



Diversification in arid mountains: biogeography and cryptic diversity of *Pristurus rupestris rupestris* in Arabia

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ABSTRACT

Aim To understand the patterns of genetic, geographical and morphological diversification in the Arabian gecko subspecies *Pristurus rupestris rupestris*, and to discuss the various processes that generate and shape diversity in arid mountain ranges.

Location Hajar Mountains, south-eastern Arabian Peninsula.

Methods We sampled 466 individuals across the entire range of *P. r. rupestris* in the Hajar Mountains and sequenced one mitochondrial gene for all the samples, and one extra mitochondrial and four nuclear genes for a subset of 75 specimens. A total of 155 individuals were characterized morphologically. Multilocus coalescent-based methods were used for species delimitation, for phylogenetic inference of species trees and to investigate the diversification dynamics. Multivariate statistics were used to analyse morphological data.

Results Our molecular methods revealed 14 candidate species hidden within the subspecies *P. r. rupestris*. Diversification started *c.* 15 Ma, but most of the lineages have evolved in the last 7 Ma of the mountains' orogeny, producing a mosaic of allopatric taxa. Secondary interchange has occurred between high and low elevation lineages, and along the mountain chain between unrelated lineages formerly separated by mountain blocks. Morphological variation between the 14 candidate species is slight, but a high elevation robust morphotype is identified.

Main conclusions Our results support the notion that mountain ranges constitute important centres of diversification in arid regions, and also act as 'species pumps' into surrounding areas. We also demonstrate that arid mountains may constitute important reservoirs of cryptic diversity, even in common and widespread species. This, and previous evidence, suggest that the Hajar Mountains are a biological hotspot and an important reservoir of diversity. We therefore suggest that these mountains should be a priority focal point for conservation in Arabia.

Keywords

allopatry, Arabia, arid mountains, biodiversity, biogeography, cryptic species, diversification, evolution, systematics

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INTRODUCTION

Mountain regions are great reservoirs of diversity, containing half of the currently defined biodiversity hotspots (Kohler & Maselli, 2012). This is because they offer great opportunities for adaptive and non-adaptive diversification. From an adaptive standpoint, mountains have strong environmental

gradients that can lead to ecological speciation (Fuchs *et al.*, 2011). From a non-adaptive (or not necessarily adaptive) perspective, allopatric or peripatric speciation may occur in mountains by isolation of populations as continuous species ranges are fragmented during mountain orogenies, interrupting gene flow (Vuilleumier & Monasterio, 1986; Randi *et al.*, 2000; Antonelli *et al.*, 2009; Schweizer *et al.*, 2011).

Such processes have an important role in arid regions, where low rainfall and plant productivity do not provide much room for high levels of diversity and diversification (Wiens *et al.*, 2013). In such extreme environments, mountain ranges potentially act as important pumps of regional biodiversity.

This study focuses on one of such mountain ranges, the Hajar Mountains, a long mountain range that borders the Arabian Sea and the Gulf of Oman in the south-eastern Arabian Peninsula (Fig. 1). These mountains are topographically complex consisting of three distinct divisions separated by lowlands, and reaching over 3000 m at Jebel Shams, in the central mountain block (Fig. 1). The highest elevations contain grassland, shrubs and an open juniper-olive tree woodland. Four distinct vegetative zones occur from *c.* 500 m to the highest summit (Ghazanfar, 1991). Their isolation, climatic diversity and complex structure make the Hajars one of the most biotically diverse regions of southern Arabia, with numerous endemic plants and animals (e.g. Arnold & Gallagher, 1977; Ghazanfar, 1991; Brinkmann *et al.*, 2009;

Carranza & Arnold, 2012; Carranza *et al.*, 2016; de Pous *et al.*, 2016).

One of the most conspicuous reptiles is the small sphaerodactylid gecko *Pristurus rupestris* Blanford, 1874. Because of its abundance and broad distribution its current conservation status is of 'Least Concern' (IUCN, 2016). *Pristurus rupestris* is characterized by being mostly diurnal, heliothermic and, like other species of the genus, individuals signal each other by waving their tails (Arnold, 1993, 2009; Badiane *et al.*, 2014). A recent taxonomic revision restricted the range of *P. rupestris* to the Hajar Mountains (subspecies *P. r. rupestris*) and some coastal areas of western Iran (subspecies *P. r. iranicus*) (Badiane *et al.*, 2014; Yousofi *et al.*, 2015).

Despite having a very broad distribution and probably being the most abundant reptile in southern Arabia, the populations of *P. r. rupestris* from the Hajar Mountains have always been considered as a single, morphologically uniform subspecies (Arnold & Gallagher, 1977; Arnold, 1980, 1986, 1993, 2009). However, many recent studies have revealed the existence of cryptic species complexes in common and widespread species

