Herpetology Notes, volume 17: 395-402 (2024) (published online on 12 June 2024)

Geckos on the borders: genetic evidence on co-occurrence of two species of wall geckos, genus *Tarentola*, on the island of Pantelleria, Italy

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Abstract. The taxonomy of wall geckos (genus *Tarentola*) in the Mediterranean basin is very complex and studies have indicated that some species are actually species complexes. We analysed mitochondrial (16S rRNA) and nuclear (MC1R, ACM4) DNA sequences of *Tarentola* samples from Pantelleria, an island in the Sicilian Channel between Tunisia and Sicily, to establish their taxonomic identity. Comparisons of our sequences with those available in GenBank indicates that four individuals were conspecific with *T. mauritanica* while a single individual was a member of the *T. fascicularis*/*deserti* complex. This documents the occurrence of the *T. fascicularis*/*deserti* complex in Pantelleria for the first time, in one of the few instances where these geckos are sympatric with *T. mauritanica*.

Keywords. Biogeography, Gekkota, herpetofauna, Mediterranean islands, Moorish Gecko, Phyllodactylidae, *Tarentola fascicularis*.

Introduction

The Mediterranean Basin harbours nine different species of *Tarentola*, namely *T. mauritanica* (Linnaeus, 1758), *T. chazaliae* (Mocquard, 1895), *T. deserti* Boulenger, 1891, *T. boehmei* Joger, 1984, *T. annularis* (Geoffroy Saint-Hilaire, 1827), *T. neglecta* Strauch, 1887, *T. mindiae* Baha El Din, 1997, *T. ephippiata* O'Shaughnessy, 1875, and *T. fascicularis* (Daudin, 1802) (Uetz et al., 2023). This taxonomic arrangement is probably not definitive since several molecular studies have indicated that some of these species are, in fact, species complexes still requiring a taxonomic assessment (Rato et al., 2012, 2016). This is certainly the case with the *T. fascicularis*/*deserti* complex, a monophyletic lineage that includes specimens assigned to either *T. fascicularis* or *T. deserti* (Rato et al., 2012). This species complex displays high genetic diversity, as indicated by nine different mitochondrial DNA sub-lineages (Lineages VII–XV; Rato et al., 2012). Phylogenetic relationships have revealed that *T. fascicularis* is paraphyletic with respect to *T. deserti* (Harris et al., 2004; Rato et al., 2012). Both genetic divergence and paraphyly indicate that the *T. fascicularis*/*deserti* complex is likely a complex of lineages, some of which may deserve species status (Rato et al., 2012).

The distribution of the *T. fascicularis*/*deserti* complex includes mainly North Africa (Tunisia, Morocco, Algeria, Libya, Egypt) but it also includes some islands in the Sicilian Channel, namely Lampedusa and its offshore islet Conigli in Italy and Chergui in Tunisia (Harris et al., 2009; Rato et al., 2012; Fig. 1). More specifically, in Lampedusa and Conigli the geckos are members of Clade VIII, assigned to *T. fascicularis*, which is also present on the border between Libya and Egypt and in western Libya. Haplotypes of Clade IX, mainly distributed in Tunisia, are found on Chergui and also on Conigli (Harris et al., 2009; Rato et al., 2012). On two other islands in the Sicilian Channel, Linosa and Malta, only geckos belonging to *T. mauritanica* have been found (Stöck et al., 2016). *Tarentola mauritanica* has a wide European distribution that also includes Peninsular Italy and Sicily (Corti et al., 2010).

In this study, we analysed mitochondrial and nuclear DNA sequences (mtDNA, nuDNA) in *Tarentola* individuals from Pantelleria, a volcanic island in the Sicilian Channel, located 100 km southwest of Sicily and 60 km east of the Tunisian coast (Figs. 1, 2). Previous investigations showed that Pantelleria's fauna was

