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Mitochondrial phylogeography of the Bedriaga's rock lizard, *Archaeolacerta bedriagae* (Reptilia: Lacertidae) endemic to Corsica and Sardinia

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ABSTRACT

Archaeolacerta bedriagae is a rock-dwelling lizard endemic to the Corso-Sardinian insular system. We investigated the phylogeography of the species by using the mitochondrial ND4 and flanking tRNAs genes from 94 specimens belonging to 19 populations. Phylogenetic, Barrier, and SAMOVA analyses revealed a highly structured pattern characterized by two levels of discontinuities in the geographical distribution of mtDNA diversity: (i) a deep phylogeographic break in Northern Corsica between Lineage A, restricted to northernmost Corsica, and Lineage B widespread all over the remaining range of the species, and (ii) some minor phylogeographic discontinuities within lineage B, which is sub-structured into six closely related haplotype clades with remarkable concordance with geography. The first evolutionary event concerning the split between the two main lineages from an ancestral population occurred in the Upper Pliocene (5.87–3.68 mya), while the divergence within lineage B would have started from the Upper Pleistocene (2.5–1.6 mya), between Corsican and Sardinian populations. Somewhat later (1.7–1.1 mya), the Sardinian ancestral population underwent fragmentation into population groups inhabiting North, Central, and South Sardinia. As inferred from previous allozyme surveys, the divergence among population groups would be driven by allopatric fragmentation, while the discrepancy concerning the major partition into two lineages inferred from mtDNA but not apparent in analysis of allozymes needs further investigation.

1. Introduction

The Mediterranean area is one of the world biodiversity 'hotspots' (Myers et al., 2000; Mittermeier et al., 2004) and within this area, insular systems such as the Tyrrhenian Islands Corsica and Sardinia are among the most important reservoirs of diversity and endemism (Médail and Quézel, 1997, 1999). This probably is due to their complex geological history and palaeogeographic evolution (Cavazza and Wezel, 2003), coupled with altitudinal and surface variation that produces a large range of habitat types. Corsica and Sardinia have been isolated from European continental landmasses following the disjunction of the Corso-Sardinian microplate(s) from the Iberian plate around 30–27 million years ago (mya) (Alvarez, 1972; Alvarez et al., 1974). The anti-clock wise rotation of the microplate and the separation of the two islands started 15 mya and ended 9 mya establishing a geographical setting roughly similar to that of the present day. It has also been hypothesised that the Corsica–Sardinia microplate remained connected with Palaeo-Europe during the rotation through a land bridge in the Tortonian period, namely 10 mya (Orszag-Sperber et al., 1993). Certain connections with Europe as well with North Africa were established during the Messinian Salinity Crisis 5.96–5.33 mya, (Duggen et al., 2003). Further sea-level oscillations occurred in the Pleistocene due to sea regressions during glacial periods and caused repeated connections and separations between Sardinia and Corsica (until 12 ka ago, Lambeck et al., 2004) and the close proximity between the latter and the Italian Peninsula.

These palaeogeological events are responsible for the origin of endemisms as well as for generalized patterns of disjunct distribution observed in various plants and animals (Various Authors, 1983). The high proportion of endemic species occurring in Corsica and Sardinia has a great relevance for their intrinsic conservation value, but also for providing an excellent model for evaluating general evolutionary and biogeographic questions, as the geological evolution of the Corso-Sardinian insular system is well known and placed in a well defined temporal framework. Indeed, by incorporating evidence from biogeographic patterns, fossil records, phylogenetic relationships, and molecular-clock divergence estimates, it has been possible to infer the biogeographic and evolutionary processes underlying the origins of various species in Corsica and Sardinia (e.g. Caccone et al., 1997; Ketmaier et al., 2003, 2006; Zangari et al., 2006; Carranza et al., 2008; Fochetti et al., 2009). However, only limited research has been devoted to intraspecific

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genetic diversity and phylogeography of Corsican/Sardinian species (Capula, 1996; Lecis and Norris, 2004; Harris et al., 2005; Ketmaier et al., 2006; Salvi et al., 2009b) and to clarify the microevolutionary processes involved in shaping diversity patterns *within* Corsica and Sardinia.

The Bedriaga's rock lizard, *Archaeolacerta bedriagae* (Camerano, 1885) represents an excellent model to test generalized patterns of endemicity and diversification in Corsica and Sardinia since it is strictly a Corso-Sardinian endemic and widespread in both islands. This lizard, according to the revision by Arnold et al. (2007), represents a monotypic genus in a monophyletic group of Palaearctic and Oriental genera of the tribe Lacertini Oppel, 1811 (subfamily Lacertinae, family Lacertidae). The phylogenetic position of this species within the Palaearctic lizard radiation and the biogeographic scenarios underlying its current distribution still remain unclear. Phylogenetic hypotheses based on morphology, as well as on nuclear and mitochondrial DNA data, recovered an ambiguous placement for this species, being unresolved or related from time to time to many different species belonging to several genera including *Algyroides* Bibron & Bory de Saint-Vincent, 1833, *Dalmatolacerta* Arnold, Arribas & Caranza, 2007, *Darevskia* Arribas, 1997, *Dinarolacerta* Arnold, Arribas & Caranza, 2007, *Podarcis* Wagler, 1830, *Sclerarcis* Fitzinger, 1843, *Takydromus* Daudin, 1802, *Teira* Gray, 1838, *Zootoca* Wagler, 1830 (Arnold, 1973; Arribas, 1999; Harris et al., 1998; Fu, 2000; Carranza et al., 2004; Arnold et al., 2007; Mayer and Pavličev, 2007; Pavličev and Mayer, 2009). Within *A. bedriagae* four subspecies have been described: *A.b. bedriagae* from Corsica, and *A.b. sardoa* (Peracca, 1903), *A.b. paessleri* (Mertens, 1927), and *A.b. ferrerae* (Stemmler, 1962) from Sardinia. However, these subspecies are not supported by genetic (Salvi et al., 2009a,b) or morphological data (Salvi et al., 2008). *Archaeolacerta bedriagae* is a strictly rock-dwelling lizard (Bombi et al., 2009a) and shows several morphological features associated with living on rocky surfaces and the use of narrow crevices as refuges, including flattened head and body, reduced cranial ossification in adults, unkeeled dorsal body scales, and laterally compressed and often kinked toes in the vertical plane (see e.g. Arnold, 1973; Arnold et al., 2007). Previous studies examined the allozyme variation of *A. bedriagae* (Salvi et al., 2009a, 2009b), revealing high polymorphism and a low differentiation among populations, without a clear geographical pattern.

