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Mitochondrial DNA and morphological analysis of hedgehogs (Eulipotyphla: Erinaceidae) in Algeria

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ABSTRACT

Algeria is the largest country in Africa and contains a rich and understudied faunal biodiversity. Two species of hedgehogs occur in the country: the North African hedgehog *Atelerix algirus* and the desert hedgehog *Paraechinus aethiopicus*. We investigated the genetic and phenotypic variation of the two species in Algeria using mitochondrial DNA and external morphological characters. The mitochondrial phylogenetic analysis identified two major clades corresponding to the two species, whereas no phylogenetic structure was observed within either species. However, analysis of the morphological data indicated the presence of two morphotypes within *A. algirus*. The more common and widespread morphotype agrees well with the standard description of *A. algirus*, while the other recognized morphotype was found almost exclusively in the Mediterranean coastal belt and is distinguishable by a combination of morphological characters. A most remarkable finding was the detection of *A. algirus* individuals, with taxonomic identity confirmed by both molecular and morphological data, at extralimital localities in oases deep in the Sahara. Two of these records in central Algeria extend the distribution of the species approximately 500 Km to the south. Further sampling in the Saharan oases is needed to map more precisely the new geographic distribution of *A. algirus* in Algeria.

1. Introduction

Hedgehogs are small insectivorous mammals of the family Erinaceidae that are native to Africa and Eurasia, with some species having wide distribution ranges (Corbet, 1988; He et al., 2012). Fifteen species within five genera have been proposed on morphological, osteological and odontological grounds (Corbet, 1988; Frost et al., 1991). Two species are known to occur in Algeria, the North African or Algerian hedgehog *Atelerix algirus* (Lereboullet 1842) and the desert hedgehog *Paraechinus aethiopicus* (Ehrenberg 1832), which can be distinguished based on morphological characteristics (Le Berre, 1990; Kowalski and Rzebik-Kowalska, 1991). *Atelerix algirus* is a hedgehog of relatively large size, with small ears, smooth spines and coarse ventral pelage, whereas *P. aethiopicus* has a comparatively smaller body, large prominent ears, grooved spines and soft ventral hair (Corbet, 1988).

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The current natural range of *A. algirus* includes the non-desert areas of North Africa from Morocco to Libya, and its preferred habitats are scrub, grasslands and around cultivation (Corbet, 1988). *Paraechinus aethiopicus* occurs in Africa, from Morocco and Mauritania through the Sahara to Egypt and Ethiopia, and in the Middle East, from Syria and Iraq to the southern Arabian Peninsula (Hutterer, 2008). The desert hedgehog is found in arid desert, semi-desert and dry steppe (Corbet, 1988), but may favour areas where food is more easily available such as oases and vegetated wadis (Harrison and Bates, 1991; Hutterer, 2008).

In Algeria, *A. algirus* is mostly found in the Mediterranean belt along the north of the country, with the southernmost records in the literature being from Ain Sefra, Ain El Orak, Laghouat and Biskra (Kowalski and Rzebik-Kowalska, 1991). *Paraechinus aethiopicus* occurs in a band of the subdesert between the Saharan Atlas and the true desert, roughly delimited by Béni Abbès to the west, Ain Sefra to the north, Biskra to the east, and El Golea in the central part of Algeria. Further south it is also present in the mountains of the Central Sahara, in the Adrar Ahnet and in the Hoggar. The two species coexist in the Hauts Plateaux (Kowalski and Rzebik-Kowalska, 1991), which indicates a parapatric distribution (Bull, 1991; Dennis and Hellberg, 2010).

