

# Genetic variation among spiny-footed lizards in the *Acanthodactylus pardalis* group from North Africa

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The systematics of the genus *Acanthodactylus* was classically based on external morphological traits, osteological characters and morphology of the hemipenes. Although the identification of species complexes is relatively easy, the distinction within some groups is difficult due to a high variability of the external morphology. Partial mitochondrial (12S and 16S rRNA) sequences (371 and 499 base pairs, respectively) were analysed from 32 specimens of the *A. pardalis* group from northern Africa including the described species *A. busacki*, *A. maculatus*, *A. mechriguensis* and *A. pardalis*. Several highly distinct genetic units were resolved, but with little support for relationships between them. These units did not coincide with current taxonomic units, but showed geographic structuring. Although the *A. pardalis* group displays significant variation, the present taxonomy of the group must be considered unsatisfactory since it is not supported by genetic evidence. For some forms, such as *A. mechriguensis* there is no support and it is suggested that it should be synonymized with *A. maculatus*. More data are clearly needed for other forms. Complex microevolutionary patterns due to the recent contraction/expansion phases of the Sahara Desert probably are related with the phylogenetic patterns observed.

**Key words:** *Acanthodactylus pardalis*, phylogeny, North Africa, mitochondrial DNA.

## INTRODUCTION

Spiny-footed lizards of the genus *Acanthodactylus* arose in the Middle East and later dispersed into Africa, probably during the mid-Miocene connection between Asia and Africa, spreading widely and evolving increasingly xeric-adapted forms (Harris & Arnold 2000). Currently, their geographic range covers the Iberian Peninsula, Africa north of the equatorial belt, and from the Middle East to western India, occurring mostly in semi-arid to desert ecosystems (Salvador 1982). *Acanthodactylus* is the most speciose genus in the Lacertidae family, with as many as 38 species listed in the E.M.B.L. Reptile Database (E.M.B.L. 2006). The systematics of the genus was classically based on external morphological traits, osteological characters and morphology of the hemipenes. Nine species groups have been recognized with such

characters (Salvador 1982). Although the identification of species groups does not present many difficulties, the distinction of taxa within some groups is difficult. There is a high intraspecific morphological variability in some traits and morphological variability is usually non-clinal. Traits that could be used for the identification of species change abruptly between adjacent populations or appear in confusing combinations (Schleich *et al.* 1996). This phenomenon has led to an unstable taxonomy, despite several revisions (Salvador 1982; Arnold 1983; Mellado & Olmedo 1990; Moravec *et al.* 1999; Harris & Arnold 2000; Crochet *et al.* 2003).

The *pardalis* group is one of the most complicated within *Acanthodactylus*. According to Salvador (1982), this group includes five species with the following ranges: 1) *A. pardalis* (Lichtenstein, 1823), occurring from Libyan Cyrenaica to southern

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Israel; 2) *A. busacki* (Salvador, 1982), restricted to the southwestern coast of Morocco and Western Sahara up to Bojador; 3) *A. maculatus* (Gray, 1838), from eastern Morocco to Libya (Tripolitania) covering the Algerian High Plateaux and Tunisia; 4) *A. bedriagai* (Lataste, 1881), restricted to the Oriental Plateaux of Algeria and westernmost central Tunisia; and 5) *A. spinicauda* (Doumergue, 1901), known only from two oases of western Algeria.

