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## Adding *Acanthodactylus beershebensis* to the mtDNA phylogeny of the *Acanthodactylus pardalis* group

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**Abstract.** The phylogenetic affinities of *Acanthodactylus beershebensis*, a highly endangered lacertid lizard endemic to the Neguev (Israel), were assessed using mtDNA markers. Fragments of 12S and 16S rRNA were analysed and compared with already published sequences of *Acanthodactylus*. Results corroborate the taxonomic placement of *A. beershebensis* as a member of the *A. pardalis* group but place it within a polytomy at the same phylogenetic level as other (unnamed) African populations. This pattern of high but poorly structured genetic diversity, previously observed for other *Acanthodactylus* complexes, has been suggested to derive from the climatic instability of North Africa and the Middle East during the humid and dry periods of the Pleistocene as well as dune migrations. In conservation terms, if *A. beershebensis* is to be prioritised, then other populations of the *A. pardalis* group inhabiting North Africa would deserve a similar status, making their species definition urgent. These results highlight the need for considering phylogeny when establishing conservation priorities.

**Keywords:** *Acanthodactylus beershebensis*, *Acanthodactylus pardalis*, Lacertidae, phylogeny, Middle East.

### Introduction

*Acanthodactylus* sp., the spiny-footed lizards, constitute a successful genus of lacertids widely distributed from the Middle East, where they originated, to India and North Africa (Harris & Arnold 2000). The 41 species described so far (Uetz 2011) have been systematically arranged into species groups mostly supported by both morphological and molecular evidence (Salvador 1982, Arnold 1983, Harris & Arnold 2000, but see Fonseca et al. 2009). In contrast, within the species groups, both kinds of evidence are often conflictive making taxonomy unstable (Mellado & Olmedo 1990, Crochet et al. 2003, Harris et al. 2004, Fonseca et al. 2008, 2009).

The forms of the *A. pardalis* group are restricted to limestone soils in semi-arid and arid regions, usually with sparse and low vegetation avoiding the hyper-arid areas (Blanc 1980, Arnold 1983, Schleich et al. 1996, Moravec et al. 1999). They, hence, occupy a narrow coastal strip, from Western Morocco to Jordan interrupted in the Sinai Peninsula, and between the Mediterranean areas and the true Sahara, Sinai and Arabic deserts

(Fig. 1). A phylogenetic study on the African members of the group (Fonseca et al. 2008) revealed considerable genetic variation related to the recent contraction/expansion phases of the Sahara desert but did not provide support for the current taxonomic arrangement. Specimens of the Middle East forms, geographically isolated from the remaining populations, were not available.

*Acanthodactylus beershebensis*, Moravec et al. (1999), was described on the basis of the morphological distinction (size, hemipenis, colouration, scalation, morphometrics) and geographic isolation of the specimens from the Neguev (Israel) from those in coastal Egypt and Libya (Moravec et al. 1999). Whereas Egyptian and Libyan specimens were included in the analysis by Fonseca et al. (2008), no samples from Israel have previously been sequenced.

*Acanthodactylus beershebensis* is now a well known model in lizard ecology and behaviour (Seligmann et al. 2003, Hawlena 2009, Hawlena et al. 2006, Hawlena & Pérez-Mellado 2009). Moreover, it is threatened in its isolated range due to habitat disturbance, reduction and fragmentation associated to massive agricultural transforma-

tions (Hawlena et al. 2010), being classified as critically endangered by the IUCN (Werner et al. 2006).

In this context, here we aim to assess the phylogenetic affinities of *A. beershebensis*, namely regarding the members of the *A. pardalis* species complex, using mtDNA sequence data in order to determine its placement in the evolution of the genus.

