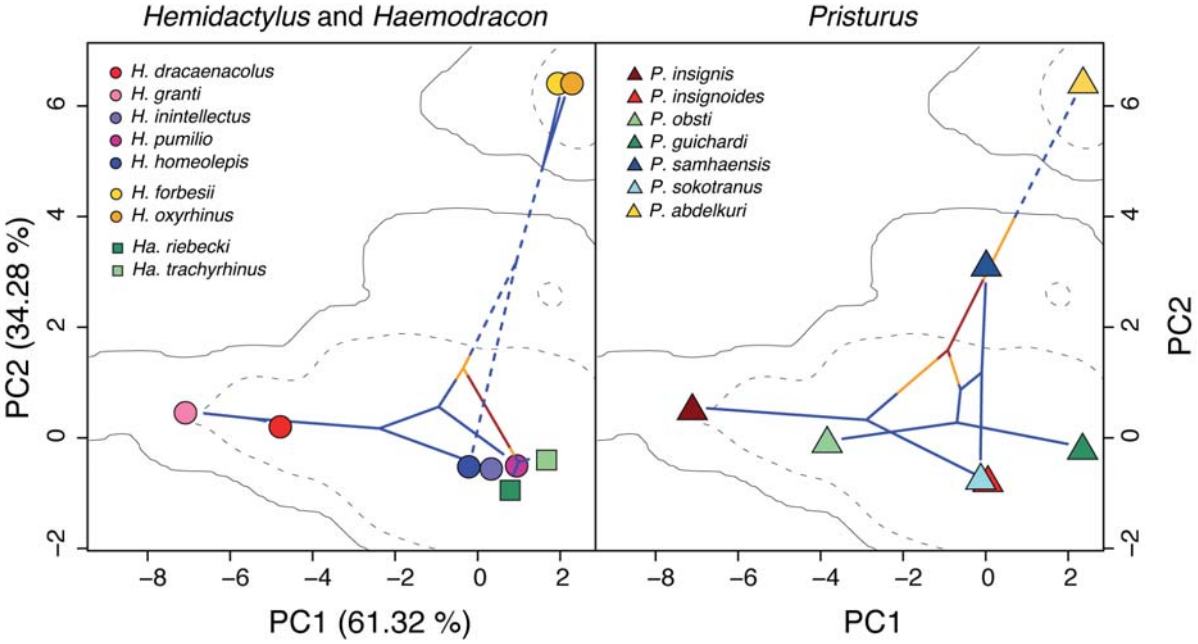

Figure 5. Relative positions of the mean values of the climatic envelopes of all the species of geckos in the Socotra Archipelago. Environmental space is defined from a PCA performed on six climatic variables (Bio 1, Bio 3, Bio 4, Bio 7, Bio 14 and Bio 16) and the climatic space provided by the archipelago is represented by the solid and dashing contour lines, illustrating, respectively, 100% and 50% of the available (background) climatic space. Also shown is the phylogeny projected into the bivariate space using the mean values of the species with the reconstructed values at the nodes. The colors shown along the branches provide a visualization of the time spent by each branch on each of the three geological stages of the island (continental, transition and oceanic, being brown, yellow and blue respectively). Discontinuous branches depict branches that although fall into the oceanic stage of the archipelago, the change along these took place outside the archipelago.

Finally, the situation in the genus *Hemidactylus* is notably more complex. On one hand in the early stages of intra-island diversification tend to show low levels of macroniche diversification (Figs. 4 and 5). This is visible from the highly overlapping climatic envelopes between *H. oxyrhinus* and *H. forbesii*, the unique speciation event that took place in Abd al Kuri (p-value = 0.99) and between *H. pumilio* and *H. inintellectus* (p-value = 0.19), the species that involve the first and the second branching event of this genus in Socotra. However, this contrasts with the amount of macroniche divergence existing between these and the two most recently appeared species, *H. dracaenacolus* and *H. granti*. These two species present similar climatic envelopes (p-value = 0.19) but both show highly divergent climatic envelopes compared to *H. inintellectus* (p-value < 0.01 and 0.04, respectively) and between *H. granti* and *H. pumilio* (p-value = 0.06) (Table 1; Figs. 4 and 5). Interestingly, *H. homoeolepis*, a recent instance

of overseas dispersal, present a wide climatic envelope, greatly overlapping with all species in the island except with *H. granti* (*p*-value = 0.005) (Table 1; Figs. 4 and 5).

SPECIES COMPARISONS	MACRONICHE OVERLAP (D)	P-VALUE
H. riebecki vs H. trachyrhinus	0,511	0,331
H. forbesii vs H. oxyrhinus	0,839	0,996
H. dracaenacolus vs H. granti	0,198	0,096
H. dracaenacolus vs H. homoeolepis	0,323	0,155
H. granti vs H. homoeolepis	0,207	0.005**
H. inintellectus vs H. granti	0,022	0***
H. inintellectus vs H. dracaenacolus	0,068	0.004**
H. inintellectus vs H. homoeolepis	0,407	0,067
H. pumilio vs H. granti	0,196	0.006**
H. pumilio vs H. dracaenacolus	0,354	0,196
H. pumilio vs H. inintellectus	0,455	0,197
H. pumilio vs H. homoeolepis	0,770	0,975
P. guichardi vs P. samhaensis	0,087	0.017*
P. guichardi vs P. sokotranus	0,674	0,988
P. insignis vs P. samhaensis	0,053	0***
P. insignis vs P. obsti	0,090	0.001**
P. insignis vs P. insignoides	0,122	0.004**
P. insignis vs P. guichardi	0,346	0,1
P. insignis vs P. sokotranus	0,542	0,162
P. insignoides vs P. obsti	0,000	0***
P. insignoides vs P. samhaensis	0,000	0***
P. insignoides vs P. sokotranus	0,404	0,215
P. insignoides vs P. guichardi	0,529	0,69
P. obsti vs P. samhaensis	0,004	0***
P. obsti vs P. guichardi	0,011	0.001**
P. obsti vs P. sokotranus	0,091	0.001**
P. samhaensis vs P. sokotranus	0,087	0***

Table 1. Values of macroniche overlap (D) between all pairwise comparisons of congeneric species coexisting in the same island. *p*-values are calculated by means of a null distribution generated by 1,000 randomizations of the localities of each of the two species involved in each pairwise comparison. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

MORPHOLOGICAL DIFFERENTIATION

Characterization of the ecomorphological variation

The PCA conducted on the morphological data, revealed that body size is the major source of variation among the species in the archipelago. This is essentially reflected in the first component (PC1), which accounted for 88% of the total variation (with all loadings in this axis being positive and presenting similar values). The second component produced in the analysis (PC2) accounted for 5.7% of the total variation and essentially reflected the variation of limb lengths, particularly BL and CL (Table S2). The

visualization of the morphospace defined by these two axes (explaining 94% of the total variation; Fig. 6) shows a strong segregation along the PC2 between *Pristurus* and the other two genera, reflecting disproportionally longer limbs in *Pristurus*.

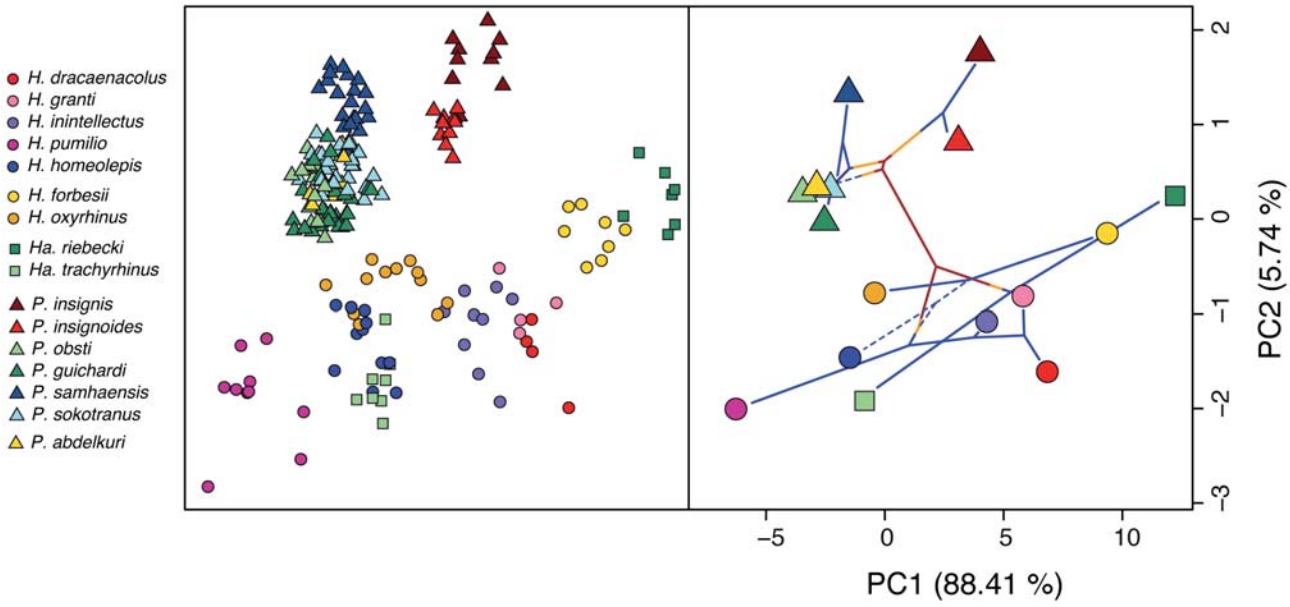

Figure 6. Morphospace occupied by the 16 species of geckos in the archipelago. This morphospace was obtained from a PCA performed on 18 measurements taken on 201 specimens. The chart on the right shows the phylogeny projected using the means of each of the species and their reconstructed values at the nodes. The colors shown along the branches provide a visualization of the time spent by each branch of the tree on each of the geological stages of the islands (continental, transition and oceanic, being brown, yellow and blue respectively). Discontinuous branches depict branches that although fall into the oceanic stage of the Archipelago, the change along these took place outside the archipelago.

The morphological differentiation between *Hemidactylus* and *Haemodracon* is less marked, presenting substantial amounts of overlap along both PC axes.

Regarding the amounts morphological variation within genera, different degrees of differentiation were observed. *Hemidactylus* and *Haemodracon* present the greatest levels of morphological differentiation. This is strong along the axis of size variation and only modest along the axis of limb length variation. Morphological variation in *Pristurus* is also notable along the axis of size variation, with *P. insignis* and *P. insignoides* ("big *Pristurus*") clearly presenting bigger sizes compared to the rest of the *Pristurus* in the archipelago ("small *Pristurus*"). Morphological divergence along the axis of limb length variation is solely worth noticing in the differentiation between *P. samhaensis* and the rest of the small *Pristurus* and between *P. insignis* and *P. insignoides*.

The visualization of morphological diversification from a phylogenetic and temporal perspective revealed that most of the size diversification in *Haemodracon* and *Hemidactylus* occurred in the context of *in situ* speciation events (Fig. 6). It is remarkable the extreme body size divergence existing between the sister species of *Haemodracon* in Socotra and the sister species of *Hemidactylus* occurring in Abd al Kuri. Likewise, it is also notable the size differentiation that took place in the frame of the intra-island split

that separated the smallest species in Socotra (*H. pumilio*) from the other species of *Hemidactylus* that diversified as a consequence of intra-island speciation events (*H. inintellectus*, *H. dracaenacolus* and *H. granti*). The recently arrived *H. homoeolepis* occupied an intermediate size between *H. pumilio* and the rest of the species in the island (Fig. 6). Finally, in *Pristurus*, the greatest size differentiation in this group involved the two independent origins of this genus in the archipelago (producing the "big" and "small" *Pristurus*) with all intra-island speciation events involving minimal amounts of body size diversification and moderate amounts of limb length differentiation.

Exploring morphological overlap within sympatric species assemblages

The assessment of congeneric species coexisting in areas smaller than 1 km^2 produced 14 different sympatric species assemblages (Table 2; Fig. 7). These consisted of five species combinations for the genus *Pristurus*, eight combinations for the genus *Hemidactylus* and a single one for the genus *Haemodracon* (containing the two unique species in this genus). The phylogenetic structure of communities varied substantially between genera and islands (Fig. 7). While in *Pristurus* sister species never coexist, in *Haemodracon* and in *Hemidactylus* from Abd el Kuri, sister species fully coexist in the same community. The situation in *Hemidactylus* in the island of Socotra is more complex: on one hand, the recently arrived *H. homoeolepis* coexists with all other species of *Hemidactylus*. On the other, all the remaining species (originated through intra-island speciation events) coexist with each other with the exception of *H. inintellectus* and *H. granti*, the sister species *H. dracaenacolus* and *H. granti* and these two with *H. pumilio* (Fig. 7).

When the morphologies of all pairwise combinations of coexisting species were compared along PC1 (the axis that explained 88% of the variation, see results), very contrasting patterns emerged between different genera (Table 2; Fig. 7). In *Haemodracon* and *Hemidactylus* coexisting species appear to be strongly segregated along the axis of body size variation, with all permutational ANOVAs producing significant differences between all coexisting species along this axis (all p-values < 0.05) (Table 2). Also, the levels of dissimilarity between coexisting species in these two genera were always extremely high, with a mean dissimilarity of 96% in *Hemidactylus* and a 100% of dissimilarity between the two species of *Haemodracon*. Interestingly, the mean dissimilarity between non-coexisting species in *Hemidactylus* (at a mean of 71%) is substantially lower. This is because among non-coexisting species we find the lowest dissimilarities computed between two species of *Hemidactylus* living in the same island: this is the case of *H. dracaenacolus* and *H. granti,* which present a 27% of dissimilarity and are the only two species that do not significantly differ along PC1 (p-value = 0.30), and the comparison between *H. inintellectus* and *H. granti*, which present a 58% of dissimilarity (although in this case the permutational ANOVA produced significant differences; p-value = 0.005).

TYPE	AXIS	GENUS	SPECIES COMPARISONS	SUM SQ1	SUM SQ2	DF1	DF2	MEAN SQ1	MEAN SQ2	F VALUE	PR(>F)	DISSIMILARITY
coexisting	PC1	*Haemodracon*	*riebecki vs trachyrhinus*	523,260	5,754	1	13	523,260	0,443	1182,134	0.001**	1,000
coexisting	PC1	*Hemidactylus*	*forbesi vs oxyrhinus*	345,813	31,989	1	18	345,813	1,777	194,584	0.001**	1,000
coexisting	PC1	*Hemidactylus*	*homoeolepis vs inintellectus*	115,816	13,243	1	18	115,816	0,736	157,418	0.001**	0,991
coexisting	PC1	*Hemidactylus*	*homoeolepis vs pumilio*	116,695	21,847	1	19	116,695	1,150	101,488	0.001**	0,938
coexisting	PC1	*Hemidactylus*	*inintellectus vs pumilio*	432,649	21,105	1	17	432,649	1,241	348,504	0.001**	1,000
coexisting	PC1	*Hemidactylus*	*granti vs homoeolepis*	131,722	9,983	1	13	131,722	0,768	171,529	0.001**	1,000
coexisting	PC1	*Hemidactylus*	*dracaenacolus vs homoeolepis*	159,321	9,005	1	13	159,321	0,693	229,996	0.001**	1,000
coexisting	PC1	*Hemidactylus*	*dracaenacolus vs pumilio*	417,607	16,867	1	12	417,607	1,406	297,108	0.001**	1,000
coexisting	PC1	*Hemidactylus*	*dracaenacolus vs inintellectus*	17,764	8,263	1	11	17,764	0,751	23,648	0.002**	0,809
coexisting	PC1	*Pristurus*	*insignis vs socotranus*	255,462	27,304	1	40	255,462	0,683	374,243	0.001**	1,000
coexisting	PC1	*Pristurus*	*obsti vs socotranus*	10,281	23,899	1	48	10,281	0,498	20,648	0.001**	0,509
coexisting	PC1	*Pristurus*	*insignoides vs socotranus*	161,927	21,210	1	40	161,927	0,530	305,377	0.001**	1,000
coexisting	PC1	*Pristurus*	*guichardi vs insignoides*	178,790	18,084	1	33	178,790	0,548	326,254	0.001**	1,000
coexisting	PC1	*Pristurus*	*guichardi vs socotranus*	2,167	37,493	1	55	2,167	0,682	3,179	0,093	0,154
coexisting	PC1	*Pristurus*	*guichardi vs insignis*	273,106	24,179	1	33	273,106	0,733	372,748	0.001**	1,000
non-coexisting	PC1	*Hemidactylus*	*dracaenacolus vs granti*	0,894	5,003	1	6	0,894	0,834	1,072	0,303	0,277
non-coexisting	PC1	*Hemidactylus*	*dracaenacolus vs pumilio*	417,607	16,867	1	12	417,607	1,406	297,108	0.003**	1,000
non-coexisting	PC1	*Hemidactylus*	*granti vs inintellectus*	9,622	9,241	1	11	9,622	0,840	11,454	0.005**	0,582
non-coexisting	PC1	*Hemidactylus*	*granti vs pumilio*	372,691	17,845	1	12	372,691	1,487	250,623	0.002**	1,000
non-coexisting	PC1	*Pristurus*	*guichardi vs obsti*	3,185	20,773	1	41	3,185	0,507	6,287	0.02*	0,381
non-coexisting	PC1	*Pristurus*	*guichardi vs samhaensis*	6,979	23,267	1	43	6,979	0,541	12,899	0.001**	0,414
non-coexisting	PC1	*Pristurus*	*insignis vs insignoides*	6,966	7,895	1	18	6,966	0,439	15,882	0.001**	0,631
non-coexisting	PC1	*Pristurus*	*insignis vs obsti*	291,613	10,584	1	26	291,613	0,407	716,355	0.001**	1,000
non-coexisting	PC1	*Pristurus*	*insignis vs samhaensis*	193,743	13,078	1	28	193,743	0,467	414,798	0.001**	1,000
non-coexisting	PC1	*Pristurus*	*insignoides vs obsti*	198,356	4,490	1	26	198,356	0,173	1148,655	0.001**	1,000
non-coexisting	PC1	*Pristurus*	*insignoides vs samhaensis*	118,189	6,984	1	28	118,189	0,249	473,842	0.001**	1,000
non-coexisting	PC1	*Pristurus*	*obsti vs samhaensis*	17,119	9,673	1	36	17,119	0,269	63,715	0.001**	0,743
non-coexisting	PC1	*Pristurus*	*samhaensis vs socotranus*	1,965	26,393	1	50	1,965	0,528	3,723	0,055	0,278

Table 2. Results of the permutational ANOVAs and dissimilarity analyses on all the possible pairwise comparisons of congeneric species coexisting in the same island and in the same locality (type = coexisting) and all congeneric species coexisting in the same island but not in the same locality (type = non-coexisting). Dissimilarity values goes from 1 (no morphological overlap) to 0 (total morphological overlap). Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

Regarding *Pristurus*, coexisting species also present significant size differences with the exception of *P. sokotranus* and *P. guichardi* in which only marginally significant differences exist (p-value = 0.055). However, despite these size differences, dissimilarities were substantially lower than in *Haemodracon* and *Hemidactylus* (at a mean of 80%). Low dissimilarities between coexisting species in this genus

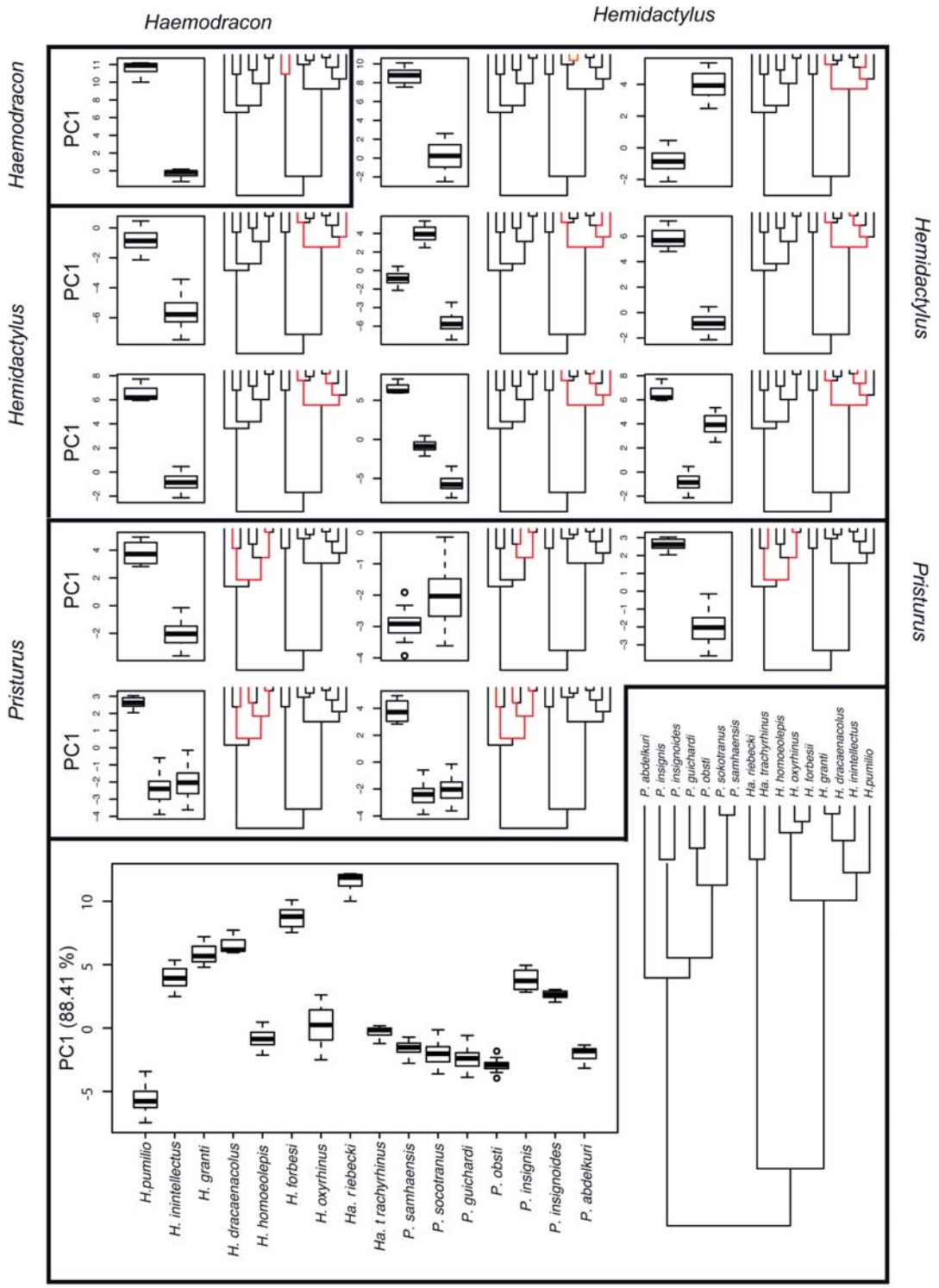

Figure 7. Visualization of the morphological variation (along PC1) and the phylogenetic structure existing in all 14 communities of congeneric species coexisting in the same island. For each community variation along PC1 is shown by means of boxplots of the coexisting species and the phylogenetic structure is represented as highlighted branches in the tree depicted next to the boxplots for each community (red for Socotra, orange for Abd al Kuri). The lower plot provides a visualization of the variation along PC1 for all the species in the archipelago.

include prominent examples as in the comparison between *P. obsti* and *P. sokotranus*, which coexist despite presenting a 50% of dissimilarity, and also the case between *P. guichardi* and *P. sokotranus* only presenting a 15% of dissimilarity. Non-coexisting species of *Pristurus* present only slightly lower amounts of dissimilarity compared to coexisting species (with a mean dissimilarity of 71%). However, in this case these differences in dissimilarities might be difficult to interpret as in both coexisting and non-coexisting species assemblages, representatives of the big and small *Pristurus* are always present. When we compared dissimilarities between coexisting and non-coexisting species within each of these size groups of *Pristurus* (which represent independent island invasions; Fig. 3), we found that although the two species of big *Pristurus* (presenting 63% of dissimilarity) never coexist, small *Pristurus* can coexist despite of presenting low levels of dissimilarity (with a mean of 33% of dissimilarity between coexisting species and a mean of 45% of dissimilarity between non-coexisting species).

In summary, coexisting species in *Haemodracon* and in *Hemidactylus* present extreme size divergences, which tend to decrease among non-coexisting species in the genus *Hemidactylus*. Size differentiation between coexisting species is mostly the consequence of intra-island speciation events, with the exception of *H. homoeolepis* in the island of Socotra, which is the consequence of a dispersal event from the continent. In *Pristurus*, two sister species originated from an intra-island speciation event never coexist in the same community. However in this genus small *Pristurus* presenting similar sizes are found to coexist in the same community.

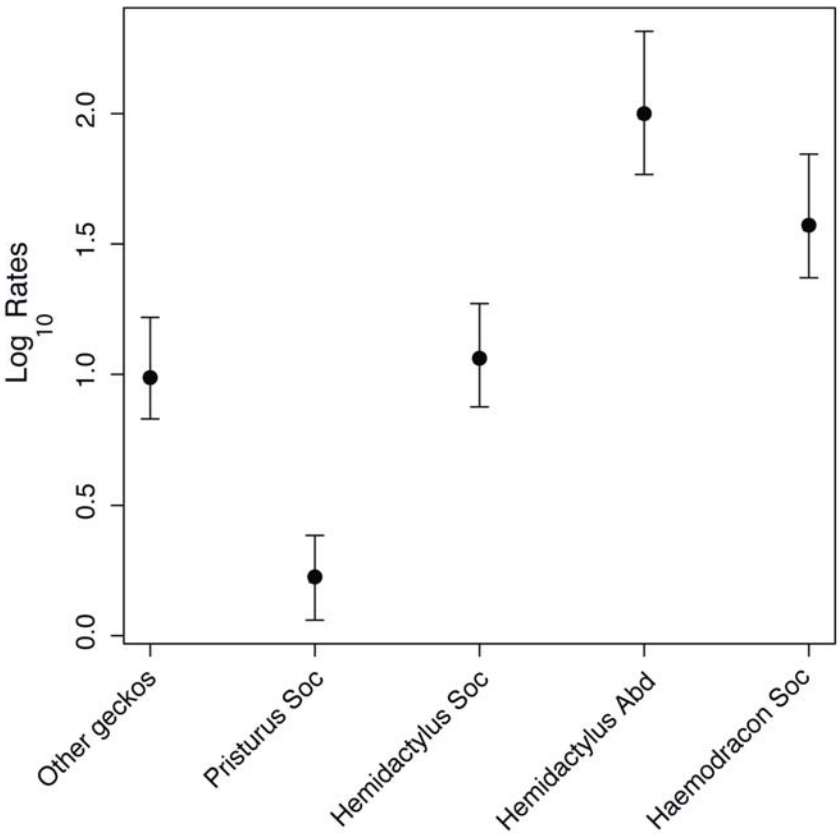

Figure 8. Plot of the mean relative rates of body-size evolution and their associated 95% confidence intervals for each category. Rates were estimated by means of a Brownian motion model assuming rate heterogeneity among categories (see text for details).

Comparing rates of body size evolution across the three genera

The model that implied different rate parameters across groups for all trees presented substantially lower AIC_c values compared to the model relying on just a single rate parameter (with the differences of AIC_c between the two models being at a mean of 11) (Fig. S3). The visualization of the rate parameters of each of the groups with their confidence intervals clearly shows that *Pristurus* from the Socotra Archipelago presents the lowest rates of body size evolution, not only when compared to the other Socotran genera, but also when compared to the average rate computed for other geckos (Fig. 8). *Hemidactylus* and *Haemodracon* present substantially higher rates compared to *Pristurus*, but only in *Hemidactylus* from Abd al Kuri and in *Haemodracon* we compute rates substantially higher than those computed for the rest of the geckos.

Exploring stages of macro and microniche diversification

The comparison between morphological and climatic divergences in diversifying groups showed clearly different patterns between genera (Fig. 9). At one extreme, in *Pristurus* diversification was mostly mediated through climatic differentiation and involved comparatively low amounts of morphological divergence. At the other extreme in *Haemodracon* and in *Hemidactylus* from Abd el Kuri, diversification was solely mediated through morphological differentiation with a minimal contribution of climatic divergence. Finally, the Socotran *Hemidactylus* represent a middle ground between the above-mentioned cases. In this group, the onset of diversification is mostly mediated through morphological divergence but at latter stages of the diversification, climatic differentiation acquires a greater weight.

DISCUSSION

In this study we explored the processes that build up and structure the diversity of island biotas using all species of endemic geckos existing in the remote Socotra Archipelago as a model. This included 16 endemic species belonging to three highly divergent genera, each presenting their unique morphology and ecology. Our results showed that in these three genera, intra-island (*in situ*) diversification was the most important process at building up the diversity as well as in mediating in the niche structure within the islands. However, not a single pattern of climatic, geographic and morphological structuration across the archipelago applied to all three genera. In the following we elaborate on the differences and similarities showed by these different genera diversifying in the same islands and propose plausible processes consistent with the observed patterns.

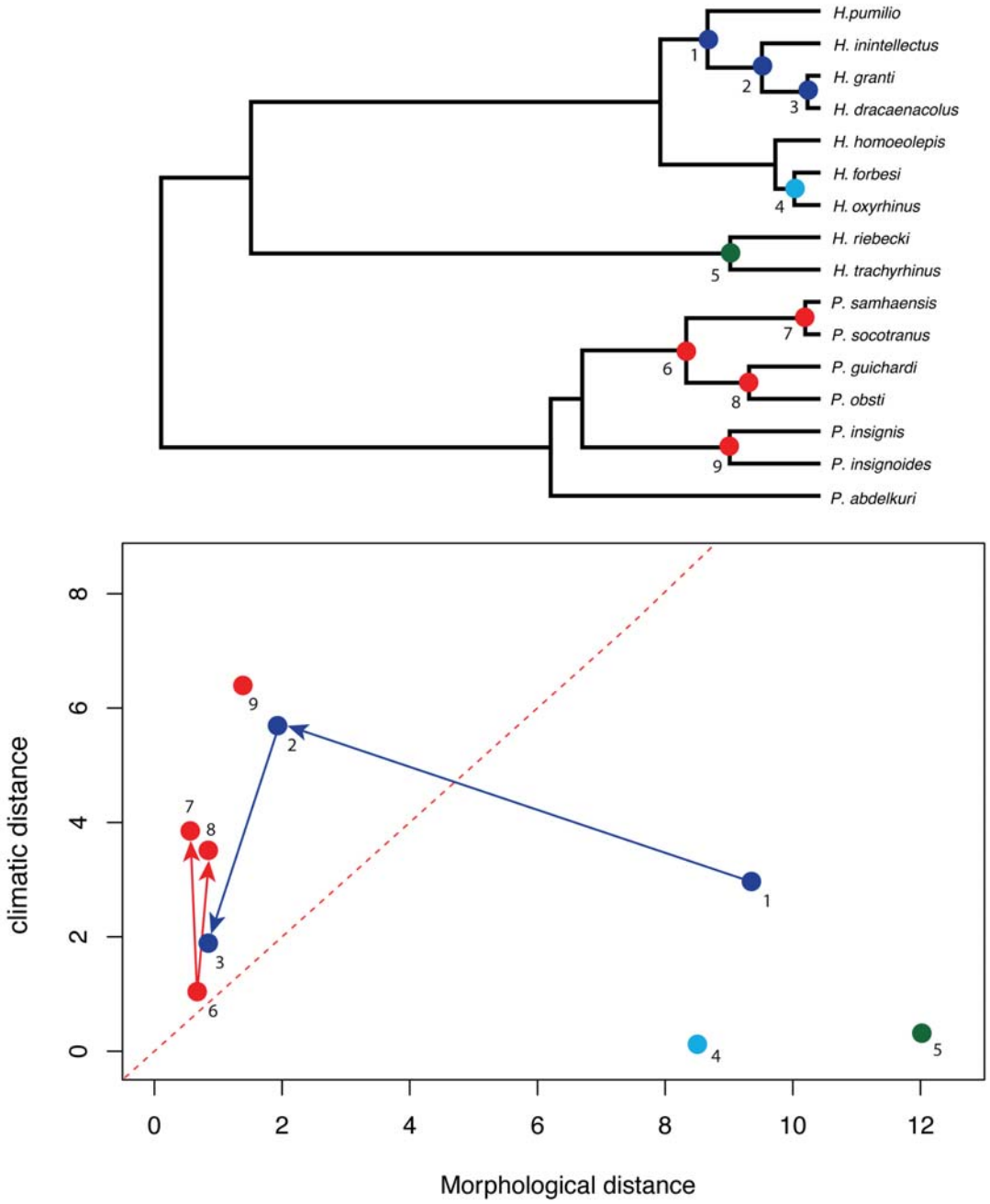

Figure 9. Plot showing the relative morphologic and climatic divergences involved in each of the intra-island speciation events that took place within the Socotra Archipelago. Numbers and colors correspond with those in the phylogeny. Arrows connect associated diversification events. The dashed line represents an equal amount of morphologic and climatic divergence.

SPECIES ASSEMBLY IN THE SOCOTRA ARCHIPELAGO

Most of the present diversity of geckos in the Socotra Archipelago originated once the archipelago was completely detached from the continent. This agrees with other cases of continental islands in which most of its diversity is clearly the consequence of *de novo* diversification once islands were in their oceanic stage. Mass extinction events are often invoked to explain the turnover from mostly vicariant species assemblages to *de novo* diversity. For instance Hedges (1996) suggested that the meteorite impact at the Cretaceous-Tertiary boundary wiped out nearly all vertebrates in many of the

continental islands of the Caribbean, therefore opening the stage for *de novo* rebuilding of their diversity by means of dispersal and *in situ* diversification. Other examples include New Caledonia, New Zealand and Chatham islands, in which a complete (or almost complete) submersion of these islands seems to have eliminated all (or most) of the old vicariant elements present in these islands (Waters & Craw, 2006; Trewick et al., 2007; Espeland & Murienne, 2011).

Nonetheless, evidence of a mass extinction event is lacking in the Socotra Archipelago, and the existence of at least some cases consistent with vicariance rejects a scenario of a complete discontinuity between vicariant and *de novo* diversity. In an alternative scenario, a continuous turnover between old and new diversity is conceivable in a situation in which the random (background) extinction of old vicariant elements produces a continuous flow of ecological vacancies, which in turn may be filled by elements derived either from immigration or by *in situ* diversification (Wittaker et al., 2008). According to this scenario, we would expect a progressive turnover from mostly vicariant species assemblages, when the island just detached from the continent, to a situation of elements with miscellaneous origins, either being vicariants, descended from immigration or generated through *in situ* diversification in a more mature state of the island. Moreover, it is also conceivable that in combination with the ecological vacancies left by extinction, continental islands can also produce their own ecological opportunity, either through orogenic processes affecting the island after its break up from the continent or by climatic change (as their oceanic analogues do; Wittaker et al., 2008).

In the particular case of the Socotra Archipelago, *in situ* diversification (as opposed to dispersal) has been, by far, the most important process of community assembly. There are a number of factors that determine whether the ecological opportunity in an island is filled by immigration or by *in situ* diversification. Arguably, one of the most important is island isolation (Losos & Parent, 2009). Essentially, the more isolated an island is, the lower is the rate of colonization from the continent (MacArthur & Wilson, 1967) and this, in turn, may increase the chances of intra-island speciation events (MacArthur & Wilson, 1963, 1967; Heaney 2000, 2007). A number of reasons explain this, for instance, the more isolated an island is, the easier for island groups is to interrupt gene flow with the conspecifics inhabiting the source region. This allows island groups the possibility to take their own evolutionary path as a completely isolated gene pool. Moreover fewer potential competitors likely reach very isolated islands, therefore facilitating a filling of the ecological vacancies through *in situ* diversification rather than through immigration (Losos & Parent, 2009).

However, island isolation is usually understood in terms of the distance of an island from its source pool (MacArthur &Wilson, 1967) and from this geographic perspective, the Socotra Archipelago might not be a very isolated archipelago (only being at 100 Km from the Horn of Africa). Despite this, only two unambiguous dispersal events have occurred from the continent since the islands completely detached from it around 20 Ma. On the other hand, not a single species of reptile is shared between the two main islands of the archipelago (Socotra and Abd al Kuri), despite being at 105 km from each other (Razzeti

et al., 2011). These very low rates of mainland-island or between-islands dispersal events outline a situation of great isolation of these islands, which may be due to factors other than geography. In either case, the low dispersal into and among the islands of the archipelago fits very well with the great contribution observed of *in situ* diversification in the building up of the diversity of socotran geckos.

MACRO AND MICRONICHE STRUCTURATION

According to our results, *in situ* diversification was the most important process mediating in the macro and microniche structuration of the geckos in the Socotra Archipelago, although it was not the only one. Dispersal or vicariant events might have also played a role. This is the case of the recently arrived *H. homoeolepis*, which while showing a very wide climatic envelope and a distribution that greatly overlaps with most of the other species in Socotra, at the same time presents a highly distinct size that does not overlap with the size of any other species in the island. Another similar case involves the two distinct "big" and "small" *Pristurus*. These, according to our dating estimates, represent two independent biogeographic origins of this genus in the archipelago and although they greatly overlap with each other in terms of climatic envelopes and distributions, at the same time they present greatly distinct sizes. As seen in both cases, we observe notable size differences between coexisting species in the same island, without the involvement of intra-island speciation events.

This provides circumstantial evidence consistent with a size assortment process, in which inter-specific competition acts as a filter, by means of extinction or failed colonizations, and only allows certain species (with certain traits) to coexist in the same community (Case & Bolger, 1991). Similar patterns consistent with species assortment are not uncommon in lizard communities in islands (Case & Bolger, 1991; Losos, 2009). It is then plausible that only species substantially differing in size, as the big and small *Pristurus*, or substantially differing from all the other species pre-existing in the island, as in the case of *H. homoeolepis*, could be able to establish themselves in Socotra. However, given that our analysis did not take into account the sizes of species other than the ones present in the Socotra Archipelago, we cannot rule out a possible size adjustment after the animals dispersed into (or were isolated in) the islands.

In either case, our results highlighted *in situ* diversification as the most important process mediating in macro and microniche structuration. However a single process of niche structuration failed to explain the patterns observed in all genera. At one extreme, in *Haemodracon* and in the *Hemidactylus* from Abd el Kuri, intra-island diversification events were associated with an extreme body size divergence and negligible amounts of climatic divergence. At the other extreme, a completely opposed pattern appeared in *Pristurus*, in which most of the splits involved climatic shifts but minimal amounts of body size divergence. Finally, the *Hemidactylus* of Socotra presented an intermediate pattern in which body size divergence was maximal at the beginning of the intra-island diversification events and progressively diminished in importance as the diversification progressed. This contrasted with the observed pattern for

climatic evolution, which showed a modest contribution at the onset of the diversification but progressed into greater contributions towards the present (particularly in the second intra-island split). Interestingly, this shows how different (but closely related) groups can take completely different evolutionary paths even when occurring in the same geographical and ecological context. Examples like this highlight the importance of group-dependent factors at determining the particular stages of diversification that each group follows.

One of them involves different trait evolvabilities among different groups. This factor has been invoked to explain why different groups fail to diversify while others do despite of being exposed to similar levels of ecological opportunity (Losos, 2010). Along these lines, if the evolutionary plasticity of body size in *Pristurus* somehow is constrained due to physiological or ecological reasons, this could explain why they have not diversified into the extent observed in *Hemidactylus* and *Haemodracon*. Having said that, from a completely different perspective, this difference might rely on smaller climatic evolvabilities in *Haemodracon* and *Hemidactylus* compared to *Pristurus*. Unfortunately the low number of species in the islands did not allow us to obtain reliable estimates of the phylogenetic signals of climatic or morphological variables. However, when we compare the rates of body size evolution between all Socotran groups and a varied representation of geckos, we found that *Pristurus* presented the lowest rates of body size evolution compared to all other island genera and to an estimate obtained for other geckos. This pattern is consistent with different evolvabilities as a lower capacity to experience evolutionary changes is usually reflected in low rates of evolution (Price et al., 2010). One possibility that could explain a scenario of different evolvabilities could be related to the different lifestyles between the genera: while *Pristurus* is diurnal, *Hemidacylus* and *Haemodracon* are nocturnal. Diurnal and nocturnal species experience different selective pressures regarding optimal body sizes. For instance, diurnal species are likely to be thermoregulators and therefore their activity patterns will strongly rely on fast rates of heat exchange with the environment. In such a situation small lizards are able to gain and lose heat more quickly and therefore small sizes tend to be the optimal ones. This contrasts with the situation of nocturnal species, which tend to be thermoconformers and therefore less affected by body-size related cooling and heating rates (Huey & Slatkin, 1976; Meiri, 2008). As a consequence, nocturnal species might be freer to evolve into a greater variety of sizes, particularly bigger sizes compared to diurnal species (Meiri, 2008). These physiological limitations could theoretically explain, not only the shorter span of sizes observed in *Pristurus* compared to *Hemidactylus* and *Haemodracon* (which never get to the big sizes attained by *Ha. riebeckii* or *H. forbesii*), but also their different predispositions to evolve along climatic or phenotypic axes.

Also related to different evolvabilities, there is an important trait difference between *Pristurus*, on one side, and *Hemidactylus* and *Haemodracon*, on the other, that can potentially play an important role: the adhesive toepads. Adhesive toepads consist of a series of modified lamellae, each one covered with millions of microscopic hair-like bristles called setae. These setae are so thin that are able to engage

Van der Waals interactions with the molecules of the substrate enabling a great adhesive power (Hiller, 1968; Autumn & Peattie, 2002). The great adhesiveness granted by toepads is supposed to allow padded species the capability to use more efficiently highly tridimensional environments (as arboreal environments) and have access to a higher variety of resources and niches compared to padless species (Losos, 2009). In turn, the increased ecological opportunity that toepads provide can be translated into the extensive amounts of evolutionary diversification experienced by the padded *Anolis* and possibly also geckos (Losos, 2009). Despite of some recent studies that show that toepads in geckos might not be as important as previously thought (Gamble, 2012; Garcia-Porta & Ord, 2013), in the present study we show how padded species are precisely the ones presenting greater disparities and higher rates of size evolution. It is plausible that toepads could allow the species possessing them the possibility to be exposed to a wider range of resources (e. g. a greater spectrum of prey size variation) therefore triggering their diversification into a greater variety of phenotypes (as different body sizes). Nonetheless, the low sample size of this study in terms of number of genera and species hamper us to test this question in a reliable way. More studies involving a greater sample size and a greater phylogenetic scale will be crucial to shed light on this issue.

Aside of the potential existence of intrinsic factors at modulating the extent of micro and macroniche diversification, the comparison between Socotra and Abd al Kuri also highlights the importance of extrinsic factors. These two islands mainly differ in their lengths of environmental gradients and therefore offer different chances of macroclimatic differentiation. In fact, macroniche differentiation was important in Socotra, particularly along an altitudinal gradient, but was almost inexistent in Abd al Kuri. Interestingly we can see a possible interplay between extrinsic and intrinsic factors in the two gecko genera existing in Abd al Kuri. *Pristurus*, despite of being a vicariant element present in the island since its detachment from the continent, solely contains a single species in the island. This contrasts with the recently arrived *Hemidactylus*, which diversified in the island producing two species greatly differing in size. It is plausible that the small climatic envelope provided by the island of Abd al Kuri has limited the chances of diversification in *Pristurus* (which tend to diversify along macroclimatic axis rather than along a morphological axis) but has not supposed any limitation to *Hemidactylus* (which tend to diversify along morphological axes).

In many groups, the existence of size diversification in the context of intra-island speciation events has been related to intra-island competition (Moen & Wiens, 2009; Losos, 2009) and this could also apply to the case on the *Hemidactylus* and *Haemodracon* in the Socotra Archipelago. It is very remarkable the case in Abd al Kuri, in which two species diversify in the context of an intra-island speciation event, greatly diverging in body size evolution (and presenting the highest rates of body size evolution computed for the three genera). In this case, given the small size of the island (therefore not allowing many opportunities of allopatric speciation), speciation and size divergence could have been simultaneous. This would be the case if size divergence were driven by strong intra-specific competition leading to disruptive

selection on body size (Nosil 2012). Disruptive selection may arise if phenotypically intermediate (and more common) phenotypes (e.g. intermediate sizes) compete more strongly for resources than those at the tails of the distribution (extreme sizes). This results in a lower fitness of intermediate phenotypes and higher fitness in the most extreme sizes, which will ultimately interrupt gene flow between each other producing speciation (Nosil, 2012). In the case of the island of Socotra, its bigger area and more complex topography could easily allow a slightly different scenario: speciation in allopatry followed by a secondary contact in which size divergence would take place by character displacement (Grant & Grant, 2009; Losos, 2009). Distinguishing between these two scenarios is beyond the scope of this study, however we provide evidence consistent with a common prediction of both: the existence of size-based resource partitioning (Stuart & Losos, 2013). Consistently with this, coexisting species of *Hemidactylus* and *Haemodracon* are always significantly different in size and present low levels of size overlap. In *Pristurus*, the same occurs between the big and small species, always found to coexist in the same community. Size differentiation is commonly associated with prey size partitioning (Fisher & Dickman, 1993; Woodward & Hildrew, 2002; Duellman, 1995; Moen & Wiens, 2008) although it can also be involved in the specialization to different structural habitats (Losos, 2009). Future studies should shed light on the precise nature of this size-mediated resource partitioning; whether it involves structural habitat, different prey sizes, or a combination of both.

Solely the species forming the group of small *Pristurus* seemingly constitutes an exception to the importance of size differentiation in the structuration of communities. In this group *P. sokotranus* coexist with *P. obsti* and *P. guichardi* despite of presenting highly overlapping body sizes. This suggests that, in this particular case, resource partitioning potentially takes place along axes other than size (or morphologic) variation. Razzetti et al., (2011) describe *P. obsti* and *P. guichardi* as purely arboreal while *P. sokotranus* is described as occupying a wide range of habitats but being mainly rock dwelling. It is plausible that these habitat differences could potentially mediate in limiting inter-specific competition between these coexisting species. More direct evidence from studies on diet, or spatial use would add an essential complement to this question.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This study used the complete diversity of geckos in the Socotra Archipelago to understand the processes of species assembly and macro and microniche structuration in the islands of this archipelago. According to our results, the Socotra Archipelago despite of being formed by islands with a continental origin, produced most of its diversity in its oceanic stage, when the islands that form the archipelago were completely surrounded by ocean. This evidence aligns well with the evidence provided by other studies that show how continental islands might not greatly differ from their oceanic counterparts in their main processes of community assembly. The little contribution of vicariant elements in the total Socotran

diversity may be due to extinction and the little contribution of dispersal events from the continent (and among the islands) might be explained by a situation of effective isolation of the islands. The study of the contributions of vicariance, dispersal and intra-island diversification in other groups in the archipelago will be crucial to assess the generality of this pattern.

Regarding the roles of these processes at producing the niche structure observed in the archipelago, our results clearly highlight intra-island diversification as the most important process. However our analyses also show that the different genera are not homogeneous in regard to the how diversification proceeds in the islands. Some groups tend to diversify along climatic axes while tend to be conserved in morphology (*Pristurus*), others tend to diversify in morphology while are conserved in macroniche (*Haemodracon* and *Hemidactylus* from Abd al Kuri) and finally, others shift from a situation of low climatic and great morphological diversification to a situation of high climatic and low morphological diversification (*Hemidactylus* from Socotra). These extreme differences between groups and islands can be explained by the interplay of different intrinsic and extrinsic factors, particularly different evolvabilities or different potentials for niche diversification existing in the different islands. Studies relying on a greater phylogenetic scale (not only relying on island species, but also including data of continental species) would be critical to test some of the hypotheses produced in the present study. For instance, a low evolvability in *Pristurus* compared to *Hemidactylus* should not only be detected in the Socotra Archipelago, but also should be detected in the continent. Also, a potential association between toepads and size diversification should be assessed in a greater phylogenetic context, involving many of the independent origins of toepads along the evolutionary history of geckos (Gamble et al., 2012). Finally, the extension of the analyses used in this study to other axes of macro and microclimatic variation would provide an important complement in the understanding of the macro and microniche structuration of the species of the islands. Macroniche structuration needs to be complemented by data on habitat variation across the island and proxies other than morphology could greatly improve our assessments of microniche structuration. Although our study highlights size as a key trait likely mediating resource partitioning in the islands, this needs to be confirmed by additional data. For instance, data on the diets of the different species would provide hard evidence supporting a hypothetical scenario of prey size-based resource partitioning (Moen & Wiens, 2009). Moreover, the existence of closely related species coexisting in the same community, almost not differing in size, shows that other traits besides size could play a role at partition resources.

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SUPPLEMENTARY FIGURES

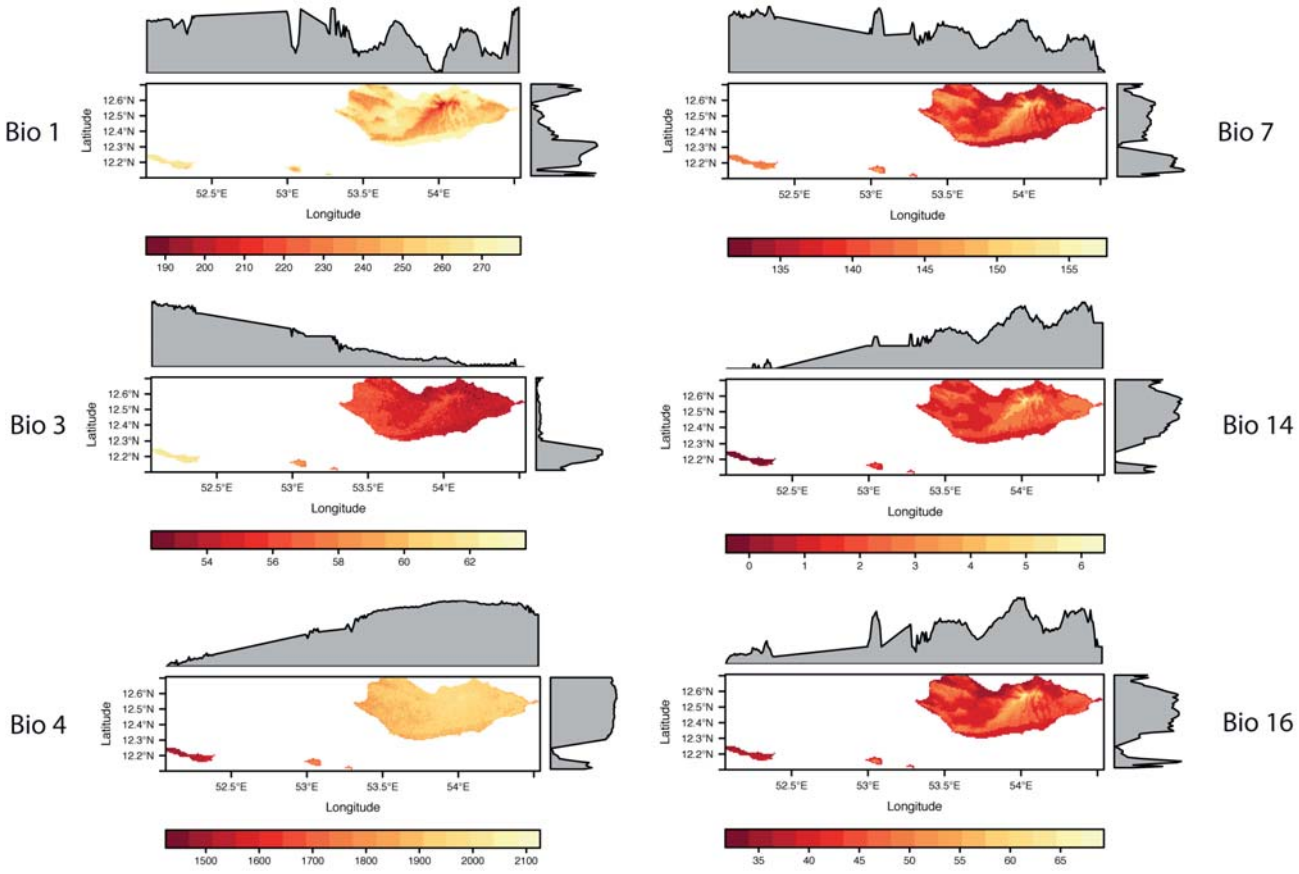

Figure S1. Plots presenting the Bioclim variables used in this study. The plots on the margins show the variability of the mean cell values along a longitudinal (upper) and latitudinal (right) gradient.

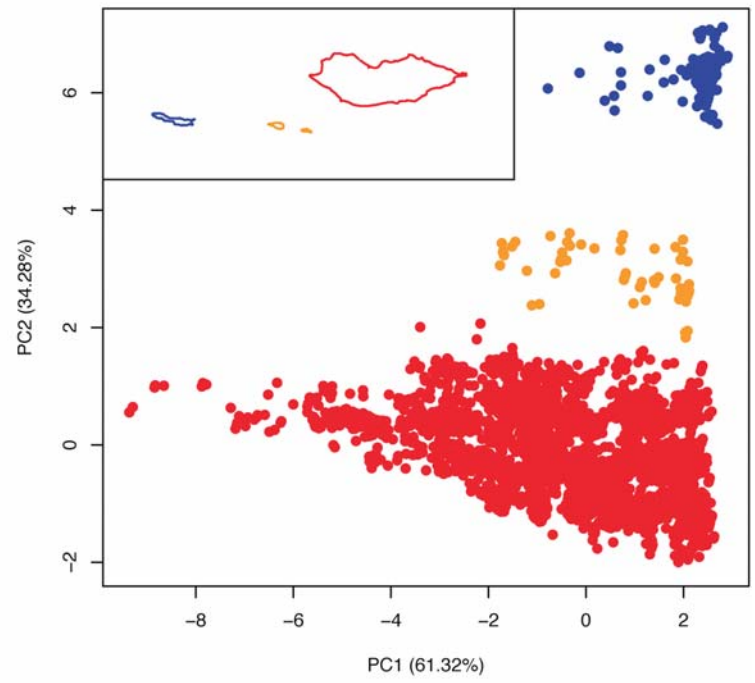

Figure S2. Plot showing the climatic space existing in the archipelago. This space is defined from a PCA performed on six climatic variables (Bio 1, Bio 3, Bio 4, Bio 7, Bio 14 and Bio 16). The climatic space provided by each of the islands is represented by different colors: blue for Abd al Kuri, orange for Samha and Darsa and red for Socotra.

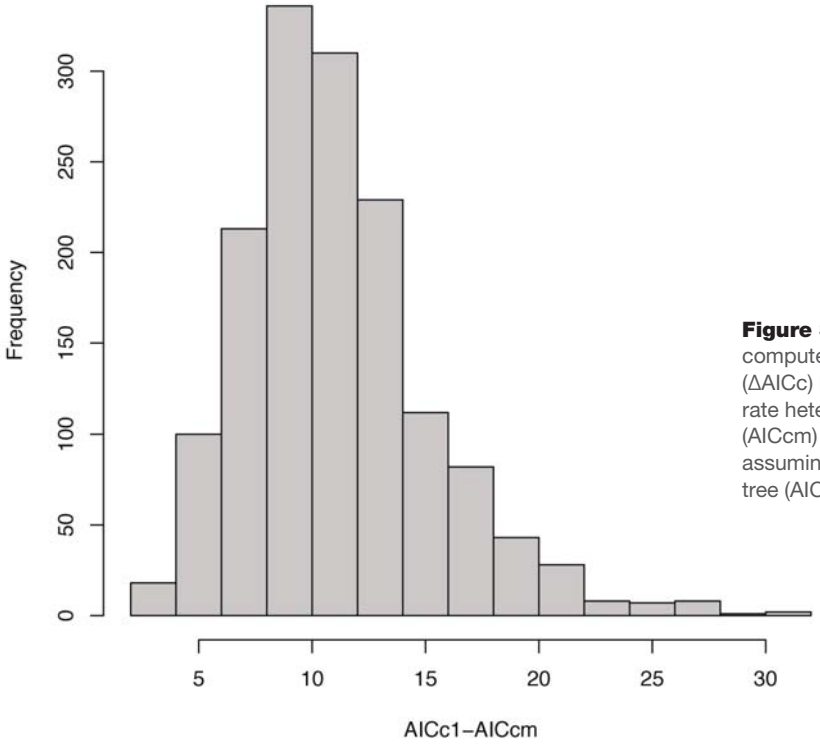

Figure S3. Distribution of the computed differences of Akaikes (ΔAICc) between a model assuming rate heterogeneity across the tree (AICcm) and an alternative model assuming a single rate across the tree (AICc1).

SUPPLEMENTARY TABLES

VARIABLE	PC1	PC2
bio1	66,51	-8,29
bio3	-3,38	66,37
bio4	-27,80	-59,39
bio7	-57,16	32,57
bio14	-61,88	-16,91
bio16	-66,48	0,91
Eigenvalue	16723,27	9348,66
Variance explained (in %)	61,32	34,28

Table S1. Results of the principal components analysis (PCA) on six climatic variables: Bio 1, Bio 3, Bio 4, Bio 7, Bio 14 and Bio 16, showing the loadings of each original variable on the two firsts principal components (PC1 and PC2) and the proportion of total variation represented by each PC axis.

VARIABLE	PC1	PC2
SVL	0,25	-0,04
HL	0,25	-0,09
HW	0,24	-0,24
HWN	0,24	-0,18
HD	0,25	0,01
HDN	0,24	-0,05
IND	0,23	-0,10
END	0,24	0,06
IOD	0,24	-0,17
OD	0,23	-0,24
EED	0,24	-0,19
AGL	0,24	-0,05
ASG	0,24	-0,14
APGd	0,24	-0,12
BL	0,22	0,42
AL	0,23	0,36
TL	0,23	0,33
CL	0,20	0,56
Eigenvalue	3,99	1,02
Variance explained (in %)	88,41	5,74

Table S2. Results of the principal components analysis (PCA) on 18 morphologic variables obtained from a total of 201 specimens. The table shows the loadings of each original variable on the two firsts principal components (PC1 and PC2) and the proportion of total variation represented by each PC axis.

