Evolutionary history of the two North African hedgehogs (Mammalia: Erinaceidae) *Atelerix algirus* and *Paraechinus aethiopicus* based on phylogeography and species distribution modelling

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Abstract

Two species of hedgehogs are known to occur in northern part of Africa: the Algerian hedgehog *Atelerix algirus* and the Ethiopian hedgehog *Paraechinus aethiopicus*. Within each species several subspecies were described based on morphometrical data and pelage coloration, but all these subspecies have enigmatic and unclear definitions. We investigated the phylogeographical history and taxonomy of these two species based on mitochondrial DNA data covering the entire geographical distribution of *A. algirus* and the North African distribution of *P. aethiopicus*. We also used climatic niche modelling to make inferences about their evolutionary history. Low genetic diversity was recovered in both species. While no phylogeographic pattern was found in *P. aethiopicus*, two haplogroups were identified within *A. algirus*. This could be explained by the fact that continuous high or moderate climatic suitability occurred throughout most of the Saharan desert since the LGM (Last Glacial Maximum) for the first species, while during the LGM there were several disconnected areas of high climatic suitability for *A. algirus*: one in South-West Morocco, one at the coastal Moroccan-Algerian border and one in Tunisia-coastal Libya. Our genetic results confirm that *A. algirus* recently colonized Spain, Balearic and Canary Islands, and that this colonization was probably mediated by humans. Suitable climatic conditions occurred throughout most of the Southern and Eastern Iberian Peninsula during the last 6,000 years which could have favored the spatial expansion of the Algerian hedgehog after its arrival in Europe. According to our molecular results subspecific recognition within North Africa is unwarranted for both species.

Key words

Climatic niche modelling; haplotype network; mammal; mitochondrial DNA; North Africa; phylogeny; taxonomy
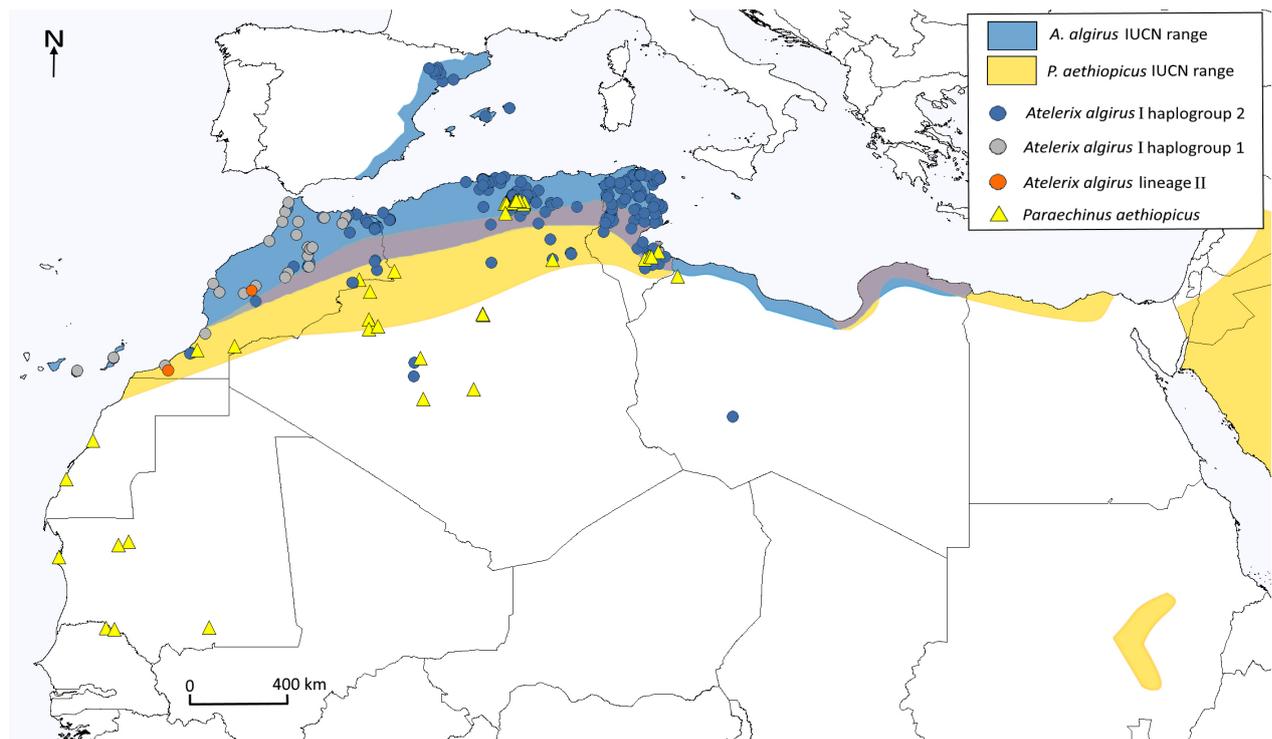
Figure 1. Geographical distribution of hedgehog DNA samples used in this study and distribution of the analyzed taxa according to IUCN (2015).

Introduction

Two species of hedgehogs are known to occur in northern part of Africa (Fig. 1): the North African or Algerian hedgehog *Atelerix algirus* (Lereboullet, 1842) and the Ethiopian hedgehog *Paraechinus aethiopicus* (Ehrenberg, 1832), which can be distinguished based on morphological characteristics (Happold and Happold 2013) and genetic data (He et al. 2012; Bannikova et al. 2014; Velo-Antón et al. 2019). The Algerian hedgehog is recorded from North Africa (Morocco, Algeria, Tunisia and Libya), in parts of the Mediterranean coastal regions of Spain, and the Balearic, Canary and Maltese islands (Best 2018). It occurs in coastal dry and warm Mediterranean scrublands and grasslands. It does not inhabit arid deserts, but it has been documented at oases in the Sahara desert (Happold and Happold 2013; Best 2018). The Ethiopian hedgehog occurs in arid and semi-arid habitats in Africa, from Senegal and Mauritania through scattered locations in the Sahara to Egypt, Sudan, and Somalia, and in the Middle East, from Syria and Iraq to the Southern Arabian Peninsula (Corbet 1988; Hutterer 2016; Best 2018).

Within each species several subspecies and forms were described based on morphometrical data and pelage coloration. According to Hutterer (2005) the Algerian hedgehog comprises three subspecies: *A. algirus algirus* (Lereboullet, 1842) from North Africa (with four synonyms: *caniculus* (Thomas, 1915), *diadematus* (Dobson, 1882), *fallax* (Dobson, 1882) and *lavaudeni* (Cabrera, 1928)), *A. a. girbaensis* (Vesmanis, 1981) from Djerba Island (Tunisia), *A. a. vagans* (Thomas, 1901) from Spain

and Minorca and other islands (with one synonym: *krugi* (Peters, 1877)). According to the same author the Ethiopian hedgehog comprises five subspecies: *P. aethiopicus aethiopicus* (Ehrenberg, 1832) from Soudan and Ethiopia, *P. a. albatus* Thomas, 1922 from Djerba island (with three synonyms: *albior* Pocock, 1934, *blancalis* (Thomas, 1921) and *brachydactylus* (Wagner, 1841)), *P. a. deserti* (Loche, 1858) from North Africa, *P. a. ludlowi* (Thomas, 1919) from Iraq and a part of Iran (with two synonyms: *oniscus* (Thomas, 1922) and *pallidus* (Fitzinger, 1867)) and *P. a. pectoralis* (Heuglin, 1861) from Arabian Peninsula (with three synonyms: *dorsalis* (Anderson and de Winton, 1901), *sennaariensis* (Hedenborg, 1839) and *wassifi* (Setzer, 1957)). However, as stated by Hutterer (2005) and by Best (2018) all these subspecies have enigmatic and unclear definitions, and subspecific taxonomy required reassessment.

