NORTH-WESTERN JOURNAL OF ZOOLOGY 7 (1): 138-142
Article No.: 111116

www.herp-or.uv.ro/nwjz

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

Miguel A. CARRETERO[1,*], Miguel M. FONSECA[1,2,3],
Enrique GARCÍA-MUÑOZ[1,4,5], José C. BRITO[1] and D. James HARRIS[1,2]

1. CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto,
Campus Agrário de Vairão, 4485-661 Vairão, Portugal.
2. Departamento de Zoologia e Antropologia, Faculdade de Ciências
da Universidade do Porto, 4099-002 Porto, Portugal
3. Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología,
Universidad de Vigo, 36310 Vigo, Spain
4. Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén,
Campus de las Lagunillas s/n, 23071 Jaén, Spain
5. CESAM, Centro de Estudios de Ambiente o do Mar, Universidade de Aveiro,
Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
* Corresponding author, M.A. Carretero, E-mail: carretero@mail.icav.up.pt

Received: 18. February 2011 / Accepted: 25. April 2011 / Available online: 01. May 2011

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 (Seligmannn 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 transformations (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

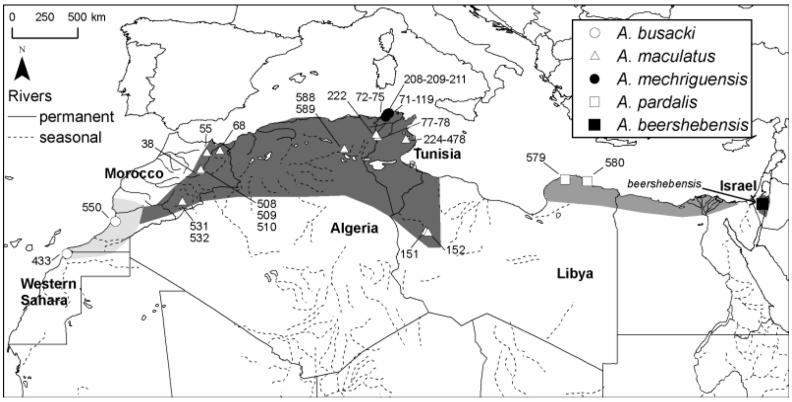

Figure. 1. Geographic location of samples of specimens from the *Acanthodactylus pardalis* group (modified from Fonseca *et al*., 2008). From west to east: *A. busacki* (light grey), *A. maculatus* (dark grey), *A. pardalis* (grey) and *A. beershebensis* (black, sample locality is marked with an arrow). *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)	68.16	Supralabials	4
Head length	25.64	Gulars	30
Head width	17.86	Plates in collar	12
Head depth	13.45	Dorsals	63
Forelimb length	33.46	Ventrals across belly	11
Hindlimb length	56.92	Transverse rows of ventrals	34
Fourth toe length	25.12	Femoral pores	19
Tail length	150.15	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 pseudoreplicates) 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. beershebensis* 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-

