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Genetic affinities of *Tarentola mauritanica* (Reptilia: Gekkonidae) from Lampedusa and Conigli islet (SW Italy)

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Abstract. Mitochondrial DNA (12S rRNA, 16S rRNA) and C-*mos* nuclear gene fragment sequences were analysed within *Tarentola mauritanica* from Lampedusa and Conigli Islet, located in the Mediterranean between Sicily and Tunisia, and compared with already published sequences. In all analyses, the five previously found genetically distinct lineages can be identified, supporting previously published estimates of relationships. The Conigli islet and Lampedusa specimens can be separated in two lineages, one nearly identical to a specimen from Libya and the other one distinct from all currently sampled specimens. These two lineages occur in a very small area and might have reached the islands from North Africa rather than Southern Europe, either by natural rafting or anthropogenic introduction. An estimate of relationships derived from C-*mos* nuclear DNA sequences supports all relationships between the samples from Lampedusa and Conigli, one *T. m. fascicularis* from Libya and one individual of *T. deserti* from Morocco. The paraphyly of *T. mauritanica* with respect to *T. deserti* and *T. angustimentalis* is demonstrated once again in this work. Additional sampling of all extant North African *Tarentola* species will be necessary to determine the evolutionary relationships of this species complex.

Key words: *Tarentola mauritanica*, 12S rRNA, 16S rRNA, C-mos, Lampedusa

The island of Lampedusa, located in the channel that separates Sicily and Tunisia, is a part of the African continental platform and represents the largest island of the Pelagian Archipelago. It is calcareous and covers an area of about 20 km^2. The Conigli islet is separated from Lampedusa by a channel of only 150 meters of shallow water (Fig. 1). The genus *Tarentola*, includes approximately 20 essentially nocturnal species (Joger 1984a, b, c, Schleich 1984, Baha el Din 1997, Sprackland & Swinney 1998). It is distributed throughout southern Europe, North Africa and Macaronesia (the islands of Madeira, Selvagens, Canary Islands and Cape Verde). Two species can be found in Cuba (Diaz & Hedges 2008) and in the Bahamas and another one, probably extinct, was described from Jamaica (Sprackland & Swinney 1998). This phyllo-

dactylid group (Gamble et al. 2008) has been widely studied employing both phylogenetic (Carranza et al. 2000, 2002, Jesus et al. 2002) and phylogeographic approaches (Gübitz et al. 2000, Harris et al. 2004a, 2004b). More specifically, the Moorish gecko *Tarentola mauritanica* appears to represent a species complex highly divergent in North Africa, with at least eight different mitochondrial DNA (mtDNA) lineages (Harris et al. 2004a, 2004b). Similarly, in both the Canary and Cape Verde islands, morphologically conservative species have been shown to contain genetically distinct lineages (Carranza et al. 2000, Jesus et al. 2002). *Tarentola mauritanica* is clearly paraphyletic with respect to *T. angustimentalis* from the Canary Islands (Harris et al. 2004a, 2004b). The previous finding of a widespread mtDNA haplotype in Portugal, Spain, Minorca, continental Italy, Tunisia, Crete and Madeira suggested *T. mauritanica* might be introduced across much of Europe. As far as Morocco is concerned, two genetically distinct lineages were identified, one in the north and other in the central and southern regions (Harris et al. 2004a, 2004b). However, previous studies on Iberian and North African *Podarcis* Wall lizards showed that distinct cryptic forms can be limited to small geographic regions (Harris et al. 2002). Additional lineages of *T. mauritanica* have recently been reported from the Iberian Peninsula (Perera & Harris 2008). Therefore additional genetic lineages within *T. mauritanica* elsewhere can be hypothesised.