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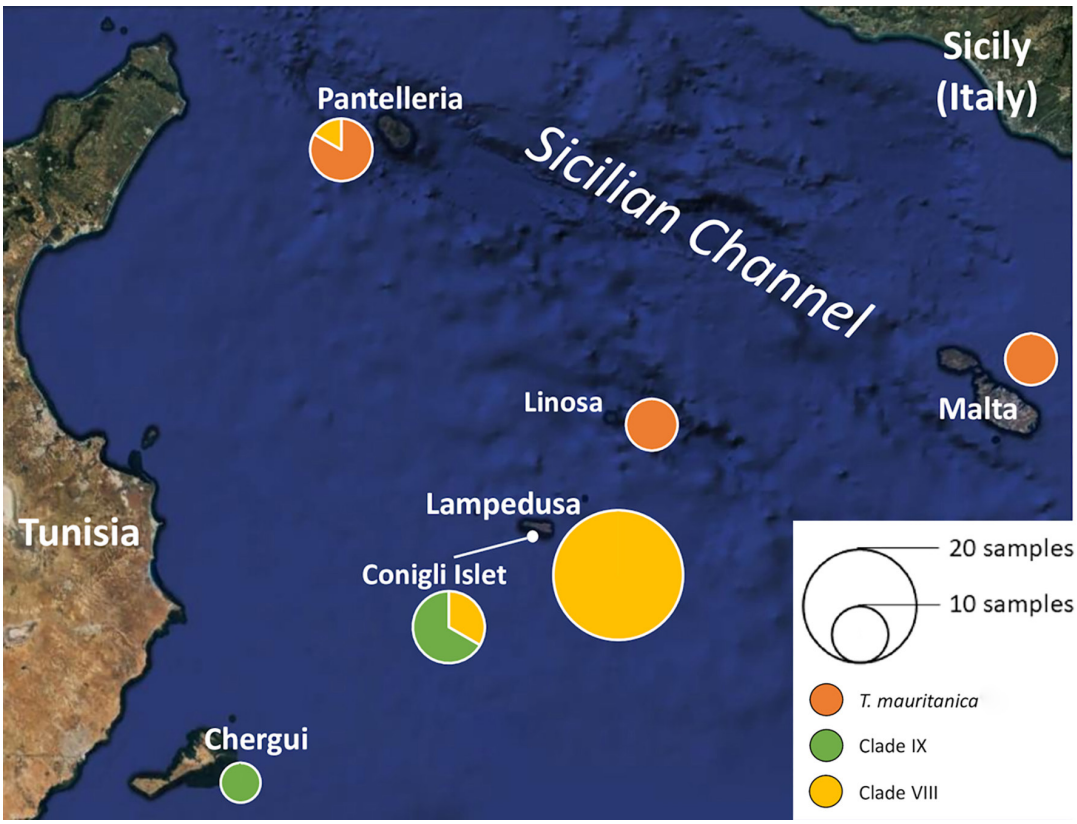

Figure 1. Map of the Sicilian Channel showing the position of the cited islands and the distribution of the different *Tarentola* clades in the area.

shaped by colonization from both Italy and North Africa (e.g., Stöck et al., 2016; Faraone et al., 2020; Fichera et al., 2022; Sciandra et al., 2022; Antinucci et al., 2023). Using newly generated sequences and those available in GenBank, we establish the taxonomic identity of *Tarentola* specimens on Pantelleria and update the distribution and frequency of the different mitochondrial lineages on islands in the Sicilian Channel.

Materials and Methods

For genetic analysis, five geckos (collection numbers PANTR 21–23, 78, 111) were collected by hand in May and September 2022 at two localities (36.8297°N, 11.9429°E; 36.8128°N, 11.9821°E) on Pantelleria (Fig. 2). Tissues were obtained from small tail tips by inducing autotomy after light pressure or released spontaneously during capture. All individuals were released at the capture site. Tail tissues were stored in 96% ethanol and deposited in the collection of the Museum of Comparative Anatomy "Giovanni Battista Grassi" at Sapienza University, Rome, Italy.

Genomic DNA was extracted from all tissue samples by means of the universal extraction protocol (Salah and Martinez, 1997). The primers 16SA-L (light chain; 5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SB-H (heavy chain; 5'-CCG GTC TGA ACT CAG ATC ACG T-3') were used to amplify a section of the mitochondrial 16S ribosomal RNA gene (Palumbi et al., 1991). For two selected samples (see results) we sequenced fragments of two nuclear genes, acetylcholinergic receptor M4 (ACM4) and melanocortin 1 receptor (MC1R). The primers MC1R-F (light chain, 5'-GGCNGCCATYGTCAAGAACCGGAACC-3') and MC1R-R (heavy chain, 5'-CTCCGRAAGGCRTAAATGATGGGGTCCAC-3') were used to amplify MC1R (Pinho et al., 2010), whereas the primers Tg-F (light chain, 5'-CAAGCCTGAGAGCAARAAGG-3') and Tg-R (heavy chain, 5'-ACYTGACTCCTGGCAATGCT-3')