The aim of this paper is to investigate intraspecific genealogy of *A. bedriagae* in an explicitly phylogeographic framework, using mtDNA sequence data to uncover the evolutionary history of this endemism, and to provide a biogeographic model to compare with other Corso-Sardinian endemisms.

2. Materials and methods

2.1. Sampling

Specimens employed in the molecular analyses with locality details and population acronyms used in the paper, are reported in Table 1 and Fig. 1. Collecting localities were selected with the aim of covering the whole range and of obtaining a representative sample. Thus, samples from each geographic region were analysed, including all four subspecies (Fig. 1). We sampled a total of 94 individuals from 19 localities.

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from alcohol-preserved tail muscle collected from live specimens following standard high-salt protocols (Sambrook et al., 1989). A fragment of 848 base pairs (bp) from mitochondrial DNA including the ND4 gene and the tRNAs for Serine ($tRNA^{Ser}$), Histidine ($tRNA^{His}$), and Leucine ($tRNA^{Leu}$) was amplified by polymerase chain reaction (PCR) using primers ND4 and Leu published by Arévalo et al. (1994). Amplifications were conducted in 25 μL volumes, containing 2.5 μL of 10× reaction buffer (Ecogen), 3.2 mM $MgCl_2$, 0.4 mM each dNTP, 0.2 μM each primer, 1 U of Ecotaq DNA polymerase (Ecogen), and approximately 50 ng genomic DNA. Amplification conditions consisted of a predenaturing step of 3 min at 94 °C followed by 40 cycles of a denaturing step of 30 s at 94 °C, annealing at 50 °C for 30 s, extension at 72 °C for 45 s, and the final extension at 72 °C for 4 min. The PCR products were enzymatically purified and sequenced on an ABI Prism 310 automated sequencer (Applied Biosystems), with the same primers used for amplification.

2.3. Phylogenetic analysis and sequence divergence

Sequence alignment was performed manually using Bioedit v. 5.0.9. (Hall, 1999). Four species belonging to two additional genera of the tribe Lacertini (*sensu* Arnold et al., 2007) were employed as outgroups in the phylogenetic analyses, *Podacis sicula* (Rafinesque-

Table 1
Geographical locations, sample sizes, names and frequencies of the haplotypes found, and estimates of haplotype diversity (h) and nucleotide (π) diversity for the studied populations of *A. bedriagae*.