However, in northern Africa, with the exception of *A. spinicauda*, the systematics is not clear and there is profound disagreement among authors. Salvador (1982) and Arnold (1983) considered two distinct clades; the oriental populations belonging to *pardalis* and the occidental populations belonging to *maculatus*, these being sister taxa although with high morphological variability among populations. However, Salvador (1982) considered the populations from southwestern Morocco as a distinct species, *A. busacki*, whereas Arnold (1983) ranked them as a subspecies of *pardalis*: *A. p. bedriagai*. Furthermore, Salvador (1982) considered *busacki* to be closely related to *bedriagai* and *pardalis* and admitted that their distinction was complicated. Mellado & Dakki (1988) and Mellado & Olmedo (1990), while analysing morphological variability within Morocco, concluded that the distinction between *busacki* and *maculatus* was very subjective and that these should be synonymies of *pardalis*, following the criteria of Pasteur & Bons (1960), which would occupy all of the study area. Harris & Arnold (2000), in a coarse-scale analysis of mitochondrial DNA variation, indicated that *busacki* (named *bedriagai* by Harris & Arnold (2000), following Arnold (1983)) and *maculatus* were sister taxa. When morphological data were added to the phylogeny, these authors related respectively *busacki* with *pardalis*, and *maculatus* with *spinicauda* as sister taxa. Recently, Nouira & Blanc (1999) described *A. mechriguensis*, endemic to coastal areas of northern Tunisia, which is characterized, in comparison with Tunisian populations of *maculatus*, by a larger size, absence of denticles covering the ear opening, a higher number of rows of supraciliar granules, increased fragmentation of the first supraocular scale and a higher frequency of specimens with more than 12 rows of transversal ventral scales. Also, Moravec *et al.* (1999) described *A. beershebensis*, endemic to the Negev Desert of Israel, with marked differences in body size, coloration, scale arrangement and sexual dichromatism from Egyptian populations of *pardalis*. Further-

more, Werner (2004) recently described *A. ahmadisii*, endemic to Jordan, with marked differences, although this was based on a single specimen.

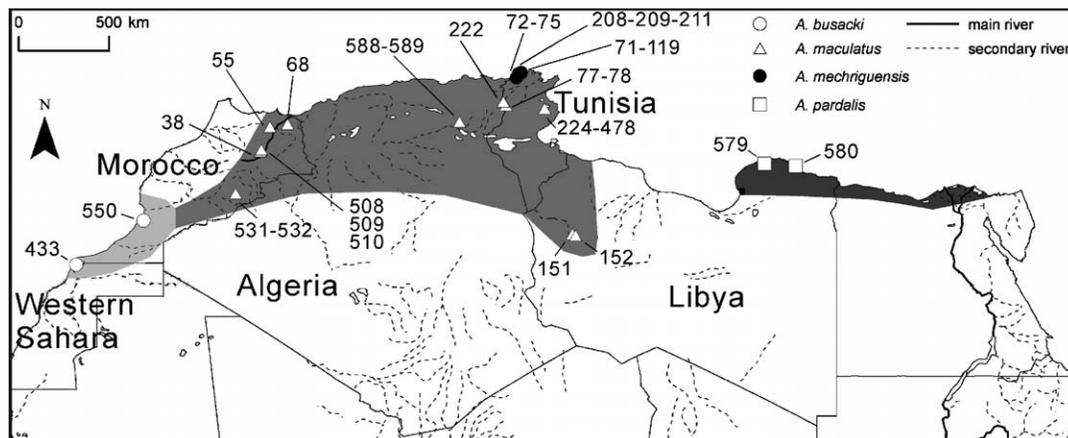
In order to identify phylogenetic relationships and to clarify the systematics of the *pardalis* group in northern Africa, portions of two mitochondrial genes (12S and 16S rRNA) were sequenced and analysed for genetic variation. Although mtDNA-based phylogenies do not necessarily correlate with species relationships (Ballard & Whitlock 2004), there is often strong congruence. These markers have been widely used in other lacertids (e.g. Harris *et al.* 1998; Carranza *et al.* 2006), and thus typical variation found between and within species is known. Several previous analyses using mtDNA markers within North African reptiles have uncovered evidence for cryptic species (Brown *et al.* 2002; Harris *et al.* 2004b; Perera *et al.* 2007), and thus assessment of the morphologically highly variable *Acanthodactylus* sp. is especially needed to assess this possibility.

## METHODS

The study area included northern Africa from Morocco to Libya (Fig. 1). In total, 30 specimens were analysed including 25 specimens captured by hand, two preserved specimens from the collections of the Natural History Museum of Crete and four that were previously published (Harris & Arnold 2000; Harris *et al.* 2004a) (Table 1, Fig. 1). Each specimen was photographed, and these photographs are available from the authors on request.