CHAPTER 4.1

PHYLOGENETIC RELATIONSHIPS OF SEMAPHORE GECKOS (SQUAMATA: SPHAERODACTYLIDAE: *PRISTURUS*) WITH AN ASSESSMENT OF THE TAXONOMY OF *PRISTURUS RUPESTRIS*

Photo credit: Salvador Carranza

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Phylogenetic relationships of Semaphore geckos (Squamata: Sphaerodactylidae: *Pristurus*) with an assessment of the taxonomy of *Pristurus rupestris*

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Abstract

A molecular phylogeny of the sphaerodactylid geckos of the genus *Pristurus* is inferred based on an alignment of 1845 base pairs (bp) of concatenated mitochondrial (*12S*) and nuclear (*acm4, cmos, rag1* and *rag2*) genes for 80 individuals, representing 18 of the 23–26 species, and the three subspecies of *P. rupestris*. The results indicate that *P. rupestris* is polyphyletic and includes two highly divergent clades: the eastern clade, found in coastal Iran and throughout the Hajar Mountain range in northern Oman and eastern UAE; and the western clade, distributed from central coastal Oman, through Yemen, Saudi Arabia and north to southern Jordan. Inferred haplotype networks for the four nuclear genes show that the eastern and western clades of "*P. rupestris*" are highly differentiated and do not share any alleles. Moreover, although the two clades are differentiated by a morphological multivariate analysis, no one character or set of characters was found to be diagnostic. Based on the molecular analysis of specimens from the type locality of *P. rupestris rupestris*, the name *P. rupestris* is applied to the eastern clade. The name that should apply to the western clade cannot be clarified until morphological and genetic data for "*P. rupestris*" is available from the vicinity of Bosaso, Somalia, and therefore we refer to it as *Pristurus* sp. 1. The phylogenetic tree of *Pristurus* supports the hypothesis that *P. celerrimus* is sister to all the other species in the analyses and that the Socotra Archipelago was independently colonized a minimum of two times.

Key words: gecko, Arabia, phylogeny, taxonomy, systematics, Socotra Archipelago, mitochondrial DNA, nuclear DNA.

Introduction

The sphaerodactylid geckos of the genus *Pristurus* Rüppell, 1835, also known as Semaphore geckos, comprise 23–26 species (Arnold 2009; Sindaco & Jeremčenko 2008; Uetz 2013), characterized by being mostly diurnal,

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heliothermic and by signaling each other by waving their tails. These features are unusual among geckos, which are mainly nocturnal, active at comparatively low temperatures and communicate predominantly by vocalization or by chemical cues. In fact, some *Pristurus* species behave more like desert agamid lizards than typical geckos (Arnold 1993). Most of the species of the genus are found in northeast Africa (7 species, 4 endemic), the Arabian Peninsula (14 species, 12 endemic) and the Socotra Archipelago (7 species, all endemic), with a widely distributed species, *P. rupestris* Blanford, 1874, extending into coastal Iran. Unexpectedly, a new isolated species, *P. adrarensis* Geniez & Arnold, 2006, was recently described from a very small area in Mauritania, constituting a 4700 km range extension to the west.

At a morphological level, *Pristurus* species are characterized externally by having simple toes without expanded adhesive pads beneath, tail compressed laterally, dorsal scaling consisting of small granules without larger tubercles on the body, flanks often with longitudinal series of short dark or reddish streaks separated by pale spots, pupil round or vertically elliptical with complete (or notchless) borders, absence of preanal and femoral pores, cloacal tubercles and postcloacal sacs (Arnold 1977, 1982, 1986a, 2009). These main features, together with other morphological, behavioral and ecological characters were used by Arnold (2009) to infer a phylogenetic analysis and suggested that *Pristurus* is most closely related to *Quedenfeldtia* of Morocco and then successively to the American Sphaerodactylini and *Saurodactylus* from the Maghreb (Arnold 2009). A recent multilocus phylogenetic analysis including representatives of 107 of 118 recognized gecko genera (Gamble *et al*. 2012) indicates that *Pristurus* is sister to *Saurodactylus* and that *Quedenfeldtia* is sister to the South American *Aristelliger*, suggesting a high degree of homoplasy in morphology, ecology and behavior in *Pristurus* and *Quedenfeldtia*.

Despite the interest in *Pristurus* from a biogeographical, evolutionary, ecological and behavioral points of view, the genus has been relatively neglected, compared to other gecko groups from the same region like *Hemidactylus* and *Stenodactylus*, for which very robust phylogenetic hypotheses and an updated taxonomic knowledge exist (Carranza & Arnold 2012; Fujita & Papenfuss 2011; Gómez-Díaz *et al*. 2012; Metallinou & Carranza 2013; Metallinou & Crochet, 2013; Metallinou *et al*. 2012; Šmíd *et al*. 2013a,b). A recent phylogeny by Arnold (2009) based on 72 morphological characters of 20 species, supported earlier hypotheses, which suggested that *Pristurus celerrimus* Arnold, 1977 is sister to all other species (Arnold 1986a, 1993). It also recovered a monophyletic assemblage of nine species distinctive in their morphology, ecology and behavior that were considered by Arnold (2009) as members of the subgenus *Spatalura* Gray, 1863. Interestingly, only two (*P. insignis* Blanford, 1881 and *P. insignoides* Arnold, 1986a) of the five endemic species from the Socotra Archipelago included in Arnold's (2009) morphological phylogeny were recovered monophyletic. *Pristurus obsti* Rösler & Wranik, 1999 and *P. samhaensis* Rösler & Wranik, 1999 were not included in Arnold's (2009) phylogenetic analysis. Relationships among other members of the genus were not very well supported and therefore were left unresolved. The only previous molecular phylogeny of *Pristurus* included 11 species that were analyzed independently for one mitochondrial fragment (1457 bp of ND2 and adjoining tRNAs) and one nuclear locus (1381 bp of RAG1) (Papenfuss *et al*. 2009). The results of this molecular phylogeny were largely congruent with the morphological results of Arnold (2009) concurring on the position of *P. celerrimus* and the monophyly of the four members of the subgenus *Spatalura* included in the analyses. Regarding the Socotran species, results differed between the two markers used by Papenfuss *et al*. (2009), but in all analyses *P. abdelkuri* Arnold, 1986a, endemic to Abd al-Kuri Island, branched independently from the other three *Pristurus* species from Socotra Island included in the study (*P. obsti* and *P. samhaensis* were not in the analyses). Despite being a partial phylogeny, the molecular tree by Papenfuss *et al.* (2009) clearly showed that the level of genetic variability within *Pristurus* was very high, some of these diverging lineages including members of *P. rupestris*.

Pristurus rupestris is the most widely distributed species in the genus, being found in northeast Africa (Eritrea, Djibouti, north of Somalia and adjoining Ethiopia), the Arabian Peninsula (southern Jordan, Saudi Arabia, Yemen, Oman, UAE, Qatar, Bahrain and various other islands in the Gulf, including Khark island, one of the original type localities), and in coastal Iran, from Bushehr in the west to Sistan and Baluchistan province in the east (Arnold 1993, 2009; Sindaco & Jeremčenko 2008; Gholamifard *et al*. 2009). Three subspecies of *Pristurus rupestris* are currently recognized: *P. rupestris rupestris* Blanford, 1874 [type locality: "insulae Kharfvel Carrack" (=Khark Island) and Maskat (= Muscat), restricted to Muscat by Schmidt 1952]; *P. rupestris iranicus* Schmidt, 1952 [type locality: "insulae Kharfvel Carrack" (=Khark Island)]; and *P. rupestris guweirensis* Haas, 1943 [described as *P. flavipunctatus guweirensis*, type locality: "Nubian sandstone desert of Guweira (=Quweira), between Ma'an and

Guweira police station", Jordan]. One species, *Pristurus migiurtinicus* Scortecci, 1933 [type locality "Bender Cassim" (= Bosaso), Somalia], was synonymized with *P. rupestris* by Loveridge (1947).

Pristurus rupestris is one of the smallest species, measuring usually less than 30 mm of snout-vent length. It is found from sea level up to 2330 m in the Jebel Akhdar, northern Oman, and to 3000 m in Yemen (pers. obs.). It is, by far, the most abundant reptile species in Oman and other areas of Arabia, where it occurs from quite mesic habitats to arid lowlands and mountains. This gecko is nearly always found on rocky or stony surfaces, mainly within a meter above the ground, but also on tree trunks and branches, especially in forested areas. Occasionally it occurs on sandy substrates but always in the vicinity of rocks. It can be abundant in villages, towns and even cities, preferring man-made walls, other constructions and planted trees. *Pristurus rupestris* is diurnal, heliothermic, and is a passive forager ("sit and wait"), spending long periods at feeding perches (Arnold 1980, 1993). The most usual refuges are rock crevices and the interstices between stones. The tail is approximately 1.5 times longer than the snout-vent length, flattened vertically, sometimes with a fringe on the upper mid-line and both sexes use it to produce a wide repertoire of signals, including curling it over the back (Arnold 1993; Ross 1990).

Here, we use molecular data to infer the phylogenetic relationships of the genus *Pristurus* and to revise the taxonomy of *Pristurus rupestris*. The results indicate that *P. rupestris* is polyphyletic and includes two highly differentiated clades: the eastern clade, distributed across northern Oman, UAE and Iran; and the western clade, which extends from coastal middle-southern Oman, to Yemen, western Saudi Arabia and up to southern Jordan (Fig.1). The two clades are also differentiated from a morphological point of view. The name *Pristurus rupestris* is applied to the eastern clade and, until more data of "*P. rupestris*" from Somalia is available, we temporarily refer to the western clade as *Pristurus* sp. 1.

Material and methods

Molecular analyses. Molecular samples, DNA extraction and amplification. A total of 80 individuals of *Pristurus* including 18 of the 23–26 species were included in the phylogenetic analyses. Two members of the family Gekkonidae available from GenBank, *Gekko gecko* (Linnaeus, 1758), *Phelsuma modesta* Mertens, 1970, and one from the family Phyllodactylidae, *Tarentola mauritanica* (Linnaeus, 1758), were used to root the tree based on published evidence (Gamble *et al*. 2011, 2012). As a result of the uncertain phylogenetic position of *Pristurus* in the tree of Gekkota (Gamble *et al*. 2011, 2012), more distant outgroups were preferred over closer ones. A list of all *Pristurus* included in the molecular analyses with their taxonomic identification, sample code, voucher code, country, and corresponding geographical distribution data and GenBank accession numbers for all sequenced genes is presented in Appendix I. To select which samples of "*Pristurus rupestris*" should be included in the molecular phylogeny but especially to analyze the distribution ranges of the two lineages, a preliminary molecular analysis was carried out sequencing one mitochondrial fragment of the gene encoding the ribosomal *12S* rRNA for 512 specimens of "*P. rupestris*" (data not shown). This included samples from across its distribution range in Arabian and one locality from Iran, including members of the three recognized subspecies (Fig. 1). The results of the preliminary analysis of the *12S* data were used to set the boundaries between the eastern (384 specimens) and western (128 specimens) clades of "*P. rupestris*" (Fig. 1). As a result of the high level of genetic variability detected within the 384 specimens of the eastern clade of "*P. rupestris*" analyzed, seven specimens, four from the type locality of *P. r. rupestris* (Specimens IBES7680, IBES6038, IBES7709 and IBES6039; see Appendix I) and two others that could be confidently assigned to the same lineage than the samples from the type locality of *P. r. rupestris*, were selected together with one sample of *P. rupestris iranicus* for further multilocus phylogenetic analyses (locs. 15–20 in Fig. 1). The high level of genetic variability within the eastern clade of "*P. rupestris*" will be addressed somewhere else (work in progress). The 128 specimens belonging to the western clade of "*P. rupestris*" were genetically uniform and therefore the 15 specimens included in the multilocus analyses were selected covering the whole distribution range of the western clade in Arabia (locs. 1–14 in Fig. 1), including two samples of *P. r. guweirensis* from Jordan (loc. 1).

Genomic DNA was extracted from ethanol-preserved tissue samples using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) or the SpeedTools Tissue DNA Extraction kit (Biotools, Madrid, Spain). Five genetic markers were PCR-amplified and sequenced: one mitochondrial fragment of the gene encoding the ribosomal 12S rRNA (*12S*; primers 12Sa and 12Sb—Kocher *et al*. 1989), and four nuclear fragments of the genes

encoding the acetylcholinergic receptor M4 (*acm4*; primers tg-F and tg-R—Gamble *et al*. 2008), the oocyte maturation factor Mos (*cmos*; primers FUF and FUR—Gamble *et al*. 2008), a short fragment of the recombination-activating gene 1 (*rag1*; primers F700 and R700—Bauer *et al*. 2007), and the recombination-activating gene 2 (*rag2*; primers PyF1 and PyR—Gamble *et al*. 2008). PCR conditions used for the amplification of the *12S* mitochondrial fragment as well as the nuclear genes *cmos*, *rag1* and *rag2* can be found in Šmíd *et al.* (2013a), and for the nuclear gene *acm4* in Barata *et al*. (2012).

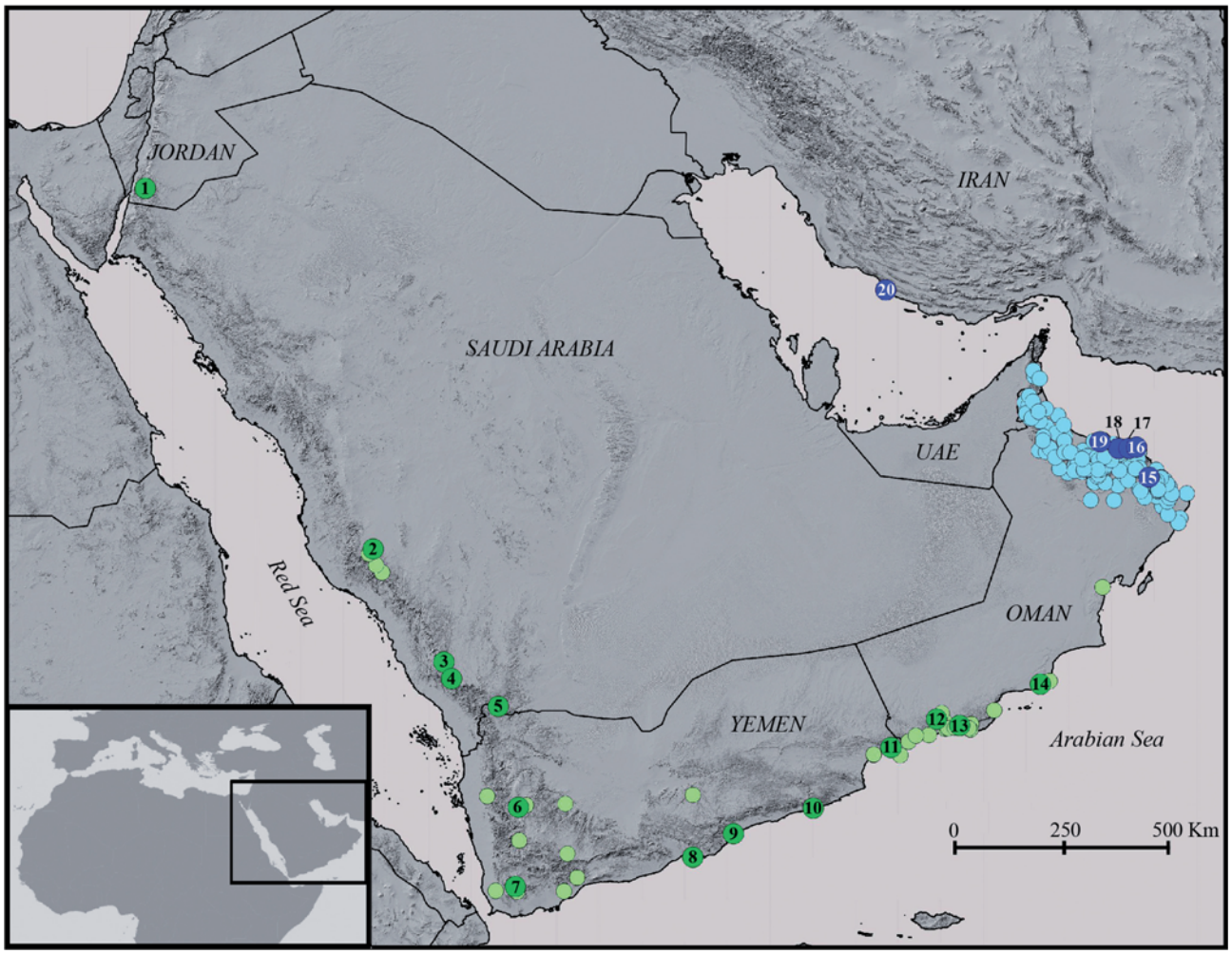

FIGURE 1. Map of localities of *Pristurus rupestris* and *Pristurus* sp. 1. Blue localities are specimens of "*P. rupestris"* referred to as the eastern clade. Dark blue corresponds to localities included in the genetic analyses (Figs. 2-3; Appendix I); light blue to specimens genotyped for the *12S* gene to determine the distribution of *P. rupestris*. Green, localities refer to *Pristurus* sp. 1, the western clade of "*P. rupestris*". Dark green corresponds to localities of specimens included in the genetic analyses (Figs. 2-3; Appendix I), and light green corresponds to specimens genotyped for the *12S* gene only to indicate the distribution of *Pristurus* sp. 1.

Sequence analysis. Geneious v. R6 (Biomatters Ltd.) was used for assembling and editing the chromatographs. Heterozygous positions for the nuclear coding gene fragment were identified based on the presence of two peaks of approximately equal height at a single nucleotide site in both strands. The nuclear coding fragments were translated into amino acids and no stop codons were observed. DNA sequences were aligned using MAFFT v.6 (Katoh & Toh 2008) applying parameters by default (Auto strategy, Gap opening penalty: 1.53, Offset value: 0.0). Phased sequences of the *acm4, cmos, rag1* and *rag2* genes were used for the network analyses and to infer independent phylogenies for each nuclear gene. SEQPHASE (Flot 2010) was used to convert the input files, and the software PHASE v. 2.1.1 to resolve phased haplotypes (Stephens *et al.* 2001). Default settings of PHASE were used except for phase probabilities that were set as ≥ 0.7 (see Harrigan *et al.* 2008). Uncorrected mean genetic distances between and within groups for the mitochondrial gene fragment were calculated using MEGA 5 (Tamura *et al.* 2011), using the *p*-distance model.

Phylogenetic and network analyses. Best-fitting models were inferred for each gene independently using jModeltest v.0.1.1 (Posada 2008) under the Akaike information criterion (AIC) (Akaike 1973). Phylogenetic analyses were performed using Maximum Likelihood (ML) and Bayesian (BI) methods. Maximum Likelihood analyses were performed in RAxML v.7.0.3 (Stamatakis 2006). The dataset was partitioned by gene and a GTR+G model was used with parameters estimated independently for each partition. Independent ML trees were also produced for each nuclear gene (datasets phased). Reliability of the ML tree was assessed by bootstrap analysis (Felsenstein 1985) including 1000 replications. Bayesian analyses were performed with BEAST v.1.6.1 (Drummond & Rambaut 2007) using the same dataset used in the ML analysis but without the outgroups. Analyses were run three times for $5x10^7$ generations with a sampling frequency of 10 000. Models and prior specifications applied were as follows (otherwise by default): model of sequence evolution for each independent partition as indicated in Table 1; coalescent constant size process of speciation; random starting tree; alpha Uniform (0, 10); fix mean rate of molecular clock model to 1. The xml file was manually modified to "Ambiguities=TRUE" for the *acm4, cmos, rag1* and *rag2* partitions to account for variability in the heterozygote positions, instead of treating them as missing data. Convergence was assessed by examining the posterior trace plots and by confirming sufficient effective sample sizes for all parameters in Tracer v1.5 (Rambaut & Drummond, 2007). The results of the individual runs were combined in LogCombiner discarding 10% of the samples and the ultrametric tree was produced with TreeAnnotator (both provided with the BEAST package). Nodes in the phylogenies were considered strongly supported if they received ML bootstrap values ≥ 70% and posterior probability (pp) support values ≥ 0.95 (Huelsenbeck & Rannala 2004; Wilcox *et al.* 2002). The branch attachment frequency (BAF) algorithm implemented in the package Phyutility 2.2.4 (Smith & Dunn 2007) was used to explore how often in the 13 500 trees from the posterior of BEAST analysis (after "burnin"; see above) the eastern and western clades of the polyphyletic "*P. rupestris*" were joined in a single clade.

TABLE 1. Information on the length of all partitions used in the phylogenetic analyses, model of sequence evolution selected by jModelTest (model), and number of variable and parsimony-informative sites.

Gene	length (bp)	N	model	variable sites		parsimony-informative sites	
				with outgroup	without outgroup	with outgroup	without outgroup
12S	405	81	GTR+I+G	246	219	199	181
acm4	429	59	HKY+I+G	95	59	57	44
cmos	372	63	HKY+I+G	107	87	80	69
rag1	279	78	HKY+G	103	84	73	65
rag2	360	76	HKY	121	94	93	80

Genealogical relationships in the four nuclear genes between the eastern and western clades of "*P. rupestris*" were assessed with haplotype networks constructed using statistical parsimony as implemented in the program TCS v.1.21 (Clement *et al.* 2000). Phased sequences were used (see above) and a connection limit of 95% was applied.

Morphological analyses. Morphological samples, variables and museums acronyms. Eighty five alcohol-preserved adult specimens of "*Pristurus rupestris"* were included in the morphological analyses. Of these, 32 specimens belonged to the eastern clade and 53 specimens to the western clade of "*P. rupestris*". Based on the *12S* preliminary study (see above) all the specimens from the eastern clade of "*P. rupestris*" were selected from the genetic lineage that included the specimens from the type locality of *P. r. rupestris*. A list of all studied specimens with their GPS localities is presented in Appendix II. Specimens were obtained from the following institutions: Museo Civico di Storia Naturale di Carmagnola, Turin, Italy (MCCI); S. Carranza's field series housed at the Institute of Evolutionary Biology (IBE), Barcelona, Spain; and Lukáš Kratochvíl's field series from Yemen (JEM), housed at the Department of Ecology, Charles University in Prague, Czech Republic. Variables for the morphological analyses were selected based on previous taxonomic studies of *Pristurus* (Arnold 1977, 1982, 1986a, 1993, 2009), and on our own personal observations. One body size and 11 shape measurements were taken by the same person (A.B.) on the left side of each specimen. As a result of the diminutive size of the specimens, measurements were obtained from highly precise picture measurements using the software ImageJ (Abràmoff *et al.* 2012). The snout-vent length (SVL), was measured from beneath as the distance from tip of snout to vent; shape

variables: head length (HL), measured laterally from tip of snout to base of the anterior ear border; head depth (HD), measured laterally from above the eyes to the commissure of mouth; maximum head width (HW), measured from beneath at its widest part, usually at the level of temporal region; transverse orbital diameter (OD); brachium length (BL), from elbow to the insertion of the forelimb on the anterior part of body; antebrachium length (AL), from wrist to elbow; thigh length (ThL), measured from knee to the insertion of the hind limb on the posterior side of body; crus length (CL), measured from ankle to knee; axilla-groin length (AGL), distance measured from beneath, between the fore and hind limb insertion points; scapular width (SW), distance measured from beneath, between the insertion of the left and right fore limbs; pelvic width (PW), distance measured from beneath, between the insertion of the left and right hind limbs.

Multivariate analyses. Statistical analyses were used to investigate differences in size and shape between the eastern and western clades of "*Pristurus rupestris*" identified by the molecular phylogenetic analyses. All measurements were $\log_{10}$-transformed to increase the homogeneity of variances. To remove the effect of body size on the shape variables, we computed the residuals of each morphometric variable regressed against SVL using ordinary least-squared regression. For shape variables we performed a principal components analysis (PCA) on the correlation matrix of the residuals to visualize the shape variation of both species in a reduced dimensional space. We tested for shape differences between both clades by means of a permutational MANOVA (PERMANOVA) on the PCA scores, using a Euclidean distance matrix with 10 000 permutations to assess significance. To assess the contribution of each shape variable at separating the two lineages, we performed a one-way ANOVA on each of the six principal components retained in the PCA analysis. Regarding body size, we tested for differences in SVL between groups using a one-way ANOVA. All analyses were performed in the R environment using the packages Stats (R development Core Team 2013) and vegan (Oksanen 2013).

Results

Molecular analyses

The dataset used for the multilocus phylogenetic analyses consisted of an alignment of 1845 base pairs (bp) of concatenated mitochondrial and unphased nuclear DNA for 80 *Pristurus,* including representatives of 18 of the 23–26 species, as well as the three subspecies of "*P. rupestris*". The eight species that could not be included in our study were: *P. gasperetti* Arnold, 1986a (not recognized by Arnold 2009; considered a synonym of *P. flavipunctatus* Rüppell, 1835); *P. longipes* Peters, 1871 (not recognized by Arnold 2009; considered a synonym of *P. crucifer* (Valenciennes, 1861), but see Schatti & Gasperetti 1994; Loveridge 1947); *P. mazbah* Al-Safadi, 1989 (not recognized by Arnold 2009; considered a synonym of *P. saada* Arnold, 1986a); *Pristurus ornithocephalus* Arnold, 1986a; *P. phillipsii* Boulenger, 1895; *P. saada*; *P. simonettai* (Lanza & Sassi, 1968); and *P. schneideri* Rösler, Köhler & Böhme, 2008. All information related to each partition including alignment length, model selected, and the number of variable and parsimony-informative sites of the molecular dataset is presented in Table 1.

The results of the ML and BI analyses are presented in Fig. 2. The two trees were almost identical and show that "*Pristurus rupestris*" is polyphyletic and includes two highly divergent and very well supported clades: the eastern clade, which includes all seven specimens from localities 15–20 (Figs. 1 and 2; Appendix I); and the western clade, which includes all 15 specimens from localities 1–14 (Figs. 1 and 2; Appendix I). The uncorrected genetic distance (*p*-distance) for the *12S* between the eastern and western clades is 13.3 ±1.6 %. The level of genetic variability for the *12S* within the western clade is 2.21 ±0.4% and within the eastern clade is 0.39 ±0.14%, although within this latter clade only specimens from the type locality of *P. r. rupestris* or from the same genetic lineage as specimens from the type locality were included. The results of the haplotype network analyses of all the specimen of "*P. rupestris*" selected are presented in Fig. 3 and clearly show that, despite the relatively large number of specimens analyzed, the eastern and western clades do not share a single haplotype in any of the four nuclear genes. Moreover, in the *cmos*, *rag1* and *rag2*, the two clades form unconnected parsimony networks under the stringent 95% threshold applied. The results of the ML analyses of the phased datasets of the four nuclear genes are presented in Appendix III and clearly show that the eastern and western clades of "*P. rupestris*" do not share nuclear gene haplotypes with any other *Pristurus* species included in the present analysis. In fact, with the only exception of *P. samhaensis* and *P. sokotranus*, which share one haplotype in the *cmos* and one in the *rag1* genes, all the other species of *Pristurus* included in the analyses present private haplotypes for all four nuclear genes (see

Appendix III). The results of the BAF analysis conducted over the 13500 trees of the BEAST posterior indicated that the eastern and western clades appear as sister clades at a very low frequency (2.11%).

Besides the polyphyly of "*P. rupestris*", the phylogenetic tree from Fig. 2 also supports that *P. celerrimus* is sister to all other *Pristurus* included in the analyses. The six species from Socotra and the satellite island of Samha form a monophyletic group, although the bootstrap and pp support for this assemblage is very low. Within this island clade, the large (*P. insignis* and *P. insignoides*) and the small (*P. obsti*, *P. guichardi* Arnold 1986a, *P. samhaensis* and *P. sokotranus* Parker, 1938) species form two very well supported reciprocally monophyletic groups. *Pristurus obsti* and *P. guichardi* are closely related sister taxa with a genetic distance in the *12S* of 6.1 ±1.1 %. According to our results, *Pristurus sokotranus* is paraphyletic, with one of the three sampled specimens from Socotra Island being more closely related to *P. samhaensis* from Samha Island than to the other two specimens of *P. sokotranus* included in the phylogenetic analyses. The clade formed by the species from Socotra and Samha Islands, is sister to a large and very well supported clade that includes 12 taxa comprising the western clade of "*P. rupestris*", five species in the subgenus *Spatalura*, the eastern clade of "*P. rupestris*", and five species distributed in Mauritania (*P. adrarensis*), Abd al Kuri Island (*P. abdelkuri*), Oman (*P. gallagheri* Arnold, 1986a), Saudia Arabia (*P. popovi* Arnold, 1982), and Yemen and eastern Africa (*P. flavipunctatus*). *Pristurus popovi* and *P. flavipunctatus* form a well supported clade, but other relationships within this group are weakly supported, like the phylogenetic position of the eastern and western clades of "*P. rupestris*", or the sister taxa relationship between the endemic species from Abd al-Kuri (*P. abdelkuri*; Socotra Archipelago) and *P. gallagheri*.

Multivariate analyses of the morphological data

For the comparison of body shape between the eastern and western clades of "*P. rupestris"*, we retained the six first PCA components explaining 83.11% of variance (Table 2). The results of the PERMANOVA revealed significant shape differences between the two clades ($F_{1,83}$ = 11.268, P < 0.0001). When each component was analyzed independently by means of ANOVAs, only the first and the second components produced significant differences between groups ($F_{1,83}$= 15.41, P < 0.0001 and $F_{1,83}$= 27.26, P < 0.0001, respectively) (Table 3; Fig. 4). These two components mostly correspond to limb and head dimensions (Table 2; Fig. 4) and accounted for approximately 50% of the total variance. Specimens from the western clade appear to have relatively wider and higher heads and shorter limbs. Regarding body size, the ANOVA revealed that there are significant size differences between the two clades ($F_{1,83}$= 8.76, P < 0.0001) (Fig. 4). Descriptive statistics for the 12 size and shape variables included in the morphological analysis are presented in Table 4.

TABLE 2. Loadings, eigenvalues, and variance explained by each of the first six components retained from the PCA performed on shape residuals.

Trait	PC1	PC2	PC3	PC4	PC5	PC6
HL	-0.26	-0.37	0.30	0.29	0.04	0.30
HD	-0.01	-0.49	0.15	-0.18	-0.52	0.44
HW	0.28	-0.45	-0.06	-0.26	-0.09	-0.18
OD	-0.06	-0.32	0.53	0.38	0.13	-0.58
BL	-0.34	-0.16	-0.36	-0.23	-0.24	-0.43
AL	-0.42	-0.06	-0.19	0.05	0.39	0.20
ThL	-0.43	-0.09	-0.09	-0.19	-0.12	-0.25
CL	-0.41	-0.21	-0.07	-0.21	0.35	0.12
AGL	-0.05	-0.12	-0.52	0.73	-0.23	0.07
SW	0.31	-0.34	-0.17	-0.06	0.55	0.11
PW	0.31	-0.32	-0.36	0.03	0.10	-0.18
Eigenvalue	3.34	2.16	1.37	0.93	0.79	0.56
Variance explained (in %)	30.32	19.63	12.43	8.47	7.15	5.10

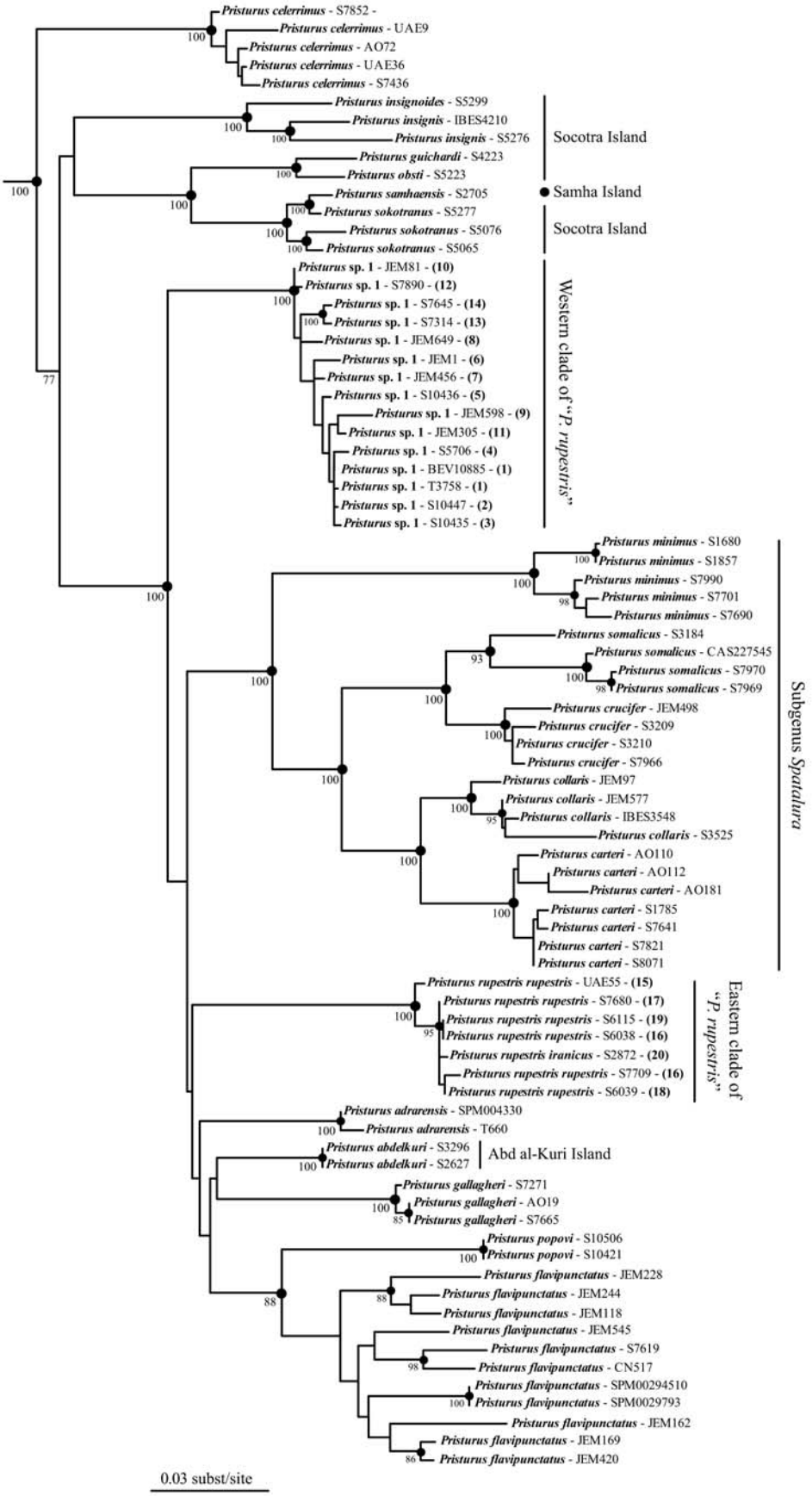

FIGURE 2. ML tree of *Pristurus* inferred using *12S* mtDNA, and *acm4, cmos, rag1, rag2* nuclear gene fragments. Bootstrap values ≥70% are shown next to the nodes. Black circles on nodes indicate posterior probability values ≥0.95. The tree was rooted using *Gekko gecko*, *Phelsuma modesta*, and *Tarentola mauritanica*. Information on the samples is given in Appendix I.

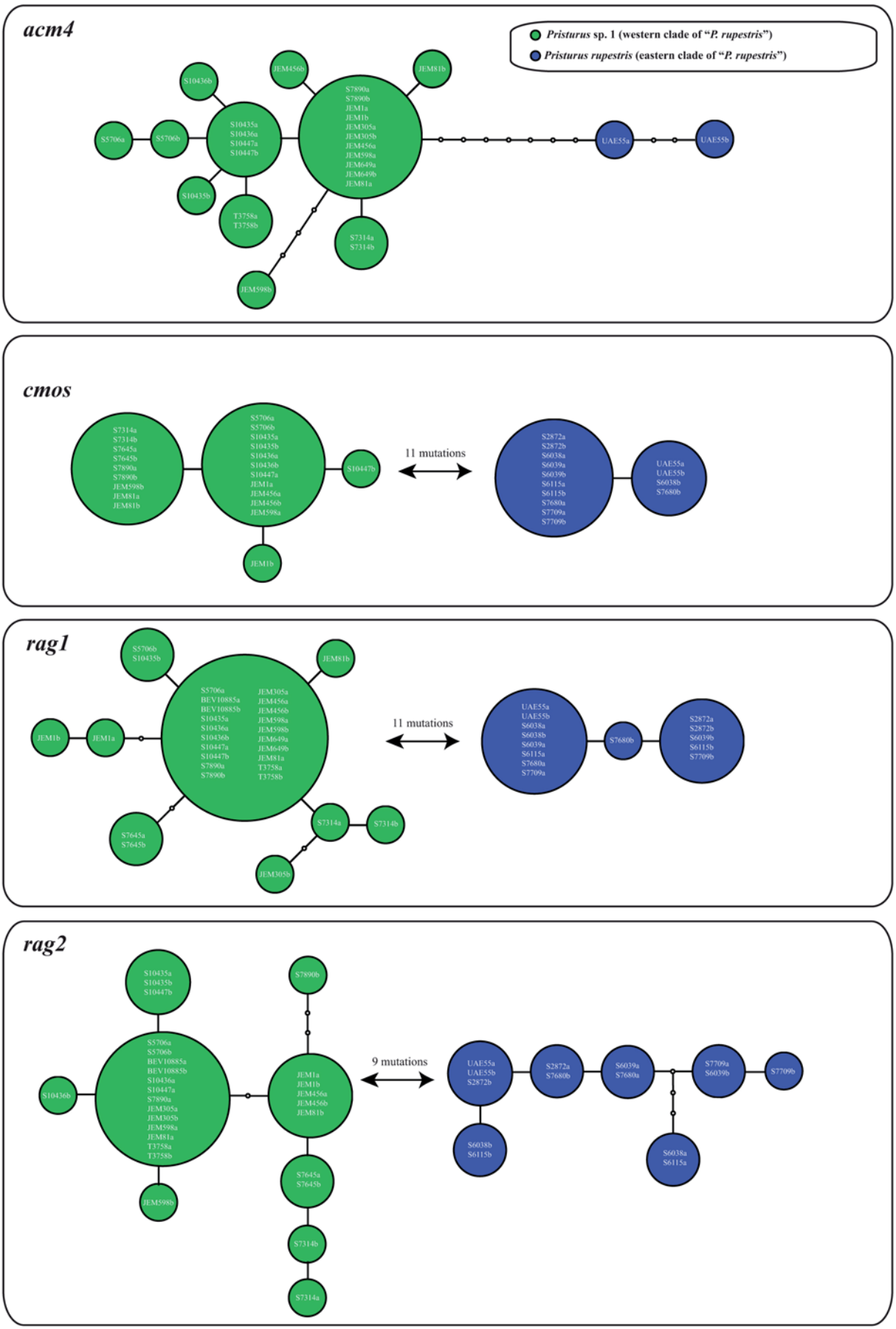

FIGURE 3. Haplotype networks of the phased sequences of nuclear markers *acm4, cmos, rag1* and *rag2*. Phase probabilities were set as ≥0.7. Information on the samples is shown in Appendix I.

TABLE 3. Results of the ANOVAs performed on each of the six components retained from the PCA analysis. F values along with their associated *P*-values are given.

	PC1	PC2	PC3	PC4	PC5	PC6
F1,84	15.41	27.26	0.109	2.933	0.01	0.429
P-value	0.000178 ***	1.29e-06 ***	0.742	0.0905	0.922	0.515

TABLE 4. Descriptive statistics for the 12 continuous variables for *P. rupestris* and *P.* sp. 1. Mean ± Standard Error of the Mean (SE) and range are given.

	Pristurus sp. 1 (n=53)	*P. rupestris* (n=32)
Variable	Mean±SE (Min–Max)	Mean±SE (Min–Max)
SVL	24.40 ± 0.33 (19.29–28.82)	25.87 ± 0.34 (22.05–30.06)
Head length (HL)	6.04 ± 0.07 (5.12–7.25)	6.28 ± 0.06 (5.34–6.84)
Head depth (HD)	2.57 ± 0.03 (2.07–3.17)	2.48 ± 0.04 (2.08–2.96)
Head width (HW)	5.28 ± 0.06 (4.23–6.50)	5.00 ± 0.07 (4.27–5.73)
Orbital diameter (OD)	1.54 ± 0.02 (1.30–1.84)	1.53 ± 0.02 (1.26–1.78)
Brachium length (BL)	2.71 ± 0.07 (1.86–3.93)	2.95 ± 0.06 (2.34–3.58)
Antebrachium length (AL)	3.50 ± 0.07 (1.81–4.57)	3.90 ± 0.07 (3.14–4.80)
Thigh length (ThL)	4.30 ± 0.10 (2.79–5.30)	4.88 ± 0.10 (3.89–6.21)
Crus length (CL)	4.96 ± 0.09 (3.68–6.06)	5.24 ± 0.08 (4.20–5.99)
Axilla-groin length (AGL)	9.69 ± 0.18 (7.38–12.32)	10.52 ± 0.22 (8.68–13.67)
Scapular width (SW)	4.89 ± 0.09 (3.46–6.55)	4.73 ± 0.10 (3.71–5.90)
Pelvic width (PW)	4.16 ± 0.08 (3.03–5.39)	4.00 ± 0.08 (3.32–5.19)

Taxonomic account

Based on the *12S* genetic distances and the results of the phylogenetic and haplotype network analyses (Figs. 2 and 3; Appendix III), it is certain that the taxon "*Pristurus rupestris*" contains two genetically well differentiated species: namely the eastern clade of "*P. rupestris*", inhabiting coastal Iran and the al Hajar Mountain range in northern Oman and eastern UAE; and the western clade of "*P. rupestris*", distributed from coastal middle Oman, through Yemen, Saudi Arabia and up to southern Jordan (Fig. 1). Specimens IBES7680, IBES6038, IBES7709 and IBES6039, are all from the type locality of *P. r. rupestris* (Muscat) and genetic sample S2872 is a *P. r. iranicus* from Iran. Therefore, the molecular results confirm that the name *Pristurus rupestris* applies to the eastern clade (Fig. 1). As for the western clade, two possible names are available *Pristurus guweirensis* Haas, 1943 (=*P. r. guweirensis* elevated to species) and *Pristurus migiurtinicus* Scortecci, 1933 (synonymized with *P. rupestris* by Loveridge 1947). Specimen BEV10885 and sample T3758 originate from the type locality of *P. r. guweirensis*, in the sandstone desert of Guweira (=Quweira), 10 km south of the city of Quweira, Jordan. Unfortunately, no samples for genetic or morphological analysis of "*P. rupestris*" are available from or near the type locality of *Pristurus migiurtinicus* ("Bender Cassim" (=Bosaso), Somalia). Moreover, the holotype of *Pristurus migiurtinicus* Scortecci, 1933 housed at the Museo Civico di Storia Naturale di Milano under reference MSNM Re97 (formerly 1323), was sent on loan to the Natural History Museum, London back in 1980 and the type is apparently lost. As a result of the impossibility of studying morphologically and genetically any specimens of *Pristurus migiurtinicus,* it is not possible at present to know which of the two names, *P. guweirensis* (=*P. r. guweirensis* elevated to species) or *P. migiurtinicus*, applies to the western clade of "*P. rupestris*". Therefore, until material of "*P. rupestris*" from Somalia is obtained and analyzed we prefer to refer to the western clade as *Pristurus* sp. 1.

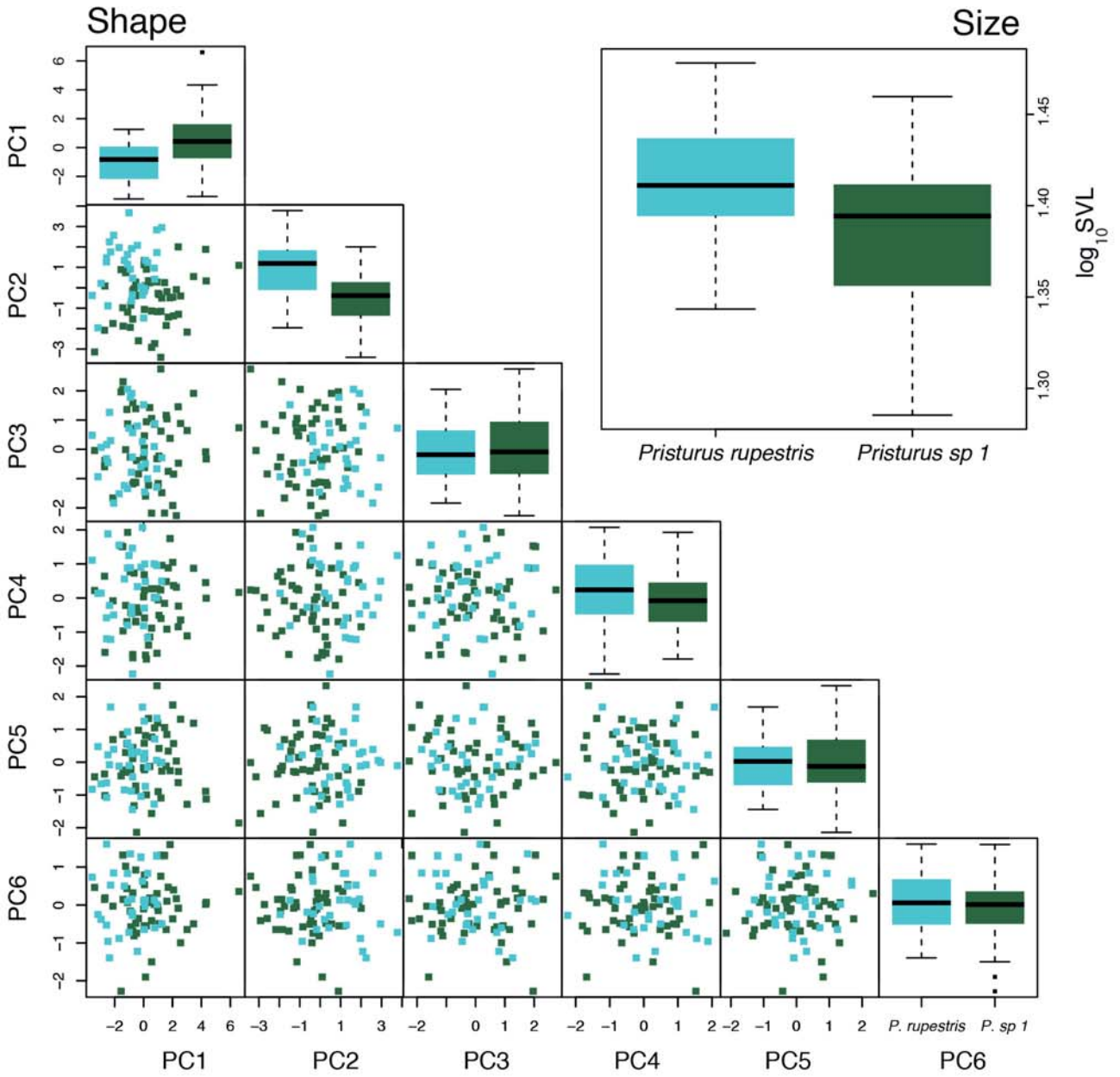

FIGURE 4. Charts presenting the size and shape variation existing between *Pristurus rupestris* (eastern clade of "*P. rupestris*") in blue and *Pristurus* sp. 1 (western clade of "*P. rupestris*") in green. The upper-right chart shows size variation by means of a boxplot on the log-transformed SVL values. The lower-left chart shows the shape variation reflected in the six components retained from the PCA analysis (accounting for 83.11% of the total variance). All bivariate combinations are presented along with a boxplot comparing the variation of both species on each component.

Discussion

The phylogenetic analyses presented in Fig. 2 clearly show that what was previously regarded as *Pristurus rupestris* includes two different species: *P. rupestris* and *Pristurus* sp. 1. Within *P. rupestris* there are two subspecies described, *P. r. rupestris* (type locality Muscat, Oman) and *P. r. iranicus* (type locality Khark Island, Iran). Our results show that these two subspecies are not differentiated genetically, and that *P. r. iranicus* makes *P. r. rupestris* paraphyletic. This suggests that the distribution of *P. rupestris* in Iran is probably the result of a recent colonization from Arabia. However, until material from the type locality of *P. r. iranicus* is analyzed, we prefer not to speculate about the origin and taxonomic validity of the Iranian subspecies. As shown in Fig. 1, we could not include specimens from Qatar, Bahrain and other islands in the Gulf, as well as from coastal eastern Saudi Arabia.

FIGURE 5. Pictures of live *Pristurus* sp. 1. A–B, specimen from 25 km south of Quweira, Jordan (photo by R. Sindaco); C, typical specimen from moist habitats in Dhofar performing characteristic tail signaling (Wadi Dharbat, Dhofar, Oman) (photo by S. Carranza).

However, based on the geographic distribution of *P. rupestris* and *Pristurus* sp. 1, we can speculate that the populations distributed across the eastern coast of the Arabian Peninsula belong to *P. r. rupestris* (work in progress).

Pristurus sp. 1 (Fig. 5) is one of the most widely distributed species in the genus, occurring from southern Jordan to central Oman (Fig. 1). Although we could not incorporate samples from Africa in our analyses, probably the populations from Eritrea, Djibouti, northern Somalia and adjoining Ethiopia, previously assigned to "*P. rupestris*", belong to *Pristurus* sp. 1, or to an undescribed taxon. Despite its large distribution range, the level of uncorrected genetic variability in the mitochondrial *12S* within *Pristurus* sp. 1 is very low (2.21 ± 0.4%), especially if compared with the level of intraspecific variability in other *Pristurus* species, like for instance *P. minimus* Arnold 1977 (4.61 ± 0.7%), *P. flavipunctatus* (7.63 ± 0.8%), *P. somalicus* Parker, 1932 (5.78 ± 1.0%) and *P. collaris* (Steindachner, 1867) (3.64 ± 0.6%). In fact, *Pristurus* sp. 1 has a similar level of intraspecific genetic variability to *P. carteri* (Gray, 1863) (2.61 ± 0.5%), *P. celerrimus* (2.58 ± 0.5%), and *P. crucifer* (1.78 ± 0.4%), all of which have much more restricted distribution ranges. The relatively low level of genetic variability within *Pristurus* sp. 1 contrasts with its rather high level of morphological variability. Over its range this diminutive gecko occupies many different habitats, from very arid open areas to moist subtropical forest, and from sea level up to 3000 m (pers. obs.). As a result of that, it exhibits marked variation, especially in coloring, size and behavior, and even neighboring populations may show some differences (Arnold 1980, 1993; pers. observ.). According to Arnold (1980) and our personal observations, in Dhofar, animals from moist habitats such as Wadi Sayq and Wadi Dharbat are relatively large, dark, with the underside of the tail opaque white and with very heavy markings on the throat. In contrast, drier habitats tend to be occupied by smaller, paler animals on which dark markings are reduced, although even here animals from dark backgrounds tend to be darker (Fig. 5). As with *P. carteri,* physiological color change is not the main cause of this variation.

A high level of morphological variability also occurs in *P. rupestris* and this seems linked to the diversity of habitats and altitudes occupied by this species, from very arid open areas to forested areas, and from sea level up to 2330 m. In fact, some of the morphs identified in *Pristurus* sp. 1 can be also identified in *P. rupestris*, which suggests a high level of phenotypic convergence between these two species (Arnold 1993; pers. obs.). Although no data are available, it is probable that both *P. rupestris* and *Pristurus* sp. 1, like other small lizards that are active by day in open places, are subject to heavy predation by visually oriented hunters, especially birds. Therefore, there must be a strong selective pressure for crypsis and camouflage, which explains the appearance of similar (convergent) morphs related to habitat in both species. This reason, together with the overall morphological similarity between *P. rupestris* and *Pristurus* sp. 1, may explain why, despite being the most abundant reptile species in Arabia, and having been collected intensively and included in some systematic revisions (Arnold 1977, 1980, 1982, 1986a,b; Leviton *et al*. 1992), their taxonomy has never been clarified. It is in cases like this, with high levels of morphological variability and convergence in very similar species, that molecular methods are more helpful (Gvoždík *et al*. 2008). Although our preliminary morphological analyses including several variables on a restricted sampling seem to suggest that the two species may be differentiated, more analyses including more variables (especially pholidotic) and many more samples will be needed in order to see the extent of morphological variability within and between both species and to try to find some clear diagnostic characters.

The phylogenetic tree presented in Fig. 2 coincides partially with Arnold's (2009) morphological tree and the molecular phylogeny by Papenfuss *et al*. (2009). All three phylogenies suggest that *P. celerrimus* is sister to the remaining *Pristurus* species. Our dataset is the first to include all seven endemic species of *Pristurus* from the Socotra Archipelago and indicates that this small radiation has a minimum of two independent origins, one for all six species from Socotra, and the satellite islands of Samha and Darsa, and another independent origin for *P. abdelkuri* from Abd al-Kuri Island. However, until a calibrated phylogeny for the genus *Pristurus* is produced (work in progress) it will not be possible to know if the deep split that separates the large (*P. insignis* and *P. insignoides*) and small (*P. sokotranus, P. obsti, P. guichardi*, and *P. samhaensis*) species endemic to the Socotra Archipelago occurred before or after the separation of Socotra from mainland Arabia. An independent origin for Abd al-Kuri reptile species has already been proposed for an endemic skink of the genus *Trachylepis* (Sindaco *et al*. 2012) and for the ancestor of the two endemic *Hemidactylus* geckos (Gómez-Díaz *et al*. 2012, Carranza & Arnold 2012, Šmíd *et al*. 2013a). These molecular studies indicate that despite being just 60 km to the west of Samha Island, at least four of the five endemic lizard species from Abd al-Kuri have had independent origins from

species of the same genus now present in Samha, Darsa and Socotra Islands (which were merged into a single island during the sea level fluctuations that ocurred during the Pleistocene). This is very unusual and contrasts with what has been found in other archipelagoes. For instance, in the Canary and Cape Verde Islands, species usually colonized one of the islands of the archipelago and, from there, spread to neighboring islands (Arnold *et al*. 2008; Carranza & Arnold 2006; Carranza *et al*. 1999, 2000, 2001; Maca-Meyer *et al*. 2003; Miralles *et al*. 2011; Vasconcelos *et al*. 2010). According to Arnold (2009), *P. obsti* and *P. samhaensis* are very similar in their morphology to *P. guichardi* and *P. sokotranus*, respectively. Our molecular data indicates that the two arboreal Socotran endemics, *P. obsti* and *P. guichardi*, are genetically well differentiated. Razzetti *et al*. (2011), state that these two species segregate altitudinally within Socotra Island, with *P. obsti* being distributed at lower altitudes than *P. guichardi*. The taxonomy of *P. sokotranus* is more complicated. According to our molecular results, including three specimens from three different localities within Socotra Island, *P. samhaensis* is more closely related to one of the specimens, making *P. sokotranus* paraphyletic. Analyses with more samples from across the distribution range of *P. sokotranus* and *P. samhaensis,* including their type localities will be necessary to clarify the taxonomy of these two species (work in progress).

Relationships between members of the subgenus *Spatalura* are very similar to those recovered by Papenfuss *et al*. (2009). In both phylogenies, *P. minimus* is sister to all the other members of *Spatalura* and *P. somalicus* and *P. crucifer* are sister taxa. Moreover, like the morphological phylogeny by Arnold (2009), our results support the close relationship between *P. carteri* and *P. collaris*. The nested position of *P. adrarensis* within the clade formed by the 12 species (Fig. 2), suggests that this 4700 km range extension occurred from east to west, after the first colonization of the Socotra Archipelago. However, until more samples are included in a calibrated phylogeny of the genus, we prefer not to hypothesize about the possible causes of the presence of this isolated species in Mauritania, or the biogeography and evolution of this interesting genus of diurnal geckos.

