Amphibia-Reptilia 32 (2011): 281-286

Preliminary survey on genetic variation within the Pygmy Algyroides, *Algyroides fitzingeri*, across Corsica and Sardinia

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Abstract. *Algyroides fitzingeri* is a Corso-Sardinian endemic lizard belonging to a relictual genus within the Lacertini radiation. In recent phylogeographic studies of Corso-Sardinian endemic lizards incongruent patterns are emerging. We investigated the mitochondrial genetic variation of *A. fitzingeri* across Corsica and Sardinia to obtain a preliminary portrait of its phylogeographic history. This species showed some polymorphism, but with low genetic differentiation between populations, that probably originated during the Pleistocene. Corsican populations are closely related to those from North Sardinia and are likely to have originated from them, given the higher diversity and deeper phylogeographic structure observed in Sardinia than in Corsica. While the phylogeographic structure of *A. fitzingeri* in Corsica is surprisingly shallow when compared with other co-distributed lizards, in Sardinia a common pattern apparently emerges. Further research is needed to confirm the hypotheses here presented and to provide a conclusive assessment of the phylogeography of this species.

Keywords: 12S rRNA gene, *Algyroides fitzingeri*, Corsica, Lacertidae, ND4 gene, phylogeography, Sardinia.

The genus *Algyroides* Bibron and Bory de Saint-Vincent, 1833 includes four Mediterranean lizard species with a relictual distribution in southern Spain, Corsica and Sardinia, and the western and southern Balkan Peninsula. They share unique morphological features among lacertid lizards including very large dorsal body scales with oblique keels (Harris, Arnold and Thomas, 1999). However, recently a molecular study questioned their monophyletic origin (Pavlicev and Mayer, 2009). The Pygmy Algyroides, *Algyroides fitzingeri* (Wiegmann, 1834) is endemic to Corsica and Sardinia where it is quite widespread (Delaugerre and Cheylan, 1992; Salvi and Bombi, 2010). It mostly inhabits Mediterranean-type shrubby vegetation as well as temperate forests, rocky habitats, and dry walls along rural areas. Little is known about the biology of this elusive species (Schneider, 1981; Capula and Luiselli, 1992; Capula et al., 2002), and its geographic variation has never been investigated.

Corsica and Sardinia host several obvious geographical barriers for species dispersion such as the Strait of Bonifacio which separates them, and high mountain chains (with peaks up to 2700 m asl in Corsica, Mount Cinto, and 1932 m asl in Sardinia, Gennargentu Mountains). These environmental discontinuities could have acted as phylogeographical barriers for most Corso-Sardinian species. However, recent studies on geographic variation of the endemic lizards *Archaeolacerta bedriagae* and *Podarcis tiliguerta* (Capula, 1996; Harris et al., 2005; Salvi et al., 2009a, 2009b, 2010) highlight that although these species are genetically structured across the geographic space, they differ in the magnitude and spatial placement of phylogeographic barriers among population groups. In this framework, data from other co-distributed species are crucial for understanding how historical biogeographic factors have shaped the genetic diversity in the Corso-Sardinian biota. In this study we report preliminary results on the genetic variation of *Algyroides fitzingeri* across the species' range at two mitochondrial gene fragments, ND4 and 12S rRNA. The observed pattern is discussed in comparison with the

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DOI:10.1163/017353711X556989

Table 1. Geographical locations, sample codes, number of specimens and haplotypes composition for the samples of *Algyroides fitzingeri* studied.

Sample	Locality	Coordinates	N	Haplotype (*n*)
C1	Ruisseau de Capronale, Ométa (Corsica)	42°19′N 8°49′E	3	*h1* (3)
C2	Gorges de la Restonica, Corte (Corsica)	42°17′N 9°08′E	2	*h1* (2)
C3	Piana, Porto (Corsica)	42°14′N 8°34′E	1	*h2* (1)
C4	Bonifacio, Bonifacio (Corsica)	41°23′N 9°09′E	1	*h1* (1)
S1	Ponte Liscia, Tempio Pausania (Sardinia)	41°10′N 9°18′E	1	*h3* (1)
S2	San Pantaleo, Olbia (Sardinia)	41°02′N 9°29′E	1	*h3* (1)
S3	Casagliana, Olbia (Sardinia)	40°59′N 9°28′E	1	*h4* (1)
S4	Monte Albo, Siniscola (Sardinia)	40°30′N 9°36′E	3	*h5* (2), *h6* (1)
S5	Padria, Pozzomaggiore (Sardinia)	40°23′N 8°37′E	1	*h7* (1)
S6	Cala Gonone, Dorgali (Sardinia)	40°16′N 9°36′E	2	*h8* (2)
S7	Passo Tascusì, Desulo (Sardinia)	40°02′N 9°14′E	1	*h9* (1)
S8	Giara di Gesturi, Gesturi (Sardinia)	39°44′N 8°59′E	1	*h10* (1)
S9	Villaggio dei Gigli, Maracalagonis (Sardinia)	39°16′N 9°18′E	1	*h11* (1)

geographic variation observed in other Corso-Sardinian endemics.