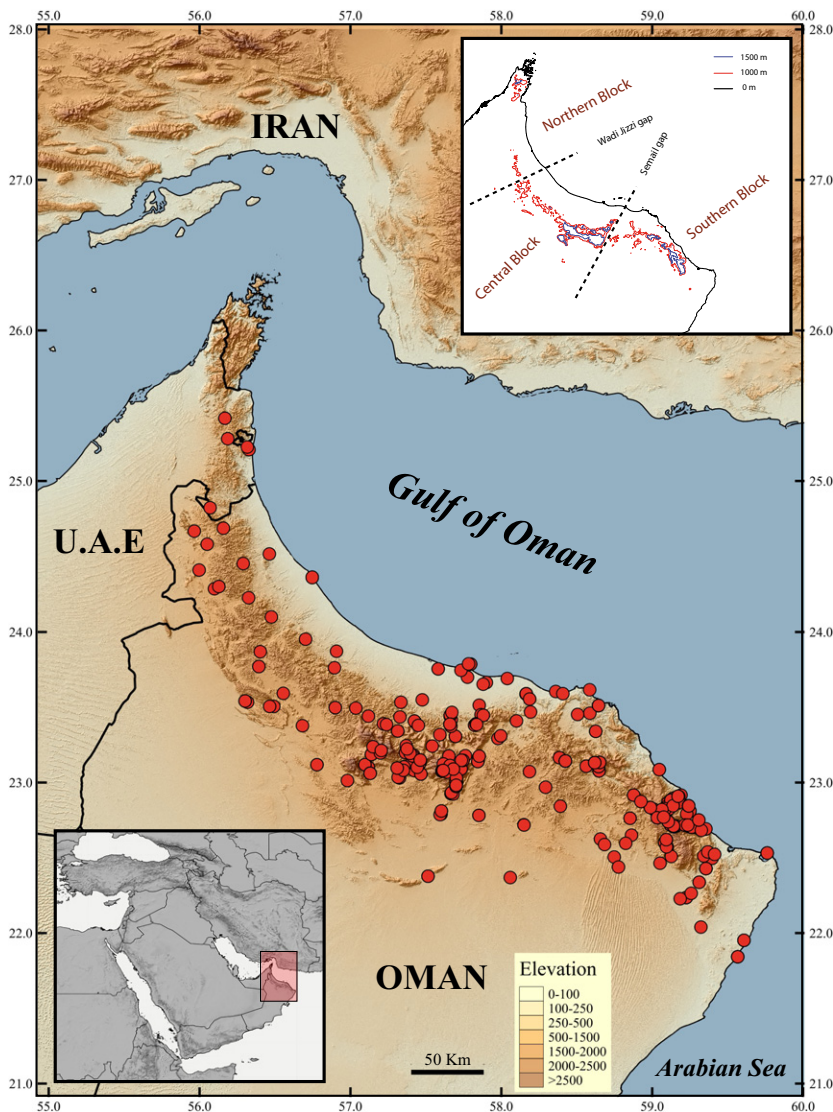


Figure 1 Map of the Hajar Mountains of south-eastern Arabia and sampling locations of *Pristurus rupestris rupestris*. The upper right plot depicts the topographic complexity of the Hajar Mountains with topographic discontinuities that dissect the range into northern, central and southern blocks, physically separated by the Wadi Jizzi and Semail gaps.

in arid mountain ranges, including the Hajar Mountains (Busais & Joger, 2011; Pepper *et al.*, 2011; Barata *et al.*, 2012a, b; Carranza & Arnold, 2012; Brito *et al.*, 2014; Metallinou *et al.*, 2015; Šmíd *et al.*, 2015; Carranza *et al.*, 2016; de Pous *et al.*, 2016), and previous preliminary studies suggested that *P. r. rupestris* could be no exception (Badiane *et al.*, 2014).

In this study we aim to explore three main questions: (1) How much genetic and phenotypic diversity exists within *P. r. rupestris*? (2) Is the phylogenetic structure and genetic divergences within *P. r. rupestris* consistent with the existence of multiple species? (3) What is the role of the Hajar Mountains in producing this phylogenetic structure?

MATERIALS AND METHODS

Sampling

Eight field campaigns were carried out between 2005 and 2014 to explore the diversity of the reptiles in Oman, including the endemic fauna from the Hajar Mountains. Numerous collections were made along the 650 km length of the mountains and the adjacent lowlands, covering the entire distribution range of *P. r. rupestris*. We selected 466 tissue samples from 233 different localities and 155 vouchers to assess the genetic and phenotypic structure of *P. r. rupestris*. Figure 1 illustrates the distribution of the sampling localities, and information on the specimens included in the molecular and morphological analyses is provided in Table S1.

Molecular analyses

Four molecular data sets were assembled for the phylogenetic, phylogeographical and species delimitation analyses. Details of the laboratory, phylogenetic and species delimitation protocols and analyses are in the Supporting Information (see Appendix S1).

Identification and delimitation of mitochondrial lineages

To obtain a first estimation of the genetic diversity within *P. r. rupestris*, the *12S* mitochondrial gene was amplified and sequenced for 466 samples. After removing identical sequences, we aligned the remaining unique 221 sequences (data set 1) with MAFFT 7 (Katoh & Toh, 2008) and produced an ultrametric tree and a set of 250 post-burn-in trees using BEAST 1.8.0 (Drummond & Rambaut, 2007). We then objectively identified and delimited deep mitochondrial lineages with the general mixed Yule-coalescent model (GMYC) (Pons *et al.*, 2006; Fujisawa & Barraclough, 2013). Using the summary tree, this model was implemented in a maximum likelihood and Bayesian framework (using the 250 post-burn trees), by means of the packages 'splits' (Ezard *et al.*, 2009) and 'bGMYC' (Reid & Carstens, 2012), respectively, both available in R (R 3.1.0, R Development Core Team, 2014).