Sample	Locality	Altitude (m a.s.l.)	Coordinates	Sample size	Haplotypes (n)	h	π [10^2]
C1	*Ruisseau de Capronale*, Ométa (Corsica)	1331	42° 19′ N 8° 49′ E	4	*c1a* (1); *c1b* (1); *c1c* (2)	0.833 (0.222)	4.093 (1.208)
C2	*Gorges de la Restonica*, Corte (Corsica)	1255	42° 14′ N 9° 02′ E	6	*c2a* (3); *c2b* (2); *c2c* (1)	0.733 (0.155)	0.110 (0.030)
C3	*Piana*, Porto (Corsica)	387	42° 14′ N 8° 34′ E	3	*c3* (3)	0.000 (0.000)	0.000 (0.000)
C4	*U' Fium'Orbu*, Ghisoni (Corsica)	428	42° 06′ N 9° 15′ E	5	*c4a* (4); *c4b* (1)	0.400 (0.237)	0.189 (0.122)
C5	*Zicavo*, Zicavo (Corsica)	1230	41° 52′ N 9° 07′ E	5	*c5* (5)	0.000 (0.000)	0.000 (0.000)
C6	*L'hospidale*, Porto Vecchio (Corsica)	1001	41° 40′ N 9° 12′ E	5	*c6a* (3); *c6b* (1); *c6c* (1)	0.700 (0.218)	0.945 (0.528)
C7	*Roccapina*, Sartène (Corsica)	83	41° 29′ N 8° 56′ E	6	*c7a* (3); *c7b* (3)	0.600 (0.129)	0.923 (0.199)
C8	*Bonifacio*, Bonifacio (Corsica)	135	41° 24′ N 9° 07′ E	6	*c8a* (3); *c8b* (3)	0.600 (0.129)	0.213 (0.046)
S1	*Punta dei Colmi*, La Maddalena Island (Sardinia)	106	41° 13′ N 9° 23′ E	4	*s1* (4)	0.000 (0.000)	0.000 (0.000)
S2	*Punta Marmorata*, S. Teresa di Gallura (Sardinia)	3	41° 15′ N 9° 14′ E	4	*s2* (4)	0.000 (0.000)	0.000 (0.000)
S3	*Petraiaccio*, Caprera Island (Sardinia)	72	41° 12′ N 9° 27′ E	4	*s3* (4)	0.000 (0.000)	0.000 (0.000)
S4	*Capo d'Orso*, Palau (Sardinia)	113	41° 10′ N 9° 24′ E	4	*s3* (4)	0.000 (0.000)	0.000 (0.000)
S5	*Monte Moro*, Cannigione (Sardinia)	419	41° 06′ N 9° 31′ E	4	*s3* (4)	0.000 (0.000)	0.000 (0.000)
S6	*Monte Limbara*, Tempo Pausania (Sardinia)	1306	40° 51′ N 9° 10′ E	6	*s6a* (4); *s6b* (1); *s6c* (1)	0.600 (0.215)	0.229 (0.078)
S7	*Nuraghe Ortachis*, Bolotana (Sardinia)	1034	40° 21′ N 8° 54′ E	6	*s7* (6)	0.000 (0.000)	0.000 (0.000)
S8	*Supramonte*, Oliena (Sardinia)	1233	40° 15′ N 9° 25′ E	6	*s8a* (3); *s8b* (3)	0.600 (0.129)	0.147 (0.032)
S9	*Gennargentu*, Fonni (Sardinia)	1783	39° 59′ N 9° 19′ E	5	*s9* (5)	0.000 (0.000)	0.000 (0.000)
S10	*Monte Genis*, Villasalto (Sardinia)	776	39° 24′ N 9° 18′ E	5	*s10* (5)	0.000 (0.000)	0.000 (0.000)
S11	*Monte Settefratelli*, Burcei (Sardinia)	483	39° 17′ N 9° 24′ E	6	*s10* (4), *s11b* (2)	0.533 (0.172)	0.315 (0.102)

