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Molecular analysis confirms Madeira as source for insular and continental introduced populations of *Teira dugesii* (Sauria: Lacertidae)

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Biological invasions are major threats to biodiversity worldwide. Species are translocated from their original regions of distribution to new environments at increasing rates, with a fraction of them having the potential of becoming harmful to native biota (SIMBERLOFF et al. 2013). Reptiles are amongst the groups more often introduced and, at the same time, more threatened by introductions (KRAUS 2009). The frequency and impact of reptile invasions are particularly important in island ecosystems where native species may have lost abilities for competing, eluding predators, and handling disease due to their evolution under insular conditions (WHITTAKER & FERNÁNDEZ-PALACIOS 2007, NOVOSOLOV et al. 2012). However, even if much rarer, cases where insular reptiles have been introduced to either the mainland or other islands have also been reported (NICHOLSON et al. 2005, KOLBE et al. 2007 and references therein). In any case, the assessment of the origin of populations of alien species is essential for inferring dispersal pathways and setting up conservation policies aimed at preventing new introductions.

Teira dugesii (MILNE-EDWARDS, 1829) (Sauria: Lacertidae) is a lacertid lizard endemic to the Madeira Archipelago (Portuguese Atlantic Islands), being present on Madeira, Porto Santo, Ilhas Desertas, and Ilhas Selvagens (JESUS 2008). It inhabits a variety of habitats, where beaches, open lands, woodlands and, more rarely, laurel forest are converted into recreational resorts, picnic areas and housing complexes, ranging from sea level to the top of the mountains (SADEK 1981, COCK 1983), and even occurring in the intertidal area (WAGNER 2001). BISCHOFF et al. (1989), based on both morphological characters and allozyme

data, proposed the existence of three subspecies, which was a notion later corrected because of a lack of congruence between morphological and genetic data (BREHM et al. 2001, 2003a, b). JESUS (2008) later concluded that *Teira d. dugesii* (MILNE-EDWARDS, 1829) would be native to Madeira, *T. d. mauli* (MERTENS, 1938) to Desertas Islands, *T. d. jogeri* (BISCHOFF, OSENEGG & MAYER, 1989) to Porto Santo, and *T. d. selvagensis* (BISCHOFF, OSENEGG & MAYERS, 1989) to Selvagens Islands.

Porto Santo might have been the oldest place of colonization from the West Africa; from here the taxon radiated to Madeira; then this larger island was the source for the lizard to arrive firstly on the Desertas and finally on the Selvagens around 1.00–0.75 million years ago (JESUS et al. 2005).

Over the last decades, this species was reported as introduced to the Azores (ULSTRAND 1961, MALKMUS 1984), Lisbon (SÁ-SOUSA 1995) and, more recently, to the Canary Islands (MATEO et al. 2011, LÓPEZ-DOS SANTOS et al. 2013). In the Azores archipelago, it is known from all the islands inhabited by humans (JESUS 2008). In Lisbon, a reproductive population was first observed in 1992 (SÁ-SOUSA 1995), near Alcântara, and the population currently persists but is still constrained to that area (SÁ-SOUSA pers. obs.). As far as the Canary Islands are concerned, this species was first recorded from La Orotava, Tenerife, in the beginning of the 20th century (BOULENGER 1920). However, a lack of observations during more than one century suggested that this population might be extinct (MATEO et al. 2011). In 2011, an adult male was found in the city of Las Palmas, Gran Canaria, and an extensive survey in 2012 revealed a

population comprising not only adults, but also juveniles still existed (LÓPEZ-DOS SANTOS 2013) and still persisted in June of 2014 (M. A. CARRETERO and B. FARIÑA pers. obs.).

In this work, we use phylogenetic analyses to assess the putative origin of the Lisbon, Azorean, and Canarian populations. The identification of the origin of these three introduced populations is expected to provide insights into the invasion pattern of this species.