The subspecies currently described for north-east Africa (SW Tunisia, Libya and Egypt) is *T. m. fascicularis* (Daudin, 1802) (Schleich et al. 1996), which is genetically divergent from *T. m. mauritanica*, with at least 8% uncorrected p-distance for 12S and 16S rRNA partial gene sequences (Harris et al. 2004a, 2004b). All of the Italian populations have been traditionally attributed to

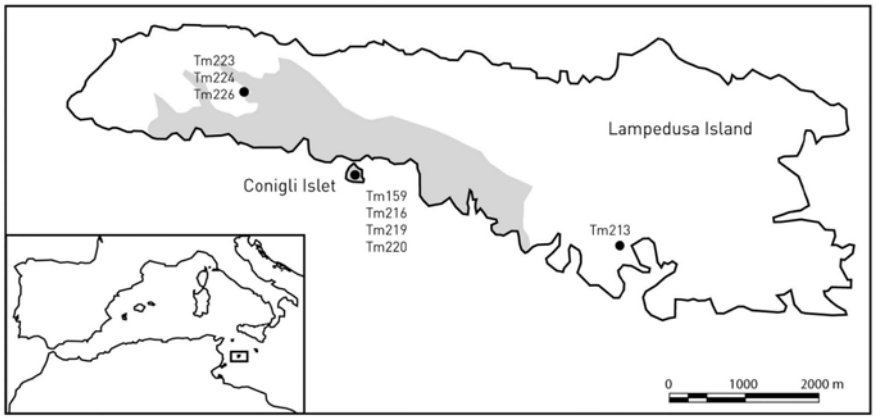

Figure 1. Map showing sampling localities of *Tarentola* sequenced in this study. Shading indicates the Riserva Naturale "Isola de Lampedusa" area. Codes for samples are given in table 1. All others are from Harris et al. (2004a, 2004b).

the nominal subspecies (Guarino & Picariello 2006). The aim of this work was to include several samples from Lampedusa and the Conigli islet (SW Italy) together with the samples already published in order to estimate the phylogenetic affinities of the Moorish geckos from those islands, and to assess the possibility of an anthropogenic introduction.

A total of 71 individuals were used in this study. Specimens were collected in the field and identified to subspecies following Bons and Geniez (1996) and Geniez et al. (1999). Of these, 63 belong to previously published studies (Harris et al. 2004a, 2004b). New samples are shown in Table 1, and localities in Figure 1. Digital photographs were taken, and then individuals were released after tail tips were collected. Total genomic DNA was extracted from 2-3 mm^3 of tail tissue 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) and G73 and G74 for C-mos (Saint et al. 1998). Amplification conditions were the same as described by Harris et al. (1998) and Saint et al. (1998). Amplified fragments were sequenced using an ABI 310 automated sequencer (Applied Biosystems), following the manufacturer's protocols. Mitochondrial DNA sequences were aligned using ClustalX (Thompson et al. 1997) with default parameters (gap opening = 10; gap extension = 0.2). Aligned sequences were 873 base pairs long. *Tarentola boettgeri* was defined as an outgroup. The data were imported into PAUP* 4.0b10 (Swofford 2002) for phylogenetic analysis. We used Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian inferences. We followed the approach outlined by Huelsenbeck and Crandall (1997) to test 56 alternative models of evolution, employing PAUP* 4.0b10 and Modeltest (Posada and Crandall 1998). Once a model of evolution was chosen, it was used to estimate a tree with both ML and Bayesian analysis. A MP analysis was carried out (100 replicate heuristic search, TBR branch-swapping) with gaps treated as missing data, and support for nodes estimated by bootstrapping with 1000 replicates (Felsenstein 1985). The Bayesian analysis was implemented using MrBayes (Huelsenbeck & Ronquist 2001) which calculates Bayesian posterior probabilities using a Metropolis-coupled, Markov chain Monte Carlo (MC-MCMC) sampling approach. Bayesian analyses were conducted with random starting trees, run 1.5 x 10^6 generations, and sampled every 100 generations using a general-time-reversible model of evolution with a gamma model of among site rate variation. In both searches stationarity of the Markov Chain was determined as the point when sampled log likelihood values plotted against generation time reached a stable mean equilibrium value; "burn-in" data sampled from generations preceding this point were discarded. All data collected at stationarity were used to estimate posterior nodal probabilities and a summary phylogeny. Two independent replicates were conducted and inspected for consistency to check for local optima (Huelsenback & Bollback 2001). Sequences of the nuclear protein-coding gene C-*mos* (356 base pairs long) were aligned against all published *Tarentola* sequences (Carranza et al. 2002, Jesus et al. 2002, Harris et al.