The two species have never been the subject of detailed population studies at local or regional scales in which samples were investigated for both morphological and genetic variation. Genetic confirmation of species identity is also relevant because previous morphological studies (Kahmann and Vesmanis, 1977) may have confounded the two species (Corbet, 1988; Kowalski and Rzebik-Kowalska, 1991). The majority of the morphological characters most useful for distinguishing the two species are anatomical, and therefore not accessible in living individuals. On the other hand, morphological identification to species of road-kills and animals found dead in the field might be difficult, even for experienced naturalists, due to deterioration of carcasses. In Algeria, previous research has examined their diet (Doumandji and Doumandji, 1992a, 1992b; Derdoukh et al., 2012), aestivation and hibernation physiology (Sayah et al., 2008), and parasites (Khaldi et al., 2012a,b; Madoui et al., 2014).

Therefore, with this study we aimed to provide the first mitochondrial DNA (mtDNA) and morphological assessment of hedgehogs across Algeria, characterize patterns of genetic and phenotypic variation, and ascertain the species distributions.

2. Materials and methods

2.1. Sampling

Sixty-seven hedgehog individuals were sampled between 2008 and 2014 in different regions of Algeria. Samples were obtained from road-kills, animals found dead of unknown causes, and specimens captured alive by hand in the field. For each individual, we recorded information on several phenotypic parameters, with some of them used to assign individuals to morphospecies, and took an ear biopsy that was stored in a vial with 96% ethanol. To reduce error, the same person (LD) collected all morphological data. After sampling, alive hedgehogs were released at capture site. The specimen codes, based on the administrative region where each individual was sampled, and the geographic location of sampling sites for the 67 analysed hedgehogs are given in Table S1 (Supplementary Data) and Fig. 1.

2.2. Mitochondrial DNA analysis

Total genomic DNA was extracted from tissue samples using the EZNA Tissue DNA kit (Omega Bio-Tek). To monitor potential contamination, we included a negative extraction control in each extraction session. We amplified a fragment of 456–465 bp of the control region (CR) with the primers Erinaceinae CR F: 5′-CATCAACACCCAAAGTTG-3′ and Erinaceinae CR R: 5′-TGAAGAAAGAACCAGATG-3′. The polymerase chain reactions (PCR) were carried out in volumes of 15 μl with 1× PCR Buffer (Finnzymes), 0.2 mM of each dNTP (Bioline), 0.5 μM of each primer, 0.3 μl of Phire®Hot Start DNA polymerase (Finnzymes), and 3 μl of DNA extract. Thermal cycling conditions consisted of an initial denaturation at 98 °C for 30 s, followed by 45 cycles of 5 s at 98 °C, 5 s at 60 °C, 12 s at 72 °C, and a final extension of 1 min at 72 °C. PCR products were purified with an Exo-SAP protocol (Hanke and Wink, 1994) and sequenced at Macrogen Inc. Sequences were edited, assembled and aligned using Sequencher 4.7 (Gene Codes Corporation).

Since insertion/deletions (indels) led to uncertainty in the CR alignment, we used M-Coffee (Wallace et al., 2006), a meta-aligner that combines the solutions of alternative alignment methods, to estimate a consensus alignment. We combined the three top-performing methods (Probcons, T-Coffee and Mafft) in a recent benchmark study of sequence alignment algorithms (Thompson et al., 2011). The derived alignment was analysed with FaBox 1.41 (Villesen, 2007) to collapse identical individual sequences into representative haplotypes for phylogenetic analysis.

We used DnaSP 5.10.1 (Librado and Rozas, 2009) to compute the number of haplotypes (n_H), haplotype diversity (h), nucleotide diversity (π), the mean number of pairwise differences (k), and the average number of nucleotide substitutions per site (Dxy) (Tajima, 1983; Nei, 1987). The phylogenetic analysis was performed using maximum likelihood (ML) as implemented in MEGA 6.0 (Tamura et al., 2013), with the best-fit model of nucleotide substitution determined using the Bayesian information criterion (BIC; Schwarz, 1978) also in MEGA. Nodal support was evaluated by 1000 bootstrap replicates. Trees were visualized and edited with TreeGraph 2.4.0 (Stöver and Müller, 2010).

