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Assessment of genetic diversity within *Acanthodactylus erythrurus* (Reptilia: Lacertidae) in Morocco and the Iberian Peninsula using mitochondrial DNA sequence data

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Spiny-footed lizards *Acanthodactylus* consist of approximately 32 ground-dwelling lizards widely distributed across northern Africa and southwestern Asia. *Acanthodactylus erythrurus* (Schinz, 1838) is the only representative of the genus occurring in continental Europe, being found in the southern and central Iberian Peninsula as well as across Morocco and a large part of northern Algeria. Taxonomically *Acanthodactylus* is a notoriously difficult group, since most species are morphologically similar and often show intraspecific variability, and *A. erythrurus* is no exception. Salvador (1982) and Arnold (1983) accepted three subspecies: *A. e. erythrurus* (Schinz, 1838) in the Iberian Penninsula, *A. e. lineomaculatus* Duméril and Bibron, 1839 in the western plains and Atlantic coast of Morocco, and *A. e. belli* Gray, 1845 elsewhere in Morocco and Algeria. Both considered *A. e. atlanticus* Boulenger, 1918 from the Moroccan Atlas mountains a morphological intermediate between *belli* and *lineomaculatus*. Bons and Geniez (1995), however, suggested that *A. e. atlanticus* was a valid subspecies, and that *A. e. lineomaculatus* deserved specific status.

Our aim was to try to clarify this situation by sequencing parts of the 12S rRNA and 16S rRNA mitochondrial genes. These have already been used in assessing relationships within lacertid lizards (Harris et al., 1998) and within *Acanthodactylus* (Harris and Arnold, 2000), and are thus known to be phylogenetically informative. An additional aim was to examine genetic variation across the Strait of Gibraltar to compare with other taxa for which data have already been published. Around 5.5 mya the Atlantic Ocean breached the

Table 1. Specimens sequenced for this study with locality and code number.

Species	Locality		Code
Acanthodactyluse. erythurus	Picote	Portugal	Aee 1
Acanthodactylus e. erythrurus	Picote	Portugal	Aee 2
Acanthodactylus e. erythrurus	Torredambarra	Spain	Aee 3
Acanthodactylus e. erythrurus	Valencia	Spain	Aee 4
Acanthodactylus e. belli	Bab Taza	Morocco	Aeb 5
Acanthodactylus e. belli	Bab Taza	Morocco	Aeb 6
Acanthodactylus e. belli	Taza	Morocco	Aeb 7
Acanthodactylus e. belli	Taza	Morocco	Aeb 8
Acanthodactylus e. belli	Taza	Morocco	Aeb 9
Acanthodactylus e. belli	Debdou	Morocco	Aeb 12
Acanthodactylus e. belli	Debdou	Morocco	Aeb 14
Acanthodactylus e. belli	Debdou	Morocco	Aeb 15
Acanthodactylus blanci	Bou Chebka	Tunisia	Abl 17
Acanthodactylus blanci	Bou Chebka	Tunisia	Abl 18
Acanthodactylus boskianus	Bou Ghanem	Tunisia	Ab 19
Acanthodactylus boskianus	Bou Ghanem	Tunisia	Ab 20
Acanthodactylus e. belli	Debdou	Morocco	Aeb 21
Acanthodactylus e. belli	Debdou	Morocco	Aeb 22
Acanthodactylus e. belli	Debdou	Morocco	Aeb 23
Acanthodactylus e. atlanticus	Ida-ou-bouzia	Morocco	Aea 24
Acanthodactylus e. lineomaculatus	Kenitra	Morocco	Ael 25
Acanthodactylus bedriagae	Taroudent	Morocco	Abd 26
Acanthodactylus bedriagae	Agadir-Tiznit	Morocco	Abd 27
Acanthodactyluse. lineomaculatus	Moussa	Morocco	Ael 28

region and refilled the Mediterranean Sea which had dried up during the Messinian salinity crisis (Hsü et al., 1977). This re-established a marine barrier between terrestrial fauna, which could then diverge allopatrically. Alternatively, genetic analyses suggest that the opening of the Straits was not the cause of divergence in *Podarcis* lizards, but instead two transmarine dispersals across the Straits were involved (Harris et al., 2002). We therefore also intended to assess how often *Acanthodactylus* had crossed the Straits, and by using rough molecular clocks to assess if fragmentation between Iberian and north African forms could be associated with the opening of the Straits.