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Species	Sample code	Voucher code	Country	Loc. code	Latitude	Longitude	*12S*	*acm4*	*cmos*	*rag1*	*rag2*
P. abdelkuri	S2627	MCCI-R1592-2	Yemen (Soc.)	-	12.18548	52.23756	KJ849839	KJ849917	KJ849973	KJ850033	KJ850108
P. abdelkuri	S3296	MCCI-R1592-1	Yemen (Soc.)	-	12.69713	53.48669	KJ849840	KJ849918	KJ849974	KJ850034	KJ850109
P. adrarensis	SPM004330	BEV5916	Mauritania	-		-13.27	KJ849841	-	-	-	-
P. adrarensis	T660	-	Mauritania	-	20.58	-13.27	KJ849842	KJ849919	KJ849975	KJ850035	KJ850110
P. carteri	AO110	-	Oman	-	20.58	54.06917	KJ849843	KJ849920	KJ849976	KJ850036	KJ850111
P. carteri	AO112	-	Oman	-	17.66028	53.90667	KJ849844	KJ849921	KJ849977	KJ850037	KJ850112
P. carteri	AO181	-	Oman	-	17.24222	54.65806	KJ849845	KJ849922	KJ849978	KJ850038	KJ850113
P. carteri	S1785	-	Oman	-	17.02583	58.81975	KJ849846	KJ849923	-	KJ850039	KJ850114
P. carteri	S7641	IBES7641	Oman	-	20.55547	59.6082	KJ849847	KJ849924	KJ849979	KJ850040	KJ850115
P. carteri	S7821	-	Oman	-	21.95181	57.52845	KJ849848	KJ849925	KJ849980	KJ850041	KJ850116
P. carteri	S8071	IBES8071	Oman	-	19.03103	57.52845	KJ849849	KJ849926	KJ849981	KJ850042	KJ850117
P. celerrimus	AO72	-	Oman	-	19.03103	57.43194	KJ849850	KJ849927	-	KJ850043	KJ850118
P. celerrimus	S7436	IBES7436	Oman	-	23.17333	56.36005	KJ849851	KJ849928	-	KJ850044	KJ850119
P. celerrimus	S7852	IBES7852	Oman	-	23.54458	57.60424	KJ849852	KJ849929	KJ849982	KJ850045	KJ850120
P. celerrimus	UAE36	-	UAE	-	23.07126	56.29833	KJ849853	KJ849930	KJ849983	KJ850046	KJ850121
P. celerrimus	UAE9	-	Oman	-	25.45417	56.23472	KJ849854	KJ849931	KJ849984	KJ850047	KJ850122
P. collaris	JEM577	JEM577	Yemen	-	25.96528	49.33	KJ849857	KJ849933	-	KJ850048	-
P. collaris	JEM97	JEM97	Yemen	-	14.63	49.03	KJ849858	KJ849934	-	KJ850049	KJ850124
P. collaris	S3525	-	Yemen	-	14.9	49.248253	KJ849855	-	-	-	-
P. collaris	S3548	SMB11549	Yemen	-	14.592317	49.248253	KJ849856	KJ849932	KJ849985	-	KJ850123
P. crucifer	JEM498	JEM498	Yemen	-	14.592317	43.25	KJ849862	KJ849938	-	KJ850053	-
P. crucifer	S3209	-	Somalia	-	13.43	44.2239	KJ849859	KJ849935	KJ849986	KJ850050	KJ850125
P. crucifer	S3210	-	Somalia	-	9.6366	44.2239	KJ849860	KJ849936	KJ849987	KJ850051	KJ850126
P. crucifer	S7966	TMHC2013.12.450	Ethiopia	-	9.6366	41.83886	KJ849861	KJ849937	KJ849988	KJ850052	KJ850127
P. flavipunctatus	CN517	IBECN517	Eritrea	-	9.57745	38.95722	KJ849863	-	KJ849989	KJ850054	KJ850128
P. flavipunctatus	JEM118	JEM118	Yemen	-	15.6103	49.08	KJ849865	-	KJ849991	KJ850056	KJ850130

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P. carteri	AO112	-	Oman	-	17.66028	53.90667	KJ849844	KJ849921	KJ849977	KJ850037	KJ850112
P. carteri	AO181	-	Oman	-	17.24222	54.65806	KJ849845	KJ849922	KJ849978	KJ850038	KJ850113
P. carteri	S1785	-	Oman	-	17.02583	58.81975	KJ849846	KJ849923	-	KJ850039	KJ850114
P. carteri	S7641	IBES7641	Oman	-	20.55547	59.6082	KJ849847	KJ849924	KJ849979	KJ850040	KJ850115
P. carteri	S7821	-	Oman	-	21.95181	57.52845	KJ849848	KJ849925	KJ849980	KJ850041	KJ850116
P. carteri	S8071	IBES8071	Oman	-	19.03103	57.52845	KJ849849	KJ849926	KJ849981	KJ850042	KJ850117
P. celerrimus	AO72	-	Oman	-	19.03103	57.43194	KJ849850	KJ849927	-	KJ850043	KJ850118
P. celerrimus	S7436	IBES7436	Oman	-	23.17333	56.36005	KJ849851	KJ849928	-	KJ850044	KJ850119
P. celerrimus	S7852	IBES7852	Oman	-	23.54458	57.60424	KJ849852	KJ849929	KJ849982	KJ850045	KJ850120
P. celerrimus	UAE36	-	UAE	-	23.07126	56.29833	KJ849853	KJ849930	KJ849983	KJ850046	KJ850121
P. celerrimus	UAE9	-	Oman	-	25.45417	56.23472	KJ849854	KJ849931	KJ849984	KJ850047	KJ850122
P. collaris	JEM577	JEM577	Yemen	-	25.96528	49.33	KJ849857	KJ849933	-	KJ850048	-
P. collaris	JEM97	JEM97	Yemen	-	14.63	49.03	KJ849858	KJ849934	-	KJ850049	KJ850124
P. collaris	S3525	-	Yemen	-	14.9	49.248253	KJ849855	-	-	-	-
P. collaris	S3548	SMB11549	Yemen	-	14.592317	49.248253	KJ849856	KJ849932	KJ849985	-	KJ850123
P. crucifer	JEM498	JEM498	Yemen	-	14.592317	43.25	KJ849862	KJ849938	-	KJ850053	-
P. crucifer	S3209	-	Somalia	-	13.43	44.2239	KJ849859	KJ849935	KJ849986	KJ850050	KJ850125
P. crucifer	S3210	-	Somalia	-	9.6366	44.2239	KJ849860	KJ849936	KJ849987	KJ850051	KJ850126
P. crucifer	S7966	TMHC2013.12.450	Ethiopia	-	9.6366	41.83886	KJ849861	KJ849937	KJ849988	KJ850052	KJ850127
P. flavipunctatus	CN517	IBECN517	Eritrea	-	9.57745	38.95722	KJ849863	-	KJ849989	KJ850054	KJ850128
P. flavipunctatus	JEM118	JEM118	Yemen	-	15.6103	49.08	KJ849865	-	KJ849991	KJ850056	KJ850130

APPENDIX 1. (Continued)

Species	Sample code	Voucher code	Country	Loc. code	Latitude	Longitude	*12S*	*acm4*	*cmos*	*rag1*	*rag2*
P. flavipunctatus	JEM162	JEM162	Yemen	-	14.65	44.2	KJ849866	-	-	KJ850057	-
P. flavipunctatus	JEM169	JEM169	Yemen	-	14.9	43.47	KJ849867	-	-	KJ850058	KJ850131
P. flavipunctatus	JEM228	JEM228	Yemen	-	13. 52	43.95	KJ849868	-	KJ849992	KJ850059	KJ850132
P. flavipunctatus	JEM244	JEM244	Yemen	-	13.87	45.8	KJ849869	-	KJ849993	KJ850060	KJ850133
P. flavipunctatus	JEM420	JEM420	Yemen	-	13.02	44.55	KJ849870	-	-	KJ850061	KJ850134
P. flavipunctatus	JEM545	JEM545	Yemen	-	15.72	43.62	KJ849871	-	KJ849994	KJ850062	KJ850135
P. flavipunctatus	S7619	TMHC2013.12.449	Somalia	-	10.03335	45.15981	KJ849864	-	KJ849990	KJ850055	KJ850129
P. flavipunctatus	SPM00294510	-	Egypt	-	22.18	36.35	KJ849872	-	-	KJ850063	KJ850136
P. flavipunctatus	SPM0029793	-	Egypt	-	22.18	36.35	KJ849873	KJ849939	KJ849995	-	KJ850137
P. gallagheri	AO19	-	Oman	-	22.78667	57.59389	KJ849874	KJ849940	-	KJ850064	-
P. gallagheri	S7271	IBES7271	Oman	-	23.18292	57.41627	KJ849875	-	KJ849996	KJ850065	KJ850138
P. gallagheri	S7665	IBES7665	Oman	-	22.92395	57.68267	KJ849876	-	-	KJ850066	KJ850139
P. guichardi	S4223	-	Yemen (Soc.)	-	12.5636	54.0418	KJ849877	KJ849941	KJ849997	KJ850067	KJ850140
P. insignis	S4210	-	Yemen (Soc.)	-	12.5353	54.3752	KJ849878	KJ849942	KJ849998	KJ850068	KJ850141
P. insignis	S5276	-	Yemen (Soc.)	-	12.5132	53.4223	KJ849879	KJ849943	KJ849999	KJ850069	KJ850142
P. insignoides	S5299	-	Yemen (Soc.)	-	12.5727	54.0464	KJ849880	KJ849944	KJ850000	KJ850070	KJ850143
P. minimus	S1680	IBES1680	Oman	-	20.55547	58.81975	KJ849881	KJ849945	KJ850001	KJ850071	KJ850144
P. minimus	S1857	-	Oman	-	20.41939	58.81237	KJ849882	KJ849946	KJ850002	KJ850072	KJ850145
P. minimus	S7690	IBES7690	Oman	-	21.95181	59.6082	KJ849883	KJ849947	KJ850003	KJ850073	KJ850146
P. minimus	S7701	IBES7701	Oman	-	20.59722	58.26669	KJ849884	KJ849948	KJ850004	KJ850074	KJ850147
P. minimus	S7990	IBES7990	Oman	-	19.03103	57.52845	KJ849885	KJ849949	KJ850005	KJ850075	KJ850148
P. obsti	S5223	-	Yemen (Soc.)	-	12.5417	53.3734	KJ849886	KJ849950	KJ850006	KJ850076	KJ850149
P. popovi	S10421	IBES10421	Saudi Arabia	-	18.20716	42.41003	KJ849887	-	-	KJ850077	KJ850150
P. popovi	S10506	IBES10506	Saudi Arabia	-	18.26042	42.37877	KJ849888	-	-	KJ850078	KJ850151
P. rupestris iranicus	S2872	-	Iran	20	27.306694	52.710806	KJ849903	-	KJ850018	KJ850094	KJ850166
P. rupestris rupestris	S6038	IBES6038	Oman	16	23.589399	58.166075	KJ849904	-	KJ850019	KJ850095	KJ850167
P. rupestris rupestris	S6115	IBES6115	Oman	19	23.74533	57.73245	KJ849906	-	KJ850021	KJ850097	KJ850169
P. rupestris rupestris	S7680	IBES7680	Oman	17	23.59032	58.4078	KJ849907	-	KJ850022	KJ850098	KJ850170
P. rupestris rupestris	UAE55	IBEUAE55	Oman	15	22.91639	58.87694	KJ849909	KJ849964	KJ850024	KJ850100	KJ850172

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APPENDIX 1. (Continued)

Species	Sample code	Voucher code	Country	Loc. code	Latitude	Longitude	*12S*	*acm4*	*cmos*	*rag1*	*rag2*
P. rupestris rupestris	S6039	IBES6039	Oman	18	23.589399	58.166075	KJ849905	-	KJ850020	KJ850096	KJ850168
P. rupestris rupestris	S7709	IBES7709	Oman	16	23.61589	58.58542	KJ849908	-	KJ850023	KJ850099	KJ850171
P. samhaensis	S2705	MCCI-R1596	Yemen (Soc.)	-	12.1731	53.0122	KJ849910	KJ849965	KJ850025	KJ850101	KJ850173
P. sokotranus	S5065	-	Yemen (Soc.)	-	12.6091	54.1559	KJ849911	KJ849966	KJ850026	KJ850102	KJ850174
P. sokotranus	S5076	-	Yemen (Soc.)	-	12.56356	54.04176	KJ849912	KJ849967	KJ850027	KJ850103	KJ850175
P. sokotranus	S5277	-	Yemen (Soc.)	-	12.491	53.5623	KJ849913	KJ849968	KJ850028	KJ850104	KJ850176
P. somalicus	CAS227545	CAS227545	Somalia	-	11.19416	49.2195	-	KJ849969	KJ850029	KJ850105	KJ850177
P. somalicus	S3184	-	Somalia	-	8.966968	46.145403	KJ849914	KJ849970	KJ850030	-	KJ850178
P. somalicus	S7969	TMHC2013.12.452	Somalia	-	10.03778	44.78831	KJ849915	KJ849971	KJ850031	KJ850106	KJ850179
P. somalicus	S7970	TMHC2013.12.453	Somalia	-	9.73055	44.419383	KJ849916	KJ849972	KJ850032	KJ850107	KJ850180
P. sp. 1	BEV10885	BEV10885	Jordan	1	29.69038	35.35802	-	-	-	KJ850079	KJ850152
P. sp. 1	JEM1	JEM1	Yemen	6	15.28	44.2	KJ849896	KJ849957	KJ850014	KJ850087	KJ850160
P. sp. 1	JEM305	JEM305	Yemen	11	16.63	53.01	KJ849897	KJ849958	-	KJ850088	KJ850161
P. sp. 1	JEM456	JEM456	Yemen	7	13.52	44.05	KJ849898	KJ849959	KJ850015	KJ850089	KJ850162
P. sp. 1	JEM598	JEM598	Yemen	9	14.77	49.6	KJ849899	KJ849960	KJ850016	KJ850090	KJ850163
P. sp. 1	JEM649	JEM649	Yemen	8	14.01	48.32	KJ849900	KJ849961	-	KJ850091	-
P. sp. 1	JEM81	-	Yemen	10	15.15	51.01	KJ849901	KJ849962	KJ850017	KJ850092	KJ850164
P. sp. 1	S10435	IBES10435	Saudi Arabia	3	18.57702	42.35605	KJ849889	KJ849951	KJ850007	KJ850080	KJ850153
P. sp. 1	S10436	IBES10436	Saudi Arabia	5	17.53827	43.6298	KJ849890	KJ849952	KJ850008	KJ850081	KJ850154
P. sp. 1	S10447	IBES10447	Saudi Arabia	2	21.22914	40.69745	KJ849891	KJ849953	KJ850009	KJ850082	KJ850155
P. sp. 1	S5706	IBES5706	Saudi Arabia	4	18.1873	42.5351	KJ849892	KJ849954	KJ850010	KJ850083	KJ850156
P. sp. 1	S7314	IBES7314	Oman	13	17.08981	54.4428	KJ849893	KJ849955	KJ850011	KJ850084	KJ850157
P. sp. 1	S7645	IBES7645	Oman	14	18.05712	56.33459	KJ849894	-	KJ850012	KJ850085	KJ850158
P. sp. 1	S7890	-	Oman	12	17.2386	53.89913	KJ849895	KJ849956	KJ850013	KJ850086	KJ850159
P. sp. 1	T3758	-	Jordan	1	29.69038	35.35802	KJ849902	KJ849963	-	KJ850093	KJ850165

APPENDIX II. Information on the *Pristurus rupestris* (eastern clade of "*P. rupestris*") and *P.* sp. 1 (western clade of "*P. rupestris*") included in the morphological analyses.

Species	Specimen Code	Country	Latitude	Longitude
P. sp. 1	IBEAO149	Oman	17.174440	54.094720
P. sp. 1	IBES10411	Saudi Arabia	21.225770	40.697860
P. sp. 1	IBES10431	Saudi Arabia	21.229140	40.697450
P. sp. 1	IBES10447	Saudi Arabia	21.229140	40.697450
P. sp. 1	IBES10455	Saudi Arabia	21.225770	40.697860
P. sp. 1	IBES10474	Saudi Arabia	21.227320	40.694220
P. sp. 1	IBES10487	Saudi Arabia	21.114850	40.599400
P. sp. 1	IBES7645	Oman	18.057120	56.334590
P. sp. 1	IBES7683	Oman	18.132160	56.551330
P. sp. 1	IBES7692	Oman	17.121420	54.714040
P. sp. 1	IBES7952	Oman	17.041300	54.326050
P. sp. 1	JEM1	Yemen	15.28	44.2
P. sp. 1	JEM171	Yemen	14.65	44.20
P. sp. 1	JEM172	Yemen	14.65	44.20
P. sp. 1	JEM193	Yemen	13.3	44.06
P. sp. 1	JEM2	Yemen	15.28	44.2
P. sp. 1	JEM20	Yemen	15.37	44.47
P. sp. 1	JEM217	Yemen	13.32	44.12
P. sp. 1	JEM243	Yemen	13.87	45.80
P. sp. 1	JEM245	Yemen	13.87	45.80
P. sp. 1	JEM249	Yemen	13.87	45.80
P. sp. 1	JEM250	Yemen	13.87	45.80
P. sp. 1	JEM3	Yemen	15.28	44.2
P. sp. 1	JEM300	Yemen	16.63	53.01
P. sp. 1	JEM305	Yemen	16.63	53.01
P. sp. 1	JEM306	Yemen	16.63	53.01
P. sp. 1	JEM458	Yemen	13.52	44.05
P. sp. 1	JEM546	Yemen	15.72	43.62
P. sp. 1	JEM547	Yemen	15.72	43.62
P. sp. 1	JEM598	Yemen	14.77	49.60
P. sp. 1	JEM6	Yemen	15.37	44.47
P. sp. 1	JEM649	Yemen	14.01	48.32
P. sp. 1	JEM7	Yemen	15.37	44.47
P. sp. 1	MCCI-R1417-1	Yemen	15.4104	44.1147
P. sp. 1	MCCI-R1417-2	Yemen	15.4104	44.1147
P. sp. 1	MCCI-R1417-3	Yemen	15.4104	44.1147
P. sp. 1	MCCI-R405	Jordan	29.5765	35.4197
P. sp. 1	MCCI-R595	Jordan	29.5765	35.4197
P. sp. 1	MCCI-R596	Jordan	29.5765	35.4197
P. sp. 1	MCCI-R896-2	Yemen	15.02	43.45
P. sp. 1	MCCI-R896-3	Yemen	15.02	43.45

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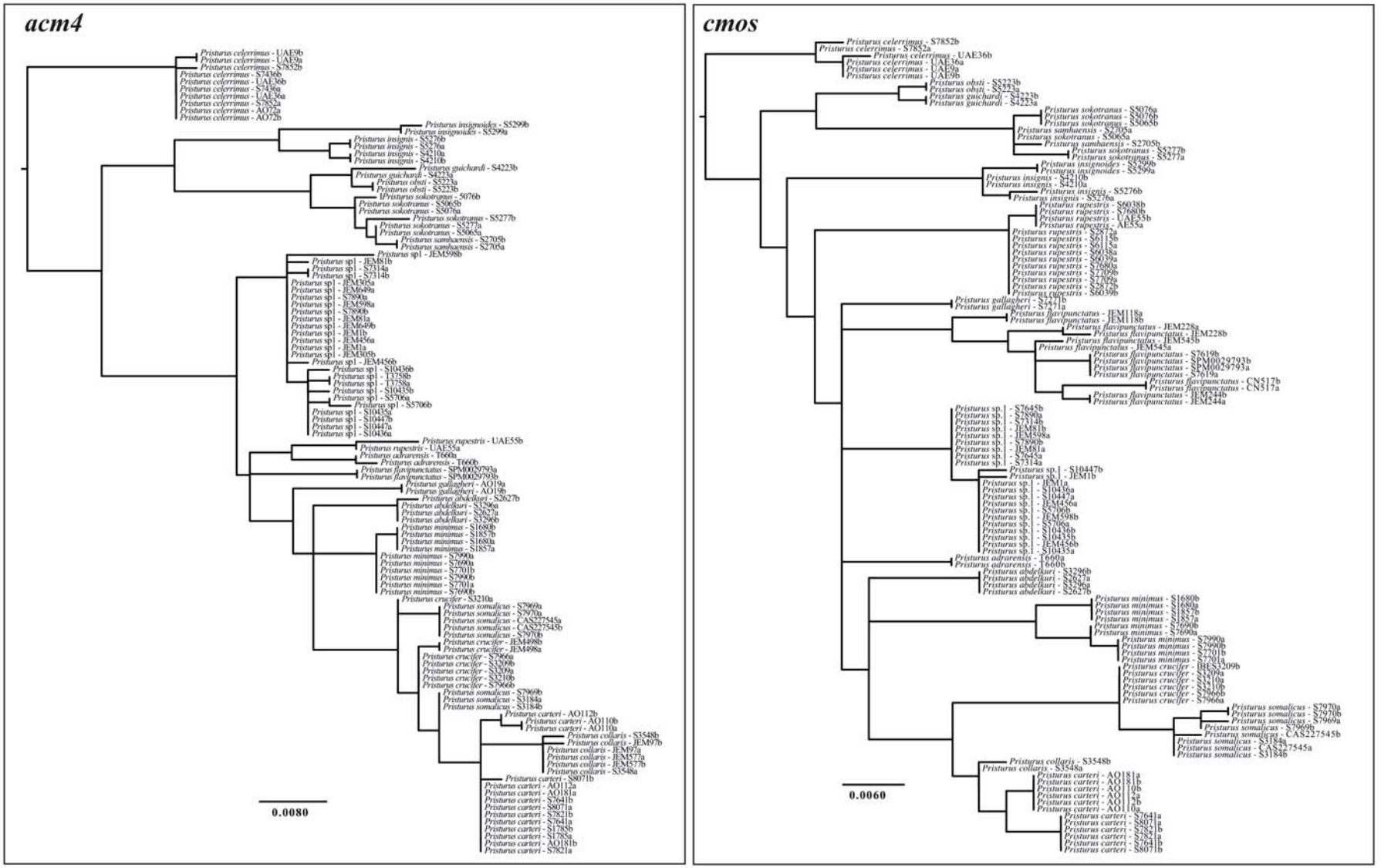
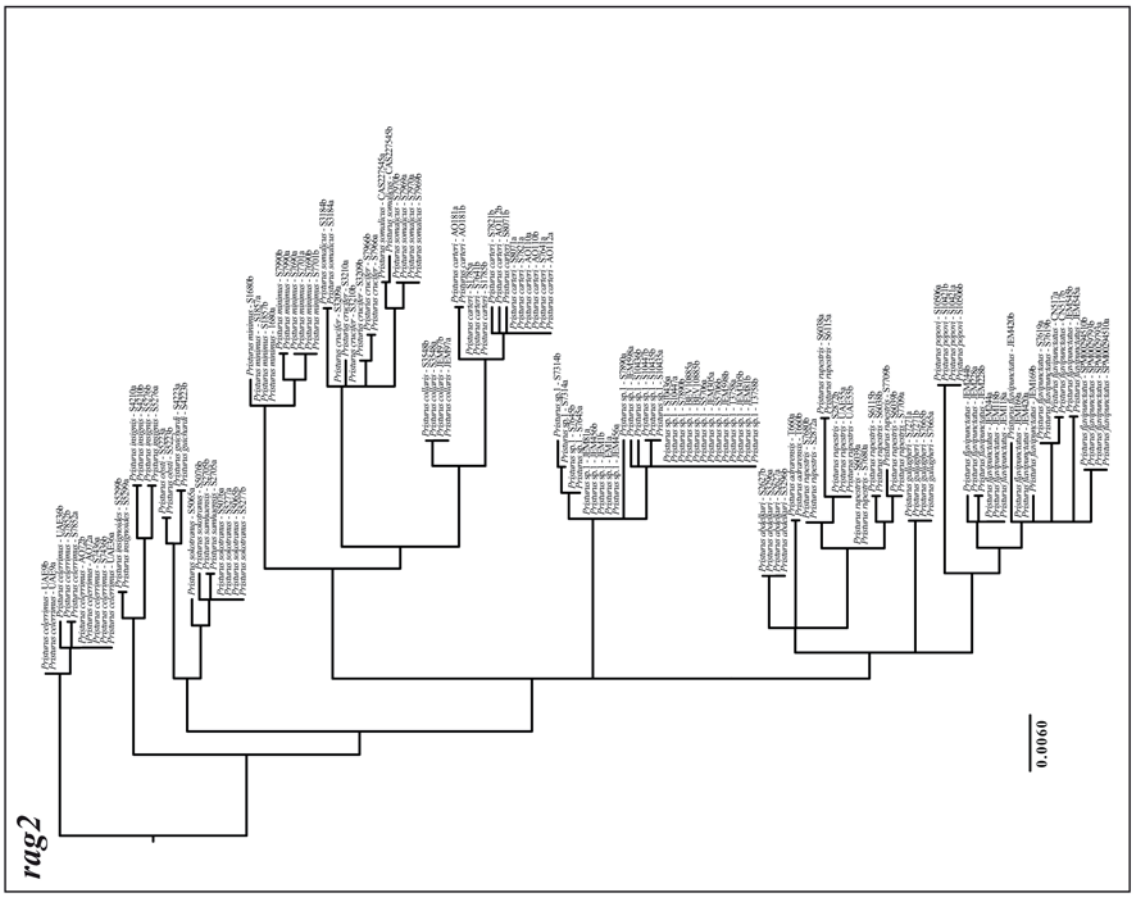
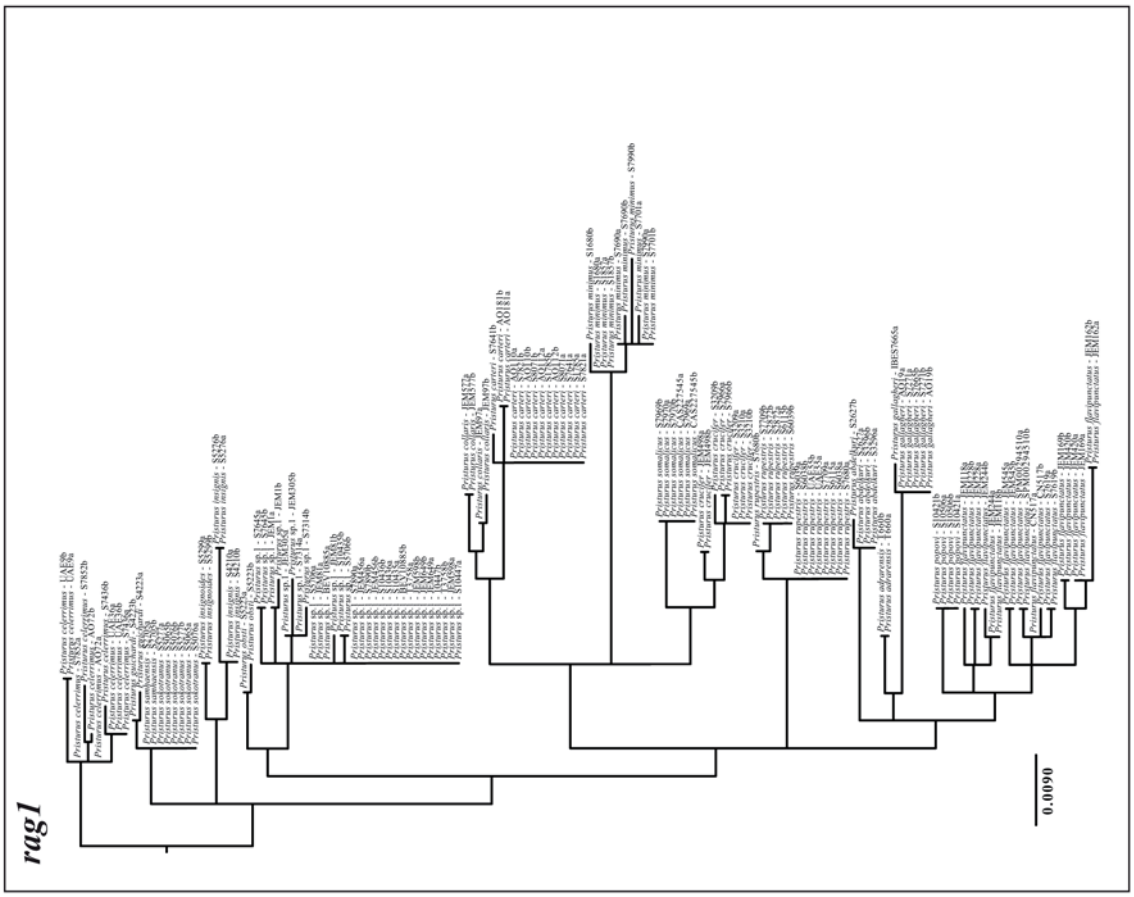
APPENDIX II. (Continued)

Species	Specimen Code	Country	Latitude	Longitude
P. sp. 1	MCCI-R897	Yemen	15.24	45.15
P. sp. 1	MCCI-R898-1	Yemen	15.01	43.44
P. sp. 1	MCCI-R898-2	Yemen	15.01	43.44
P. sp. 1	MCCI-R899-1	Yemen	15.54	48.47
P. sp. 1	MCCI-R899-2	Yemen	15.54	48.47
P. sp. 1	MCCI-R899-3	Yemen	15.54	48.47
P. sp. 1	MCCI-R899-4	Yemen	15.54	48.47
P. sp. 1	MCCI-R899-5	Yemen	15.54	48.47
P. sp. 1	MCCI-R900-1	Yemen	12.7747	45.0231
P. sp. 1	MCCI-R900-2	Yemen	12.7747	45.0231
P. sp. 1	MCCI-R900-3	Yemen	12.7747	45.0231
P. sp. 1	MCCI-R900-4	Yemen	12.7747	45.0231
P. rupestris	IBES6038	Oman	23.589399	58.165075
P. rupestris	IBES6039	Oman	23.589399	58.165075
P. rupestris	IBES6040	Oman	23.589399	58.165075
P. rupestris	IBES6131	Oman	23.589399	58.165075
P. rupestris	IBES6135	Oman	23.085750	59.046683
P. rupestris	IBES6140	Oman	23.589399	58.165075
P. rupestris	IBES7068	Oman	23.554560	58.187460
P. rupestris	IBES7398	Oman	23.293080	57.977470
P. rupestris	IBES7409	Oman	23.786010	57.795320
P. rupestris	IBES7416	Oman	23.786010	57.795320
P. rupestris	IBES7418	Oman	23.310270	57.995840
P. rupestris	IBES7422	Oman	23.513720	57.853400
P. rupestris	IBES7439	Oman	23.786010	57.795320
P. rupestris	IBES7446	Oman	23.786010	57.795320
P. rupestris	IBES7473	Oman	22.895140	59.137610
P. rupestris	IBES7525	Oman	23.164830	58.385300
P. rupestris	IBES7527	Oman	22.823740	59.007590
P. rupestris	IBES7534	Oman	23.131670	58.618890
P. rupestris	IBES7535	Oman	23.106030	58.644440
P. rupestris	IBES7553	Oman	23.131670	58.618890
P. rupestris	IBES7561	Oman	22.896280	59.160190
P. rupestris	IBES7675	Oman	23.589399	58.165075
P. rupestris	IBES7680	Oman	23.590320	58.407800
P. rupestris	IBES7709	Oman	23.615890	58.585420
P. rupestris	IBES7729	Oman	23.072330	58.186680
P. rupestris	IBES7738	Oman	23.452280	58.505340
P. rupestris	IBES7749	Oman	23.754680	57.581270
P. rupestris	IBES7864	Oman	23.690940	58.039380
P. rupestris	IBES7874	Oman	23.310270	57.995840
P. rupestris	IBES9018	Oman	22.833260	58.988210
P. rupestris	IBEUAE55	Oman	22.916390	58.876940
P. rupestris	IBEUAE58	Oman	23.108060	58.562500

Appendix III.- Phylogenetic analyses of the four nuclear genes independently (*acm4, cmos, rag1* and *rag2*). The dataset used were phased in order to show the two alleles of each specimen. All the haplotypes of the four nuclear gene are private for all *Pristurus* species, with the only exception of *P. samhaensis* and *P. sokotranus*, which share one haplotype in the *cmos* gene and one haplotype in the *rag1* gene.




rag2
0.0060


rag1
0.0090

CHAPTER 4.2

DIVERSIFYING IN THE SKY ISLANDS OF ARABIA: THE CASE OF THE HIDDEN DIVERSITY WITHIN THE SUBSPECIES *PRISTURUS RUPESTRIS RUPESTRIS*

Photo credit: Joan Garcia Porta

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ABSTRACT

Biodiversity in this planet is largely underestimated. This naturally applies to intertropical regions, but this may also be the case in the arid regions, not traditionally considered as high diversity reservoirs. Recent studies show that in these regions mountain ranges can potentially play a very important role as pumps of diversity, likely keeping great amounts of unknown species. The aim of this study is to expose one of these cases of hidden diversity in what is nowadays a gecko subspecies *Pristurus rupestris rupestris.* This subspecies is distributed across one of the most arid mountain ranges of the World, the Hajar Mountains in southeastern Arabia and preliminary studies showed that it might actually be formed by highly divergent lineages. According to our results based on the latest species delimitation approaches, *Pristurus r. rupestris* actually consists of 14 species, most of them presenting deep divergences highly overlapping with those existing between other species of geckos. We found that most of the diversification postdated the mountain orogeny and likely took place as a consequence of high rates of climate-mediated range expansions and contractions involving the different blocks that form the Hajar Mountains. Phenotypic diversification was very subtle probably due to the low distributional overlap existing between the different species. However body shape seems to obey an altitudinal gradient, with high altitude species presenting comparatively more robust morphologies. Dynamics of diversification were found to be expansive not showing any sign of saturation and therefore indicates that diversification may still be in an active process.

KEY WORDS: gecko, *Pristurus rupestris*, diversification, mountains, sky island, Arabia.

INTRODUCTION

Mountain regions are great reservoirs of diversity, containing half of the currently defined biodiversity hotspots (Kohler, 2009). This is because mountain ranges offer great opportunities for both adaptive and non-adaptive diversification. From an adaptive standpoint, mountain ranges provide strong environmental gradients that may be associated with different selective regimes. As a consequence ecological speciation can easily take place when different populations are subjected to divergent regimes along, for instance, an elevational gradient (Fuchs et al., 2011). From a non-adaptive (or not necessarily adaptive) perspective, speciation in mountains can take place by isolation in allopatric and/or parapatric scenarios (Vuilleumier & Monasterio, 1986; Randi et al., 2000; Antonelli et al., 2009). These are particularly important during mountain orogenies, when continuous species ranges are easily fragmented producing an interruption of gene flow between populations (Schweizer et al., 2011). Finally, another important diversification mechanism operating in mountains lies in middle grounds between adaptive and non-adaptive processes and stems from the interaction between topography and climate

change. In a situation of a climatic niche extending continuously between different mountains, a species adapted to such niche is usually able to maintain a continuous gene flow between all mountains. However if this climatic niche experiences a shift towards higher altitudes and becomes interrupted at lower altitudes, a once continuous species range may potentially be fragmented in different allopatric domains (i.e. one on each mountain), setting the stage for non-adaptive speciation (Wiens et al., 2006; Nosil, 2012).

These different mechanisms of diversification in mountain ranges are not exclusive between each other, so this leads to the following question: which is the contribution of these processes at generating mountain diversity? This is the core question of this study, which is intended to explore the processes that have produced diversity in one of the most arid mountain ranges of the planet: the Hajars Mountains in southeast Arabia. The Hajars are located in the southeast corner of the Arabian Peninsula, where they form an arcuate range bordering the Gulf of Oman (Fig. 1). These form an extremely isolated mountain system, with the nearest Arabian mountain range, the Dhofar Mountains, being approximately 900 km away. Their orogenic history is very complex, involving two different orogenic cycles: the first one took place between the Late Permian and Late Cretaceous but the major uplift took place during the Oligocene and continued through the Miocene (although vertical movements of the mountain range, have continued until the present) (Glennie et al., 1974; Robertson et al., 1990). Interestingly, the Hajars do not consist of a compact mountain system but are divided by three distinctive topographic discontinuities. These dissect the range in Northern, Central and Southern blocks, the last two presenting large areas of high-elevation habitats (with altitudes surpassing 1500 m) (Fig. 1). These particular geographic features make the Hajar Mountains a “mountain archipelago” containing a number of isolated high altitude environments (often referred to as “sky islands”; McCormack et al., 2009). Their high degree of isolation and their complex structure make the Hajars one of the most diverse regions of Arabia, with numerous endemic plants and animals (Arnold & Gallagher, 1977, and other articles in the same volume).

One of the most conspicuous and abundant reptiles of the Hajar Mountains is the small sphaerodactylid gecko *Pristurus rupestris*. Like all the other members of the genus *Pristurus*, this species is characterized by being mostly diurnal, heliothermic and by signaling each other by waving their tails (Arnold, 1993; 2009). Until very recently *P. rupestris* was considered the most widely distributed species in the genus. However, a recent systematic revision using molecular and morphological data clearly showed that *P. rupestris* was polyphyletic and included two morphologically very similar but genetically highly divergent clades: the Western clade, *Pristurus* sp. 1, which extends from coastal middle-southern Oman, to Yemen, western Saudi Arabia and up to southern Jordan; and the Eastern clade, which includes two subspecies, *P. rupestris rupestris* from the Hajar Mountains and *P. rupestris iranicus* from coastal Iran (Badiane et al., 2014). Preliminary data suggested that *P. r. rupestris* could actually be constituted by deeply divergent lineages, however until now no exhaustive analysis has been carried out to elucidate the real diversity contained in this "subspecies".

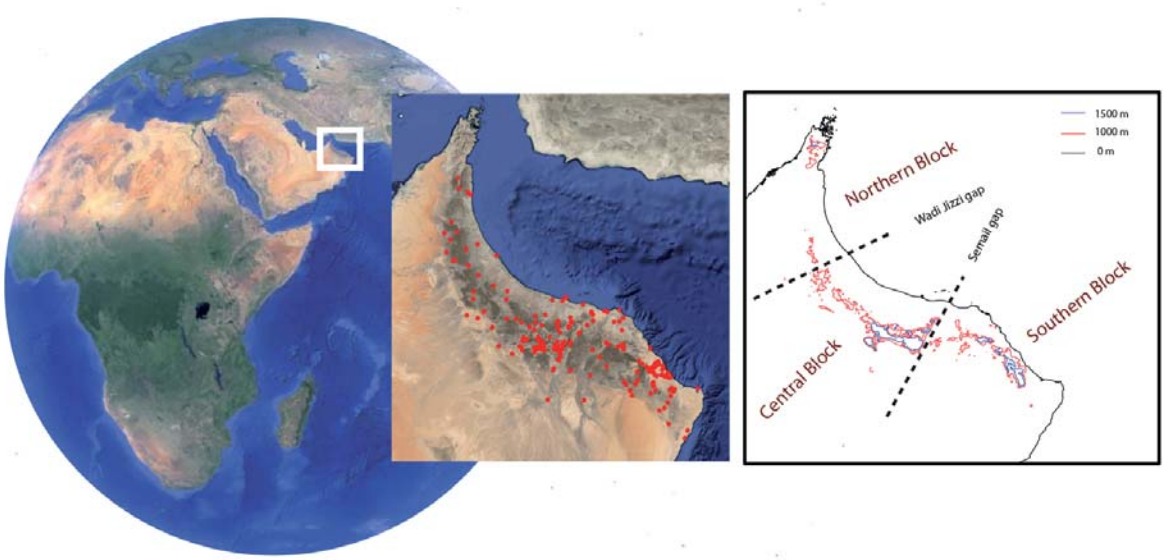

Figure 1. Map showing the geographic limits of this study. From left to right, the enlarged maps show the sampling of *P. r. rupestris* used in this study (left) and the topographic complexity of the Hajar Mountains (right). Three main blocks with altitudes above 1,000 m are separated by low-lying discontinuities.

The aims of this study are two-fold, on one hand our goal is to uncover the real species diversity within the "subspecies" *P. r. rupestris* from the Hajar Mountains applying the recent-most species delimitation methods based on molecular data. On the other, is also our aim to explore the eco-geographical drivers that generated this diversity in terms of number of species and phenotypic variation.

MATERIAL AND METHODS

GENETIC SAMPLING AND PRELIMINARY SPECIES DELIMITATION HYPOTHESIS

A total of five field campaigns were carried out between 2005 and 2011 aimed to explore the diversity of the reptiles of Oman, including the interesting fauna from the Hajar Mountains. This consisted in prospections along the 650 km of mountain range and adjacent lowland areas, spanning from the Strait of Hormuz (in the north) to the foot-hills in Ras al Hadd (in the southwest), covering the totality of the distribution range of *Pristurus r. rupestris*. Sampling was particularly intensified in the Central and Southern blocks, the ones likely presenting the longest altitudinal gradients based on their maximum altitudes. After these field campaigns, our sampling consisted of 366 tissue samples (Fig. 1) and 104 vouchers that were used to assess the phenotypic variation existing in the "subspecies" (see below).

Genomic DNA was extracted from ethanol-preserved tissue samples using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) and to obtain a first estimation on the genetic diversity within *P. r. rupestris*, we amplified and sequenced a short segment of the mitochondrial fragment of the gene encoding the ribosomal 12S rRNA (*12S*; primers 12Sa and 12Sb – Kocher et al.,1989) for all 366 samples (using the same PCR conditions as in Šmíd et al., (2013)). After removing identical sequences,

we aligned the remaining unique 168 sequences with the online version of MAFFT v.6 (Katho & Toh, 2008) and we then produced a summary tree by means of the package BEAST 1.8.0. (Drumond & Rambaud, 2007). This analysis relied on an uncorrelated log-normal relaxed clock, a coalescent tree prior and was run in two independent MCMC chains during $5*10^6$ generations each one. This summary tree was then used to generate our first species delimitation hypothesis, which was informed by the general mixed Yule-coalescent model (GMYC) (Pons et al., 2006; Fujisawa & Barraclough, 2013). This model essentially detects the most likely tree depth (in the single-threshold approach) at which the pattern of tree branching shifts between a Yule process (reflecting inter-specific phylogenetic structure) to a coalescent process (reflecting intra-specific structure). The analysis was performed using the package "splits" (Ezard et al., 2009) in R (R Development Core Team, 2014) and it allowed us to objectively identify highly divergent lineages existing within *P. r. rupetris*.

In order to examine whether the divergences between the putative species derived from GMYC were in the range of the divergences existing between valid species of geckos, we obtained sequences homologous to our *12S* fragment for all species available in GenBank (Benson et al., 2012). We then calculated the pairwise sequence distances (p distances) between all combinations of species within each genus and we compared them with the distances computed between all combinations of each of the putative species found by the GMYC model.

COALESCENT-BASED SPECIES DELIMITATION USING NUCLEAR DATA

For all 16 putative species defined by the GMYC model (see results), we selected a subset of at least four specimens per species (with the exception of one of the species, which was represented by a single specimen), for which we amplified and sequenced five additional genes: one mitochondrial fragment of the gene encoding the cytochrome *b* (*cytb*; primers Cytb1 and Cytb2 – Kocher et al.,1989), and four nuclear fragments of the genes encoding the oocyte maturation factor Mos (*cmos*; primers FUF and FUR – Gamble et al., 2008), a short fragment of the recombination-activating gene 1 (*rag1*; primers F700 and R700 – Bauer et al., 2007), the recombination-activating gene 2 (*rag2*; primers PyF1 and PyR – Gamble et al., 2008) and the melano-cortin 1 receptor (*mc1r*) gene (PCR conditions used for the amplification all gene fragments can be found in Šmíd et al., (2013)). These four nuclear genes were used to test whether the species defined by the GMYC model were also supported by nuclear data. This was assessed using a coalescent-based species delimitation approach as implemented in the program Bayesian Phylogenetics and Phylogeography (BP&P) version 2.2 (Yang & Rannala, 2010). This program essentially uses a reversible-jump Markov chain Monte Carlo (rjMCMC) to estimate whether two or more putative species in a given species tree can be differentiated from each other accounting for the coalescent uncertainty provided by multiple independent loci. This method has shown to perform very robustly when multiple specimens and independent loci are used, even in the instances of low levels of migration between species (Zhang et al., 2011). However, this approach is very sensitive to the

species tree used to guide the species delimitation algorithm (Leaché & Fujita, 2010). In order to supply the analysis with the best possible tree containing all putative species defined by the GMYC model, we used *BEAST (as implemented in the package BEAST 1.8.0) to calculate a species tree (guide tree) informed by all possible molecular evidence: the two mitochondrial genes (*12S* and *cytb*) and the four nuclear genes (*cmos, rag1, rag2*, and *mc1r*). This method essentially implements a multispecies coalescent model to estimate the species tree from multiple genes and multiple individuals per species, while taking into account incomplete lineage sorting (Ruane et al., 2013). For this analysis, nuclear data were phased using the program PHASE v. 2.1 (Stephens et al., 2001; Stephens & Scheet, 2005) and input/output files were interconverted with SeqPHASE (Flot, 2010) with phase probabilities set at 0.7. The analysis was run using two MCMC chains, each one with a length of $100*10^6$ generations and a sampling frequency of 5,000 generations.

We ran two sets of BP&P analyses: one set of analyses was informed by a combination of mitochondrial and nuclear loci and involved *cytb*, *cmos, rag1, rag2*, and *mc1r* (we excluded *12S* as this gene was the one used to generate the first species delimitation hypothesis). The other set of analyses was based solely on the nuclear loci, which allowed us to evaluate the species delimitation hypothesis with loci not used to generate it. Within each of the two previously described sets, we ran six different types of analyses, each one having a different combination of prior settings and rjMCMC algorithms. Regarding the algorithms, we used the two types available in the program BP&P (0 and 1). Regarding the prior settings, following previous studies (Leaché & Fujita, 2010; Burbrink et al., 2011; Cox et al., 2012; Ruane et al., 2013) we parameterized the prior distributions of ancestral population sizes and root age using a gamma distribution with the following combinations of alpha and beta parameters: large ancestral populations sizes and deep divergences (alpha = 1, beta = 10 for both priors); small ancestral populations and shallow divergences (alpha = 2, beta = 2000 for both priors) and finally, large ancestral population sizes (alpha = 1, beta = 10) with shallow divergences (alpha = 2, beta= 2000). Each combination of prior settings and algorithms was run in two independent rjMCMC chains, for 500,000 generations, each time using a random species delimitation scheme as starting point. All runs were monitored during the initial few thousand generations to assess whether the acceptance proportions for the MCMC moves were in the recommended range between 0.3 and 0.7. If in some of the runs any acceptance proportion was outside this range, the runs were stopped and the finetune variables were modified accordingly before restarting the runs. After confirming convergence and good mixing in each of the analysis we evaluated the posterior probabilities (pp) of all putative speciation events recovered in our guide tree. Any putative speciation event presenting a pp smaller than 0.9 in at least one of the runs was considered as not reliable and its descendant tips were collapsed into the same species.

DATING THE ONSET OF DIVERSIFICATION

In order to date the onset of diversification within *Pristurus r. rupestris*, we placed the 14 species

delimited by BP&P (see results) in the phylogenetic context of a wide representation all species of geckos available in GenBank. Working at this large phylogenetic scale allowed the possibility to use a number of calibration points scattered across the phylogeny of Gekkota (see below), all external to our group of interest. On the other hand, the comparison of the divergence times within the *Pristurus r. rupestris* compared to the divergence times computed for other species of geckos served as an alternative way to assess the reliability of the species delimited by BP&P.

To assemble this dataset we BLASTed (Altschul et al., 1997) all six genes sequenced for this study against all gecko sequences available in GenBank. We then retrieved the longest sequence for each species of gecko with the additional requirement that sequences had to contain 200 bp or more to be selected (the search in GenBank was conducted during March 2014). After this procedure, we opted to exclude the nuclear gene *mc1r* due to its low number of hits in GenBank. For the remaining genes, in order to minimize the amount of missing data, we included the sequences of all species for which at least three genes were available. This resulted in a dataset of 435 species spanning 117 different genera. We then combined this dataset with a molecular dataset that included the longest sequence of each of the 14 species delimited in *P. r. rupestris* by BP&P. Each gene was then aligned using two procedures: the ribosomal coding *12S* was aligned by means of MAFFT v6 (http://www.ebi.ac.uk/Tools/msa/mafft/; (Katoh et al., 2002) and the protein coding genes (*cytb*, *cmos, rag1, rag2*, and *mc1r*) were aligned using the translation alignment algorithm implemented in the software Geneious (Drummond et al., 2010). The final alignment included 449 species and consisted in a total of 2,467 bp distributed in each gene as follows: *12S* (391 bp), *cytb* (306 bp), *cmos* (414 bp), *rag1* (280 bp) and *rag2* (410 bp).

The phylogenetic analysis was conducted by means of the package BEAST v1.8.0 following the same setting and parameters as in *BEAST (see above) but with the nuclear genes unphased.

Five calibrations were used to estimate branch lengths in units of time:

1. The onset of the diversification in the Caribbean *Sphaerodactylus*. This calibration was set to a minimum of 20 Ma based on an amber fossil from the Dominican Republic (Daza & Bauer, 2012). The maximum age of this radiation was set conservatively to a soft maximum of 70 Ma. This was done by means of a gamma distribution (α=2, β=10).
2. The split between *Teratoscincus scincus* and the clade formed by *T. przewalskii* and *T. roborowskii*. This was set to an age of 10 Ma (with a standard deviation of 1 Ma), based on the age of the Tien Shan-Pamir uplift in western China, considering that this split originated via vicariance as a result of this geologic event (Macey et al.,1999).
3. The onset of diversification of the diplodactyloid radiation in New Caledonia. This was set to a soft maximum of 37 Ma. This is based on several lines of evidence (geological and biological) that show that the island was under water until this approximate time (Nattier et al., 2011; Pillon, 2012; Papadopoulou et al., 2013; Garcia-Porta & Ord, 2013). A normal distribution with a mean at 20 Ma and a standard deviation of 10 Ma was used to set the prior of this calibration point.

4. The split between *Phelsuma ornata* from the island of Mauritius and *Phelsuma inexpectata* from the island of Reunion. This split was set to a soft maximum of 8.9 Ma based on the age of the oldest rocks of Mauritius (the oldest island in the Mascarenes, including both Mauritius and Reunion) (Moore et al., 2011). The prior was set by means of an exponential distribution with an offset of 0 and a mean at 3 Ma.
5. Finally, the deep-most split in the diplodactyloid radiation of New Zealand was set to a minimum of 19 Ma based on the oldest fossils of geckos in the archipelago (Lee et al., 2009) with a conservative soft maximum of 65 Ma. This was set by means of a gamma distribution (α=3, β=7).

SPECIES TREE ESTIMATION

A species tree that included all species detected within *Pristurus r. rupestris* (only including all species that presented high support values across all BP&P runs), was estimated with *BEAST following the same settings and parameters previously described. The analysis was based on all six genes used in this study including the phased sequences for all nuclear genes (see above). In order to obtain the species tree in units of time, we used two different approaches. First we ran five runs in which the priors of the substitution rates for *12S*, *cytb*, *cmos*, *rag1* and *rag2* were established as the rates estimated from the above-described BEAST analysis (conducted on 449 species of geckos and five calibration points). Secondly, we ran another five runs in which, in addition to implementing these substitution rates, we also calibrated the root of the tree with the estimated age for this same node in the phylogeny spanning all geckos.

GEOGRAPHIC STRUCTURE AND MACROCLIMATIC DIVERSIFICATION

To study the geographic structure within *P. r. rupestris*, we assigned species to different mountain blocks in the Hajar Mountains by calculating the distribution centroids of all species and placing them relative to the two great mountain discontinuities existing in the mountain range: the Wadi Jizzi line, separating the Northern and Central mountain blocks and the Semail gap, separating the Central and Southern blocks (Fig. 1). We then visualized the distribution of the centroids in a phylogenetic perspective, assessing whether the different mountain blocks in the Hajar Mountains formed monophyletic species assemblages (situation compatible with a founder event followed by *in situ* diversification) or whether the different mountain blocks consisted in polyphyletic species assemblages (situation consistent with multiple dispersals between the different mountain blocks). We also evaluated the amount of sympatry by dividing the region in cells of 1 km^2 and counting the number of cells in which more than one species coexisted. In order to study macroclimatic diversification of the species constituting *P. r. rupestris*, we first filtered all sampling localities by means of a grid of 1 km x 1 km of cell size, extracting one locality per species and cell. This resulted in 207 localities with a mean of 15 localities per species. We then compared the occurrence-based climatic envelopes between all species considering all possible climatic conditions existing in the region. Climatic conditions in southwestern Arabia were informed by

all 19 Bioclim variables plus altitude at 30 arc-seconds of spatial resolution (available from http://www.worldclim.org; Hijmans et al., 2005). The climatic space existing in the region and the climate envelope of each species were visualized by means of a PCA (Broenninan et al., 2012). We also obtained a point estimate of the niche position of each species by calculating the mean values on the first and the second PCA axes.

We integrated macrolimatic data with the phylogenetic data in the following ways: we first estimated the rates of climatic evolution and the phylogenetic signal along the PC1 and PC2. Rates of evolution were calculated as the mean square of all independent contrasts computed from the tree (Felsenstein, 1985; Garland, 1992; Garland et al., 1992; Martins, 1994; Revell et al., 2007). Phylogenetic signal was calculated by means of Blomberg's K statistic (Blomberg et al., 2003). This provides an estimate of the amount of phylogenetic signal in the tip data relative to the expectation under a Brownian motion (BM) scenario. A K =1, indicates an amount of phylogenetic signal equal to the expectation under a BM model, a K < 1 indicate less climatic resemblance between close relatives than the Brownian expectation (indicating that climatic envelopes of closely related species tend to diverge). Finally, values > 1 indicate a phylogenetic signal stronger than the expected in a Brownian model (suggesting a great convergence in the climatic envelopes exhibited by closely related species). We assessed whether our empirical K value was significantly different from 1 by conducting 1,000 simulations under a BM regime. These simulations were based on the rate estimate calculated for each PC axis (see above). From the distribution of simulated K values we derived the p-values of our empirical estimates of K for each PC axis. Finally we visualized macroniche variation in a phylogenetic context by projecting the species tree into a bivariate space represented by the values of each species in the climatic space and the reconstructed values at the nodes (here and after "phyloclimatic space" by analogy to the "phylomorphospace"; Sidlauskas, 2008).

All calculations and data manipulations described in this section were conducted in R (R Core Team, 2014) using the packages "raster" (Hijmans, 2014), "dismo" (Hijmans et al., 2012), "ade4" (Dray & Dufour, 2007), "adehabitat" (Calenge, 2006), "sp" (Pebesma & Bivand, 2005; Bivand et al., 2008) and "phytools" (Revell, 2012).

PHENOTYPIC DIVERSIFICATION

We characterized the morphology of each of the species within *P. r. rupestris* in the Hajar Mountains by means of 12 different measurements. Body size was measured as the length between the snout and the opening of the cloaca (snout vent length = SVL), head shape was characterized by its length measured from the snout to the auricular opening (HL), its maximum width (HW), its maximum height (HD). Body proportions were measured as the axilla to groin distance (AGL) and the body amplitude at the level of the scapular and pelvic girdles (ASG and APG, respectively). Regarding limb proportions, forelimbs were measured as the length of the brachium (BL), length of the ante brachium (AL) and hindlimbs proportions

were quantified as the thigh length (TL) and the crus length (CL). All measurements were taken three times using a digital caliper (to the nearest 0.1 mm) with the average of the three replicates used as the final value. These were then $\log_{10}$-transformed to improve normality and homoscedasticity. A total of 102 specimens were measured, with a mean of seven specimens per species. Only adult specimens were measured and given that preliminary analyses showed no significant differences between males and females in any of the measurements taken (data not shown), both sexes were pooled together. To remove the effect of body size on the shape variables, we computed their residuals against SVL. We then characterized the morphospace occupied by all species delimited within *P. r. rupestris* by means of a PCA (conducted in the package "ade4"; Dray & Dufour, 2007). To assess whether size and shape differences existed between the species, we used a permutational ANOVA on body size (using the R package "lmPerm", Wheeler, 2010) and a perMANOVA on the first five PC axes of shape variation (using the R package "Vegan"; Oksanen et al., 2013). Furthermore, as described for the macroclimatic variables (see previous section) we calculated the rates of evolution and the phylogenetic signal on body size and on the first three axis of shape variation (the axes that allowed an easier interpretation, see results).

We used the function "contMap" in the R package "phytools" (Revell, 2012) to visualize size and shape variation across the phylogeny. This method essentially reconstructs the ancestral states of continuous characters at the nodes and interpolates the values along the branches ("Method 2" in Revell, 2013). In the particular case of shape, we also visualized the morphospace by projecting the phylogeny into a bivariate space represented by the species values and the reconstructed states at the nodes for each combination of three first PCA components (here and after: "phylomorphospace" (Sidlauskas 2008). This representation was very useful to visualize the shape morphospace in a phylogenetic context, allowing us to identify the major trends of shape change across the tree, as well as the different magnitudes of shape variation (proportional to the branch lengths in the phylomorphospace; Sidlauskas 2008). Finally, in order to assess whether climatic and morphologic variables were correlated, we regressed each morphological variable against each axis of climatic variation. Significance was assessed by a permutation approach as implemented in the R package "lmPerm".

SPECIES DIVERSIFICATION

To examine the diversification dynamics within *P. r. rupestris* we used the coalescent-based approach described by Morlon *et al.* (2010). This method basically models the internode distances of a phylogeny assuming that they are distributed according to a standard coalescent approximation (Griffiths & Tabaré, 1994). This has the advantage of modeling species diversity from the present to the past assuming that it can take any value at any point in time. Six models of diversification that differed in their assumed diversity dynamics were applied to the species summary tree: Models 1 and 2 assumed that speciation rates were constant through time according to a constant birth-death and a Yule process, respectively.

The rest of the models assumed that speciation rates were varying exponentially through time and differed in the dynamics of the extinction rates: Model 3 assumed a constant extinction rate, Model 4 assumed a extinction rate that varied as a function of the speciation rate, Model 5 assumed an exponential change in extinction rate over time, and finally Model 6 assumed no extinction rates. The parameters and likelihood of each model were estimated using the R code provided in Morlon et al., (2010). The best supported model was identified as the model with the highest computed Akaike weight (AICw) (Morlon et al., 2010).

RESULTS

GENETIC SAMPLING AND SPECIES DELIMITATION

Specimens of *P. r. rupestris* were found extensively distributed in the Hajar Mountains and adjacent lowland areas, with the exception of the northern-most region (above 25º 25' of latitude, including the Musandam Peninsula), where not a single specimen was detected (Fig. 1). The GMYC analysis implemented on the *12S*-based summary tree that included the unique sequences existing across all 366 genetic samples collected, proposed 16 putative species in its maximum likelihood (ML) estimate (Fig. S1). The distributions of these putative species were geographically consistent, each one formed by specimens collected in the same area or neighboring regions. Moreover, the mean pairwise genetic distances between putative species (at a lower estimate of 0.0069, an upper estimate of 0.15 and a mean of 0.08) were in the range of the distances computed between valid species of geckos (Fig. S2). Based on the previously exposed evidence, we adopted the 16 species detected by the GMYC model as our working hypothesis.

The coalescent-based analyses using nuclear evidence produced different results depending on whether solely nuclear loci were used or whether aside of the nuclear loci, the mitochondrial *cytb* was also used. All analysis that involved *cytb* supported all species defined by our GMYC model (with all species splits receiving supports higher than 0.99), while all analysis involving only nuclear loci, produced low support values on some of the species splits (Fig. S3). The topological placement of the splits that presented low supports varied depending on the prior parameterization: when big populations sizes were combined with deep divergence times, the node separating the putative species 4 and 5, received a very low support (with pp always below 0.65). However when big population sizes were combined with shallow divergences times, or when small population sizes were combined with shallow divergences, the split separating the species 10 and 11 was the one presenting a low support (with pp values always below 0.76) (Fig. S3). Considering this, we conservatively pooled species 4 and 5 and species 10 and 11 in a single species (species 4.5 and species 10.11, respectively). Therefore, after the BP&P analyses the 16 species delimited by the GMYC model were reduced to 14.

DATING THE ONSET OF DIVERSIFICATION AND SPECIES TREE ESTIMATION

The summary tree encompassing 449 species of geckos was found to be generally consistent with previous published phylogenies of Gekkota (Gamble et al., 2008, 2011, 2012; Pyron et al., 2013), with 62% of the nodes presenting a posterior probability (pp) greater than 0.90 (Fig. 2). According to our dating estimates, the crown radiation of Gekkota dated from 65 to 114 Ma (with a median of 96.6 Ma), and therefore is in the range of most of the estimates provided by previous studies (Vidal & Hedges, 2005; Wiens et al., 2006; Hugall et al., 2007; Gamble et al., 2008; Jones et al., 2013). The onset of the diversification in the genus *Pristurus* was dated approximately from 30 to 47 Ma (with a median at 37 Ma) and the onset of diversification within *P. r. rupestris* dated from 10 to 22 Ma, with the median estimate computed at 15.6 Ma. Remarkably, the visual comparison between the divergences times within *P. r. rupestris* were in the range of the time divergences found between other species of *Pristurus* and other species of geckos, therefore independently validating the deep structure found within this "subspecies" (Fig. 2).

