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Genetic insights into the identity and distribution of *Tarentola* spp. geckos on Lampedusa island

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SUMMARY

The Sicily Channel islands are known as biogeographic crossroads between European and African fauna. In this context, Lampedusa hosts a mainly Maghrebian herpetofauna, among which a Moorish gecko (*sensu lato*) belonging to a North African species-rank clade, provisionally named "*Tarentola fascicularis/deserti*" (*sensu* Rato et al., 2017). Here, we investigated the distribution of *Tarentola* spp. using the 16S mitochondrial DNA fragment on individuals from Lampedusa and verified the possible occurrence of *T. mauritanica*. This is a widely introduced species, hardly distinguishable based on morphological traits from *T. fascicularis/deserti*. Our molecular results support the hypothesis that both *T. fascicularis/deserti* and *T. mauritanica* occur in Lampedusa. Furthermore, nine out of eleven sequences are lumped together with *T. fascicularis/deserti* clade, suggesting the natural biogeographic connection between Tunisia and Lampedusa. Conversely, the detection of *T. mauritanica* haplotypes, exclusively within the Lampedusa port area, strongly point out a recent human-mediated introduction of the species. The finding of these two distinct mitochondrial lineages suggests the co-occurrence of African and European dispersion routes in the extant Lampedusa herpetofauna. In light of this, it is necessary to increase sampling efforts to other islands across Sicily Channel to better assess the origin and phylogenetic relationships of gecko populations.

INTRODUCTION

Islands, often described as natural laboratories, actually represent a unique context to understand biogeographic patterns and ecological processes (Whittaker et al., 2017). These isolated ecosystems, characterised by high levels of endemism and unique speciation events (Matthews and Triantis, 2021), are often also biodiversity hotspots. They also play a pivotal role as refuges and stopover sites for migratory species, thus contributing to global biodiversity networks (Moore et al., 1990; Sheehy et al., 2011). However, their sensitivity to environmental changes, particularly climate change and sea-level rise, makes them early indicators of broader global impacts (Kelman and West, 2009). The Mediterranean region, with its numerous small islands and archipelagos, offers a paramount example of these ecological dynamics. The Sicily Channel serves as a biogeographic crossroads, hosting a unique admixture of European and African flora and fauna (Fichera et al., 2022; Antinucci et al., 2024; Belluardo et al., 2024). These islands, though biogeographically significant, face increasing pressure from human activities, such as tourism, climate change-induced challenges, and possibly alien species introduction (Faraone et al., 2019; Surico, 2020).

Despite their ecological importance, detailed knowledge about the faunal and floral assemblages of these Palearctic islands remains limited (Angelici et al., 2009; Fichera et al., 2022; Sciandra et al., 2022). Comprehensive studies are necessary to understand their role as corridors for species dispersal and potential outposts for colonisation, especially in the context of climate change.