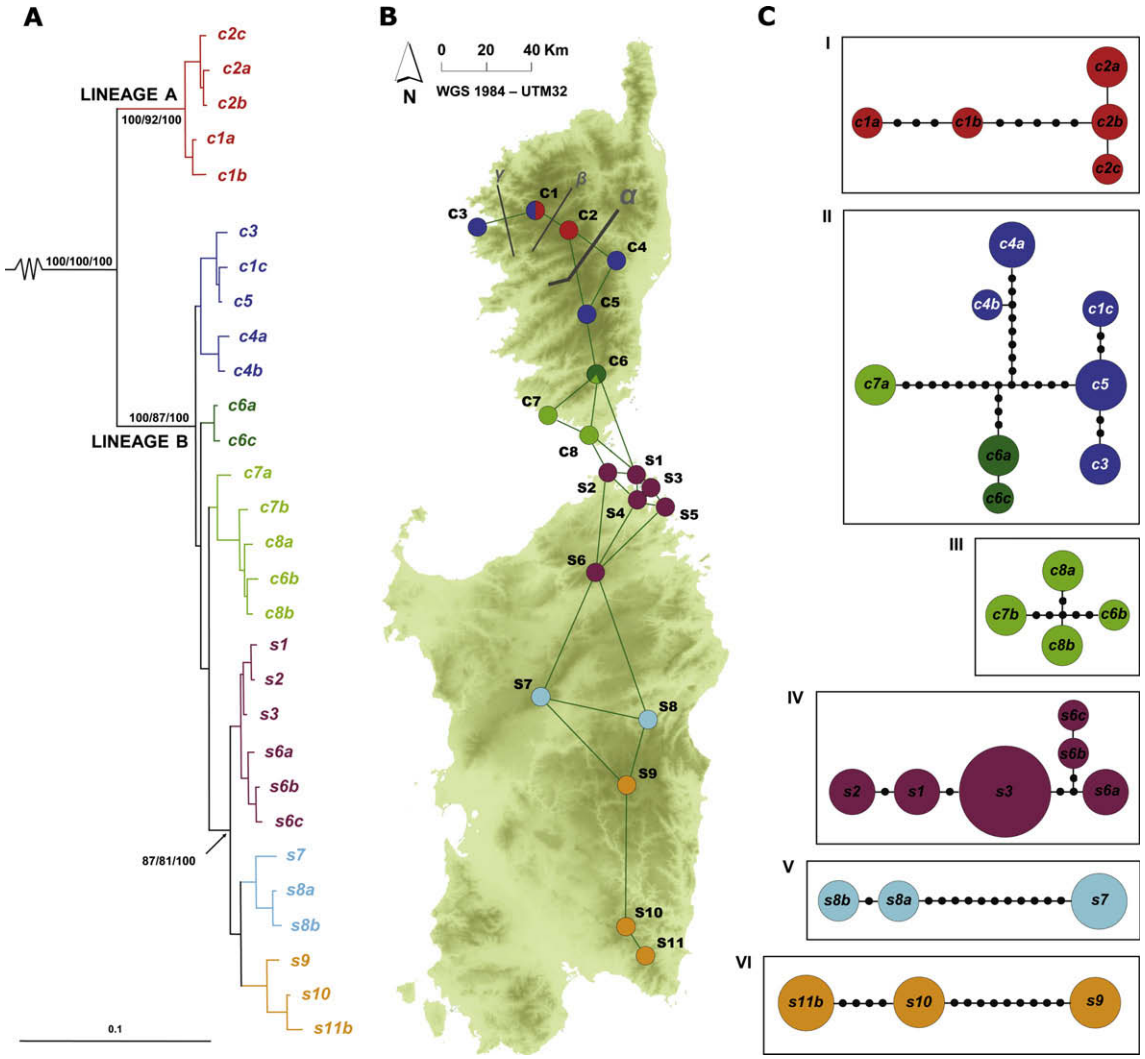

Fig. 1. (A) Phylogenetic relationships among the 29 *Archaeolacerta bedriagae* haplotypes based on Bayesian analysis of the ND4 and flanking tRNAs genes (outgroup species not shown). The topology is equivalent in MP and ML analyses. Bootstrap values and Bayesian Posterior Probabilities for the main nodes are shown (MP/ML/BPP). The two main lineages are indicated and the sub-clades found in Lineage B are referred in the text and coloured in the map as follow: clade b1: blue; clade b2: dark green; clade b3: light green; clade b4: amaranthine; clade b5: light blue; clade b6: orange. (B) Geographical location of the sampled populations and their haplotype composition, and results of the barrier analysis. Populations are coloured according to the frequency of the haplotypes clades identified by phylogenetic analyses. The genetic barriers α, β, and γ are represented by lines which thickness is proportional to the ratio between genetic distance values between populations on both side of each barrier to the average genetic distance among populations in the whole dataset. (C) Haplotypes networks recovered by statistical parsimony analysis. Haplotypes are represented by circles with size proportional to their frequencies and coloured according to clades recovered in phylogenetic analyses. Black circles represent missing haplotypes (extinct or not sampled). For geographical detail on sampling locations and haplotype codes see Table 1.

Schmalz, 1810), *P. muralis* (Laurenti, 1768), *P. hispanica* (Steindachner, 1870), and *Lacerta viridis* (Laurenti, 1768) (Accession Numbers DQ001021, DQ001020, EU269589, and AM176577, respectively). Although the systematic position of *A. bedriagae* is still far from being clear (see Harris et al., 1998; Fu, 2000; Arnold et al., 2007; Mayer and Pavličev, 2007; Pavličev and Mayer, 2009), we used as outgroups representatives of both the likely-allied genus *Lacerta* Linnaeus, 1758, and the less related *Podarcis* Wagler, 1830. We used multiple methods to infer phylogenetic relationships among *A. bedriagae* haplotypes: Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian inference (BI). In addition to these tree-building methods we analysed the genealogical relationships among haplotypes by means of a statistical parsimony network (Templeton et al., 1992). MP analyses were performed in PAUP* 4.0b10 (Swofford, 2002) (ACCTRAN optimization, gaps coded as a new state, TBR branch swapping), using heuristic searches. Bootstrap support for MP trees was calculated using 10,000 replicates. For ML analyses a model of evolution was chosen through the Akaike information criterion (AIC) implemented in Modeltest 3.7 (Posada and Crandall, 1998). ML analyses were performed using the software PAUP* 4.0b10, with a heuristic search strategy, and bootstrap support was calculated using 100 replicates (heuristic search, stepwise addition, 10 random-sequence addition replicates, TBR branch swapping). BI was performed using the software MrBayes 3.1 (Huelsenbeck and Ronquist, 2001), which employs a Metropolis-coupled, Markov chain Monte Carlo (MC-MCMC) sampling approach. A four-chain MC-MCMC analysis was run twice in parallel (with the default heating values) for 2.1×10^6 generations starting with random trees, and trees were sampled every 100 generations. The model of evolution was the one chosen by Modeltest and used for ML analysis. The log-likelihood values of the sample point were plotted against the generation time, and "burn-in" data sampled from generations preceding the stationarity of the Markov Chain were discarded. Combining the remaining trees, a 50%

majority consensus tree was generated. The frequency of any particular clade of the consensus tree represents the posterior probability of that clade (Huelsenbeck and Ronquist, 2001). Two independent replicates were conducted and inspected for consistency to avoid being trapped on local *optima* (Huelsenbeck and Bollback, 2001). The statistical parsimony network was constructed under the 95% probability criterion using the software TCS 1.1. (Clement et al., 2000). The amount of genetic variation of populations was estimated as haplotype (h) and nucleotide (π) diversity using the software DNASP 4.0 (Rozas et al., 2003).

2.4. Phylogeographic analysis

An analysis of molecular variance (AMOVA) was used to examine the amount of genetic variability partitioned within and among populations using the software Arlequin 3.1 with significances assessed by 10,100 permutations (Excoffier et al., 2005). The genetic structure was investigated through the spatial analysis of molecular variance (SAMOVA) with SAMOVA 1.0 (Dupanloup et al., 2002). This method is based on a simulated annealing procedure aimed at identifying groups of populations that are geographically homogeneous and maximally differentiated in terms of among-group component (F_{CT}) of the overall genetic variance, without the prior assumption of group composition that is necessary for AMOVA. The program was run for 10,000 iterations from each of 100 random initial conditions, and testing all the grouping options (predefined number of groups (K) ranging from 2 to 18). The relationship between genetic differentiation (uncorrected-distance) among populations and their geographical distances (linear distance in km) was evaluated through a Mantel test with 30,000 permutations, as implemented in the software IBDWS 3.15 (Jensen et al., 2005). To provide a geographic representation of genetic discontinuities between populations, we identified the geographic areas associated with a significant genetic change using the methods of Manni et al. (2004) implemented in the software BARRIER 2.2. Following this method, a geometric network connecting all the adjacent sampled populations is computed using Delaunay triangulation, and from the intersection of the medians of each triangle a Voronoï tessellation is obtained. Genetic distances are calculated for all neighboring populations and associated to each edge of the network. We used uncorrected-pairwise genetic distance as a measure of genetic differentiation between populations. Then a number of barriers were identified through the Monmonier's algorithm across the edge of the network associated with higher values of genetic distance between populations. The number of barriers needs to be defined *a priori*. We represented only barriers including values of pairwise genetic differentiation higher than the average calculated on the whole samples.