Multilocus coalescent-based species delimitation

From the 16 lineages defined by GMYC (see Results), we amplified and sequenced the mitochondrial *cytb* and the nuclear *c-mos*, *rag1*, *rag2* and *mc1r* genes for a subset of specimens and assembled data set 2. This data set included 2,459 bp from 75 specimens, including at least four specimens from each GMYC lineage (except lineage 16, from which only a single specimen could be amplified for the nuclear genes; see Table S1). The four nuclear loci were used to test whether the mitochondrial lineages defined by the GMYC model were also supported by nuclear data. We assessed this using a coalescent-based species delimitation approach in the program Bayesian Phylogenetics and Phylogeography (BP&P) 2.2 (Yang & Rannala, 2010; see also Olave *et al.*, 2014). To obtain the best tree for the analysis containing all 16 lineages defined by the GMYC model, we used *BEAST 1.8.0 to calculate a species tree (guide tree; see Fig. S2) from data set 2. We ran BP&P analyses with the four nuclear genes (*c-mos*, *rag1*, *rag2*, and *mc1r*), which allowed us to evaluate the GMYC lineage delimitation hypothesis with loci not used to generate it. We ran six different types of analyses, each one having a different combination of prior settings and rjMCMC algorithms. After confirming convergence and good mixing in each analysis we evaluated the posterior probabilities (pp) of all cladogenetic events recovered in our guide tree. We only considered as candidate species those that presented high support values across all different runs (pp > 0.95).

Dating the radiation of *P. r. rupestris*

To date the onset of diversification within *P. r. rupestris*, we assembled data set 3 by placing one representative from each of the 14 candidate species delimited by BP&P (see Results) in the phylogenetic context of a wide representation of all species of geckos available in GenBank. Working at this large phylogenetic scale allowed using five calibration points scattered across the phylogeny of Gekkota, all external to our group of interest. Data set 3 consisted of up to 1614 bp of the mitochondrial *12S* and *cytb* and the nuclear *c-mos*, *rag1* and *rag2* genes for one representative of each of the 14 candidate species of *P. r. rupestris* plus 435 species downloaded from GenBank, including representatives of 117 of the 123 recognized gecko genera (see Table S2; Uetz *et al.*, 2016). Phylogenetic analysis and divergence times were estimated in BEAST 1.8.0 using methods and calibration points specified in the electronic supplementary material.

Inference of a calibrated coalescent-based species tree for the *P. r. rupestris* radiation

A species tree of the 14 candidate species of *P. r. rupestris*, was estimated with data set 2 (see above) using *BEAST (Heled & Drummond, 2010). To obtain the species tree in units of time, we imported into our *BEAST analysis the

substitution rates for *12S*, *c-mos*, *rag1* and *rag2* genes and the age of the root of the *P. r. rupestris* radiation obtained from the BEAST analysis of data set 3 (see above).

Genetic distances

Uncorrected genetic distances (*P*-distances) of the *12S* mitochondrial gene for the 14 candidate species were inferred from data set 1 with MEGA 7.0 (Kumar *et al.*, 2016).

Geographical structure

We used three different approaches to understand the geographical structure within *P. r. rupestris*. First, to infer the phylogeographical history and reconstruct the ancestral origin of *P. r. rupestris* in the Hajar Mountains we used the Bayesian stochastic search variable selection (BSSVS; Lemey *et al.*, 2009) of the discrete phylogeographical model as implemented in BEAST 1.8.0. To maximize geographical representation we used data set 4, which included all 466 sequences of *12S* with the tree topology of the main lineages constrained to match the *BEAST tree topology obtained from the analysis of data set 2 (see Fig. S2). We established the phylogeographical traits according to three discrete topographic discontinuities of the Hajar Mountains, the northern, central and southern blocks, which are physically separated by the Wadi Jizzi and Semail gaps (Fig. 1). Models, prior settings and parameters were the same used for the BEAST analysis of data set 1 (see the electronic supplementary material). Secondly, to assess if diversification had occurred along altitudinal gradients (Kozak & Wiens, 2007; Cadena *et al.*, 2012), we compared the mean altitudes and ranges of the candidate species in a phylogenetic framework by mapping their means onto the phylogeny. To approximate altitudinal change through time, we used the function 'phenogram' of the R package 'phytools' (Revell, 2012) to reconstruct ancestral altitudes. Finally, we evaluated the degree of range overlap between candidate species by creating a 100 m buffer around each one of the 233 unique localities with QGIS 2.6.1 and checking the number of different candidate species coexisting within each buffered locality. The 100 m buffer was selected because the Hajar Mountains can have dramatic altitudinal and topographic changes within a few hundred metres (Mandaville, 1977).