Lampedusa, the largest of the Italian Pelagie islands, provides an ideal model for such investigations (Surico, 2020), as hosting a unique herpetofauna, mostly composed by African taxa showing their only Italian population on the island [i.e., the Algerian psammodromus *Psammodromus algirus* (Linnaeus, 1758), the North African green toad *Bufotes boulengeri boulengeri* (Lataste, 1879), the Eastern Montpellier snake *Malpolon insignitus* (Geoffroy Saint-Hilaire, 1809) and the false smooth snake *Macroprotodon* cf. *cucullatus* (Geoffroy Saint-Hilaire, 1827) (Harris et al., 2009a; Nicolas et al., 2018; Faraone et al., 2020a, 2022)]. Gekkota species occurring in the island include the Mediterranean Turkish gecko *Hemidactylus turcicus* (Linnaeus, 1758) and a Moorish gecko (*sensu lato*) belonging to an unresolved North African clade here named "*Tarentola fascicularis/deserti*" following Rato et al. (2012) (see also Harris et al., 2009b; Belluardo et al., 2024). Recently, Rato et al. (2012) highlighted a great cryptic diversity within the genus *Tarentola* across the Mediterranean basin, including the "*Tarentola fascicularis/deserti*" clade, a monophyletic unit which encompasses specimens previously assigned to both *T. fascicularis* (Daudin, 1802) and *T. deserti* Boulenger, 1891. A strong pattern of hidden diversity is also noticeable for *T. mauritanica* (Linnaeus, 1758), which subsequently turned out to be a complex of six species-rank clades (Rato et al., 2016). Colonisation routes of *T. mauritanica* suggest the possible occurrence of this taxon in Lampedusa too (Rato et al., 2023). On two other islands located in the Sicily Channel, Linosa and Malta, only *T. mauritanica* is known to occur (Stöck et al., 2016), and in Pantelleria both *Tarentola* species have been recently recorded (Antinucci et al., 2024). Morphological features to distinguish between *T. mauritanica* and *T. fascicularis/deserti* are subtle and to date debatable (Sarra et al., 2013). For instance, *T. fascicularis/deserti* is generally more brightly coloured than *T. mauritanica*, but it often exhibits a more uniform coloration with less variation in pattern. Conversely, *T. mauritanica* can have more variable coloration with darker markings and spots. In *T. fascicularis/deserti*, the rostral scale is in direct contact with the nostril (Fig. 1), whereas the average number of interorbital scales is lower compared to *T. mauritanica*. *Tarentola fascicularis/deserti* tends to have a slender body shape compared to *T. mauritanica*, which is typically more robust. Lastly, *T. fascicularis/deserti* often have golden/brownish eyes, lighter in colour than *T. mauritanica* (Joger and Bshaenia, 2010; Aprea et al., 2011; Sarra et al., 2013) (Fig. 1). Since some of these traits are not strongly diagnostic, genetic analyses emerge as the most reliable system to distinguish between these species.