The species tree produced with *BEAST based on the substitution rates estimated in the previous BEAST analysis and the one based additionally on the calibration of the root of the tree, were generally consistent with each other and topologically consistent with the tree computed in the BEAST analysis for all geckos. Given that the species tree informed by substitution rates plus the calibration at the root presented the shortest high posterior density (HPD) intervals, it was the one chosen. This species tree revealed four highly supported clades: Clade 1 formed by species 1, 2, 3, and 4.5 was mostly distributed in the Central and Western region of the distribution area; Clade 2, formed by species 10.11, 12, 13, 14 and 15, was distributed throughout the whole region, including the Northern, Central and Southern blocks; Clade 3, formed by the species 8 and 9, was mostly limited to the lowland areas South of the Hajar Mountains; Clade 4 was constituted by species 6 and 7, both endemics from the Southern block; and finally, species 16, which appeared as an isolated lineage was associated with low support to Clade 1 and was constituted by a unique specimen collected in the Southern block (Fig. 3).

GEOGRAPHIC STRUCTURE AND MACROCLIMATIC DIVERSIFICATION

The centroids of the distributions for all 14 species found within *P. r. rupestris* allowed the assignation of six species to the Central Hajars and eight species to the Southern Hajars. The visualization of the species distributions from a phylogenetic perspective revealed highly polyphyletic species assemblages in both mountain blocks (Fig. 4). Sympatry was very low, with only 7% of all cells of 1 km^2 presenting more than one species in it. These consisted in nine unique species assemblages, all of them formed by two species belonging to different clades, with the exception of species 6 and 7 that were found to coexist in one of the cells.

Regarding the analysis on the climatic variables across southeast Arabia, the first two components produced by the PCA explained a 65.62% and 21.86% of the total variance respectively (Table S1).

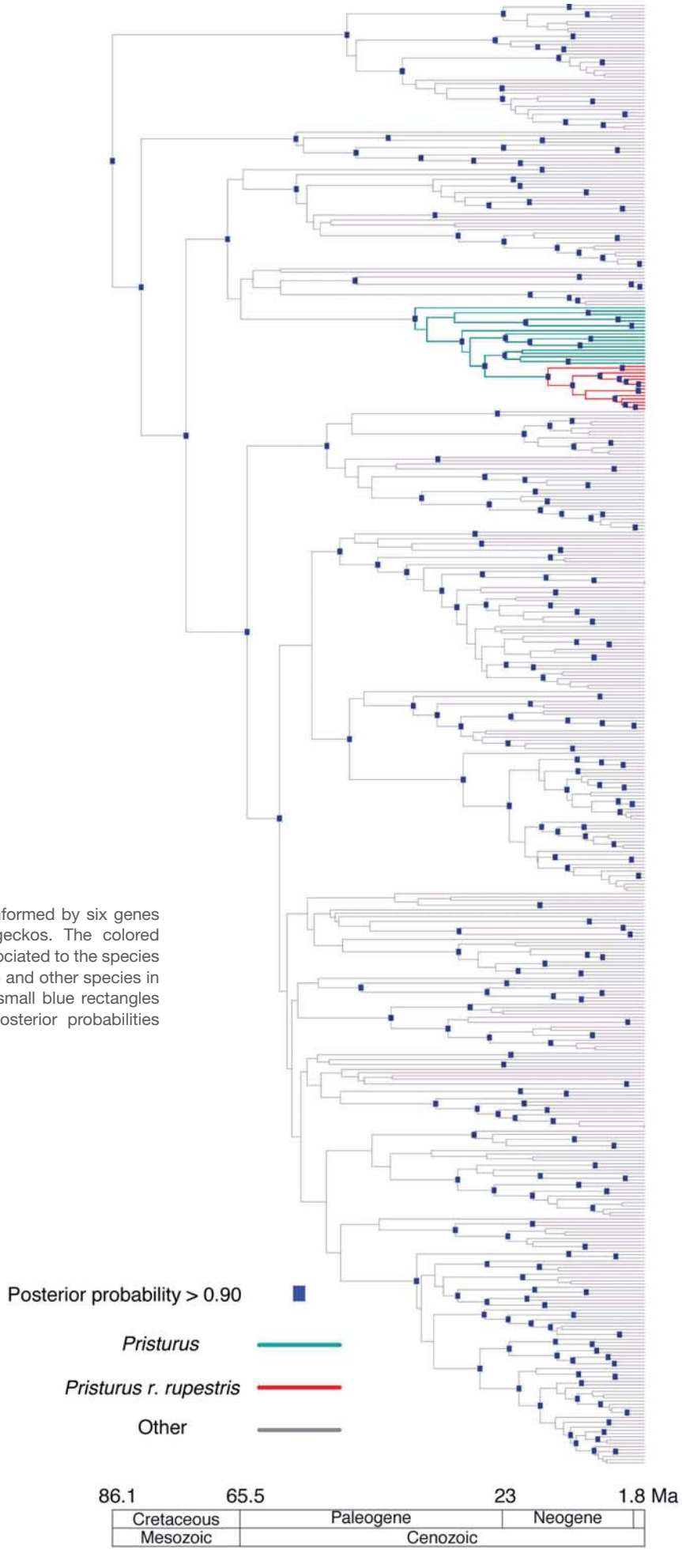

Figure 2. Time-calibrated tree informed by six genes and including 449 species of geckos. The colored branches refer to all branches associated to the species delimited within *P. r. rupestris* (red) and other species in the genus *Pristurus* (green). The small blue rectangles highlight all nodes presenting posterior probabilities higher than 0.90.

PC1 essentially reflected an altitudinal gradient and was highly correlated with altitude, amount of precipitation and inversely correlated to temperature. PC2 reflected variation along a horizontal axis (mostly longitudinal) and was inversely correlated with the mean diurnal range, temperature seasonality, temperature annual range and precipitation seasonality (Table S1). The visualization of the climatic envelopes of all 14 species within *P. r. rupestris* species complex revealed a great climatic heterogeneity among species although some of them presented extensive levels of niche overlap (Fig. 5).

The visualization of the "phyloclimatic" space revealed that the highest magnitudes of change across the climatic space took place along the altitudinal gradient of variation, being the magnitudes of change along PC2, comparatively smaller in extent (with the exception of "sp 10.11") (Fig. 6). This is consistent with the computed rates of macroniche evolution that show how the rates of evolution along PC1 (at a mean of 7.70) were more than 15 times larger than the rates computed along PC2 (at a mean of 0.40).

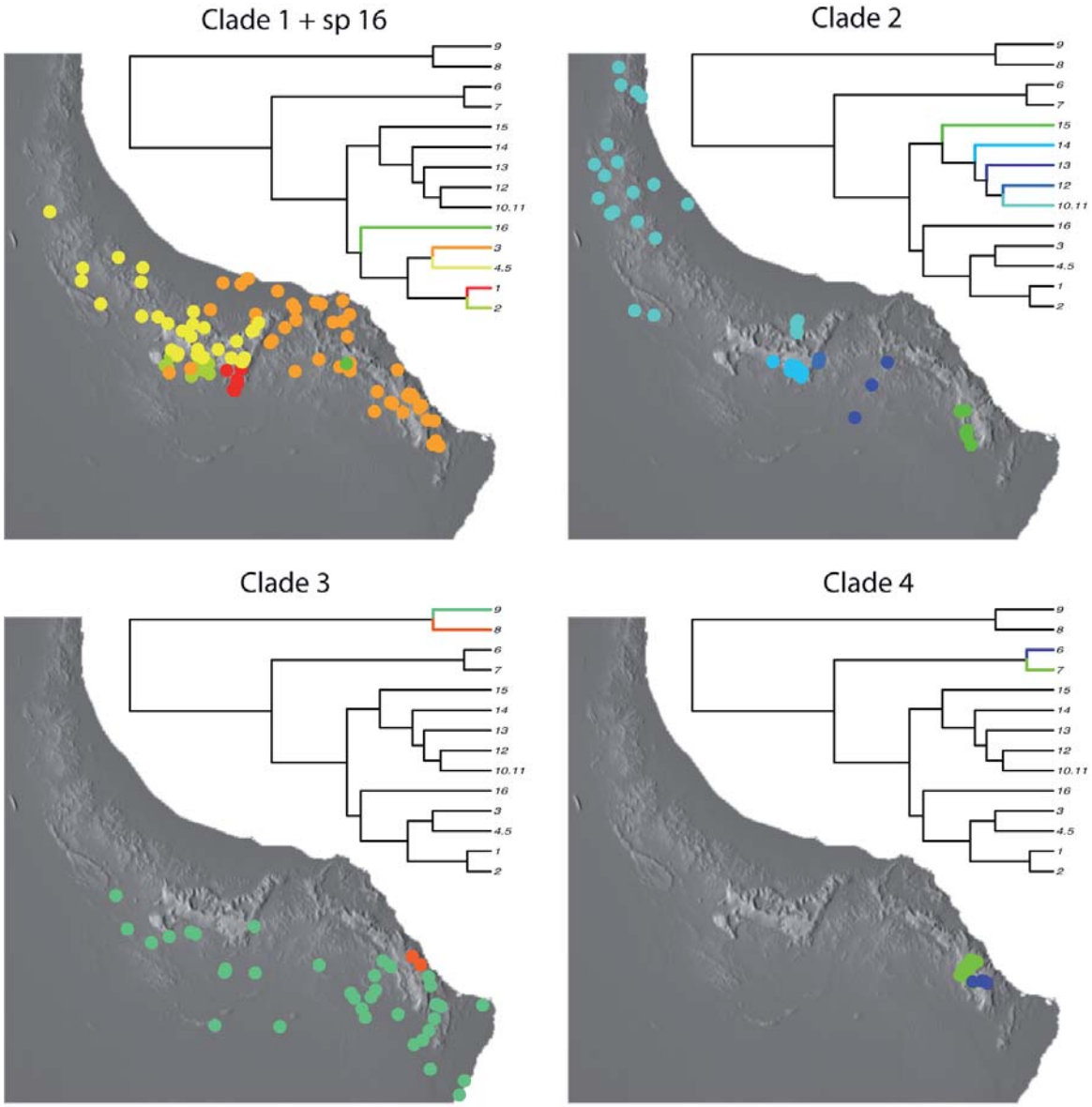

Figure 3. Distributions of the 14 species existing within *P. r. rupestris*, separated in each of the main clades. Colors on the map correspond with the colored branches in the species tree (derived from the *BEAST analysis).

However, when we computed the phylogenetic signal along both axes, PC2 presented a comparatively smaller phylogenetic signal, which was significantly different from the null expectation (K = 0.32, *p*-value = 0.02). By contrast, PC1 showed a higher phylogenetic signal not significantly different from the null expectation (K = 0.55, *p*-value = 0.30). Given that PC2 reflects climatic variation along a horizontal axis (mostly variation along longitude), its low phylogenetic signal likely reflects the intense geographic motility of this group of species, involving several jumps between the Central and Southern blocks (which present different climatic conditions). It is also interesting to note that high altitude species form a polyphyletic assemblage, indicating that high altitude environments (above 1000 m) were accessed at least three times independently (Fig. 6).

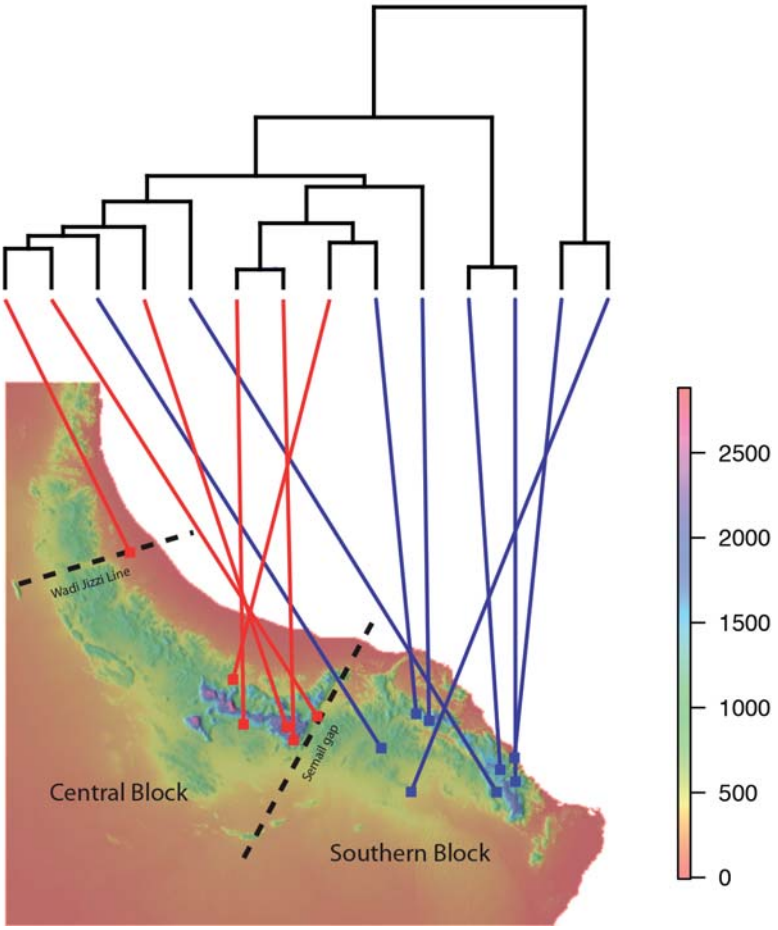

Figure 4. Map showing the centroids of the distributions of each of the 14 species found within *P. r. rupestris* and its geographic assignment to the Central (red) or Southern block (blue). This figure also provides a visualization of the phylogeographic structure of the species complex, with each centroid linked to the corresponding tip in the species tree.

PHENOTYPIC DIVERSIFICATION

We found significant, although subtle, differences in the morphologies of the species forming *P. r. rupestris*. Regarding body size, differences were significant (*p*-value = 0.0026) despite of the great size overlap existing between the different species (Fig. 7).

Regarding shape variables, the perMANOVA performed on the first five components of the PCA (explaining 77% of variance) also showed significant differences among species (*p*-value = 0.003). The plot of the first three components showed that, although most of the species presented an extensive overlap (Fig. 8), three species occupied a distinct portion of the morphospace. These were "sp 6", "sp 7", "sp

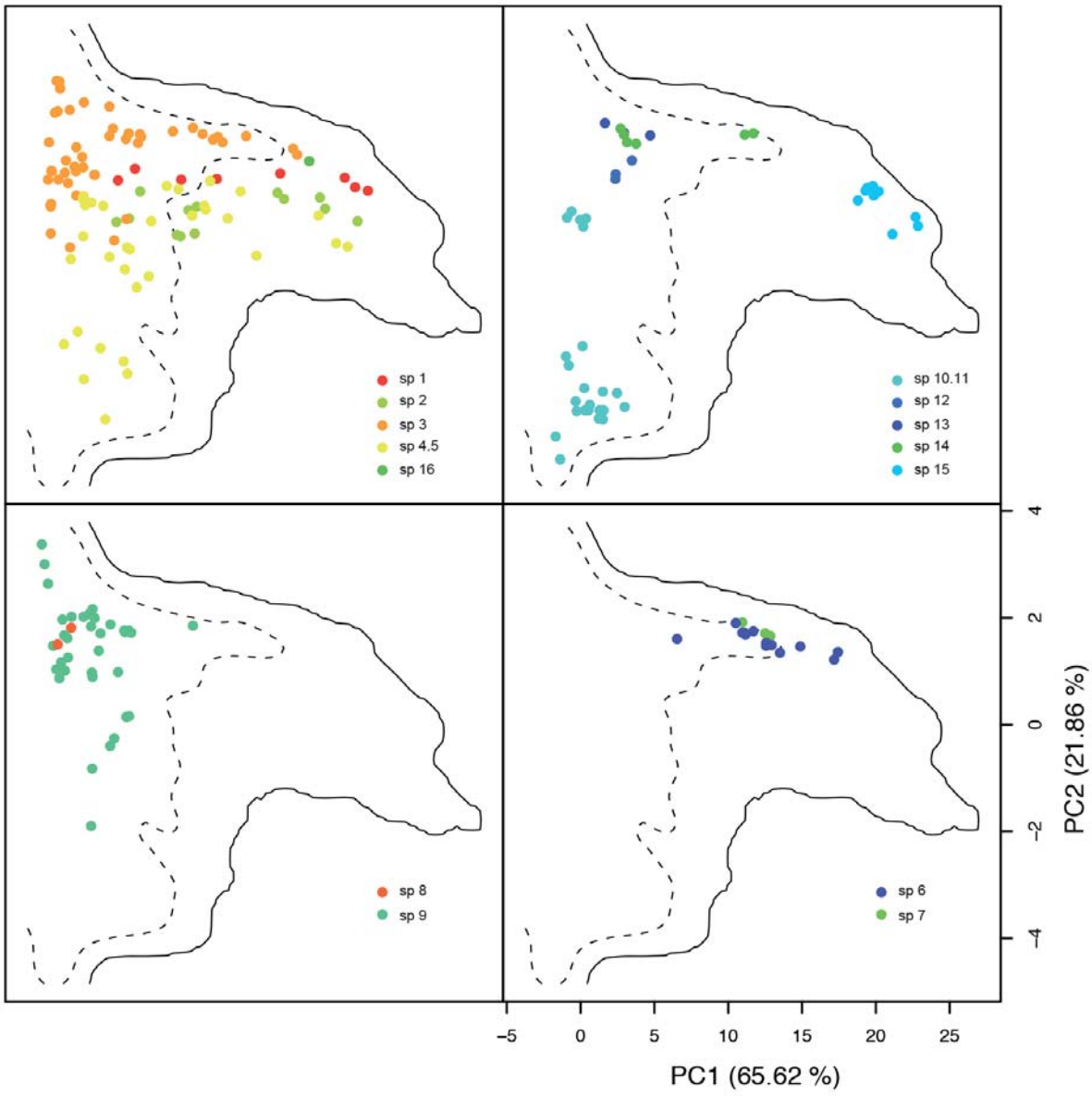

Figure 5. Relative positions of the climatic envelopes of all the species existing within *P. r. rupestris*. Environmental space is defined from a PCA performed on altitude plus 19 climatic variables (Bio 1-19). The climatic space provided by the region is represented by the solid and dashing contour lines, illustrating, respectively, 100% and 50% of the background climatic space.

14" and "sp 16" and presented comparatively more robust morphologies, presenting disproportionally wider and higher heads, wider scapular and pelvic girdles (variation along PC1), shorter legs (variation along PC2) and shorter axilla-groin lengths (variation along PC3) (Table S2). The visualization of the phylomorphospace showed how this morphology was acquired by three clades independently (Fig. 8). Interestingly, these species presenting robust morphologies were also the ones reaching high altitudes according to the phyloclimatic space (Fig. 6). This observation was validated by our regression analyses between the different axes of morphological variation and the axes of climatic variation. According to these, the morphological PC1 and PC3 were highly correlated with the climatic PC1 (p-values = 0.06 and 0.0009 respectively), reflecting a possible association between a robust phenotype and altitude (Fig. 9).

The computed Brownian rates of body size evolution were extremely low (at a mean of 7.07e-05). This contrasted with the rates computed for shape variables that showed means ranging from 0.13 to 0.18. Phylogenetic signals, in both size and shape, ranged from 0.40 to 0.45 and in all cases were not significantly different from the value expected under the null model (with *p*-values ranging from 0.10 to 0.19).

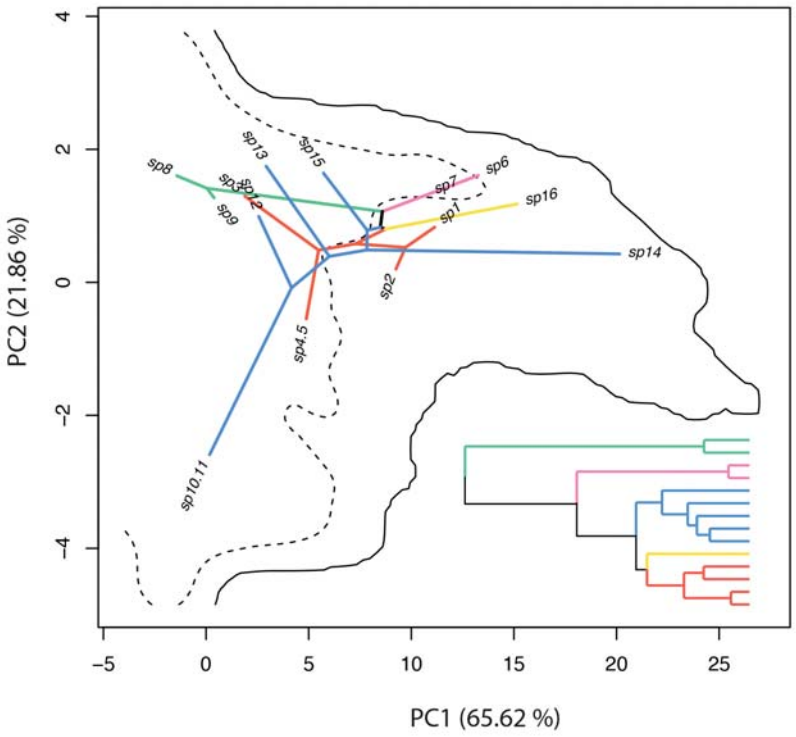

Figure 6. Phylogeny projected into the bivariate climatic space using the mean values of each of the species and the reconstructed values at the nodes (phyloclimatic space). The different colors shown along the branches correspond to the main clades existing in *P. r. rupestris*.

SPECIES DIVERSIFICATION

The comparison of the six alternative diversification models fitted on the summary species tree highlighted Model 2 as the best supported model (AICw = 0.61) (Fig. S4). According to this model, diversification is described as a pure birth process with constant speciation (with an estimated speciation rate of 0.20). The two other best supported models were Model 1 and Model 6 (both with AICw = 0.16). Model 1 implies a birth and death model with constant speciation and extinction (with speciation and extinction rates computed at 0.25 and 0.08 respectively). According to Model 6, diversification follows a pure birth process with varying speciation, with a speciation rates computed at 0.24 and a parameter of exponential variation estimated at -0.02 (speciation rate increasing exponentially through time). Overall the three most supported models agree in that diversification in *P. r. rupestris* is in its expansive phase, with constant or increasing speciation rates.

DISCUSSION

Our study exposed the greatest diversification of a vertebrate so far discovered in the mountains of Arabia, with 14 highly divergent species previously thought to be part of the same subspecies, *Pristurus*

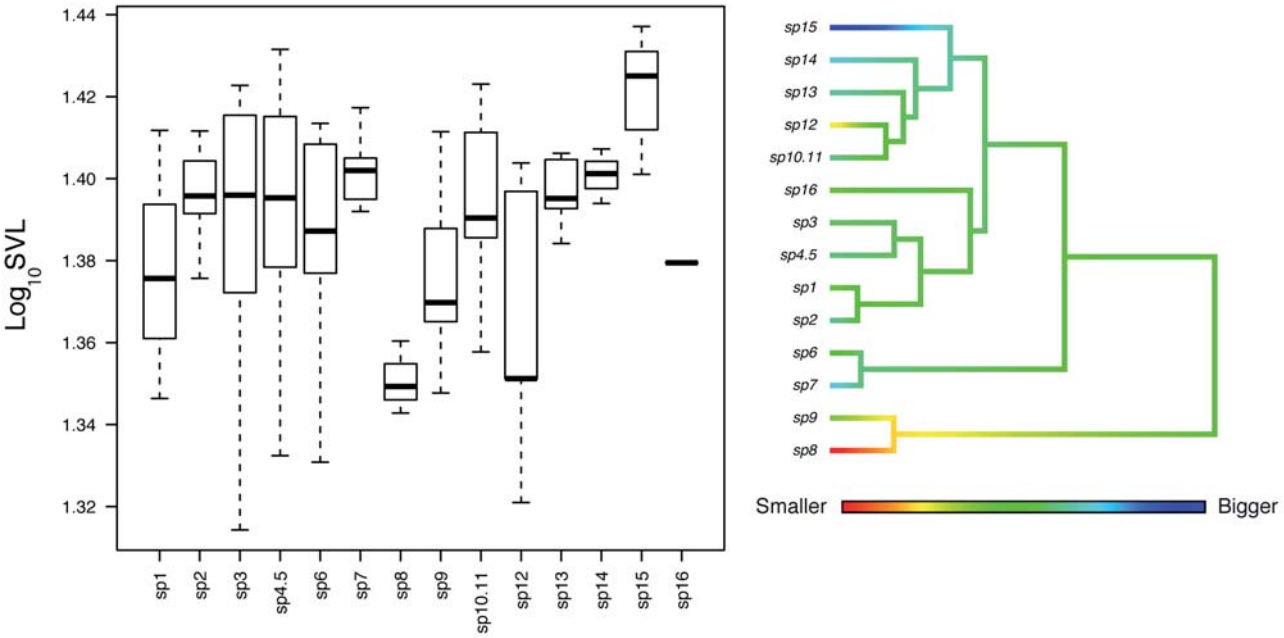

Figure 7. (Left) Boxplot showing the log_{10}SVL variation existing within *P. r. rupestris*. (Right) Visualization of the size variation across the species tree. This is based on a size reconstruction along the nodes and branches of the tree as implemented by the Method 2 described in Revell (2013).

r. rupestris. This constitutes a prominent example of the unknown diversity still to be discovered in the frequently overlooked arid regions of the Planet. In these regions, mountain ranges likely act as diversity reservoirs, as many species benefit from the mild climatic conditions provided by high altitudes in an otherwise torrid environment (Sanders et al., 2003; McCain, 2007). One of the most important effects of mountain ranges is their topographic complexity, offering great chances of range fragmentation, particularly during mountain orogeny (Antonelli et al., 2009). Although the association between orogeny and diversification has been detected in a number of groups (Liu et al., 2006; Bunce et al., 2009; Antonelli et al., 2009), it does not seem to be the case for the species forming *P. r. rupestris*. Although the major uplift of the mountains began during the Oligocene and continued through the Miocene (Glennie et al., 1974) most of the diversification events took place in the last 10 Ma (particularly peaking in the last 6 Ma), therefore postdating the major orogenic phase of the mountains. Consequently, most of the diversification existing in *P. r. rupestris* took place once the Central and Southern blocks were already uplifted and isolated by the Semail gap (Glennie et al., 1974).

In these circumstances, a plausible scenario could imply the existence of founder events in each of the mountain blocks followed by intra-mountain diversification. The strong environmental gradients offered by mountains may offer chances for adaptive diversification, for example along altitudinal gradients (Kozak & Wiens, 2007; Cadena et al., 2012). However a phylogenetic pattern consistent with this scenario would be the existence of monophyletic species assemblages in each of the mountain blocks and great levels of phylogenetic overdispersion along the altitudinal gradient (low phylogenetic signal).

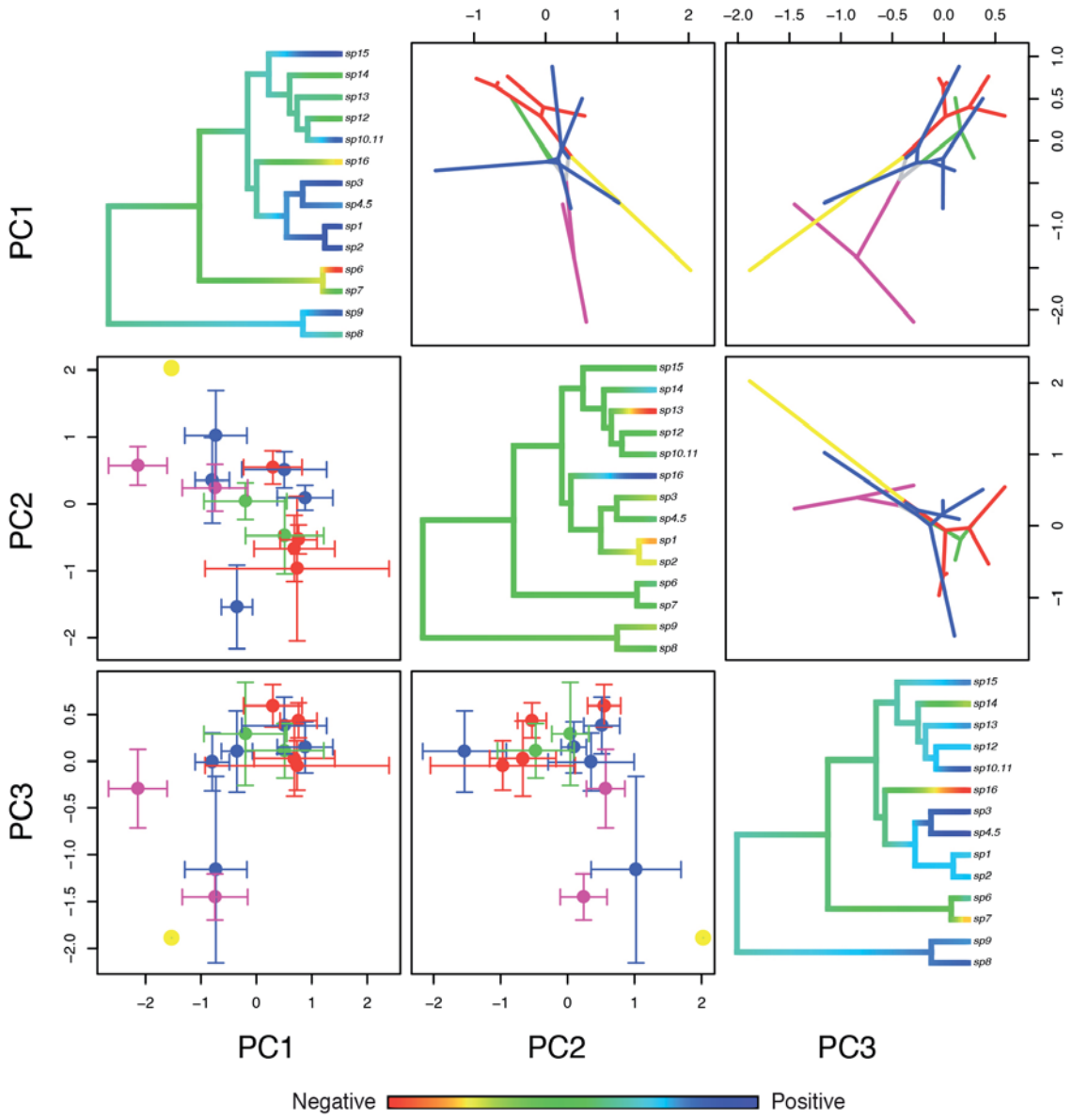

Figure 8. Visualization of the morphospace occupied by the 14 species existing within *P. r. rupestris*. This morphospace is defined by the three first components of a PCA performed on 10 body measurements. The upper-right portion of the panel represents the phylomorphospace derived from mean scores of each species and the reconstructed values at the nodes. The lower-left portion provides a visualization of the intra-specific variation of each species with a plot of the mean scores and associated standard errors on the PC axes. The trees in the diagonal show the residuals of each shape dimension reconstructed along the nodes and branches of the summary tree using the Method 2 described in Revell (2013). Species belonging to different clades are highlighted in different colors (following the color code used in Fig. 6).

Our results do not point into this direction, in fact show how species assemblages within each of the mountain blocks are highly polyphyletic, implying the existence of frequent movements between the different mountain blocks. This pattern is also supported by the low phylogenetic signal existing along all horizontal environmental gradients (climatic PC2). A possible scenario that could explain the existence of polyphyletic assemblages in each of the mountain blocks involves the interaction between topography and niche conservatism. For instance, topography can isolate two species in the Central and Southern blocks due to the unsuitability existing across lower altitudes. However in a situation of

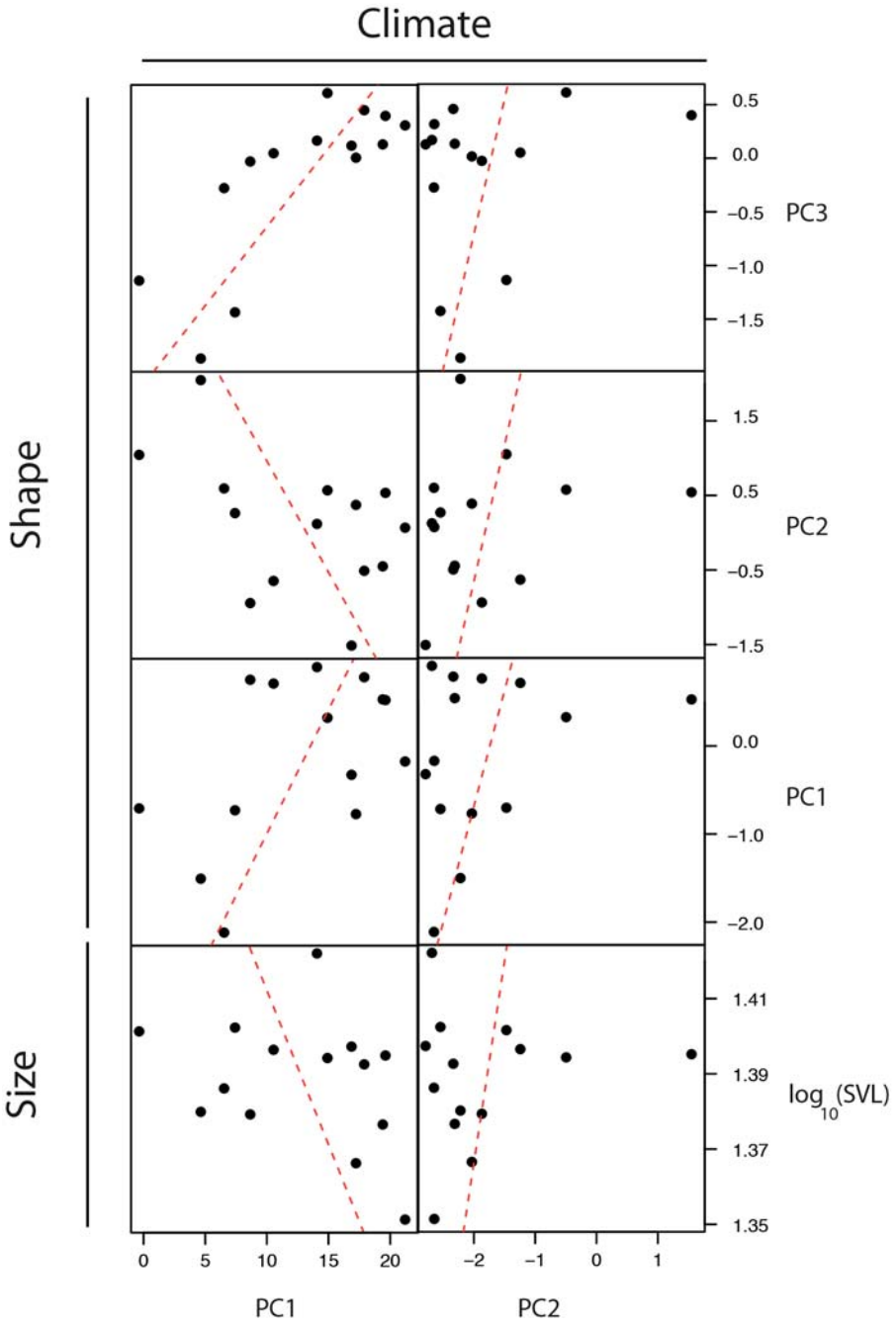

Figure 9. Bivariate plots showing the relationships between morphological and climatic axes of variation. The regression line is shown in each of the bivariate plots.

climatic change (e. g. during glaciations), the niche of both species may expand toward lower latitudes possibly overlapping both mountain blocks. To this, range expansion of both species may follow, potentially producing continuous species ranges between mountain blocks. If overall climatic conditions return to their initial state, ranges might be fragmented separating closely related species in different mountain blocks (see Wiens, 2004; Nosil, 2012). These pulses of range expansion and retraction can potentially produce great amounts of diversification (Wiens, 2004), generating a pattern consistent with our results. However, one of the premises of this mechanism is the existence of niche conservatism in the newly formed species, something not widely shown by all species found by all species forming *P. r. rupestris*. Actually, some of the species present wide climatic envelopes, particularly along the altitudinal

axis, occurring both at high and low elevations. These examples caution that this process might not be the unique mechanism driving diversification in the Hajar Mountains. In any case, the fact that the dynamics of diversification in this group are expansive, points towards a mechanism that might still be active. This is consistent with the fact that the species complex is practically absent from the Nothern block of the Hajars and suggests that the group might still be in an active process of range expansion. Regarding phenotypic diversification, most of the species presented high levels of morphological overlap, suggesting that phenotype was not an important axis of diversification. This can be explained by the low levels of sympatry observed, which reduces the need for resource partitioning between species. Resource partitioning is very often mediated by morphological diversification (Losos, 2009) and therefore low levels of morphological diversification may be a common pattern between strictly allopatric species (Harmon et al., 2008). However, although phenotypic diversification does not seem to obey to interspecific interaction, it might be associated to environmental conditions. Our study detected an association between high altitude environments and a robust morphology. More study is needed to explore this association and shed light on its functional basis.

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SUPPLEMENTARY FIGURES

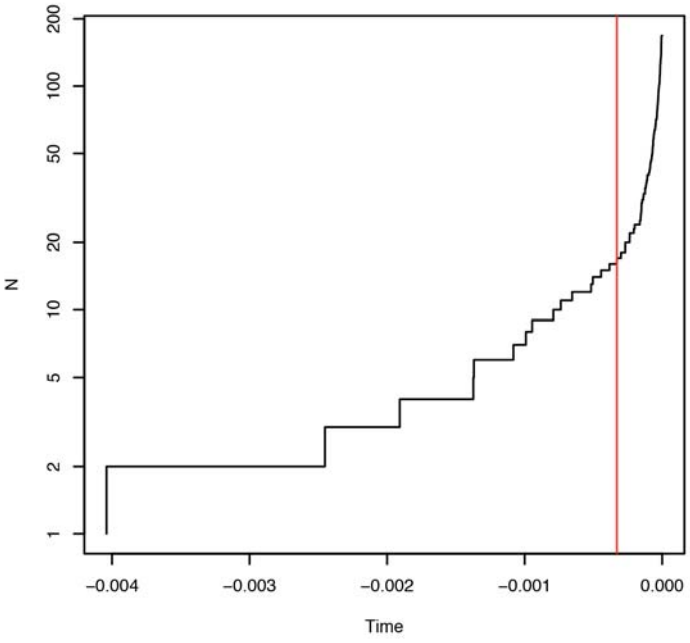

Figure S1. Provisional species delimitation derived from applying the GMYC model to a summary tree spanning 198 unique 12S sequences obtained from 366 specimens. The red clades highlight each putative species according to the model. The lower-left plot shows a lineage through time plot on which the most likely threshold separating a Yule process from a Coalescent process is placed.

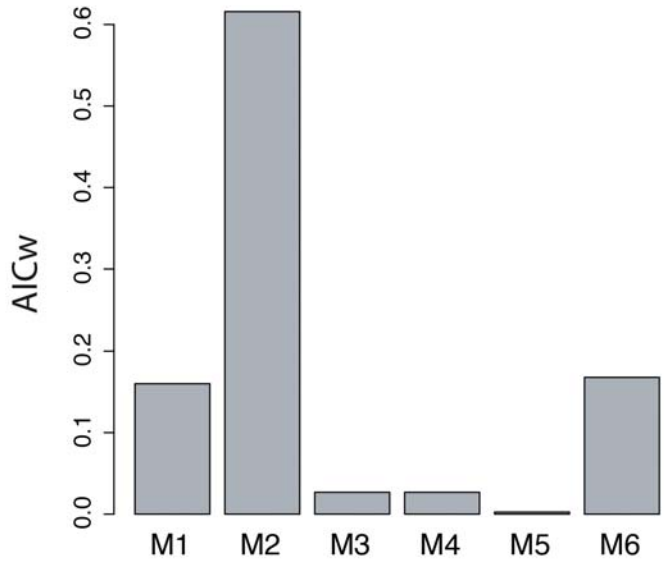

Figure S4. Barplot showing the relative support of each of the six diversification models fitted in our species tree. Supports are based on the computed AICw values.

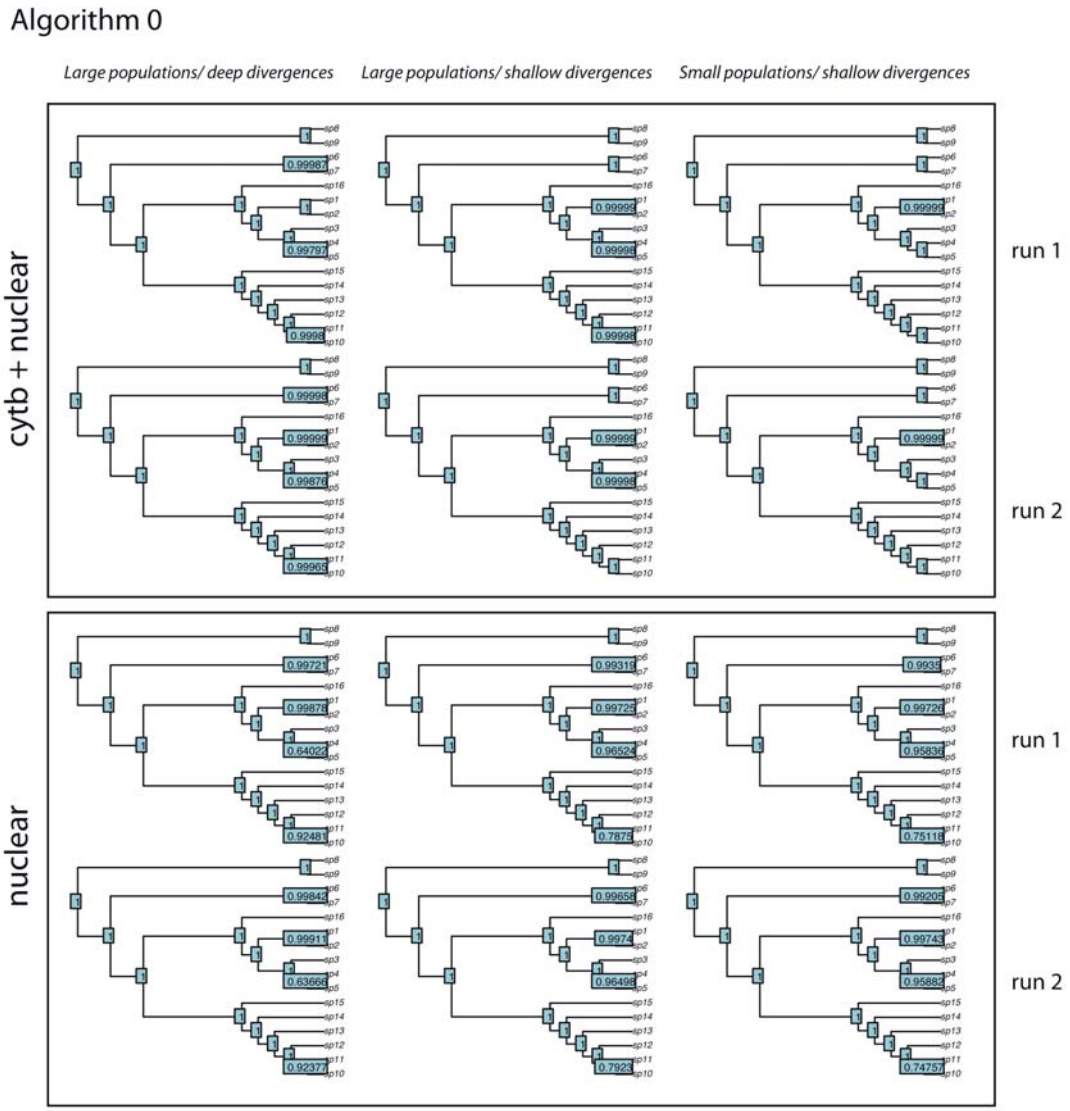

Figure S3. Chart showing the posterior probabilities computed by BP&P for each node based on different molecular evidence and different prior parametrizations. The results shown are for algorithm 0. Algorithm 1 produced almost identical results.

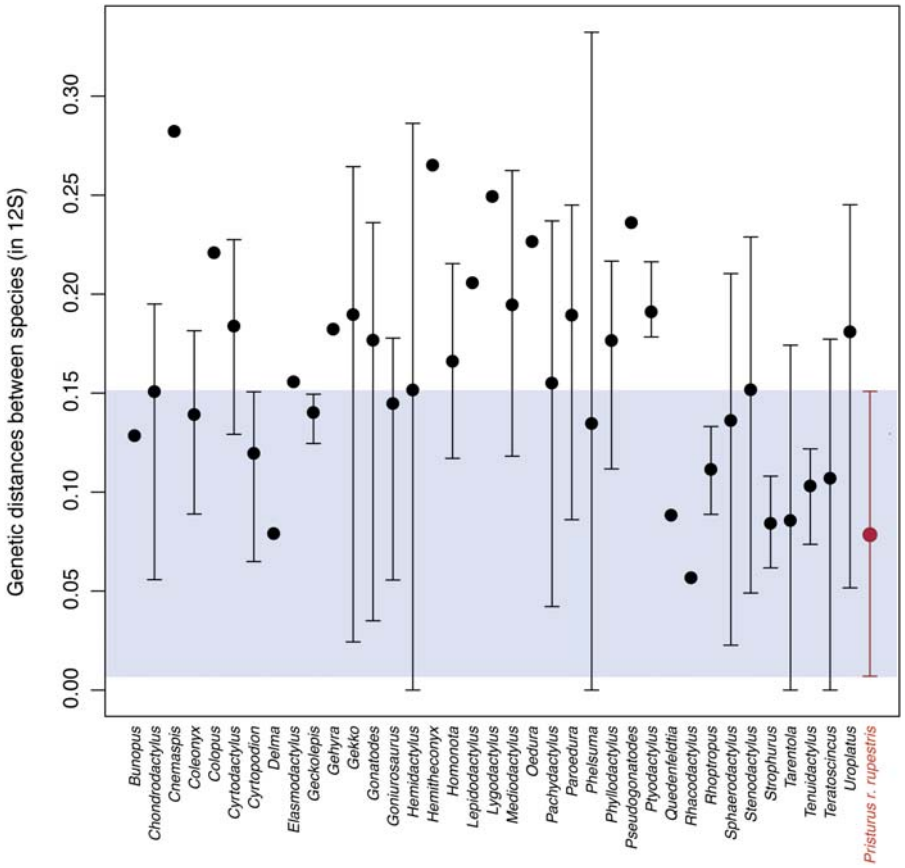

Figure S2. Plot showing the minimum, maximum and mean genetic divergences within *P. r. rupestris* compared to the genetic divergences existing in other genera of geckos. Divergences are calculated as "p-distances" in 12S.

SUPPLEMENTARY TABLES

Variable	PC1	PC2
alt	347,19	-17,65
bio1	-352,26	20,14
bio2	-123,04	-316,06
bio3	-239,62	-162,50
bio4	198,09	-247,71
bio5	-338,82	-100,94
bio6	-310,15	163,21
bio7	20,52	-343,38
bio8	-310,32	97,01
bio9	-264,64	-192,77
bio10	-343,46	-25,23
bio11	-348,49	65,16
bio12	351,58	-7,51
bio13	278,70	-212,85
bio14	287,79	83,72
bio15	-176,42	-290,26
bio16	337,17	-94,73
bio17	331,60	68,88
bio18	318,97	81,13
bio19	273,40	-106,25
Eigenvalue	1688924,83	562782,85
Variance explained (in %)	65,62	21,86

Table S1. Results of the principal components analysis (PCA) on the 19 bioclimatic variables plus altitude. Also shown are the loadings of each original variable on the two firsts principal components (PC1 and PC2) and the proportion of total variation represented by each PC axis.

Variable	PC1	PC2	PC3	PC4	PC5
HL	-0,65	-0,26	-0,33	0,35	0,06
HW	-0,77	0,34	-0,21	0,10	-0,06
HD	-0,80	0,19	0,08	0,15	0,29
SC	-0,78	0,05	-0,19	-0,16	-0,17
PL	-0,69	0,23	-0,23	-0,41	-0,10
AG	-0,29	0,41	0,63	-0,27	-0,34
BL	-0,38	-0,40	0,41	-0,37	0,49
AL	-0,33	-0,51	0,28	0,28	-0,54
TL	-0,09	-0,68	-0,29	-0,48	-0,17
CL	-0,57	-0,31	0,38	0,27	0,13
Eigenvalue	3,41	1,42	1,12	0,94	0,83
Variance explained (in %)	34,06	14,16	11,18	9,39	8,25

Table S2. Results of the PCA on 10 morphometric measurements, showing the loadings of each original variable on the first five componets and the proportion of variance represented by each one.

GENERAL DISCUSSION

Throughout these four chapters we have explored the extent in which island colonization and key innovations, have driven evolutionary diversification in two different continental-island systems and using geckos as model organisms.

From these four studies we can extract some generalities, some of them match with expected theoretical scenarios and others do not. In the following we expose these generalities stressing how they match or disagree with the theoretical expectations and highlighting potential avenues for future research.

ISLANDS AS DRIVERS OF EVOLUTIONARY DIVERSIFICATION

As exposed in the introduction, islands are thought to provide a context of many available resources with few competitors and predators. This allows to colonizing groups the possibility to experience an "ecological release" and use a wider array of niches compared to their continental close-relatives (Losos & Ricklefs, 2009). In such a situation, we expect an expansion of the morphospace in island groups typically associated with high rates of phenotypic and species diversification (Losos & Ricklefs, 2009; Schluter, 2000).

We have found compelling evidence for this in both of the mainland-island systems studied. The Australasian diplodactyloid geckos clearly expanded the range of phenotypic variation existing in the continent, producing the biggest (*Hoplodactylus delcourti* from New Zealand) and the smallest species (*Dierogekko poumensis* from New Caledonia) in the radiation **(Chapter 2)**. Likewise, in the *Hemidactylus* geckos from Arabia-Socotra, island species also produced the most extreme sizes in the radiation with *Hemidactylus forbesii* from Abd al Kuri reaching the biggest size and *H. pumilio* from Socotra reaching the smallest **(Chapter 1)**. In this last mainland-island system, aside of reaching the most extreme sizes, the disparities in insular species assemblages were always significantly greater than the disparities computed by continental species assemblages. All this is consistent with the expected morphospace expansion that follows island colonization as it has been reported for several groups (Gillespie, 2004; Grant & Grant, 2011; Harmon et al., 2010). However, we also showed that not all traits experience similar amounts of phenotypic expansion after island colonization. For instance, in the particular case of the Arabian and Socotran *Hemidactylus*, head proportions showed significantly greater disparities than in the continent only in the island of Abd al Kuri **(Chapter 1)**.

Regarding the rates of phenotypic and species diversification, once again our results entirely matched the theoretical expectations. The colonization of New Caledonia and New Zealand were associated

with significant accelerations in the rates of phenotypic and species diversification compared to their immediate close relatives in the continent **(Chapter 2)**. Similarly, in the Arabian and Socotran *Hemidactylus*, the highest computed rates of size diversification in the radiation were detected after the colonization of Abd al Kuri and at the onset of the diversification in Socotra **(Chapter 1)**. Again, this pattern was not replicated for head shape, which always presented rates in the range of those computed in the continent. These differences in the evolutionary patterns exhibited for different traits likely reflect their relative importance at partitioning resources. The pattern found in *Hemidactylus* and in the diplodactyloid geckos of Australasia suggests that size variation is likely one of the major axis of resource partitioning on these islands. This has also been reported in other island taxa and it has been hypothesized that this allows partitioning across different prey sizes (Losos, 2009; Moen et al., 2009). Additional evidence on the diets of the different species would be extremely important to confirm this interpretation.

Nonetheless, when we compare *Hemidactylus* to other groups diversifying in the same islands, it appears that not all groups equally tend to diversify in body size. In fact, the comparison of the stages of diversification between the three gecko genera occurring in the Archipelago of Socotra revealed that not a single path of intra-island diversification was shared by all genera **(Chapter 3)**. *Hemidactylus* and *Haemodracon* greatly diversified in body size. However, in *Pristurus* diversification was strongly mediated by climatic shifts involving very low size diversification. This is an important result as suggests that not all groups respond in the same way to similar amounts of ecological opportunity and that group-dependent (intrinsic) components can potentially play a role at defining the different stages of diversification (Losos, 2010).

One possibility lies on the different morphological or climatic evolvabilities existing in different groups. For instance, in some groups diversifying among different climatic niches may be easier than diversifying in different shapes or sizes. Interestingly, when we compare the rates of body size evolution across the three genera and other geckos we find a situation consistent with different size evolvabilities: *Pristurus*, the genus that mostly diversifies along climatic axes is also the one presenting the lowest computed rates of body size evolution, not only compared to the other Socotran genera, but also compared to other geckos. The functional basis for this apparent low size evolvability in *Pristurus* is not obvious at first sight, and this could be an interesting question to explore in the future, not solely including island species, but extending the analyses to other species in the genus.

It is interesting to remark that these exposed examples of phenotypic, climatic and/or species diversification took place in continental islands, all of them once part of the continent. This demonstrates that continental islands have more in common with oceanic islands than with the continents they originate from. In the case of New Zealand and New Caledonia this is in line with the evidence that suggests

that these islands were partially or totally submerged during a long period of time (Trewick et al., 2007; Espeland & Murienne, 2011). Is therefore conceivable that they were completely recolonized after their re-emergence (as typical oceanic islands). This is also consistent with our dating estimates, which place the age of the onset of the diversification in New Caledonia and New Zealand shortly after the most likely moment of re-emergence of both islands **(Chapter 2)**. However, in the case of the Socotra Archipelago at least some lineages existing in the archipelago can be traced back to the continental-island detachment event **(Chapter 3)**. This implies that this archipelago behaves biogeographically as oceanic despite of likely having been originated as an ecologically filled continental fragment.

In all islands studied (Abd al Kuri, Socotra, New Caledonia and New Zealand), intra-island diversification (as opposed to vicariance or dispersal events) has had the most important role at generating the diversity in the islands **(Chapter 2, Chapter 3)**. A key feature that may have contributed to this situation on all these archipelagos is their isolation. New Zealand and New Caledonia are very far from their closest mainland (Australia) and diplodactyloid geckos have dispersed into these islands (at least successfully) only in the colonization events. In the case of the Archipelago of Socotra, at first sight, its geographic closeness to Africa and Arabia seems to indicate that these islands are not isolated. However, only two unambiguous dispersal (at least successful) events occurred from the continent and not a single dispersal event took place between the two main islands in the archipelago **(Chapter 3)**. This outlines a situation of great isolation in these islands and this could have contributed to explain their great levels of *in situ* diversification (Losos & Parent, 2009). More research on the dynamics of diversification of other groups in the archipelago will be crucial to shed light on this and confirm whether this "isolation" of the Socotra Archipelago is also detected in other groups.

In both island-mainland systems, our analyses provided compelling evidence that island colonization played a key role at producing great amounts of evolutionary diversification. However the studies presented in this thesis also provide examples of high rates of phenotypic diversification in some groups in the continent. This is the case for the Australasian Pygopodidae, which attained rates of phenotypic diversification (possibly species diversification too) comparable to insular groups **(Chapter 2)**. This was also the case of a small clade of recently evolved continental species in the *Hemidactylus* radiation **(Chapter 1)**. Another remarkable example of continental diversification revealed in this thesis is the hidden diversity found within the "subspecies" *Pristurus r. rupestris* **(Chapter 4)**. This diversification took place in a truly “island-like” setting, the Hajar Mountains in southeastern Arabia, formed by three main isolated blocks or “sky islands”. However, in this case diversification failed to take place within each of the “islands” and mostly was driven through intermittent pulses of dispersal and isolation taking place between the two main mountain blocks. Examples like this provide an example of how continental groups in some contexts, as the ones offered by mountain ranges, can fuel substantial amounts of diversification. In this case the greatest vertebrate diversification in Arabia.

KEY INNOVATIONS AS DRIVERS OF DIVERSIFICATION

Along the chapters that form this thesis, when possible, we have integrated the effects of other sources of ecological opportunity with the effects of island colonization. Given that toepads and the snake-like phenotype are found in the geckos of Arabia, Socotra and Australasia, we used this chance to explore the contributions (and interactions) of key innovations and island colonization at producing evolutionary diversification. According to our results, only one of the innovations examined can be compared to the effects caused by island colonization. This is the acquisition of the snake-like phenotype in the Australasian geckos **(Chapter 2)**. The high rates detected in the Australasian Pygopodidae are likely adaptive, consequence of their remarkable innovation consisting in the snake-like phenotype. Aside of the evidence provided by the rates of evolution, this interpretation is also supported by the ancestral state reconstructions. These placed the origin of the snake-like phenotype before the arrival of the snake-like groups nowadays inhabiting Australia, suggesting that these snake-like creatures evolved in a context of low competition. This contrasts with our results regarding the evolutionary effects of toepads. According to our analyses conducted on the Australasian diplodactyloid geckos, toepads, despite being a paradigmatic example of key innovation, did not have a major role at producing higher rates of phenotypic and species diversification. This is congruent with evidence provided by a recently published study that tackled this question using a global dataset (Gamble et al. 2012).

However, this contrast with the picture that emerges from our comparisons between different genera evolving within the Socotra Archipelago: the two padded genera (*Hemidactylus* and *Haemodracon*) presented significantly higher rates of phenotypic evolution than the species that lacked toepads (the genus *Pristurus*) **(Chapter 3)**. These two apparently contradicting pieces of evidence suggest that the effects of key innovations might depend on the particular taxonomic group or ecological context. A new study exploring rates of phenotypic evolution using comprehensive datasets (including many groups of geckos and different ecological settings) will be crucial to shed light on this matter.

In either case, the fact that in all mainland-island systems studied in this thesis, padded groups were able to reach islands raises a cautionary point: island colonization (or the invasion to new environments in general) can constitute an important confounding factor in the studies that explore the effects of toepads (or any other key innovation) on evolutionary diversification. Indeed, if in the Australasian geckos we had pooled continental and island species into the same toepad category, the effect of island colonization would have inflated the rates of evolutionary diversification in this category supporting a link between the evolution of a key innovation and subsequent evolutionary diversification. Therefore, potential interactions or synergies between different sources of ecological opportunity might obscure the contribution of each one in the total evolutionary diversification experienced in a given group. This is something that should be taken into account in any future study focusing on the effects of ecological opportunity on evolutionary diversification.

CONCLUSIONS

1- The genus *Hemidactylus* in the Socotra Archipelago and the diplodactyloid geckos in New Caledonia and New Zealand expanded the range of body size variation existing in their continental close relatives, reaching the maximum and the minimum sizes of the radiations in both geographical settings. This is therefore consistent with the expansion of the morphospace that is expected to follow island colonization, given the context of low competition and predation provided by islands.