Morphological differentiation

We characterized the morphology of each candidate species with 11 different measurements. Body size was measured from the snout to the cloaca (snout-vent length = SVL); head shape was defined by the length from the snout to the auricular opening (HL), by the maximum width (HW) and by the maximum depth (HD). Body proportions were quantified by the axilla to groin distance (AGL) and by the body width at the level of the scapular and pelvic girdles (ASG and APG respectively). Forelimb proportions included measurements

of the length of the brachium (BL), length of the antebrachium (AL) and hindlimb proportions of the thigh (TL) and crus lengths (CL). Tail length was not measured because most of the specimens had regenerated tails. All measurements were taken three times by the same person using a digital calliper (± 0.1 mm). To improve normality and homoscedasticity, data were \log_{10} -transformed and the means were used in the analysis.

A total of 155 specimens (all adults) were measured, with a minimum of two, a maximum of 24 and a mean of 11 specimens per candidate species (see Table S3). To remove the effect of body size on the shape variables, we computed their residuals against SVL. We tested for sex differences in SVL and in each of the shape measurements (using the residuals) by fitting linear models using each body measurement as dependent variable and sex as the independent variable (using the package 'stats'). The morphospace occupied by each candidate species was determined by a Principal Component Analysis (PCA; package 'ade4'; Dray & Dufour, 2007). To assess whether size and shape differences existed between the candidate species, we conducted a permutational ANOVA on body size (using the package 'stats') and a permutational multivariate ANOVA (perMANOVA) on the first eight PC axes of shape variation (using the R package 'vegan' Oksanen *et al.*, 2013). Finally, a linear discriminate function analysis with all \log_{10} -transformed measurements was used to determine if specimens could be correctly assigned to the candidate species identified by molecular data, with the R package 'MASS' (Venables & Ripley, 2002).

RESULTS

Identification and delimitation of mitochondrial lineages and multilocus coalescent-based species delimitation

Pristurus rupestris rupestris is extensively distributed in the Hajar Mountains and adjacent lowlands, with the exception of the northern region above 25°25' N latitude, where not a single specimen was found (Fig. 1). The results of the GMYC and bGMYC analyses supported 16 different mitochondrial lineages (see Fig. S1). The coalescent-based species delimitation analyses (BP&P) produced different results depending on the prior settings and rjMCMC algorithms, giving low support values for some of the splits between the deep mitochondrial lineages (see Fig. S2). The topological placement of the splits having low support varied depending on the prior parameterization: when big population sizes were combined with deep divergence times, the nodes separating lineages 4 and 5, and 10 and 11 received low support ($pp < 0.93$). However, when big population sizes were combined with shallow divergences times, or when small population sizes were combined with shallow divergences times, only the split separating lineages 10 and 11 had low support ($pp < 0.80$). Consequently, we conservatively collapsed the mitochondrial lineages 4 and 5 and 10 and 11 into putative species 4.5 and 10.11, respectively.

Genetic distances for the 12S mitochondrial gene between all 14 candidate species resulting from the BP&P analysis were very high in most comparisons (5–20%), with the only exception of sister species 1 and 2 (2.9%), and 6 and 7, (2.8%), which present moderate levels of genetic divergence (see Table S4).

Dating the radiation of *P. r. rupestris*

The topology of the phylogeny inferred with data set 3 including the 14 candidate species detected in *P. r. rupestris*, plus 435 additional species of geckos was generally consistent with previously published phylogenies of Gekkota (Gamble *et al.*, 2008, 2011, 2012; Pyron *et al.*, 2013), with 62% of the nodes having pp support values > 0.90 (see Fig. S3). According to our estimates, the age of the crown radiation of Gekkota occurred around 96.6 Ma [95% high posterior density interval (HPD), 65–114 Ma], which is in the range of most estimates from previous studies (Vidal & Hedges, 2005; Hugall *et al.*, 2007; Gamble *et al.*, 2008; Jones *et al.*, 2013; Garcia-Porta *et al.*, 2016). The onset of diversification in the genus *Pristurus* began *c.* 37 (HPD 30–47) Ma and in *P. r. rupestris* began at *c.* 16 (HPD 10–22) Ma. Remarkably, divergence times within *P. r. rupestris* are similar to those between other species of *Pristurus* and to other species of geckos, independently supporting the deep structure found within this ‘subspecies’ (see Fig. S3).

Inference of a calibrated coalescent-based species tree for the *P. r. rupestris* radiation

The species tree of the *P. r. rupestris* species complex produced with *BEAST (Fig. 2) is consistent with the phylogenetic relationships of this radiation recovered in the large phylogenetic tree that includes most gecko genera (see Fig. S3). The species tree revealed four highly supported clades whose detailed distribution ranges are shown in Fig. S4. Clade 1 (candidate species 8 and 9) is mostly limited to lowland areas of the southern block, clade 2 (candidate species 6 and 7) is endemic to high elevations in the southern block, clade 3 (candidate species 10.11, 12, 13, 14 and 15) is distributed throughout the whole region, including the southern, central and northern blocks, and clade 4 (candidate species 1, 2, 3 and 4.5) is mainly distributed in the central and southern blocks. Finally, candidate species 16, is an isolated lineage from the southern block, and is associated with low support to clade 4 in both *BEAST and BEAST analyses of data sets 2 and 3 respectively (Fig. 2 and see Fig. S3).