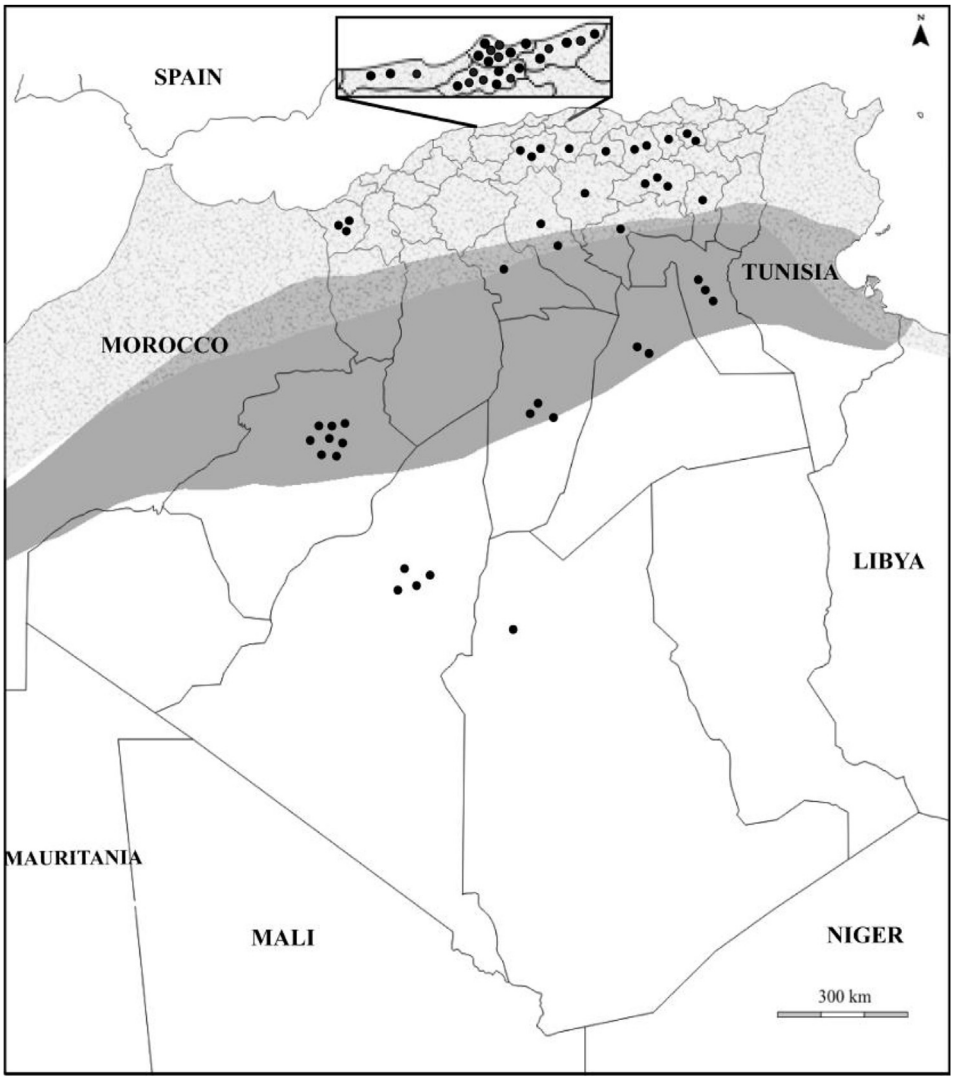

Fig. 1. Map showing the location in Algeria of the samples collected in this study (black dots) and the distributions of the desert hedgehog *Paraechinus aethiopicus* (dark grey) and North African hedgehog *Atelerix algirus* (stippled light grey) following Hutterer (2008) and Amori et al. (2008), respectively.

