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Genetic variation in *Tarentola mauritanica* (Reptilia: Gekkonidae) across the Strait of Gibraltar derived from mitochondrial and nuclear DNA sequences

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Introduction

The Moorish gecko *Tarentola mauritanica* L. is widespread in North Africa from the Mediterranean to the Sahara and also in the Iberian Peninsula, the southern coast of France, Italy and has isolated populations in Greece, Israel and many Mediterranean islands (Martínez-Rica, 1997). Recent studies have shown it is paraphyletic with respect to *Tarentola angustimentalis* Steindachner, 1891 from the Canary Islands (Carranza et al., 2000; Harris et al., 2004), and may be a species complex (Harris et al., 2004). Identification of a single mitochondrial DNA (mtDNA) haplotype in Portugal, Spain, Italy,

Tunisia, Menorca, Crete and Madeira indicated that *T. mauritanica* might be introduced across Europe (Harris et al., 2004). Two genetically distinct mtDNA lineages were identified in Morocco, one in the north and one in the central and southern regions. However studies of Iberian and North African Wall lizards *Podarcis* have shown that genetically distinct cryptic forms can be limited to small geographic regions (Harris et al., 2002), while Chamaeleons were apparently introduced twice to the Iberian Peninsula from genetically distinct source populations (Paulo et al., 2002). Thus to understand evolutionary relationships in this biogeographically complex region extensive sampling is critical. Therefore we have greatly increased sampling in this area, both to further test the hypothesis that *T. mauritanica* is introduced in Europe and also to re-evaluate the taxonomy of *T. mauritanica* in Morocco where three subspecies, *T. m. mauritanica* (Linnaeus, 1758), *T. m. juliae* Joger, 1984 and *T. m. pallida* Geniez et al., 1999 have been described. We have also analysed a segment of the nuclear gene C-*mos* to enable us to compare relationships based on an independent marker.

Methods

Specimens collected in the field were identified to subspecies following Bons and Geniez (1996) and Geniez et al. (1999) (table 1 and fig. 1). Digital photographs were taken, and then individuals were released after tail tips were collected. Total genomic DNA was extracted from these small pieces of tail using standard methods, following Harris et al. (1998). Polymerase Chain Reaction primers used in both amplification and sequencing were 12Sa and 12Sb and 16SL and 16SH from Kocher et al. (1989) and G73 and G74 for C-*mos* from 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 on a 310 Applied Biosystem DNA Sequencing Apparatus.

Mitohcondrial DNA sequences were aligned using Clustal W (Thompson et al., 1994). Previously published sequences were included (Harris et al., 2004). Aligned sequences were 873 base pairs long. GenBank accession numbers are AY828448 to AY828499.

The data were imported into PAUP* 4.0b10 (Swofford, 2002) for phylogenetic analysis. For the phylogenetic analysis of the mtDNA data we used maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference. 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) discussed in detail in Posada and Crandall (2001). Once a model of evolution was chosen, it was used to estimate a tree using ML. 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 and Ronquist, 2001) which calculates Bayesian posterior probabilities using a Metropolis-coupled, Markov chain Monte Carlo (MC-

Table 1. Specimens sequenced for this analysis with locality and specimen voucher number. The individual marked with an * could not be identified to the subspecific level, although from the distribution it would be expected to be *T. m. pallida*.

