Genetic identification and distribution of two hedgehog species (*Atelerix algirus, Hemiechinus aethiopicus*) in Algeria.

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Abstract

The various studies carried out on the *Erinaceidae* to date show that Algeria is home to two species of hedgehog belonging to two different genera, the Mediterranean species *Atelerix algirus* and the desert species *Hemiechinus aethiopicus*. Based on the literature, it would appear that these two species are allopatric, the former being found in the northern regions of the country while the latter is native to southern Algeria. However, our recent investigations reveal the presence of overlapping zones where the two species coexist in sympatry in certain arid zones (Ain Sefra, Aïn el Orak, Laghouat, Biskra). In order to distinguish these two species, which are protected in Algeria, genetically and to detect any possible hybridisation between them, particularly in contact zones (hybridisation zone), a study of mitochondrial and nuclear DNA was carried out using specific molecular markers (myoglobin, D-Loop). The results obtained provide information on the characteristics of the two species (*Atelerix algirus*, *Hemiechinus aethiopicus)* in relation to the large family *Erinaceidae*, and propose a well-founded geographical distribution model for the hedgehog in Algeria.

Keywords: Hedgehog, distribution, genetics, Algeria.

Introduction

The hedgehog is a small insectivorous mammal belonging to the *Erinaceidae* family, with a very wide distribution (Corbet, 1988 ; He et *al.,* 2012). Seventeen species, currently divided into five genera, are found in Europe, Asia, Africa and New Zealand (by importation). Each genus has its own morphological, craniometric, osteological and odontological characteristics (Frost et *al.,* 1991). In Algeria, work to date reports the presence of two different species (Leberre, 1990), the Mediterranean species *Atelerix algirus* (Lereboull et, 1842) and the desert species *Hemiechinus aethiopicus* (Ehrenberg, 1832).

As for its geographical distribution, the desert hedgehog inhabits a fairly narrow band (sub-desert) between the Saharan Atlas and the great desert. In the north, it is found in the Ain Sefraregion, on the northern slopes of the Saharan Atlas, in Ain Ouarka, Brezina, Laghouat and Biskra. It is also found in Beni Abbès in the west and El Golea in central Algeria. This desert species is widely found in the mountains of the central Sahara, and has been observed in Ahnet Adrar and in and around the Hoggar. The range of the Mediterranean species *Atelerix algirus*covers the whole of northern Algeria. It is present in the High Plateaux, where it coexists with *Hemiechinus aethiopicus* south of Ain Sefra, Aïn el Orak, Laghouat and Biskra (Kowalski et Rzebik-Kowalska, 1991).

According to the data reported in the literature, it is likely that the geographical distribution of the two species follows the allopatric distribution, but there is no doubt that there is a hybridisation zone (Bogdanov et *al.,* 2009) where the Mediterranean species and the desert species coexist together in a parapatric distribution (Bull, 1991 ; Dennis et Hellberg, 2010). With this in mind, in order to gain a better understanding of the distribution area of these two protected species in Algeria and to detect any hybridisation between them, we carried out this study based on the study of mtDNA and nuclear DNA and, more importantly, the genetic identification of the two species, *Atelerix algirus* and *Hemiechinus aethiopicus*, collected in different regions of the country.

Materials and methods

Sampling

Hedgehog samples were collected in different regions of Algeria from corpses found killed on roads, dead in the wild in the field or from animals caught living in forests. For each sample, a phenotypic identification was carried to determine the species, *Atelerix algirus*or *Hemiechinus aethiopicus*. An ear biopsy was taken and preserved in DMSO (salt solution) (Seutln et *al.,* 1991). After sampling, the live hedgehogs are released back into their native environment. Table I below shows the sampling sites of the 44 hedgehogs studied in this study.

Extraction

DNA was extracted from tissue samples using the DNeasyTissue Kit (Qiagen, Crawley, UK), following the manufacturing instructions; the final volume of DNA extract was 200 µl for each sample, contamination was monitored by including two extraction vials in each extraction round.

