Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 30 (2004) 855–859
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Short Communication

Complex estimates of evolutionary relationships in *Tarentola mauritanica* (Reptilia: Gekkonidae) derived from mitochondrial DNA sequences

D. James Harris,[a,*] Vasco Batista,[a] Petros Lymberakis,[b] and Miguel A. Carretero[a]

[a] *Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO\UP), ICETA, Campus Agrario de Vairão, 4485-661 Vila do Conde, Portugal*
[b] *Natural History Museum of Crete, University of Crete, 157, Knossou Avenue, Irakleio, Crete, Greece*

Received 17 March 2003; revised 2 July 2003

Abstract

Mitochondrial DNA (12S rRNA, 16S rRNA) sequences were analysed within *Tarentola mauritanica* and other selected species of *Tarentola*. Several highly genetically distinct lineages occur in North Africa, revealing phylogroups in southern and central Morocco, northern Morocco, Algeria, Tunisia, and Libya. A single haplotype characterizes populations across Spain, Portugal, Italy, Menorca, Crete, and Tunisia raising the possibility of an anthropogenic introduction followed by rapid population expansion throughout southern Europe. *T. mauritanica* is paraphyletic with respect to *T. angustimentalis*, a Canary islands endemic. The high genetic diversity observed across North Africa suggests *T. mauritanica* may represent a species complex.

Keywords: 12S rRNA; 16S rRNA; *Tarentola mauritanica*; Phylogeny

1. Introduction

Tarentola (Reptilia, Gekkonidae) contains some 22 morphologically similar species, which occur in North Africa, coastal regions of the Mediterranean sea, Macaronesian islands (Selvagens, Canaries, and the Cape Verde) and also Cuba and the Bahamas. *Tarentola mauritanica* is widespread in North Africa from the Mediterranean to the Sahara, and similarly in the Iberian Peninsula, the Mediterranean coast of France, Italy, and has isolated populations in Greece, Israel, and many Mediterranean islands (Martínez-Rica, 1997). While some populations are known to be introduced, e.g., Madeira and South America, there is speculation that some of the eastern European populations also represent anthropogenic introductions (Joger, 1984; Martínez-Rica, 1997). The genus *Tarentola* has been widely studied phylogenetically (Carranza et al., 2000, 2002; Jesus et al., 2002), as well as phylogeographically for some species (Gübitz et al., 2000). 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* appears to be paraphyletic with respect to *T. angustimentalis* from the Canary islands, although intraspecific sampling in this survey was limited (Carranza et al., 2000). Therefore, we attempted to sample *T. mauritanica* across its range to test more rigorously the monophyly of the species. Assessment of levels of genetic variation might also give an indication whether some populations were introduced.

2. Materials and methods

The number and geographic locations of the specimens used in this study are given in Table 1 and Fig. 1. We analysed all four accepted subspecies of *T. mauritanica*, and also the sympatric species *T. deserti* and *T. boehmei*. Total genomic DNA was extracted from small pieces of tail using standard methods (Sambrook et al.,

* Corresponding author. Fax: +351-252-661780.
E-mail address: james@mail.icav.up.pt (D. James Harris).

1055-7903/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S1055-7903(03)00260-4