Total genomic DNA was extracted from small pieces of clipped tails, following standard methods (Sambrook *et al.* 1989). Primers used in both amplification and sequencing were 12Sa and 12Sb and 16SL and 16SH from Kocher *et al.* (1989). Amplification conditions were those described by Harris *et al.* (1998). Amplified fragments were sequenced on a 310 Applied Biosystem DNA Sequencing Apparatus. Sequences from three additional *Acanthodactylus* species (*A. tristami*, *A. aureus*, *A. erythrurus*) and two *Mesalina* species (*M. guttulata* and *M. adramitana*) were downloaded from GenBank and included as outgroups, following Harris *et al.* (1998). Sequences were aligned using Clustal W (Thompson *et al.*, 1994) and adjusted manually. The 12S rRNA and 16S rRNA were, respectively, 371 and 499 base pairs long. New sequences were deposited on GenBank, accession numbers EU086854–EU086957.

Combined sequences were imported into



**Fig. 1.** Geographic location of specimens from the *Acanthodactylus pardalis* group used in the study and distribution of currently described species. From west to east: *A. busacki* (light grey), *A. maculatus* (grey), *A. pardalis* (dark grey). *A. mechriguensis* is restricted to coastal northern Tunisia (area not shown). Numbers refer to sample codes.

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. We used the approach outlined by Huelsenbeck & Crandall (1997) to test 56 alternative models of evolution, employing PAUP\* 4.0b10 and Modeltest (Posada & Crandall 1998). 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 with TBR – branch swapping). The MP analysis was also carried out with random sequence addition (100 replicate heuristic search, TBR branch swapping). For MP, support for nodes was estimated using the bootstrap technique with 1000 replicates. The Bayesian analysis was implemented using MrBayes 3.0b4 (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  $1 \times 10^6$  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 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 (1000) were discarded. All data collected at stationarity were used to estimate posterior nodal probabilities and a summary phylogeny.

## RESULTS

All phylogenetic methodologies produced similar estimates of relationships (Fig. 2). While ML and Bayesian analyses recovered a single, identical tree, 33 most parsimonious trees were found (of 848 characters, 145 were variable and 89 informative under parsimony), the strict consensus of which differed in lack of resolution of most of the short branches separating several well supported groups identified by all analyses (Fig. 2). One group consisted of all Tunisian samples (including both *A. maculatus* and *A. mechriguensis*). This group appears to be the sister taxon to *A. maculatus* from Eastern Algeria. Four samples of *A. pardalis* formed a clade, with the two newly sequenced specimens from Libya being related to the previously published specimens of *A. maculatus* from Algeria (Harris & Arnold 2000). Samples of *A. maculatus* from Morocco clearly formed two distinct clades, one of which may be the sister taxon of all other members of the *A. pardalis* group. The specimen of *A. busacki* from Western Sahara was very divergent from the two samples of *A. busacki* from Morocco, making *A. busacki* paraphyletic. The two specimens from southwestern Libya formed another distinct clade. All of these groups arose from a polytomy, with extremely short branches giving little information as to how the groups are related to each other as indicated by low Bayesian probabilities, and collapse of nodes in the MP consensus tree.

## DISCUSSION

Overall, there is geographic substructuring with several well-supported groups identified in all

**Table 1.** Geographic coordinates (WGS84 datum) and location of specimens from the *Acanthodactylus pardalis* group sequenced for this study and current species assignment according to Salvador (1982), Schleich *et al.* (1996) and Nouria & Blanc (1999). NHMC, Natural History Museum of Crete; MB, Museum Bocage (Lisbon); SLC, Saïd Larbes collection; MKC, Moshen Kalboussi collection; CR, captured and released.

