©Österreichische Gesellschaft für Herpetologie e.V., Wien, Austria, download unter www.biologiezentrum.at

Phylogeography of *Macroprotodon*: mt DNA sequences from Portugal confirm European populations arrived recently from NW Africa

The False Smooth Snakes, *Macroprotodon*, occur in North Africa from Morocco to Palestine (BONS & GENIEZ 1996) as well as in the Iberian Peninsula and in the western Mediterranean islands of Lampedusa, Mallorca and Menorca (GASC et al. 1997; WADE 2001). In the most recent morphological review, WADE (2001) recognized four species of *Macroprotodon*: *M. cucullatus* (GEOFFROY, 1827), *M. mauritanicus* GUICHENOT, 1850, *M. abubakeri* WADE, 2001 in North Africa and *M. brevis* (GÜNTHER, 1862) in both Africa and the Iberian Peninsula.

Based on allozymes eletrophoretic data, BUSACK (1986) postulated an African origin for the Iberian populations suggesting they colonized the Iberian Peninsula by rafting across the Strait of Gibraltar during the Pleistocene. Alternatively, CARRANZA et al. (2004), using mitochondrial DNA sequence data, suggested that *M. brevis*, from extreme Northern Morocco extended its distribution in very recent times to Iberia either as the result of a natural colonization or anthropogenic introduction. This hypothesis was based on genetic uniformity of the Spanish samples and their great similarity to the Northern Moroccan ones. However, for their continental Iberian Peninsula sampling, they only included individuals from Andalusia and one from Extremadura, that is, from the extreme south and central Iberia. In this way, when concluding low genetic diversity for the Iberian specimens of *M. brevis ibericus* BUSACK & MCCOY, 1990, CARRANZA et al. (2004) assumed no variation in the rest of Iberia. Similar assumptions were proved to be erroneous or too straightforward to explain the phylogeographic histories of *Chamaeleo chamaeleon* (LINNAEUS, 1758) and *Podarcis hispanica* (STEINDACHNER, 1870) species complex when more extensive geographic sampling was included. In the first case, PAULO et al. (2002) using mitochondrial 16S ribosomal RNA gene revealed a double origin for the Iberian population, since two introduction events from different Moroccan populations had occurred – the population from Malaga being closely related to Mediterranean North African populations and the Atlantic Iberian populations more closely related to populations of the Atlantic North African coast. In the second case, in the first studies about *Podarcis* sp. phylogeography based on protein data, BUSACK (1986) found low genetic distances between populations from southern Spain and Morocco. However, it is now known, after a wider sampling effort, that *P. hispanica* has a much more complex evolutionary history with a distinct lineage that is present both in Iberia and North Africa, and others are unique to each distribution region (HARRIS et al. 2002; PINHO et al. 2006). These cases underline the importance of sufficient and adequate sampling when investigating the role of geographical barriers in creating differentiation within clades. This may be particularly relevant for *Macroprotodon*, since ALMEIDA & ALMEIDA (1986) reported a morphological difference present in an isolated population from northeastern Portugal - 19 rows of dorsal scales at mid-body instead of 21, as known for the rest of the Iberian specimens. This morphological character was also found in northeastern Africa, from eastern Morocco to Egypt (ALMEIDA & ALMEIDA 1986).

The aim of this work was to test the hypothesis of a recent arrival of *Macroprotodon* from northwest African populations to Iberia by extending the sampling area in order to cover more of its distribution range, especially in the Iberian Peninsula and including samples from the apparently isolated Portuguese populations (GASC et al. 1997; BARBADILLO et al. 1999) where the morphological differences were observed.

Macroprotodon specimens were collected in the field from Portugal and Morocco and released after a small clip of material was taken and stored in ethanol. Genomic DNA was extracted following a standard high-salt protocol. Part of the cytochrome *b* gene was amplified by PCR using cytochrome *b1* and *b2* (KOCHER et al. 1989) and conditions described by HARRIS (2001). This region was chosen to be amplified as it typically evolves faster than 12S or 16S and because it had also been analyzed

©Österreichische Gesellschaft für Herpetologie e.V., Wien, Austria, download unter www.biologiezentrum.at

Table 1: Locations, sample codes and GenBank numbers of the new samples of *Macroprotodon brevis ibericus* BUSACK & MCCOY, 1990 used in this study.