Until recently, virtually nothing was known on the genetic variability within these two species. However, during the last five years, several studies were published. Based on one mitochondrial (Cytochrome b) and one nuclear (BFIBR) genes Khaldi et al. (2016) showed low genetic diversity across the geographical range of *A. algirus* and a recent colonization of Spain, Balearic and Canary Islands by this species, probably mediated by humans. Following these results, Best (2018) proposed to consider *A. a. algirus* and *A. a. vagans* as a single subspecies: *A. a. algirus*. Derouiche et al. (2016) also found low genetic diversity within this species in Algeria based

on a fragment of the control region. However, with the same DNA fragment Velo-Antón et al. (2019) found three distinct genetic lineages within this species: two lineages endemic to Morocco and one lineage widely distributed from Eastern Morocco to Libya. This result suggests that the control region could be a suitable marker to study the phylogeographical structure and evolutionary history of this species across its distribution range, owing to its fast evolutionary rate and presumed selective neutrality as a non-coding region. Molecular studies of *P. aethiopicus* found no phylogeographic structure within North Africa, whatever the DNA fragment used (Derouiche et al. 2016; Velo-Antón et al. 2019), and genetic differentiation between North African and Arabian populations (Derouiche et al. 2017).

The aim of this study is to investigate the phylogeographical history and taxonomy of these two species based on improved sampling covering the entire geographical distribution of *A. algirus* and the North African distribution of *P. aethiopicus*. New samples from Morocco, Algeria, Tunisia, peninsular Spain, and Balearic and Canary Islands were included. We also used ecological niche modelling to make inferences about the evolutionary history of these lineages and the potential impact of paleoclimatic events on the current genetic variability and distribution of each species.

Material and methods

Sampling

A total of 126 new samples (See supplementary file 1) were collected in the field, either from road-killed animals or live-trapping or from museum collections (Museum of Natural Science of Barcelona) and stored in 96% ethanol. These specimens come from Tunisia (72 specimens), Algeria (32), Morocco (2 specimens), Spain (15), Balearic (2) and Canary Islands (3). Geographical coordinates for the collected samples were recorded with a Global Positioning System. Species identification in the field was based on morphological characteristics, namely ventral, leg and facial color patterns, as well as ear length (Aulagnier et al. 2008).

DNA extraction, amplification and sequencing

Whole genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN), following the manufacturers' protocols. We amplified a fragment of 456–465 bp of the mitochondrial control region (CR) using the primers developed by Derouiche et al. (2016): CRF: 5'-CATCA-ACACCCAAAGTTG-3'and Erinaceinae CRR: 5'-TGA-AGAAAGAACCAGATG-3'. The amplification conditions consisted of an initial denaturation phase at 94 °C for 3 min, followed by 40 cycles each of denaturation at

94 °C for 40 s, hybridization at 53 °C for 40 s and elongation at 72 °C for 60 s; then a final elongation phase ran at 72 °C for 5 min. The quality and size of the PCR products were then verified by electrophoresis on 1% agarose and ethidium bromide-supplemented gel. The double-stranded PCR products were sent for purification and Sanger sequencing at EUROFINS. All newly determined sequences were submitted to GenBank (accession numbers OL692499–OL692624).

Phylogenetic analyses and genetic diversity

Chromatograms were checked and sequences manually corrected in CodonCode Aligner 8.0.2. In our phylogenetic analyses, we included all the newly sequenced specimens and all specimens of *Atelerix* and *Paraechinus* available in the GenBank database for which the control region was sequenced. We also added several references sequences for the hedgehog species *Erinaceus europaeus*, *E. roumanicus* and *E. concolor*. Sequences were aligned with ClustalW implemented in Geneious 4.6.4., and the alignment was corrected by eyes. The final alignment comprised 283 sequences (see supplementary file 1 for specimens list, and see supplementary file 2 for sequence alignment). Sequences of 423 bp were retained for the final analyses as they were available for most specimens.

We estimated evolutionary relationships among sequences by constructing phylogenetic trees using maximum-likelihood (ML) and Bayesian inference (BA). The computer program MrModeltest ver.2 (Nylander 2004) was used to evaluate the fit of 24 nested models of nucleotide substitution to the data. The model (HKY+I+G) chosen by MrModeltest according to the Akaike Information Criterion was then used in ML and BA analyses. The ML analysis was performed with PhyML ver. 3.0 (Guindon et al. 2010) on the PhyML online web server (Guindon et al. 2005). In the PhyML procedure, we used the BIONJ distance-based tree as the starting tree and 100 bootstrap replicates. Bayesian inference was performed with MrBayes ver. 3.2.6 (Ronquist et al. 2012) using 7,000,000 generations (burnin = 25%), sampling every 1,000 steps. Convergence between chains was checked by manual inspection using the 'sump' command and by using the mean SD of split frequencies, which was below 0.01 in our runs, as an indicator of convergence.

A haplotype network for each species (See supplementary file 3 for *A. algirus* sequences alignment, and see supplementary file 4 for *P. aethiopicus* sequences alignment) was constructed in TCS 1.2.0 (Clement et al. 2000) using statistical parsimony to calculate the minimum number of mutational steps among haplotypes with connections based on 95% confidence interval. Gaps were treated as 5th state. The web-based program tcsBU was used to draw the network (Murias dos Santos et al. 2016).

Nucleotide diversity, haplotype diversity (Nei 1987) and the mean number of pairwise differences were calculated using DnaSP 6.12 (Librado and Rozas 2009).

Inferences of population expansion were made using two different methods: the Fs statistic (Fu 1997) and the R2 statistic (Ramos-Onsins and Rozas 2002). Ramos-Onsins and Rozas (2002) demonstrated that these statistics have the greatest power to detect population expansion for nonrecombining regions of the genome under a variety of different circumstances, especially when population sample sizes are large or when sample sizes are small. The significance of Fu's Fs and R2 was obtained by examining the null distribution of 1,000 coalescent simulations of these statistics using DnaSP 6.12 (Rozas et al. 2017). Significantly large negative Fu's Fs values and significantly positive R2 values were taken as evidence of a population expansion.

Species distribution modelling

To evaluate current and past habitat suitability for the two species of hedgehogs (*A. algirus* and *P. aethiopicus*) we produced distribution models under three different climatic conditions (current, mid-Holocene and Last Glacial Maximum [LGM]) using a distribution modeling approach. Species occurrence data were assembled from the literature (Derouiche et al. 2016; Aulagnier et al. 2017; Deef 2020; El-Farhati et al. 2020; de Smet et al. 2021), museum collections (Global Biodiversity Information Facility, GBIF.org (2 December 2020) GBIF Occurrence Download https://doi.org/10.15468/dl.gkz4ya and https://doi.org/10.15468/dl.m8y5zz) and our own records (See supplementary file 5). Datasets of this type often exhibit sampling bias, which can lead to unreliable ecological niche modeling results (Fourcade et al. 2014). To correct sampling bias, a subsample of records regularly distributed in the geographical space was selected. The resolution of the reference grid was set to 2.5 arc min; i.e. the same as that used for the environmental layers. This coarse resolution was chosen to better match the coordinate uncertainties associated with georeferenced, textual localities of museum specimens. After filtering, the final dataset used for modeling consisted of 791 distribution records for *A. algirus* and 481 distribution records for *P. aethiopicus*.