Amplification

A 473 bp nuclear DNA fragment (base pair), the myoglobin gene, and a 460 bp mitochondrial DNA fragment, the D-Loop control region, were amplified by Polymerase Chain Reaction (PCR) using two different primer pairs (Krettek et al., 1995; Madsen et al., 2001; HE et al., 2012; Dubey et al., 2007).

Myoglobin PCR was conducted with a reaction mixture containing 50 mM Tris-HCl (pH 8.9), 20 mM ammonium sulphate, 20 mM EDTA, 170 mg/ml bovine serum albumin, 200 mM of each dNTP, 2 mM MgCl2, 0.6 mM of each primer, 0.1 to 0,2 mg of DNA and 2 units of Taq polymerase while the D-Loop PCR was conducted with the reaction mixture containing 50 mM Tris-HCl (pH 8.9), 20 mM ammonium sulphate, 20 mM EDTA, 170 mg/ml bovine serum albumin, 200 mM of each dNTP, 2 mM MgCl2, 0.6 mM of each primer, 0.1 to 0.2 mg of DNA and 2 units of Taq polymerase.

The thermal cycle results in an initial denaturation at 94° C for 2 min, 30 cycles (94° C for 30 s, 55° C for 30 s, 72° C for 45 s), and a final extension at 72° C for 7 min, or alternatively, the amplification phase is 35 cycles (94° C for 20 s, 55° C for 20 s,72° C for 30 s) for myoglobin but for D-Loop amplification the initial denaturation was at 94° C for 2 min, 30 cycles (94° C for 30 s, 55° C for 30 s, 72° C for 45 s), and the final extension was at 72° C for 7 min, or alternatively, the amplification phase was 35 cycles (94° C for 20 s, 55° C for 20 s, 72° C for 30 s).

PCR products were cleaned using the Qiagen PCR Cleaning Kit (Valencia, CA).

Séquence processing

MEGA5 (Evolutionary Molecular Genetic Analysis) software version 6.0 (Tamura et al., 2013) was used to process the sequences and construct the dendrogram using the UPGMA (Unweighted Pair Group Method with Arithmetic mean) method. This software has a wide variety of data editing tasks, sequence alignment using ClustalW, molecular clock testing and single-branch group significance tests (Tamura et al., 2007; Kumar et al., 2008).

Results

Phylogeny of the Algerian hedgehog

Analysis of the 44 samples from 21 wilayas in Algeria identified 2 very distinct groups based on morphological criteria: the *Atelerixalgirus* group (Figure 1) with 29 samples from the following regions: Bordj Bou Arreridj, Boumerdès, Médéa, Tipaza, Bouira, Alger, Adrar, Djelfa, El Oued, Biskra, Blida, Khenchela, Tlemcen, Ouargla, Laghouat, Batna and Constantine and the *Hemiechinusaethiopicus* group (Figure 2) with 15 samples from the following regions: Adrar, Bechar, Ghardaïa, Ouargla, Tamanrasset and M'Sila.

Figure 01: *Atelerix algirus.*

Figure 02: *Hemiechinus aethiopicus.*

The results of the molecular study, based on the nuclear DNA (myoglobin) used to determine the species and the mitochondrial DNA (D-Loop) used to detect intra-specific variations, were analysedusing the MEGA5 programme and gave a very representative dendrogram (UPGMA) (Figure 3). This tree shows the presence of two very distinct groups, each corresponding to a species; the first group with 29 samples represents *Atelerixalgirus* and the second group with 15 samples represents *Hemiechinusaethiopicus*.

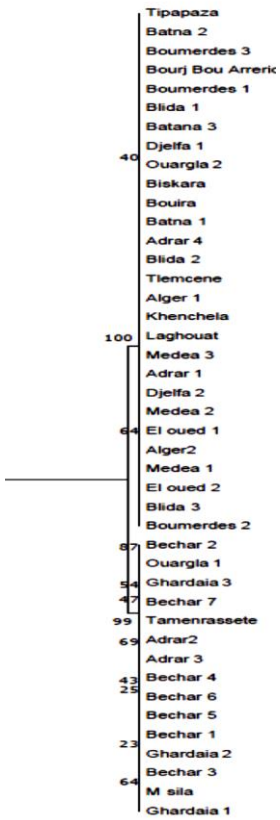

Figure 03. Phylogenetic tree showing the structure of the hedgehogs studied. This tree, which is the basis of the UPGMA method, was constructed using K-2P distances and the figures are bootstrap values (1000 replicates).