Species	Locality	Code
Tarentola m. mauritanica	Zafra, Spain	Tm27
Tarentola m. mauritanica	Zafra, Spain	Tm29
Tarentola m. mauritanica	Valdezufre, Spain	Tm30
Tarentola m. mauritanica	Al Jadida, Morocco	Tm33
Tarentola m. mauritanica	Al Jadida, Morocco	Tm34
Tarentola m. mauritanica	Oulad Brahim, Morocco	Tm36
Tarentola m. mauritanica	Oulad Brahim, Morocco	Tm37
Tarentola m. mauritanica	Oulad Brahim, Morocco	Tm38
Tarentola m. mauritanica	Oulad Brahim, Morocco	Tm39
Tarentola m. mauritanica	Oulad Brahim, Morocco	Tm40
Tarentola m. juliae	31 km S. of Azni, Morocco	Tm42
Tarentola m. juliae	Azar, Morocco	Tm43
Tarentola m. juliae	Azar, Morocco	Tm44
Tarentola m. juliae	Azar, Morocco	Tm45
Tarentola m. juliae	Azar, Morocco	Tm46
Tarentola m. juliae	Agadir-Tizni Rd, Morocco	Tm48
Tarentola m. juliae	Agadir-Tizni Rd, Morocco	Tm49
Tarentola m. pallida	Massa, Morocco	Tm50
Tarentola m. pallida	Massa, Morocco	Tm51
Tarentola m. pallida	Merght, Morocco	Tm52
*Tarentola mauritanica**	Guelmine, Morocco	Tm53
Tarentola m. juliae	Argana, Morocco	Tm54
Tarentola m. mauritanica	Taza, Morocco	Tm56
Tarentola m. mauritanica	Taza, Morocco	Tm57
Tarentola m. mauritanica	Assilah, Morocco	Tm58
Tarentola m. mauritanica	Galera, Spain	Tm65
Tarentola m. mauritanica	Maquilla, Spain	Tm66
Tarentola m. mauritanica	Maquilla, Spain	Tm67

MCMC) sampling approach. Bayesian analyses were conducted with random starting trees, run 0.5×10^6 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 and Bollback, 2001).

New sequences from the nuclear protein coding gene C-*mos* were aligned against all published *Tarentola* sequences (Carranza et al., 2002; Jesus et al., 2002). There were no indels. Because variation is low, the sequences were joined in a median network (Bandelt et al., 2000).

Results

Including the outgroups 63 combined mtDNA sequences were analyzed. We concluded that the GTR model (base frequencies A 0.31, C 0.30, G 0.21, T 0.18) with a gamma distributed rate heterogeneity model (4 rate categories, G = 0.8415) and an estimated proportion of invariable sites (0.52) was the most appropriate model of evolution for these data. A ten replicate heuristic search incorporating this model found two trees of − ln 3389. Maximum parsimony analysis found 897 trees of 430 steps, the strict consensus of which was identical to the ML analysis, but less well resolved (fig. 2). One hundred and sixty-nine characters were parsimony informative. The estimate of phylogeny obtained using Bayesian analyses was very similar to the ML tree, with no branches with higher than 50% support in conflict (fig. 2).

For the assessment of C-*mos* variation 37 taxa were analysed, with a length of 375 base pairs, including ten new sequences for *Tarentola mauritanica*. In the median spanning network two sites are homoplastic (fig. 3). A single individual (Tm8) was heterozygous, and both haplotypes are represented separately in the network.