Table 1
Sample code and locality of specimens used for this study

Species	Locality	Code
Tarentola m. mauritanica	Zahara de los Atunes, Spain	Tm1
Tarentola m. mauritanica	Zahara de los Atunes, Spain	Tm2
Tarentola m. mauritanica	Barcelona, Spain	Tm3
Tarentola m. mauritanica	Barrosa, Spain	Tm4
Tarentola m. mauritanica	Barrosa, Spain	Tm5
Tarentola m. mauritanica	Monte Clerigo, Portugal	Tm6
Tarentola m. mauritanica	Monte Clerigo, Portugal	Tm7
Tarentola m. mauritanica	Bab Taza, Morocco	Tm8
Tarentola m. mauritanica	Bab Taza, Morocco	Tm9
Tarentola m. mauritanica	Bab Taza, Morocco	Tm10
Tarentola m. juliae	Tizi-n-Test, southern Morocco	Tm11
Tarentola m. mauritanica	Jebel Zebla, Tunisia	Tm12
Tarentola m. mauritanica	Kebir, Tunisia	Tm13
Tarentola m. mauritanica	Tabarka, Tunisia	Tm14
Tarentola m. mauritanica	Ercolano, Italy	Tm15
Tarentola m. mauritanica	Taza, Morocco	Tm16
Tarentola m. mauritanica	Garajau, Madeira	Tm17
Tarentola boettgeri	Gran Canaria	Tb18
Tarentola angustimentalis	Lanzarote	Ta19
Tarentola angustimentalis	Lanzarote	Ta20
Tarentola m. fascicularis	Libya/Egypt border	Tm21
Tarentola m. fascicularis	Maquis, Libya	Tm22
Tarentola m. fascicularis	Om Arazam, Libya	Tm23
Tarentola m. mauritanica	Moni Vosakou, Crete	Tm24
Tarentola m. mauritanica	Kolokytha island, Crete	Tm25
Tarentola m. fascicularis	Tobruk, Libya	Tm26
Tarentola m. mauritanica	Al Jadida, Morocco	Tm32
Tarentola m. mauritanica	Oulad Brahim, Morocco	Tm35
Tarentola m. mauritanica	Oulad Brahim, Morocco	Tm36
Tarentola m. pallida	Massa, southern Morocco	Tm51
Tarentola m. pallida	Merght, southern Morocco	Tm52
Tarentola m. mauritanica	Lithica, Menorca	Tm59
Tarentola m. mauritanica	Lithica, Menorca	Tm60
Tarentola m. mauritanica	Lithica, Menorca	Tm61
Tarentola deserti	Erfoud, Morocco	Td55
Tarentola deserti	Erfoud, Morocco	SC62
Tarentola m. mauritanica	Abdelmaleh Rahmd, Algeria	SC63
Tarentola boehmei	Akka Ighane, Morocco	SC64

1989). Primers used in both amplification and sequencing were 16SL and 16SH and 12Sa and 12Sb from Kocher et al. (1989). Amplification conditions were the same as described by Harris et al. (1998). Amplified fragments were sequenced on a 310 Applied Biosystem DNA Sequencing Apparatus. Sequences were aligned using Clustal W (Thompson et al., 1994). The 16S rRNA and 12S rRNA sequences were, respectively, 521 and 366 base pairs long. The 12S rRNA sequences from one individual of *T. mauritanica* from Algeria, *T. deserti* and *T. boehmei* had been previously published (Carranza et al., 2002).

Sequences were imported into PAUP* 4.0b10 (Swofford, 2003) for phylogenetic analysis. For the phylogenetic analysis of the combined data, we used maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference. When estimating phylogenetic relationships among sequences, one assumes a model of evolution. We used the approach outlined by Huelsenback and Crandall (1997) to test 56 alternative models of evolution, employing PAUP* 4.0b10 and Modeltest [Posada and Crandall (1998) described in detail in Posada and Crandall (2001)]. Once a model of evolution was chosen, it was used to estimate a tree using ML (Felsenstein, 1981) with random sequence addition (10 replicate heuristic search). The MP analysis was also carried out with random sequence addition (100 replicate heuristic search), and support for nodes estimated using the bootstrap technique (Felsenstein, 1985) with 1000 replicates. The Bayesian analysis was implemented using MrBayes (Huelsenback and 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 0.5×10^6 generations, and sampled every 10 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).

3. Results

In total, 38 taxa were included for a total of 887 base pairs; ML, MP, and Bayesian analyses gave congruent estimates of relationships (Fig. 2). Following Carranza et al. (2002), *T. boettgeri* was used to root the trees, as it is clearly separate from the *T. mauritanica*/*T. angustimentalis* group. The most appropriate model for the combined data was the GTR model with an estimate of invariable sites (0.512) and a discreet approximation of the gamma distribution (0.822). The ML heuristic search using this model found a single tree of −ln 3253. Bayesian analysis produced an identical estimate of relationships. For MP 159 characters were informative, and the MP search found two trees of 405 steps. The 50% bootstrap consensus tree derived from the MP