We sampled 19 individuals of *A. fitzingeri* from 13 localities including Corsica (N = 7) and Sardinia (N = 12). Specimens employed in the analyses along with locality details and population acronyms used in the paper are reported in table 1 and fig. 1. Tail tips from each specimen were obtained in the field and stored in pure ethanol.

Total genomic DNA was extracted from tail muscle following standard high-salt protocols (Sambrook, Fritsch and Maniatis, 1989). Two mitochondrial gene fragments were amplified by polymerase chain reaction (PCR): one including the ND4 gene and flanking tRNAs (tRNA^{Ser}, tRNA^{His}, and partial tRNA^{Leu}) and the other one including partial ribosomal 12S rRNA gene. Primers used for PCR and sequencing are ND4 and Leu (Arévalo, Davis and Sites, 1994) for the ND4 fragment, and 12Sa and 12Sb for the 12S rRNA (Kocher et al., 1989). Amplifications were conducted in 25 μl volumes, following conditions described in Salvi et al. (2010) for the ND4 fragment and Harris et al. (1998) for the 12S rRNA fragment. Purification and sequencing of PCR products were carried out by a commercial company, Macrogen (www.macrogen.com).

Sequence alignment was performed manually using Bioedit v. 5.0.9. (Hall, 1999). Sequence divergence was assessed by the software MEGA v. 4 (Tamura et al., 2007). The amount of genetic variation within *A. fitzingeri* was estimated as haplotype (h) and nucleotide (π) diversity using the software DNASP 4.0 (Rozas et al., 2003).

We inferred the genealogical relationships among haplotypes by means of a statistical parsimony network (Templeton, Crandall and Sing, 1992) under the 95% probability criterion for a parsimonious connection, using the software TCS 1.21 (Clement, Posada and Crandall, 2000). Intraspecific genealogies are better represented using a network approach rather than by tree-building methods since intraspecific gene evolution is often multifurcated, ancestral and descendant genes coexist, and usually few characters for phylogenetic analysis are available due to shallow levels of divergence (Posada and Crandall, 2001).

The most appropriate haplotypes clustering solution was inferred using the non-hierarchical Bayesian approach implemented in BAPS (Corander, Sirén and Arjas, 2008). This method uses prior knowledge of sampling location and estimates posterior probabilities for all different clusterings of individual nucleotide sequences. We ran the BAPS analysis five times for each value of the maximum number of clusters (Kmax from 2 to 12) to ensure stability of the results.

DNA sequences from the two mitochondrial gene fragments were concatenated, resulting in a combined alignment of 1257 base pairs (bp) (876 bp from ND4 and flanking tRNAs genes and 381 bp from 12S rRNA). Aligned sequences contained no indels. There were 27 variable sites, of which 15 were informative. A total of 11 haplotypes were identified (GenBank Accession Numbers: FR821067-FR821088), two in Corsica (*h1-2*) and nine in Sardinia (*h3-10*). Haplotypes were connected into a single network and the maximum number of mutational steps allowing for a 95% parsimonious connection between them was estimated to be 15 (fig. 1). The distribution of haplotypes and their relatedness were geographically structured, with closely related haplotypes grouping into distinct geographical clusters, but often without any association between genetic distance between haplotypes and geographical distance (fig. 1). In Corsica, the haplotype *h1* is widespread from North to South (sites C1, C2, and C4) and is by far the most common being shared by 86% of the specimens analysed. The haplotype *h2* is restricted to the north-