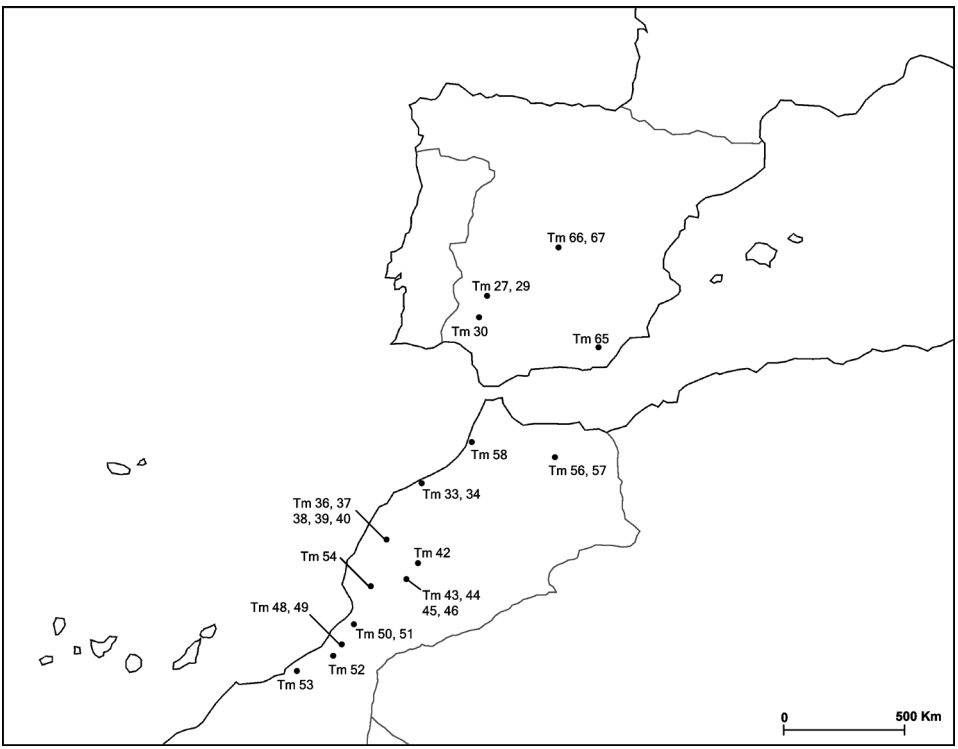

Figure 1. Map showing sampling localities of *Tarentola* sequenced in this study. Codes for samples are given in table 1. All others are from Harris et al. (2004).