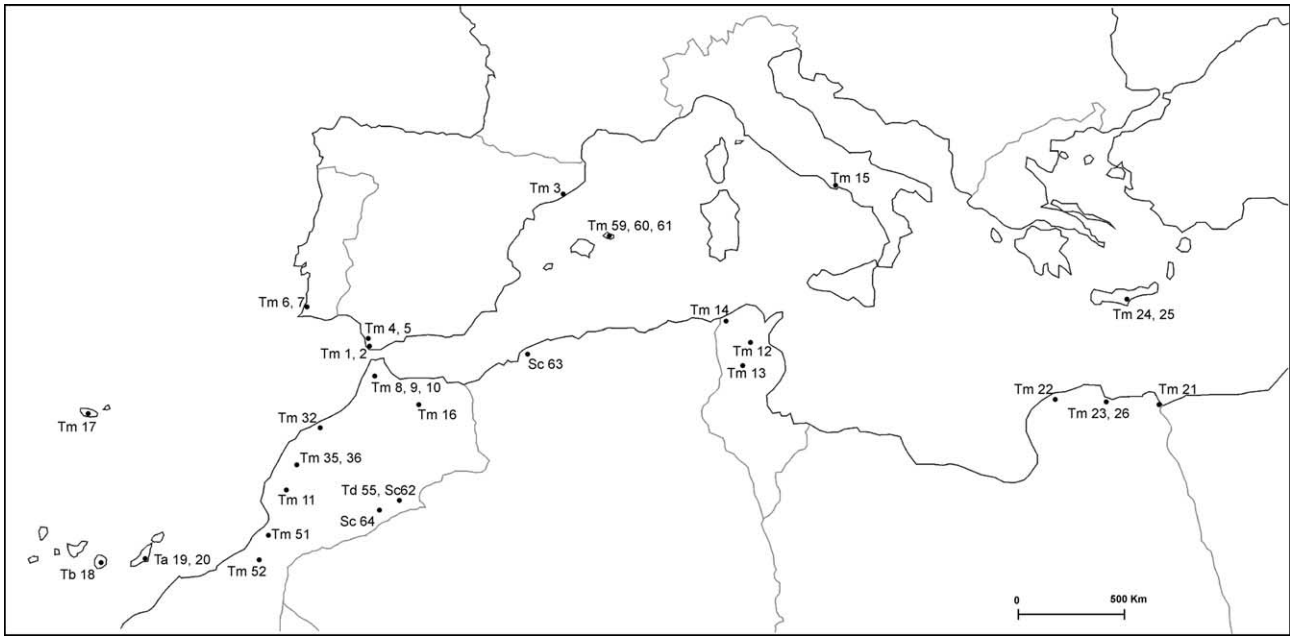

Fig. 1. Map showing the sampling localities of *Tarentola* sequenced for this study. Codes are given in Table 1. The 12S rRNA sequences from samples SC62, 63, and 64 were previously published (Carranza et al., 2002).

analysis differed only from the ML tree in that it was less well resolved (Fig. 2). In all analyses five clades, all with 100% Bayesian support, can be identified. One group is made up of specimens of *T. m. mauritanica* from across Europe, including Spain, Portugal, Menorca, Italy, and Crete, and of those samples from Tunisia. All are identical for these regions of the mtDNA. Sister taxa to these are samples from southern and central Morocco, including three subspecies, *T. m. mauritanica*, *T. m. juliae*, and *T. m. pallida*. A third clade includes the two samples of *T. angustimentalis* from the Canary islands, while a fourth includes samples from northern Morocco. These four clades are strongly supported as a group. The fifth clade can also be divided into subclades. One comprises three individuals of *T. mauritanica* from Libya, another one *T. mauritanica* from Libya and one *T. deserti* from Morocco, and separately an individual of *T. mauritanica* from Algeria.

4. Discussion

Based on our analysis of 12S and 16S rRNA *T. mauritanica* is clearly paraphyletic with respect to *T. augustimentalis*. In all analyses, *T. augustimentalis* is more closely related to most *T. mauritanica* populations relative to those from Libya and Algeria. Several genetic lineages can be identified most of which have some geographical coherency. These lineages are highly genetically differentiated. For example based only on the 16S rRNA the uncorrected genetic distance between samples from Libya and those from Tunisia is over 8%, and between North and South Morocco 5%. In a phylogeographic study of the Agamid lizard *Agama impalearis* in Morocco, maximum intraspecific sequence variation for the same region of 16S rRNA was 2.6% (Brown et al., 2002) and this was considered "surprisingly large and represents one of the most substantial within-species divergences described." Thus the genetic variation between these lineages is far higher than typically found within other reptile species. However, all the samples from Europe, including Portugal, Spain, Menorca, Italy, and Crete shared a single haplotype that was also found in Tunisia. Populations spanning such a widespread, disjunct area sharing a single haplotype for these respective gene regions is similarly extraordinary. However, geckos are often introduced by man and these colonizing populations can spread rapidly; *T. mauritanica* was introduced to Madeira only 15 years ago, and has already spread over a 16 km stretch of coast (D.J.H., pers. obs.). It is quite feasible that *T. mauritanica* was introduced to Europe from North Africa anthropogenically thousands of years ago, and has thus had plenty of time to expand. Was this the case, it would represent the largest colonization of any non-indigenous reptile in Europe. Highly variable markers, such as microsatellites, would be needed to confirm this. The more recent colonization of Madeira clearly involved the European mtDNA lineage, rather than a closer North African lineage.