2.5. Divergence time estimates

Calibrating the molecular clock based on fossils is not possible for *A. bedriagae* due to a lack of such records. However, calibration for the ND4 region is available for several reptiles including Lacertidae. To get a rough approximation of the divergence time for the main phylogroups, we used both the higher and the lower sequence divergence rates proposed by Pinho et al. (2007) for the ND4–tRNA^{Leu} mitochondrial fragment (2.78% and 1.74% per million years, respectively). These evolutionary rates have been estimated from molecular clocks calibrated for *Podarcis* species on independent geological events (Poulakakis et al., 2005). We used a relaxed molecular clock and Bayesian coalescent approach as implemented in beast 1.4 (Drummond and Rambaut, 2006). We performed two different searches under the HKI + G model (previously estimated by Modeltest excluding from the dataset the outgroup sequences) assuming the two ND4 specific divergence rates proposed by Pinho et al. (2007) for lacertids. For each search three independent MCMC analyses 3×10^7 steps long were performed sampling every 1000 generations. Samples from independent runs, which yielded similar results, were combined and convergence of the chains was checked using the program Tracer 1.3 (Rambaut and Drummond, 2004).

3. Results

We obtained a fragment of 848 base pairs (bp) of the mitochondrial ND4 and flanking tRNAs genes from all 94 specimens studied. The ND4, tRNA^{SER}, and tRNA^{LEU} sequences contained no indels. Alignment of tRNA^{HIS} required insertion/deletion in one place among *Podarcis* species sequences employed as outgroup and the ingroup sequences. There were 118 variable sites (13.9% of the total nucleotide positions), of which 95 were parsimony informative. Concerning the ND4 fragment (678 bp), 110 nucleotide positions were variable, of which 27 were in the first, 9 in the second, and 74 in the third codon position. Twenty-two out of 225 amino acid sites (corresponding to 9.78% of the total amino acid sites) were variable. All four outgroup sequences represented unique haplotypes. Including outgroup taxa, 308 nucleotide positions (36.06% of the total nucleotide positions) were variable, and 214 were parsimony informative.

A total of 29 haplotypes were identified (GenBank Accession Numbers: FN667883–FN667911) among the 94 ingroup sequences. The number of observed haplotypes within populations ranged from one (10 populations) to three (four populations). Estimates of haplotype (h) and nucleotide (π) diversity for each population are given in Table 1. The highest values of both haplotype and nucleotide diversity occurred in sample C1. Relatively high values of haplotype and nucleotide diversity occurred also in populations C2, C6, C7. Ten populations showed a complete lack of genetic diversity (C3, C5, S1–S5, S7, S9–S10).

3.1. Phylogenetic analysis and sequence divergence

The MP analysis identified eight equally most parsimonious trees (tree length = 539; consistency index excluding uninformative sites = 0.634; retention index = 0.807). The ML tree was estimated with the best-fit model GTR + G + I (proportion of invariant sites = 0.50; shape parameter α = 1.73; four rate categories). The log-likelihood (−LnL) of the best ML tree obtained was −LnL = 3642.22.

All the phylogenetic trees (MP, ML, and BI) and the statistical parsimony network supported the same phylogenetic grouping of haplotypes (Fig. 1). In particular, the consensus of the MP trees differed from the BI tree only in being less resolved and the ML tree showed only one minor difference of a short terminal branch with respect to the BI tree.

The 29 *A. bedriagae* haplotypes clustered in two main mtDNA clades, namely Lineage A and B, the latter of which can be further subdivided into six (b1–b6) sub-clades with remarkable concordance with geography. Lineage A is geographically restricted to northern Corsica and includes haplotypes from Restonica valley and Ométa populations (samples C2 and C1, respectively). Lineage B is widespread in Corsica and Sardinia and is sub-structured into six closely related haplotype clades consistently distributed from North to South: one (b1) comprising haplotypes from three central Corsica populations and those from Ométa, the second and the third (b2, b3) including haplotypes from three southern Corsica populations, and the last three (b4, b5, b6) grouping haplotypes from North, Central and South Sardinia, respectively. These Sardinian haplotype groups form a statistically supported monophyletic unit (Fig. 1).

The two main lineages, A and B, are supported by very high bootstrap values (Fig. 1) as well as most of their sub-clades, while relationships between sub-clades within lineage B are poorly resolved.

All haplotypes found in one population were exclusive to it, except the haplotype *s3*, which is carried by individuals from three very close populations of North-eastern Sardinia (populations S3, S4, and S5), and the haplotype *s10*, distributed in the southernmost Sardinian populations (populations S10 and S11). Only in the population from Ométa (sample C1) were haplotypes found that were included in different mitochondrial lineages (haplotypes *c1a* and *c1b* in Lineage A; haplotype *c1c* in Lineage B).