2.3. Morphological analysis

The variables used in the morphological analysis were selected following Corbet (1988), Bretagnolle and Attié (1989) and Ulutürk and Coskun (2011). We only wanted to use characters that could be measured in living animals. Qualitative variables included the colouration of the body and spines and the size and shape of the ears. Qualitative variables were coded as binary in the data matrix based on its presence (1) or absence (0). The quantitative variables were body weight, head and body length, hind foot length, tail length and spine length (see Table S2, Supplementary Data). Individuals were measured to the nearest millimetre using a flexible ruler and weighed to the nearest gram using a digital electronic balance. For some individuals, length measurements could only be made after a few days in captivity to accustom them to handling. To reduce age-related biases in the results we only analysed adult specimens.

We used principal coordinate analysis (PCoA), nonmetric multidimensional scaling (NMDS) and hierarchical clustering by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA, Sokal and Michener, 1958) on a matrix of pairwise Gower distances (Gower, 1971) to analyse the full morphological data set. PCoA, NMDS and the Gower coefficient are suitable for data with a mixture of quantitative and qualitative variables (Legendre and Legendre, 2012). The fit of the data to the NMDS solution on two dimensions was measured by the stress value (Kruskal, 1964). The multivariate analyses were conducted in the software PAST version 3.04 (Hammer et al., 2001).

3. Results

3.1. Mitochondrial DNA analysis

We obtained CR sequences for 56 individuals, of which 42 were inferred to belong to *A. algirus* and 14 to *P. aethiopicus*. The *A. algirus* alignment contained four sites with alignment gaps and nine polymorphic sites, of which six were parsimony informative, and yielded 15 haplotypes; the mean number of pairwise differences was 1.236, the haplotype diversity was 0.774, and the nucleotide diversity was 0.3%. The *P. aethiopicus* alignment contained one site with alignment gaps and 14 polymorphic sites, of which 11 were parsimony informative, and yielded 11 haplotypes; the mean number of pairwise differences was 4.297, the haplotype diversity was 0.956, and the nucleotide diversity was 1.1%. Between the two species, the mean number of pairwise differences was 135.8 and the average number of nucleotide substitutions per site was estimated at 0.336. New haplotypes were deposited in GenBank (accession numbers KU179768-KU179793).

The best-fit model of nucleotide substitution as determined by MEGA was the HKY+Γ (Hasegawa et al., 1985), with gamma shape parameter = 0.09. The ML phylogenetic tree recovered two main clades corresponding to the two hedgehog species, while essentially no strongly supported phylogenetic structure was observed within either species (Fig. 2).

The molecular results confirmed the morphological identification of the specimens. Notably, the mtDNA sequences of individuals Ouargla2, Adrar3 and Adrar4 supported their preliminary phenotypic assignment to *A. algirus*, which initially was considered tentative because Adrar and Ouargla are extralimital to the known distribution of the species in Algeria (Le Berre, 1990; Kowalski and Rzebik-Kowalska, 1991).

3.2. Morphological analysis

The PCoA and NMDS plots showed a clear separation between hedgehogs identified as *P. aethiopicus* and as *A. algirus*, and a subdivision of the latter into two groups, hereafter designated I and II (Figs. 3 and S1). The same pattern of morphological differentiation was revealed by the UPGMA tree (Fig. S2), which also suggested further splitting within each of the three main clusters but this partitioning was only relatively well supported for *A. algirus* I, with bootstrap values for its subgroups of 65% and 72%.