While this study concentrated on *T. mauritanica*, we included two individuals of *T. deserti*, one of which had been included in a previous phylogenetic analysis (Car-

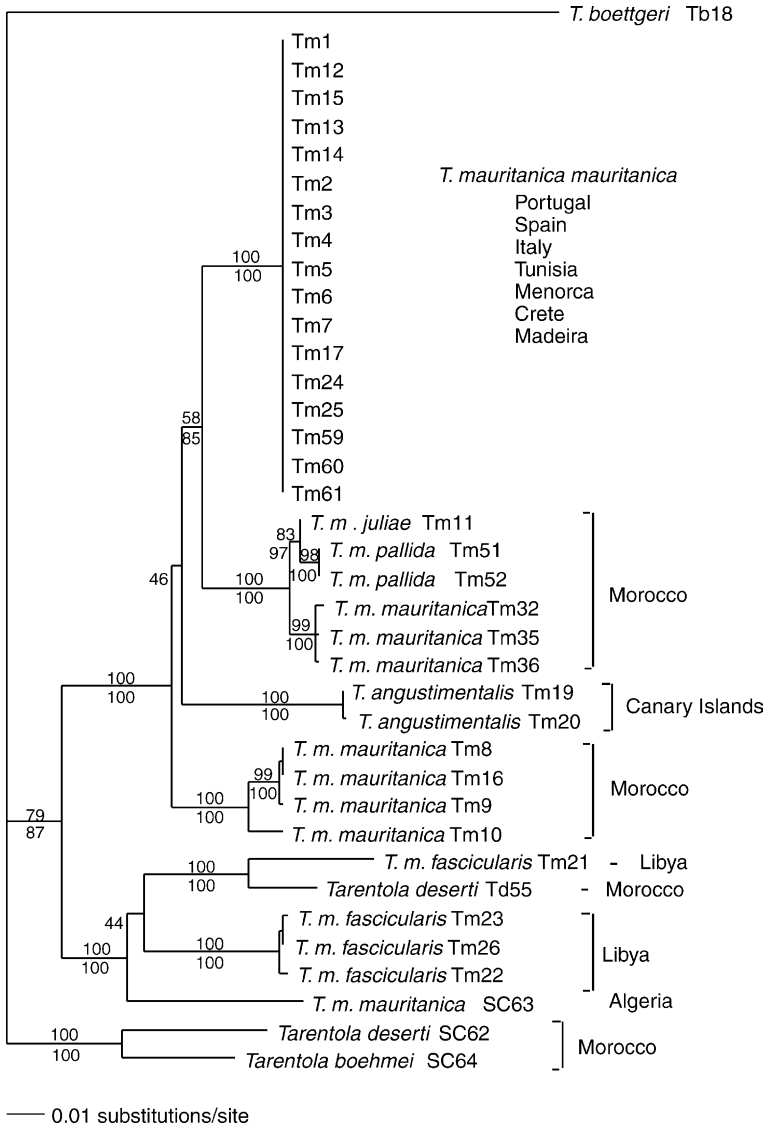

Fig. 2. Tree derived from an ML search using the model described in the text for the combined 12S and 16S rRNA sequences. Bayesian analysis gave an identical estimate of relationships. Bootstrap values (>50%) for MP are given above the nodes, and Bayesian probabilities are given below the nodes. The tree was rooted using *T. boettgeri*.

ranza et al., 2002). Both were collected from the same area of southern Morocco, but while one is closely related to *T. boehmei* the other appears in a separate clade more closely related to *T. mauritanica* from Libya. While it is impossible to draw substantial conclusions from only two individuals, this species deserves further investigation.

On average geckos appear to have higher levels of mtDNA divergence between species than other lizards (Harris, 2002), and several studies since then have shown intra-specific variation in different gecko groups far higher than is typically seen between species of vertebrates (e.g., Lamb and Bauer, 2000, 2002). Jesus et al. (2002) compared mtDNA variation in *Tarentola* from the Cape Verde islands and concluded that mtDNA variation was high between species relative to other reptiles from the same islands. The same is seen in this study. This could be an artefact of taxonomy—perhaps geckos are more morphologically conservative so that cryptic species have been overlooked. This phenomenon deserves further investigation.