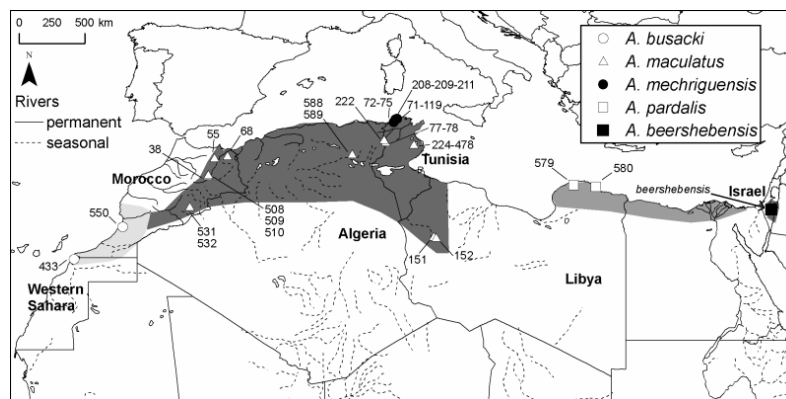
#### Material and Methods

An adult male *Acanthodactylus* sp. was found in the herpetological collection of the Department of Animal Biology, University of Barcelona (Spain) labelled as *Acanthodactylus* sp. with the voucher code DBA-7004. After inquiries on the origin of the specimen, the collector (L. Mestre) indicated that it had been found accidentally dead inside a spider pitfall trap set in Sde Boquer (30.873377 N, 34.781689 E, altitude 489 m, Neguev Desert, Israel, Fig. 1) in 2008. The finding took place in the early morning when routinely checking the traps after night

sampling. Considering its morphological traits, typical of the *A. pardalis* species group (Scheleich et al. 1996) and falling within the variability of *A. beershebensis* (Moravec et al. 1999, Table 1), and the location of this site, 40 km south of Be'er Sheva, the type locality of *A. beershebensis* (Moravec et al. 1999), it was ascribed to this species. The specimen was photographed, and pictures are available from the authors on request.

In the lab, total genomic DNA was extracted from the tail tip of the specimen using standard methodologies (Harris et al. 1998). Fragments of 12S and 16S rRNA genes were amplified using published primers 12Sa and 12Sb for 12S rRNA gene (Kocher et al. 1989) and 16SL1 and 16SH2 for 16S rRNA gene (Hedges & Bezy 1993). The PCR products were purified by enzymatic cleaning and sequenced using the ABI Prism Big Dye Terminator Cycle sequencing protocol on an ABI Prism 310 automated sequencer (Applied Biosystems).

Sequences obtained were aligned with a total of 22 published sequences belonging to the *A. pardalis* group (Fonseca et al. 2008) plus three additional sequences from other *Acanthodactylus* species groups (*A. tristami*, *A. boskianus*, *A. erythrurus*) as outgroups following Harris et al. (1998), all downloaded from GenBank. The multiple



**Figure 1.** Geographic location of samples of specimens from the *Acanthodactylus pardalis* group (modified from Fonseca et al., 2008). From west to east: *A. busacki* (light grey), *A. maculatus* (dark grey), *A. pardalis* (grey) and *A. beershebensis* (black, sample locality is marked with an arrow). *A. mechriguensis* is restricted to coastal northern Tunisia (area not shown).

**Table 1.** Morphological traits of the specimen of *Acanthodactylus beershebensis* analysed (according to Moravec et al, 1999). Metric characters were measured with a digital calliper to nearest 0.01 mm.

Metric characters	Meristic characters	
ra (SVL)	Supralabials	4
Head length	Gulars	30
Head width	Plates in collar	12
Head depth	Dorsals	63
Forelimb length	Ventrals across belly	11
Hindlimb length	Transverse rows of ventrals	34
Fourth toe length	Femoral pores	19
Tail length	Scales betw. rows of femoral pores	0
	Preanals	7
	Subdigital lamellae	20

alignments of the DNA sequences were performed using MAFFT v.5 (Katoh et al. 2005) with the "Q-INS-i" strategy. New sequences were deposited in GenBank, accession numbers JF912448-JF912449.