were used to amplify ACM4 (Gamble et al., 2008). All PCRs were carried out in 25 µl final volume using Bioline MyTaq DNA Polymerase and Bioline MyTaq Reaction Buffer following the supplier's suggested instructions for reagents protocols. The PCR cycling procedure was performed as follows for the 16S gene: initial denaturation at 94°C for 90 s followed by 34 cycles with denaturation at 94°C for 45 s; annealing at 55°C for 45 s; elongation at 72°C for 90 s; and a final extension at 72°C for 10 min (Palumbi et al., 1991). For nuclear markers, we used two different PCR cycling programs. For MC1R we used initial denaturation at 92°C for 5 min followed by 40 cycles with denaturation at 92°C for 30 s; annealing at 51.5°C for 30 s; elongation at 72°C for 90 s; and a final extension at 72°C for 5 min (Pinho et al., 2010). For ACM4 we used initial denaturation at 94°C for 5 min followed by 32 cycles with denaturation at 94°C for 30 s; annealing at 52°C for 45 s; elongation at 72°C for 60 s; and a final extension at 72°C for 5 min (Gamble et al., 2008). PCR products were sequenced using an external service (Macrogen, Milan, Italy). Obtained sequences were deposited in GenBank (Accession numbers: PP338261–2, PP339746–9).

The obtained 16S sequences (439 bp) were compared to the GenBank database using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to search for highly similar sequences (Mega BLAST) in the entire nucleotide collection database. Based on BLAST results (see below), we decided which GenBank sequences should be used as comparative material to assess the phylogenetic position of the Pantelleria geckos. Subsequently phylogenetic analyses were performed in MEGA v11.0.13 (Tamura et al., 2021). Specifically, an alignment was made with MUSCLE (Edgar, 2004) using 93 sequences (Appendix 1; length of alignment 402 bp) from the *T. fascicularis*/*deserti* complex. Sequences of *T. mindiae* and *T. neglecta* were also included and designated as outgroups (Harris et al., 2009; Rato et al., 2012; Stöck et al., 2016).

Relationships were estimated using the Maximum Likelihood (ML) algorithm in MEGA using the HKY+G model. This model was chosen among 24 evolutionary nucleotide models under a Bayesian Information Criterion (BIC). Node support was obtained through bootstrap re-sampling (500 replicates). No further phylogenetic methods were applied as our goal was to assign our specimens to already defined clades and not to study the phylogenetic relationships among clades.

Sequences of the two nuclear genes were aligned with orthologous GenBank sequences of specimens from a wide geographic range around the Mediterranean Basin (Appendices 2, 3; Rato et al., 2010, 2012, 2016). These included members of the *T. fascicularis*/*deserti* complex as well as representatives of *T. mauritanica*. Final alignments included 152 total sequences for ACM4 (302 bp) and 151 sequences for MC1R (522 bp). Minimum spanning networks were built for each alignments using PopArt v1.7 (Leigh and Bryant, 2015) to visualize the relationships between nuclear haplotypes.

Results

The five geckos from Pantelleria had two different mtDNA haplotypes. One of the haplotypes (PANT1) was found in four individuals from Pantelleria town, while the other (PANT2) was found in a single individual (PANTR 78) from Bagno dell'Acqua (Fig. 2). The two haplotypes diverged by 40 SNPs and three indel region (1–7 bp) over 401 bp (p distance = 0.10). BLAST analysis revealed that PANT1 is found in *T. mauritanica* and is identical to one of the most widespread haplotypes of this species (Clade A; Rato et al., 2010), while PANT2 is identical to some haplotypes (over 390–400 bp) found in Clade VIII (sensu Rato et al., 2012) of the *T. fascicularis*/*deserti* complex.

Figure 2. Sampling locations on Pantelleria Island. Map from the open-source database of the Italian Institute for Environmental Protection and Research (http://sgi2.isprambiente.it/mapviewer), modified by the authors.