Location	Sample code	GenBank number
Portugal, Alijó	Mc 067	DQ324857
Portugal, Serra do Monfurado	Mc 094	DQ324858
Portugal, Évora	Mc 095	DQ324859
Portugal, Tua	Mac 01	DQ324860
Morroco, Taza Caves	Mac 02	DQ324861
Morroco, Balcon D'ito	Mac 03	DQ324862

by CARRANZA et al. (2004). The amplified products were sequenced on an automated sequencer (ABI 310 ® by Amersham Biosciences) and the new sequences – four from Portugal and two from Morocco – were checked by eye and aligned against others previously published using BioEdit (HALL 1999) – one from Tunisia (NAGY et al. 2003), one from Spain (SLOWINSKI, unpublished sequence from GenBank) and 34 analysed by CARRANZA et al. (2004). Details of the new samples and sequences used in this study are presented in the table 1. The new sequences were deposited on GenBank, accession numbers DQ324857 to DQ324862.

Including the outgroups, 50 sequences of 291 base pairs length were analyzed. The approach outlined by HUELSENBECK & CRANDALL (1997) was used to test 56 alternative models of evolution, employing PAUP* 4.0b10 (SWOFFORD 2002) and Modeltest (POSADA & CRANDALL 1998). Once a model of evolution was chosen, it was used to estimate a tree using maximum likelihood (ML). Support for nodes was estimated using bootstrapping (FELSENSTEIN 1985) with 200 replicates. Maximum parsimony (MP) analysis was also performed, with a 10 replicate heuristic search, and support for nodes estimated using bootstrapping with 1000 replicates. To analyse the low level of variation within *M. brevis ibericus*, all haplotypes of this subspecies were also joined in a median-joining network (fig. 1).

The most appropriate model of evolution for the data was the HKY model (transition/transversion ratio 4.79) with an estimate of invariable sites (0.48) and a discrete approximation of the gamma distribution (1.08). Using this model, the heuristic search recovered a single tree of -ln 1847 (fig. 2). Using MP, the heuristic search recovered 74 equally parsimonious trees of 335 steps. The strict consensus of the trees derived from the ML analysis differed from this only in being less well resolved (fig. 2).

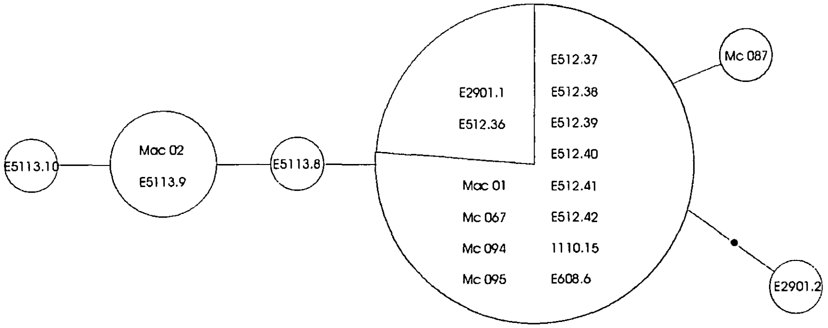

Fig. 1: Median-joining network of the cytochrome *b* sequences for *Macroprotodon brevis ibericus* BUSACK & MCCOY, 1990. The Iberian samples are in grey and the Moroccan ones in white. Codes for new sequences are given in table 1. All others are from CARRANZA et al. (2004), except Mc087 (SLOWINSKI, unpublished sequence from GenBank) and Mc026 (NAGY et al. 2003).

©Österreichische Gesellschaft für Herpetologie e.V., Wien, Austria, download unter www.biologiezentrum.at

The level of divergence found within cytochrome *b* sequences of *M. brevis ibericus* from Portugal, Spain and Morocco was very low or nonexistent, with a maximum difference of three mutational steps (fig. 1). All individuals from Portugal, including the one presenting the morphological difference, shared the same haplotype for this gene, which was also shared by some Spanish and Moroccan individuals. A higher number of haplotypes (five) was revealed within cytochrome *b* sequences from Moroccan samples.