As environmental layers, we used available climatic data from the WorldClim database (Hijmans et al. 2005). We removed from our analyses variables BIO2, BIO3, BIO5, BIO7 and BIO15 because those variables show a high level of discrepancy between general circulation models (GCMs) for North Africa (Varela et al. 2015). Colinearity of the initial variables was measured by Pearson's correlation coefficient. We retained six variables that are considered ecologically important for hedgehogs and for which pairwise correlations were mostly smaller than 0.75: annual mean temperature (BIO1), seasonality of temperature (BIO4), minimal temperature for coldest period (BIO6), mean temperature of the warmest quarter of the year (BIO10), mean temperature of the coldest quarter of the year (BIO11), annual precipitation (BIO12). These climatic variables are ecologically important for hedgehogs because temperature affects hedgehogs' prey

activity, and hedgehogs are known to hibernate in cold winters (Mouhoub-Sayah 2009; Mouhoub Sayah et al. 2009). Climatic variables were downloaded for present conditions, for mid-Holocene (a wetter period, about 6,000 years ago) and for the LGM (a cold and dry period, about 22,000 years ago). Mid-Holocene and LGM climate data were drawn from General Circulation Model (GCM) simulations from two climate models: the Community Climate System Model (CCSM ver. 3) (Collins et al. 2006) and the Model for Interdisciplinary Research on Climate (MIROC ver. 3.2) (Hasumi and Emori 2004). These climate models differ in temperature and precipitation patterns. For a discussion of the uncertainties associated with the climatic data see Schorr et al. (2012) and Varela et al. (2015). The use of these two different climate models enabled us to assess and account for modeling uncertainty due to LGM climate data. To predict the potential distribution of the species in current conditions and in the past, we used Maxent ver. 3.3.3 (Phillips et al. 2006), which outputs a model with relative occurrence probability of a species within the grid cells of the study area. To ensure consistency of model predictions among repeated runs, we performed a 50-fold cross-validation with random seed. To determine whether the predictions for current conditions generated by Maxent were better than random predictions we used the area under the receiver operating characteristic curve (AUC), a commonly used measurement for comparison of model performance (Elith et al. 2006). The AUC ranges from 0 to 1, with greater scores indicating better discrimination ability; an AUC greater than 0.5 indicates that the model discriminates better than random.

Results

Phylogenetic analyses and genetic diversity

The final alignment contained 283 sequences of 423 pb long with 32 sites with alignment gaps. ML and BA analyses resulted in a well resolved tree (Fig. 2) supporting the monophyly of the three included genera (*Atelerix*, *Erinaceus* and *Paraechinus*). All *P. aethiopicus* specimens cluster in a well-supported lineage. Specimens of *A. algirus* cluster in two distinct well supported lineages (I and II), which are not sister lineages. The first lineage includes most specimens and is distributed throughout the geographical range of the species. The second lineage includes only two Moroccan specimens. The average number of nucleotide differences between these two lineages is 27.574 with 22 fixed differences. As a matter of comparison, it is 27.000 with 27 fixed differences between *A. algirus* lineage II and *A. albiventris* lineage 1, and it is 27.667 with 26 fixed differences between *A. algirus* lineage II and *A. albiventris* lineage 2. The two sequences of *A. algirus* lineage II were previously published by Velo-Antón et al. (2019). Considering the high
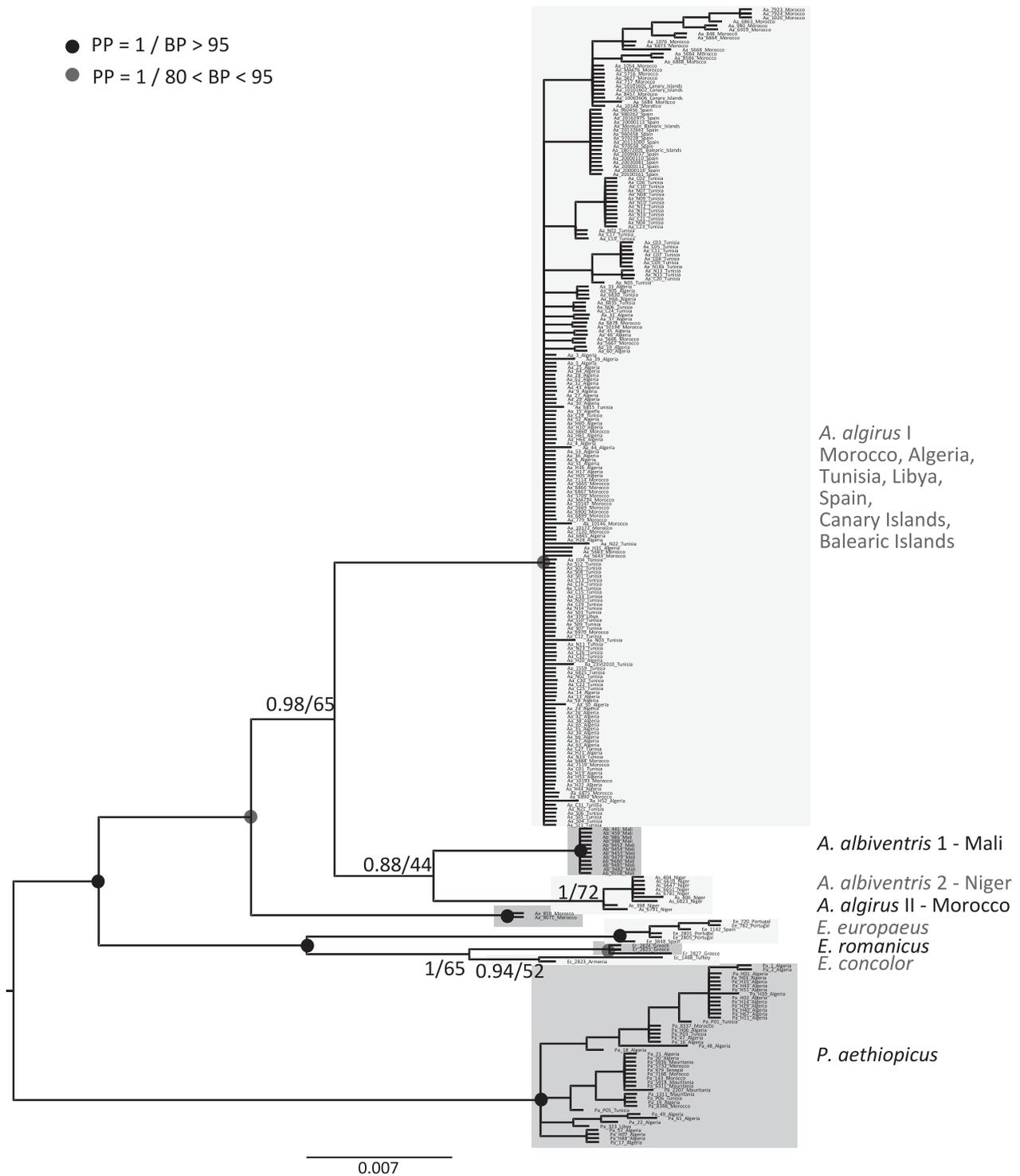
Figure 2. Bayesian phylogenetic tree generated in MrBayes for the hedgehogs. Circles and numbers at nodes represent posterior probabilities (Bayesian analysis) and bootstrap support (Maximum Likelihood analysis). Branch lengths are in substitutions/site.

genetic divergence between these two lineages and the topology of the tree, doubts on the species identification and/or validity of these sequences are raised (see discussion part). Thus, they are not considered as belonging to *A. algirus* in subsequent analyses.

