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Genetic variation within African spiny-tailed lizards (Agamidae: *Uromastyx*) estimated using mitochondrial DNA sequences

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Abstract. African spiny-tailed lizards (*Uromastyx*) are large, herbivorous lizards extensively traded locally for food and internationally as pets. Several species have recently been described, although some remain controversial. To determine relationships within North African forms, twenty individuals were analysed for over 1000 bases of mitochondrial DNA sequences. Phylogenetic analyses indicate four deeply divergent lineages that correspond to sampling areas, but not to current species designations. These results indicate that present taxonomy does not reflect the evolutionary history of these species.

Introduction

Spiny-tailed lizards of the genus *Uromastyx* are large generalist herbivores distributed in arid regions from India to the Arabian Peninsula and across the Saharan region of Africa. *Uromastyx* is registered in appendix II of CITES due to their being collected for food as well as extensively for the pet trade – based on CITES data over 215 000 *Uromastyx* were legally traded between 1977-2001, almost all wild-caught and primarily from Egypt and Mali (Knapp, 2004). Because of this, there are many publications regarding captive breeding and husbandry, but few related to ecology, behaviour in the wild, or phylogenetic relationships. Currently 16 species are recognized (Wilms, 2001), with several including *Uromastyx geyri* (Joger and Lambert, 1986), *Uromastyx flavifasciata*, *Uromastyx occidentalis* (Mateo et al., 1998), *Uromastyx alfredschmidtii* (Wilms and Böhme, 2001) and *Uromastyx leptiens* (Wilms and Böhme, 2000) being recently described. The acceptance of the specific status of some forms is not universal (e.g. *U. flavifasciata*, reviewed in Geniez et al., 2004). Amer and Kumazawa (2005) presented the first phylogeny based on mitochondrial DNA (mtDNA) sequences that included several African and Arabian taxa. They clearly showed that the African forms were a clade, but included only four species within this clade and none of the more controversial recently described forms. Further, many were captive bred individuals, so geographic variation within species could not be assessed. To determine relationships within the North–West African species, 20 individuals were analysed, all sampled in the field, from five species for approximately 1050 base pairs of mtDNA from three genes (cytochrome *b*, 12S rRNA and 16S rRNA). Genetic variation was then compared to the current taxonomic status.

Methods

Specimens were identified in the field using discriminant characters published in available guides (Geniez et al., 2004), digital photographs taken (available though the authors by request), and a toe or piece of tail was removed and stored in 100% ethanol (fig. 1 and table 1). Some specimens were roadkills. All live specimens were released immediately after sampling. Total genomic DNA was extracted from these small pieces of tissue using standard methods, following Harris et al. (1998). Polymerase Chain Reaction primers used in both amplification and sequencing were 12Sa and 12Sb, 16SL and 16SH, and cytochrome *b*1 and cytochrome *b*3 from Kocher et al. (1989), Simon et al. (1990) and Palumbi et al. (1991). Amplified fragments were sequenced on a 310 Applied Biosystem DNA Sequencing Apparatus.

Mitochondrial DNA sequences were aligned using Clustal W (Thompson et al., 1994). Since North African *Uromastyx* are known to form a clade (Amer and Kumazawa, 2005), the *Uromastyx* sp. from Yemen was designated as an outgroup. A total of 20 taxa were analysed. Aligned