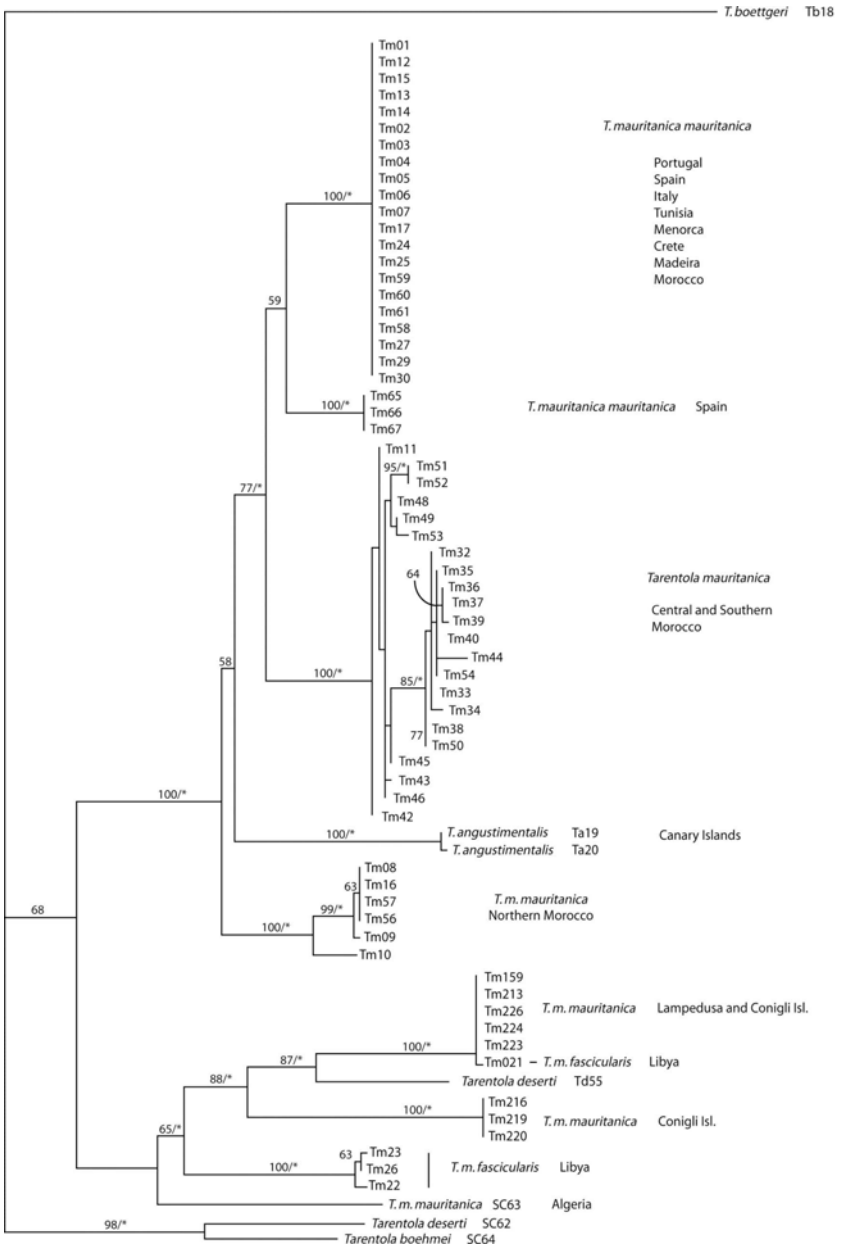

Figure 2. Tree derived from ML analysis using the model explained in the text, based on partial 12S rRNA and 16S rRNA sequences. Numbers above the nodes correspond to bootstrap support from MP analysis, * correspond to Bayesian probabilities superior to 95.