Geographical structure

The results of the discrete phylogeographical analysis carried out with data set 4 using the BSSVS model are presented in Fig. 3a (see also Fig. S5) and suggest that diversification likely started in the southern block (68%, 76%, 74% and 65% of probability in the four deepest nodes of the phylogenetic tree;

from deepest to shallowest). Diversification in clades 1, 2 and 3 also started in the southern block (92%, 99% and 73% of probability respectively) with some specimens within clade 1 (candidate species 9), and several specimens within clade 3 (all specimens from candidate species 14 and most specimens of candidate species 10.11) having colonized the central block from the southern block. Moreover, within candidate species 10.11 there are some individuals that apparently have recently colonized the northern block from the central block (91% of probability). The origin of clade 4 is inferred to have been in the central block (62% of probability), where candidate species 1, 2 and 4.5 are currently distributed. Candidate species 3 colonized the southern block from the central block (63% of probability) and very recently it has back colonized the central block (93% of probability).

Range overlap between candidate species within the 100 m buffer zone is uncommon, occurring in only 12 of the 233 localities (see Table S5). The great majority of the candidate species have disjunct distributions (94.9%), consistent with a prevalent situation of allopatry in this species complex (Rivas, 1964; Barraclough & Vogler, 2000). Moreover, all sympatric localities had a maximum of two candidate species: only one case included two sister candidate species (6 and 7) and the remaining 11 sympatric localities had pairs of candidate species from different clades (see Table S5).

Morphological differentiation

Morphological differentiation was negligible between sexes and therefore we pooled all sexes together in all posterior analyses (results not shown). Our analyses detected some morphological differentiation among candidate species. The permutational ANOVA on body size was significant (P -value < 0.00001) showing some size variation despite of the high levels of size overlap observed among species (Fig. 4). The perMANOVA analysis of the first eight components of the PCA (explaining 96% of the shape variance) identified significant differences among several candidate species (P -value < 0.00001). The PCA analysis of body shape concluded that most species overlap extensively in their morphologies (Fig. 4), but candidate species 6, 7, 14 have more robust phenotypes, with wider and deeper heads, wider scapular and pelvic girdles (PC1), shorter extremities (PC2) and shorter axilla-groin lengths (PC3) (see Table S6). The position of candidate species 16 in the PCA is uncertain because only two specimens were included in the analysis (see Table S3). All three taxa with robust phenotypes live at higher altitudes according to our elevational reconstructions (Fig. 3b). However, the linear discriminant function analysis failed to assign individual specimens to their respective candidate species in more than 70% of the comparisons (mean correct assignment 26%) (see Table S7). Robust phenotypes of candidate species 6, 7 and 14 were assigned to other robust phenotypes in 64%, 90% and 80% of the comparisons, respectively, and correctly assigned to their respective candidate species in 40% of the comparisons. Although the