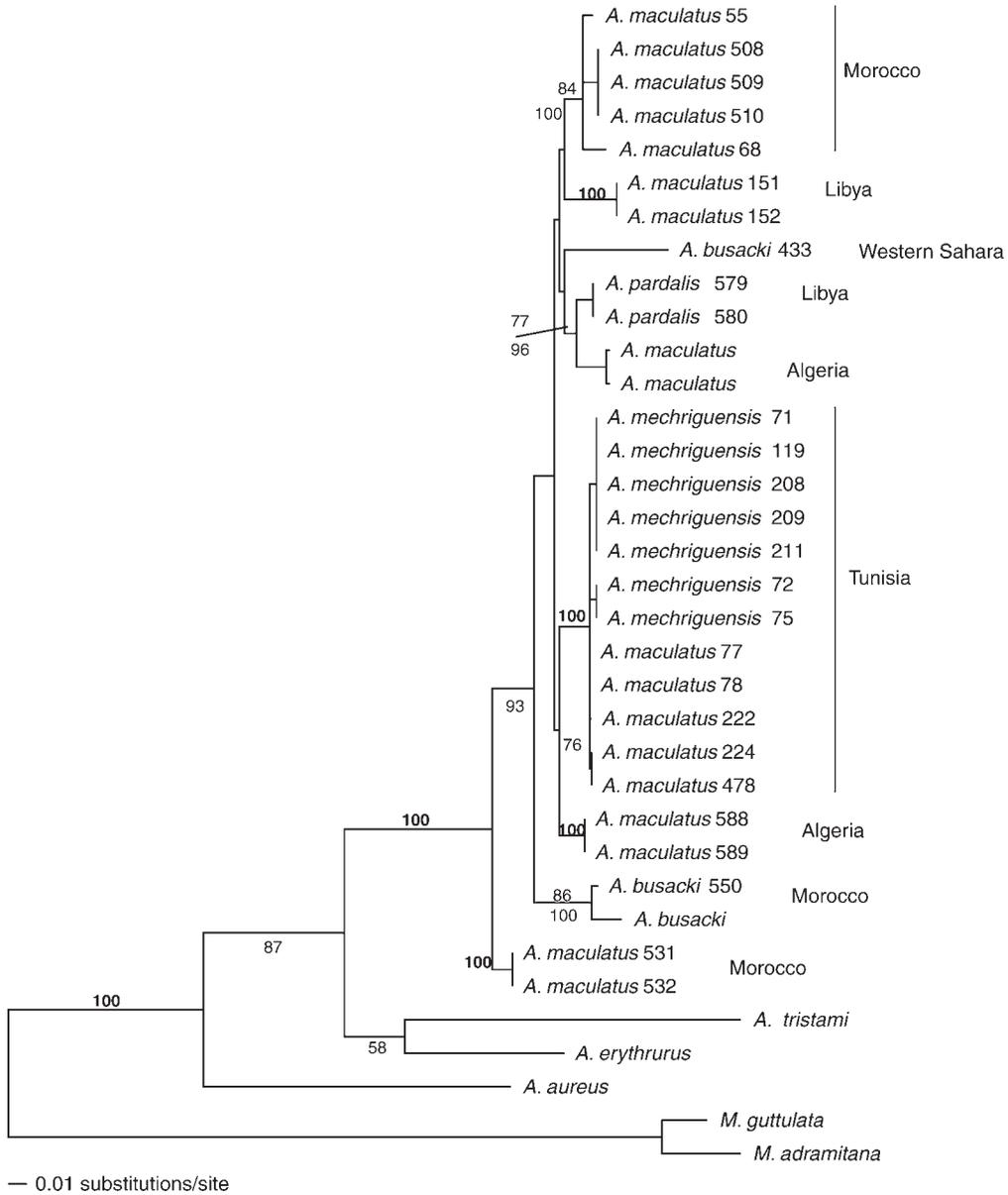
Current species assignment	Country	Location	Latitude	Longitude	Code	Voucher code
<i>A. busacki</i>	Morocco	Oued Massa	N29°48.369'	E09°38.85'	550	MB-07-881-02
	Western Sahara	NW margin of sebkha Oum Dba	N27°35.739'	E12°59.902'	433	MB-07-881-01
<i>A. maculatus</i>	Algeria	Ain Naga; Roman ruins of Zana	N34°45.29'	E06°09.093'	588	SLC Zana1
		Ain Naga; Roman ruins of Zana	N34°45.29'	E06°09.093'	589	SLC Zana2
	Libya	Hamadath al Hamrah; 172 km SE of Derj	N29°07.723'	E11°47.109'	151	MB-07-880-01
		Hamadath al Hamrah; 190 km SE of Derj	N29°03.633'	E11°57.269'	152	MB-07-880-02
	Morocco	10 km E of Alnif	N31°09.609'	E05°02.237'	531	CR
		10 km E of Alnif	N31°09.609'	E05°02.237'	532	CR
		Outat-Oulad-El-Haj	N33°21.198'	E03°45.63'	508	CR
		Outat-Oulad-El-Haj	N33°21.198'	E03°45.63'	509	CR
		Outat-Oulad-El-Haj	N33°21.198'	E03°45.63'	510	CR
		15 km south of Saka	N34°29.801'	E03°19.564'	55	MB-07-883-02
		10 km north of El Aioun	N34°38.666'	E02°26.471'	68	MB-07-883-05
	Tunisia	3 km E of Haidra; W of Thala	N35°34.999'	E08°28.958'	77	CR
		3 km E of Haidra; W of Thala	N35°34.999'	E08°28.958'	78	CR
Jugurtha Table		N35°45'	E08°21'	222	MKC F75	
13 km SE Kairouan		N35°25.462'	E10°24.852'	224	MB-07-880-03	
13 km SE Kairouan		N35°25.462'	E10°24.852'	478	CR	
<i>A. mechriguensis</i>	Tunisia	South of el Berrak dam; 6 km W of Nefza	N36°58.331'	E09°00.405'	72	CR
		South of el Berrak dam; 6 km W of Nefza	N36°58.331'	E09°00.405'	75	CR
		Cape Serrat beach	N37°12.893'	E09°14.771'	71	CR
		Cape Serrat beach	N37°12.893'	E09°14.771'	119	CR
		Sidi Mechrig	N37°09.795'	E09°07.405'	208	CR
		Sidi Mechrig	N37°09.795'	E09°07.405'	209	CR
		Sidi Mechrig	N37°09.795'	E09°07.405'	211	MKC 5
<i>A. pardalis</i>	Libya	Igdeida semidesert	N32°39.12'	E21°24.038'	579	NHMC: 80.3 73.1
		2 km west of Om Arazam	N32°32.559'	E22°57.022'	580	NHMC: 80.3 73.2