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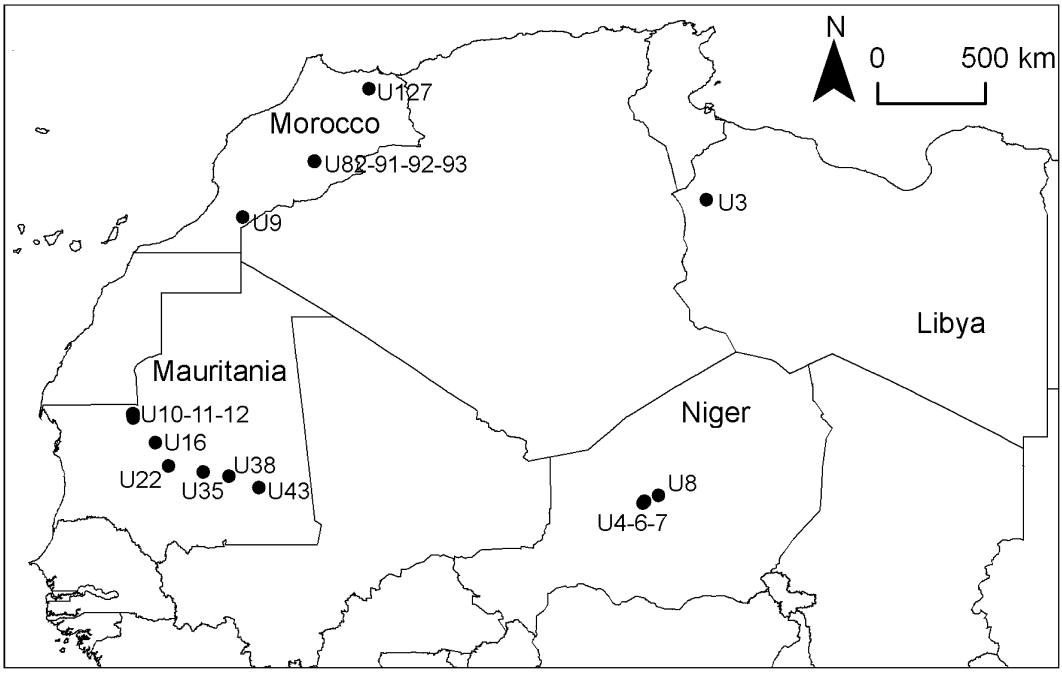

Figure 1. Map showing sampling localities. Codes relate to table 1 and figure 2.

Table 1. Specimens sequenced for this analysis with locality, coordinates and sample code numbers.

Species	Locality	Coordinates	DNA code
Uromastyx sp.	Yemen		U2
U. acanthinura	Derj-Idri, Libya	N29°52.8″ E10°45.4″	U3
U. acanthinura	Guelmine, Morocco	N29°9.3″ W8°35.6″	U9
U. acanthinura	Tinerhir, Morocco	N31°27.5″ W5°37.0″	U82
U. acanthinura	Tinerhir, Morocco	N31°27.5″ W5°37.0″	U91
U. acanthinura	Tinerhir, Morocco	N31°27.5″ W5°37.0″	U92
U. acanthinura	Tinerhir, Morocco	N31°27.5″ W5°37.0″	U93
U. acanthinura	Saka, Morocco	N34°29.8″ W3°19.6″	U127
U. geyri	Agadez-Timia, Niger	N17°13.2″ E8°6.0″	U4
U. geyri	Agadez-Timia, Niger	N17°18.2″ E8°10.2″	U6
U. geyri	Agadez-Timia, Niger	N17°18.2″ E8°10.5″	U7
U. geyri	Agadez-Timia, Niger	N17°33.3″ E8°44.9″	U8
U. dispar	Tenechfeil, Mauritania	N20°56.7″ W13°11.2″	U10
U. dispar	Gleibet Ahmed el'Abeidena, Mauritania	N20°46.6″ W13°10.4″	U12
U. dispar	Irmechat, Mauritania	N19°45.4″ W12°14.4″	U16
U. flavifasciata	Foum Ajar, Mauritania	N18°32.1″ W10°15.5″	U35
U. flavifasciata	Arhrijit, Mauritania	N18°21.2″ W9°10.3″	U38
U. flavifasciata	Oujaf well, Mauritania	N17°52.4″ W7°55.5″	U43
U. flavifasciata/dispar	Tenechfeil, Mauritania	N20°57.3″ W13°11.0″	U11
U. flavifasciata/dispar	Rachid, Mauritania	N18°47.0″ W11°41.5″	U22

sequences for 12S, 16S and cytochrome *b* were 346, 396 and 312 base pairs long (1054 bp in total), respectively.

