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Rapid Communication

Alien but not that one: a molecular reassessment of the Alborán Island's *Saurodactylus* Fitzinger, 1846 gecko

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Abstract

Alborán Island is located in the centre of the Alboran Sea in the Western Mediterranean, between the Iberian Peninsula and North Africa. Due to its geostrategic position and rich fishing grounds, this island has been frequently visited by humans throughout history from both coasts of the Alborán Sea, leading to the accidental introduction of many reptile species. However, during one field expedition a few specimens morphologically identified as *Saurodactylus mauritanicus* geckos were found at several areas of the island and considered as native to the region. This classification carried several nomenclature and conservation consequences to both species and Alborán Island. To clarify the taxonomic identification of the specimens found in Alborán and to evaluate its alien status in the island, we assessed its phylogenetic relationships among all *Saurodactylus* taxa under a mitochondrial genetic approach. We found that the sequenced specimen from Alborán, clusters with individuals of the *S. fasciatus* clade, instead of *S. mauritanicus*. Hence, this study does not support the proposed nomenclature change, neither the species nor the island's conservation status based solely on *S. fasciatus* from Alborán.

Key words: phylogeny, conservation, mtDNA, reptile, human introduction

Introduction

The deliberate or accidental introduction of new species to areas outside their native range has been taking place over millennia. The reshuffling of species ranges is in some cases so great, that it becomes uncertain what is the geographic origin of the introduced taxa. Fortunately, the routine procedure of molecular tools has led to a shift from a purely phenotypic approach to the inclusion of molecular characters in the investigation of the evolutionary history, and invasion pathways of taxa (e.g., Rato et al. 2021a, b; Silva-Rocha et al. 2015).

The Mediterranean region, in particular, has one of the longest histories of human settlements and trade, as well as of interaction between humans and biodiversity, with multiple biological introductions occurring over

thousands of years (Blondel et al. 2010). In that sense, and at least for reptiles, humans have been key drivers for their dispersal on Mediterranean islands (Silva-Rocha et al. 2019). However, their distributions are determined by a complex interplay between human activities, geographic factors and species features (Silva-Rocha et al. 2019).

Although largely sedentary, reptiles are frequently introduced by humans during transport of building material, soil or cultivated plants (Kraus 2009). Islands, in particular, are not hosting a large number of alien reptiles (Pitt et al. 2005), which are among the most successful and abundant vertebrates in small islands (Novosolov et al. 2016). Moreover, small islands appear to be more prone to be successfully invaded than larger land masses (references in Kraus 2009). Besides, insular regions where biota have evolved within simplified ecosystems with few biological interactions, are especially prone to appearance and extinction of endemic taxa, while invasion episodes are often repeated (Whittaker and Fernández-Palacios 2007).

The Alborán Island (35°56′24″N; 3°02′04″W) is located in the centre of the Alborán Sea Western Mediterranean, 85 km from Punta Entinas (Spain) and 55.5 km from Cabo Tres Forcas (Morocco), the two closest continental shores in the enclave (Figure 1). This small volcanic island is only 7.12 ha, with 15 m of maximum altitude, 605 m long and 265 m wide. The emerged part of the island is from the late Early Pleistocene (800,000 years) but the volcanic base is from the Middle-Late Miocene (7–18 Mya). It is surrounded by sea depths of 1,000–2,000 m and has not been connected to the mainland since the Messinian Salinity Crisis, 5–6 Mya (Yus et al. 2013).

Due to its geostrategic position and rich fishing grounds, this island has been frequently visited throughout history by fishermen, pirates, explorers and travellers from both coasts of the Alborán Sea. Hence, the island has maintained an intermittent human presence. Since 1936, the human presence in Alborán has been mainly military and, after several periods of absence (1939–1941, 1964–1967 and 1992–1997), the Alborán Military Detachment became permanent on the island since 1997. However, during the last absence period there were assiduous visits, dockings and repeated landings of ships coming, among other places, from North Africa (Paracuellos et al. 2005; Rubio 2001). Furthermore, in 1997 the enclave became protected as a Marine Reserve and Fishing Reserve, and since 2003 as Natural Area. Such events have meant that, to this day, there are constant movements of cargo shipments between Alborán and, mainly, the south of the Iberian Peninsula transporting personnel, building materials and equipment (Acosta 1996; Cano et al. 2006; Rubio 2001; Salvator 1898; Sola et al. 2006). Not surprisingly, a recent molecular study identified southern Iberia as the source area of the introduced lizard populations of *Tarentola mauritanica*, *Hemidactylus turcicus* and *Podarcis vaucheri* in Alborán Island (Rato et al. 2021a).