The 204 specimens of *A. algirus* lineage I resulted in 58 haplotypes (Table 1). The nucleotide diversity is 0.00745 ± 0.00037, haplotype diversity is 0.924 ± 0.008 and the average number of nucleotide differences is 3.010. No strong phylogeographic structure is recorded within

this species (Fig. 3). One abundant haplotype is shared between specimens from Tunisia, Algeria and Morocco, and several haplotypes are shared between specimens from Algeria and Morocco, or from Algeria and Tunisia. One haplotype is shared between specimens from Libya and Tunisia. High genetic diversity is observed within Morocco (51 specimens, 23 haplotypes) with numerous endemic haplotypes. Haplotypes from Morocco can be divided in two groups: the first haplogroup (haplotypes A01 to A13) is composed exclusively of haplotypes en-

Table 1. Diversity and neutrality estimates for the two species and main haplogroups identified in the Statistical parsimony haplotype networks. Significant values are indicated by asterix (* for P < 0.05, ** for P < 0.01, *** for P < 0.001). Number of sequences (N), number of distinct haplotypes (Nh), haplotype diversity (Hd), nucleotide diversity (Pi), average number of nucleotide differences (k) and results of the Fu's Fs and R2 tests are given.

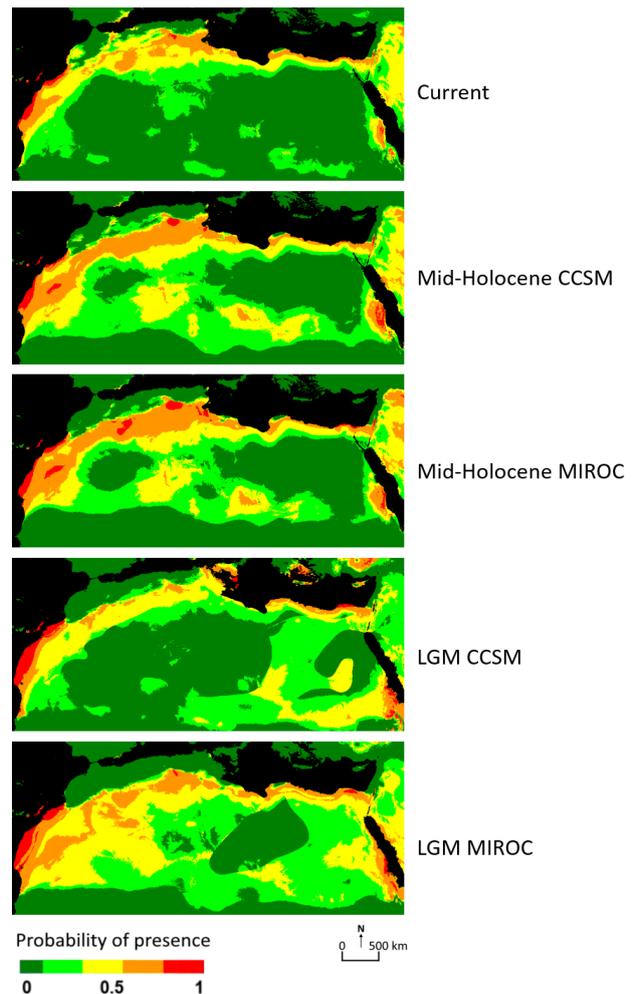|	N	Nh	Hd	Pi	k	Fu's Fs	R2
All *A. algirus* lineage I	204	58	0.924 ± 0,008	0.00745 ± 0,00037	3.010	−28,426***	0.057
Haplogroup 1	25	13	0.827 ± 0.067	0.00514 ± 0.00069	2.077	−3.555*	0.110
Haplogroup 2	179	43	0.904 ± 0.010	0.00592 ± 0.00030	2.399	−19.395***	0.053
All *P. aethiopicus*	45	19	0.905 ± 0.026	0.01086 ± 0.00050	4.386	−3.846	0.133
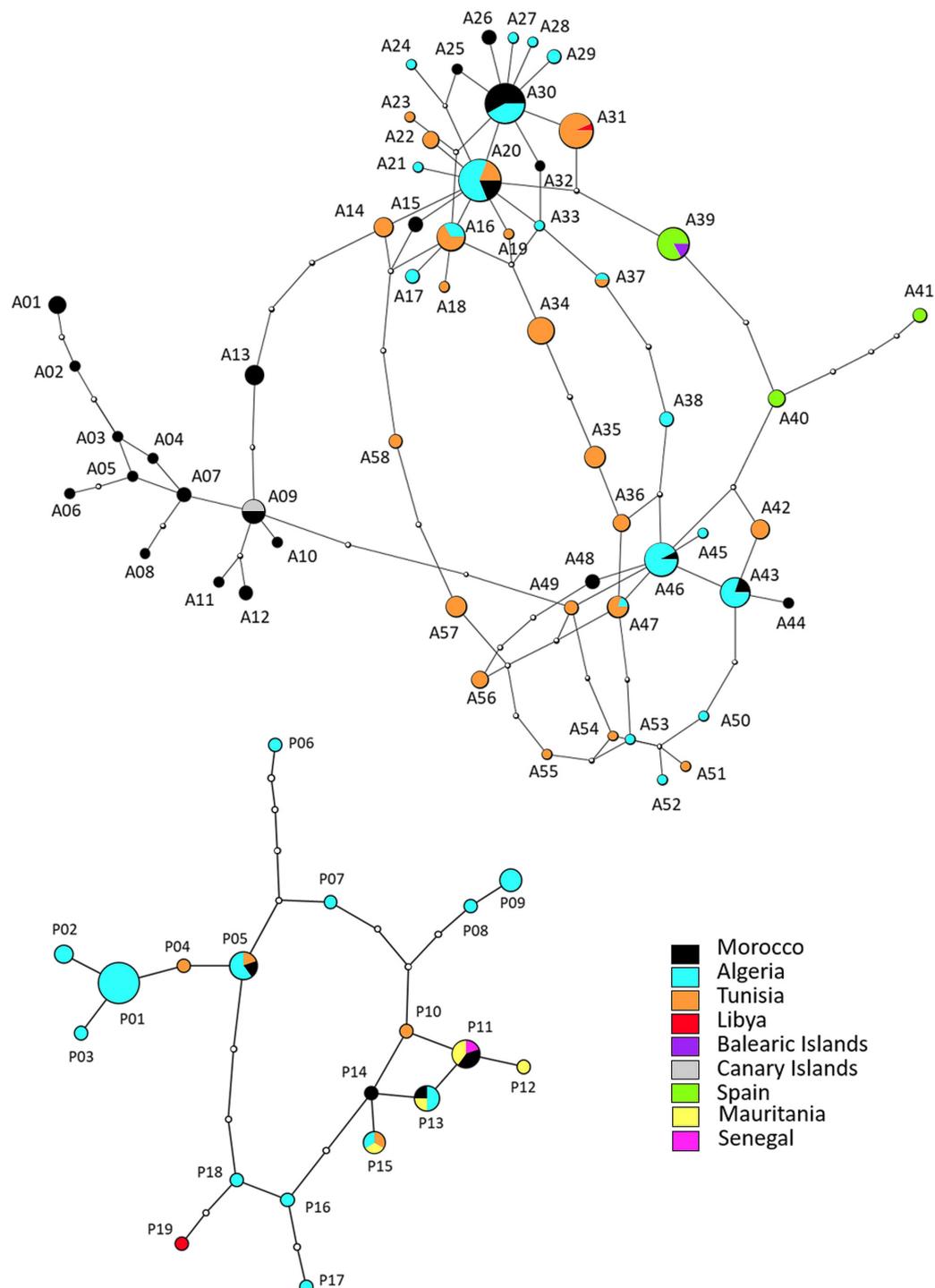
Figure 3. Statistical parsimony haplotype networks for *Atelerix algirus* (top) and *Paraechinus aethiopicus* (bottom) based on the control region. Codes refer to haplotype numbers (see Appendix S1 for the correspondance between haplotypes numbers, specimens numbers and geographic origin).
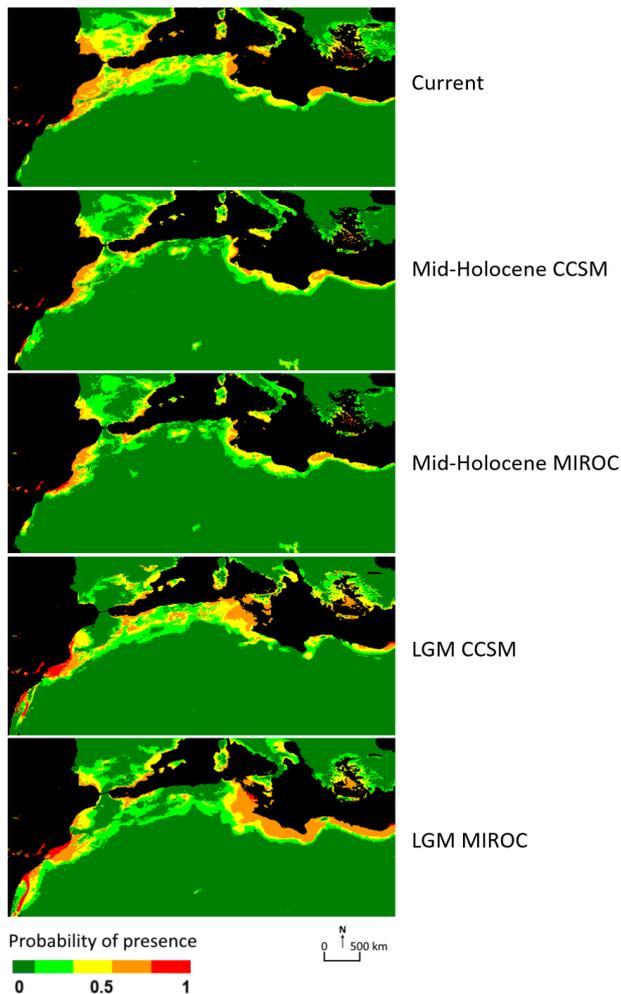
Figure 4. Species distribution modeling of *Atelerix algirus* as estimated by Maxent for present-day conditions (current), for Holocene and the Last Glacial Maximum (LGM) based on the Model for Interdisciplinary Research on Climate (MIROC) and on the Community Climate System Model (CCSM) paleoclimatic models. Warmer colors show areas with higher probability of presence.