analyses. One group consisted of all samples from Tunisia, with others from Algeria, from southeastern Morocco (Alnif region), from southwestern Morocco (oued Massa), from northern Morocco, from southwestern Libya (Hamadath al Hamrah plateau), from northeastern Libya (Cyenaica peninsula), and from the Western Sahara (Oum Dba sebkha).

### Which historical factors could be involved in this complex scenario of species evolution?

The North African forms of the *pardalis* group are largely confined to relatively hard compact substrates in semi-arid and arid regions, usually with sparse and low vegetation cover (Blanc 1980; Arnold 1983; Schleich *et al.* 1996). However, they tend to avoid the hyper-arid areas (*sensu* Le Houérou 1996). Thus they occur in a relatively narrow strip between the northern humid areas of Mediterranean climate and the arid true Sahara

Desert (Fig. 1). Throughout time, the Sahara exhibited drier periods alternated with humid periods. The drier periods occurred during the Cambrian–Ordovician (500 million years ago, Mya), Westphalian–Albien (120 Mya) and mid-Miocene (15 Mya) (Bons 1973 and references therein; Le Houérou 1997; Zachos *et al.* 2001). Between the Miocene of hyper-arid desert habitats and the Pleistocene (1.6 Mya) of steppe habitats, at least four humid periods were alternated by periods of desertification (Le Houérou 1997). At the Last Glacial Maximum (18000 y) the climate was again arid with sand dunes much more widespread than today (Sarnthein 1978; Schuster *et al.* 2006). But at about 6000 y BP the Sahara Desert was replaced by steppes in many low-altitude sites, and temperate Xerophytic woods and warm mixed forest in the Saharan mountains (Prentice *et al.* 2000). Later, there was an increase in aridity resulting in current conditions. Extraordinarily, these climatic changes from desert to vegetated land and *vice versa* were



**Fig. 2.** Estimate of relationships among *Acanthodactylus pardalis* group derived from the combined mtDNA data using ML. Many short branches collapsed in the MP strict consensus, otherwise estimate of relationships derived from different methods were the same. Bootstrap values and Bayesian probabilities above 50% are indicated above and below nodes respectively. 100 in bold indicates both methods had 100% support. The tree was rooted using two species of *Mesalina*.