The maximum parsimony (MP) analyses and alternative tree topology tests (Shimodaira-Hasegawa test using 1000 bootstrap replicates with full optimization - one-tailed test, Shimodaira & Hasegawa 1999) were performed with PAUP\* version 4.0b10 (Swofford 2003) using a heuristic search algorithm with 100 random addition replicates. All changes had the same weight and gaps were treated as a fifth state. The most appropriate model of sequence evolution (GTR+I+G) was selected employing jMODELTEST v.1.0.1 (Posada 2008) under the corrected Akaike's information criterion. The Maximum Likelihood (ML) analyses were performed using *PhyML* (Guidon & Gascuel 2003). Bootstrapping (1000 pseudo-replicates) was used to evaluate the stability of nodes (Felsenstein 1985) for MP and ML analyses. The Bayesian inference (BI) was implemented using MrBayes v.3.1.2. (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Two independent runs were generated for the dataset. Both runs reached stationarity and showed convergence. For all the analyses the burn-in period covered the first 20%. A 50% majority-rule consensus tree was generated combining the remaining trees of the analyses.

## Results

The final alignment consisted of partial sequences of 35 lizards and included 371 and 514 positions for the 12S and 16S rRNAs fragments, respectively, of which 111 were parsimony informative. These amplified fragments correspond approximately to the 3' end of the complete rRNA genes: the second half of 12S and the last third of 16S rRNAs genes were sequenced. The variation within the sequences was found mainly in loops or at the terminal positions of stems of the rRNAs. Around 5% of the alignment was variable. Within these, transitions were almost 3 times more frequent than transversions. The variation of the fragments of *A. beershebensis* was similar to the remaining sequences, i.e. the variation is found in the same regions in *A. beershebensis* as in other species. The dataset used in this study only differs from the one used by Fonseca et al. (2008) by the inclusion of novel mitochondrial DNA (mtDNA) sequences from *A. beershebensis*. Therefore, we will focus on the results concerning this species.

The majority-rule (50%) consensus trees of all analyses (MP, ML and BI) were topologically identical (see Fig. 2), only differing in the supporting values of some branches. The monophyly of the *A. pardalis* group was strongly supported by the phylogenetic trees, as well as the placement of *A. beer-*

*shebensis* included within this group. The lack of resolution at the base of the tree makes it unfeasible to determine the closest African population/species to *A. beershebensis*. Nonetheless, the latter is genetically distinct from the other specimens, in contrast to what is shown by the Tunisian samples of *A. maculatus* and *A. mechriguensis*.

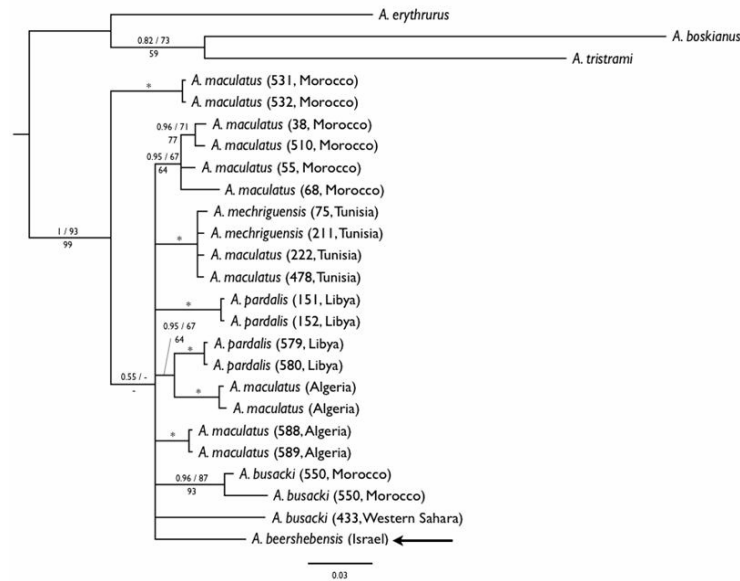
To test the monophyly of the *A. pardalis* group species, the optimal topology obtained with the ML analysis was compared with alternative topological arrangements (see Table 2). The results reject *A. maculatus* and *A. pardalis* as monophyletic species.