5. Conclusions

The strongly supported paraphyly of *T. mauritanica* combined with the high levels of mtDNA divergence among populations is suggestive of a species complex. It appears likely that *T. mauritanica* was introduced in Europe. Other gecko species, e.g., *Hemidactylus turcicus*, should also be analysed genetically to see if they too are anthropogenic introductions in Europe.

Acknowledgments

This project was supported by grants from Fundação para a Ciência e Tecnologia (FCT) POCTI/41912/BSE/2001 and SFRH/BPD/5702/2001 (to D.J.H.). Thanks to C. Pinho and D. Barbosa for assistance during fieldwork in North Africa, to A. Brehm and J. Jesus for Madeiran samples, to A. Perera for Menorcan samples, and to S. Carranza for sending several critical samples. Thanks also to G. Caccone and the annonymous reviewers who made constructive comments on an earlier version of the manuscript.

References

Brown, R.P., Suárez, N.M., Pestano, J., 2002. The atlas mountains as a biogeographical divide in North-West Africa: evidence from mtDNA evolution in the Agamid lizard *Agama impalearis*. Mol. Phylogenet. Evol. 24, 324–332.

Carranza, S., Arnold, E.N., Mateo, J.A., López-Jurado, L.F., 2000. Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. Proc. R. Soc. Lond. B 267, 637–649.

Carranza, S., Arnold, E.N., Mateo, J.A., Geniez, P., 2002. Relationships and evolution of the North African geckos *Geckonia* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial and nuclear DNA sequences. Mol. Phylogenet. Evol. 23, 244–256.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Gübitz, T., Thorpe, R.S., Malhotra, A., 2000. Phylogeography and natural selection in the Tenerife gecko *Tarentola delalandii*: testing historical and adaptive hypotheses. Mol. Ecol. 9, 1213–1221.

Harris, D.J., 2002. Reassessment of comparative genetic distance in reptiles from the mitochondrial cytochrome *b* gene. Herpetol. J. 12, 85–86.

Harris, D.J., Arnold, E.N., Thomas, R.H., 1998. Relationships of the lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. Proc. R. Soc. Lond. B 265, 1939–1948.

Huelsenback, J.P., Bollback, J.P., 2001. Emperical and hierarchical Bayesian estimation of ancestral states. Syst. Biol. 50, 351–366.

Huelsenback, J.P., Crandall, K.A., 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Ann. Rev. Ecol. Syst. 28, 437–466.

Huelsenback, J.P., Ronquist, F., 2001. MR-BAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Jesus, J., Brehm, A., Harris, D.J., 2002. Relationships of *Tarentola* (Reptilia: Gekkonidae) from the Cape Verde islands estimated from DNA sequence data. Amphibia-Reptilia 22, 235–242.

Joger, U., 1984. Taxonomische revision der Gattung *Tarentola* (Reptilia, Gekkonidae). Bonner Zool. Beitr. 35, 129–174.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial evolution in animals: amplification and sequencing with conserved primers. Proc. Nat. Acad. Sci. USA 86, 6196–6200.

Martínez-Rica, J.P., 1997. *Tarentola mauritanica*. In: Gasc, J.-P., Cabela, A., Crnobrnja-Isailovic, J., Dolmen, D., Grossenbacher, K., Haffner, P., Lescure, J., Martens, H., Martinez Rica, J.P., Maurin, H., Oliveira, M.E., Sofianidou, T.S., Veith, M., Zuiderwijk, A. (Eds.), Atlas of Amphibians and Reptiles in Europe. Societas Europaea Herpetologica and Muséum National d'Histoire Naturelle, Paris.

Lamb, T., Bauer, A.M., 2000. Relationships of the *Pachydactylus rugosus* group of geckos (Reptilia: Squamata: Gekkonidae). Afr. Zool. 35, 55–67.

Lamb, T., Bauer, A.M., 2002. Phylogenetic relationships of the large-bodied members of the African lizard genus *Pachydactylus* (Reptilia: Gekkonidae). Copeia 2002, 586–596.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Posada, D., Crandall, K.A., 2001. Selecting models of nucleotide substitution: an application to human immunodeficiency virus 1 (HIV-1). Mol. Biol. Evol. 18, 897–906.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, second ed. Cold Spring Harbour Press, Cold Spring Harbour, NY.

Swofford, D.L., 2003. PAUP*: Phylogenetic Analysis Using Parsimony (and other methods) 4.0.b10. Sinauer Associates, Sunderland, MA.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acid Res. 22, 4673–4680.