

## Unexpected phylogeographic affinities of *Psammodromus algirus* from Conigli islet (Lampedusa)

MIGUEL A. CARRETERO<sup>1</sup>, ANNA PERERA<sup>1</sup>, PIETRO LO CASCIO<sup>2</sup>, CLAUDIA CORTI<sup>3</sup>, D. JAMES HARRIS<sup>1</sup>

<sup>1</sup> CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal. Corresponding author. E-mail: carretero@mail.icav.up.pt

<sup>2</sup> Associazione "Nesos", Via Vittorio Emanuele 24, I-98055 Lipari (ME), Italy.

<sup>3</sup> Museo di Storia Naturale dell'Università di Firenze, Sezione di Zoologia "La Specola", Via Romana 17, I-50125 Firenze, Italy.

**Abstract.** The only Italian population of the lacertid *Psammodromus algirus* is found in Conigli islet whereas the species is absent from the nearby island of Lampedusa. The phylogeographic relationships of this population were investigated. Mitochondrial DNA (12S rRNA and 16S rRNA) fragment sequences were analysed and compared with already published sequences from the whole species range. In all the analyses, the sample from Conigli grouped with those from Morocco and not with the closer Tunisian ones. Such surprising result poses serious doubts to the traditional interpretation of the enigmatic distribution pattern of this species in Italy suggesting a recent colonisation of the islet from NW Africa, probably human-mediated, rather than a land crossing from Tunisia during the Pleistocene.

**Keywords.** Phylogeography, *Psammodromus algirus*, Lacertidae, Conigli islet.

---

### INTRODUCTION

The Large *Psammodromus*, *Psammodromus algirus*, is a medium-sized lacertid with ground-dwelling habits extensively distributed on both sides of the Western Mediterranean Basin (Fig. 1). Throughout its range, this generalist species is usually very common (Carretero et al., 2002; Sindaco, 2006) constrained only by the availability of some plant cover (Carretero and Llorente, 1997). However, in Italy, the species is restricted to the small islet of Conigli (Lampedusa, Pelagian Islands), where it occupies an area of 3 ha covered by nitrophilous vegetation. Since its discovery (Zavattari, 1954), the presence of this population has remained biogeographically unexplained since the islet is separated only by a channel of 30 m width and 1.5 m depth from the main island of Lampedusa where the species is completely absent (Sindaco, 2006). Distance to the closest African population on the Tunisian coast is 130 km. Recent literature tentatively invokes historical

changes in the landscape and predators due to human introductions to justify the absence from Lampedusa (Corti and Lo Cascio, 2002).

The species seems, in fact, a poor islet colonizer (Carretero et al., 1993), at least in comparison with *Podarcis* sp. (Corti et al., 1999; Poulakakis et al., 2003; Harris et al., 2005). However, phylogeographic evidence indicates that transmarine colonisations in this species are not unrealistic (Carranza et al., 2006). Using mitochondrial markers, a recent study (Carranza et al., 2006) detected strong phylogeographic structure in the species. Two clades separated by 3.6 My, one in East Iberia and the other in West Iberia and North Africa, were detected. Separation between Iberia and North Africa (1.9 My) indicates a crossing of the Gibraltar Strait, after the refilling of the Mediterranean at the end of the Messinian (5.6 My, Hsü et al., 1977). Moreover, within North Africa, the separation between the populations from Morocco and Tunisia is estimated to be 1.5 My (Carranza et al., 2006).

In this context, here we aim to assess the phylogeographic affinities of the Conigli population through comparison with the lineages previously described using mtDNA sequence data in order to determine the origin of this colonization.