Material and Methods. Specimens examined and localities are shown in table 1 and figure 1. We analysed two individuals of *A. boskianus* Daudin, 1802 and *A. bedriagae* Lataste, 1881 as outgroup material and two *A. blanci Domergue*, 1901 that are thought to be closely related to *A. erythrurus* (Arnold, 1983). Specimens collected in the field were identified to the subspecific level following Bons and Geniez (1996). Some voucher specimens were taken, but in most cases individuals were released after tail tips were taken. Digital photographs were taken of all individuals. Total genomic DNA was extracted from 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). Amplification conditions were the same as described by Harris et al. (1998). Amplified fragments were sequenced on a 310 Applied Biosystem DNA Sequencing Apparatus.

Sequence analysis. Sequences were aligned using Clustal W (Thompson et al., 1994) and adjusted by eye. Two previously published *A. e. belli* (Fu, 2000; Harris and Arnold, 2000) were included in the analysis. Single individuals of *A. bedriagae*, *A. maculatus* and *A. boskianus* (Harris and Arnold, 2000) were also included. Aligned
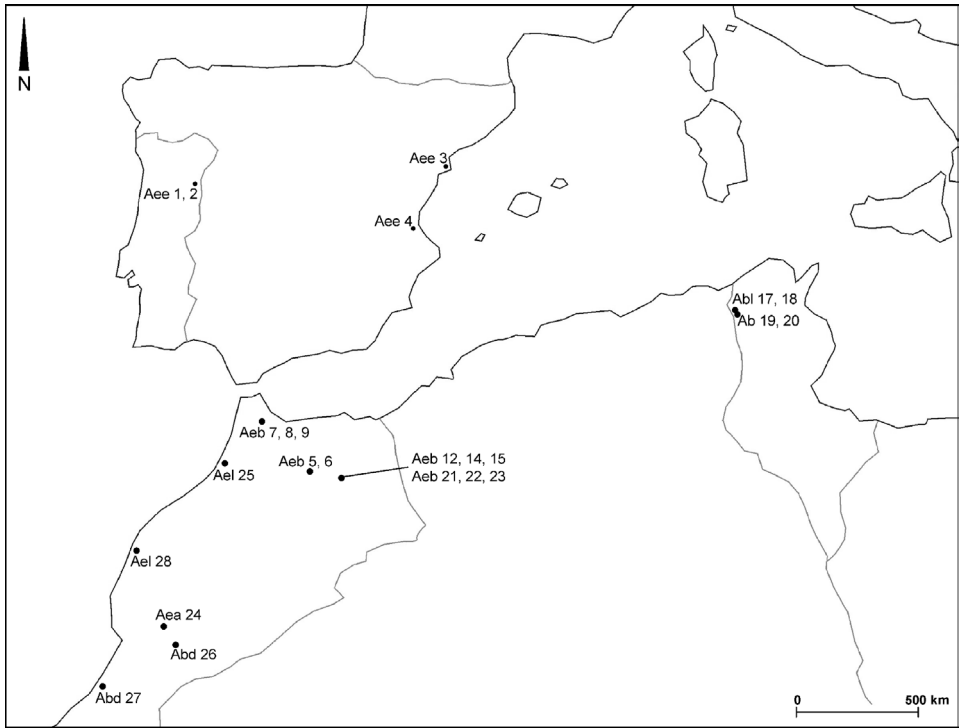
Figure 1. Map showing the sampling localities of *Acanthodactylus* sequenced for this study. Codes are given in table 1.

sequences of the combined partial gene regions were 865 base pairs long. A single 41 base pair loop region of the 16S rRNA could not be unambiguously aligned, and so was excluded from the analyses. GenBank accession numbers are AY633396 to AY633443.