Dispersion model for the samples studied

Figure 04 shows the identification of the groups of samples analysed by morphological and molecular methods and their distribution in the 21 wilayas of Algeria.

The characteristics of the two ecosystems to which each group of hedgehogs belongs, with a very narrow contact zone or parapatry zone (Dennis et al., 2010), has resulted in two highly developed ecotypes that are well adapted to their living environment, represented by two species, the Mediterranean species *Atelerix algirus* and the desert species *Hemiechinus aethiopicus*. The morphological study shows the differences specific to each species, despite the fact that there are similarities in certain characteristics such as the spines that cover the animal's body, the tactile vibrissae, the dental formula and the absence of sexual dimorphism (Leberre, 1990; Kowalski and Rzebik-Kowalska, 1991). The molecular results reflect the same divergences observed in the two representative groups of the two species.

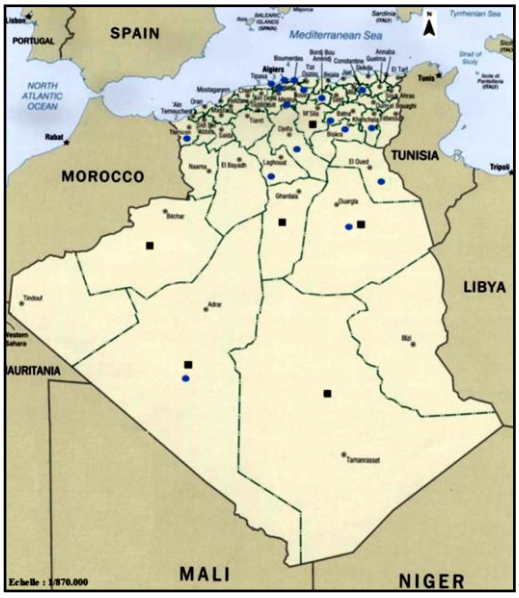

Figure 04: Comprehensive map showing the identification of sample groups studied in 21 wilayas. (*Atelerix algirus*; *Hemiechinus aethiopicus*).

In our opinion, the arid zones studied represent contact zones between the two species and also reflect the geographical limits between the two species studied. Three hedgehogs do not seem to corroborate the observed distribution pattern, two hedgehogs from the wilaya of Adrar and one hedgehog from the wilaya of Ouargla which are considered, according to our study and all previous studies, to be distribution areas for *Hemiechinus aethiopicus* show a phenotype similar to that of *Atelerix algirus*. The presence of these specimens in cohabitation with the desert hedgehog, which has a distinct phenotype, could be explained by the presence of hybrids, but this hypothesis is rejected by the molecular study, which confirmed that they are pure *Atelerix algirus* identical to the Mediterranean species.

Position of the Algerian hedgehog in the family *Erinaceidae*

In order to find the position of the Algerian hedgehog in the phylogenetic tree of the *Erinaceidae* family on the basis of the genetic identification of species, we used the data available on the GenBank. We introduced our sequences from the control region and with the BLAST function, which means comparing a nucleic sequence to a nucleic library, we looked for the species in the library that has the sequence closest to ours, we found 10 sequences of *Atelerix algirus* and 19 sequences of *Hemiechinus aethiopicus* which represent the results of other work carried out on these two species, we also had as results the European hedgehog *Erinaceus euraopaeus*, *Erinaceus concolor*, *Erinaceus roumanicus*, *Erinaceus amurensis*, *Hemiechinus auritus*; The results are shown in Table II.

By analysing the percentages of similarity between the different species (Table II) we found that contrary to the morphological results where several studies classified the European hedgehog *Erinaceus euraopaeus* as the hedgehog closest to *Atelerix algirus* (Bretagnoulle and Attie, 1989) the molecular results (for myoglobin and D-Loop) gave a similarity rate of 90% between the sequences of *Atelerix algirus* and the sequences of the Eastern European hedgehog *Erinaceus concolor*, and 87% with the sequence of the Western European hedgehog *Erinaceus euraopaeus*, which leads us to conclude that *Atelerix algirusis* closer to *Erinaceus concolor* than to *Erinaceus euraopaeus*.