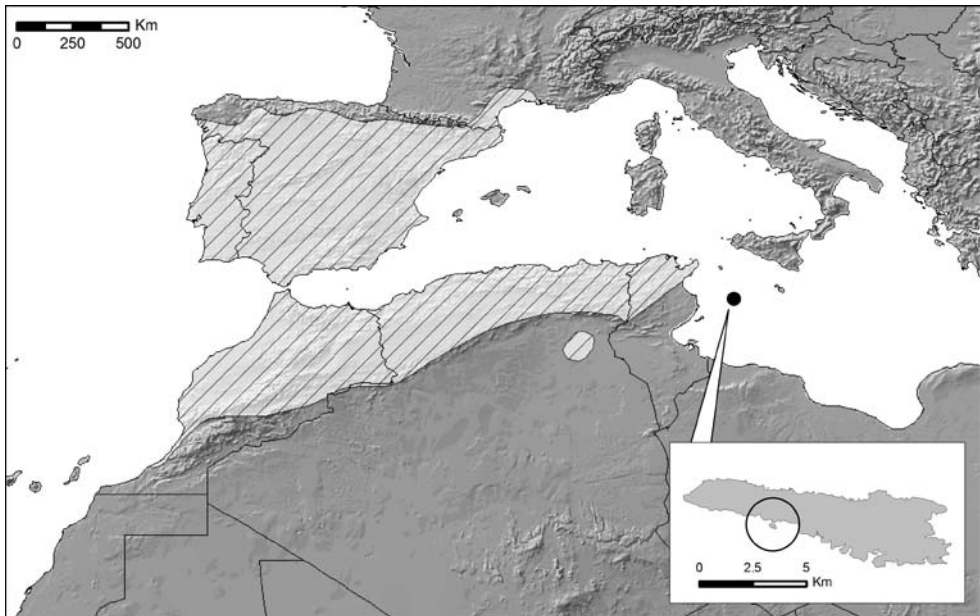
## MATERIAL AND METHODS

A juvenile specimen of *P. algirus* was collected from Conigli islet (35°30'37"N, 11°33'30"E, Fig. 1) in September, 2005. Once digital photographs were taken and a tail tip was collected, the lizard was released in the site of capture. In the lab, total genomic DNA was extracted using standard methods, following Harris et al. (1998). Polymerase Chain Reaction primers used in both amplification and sequencing were 12Sa and 12Sb for the 12S rRNA gene and 16SL and 16SH for the 16S rRNA gene (Kocher et al., 1989). Amplification conditions were the same as described by Harris et al. (1998). Amplified fragments were sequenced using a 310 Applied Biosystem DNA Sequencing Apparatus following the manufacturer's protocols. Mitochondrial DNA sequences were aligned by eye. Aligned sequences were 901 base pairs long. GenBank accession numbers of new sequences are FJ799792 (16S rRNA) and FJ799793 (12S rRNA).

The data were imported into PAUP\* v. 4.0b10 (Swofford, 2002) for phylogenetic analysis. Both Neighbour Joining (NJ) and Maximum Parsimony (MP) were used to estimate relationships. Representatives of all major lineages found by Carranza et al. (2006) were included. *Psammodromus blanci*, *P. h. hispanicus* and *P. h. edwardsianus* were included as outgroups. For MP a 10 replicate heuristic search was used. In both cases support for nodes was estimated using the bootstrap technique (Felsenstein, 1985) with 1000 replicates. Additionally relationships were estimated in a Bayesian framework, using MrBayes v. 3.1.2 (Huelsenback and Ronquist, 2001), with  $1 \times 10^6$  generations using a general-time-reversible model of evolution with a gamma model of among site rate variation and an estimated proportion of invariable sites. After the burning period remaining trees were used to estimate posterior nodal probabilities.

## RESULTS

Including the outgroups, 31 combined mtDNA sequences were analyzed. MP analysis found 5826 equally parsimonious trees of 234 steps. All estimates of relationships differed only at nodes without strong support (Fig. 2). All analyses recovered the sample from