The data were imported into PAUP* 4.0b10 (Swofford, 2002) for phylogenetic analysis. For the phylogenetic analysis of the mtDNA data maximum likelihood (ML) and maximum parsimony (MP) were used. The approach outlined by Huelsenbeck and Crandall (1997) was used 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 us-

ing ML, and support for nodes 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.

Results and discussion

Including the outgroup, 20 combined mtDNA sequences were analyzed. It was concluded that the GTR model (with a gamma distributed rate heterogeneity model (4 rate categories, G = 0.487) and an estimated proportion of invariable sites (0.45)) was the most appropriate model of evolution for these data. A ten replicate heuristic search incorporating this model found one tree of $-\ln 2623$. Maximum parsimony analysis of 86 informative characters found six trees of 241 steps, the 50% bootstrap consensus of which was similar to the ML analysis, but differed in weakly supported nodes (fig. 2).

Four genetically distinct units can be identified from these analyses, all of which are geographically coherent, although relationships between these units are not well supported. One group corresponds to *U. geyri* from Niger (Group B, fig. 2). The question of if *geyri* should be considered a separate species or a subspecies of *U. acanthinura* has been widely debated. Immunological data supported a separate species status (Joger, 1986), although an intergredation zone has also been reported which might suggest subspecific status is more appropriate (Wilms and Böhme, 1993). The degree of genetic divergence found in this analysis (9.3% for cytochrome *b* sequences) is slightly less than typically found between congeneric reptiles species, but would be an unusually high level of intraspecific variation (Harris, 2002). All the specimens of *U. acanthinura* from Morocco form a second unit (group C), that contains quite high within-group variation (up to 4.1% for cytochrome *b*). However, the specimen of *U. acanthinura* from Libya forms a third distinct unit (group A), that is not the sister taxa to *U. acanthinura* from Morocco, indicating that *U. acanthinura* is paraphyletic. The fourth clade (group D) contains all the specimens collected from Mauritania, belonging to two described species; *U. flavifasciata* and *U. dispar*. Genetic variation within these forms is extremely low, and the limited substructuring observed does not relate to the presently assigned specific status of the forms (fig. 2). Additionally, two specimens captured in Mauritania (U11 and U22), could not be assigned distinctively to a species as they shared common morphological characters with *U. flavifasciata* and *U. dispar* (fig. 3). These specimens have a colour pattern similar to *U. flavifasciata* but have a relatively small body size and less than 175 scales between the neck and cloaca, a feature of *U. dispar* (Geniez et al., 2004) (fig. 3). Combined with the lack of evidence of genetic differentiation based on the mtDNA sequences, this indicates the two forms are probably conspecific. The estimate of relationships derived using MP supported the same groups, but relationships between them were: ((D, C), A), B)). Thus, while the four groups are all clearly distinct, relationships between them remain ambiguous.

These results suggest the current taxonomy does not reflect the phylogenetic history of the species, nor the degree of genetic differentiation between forms. If *U. geyri* is accepted as a species distinct from *U. acanthinura*, then the latter species is paraphyletic and would need to be split into two separate species. The range of these two species would remain largely unknown. On the other hand, at least one of the recently described forms from Mauritania (*U. flavifasciatus*) does not appear to warrant separate species status and could be referred to as *U. dispar*. However, there are many difficulties in using a single marker such as mtDNA sequences to address taxonomic issues (reviewed in Ballard and Whitlock, 2004). Therefore although the present taxonomy is clearly inadequate, we recommend that further sampling and analysis of nuclear markers is performed prior to making formal taxonomic changes.