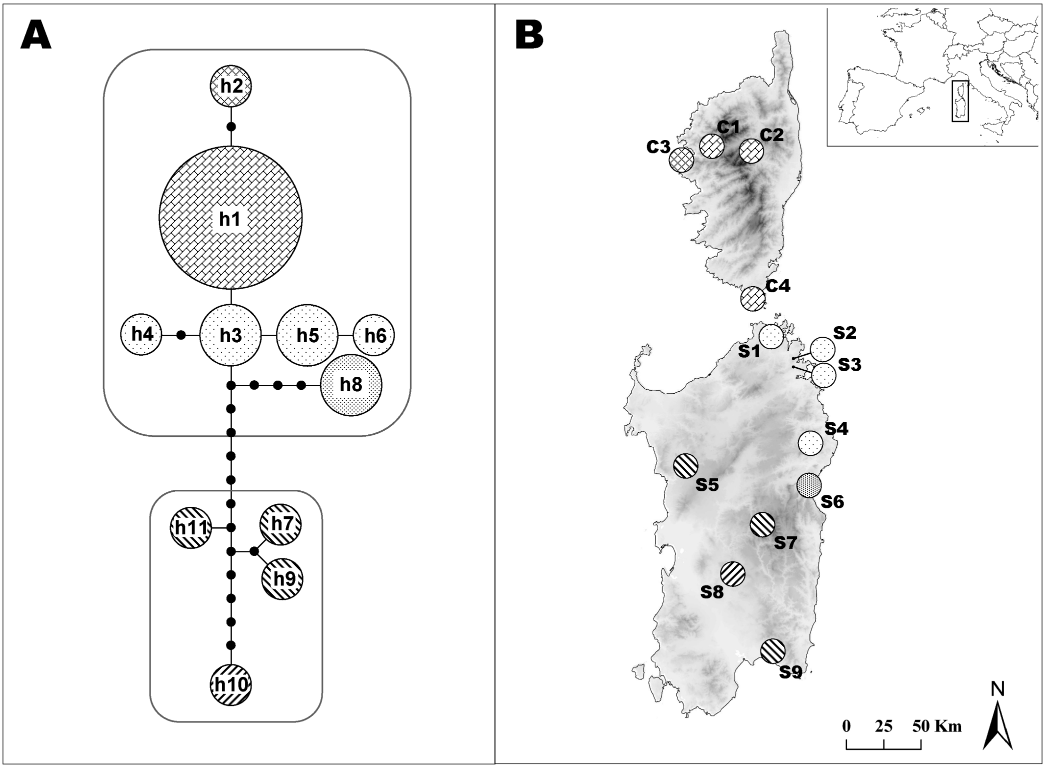

Figure 1. (A) Phylogenetic relationships among the 11 haplotypes from *Algyroides fitzingeri* based on the statistical parsimony network. Haplotypes are represented by circles with size proportional to their frequency and grouped into boxes according to the results of BAPS clustering analysis. Black circles represent missing haplotypes. (B) Map showing the geographical location of the sampling sites represented by circles where the motif depicts their haplotype composition according to the network. For details on geographical locations and haplotypes codes see table 1.

western locality (C3). Corsican haplotypes are very closely related to those found in North Sardinia (*h3-6*) from Gallura coast (site S1) to Monte Albo (site S4). South of this area, five haplotypes were found, one in the eastern coast (*h8*, site S6), one in the Giara di Gesturi plateau (*h10*, site S8) and three spanning from the North West to the South East and closely related to each other (*h7*, *h9*, *h11*, sites S5, S7, S9).

The sequence divergence (uncorrected *p*-distance) among haplotypes ranged from 0% to 1.10%. The average *p*-distance within Corsica was 0.07% and within Sardinia was 0.60%. Haplotype diversity (h) and nucleotide diversity (π) were quite high in *A. fitzingeri* ($h = 0.895$, SD = 0.057; $\pi = 4.62 \times 10^{-3}$, SD = 0.90×10^{-3}). Both estimates of genetic variation were much higher in Sardinia ($h = 0.955$, SD = 0.002; $\pi = 5.87 \times 10^{-3}$, SD = 0.88×10^{-3}) than in Corsica ($h = 0.286$, SD = 0.038; $\pi = 0.45 \times 10^{-3}$, SD = 0.31×10^{-3}). The Bayesian clustering analysis partitioned the haplotypes into two groups ($K = 2$; posterior probability = 0.78), one grouping haplotypes from North Corsica to central Sardinia (*h1-h6*, and *h8*, from sites C1-4, S1-4, and S6) and the other one including haplotypes from central to South Sardinia (*h7*, and *h9-h11*, from sites S5, and S7-9) (fig. 1).

Algyroides fitzingeri showed quite high genetic diversity which is geographically, although shallowly, structured. The polymorphism in this species is much higher in Sardinia than in Corsica where one of the two haplotypes found is widespread from North to South. In Sardinia four haplogroups where found, one in the North and closely related to Corsican haplotypes, one in central-eastern Sardinia, one spanning form North West to South East, and one from the Giara plateau. These groups are fairly differentiated from each other (from 5 to 16 mutational steps), much more than the northern Sardinian group with respect to the Corsican one (1 mutational step). The overall pattern of shallow genetic differentiation among populations observed in *A. fitzingeri* is likely to have a very recent origin. By taking into account the highest and the lowest lizard specific divergence rates for the two mitochondrial fragments (0.5 and 2.74% per million years, respectively; see Pinho, Harris and Ferrand, 2007 and references in Rastegar-Pouyani et al., 2010), and assuming a linearity of accumulation of substitutions over time in *A. fitzingeri*, the intraspecific genetic differentiation would have originated during the Pleistocene (starting from 2.2 or 0.4 Mya, assuming the highest and the lowest divergence rate, respectively).