Figure 5. Species distribution modeling of *Paraechinus aethiopicus* as estimated by Maxent for present-day conditions (current), for Holocene and the Last Glacial Maximum (LGM) based on the Model for Interdisciplinary Research on Climate (MIROC) and on the Community Climate System Model (CCSM) paleoclimatic models. Warmer colors show areas with higher probability of presence.

demic to Morocco that differ from haplotypes from other countries by more than three mutations (except one haplotype shared between Morocco and Canary Islands), and the second haplogroup is composed of haplotypes shared (or closely related: one mutation) with specimens from Algeria and Tunisia. The first Moroccan haplogroup is mainly distributed in Atlantic plains of Morocco, and the second one is mainly distributed in southern and eastern Morocco. Both haplogroups occur in Atlas and Rif mountains (Fig. 1). Three haplotypes are found in Spain, and one of them is shared with Balearic Islands. We obtained significant negative values of Fu's Fs for all haplogroups (Table 1). Positive values of R2 were obtained for all haplogroups, but they were not significant.

The 45 specimens of *P. aethiopicus* resulted in 19 haplotypes. Nucleotide diversity is 0.01086 ± 0.00050, haplotype diversity is 0.905 ± 0.026 and the average number of nucleotide differences is 4.386. A higher number of haplotypes is found in Algeria, but this is probably due to the greater sample size in this country (Fig. 3). No phy-

logeographic structure is apparent in the network: specimens from Morocco, Mauritania and Senegal can share the same haplotype, and this is also true for specimens from Algeria, Morocco and Mauritania, from Tunisia, Algeria and Mauritania, and from Algeria, Morocco and Tunisia. We obtained a negative value of Fu's Fs and a positive value of R2, but they were not significant (Table 1).

Species distribution modelling

We built species distribution models for the two African hedgehog species based on the known presence localities. The AUC values were 0.935 and 0.808 for *A. algirus* and *P. aethiopicus*, respectively. Thus, they are considered to correspond to a useful predictive model.

Under present-day climatic conditions the model revealed climatic suitability for *A. algirus* across all the Mediterranean Basin (Fig 4). This area is larger than the currently known distribution of this species, as it is not

present in Eurasian coasts of the Mediterranean basin (except in the Iberian Peninsula). In North Africa, the model shows three patches of high climatic suitability: one in Western Morocco isolated by High Atlas, one in northern part of Eastern Morocco and Northern Algeria and one in Tunisia and coastal part of Libya. At the mid-Holocene, according to both CCSM and MIROC models, areas of high climatic suitability were localized more or less at the same places than at present-day conditions but their size were smaller. During the LGM three areas of high climatic suitability were present: one in south-west Morocco, one in Tunisia and coastal Libya, and one on the coast at the border between Morocco and Algeria.

For *P. aethiopicus*, in North Africa, the model revealed continuous high climatic suitability under present-day bioclimatic conditions in Western part of the Sahara desert from Mauritania to Morocco limited by the Anti-Atlas and the High Atlas (Fig 5). Continuous high climatic suitability was predicted from Saharan Atlas (Algeria), Southern Tunisia and all coastal parts of Libya and Egypt to the Arabian Peninsula. The distribution predicted for *P. aethiopicus* during the mid-Holocene is remarkably similar to the present distribution model with more climatic suitability in Algeria and North-Western part of Mauritania according to both CCSM and MIROC data. For LGM period, CCSM and MIROC model show a high climatic suitability in Western part of Sahara desert, Libyan an Egyptian coastal parts.