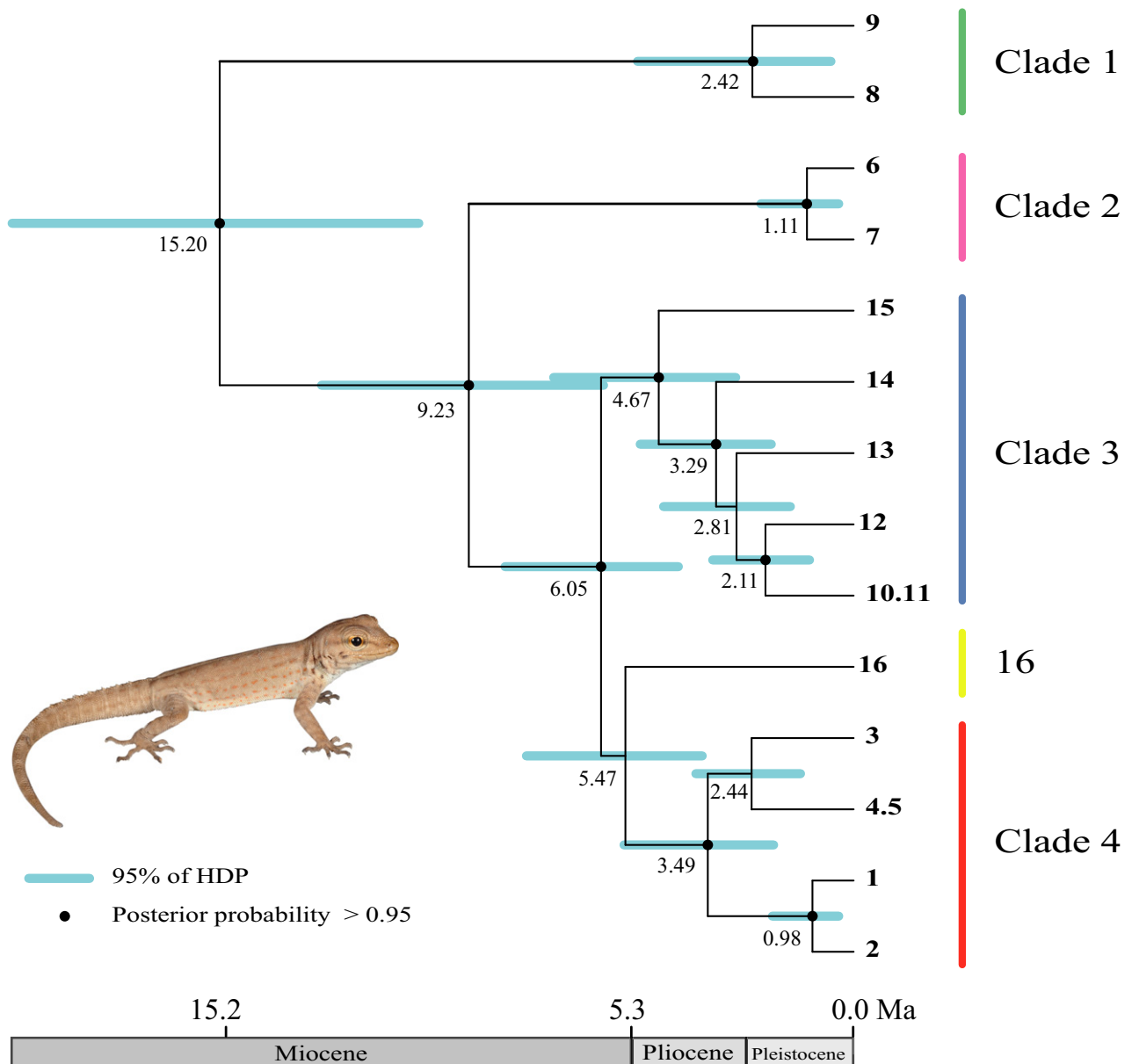


Figure 2 Time-calibrated multilocus coalescent-based species tree of *Pristurus rupestris rupestris* inferred in *BEAST with data set 2 (two mitochondrial and four nuclear genes, 2459 bp) and supported by the BP&P species delimitation analysis. Numbers at nodes indicate the mean divergence time and bars represent the 95% high posterior density interval of the time estimates. Filled circles at nodes indicate posterior probability support values > 0.95. The photograph is *P. r. rupestris* from candidate species 7. See Supporting information for more information on the genes, parameters and calibrations used.

robust and slender phenotypes are differentiated, most candidate species within each of these morphotypes are indistinguishable.

DISCUSSION

This study uncovers an unparalleled degree of species-level diversification in an Arabian vertebrate, identifying 14 candidate species within a single subspecies of gecko inhabiting an area of less than 25,000 km². This illustrates the unknown diversity yet to be discovered in the planet's desert regions.

Moreover, these results highlight the role of mountain ranges in generating and preserving diversity in arid environments (Sanders *et al.*, 2003; McCain, 2007). Mountains provide numerous possibilities for smaller scale geographical isolation of demes and allopatric speciation, particularly during periods of orogeny (Antonelli *et al.*, 2009), a relationship that has been detected in a number of groups (Liu *et al.*, 2006; Antonelli *et al.*, 2009; Bunce *et al.*, 2009; Toussaint *et al.*, 2014; Merckx *et al.*, 2015), and has probably contributed significantly to cladogenesis in *P. r. rupestris*. Indeed, most of its diversification occurred during the latter stages of