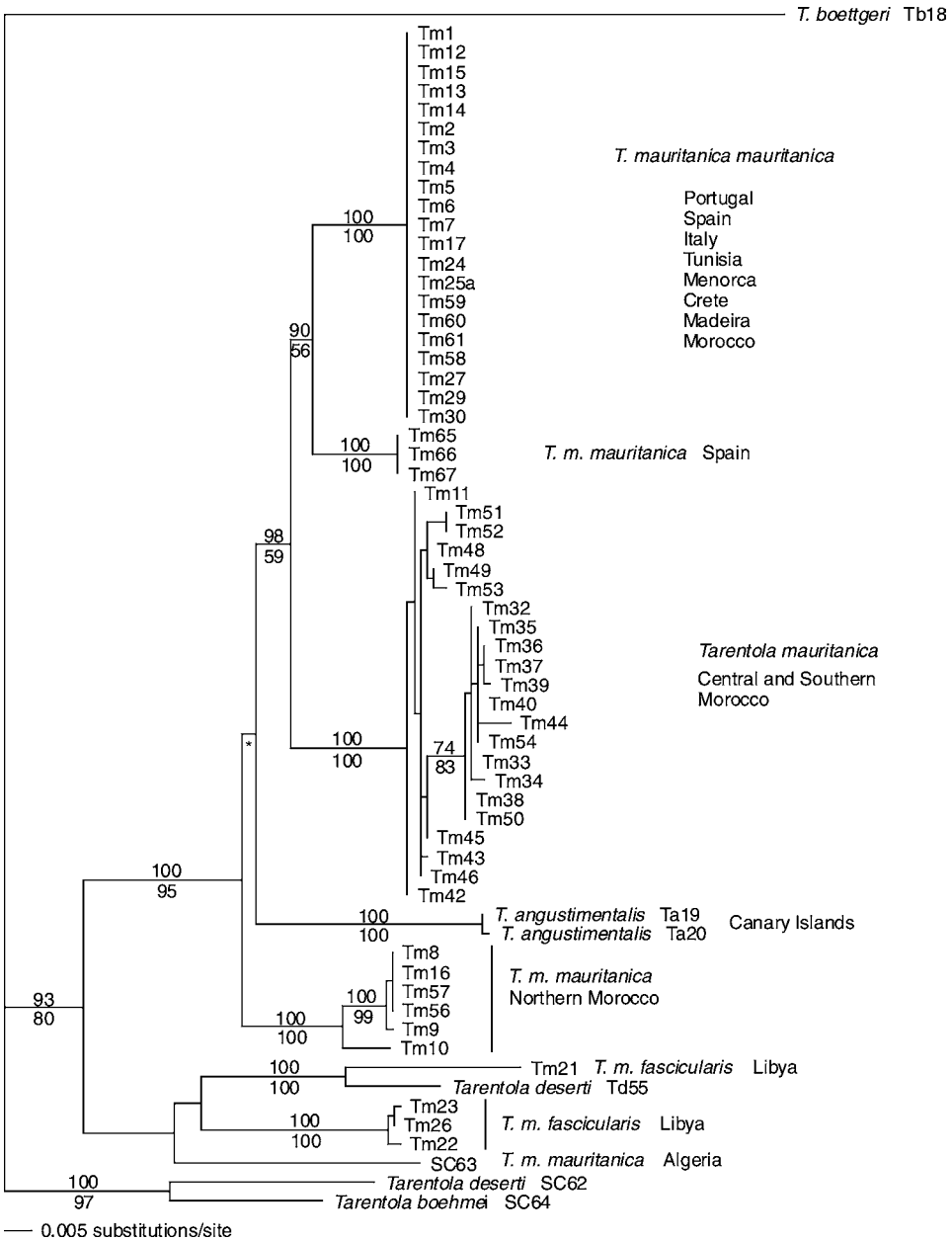

Figure 2. One of two trees derived from a ML analysis using the model described in the text. All analyses produced almost identical estimate of relationships to the one shown, except for rearrangements of minor branches within the *T. mauritanica* from Central and Southern Morocco clade. The node indicated by a * collapsed in the MP strict consensus and differed in the ML and Bayesian analyses, although support levels were low. Posterior node probabilities from the Bayesian analyses are indicated above nodes and bootstrap values for MP are given below nodes.

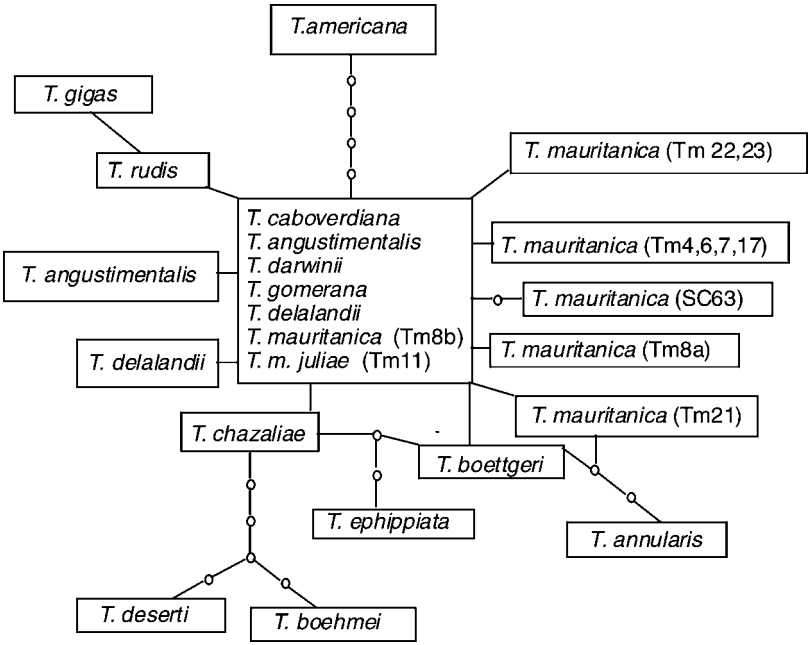

Figure 3. Median-joining network of the C-*mos* sequences for *Tarentola.* Circles indicate presumed missing haplotypes. *Tarentola mauritanica* from Morocco (Tm8) was a heterozygote, so both haplotypes are indicated. Six individuals of *T. boettgeri*, two of *T. ephippiata* and two of *T. chazaliae*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

Discussion

Our extended results support most of the conclusions of Harris et al. (2004), in particular the paraphyly of *T. mauritanica* with respect to *T. angustimentalis*. However our extended sampling around the Strait of Gibraltar alters some of the previous conclusions.