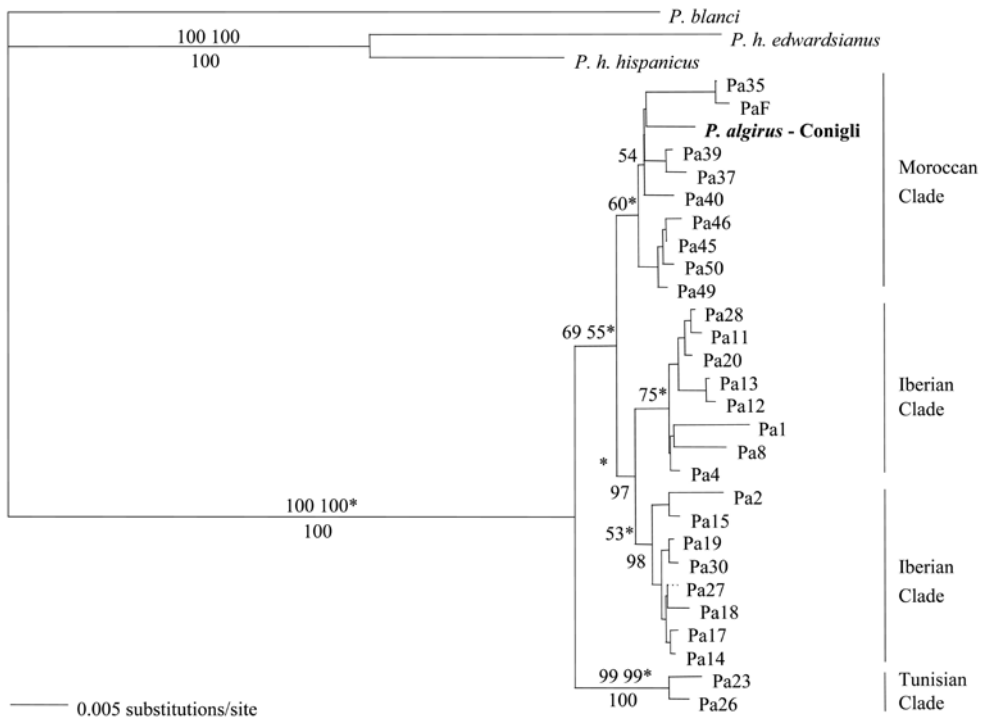


**Fig. 1.** Map showing distribution range of *P. algirus* in shade (after Miras et al., 2006). In the square, Lampedusa island with Conigli islet marked with a circle.

Conigli islet as a branch within a group otherwise comprised of samples from Morocco. Relationships between major clades differed slightly from those proposed by Carranza et al. (2006) probably due to the weak resolution of these nodes.

## DISCUSSION

Although only a single specimen was sequenced, the two different mitochondrial markers undoubtedly indicate a phylogeographic affinity with the Moroccan populations. Such result not only allow ascribing the Conigli population to the nominal subspecies (*Terra typica*: 'Mauritania' Linnaeus, 1758) but also poses serious doubts on the traditional interpretation of the enigmatic distribution pattern of *P. algirus* in the Pelagian archipelago. Certainly, the bathymetry indicates that Lampedusa was connected with Tunisia during the last glacial maximum (Masseti, 2002) and the small islet of Conigli seems to have been united to Lampedusa until the Roman time (Corti and Lo Cascio, 2002), so that both could have easily been colonised by land from Tunisia during the Pleistocene. The absence of the species from Lampedusa was usually attributed to the degradation of the vegetation by imported herbivores together with the probable increase of predation pressure derived from the introduction of saurophagous snakes of the genera *Malpolon* and *Macroprotodon* (Corti and Lo Cascio, 2002), both absent in Conigli. However, in other regions of its distribution range, *P. algirus* occurs in habitats with low



**Fig. 2.** Tree derived from NJ analysis, based on partial 12S rRNA and 16S rRNA sequences. Sample from Conigli islet in bold, all others from Carranza et al. (2006). Nodes found in the strict consensus of 5826 equally parsimonious trees are indicated with an \*. Bootstraps from NJ and MP are given above, and Bayesian probabilities below the nodes.

plant cover and coexists with those snakes. Conversely, the unexpected phylogeographic results obtained here suggest a more recent colonization from a distant area (NW Africa), probably human-mediated. Although infrequent, there is at least one case of introduction reported for Mallorca, Balearic Islands (Masius, 1999; Vicens, 2005). This evidence, does not support the previously accepted theory of extinction, but makes it more feasible that the species had never existed on Lampedusa (Sindaco, 2006). Further analyses of fossil remains could confirm or discard this last hypothesis. In conservation terms, the previously suggested introduction of this species in the main island (Padoa-Schioppa and Massa, 2001) is, hence, not recommended.

#### ACKNOWLEDGEMENTS

The lab work was funded by the project POCI/BIA-BDE/55865/2004; M.A.C. and A.P. held the post-doctoral grants SFRH/BPD/27025/2006 and SFRH/BPD/26546/2006, all from Fundação para a Ciência e a Tecnologia, FCT (Portugal). Fieldwork funding and collecting permits were pro-

vided by Riserva Naturale Orientata "Isola di Lampedusa" (management Legambiente). Thanks are especially due to Giuseppina Nicolini (Legambiente) for her enthusiastic support and to Vasco Batis-ta for field assistance.