Three samples of *T. dugesii* were collected in Alcântara, Lisbon (38°42'12.096" N, 9°9'59.544" W), three samples on Santa Maria Island, Azores Archipelago (36°58'44.73" N, 25°6'22.50" W), and two samples in Las Palmas city, Gran Canaria, Canary Islands (38°42'12.096" N, 9°9'59.544" W). Total genomic DNA was extracted from tail tissue following the standard saline method (SAMBROOK et al. 1989). We amplified both fragments of the mitochondrial gene *cyt-b* and of the ribosomal gene 12S for all the samples. A fragment of 154 base pairs of the *cyt-b* was amplified by PCR, using the GluDG and Cytb1 primers under the conditions described by SILVA-ROCHA et al. (2012). For the ribosomal gene 12S, a fragment of 337 base pairs was amplified by PCR using the 12Sa and 12Sb primers (KOCHER et al. 1989). The amplification consisted of an initial step of denaturing for 3 min at 94°C, followed by 35 cycles of denaturation for

30 s at 94°C, annealing for 30 s at 50°C, extension for 45 s at 72°C, and a final extension step for 5 min at 72°C. The PCR products were purified and sequenced by an external service (company Beckman).

The sequences generated in the present study (accession numbers: KP668869–KP668884) were aligned with seven sequences of each gene downloaded from GenBank: four from Madeira Island (accession numbers (12S/*cyt-b*): AF080313/AF080314, AF543306/AF543302, GQ142073/GQ142121, Z48041/Z48037), one from Desertas (accession numbers (12S/*cyt-b*): AF543308/AF543304), one from Selvagem Grande (accession number (12S/*cyt-b*): AF543307/AF543303), and one from Porto Santo (accession number (12S/*cyt-b*): AF543309/AF543305). Two sequences of *Podarcis hispanica* (STEINDACHNER, 1870) and *Scelarcis perspicillata* (DUMÉRIL & BIBRON 1839), were also downloaded from GenBank and used as outgroups (accession numbers AF469459 and AF080303, respectively), following BREHM et al. (2003a, b). We performed a Maximum Likelihood (ML) phylogenetic analysis to infer the relationships between the *cyt-b* haplotypes using the software Mega 6 (TAMURA et al. 2013). The Tamura-Nei model (TAMURA & NEI 1993) with gamma-distributed rate variation among sites was selected as the best model of sequence