Comparative phylogeography of European biota indicates a typical pattern of repeated

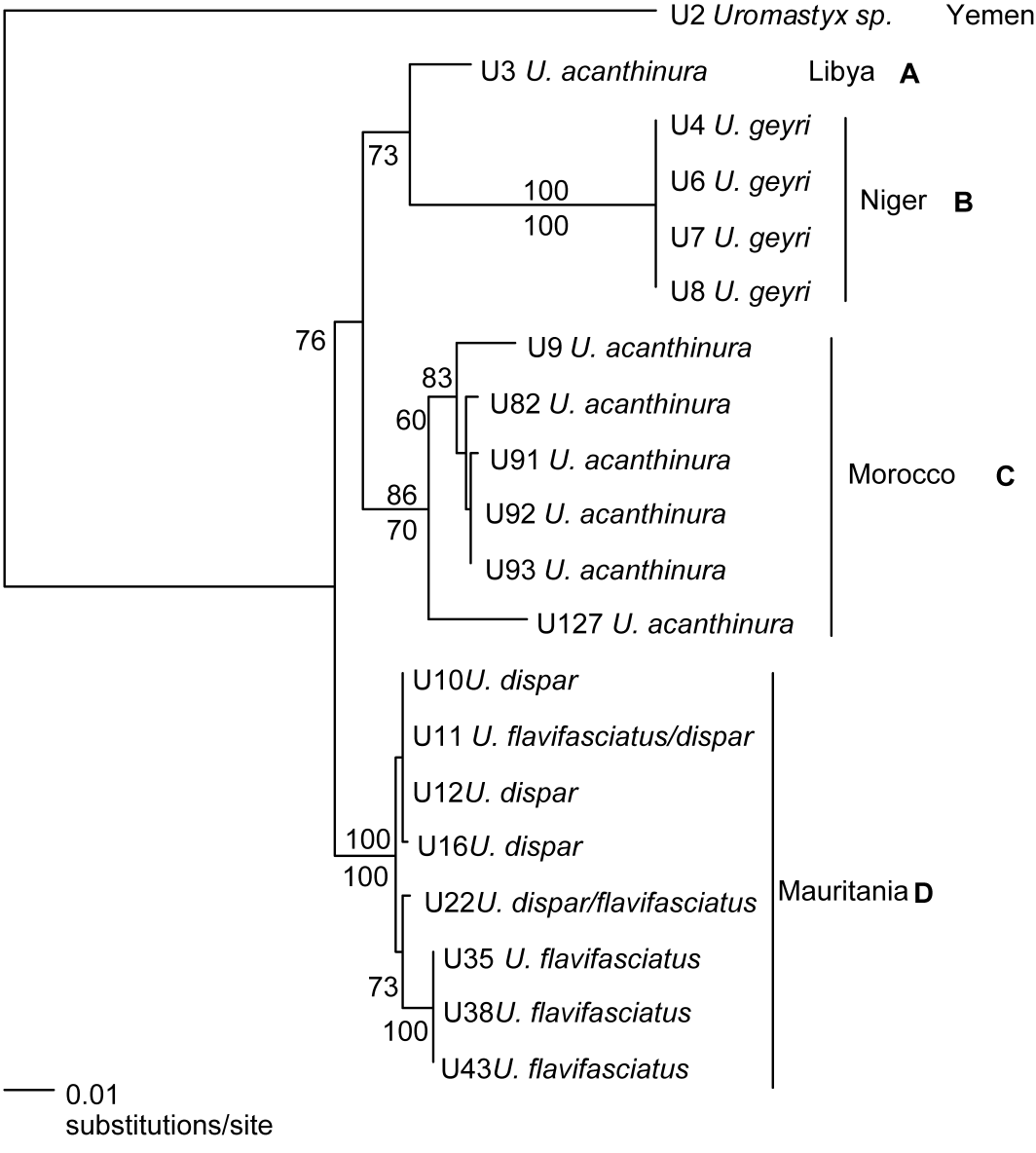

Figure 2. Single tree derived from a ML analysis using the model described in the text. Differences between the topology obtained by MP are discussed in the text. Bootstrap values for MP and ML and given above and below the nodes respectively. The four main groups are labeled as A-D. The tree was rooted using the specimen from Yemen.