2- Rates of species diversification (in the diplodactyloid geckos of New Caledonia and New Zealand) and rates of body size evolution (in the diplodactyloid geckos of New Caledonia and New Zealand and in the *Hemidactylus* geckos of the Socotra Archipelago) were generally accelerated compared to most of the continental groups. This is an expected outcome of the diversification in islands where insular groups tend to rapidly occupy the new adaptive landscape that islands provide.

3- In the *Hemidactylus* of the Socotra Archipelago, not all traits experienced equal rates and magnitudes of diversification. While body size involved the greatest acceleration and disparification (presenting significantly greater disparities compared to continental groups), head shape solely presented a similar pattern in Abd al Kuri. This shows how the patterns of evolution of different traits can be decoupled in the same group and in the same island.

4- Within the Australasian diplodactyloid geckos, the acquisition of the snake-like phenotype by the family Pygopodidae took place before the arrival of most of the species of snake-like reptiles living nowadays in Australia and triggered accelerated rates of body size (and possibly also species) diversification.

5- Within the Australasian diplodactyloid geckos, adhesive toepads, despite of being a paradigmatic example of key innovation, failed to induce accelerated rates of phenotypic or species diversification compared to padless species. Examples like this demonstrate that the acquisition of a key innovation, while enabling a new interaction with the environment, not necessarily induce high levels of evolutionary diversification

6- The invasion of new environments can act as a confounding factor in studies that seek to explore the evolutionary effects of the acquisition of key innovations. Comparative methods aimed at recognizing patterns of diversification not depending on any pre-conceived partition of clades (like "auteur") are crucial to avoid the effects of interactions and synergies between different sources of ecological opportunity.
7- The diversity of the endemic geckos of the Socotra Archipelago involved five independent phylogenetic origins and was mostly generated through *in situ* diversification, with dispersal and vicariance presenting a minimal contribution.

8- According to our dating estimates, the deep-most split within the *Pristurus* clade of Socotra took place before the island was completely detached from the continent. Examples like this cautions that not all nodes separating island species in a phylogeny are necessarily the consequence of intra-island events of speciation.

9- According to our dating estimates, the split that separates the island genus *Haemodracon* from the continental *Assacus*, predates the moment in which the Socotra Archipelago detached from Arabia. This and other examples caution that a mainland-island split not necessarily involves a vicariant or dispersal event.

10- Not all three genera of geckos existing in the Socotra Archipelago evolved into the same stages of diversification. While in *Haemodracon* and *Hemidactylus*, most of the intra-island diversification was mostly mediated by body size diversification, in *Pristurus* most of the intra-island diversification was mediated through climatic diversification without substantial amounts of body size differentiation. This shows how different groups may substantially differ in their patterns of macro and microniche structuration in the same archipelago and calls into question the existence of a general theory of island diversification applying to a wide range of groups.

11- The species *Pristurus rupestris* forms a polyphyletic clade with two highly divergent lineages. One inhabiting coastal Iran and the Hajar Mountain range in northern Oman and eastern UAE (eastern lineage), another distributed from coastal middle Oman, through Yemen, Saudi Arabia and up to southern Jordan (western lineage). Therefore *P. rupestris* needs to be split in two species: the proper *P. rupestris* in the east and a new species in the west that we refer to it as *Pristurus* sp 1.

12- The "subspecies" *Pristurus r. rupestris* forms in the Hajar Mountains of southeastern Arabia a species complex with 14 highly divergent species that started to diversify in the region around 15 Ma. This is the greatest diversification so far described for a vertebrate in Arabia and demonstrates that diversity in arid regions such Arabia may be still be very underestimated.

13- The 14 species forming *P. r. rupestris* make two highly polyphyletic assemblages in the Central and Southern blocks of the Hajar Mountains. These are likely the consequence of several pulses of range expansions and retractions involving the two mountain blocks.

14- High altitudes (above 1500 m) were reached at least four times independently within the species complex existing in *P. r. rupestris*. Three of these produced a "robust" phenotype consisting in disproportionally big heads and longer axilla-groin lengths. This suggests a plausible correlation between phenotypes and the altitudinal gradient in the Hajar Mountains.

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RESUMEN DE LA TESIS

INTRODUCCIÓN

La diversidad de formas de vida es espectacular, no sólo en términos de número de especies, sino también en términos de colores, tamaños, formas, historias vitales e incluso metabolismos diferentes. La contemplación de esta belleza ha inspirado a muchas generaciones de naturalistas, filósofos y científicos desde el inicio de nuestra civilización, impulsando su curiosidad ya natural en la tarea de entender cómo surgió toda esta variedad de formas.

Este empeño dio un gran paso hacia delante el 24 de Noviembre de 1859 con la publicación del Origen de las Especies. En este libro, Charles Darwin propuso un mecanismo que revolucionó su tiempo: la teoría de la evolución por medio de la selección natural. Darwin sostenía que las distribuciones de los caracteres en las poblaciones naturales cambian a lo largo del tiempo, dado que los individuos con las combinaciones de caracteres mejor adaptadas al ambiente tienden a reproducirse a una tasa más alta que aquellos individuos con caracteres no tan bien adaptados (en el caso de que estos caracteres sean heredables) (Darwin, 1859). De esta idea deriva un simple pero potente razonamiento: si diferentes poblaciones dentro de una especie están expuestas a diferentes ambientes, la combinación particular de caracteres que maximice la eficacia biológica en cada uno de los ambientes puede no ser la misma. Esto podría generar presiones selectivas divergentes existentes entre diferentes ambientes que, a su

vez, podrían inducir una diferenciación fenotípica entre las poblaciones. (Mayr, 1942; Huxley et al., 2010). George G. Simpson trabajó posteriormente a fondo en esta idea, y proporcionó un concepto simple en el cual estas presiones selectivas divergentes podría ser fácilmente visualizadas: el paisaje adaptativo. En este concepto, inspirado por los paisajes adaptativos de Wright para frecuencias génicas (Wright, 1984), la eficacia biológica se visualiza como la altura en una superficie que varía en función de los valores de dos o más caracteres fenotípicos (Simpson 1944; 1965). De esta manera, la combinación de los valores de los caracteres que determinan una elevada eficacia biológica se visualizan como "cumbres", mientras que los valores que los valores que caracteres que determinan una baja eficacia biológica son visualizados como "valles". En tal paisaje formado por cumbres y valles, las poblaciones divergen porque son empujadas hacia cumbres adaptativas diferentes y repelidos de los valles de menos eficacia biológica. Desde una perspectiva ecológica, cada uno de estos picos puede ser visto como una combinación particular de valores de caracteres que permite un uso eficiente de un nicho en particular (Schluter, 2000).

En ocasiones, la naturaleza expone grupos a una gran variedad de nichos vacíos, que pueden ser visualizados cono paisajes adaptativos con muchas cumbres adaptativas desocupadas (Martin y Wainwright, 2013). En un posible escenario derivado de esta situación, los regímenes selectivos divergentes que operan en el paisaje adaptativo pueden rápidamente empujar las diferentes poblaciones a diferentes cumbres adaptativas produciendo divergencias fenotípicas (y genéticas) entre las poblaciones. Asimismo, en este este escenario, la especiación ocurre como un efecto colateral de la divergencia adaptativa (Nosil, 2012).

De esta manera, la nueva disponibilidad de nichos o en las palabras de Simpson: "oportunidad ecológica", puede, teoréticamente, generar niveles elevados de diversificación fenotípica y de especies. De hecho, la oportunidad ecológica se considera como uno de los motores más importantes de diversificación en muchos de los ejemplos más notables en radiaciones adaptativas, desde los pinzones de Darwin a la gran diversidad existente de cetáceos (Schluter 2000; Slater et al. 2010; Grant y Grant 2011a).

Desde una perspectiva dinámica, este proceso predice diferentes fases que difieren en su ritmo de diversificación. Las tasas de diversificación fenotípica y de especies se predicen como máximas al comienzo de la diversificación cuando los grupos están en el proceso de ocupar rápidamente todo el espacio disponible y, a partir de ahí, tienden a disminuir a medida que la disponibilidad de nichos disminuye progresivamente. Dicha aceleración en las tasas evolutivas al comienzo de la diversificación por oportunidad ecológica se conoce como "explosión temprana", fenómeno muy común en muchas radiaciones adaptativas (Schluter, 2000; pero ver también Harmon et al., 2010).

Simpson propuso 3 escenarios diferentes en los cuales altos niveles de oportunidad ecológica podrían teóricamente llevar a altos niveles de diversificación evolutiva (Simpson, 1944; 1965). Estos escenarios son los siguientes:

1. **Extinción de antagonistas**. Esto ocurre cuando un número de nichos se vuelven disponibles después de la extinción de las especies que los ocupan. Este es el típico caso que ocurre tras un evento de extinción masiva en el cual un gran número de nichos pasan a estar de repente vacíos. En estas situaciones, los grupos supervivientes normalmente ocupan de manera muy rápida las vacantes ecológicas, produciendo niveles espectaculares de diversidad fenotípica y de especies.
2. **Exposición a nuevos ambientes**. Esto puede ser bien la consecuencia de la dispersión de un grupo a una nueva área o la consecuencia de una cambio en el ambiente donde habita el grupo. El ejemplo más obvio de una diversificación evolutiva producida por nuevos ambientes son las grandes diversificaciones que siguen a la colonización de islas (Losos y Ricklefs, 2009).
3. **Adquisición de una innovación clave**. En su definición más antigua, una innovación clave es un carácter que permite una nueva interacción con el ambiente. Muy a menudo, esto garantiza el acceso a número de nuevos, anteriormente inaccesibles, nichos que, a su vez, pueden desencadenar tasas elevadas de diversificación fenotípica y de especies. Uno de los ejemplos más destacables de las espectaculares diversificaciones que siguen a la adquisición de una innovación clave, es la diversidad de aves derivada de la adquisición de plumas por los dinosaurios terópodos (Hunter, 1998).

El principal objetivo de esta tesis es examinar las dos últimas fuentes de oportunidad ecológica - específicamente, la colonización de islas y la evolución de innovaciones clave - y explorar la magnitud en la que esto conduce a diversificación fenotípica y de especies en diferentes contextos taxonómicos y geográficos. A continuación, desarrollaré cómo, a nivel teórico, estas dos fuentes de oportunidad ecológica pueden potencialmente generar diversificación evolutiva.

ISLAS COMO IMPULSORES DE DIVERSIFICACIÓN EVOLUTIVA

Las islas son ampliamente conocidas por sus espectaculares diversificaciones. Las diversidades tan increíbles en los mieleros hawaianos o en los pinzones de Galápagos son ejemplos de los tipos de diversificaciones que ocurren después de la colonización de una isla (Losos y Ricklefs, 2009). La idea de oportunidad ecológica está íntimamente unida al nexo entre colonización de islas y diversificación evolutiva. La oportunidad ecológica deriva del gran número de vacantes ecológicas existentes en las primeras fases de la evolución de una isla (Whittaker et al., 2010). En este contexto de muchos recursos disponibles con pocos competidores, los grupos colonizadores tienden a usar un conjunto amplio de nichos, mas amplio que el que podrían acceder en el continente. Esto se debe a que en las islas, las especies pueden usar recursos nuevos, recursos que en continente serían ocupados por otras especies (fenómeno conocido como “liberación ecológica”; Yoder et al., 2010). Una de las consecuencias de esta expansión de nicho es que las especies pueden también experimentar un aumento de la heterogeneidad de regímenes selectivos que actúan ellas.

Tal y como se ha expuesto anteriormente, estos diferentes regímenes selectivos actuando en poblaciones diferentes dentro de la misma especie pueden potencialmente inducir divergencia fenotípica que puede llevar a la especiación (Schluter, 2000).

Sumado a la ausencia de competidores, la ausencia de depredadores es también un aspecto clave para explicar las diversificaciones en islas. Los depredadores en el continente normalmente mantienen a sus presas a unos niveles de densidad baja (Millien, 2011) y, muy a menudo, imponen una fuerte selección estabilizadora en muchos de los caracteres de sus presas (Yoder et al., 2010). Por ejemplo, en el continente, las especies de presas pueden tender a evolucionar hacia un tamaño corporal óptimo que maximizan las oportunidades de escaparse o esconderse de los depredadores. En islas, debido a la baja densidad y diversidad de depredadores, las presas se liberan de repente de estas limitaciones y, de esta manera, pueden diversificar en una gran variedad de fenotipos (como por ejemplo aumentando sus tamaños comparándolos con sus parientes en el continente) (Yoder et al., 2010).

Asimismo por la ausencia de depredadores (en combinación con la ausencia de competidores), las especies en las islas suelen presentar densidades poblacionales más altas comparándolos con el continente, fenómeno conocido como "compensación por densidad" (Case 1975; Bennett and Gorman, 1979; Rodday Bradley, 2002; Buckley y Jetz, 2007). En tales situaciones, selección disruptiva puede aparecer en el caso de que los fenotipos intermedios compitan más fuertemente por los recursos que aquellos fenotipos situados en las colas de la distribución. Esto resulta en una eficacia ecológica más baja en los fenotipos intermedios propiciando una expansión de las poblaciones hacia nuevos y menos explotados recursos. De este proceso también se espera una gran divergencia fenotípica, la cual puede finalmente llevar a la especiación (Nosil, 2012).

Todos los procesos explicados anteriormente delimitan un marco teórico que ayuda a explicar por qué las islas presentan ejemplos tan alucinantes de diversificación. No obstante, la evidencia empírica muestra que no todos los grupos de islas experimentan niveles elevados de diversificación adaptativa. Hay ocasiones en las cuales grupos insulares ni siquiera experimentan una mínima diversificación evolutiva. Un ejemplo de esto es el pinzón de Darwin de la Isla de Cocos. Aunque las poblaciones de estos pinzones muestran un nicho claramente expandido en comparación con especies continentales (Wener y Sherry, 1987), estas no han logrado diversificarse en la variedad de eco-morfologías presentadas en sus parientes más cercanos de Galápagos. Estos ejemplos demuestran que en ciertas circunstancias las islas pueden no generar elevados niveles de diversificación evolutiva.

Existen un número de factores que, potencialmente, juegan un papel en determinar si grupos insulares tomarán o no el camino de la diversificación evolutiva. A continuación, paso a introducir los más importantes, separándolos en dos grupos principales: factores extrínsecos, modulados mayoritariamente por la geografía o geología de la isla y factores intrínsecos, determinados mayoritariamente por las características biológicas de los grupos que colonizan las islas.

FACTORES QUE MODULAN LA DIVERSIFICACIÓN EN ISLAS

FACTORES EXTRÍNSECOS

Aislamiento en islas

El nexo teórico entre aislamiento de islas y la riqueza de especies, se remonta a los trabajos de MacArthur y Wilson sobre biogeografía de islas. De acuerdo con su teoría, cuanto menor es el aislamiento, mayor es la tasa de colonización esperada desde el continente y, como consecuencia, mayor es la riqueza esperada en el equilibrio de la isla (MacArthur, 1967).

Asimismo, las islas que están expuestas a un elevado flujo de inmigrantes también limitan las oportunidades de diversificación dentro de la isla (Losos y Parent, 2009). Esto se explica dado que las vacantes ecológicas en la isla serán mas fácilmente ocupadas por especies continentales que ya tengan todas las adaptaciones requeridas para usar de manera eficiente esas vacantes, que por especies generadas a partir de especiación intra-isla, cuyas adaptaciones para usar un nicho en particular van a necesitar tiempo para evolucionar. Es por ello, que se puede decir que, en general, cuanto más aislada esté una isla, mayor es la oportunidad ecológica que ofrece ya que menos competidores potenciales probablemente alcancen esa isla. El aislamiento por si solo puede también juntar fuerzas con la oportunidad ecológica para impulsar diversificación *in situ*: cuanto más aislada esté la isla, más fácil será para los grupos de la isla interrumpir el flujo genético con sus parientes del continente (Losos y Parent, 2009). Esto permite a los grupos de islas la posibilidad de tomar su propio camino evolutivo como un acervo genético completamente independiente e iniciar el camino de la diversificación *in situ*.

Área de la isla

Esta es otra de las características insulares clásicamente investigadas por biogeógrafos. De acuerdo con MacArthur y Wilson, el área de una isla es uno de los mejores indicadores de su riqueza de especies. Esto es porque los grupos habitantes de islas más grandes tienden a experimentar menores tasas de extinción, permitiendo así valores de riqueza de especies mas elevados en el equilibrio de la isla (MacArthur y Wilson, 1963). Recientes estudios han demostrado que el área de una isla, no solo es un buen predictor de su riqueza de especies, sino también es un buen predictor de la capacidad que los grupos insulares tienen de diversificar *in situ* (Steppan y Zawadzki, 2003; Gillespie, 2004; Parent y Crespi, 2006; Losos y Parent, 2009). Esto se demostró por primera vez en los *Anolis* del Caribe (Losos y Ricklefs, 2009) donde se sugirió que la relación entre el área y la diversificación resultan fundamentalmente de un aumento en la tasa de especiación con el área más que en una disminución de la tasa de extinción. Pero, ¿cómo un aumento del área puede hacer aumentar la diversificación? Existen diferentes teorías para explicar esto, aunque no todas requieren de un componente adaptativo. Por ejemplo, el potencial

para especiación alopátrica puede aumentar con el área de la isla (no necesariamente implicando diversificación adaptativa). Pero también el área de la isla está correlacionada con la heterogeneidad ecológica (revisado en Ricklefs y Lovette, 1999; Whittaker y Fernandez-Palacios, 2007). A su vez, esta puede aumentar el espacio del nicho disponible en islas más grandes aumentando también las posibilidades de diversificación adaptativa. Encontramos un ejemplo de esto en los caracoles bulimúlidos en las islas Galápagos. En estos, la especiación in situ esta correlacionada con el área, pero está aún más correlacionada con la diversidad de la vegetación, una aproximación a la diversidad de nicho (Losos y Parent, 2009)

Edad de la Isla

La edad de una isla se ha demostrado que es uno de los más importantes moduladores de diversificación *in situ* (Losos y Parent, 2009). La edad puede estar relacionada con la diversificación de una isla en dos principales (y opuestas) maneras. En primer lugar, las islas más antiguas normalmente tienden a tener también grupos más antiguos, los cuales sencillamente han tenido mas tiempo para diversificar *in situ* (Heaney, 2000). Esto se demuestra empíricamente en estudios que, por ejemplo, sugieren una asociación positiva entre la edad de una isla y los niveles de diversificación *in situ* (Sequeira et al., 2008; Losos y Parent, 2009). No obstante, la edad de una isla también puede tener un papel en contra de la diversificación. Esto es porque la oportunidad ecológica proporcionada por una isla puede disminuir con el tiempo, ya que las fuerzas de erosión tienden a simplificar su topografía y reducir su área (Whitaker et al., 2008). Una evidencia de una relación negativa entre la edad y la diversificación es el caso de los escarabajos *Tarphius* de las Islas Canarias.
En estas especies, la mayor parte de la diversificación ocurre en las islas de edad intermedia y disminuye en las islas más antiguas (Emerson y Oromí, 2000).

Origen geológico de las islas

En cuanto a su origen geológico, las islas se pueden clasificar en dos amplios grupos: islas oceánicas e islas continentales. Las islas oceánicas están originadas típicamente por procesos volcánicos (normalmente implicando a la corteza oceánica) y genera masas de tierra aisladas del continente por océano. Por el contrario, las islas continentales típicamente se originan o bien por haber estado unidas al continente en el pasado por descensos del nivel del mar (islas de puente de tierra) o bien por formar fragmentos continentales que se han separado del continente por procesos tectónicos (fragmentos continentales) (Whittaker y Fernandez-Palacios, 2007). Ambos tipos de islas presentan importantes diferencias en cuanto a los procesos que generan sus diversidades. En las islas oceánicas, la diversidad aparece *de novo* por la interacción de dos procesos: dispersión desde el continente (u otra isla) y/o diversificación

in situ. Sin embargo, en las islas continentales, a parte de estos dos procesos biogeográficos, parte de la diversidad puede haber sido pasivamente heredada desde el estado continental de estas islas, cuando parte de la diversidad continental quedó aislanda en las islas en el momento de la separación continente (vicarianza) (McDowall, 2004). En este caso las islas continentales podrían estar podrían tener ya todos sus nichos llenos al comienzo de su existencia como masas de tierra independientes. Como consecuencia, las islas continentales podrían ofrecer menos oportunidad ecológica ya que sus nichos podrían estar ya ocupados por los componentes vicariantes de su diversidad.

Islas versus archipiélagos

Las islas pueden existir como masas de tierra aisladas o en archipiélagos, formando conjuntos de masas de tierra próximos los unos a los otros. La evidencia muestra que estos diferentes escenarios geográficos pueden potencialmente influir en las oportunidades de eventos de diversificación adaptativa. Encontramos uno de los ejemplos más extraordinarios de esto en los famosos pinzones de Darwin en las Islas Galápagos. Análisis detallados de la variación entre poblaciones muestran que las 13 especies en el archipiélago especiaron probablemente en diferentes islas, volviéndose posteriormente simpátridas como resultado de los eventos de dispersión entre islas. Una vez en simpatría, si las especies coexistentes no diferían aún en sus fenotipos y ecologías, probablemente empezaron a divergir como consecuencia de desplazamiento de carácter (Losos y Ricklefs, 2009; Grant y Grant, 2011b).

Así, en este caso, la especiación alopátrida ocurrida en islas diferentes en un archipiélago seguida por invasiones secundarias puede resultar en la aparición de gran diversidad de especies a través de procesos adaptativos.

Factores intrínsecos

¿Diferentes grupos al llegar a la misma isla o archipiélago, experimentarán niveles parecidos de diversificación evolutiva? No hay muchos estudios que se centren en detalle en esta cuestión, no obstante, evidencias circunstanciales muestran que este puede no ser el caso. En las Islas Galápagos, por ejemplo, los pinzones de Darwin son los únicos pájaros que han diversificado en modo extensivo (Jackson, 1993). Así mismo, en el Caribe, a parte de los gecos del género *Sphaerodactylus,* ningún otro grupo de reptiles ha diversificado en términos de especies y fenotipos como lo han experimentado los *Anolis* (Losos, 2009). Estos ejemplos muestran que los atributos físicos y geográficos de una isla probablemente no son la historia completa. Deben haber factores, intrínsecos a los diferentes grupos colonizadores que determinan si tomarán o no el camino de la diversificación en una isla.

Moduladores de la oportunidad ecológica grupo-dependientes

Hemos visto que la cantidad de oportunidad ecológica ofrecida por una isla está fuertemente determinada por sus atributos geográficos, geológicos y físicos. No obstante, siendo estos iguales, es razonable pensar que no todos los grupos experimenten los mismos niveles de oportunidad ecológica. Esto es porque la cantidad de nichos accesible por las especies depende en último término de su anatomía, fisiología y comportamiento particular. Como consecuencia los recursos apropiados puede ser que no estén disponibles para todos los grupos de organismos. Esto podría explicar la falta de diversificación sustancial en los pájaros del género *Certhidea* en las Islas Galápagos. La ausencia de recursos discretos a los cuales las diferentes especies de este genero se podrían adaptar, explicaría la baja diversificación en este grupo (Rundell y Price 2009; Grant y Grant 2011a). Otra variable importante que influye en los niveles de oportunidad ecológica que experimenta un grupo es su tiempo de llegada a la isla. Colonizadores tempranos experimentarán probablemente niveles elevados de oportunidad ecológica mientras que grupos colonizadores que lleguen a la isla en una fase más tardía, puede que encuentren con un escenario con muchos competidores y pocos nichos a ocupar (Losos, 2010).

Predisposiciones diferentes a la especiación

Es posible que no todos los grupos colonizadores presenten la misma propensión a especiar en una isla todo y estando expuestos a altos niveles de oportunidad ecológica. Por ejemplo, cortejos complejos, o que dependen de señales acústicas, cinéticas o visuales complejas podrían aumentar la probabilidad de que poblaciones expuestas a diferentes ambientes (o usando diferentes recursos) se aíslen reproductivamente (Losos, 2009). Por otro lado, la expansión de nicho podría estar mayormente mediada por plasticidad comportamental, no implicando necesariamente (al menos a corto plazo) especialización de diferentes poblaciones a diferentes partes del espectro de recursos ofrecidos por la isla. En tales situaciones, con individuos moviéndose constantemente entre diferentes ambientes y usando alternativamente diferentes recursos, es esperable que las oportunidades de acabar aislados reproductivamente puedan ser más limitadas.

Diferentes capacidades evolutivas

Las diferentes capacidades para evolucionar en diferentes formas (Schluter, 2000) pueden ser también cruciales para explicar que no todos los grupos diversifiquen del mismo modo en las mismas islas. Por ejemplo, es posible que algunos grupos posean limitaciones anatómicas, fisiológicas o biomecánicas que los hagan incapaces de entrar en dinámicas de elevada diversificación evolutiva, a pesar de estar expuestos a niveles elevados de oportunidad ecológica. Se ha recurrido a esta idea, por ejemplo para

explicar por qué los *Anolis* en el Caribe han producido tal inmensa diversificación fenotípica mientras que otro grupo de lagartos, habitantes de islas tropicales parecidas, el género *Phelsuma,* muestra tan sólo cantidades moderadas de diversificación fenotípica (Losos, 2010). Los gecos, especialmente las especies arbóreas, presentan las extremidades orientadas lateralmente con respecto al cuerpo y forman un ángulo muy bajo con el sustrato, manteniendo su centro de gravedad cerca de este. Este tipo de diseño corporal en estos gecos arbóreos, puede haber limitado las capacidades en las que estos gecos podrían adaptarse a distintos micro-hábitats (Losos, 2010). Los *Anolis*, sin embargo, están libres de esta limitación anatómica, lo cual parece haberles permitido diversificar en las innumerables formas y tamaños existentes en las Islas Caribeñas (Losos, 2009)

DIVERSIFICACIÓN EVOLUTIVA EN ISLAS VERSUS CONTINENTE

Los colonizadores de islas normalmente implican el salto entre un contexto de elevados niveles de competencia y depredación a un contexto prácticamente carente de competidores y depredadores. Esta gran asimetría ecológica entre ambos ambientes probablemente queda reflejada en como la diversificación evolutiva se lleva a cabo en ambos dominios. En el continente, las comunidades son normalmente complejas y compuestas por muchas especies que comparten típicamente una larga historia de coexistencia. En tal escenario, la mayoría de los nichos continentales estarán probablemente ocupados, dejando poco espacio ecológico para las especies recién formadas (Losos y Ricklefs, 2009). En tal contexto, se esperan niveles elevados de competición inter-específica los cuales, a su vez, tenderán a limitar una eficiente expansión de nicho (y del morfoespacio). Además, la depredación, normalmente muy intensa en comunidades continentales, probablemente contribuirá a limitar la diversificación morfológica induciendo selección estabilizadora en muchos de los caracteres de sus presas (Yoder et al., 2010).

Como resultado de los efectos combinados de competición inter-específica y depredación, la diversificación evolutiva en continentes se espera que produzca pequeñas variaciones de los temas adaptativos ya exitosos, implicando por tanto un gran conservadurismo morfológico (Moen et al. 2009; Losos y Ricklefs, 2009). Esto contrasta con la situación que experimentan los colonizadores de islas; por todas las razones expuestas anteriormente, los grupos insulares en muchos casos pueden diversificar de manera extensiva dado que se mueven a través del nuevo paisaje adaptativo que las islas les proporcionan (Losos y Ricklefs, 2009). Esto probablemente produce dos importantes diferencias entre diversificaciones insulares y continentales: en primer lugar, los grupos de las islas experimentarán elevadas tasas de diversificación fenotípica y/o de especies comparado con los grupos continentales. Esto es consecuencia del fenómeno de “explosión temprana” anteriormente explicado (Schluter, 2000). En segundo lugar, dado que las comunidades insulares están necesariamente formadas por un subconjunto de comunidades continentales, los grupos insulares pueden usar nichos que en el

continente estarían ocupados, incluso, por grupos filogenéticamente distantes. En palabras de Darwin (1859):

"Las islas oceánicas, a veces, son deficientes en ciertas categorías, y sus lugares son aparentemente ocupados por oros habitantes; en las Islas Galápagos reptiles, y en Nueva Zelanda pájaros gigantes sin alas, ocupan el lugar de los mamíferos."

Como consecuencia, la diversificación evolutiva en islas podría dar pie a una expansión adaptativa del morfoespacio, permitiendo la gran disparidad de fenotipos típica de grupos insulares (Carlquist, 1974). Esta expansión del morfoespacio puede llevar a grupos insulares incluso a traspasar los límites del morfoespacio continental, produciendo tantos ejemplos de tamaños y formas de especies insulares radicalmente divergentes a aquellos encontrados en sus parientes cercanos del continente (Losos y Ricklefs 2009).

Uno de los ejemplos más extremos de esto, lo encontramos en la comparación entre los mieleros hawaianos con sus parientes del continente (subfamilia Carduelinae). Las especies continentales han diversificado en un morfoespacio muy reducido de formas de pico (esencialmente variaciones de tamaño en la morfología de tipo pinzón estándar). Sin embargo, los mieleros hawaianos han expandido asombrosamente su morfoespacio, replicando, sólo en Hawai, la mayoría de la variación existente en el orden entero de los paseriformes (Lovette y Bermingham 2002; Losos y Ricklefs 2009). El morfoespacio ocupado por los mieleros contiene morfologías que incluso van más allá de los límites alcanzados por ningún paseriforme continental. Este es el caso de akiapoolau (*Hemignathus munroi*) que exhibe un extraño pico, con una fuerte asimetría en la longitud de las mandíbulas superior e inferior. Esta especie ocupa el nicho que ocupan los ausentes pájaros carpinteros en Hawai, aunque usa una estrategia completamente diferente: Akiapoolau extrae larvas de la corteza de los árboles haciendo agujeros usando su corta mandíbula inferior. Entonces, extrae éstas usando su larga y curvada mandíbula superior (Losos y Ricklefs, 2009).

Otros ejemplos de Hawai los encontramos en las arañas del género *Tetragnata* y en grupos de gorgojos, los cuales han producido fantásticas diversificaciones en estas islas sobrepasando ampliamente la diversificación existente entre sus parientes continentales (Gillespie y Croom, 1994; Paulay, 1994).

Pero, ¿necesariamente los grupos insulares siempre diversificarán a tasas elevadas y expandirán sus morfoespacios de sus parientes continentales? Comparar grupos de las islas y continentales no es una tarea fácil y el problema es siempre obtener un muestreo representativo de la diversidad real en el continente. Pero cuando un estudio tal había sido llevado a cabo con uno de los ejemplos más importantes de diversificación en islas, los *Anolis* el Caribe, los resultados fueron bastante inesperados. A pesar de constituir uno de los ejemplos más impresionantes de diversificación en islas, sus tasas de

diversificación fenotípica y disparidad no logran ser sustancialmente diferentes a los niveles encontrados en los *Anolis* continentales (Pinto et al., 2008).

Casos como este ponen en cuestión que las diversificaciones insulares siempre conlleven niveles de diversificación superiores a los observados en el continente.

Por ejemplo, es concebible que patrones insulares puedan aparecer también en grupos continentales aunque sean más difíciles de detectar debido a las grandes escalas geográficas y taxonómicas implicadas (Claramunt et al., 2012). Factores extrínsecos como cambios climáticos, procesos orogénicos y episodios de extinciones masivas pueden proporcionar nuevos nichos que pueden potencialmente estimular niveles altos de diversificación también en el continente (Simpson, 1944). Además, factores intrínsecos como la aparición de innovaciones clave pueden facilitar el acceso a una amplia gama de nichos en grupos continentales produciendo patrones de diversificación fenotípica similares a los esperados en islas (Simpson, 1944; Claramunt et al., 2012). Más estudios empíricos basados en sistemas continente-isla bien muestreados serian cruciales para aclarar esta cuestión.

INNOVACIONES CLAVE COMO MOTORES DE LA DIVERSIFICACIÓN EVOLUTIVA

Desafortunadamente, el concepto de innovación clave es uno de los conceptos más ambiguos en biología evolutiva. En su definición más tradicional, las innovaciones clave son rasgos que permiten a los grupos interactuar con el medio de nuevas maneras. Uno de los aspectos más importantes de las innovaciones clave es que estas nuevas interacciones con el ambiente pueden permitir el acceso a tipos de recursos completamente nuevos y pueden exponer los grupos a niveles elevados de oportunidad ecológica (Miller, 1949; Hunter, 1998; de Queiroz, 2002; Losos, 2009).

De una manera similar a la descrita para islas, las innovaciones clave pueden mover grupos de contextos de baja oportunidad ecológica a contextos de elevada oportunidad. A su vez, esto puede inducir la aparición de granes cantidades de diversificación fenotípica y de especies, como se ha demostrado para muchos grupos (Galis, 2001).

Ejemplos clásicos de innovaciones clave son la evolución de las plumas y las alas en dinosaurios (que les permitieron volar; Hunter, 1998) y la aparición de las flores en las plantas (que permitieron la polinización animal; Vamosi y Vamosi, 2010). Los conceptos de innovación clave y radiación adaptativa están fuertemente vinculados en la bibliografía (Losos 2009, 2010). No obstante, muchos son los ejemplos de innovaciones clave que no han derivado en grandes diversificaciones (Hodges, 1997; Price et al., 2010; Claramunt et al., 2012). Ejemplos destacables son taxones como el cerdo hormiguero (familia Orycteropodidae) o incluso nosotros mismos, los humanos. Ambos grupos poseen una gran variedad de innovaciones clave, aunque exhiben una baja diversidad morfológica y de especies (Hunter, 1998; Wood y Collard, 1999). Tales ejemplos advierten que la evolución de las innovaciones clave no

necesita siempre derivar en grandes diversificaciones evolutivas (Fürsich y Jablonski, 1984).

El fracaso de una innovación clave para mediar en diversificación evolutiva puede ser explicada de diversas maneras. Una de ellas podría ser el escenario ecológico particular en el cual una innovación ecológica se origina (Hodges, 1997; de Queiroz, 2002). Por ejemplo, la evolución de la mandíbula faríngea en los cíclidos Africanos es una innovación clave que sólo ha llevado a radiaciones adaptativas en lagos tectónicos de formación reciente y libres de competidores (Liem, 1973). Además, limitaciones intrínsecas, morfológicas o genéticas (falta de capacidad de evolucionar) han sido propuestas para explicar ejemplos de innovaciones clave asociadas con baja diversificación evolutiva (Schluter, 2000; Price et al. 2010). Se han recurrido a tales limitaciones para explicar, por ejemplo, por qué las innovaciones en el diseño de la mandíbula de los peces loro no han derivado en gran diversidad morfológica (Price et al. 2010) o por qué los gecos con lamelas adhesivas (otra innovación clave) no han experimentado niveles de diversificación fenotípica comparable a *Anolis* (Losos, 2010).

APROXIMACIONES METODOLÓGICAS PARA EL ESTUDIO FENOTÍPICO Y DE DIVERSIFICACIÓN DE ESPECIES

¿La colonización de islas o la adquisición de innovaciones clave inducen niveles altos de diversificación evolutiva? Para responder a esta pregunta necesitamos métodos para cuantificar y comparar diversidad fenotípica entre diferentes clados. En una aproximación ingenua, podríamos simplemente comparar la disparidad fenotípica y la riqueza de especies entre clados continentales e insulares o entre clados que posean innovaciones clave y clados que carezcan de ella. Sin embargo, estas comparaciones no serían correctas puesto que no incorporan un componente crucial: las relaciones evolutivas entre especies, en otras palabras, la filogenia. Afortunadamente, hoy en día vivimos en una edad de oro en el desarrollo de aproximaciones metodológicas que integren datos ecológicos y fenotípicos con información filogenética. A continuación, paso a destacar algunas de las aproximaciones metodológicas usadas mas extensamente en los capítulos de esta tesis, centrándome particularmente en la estimación de tasas de diversificación, tanto fenotípica como de especies.

TASAS DE DIVERSIFICACIÓN FENOTÍPICA

Una de las posibles consecuencias derivadas de la colonización de islas o de la adquisición de innovaciones clave es un incremento en las tasas de evolución fenotípica ya que los grupos, como se ha mencionado, en una aproximación incompleta podríamos simplemente comparar la disparidad fenotípica, comparando las varianzas de los caracteres entre, por ejemplo, clados de las islas y clados continentales. Si encontráramos una disparidad fenotípica mayor en los grupos de las islas, ¿esto nos permitiría concluir que los grupos insulares experimentan tasas de diversificación fenotípica mayores? La respuesta es: no, necesariamente, ya que esta comparación no tiene en cuenta algunos componentes

clave que pueden actuar como factores de confusión. Estos son el tiempo y la historia compartida entre especies.

Los efectos del tiempo son muy intuitivos: los fenotipos en cada grupo han diversificado desde el tiempo de su antecesor común más reciente de todo el grupo (MRCA). Si la edad del MRCA de un clado de una isla (el clado que, por ejemplo, presenta la varianza fenotípica mayor) es considerablemente mayor que la edad del MRCA del clado continental (por ejemplo con una disparidad menor), entonces esta diferencia en los tiempos de evolución entre ambos grupos podría ser suficiente para explicar las distintas disparidades de caracteres (Wainwright, 2007). Simplemente, cuánto más tiempo tenga que diversificar un clado, mayor será el nivel de diferenciación que podrá alcanzar (Fiq. 1A).

Pero dada una misma edad del MRCA de ambos clados, podríamos también tener otro potencial factor de confusión: el tiempo de historia compartida dentro de cada clado. Cuanto más corto sea el tiempo de historia compartida entre las especies de un clado, mayor será la disparidad fenotípica esperada en ese clado (O'Meara, et al. 2006; Wainwright, 2007). La razón es que cuanto más corta sea la historia compartida entre las especies de un clado, más largo será el tiempo en el que cada especie o linaje ha evolucionado de modo independiente. A su vez, esto tiende a incrementar la disparidad de ese clado (Fig. 1B).

Es, por ello, razonable que la disparidad medida entre las especies en un clado no sólo dependa en las tasas de evolución fenotípica, sino también que también sea función de la profundidad del clado (la edad del MRCA) y el tiempo de historia compartida entre linajes (Garland, et al., 1992; Gittleman, et al. 1996; Mooers, et al., 1999; Ackerly y Nyffeler, 2004; O'Meara et al., 2006; Thomas y Freckleton, 2006). Estos tres componentes juegan un papel fundamental al determinar los niveles de diferenciación fenotípica de distintos grupos y se encuentran bien integrados en un simple modelo evolutivo: el modelo Browniano (modelo "BM").

De acuerdo con este modelo, el cambio en un carácter a lo largo del tiempo puede ser descrito mediante la siguiente ecuación (Butler y King 2004):

$$dX(t) = \sigma dB(t)$$

Donde $dX(t)$ es el cambio en el carácter X a lo largo de tiempo, $dB(t)$ se refiere al "ruido blanco" esto es, variables aleatorias con media 0 y varianza dt y finalmente, el parámetro σ es el parámetro que determina como de grande será la varianza de la distribución aleatoria. Tal y como se especifica en la ecuación, en cualquier momento en el tiempo:

- El carácter X puede aumentar, disminuir o permanecer igual.
- La dirección y magnitud del cambio es independiente al estado del carácter en un momento dado.
- La varianza de cambio es contante e igual a la varianza de la distribución aleatoria (tasa constante).

Estos puntos básicos producen una interesante propiedad cuando muchos procesos Brownianos son simulados desde un punto común (equivalente a múltiples linajes evolucionando de manera independiente desde un único antecesor): la varianza esperada (o disparidad) aumentará con el tiempo (Fig. 2) pero, además, la tasa a la cual la varianza aumenta con el tiempo será modulada por el parámetro σ (cuanto más alta sea σ, más rápidamente aumentará la varianza con el tiempo) (Fig. 3). En otras palabras, dada la misma cantidad de tiempo, el clado que presente la disparidad más grande será el que tenga la σ mayor. Es evidente, entonces, que los valores del parámetro σ (también llamado parámetro de tasa) pueden verse como un proxy para las tasas de evolución fenotípica.

En el caso de tener múltiples linajes evolucionando a lo largo de la filogenia, como hemos visto, la disparidad esperada, no sólo dependerá del tiempo y del parámetro de tasa, sino que también estará en función del tiempo de historia compartida entre las especies de un clado. Esto puede ser fácilmente adaptado en el modelo BM mediante la siguiente ecuación (derivada en O'Meara et al., 2006):

$$E(\textit{disparidad}) = \sigma^2[(1/N)tr(C) - (1/N^2)1'C1]$$

Donde σ es el parámetro de tasa, N es el número de puntas en el árbol (número de especies) y C es la matriz de varianza-covarianza filogenética. Esta matriz, esencial en muchos métodos comparativos filogenéticos, describe numéricamente el patrón de tiempos compartidos entre las especies de un clado.

Es importante señalar que a pesar de la naturaleza aleatoria del modelo Browniano, este modelo no es sólo válido para describir procesos evolutivos puramente aleatorios (por ejemplo, deriva genética). Tambien es un modelo razonablemente bueno en un contexto de diversificación adaptativa con óptimos fluctuantes (O´Meara et al., 2006).

TASAS DE DIVERSIFICACIÓN DE ESPECIES

Como en el caso de evolución fenotípica, diferentes riquezas de especies no necesariamente reflejan diferentes tasas de diversificación entre clados. Como para la disparidad, cuanto más tiempo haya tenido que evolucionar un clado, más tiempo ha tenido para producir más especies. Necesitamos por tanto métodos para estimar tasas de diversificación y, afortunadamente, el patrón de ramificación en un árbol filogenético contiene toda la información que puede ser usada, no sólo para estimar tasas de diversificación, sino para separarla en sus componentes de especiación y extinción (Ricklefs, 2007).

La estimación de tasas de diversificación (el balance entre las tasas de especiación y extinción) a partir de filogenias depende del modelo de diversificación asumido. Por ejemplo, de acuerdo con el modelo más simple de diversificación, el proceso de Yule, las tasas de diversificación son equivalentes a una tasa de

especiación constante sin extinción. En este caso, el tamaño del clado aumentará exponencialmente con el tiempo de acuerdo con la siguiente simple ecuación (Nee, 2006):

$$E(n) = \exp(\lambda t)$$

Donde E(*n*) es el número esperado de linajes de un clado, t es el tiempo y λ es un posible proxy de tasa de diversificación, ya que es el parámetro que modela el incremento del número de especies con el tiempo.

Sin embargo, si añadimos a este proceso el componente de extinción, la situación se complica, ya que, en este caso, las diferencias en riqueza de especies entre dos clados pueden resultar de diferencias en las tasas de especiación, de extinción o ambas (sumándose tambien las fluctuaciones aleatorias).
De acuerdo con el modelo más simple de diversificación que con extinción, el modelo de nacimiento y muerte ("birth and death"), tasas constantes de especiación (λ) y extinción (μ) cuantifican las probabilidades de que un evento de especiación o una extinción ocurra dentro de un intervalo de tiempo (t).
En este caso, el tamaño esperado del clado seguiria la siguiente ecuación (Ricklefs, 2007):

$$E(n) = \exp[(\lambda - \mu)t]$$

Obviamente, en este caso la tasa de diversificación corresponde a la diferencia entre la tasa de especiación y la de extinción (λ - μ). Sin embargo, este modelo asume que las tasas de especiación y extinción son las mismas para todos los linajes y no varían con el tiempo y la violación de este supuesto puede sesgar drásticamente nuestras estimas de tasas de diversificación. Esto es particularmente problemático en el marco de preguntas evolutivas tratadas en esta tesis, ya que después de la colonización de una isla o después de la adquisición de una innovación clave esperamos un patrón que explicitamente implica tasas no constantes de diversificación: tasas elevadas al comienzo de la diversificación ("explosión temprana") y un paulatino descenso de las tasas hacia el presente (Schluter, 2000). Por ello, con el fin de calcular y comparar tasas de diversificación necesitamos ser capaces de seleccionar el modelo correcto de diversificación en cada caso.
Existen diferentes aproximaciones orientadas a comprobar si las tasas en un clado son constantes o varían con el tiempo. Uno de las más clásicas es el estadístico Gamma (Pybus y Harvey, 2000). Este estadístico describe el patrón de distribución de los nodos en un árbol, con lo que este puede ser comparado con el esperado por un proceso de Yule de tasas constantes. Las filogenias con valores negativos para este estadístico indican que la mayoría de los nodos están situados cerca de la raíz del árbol y pueden ser interpretados como una muestra de disminución temporal de velocidad de las

tasas de especiación. Sin embargo, uno de los problemas de esta aproximación (entre otras) es que no permite detectar disminuciones de las tasas de especiación en situaciones de tasas de extinción no nulas (Rabosky y lovette, 2008). Recientemente, se han desarrollado métodos basados en coalescencia para distinguir entre modelos alternativos de diversificación (Morlon et al., 2010). Estos métodos modelan las distancias inter-nodo de una filogenia asumiendo que están distribuidos de acuerdo con una aproximación estándar de coalescencia (Griffith y Tavaré, 1994). Esto tiene la ventaja de modelar la diversidad de especies desde el presente al pasado asumiendo que esta puede tomar cualquier valor en cualquier momento del tiempo (incluyendo diversidad constante en el tiempo). Esta aproximación también permite acomodar fácilmente filogenias muestreadas de manera incompleta (otra gran fuente de sesgo en análisis de diversificación) dado la teoría de la coalescencia se basa de la teoría de las muestras (Morlon et al., 2010).

INTRODUCCIÓN A NUESTRO ORGANISMO MODELO: LOS GECOS DE ARABIA Y AUSTRALASIA

Los gecos (infraorden : Gekkota) con más de 1500 especies de 118 géneros constituyen uno de los más diversos grupos de reptiles (suponiendo el 25% de todas las especies de lagartos) (Gamble et al., 2012). De acuerdo con la mayoría de las filogenias moleculares que incluyen a todos los escamosos, los gecos son el grupo hermano de todos los escamosos con la excepción de los dibámidos (Hedges y Vidal, 2009). La edad del inicio de la diversificación de los gecos actuales varía dependiendo de las diferentes estimas pero la mayoría de ellas coinciden en situar el MRCA de los gecos actuales en algún momento durante el Cretácico (145-66 Ma). El fósil más antiguo atribuíble a los gecos también data de esa época (Daza et al., 2014).

Una de las características más importantes de los gecos fue ya señalada por Aristóteles más de 2000 años atrás, quien escribió: “Puede recorrer un árbol de arriba debajo de cualquier manera, incluso con la cabeza hacia abajo” (Aristóteles/Thompson, 1918). Aristóteles se refería a la famosa habilidad de lo gecos de desafiar a la gravedad y a correr incluso en superficies verticales lisas. Ahora sabemos que el secreto de tales capacidades está en una estructura muy especial existente debajo de sus dedos: las lamelas adhesivas (“toepads”). Estas contienen cada una millones de estructuras filiformes microscópicas llamadas *setae*. Al principio, se propuso la hipótesis de que estos *setae* producían adhesión actuando como micro-ganchos, agarrándose a las irregularidades de las superficies (Dellit, 1933). Sin embargo, el mecanismo verdadero de adhesión és mucho más espectacular: estas estructuras filiformes son tan finas y pequeñas que los átomos de la punta de cada una de ellas son capaces de establecer enlaces químicos débiles (fuerzas de Van der Waals) con lo átomos del sustrato. Es, entonces, la suma de todas estas fuerzas débiles sobre el total de la superficie de cada uno de los dedos lo que produce la extraordinaria capacidad adhesiva de los gecos (Hiller, 1968; Autumn y Peattie, 2002).

Las lamelas adhesivas están presentes en el 60% de las especies de gecos y hay evidencia de que han sido adquiridas de manera independiente varias veces a lo largo de la historia evolutiva de los gecos (Gamble et al., 2012). Es obvio que un mecanismo tan extraordinario ofrece a los gecos la posibilidad de interactuar con el ambiente de una manera completamente nueva, permitiéndoles un uso más eficiente de los hábitats altamente tridimiensionales (como hábitats arbóreos). Por esta razón, las lamelas adehesivas ("toepads") son un ejemplo paradigmático de innovación clave y su adquisicón en varios linajes de gecos ha sido propuesta como el factor principal que explica la gran diversificación existente en los gecos (Losos, 2009; 2010).

Otro aspecto destacable de los gecos es su gran capacidad de dispersión. Esto se ve reflejado en su distribución mundial, habitando todos los continentes, excepto la Antártida. Dentro de la gran capacidad de dispersión de los gecos, hay que resaltar su capacidad para llevar a cabo eventos de dispersión marítima (Gamble et al., 2011) lo que los hace muy buenos colonizadores de islas remotas (Bauer, 1994; Carranza y Arnold, 2000; Austin et al., 2004; Rocha et al., 2007) y, por ello mismo, también los hace un buen modelo para estudiar procesos evolutivos insulares.

En esta tesis, me dedico a explorar los efectos de la colonización de islas y otras fuentes de oportunidad ecológica (como la adquisición de toepads adhesivos) usando tres géneros de gecos de Arabia y toda a diversidad de gecos diplodactiloides Australasiáticos. A continuación, proporciono un breve introducción de estos grupos desde un punto de vista taxonómico, ecológico y biogeográfico.

LOS GECOS DE ARABIA Y EL ARCHIPIÉLAGO DE SOCOTRA

En el sistema isla-continente Arabia-Socotra, nos centramos en tres géneros muy dispares: *Pristurus, Hemidactlus* y *Haemodracon*.

El género *Pristurus*, también conocido como los gecos semáforo, pertenece a la familia *Sphaerodactylidae*, aunque su posición filogenética dentro de ésta es incierta. Esta familia contiene entre 23 y 26 especies (Arnold, 2009; Sindaco y Jerecenko; Uetz, 2013) y, a diferencia de la mayoría de los gecos, son diurnos y heliotérmicos. Una particularidad destacable de este género es que la mayoría de las especies presentan un método de señalización muy conspicuo y elaborado consistente en movimientos de cuerpo y cola. Estas características no son muy comunes dentro de los gecos, ya que la mayoría son nocturnos y se comunican mayoritariamente por medio de vocalizaciones o señales químicas. En realidad, la mayoría de las especies de *Pristurus* se comportan más como agámidos deserticolas que como gecos típicos (Arnold, 2009). La mayoría de las especies de gecos semáforo se encuentran en el noreste de África (7 especies con 4 endemismos), la Península Arábica (14 especies con 12 endemismos) y el Archipiélago de Socotra (7 especies endémicas) con unas de las especies arábicas *P. rupestris* extendiéndose hasta las regiones costeras de Irán. Como una rareza biogeográfica fascinante, una especie aislada de este género, *P. adrarensis*, se encuentra en un área muy pequeña de Mauritania, separado 4700 km del área de la zona donde se distribuyen la mayoría de especies del

género. Las especies de este género ocupan una gran variedad de hábitats, desde zonas rocosas a arenosas y no presentan lamelas adhesivas. El género *Hemidactylus* con 122 especies distribuidas por todo el mundo, constituye uno de las géneros de gecos más diversos. Pertenecen a la familia Gekkonidae y dentro de esta familia son grupo hermano del género *Cyrtodactylus*. En esta tesis, nos hemos centramos en el llamado clado árido, una radiación monofilética de más de 40 especies distribuidas por las zonas áridas del noreste de África, el Levante, Arabia, zonas colindantes de suroeste de Asia y, interesantemente para nuestros objetivos, también en el Archipiélago de Socotra (Carranza y Arnold, 2006; Moravec et al., 2011; Carranza y Arnold 2012; Gómez-Díaz et al., 2012; Šmíd et al., 2013).

Todas estas especies son nocturnas, depredadores y ocupan una gran variedad de hábitats: desde llanuras tórridas hasta zonas montañosas. Poseen lamelas adhesivas altamente desarrolladas, aunque el área que cubren varía de una especie a otra.

Finalmente, el género *Haemodracon* pertenece a la familia Phyllodactylidae y está formada por dos especies endémicas del Archipiélago de Socotra. Tienen lamelas adhesivas bien desarrolladas y se encuentran en hábitats muy variados. *H. riebecki* normalmente se encuentra en acantilados, rocas, cuevas y troncos de árboles, mientras que *H. trachyrhinus* es más un habitante del suelo.

LOS GECOS DIPLODACTÍLIDOS AUSTRALASIÁTICOS

Con finalidad comparativa, aparte de estudiar los gecos de Arabia y el archipiélago de Socotra, también trabajamos con otro interesante sistema continente-isla: los gecos diplodactílidos Australasiáticos. Estos forman una radiación de más de 200 especies distribuidas en Australia, Nueva Caledonia y Nueva Zelanda (Uetz, 2014) y contienen tres familias independientes: Diplodactylidae, Carphodactylidae y Pygopodidae, las cuales se sitúan filogenéticamente como grupo hermano del resto de los gecos (Hedges y Vidal, 2009).

Aparte de haber colonizado de forma independiente dos archipiélagos insulares, este grupo también se caracteriza por su gran diversidad ecológica y morfológica (Oliver y Sanders, 2009).

La mayoría-pero no todas- de las especies poseen una de dos importantes innovaciones clave: lamelas adhesivas o un fenotipo serpentiforme. Este fenotipo consiste en un cuerpo alargado sin extremidades anteriores y sólo pequeñas aletas escamosas como miembros posteriores (Shine, 1986). Este fenotipo se encuentra en todas las especies pertenecientes a la familia Pygopodidae y tiene una serie de ventajas importantes para las especies que lo presentan, entre ellas: 1) una locomoción más eficiente; 2) la capacidad de utilizar los espacios estrechos, como grietas para la obtención de alimentos, termorregulación, o refugio, 3) la capacidad de excavar en la tierra o arena; y, a menudo, 4) la capacidad para ingerir presas más grandes que ellos mismos (Gans, 1975; Shine 1986). La presencia en este grupo de dos innovaciones clave independientes y de dos casos de colonización insular nos ofrece una gran oportunidad para estudiar la contribución relativa de cada una de estas fuentes alternativas de oportunidad ecológica como motor de la diversificación evolutiva.

OBJETIVOS GENERALES

El objetivo principal de esta tesis es el de examinar cómo la oportunidad ecológica – específicamente, la colonización de islas (en contextos oceánicos y continentales) y la evolución de las innovaciones clave – ha impulsado la diversidad fenotípica y de especies en los gecos de Arabia y del Archipiélago de Socotra, comparados con otro sistema continente-isla: los diplodactílidos australasiáticos.

Ocho son las preguntas generales sobre las cuales esta tesis pretende aclarar:

1. ¿Los grupos colonizadores de islas experimentan una expansión del morfoespacio comparado con los grupos continentales?
2. ¿Los grupos colonizadores de islas experimentan tasas aceleradas de diversificación fenotípica y de especies?
3. ¿Cuál es la contribución relativa de innovaciones clave y la colonización de islas para impulsar diversificación evolutiva?
4. ¿Diferentes caracteres responden de la misma manera a la oportunidad ecológica que las islas proporcionan?
5. ¿Por qué procesos (dispersión, diversificación intra-isla o vicarianza), las islas continentales han desarrollado su biodiversidad?
6. ¿Cuál es la contribución relativa de dispersión, diversificación intra-isla o vicarianza para producir la estructuración de nichos observada en islas?
7. ¿Cómo ocurre la diversificación en entornos “insulares” continentales?

OBJETIVOS ESPECÍFICOS

1. Explorar los efectos de la colonización de las islas de Socotra y Abd al Kuri en la diversificación fenotípica experimentada en los gecos *Hemidactylus* pertenecientes al clado árido. Particularmente, se busca estudiar si las especies en islas tienden a expandir los morfoespacios de sus parientes cercanos en el continente y comprobar si los linajes insulares experimentan aceleraciones de las tasas de diversificación morfológica.
2. Comparar los efectos de dos innovaciones clave (las lamelas adhesivas y el fenotipo en forma de serpiente) y la colonización de islas (concretamente, la colonización de Nueva Caledonia y Nueva Zelanda) en la diversificación de los gecos diplodactílidos australasiáticos. Asimismo, estudiar si los grupos que presentan una innovación clave o llegan a islas experimentan aceleraciones de las tasas de diversificación fenotípica o de especies.

3. Evaluar la contribución relativa de vicarianza, dispersión o diversificación *in situ* en el ensamblaje de la diversidad de gecos del archipiélago de Socotra, así como en la estructuración de los nichos de sus especies.
4. Examinar si en todos los casos independientes de diversificación *in situ* existentes en los geckos del archipiélago de Socotra, se dan patrones equivalentes de diversificación macro y microecológica.
5. Incrementar el muestreo y avanzar en la comprensión de la diversidad genética existente dentro de la especie *Pristurus rupestris*.
6. Comprender los efectos de las montañas de Arabia al producir la diversidad fenotípica y de especies existente dentro del complejo de especies *Pristurus rupestris.*

DISCUSIÓN

A través de los cuatro capítulos que componen esta tesis, he explorado los efectos de la colonización de islas y las innovaciones clave como impulsores de diversificación evolutiva. Para ello, he usado los gecos como modelo y dos sistemas diferentes de continente-isla con fines comparativos.

De estos cuatro estudios, podemos extraer algunas generalidades; algunas de ellas concuerdan con los escenarios teóricamente esperados mientras que otras no lo hacen. A continuación, expongo estas generalidades, enfatizando en qué medida concuerdan o no con las expectativas teóricas, resaltando también caminos futuros de investigación potencialmente interesantes.

ISLAS COMO MOTORES DE DIVERSIFICACIÓN EVOLUTIVA

Tal y como se ha expuesto en la introducción, se piensa que las islas proporcionan un contexto de muchos recursos disponibles con pocos competidores y depredadores. Esto permite a grupos colonizadores experimentar una "libreación ecológica" y usar un conjunto más amplio de nichos comparado con sus parientes más cercanos en el continente (Losos y Ricklefs, 2009). En esta situación esperamos una expansión del morfoespacio en grupos de las islas, típicamente asociados con altas tasas de diversificación fenotípica (Losos y Ricklefs, 2009; Schluter, 2000).