The sequence divergence (uncorrected *p*-distance) among haplotypes ranged from 0.13% to 7.64%. The average *p*-distance between Lineages A and B is 6.85%. The within-group average is 0.74% and 2.57% for Lineages A and B, respectively.

The statistical parsimony analysis confirmed the results of the phylogenetic analyses, but it provided more resolution on relationships among haplotypes within sub-clades. The 29 haplotypes, into which the sequences of *A. bedriagae* were collapsed, were grouped into six unconnected networks. The maximum number of mutational steps allowing for a 95% parsimonious connection between haplotypes was estimated at 12. Two ambiguous connections within Network I and II were removed. Networks nearly correspond to phylogenetic clades identified by the MP, ML and BI analyses. Network I corresponds to Lineage A, Network II–VI to Lineage B. However, Network II includes haplotypes clustered in clade b1, b2 and haplotype *c7a* from b3; Network III includes the remaining haplotypes from b3 clade; Networks IV, V, and VI correspond to clade b4, b5, b6, respectively.

3.2. Phylogeographic analysis

The overall differentiation among populations was very high and statistically significant (F_{ST} = 0.89, P < 0.001). The AMOVA revealed that differences among populations accounted for 88.55% of the overall genetic variance observed and differences within population for 11.45%.

The best partitioning of the genetic diversity by SAMOVA was obtained when samples were grouped from two to six groups (see Table 2 and Fig. 2). After this threshold further increase of K would remove one population at a time from groups, thus not producing further informative-clusters. The genetic structure captured by SAMOVA identified the same geographic discontinuities found among haplotype clades delineated in phylogenetic analyses. Moving from K = 2 to K = 6, different levels of SAMOVA closely reflect the geographic associations of haplotypes at different clade levels. With K = 2 the deep partition between northern Corsican populations (samples C1 and C2) and the others (sample C3–C8, S1–S11) corresponding to the two main haplotype lineages (Lineage A and B) is recovered, while with K = 6, populations are grouped in North/Central/South Corsica and North/Central/South Sardinia according to the lowest haplotype-clade level. Population C1, where haplotypes from both Lineage A and B occur, clustered to C2 (where only haplotypes from Lineage A occur) or to other Corsican/Sardinian population groups (where haplotypes from Lineage B occur) in different levels of SAMOVA, reflecting an ambiguous assignment to the main haplotype/population groups. Results of SAMOVA analysis are reported in Fig 2 with different grouping levels, from K = 2 to K = 6, indicated by contour lines. The components of genetic variance explained for each grouping option are presented in Table 2.

Mantel tests revealed a significant correlation between genetic differentiation and geographic distance between populations (r = 0.52; P < 0.001). This correlation increased (r = 0.77; P < 0.0001) when excluding from the analysis the most differentiated populations (sample C1 and C2) to take into account long-term historical divergence among populations as suggested by Telles and Diniz-Filho (2005).

Three areas of relevant genetic discontinuity (α, β, and γ) have been identified by the Monmonier's method with the software BARRIER (Fig. 1). All these barriers are located in northern Corsica and separate populations C2 and C1 from each other and from the remaining populations. In particular, the most significant barrier (α), associated with the highest values of genetic distance between populations, is located south of population C2.

3.3. Divergence time estimates

Considering a divergence rate of 2.74% or 1.78% per million years, the two main mitochondrial lineages found in *A. bedriagae* (Lineage A and B) diverged at 3.68/5.87 mya (95% high posterior density (HPD) interval of 1.72–6.13/2.80–5.49 mya). The most recent common ancestor (TMRCA) of the Lineage A was estimated at 0.53/0.84 mya (95% HPD: 0.18–0.98/0.30–1.54 mya); the TMRCA of the Lineage B was estimated at 1.59/2.53 mya (95% HPD:

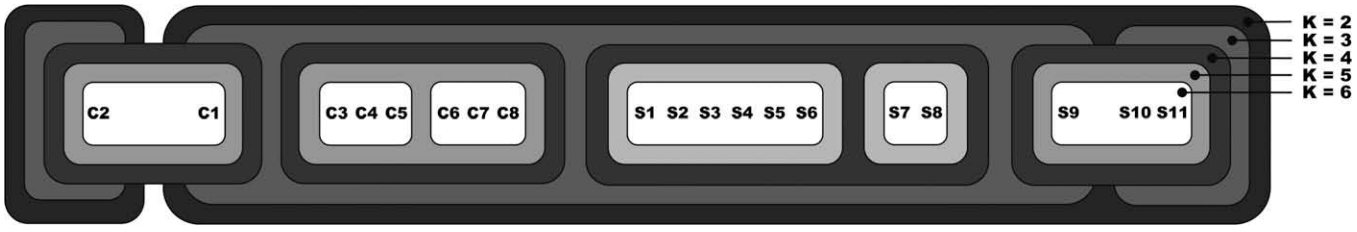

Fig. 2. Group structures defined by the different levels of SAMOVA. Counter lines and polygons identify population clusters derived from SAMOVAs with different pre-defined numbers of clusters (K). See Table 2 for further details.

Table 2
Results from SAMOVA analysis with different pre-defined numbers of groups. Group composition (see also Fig. 2), fixation indices, and associated P values are reported.