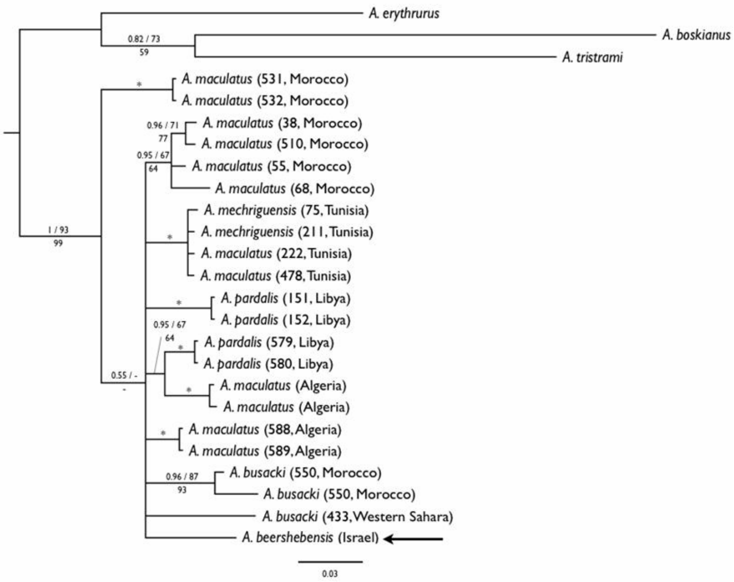

Figure 2. Phylogenetic relationships (50% majority-rule consensus) of *A. beershebensis* within the *A. pardalis* group derived from partial 12S and 16S rRNAs sequences (all analyses showed the same consensus tree). The tree was rooted using *Acanthodactylus erythrurus*, *A. boskianus* and *A. tristrami*. 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:	Δ –Log Likelihood:	SH *P*:
Unconstrained ML tree (Fig.2)	2861.00562	(best)	
Constrained (*A. beershebensis* sister taxon to the remaining *A. pardalis* group species)	2866.30908	5.30346	0.625
Constrained (*A. busacki* monophyletic)	2863.12306	2.11744	0.798
Constrained (*A. maculatus* monophyletic)	2954.36624	93.36062	0.000*
Constrained (*A. mechriguensis* monophyletic)	2875.55382	14.54820	0.280
Constrained (*A. pardalis* 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).

Acknowledgements. This work was supported by the project PTDC/BIA-BDE/67678/2006 (to MAC), the grant SFRH/BD/31158/2006 (to MMF), the post-doctoral grant SFRH/BPD/72806/2010 (to EGM) and Programme Ciência (to

MAC, JCB and DJH) from Fundação para a Ciência e a Tecnologia, FCT (Portugal). Thanks are due to Laia Mestre, Guillem Pascual and Gustavo A. Llorente (University of Barcelona) for the loan of the specimen.

References

Arnold, E.N. (1983): Osteology, genitalia and the relationships of *Acanthodactylus* (Reptilia: Lacertidae). Bulletin of the British Museum of Natural History (Zoology) 44: 291-339.

Blanc, C.P. (1980): Studies on the *Acanthodactylus* of Tunisia. IV. Geographic distribution and habitats. Journal of Herpetology 14: 391-398.

Cox, N., Chanson, J., Stuart, S. (2006): The Status and Distribution of Reptiles and Amphibians of the Mediterranean Basin. IUCN, Gland, Switzerland and Cambridge, U.K.

Crochet, P.A., Geniez, P., Ineich, I. (2003): A multivariate analysis of the fringe-toed lizards of the *Acanthodactylus scutellatus* group (Squamata: Lacertidae): systematics and biogeographical implications. Zoological Journal of the Linnean Society 137: 117-155.

Drake, N.A., El-Awat, A.S., Turner, P., Armitage, S.J., Salem, M.J., White, K.H., McLaren, S. (2008): Palaeohydrology of the Fazzan Basin and surrounding regions: the last 7 million years. Palaeogeography, Palaeoclimatology, Palaeoecology 263: 131-145.

Felsenstein, J. (1985): Confidence limits on phylogenies: an approach using bootstrap. Evolution 39: 783-791.

Fonseca, M.M., Brito, J.C., Paulo, O., Carretero, M.A., Harris, D.J. (2009): Systematic and phylogeographical assessment of the *Acanthodactylus erythrurus* group (Reptilia: Lacertidae) based on phylogenetic analyses of mitochondrial and nuclear DNA. Molecular Phylogenetics and Evolution 51: 131-142.

Fonseca, M., Brito, J.C., Rebelo, H., Kalboussi, M., Larbes, S., Carretero, M.A., Harris, D.J. (2008): Genetic variation of spiny footed lizards in the *Acanthodactylus pardalis* group in North Africa. African Zoology 43: 8-15.

Gasse, F. (2006): Climate and hydrological changes in tropical Africa during the past million years. Comptes Rendus Palevolution 5: 35-43.

Guidon, S., Gascuel, O. (2003): A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematics Biology 52: 696-704.

Harris, D.J., Arnold, E.N. (2000): Elucidation of the relationships of spiny-footed lizards, *Acanthodactylus* spp. (Reptilia: Lacertidae) using mitochondrial DNA sequence, with comments on their biogeography and evolution. Journal of Zoology 252: 351-362.

Harris, D.J., Arnold, E.N., Thomas, R.H. (1998): Relationships of the lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. Proceedings Royal Society London B. 265: 1939-1948.