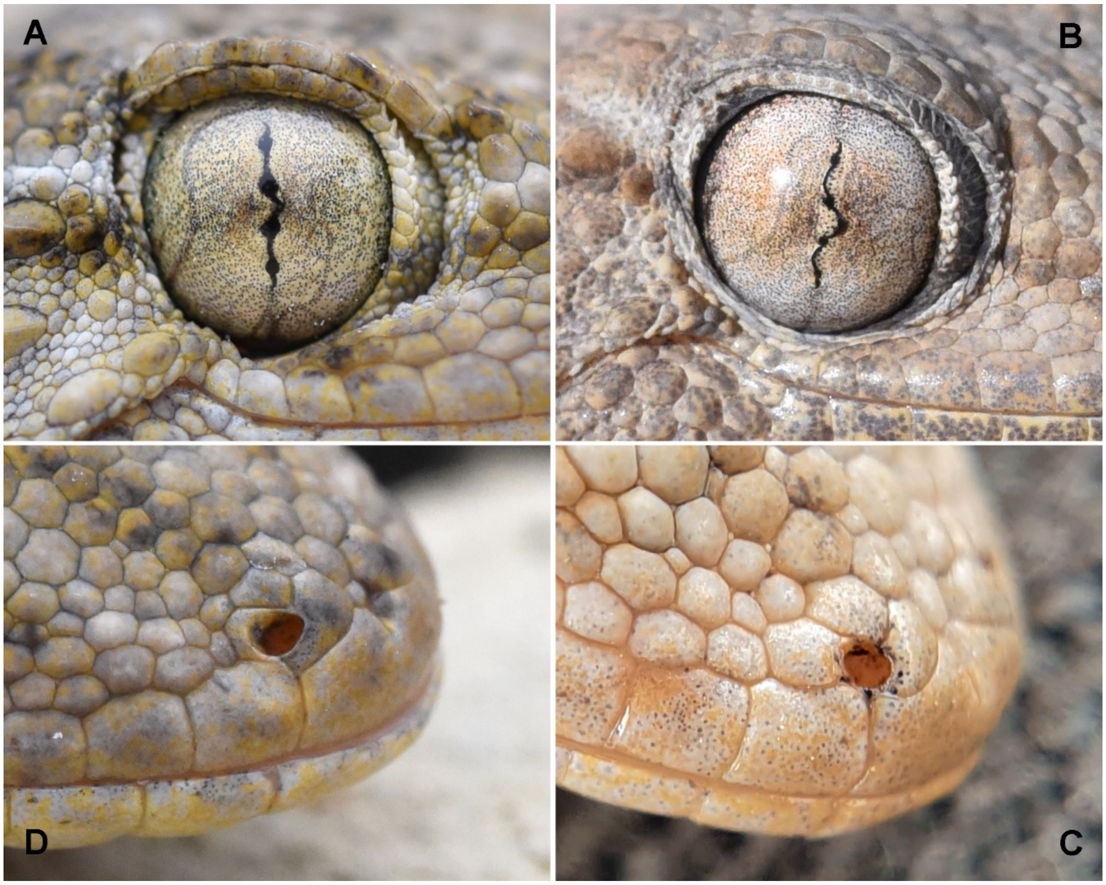

Figure 1. Photo details of the two "Moorish gecko" species present in Lampedusa. **A**, *Tarentola mauritanica* eye detail; **B**, *T. fascicularis/deserti* eye detail; **C**, *T. fascicularis/deserti* nostril and rostral scale detail; **D**, *Tarentola mauritanica* nostril and rostral scale detail.

In the present study, we increased the sampling of *Tarentola* specimens from Lampedusa to investigate the potential distribution of different genetic clades in the study area. Individuals were sampled both in urbanised (where introductions may have occurred) and in natural habitats. We hypothesised that both *Tarentola* taxa may occur in Lampedusa, with *T. mauritanica* restricted to urban environments (e.g., due to its putative recent, human-mediated introduction, see also Harris et al., 2004).

MATERIALS AND METHODS

Sampling

To build a genetic dataset, we sampled 11 *Tarentola* individuals from 11 locations within Lampedusa island, spanning from Spring 2023 to Autumn 2024, including the port and the town centre (Fig. 2). The individuals were captured by hand during both nocturnal and diurnal searches by hand. After capture, the terminal 2 cm of the tail was clipped and preserved in absolute ethanol. No animals were sacrificed to accomplish this study.

Figure 2. Distribution of sampled localities in Lampedusa and Conigli islands. Yellow dots refer to *Tarentola fascicularis/deserti* previously published records (Harris et al., 2009b; Rato et al., 2010, 2012; Stöck et al., 2016); yellow squares refer to *T. fascicularis/deserti* novel records; red triangles refer to *T. mauritanica* novel records.

Genetic analyses

DNA extraction was performed on a small tissue sample taken from the tails of each of the collected specimens. Prior to DNA extraction, the tissue was carefully cleaned and soaked in double-distilled water for 5 minutes, followed by processing using the BIORON GmbH "Ron's Tissue DNA Mini Kit". The primer pair 16sar-L and 16sbr-H (Palumbi et al., 1991) was used to amplify a fragment of the large ribosomal subunit 16S rRNA (16S). The composition of the polymerase chain reaction (PCR) mix and thermal cycling were performed according to Antinucci et al. (2024). The obtained chromatograms showed no peak ambiguities. All novel 16S rRNA sequences were uploaded to GenBank (Accession Numbers, A.N.s: PV715685-PV715695). These sequences were analysed and manually proofread using Chromas v. 2.6.2 (Technelysium Pty. Ltd., 1998, Queensland, Australia) and aligned using MEGA12 (Kumar et al., 2024).

The molecular identification of the specimens and the reconstruction of their phylogenetic relationships were performed using Bayesian Inference (BI) and Maximum Likelihood (ML) methods, implemented in MrBayes v. 3.2.7 (Ronquist et al., 2012) and PhyML v. 3.0 (Guindon et al., 2010), respectively. Branch support was assessed by reporting posterior probability values on the BI tree and calculating bootstrap values with 1000 replicates on the ML tree (Felsenstein, 1985). The optimal evolutionary model was selected from those analysed by MrBayes, using the Bayesian model choice criteria (nst = mixed, i.e., GTR+I+Γ) and then selected also in the ML analysis. In the BI analyses, two independent Markov Chain Monte Carlo analyses were run with one million generations (4 chains, temp.: 0.2; default priors). Trees and parameter values were sampled every 100 generations, resulting in 10.000 saved trees per analysis. Once convergence was reached (Effective Sample Size above 200), 2500 trees were conservatively discarded as "burn-in".