The topology of the ML tree (Fig. 3) retrieves the same clustering of haplotypes as previous works, confirming the existence of nine clades that in our ML tree are supported by bootstrap values between 65 and 99%, with the exception of Clade X that is not supported. Within Clade VIII, PANTR 78 shows a haplotype identical to the most common haplotype found on Lampedusa and Conigli. This haplotype diverges by 1.2% from members of the same clade found in Libya. We update the distribution of the different mitochondrial lineages of *Tarentola* in the Sicilian Channel using the data from Rato et al. (2012), Stöck et al. (2016), and this study (Fig. 1). Nuclear DNA sequences were obtained from two of our samples that belonged to the two different mtDNA lineages: PANTR 22 and PANTR 78. The two specimens diverged by four SNPs in ACM4 and three SNPs in MC1R. For both nuclear markers, the networks reveal shallow divergence among haplotypes and low phylogenetic resolution compared to mtDNA (Fig. 4). PANTR 78 shares its MC1R and ACM4 haplotypes with specimens from Conigli and Lampedusa, where the *T. fascicularis*/*deserti* complex is found based on mtDNA results. Similarly, the MC1R and ACM4 haplotypes found for PANTR 22 are close (only one SNP of divergence) to the ones observed in specimens belonging to peninsular Italy where *T. mauritanica* is found (Fig. 4).

Discussion

Given its geographical position halfway between Africa and Europe, Pantelleria is of considerable interest from a biogeographical point of view. Previous studies have shown that both the Ocellated Skink, *Chalcides ocellatus* (Forskål, 1775) and the Horseshoe

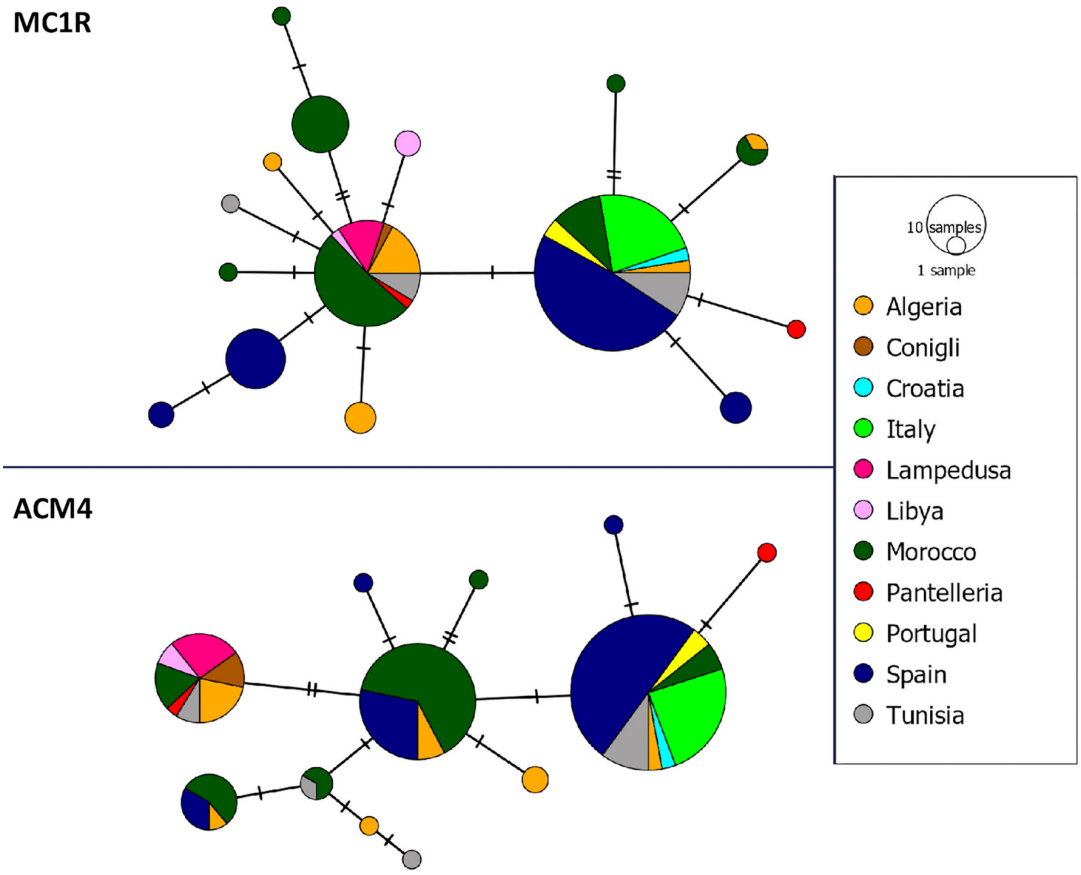

Figure 4. Minimum spanning networks based on MC1R and ACM4 nuclear markers data. Sequences from mainland Italy and Sardinia are all labelled together as "Italy", while the islands of the Sicilian channel Lampedusa, Conigli and Pantelleria are labelled separately.