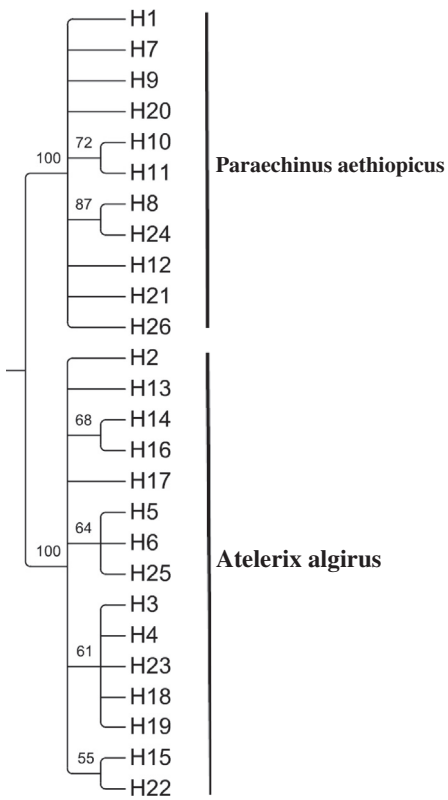

Fig. 2. Condensed tree of the maximum likelihood analysis of control region haplotypes. Numbers above branches indicate the percentage of bootstraps (cut-off value of 50%) based on 1000 replicates. The correspondence between haplotypes and samples is given in Table S1.

The morphological analyses confirmed the initial phenotypic identification of the specimens Ouargla2, Adrar3 and Adrar4 as *A. algirus* and matched the identification inferred from the mtDNA data. This result is important because these records significantly extend southwards the known geographic range of the species in the centre and east of Algeria (Fig. 4) (see Kowalski and Rzebik-Kowalska, 1991). Interestingly, all those three specimens belonged to morphotype I that was found to be widely distributed in northern Algeria, whereas morphotype II was almost exclusively restricted to the Mediterranean coastal belt (Fig. 4).

Morphotype I is characterized by the whitish colouration of the forehead and from the chin to the abdomen, while the ears, muzzle, limbs and tail are brown to black; ears are small and pointed and spines are either black and white or brown and beige (Fig. S3 A and B). The more common and widespread morphotype I agrees well with the standard description of *A. algirus* (Corbet, 1988). The other recognized morphotype (II) is distinguishable by the combination of beige or brown colour of the body and limbs, small rounded ears, and beige and brown spines (Fig. S3 C and D). Characteristically, specimens of morphotype II lack the whitish band from the forehead to the chest, a feature typical of morphotype I (Fig. S3 A and B) and that Corbet (1988) called the 'white mask'.

Individuals of *P. aethiopicus* were recognisable by a narrow longitudinal dark stripe from the snout across the white forehead to the crown of the head and by the large pointed ears (longer than the spines, Corbet, 1988); spines are either black and white or brown and beige (Fig. S3 E and F).

4. Discussion

This mtDNA and morphological analysis of hedgehogs in Algeria, the North African hedgehog *A. algirus* and the desert hedgehog *P. aethiopicus*, is to our knowledge the first study in any of the species in which patterns of population variation were examined using both genetic and morphological characters.

We uncovered evidence for the presence of two morphotypes of *A. algirus*, one apparently more frequent and geographically widespread and another that was essentially found in the Mediterranean ecoregions, where therefore both morphotypes co-occur. Future studies with more samples are needed to confirm this geographical pattern in Algeria, and the frequency and distribution of the two morphotypes elsewhere in the native range of the species should also be investigated and compared with previous studies of morphological variation (Vesmanis, 1979).

A major finding of this study, supported by both the molecular and morphological data, was the presence of *A. algirus* deep in the Sahara (Adrar and Ouargla). This result was surprising because, although *A. algirus* was know to occur in semi-desert areas (Amori et al., 2008), aridity is considered a limiting factor for its distribution in North Africa (Corbet, 1988). Our study