## REFERENCES

- Carranza, S., Harris, D.J., Arnold, E.N., Batista, V., González de la Vega, J.P. (2006): Phylogeography of the lacertid lizard, *Psammodromus algirus*, in Iberia and across the Strait of Gibraltar. *J. Biogeog.* **33**: 1279-1288.
- Carretero, M.A., Bosch, M., Pedrocchi, V. (1993): Nuevos datos herpetológicos de la Meda Gran (Islas Medes, Girona). *Bol. Asoc. Herpetol. Esp.* **4**: 9-11.
- Carretero, M.A., Llorente, G.A. (1997): Habitat preferences of two sympatric lacertids in the Ebro Delta (NE Spain). In: *Herpetologia Bonnensis*, p. 51-62. Böhme, W., Bischoff, W., Zeigler, T., Eds, Societas Europaea Herpetologica, Bonn.
- Carretero, M.A., Montori, A., Llorente, G.A., Santos, X. (2002): *Psammodromus algirus*. In: *Atlas y Libro Rojo de los Anfibios y Reptiles de España*, p. 259-261. Pleguezuelos, J.M., Márquez, R., Lizana, M., Eds, Dirección General de Conservación de la Naturaleza-Asociación Herpetológica Española, Madrid.
- Corti, C., Lo Cascio, P. (2002): Anfibi e Rettili. In: *Storia naturale delle isole Pelagie*, p. 79-84. Corti, C., Lo Cascio, P., Masseti, M., Pasta, S., Eds, L'Epos, Palermo.
- Corti, C., Masseti, M., Delfino, M., Pérez-Mellado, V. (1999): Man and herpetofauna of the Mediterranean islands. *Rev. Esp. Herpetol.* **13**: 83-100.
- Felsenstein, J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.
- Harris, D.J., Arnold, E.N., Thomas, R.H. (1998): Relationships of the lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proc. R. Soc. B-Biol. Sci. Lond.* **265**: 1939-1948.
- Harris, D.J., Pinho, C., Carretero, M.A., Corti, C., Böhme, W. (2005): Determination of genetic diversity within the insular lizard *Podarcis tiliguerta* using mtDNA sequence data, with a reassessment of the phylogeny of *Podarcis*. *Amphibia-Reptilia* **26**: 401-407.
- Hsü, K.J., Montadert, L., Bernoulli, D., Bianca, C.M., Erickson, A., Garrison, R.E., Kidd, R.B., Mélières, F., Müller, C., Wright, R. (1977): History of the Mediterranean salinity crisis. *Nature* **267**: 399-403.
- Huelsenback, J.P., Ronquist, F. (2001): MR-BAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754-755.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989): Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Nat. Acad. Sci. USA* **86**: 6196-6200.
- Masius, P. (1999): First record of *Psammodromus algirus* on Mallorca Island. *Die Eidechse* **10**: 64.
- Masetti, M. (2002): Inquadramento, paleontologico, archeozoologico e paleobiogeografico. In: *Storia naturale delle isole Pelagie*, p. 27-30. Corti, C., Masseti, M., Pasta, S., Eds, L'Epos, Palermo.

- Miras, J.A.M., Cheylan, M., Nouira, M.S., Joger, U., Sá-Sousa, P., Pérez-Mellado, V. (2006): *Psammodromus algirus*. In: 2007 IUCN Red List of Threatened Species. IUCN. [www.iucnredlist.org](http://www.iucnredlist.org).
- Padoa-Schioppa, E., Massa, R. (2001): Possibile effetto della predazione di ofidi sull'abbondanza e sulla taglia media dei Sauri di Lampedusa. *Nat. Sicil.* **25** (suppl.): 99-110.
- Poulakakis, N., Lymberakis, P., Antoniou, A., Chalkia, D., Zouros, E., Mylonas, M., Valakos, E.D. (2003): Molecular phylogeny and biogeography of the wall-lizard *Podarcis ehrardii* (Squamata: Lacertidae). *Mol. Phyl. Evol.* **28**: 38-46.
- Sindaco, R. (2006): *Psammodromus algirus*. In: Atlante degli Anfibi e dei Rettili d'Italia / Atlas of Italian Amphians and Reptiles, p. 504-507. Sindaco R., Doria, G., Razzetti, E., Bernini, F., Eds, Societas Herpetologica Italica, Polistampa, Firenze.
- Swofford, D.L. (2002): PAUP\*: Phylogenetic Analysis Using Parsimony (and other methods) 4.0.b10. Sinauer Associates, Sunderland, Massachusetts, USA.
- Vicens, P. (2005): Sobre la presència de *Psammodromus algirus* Linnaeus, 1759 (Sauria, Reptilia) a Mallorca. *Boll. Soc. Hist. Nat. Balears* **48**: 109-112.
- Zavattari, E. (1954): Rinvenimento di *Psammodromus algirus* nell'Isola dei Conigli di Lampedusa. *Boll. Zool.* **21**: 93-98.