RESULTS

Overall, 11 novel 16S sequences were obtained and included in the analyses, along with an additional 119 comparison sequences previously published and downloaded from GenBank (see Fig. 3 for their A.N.s). The length of the readable 16S PCR products ranged from 510 to 570 bp. After trimming the tails of the sequences, a 16S alignment of 464 bp was obtained. The phylogenetic trees based on BI and ML analyses, rooted on *Tarentola chazaliae* (Mocquard, 1895) (A.N., JQ300955 see also Fig. 3), showed topologies congruent with the currently known phylogenetic relationships of the analysed taxa (see Rato et al., 2012; Antinucci et al., 2024).

Nine of the 11 novel sequences from Lampedusa clustered with the clade VIII (*sensu* Rato et al., 2012) of the *Tarentola fascicularis/deserti* lineage, along with other samples from Lampedusa, Libya and Tunisia. Conversely, two sequences (GenBank A.N.s, PV715694-PV715695) fell into clade VI, which includes *T. mauritanica* samples from Morocco, Tunisia and all the European populations analysed (Italy, Malta, Spain), sharing the same haplotype. Both sequences in clade VI were obtained from individuals sampled at the port of Lampedusa (Fig. 2, 3).

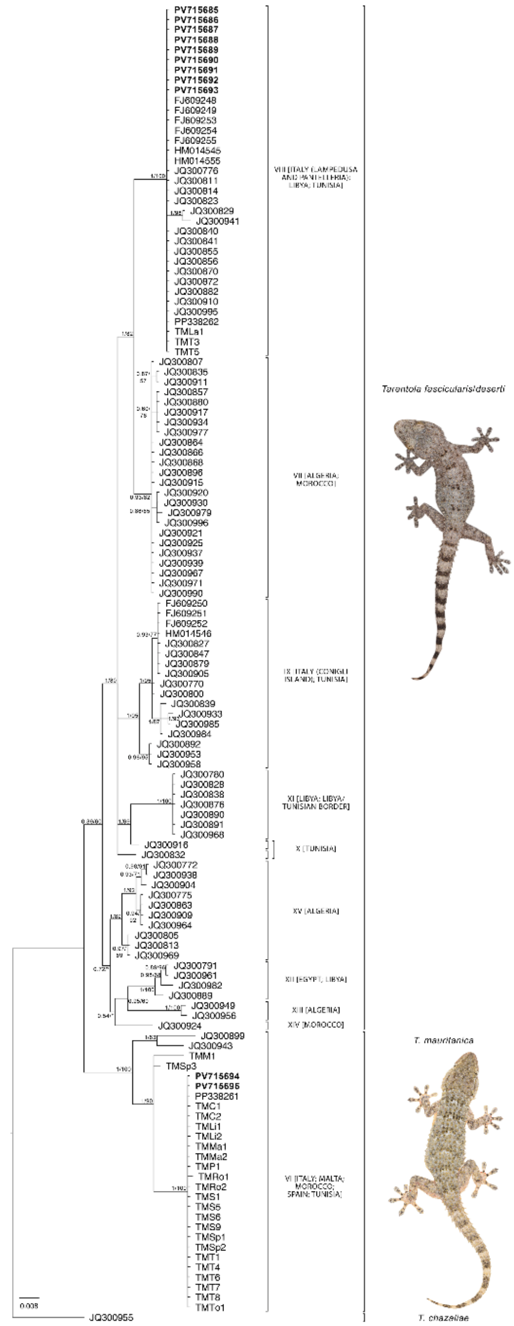

Figure 3. Bayesian phylogram of *Tarentola* spp. based on a 464-bp fragment of the mtDNA 16S. A sample of *T. chazaliae* (Mocquard, 1895) (GenBank Accession Number, A.N.: JQ300955) was used as outgroup to root the tree. Node statistical support is reported as nodal posterior probabilities (Bayesian inference of phylogeny, BI)/bootstrap values (Maximum Likelihood, ML). Asterisks indicate bootstrap support values lower than 50. Rectangles group the samples according to their major clustering reported in Rato et al. (2012). The analysed specimens are reported using the A.N.s. The novel sequences are reported in bold.