Five clades were found, three of them well supported (fig. 2). In the first one, even with a low bootstrap value, *M. brevis* ssp. from Morocco sequences grouped with *M. cucullatus textilis* (DUMÉRIL & BIBRON, 1854) from Morocco. In the second one, all *M. brevis ibericus* sequences from Morroco grouped with the ones from Spain and Portugal. The third one included *M. cucul-*

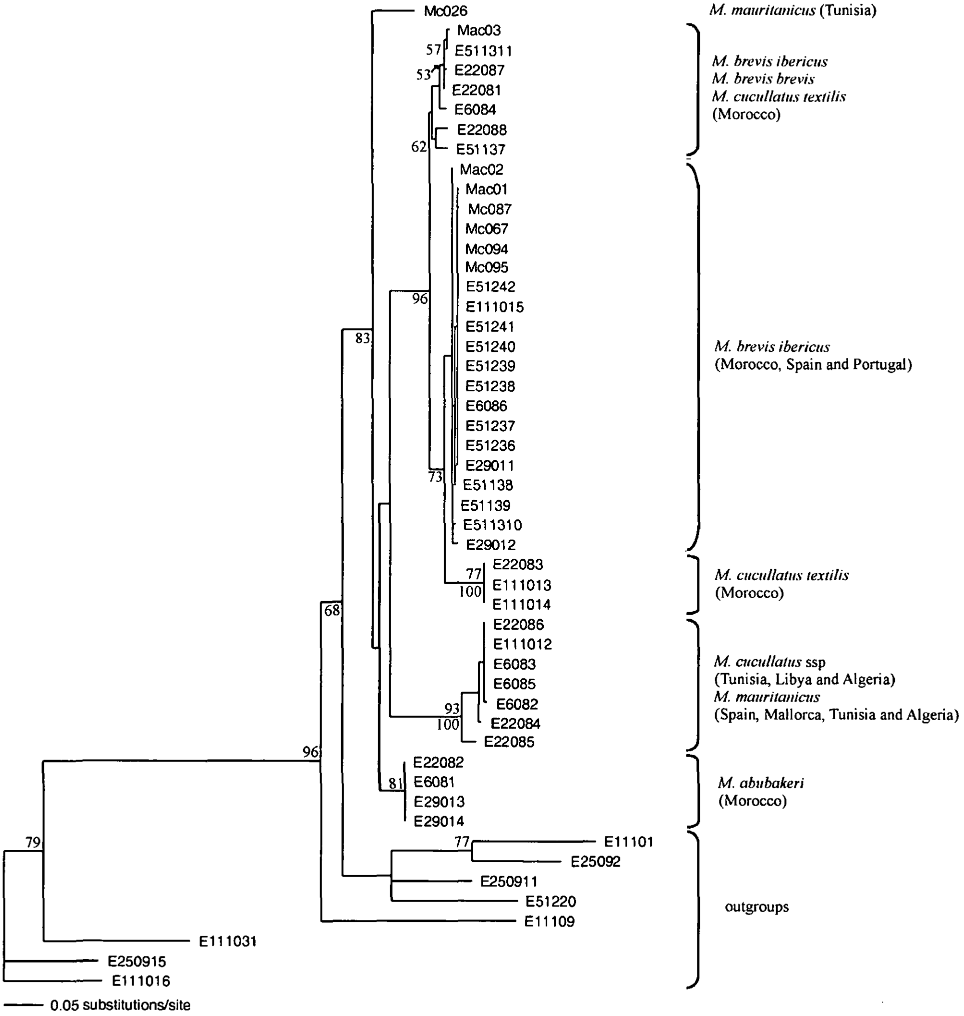

Fig. 2: Single Maximum Likelihood (ML) tree of *Macroprotodon* inferred from cytochrome *b* mitochondrial genes. Bootstrap support for Maximum Parsimony (MP) and ML analyses are indicated above and below nodes respectively. Codes for new sequences are given in table 1. All others are from CARRANZA et al. (2004), except Mc087 (SLOWINSKI, unpublished sequence from GenBank) and Mc026 (NAGY et al. 2003).

©Österreichische Gesellschaft für Herpetologie e.V., Wien, Austria, download unter www.biologiezentrum.at

latus textilis from Morocco that are clearly separated from *M. cucullatus* ssp. from Tunisia, Algeria and Libya that form the fourth group together with *M. mauritanicus* from Mallorca, Tunisia and Algeria. In the last group, *M abubakeri* specimens appear clustered with a high convergence value indicating a well-supported relationship.

Macroprotodon cucullatus appears as a polyphyletic taxon given that some individuals appear as a well isolated group (with 81 to 100% bootstrap) and others associated with *M. brevis brevis* from Morocco and also with *M. mauritanicus* from Tunisia, Algeria and Mallorca, Spain, within very distant groups.

The Tunisian sample of *M. mauritanicus* in the analysis of CARRANZA et al. (2004) did not group within any clade, but appeared to be sister taxa to all other *Macroprotodon*. That is, *M. mauritanicus* no longer forms a monophyletic group, as suggested by these authors, since Mc 026 appeared as a separate lineage in this analysis (fig. 2).