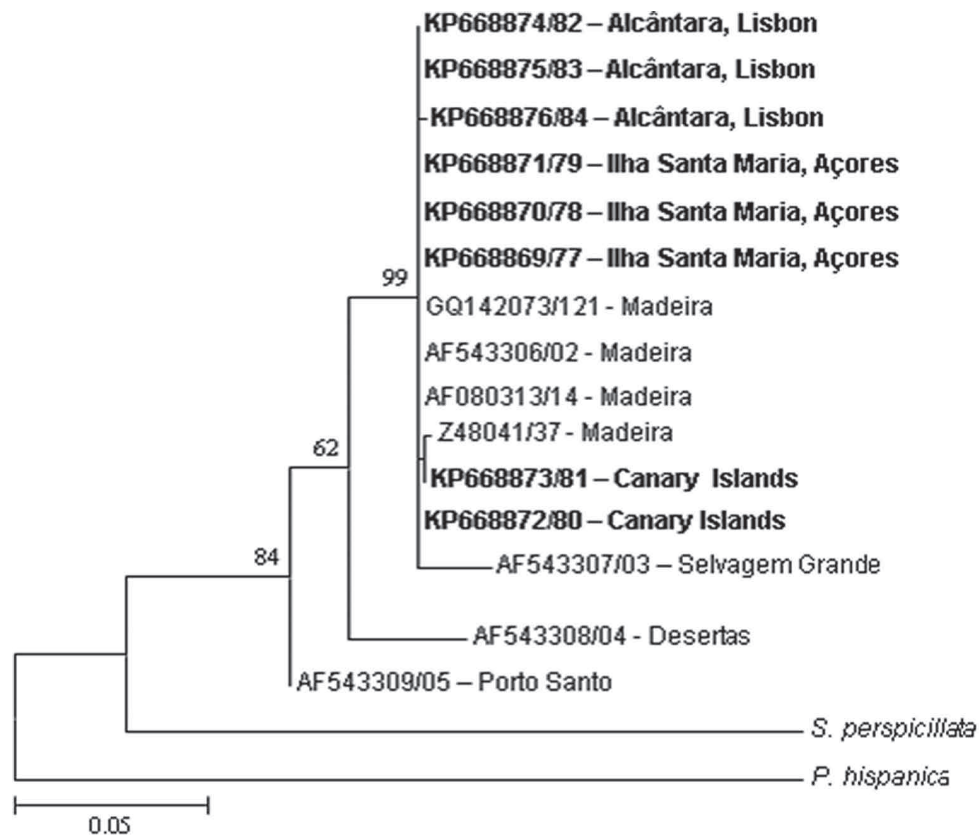


Figure 1. ML tree depicting the relationships between the haplotypes from the native region, Madeira Archipelago, and the introduced populations from the Azores, Lisbon, and the Canary Islands. *Podarcis hispanica* and *Scelarcis perspicillata* were used as outgroups. Sequences downloaded from GenBank are named according to their accession number and their source region. Introduced populations are in bold. Bootstrap support values are indicated above the nodes of interest.

evolution under the Bayesian Information Criterion (BIC). Tree searches were performed using the heuristic search mode. Node support was calculated over 1,000 bootstrap replicates.

The final alignment included 17 sequences and 599 base pairs. A total of nine haplotypes were identified amongst which one is shared by all the introduced populations and the Madeiran samples.

The estimate of relationships based on ML indicates that lizards from all three introduced populations (Azores, Lisbon, and Canary Islands) clustered together with samples from Madeira (Fig. 1). This result is coherent with the suggestions by SÁ-SOUSA (1995) for the Lisbon population and the findings by BREHM et al. (2003b) for the population of São Miguel in the Azores. For all three allochthonous populations, ship cargo seems to be the main vector by which this species is transported from one region to another (SÁ-SOUSA 1995, BREHM et al. 2003b, LÓPEZ-DOS SANTOS et al. 2013). In fact, all three populations occur in close geographical proximity (< 500 m) to respective commercial harbours.

Regarding the conservation issues inherent to species introductions, there are no reports on the impact of *T. dugesii* in the new environments (KRAUS 2009, MATEO et al. 2011). The stability of the population in Lisbon (SÁ-SOUSA pers. obs.) and extinction of that on Tenerife (MATEO et al. 2011) suggest that this species is not likely to become invasive. A similar pattern is indicated for the lacertid *Podarcis pityusensis* (BOSCÁ, 1883), which was introduced from its native range in Ibiza and Formentera (Balearic Islands) to Mallorca Island, Barcelona and the Basque Country, which are localities where the species remains relatively constrained after one or more decades (MATEO et al. 2011).

Nevertheless, such insular invaders cannot be by default considered completely harmless. If translocated in a secondary dispersal to other islands, competition, parasite transfer, and/or loss of genetic identity of native micro-insular populations may ensue (MATEO et al. 2011). Thus, it is here recommended that a mid-term monitoring programme be established for these populations to prevent such menaces. Additionally, a general quarantine area for cargo should be set up on Madeira to prevent new introductions of this species in other places or other species to Madeira (WAGNER et al. 2012). For instance, *Podarcis sicula* (RAFINESQUE-SCHMALTZ, 1810), a lacertid prone to introduction by human mediation and with a potential of becoming invasive in island situations (SILVA-ROCHA et al. 2012), is also present in the Lisbon area, with a population being located only 10 km NW from the site at which *T. dugesii* is found (GONZÁLEZ DE LA VEGA 2011).

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