Is T. mauritanica *introduced in Europe?*

Previous finding of a single haplotype in all individuals sampled across Spain, Portugal, Italy, Menorca, Crete and Tunisia was interpreted as due to an anthropogenic introduction (Harris et al., 2004). Since the majority of variation within *T. mauritanica* is in North Africa, and since most species of *Tarentola* occur in this region it was suggested that the introduction was probably from Tunisian populations to Europe. However in our extended analysis we here report a new genetically distinct lineage found so far only in Spain. This means that while the majority of populations in Europe are probably introduced, at least some populations in Spain are not. Further sampling will be needed in other parts of southern Europe to determine if other natural populations exist. The genetic lineage found in southern Spain is the sister taxa to the haplotype found across the rest of Europe and

in Tunisia. This also raises the possibility that this lineage originated in Europe and was introduced to other parts of Europe and to Tunisia. If this is the case genetic diversity in Tunisia will be low. If the original hypothesis was correct, further sampling in Tunisia should uncover additional haplotypes. An individual containing the common European mtDNA haplotype was also sampled from Asilah on the northwest coast of Morocco (Tm58). One interpretation of this would be that the source population for the introduced European haplotypes was northwest Morocco. This would then imply that the Tunisian populations were introduced. However, Asilah was an important historical trading port, and it is therefore highly possible that *Tarentola* from Europe were introduced to Asilah.

Genetic differentiation between subspecies in Morocco

In this analysis we included several individuals of each of the three subspecies reported from Morocco, *T. m. juliae*, *T. m. mauritanica* and *T. m. pallida*. While *T. m. mauritanica* from northern Morocco are clearly a distinct genetic lineage individuals from central and southern Morocco representing all three subspecies form a separate lineage. Within this lineage there is no differentiation between the different subspecies. Thus the present subspecific taxonomy does not represent the genetic diversity of the species in Morocco.

Relationships between major clades

Apart from reporting an additional genetic lineage within Spain our additional sampling does not alter the conclusions of Harris et al. (2004). *T. mauritanica* is paraphyletic with respect to *T. angustimentalis* with strong (100% bootstrap) support. *Tarentola deserti* also appears to be paraphyletic, with one individual more closely related to *T. boehmei* and another individual more closely related to *T. m. fascicularis* from Libya. Additional sampling within these *Tarentola* species will also be necessary to fully determine the evolutionary relationships of *Tarentola mauritanica*.

Estimate of relationships derived from C-mos *nuclear DNA sequences*

Although variation within C-*mos* is low at this taxonomic level, this nuclear marker corroborates some of the estimates of relationships derived from the mtDNA sequences. Individuals from several species of *Tarentola*, including *T. mauritanica*, *T. darwinii*, *T. caboverdiana*, *T. gomerana* and *T. delalandii*, share a single haplotype. This is at the centre of the network, and is predicted to be the ancestral haplotype using the program TCS (Clement et al., 2000). None of the haplotypes derived from this are shared between species. The *T. mauritanica* specimens from Algeria and Libya, which are highly divergent mtDNA lineages, also have unique haplotypes for this nuclear marker. While specimens of *Tarentola* from Morocco shared the common presumably ancestral haplotype, specimens containing the mtDNA lineage which is found in Tunisia and widely in Europe had a separate haplotype.

Conclusions

Our additional sampling indicates that, while most European populations of *T. mauritanica* are probably introduced at least some populations in Spain are not. The direction of the intoduction, previously predicted to be from Tunisia to Europe needs further data to be validated. Our results suggest that *T. mauritanica* is a species complex with at least eight highly genetically distinct mitochondrial DNA lineages. There is considerable concordance between the trees derived from mtDNA sequences and the network based on nuclear DNA sequences. More data, both morphological and molecular, is needed prior to a reassessment of the taxonomy of the group.

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Patterns of road mortality in *Vipera latastei* and *V. seoanei* from northern Portugal

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The ecological effects of roads and traffic on wild animal and plant species are vast and complex (Spellerberg, 1998), as they usually increase mortality during and after road construction, change animal behaviour, fragment habitats and create barriers, change the chemical environment, enhance the spread of exotic species and increase the human use of the landscape (Trombulack and Frissel, 2000). Particularly, road killing is one of the most important sources of unnatural mortality in wildlife species, affecting from invertebrates (Seibert and Conover, 1991) to large mammals (Groot Bruinderink and Hazebroek, 1996), including ground, air-dwelling and aquatic species (Dodd et al., 1989; Mumme et al., 2000; Carr and Fahrig, 2001). Road mortality modifies the demography and population structure of species, and in certain circumstances, it can be the primary source of mortality