Number of groups (K)	Group composition	F_{CT}	F_{SC}	F_{ST}
2	(C2) (C1, C3, C4, C5, C6, C7, C8, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11)	0.600*	0.864*	0.946*
3	(C2) (C1, C3, C4, C5, C6, C7, C8, S1, S2, S3, S4, S5, S6, S7, S8, S9) (S10, S11)	0.518*	0.845*	0.925*
4	(C1, C2) (C3, C4, C5, C6, C7, C8) (S1, S2, S3, S4, S5, S6, S7, S8) (S9, S10, S11)	0.559*	0.779*	0.902*
5	(C1, C2) (C3, C4, C5, C6, C7, C8) (S1, S2, S3, S4, S5, S6) (S7, S8) (S9, S10, S11)	0.624*	0.732*	0.899*
6	(C1, C2) (C3, C4, C5) (C6, C7, C8) (S1, S2, S3, S4, S5, S6) (S7, S8) (S9, S10, S11)	0.667*	0.688*	0.896*

* P < 0.01.

0.98–2.34/1.55–3.73 mya); and the TMRCA of the Sardinian clade was estimated at 1.11/1.77 mya (95% HPD: 0.67–1.62/1.08–2.59 mya).

4. Discussion

The analysis of the mitochondrial DNA genealogies across the entire distribution of *A. bedriagae* revealed a highly structured pattern of geographic differentiation with the occurrence of two main evolutionary lineages, Lineage A restricted to northernmost Corsica and Lineage B widespread all over the remaining range of the species. Almost 90% of the overall mtDNA variation is accounted for by differences among populations. The distribution of haplotypes and their relatedness were geographically structured, with each population carrying exclusive haplotypes (with two slight exceptions in which the same haplotype is shared by neighbouring populations), and with haplotypes clustering with strong correspondence between the clades and their geographical location The genetic differentiation between populations increases with geographic distance following the isolation-by-distance model. The deep phylogeographic break between the two main lineages is placed in Northern Corsica around the Restonica Valley (where haplotypes of Lineage A occur), as revealed by haplotype genealogies and the analysis of putative genetic barriers between populations. Close to Restonica, in the population from Ométa, the syntopic occurrence of haplotypes from both mtDNA lineages A and B suggests the presence of an incomplete phylogeographic barrier between these two lineages occurring from northern inland Corsica to the eastern coast. Based on phylogenetic, Barrier, and SAMOVA analyses, many minor phylogeographic discontinuities exist within Lineage B between Corsican and Sardinian populations and between geographically coherent groups of populations within both islands.

Thus, overall, the pattern of *A. bedriagae* is characterized by two levels of discontinuities in the geographical distribution of mtDNA diversity: (i) the deep phylogeographic cleft *between-lineages* in Northern Corsica (Lineage A and B), and (ii) some minor phylogeographic discontinuities *within-lineages* all over the range of the species, suggesting a complex evolutionary scenario that involves different time-scale processes. According to the sequence divergence between the two lineages and to the two calibrations proposed by Pinho et al. (2007), the first evolutionary event concerning the split between lineages A and B from an ancestral population stock would have likely occurred in the Upper Pliocene (5.87–3.68 mya). The within-lineage divergences would have started from the Upper Pleistocene for Lineages B (2.5–1.6 mya), and from the Lower Pleistocene for the Lineage A (0.84–0.53 mya). These temporal inferences are to be treated with caution given the intrinsic inaccuracy of divergence time estimates based on molecular data (Ayala, 1997; Rodriguez-Trelles et al., 2004; Heads, 2005; Welch and Bromham, 2005). Indeed we considered different available calibrations, with the intention of placing the main events of the evolutionary history of *A. bedriagae* in a relative time frame rather than to obtain exact dating.

Regarding the evolution of the two main lineages, the phylogeographic discontinuity found in Northern Corsica would suggest long-term extrinsic barriers to genetic exchange between two evolutionarily independent population stocks. The finding of well differentiated evolutionary lineages within Corsica has been observed also in the land snail *Solatopupa guidoni* (Caziot, 1903) (Gastropoda Pulmonata) based on allozyme and mtDNA data (Boato, 1988; Ketmaier et al., 2010). Interestingly, also in this species the phylogeographic break between two well differentiated haplogroups is placed in North Corsica, separating inland and coastal populations. As in *A. bedriagae*, in *S. guidoni* the diversification between Corsican lineages pre-dated the origin and the diversification of the Sardinian populations, suggesting a deeper evolutionary history in Corsica than in Sardinia for both species. By contrast, in several other Corsican/Sardinian species found in Corsica and Sardinia the major segregation among groups of populations or related species is placed between islands (e.g. Various Authors, 1983, 1996). This pattern has been evidenced for example in the endemic salamander genus *Euproctus* Gené, 1839 including two vicariant species from Corsica and Sardinia (*E. montanus* (Savi, 1838) and *E. platycephalus* (Gravenhorst, 1829), respectively), or in the lizard *Podarcis tiliguerta* (Gmelin, 1789), which likely represents a species complex with at least two evolutionarily independent lineages from Corsica and Sardinia (Steinfartz et al., 2002; Harris et al., 2005; Vasconcelos et al., 2006). The emerging discordances in phylogeographic patterns of Corso-Sardinian elements indicate that different evolutionary scenarios underlie their shared distribution pattern. Concerning the unusual pattern of diversity found within Corsica for both *S. guidoni* and *A. bedriagae*, it could be tentatively explained by a process of allopatric diversification (Ketmaier et al., 2010), drawing attention to the role and the extent of putative barriers within Corsica in shaping patterns of distribution and differentiation among related species or groups of populations within them. However, it is difficult to identify what determined the prolonged allopatry between population groups in inland Northern Corsica. The occurrence of palaeogeographical barriers can be easily ruled out given the orography and the ancient geological stability of Corsica, suggesting a negligible effect of eustatic sea-level change or of tectonic events in the isolation between regional sets of populations. Given the high altitude of the peaks in North Corsica (2706 m, Monte Cinto), a possible extrinsic barrier to gene flow between the two lineages of *A. bedriagae* could have been represented by glacier development during cold periods. The occurrence of glaciers has been demonstrated for both Restonica and Tavignano valley (near the phylogeographic barrier observed between Lineage A and B) during the Pleistocene since the maximum glaciation, about 400 or 620 ka ago (J. Kuhlemann, pers. comm.), to the last glacial maximum, 19–23 ka ago (Kuhlemann et al., 2005, 2008). However, we can firmly exclude the presence of glaciers in Corsica during the earliest Pleistocene, 2.5–2.0 mya and Pliocene (Muttoni et al., 2003, 2007), and thus reject the hypothesis of their role in the evolution of the two major lineages of *A. bedriagae*. Since *A. bedriagae* has well defined climatic and habitat requirements (Bombi et al 2009a,b), palaeoclimatic and palaeoenviromental settings must be considered when analysing the historical onset of putative extrinsic barriers to the dispersion of this lizard. Unfortunately, detailed information about Pliocene climate and vegetation, such as those gathered with palynological records, are not available for Corsica and Sardinia (A. Bertini, pers. comm.; Favre et al., 2007), and we thus cannot compare the pattern of palaeoenvironment distribution with the observed phylogeographic discontinuities.