Discussion

Phylogeography

One of the most remarkable finding from this study is the discrepancy between our results and those published by Velo-Antón et al. (2019) for *A. algirus*. These authors found this species to be monophyletic and to include three distinct genetic lineages (lineages 1, 2 and 3), while we did not recover the monophyly of this species and found two distantly related lineages within it (lineages I and II). Our lineage II corresponds to the lineage 3 of Velo-Antón et al. (2019). It is composed of only two specimens from Morocco. In our phylogenetic tree this lineage does not cluster with lineage I and the genetic distance between these lineages is comparable to those found between lineage II and the two lineages of *A. albiventris*. Thus, the question of the species identification of these specimens is raised. Velo-Antón et al. (2019) also published the nuclear RAG1 sequences of these two specimens. They are both identical, with no polymorphic sites, and identical to the most frequent RAG1 haplotype found in *A. algirus*. They differ by several mutations from *A. albiventris* haplotypes. This mitonuclear discordance could be the result of an ancient hybridization event between the two species leading to a phenomenon of mitochondrial introgression. Alternatively, the presence of a new cryptic species in Morocco is possible. Furthermore, some species exhibit

segmental duplications involving the control region, that, in some cases, leads to the formation of pseudogenes that may co-amplified by PCR (Bensch and Harlid 2000; Cadahia et al. 2009). Multilocus data are necessary to test these alternative hypotheses. If these hypotheses could explain the discrepancy between mitochondrial and nuclear data, we still do not understand why our mitochondrial results differ from those of Velo-Antón et al. (2019), given that they are based on the same DNA fragment.

Our *A. algirus* lineage I corresponds to the two sister lineages *A. algirus* 1 and *A. algirus* 2 of Velo-Antón et al. (2019). In their study, haplotypes from lineages 1 and 2 differ by at least 22 mutations. In our study these two lineages are not recovered in the phylogenetic tree, and they can only be identified in the haplotype network because the minimum number of mutations observed between haplotypes of these two lineages is only of three mutations (between haplotypes A13 and A14, or haplotypes A09 and A49). This discrepancy between the results of these two studies is striking. It is not only due to our greater sample size (allowing the recovery of more haplotypes), but may also reflect alignment differences and/or differences in the way of treating gaps and missing data (our network analyses are based on complete sequences only, the alignment included 7 gaps and gaps were treated as a fifth state; see Appendix S3).

To sum up, our results are congruent with the study of Khaldi et al. (Khaldi et al. 2016) in showing low genetic diversity in *A. algirus* across its geographic range. However, our more extensive sampling scheme and the use of a more rapidly evolving genetic marker allows us to highlight mtDNA phylogeographic structure within this species: one haplogroup is endemic to Morocco and Canary Islands, and another haplogroup is widely distributed from Morocco to Libya, and through Spain and Balearic Islands. These results suggest that the mitochondrial phylogeographic structure of the species has a recent origin. This low mitochondrial structure at continental scale contrasts with the marked east–west divergence that has been commonly found in previous phylogeographic studies of North African organisms and which identified two common disjunction areas: one around the Algeria-Morocco border and the other one in Kabylia (central Algeria) (Nicolas et al. 2015; Beddek et al. 2018). It is interesting to note that the two haplogroups co-occur in Atlas and Rif mountains. Two hypotheses can be proposed to explain this pattern: (i) vicariance of an eastern and a western haplogroup followed by secondary expansion or (ii) retention of deep mitochondrial DNA diversity in Morocco followed by loss of diversity during eastward expansion of the species out of Morocco. Note that both scenarios imply a population expansion of haplogroup 2, for which, we find evidence only with Fu's Fs test. Our climatic niche modeling results support the first hypothesis since they show that during the LGM there were several disconnected areas of high climatic suitability for *A. algirus*: one in South-West Morocco, one at the coastal Moroccan-Algerian border and one in Tunisia-coastal Libya.

Our genetic results confirm that the Algerian hedgehog recently colonized Spain, Balearic and Canary Islands,

and that this colonization was probably mediated by humans, as already suggested by several authors (Hutterer 1983; Borg 2005; Morales and Rofes 2008; Khaldi et al. 2016). Our ecological niche modeling analyses suggest that suitable climatic conditions occurred throughout most Southern and Eastern lowland Iberian Peninsula during the last 6000 years. This would have favored the spatial expansion of the Algerian hedgehog after its arrival in Europe. It is interesting to note that the current distribution of this species in Spain is much more reduced than expected based on climatic niche modelling. Species interactions (e.g. mutual competitive exclusion, predation, parasitism, and mutualism) are also important in determining species distribution (Pearman et al. 2008; Sinclair et al. 2010; Giannini et al. 2013). Competition with the European hedgehog, *Erinaceus europaeus*, a species already well established in Spain before the introduction of the Algerian hedgehog, may explain partially its restricted distribution in Spain.

Our study shows no phylogeograhic structure in North African *P. aethiopicus*. This is consistent with our climatic niche modeling results showing a more or less continuous high or moderate climatic suitability for this species throughout most of the Saharan desert since the LGM (except in the most central parts). This study significantly increases our knowledge of the distribution of *P. aethiopicus* expanding currently recognized ranges (IUCN, 2015; Best, 2018). An extension of its distribution range in Atlantic Sahara was already proposed by Aulagnier et al. (2017). Velo-Antón et al. (2019), based on molecular data, proposed its extension in Mauritania, Senegal, Atlantic Sahara and Libya. We show that its distribution range should also be extended in Algeria since we captured it in the M'sila region, up to 35.748°N, confirming, with molecular data, the previous observation of Sellami et al. (1989).

Taxonomy

Several subspecies have been described for *Atelerix algirus*, either from the mainland or islands, *Atelerix algirus algirus* from North Africa, *A. algirus vagans* from Spain, Minorca and Balearic Islands and *A. algirus girbaensis* from Djerba Island (Tunisia). Given that the differential diagnosis is mainly based on pelage variations (hairs, spines), the validity of these subspecies has been regularly questioned (Corbet 1988; Hutterer 2005; Happold 2013). Our results do not show any strong genetic discontinuity between the Algerian, Moroccan, Tunisian, Libyan, Spanish, Balearic and Canarian populations, suggesting that subspecific recognition may be unwarranted. The status of the subspecies from Djerba Island (*A. a. girbaensis*) still needs to be assessed by genetic data. Several subspecies were also described within *P. aethiopicus*: *P. a. aethiopicus* from Soudan and Ethiopia, *P. a. pectoralis* from Arabian Peninsula, *P. a. deserti* from North Africa, *P. a. albatus* from Djerba Island and *P. a. ludlowi* from Iraq. Our genetic data do not show any genetic discontinuity throughout North Africa, from Sene-

gal to Libya, confirming the results previously obtained by Derouiche et al. (2016) and Velo-Antón et al. (2019). Derouiche et al. (2017), using a combination of nuclear and mitochondrial markers, studied the genetic differentiation between northwest African populations (*deserti*) and Arabian populations (*dorsalis*) of Ethiopian hedgehog. They showed a level of genetic differentiation that is compatible with their taxonomic separation postulated from morphology. Additional sampling in Djerba Island, Soudan, Ethiopia, and in the Middle East is needed to test the genetic differentiation of others proposed subspecies.