very rapid, and in some cases they spanned no more than a few hundred years (Sarnthein 1978). The consequences of these changes were dramatic in fauna and flora, enabling speciation events by vicariance and rapid range changes (e.g. Douady *et al.* 2003). Concerning the lizards of the *pardalis* group, these changes probably shaped species

range inducing isolation/connectivity among populations as the arid periods alternated with less arid periods. Probably, during the more arid periods their range shifted northwards, while in more humid periods it increased southwards. Therefore, the complex microevolutionary patterns found are probably related with the contraction/

expansion phases of the Sahara Desert. These have led to several events of isolation and differentiation followed by connectivity and admixture over short periods of time. This extremely dynamic process conceals the evolutionary patterns, making it hard to recover the phylogeny of the group. Even approximate timing of the major phylogenetic splits within the group is difficult, both because of the general problems of using a molecular clock (Pulquério & Nichols 2007), and the lack of fossil records to enable calibration within this group. However, most of the major clades identified are separated by 5–7% sequence divergence. This is in the same range of variation found using the same markers between populations of *A. erythrurus* from the Iberian Peninsula and North Africa that were presumed to have been separated during the Messinian Salinity Crisis around 5.5 million years ago (Harris *et al.* 2004a). Therefore it is probable that at around this time major diversification began within the *A. pardalis* group.

### Taxonomic implications

Although the *A. pardalis* group displays significant variation, the present taxonomic arrangement must be considered unsatisfactory since it is not supported by genetic evidence. Many populations of *Acanthodactylus* were originally described based on qualitative colour patterns and assigned as forms or varieties (e.g. Boulenger 1918, 1921). These were later ranked as species, causing a proliferation of names that obscured the true differentiation pattern between populations (Mellado & Olmedo 1990). Recently, other restricted-range forms were assigned with a specific status, but some of them were based on a low number of specimens (Werner 2004) or without sampling all the range of sister-taxa to detect morphological variation overlap (Nouira & Blanc 1999).

Interpretation of the results from the mtDNA-based estimate of phylogeny relative to the taxonomy is complex. For some forms, such as *A. mechri-guensis*, there is no support and this form should be synonymized with *A. maculatus*. *A. busacki* is paraphyletic, consisting of at least two divergent genetic lineages. Previous morphological analyses have also found little support for *A. busacki* (Arnold 1983; Mellado & Olmedo 1990). Furthermore, divergence estimates (ML) between *A. busacki* and *A. maculatus* from Morocco (between 5.4 and 7.2%) are not particularly different from that

between populations of *A. maculatus* within Morocco (up to 6%). This level of variation is high, especially, for these relatively slowly encoding rRNA genes, although less than that found within some other North African reptile species (e.g. Harris *et al.* 2004b).

The genetic data presented here indicate that alternative taxonomic interpretations can be made. All lineages could be subsumed into a single highly variable species (*A. pardalis*), but this would mask considerable morphological and genetic variation. In particular, according to Arnold, (1983), hemipenis are very distinct in *A. spinicauda* and *A. maculatus* and therefore at least these two species cannot easily be synonymized into *A. pardalis*, which shares a different type of hemipenis with *bedriagai* and *busacki*. Similarly, Arnold (1983) considers differences in the hemipenis of *A. pardalis* and *A. maculatus* as ‘prima facie evidence of separate species status’. However, the acceptance of all major genetic units as full species would create considerable taxonomic rearrangements, and the range of many groups would be poorly known. Therefore we recommend referring to a ‘*A. pardalis* species complex’ until units can be defined more precisely. In particular, more data is clearly needed for the Algerian forms *A. spinicauda* and *A. bedriagai*. Furthermore, the phylogenetic status of the Asiatic forms *A. ahmad-disii* and *A. beershebensis*, which were not available for this study, could also contribute to the clarification of the situation of this species group, especially, regarding the definition of *A. pardalis sensu stricto*. The inclusion of nuclear markers could help resolve the systematics of the group, but given the complex evolutionary history of the group, it is possible that no single taxonomic arrangement can be entirely satisfactory. If the complex geological history of the region has led to other widespread Saharan species to present similar phylogenetic patterns remains to be investigated, although deep phylogenetic splits have been reported for *Agama* (Brown *et al.* 2002), *Uromastyx* (Harris *et al.* 2007) and *Psammophis* (Rato *et al.* 2007). Whether these groups correspond geographically can only be understood as more taxa are included.

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