Regarding the differentiation observed within the two main lineages, the relationships among haplotypes reflect the geographic ordering of localities where haplotypes were found. A fine analysis of the arrangement of clades belonging to Lineage B in the phylogenetic tree topology reveals a trend of North–South structuring of haplotype groups from central Corsica to South Sardinia (subclades b1–b6). This pattern, coupled with the monophyly of the Sardinian populations contrasting to the paraphyletic arrangement of the Corsican population groups, could be interpreted as the result of a "step-by-step" dispersion process of populations from South Corsica to Sardinia, followed by allopatric differentiation. Nevertheless the branching pattern of the haplotype tree within Lineage B shows very short branches among groups of populations. The hierarchical relationships among clades within Lineage B are not well resolved, and the southern Corsican clade (sub-clade b3) cannot be considered the sister taxon to the Sardinian group (sub-clades b4–b6). Moreover a dispersal hypothesis accounting

for the origin of Sardinian populations is not supported by the fact that we did not recover a clinal distribution of alleles and southward loss of genetic diversity either at mitochondrial (this study) or allozyme levels (Salvi et al., 2009a, 2009b). The observed pattern is instead congruent with an almost simultaneous split among population groups driven by allopatric fragmentation. According to molecular clock estimates, the allopatric divergence between Corsican and Sardinian populations started in the Upper Pleistocene and afterwards (1.7–1.1 mya); the Sardinian ancestral population underwent fragmentation into population groups inhabiting North, Central, and South Sardinia. The peculiar biology of *A. bedriagae* could have played a role in the development of the strong phylogeographic structure resulting from allopatric fragmentation. In fact, as a consequence of its rupiculous habits and its bioclimatic requirements (Bombi et al., 2009a, 2009b), populations tend to be sparsely distributed, with small local effective population sizes, leading to strong genetic-drift effects (Patton and Feder, 1981).

The evolutionary scenario underlying the within Lineage B pattern of mtDNA variation is consistent with allozyme data reported in Salvi et al. (2009b). The overall pattern of genetic structure of *A. bedriagae* based on allozyme data is characterized by high polymorphism and slight differentiation among populations, which is congruent with a Pleistocenic divergence among them, driven by allopatric fragmentation (Salvi et al., 2009b).

On the other hand, allozyme and mitochondrial data show a noteworthy discrepancy concerning the major partition into two lineages inferred from mtDNA but not evidenced by allozymes. The allozyme dataset included the same Sardinian populations employed in the present study (sample S1–S11) but only two populations from Corsica, one from the South (sample C7) and the other one from the Restonica Valley in the Northern inland (sample C2). This latter population belongs to a different mtDNA lineage than do the Southern Corsican and Sardinian populations, while appearing slightly differentiated from those populations in analyses of allozyme variation (average Nei (1972) standard genetic distance = 0.022; Salvi et al., 2009b). Although, on average, allozymes require much more time than mtDNA to allow the alleles (or their frequencies) to diverge appreciably, given the estimated time elapsed since the split of the two mtDNA lineages, we would expect some differentiation between these two lineages to occur based on analysis of allozyme variation. The discordance between allozyme and mtDNA can be explained by several processes including differential introgression between nuclear and mitochondrial markers following the secondary contact between the two lineages (Barton and Jones, 1983; Ballard and Whitlock, 2004). However, data on genetic diversity of northernmost Corsican populations are needed to assess the extent and the magnitude of introgressive phenomena for both markers as well as on the occurrence and placement of the hybrid zone between the two evolutionary lineages found in *A. bedriagae*.

Finally, the deep phylogeographic breaks observed in unrelated organisms in Northern Corsica is an issue that certainly deserves further investigation, including phylogeographic data on additional Corsican animal and plant species, to shed light on the biogeographic and evolutionary processes underlying the origins of the observed patterns of diversity in Corsica.

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