Harris, D.J., Batista, V., Carretero, M.A. (2004): Assessment of genetic diversity within *Acanthodactylus erythrurus* (Reptilia: Lacertidae) in Morocco and the Iberian Peninsula using mitochondrial DNA sequence data. Amphibia-Reptilia 25: 227-232.

Harris, D.J., Froufe, E. (2005): Taxonomic inflation: species concept or historical geopolitical bias? Trends in Ecology and Evolution 20(1): 6-7.

Hawlena, D. (2009): Colorful tails fade when lizards adopt less risky behaviors. Behavioural Ecology and Sociobiology 64: 205-213.

Hawlena, D., Bouskila, A. (2006): Land management practices for combating desertification cause species replacement of desert lizards. Journal of Applied Ecology 43: 701-709.

Hawlena, D., Pérez-Mellado, V. (2009): Change your diet or die: predator-induced shifts in insectivorous lizard feeding ecology. Oecologia 161: 411-419.

Hawlena, D., Boochnik, R., Abramsky, Z., Bouskila, A. (2006): Blue tail and striped body: why do lizards change their infant costume when growing up? Behavioural Ecology 17: 889-896.

Hawlena, D., Saltz, D., Abramsky, Z., Bouskila, A. (2010): Ecological trap for desert lizards caused by anthropogenic changes in habitat structure that favor predator activity. Conservation Biology 24: 803-809.

Hedges, S.B., Bezy, R.L. (1993): Phylogeny of xantusiid lizards: concern for data and analysis. Molecular Phylogenetics and Evolution 2: 76-87.

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

Katoh, K., Kuma, K., Toh, H., Miyata, T. (2005): MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33: 511-518.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989): Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National. Academy of Sciences USA 86: 6196-6200.

Losos, J. B., Creer, D. A., Glossip, D., Goellner, R., Hampton, A., Roberts, G., Haskell, N., Taylor, P., Ettling, J. (2000): Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. Evolution 54:301-305.

Losos, J.B., Warheitt, K.I., Schoener, T.W. (1997): Adaptive differentiation following experimental island colonization in *Anolis* lizards. Nature 387: 70-73.

Mellado, J., Olmedo, G. (1990): El género *Acanthodactylus* en Marruecos: problemas de identificación en los grupos de especies *A. pardalis* y *A. scutellatus*. Amphibia-Reptilia 11: 131-146.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G..A.B., Kent, J. (2000): Biodiversity hotspots for conservation priorities. Nature 403: 853-858.

Moravec, J., Baha El Din, S., Seligmann, H., Sivan, N., Werner, Y.L. (1999): Systematics and distribution of the *Acanthodactylus pardalis* group (Lacertidae) in Egypt and Israel. Zoology in the Middle East 17: 21-50.

Posada D. (2008): jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution 25: 1253-1256.

Ronquist, F., Huelsenbeck, J.P. (2003): MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Salvador, A. (1982): A revision of the lizards of the genus *Acanthodactylus* (Sauria: Lacertidae). Bonner Zoologische Monographien 16: 1-167.

Schleich, H.H., Kästle, W., Kabisch, K. (1996): Amphibians and Reptiles of North Africa. Koenigstein: Koeltz Scientific Publishers.

Seligmann, H., Beiles, A., Werner, Y.L. (2003): Avoiding injury and surviving injury: two coexisting evolutionary strategies in lizards. Biological Journal of the Linnean Society 78: 307-324.

Shimodaira, H., Hasegawa, M. (1999): Multiple comparisons of Log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution 16: 1114-1116.

Smith, H.T.U. (1969): Photo-Interpretation Studies of Desert Basins of Northern Africa (U.S. Air Force - Cambridge Res. Lab. contract No. AF 19 (628) 2486, Final Rep.) 1-77.

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

Uetz, P. (2011): The Reptile Database, <http://www.reptile-database.org>, accessed May 02, 2011.

Werner, Y.L., Disi, M., Disi, A.M. (2006): *Acanthodactylus beershebensis*. IUCN. IUCN Red List of Threatened Species. Version 2010.1. <www.iucnredlist.org>. Downloaded: 22-3-2010.