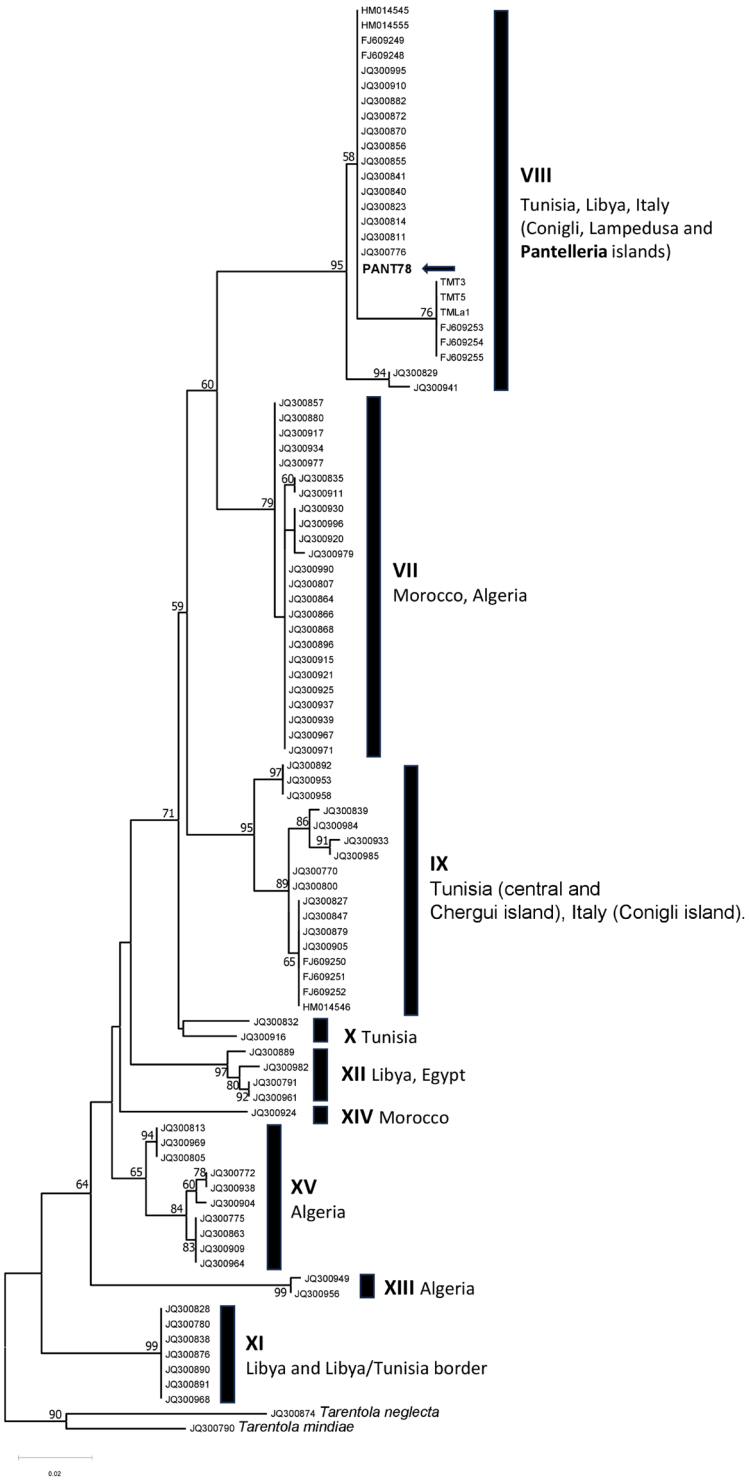

Figure 3. Maximum Likelihood tree using 16S sequence data (402 bp) and the HKY+G model, including sequences of the *Tarentola fascicularis*/*deserti* complex clades and two outgroup species (*T. neglecta*, *T. mindiae*). Nodal support produced by 500 bootstrap replicates is indicated. The clades are named using Roman numerals according to Rato et al. (2012).