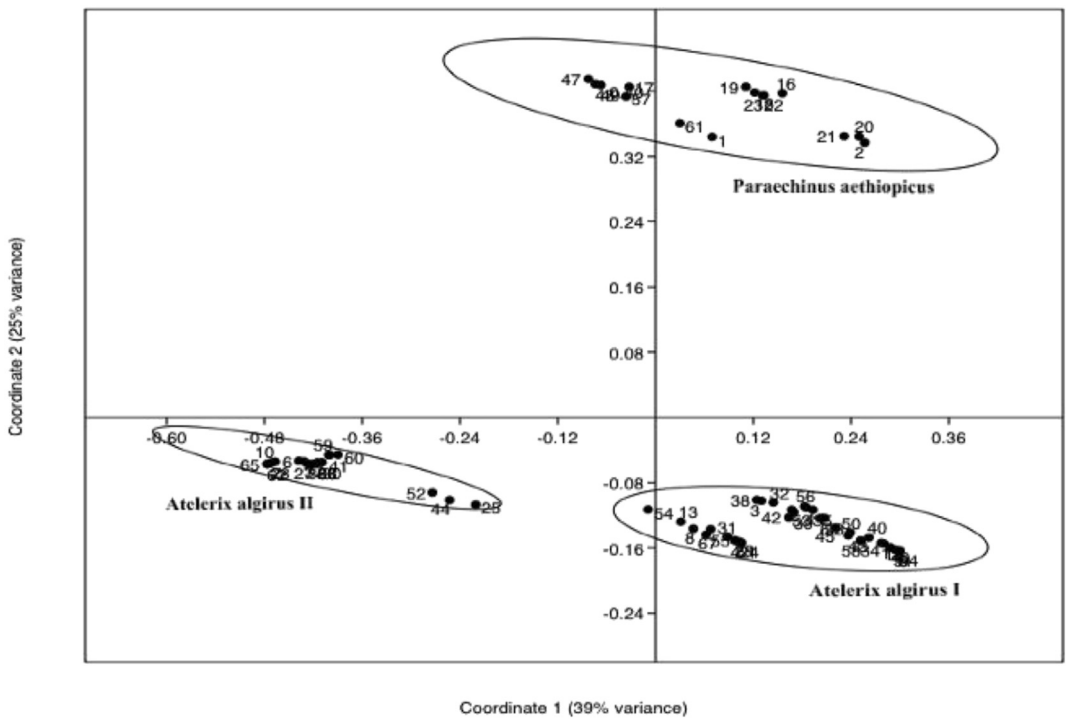

Fig. 3. Two-dimensional scatter plot depicting morphological relationships among hedgehog individuals as estimated by principal coordinate analysis (PCoA) of a matrix of pairwise Gower distances. The distribution of the discrete morphological groups is represented by bivariate 95% concentration ellipses.