The data were imported into PAUP* 4.0b10 (Swofford, 2002) for phylogenetic analysis. For the phylogenetic analysis we used maximum likelihood (ML) and maximum parsimony (MP). We used 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. Support for nodes was estimated by bootstrapping with 200 replicates (Felsenstein, 1985). 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).

Results.   Including the outgroups, 29 sequences were analyzed. We concluded that the general-time-reversible model with a gamma distributed rate heterogeneity model and an estimated proportion of invariable sites was the most appropriate model of evolution for these data. Maximum parsimony analysis found a single tree of 336 steps (Fig. 2). One hundred and forty-nine characters were parsimony informative.

Discussion.   Mitochondrial DNA sequences suggest that *A. e. erythrurus* in the Iberian is a monophyletic lineage relative to northern African *Acanthodactylus*. To estimate the
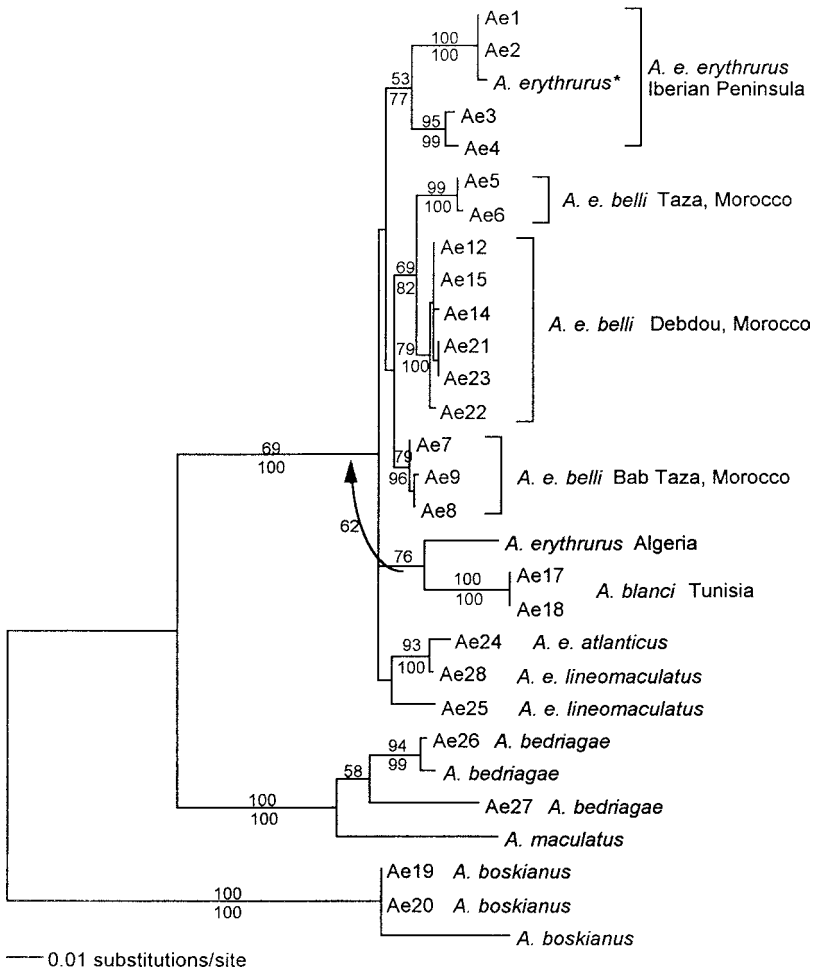
Figure 2. Tree derived from a ML analysis using the model described in the text. Bootstrap values for ML and MP are given above and below nodes respectively. MP analysis differed from ML in that *A. blanci* and *A. erythrurus* from Algeria were sister taxa to the remaining *A. erythrurus* (62% bootstrap support) indicated by the arrow. Coded samples are from this study, uncoded ones from Harris and Arnold (2000) except *A. erythrurus** that is from Fu (2000).