Data on genetic diversity are available for other two Corso-Sardinian endemic lizards, *Podarcis tiliguerta* and *Archaeolacerta bedriagae*. These species show remarkable fragmentation but very different patterns of genetic and geographic variation. The rock lizard *A. bedriagae* shows a clearly divergent lineage from northern Corsica, and several minor phylogeographic discontinuities between population groups from the remainder of the species' range (Salvi et al., 2009a, 2010). The wall lizard *P. tiliguerta* apparently is a paraphyletic species complex with very high differentiation found between (and within) populations from Corsica and different areas of Sardinia (Capula, 1996; Harris et al., 2005), but the number of evolutionary entities involved and their distribution need further research.

The overall phylogeographic pattern of *A. fitzingeri* in Sardinia is roughly congruent with that of *A. bedriagae* (Salvi et al., 2010), both in terms of placement and depth of the genetic discontinuities among populations. In these two species, populations from the eastern Sardinia clustered into three main groups from North, centre, and South, with samples from Gennargentu Massif (site S7 in this study) related to those from the southern mountains (site S9 in this study), namely Sette Fratelli Mts. These preliminary results point to a common, although recent, geographic partition of populations of these lizards in Sardinia, likely due to similar biogeographic factors affecting the palaeoenvironments distribution during Pleistocene.

On the other hand, the low genetic diversity observed in *A. fitzingeri* from Corsica is surprising when compared to the high mitochondrial diversity and deep phylogeographic structure observed in Corsica in *A. bedriagae* and *P. tiliguerta* (Harris et al., 2005; Salvi et al., 2010). The low genetic diversity of *A. fitzingeri* observed in Corsica relative to Sardinia, the close relation between Corsican and northern Sardinian haplotypes, and the deeper phylogeographic structure in Sardinia relative to Corsica, are consistent with a recent gene flow between populations from both islands and with a recent origin of Corsican populations from North Sardinia. This is in agreement with biogeographic evidence documenting repeated connections between Corsica and Sardinia during Pleistocene sea regressions until 12 000 years ago (Lambeck et al., 2004). Although for Corsican terrapins and tortoises, *Emys orbicularis* and *Testudo hermanni*, molecular data suggested a likely recent (re-)introduction in Corsica by man (Fritz et al., 2006; Pedall et al., 2011), we discard a scenario of human-mediated arrival of *A. fitzingeri* in Corsica because of two different lines of evidence. First, under a human-introduction scenario we expected to find a sub-set, likely few variants, of Sardinian haplotypes in Corsica. However, in Corsica we found two haplotypes which are both exclusive to this island and not shared with Sardinian groups. Moreover, the haplotype *h*2 is likely derived from the widespread haplotype *h1* suggesting that Corsican population behave as an evolutionary independent unit. Second, *A. fitzingeri* is a very elusive species, occurring in shrub vegetation, where it is difficult to capture making it unlikely to have been spread by human activities in Corsica where it occurs from the extreme North (Cape Corse) to the southernmost region of this island.

However, because of the limited number of individuals studied, these preliminary hypotheses needs to be confirmed by further research including higher number of samples from both islands as well as the assessment of the historical demographic trends in this species in order to obtain a conclusive phylogeographic appraisal.

Acknowledgements. We are grateful to Arie van der Meijden, Chiara Settanni, and Ernesto Recuero, who provided samples, and to the Ente Foreste della Sardegna for the logistic support in South Sardinia. Lizards were captured under permits from the Italian Ministry of Environment DPN/2D/2003/2267, and from the Direction régionale de l'environnement, de l'aménagement et du logement (DREAL) de Corse. This study was funded by the FCT projects PTDC/BIA-BDE/74349/2006 (to DJH), PTDC/BIA-BDE/74349/2006 and PTDC/BIA-BEC/101256/2008 (to MAC) from Fundação para a Ciência e a Tecnologia (FCT, Portugal), and by the PRIN project 20085YJMTC (to MAB and DS) from Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR, Italy). DS and AP are supported by FCT post-doctoral grants SFRH/BPD/66592/2009 and SFRH/BPD/26546/2006, respectively.

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Received: October 27, 2010. Accepted: December 28, 2010.