DISCUSSION

Our work provided additional information on the genetic diversity of *Tarentola* geckos in the southernmost islands of Europe, highlighting the importance of these areas as a potential zone of species overlap and introduction. The obtained molecular results confirm the presence of two distinct genetic lineages of species rank within Lampedusa, supporting the hypothesis that both *T. fascicularis/deserti* and *T. mauritanica* occur on the island. The clustering of nine out of eleven sequences with *T. fascicularis/deserti*, along with samples from Libya and Tunisia, confirmed the natural biogeographic connection between North Africa and Lampedusa. This observation aligns with previous studies which emphasised the role of the Sicily Channel as a transitional zone between European and African fauna (Ancillotto et al., 2020; Faraone et al., 2020b; Fichera et al., 2022; Antinucci et al., 2024). Conversely, the biogeographic pattern of *Tarentola* geckos on Lampedusa is the opposite of the one observed in Pantelleria, which is located 160 km north to Lampedusa, where the European species is the most widely distributed and the African clade is very localised (Antinucci et al., 2024). The detection of *T. mauritanica* haplotypes exclusively within Lampedusa human settlements (i.e., in the port area) strongly suggests a recent human-mediated introduction of the species, likely through maritime routes (Mori and Plebani, 2012; Mori et al., 2022; Rato et al., 2023; Giacalone et al., 2024), as occurred with other lizards on the island (Lo Valvo and Nicolini, 2001; Lo Cascio et al., 2005). This is consistent with prior research, which has documented the anthropogenic dispersal of *T. mauritanica* across Mediterranean islands, particularly in urbanised settings (Harris et al., 2004). Such introductions raise important issues about potential ecological interactions between native and introduced species, as well as the long-term implications for Lampedusa herpetofauna assemblage. While *T. fascicularis/deserti* appears to be the naturally occurring taxon in the island's less disturbed environments, further studies are needed to assess whether competitive displacement or potential hybridisation events may occur between the two species (Di Nicola et al., 2021).

The morphological similarities between *Tarentola mauritanica* and *T. fascicularis/deserti* add another layer of complexity to species identification in the field. As previously noted, minor variations in coloration, body shape, and head scales are often subtle and subject to individual variation (Joger and Bshaenia, 2010; Aprea et al., 2011; Sarra et al., 2013). Consequently, the use of genetic analyses is confirmed to be the most reliable method currently available for distinguishing amongst these taxa. The molecular approach used in this study not only reinforces the taxonomic status of the sampled individuals but also demonstrates the necessity of expanding genetic surveys to other nearby islands, e.g., Pantelleria, where similar taxonomic uncertainties occur (Rato et al., 2023; Antinucci et al., 2024;).

Our findings carry some potential conservation implications. The introduction of non-native species, particularly in ecologically fragile island environments, can lead to unforeseen disruptions in local ecosystems. Although *Tarentola mauritanica*, with few recent exceptions (see Renet et al., 2024), is not typically considered an invasive threat, its presence in Lampedusa raises concerns about potential competition with *T. fascicularis/deserti*, which has a more restricted range in the Mediterranean region (Di Nicola et al., 2021). Future monitoring efforts should focus on understanding population dynamics, habitat preferences, and potential interspecific interactions to evaluate whether conservation measures, such as controlling human-mediated dispersal, are necessary.

Additionally, the study highlights the broader importance of Mediterranean islands in tracking historical biogeographic patterns (Sciandra et al., 2022). The genetic links between *Tarentola fascicularis/deserti* populations from Lampedusa and North Africa provide further evidence of past dispersal routes and colonisation events during the Pleistocene (Rato et al., 2023). These findings enhance our understanding of how climate oscillations and sea-level fluctuations have historically shaped species distributions in the region. Given that island ecosystems are particularly vulnerable to climate change and anthropogenic disturbances, continued research is essential to assess how future environmental shifts may influence the composition and survival of native and introduced taxa.

In conclusion, this study confirmed the role of Lampedusa as a milestone for understanding Mediterranean biogeography and species dispersal in reptile taxa. The identification of two distinct genetic lineages within *Tarentola* populations not only confirms the co-occurrence of African and European dispersion routes in Lampedusa herpetofauna but also underscores the need for ongoing conservation and monitoring efforts. Future studies should aim to expand sampling efforts to other islands across the Sicily Channel to better define the phylogenetic relationships and origin of their gecko populations.

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