## Discussion

Our molecular results corroborate the taxonomic placement of *A. beershebensis* as a member of the *A. pardalis* group but places it within a polytomy at the same phylogenetic level as other (unnamed) African populations and the identity of the closest relative remains uncertain. In terms of conservation, the definition of these unnamed populations seems important.

Previous phylogenetic studies (using mitochondrial and nuclear markers) on the *A. erythrurus* group also showed a similar panorama where distinct genetic units with unclear relationships not coincident with the morphological taxonomy were found (Harris et al. 2004, Fonseca et al. 2009). The climatic instability of North Africa and the Middle East during the Pleistocene, oscillating phases of humid and dry periods (Gasse 2006, Drake et al. 2008) as well as dune migrations (Smith 1969) has already been suggested as responsible for these complex patterns in the *pardalis* group (Fonseca et al. 2008) which could now be extended to the Middle East. Alternatively, an interpretation of the results as deriving from ancestral genetic polymorphism cannot be completely discarded. Finally, local adaptation acting on some traits like body size and shape, fixation of rare traits in isolated populations or even phenotypic plasticity could have promoted strong phenotypic divergence not resulting from the fixed genetic changes as found in insular lizard populations (Losos et al. 1997, 2000).

*Acanthodactylus beershebensis* is now considered one of the conservation priority reptile species (Cox et al. 2006) inhabiting one of the world biodiversity hotspots (Myers et al. 2006), certainly, the one undergoing the most ancient human distur-



**Figure 2.** Phylogenetic relationships (50% majority-rule consensus) of *A. beershebensis* within the *A. pardalis* group derived from partial 12S and 16S rRNAs sequences (all analyses showed the same consensus tree). The tree was rooted using *Acanthodactylus erythrurus*, *A. boskianus* and *A. tristrami*. Branch supporting values are given in the order: Bayesian posterior probabilities; ML bootstrap values; MP bootstrap values. Values under 50% are represented by “-” and asterisks (\*) indicate maximum supporting values for all analyses. The arrow highlights the position of *A. beershebensis*.

**Table 2.** Statistical support for alternative topological hypothesis of relationships of different taxonomic units in *A. pardalis* group (SH, Shimodaira-Hasegawa test; \* indicates  $p < 0.05$  and suggests that the unconstrained and constrained topologies differ significantly).

Tree mtDNA:	-Log likelihood:	$\Delta$ -Log Likelihood:	SH <i>P</i> :
Unconstrained ML tree (Fig.2)	2861.00562	(best)	
Constrained ( <i>A. beershebensis</i> sister taxon to the remaining <i>A. pardalis</i> group species)	2866.30908	5.30346	0.625
Constrained ( <i>A. busacki</i> monophyletic)	2863.12306	2.11744	0.798
Constrained ( <i>A. maculatus</i> monophyletic)	2954.36624	93.36062	0.000*
Constrained ( <i>A. mechriguensis</i> monophyletic)	2875.55382	14.54820	0.280
Constrained ( <i>A. pardalis</i> monophyletic)	2876.12492	15.11930	0.043*

bance. Since conservation actions will fall, firstly, upon endemic species with restricted spatial distribution, it is imperative that the taxonomy of the whole *Acanthodactylus* genus is clarified in order to properly allocate limited conservation resources. For example, the specific status of the Tunisian endemic *A. blanci* (*A. erythrurus* group) is not supported either by mitochondrial or nuclear markers (Fonseca et al. 2009). A similar situation might be happening within the *A. pardalis* group with *A. mechriguensis* and *A. busacki* (based on mtDNA). However, this is not the case of *A. beershebensis*. Nevertheless, this taxonomic instability does not prioritise other populations of *A. pardalis* group

from Morocco, Tunisia and especially coastal Libya and Egypt. In fact, these populations suffer similar conservation threats (Moravec et al. 1999) and, according to our (mitochondrial) phylogeny, would deserve a similar taxonomic status. These results therefore further highlight the need for considering phylogenetic aspects when establishing conservation priorities (Harris & Froufe 2005).

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