En los estudios de esta tesis se ha encontrado evidencia convincente de que esto ocurre para los dos sistemas continente-isla estudiados. Los geckos diplodactiloides australasiáticos experimentaron una clara expansión del rango de la variación fenotípica existente en el continente, produciendo la especie más grande (*Hoplodactylus delcourti* de Nueva Zelanda) y la más pequeña (*Dierogekko poumensis* de Nueva Caledonia) en la radiación (Capítulo 2). Asimismo, en los gecos *Hemidactylus* de Arabia-Socotra, las especies de islas también produjeron los tamaños más extremos en la radiación con *Hemidactylus forbesii* de Abd al Kuri alcanzo el tamaño máximo y *H. pumilio* de Socotra alcanzando el más pequeño

(Capítulo 1). En este último sistema continente-isla, a parte de haberse alcanzado los tamaños máximos y mínimos, las disparidades existentes en las especies insulares fueron, en todos los casos, significativamente mayores que las disparidades calculadas para las especies continentales. Todo lo expuesto es consistente con la expansión del morfoespacio esperada después de la colonización de islas (Gillespie, 2004; P. R. Grant y Grant, 2011; Harmon et al., 2010). Sin embargo, no todos los caracteres experimentan niveles similares de expansión fenotípica después de colonización de islas. Por ejemplo, en el caso particular de *Hemidactylus* de Arabia y Socotra, las proporciones de la cabeza experimentaron significativamente disparidades mayores que en el continente solo en la isla de Abd al Kuri **(Capítulo 1)**.

De acuerdo con las tasas de diversificación fenotípica y de especies, una vez más nuestros resultados concuerdan completamente con las expectativas teóricas. Las colonizaciones de Nueva Caledonia y Nueva Zelanda estuvieron asociadas con aceleraciones significativas en las tasas de diversificación fenotípica y de especies comparándolos con sus parientes cercanos más inmediatos en el continente **(Capítulo 2)**. De manera similar, en los *Hemidactylus* de Arabia y Socotra, las tasas de diversificación del tamaño del cuerpo más altas elevadas fueron detectadas después de la colonización de Abd al Kuri y al inicio de la diversificación en Socotra **(Capítulo 1)**. Una vez más, este patrón no se replicó para la forma de la cabeza, para la cual, tanto en Abd al Kuri como en Socotra presentó tasas en el mismo intervalo de las calculadas para el continente. Estas diferencias en patrones evolutivos existentes para diferentes caracteres reflejan posiblemente la importancia relativa de cada carácter en partición de recursos. Este patrón tan particular encontrado en *Hemidactylus* y los geckos diplodactiloides de Australasia, sugiere que la variación del tamaño parece ser uno de los mayores ejes de repartición de recursos en estas islas. Esto ha sido demostrado y publicado para otros grupos de islas, desde ranas a reptiles y se ha hipotetizado que esto principalmente permite la partición a través de los diferentes tamaños de presa (Losos, 2009; Moen et al., 2009). Evidencia adicional proveniente de la dieta de diferentes especies podría ser extremadamente importante para confirmar la interpretación de la gran variación de tamaño existente en estos grupos.

No obstante, cuando comparamos *Hemidactylus* con otros grupos que diversificaron en las mismas islas, parece que no todos los grupos tienden a diversificar en el tamaño del cuerpo. De hecho, la comparación de las fases de la diversificación entre los tres géneros de gecos que ocurre en el Archipiélago de Socotra revela que los distintos géneros no diversificaron siguiendo patrones comunes **(Capítulo 3)**. *Hemidactylus* y *Haemodracon* diversificaron notablemente en tamaño del cuerpo. Sin embargo, la diversificación de *Pristurus* fue fuertemente mediada por cambios climáticos siendo la diversificación en tamaño casi inapreciable. Esto es un resultado importante ya que sugiere que no todos los grupos responden de la misma manera a niveles similares de oportunidad ecológica y que

los ciertos componentes grupo-dependientes (intrínsecos) pueden potencialmente tener un papel importante a la hora de definir las diferentes fases de la diversificación (Losos, 2010).
Uno de estos componentes podría ser las diferentes capacidades para evolucionar, ya sea a nivel morfológico o climático, en los diferentes grupos. Por ejemplo, en algunos grupos diversificar a través de diferentes nichos climáticos puede ser más fácil que diversificar en diferentes formas o tamaños. Interesantemente, cuando se comparan las tasas de la evolución del tamaño del cuerpo entre los tres géneros y otros gecos de fuera del archipiélago, encontramos una situación consistente con las diferentes capacidades evolutivas para el tamaño del cuerpo: *Pristurus*, el género que mayormente diversifica a través de ejes climáticos es también el que presenta las tasas de evolución más bajas.

Es interesante resaltar que el "efecto isla" encontrado para los dos sistemas continente-isla, en ambos casos, involucra islas continentales. Esto demuestra que las islas continentales tienen más en común con las islas oceánicas que con los continentes a partir de los cuales se originan. En el caso de Nueva Zelanda y Nueva Caledonia, esto concuerda con la evidencia que sugiere que estas islas estuvieron parcial o totalmente sumergidas durante un largo período de tiempo (Trewick et al., 2007;Espeland & Murienne, 2011). Es por ello, razonable que hubiesen sido colonizadas después de su re-emergencia **(Capítulo 2)**. Sin embargo, en el archipiélago de Socotra encontramos componentes vicariantes. Esto implica que este archipiélago se comporta biogeográficamente como oceánico, a pesar de haber sido originado como un fragmento continental ecológicamente ocupado **(Capitulo 3)**.
En todas las islas estudiadas (Abd al Kuri, Socotra, Nueva Caledonia y Nueva Zelanda), la diversificación intra-isla (en oposición a eventos de vicarianza y dispersión) ha tenido el papel más importante en la generación de la diversidad en las islas **(Capítulo 2, Capítulo 3)**. Una característica clave que puede haber contribuido a esta situación en todos estos archipiélagos es su aislamiento. Nueva Zelanda y Nueva Caledonia están muy lejos de su continente más cercano (Australia) y los gecos diplodactiloides han dispersado a estas islas (al menos exitosamente) tan sólo en un evento de colonización. En el caso del archipiélago de Socotra, a primera vista, su cercanía geográfica con África y Arabia parece indicar que estas islas no se encuentran muy aisladas. Sin embargo, solamente dos eventos claros de dispersión (al menos exitosos) han ocurrido desde el continente y ni un solo evento de dispersión tuvo lugar entre las dos islas principales en el Archipiélago **(Capítulo 3)**. Esto señala una situación de gran aislamiento en estas islas y puede haber contribuido a explicar los grandes niveles de diversificación *in situ* (Losos y Parent, 2009). Más investigación sobre la dinámica de la diversificación en otros grupos del Archipiélago será crucial para confirmar si este posible aislamiento del Archipiélago de Socotra también se detecta en otros grupos.

En ambos sistemas isla-continente, nuestros análisis proporcionan evidencias convincentes de que la colonización insular ha jugado un papel clave para producir grandes cantidades de diversificación

evolutiva. Sin embargo, los estudios presentados en esta tesis también proporcionan ejemplos de altas tasas de diversificación fenotípica en algunos grupos del continente. Esto es claramente el caso de los pigopódidos Australianos, que experimentaron tasas de diversificación fenotípica (posiblemente diversificación de especies también) comparables a los grupos insulares (ver más abajo) **(Capítulo 2)** y de un pequeño clado de especies continentales recientemente originado en la radiación de *Hemidactylus* **(Capítulo 1)**. Otro ejemplo destacable de diversificación continental revelado en esta tesis es el complejo de especies *Pristurus rupestris* **(Capítulo 4)**. Esta diversificación tuvo lugar en un escenario tipo insular, en las Montañas Hajar en el sureste de Arabia, formadas por tres bloques principales aislados. Sin embargo, en este caso la diversificación no tuvo lugar dentro de cada bloque (como equivalente a isla) y fue mayoritariamente impulsada por pulsos intermitentes de aislamiento y dispersión que tuvieron lugar entre dos de principales bloques de montañas. Ejemplos como éste, proporcionan evidencia de cómo grupos continentales en algunos contextos, como los ofrecidos por dicha cordillera, pueden originar niveles sustanciales de diversificación. En este caso, la mayor diversificación de vertebrados en Arabia.

INNOVACIONES CLAVE COMO MOTORES DE LA DIVERSIFICACIÓN

A lo largo de los capítulos que forman esta tesis, cuando ha sido posible, he integrado los efectos de otras fuentes de oportunidad ecológica con los efectos de la colonización de islas. Éstas fueron dos ejemplos de innovaciones clave: las lamelas adhesivas y el fenotipo en forma de serpiente. De acuerdo con mis resultados, sólo una de las innovaciones examinadas generó niveles de diversificación comparables a los efectos causados por la colonización de islas: la adquisición de fenotipo de serpiente en los pigopódidos Australasiáticos **(Capítulo 2)**. Además, las elevadas tasas detectadas se dieron como consecuencia de adquirir este fenotipo en un momento en el que aún no habían llegado las especies serpentiformes actuales al continente Australiano, lo cual es también consistente con un contexto de muy baja competencia. Esto contrasta con nuestros resultados para las lamelas adhesivas. De acuerdo con nuestros análisis usando los diplodactiloides australasiáticos como modelo, las lamelas adhesivas, a pesar de ser un ejemplo paradigmático de innovación, no tuvieron un papel importante a la hora de generar elevadas altas tasas de diversificación fenotípica y de especies. Esto es congruente con la evidencia proporcionada por un estudio publicado recientemente que analiza esta misma cuestión con a nivel de todos los gecos (Gamble et al., 2012).

Sin embargo, esto no concuerda con los resultados obtenidos en la comparación de distintos géneros dentro del Archipiélago de Socotra. En este caso, los dos géneros con lamelas adhesivas (*Hemidactylus* y *Haemodracon*) fueron precisamente los grupos que experimentaron mayores tasas de diversificación del tamaño del cuerpo, mientras que las especies sin lamelas (*Pristurus*) presentaron tasas muchos menores **(Capítulo 3)**. Estas dos piezas de evidencia aparentemente contradictorias sugieren que los

efectos de las innovaciones clave pueden depender de cada contexto ecológico o taxonómico. Nuevos estudios que exploren tasas de evolución fenotípica ampliando la escala filogenética (incluyendo muchos grupos de geckos y diferentes escenarios ecológicos) seria crucial para aclarar esta materia.

En cualquier caso, el hecho de que en todos los sistemas continente-isla estudiados en esta tesis haya grupos con lamelas adhesivas que hayan llegado a las islas sugiere que la colonización de islas podría ser un factor de confusión importante en todos aquellos estudios orientados a buscar un nexo entre innovaciones clave y diversificación evolutiva.

CONCLUSIONES

1. El género *Hemidactylus*, en el archipiélago de Socotra, y los geckos diplodactiloides, en Nueva Caledonia y Nueva Zelanda, amplían el rango de variación de tamaño corporal con respecto a sus parientes cercanos en el continente, alcanzando el máximo y el mínimo en ambos sistemas continente-isla. Esto concuerda con la expansión del morfoespacio esperada tras la colonización de islas, dado el contexto de baja competencia y depredación existente en las mismas.
2. La tasa de diversificación de especies (en los geckos diplodactiloideos de Nueva Caledonia y Nueva Zelanda) y las tasas de evolución del tamaño corporal (en los geckos diplodactiloideos de Nueva Caledonia y Nueva Zelanda y en los geckos del género *Hemidactylus* del archipiélago de Socotra) se vieron, generalmente, aceleradas con respecto a la mayoría de los grupos continentales. Este es un resultado esperado en el caso de la diversificación en las islas, en las que los grupos tienden rápidamente a ocupar el nuevo paisaje adaptativo que las islas ofrecen.
3. En las especies del género *Hemidactylus* del archipiélago de Socotra, no todos los caracteres experimentaron tasas y magnitudes de diversificación equivalentes. Mientras que el tamaño del cuerpo se aceleró y experimentó una expansión del rango de variación en los clados insulares. En el caso de la forma de la cabeza, no se detectó aceleración alguna respecto al continente aunque las disparidades en la isla de Abd al Kuri presentaban significativamente mayor disparidad en comparación con el continente.
4. Dentro del grupo de los geckos diplodactiloideos australasiáticos, la adquisición del fenotipo de serpiente en la familia Pygopodidae tuvo lugar antes de la llegada de la mayoría de las especies de reptiles con fenotipo de serpiente actualmente presentes en Australia, desencadenando tasas aceleradas de diversificación del tamaño corporal (y también, posiblemente, de especiación).
5. Dentro del grupo de los geckos diplodactiloideos australasiáticos, las lamelas adhesivas, a pesar de ser un ejemplo paradigmático de innovación clave, no indujeron tasas aceleradas de diversificación de fenotípica o de especiación. Ejemplos como estos demuestran que la adquisición de una innovación clave, aunque permite una nueva interacción con el ambiente, no tiene porqué inducir necesariamente altos niveles de diversificación evolutiva.

6. La invasión de nuevos ambientes puede actuar como un factor de confusión en estudios interesados en explorar los efectos evolutivos de la adquisición de innovaciones clave. Los métodos destinados a reconocer patrones de diversificación que no dependan de ninguna delimitación de clados pre-concebida (como Auteur) son cruciales para evitar los efectos de las interacciones y sinergias entre diferentes fuentes de oportunidad ecológica.
7. La diversidad de geckos endémicos del archipiélago de Socotra incluye cinco orígenes filogenéticos independientes y se generó, principalmente, a través de diversificación *in situ*, con una mínima contribución de la dispersión y vicarianza.
8. De acuerdo con nuestras estimas de divergencia, el nodo más profundo dentro del clado de *Pristurus* en Socotra, tuvo lugar antes de que la isla se separara por completo del continente. Ejemplos como éste, advierten que no todos los nodos que separan dos especies de una misma isla son necesariamente consecuencia de eventos de especiación intra-isla.
9. De acuerdo con nuestras estimas de divergencia, el nodo que separa el género de insular *Haemodracon* del género continental *Assacus*, precede el momento en el que el archipiélago de Socotra se separó de Arabia. Éste y otros ejemplos indican que un nodo continente-isla no necesariamente implica un evento de vicarianza o dispersión.
10. No todos los géneros de gecos existentes en Socotra han evolucionado siguiendo los mismos estadios de diversificación. Mientras que en Hemidactylus y en Haemodracon, la mayor parte de la diversificación fue mediada por cambios en el tamaño del cuerpo. En *Pristurus*, la mayor parte de la diversificación intra-isla tuvo lugar a través de ejes de variación climática implicando niveles mínimos de evolución del tamaño del cuerpo. Estos resultados muestran como distintos grupos pueden ampliamente diferir en sus patrones de estructuración ecológica dentro de las islas. A su vez, resultados como estos ponen en cuestión la existencia de patrones generales que puedan ser aplicados a una gran variedad de grupos.
11. La especie *Pristurus rupestris* forma un clado polifilético con dos linajes altamente divergentes. Uno que se distribuye por la costa de Irán y las Montañas Hajar en Oman y el este de Emiratos Árabes (linaje oriental), otra distribuida desde la costa central de Oman hasta Jordania pasando por Yemen y Arabia Saudita (linaje occidental). Por lo tanto podemos decir que "*P. rupestris*" debe ser fragmentado en dos especies: el verdadero *P. rupestris* en el este y una nueva especie en el oeste que referimos como P*risturus sp 1* hasta que se solucionen problemas de nomenclatura.
12. La especie *Pristurus rupestris* en las montañas Hajar (Arabia suroccidental), se trata de un complejo de 14 especies altamente divergentes que empezaron a diversificar hace alrededor de 15 Ma. Esta es la diversificación más grande descrita para un vertebrado en Arabia y demuestra que la diversidad de las regiones Áridas del planeta aun puede estar muy subestimada.

13. Las 14 especies que forman el complejo de especies *P. rupestris* forman dos conjuntos de especies altamente polifiléticos en los bloques central y sureño de las montañas Hajar. Estos son probablemente la consecuencia de pulsos de expansión y retracción de las distribuciones de las especies, implicando los dos bloques.
14. Hábitats de elevada altitud (por encima de 1,500 m) fueron accedidos cuatro veces independientemente en el complejo de especies *P. rupestris*. Tres de las especies de elevada altitud presentan un fenotipo “robusto” compartido, consistiendo en cabezas grandes y distancias largas entre extremidades anteriores y posteriores. Esto sugiere una plausible correlación entre fenotipo y altitud en las montañas Hajar.

ANNEX

(Other papers published during this PhD)

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Molecular phylogenetics and historical biogeography of the west-palearctic common toads (*Bufo bufo* species complex)

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ABSTRACT

In most pan-Eurasiatic species complexes, two phenomena have been traditionally considered key processes of their cladogenesis and biogeography. First, it is hypothesized that the origin and development of the Central Asian Deserts generated a biogeographic barrier that fragmented past continuous distributions in Eastern and Western domains. Second, Pleistocene glaciations have been proposed as the main process driving the regional diversification within each of these domains. The European common toad and its closest relatives provide an interesting opportunity to examine the relative contributions of these paleogeographic and paleoclimatic events to the phylogeny and biogeography of a widespread Eurasiatic group. We investigate this issue by applying a multiproxy approach combining information from molecular phylogenies, a multiple correspondence analysis of allozyme data and species distribution models. Our study includes 304 specimens from 164 populations, covering most of the distributional range of the *Bufo bufo* species complex in the Western Palearctic. The phylogenies (ML and Bayesian analyses) were based on a total of 1988 bp of mitochondrial DNA encompassing three genes (*tRNAval*, *16S* and *ND1*). A dataset with 173 species of the family Bufonidae was assembled to estimate the separation of the two pan-Eurasiatic species complexes of *Bufo* and to date the main biogeographic events within the *Bufo bufo* species complex. The allozyme study included sixteen protein systems, corresponding to 21 presumptive loci. Finally, the distribution models were based on maximum entropy. Our distribution models show that Eastern and Western species complexes are greatly isolated by the Central Asian Deserts, and our dating estimates place this divergence during the Middle Miocene, a moment in which different sources of evidence document a major upturn of the aridification rate of Central Asia. This climate-driven process likely separated the Eastern and Western species. At the level of the Western Palearctic, our dating estimates place most of the deepest phylogenetic structure before the Pleistocene, indicating that Pleistocene glaciations did not have a major role in splitting the major lineages. At a shallow level, the glacial dynamics contributed unevenly to the genetic structuring of populations, with a strong influence in the European–Caucasian populations, and a more relaxed effect in the Iberian populations.

1. Introduction

Throughout the Neogene (23–2.6 Ma), the Palearctic region has experienced several climatic and physiographic changes that have modulated the diversification of its biotas and shaped their distributions. This is particularly true for pan-Eurasiatic groups with distributions extending from the Western to the Eastern Palearctic; several paleoclimatic or paleogeographic events ranging from a regional to a global scale (Blondel and Aronson, 1999; Azanza et al., 2000; Fortelius et al., 2002; Melville et al., 2009) have likely structured these populations. Singularly, the rise of the Himalayas is one of the most important landmarks for understanding the distribution patterns in the Palearctic. This process, initiated 45–55 Ma, is considered the continents' largest perturbation to atmospheric circulation, ultimately originating the Central Asian Deserts and the monsoon-like climate in Eastern Asia (Molnar et al., 2010). Several cases of sister-species complexes at both sides of the deserts have led to the hypothesis that the origin of the Central Asian Deserts separated many Eastern and Western species complexes by

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vicariance (Savage, 1973; Borkin, 1984; Voelker, 1999). For amphibians, Savage (1973) and Borkin (1984) hypothesized that the progressive aridification of Central Asia coupled with global cooling trends during the Miocene (23–5.3 Ma) forced the amphibian faunas to retract their ranges to the South, forming isolates at both sides of the great Central Asian Deserts.

After the above-mentioned splits between Eastern and Western domains, each lineage diversified regionally throughout the rest of the Neogene. However, the major causes of these cladogenetic events in most cases are debated. In the Western Palearctic, the classic "glacial refugia" theory attempts to explain most of these cladogenetic events as a consequence of shifts in the distributional ranges towards the South during the glacial maxima, leading to subsequent allopatric isolation and genetic differentiation in the Mediterranean Peninsulas (Hewitt, 2000). The existence of species or subspecies broadly dividing into Eastern and Western groups backed this theory (e. g. *Pelobates cultripes/Pelobates fuscus*), suggesting that both groups were derived from refugia located in different Mediterranean Peninsulas (mainly Iberian Peninsula, Italian Peninsula and the Balkans) (Llorente et al., 1995). However, dating estimates revealed that although some of the splits were associated with the glacial cycles, this was not a general rule and many splits could be firmly placed in Pre-Pleistocene times (Seddon et al., 2001; Babik et al., 2007). Therefore, the role of Pleistocene glacial cycles shifted from being one of the most important processes for explaining the current diversity of species in the Palearctic to a more labile process with different degrees of relevance depending on the particular organism and the temporal scale considered (Klicka and Zink, 1997; Soria-Carrasco and Castresana, 2011). A more modern view is that the phylogeographic structure of most Paleartic groups is actually a combination of deep splits during the Miocene or Pliocene, followed by a re-structuring caused by fluctuations in population sizes experienced during the Quaternary (e.g. Paulo et al., 2001; Mattoccia et al., 2005; Nascetti et al., 2005; Ursenbacher et al., 2008). Nevertheless, in most cases the historical causes of these deep splits usually remain elusive.

Amphibians constitute a very good model to explore the historical aspects of species distributions due to their low dispersal capacity and retention of a strong phylogeographic signal. Moreover, they are very sensitive to climatic changes, which make them optimal organisms for discriminating the effects of glacial cycles and other environmental changes upon their genetic structure and biogeographic patterns (Zeisset and Beebee, 2008). The European common toad belongs to the genus *Bufo* (*sensu stricto*), a pan-Eurasiatic group comprising two species complexes. Eastern Eurasia contains the greatest species richness of the genus, with 13–14 recognized species distributed across Central and Eastern China, Northern Vietnam, Korea, far Eastern Russia, and Japan (here and after the *Bufo gargarizans* species complex) (Frost, 2011; see also Zhan and Fu, 2011).

The second complex occurs in the Western Palearctic, and only two or three valid species are currently recognized (here and after the *Bufo bufo* species complex) (Litvinchuk et al., 2008; Frost, 2011): the Eichwald toad (*Bufo eichwaldi* Litvinchuk et al., 2008), restricted to the Talysh mountains of the Southeastern Caucasus; the Caucasian toad (*Bufo verrucosissimus* (Pallas, 1814), not recognized by e.g. Crochet and Dubois, 2004), which inhabits the Caucasus and Anatolia; and the European common toad (*Bufo bufo* (Linnaeus, 1758)), the Palearctic anuran with the largest distributional range, spanning from North Africa to the Polar circle and from the Western Iberian Peninsula to the Baikal Lake in Siberia (Lizana, 2002). Despite this huge distributional range, according to Mertens and Wermuth (1960), the European common toad is a single species with three subspecies: (1) the nominate subspecies *Bufo bufo bufo*, the Eurosiberian form, distributed across Northern and Central Europe, Western Siberia, the British Islands and the Eurosiberian enclaves of the Mediterranean peninsulas, (2) *Bufo bufo spinosus* Mertens, 1925, considered the Mediterranean counterpart of the nominal subspecies, occupying the Mediterranean margins of Europe, North Africa and most parts of Western and Central France (Geniez and Cheylan, 2005, in press), and (3) *Bufo bufo gredosicola* Müller & Hellmich, 1925, with a very limited distributional range restricted to the highest prairies and lakes of the Sierra de Gredos, in Central Iberian Peninsula.

Since the European common toad and its closest relatives present a disjunct distribution across Eurasia (Lizana, 2002), and also show regional structure in the Western Palearctic, they provide an interesting opportunity to examine the importance of the Central Asian Deserts as the vicariant event that separated Eastern and Western species complexes, and secondly to assess the relative contribution of both glacial and preglacial events in the regional structure of the Western Palearctic.

The aim of the present study is to combine data from molecular phylogenies, multiple correspondence analyses of allozyme data and species distribution models, to unravel the historical processes that have contributed to shaping the biogeography and cladogenesis of the most abundant and widely distributed amphibian genus in the Palearctic.

2. Methods

2.1. Taxon sampling, DNA extraction, amplification and sequencing

A total of 151 specimens were included in the mitochondrial DNA study, covering the entire distribution range of the species complex in the Western Palearctic (Table 1 and Fig. 1). Of these, 147 are members of the *Bufo bufo* species complex, with four specimens obtained from GenBank (Benson et al., 2008). The remaining four specimens belong to the *Bufo gargarizans* species complex and were used as outgroups (all obtained from GenBank). A list of all the samples used in the present work with their extraction codes, voucher references, corresponding localities and GenBank accession numbers can be found in Table 1. Genomic DNA was extracted from ethanol-preserved tissue samples using the Qiagen DNeasy Blood & Tissue Kit. A total of 1988 bp of mitochondrial DNA were sequenced for most of the specimens (5.8% of missing data), encompassing fragments of three genes: *tRNAval* (48 bp), *16SrRNA* (1386 bp) and *ND1* (554 bp). Already published primers for the amplification and sequencing of the mitochondrial gene fragments included in the present study as well as PCR conditions used are given in detail in Biju and Bossuyt (2003) and Roelants and Bossuyt (2005). All amplified fragments were sequenced for both strands. Contigs were assembled in Geneious v. 5.3.6 (Biomatters Ltd.).

2.2. Phylogenetic analyses of mitochondrial DNA

The sequences obtained were aligned using the online version of MAFFT 6.240 (Katoh et al., 2002) (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/), following a FFT-NS-i strategy (slow, iterative refinement method) with the rest of the settings left by default (scoring matrix 200PAM ($k = 2$), gap opening penalty = 1.53). The gaps generated by the process of alignment were considered missing data in all the following analyses.

Two methods of phylogenetic analysis, namely maximum likelihood (ML) and Bayesian analysis (BI), were employed and their results compared. The ML analysis was performed using RaxML 7.0.4 (Stamatakis, 2006) with the dataset split in two partitions: one partition including the RNA-coding genes and the other including the protein-coding gene (*ND1*). JModeltest (Posada, 2008) was used to select the most appropriate model of sequence evolution

Table 1
Sampling localities for the mitochondrial phylogeny and allozyme analysis (including geographic coordinates and country), taxonomic assignation and clade or group assignation according to the molecular phylogeny or the MCA analysis, respectively. A map with the geographic distribution of all the representatives of the *Bufo bufo* species complex included in our analyses is shown in Fig. 1.

Specimen number	Data Type	Latitude	Longitude	Locality	Country	Taxon	Clade/ MCA group	VOUCHER	GenBankt RNA-16S 1st part	GenBank16S 2nd part	GenBank ND1
1	DNA	42.42139	-3.6445	Zuriza	Spain	*B. b. bufo*	Iberian		JQ348599	JQ348742	JQ348501
2	DNA	43.1224	-3.7149	Vega de Pas 1	Spain	*B. b. bufo*	Iberian		JQ348581	JQ348724	JQ348497
3	DNA	43.1272	-3.7263	Vega de Pas 2	Spain	*B. b. bufo*	Iberian		JQ348585	JQ348728	JQ348527
4	DNA	43.3202	-5.3521	Valle del Tendi	Spain	*B. b. bufo*	Iberian		JQ348563	JQ348706	JQ348500
5	DNA	42.4514	-3.6450	Udiema river	Spain	*ambiguous*	Iberian		JQ348550	JQ348693	JQ348524
6	DNA	42.3414	1.7586	Torrent del Pi	Spain	*B. b. spinosus*	Iberian		JQ348556	JQ348699	JQ348529
7	DNA	39.7717	-6.0143	Torrejon el Rubio	Spain	*B. b. spinosus*	Iberian		FJ882841		
8	DNA	45.8162	2.3252	Tigouleix	France	*B. b. spinosus*	Iberian			JQ348687	
9	DNA	41.9215	0.7185	Tartareu	Spain	*B. b. spinosus*	Iberian		JQ348560	JQ348703	JQ348486
10	DNA	42.3686	2.9807	St. Climent Sescebes	Spain	*B. b. spinosus*	Iberian		JQ348596	JQ348739	JQ348485
11	DNA	42.6253	1.0864	Son	Spain	*B. b. bufo*	Iberian		JQ348573	JQ348716	JQ348528
12	DNA	38.7985	-9.3881	Sintra	Portugal	*B. b. spinosus*	Iberian		JQ348558	JQ348701	JQ348506
13	DNA	43.7206	3.1517	Serieys	France	*B. b. spinosus*	Iberian		JQ348602	JQ348745	
14	DNA	41.7600	2.3949	Montseny 1	Spain	*B. b. spinosus*	Iberian		JQ348549	JQ348692	JQ348522
15	DNA	41.7686	2.4699	Montseny 2	Spain	*B. b. spinosus*	Iberian		JQ348575	JQ348718	JQ348478
16	DNA	42.1858	-6.8684	Sanabria	Spain	*B. b. spinosus*	Iberian		JQ348559	JQ348702	JQ348503
17	DNA	43.1565	-3.8213	San Pedro del Romeral 1	Spain	*B. b. spinosus*	Iberian		JQ348579	JQ348722	JQ348491
18	DNA	43.1565	-3.8213	San Pedro del Romeral 2	Spain	*B. b. spinosus*	Iberian		JQ348580	JQ348723	JQ348496
19	DNA	43.8600	3.3807	Saint-Michel	France	*B. b. spinosus*	Iberian	BEV.1271-1272	JQ348547	JQ348690	JQ348481
20	DNA	36.0917	-5.4455	Riogetares	Spain	*B. b. spinosus*	Iberian		JQ348564	JQ348707	JQ348507
21	DNA	43.0995	-5.0109	Retuerto	Spain	*B. b. bufo*	Iberian		JQ348582	JQ348725	JQ348492
22	DNA	42.7154	-3.0592	Santa Gadea del Cid	Spain	*B. b. spinosus*	Iberian		JQ348562	JQ348705	JQ348488
23	DNA	43.0646	-5.3884	Puerto de San Isidro	Spain	*B. b. spinosus*	Iberian		JQ348590	JQ348733	JQ348490
24	DNA	40.9468	-3.7600	Puerto de Navacerrada	Spain	*B. b. spinosus*	Iberian		JQ348591	JQ348734	JQ348516
25	DNA	36.9166	-3.0423	Darrical	Spain	*B. b. spinosus*	Iberian		JQ348565	JQ348708	JQ348515
26	DNA	43.0107	-4.7463	Pozo de las Lomas	Spain	*B. b. bufo*	Iberian		JQ348600	JQ348743	JQ348523
27	DNA	43.0567	-3.8412	Penilla	Spain	*B. b. spinosus*	Iberian		JQ348583	JQ348726	JQ348493
28	DNA	44.8064	1.4554	Payrac	France	*B. b. spinosus*	Iberian	BEV.680	JQ348555	JQ348698	JQ348483
29	DNA	36.3649	-6.0718	Pago del Humo	Spain	*B. b. spinosus*	Iberian		JQ348567	JQ348710	JQ348525
30	DNA	47.5543	-1.6529	Nozay	France	*B. b. spinosus*	Iberian		JQ348601	JQ348744	JQ348504
31	DNA	43.5755	3.7195	Murviel-lès-Montpellier	France	*B. b. spinosus*	Iberian	BEV.682			JQ348415
32	DNA	43.2275	3.1938	Mire l'Etang	France	*B. b. spinosus*	Iberian	BEV.8851	JQ348684	JQ348689	
33	DNA	43.8867	3.5680	Rogues	France	*B. b. spinosus*	Iberian	BEV.1456		JQ348688	
34	DNA	43.0897	-1.3034	Luzaide	Spain	*ambiguous*	Iberian		JQ348589	JQ348732	JQ348499
35	DNA	37.8801	-6.6210	Linares de la Sierra	Spain	*B. b. spinosus*	Iberian		JQ348557	JQ348700	JQ348505
36	DNA	42.5652	2.1004	Les Angles	France	*B. b. spinosus*	Iberian		JQ348595	JQ348738	JQ348479
37	DNA	36.8343	-3.6744	Lentegi	Spain	*B. b. spinosus*	Iberian		JQ348568	JQ348711	JQ348520
38	DNA	42.3693	-8.000	Punxin	Spain	*ambiguous*	Iberian		JQ348577	JQ348720	JQ348502
39	DNA/ allozymes	39.6667	-9.000	Pataias	Portugal	*B. b. spinosus*	Iberian		JQ348597	JQ348740	
40	DNA	43.5248	5.5470	Le Tholonet	France	*B. b. spinosus*	Iberian		JQ348553	JQ348696	JQ348480
41	DNA	43.0462	-1.0733	Larrau	France	*B. b. bufo*	Iberian		JQ348682	JQ348827	JQ348498
42	DNA	40.2841	-5.2497	Gredos 1	Spain	*B. b. gredosicola*	Iberian		JQ348592	JQ348735	JQ348512
43	DNA	40.2841	-5.2497	Gredos 2	Spain	*B. b. gredosicola*	Iberian		JQ348593	JQ348736	JQ348513
44	DNA	40.2841	-5.2497	Gredos 3	Spain	*B. b. gredosicola*	Iberian		JQ348569	JQ348712	JQ348511
45	DNA/ allozymes	43.5547	2.7941	Lac du Saut de Vésoles	France	*B. b. spinosus*	Iberian		JQ348548	JQ348691	JQ348482
46	DNA	40.8640	-3.6156	La Cabrera	Spain	*B. b. spinosus*	Iberian		JQ348584	JQ348727	JQ348494
47	DNA	36.5444	-5.6616	24 km NE of medina-Sidonia	Spain	*B. b. spinosus*	Iberian		JQ348566	JQ348709	JQ348508
48	DNA	43.0556	-5.3261	Isoba	Spain	*B. b. bufo*	Iberian		JQ348586	JQ348729	JQ348495
49	DNA	42.8816	-0.7153	Lac d'Ansabère	France	*ambiguous*	Iberian		JQ348574	JQ348717	JQ348521
50	DNA	41.7867	1.2908	Guissona	Spain	*B. b. spinosus*	Iberian		JQ348588	JQ348731	JQ348489
51	DNA	37.6568	-5.5224	Lora del Rio	Spain	*B. b. spinosus*	Iberian		JQ348578	JQ348721	JQ348510
52	DNA	36.2767	-6.0884	Conil de la Frontera	Spain	*B. b. spinosus*	Iberian		JQ348587	JQ348730	JQ348519
53	DNA	43.9638	3.3232	Combe-Redonde	France	*B. b. spinosus*	Iberian		JQ348554	JQ348697	
54	DNA	42.4728	-7.9853	San Cristovo de Cea	Spain	*ambiguous*	Iberian		JQ348551	JQ348694	JQ348476
55	DNA	36.9612	-3.3586	Capileira	Spain	*B. b. spinosus*	Iberian		JQ348594	JQ348737	JQ348509
56	DNA	42.3719	2.9221	Capmany	Spain	*B. b. spinosus*	Iberian		JQ348576	JQ348719	JQ348484

(*continued on next page*)

Table 1 (*continued*)

Specimen number	Data Type	Latitude	Longitude	Locality	Country	Taxon	Clade/ MCA group	VOUCHER	GenBankt RNA-16S 1st part	GenBank16S 2nd part	GenBank ND1
57	DNA	38.1479	-6.5601	Bodonal de la Sierra	Spain	*B. b. spinosus*	Iberian		JQ348572	JQ348715	JQ348518
58	DNA	43.0490	-1.6145	Puerto de Belate	Spain	*B. b. bufo*	Iberian		JQ348561	JQ348704	JQ348487
59	DNA	41.4501	2.2474	Badalona	Spain	*B. b. spinosus*	Iberian		JQ348552	JQ348695	JQ348477
60	DNA	39.3037	-0.5859	Catadau	Spain	*B. b. spinosus*	Iberian	BEV.7287	JQ348598	JQ348741	JQ348514
61	DNA	37.8744	-6.6661	Alajar	Spain	*B. b. spinosus*	Iberian		JQ348571	JQ348714	JQ348526
62	DNA	41.8077	-2.7856	Abejar	Spain	*B. b. spinosus*	Iberian		JQ348570	JQ348713	JQ348517
63	DNA	40.6996	39.4678	Anayurt	Turkey	*B. b. spinosus*	European	BEV.7627-7628	JQ348659	JQ348804	JQ348447
64	DNA	44.1167	15.2333	Zadar	Croatia	*B. b. spinosus*	European		JQ348657	JQ348802	JQ348456
65	DNA/ allozymes	45.400	29.600	Vilkovo	Ukraine	*B. b. bufo*	European		JQ348660	JQ348805	JQ348469
66	DNA	40.3796	15.5310	Teggiano	Italy	*B. b. spinosus*	European		JQ348648	JQ348793	JQ348466
67	DNA	43.3187	11.3305	Siena	Italy	*B. b. spinosus*	European		JQ348667	JQ348812	JQ348436
68	DNA	52.7066	1.3993	Wroxham	UK	*B. b. bufo*	European		JQ348661	JQ348806	JQ348445
69	DNA/ allozymes	39.6167	19.7833	Ropa, Kerkira island	Greece	*B. b. spinosus*	European		JQ348668	JQ348813	JQ348432
70	DNA	56.9465	24.1048	Riga	Latvia	*B. b. bufo*	European		AY325988		
71	DNA	41.4135	26.6289	Pythio	Greece	*B. b. spinosus*	European		JQ348658	JQ348803	JQ348446
72	DNA	46.0808	12.5378	Piancavallo	Italy	*B. b. spinosus*	European		JQ348622	JQ348767	JQ348458
73	DNA	40.3490	15.4383	Piaggine	Italy	*B. b. spinosus*	European		JQ348649	JQ348794	JQ348463
75	DNA	48.7406	22.4890	Perechin	Ukraine	*B. b. bufo*	European		JQ348636	JQ348781	JQ348438
76	DNA	36.6910	15.0692	Pachino	Italy	*B. b. spinosus*	European		JQ348654	JQ348799	JQ348474
77	DNA	40.0436	22.3002	Olympus mt.	Greece	*B. b. spinosus*	European		JQ348627	JQ348772	JQ348431
78	DNA	52.6281	1.2993	Norwich	UK	*B. b. bufo*	European		JQ348632	JQ348777	
79	DNA	43.7734	7.2241	Nice	France	*B. b. spinosus*	European	BEV.T2997	JQ348683		JQ348452
80	DNA	42.9244	12.0579	Monteleone d'Orvieto	Italy	*B. b. spinosus*	European		JQ348643	JQ348788	JQ348434
81	DNA	38.8723	23.2389	Marouli	Greece	*B. b. spinosus*	European		JQ348631	JQ348776	JQ348430
82	DNA	41.5516	13.1711	Maenza	Italy	*B. b. spinosus*	European		JQ348640	JQ348785	JQ348433
83	DNA	50.7635	4.27931	Lot	Belgium	*B. b. bufo*	European		JQ348665	JQ348810	JQ348470
84	DNA	51.0020	4.3019	Londerzeel	Belgium	*B. b. bufo*	European		FJ882806		
85	DNA	37.9208	13.3732	Lago Scanzano 1	Italy	*B. b. spinosus*	European		JQ348651	JQ348796	JQ348460
86	DNA	37.9208	13.3732	Lago Scanzano 2	Italy	*B. b. spinosus*	European		JQ348653	JQ348798	JQ348472
87	DNA	54.7333	49.2333	Kokryad	Russia	*B. b. bufo*	European		JQ348637	JQ348782	JQ348440
88	DNA	40.0764	22.2269	Kokkinopilos	Greece	*B. b. spinosus*	European		JQ348625	JQ348770	JQ348426
89	DNA	55.4580	12.1821	Køge	Denmark	*B. b. bufo*	European		JQ348635	JQ348780	JQ348437
90	DNA	39.6358	21.2182	Katafyto	Greece	*B. b. spinosus*	European		JQ348628	JQ348773	JQ348428
91	DNA	53.5757	7.9003	Jever	Germany	*B. b. bufo*	European		JQ348633	JQ348778	
92	DNA	57.1601	18.3362	Havdhem, Gotland	Sweden	*B. b. bufo*	European	BEV.7720	JQ348634	JQ348779	
93	DNA	36.9664	21.6989	Gialova	Greece	*B. b. spinosus*	European		JQ348626	JQ348771	JQ348427
94	DNA	44.5769	6.0532	Gap	France	*B. b. spinosus*	European	BEV.1259	JQ348672	JQ348817	JQ348443
95	DNA/ allozymes	49.65	36.26	Haidary	Ukraine	*B. b. bufo*	European	ZISP.7282	JQ348673	JQ348818	JQ348442
96	DNA	46.6468	6.0088	Foncine-le-Bas	France	*B. b. bufo*	European	BEV.8928	JQ348675	JQ348820	JQ348455
97	DNA	37.9871	14.9083	Floresta	Italy	*B. b. spinosus*	European		JQ348656	JQ348801	JQ348462
98	DNA	40.9272	37.9523	Kayabaşı	Turkey	*B. b. spinosus*	European	BEV.7656	JQ348679	JQ348824	JQ348449
99	DNA	39.5528	16.0222	Lago dei Due Uomini	Italy	*B. b. spinosus*	European		JQ348655	JQ348800	JQ348465
100	DNA	39.5604	21.3719	Desi	Greece	*B. b. spinosus*	European		JQ348630	JQ348775	JQ348429
101	DNA	45.2868	5.9067	Crolles	France	*ambiguous*	European	BEV.T2998	JQ348623	JQ348768	JQ348451
102	DNA	44.4722	9.0083	Creto	Italy	*B. b. spinosus*	European		JQ348642	JQ348787	JQ348471
103	DNA	45.7725	4.1759	Cleppé	France	*B. b. spinosus*	European	BEV.10226	JQ348624	JQ348769	
104	DNA	39.5167	15.9500	Cetraro	Italy	*B. b. spinosus*	European		JQ348686	JQ348763	JQ348464
105	DNA	37.8835	14.6564	Cesaro	Italy	*B. b. spinosus*	European		JQ348652	JQ348797	JQ348461
106	DNA	38.0825	14.8162	Castell'Umberto	Italy	*B. b. spinosus*	European		JQ348676	JQ348821	JQ348473
107	DNA	41.1260	16.8693	Bari	Italy	*B. b. spinosus*	European		JQ348650	JQ348795	JQ348467
108	DNA/ allozymes	41.6667	12.9833	Campa di Segni	Italy	*B. b. spinosus*	European	ZISP.9534	JQ348641	JQ348786	JQ348435
109	DNA	37.7597	13.8930	Caltavuturo 1	Italy	*B. b. spinosus*	European		JQ348685	JQ348762	JQ348459
110	DNA	37.7597	13.8930	Caltavuturo 2	Italy	*B. b. spinosus*	European		JQ348678	JQ348823	JQ348468
111	DNA	40.1833	28.8905	Bursa	Turkey	*B. b. spinosus*	European		DQ158438		
112	DNA	46.3667	14.1085	Bled	Slovenia	*ambiguous*	European		JQ348664	JQ348809	JQ348450
113	DNA	48.6	35.6	Balakhovka	Ukraine	*B. b. bufo*	European		JQ348638	JQ348783	JQ348441
115	DNA	41.5548	36.1127	Bafra	Turkey	*B. b. spinosus*	European	BEV.7635	JQ348671	JQ348816	JQ348448
116	DNA	47.2802	13.2313	Au	Austria	*B. b. bufo*	European		JQ348620	JQ348765	JQ348453
117	DNA/ allozymes	38.0333	23.7167	Athens	Greece	*B. b. spinosus*	European		JQ348629	JQ348774	JQ348475
118	DNA	45.9675	11.4142	Asiago	Italy	*ambiguous*	European		JQ348621	JQ348766	JQ348457
119	DNA	44.4742	3.8612	Altier	France	*B. b. spinosus*	European	BEV.10238	JQ348677	JQ348822	JQ348444
120	DNA	44.9298	4.8899	Valence	France	*B. b. spinosus*	European		JQ348663	JQ348808	JQ348454
121	DNA	41.65	41.80	Tirala Mt.	Russia	*B. verrucosissimus*	Caucasian	ZISP.6534	JQ348644	JQ348789	JQ348416

122	DNA/ allozymes	44.0833	40.7667	Psebai	Russia	*B. verrucosissimus*	Caucasian	ZISP.6547	JQ348647	JQ348792	JQ348424
123	DNA	41.8262	46.2697	Lagodekhi	Georgia	*B. verrucosissimus*	Caucasian	ZISP.4963	JQ348645	JQ348790	JQ348417
124	DNA	45.4167	40.6167	Kyurdzhinovo	Russia	*B. verrucosissimus*	Caucasian	ZISP.6541	JQ348619	JQ348764	JQ348420
125	DNA/ allozymes	44.7167	38.6833	Krepostnaya	Russia	*B. verrucosissimus*	Caucasian		JQ348662	JQ348807	JQ348425
126	DNA	42.9053	41.9833	Katkova	Georgia	*B. verrucosissimus*	Caucasian		JQ348670	JQ348815	JQ348423
127	DNA/ allozymes	41.7167	46.6	Katekh	Azerbaijan	*B. verrucosissimus*	Caucasian		JQ348674	JQ348819	JQ348418
128	DNA	44.83	40.20	Guzeripl	Russia	*B. verrucosissimus*	Caucasian	ZISP.6614	JQ348669	JQ348814	JQ348421
129	DNA	44.5777	38.0802	Gelenjik	Russia	*B. verrucosissimus*	Caucasian		JQ348646	JQ348791	JQ348422
130	DNA/ allozymes	38.45	48.73	Sim	Azerbaijan	*B. eichwaldi*	Caspian		JQ348681	JQ348826	JQ348546
131	DNA/ allozymes	38.65	48.8	Avrora	Azerbaijan	*B. eichwaldi*	Caspian	ZISP.7185	JQ348680	JQ348825	JQ348545
132	DNA	35.3689	-5.5402	Zinat	Morocco	*B. b. spinosus*	African		JQ348614	JQ348757	JQ348541
133	DNA	34	-4	Tazeka 1	Morocco	*B. b. spinosus*	African		JQ348608	JQ348751	JQ348535
134	DNA	34	-4	Tazeka 2	Morocco	*B. b. spinosus*	African		JQ348609	JQ348752	JQ348536
135	DNA	35.0706	-5.1742	3 km NE of Bab Taza	Morocco	*B. b. spinosus*	African		JQ348615	JQ348758	JQ348537
136	DNA	33.5161	-4.5322	Skoura M'daz	Morocco	*B. b. spinosus*	African		JQ348613	JQ348756	JQ348539
137	DNA	35.2801	-5.4018	Souk-el-Arba-des-Beni-Hassan	Morocco	*B. b. spinosus*	African		JQ348612	JQ348755	JQ348544
138	DNA	35.3335	-5.5382	Moulay Abdeslam	Morocco	*B. b. spinosus*	African		JQ348611	JQ348754	JQ348542
139	DNA	34.8759	-6.2495	Merja Zerga	Morocco	*B. b. spinosus*	African		JQ348617	JQ348760	JQ348540
140	DNA	34.05	-3.7667	Ifrane	Morocco	*B. b. spinosus*	African		JQ348618	JQ348761	JQ348538
141	DNA/ allozymes	34.95	-5.23	Fifi	Morocco	*B. b. spinosus*	African		JQ348607	JQ348750	JQ348533
142	DNA	35.1242	-5.7749	3 km W of Hamaïmoun	Morocco	*B. b. spinosus*	African		JQ348610	JQ348753	JQ348534
143	DNA	32.4908	-5.2347	Tounfite	Morocco	*B. b. spinosus*	African		JQ348616	JQ348759	JQ348543
144	DNA/ allozymes	36.7304	8.7080	Ain Draham 1	Tunisia	*B. b. spinosus*	African	ZISP.7523	JQ348603	JQ348746	JQ348532
145	DNA	36.776	8.6917	Ain Draham 2	Tunisia	*B. b. spinosus*	African		JQ348606	JQ348749	JQ348531
146	DNA	36.7304	8.7080	Ain Draham 3	Tunisia	*B. b. spinosus*	African		JQ348605	JQ348748	
147	DNA	36.7801	8.8183	Ain Draham 4	Tunisia	*B. b. spinosus*	African		JQ348604	JQ348747	JQ348530
148	Allozymes	57.5833	9.9667	Hirtshals	Denmark	*B. b. bufo*	European	ZISP.8525-8526			
149	Allozymes	55.6833	13.1667	Lund	Sweden	*B. b. bufo*	European				
150	Allozymes	52.6000	13.6167	Blumberg	Germany	*B. b. bufo*	European				
151	Allozymes	45.5000	17.5167	1.5 km N of Mrkoplje	Croatia	*B. b. spinosus*	European				
152	Allozymes	54.7333	28.3333	Domzheritsy	Byelorussia	*B. b. bufo*	European				
153	DNA/ allozymes	54.38	20.64	Bagrationovsk	Russia	*B. b. bufo*	European	ZISP.7048	JQ348639	JQ348784	JQ348439
154	Allozymes	59.56	30.12	Gatchina	Russia	*B. b. bufo*	European	ZISP.6992, 7259, 7508			
155	Allozymes	59.45	29.37	6 km W Volosovo	Russia	*B. b. bufo*	European				
156	Allozymes	61.50	29.98	Ikhala	Russia	*B. b. bufo*	European				
157	Allozymes	55.60	40.67	Gus-Khrustalnyi	Russia	*B. b. bufo*	European				
158	Allozymes	54.2167	37.6167	Tula	Russia	*B. b. bufo*	European				
159	Allozymes	51.48	23.85	Svityaz'	Ukraine	*B. b. bufo*	European				
160	Allozymes	50.43	25.74	Dubno	Ukraine	*B. b. bufo*	European				
161	Allozymes	48.7833	29.5167	Chechelivka	Ukraine	*B. b. bufo*	European				
162	Allozymes	46.65	29.75	Laptura Lake	Moldavia	*B. b. bufo*	European				
163	Allozymes	36.6948	-5.7733	San Jose del Valle	Spain	*B. b. spinosus*	Iberian				
165	Allozymes	35.4920	-5.8227	5 km NE of Charkia	Morocco	*B. b. spinosus*	African				
166	Allozymes	40.6167	31.2833	Abant Lake	Turkey	*B. b. spinosus*	Caucasian	ZISP.8101-8103			
167	DNA/ allozymes	36.55	32.1167	Alanya	Turkey	*B. b. spinosus*	Caucasian		JQ348666	JQ348811	JQ348419
168	Allozymes	42.98	41.10	Bagmaran	Abkhazia	*B. verrucosissimus*	Caucasian				
169	Allozymes	43.17	40.35	Lidzava	Abkhazia	*B. verrucosissimus*	Caucasian				
170	Allozymes	43.80	40.65	Nikitino	Russia	*B. verrucosissimus*	Caucasian				

OUTGROUPS Species	Locality	Country	Data Type	GenBank ref
B. gargarizans	Chusan Island	China	DNA	NC008410.1
B. gargarizans	Song	China	DNA	FJ882843.1
B. gargarizans	Bonevurovka	Russia	allozymes	Allozymes - 171
B. gargarizans	Krym	Russia	allozymes	Allozymes - 172
B. gargarizans	Novoaleksandrovsk (Sakhalin Island)	Russia	allozymes	Allozymes - 173
B. gargarizans	Quingcheng Mt. (Sichuan Province)	China	allozymes	Allozymes - 174
B. japonicus	Hiroshima	Japan	DNA	NC009886.1
B. andrewsi	Yunnan	China	DNA	FJ882808

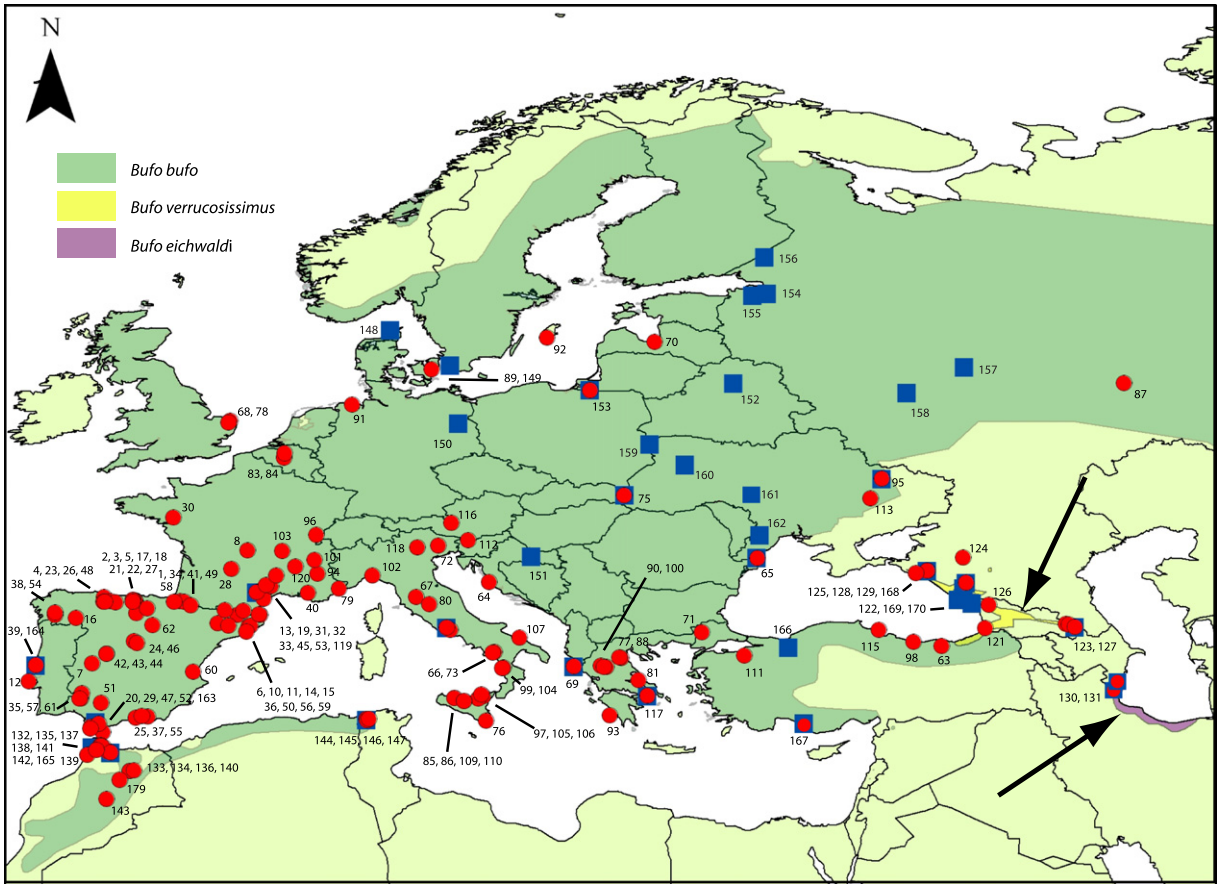

Fig. 1. Sampling localities included in the present study. Red circles indicate DNA sampling localities; blue squares indicate allozyme sampling localities. The background colors indicate the known distribution of *Bufo bufo* (green), *Bufo verrucosissimus* (yellow) and *Bufo eichwaldi* (purple). Map numbers refer to specimens listed in Table 1 and Fig. 2. Arrows highlight the distribution ranges of *B. verrucosissimus* and *B. eichwaldi*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for the ML and Bayesian analyses under Akaike's information criterion. This was the GTR model taking into account the gamma distribution and the number of invariant sites for each of the two independent partitions. To explore the topological space, we performed 1000 independent heuristic searches each one starting from a parsimony tree. The likelihoods of the resulting trees of each run were compared and the one with the highest $-\log L$ was selected. Reliability of the ML searches was assessed by bootstrap analysis (Felsenstein, 1985), involving 1000 replications. Support values for every node were superimposed onto the best tree topology.

BI analysis was conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The dataset was split into the same two partitions mentioned before (protein-coding and RNA-coding mitochondrial regions) and two independent runs of four Markov chain Monte Carlo (MCMC) chains were executed in parallel for 10 million generations, with a sampling interval of 1000 generations. Preliminary analyses (data not shown) revealed that our dataset was extremely sensitive to the branch-length prior, producing trees with very long branches (several orders of magnitude longer than the branches of the ML tree). This is not a rare phenomenon, which has been reviewed and analyzed in several recent papers (e.g. Brown et al., 2010; Marshall, 2010). To solve this problem, we followed the recommendations by Brown et al. (2010) and specified a branch-length prior with a smaller mean to reduce the posterior probability of the long-tree region in the branch-length space. The new mean was calculated using the formula proposed by the authors and was set to 1/379. Convergence of the two runs was evidenced by a split frequency standard deviation lower than 0.01 and by potential scale-reduction factors to 1 for all model parameters as shown by the command "sump" in MrBayes (Ronquist and Huelsenbeck, 2003). Posterior probabilities (pp) for every clade were obtained by combining the sampled trees from the two parallel runs excluding a relative burn-in of 30% of the trees from each run. In both analyses three species of the *Bufo gargarizans* species complex were used to root the tree (*Bufo gargarizans, Bufo japonicus* and *Bufo andrewsi*).

To determine whether the mtDNA data supported the currently established taxonomic partitions proposed by Mertens and Wermuth (1960), we used topological constraints to enforce monophyly of the subspecies as currently defined (see Table 1), using the package Mesquite v2.78 (Maddison and Maddison, 2009). Topological constraints were compared to optimal topologies using the Approximately-Unbiased (AU) (Shimodaira, 2002) and Shimodaira-Hassegawa tests implemented in CONSEL (Shimodaira and Hasegawa, 2001). The subspecies were assigned to our specimens following Mertens and Wermuth (1960), Lizana (2002) and Muratet (2008) and on information on the morphological variation of the species (PAC and PG, unpublished results). The specimens of dubious taxonomic assignment were excluded from the test.

2.3. Allozymes

Sixteen protein systems, corresponding to 21 presumptive loci were examined for 172 specimens from 40 different localities, including *Bufo gargarizans* and representatives from all the species

and subspecies of the *Bufo bufo* species complex, with the only exception of *B. b. gredosicola*. Electrophoretic conditions for the proteins studied were as described by Litvinchuk et al. (2008). The populations of the *Bufo bufo* species complex included in the allozyme analysis are shown in Fig. 1, and the corresponding locality names and taxonomic assignment are presented in Table 1.

The software BIOSYS-1 (Swofford and Selander, 1981) was used to calculate the average expected and observed heterozygosity per locus (Hexp and Hobs), the percentage of polymorphic loci (P), as well as Nei's genetic distances (Nei, 1978). The matrix of Nei's genetic similarities was converted into a neighbor-joining tree (NJ; Saitou and Nei, 1987) using MEGA 5.0 (Tamura et al., 2011). This method of phylogenetic reconstruction is known to perform well for allozyme data (Wiens, 2000).

A Multiple Correspondence Analysis (MCA) on the population frequency data was performed with the computer software Statistica Kernel version 5.5 (StatSoft, Inc.; Tulsa; USA). For this analysis each row in the dataset was a population and each column represented the frequency of the *i*th allele of the *j*th locus.