Whipsnake, *Hemorrhois hippocrepis* (Linnaeus, 1758) have a North African origin that appears to be very recent for *H. hippocrepis* and more ancient for *C. ocellatus* (Stöck et al., 2016; Faraone et al., 2020; Mori et al., 2022). Conversely, the Italian Wall Lizard, *Podarcis siculus* (Rafinesque-Schmaltz, 1810), seems to represent a recent introduction from western Sicily (Antinucci et al., 2023).

Our genetic evidence documents the presence of two different species of wall geckos on the island. Our mtDNA data clearly shows that geckos on Pantelleria belong to two divergent lineages that are considered two different species (Rato et al., 2012). However, the differences at the mtDNA level alone cannot be indicative of two sympatric species because hybridization between lineages cannot be ruled out. Nonetheless, the results emerging from nuclear networks, albeit with lower resolution, match with those of the mtDNA. In fact, for both nuclear markers, the haplotypes are identical or strictly related to those expected on the basis of the mtDNA data. The first species can be identified as *T. mauritanica*, and the corresponding mtDNA haplotype is found in Malta, Linosa (Figs. 1, 3), and also across Europe, including Peninsular Italy and Sicily, Morocco, and Tunisia (Rato et al., 2012; Stöck et al., 2016). Given the wide distribution of this haplotype, it is not possible to establish if the species colonised Pantelleria from Italy or from North Africa. The nuclear haplotypes in the analysed individual are very similar to the ones found in Peninsular Italy and North Africa, confirming its genetic identification at the level of nuDNA.

The second species, represented by PANTR 78, belongs to Clade VIII and is part of the *T. fascicularis*/*deserti* complex (Rato et al., 2012). Members of the same mtDNA clade have been found on Lampedusa and Conigli (Fig. 1) as well as in Tunisia and Libya. The two nuclear haplotypes found in this individual match the mtDNA data, since they are identical to those in Lampedusa and Conigli and in many North African countries. Therefore, it seems likely that this species colonised Pantelleria from North Africa. Unfortunately, this particular gecko escaped capture, leaving only its tail to allow identification, making the establishment of diagnostic morphological characters impossible.

Instances of sympatry between *T. mauritanica* and members of the *T. fascicularis*/*deserti* complex, as found on Pantelleria, are uncommon. As already pointed out, two clades (VIII and IX) of the *T. fascicularis*/*deserti* complex coexist on the small Conigli Islet (Harris et al., 2009), and the only documented case of sympatry between *T. mauritanica* (Clade III) and a member of the *T. fascicularis*/*deserti* complex (Clade X) was found at a single North African locality (Rato et al., 2012: Fig. 2). It cannot be excluded that similar situations of sympatry may also occur elsewhere, especially on islands in the Sicilian Channel, given the low number of individuals studied. Pantelleria therefore represents an interesting site to study the ecological and genetic interactions of these two gecko species. Future studies based on a larger number of specimens may be able to evaluate the level of hybridization between the two species and to investigate any type of differentiation of the ecological niche in syntopy (e.g., Fulgione et al., 2019).

Acknowledgements. We thank the staff of Pantelleria National Park for logistically supporting our research. Thanks are extended to Flavia Annesi for her help in the laboratory, and to three anonymous reviewers for their helpful comments. This research was partially founded by the project "Conservazione della Biodiversità del Lago Bagno dell'Acqua (Isola di Pantelleria)" (Prot. 0000645; 01/03/2022) funded by Pantelleria National Park. RC received funding from the National Biodiversity Future Center's National Recovery and Resilience Plan (Mission 4, Component 2, Investment 1.4; call for tender No. 3138 of 16 December 2021, rectified by Decree 3175 of 18 December 2021, Italian Ministry of University and Research funded by the European Union under Next Generation EU [Project code CN_00000033]). Handling of animals was permitted by the Ministero della Transizione ecologica (MITE prot. 26489; 24/05/21).

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Accepted by Idriss Bouam

Appendix 1. Localities and GenBank accession numbers for 16S rDNA data of the *Tarentola fascicularis*/*deserti* complex and the outgroup taxa *T. mindiae* and *T. neglecta* used for phylogenetic reconstruction (Harris et al., 2009; Rato et al., 2012). Each locality is listed only by country except for islands in the Sicilian Channel. Three sequences (TM) were directly obtained from the supplementary material in Stöck et al. (2016).