Prior to the finding of these introduced reptile species, a few specimens, morphologically identified as the gecko *Saurodactylus mauritanicus,* were recorded under rocks at the northern and southern parts of the island (Barbadillo et al. 1999; Mateo 2002a). These records occurred in 1997 coinciding with the final of the last period when Alborán Island was unoccupied and frequently visited by boats coming from North Africa, suggesting that the introduction was recent (Paracuellos et al. 2005). However, the authors responsible for the discovery of these specimens (1) considered it as native to the island; (2) classified it as threatened and vulnerable to extinction in Andaluzía; (3) proposed a change of its common name from "Maghreb gecko" to "Alborán gecko"; and, finally (4) classified Alborán as an important conservation area in Spain, given that it is the only place in Europe where this reptile species is present (Mateo 2001, 2002a, b). Currently, *Saurodactylus* is considered extinct due to the lack of observations during all the intensive and numerous surveys of the island before and after 1997 to date, which instead detected other alien lizards (Paracuellos et al. 2005; M. Paracuellos *pers. com*.).

Geckos of the genus *Saurodactylus*, an extremely old taxon belonging to the Family Sphaerodactylidae (Gamble et al. 2011), are endemic to the Western Maghreb region, encompassing Morocco and Algeria (Figure 1). Before the study from Javanmardi et al. (2019), the genus was composed of three species: *S. mauritanicus* (Duméril and Bribon, 1836), *S. fasciatus* Werner, 1931 and *S. brosseti* Bons and Pasteurs, 1957. However, based on molecular markers and colouration Javanmardi et al. (2019) split the later into five species: *S. brosseti*, *S. elmoudenii*, *S. slimanii*, *S. harrisii*, and *S. splendidus*, resolving the *S. brosseti* species complex. *Saurodactylus mauritanicus* occurs in North-East Morocco and Western Algeria, while all remaining species of the genus have an endemic Moroccan and Western Sahara distribution (Bons and Geniez 1996).

To clarify the taxonomic identification of the specimens found in Alborán Island and to evaluate the alien status in the island, we assessed its phylogenetic relationships among all *Saurodactylus* taxa using a genetic approach.

Materials and methods

DNA extraction, amplification and sequencing

Two adult specimens identified in the field as *Saurodactylus mauritanicus*, collected in 1997 in Alborán Island (Figure 1) and preserved in 96% ethanol, were examined. Because of the importance of these findings, voucher specimens are currently deposited in loan in the herpetological collection of CIBIO-InBIO, available for examination.

Considering the age of the preserved specimens, genomic DNA was extracted from tail muscle using the DNAeasy Qiagen® extraction kit, following the manufacturer's protocol.