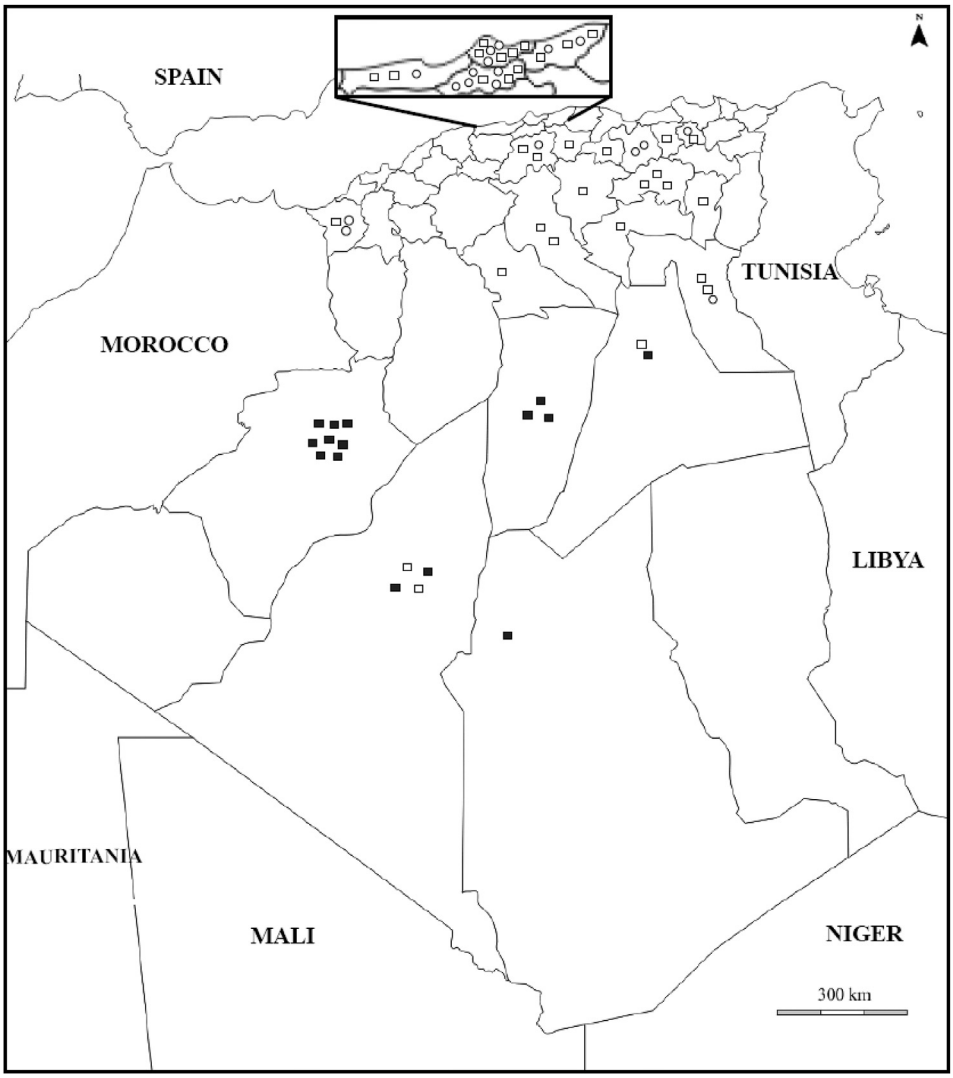

Fig. 4. Geographical distribution in Algeria of the hedgehog individuals identified as *Atelerix algirus* (white rectangles, '*algirus* I'; white circles, '*algirus* II') and as *Paraechinus aethiopicus* (black rectangles).

thus extends the distribution of *A. algirus* in Algeria approximately 500 Km to the south, the distance between Adrar and Ain El Orak (see Kowalski and Rzebik-Kowalska, 1991). Interestingly, Brahmi et al. (2010) also detected in oases in the Ouargla region the extralimital presence of two other small terrestrial mammals, the white-toothed pigmy shrew *Suncus etruscus* and the Algerian mouse *Mus spretus*. The same authors suggested that this extralimital occurrence of the two species could be due to unintentional translocations since it is known that they have been accidentally introduced in other regions. The hypothesis of human transport is also feasible for *A. algirus*, particularly given the tradition across the region of using this species for food or as a traditional medicine (Amori et al., 2008), which stimulates trade (Nijman and Bergin, 2015).

An intriguing possibility, however, for *A. algirus* and other species is that population pockets surviving in Saharan oases are relicts of a wider past distribution southwards during a green Sahara period in which the region was a savannah grassland (Rognon, 1987; Fontes and Gasse, 1991; Shaibi and Moritz, 2010). The fossil record indicates that *A. algirus* was already present in Algeria by the Late Pleistocene (Corbet, 1988). However, the observed nucleotide diversity and mean number of pairwise differences were low, for example compared to those in *P. aethiopicus* for which the sample size was three times smaller. This suggests that *A. algirus* populations in Algeria may have suffered a demographic contraction, which agrees with the scenario of a past larger distribution that shrank dramatically after the African humid period (ca. 15,000–5500 years BP). Further sampling in the oases of central Algeria is needed to map more precisely the new geographic distribution of *A. algirus* in the country, and to undertake a detailed phylogeographic and population genetic analysis to elucidate the age and origin of the isolates surviving in the Saharan oases.

The results of this work also suggest that the use of microsatellites may be a crucial strategy in future research aimed at further evaluating population genetic structure of the two hedgehog species in Algeria. No microsatellite markers have been described for any of the two species, but loci developed for the Western European hedgehog (*Erinaceus europaeus* Linnaeus, 1758) could be tested (Becher and Griffiths, 1997; Henderson et al., 2000).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2015.11.014.

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