***Tarentola fascicularis*/*deserti* complex.** ALGERIA: JQ300772, 300775, 300805, 300813, 300835, 300848, 300863–64, 300896, 300904, 300909, 300911, 300949, 300956, 300964, 300967, 300969, 300990. EGYPT: JQ300791, 300961. ITALY, Conigli Islet: FJ609248, 609250–52, HM014545, 014555, JQ300814, 300827, 300847, 300879, 300905, 300938; Lampedusa Island: FJ609249, 609253–55, HM014546, JQ300776, 300811, 300823, 300840–41, 300855–56, 300870, 300872, 300882, 300910, 300995, TMLa1. LIBYA: JQ300780, 300828–29, 300838, 300876, 300889–91, 300941, 300968, 300982. MOROCCO: JQ300807, 300857, 300866, 300868, 300880, 300899, 300915, 300917, 300920–21, 300924–25, 300930, 300934, 300937, 300939, 300943, 300971, 300977, 300979, 300996. TUNISIA: JQ300832, 300839, 300892, 300916, 300933, 300953, 300958, 300984–85, TMT3, 5; Chergui Island: JQ300770, 300800.

***Tarentola mindiae*.** EGYPT: JQ300790.

***Tarentola neglecta*.** TUNISIA: JQ300874.

Appendix 2. Details of ACM4 sequences (locality and accession numbers) used to build minimum spanning network. Specimens belong to *Tarentola mauritanica* and the *T. fascicularis*/*deserti* complex from the Mediterranean area (Rato et al., 2010, 2012, 2016).

ALGERIA: JQ301016, 301019, 301028, 301032, 301034, 301044, 301046, 301049–50, 301052, 301063, 301077, 301097, 301102. CROATIA: JQ301030, 301060. ITALY: JQ301002, 301008–09, 301011, 301014, 301024, 301039, 301041, 301065, 301068, 301071, 301079–80, 301085, 301090, 301094, 301098; Conigli Islet: HM014596–97, JQ301037; Lampedusa Island: HM014601, JQ301013, 301021, 301038, 301074, 301093. LIBYA: HM014627, JQ301025. MOROCCO: HM014590, 014598–600, 014605, 014624, 014634–35, 014637–38, JQ301003, 301005, 301015, 301018, 301027, 301033, 301040, 301043, 301045, 301047, 301056, 301059, 301066, 301067, 301072, 301075, 301091–92, 301099, 301100, 301103, 357017–18, KT357020–21, 357023–26, 357031–32. PORTUGAL: HM014612, 014620, 014640. SPAIN: HM014594–95, 014602–04, 014606–11, 014613–19, 014621, 014625, 014629–33, 014636, 014639, JQ301001, 301004, 301006, 301010, 301022, 301031, 301048, 301051, 301053, 301055, 301062, 301064, 301069–70, 301073, 301076, 301078, 301082, 301084, 301086, KT357022, 357027, 357028, 357030. TUNISIA: HM014591–93, 014622–23, 014626, JQ301007, 301012, 301020, 301023, 301026.

Appendix 3. Details of MC1R sequences (locality and accession numbers) used to build minimum spanning network. Specimens belong to *Tarentola mauritanica* and the *T. fascicularis*/*deserti* complex from the Mediterranean area (Rato et al., 2010, 2012, 2016).

ALGERIA: JQ301104, 301107, 301113–15, 301150–51, 301156, 301171, 301189, 301197, 301202, 301206. CROATIA: JQ301116, 301195. ITALY: JQ301105, 301109–10, 301117, 301131–33, 301138, 301148, 301162, 301164, 301167, 301177, 301186, 301190, 301192, 301194; Conigli Islet: JQ301158; Lampedusa Island: HM014652, JQ301111, JQ301120, JQ301136, JQ301205. LIBYA: HM014678–79, JQ301201. MOROCCO: HM014641, 014649–51, 014675, 014685, 014686, 014688–89, JQ301112, 301118–89, 301128–30, 301134, 301137, 301142–43, 301145, 301147, 301154, 301157, 301169, 301173, 301175, 301193, 301200, 301203, KT357033–37, 357039–42, 357047–49. PORTUGAL: HM014663, 014671, 014691. SPAIN: HM014645–46, 014653–62, 014664–70, 014672, 014676, 014680, 014681–84, 014687, 014690, JQ301108, 301121–22, 301126–27, 301135, 301140–41, 301149, 301153, 301155, 301161, 301165, 301168, 301180, 301183, 301187, 301198–99, 301207, KT357038, 357043–46. TUNISIA: HM014642–44, 014673–74, 014677, JQ301106, 301163, 301184, 301188, 301208.