2.4. Dating estimates

Divergence dates for our dataset were estimated using a Bayesian relaxed molecular clock approach (BRMC) by means of the package BEAST v. 1.5.2 (Drummond and Rambaut, 2007). Given that some priors (e.g. tree priors) do not adequately account simultaneously for both interspecific (phylogenetic priors) and intraspecific data (coalescent priors), we opted for a reduction of our dataset maintaining solely one representative of each clade as appeared in our ML phylogeny. In this way we also reduced the amount of intraspecific polymorphism, which yields an overestimation of the divergence times when deep and external calibration points are used (Ho et al., 2008).

In order to introduce the calibration points, all external to our ingroup, we included the *Bufo bufo* species complex in the phylogenetic context of 173 species belonging to the family Bufonidae (approximately 30% of the species of the family) plus six species that were used as outgroups (Supplementary material I and II).

Four external calibration points were used, all of them already employed in previous studies to calibrate timescales encompassing the whole family Bufonidae (Pramuk et al., 2007; Van Bocxlaer et al., 2009, 2010, see references therein) (Supplementary material II):

1. A soft maximum of 49 Ma for the split between the Caribbean Bufonidae (genus *Peltophryne*) and their closest mainland relatives (genus *Rhaebo*). This calibration is based on the geological evidence implying that the existence of emerged land in the Caribbean Sea is not older than 37–49 Ma. The soft maximum was obtained by means of a lognormal distribution with a mean placed in 27 Ma, a standard deviation of 0.35 and an offset of 5 Ma.
2. The oldest fossil attributable to the family Bufonidae in North America (20 Ma), was used as a minimum age for the split between the North American toads (genus *Anaxyurus*) and their sister group, the Central-American toads of the genus *Incilius*. This was set using a gamma distribution (alpha = 1.2, beta = 4) starting at 20 Ma.
3. The oldest fossils belonging to the *Bufotes viridis* species complex, all dating from the Lower Miocene of Southeastern France, Greece, Northern Turkey and Southern Germany, are assumed in this study to belong to the lineage leading to the European *Bufotes viridis* complex, therefore representing a minimum age for the split between this lineage and the lineage leading to *Bufotes surdus*. The calibration point was associated to a Gamma distribution (alpha = 1.2, beta = 4) with an offset placed in 18 Ma.
4. The oldest fossil attributable to the *Rhinella marina* species complex (11 Ma), was established as a minimum age for the split between the *Rhinella marina* species complex and its sister group, the *Rhinella granulosa* species complex. This was set by means of a Gamma distribution (alpha = 1.2, beta = 4) with an offset established in 11 Ma.

Preliminary analyses showed that our mitochondrial markers (*tRNAval*, *16S* and *ND1*) could not resolve deep nodes, so we concatenated two nuclear genes CXCR4 (688 pb) and NCX1 (1285 pb) downloaded from GenBank to improve the resolution of the deep splits (see Supplementary material I). The unalignable regions of the noncoding mitochondrial markers were removed by means of Gblocks (Castresana, 2000), eliminating the misaligned regions and the positions with more than 50% missing data (36% of the original mitochondrial dataset).

A Yule branching process with a uniform prior and an uncorrelated branch rate variation was modeled by means of a resampling from a lognormal distribution. The model of evolution was set to GTRGAMMAI. The clock model and the evolutionary models were applied independently to the four partitions: (1) mitochondrial protein-coding; (2) mitochondrial RNA-coding; (3) nuclear CXCR4; and (4) nuclear NCX1 (Van Bocxlaer et al., 2009).

The analysis consisted of five independent Markov chain Monte Carlo (MCMC) analyses; each chain was run for 25,000,000 generations with parameters and trees sampled every 1000 generations. These five independent runs converged on very similar posterior estimates and were combined using LogCombiner version 1.4 after excluding the first 5,000,000 generations in each one (Rambaut and Drummond, 2007). Tracer 1.2 (Rambaut and Drummond, 2007) was used to confirm convergence and good mixing of the five combined MCMC chains. Finally we generated the maximum clade credibility consensus tree with median node heights using the TreeAnnotator program (also included in BEAST package), setting the posterior probability limit to 0.5.

2.5. Species distribution modeling

Distribution models were used to tackle two questions: first, to test the role of the Central Asian Deserts as a biogeographic barrier; and secondly to examine whether the climate-based potential distribution of the species in the Last Glacial Maximum (LGM) could explain the shallow structure found in the most structured parts of our phylogeny.

2.5.1. Testing the Central Asian Deserts as a biogeographic barrier

Correlative distribution models can be very useful tools for testing the existence of environmental barriers to dispersal and gene flow, particularly when we suspect, as in this case, that the distribution limits can be greatly determined by climatic causes (the existence of the Central Asian Deserts) (Kozak et al., 2008; Sexton et al., 2009).

To accomplish this, we modeled the current distribution of both species complexes in Eurasia. The *Bufo bufo* species complex was modeled using the localities included in this study (168 localities) to which we added 269 georeferenced localities of the species complex in Russia and adjacent countries (unpublished data from SNL) plus 23,803 localities from its entire distribution range obtained from Gbif (http://data.gbif.org). To produce the distribution models of the *Bufo gargarizans* species complex, we downloaded from Gbif all available localities of *Bufo tibetanus* (511 localities), *Bufo andrewsi* (1959 localities), *Bufo tuberculatus* (9 localities), *Bufo cryptotympanicus* (4 localities) and *Bufo gargarizans* (787 localities). The localities belonging to *Bufo japonicus* (18 localities) were obtained from Igawa et al. (2006).

The models were generated by Maxent 3.3.1 (Phillips et al., 2006; Phillips and Dudík, 2008). To avoid highly correlated and redundant climatic variables in our climatic dataset, which can cause over-parametrization and loss of predictive power (Williams et al., 2003; Buermann et al., 2008), the environmental data from 10,000 randomly generated points from across the study area were extracted and, from there, the level of correlation between pairs of variables was analyzed using the Pearson correlation coefficient. When two variables shared a correlation coefficient of 0.80 or higher, these were considered highly correlated, and the most meaningful variable was selected according to the physiological requirements of a typical mesophilic amphibian. Following this criterion, of the 19 variables available, eight variables were retained as input data for the distribution models: Bio1 (annual mean temperature), Bio2 (mean diurnal range), Bio7 (temperature annual range), Bio8 (mean temperature of wettest quarter), Bio12 (annual precipitation), Bio13 (precipitation of the wettest period), Bio15 (precipitation seasonality) and Bio18 (precipitation of the warmest quarter).

We generated models for each species complex independently and models pooling the localities of both species complexes. In both cases, we generated eight sets of 50 replicates of the distribution models, every set with increasing regularization values (1, 3, 5, 9, 20, 30, 40 and 50). By doing so, we progressively reduced the overfitting of our data in every replicate, producing more spread-out potential distributions (Phillips et al., 2006). The rationale is to extend as much as possible the distribution of both species complexes in all possible directions to test the stability of the Central Asian Deserts as a biogeographic barrier. Convergence threshold and maximum number of iterations corresponded to default settings of the program (0.00001, 500 respectively). For each set, we considered the mean of the 50 models the best estimate for the potential limits for both species. Model performance was evaluated using the AUC and the threshold-dependent binomial omission tests calculated by Maxent.

2.5.2. Testing the effects of glaciations

We explored whether the range shifts and population fragmentation experienced by the species during the Late Quaternary (0.0117–0.126 Ma) were congruent with the shallow phylogenetic structure of the European–Caucasian clade (the clade with the highest degree of geographic structure, see results). Localities corresponding to the European–Caucasian clade were projected onto the current climate and, assuming niche stability during the last 18,000 years (Nogués-Bravo, 2009), onto two possible reconstructions of the climatic conditions during the last glacial maximum (LGM), which were based on two models: the Community Climate System Model (CCSM) and the Model for Interdisciplinary research on Climate (MIROC). The climatic layers from the current and past scenarios were downloaded from the WorldClim (http://www.worldclim.org) database at 2.5' spatial resolution. The methodology used to generate the layers can be found in Waltari et al. (2007) and Hijmans et al. (2005).

Both present-day and past distribution models were generated through 100 replicates with Maxent 3.3.1 (Phillips et al., 2006; Phillips and Dudík, 2008) using the same climatic layers previously selected. To the 89 georeferenced localities of the European–Caucasian clade obtained by ourselves, we added 30 localities selected from Gbif (http://www.gbif.org) to cover the less-sampled areas. In each replicate, 70% of the localities were used to train the model and 30% to test it. The model calibrated with the present-day occurrence data was projected onto the current climate and past climatic conditions. Convergence threshold, maximum number of iterations, regularization values and features were set to default values. The output probability of presence of the species was set to logistic, and a threshold of the 10th percentile of training presence was used to generate binary layers. Finally, the binary maps of both models were superimposed by means of the program ArcMap v.9.3. The model performance was evaluated using the AUC and the threshold-dependent binomial omission tests calculated by Maxent.

3. Results

3.1. Patterns of mitochondrial sequence variation and phylogeographic structure

The resulting mitochondrial dataset contained 1988 bp of which 289 bp were variable and 245 parsimony-informative (excluding the outgroups). The phylogenetic tree resulting from the analysis of the mitochondrial data is presented in Fig. 2 and reveals five major haplotype clades with the following geographic delimitations (see Figs. 2 and 3): (1) Caspian clade, the basal-most split within the *Bufo bufo* species complex, corresponding to the species *Bufo eichwaldi*. It is distributed along the Southern shore of the Caspian Sea (Southeastern Azerbaijan and probably Iran); (2) European clade, corresponding to *Bufo bufo* sensu stricto (includes specimens from the type locality of *Bufo bufo*). According to Fig. 2 and Table 1, this clade appears to include specimens classified as both *B. b. bufo* and *B. b. spinosus*. It is the sister group to the Caucasian clade and is the clade with the largest distributional range, encompassing most of the currently known distribution of the species with the exception of Southern and Western France, Iberian Peninsula, North Africa and the Caucasus; (3) Caucasian clade, the sister group to the European clade, includes the taxon *Bufo verrucosissimus* and one population (locality 167) assigned to *B. b. spinosus* (Table 1). It is distributed across the Caucasus, with one population in Anatolia; (4) Iberian clade, distributed across the entire Iberian Peninsula and Southern and Western France, includes populations classified as *B. b. spinosus* and *B. b. gredosicola*; and (5) African clade, the sister group to the Iberian clade, includes specimens assigned to *B. b. spinosus* only and it is distributed across the mountain ranges and humid areas of Morocco, Algeria and Tunisia.

The average uncorrected sequence divergence (*p*-distance) among these five major clades is 5.42%. Table 2 shows the pairwise distances among clades for the 16S and ND1 genes.

As stated above, *Bufo b. spinosus* and *B. b. bufo* were not monophyletic in our analyses of the mtDNA data (Fig. 2 and Table 1). The results of both the SH and AU tests indicate that the best tree enforcing monophyly of the currently defined subspecies has significantly less likelihood ($P < 0.0001$) than the unconstrained ML topology shown in Fig. 2 ($\mathrm{Log}\,L_{\mathrm{unconstrained}} = -7570.303$; $\mathrm{Log}\,L_{\mathrm{constrained}} = -8034.871$).

Regarding the shallow phylogenetic structure, the different clades show different degrees of intraclade structuring. The Iberian clade does not have a well-supported structure in either ML or BI analyses, and this structure lacks correlation with geography (Figs. 1 and 2). By contrast, the European, Caucasian and African clades show an explicit degree of geographic structuring in both ML and BI analyses. Indeed, in the European clade seven subclades are recovered in our analysis, corresponding to the following geographic regions (see Figs. 2 and 3): Southern Italy (e7), North-Central Italy (e6), Greek Peninsula (e5), North-Central Europe (e1), South-Central Europe (e3), Southwestern Europe (e2) and Anatolia (including Northeastern Greece) (e4). All of them have high pp and bootstrap support values with the only exception of the Anatolian clade (despite being recovered by both ML and Bayesian analyses).

In addition, the Caucasian clade is composed of three well-supported subclades: North Caucasus, South Caucasus and Central Anatolia (c1, c2 and c3 respectively), and the African clade includes two subclades, a Moroccan clade and a Tunisian clade (a1 and a2 respectively). The lack of sampling in Algeria hampers resolution

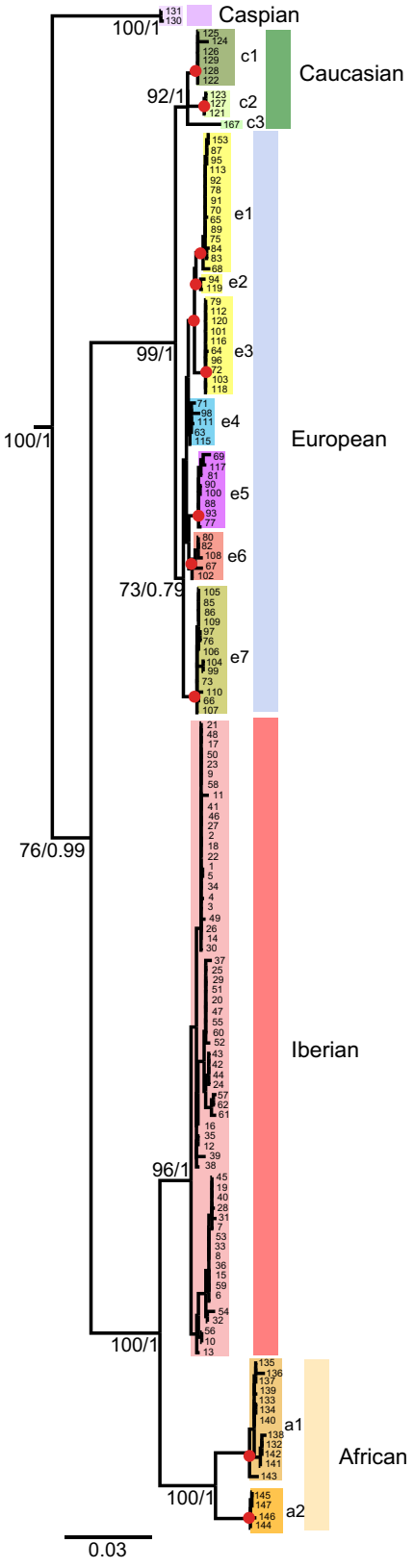

Fig. 2. Phylogenetic relationships of the *Bufo bufo* species complex in the Western Palearctic, as inferred from the ML analysis of 1988 bp of mtDNA. The support values (bootstrap/pp) are shown next to the nodes of the major splits. The red dots depicted in the shallow splits denote a bootstrap value equal or higher than 80% and simultaneously a posterior probability equal or higher than 0.90. The reference bar is expressed in substitutions per site. Numbers at the tips of the tree refer to specimens listed in Table 1. The outgroups have been removed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of the precise geographic contact between these two clades. The Moroccan clade presents some genetic differentiation between the population from the Great Atlas and the populations from the Rif/Middle Atlas.

3.2. Allozymes

Allele frequencies, sample size, percentage of polymorphic loci, and average heterozygosity are presented in Supplementary material III. Among 21 presumptive loci, 16 are polymorphic and include between two and seven alleles. The MCA analysis of allozyme variation is presented in Fig. 4. The populations are grouped according to the five mtDNA clades (Fig. 2) and not according to the current taxonomy (see Table 1). Specimens from localities 69, 108 and 117 (Table 1), classified as *B. b. spinosus*, in fact present mtDNA and allozymes of *B. b. bufo*, while specimens from localities 166 and 167, also classified as *B. b. spinosus*, group with *B. verrucosissimus*. Based on the fact that multiple independent nuclear loci are more likely to reflect evolutionary history than the mitochondrial locus, the six main mtDNA clades thus seem to correspond to the real evolutionary history of the *Bufo bufo* species complex. However, the NJ tree (Fig. 4c) based on Nei's genetic distances among populations (Supplementary material IV) does not recover the African and Iberian populations as monophyletic.

A number of populations occupy an intermediate position in the MCA scatter plots (Fig. 4a and b) and exhibit a mix of alleles typical of more than one lineage (Supplementary material III; see also Supplementary material V for scores along the first four axes for the MCA analyses). The Greek populations 69 and 117 have an intermediate position between the Caucasian and European groups, and it is clearly not an artifact, as these populations exhibit a mix of Caucasian and European alleles, indicating mixed ancestry of the Greek populations (Supplementary material III). The single individual analyzed from locality 108 (Italy) carries mostly European alleles, confirming its assignment based on mtDNA (Fig. 2), but it also presents at two loci (*Est-3*, *G6pdh*) Iberian alleles that are not found in any other individual of the Caucasian or European populations. Specimens from another locality (locality 45; Lac du Saut de Vésoles, S. France) occupy an intermediate position in the MCA scatter plots between the European and Iberian clades (Fig. 4). Its mtDNA places it with the Iberian clade, but its nuclear DNA exhibits both European and Iberian alleles (Supplementary material III). Both the Caspian and Caucasian populations are well separated in the MCA analysis.

The average Nei's genetic distances among the five clades are shown in Supplementary material VI. All the clades are genetically very well differentiated (Nei's genetic distance: 0.196–0.632). The lower genetic distance corresponds to the comparison between the Caucasian and European clades and the highest to the comparison between the Caucasian and African clades. The European populations sampled for the allozyme study (see Fig. 1 and Table 1) present a low level of genetic differentiation (average Nei's genetic distance: 0.010), despite being from localities as far away as Denmark, Sweden, Germany, Croatia, Russia, Belarus, Moldova and Ukraine. In contrast, the Moroccan and Tunisian populations of the African mtDNA clade are genetically very distinct (Nei's genetic distance: 0.349). This value is comparable to the genetic distance between the Iberian and African populations (see Supplementary material VI) and supports the distinctiveness of these three lineages obtained in the mtDNA phylogenetic analysis.

3.3. Dating estimates

Our BRMC calibrated ultrametric tree for all Bufonidae yielded most of the clades compatible with previously published phyloge-

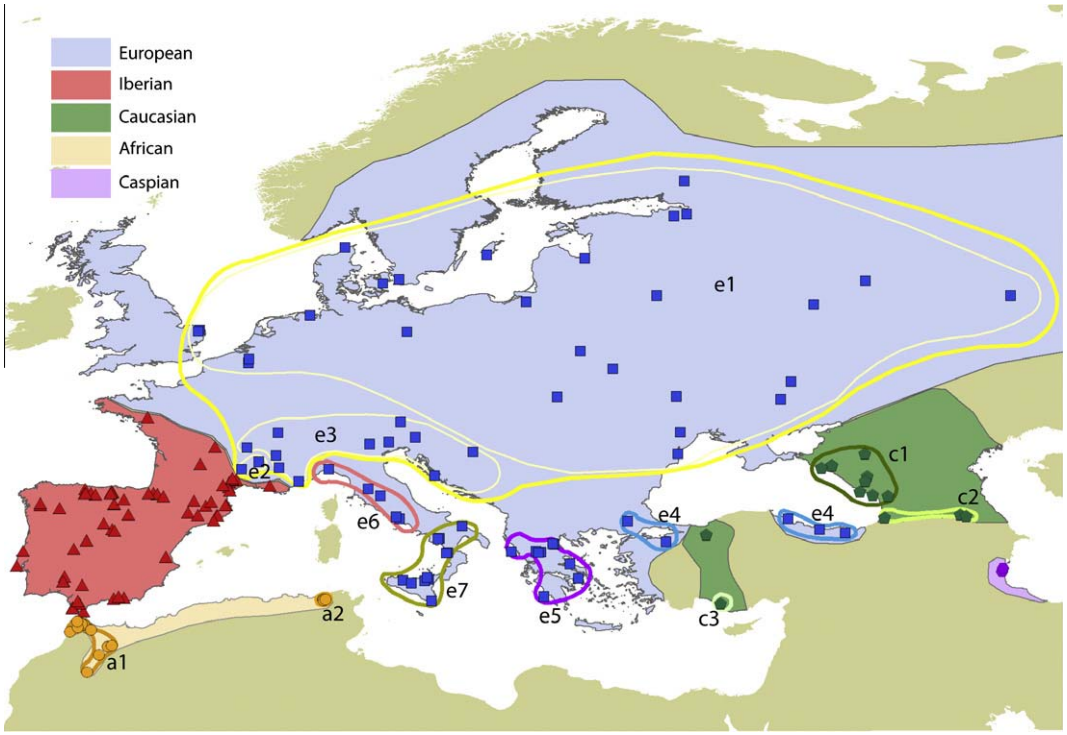

Fig. 3. Map showing the geographic distribution of the major clades (mitochondrial) and MCA groups (allozymes) recovered in our analyses (background colors). The outlines depict the shallow phylogenetic structure. The colors and codes have correspondence with those employed in Fig. 2 and in the text. Morocco (a1), Tunisia (a2), North-Central Europe (e1), Southwestern Europe (e2), South-Central Europe (e3), Anatolia (including Northeastern Greece) (e4), Greek Peninsula (e5), North-Central Italy (e6), Southern Italy (e7), North Caucasus (c1), South Caucasus (c2), Central Anatolia (c3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Estimates of evolutionary divergence over sequence pairs between clades in terms of the number of base differences per site (*p*-distance) from averaging over all sequence pairs. Upper right, distances for the 16S; lower left, distances for the ND1.

Clades	Caucasian	European	Iberian	African	Caspian
Caucasian	–	0.009	0.041	0.049	0.038
European	0.034	–	0.043	0.050	0.039
Iberian	0.100	0.093	–	0.034	0.047
African	0.096	0.093	0.054	–	0.057
Caspian	0.116	0.117	0.095	0.099	–

nies of this family (Van Bocxlaer et al., 2009, 2010), and all the nodes involving the *Bufo bufo* species complex were recovered with the highest possible support (posterior probability of 1) (Supplementary material II).

The crown age of the family Bufonidae was estimated at approximately 60 Ma (95% HDP = 47.94–75.48 Ma), compatible with previous estimates (Roelants et al., 2007; Van Bocxlaer et al., 2009, 2010) and with the age of the oldest fossil attributable to the family Bufonidae (55 Ma old) (Báez and Nicoli, 2004).

Regarding our ingroup (Fig. 5), the age of the split between the *Bufo bufo* and the *Bufo gargarizans* species complexes was estimated at 12.33 Ma (95% Highest Posterior Density = 8.81–16.36 Ma). The separation between *Bufo eichwaldi* and the main European populations occurred approximately 7.42 Ma (95% HPD = 5.15–9.99 Ma) and was followed by the split between the ancestor of the European and Caucasian populations and the ancestor of the Iberian and African populations, which occurred around 5.21 Ma (95% HPD = 3.67–7.11 Ma). Finally, our dating estimates place the separation between the Iberian and the African populations at 3.07 Ma (95% HPD = 1.91–4.36 Ma), with the remaining splits occurring within the Pleistocene (Fig. 5).

3.4. Distribution modeling

3.4.1. The Central Asian Deserts as a biogeographic barrier

All distribution models of both species complexes across Eurasia produced either independently or pooled provided mean AUC values beyond 0.9 and significance for all binomial omission tests, indicating a good performance of the models (data not shown). As shown in Fig. 6, although suitable climatic conditions for both species complexes seem to exist along the Himalayan range, implying a possible contact zone, the genus *Bufo* has never been reported in this region, which instead is occupied by the Indian-radiated genus *Duttaphrynus* and the widespread Palearctic genus *Bufotes* (Van Bocxlaer et al., 2009; Frost, 2011). Therefore, excluding this predicted contact zone, the distributions of both species complexes appear to be disjoint and nowadays completely isolated by the hyperarid, arid and semiarid regions of Central Asia. This pattern of isolation between both species complexes was resilient to the increase of the regularization values from 1 to 50, although each increment of the value produced more spread-out distributions.

3.4.2. Glaciations as drivers of phylogeographic structure

Modeling of distribution of populations of the European mtDNA clade yielded, on the current climate conditions, a mean test AUC score of 0.856, and all thresholds measured by the binomial omission tests were significantly nonrandom (data not shown). A visual inspection of the predicted distribution under the current climatic conditions showed overall an adequate fit to the distributions of the species as presented in Gasc et al. (1997) (data not shown).

The distribution models based on the LGM conditions indicate a substantial southward retraction of the ranges for the European

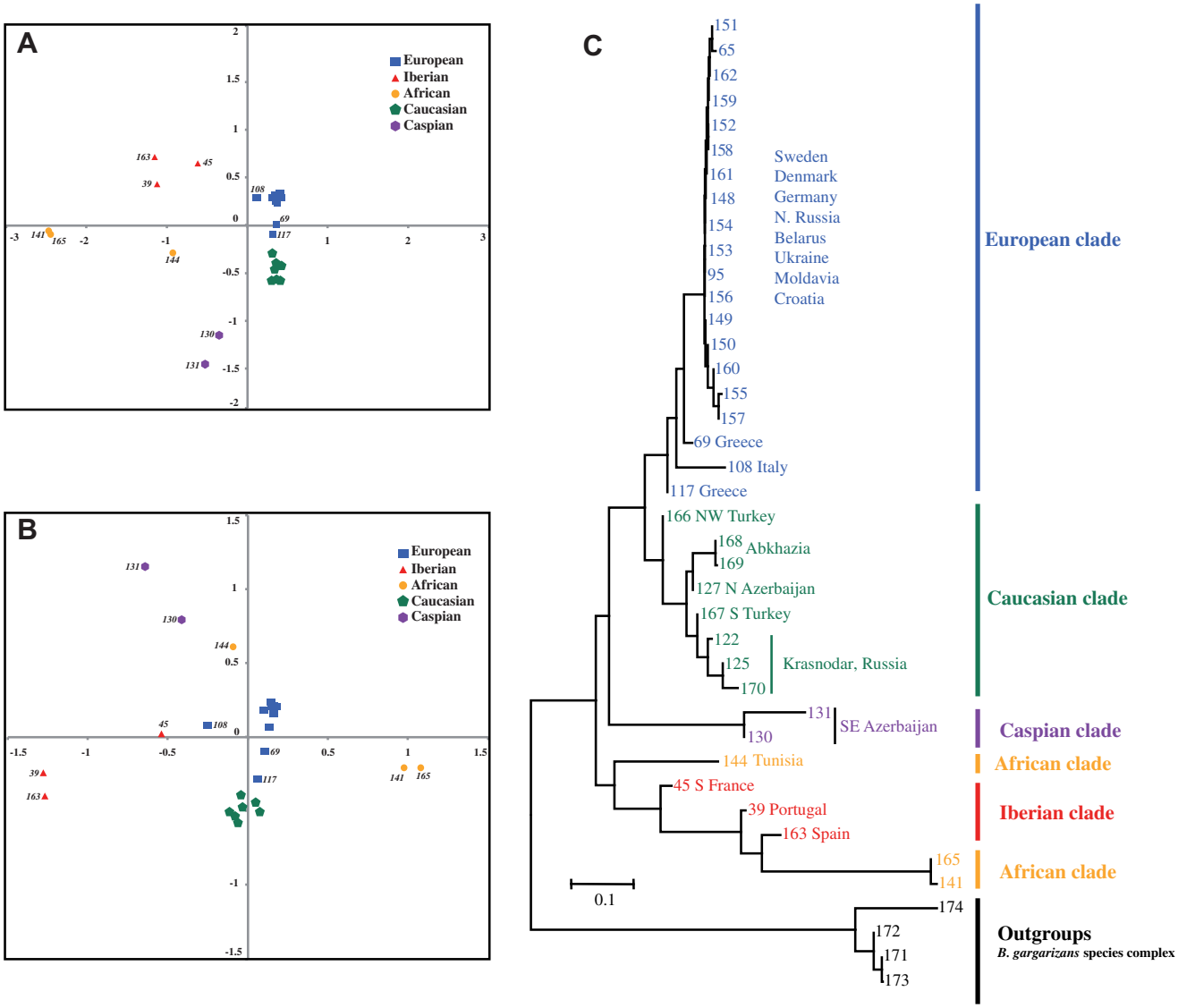

Fig. 4. Multiple Correspondence Analysis (MCA) of *Bufo* populations and neighbor-joining tree based on Nei's genetic distances among populations based on allozyme data. Data on the allozyme frequencies can be found in Supplementary material III. Information on the specimens analyzed can be found in Table 1 and their geographic position is shown in Fig. 1. (A) Biplot of factor 1 and 2 scores of the MCA analysis; (B) biplot of factors 3 and 4 of the MCA analyses. Scores along the first four axes for the MCA analyses can be found in Supplementary material V. (C) Neighbor-joining tree. The colors and codes have correspondence with those employed in Figs. 2 and 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

clade, with no suitable climate predicted above 53° of latitude in Central Europe (Fig. 7). The extent of range shrinkage is variable depending on the climatic model, being more severe under the CCSM and more relaxed under the MIROC. In both cases all Mediterranean Peninsulas, Anatolia and Caucasus appear suitable with different degrees of range fragmentation depending on the model. A substantial area in central Europe appears to be appropriate for the species according to the MIROC model.

4. Discussion

4.1. The role of the Central Asian Deserts as a biogeographic barrier

Our calibrated timetree sets the age of the split between *Bufo bufo* and *Bufo gargarizans* species complexes around 12.33 Ma (95% Highest Posterior Density = 8.81–16.36 Ma), and this is compatible with the oldest fossil attributable to the genus *Bufo* sensu stricto. This fossil from Suchomasty (Czech Republic) was dated to the MN9 (11.1–9.7 Ma) by Rage and Roček (2003), although Mein (1999), based on mammal faunas, assigned the locality to the MN10 (9.7–8.7 Ma) (Agustí et al., 2001).

Our distribution models (Fig. 6) show that both species complexes are completely isolated by the arid areas of Central Asia, a result congruent with a scenario of a climate-driven isolation between these two species complexes assuming niche conservatism during the last 10 My. However, our estimate of 12.33 Ma for the timing of this vicariant event (Fig. 5) does not match the most recent geological studies based on Loess deposits in China, which show that the Central Asian inland deserts originated in the Early Miocene, 22 Ma (Guo et al., 2008) or even 24 Ma (Sun et al., 2010). Despite that, the event that separated these two species complexes was probably not the geological origin of the Central Asian Deserts but their emergence as biogeographic barriers, and these two phenomena do not necessarily have to be synchronous. In fact, mineralogical and sedimentological records of the Northern-South China Sea show that the aridification process of Central Asia was not a homogeneous process but encompassed several pulses of accelerated aridification, with two great pulses at approx-

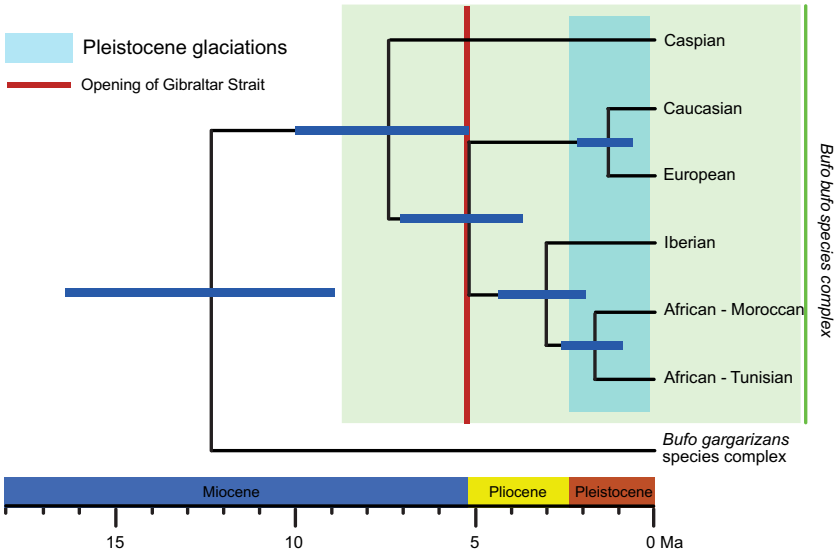

Fig. 5. Enlarged view of the Bayesian time-calibrated tree (Supplementary material II) showing the divergence times within the *Bufo bufo* species complex. The horizontal bars indicate the 95% posterior age intervals.

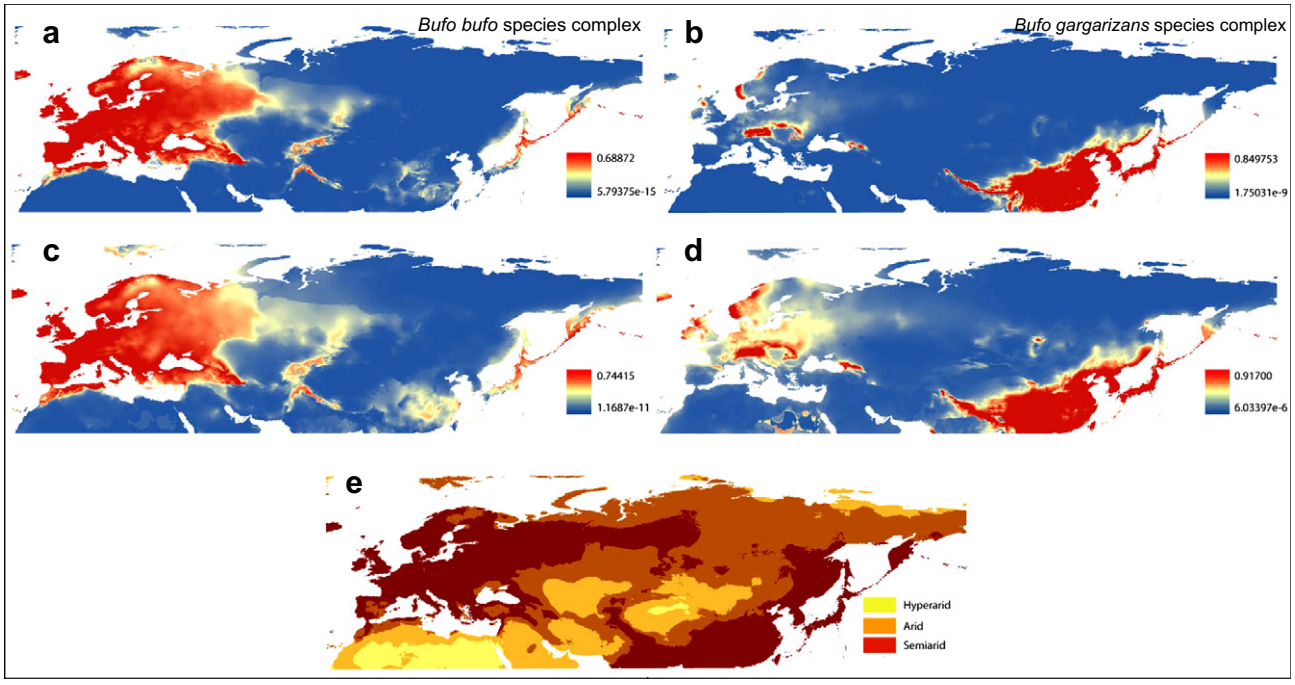

Fig. 6. Potential distribution of the *Bufo bufo* species complex and the *Bufo gargarizans* species complex across Eurasia, with different regularization factors: *a* and *b* = 9, *c* and *d* = 50. The map *e* shows the current extent of the desertic and subdesertic areas in Central Asia. The color gradient next to each map indicates the predicted probability that environmental conditions are suitable for the species. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

imately 15 and 8 Ma (Wan et al., 2007). This estimate matches those of several studies from widely separated places in the Eastern part of China's Loess Plateau, which show initial accumulation of loess between 10–8 Ma (Donghuai et al., 1998; Ding et al., 1999; Qiang et al., 2001).

From a paleobiological perspective, major levels of mean hypsodonty (dentition characterized by high-crowned teeth, typical of species inhabiting arid habitats) are observed in the large mammal faunas of Central China during the early Late Miocene (11.1–9.7 Ma), indicating an increase in aridity during this time interval (Liu et al., 2009). Moreover, the palynological record of the Northeastern margin of Tibet also reflects a marked shift towards more arid landscapes around 9 Ma (Ma et al., 1998; Sun and Wang, 2005).

Other diversification events fully agree with the development of the Central Asian Deserts during the Middle Miocene (15.9–11.6), either as an effective biogeographic barrier or as a source of new habitats that enhanced arid-adapted species radiations. An example of the latter is the origin of the radiation of desert-adapted lizards of the genus *Phrynocephalus* which, for Central Asia, dates between 17 and 11 Ma (Melville et al., 2009), according to the existing overlap of the 95% HPD between the estimates of the mitochondrial and nuclear markers. Moreover, the dating of the split between the Eastern and Western species complexes of *Bombina*,

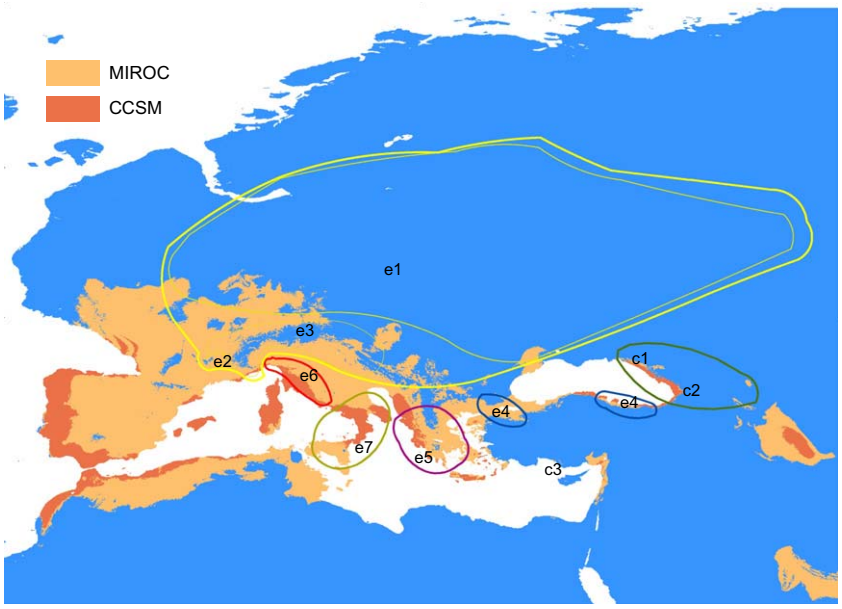

Fig. 7. Potential distribution of the European–Caucasian clade on two paleoclimatic scenarios during the LGM: MIROC (light orange) and CCSM (dark orange). The outlines represent the internal structure of the European–Caucasian clades. The colors and codes of the outlines have correspondence with those employed in Figs. 2 and 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

another case of Central Asian vicariance, has been established between 13.8 and 6.6 Ma (Roelants et al., 2007) and between 20.9 and 5.1 Ma (Zheng et al., 2009). These values are also compatible with our dating estimates.

All this evidence points to the complete isolation of lineages triggered by an increase in the arid conditions in the Central Asian region, supporting a climate-driven isolation between Eastern and Western species complexes as proposed by Savage (1973) and Borkin (1984).

4.2. Major splits in the Western Palearctic

Between the Late Miocene and Early Pliocene (11.6–3.6 Ma) two successive splits occurred: the separation of the lineage leading to *Bufo eichwaldi* (Caspian clade) from the rest and the separation between the ancestor of the European and Caucasian populations and the ancestor of the Iberian and African populations (Fig. 5). Several other cladogenetic events in amphibians have been reported in Europe within the same temporal frame: *Bombina bombina/Bombina variegata* (Fromhage et al., 2004), *Triturus vulgaris/Triturus montandoni* (Zajc and Arntzen, 1999), *Pelophylax fortis/Pelophylax ridibundus* (Akin et al., 2010), *Hyla arborea/Hyla orientalis* (Stöck et al., 2008a,b), and possibly the Eastern and Western lineages of *P. fuscus* (Crottini et al., 2007). The existence of the Parathetys Sea following an Eastern-Western axis in Central-Eastern Europe during the Miocene has been proposed to explain the split between the snakes, *Natrix natrix* and *Natrix tessellata* (Guicking et al., 2006), as a consequence of a North–South vicariance. This same vicariance could also explain the divergence between the Caspian population from the remaining populations of the *Bufo bufo* species complex given that the Paratethys Sea partially isolated what are now the Caucasus and the Southern Caspian shore from the rest of Europe during the Late Miocene (Popov et al., 2006).

However, the Late Miocene is also a period of a severe climatic and ecological reorganization already initiated around 10 Ma, when the European continent progressively shifted from more or less homogeneous humid conditions to drier conditions with higher levels of climatic and ecological heterogeneity. In fact, the Middle Turolian (7.6–6.8 Ma) is a period of upsurge of dry and open habitats as can be deduced from the great expansion of the savanna-adapted Pikermian faunas (Solounias et al., 1999) and the paleopalinologic record (fossil pollen and spores) (Fauquette et al., 2006). However it is between the Middle and Late Turolian (7–5 Ma) when the major break in climatic conditions occurred according to the levels of mammalian hypsodonty, with a transient phase of strong aridity dominating a large part of continental Europe (Fortelius et al., 2002, 2006; Van Dam, 2006). We hypothesize that this paleoclimatic scenario is congruent with severe changes in the population ranges of mesophilic amphibians, which eventually could lead to cladogenetic events.

4.3. Overseas dispersal across the Strait of Gibraltar and North African genetic structure

According to our mtDNA phylogenetic results (Fig. 2), North Africa was colonized from the Iberian Peninsula. Our dating estimates place the split between the Iberian and African clades clearly after the reopening of the Gibraltar Strait, implying overseas dispersal (Fig. 5). So far, this same mode of dispersal has been proposed for some mammals (Cosson et al., 2005), many reptiles (Carranza et al., 2004; Carranza et al., 2006; see Pleguezuelos et al., 2008 for a review), some invertebrates (e. g. Horn et al., 2006) and, regarding amphibians, it has been suggested for some lineages of *Pleurodeles waltl* (Veith et al., 2004; Carranza and Arnold, 2004; Carranza and Wade, 2004) and *Hyla meridionalis* (Recuero et al., 2007), although in both cases human-mediated dispersal cannot be fully excluded. In the Mediterranean basin, overseas dispersal from Africa to Europe has been suggested for the *Bufotes viridis* species complex (Stöck et al., 2008b).

The dispersal capabilities of amphibians across the sea have been evidenced several times despite the apparent severe limitations that salt water imposes to their physiology (Measey et al., 2007; Vences et al., 2003). Some of the best known events of overseas dispersal by amphibians seem to be mediated by the combination of two factors: (1) great rivers able to expel islets of soil and vegetation a long way into the open sea; and (2) long-persisting torrential rains that can greatly reduce the salinity across the oceanic dispersal path (Measey et al., 2007). Although during the Pli-

ocene Western Europe was overall wetter than today (Jost et al., 2009), probably rains were never intense enough to produce a substantial decrease in seawater salinity. However great Iberian rivers, such as the Guadalquivir, could have had an important role in projecting rafts into the open water, which afterwards could have reached the North African coast through the currents. In the particular case of *Bufo bufo*, it is known that brackish waters do not prevent animals from swimming in open water in the North Baltic Sea, even allowing gene flow between islands (Seppa and Laurila, 1999), indicating a certain degree of salt tolerance in this species.

The African group comprises two highly supported subgroups corresponding to Moroccan versus Tunisian populations (Figs. 2 – 4), although in the NJ tree from Fig. 4c the African group is not monophyletic. This same pattern of high level of genetic variability in North Africa has been observed in several other groups, as for instance the lacertid lizard *Timon tangitanus*/*Timon pater* (Paulo et al., 2008), the frogs *Hyla meridionalis* (Recuero et al., 2007; Stöck et al., 2008a) and *Pelophylax saharicus* (Harris et al., 2003), the toad *Discoglossus scovazzi*/*Discoglossus pictus auritus* (Zangari et al., 2006), and the snakes *Natrix maura* (Guicking et al., 2006), *Natrix natrix* and *Coronella girondica* (pers. observ.). The arid conditions of the Moulouya Basin could explain this dichotomy, as evidenced by the gap in the potential distribution of the *Bufo bufo* species complex in North Africa (results not shown). However, because of our lack of samples in the wide area existing between our Easternmost Moroccan samples and our Tunisian samples (mainly Algeria), we cannot know with certainty whether the Moulouya Basin represents the vicariant event that separated these populations.

4.4. Effects of glaciations

The Caucasian populations comprise three distinct subgroups (c1–c3; Fig. 2) and the European populations up to seven subgroups (e1–e7; Fig. 2). According to our dating estimates (Fig. 5) this structuring occurred during the Pleistocene and can be interpreted as signatures of the Quaternary glacial events, implying several cycles of retraction/expansion of the population ranges accompanied by strong effects of sorting of ancestral polymorphisms (Hewitt, 2000). The nested pattern observed in the European–Caucasian group suggests that at least two heterochronous events shaped its inner structure. The first event could have involved the Caucasian region as Pleistocene refugia, although we cannot exclude the possibility of an extra-Caucasian split with a posterior population retraction into the Caucasus. Secondary glacial events produced the youngest fragmentations, which, based on their strong geographic association, suggest that up to seven refugia could have existed (Figs. 2, 3 and 7).

The Caucasus appears in many phylogeographies as a source of distinctive lineages, involving cases of recent glacial-driven splits (Grassi et al., 2008). Paleopalinological data indicate the presence of a mild climate in the area during the LGM, as can be inferred from the presence of conifer and mixed forests in the Western Caucasus 18 Ka (Tarasov et al., 2000). This is congruent with our LGM projections that show the area as climatically suitable for the species and greatly isolated, supporting the hypothesis that it could have acted as a Pleistocene refugium (Fig. 7).

The structuring of the European populations is congruent with the existence of four Pleistocene refugia in the Mediterranean region and three refugia in Central Europe (see Figs. 2, 3 and 7). The Mediterranean refugia encompass Southern Italy (e7), Central/Northern Italy (e6), Greece (e5) and Anatolia (e4), and all of them have been reported as Pleistocene refugia for many groups, conforming to the Adriatic-Mediterranean and Pontic-Mediterranean elements based on chorological analysis (Schmitt, 2007). The existence of more than one phylogroup in the Italian Peninsula is a very common pattern in other Mediterranean Peninsulas, revealing so-called "refugia within refugia" (Gómez and Lunt, 2007). The main phylogeographic feature found in many Italian taxa is the presence of distinctive lineages in the Sicilian-Southern Italian region, as observed for instance in the mammal *Sciurus vulgaris* (Grill et al., 2009), reptiles *Hierophis viridiflavus* and *Zamenis longissimus*/*Zamenis lineatus* (Joger et al., 2010) and amphibians *Bombina pachypus* (Canestrelli et al., 2006) and *Hyla intermedia* (Canestrelli et al., 2007). Our results match this pattern, with a clearly differentiated Southern Italian haplotype group distributed across Sicily and Southern Italy.

The genetic differentiation of the South Italian biotas has been attributed to two major physiographic features in the region, the Crati-Sibari graben and the Catanzaro graben, which have been repeatedly marine-flooded following glacio-eustatic sea-level fluctuations during the Upper Pliocene and Pleistocene, interrupting or reducing the genetic exchange with the rest of the Italian Peninsula (Santucci and Nascetti, 1996; Canestrelli et al., 2007; and references therein). However, considering the patchy distribution pattern observed in our climatic projection under the CCSM paleoclimatic scenario, we do not exclude a climatically driven isolation during the Pleistocene glaciations.

The Anatolian refugium was already suggested for *Bufo bufo* by Kutrup et al. (2006), and this is consistent with our Anatolian haplotype group, which is distributed from the Eastern Balkans-Western Anatolia to Eastern Anatolia (e4; Figs. 2 and 3). A similar phylogeographic pattern occurs in the reptile *Zamenis longissimus* (Joger et al., 2010) and several species of mammals (Randi, 2007; and references therein). The presence of a cool temperate forest has been proposed for a narrow band along the southern shore of the Black Sea 18 ka (Adams and Faure, 1997), supporting the putative role of this region as a glacial refugium.

Three subclades can be geographically associated with Central Europe, all of them phylogenetically closely related (see Figs. 2, 3 and 7): a South-Central European clade (e3) including Southern France, the Alpine region and Northern Balkans, a North-Central European clade (e1) encompassing Great Britain, Scandinavia, the North-Central European mainland reaching Western Russia and Ukraine, and a Southwestern European clade (e2) containing the localities of Gap and Altier in Southwestern France. Clades tightly associated with Central Europe have been reported for many groups. In the case of amphibians, these have been noted for *Rana arvalis* (Babik et al., 2004), *Rana temporaria* (Palo et al., 2004) and even in thermophilous species such as *Epidalea calamita* (Rowe et al., 2006). These phylogeographic patterns suggest the existence of Northern "cryptic" refugia for several species, and the existence of these refugia has been unambiguously corroborated on the basis of paleopalynology, paleontology (Stewart and Lister, 2001) and paleoclimatic modeling (Svenning et al., 2008). Our niche projection onto the MIROC paleoclimatic scenario indicates that suitable LGM climatic conditions could have existed across Central Europe for the European populations, supporting the possibility that the Central European populations could correspond to Northern "cryptic" refugia for the species; however, we cannot exclude the possibility that these populations could have originated from refugia located in the Northern Balkans (North-Central European clade) or the submediterranean areas of South-Central Europe (Southwestern and South-Central European clades), which also contained suitable areas for the species during the LGM according to our paleoclimatic projection (Fig. 7).

In the Iberian Peninsula, the Iberian populations fail to show a clear phylogeographic pattern, and this agrees with a previous genetic survey of Iberian populations using microsatellites and the mitochondrial control region revealing little population differentiation and extensive gene flow at a wide spatial scale (Martinez-Solano and Gonzalez, 2008). This suggests that the Iberian populations did not experience a great amount of population

fragmentation during the Pleistocene glaciations, possibly as a consequence of a single and wide Pleistocene refugium preserving a great amount of haplotypic diversity with little geographic fragmentation.

4.5. Implications for the systematics of the Bufo bufo species complex

Although a subspecies or species is not necessarily monophyletic for mtDNA haplotypes, when monophyly of mtDNA haplotypes characterizes a geographic population, that observation serves as a strong diagnostic criterion for a historically distinct lineage. The results of both mtDNA and allozyme analyses support the same five main population lineages within the *Bufo bufo* species complex but do not show congruence with the currently accepted taxonomy of the group. The monophyly tests performed with the mtDNA data set clearly rejected monophyly of *Bufo bufo spinosus* and *Bufo bufo bufo* as currently defined (see above). The three samples of *Bufo b. gredosicola* included in the mtDNA study branched within *B. b. spinosus* from the Iberian haplotype clade. However, further studies including morphology, fast-evolving nuclear markers and a better sampling at the population level are needed to assess the taxonomic validity of the population of *Bufo bufo* from the Sierra de Gredos (Lizana, 2002). Populations assigned to *B. bufo spinosus* based on their morphology and geographic distribution presented mtDNA and/or allozymes typical of *B. b. bufo* (Greece and Italy: localities 69, 108 and 117; Table 1; Figs. 1, 2 and 4) or *Bufo verrucosissimus* (Western Anatolia, localities 166 and 167; Table 1; Figs. 1, 2 and 4). *Bufo b. spinosus* is traditionally diagnosed mainly based on its body size and the degree of development of the keratinous warts. Our results suggest that these characters evolved independently multiple times towards the Peri-Mediterranean area, possibly as an adaptation to dry environments. The relationship between moisture and size has been proposed by Duellman and Trueb (1994), with larger animals having greater desiccation tolerance due to the decrease in body surface (especially so in "spherical" shapes like the toads). Moreover, the level of keratinization could be related to desiccation tolerance as well.

Based on the mtDNA and allozymic results, each one of the five main population lineages of the *Bufo bufo* species complex represent a different taxon. According to the phylogeny from Fig. 2 and the taxonomy of the group, the Caspian population should be recognized at the specific level as *Bufo eichwaldi*. The results of the MCA analyses (Fig. 4) and a close inspection of the allozyme frequency table (Supplementary material III) clearly show that some of the populations present a mixed ancestry indicating extensive past or ongoing introgression. For instance, Greek populations 69 and 117, which carry mtDNA of the European haplotype clade (Fig. 2), have an intermediate position between the Caucasian populations and the European populations in the MCA allozyme analysis (Fig. 4). The same occurs with specimens from locality 108, assigned to the European population based on their mtDNA but with some Iberian alleles at some loci (*Est-3*, *G6phd*; see Fig. 4 and Supplementary material III), or with specimens from locality 45, classified as belonging to the Iberian population based on their mtDNA but with European alleles in some loci (Fig. 4; Supplementary material III). As a result of the observed introgression between the different populations and until a more detailed study reveals the contact zones, we prefer to regard the Caucasian, European, Iberian and African populations as different subspecies of *Bufo bufo*. Moreover, any future analyses should attempt to add information on the morphology and bioacoustics of the different populations.

Based on the information on the type localities, the name *Bufo bufo verrucosissimus* should be used for the Caucasian population, *Bufo bufo bufo* for the European population, *Bufo bufo spinosus* should be restricted to the Iberian population and, until a new subspecies is described, *Bufo bufo ssp.* should be used for the African population. This latter population might, in fact, include two different subspecies, one in the Western Maghreb and another one in the Eastern Maghreb.

5. Conclusions

According to our results, the *Bufo bufo* and the *Bufo gargarizans* species complexes diverged during the Middle Miocene, most probably as a consequence of a climate-driven isolation coincident with an increase in the aridification of Central Asia. After this split between Eastern and Western complexes, at least three main lineages were generated within the *Bufo bufo* complex: (1) Caspian lineage (*Bufo eichwaldi*); (2) the Iberian-African lineage; and (3) the European–Caucasian lineage (Fig. 2). All these three splits were unambiguously placed in Pre-Pleistocene times according to our dating estimates, probably in the Late Miocene. We propose here that the cladogenetic events leading to these lineages could be mediated by the combination of paleogeographic features (as the Parathetys Sea) and the climate shift that occurred in Europe in the Late Miocene towards major levels of aridity and savanna-like environments.

A dispersion event leading to the colonization of Northern Africa occurred during the Pliocene, after the opening of the Gibraltar Strait, and this could be the first unambiguous case of overseas dispersal across the Gibraltar Strait for an amphibian.

The structure of the European–Caucasian populations involved at least two major reorganizations during the Pleistocene, with an older split between the European lineage and the Caucasian lineage, possibly involving the Caucasus as a Pleistocene refugium, and other shallower structuring mediated by more recent Pleistocene glaciations. These promoted high levels of geographic fragmentation and genetic differentiation in the European and Caucasian lineages. This more recent structuring is compatible with the range fluctuations experienced by Paleartic faunas during the Pleistocene concomitant to glacial events. We propose seven Pleistocene refugia, four Mediterranean refugia involving the Iberian and Italian peninsulas, the South Balkanic region and Anatolia, and three refugia with diffuse locations that together form an extensive Central European group (see Figs. 2, 3 and 7). These latter refugia were the most probable source for the recolonization of northern Europe after the ice withdrew. In contrast, the Iberian Peninsula seems not to have any structure coupled with geography, and this is probably the consequence of a single wide refugium retaining great amounts of ancestral polymorphism.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.12.019.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Is sociality required for the evolution of communicative complexity? Evidence weighed against alternative hypotheses in diverse taxonomic groups

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Research

Is sociality required for the evolution of communicative complexity? Evidence weighed against alternative hypotheses in diverse taxonomic groups

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Complex social communication is expected to evolve whenever animals engage in many and varied social interactions; that is, sociality should promote communicative complexity. Yet, informal comparisons among phylogenetically independent taxonomic groups seem to cast doubt on the putative role of social factors in the evolution of complex communication. Here, we provide a formal test of the sociality hypothesis alongside alternative explanations for the evolution of communicative complexity. We compiled data documenting variations in signal complexity among closely related species for several case study groups—ants, frogs, lizards and birds—and used new phylogenetic methods to investigate the factors underlying communication evolution. Social factors were only implicated in the evolution of complex visual signals in lizards. Ecology, and to some degree allometry, were most likely explanations for complexity in the vocal signals of frogs (ecology) and birds (ecology and allometry). There was some evidence for adaptive evolution in the pheromone complexity of ants, although no compelling selection pressure was identified. For most taxa, phylogenetic null models were consistently ranked above adaptive models and, for some taxa, signal complexity seems to have accumulated in species via incremental or random changes over long periods of evolutionary time. Becoming social presumably leads to the origin of social communication in animals, but its subsequent influence on the trajectory of signal evolution has been neither clear-cut nor general among taxonomic groups.

Keywords: animal communication; phylogenetic comparative methods; adaptation; sexual selection; natural selection

1. INTRODUCTION

Complex communication is classically linked to the evolution of complex animal societies [1]: as the number and context of social interactions increase, communication mediating those interactions tends to become increasingly elaborate. Indeed, it is difficult to think of any highly social animal that does not possess a complex system of communication. Humans are an obvious example, as are many other primates and long-lived mammals. Chimpanzees form complex hierarchies and alliances among troop members and rely on an elaborate array of vocal and visual signals to mediate those relationships [2–5]. Elephants have similarly complex social interactions and use an extensive repertoire of social signals as an apparent consequence [6–9]. But is it correct to say that sociality is a necessary prerequisite for the evolution of communicative complexity? Are less social species really predisposed to basic forms of communication more than socially complex species?

These questions lie at the very foundation of how we believe communication evolved in animals. If communicative complexity and sociality are tightly coupled, then both the origin and direction of communication evolution has essentially been a by-product of factors driving the evolution of sociality more generally. Alternatively, communication might have originated to mediate social interactions among conspecifics, but its ultimate elaboration has been driven by other factors independent of changes in sociality. Freeberg *et al.* [1] have discussed in detail the possible non-social pressures that might produce communicative complexity. These include environmental factors that influence signal fidelity, the need for reliable species recognition and neutral or non-adaptive evolutionary processes. Some of these factors have empirical support (species recognition; [10,11]), whereas others have very little (neutral processes; reviewed by Freeberg *et al.* [1]). The challenge is to

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test the sociality hypothesis alongside its alternatives and in a way that allows the weight of evidence for each hypothesis to be directly compared.

One can try to make broad taxonomic comparisons to argue that group-living primates, for example, have significantly more elaborate systems of communication relative to claw-waving territorial crabs because primates form complex societies, whereas crabs do not (by comparison). Yet there are many other non-primate groups that do exhibit extraordinary complexity in their communication, and some researchers have even gone as far as to say have levels of signal complexity comparable to primates [12,13]. Consider the exquisite song and courtship dances of many birds [14], the elaborate headbobbing and colourful dewlap displays of anole lizards [11,15], the rippling colour and movement displays of cuttlefish [16,17] or the elaborate foot thumping signals of ornate wolf spiders [18,19]. These are all examples of complex communication—complex in repertoire, the number and type of components making up a signal and, in some cases, the number of sensory modalities used for communication (e.g. signals that are both auditory and visual). Yet, these animals do not exhibit the same level of sociality seen in primate societies.