Table II: Results of comparison between the two species of Algerian hedgehog and other species of the family *Erinaceidae*.

Family Erinaceidae	***Atelerix algirus***	***Hemiechinus aethiopicus***
Erinaceus concolor	90 %	87 %
Erinaceus roumanicus	88 %	86 %
Erinaceus amurensis	88 %	85 %
Erinaceus europaeus	87 %	84 %
Hemiechinus auritus	+ more than20	87 %

Hedgehog hybridisation

Reports of hybridisation and introgression between wild animal species have increased considerably in recent decades (Allendorf et al., 2001; Grant et al., 2005; Mallet, 2005). Many cases are still unknown because of the difficulties in detecting and recognising their occurrence, especially when morphologically overlapping phenotypes are involved (Largiader, 2007). Hybridisation is the result of mating between individuals belonging to genetically distinct populations, whatever the current taxonomic status of these populations (Rhymer and Simberloff, 1996). Introgression is the result of gene flow between populations in the form of backcrossing between hybrids and one or two parental species (Rhymer and Simberloff, 1996).

Natural hybridisation is an important process in the evolution and diversification of species (Arnold, 1997; Dowling and Secor, 1997; Barton, 2001). However, hybridisation can also contribute to the decline of species, either through the loss of genetically different traits when hybrids are fertile or through the loss of reproduction when hybrids are partially or totally sterile, and can ultimately lead to extinction (Allendorf and Luikart, 2007).

The presence of reproductive barriers is also associated with the loss and fragmentation of habitats and/or the introduction of species (Allendorf et al., 2001). In populations where hybridisation has already been impoverished, individuals are unable to find partners of the same species (reproductive barriers), thus increasing the risk of mating with specimens from closely related species (Kyle et al., 2003; Lode et al., 2005; Cabria et al., 2011).

Hybridisation is common in mammals and has been detected in monkeys (Roos et al., 2011), wild goats (Ropiquet and Hassanin, 2006), hares (Melo-Ferreira et al., 2007; Liu et al, 2011), carnivores (Kyle et al., 2003; Schwartz et al., 2004; Adams et al., 2007; Trigo et al., 2008; Cabria et al., 2011) and rodents (Ermakov et al., 2002; 2006; Spiridonova et al., 2006; Tsvirka et al., 2006). This phenomenon

has also been observed in Western European hedgehogs and Northern European hedgehogs, between *Erinaceus europaeus* and *Erinaceus roumanicus*. The morphological study of one specimen classified it as *Erinaceus roumanicus*, but molecular analysis using a nuclear marker and a mitochondrial marker revealed that it was a hybrid species with nuclear DNA from *E. roumanicus* and mitochondrial DNA from *E. europaeus*, resulting from one or more crosses between the two (Bogdanov et al., 2009).

Although the samples we studied come from different regions of Algeria, taking into account possible areas of hybridisation between the two species or areas of parapatry (Dennis et al., 2010), which correspond to semi-arid and arid regions (Djelfa, M'sila, Batna, Khenchela, Laghouat). Analysis of the phylogenetic tree, which shows the structure of the samples, reveals a hierarchical classification into two distinct groups. These groups would certainly correspond to the two species mentioned above, which leads us to suggest two propositions: 1) there would be an absence of hybrids between the two species 2) the sampling carried out is outside the hybridisation zone, which has yet to be located.

Conclusion

Molecular analysis has enabled us to clarify the genetic identity of the two hedgehog species found in Algeria and their position in the phylogenetic tree of the large family *Erinaceidae*. The presence of *Atelerix algirusin* desert areas that are, according to their nature, regions specific to *Hemiechinus aethiopicus* means that we need to launch further studies and review their geographical distribution by means of a more detailed phylogeographical study in order to propose a distribution model for better conservation of these protected insectivores in Algeria.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflicts of interest.

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