Table 1. New specimens sequenced for this analysis with locality and specimen voucher number. From their distribution, individuals would be expected to be *T. m. mauritanica.*

Species	Locality	Code	Accession numbers (12S, 16S)
T. mauritanica	Conigli islet	Tm159	FJ609240, FJ609248
T. mauritanica	Lampedusa village, Lampedusa	Tm213	FJ609241, FJ609249
T. mauritanica	Conigli islet	Tm216	FJ609242, FJ609250
T. mauritanica	Conigli islet	Tm219	FJ609243, FJ609251
T. mauritanica	Conigli islet	Tm220	FJ609244, FJ609252
T. mauritanica	Sanguedolce, Lampedusa	Tm223	FJ609245, FJ609253
T. mauritanica	Sanguedolce, Lampedusa	Tm224	FJ609246, FJ609254
T. mauritanica	Sanguedolce, Lampedusa	Tm226	FJ609247, FJ609255

2004b). There were no indels. Because variation is low, the sequences were joined in a median network (Bandelt et al. 2000).

Including the outgroups 71 combined mtDNA sequences were analyzed. It was found that the GTR model (base frequencies A 0.31, C 0.30, G 0.20, T 0.18) with a gamma distributed rate heterogeneity model (4 rate categories, G = 0.8535) and an estimated proportion of invariable sites (0.49) was the most appropriate model of evolution for these data. A ten replicate heuristic search incorporating this model found one tree of –ln 3556. For MP 178 characters were informative, and the MP analysis found 840 trees of 466 steps. The 50% majority-rule consensus tree derived from the MP analysis differed from the ML tree only in that it was less well resolved (Fig. 2). In all analyses seven clades, all with 100% Bayesian support can be identified. One group is composed of specimens of *T. m. mauritanica* from across Europe, including Spain, Portugal, Minorca, continental Italy, and Crete, and of those samples from Tunisia and Morocco. All are identical for the fragments of 12S rRNA and 16S rRNA analysed (Fig. 2). Sister taxa to these are samples from a genetically distinct lineage found so far only in Spain. A third clade includes samples from southern and central Morocco, comprising three subspecies, *T. m. mauritanica*, *T. m. juliae* and *T. m. pallida*. The fourth clade includes two samples of *T. angustimentalis* from the Canary Islands. A fifth group comprises samples from northern Morocco. These five clades are strongly supported as a group. All these results are consistent with previously published estimates of relationships (Harris et al. 2004a, 2004b). The sixth clade can be divided into subclades. One includes five *T. mauritanica* samples from Conigli islet and Lampedusa and also one of the *T. m. fascicularis* from Libya. The samples from Conigli islet and Lampedusa are identical, and differ from the Libyan sample by just 4 nucleotide substitutions. Sister taxon to these is a Moroccan specimen identified as *T. deserti*. The remaining samples from Conigli islet represent a further subclade, distinct from all other known mtDNA lineages. Other subclades include the remaining *T. m. fascicularis* representatives from Libya, and separately an individual of *T. mauritanica* from Algeria. For the

assessment of C-*mos* variation 49 taxa were analysed, including new sequences for *T. mauritanica* from Lampedusa and Conigli. Two individuals were heterozygotes, and each allele is represented in the network (Fig. 3).

These results support most of the conclusions reported in previous works (Harris et al. 2004a, 2004b), showing once again the paraphyly of *T. mauritanica* with respect to *T. angustimentalis* as well as the presence of two genetically distinct lineages in Europe. However, according to the data obtained from the samples collected from Conigli islet and Lampedusa, new evolutionary relationships may be hypothesised. The currently described species for these islands is *T. mauritanica* (Corti & Lo Cascio 2002), and it has been assumed to belong to the nominal subspecies (Guarino & Picariello 2006), which is also present in the nearest North African country, Tunisia. Nevertheless, our results show that the island specimens are more closely related to those from Libya, which are genetically described as *T. m. fascicularis*. Interestingly, in the tiny islet of Conigli, two divergent mtDNA lineages were found, that differ from each other by 5%. Such a divergence, with relatively slowly evolving rRNA genes, represents extremely high intraspecific variation. In other vertebrate groups, this amount of variation has been considered as sufficient to constitute different species (Brown et al. 2002). Taking into account the results obtained in this work, a taxonomic revision