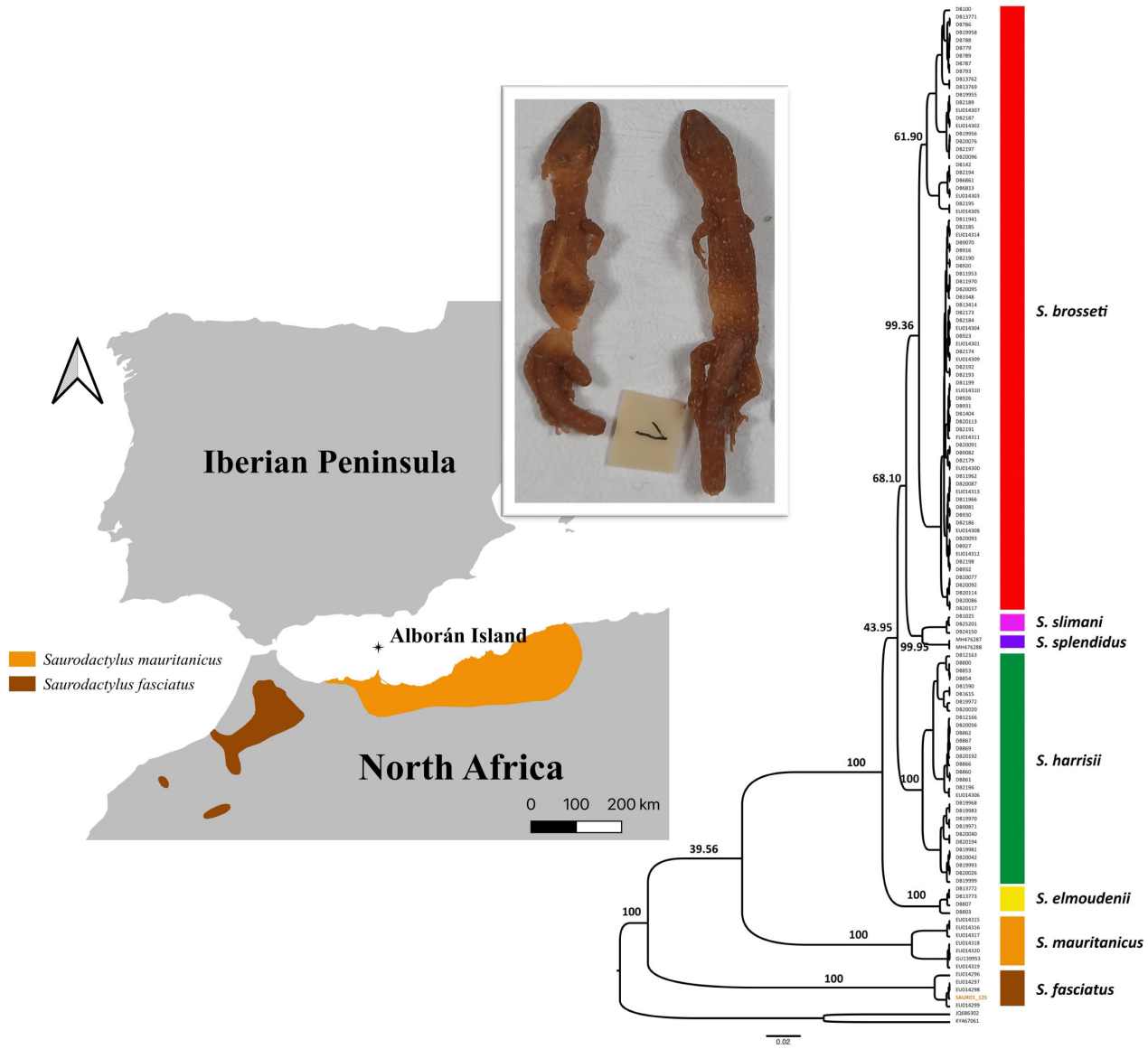

Figure 1. Inset map depicting the geographic location of Alborán Island, and the distribution range of both *Saurodactylus mauritanicus* and *S. fasciatus*. The figure with the two specimens corresponds to the original *Saurodactylus* sp. individuals collected in the island in 1997 (Carlos Bazaga Miras leg.), used in this study. On the right is represented the 12SrRNA mitochondrial phylogenetic topology under a Bayesian inference approach (BEAST), using *Quedenfeldtia moerens* as outgroup. The *Saurodactylus* specimen used in this study is highlighted in orange nesting within the *S. fasciatus* clade. Values next to the nodes correspond to posterior probabilities.

This was followed by the amplification of the 12SrRNA gene fragment by Polymerase Chain Reaction (PCR), using the primers 12Sa/12Sb from Kocher et al. (1989). PCR conditions were the same as those described in Harris et al. (1998). After several repeated trials, Sanger amplification was only successful for one of the specimens, which will be the one used in the following analyses (GenBank assession number will be provided after acceptance of the manuscript).

Gene genealogies

Additional 12S fragments assigned as *S.mauritanicus*, *S. brosseti* sensu latu and *S. fasciatus* were downloaded from GenBank (Javanmardi et al. 2019;

Rato and Harris 2008; Rosado et al. 2017), as well as two 12S sequences from *Quedenfeldtia moerens*, another member of the Sphaerodactylidae family (Barata et al. 2012; Harris et al. 2017). The obtained sequences were imported into the software Geneious Prime v.2022.0.1 (https://www.geneious.com) where alignment was performed with MAFFT v.7.017 (Katoh and Standley 2013), under the default parameters (auto algorithm; scoring matrix = 200PAM/k = 2; gap open penalty = 1.53; and offset value = 0.123).

To determine the genetic affinity of Alborán's *Saurodactylus* specimen, a phylogenetic analysis was carried out under a Bayesian Inference (BI) methodology. To build this genealogy, the sequences of *Q. moerens*, were used as outgroup.

To determine the best fitting nucleotide model to be applied in the BI analysis, the software PartitionFinder v.1.1 was used (Lanfear et al. 2012). Parameters used were *branchlengths* = linked and *modelselection* = BIC.