contractions to southern refugia during ice ages, followed by northwards expansions during interglacial periods (Hewitt, 2001). However, much less is understood concerning the evolutionary history of North African fauna during this period. North Africa experienced alternate humid and arid periods that appeared to affect many terrestrial species, from snails (Guiller et al., 2001) to mammals (Cosson et al., 2005). Of the reptiles studied, *Agama* (Brown et al., 2002), *Acanthodactylus* (Harris et al., 2004a), *Blanus* (Vaconcelos et al., 2006), *Lacerta perspicillata* (Harris et al., 2003) and *Tarentola* (Harris et al., 2004b) all show deep phylogenetic divisions, and the same is demonstrated here in *U. acanthinura*. Whether this is a com-

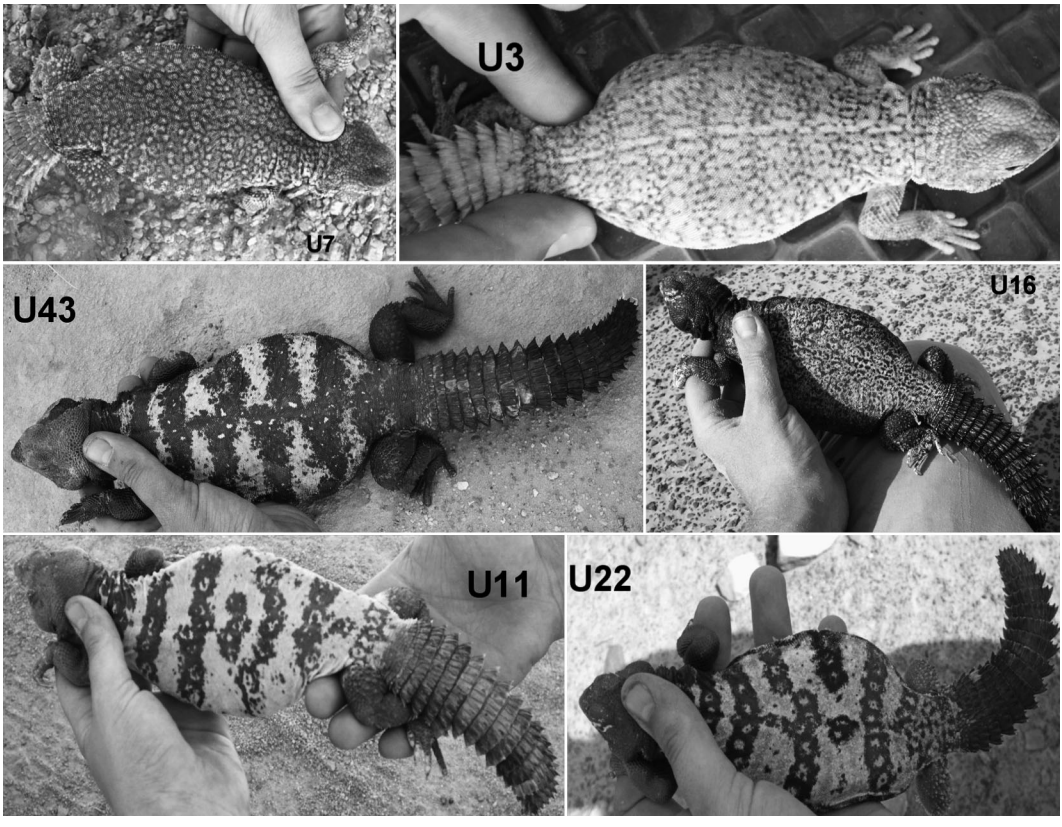

Figure 3. Specimens of some of the *Uromastyx* used in this study. Codes relate to table 1 and figure 2.

mon phylogeographic pattern of North African reptiles deserves to be further investigated.

Conclusions

Despite being extensively traded, this analysis shows that the relationships of North African *Uromastyx* are poorly understood and that current taxonomy is inadequate for describing patterns of genetic variation. Such knowledge is essential if sustainable exploitation of these lizards is to be developed. At least four separate genetic units can be identified within North Africa, corresponding to specimens from Niger, Libya, Morocco and Mauritania. Further sampling is needed to determine if other units exist, and to establish the ranges of the forms so far uncovered.

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