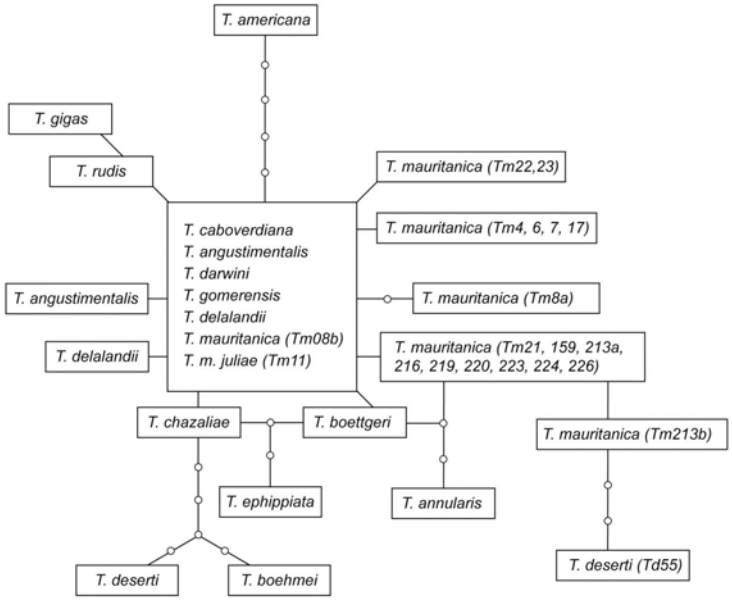

Figure 3. Median-joining network of the C-*mos* sequences for *Tarentola*. Circles indicate presumed missing haplotypes. *Tarentola mauritanica* from Morocco (Tm8) and Lampedusa (Tm213) were heterozygotes, so both alleles are indicated. Six individuals of *T. boettgeri*, two of *T. ephippiata* and two of *T. chazaliae* had three respectively identical haplotypes.

should be considered, at least for the subspecies *T. m. fascicularis*. There is also the possibility that these genes evolve at a faster rate in geckos than in other groups of organisms, and therefore higher levels of genetic distances are observed (Jesus et al. 2005a, 2006, Rato & Harris 2008). Information on other mitochondrial genes, and in particular from additional nuclear markers, should be assessed in order to determine if the amount of variation is due to relatively fast evolving mitochondrial genes or truly divergent lineages.

The two lineages were found in a remarkably small area (the islet of Conigli is separated from Lampedusa by only 150 meters of shallow water). Thus, a more extensive sampling is needed in order to uncover possible new genetically distinct lineages on surrounding islands and continental shore. One lineage is nearly identical to a specimen from Libya, but the other is distinct from all currently sampled specimens. These individuals might have reached the islands originating from North Africa and not from the Italian Peninsula, either by natural rafting or anthropogenic introduction. Either way, two separate colonization events are needed to explain the data. Given the genetic distinctiveness of the two lineages, assessments of interactions and possible hybridization within Conigli islet would be extremely useful in helping to determine the taxonomic level at which such subclades should be recognized. Further continental sampling in North Africa and in Italy, particularly the adjacent Italian islands including Sicily, is also needed to determine the fully range of these subclades. This work also includes an estimate of relationships derived from C-*mos* nuclear DNA sequences. All the major conclusions of Harris et al. (2004b) were supported. The individuals from Conigli and Lampedusa share the same haplotype as one of the Libyan samples, although they form two separate mtDNA lineages. Although slowly evolving, analysis of the C-*mos* sequences confirms the relationship between the samples from Lampedusa and Conigli, one *T. m. fascicularis* from Lybia (Tm21) and one individual of *T. deserti* (Td55) from Morocco. As in the analysis based on mtDNA, *T. deserti* is paraphyletic with one individual being more similar to *T. boehmei*, and the other associated with *T. mauritanica* from Libya and Lampedusa. Thus while C-*mos* is typically used for reconstructing higher level phylogenies, it is also a useful marker for assessing variation within species with divergent mtDNA lineages (Harris et al. 2004b, Jesus et al. 2005b, Godinho et al. 2006). Results obtained in this work again demonstrate the paraphyly of *T. mauritanica* with respect to *T. deserti* and *T. angustimentalis* (Harris et al. 2004a, 2004b) and also of the subspecies *T. m. mauritanica* and *T. m. fascicularis*. Additional sampling of all extant North African *Tarentola* species will be necessary to fully determine the evolutionary relationships of *T. mauritanica*, which is clearly a species complex. More data, both morphological and molecular, are clearly needed prior to a reassessment of the taxonomy of the group.

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