Conclusion

In the two studied hedgehog species low genetic diversity was found in North Africa. However, while no phylogeographic pattern was found in *P. aethiopicus*, two haplogroups were identified within *A. algirus*. This could be explained by the fact that a more or less continuous high or moderate climatic suitability occurred throughout most of the Saharan desert since the LGM for the first species, while during the LGM there were several disconnected areas of high climatic suitability for *A. algirus*: one in South-West Morocco, one in coastal Moroccan-Algerian border and one in Tunisia-coastal Libya. Our genetic results confirm that the Algerian hedgehog colonized recently Spain, Balearic and Canary Islands, and that this colonization was probably mediated by humans. Suitable climatic conditions occurred throughout most lowland Southern and Eastern Iberian Peninsula during the last 6000 years which could have favored the spatial expansion of the Algerian hedgehog after its arrival in Europe.

Several subspecies have been described within the two African hedgehog species. Our results do not show any strong genetic discontinuity within either species in North Africa, suggesting that subspecific recognition within this region may be unwarranted. Additional sampling in Djerba Island (for both species), Soudan, Ethiopia and in the Middle East (for *P. aethiopicus*) is needed to test the genetic differentiation of other proposed subspecies.

Our phylogeographic and taxonomic conclusions are only based on short mitochondrial fragment, which could explain the low bootstrap support of several important nodes in our phylogeny. Increasing the amount of genetic data frequently allows resolution of even the most problematic phylogenetic relationships (DeFilippis and Moore 2000; Rokas and Carroll 2005; Fabre et al. 2017). One such approach is based on sequencing of complete mitochondrial genomes ("mitogenomics"). However, due to its maternal inheritance, it only estimates the matrilineal histories of individuals and populations. Because of the absence of recombination, the mitochondrial DNA should be still considered as a single locus and reconstructed phylogenies represent only "single gene" trees. There are many reasons why historical inferences derived directly from a gene genealogy may be inaccurate or misleading (Knowles 2004, 2009a, b). Each gene has its own history,

and gene trees can be different from the species tree. To address this problem, recent phylogenomic approaches target multiple nuclear fragments, which allow to infer a phylogenetic tree that accounts for discord among hundreds of independent loci at nuclear DNA (Lemmon et al. 2012). To better investigate the evolutionary history of the African hedgehogs such multi-locus data are needed. Moreover, integrative taxonomic studies combing these multilocus data and morphometric, behavioral and ecological data are required to test our taxonomic conclusions.

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References

Aulagnier S, Cuzin F, Thévenot M (2017) Mammifères sauvages du Maroc: peuplement, répartition, écologie. Société Française pour l'Etude et le Protection des Mammifères, Paris, 340 pp.

Aulagnier S, Haffner P, Mitchell-Jones AJ, Moutou F, Zima J (2008) Guide des mammifères d'Europe, d'Afrique du Nord et du Moyen-Orient. Delachaux et Niestlé, Paris, 271 pp.

Bannikova AA, Lebedev VS, Abramov AV, Rozhnov VV (2014) Contrasting evolutionary history of hedgehogs and gymnures (Mammalia: Erinaceomorpha) as inferred from a multigene study. Biological Journal of the Linnean Society 112: 499–519.

Beddek M, Zenboudji-Beddek S, Geniez P, Fathalla R, Sourouille P, Arnal V, Dellaoui B, Koudache F, Telailia S, Peyre O, Crochet PA (2018) Comparative phylogeography of amphibians and reptiles in Algeria suggests common causes for the east-west phylogeographic breaks in the Maghreb. PloS one 13: e0201218. https://doi.org/10.1371/journal.pone.0201218

Bensch S, Harlid A (2000) Mitochondrial genomic rearrangements in songbirds. Molecular Biology and Evolution 17: 107–113.

Best TL (2018) Family Erinaceidae (Hedgehogs and Gymnures). In: Wilson DE, Mittermeier RA (Eds) Handbook of the Mammals of the World. Lynx Edicions, Barcelona, 288–331.

Borg JJ (2005) Technical Appendix 7: Mammalia Baseline Survey on the Xaghra l-Hamra Golf Course Proposal. Malta Tourism Authority, Malta, 40 pp.

Cadahia L, Pinsker W, Negro JJ, Pavlicev M, Urios V, Haring E (2009) Repeated Sequence Homogenization Between the Control and Pseudo-Control Regions in the Mitochondrial Genomes of the Subfamily Aquilinae. Journal of Experimental Zoology Part B-Molecular and Developmental Evolution 312b: 171–185.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1659.

Collins WD, Bitz CM, Blackmon ML, Bonan GB, Bretherton CS, Carton JA, Chang P, Doney SC, Hack JJ, Henderson TB, Kiehl JT, Large WG, McKenna DS, Santer BD, Smith RD (2006) The Community Climate System Model Version 3 (CCSM3). Journal of Climate 19: 2122–2143.

Corbet GB (1988) The family Erinaceidae: a synthesis of its taxonomy, phylogeny, ecology and zoogeography. Mammal Review 18: 117–172.

de Smet K, Loumassine H-E, Bounaceur F (2021) Le hérisson du désert: un manque de données malgré sa large distribution. Sahara et Sahel 237: 16–23.

Deef LEM (2020) Using DNA Barcoding to Study Hedgehog Community in Egypt. Russian Journal of Genetics 56: 226–233.

DeFilippis VR, Moore WS (2000) Resolution of phylogenetic relationships among recently evolved species as a function of amount of DNA sequence: an empirical study based on woodpeckers (Aves: Picidae). Molecular Phylogenetics and Evolution 16: 143–160.

Derouiche L, Bouhadad R, Fernandes C (2016) Mitochondrial DNA and morphological analysis of hedgehogs (Eulipotyphla: Erinaceidae) in Algeria. Biochemical Systematics and Ecology 64: 57–64.

Derouiche L, Vercammen P, Bouhadad R, Fernandes C (2017) Genetic evidence supporting the taxonomic separation of the Arabian and Northwest African subspecies of the desert hedgehog (*Paraechinus aethiopicus*). Gene 620: 54–65.

El-Farhati H, Jaziri B, Hizem MW, Nouira S (2020) Distribution, bioclimatic niche and sympatry of two Erinaceidae in Tunisia. African Journal of Ecology 58: 193–210.

Elith J, Graham CH, Anderson RP, Dudik M, Ferrier S, Guisan A, Hijmans RJ, Huettmann F, Leathwick JR, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, Nakamura M, Nakazawa Y, Overton JM, Peterson T, Phillips SJ, Richardson K, Scachetti-Pereira R, Schapire RE, Soberón J, Williams S, Wisz MS, Zimmermann NE (2006) Novel methods improve prediction of species' distributions from occurrence data. Ecography 29: 129–151.

Fabre PH, Upham NS, Emmons LH, Justy F, Leite YL, Loss AC, Orlando L, Tilak MK, Patterson BD, Douzery EJ (2017) Mitogenomic phylogeny, diversification, and biogeography of South American spiny rats. Molecular Biology and Evolution 34: 613–633.

Fourcade Y, Engler JO, Rodder D, Secondi J (2014) Mapping species distributions with MAXENT using a geographically biased sample of presence data: a performance assessment of methods for correcting sampling bias. PloS One 9: e97122. https://doi.org/10.1371/journal.pone.0097122

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915–925.

Giannini TC, Chapman DS, Saraiva AM, Alves-dos-Santos I, Biesmeijer JC (2013) Improving species distribution models using biotic interactions: a case study of parasites, pollinators and plants. Ecography 36: 649–656.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology 59: 307–321.

Guindon S, Lethiec F, Duroux P, Gascuel O (2005) PHYML Online- a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Research 33: W557–W559.