Such examples seem to weaken the putative link between communicative complexity and sociality. However, broad comparisons among disparate groups like these (primates versus crabs or birds) actually provide little insight into the evolution of animal communication. The vast number of attributes that vary among species at these broad phylogenetic scales make it virtually impossible to determine what factors might or might not account for taxonomic differences in communication. More informative are comparisons among closely related species within broad taxonomic groups. In almost any group that depends on social communication, closely related species differ, to a lesser or greater degree, in their social behaviour and complexity of communication. Furthermore, at this finer phylogenetic scale, the specific selection pressures that direct the evolution of communicative complexity become more apparent (reviewed by Ord [20]). Rather than conducting a literature review of the evidence for and against the sociality hypothesis, we chose to test the hypothesis directly alongside other potential causal factors, using empirical data collected for closely related species.

To this end, we developed six datasets or 'case studies' to represent a variety of taxonomic groups (frogs, birds, lizards and ants) and signal classes (acoustic, visual and chemical). The data for each case study documented variations among closely related species in signal complexity, social behaviour and other factors predicted to influence the evolution of communicative complexity (see §1*a–d*). We then used new phylogenetic comparative approaches and assembled new phylogenies to evaluate alternative evolutionary models of how communicative complexity might have evolved in each case study. The models corresponded to one of the four hypotheses and were not mutually exclusive. We assembled findings across the case studies to determine the generality of each hypothesis in describing the evolution of communicative complexity over diverse taxa and signal classes. A secondary goal of our study was to provide communication biologists, who might not be familiar with recent advances in phylogenetic techniques, with a heuristic example of how multiple hypotheses can be considered jointly, using methods that also inform on the probable mode of evolution.

(a) *Social drivers of signal complexity*

When the frequency or importance of social interactions increases, signals used to mediate those interactions should become more complex for a range of reasons: to convey information more reliably, to manipulate social partners more effectively or to provide relevant social cues in different contexts [1]. For instance, as sexual selection intensifies, either because mates become choosy in what they find attractive or as competition for mates and territories increases, signal repertoires are expanded or the design of signals are elaborated to help the signaller outcompete courtship and territorial rivals [11,21–25]. For species living in groups or otherwise interacting with a range of different social partners (e.g. mates, rivals, juveniles, adults, dominates and subordinates), different signals or cues are often required for different contexts, leading to an increase in repertoire size or the complexity of existing signals to convey multiple messages [26]. The sociality hypothesis predicts variation among species in the design or repertoire of signals whenever species differ in the frequency or context of social interactions.

(b) *Ecological influences on signal complexity*

The range of factors that make up the ecology of species is diverse and there are a number of possible ways in which ecology can direct the evolution of communicative complexity. The influence of the environment on the transmission of animal signals is well documented [27–30]. Background acoustic or visual noise masks calls [31,32] and displays [33,34] and obstructions in the environment deflect and scatter sound waves [28] and obscure visual signals [35,36], as does the reduced visibility imposed by poor light [37–39]. The strategies animals adopt to enhance signal fidelity in noisy, cluttered or dim habitats are varied. Background noise can limit the range of frequencies heard by birds [40] or the types of movements that can be seen by lizards [34], and this restricts the range of signal designs that can be readily detected by receivers. Namely, difficult environmental conditions limit signals to those that are simple in design. There are, however, some instances where noisy environments can facilitate signal complexity by promoting the evolution of alert components. These are new components added to signals to attract the attention of receivers, before the more information rich portion of the signal is delivered [39,41]. Here, poor signal environments promote the evolution of more complex signals (i.e. increases in component number through the addition of alerts and other components to enhance signal detection).

Microhabitat or the site within the environment from which animals communicate with one another

can increase or decrease the severity of environmental conditions that affect signal efficiency [42]. Acoustically communicating species living near streams are susceptible to noise generated by flowing water in what otherwise might be a quiet macrohabitat (e.g. a sheltered temperate forest). In the case of some frogs, species near streams facilitate call detection by producing calls at ultrasonic frequencies above the broadband frequency of running water [43]. Calls given near the ground are also more susceptible to degradation through muffling and deflection [28]. Likewise, the visibility of displays is enhanced from perches above undergrowth and other low-lying visual obstructions [44]. On the one hand, the constraints acting on signal efficiency in microhabitats near noise sources or physical obstructions limit the range of signal designs that can be detected by receivers. On the other hand, animals can minimize masking by adding alert or amplifier components to signals and, in the process, increase the complexity of their signals [39,41]. Whether environmental variables constrain or promote signal complexity is unclear (but see [35]). Yet it is reasonable to expect environmental conditions to play some part in directing the evolution of communicative complexity.

Finally, when animals frequently encounter heterospecific congeners in the environment and are not in direct competition with those congeners for resources (e.g. mates), the need for accurate species recognition becomes important (reviewed by Ord *et al.* [45]). The design of social signals often provides the best cues for ascertaining species identity. The number of sympatric species an animal encounters should prompt increases in signal complexity to facilitate recognition [10,11]. That is, signal elaboration results in a unique, species-typical signal that can be easily distinguished from the signals of sympatric congeners.

(c) *The allometry of signal complexity*

Body size influences numerous features of an animal. Of special relevance to communication is the allometry of physical structures and the physiological mechanisms that govern signal production. For example, the length of the vocal tract is heavily dependent on body size (larger animals have longer vocal tracts [46]). The vocal tract in turn determines the types of sounds that can be produced by an animal [47,48]. The allometry of the vocal tract should therefore lead to disparity in vocal signals among species that differ in size. In addition, size-specific metabolic rate seems to explain a large portion of the variance among species in the rate, frequency and duration of acoustic signals in groups as diverse as insects and mammals: large species typically produce low-frequency calls of long duration and at low rates [49]. A similar physiological mechanism has been implicated in the evolution of movement-based displays in lizards [50]. Larger lizards have higher energetic costs associated with movement compared with smaller lizards, and this has been suggested to constrain the number, duration or type of movements that large lizards can include in displays [50]. However, in the case of static visual signals, possessing a large body might instead provide more surface area for the expression of big or elaborate ornaments.

Taken together, the allometry of communicative complexity will depend on the modality and type of signal characteristic examined. Larger bodied acoustically communicating species will have vocalizations of longer duration than smaller bodied species [49]. Whether size-specific metabolic rate, or body size more generally, influences other indices of vocal complexity such as repertoire size or note number is unknown. In visually communicating lizards, size-specific energetic costs should limit the evolution of movement-based display complexity [50], but larger body sizes should allow the evolution of large or more numerous ornaments because of increased surface area.

(d) *Non-adaptive signal complexity*

Divergence in song complexity can occur 'passively' among populations in wide-ranging species through cultural or genetic drift (e.g. birds: [51,52]; mammals: [53]). This leads to the unusual hypothesis that stochastic processes can generate signal complexity in the absence of selection (see also [54]). Genetic drift and neutral mutation (mutations that are neither deleterious nor beneficial) may incidentally increase signal complexity over evolutionary time or following bouts of rapid evolution (e.g. during speciation). If true, variations in communicative complexity will tend to track phylogeny (closely related species will tend to share similar levels of signal complexity, whereas distantly related species will not) and, in particular, match a pattern of stochastic evolution. Recent developments in phylogenetic comparative analyses allow the joint estimation of both phylogenetic inertia and stochasticity in evolutionary diversification [55]. This neutrality hypothesis will be novel to most communication biologists, but it is an important null model missing from most studies of signal adaptation.

2. MATERIAL AND METHODS

We identified recent studies in which the design or repertoire of communication had been surveyed for a large number of closely related species. Our criterion for selecting case studies was dependent on several factors. First, we needed to be fairly certain that we could obtain adequate social, ecological or morphological data for the species in question. Some of this information was reported in the original sources, or the authors of those sources were willing to share unpublished data with us when contacted directly (see Acknowledgements). In other cases, we used electronic databases and other published sources to supplement datasets. Second, to construct phylogenies for each case study, there had to be adequate and comprehensive genetic markers for species in GenBank. Third, we wished to cover several diverse taxonomic groups and obtain representatives of both the most commonly studied and least-studied forms of communication (in decreasing order of research attention: acoustic signals, static visual ornaments, movement-based displays and chemical signals). We excluded primates and other mammals because these systems were either well represented in earlier tests of

the sociality hypothesis (e.g. [56–58]; reviewed in Freeberg *et al.* [1]) or were the focus of other studies included in this theme issue [59–61]. Finally, in some cases, we selected two case studies for the same taxonomic group (specifically birds and lizards), one that represented phylogenetically diverse species from multiple genera and families, while the second consisted of closely related species from the same genus or phylogenetically adjacent genera. We refer to these case studies as 'distantly related' and 'closely related' examples, respectively. Our motivation here was to assess the sensitivity of our estimates of phylogenetic inertia and stochasticity to taxon sampling.

Details on the data and analyses used are described in the following sections. All data and GenBank accession numbers for DNA sequences used to assemble phylogenies have been deposited in files assigned to this article in the public electronic database Dryad Digital Repository (see Acknowledgements).

(a) *Communication data and social indicators*

We compiled data on signal and social characteristics that we believed were consistent with current definitions of complexity [1]. Indices of signal complexity were: call amplitude modulation (frogs); call, song or display duration (frogs, birds and lizards); song or syllable repertoire size (birds); number of ornaments (lizards); number of separate components making up signals (lizards and ants; this includes colour dichromatism in lizards, measured as the number of colourful body patches exhibited by males but not seen in females [62]). Indices of sociality were: sexual size dimorphism (frogs and lizards; in these taxa, size dimorphism is believed to reflect the intensity of competition among males for mates and territories [24]); levels of extra-pair paternity (birds); mating system (in birds this was coded as 'monogamy', 'irregular polygyny' and 'regular polygyny'; in ants, 'no polygyny or polyandry' versus 'polygyny or polyandry present'); and colony size (ants). All data on signal complexity were compiled directly from published studies [25,62–67] except for frogs (see below). Indices of sociality were obtained from the same sources used for communication data (frogs [68]; lizards, distantly related [25]; birds [66]), unpublished data from the authors of these studies (ants—E. van Wilgenburg, M. R. E. Symonds & M. A. Elgar 2011, unpublished data) or compiled separately from other literature (lizards, closely related [69]).

To obtain data on the signals of frogs, we used oscillograms of species-typical frog calls to estimate call duration and amplitude modulation from a comprehensive field guide on the anuran fauna of the Kaiteur National Park in Guyana [68]. Oscillograms were digitally scanned and ImageJ v. 1.42q (W. Rasband 1997–2009, NIH) used to measure the duration of calls in seconds from the start of the first note to the end of the last note. Call amplitude modulation was estimated as the coefficient of variation (CV) of the peak sound pressure computed across pulses making up a call. That is, a call with many pulses varying in peak sound pressure had a higher estimated CV than a call with pulses that peaked at consistent sound pressure levels.

(b) *Ecological and morphological data*

Most ecology data were obtained from sources reporting communication data and included: macrohabitat for the 'distantly related' lizards [25]; microhabitat for both lizard case studies [63,64] as well as the frogs [68]; environmental noise for the frogs [68]; whether species were migratory for the 'closely related' bird case study [65]; species geographical range for the 'distantly related' lizard case study [62]; the number of sympatric species for frogs [68], and two climatic variables for ants [67]. Macrohabitat was generally coded as 'open' (e.g. grasslands, deserts and plains) or 'closed' habitats (e.g. forests and woodlands). This is a biologically relevant categorization of habitat for communication because closed environments generally reduce the range of detection for acoustic and visual signals compared with open environments (see §1). Microhabitat in frogs was the average height above ground of call sites for each species, while in lizards it was whether species were arboreal or terrestrial. In frogs, species that were reported preferring environments near streams were assumed to experience environmental noise from running water and categorized as occupying 'high noise' environments, whereas all other species were categorized as occupying 'low noise' environments. Whether species were migratory or occupying large geographic ranges was used as an index of the likely habitat heterogeneity experienced by species, as well as the likelihood of interacting with heterospecifics. Habitat heterogeneity was expected to influence the evolution of communicative complexity because heterogeneity will either truncate the set of signals that are detectable across different habitats or because heterogeneity facilitates the evolution of new signal components or larger repertoire sizes (see §1). Widely distributed species can also be expected to interact with more sympatric species compared to localized species, and sympatry is in turn predicted to promote the evolution of signal complexity (see §1). In frogs, the number of sympatric species was estimated as the number of co-occurring frog species reported at sites where a species was stated to occur. Climatic variables expected to influence the composition of cuticular hydrocarbons in ants are rainfall and temperature [67], which may limit the range of chemical components included in an olfactory signal.

We used a GIS approach to obtain information on the ecology of species for two case studies for which data were not reported in original sources. For one lizard case study (communication data from Martins [63]), we used GIS distribution data to compute the geographic range of species, the percentage range overlap with congeneric heterospecifics (species of the same genus; this index is hereafter referred to as 'sympatry: overlap'), the number of sympatric species (hereafter 'sympatry: number') and the level of habitat heterogeneity likely experienced by species. Specifically, geographical distribution data for 79 species of *Sceloporus* (88% of described species within the genus) were obtained from the IUCN red list database (http://www.iucnredlist.org/technical-documents/spatial-data) and mapped using a cylindrical equal area projection. The range for the species of interest

was measured in square kilometres and estimated as the area enclosed by the polygon corresponding to each species distribution. We then calculated the percentage area of a species range that was overlapped by at least one *Sceloporus* congener. We also estimated the number of sympatric species by tallying the number of overlapping species. Habitat heterogeneity was computed by intersecting the ecoregion GIS layer described in Olson *et al.* ([70]; available electronically at http://www.worldwildlife.org/science/data/item1875.html) with the projected distribution of species. Initially, the percentage overlap of ecoregions for a given species was grouped into four broad categories—forest, shrubland, grassland and desert—but these were later merged into 'forest' and 'non-forest' environments (forest versus all others) because this dichotomous categorization was more biologically appropriate for testing our hypotheses (see opening paragraph in this section).

The same methods were used to obtain ecological data for one bird case study (communication data from Soma & Garamszegi [66]). In this case, species distributions were obtained from the BirdLife International and NatureServe database ([71]; http://www.birdlife.org/datazone/info/spcdownload3, accessed on August 19, 2011). Our projected distributions were based on the known 'breeding range' if species were migratory (given that song charactersitics were used in mate choice and territory defence [66], activities specific to the breeding season) or the 'resident range' if species were non-migratory. Ecoregion data and analyses were the same as those of the lizard example described earlier. However, we were unable to obtain data on sympatric species for this bird case study, so sympatry was not evaluated. All GIS analyses were performed using ArcGIS v. 9.3 (ESRI, Redlands CA).

Data on body size were largely obtained from studies reporting communication data (the only exception being the Soma & Garamszegi [66] case study; see above). Measures of size included snout-to-vent length for frogs and lizards, and tarsus length in birds (tarsus length is a common metric of size in birds—e.g. [72,73]—and is a useful measure because it reflects both the overall size and mass of the bird).

(c) *Phylogenies*

We developed phylogenies for each case study using the two most comprehensive mitochondrial DNA markers deposited in GenBank [74]. The longest sequences available for a given species were selected and aligned using the program Mafft v. 6 ([75]; http://www.ebi.ac.uk/Tools/msa/mafft/) with the following parameter settings: gap penalty = 1.53; gap extension = 0.123; tree rebuilding number = 100; maximum number of iterations = 100; and fast Fourier transforms (FFTS) = local pair. When sequence data for a given species were not available in GenBank, we used surrogate sequences from two species of the same genus (when possible; in rare instances only one species of the same genus was represented in GenBank). Surrogate sequences were required only for five of the 199 species included in our study.

For every case study, we generated two ultrametric phylogenies using BEAST v. 1.6.1 [76]. We used two trees rather than a single phylogeny for each case study to incorporate alternative phylogenetic hypotheses into our analyses (results from comparative analyses are partly dependent on the phylogeny used). The first tree was based on species relationships informed exclusively by the two mitochondrial DNA genes downloaded from GenBank. The second tree used additional information from the most recently published phylogenies for a given group to 'constrain' the topology of the tree such that only branch lengths were estimated in our phylogeny using the mitochondrial DNA genes from GenBank. This lead to an overall topology in the second tree that was congruent with existing phylogenies (frogs: [77]; birds: [78–83]; lizards: [84–88]; ants: [67]). We refer to these trees as 'unconstrained' and 'constrained' phylogenies, respectively.

Topology, node supports (in the unconstrained trees) and branch lengths (in both the unconstrained and constrained trees) were inferred using the Bayesian algorithms implemented in BEAST. A Yule branching process with a uniform prior was used and an uncorrelated branch rate variation was modelled using a lognormal distribution. For both genes, the model of evolution was set to GTRGAMMAI. The mean global substitution rate was set to unity and produced ultrametric trees with branch lengths expressed in units of substitutions per site. The analysis consisted of two to four independent Markov Chain Monte Carlo (MCMC) chains that ran for 20 000 000 generations with parameters and trees sampled every 3000 generations. Independent runs in all cases converged on very similar posterior estimates and were combined using LogCombiner v. 1.5.4 (included in the package BEAST). In all runs, the first 10 per cent of generations were considered to belong to the burn-in phase of the analysis and were excluded. The program Tracer v. 1.2 [89] was then used to confirm convergence and good mixing of the combined MCMC chains. Finally, summary trees were obtained with mean node heights computed using TreeAnnotator v. 1.5.4 (in the package BEAST), with a posterior probability limit set to 0.5.

(d) *Analysis*

We used model fitting within a phylogenetic framework to assess the extent to which different predictor variables explained variation among species in communicative complexity. We used the second-order Akaike's Information Criterion (AIC_c) to determine the level of support for each model. AIC_c is a modification of AIC that corrects for sample size; as sample size increases, AIC_c values converge on those of AIC [90]. As applied here, an AICc value reflects the likelihood that a given model fits the observed variation in signal complexity among species, given the phylogenetic relationships among those species. The model with the lowest AIC_c value is the model that best fits the data, although any model within two AIC_c units of this lowest value is by convention considered to fit the data just as well [90]. We also computed model

weights, AIC_w, to provide a more intuitive indication of the support of a given model relative to all models considered. See Burnham & Anderson [90] for details on the calculation of AIC_c and AIC_w.

Model fitting and subsequent selection using information and likelihood theory is becoming increasingly common in evolutionary ecology studies (see [91] for review). This is because it offers an enticing alternative approach to traditional *p*-value-driven statistical analyses, with several statistical advantages that are particularly amenable for biological study. First, there is often a range of biologically plausible variables that might account for the observed patterns we see in the natural world. These variables might be alternative indices associated with a specific hypothesis or correspond to different hypotheses in their own right. Model fitting incorporates this notion of multiple potential causal factors as an explicit part of its analytic philosophy by allowing simultaneous evaluation of a number of alternative models. Second, such multiple comparisons are a menace for the interpretation of *p*-values because of the increased chance of falsely concluding a 'significant' relationship when many statistical comparisons are performed (i.e. type II error rates increase with the number of statistical analyses performed on a dataset). While there are various corrections that can be applied to *p*-value thresholds for judging significance (Bonferroni, false discovery rate), the problem is circumvented entirely using model fitting methods such as AIC.

This ability to consider a range of different models of how evolution might have occurred in a group is especially useful for phylogenetic comparative analyses in which there are typically several potential causal factors. In our study, we had a number of predictor variables associated with several hypotheses. These hypotheses were not mutually exclusive. While the model fitting framework allows all possible multivariate combinations of variables to be considered, we choose to focus on a relatively simple set of models to facilitate interpretation and avoid generating large unwieldy AIC_c tables. We therefore relied on model weights to identify which hypothesis, or combination of hypotheses, most likely accounted for the evolution of signal complexity in a given case study.

Models were fitted individually to the communication data using the phylogenetic comparative software SLOUCH v. 1.1 [55] run in R v. 2.8.1 (R Development Core Team). Thomas Hansen provides an introduction to his program and its analyses in accessible non-technical language in the accompanying user manual. A more detailed description of his approach is given in Hansen *et al.* [55] (see also [92]). We elaborate here on some of the key aspects of the program that are relevant for the interpretation our results.

SLOUCH has several features that make it especially attractive for comparative study among the daunting array of methods currently available. In particular, rather than assume a particular mode of evolution at the outset (a critical flaw of the popular independent contrasts method ([93]; see discussion in Ord & Martins [94]), SLOUCH uses likelihood to estimate the level of phylogenetic inertia and stochasticity in the evolution process based on the distribution of species data across the tips of the phylogeny and the nature of the phylogeny itself (e.g. its topology, length of branches). In the case of phylogenetic inertia, SLOUCH computes the phylogenetic half-life, $t_{1/2}$, of the phenotype (here signal complexity), which is the time the phenotype would likely take to evolve halfway towards an adaptive optimum. The value of $t_{1/2}$ is a direct function of phylogenetic inertia: strong phylogenetic inertia is reflected in large values of $t_{1/2}$ and is consistent with incremental phenotypic change over long periods of evolutionary time, whereas weak phylogenetic inertia is reflected in small values of $t_{1/2}$ and bursts of phenotypic change over short periods of evolutionary time. Physiological or morphological constraints, genetic correlations or low mutation rate are some of the factors that might contribute to phylogenetic inertia and large values of $t_{1/2}$.

SLOUCH parametrizes $t_{1/2}$ from zero to infinity, a range that includes a rapid and directed phenotypic change in response to selection, to gradual phenotypic change occurring via a process of Brownian motion (which may or may not be directed by selection). In the instance of adaptive evolution, the phenotype might track a continuously shifting selection regime or be maintained at an optimum phenotype through stabilizing selection. This provides considerable versatility because it allows for the possibility of non-adaptive evolutionary change, adaptive evolutionary change through directional selection and adaptive evolutionary change through stabilizing selection.

In the context of signal evolution, values of $t_{1/2}$ approaching infinity imply that signals have evolved via an incremental change culminated over long periods of evolutionary time. In this scenario, species tend to share similar levels of signal complexity as a function of phylogenetic relatedness. Adaptive evolution may have occurred, but the process has been slowed by strong phylogenetic inertia. Intermediate values of $t_{1/2}$ imply that signal evolution has tended to proceed towards an optimal phenotype via Brownian motion and, if reached, stabilizing selection has kept signal designs at or near this optimum. Values of $t_{1/2}$ approaching zero suggest that signal designs among species are effectively independent and have little relationship to phylogeny. In this situation, animal signals have been free to vary adaptively and retain no signature of evolutionary history.

There are other phylogenetic comparative methods that use likelihood to estimate parameters reflecting the extent phenotypic evolution has been influenced by factors associated with phylogeny. For example, Martin & Hansen's [95] phylogenetic generalized least squares model (PGLS) estimates α, or the rate of adaptation, which can be used to compute $t_{1/2}$ [55]. Other methods compute parameters such as K [96] or λ [97] that estimate the extent phenotypic evolution has followed a Brownian motion process, commonly interpreted as the level of phylogenetic signal in phenotypic traits. The utility of SLOUCH lies in its foundation on the Ornstein–Uhlenbeck process, which includes both Brownian motion and the possibility of adaptive evolution towards an optimum. Furthermore, unlike other methods, SLOUCH

computes a separate estimate of stochasticity. Stochasticity can generate non-adaptive phenotypic variations among living species despite the presence of strong directional or stabilizing selection and contributes to a random phenotypic change under Brownian motion.

SLOUCH estimates stochasticity, v_y, over a range from zero to infinity. Values approaching infinity imply that stochastic processes have been highly influential in the course of phenotypic evolution. Stochasticity can be generated by factors such as genetic drift or secondary selection pressures acting on other traits that are genetically correlated or during the process of evolutionary change more generally. Values approaching zero imply that there have been few perturbations from an adaptive optimum or during the process of evolutionary change generally.

There is always the danger of over interpreting $t_{1/2}$ and v_y parameters and others like them in phylogenetic comparative methods (K or λ). It must be kept in mind that these parameters are not direct measures of the evolutionary process. Rather they are statistical parameters measuring patterns in the data that are *consistent* with phylogenetic inertia and stochasticity in phenotypic evolution. But these parameter values can reflect other non-biological factors, such as the level of measurement error in data or patchiness in taxon sampling. Practitioners of phylogenetic comparative methods should consider carefully the warnings of Revell *et al.* [98] and Freckleton [99]. With a reasonable degree of caution, parameters potentially reflecting phylogenetic inertia and stochasticity can still provide some insight into the evolutionary processes that might have lead to species variation in signal complexity. To facilitate interpretation, we provide a measure of confidence in $t_{1/2}$ and v_y estimates by reporting the range of values within two support units of the best estimate [55]. As with AIC_c, values within this two-unit range are those that are essentially equally well supported.

Finally, SLOUCH offers two methods for examining phenotypic evolution. The first assumes that the phenotype has evolved towards discrete stationary optima (either a common optimum or several alternative optima; e.g. a specific phenotype selected for and maintained in a given environment via stabilizing selection). The method relies on ancestor state reconstructions of a categorical predictor variable onto the phylogeny (e.g. habitat type), which is then used to assess whether different phenotypes have been favoured in different regions of the phylogeny specified by that predictor variable. We used this approach to fit models with the following variables: open versus closed macrohabitats, arboreal versus terrestrial microhabitats, high-noise versus low-noise environments, migration and mating system. These categories were reconstructed onto the phylogeny using parsimony in the program MESQUITE v. 2.74 [100]. Ideally, likelihood reconstructions should be used here, but likelihood assigns a probability that a categorical variable is present in a given ancestor. To implement the optimality analysis in SLOUCH, these probabilities would have to be manually assigned using some arbitrary cut-off (e.g. an ancestor with a probability greater than 50 per cent coded as having lived in an open rather than closed habitat). To avoid this, we followed current convention and assigned ancestor states as present or absent using parsimony [101,102]. The method of reconstruction does influence ancestor assignments. However, our preliminary analyses showed that changes to the phylogeny had a greater effect on ancestor reconstructions than the method of assignment (parsimony versus likelihood). All of our analyses were repeated on alternative phylogenies for each case study.

Reconstructions were imported into SLOUCH and used to fit models that assumed that reconstructed variables (e.g. open versus closed habitats) have selected for different adaptive optima in signal complexity (e.g. complex signals in open habitats, simple signals in closed habitats).

The second method in SLOUCH is similar in the respect that it also assumes that the phenotype has evolved towards an adaptive optimum, but differs in the sense that this optimum is not stationary and varies as a function of fluctuations in a continuous predictor variable. The model fitted is essentially a regression of signal complexity on the predictor variable and was used for all continuous predictors. SLOUCH also provides output for an optimal and evolutionary regression. The optimal regression depicts the relationship between the predictor variable and signal complexity assuming phylogenetic inertia was absent, whereas the evolutionary regression provides the 'observed' relationship between the predictor and signal complexity as a function of both adaptation and the constraining force of phylogenetic inertia. We report the results from evolutionary regressions only.

For all best supported models, we report phylogenetic effect sizes (r-values) to provide an indication of the direction and magnitude of relationships in the data.

3. RESULTS

Support for models applied to each case study are reported in tables 1–3. We provide a brief summary of key findings below and in figures 1 and 2, and elaborate on the combined findings of the analyses in the discussion (§4).

(a) *Complexity in vocal communication*

There was little support for the role of sociality in the evolution of complexity in vocal signals (mean $\pm$ s.e. $AIC_w = 0.07 \pm 0.01$). Instead, phylogenetic null models and a range of different ecological models—the probability of encountering heterospecifics, migration/geographic range and habitat preference—were among the best-supported models (table 1). For example, the phylogeny of frogs, their probable encounter rate with heterospecifics and call site position above ground fit variations in call complexity well. Plots of sympatry and call modulation revealed several prominent outliers (figure 1*a*), but the overall trend was consistent with the species recognition hypothesis. Call site was positively correlated to call duration and, to some extent, levels of call modulation, implying that calling high above the ground has

Table 1. Factors influencing the evolution of complexity in vocal communication. SSD, sexual size dimorphism.

		constrained tree					unconstrained tree				
case-study, signal variable	model, rank	AIC_c	AIC_w	r	$t_{1/2}$ (support region)	v_y (support region)	AIC_c	AIC_w	r	$t_{1/2}$ (support region)	v_y (support region)
frogs, $n = 32$ *species*											
call amplitude modulation	1. phylogeny: null	292.1	0.37	n.a.	0 (0–10)	440 (260–760)	290.5	0.37	n.a.	20 (0–140)	440 (300–∞)
	2. ecology: sympatry	294.4	0.12	~0.16[a]	1 (0–10)	420 (260–720)	292.9	0.11			
	3. ecology: microhabitat[b]	294.2	0.13				292.5	0.14	−0.14	20 (0–160)	430 (280–∞)
	4. ecology: noise[c]	294.2	0.13				292.8	0.12			
	5. social: SSD	294.5	0.11				293.1	0.10			
	6. allometry: body size	294.7	0.10				292.6	0.13			
	7. ecology: macrohabitat	296.7	0.04				294.8	0.04			
call duration	1. ecology: microhabitat[b]	−11.82	0.81	0.45	900 (170–∞)	30 (0–70)	57.1	0.12			
	2. phylogeny: null	−7.20	0.08				54.6	0.42	n.a.	460 (870–∞)	10 (0–20)
	3. ecology: sympatry	−5.16	0.03				57.3	0.11			
	4. allometry: body size	−5.20	0.03				57.2	0.11			
	5. ecology: noise[c]	−4.63	0.02				57.3	0.11			
	6. social: SSD	−4.59	0.02				57.3	0.11			
	7. ecology: macrohabitat	−3.53	0.01				60.0	0.03			
birds (*distantly related*), $n = 23$ *species*											
syllable repertoire size	1. allometry: body size	46.2	0.35	0.35	75 (25–∞)	25 (0–700)	47.4	0.34	0.34	500 (25–∞)	175 (0–700)
	2. phylogeny: null	46.3	0.33	n.a.	300 (25–∞)	125 (0–825)	47.4	0.34	n.a.	175 (25–∞)	75 (0–825)
	3. ecology: breeding range	48.6	0.11				49.7	0.11			
	4. ecology: macrohabitat	48.7	0.10				50.0	0.09			
	5. social: extra-pair paternity	48.9	0.09				49.9	0.10			
	6. social: mating system	52.1	0.02				53.0	0.02			
song repertoire size	1. phylogeny: null	55.0	0.46	n.a.	975 (0–∞)	600 (0–∞)	56.3	0.46	n.a.	975 (0–∞)	575 (0–∞)
	2. ecology: breeding range	57.0	0.17	0.21	950 (0–∞)	550 (0–∞)	58.3	0.17	0.20	975 (0–∞)	575 (0–∞)
	3. allometry: body size	57.9	0.11				59.2	0.11			
	4. social: extra-pair paternity	58.0	0.10				59.2	0.11			
	5. ecology: macrohabitat	58.0	0.10				59.1	0.11			
	6. social: mating system	59.2	0.06				61.3	0.04			
birds (*closely related*), $n = 23$ *species*											
syllable repertoire	1. ecology: migration	−41.4	0.42	~0.52[d]	∞ (630–∞)	10 (0–20)	−37.5	0.13			
	2. phylogeny: null	−41.1	0.36	n.a.	940 (500–∞)	10 (0–30)	−40.5	0.57	n.a.	910 (480–∞)	10 (0–30)
	3. ecology: macrohabitat	−38.7	0.11				−37.8	0.15			
	4. allometry: body size	−38.7	0.11				−38.0	0.16			
syllable duration	1. phylogeny: null	−64.1	0.66	n.a.	∞ (830–∞)	10 (0–20)	−64.2	0.66	n.a.	∞ (830–∞)	10 (0–20)
	2. ecology: macrohabitat	−61.2	0.15				−61.3	0.16			
	3. allometry: body size	−61.2	0.15				−61.2	0.15			
	4. ecology: migration	−57.9	0.03				−58.1	0.03			

(*Continued.*)

Table 1. (*Continued.*)

case-study, signal variable	model, rank	constrained tree					unconstrained tree				
		AIC_c	AIC_w	r	$t_{1/2}$ (support region)	v_y (support region)	AIC_c	AIC_w	r	$t_{1/2}$ (support region)	v_y (support region)
song duration	1. phylogeny: null	−57.8	0.64	n.a.	∞ (780–∞)	10 (0–20)	−52.2	0.11			
	2. allometry: body size	−55.2	0.27				−55.0	0.43	0.28	∞ (780–∞)	10 (0–20)
	3. ecology: macrohabitat	−54.9	0.15				−54.6	0.35	0.05	∞ (780–∞)	10 (0–20)
	4. ecology: migration	−52.1	0.06				−52.2	0.11			

[a]There are several prominent outliers making direct interpretation of this coefficient misleading (figure 1*a*).
[b]Microhabitat was defined as the height (m) of call sites above ground.
[c]The level of background noise potentially experienced by a species was determined by whether species were reported to call near streams.
[d]The standard errors of parameter values for migration were very large making biological interpretation difficult.

facilitated the evolution of more complex calls. There was some support for the allometry hypothesis in birds (e.g. body size correlated positively with syllable repertoire size; figure 1*b*).

There were high levels of possible phylogenetic inertia in most vocal signals. It was particularly striking that the estimates of phylogenetic inertia (implying evolution via Brownian motion) were consistent across measures of signal complexity and for datasets in which species were sampled quite differently (distantly related versus closely related). Figure 2*a*,*b* illustrates repertoire size in birds as it relates to phylogeny. Both examples recorded high phylogenetic inertia, but differed in estimates of stochasticity: stochasticity in repertoire evolution was low for the closely related species (figure 2*a*), but high among distantly related species (figure 2*b*). This difference in stochasticity probably reflects differences in taxon sampling between the two case studies.

(b) *Complexity in visual communication*

Visual communication in lizards provided the only compelling support for models of sociality (mean ± s.e. $AIC_w = 0.44 \pm 0.13$). Effect sizes showed that sexual size dimorphism, a proxy for the intensity of male–male competition within species, was positively correlated with the number of ornaments (e.g. horns, spines, tail crests; figure 1*c*), colour dichromatism and headbob display duration (table 2). In the case of colour dichromatism, our analyses were focused on colour signals occurring on two separate body regions because previous studies have shown that the selection pressures acting on dorsal coloration ('exposed'; these signals are visible to aerial predators) have been different from those acting on ventral coloration ('concealed'; these signals are only exposed during headbob displays to territorial rivals [25]). Phylogeny and whether species were arboreal or terrestrial were other models that received good support. On the latter, consistent with call evolution in frogs, headbob display evolution in lizards seems to have been facilitated by an arboreal lifestyle (figure 1*d*).

Visual signal evolution in lizards was associated with estimates of phylogenetic inertia close to zero. Our analyses also suggested very little stochasticity in evolutionary diversification. Taken together, visual signals have potentially been free to respond quite rapidly to selection.

(c) *Complexity in chemical communication*

We found little support for the role of sociality in the evolution of signal complexity in ants (mean ± s.e. $AIC_w = 0.14 \pm 0.003$). The ecological models faired no better (table 3). Phylogeny appeared to be the only model that fit the data at all and even then the level of support was only marginally better than the other models considered (table 3). Almost no phylogenetic inertia or stochasticity was detected in the data. That is, evolutionary changes in the number of cuticular hydrocarbons appear to have occurred extremely rapidly and not stochastically. This implies that these signals could have been targets for selection. What selection pressure(s) this might have been is unknown.

Table 2. Factors influencing the evolution of complexity in visual communication.

		constrained tree					unconstrained tree				
case-study, signal variable	model, rank	AIC_c	AIC_w	r	$t_{1/2}$ (support region)	v_y (support region)	AIC_c	AIC_w	r	$t_{1/2}$ (support region)	v_y (support region)
lizards (*distantly related*), $n = 55–59$ *species*											
no. ornaments	1. social: SSD	210.7	1.00	0.58	40 (0–450)	100 (20–∞)	208.8	1.00	0.58	60 (0–400)	140 (20–∞)
	2. allometry: body size	230.7	0				228.6	0			
	3. phylogeny: null	234.4	0				231.9	0			
	4. ecology: macrohabitat	236.5	0				233.8	0			
	5. ecology: range	236.6	0				221.1	0			
colour dichromatism, exposed	1. phylogeny: null	161.8	0.37	n.a.	125 (0–800)	225 (25–∞)	150.6	0.39	n.a.	50 (0–∞)	75 (25–∞)
	2. social: SSD	162.6	0.24	0.15	100 (0–825)	175 (25–∞)	152.2	0.17	0.11	175 (0–∞)	250 (25–∞)
	3. allometry: body size	163.8	0.13	0.07	277 (0–800)	225 (25–∞)	152.6	0.14	0.07	50 (0–∞)	75 (25–∞)
	4. ecology: macrohabitat	163.9	0.13				152.7	0.14			
	5. ecology: range	163.9	0.13				152.4	0.16		50 (0–∞)	75 (25–∞)
colour dichromatism, concealed	1. social: SSD	172.2	0.79	0.31	25 (0–800)	50 (25–∞)	169.7	0.76	0.31	25 (0–800)	50 (25–∞)
	2. phylogeny: null	176.7	0.08				174.0	0.09			
	3. allometry: body size	177.1	0.07				174.1	0.08			
	4. ecology: range	178.8	0.03				175.8	0.04			
	5. ecology: macrohabitat	178.9	0.03				176.2	0.03			
lizards (*closely related*), $n = 22$ *species*											
bob no.	1. ecology: microhabitat[a]	125.3	0.67	0.49	0 (0–10)	10 (0–30)	125.3	0.69	0.49	0 (0–10)	10 (0–30)
	2. ecology: sympatry, overlap	128.5	0.14				128.5	0.14			
	3. phylogeny: null	129.3	0.09				129.3	0.09			
	4. social: SSD	131.2	0.04				132.3	0.02			
	5. ecology: sympatry, no.	131.2	0.04				132.4	0.02			
	6. allometry: body size	132.4	0.02				132.4	0.02			
	7. ecology: macrohabitat	134.1	0.01				132.3	0.02			
display duration	1. phylogeny: null	132.7	0.35	n.a.	0 (0–10)	20 (5–35)	132.7	0.35	n.a.	0 (0–50)	20 (0–∞)
	2. social: SSD	134.1	0.18	0.29	1 (0–50)	20 (0–∞)	134.1	0.17	0.29	0 (0–70)	20 (0–∞)
	3. allometry: body size	134.8	0.12				134.8	0.12			
	4. ecology: sympatry, overlap	135.0	0.11				135.0	0.11			
	5. ecology: microhabitat[a]	135.2	0.10				135.1	0.10			
	6. ecology: sympatry, no.	135.6	0.08				135.4	0.09			
	7. ecology: macrohabitat	136.7	0.05				136.4	0.05			

[a]Microhabitat was defined as arboreal or terrestrial (that is, typically displaying from trees or from the ground).

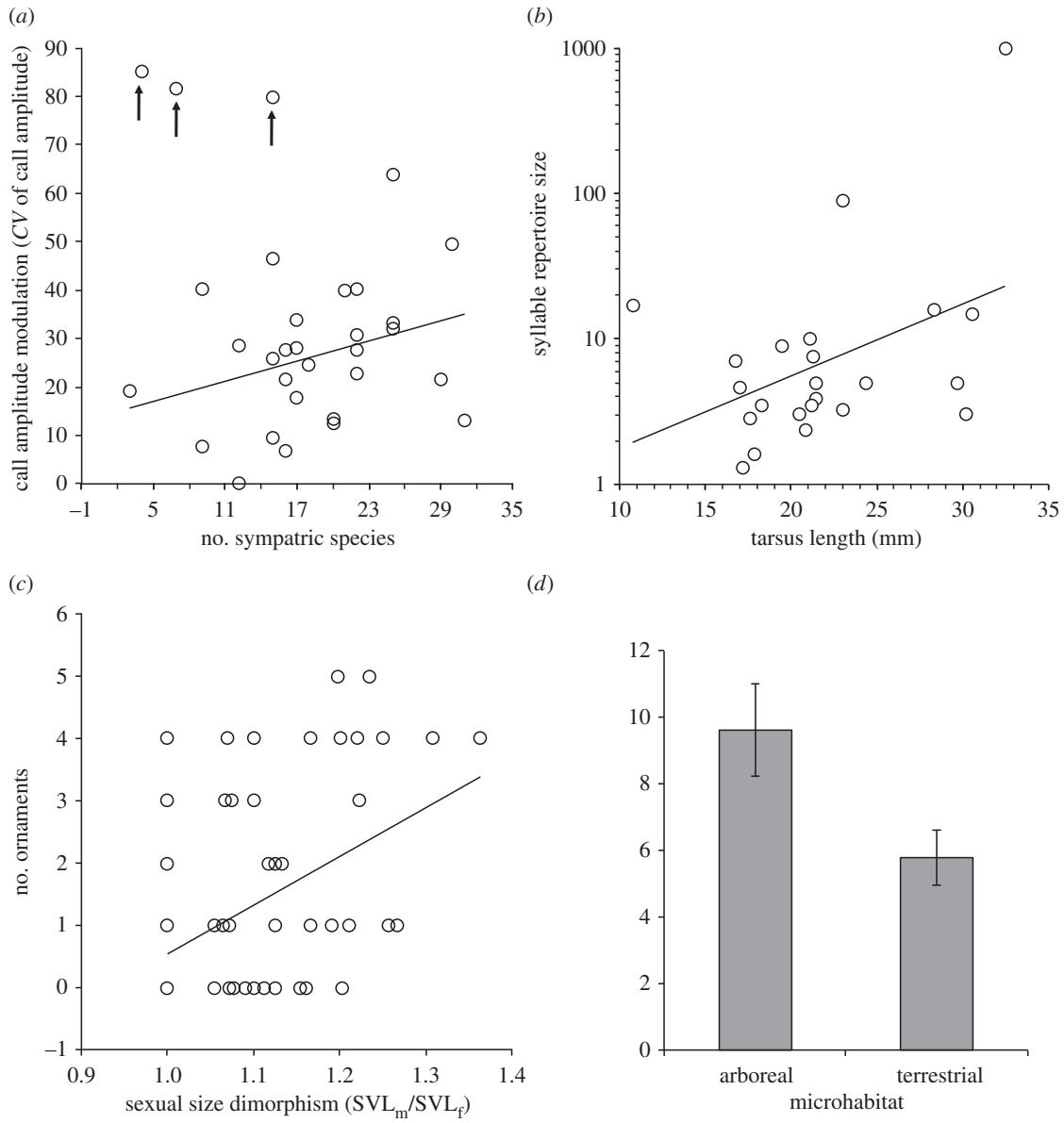

Figure 1. Factors predicting variations in signal complexity in (*a*) frogs, (*b*) distantly related birds, (*c*) distantly related agamid lizards and (*d*) closely related *Sceloporus* lizards. The arrows in (*a*) highlight outliers not included in the computation of the trend line depicted in the plot.

Table 3. Factors influencing the evolution of complexity in chemical communication. CHCs, cuticular hydrocarbons.

		constrained tree					unconstrained tree				
case-study, signal variable	model, rank	AIC_c	AIC_w	r	$t_{1/2}$ (support region)	v_y (support region)	AIC_c	AIC_w	r	$t_{1/2}$ (support region)	v_y (support region)
ants, $n = 40$ species											
no. different CHCs	1. phylogeny: null	298.9	0.46	n.a.	0 (0–10)	90 (50–150)	298.9	0.46	n.a.	0 (0–10)	90 (50–150)
	2. social: colony size	301.2	0.14				301.2	0.14			
	3. ecology: rainfall	301.3	0.14				301.3	0.14			
	4. social: mating system	301.4	0.13				301.4	0.13			
	5. ecology: temperature	301.4	0.13				301.4	0.13			

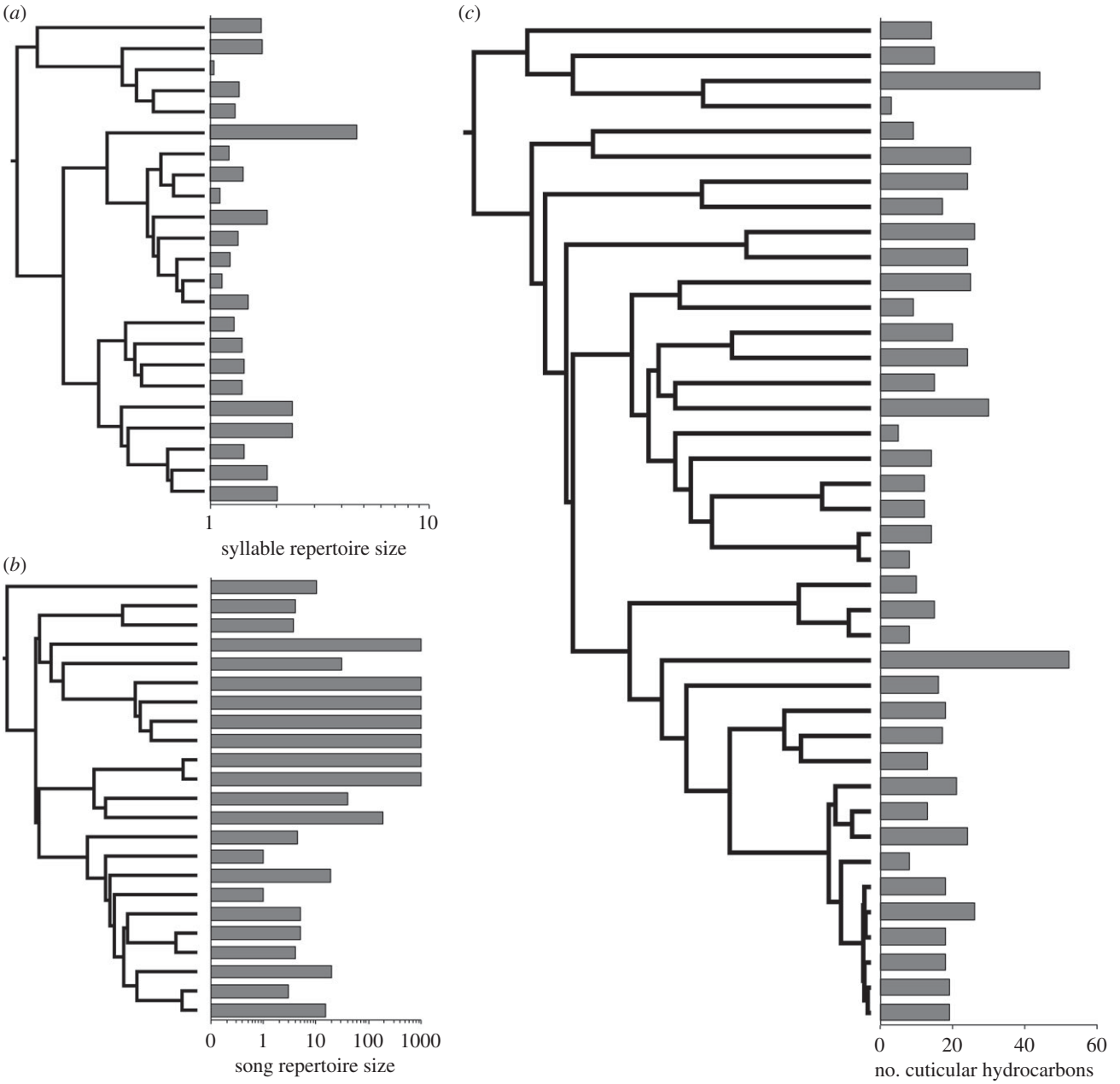

Figure 2. The phylogeny of signal complexity in (*a*) closely related birds, (*b*) distantly related birds and (*c*) ants. Strong phylogenetic inertia was estimated for the repertoires of both bird groups, while virtually no phylogenetic inertia was found for the pheromones of ants.

Variations in signal complexity across the ant phylogeny is depicted in figure 2*c*.

4. DISCUSSION

The goal of our study was twofold: (i) to evaluate the relative support of several hypotheses for the evolution of communicative complexity, and (ii) to illustrate how new phylogenetic approaches can be incorporated into the study of animal communication more generally. With this second goal in mind, we briefly discuss some practical points first to provide readers who are not familiar with the techniques used with a better perspective on how to interpret our findings, before elaborating on the broader implications of our study.

An important point to remember for any statistical analysis is that the validity of results is contingent on the extent to which the data accurately reflect the biological variables being investigated. For example, the particular index of signal complexity we used for a given taxonomic group may or may not be functionally relevant for the species in question. Communication systems are also often complex in ways that are not easily quantified by a single metric. For example, males might be the predominant signallers in one species, whereas both sexes might rely heavily on social communication in another species. In this instance, it is not immediately obvious whether repertoire size (for example) should be summed across the sexes to provide a common metric for both species, or whether each should be sex-evaluated separately. Similar difficulties can exist for metrics of sociality. As an example, the evolution of sexual size dimorphism can reflect other factors aside from sexual selection.

Table 4. A summary of the relative support for factors expected to influence the evolution of communicative complexity. Shown are median ranks of the highest ranked model for a given hypothesis for each case study based on the results from the constrained trees only or both the constrained and unconstrained trees. Calculations used the actual ranks of models when those models were ranked within two AIC_c units of the best-supported model or an assigned rank of 100 if models fell outside this region. Subsequent median ranks of 100 were then classified as 'unranked'.

	signal modality			
hypothesis	acoustic	visual	chemical	all modalities
constrained trees only				
phylogeny	1	1	1	1
ecology	1	1-unranked	unranked	1–2
social	unranked	1–2	unranked	unranked
allometry	unranked	3-unranked	not tested	unranked
constrained or unconstrained trees				
phylogeny	1	1	1	1
ecology	1	2	unranked	1–2
allometry	1	3-unranked	not tested	3
social	unranked	1–2	unranked	unranked

The sexes might appear monomorphic despite male size being the target of sexual selection because natural selection on females has lead to the evolution of large female body size for enhanced fecundity [103]. Alternatively, the presence of size dimorphism might reflect sex-specific divergence in the ecological resources exploited (e.g. differences in the size of prey eaten by the sexes [104]). In comparative analyses, errors in the phylogeny can influence findings as well, as can the assumed mode by which evolution has proceeded. To circumvent these latter issues, we relied on alternative phylogenies and a comparative method that assessed the fit of a range of parameter values designed to assay varying levels of phylogenetic inertia and stochasticity in the evolution process.

The consequence of error in either the phenotypic data or the phylogeny is the generation of noise in analyses. Data noise makes it difficult to detect relationships of small effect that might otherwise exist in nature (i.e. enhanced type I statistical error). For this reason, it is hard to conclusively reject a hypothesis if it does not gain compelling support in a comparative analysis. This is exacerbated when data are complied from a range of different sources (noise in the data can increase because sources define variables differently or use different methods for measuring variables). By the same token, broad trends that are in fact revealed in comparative analyses will probably reflect major evolutionary phenomena because only factors leading to strong biological effects will tend to be detected. In this respect, phylogenetic comparative analyses can offer conservative support for a hypothesis. This support can then be used to justify a more refined comparative analysis in which direct measures or more accurate data are collected by the comparative biologists themselves (e.g. see exploratory literature-based analysis of Ord & Martins [11] and subsequent follow-up field study by Ord *et al.* [105]) or focused experimental research that confirms causal links between a putative selective force and its adaptive outcome [39,106].

Our study is therefore an exploratory analysis that identifies potentially productive avenues for future research. Our findings are not meant to provide definitive conclusions on the specific evolutionary causes of signal complexity in the groups studied (although several strong candidates are highlighted (figure 1*a–d*). Rather the purpose of our study was to use these diverse groups as case studies to offer broader insight into the evolution of communicative complexity generally. On this front, we found that sociality – based on the metrics we were able to compile from the literature and electronic databases – was not as influential in the evolution of signal complexity as we had anticipated. Indeed, it appears to have only been an important factor in the evolution of signal complexity in lizards (table 2 and figure 1*c*; this is consistent with earlier comparative analyses [24,25]).

Table 4 provides an overview of the median model rank associated with each of the four hypotheses. It should be noted that the table weighs evidence from each of the case studies equally, and the communication systems and ecology of these groups do differ in a number of potentially important ways (e.g. dueting songbirds versus chorusing male frogs; colonially living ants versus territorial lizards). However, if a hypothesis is a general explanation for the evolution of communicative complexity, then it should be largely independent of the social or ecological peculiarities of a given species or taxonomic group. That is, support for a given hypothesis should be apparent across broad taxonomic groups rather than exclusive to select species. Table 4 shows that the phylogenetic null model, not social factors, was most often the best-supported model, followed closely by models reflecting ecology. In some instances, phylogenetic inertia and stochastic processes can explain variations in signal complexity among species. Signal complexity therefore has the potential to accumulate in lineages via neutral or non-adaptive processes (e.g. syllable duration in birds; table 1). When evidence of adaptation was found, it was more often associated with variations in ecological factors and (to a lesser extent) allometry than it was with social pressures. For example, signal complexity increased in frogs as a possible function of the number of sympatric species encountered (table 1

Table 5. A summary of phylogenetic patterns associated with estimates of communicative complexity. CHCs, cuticular hydrocarbons.

case-study, signal variable	$N_{species}$	phylogenetic inertia ($t_{1/2}$)	stochasticity (v_y)
vocal signals			
frogs			
call amplitude modulation	32	low	high
call duration	32	high	low
birds (distantly related)			
syllable repertoire size	23	moderate	moderate
song repertoire size	23	high	high
birds (closely related)			
syllable repertoire	23	high	low
syllable duration	23	high	low
song duration	23	high	low
visual signals			
lizards (distantly related)			
number of ornaments	59	low	low–moderate
colour dichromatism, exposed	55	low–moderate	low–moderate
colour dichromatism, concealed	55	low	low
lizards (closely related)			
bob number	22	virtually zero	low
display duration	22	virtually zero	low
chemical signals			
ants			
no. different CHCs	40	virtually zero	low

and figure 1*a*) or the height of call sites above the ground (table 1). In lizards, the switch from a terrestrial lifestyle to being arboreal appears to have facilitated the evolution of more elaborate territorial displays (table 2 and figure 1*d*; see also [63]). The difficulty in interpreting the role of ecology here is whether variables such as call or display site drive or constrain signal complexity. On the one hand, the range over which signals must remain effective increases with perch elevation, leading to potential directional selection on signals for longer duration or more components to facilitate detection by distant receivers. On the other hand, the environmental constraint leading to simple signals when communication is conducted close to the ground is no longer present or reduced for species signalling from elevated perches, opening the door for more complex signals to evolve via other factors. Semantically the distinction is subtle, but biologically it is important for inferring causality. But again, causality can only really be confirmed by means of empirical study and experimental manipulation [106].

That said, empirical and experimental studies within species alone do not adequately identify the selection pressures that have directed signal evolution. Experimental studies can demonstrate current utility, but they cannot reveal the evolutionary history of communication, which in itself can have important consequences on how species adapt to contemporary selection pressures [107]. Consider the phylogeny of signal complexity in the case studies we examined. Table 5 illustrates remarkable consistency within signal classes in possible levels of phylogenetic inertia. If these estimates are accurate, they imply that the evolution of complexity in vocal signals exhibits far greater phylogenetic inertia, and subsequently lower rates of adaptation, than other signal modalities. This could reflect major physiological or metabolic constraints on the production of auditory signals [49,108,109]. We also found evidence for possible selection on the pheromone signals of ants, even though none of our selection models obtained any compelling support. The evolution of pheromone complexity appears to have been non-random and not the product of the gradual accumulation of cuticular hydrocarbons over long periods of evolutionary time (indicated by low values of stochasticity in the evolution process and low values of phylogenetic half-life, respectively). We reiterate that the interpretation of statistical parameters reflecting phylogenetic patterns need to be made with caution. With this in mind, our results are consistent with the notion that an unknown selection pressure has promoted rapid, predictable changes in pheromone complexity in ants. However, these results may also reflect that the hydrocarbons assayed have little functional relevance for communication or fitness generally.

Future research will be needed to clarify the significance of our findings in relation to the specific case studies examined. But the general outcome of our investigation is that sociality is not always required for the evolution of communicative complexity. Or at least, communicative complexity is the product of an intricate evolutionary process that cannot be distilled to a single factor. The complexity of form in the way animals communicate with one another fascinates biologists and amateur naturalists alike. On an informal level, communicative complexity implies richness in the social lives of animals. This sophistication in social behaviour probably enticed many of us into a career of studying animal communication in the first place. Indeed, in some regards, the study of communication in non-avian and non-primate species might be

under-represented in the literature because of the perceived notion that such systems lack a degree of sociality and are therefore less attractive systems for the study of communication. Yet the generality of any hypothesis of why and how animal communication evolved is reliant on testing hypotheses on diverse taxa. We conducted such a study here and found compelling evidence—ironically, it seems—only for social factors in the evolution of visual signals in lizards. Whether sociality is a prerequisite for the evolution of communicative complexity in other systems awaits further investigation. Our results suggest that it may not be (see [56–58] for positive tests in mammals).

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ZOOTAXA

Review of the systematics, distribution, biogeography and natural history of Moroccan amphibians

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Table of contents

Abstract

The amphibian fauna of the Kingdom of Morocco was traditionally regarded as poor and closely related to its European counterpart. However, an increase in research during the last decades revealed a considerable degree of endemism amongst Moroccan amphibians, as well as phenotypic and genotypic inter- and intraspecific divergence. Despite this increase in knowledge, a comprehensible overview is lacking while several systematic issues have remained unresolved. We herein present a contemporary overview of the distribution, taxonomy and biogeography of Moroccan amphibians.

Fourteen fieldtrips were made by the authors and colleagues between 2000 and 2012, which produced a total of 292 new distribution records. Furthermore, based on the results of the present work, we (i) review the systematics of the genus *Salamandra* in Morocco, including the description of a new subspecies from the Rif- and Middle Atlas Mountains, *Salamandra algira splendens* ssp. nov.; (ii) present data on intraspecific morphological variability of *Pelobates varaldii* and *Pleurodeles waltl* in Morocco; (iii) attempt to resolve the phylogenetic position of *Bufo brongersmai* and erect a new genus for this species, *Barbarophryne* gen. nov.; (iv) summarize and assess the availability of tadpole-specific characteristics and bioacoustical data, and (v) summarize natural history data.

Key words: North Africa, Maghreb, Amphibia, Anura, Urodela, tadpole, identification key

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