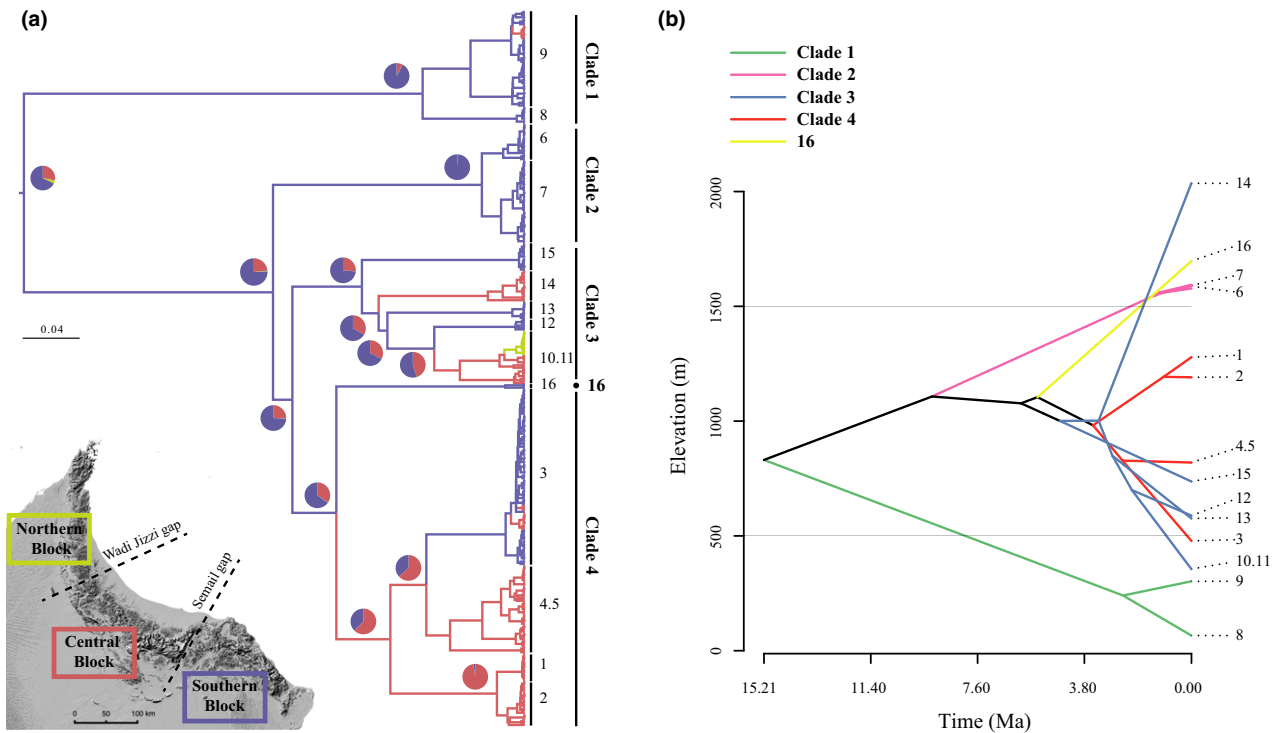


Figure 3 Map of the Hajar Mountains of south-eastern Arabia showing the geographical and altitudinal distribution of the 14 candidate species of *Pristurus rupestris rupestris*. (a) BEAST summary tree of *P. r. rupestris* using the BSSVS method of ancestral area reconstruction. Branch colour indicates inferred ancestral range (southern block, central block or northern block). A pie chart describing the probability of each inferred area is presented near the relevant nodes. A more detailed version of this figure with the probability values is shown in Fig. S5. (b) Altitudinal diversification through time of *P. r. rupestris*, based on ancestral state reconstructions of the elevation in the nodes.

mountain uplift, beginning in the late Miocene and continuing through the Pliocene and Quaternary, a time when elevations increased from 200 to 500 m (Kusky *et al.*, 2005). The appearance of new climatic environments and topographic discontinuities, combined with repeated altitudinal shifts of biotic communities during arid (glacial) and pluvial (interglacial) phases of the Arabian Pleistocene (Burns *et al.*, 2001) likely stimulated multiple dispersal events from areas of origin and fragmented populations into isolated demes leading to genetic divergence. Consistent with this, most of the sister candidate species date from the early and middle Pleistocene (Fig. 2).

Our ancestral area reconstruction (Fig. 3a and see Fig. S5) suggests that range expansion first initiated in the southern block and later proceeded northward, a pattern also reported for *Trachydactylus hajarensis* (de Pous *et al.*, 2016), and *Asacus gallagheri* (work in progress), two gecko species endemic to the Hajar Mountains. During their movements towards north, Clades 3 and 4 have independently crossed the Semail gap to the central block, creating an assemblage of polyphyletic candidate species on both mountain blocks.

Several lineages have undergone genetic divergence and morphological diversification along elevational gradients (Figs 3 & 4). High altitudes were reached three times independently, and a robust morphology has convergently

evolved consisting of wide heads, wide scapular and pelvic girdles, short legs and short axilla-groin lengths. Nevertheless, the morphospace of the majority of the candidate species greatly overlaps within the robust and slender phenotypes, resulting in a high degree of cryptic.

The lowlands (< 500 m), which are occupied by candidate species 8 and 9 (clade 1), have been independently colonized two times by high-altitude ancestors, candidate species 3 (clade 3) and 10.11 (clade 4). This pattern illustrates that mountains are not only centres of diversification (Toussaint *et al.*, 2014), but also act as 'species pumps', as taxa disperse into the surrounding lowlands (Wollenberg *et al.*, 2008).

Similar radiations of genetically divergent but morphologically cryptic populations may evolve in other lizards living on widespread but similarly coloured substrates that cover geologically unstable and irregular mountain topographies. In fact, radiations of cryptic mountain taxa have been reported for the lacertid lizards of the genus *Atlantolacerta* (Barata *et al.*, 2012a, 2014) the diurnal geckos of the genus *Quedenfeldtia* (Barata *et al.*, 2012b) and the nocturnal geckos of the genus *Ptyodactylus* (Perera & Harris, 2010) from the Atlas Mountains, and more recently for the extensive radiation of the *Ptyodactylus* species complex across the main mountain ranges of Arabia (Metallinou *et al.*, 2015).