The BI analysis was performed with the software BEAST v.1.10.4 (Suchard et al. 2018), running the dataset twice for 10×10^6 generations with a sampling frequency of 1,000 generations. Models and prior specifications applied were as follows (otherwise by default): GTR substitution model; Strict Clock; Coalescent with Constant Population Size; random starting tree. Convergence for all model parameters was assessed by examining trace plots and histograms in Tracer v.1.7.1 (Rambaut et al. 2018) after obtaining an effective sample size (ESS) > 200. The initial 10% of samples were discarded as burn-in. Runs were combined using LogCombiner, and maximum credibility trees with divergence time means and 95% highest probability densities (HPDs) were produced using Tree Annotator (both part of the BEAST package). Trees were visualized using FigTree v.1.4.4 (Rambaut 2009).

Results and discussion

The specimen found and collected in Alborán island clusters with the *S. fasciatus* clade (Figure 1), matching in 100% with the closest sequences (EU014297, EU014298) in terms of nucleotide base pairs.

The obtained phylogenetic topology coincides almost entirely with the ones obtained in previous studies (Javanmardi et al. 2019; Rato and Harris 2008); *S. fasciatus* occupies a basal position, with *S. mauritanicus* and the remaining members of the *S. brosseti* species complex as sister taxa. As we only used a single mitochondrial gene fragment, the relationships among the *S. brosseti* species complex are different from Javanmardi et al. (2019), and some of the nodes not that highly supported: the splitting of *S. harrisi* (43.95), and the divergence of *S. slimani* and *S. splendidus* from the remaining taxa (68.10). Nevertheless, the assessment of the phylogenetic relationships within all the *Saurodactylus* genus is not the scope of this study, but the genetic assignment of the individuals found in Alborán island.

Considering the geographic range of each species, it is reasonable to think that the *Saurodactylus* specimens found in Alborán could belong to *S. mauritanicus* and were allochthonous. Moreover, apart from *S. brosseti*, which clearly differs morphologically from the other species in terms of head shape, pholidosis and colour pattern, all remaining taxa have a considerable similar morphology (Bons and Geniez 1996; Schleich et al. 1996). Nevertheless, a morphological examination of the two specimens (in poor preservation state), indicates a temporal band with a white edge, apart from 48–50 scales between the eyes, which are both more compatible with *S. fasciatus* and not with *S. mauritanicus* (Schleich et al. 1996).

Moreover, the taxonomic result obtained in this study, raises the question that the discovered *Saurodactylus* specimens analysed here could have been collected from its natural distribution and then mislabelled. This is very unlikely, since the collector of the specimens (Carlos Bazaga, nationality Spanish) was a customs ship pilot, who had no jurisdiction to disembark in a foreign country, such as Morocco. Contacted by the authors, he declared he usually moved through the Alboran Sea, so apart from Alborán Island, the specimen could only have caught in either Melilla or Chafarinas, but in these islands, the recorded species is *Saurodactylus mauritanicus* (Mateo 1997; Vargas and Antúnez 1982). In the remote hypothetical case that the specimens were captured there, it would also be an introduced species.

Overall, the result obtained here is quite unexpected carrying with it conservation implications. When Barbadillo et al. (1999) reported the *Saurodactylus* specimens in Alborán island, apart from identifying it as *S. mauritanicus*, they interpreted the arrival of this species as ancient and naturally driven, considering it as native to the island. These assumptions led to a series of conservation and nomenclature proposals, such as the classification of the species as vulnerable in Spain and the change of the common name from "Maghreb gecko" to "Alborán gecko", which in turn supported the consideration of Alborán Island as an important conservation area to the herpetology in Spain (Mateo 2001, 2002a, b).

According to the current genetic results, not only we confirm that the specimens from Alborán belong to *S. fasciatus*, but given the high genetic similarity with the closest genetic sequences, the possible arrival to the island is recent and likely human-mediated. Indeed, it could have taken place between 1992 and 1997 when Alborán was abandoned by the Spanish military detachment and illegal ship traffic was common between North Africa (Paracuellos et al. 2005; Rubio 2001).

Hence, this study does not support the proposed nomenclature change, neither the species nor the island's conservation status based solely on *S. fasciatus* from Alborán, something already proposed by Paracuellos et al. (2005). Whatever the case, this is another example suggesting that native, alien or absent biodiversity should be carefully evaluated based on multiple sources of evidence. Other doubtful findings in the biogeographically complex Mediterranean Basin may be still awaiting proper analysis.

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Authors' contribution

Catarina Rato: Conceptualization, Methodology, Software, Validation, Formal analysis, Writing – Original Draft, Writing – Review and Editing, Project administration, Funding acquisition. Lekshmi B Sreelatha: Laboratory Work, Writing – Review and Editing. José A. Mateo: Sample collection, Writing – Review and Editing. Miguel A. Carretero: Conceptualization, Writing – Review and Editing, Project administration, Funding acquisition.

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