Happold DCD (2013) *Atelerix algirus* Algerian Hedgehog. In: Happold M, Happold DCD (Eds) Mammals of Africa, volume IV: Hedgehogs, shrews and bats. Bloomsbury Publishing, London, U.K., 33–34.

Happold M, Happold DCD (2013) Mammals of Africa, volume IV: Hedgehogs, Shrews and Bats. Bloomsbury Publishing, London, 800 pp.

Hasumi H, Emori S (Eds) (2004) K-1 Coupled GCM (MIROC) Description. K-1 Model developers tech Rep 1, Center for Climate System Research, University of Tokyo, Tokyo, Japan, 34 pp.

He K, Chen JH, Gould GC, Yamaguchi N, Ai HS, Wang YX, Zhang YP, Jiang XL (2012) An estimation of Erinaceidae phylogeny: a combined analysis approach. PLoS ONE 7: e39304. https://doi.org/10.1371/journal.pone.0039304

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965–1978.

Hutterer R (1983) Über den Igel (*Erinaceus algirus*) der Kanarischen Inseln. Zeitschrift für Säugetierkunde 48: 257–265.

Hutterer R (2005) Order Erinaceomorpha. In: Wilson DE, Reeder DM (Eds) Mammals species of the World. A taxonomical and geographical reference. 3rd edn. The John Hopkins University Press, Baltimore, 212–219.

Hutterer R (2016) *Paraechinus aethiopicus*. The IUCN Red List of Threatened Species 2016: e.T40606A22326233. https://doi.org/10.2305/IUCN.UK.2016-2.RLTS.T40606A22326233

Khaldi M, Ribas A, Arrizabalaga A, Barech G, Hugot J-P, Benyettou M, Albane L, Arrizabalaga A, Nicolas V (2016) Molecular evidence supports recent anthropogenic introduction of the Algerian hedgehog *Atelerix algirus* in Spain, Balearic and Canary islands from North Africa. Mammalia 80: 313–320.

Knowles LL (2004) The burgeoning field of statistical phylogeography. 17: 1–10.

Knowles LL (2009a) Estimating species trees: methods of phylogenetic analysis when there is incongruence across genes. Systematic biology 58: 463–467.

Knowles LL (2009b) Statistical phylogeography. Annual Review of Ecology, Evolution, and Systematics 40: 593–612.

Lemmon AR, Emme S, Lemmon EM (2012) Anchored hybrid enrichment for massively high-throughput phylogenetics. Systematic Biology 61: 721–744.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.

Morales A, Rofes J (2008) Early evidence for the Algerian hedgehog in Europe. Journal of Zoology 274: 9–12.

Mouhoub-Sayah C (2009) Ecophysiologie du Hérisson d'Algérie *Atelerix algirus* Lereboullet, 1842 (Mammalia, Insectivora) dans quelques stations du Djurdjura et dans la vallée de la Soummam. PhD Thesis, Ecole Nationale Supérieure Agronomique El Harrach, Alger.

Mouhoub Sayah C, Robin JP, Pévet P, Monecke S, Doumandji S, Saboureau M (2009) Road mortality of the Algerian hedgehog (*Atelerix algirus*) in the Soummam Valley (Algeria). Revue d'écologie La Terre et la Vie 64: 145–156.

Murias dos Santos A, Cabezas MP, Tavares AI, Xavier R, Branco M (2016) tcsBU: a tool to extend TCS network layout and visualization. Bioinformatics 32: 627–628.

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York, 512 pp.

Nicolas V, Mataame A, Crochet PA, Geniez P, Ohler A (2015) Phylogeographic patterns in North African water frog *Pelophylax saharicus* (Anura: Ranidae). Journal of Zoological Systematics and Evolutionary Research 53: 239–248.

Nylander JA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.

Pearman PB, Guisan A, Broennimann O, Randin CF (2008) Niche dynamics in space and time. Trends in Ecology & Evolution 23: 149–158.

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modelling of species geographic distributions. Ecological Modelling 190: 231–239.

Ramos-Onsins SE, Rozas J (2002) Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19: 2092–2100.

Rokas A, Carroll SB (2005) More genes or more taxa? The relative contribution of gene number and taxon number to phylogenetic accuracy. Molecular Biology and Evolution 22: 1337–1344.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Molecular Biology and Evolution 34: 3299–3302.

Schorr G, Holstein N, Pearman PB, Guisan A, Kadereit JW (2012) Integrating species distribution models (SDMs) and phylogeography for two species of Alpine Primula. Ecology and Evolution 2: 1260–1277.

Sellami M, Belkacemi H, Sellami S (1989) Premier inventaire des mammifères de la réserve naturelle de Mergueb (M'Sila, Algérie). Mammalia 53: 116–119.

Sinclair SJ, White MD, Newell GR (2010) How useful are species distribution models for managing biodiversity under future climates? Ecology and Society 15: 8.

Varela S, Lima-Ribeiro MS, Terribile LC (2015) A Short Guide to the Climatic Variables of the Last Glacial Maximum for Biogeographers. PLoS One 10: e0129037. https://doi.org/10.1371/journal.pone.0129037

Velo-Antón G, Boratyński Z, Ferreira CM, Lima VO, Alves PC, Brito JC (2019) Intraspecific genetic diversity and distribution of North African hedgehogs (Mammalia: Erinaceidae). Biological Journal of the Linnean Society 127: 156–163.

Supplementary material 1

List of specimens

Authors: Haithem El-Farhati, Mourad Khaldi, Alexis Ribas, Mohamed Wassim Hizem, Saïd Nouira, Violaine Nicolas (2021)

Data type: .xlsx

Explanation note: List of specimens included in the mitochondrial DNA phylogenetic.

Link: https://doi.org/10.3897/vz.71.e70989.suppl1

Supplementary material 2

Sequence alignment – phylogenetic tree analyses

Authors: Haithem El-Farhati, Mourad Khaldi, Alexis Ribas, Mohamed Wassim Hizem, Saïd Nouira, Violaine Nicolas (2021)

Data type: .csv

Explanation note: Sequence alignment used for the phylogenetic tree analyses.

Link: https://doi.org/10.3897/vz.71.e70989.suppl2

Supplementary material 3

Sequence alignment used for haplotype network analysis of *A. algirus*

Authors: Haithem El-Farhati, Mourad Khaldi, Alexis Ribas, Mohamed Wassim Hizem, Saïd Nouira, Violaine Nicolas (2021)

Data type: .csv

Explanation note: Sequence alignment used for haplotype network analysis of *A. algirus*.

Link: https://doi.org/10.3897/vz.71.e70989.suppl3

Supplementary material 4

Sequence alignment used for haplotype network analysis of *P. aethiopicus*

Authors: Haithem El-Farhati, Mourad Khaldi, Alexis Ribas, Mohamed Wassim Hizem, Saïd Nouira, Violaine Nicolas (2021)

Data type: .csv

Explanation note: Sequence alignment used for haplotype network analysis of *P. aethiopicus*.

Link: https://doi.org/10.3897/vz.71.e70989.suppl4

Supplementary material 5

Species occurrence data

Authors: Haithem El-Farhati, Mourad Khaldi, Alexis Ribas, Mohamed Wassim Hizem, Saïd Nouira, Violaine Nicolas (2021)

Data type: .xlsx

Explanation note: Species occurrence data used in our climatic niche modelling analyses.

Link: https://doi.org/10.3897/vz.71.e70989.suppl5