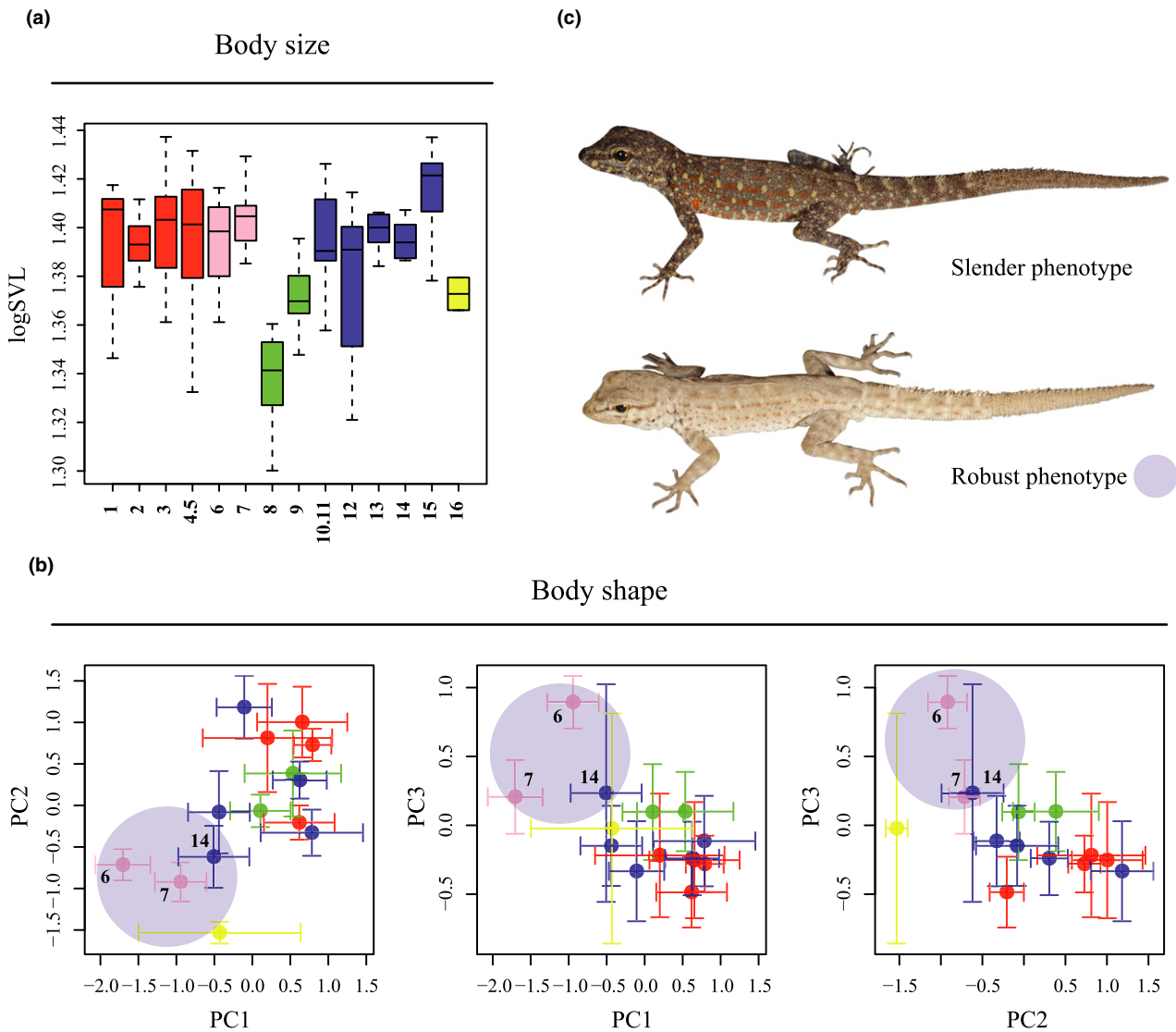


Figure 4 (a) Boxplot showing the $\log_{10}SVL$ variation of the 14 candidate species of *Pristurus rupestris rupestris* and (b) their morphospace. The morphospace was obtained from the three first components of a PCA (reflecting 58% of the total variation) performed on 10 body measurements. The three robust phenotypes of *P. r. rupestris* are indicated by their candidate species codes. (c) The slender (candidate species 12) and robust (candidate species 7) phenotypes.

Overall, our results stress the importance of mountain ranges as centres of speciation and diversification, especially when compared to the surrounding low elevation arid environments, where reduced ecological complexity and extreme climatic conditions limit biodiversity. Thus, previous and ongoing research indicate that the Hajar Mountains are a biological hotspot and an important reservoir of diversity, and therefore should be a priority conservation focal point (Arnold & Gallagher, 1977 and other articles in the same volume; Carranza & Arnold, 2012; Carranza *et al.*, 2016; de Pous *et al.*, 2016). For example, candidate species 9, 3, 4.5 and 10.11 have wide distributions, but the remaining 10 candidate species appear to have very restricted ranges, sometimes being known from a single valley or slope and are likely populations of high conservation concern (see Fig. S4).

The cryptic diversity discovered in *P. r. rupestris* not only points to the need for a taxonomic revision, but it also highlights the problems faced by conservation planners. Without knowledge of the hidden biodiversity in a region like the Hajar Mountains, it is not possible to make meaningful conservation plans to protect these evolutionarily significant units. Similar analytical methodology, and importantly, fine-scale sampling is likely to uncover unknown diversity even in the most widespread and common species.

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SUPPORTING INFORMATION

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

Appendix S1 Extended methods.

Appendix S2 Supplementary tables.

Appendix S3 Supplementary figures.

DATA ACCESSIBILITY

All sequences generated for this study are deposited in GenBank with accession numbers listed in the supporting information (see Tables S1 and S2). All specimen measurements are available in Table S3.

BIOSKETCH

One of the main research lines of the Systematics, Biogeography and Evolution of Reptiles and Amphibians lab at the Institute of Evolutionary Biology (CSIC-Pompeu Fabra University) in Barcelona, Spain is the study of the processes that shape biodiversity across space and time, using Afro-Arabian reptiles as model organisms.

<http://molevol.cmima.csic.es/carranza/index.html>

Author contributions: J.G.P. and S.C. conceived the study; J.G.P., M.R. and S.C. collected samples; M.S.R. carried out the molecular lab work; J.G.P. and M.S.R. measured specimens and carried out the phylogenetic analyses; J.G.P. carried out the statistical analyses, all authors drafted the manuscript and gave final approval for publication.

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