age of separation between these two groups we can use a molecular clock calibrated using colonization events within the Canary Islands for the lacertid lizard *Gallotia*, and which was also used within *Tarentola* (Carranza et al., 2000). A 1.96% genetic divergence per million years was estimated across two mtDNA gene fragments, 12S rRNA and cytochrome *b*. We plotted the pairwise divergence for 12S rRNA against cytochrome *b* for *Tarentola* to estimate the proportion of divergence that was due to the variation within 12S rRNA relative to the combined data. This indicates that the partial 12S rRNA alone evolves

at a rate of approximately 0.5% per million years in these lizards. Using our estimate of phylogeny this indicates that the Iberian *Acanthodactylus* mtDNA lineage has been isolated from the northern African lineage for circa 5 million years. Thus the re-opening of the Strait of Gibraltar could possibly have been the cause agent for the separation of these groups. Our results indicate the Iberian Peninsula was colonized from north Africa and not vice versa. This is expected, since the majority of extant *Acanthodactylus* species are from this region, and has also been indicated by protein electrophoretic data with ten times greater genetic distances reported from within Morocco compared to within Spain (Busack, 1986). Within the Iberian Peninsula there are two genetically distinct forms, separating individuals from eastern Spain from those in northern Portugal and an individual from Cadiz, southern Spain (Fu, 2000).

Within North Africa several genetically distinct groups can be identified. Samples of *A. e. belli* form a weakly supported unit with geographical substructuring between populations from Debdou, Taza and Bab Taza. The individual from Algeria, also *A. e. belli*, is not a part of this lineage, but instead is more closely related to two individuals of *A. blanci*. This makes both *A. e. belli* and *A. erythrurus* as a whole paraphyletic. In the topology derived using MP, the *A. erythrurus* from Algeria and *A. blanci* are sister taxa to the remaining *A. erythrurus*. The two samples of *A. e. lineomaculatus* are similarly paraphyletic, with one more closely related to an individual of *A. e. atlanticus*. Relationships between these lineages are weakly supported by the data, with none having >50% bootstrap support in the ML analysis. Genetic divergence between all populations of *A. erythururus* in North Africa is high, ranging from 0.08% between Debdou and Taza to a maximum of 3.1% between *A. e. belli* and *A. e. lineomaculatus* for 12S rRNA. While not as high as typically seen between lacertid species, there is substantial genetic variation within *A. erythururus*, and this shows geographic structuring. Although structuring within the *A. erythrurus* group (in this analysis including *A. erythrurus* and *A. blanci*) is not strongly supported by our analyses the unity of the group is. The separation of the *A. pardalis* group (samples of *A. bedriagae* and *A. maculatus*) relative to *A. boskianus* is similarly supported.

Conclusions. Our analyses indicate that Iberian *A. e. erythrurus* were separated from northern African forms around the time of the re-opening of the Straits of Gibraltar. This differs from *Podarcis*, which appear to have made two natural transmarine crossings (Harris et al., 2002) and from chamaeleons which were anthropogenically introduced to the Iberian Peninsula twice from genetically distinct source populations in North Africa (Paulo et al., 2002). As stated before, all taxa are not uniformly affected by the same geological event (Busack, 1986).

More sampling from Algeria will be crucial in evaluating the distinctiveness of *A. blanci*. Within Morocco our data do not support the separation of *A. e. lineomaculatus* as a distinct species. However, patterns of relationships are not strongly supported by our preliminary data, and clearly more data will be needed to define more precisely the North African genetic lineages within the *A. erythrurus* species group.

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