Since cytochrome *b* is a fast-evolving gene – around 2,2-3.2% sequence divergence per million years (CARRANZA et al. 2004) - the small genetic variation between *M. b. ibericus* sequences from Portugal, Spain and Morocco and the almost absence of variation between the Iberian ones, supports the hypothesis of a very recent colonization of the Iberian Peninsula by *Macroprotodon* from northwest Africa, as predicted by CARRANZA et al. (2004). This suggests that the reduction of dorsal scale count arose very recently in the isolated Portuguese populations and that this character does not indicate any close phylogenetic relationship with North African populations with similarly reduced scale counts.

These results generally support the conclusions of CARRANZA et al. (2004). However, the inclusion of an additional sample from GenBank of *M. mauritanicus* indicates that the phylogeny of *Macroprotodon* is more complex than assumed before and only further morphological and molecular studies in broader areas of North Africa will help to clarify *Macroprotodon*'s phylogeny and evolutionary history.

ACKNOWLEDGEMENTS: This project was supported by grants from Fundação para a Ciência e Tecnologia POCTI/41912/BSE/2001 and SFRH/BPD/5702/2001 (to DJH). Thanks to C. Pinho, J. C. BRITO, M. A.CARRETERO (CIBIO/UP – Centro de Investigação em Biodiversidade e Recursos Genéticos / Universidade do Porto) and A. PERERA for help during fieldwork in Portugal and Morocco. Thanks to Armando LOUREIRO and the BTVS do ICN – Banco de Tecidos de Vertebrados Selvagens do Instituto de Conservação da Natureza – for donating some of the samples.

REFERENCES: ALMEIDA, N. F. & ALMEIDA, F. F. (1986): On the occurrence and feeding habits of the False Smooth Snake *Macroprotodon cucullatus* (GEOFFROY, 1827) in Portugal (Serpentes: Colubridae).– Amphibia-Retilia, Leiden; 7: 75-81. BARBADILLO, L. J. & LACOMBA, J. I. & PÉREZ-MELLADO, V. & SANCHO, V. & LÓPEZ-JURADO, L. F. (1999): Anfibios y reptiles de la Península Ibérica, Baleares e Canarias. Guía ilustrada para identificar y conocer todas las especies. Barcelona (GeoPlaneta, S. A.), 419 pp. BONS, J. & GENIEZ, P. (1996): Amphibiens et reptiles du Maroc (Sahara Occidental compris). Atlas biogéographique. Barcelona (Asociación Herpetológica Española), 320 pp. BUSACK, S. D. (1986): Biogeographic analysis of the herptofauna separated by the formation of the Strait of Gibraltar.– National Geographic Research Journal, Washington; 2 (1): 17-36. CARRANZA, S. & ARNOLD, E. N. & WADE, E. & FAHD, S. (2004): Phylogeography of the false smooth snakes, *Macroprotodon* (Serpentes, Colubridae): mitochondrial DNA sequences show European populations arrived recently from Northwest Africa.– Molecular Phylogenetics and Evolution, Chicago; 33: 523-532. FELSENSTEIN, J. (1985): Confidence limits on phylogenies: an approach using the bootstrap.– Evolution, Lawrence; 39, 783–791. GASC, J. P. & CABELA, A. & CCROBRNJA-ISAILOVIC, J. & DOLMEN, D. & GROSSENBACHER, K. & HAFFNER, P. & LESCURE, J. & MARTENS, H. & MARTÍNEZ RICA, J. P &, MAURIN, H. & OLIVEIRA, M. E. & SOFIANIDOU, T. S. & VEITH, M. & ZUIDERWIJK, A. (eds.) (1997): Atlas of amphibians and reptiles in Europe. Paris, (Societas Europaea Herpetologica & Muséum National d'Histoire Naturelle - IEGB/SPN), 494 pp. HALL, T. A. (1999): Bioedit: a user friendly biological sequence alignment editor and ananysis program for Windows 95/98/NT.- Nucleic Acids Symposium Series, London; 41: 95-98. HARRIS, D. J. (2001): Re-evaluation of 16S ribosomal RNA variation in *Bufo* (Anura: Amphibia).– Molecular Phylogenetics & Evolution, Chicago; 19: 326-329. HARRIS, D. J. & CARRANZA, S. & ARNOLD, E. N. & PINHO, C. & FERRAND, N. (2002): Complex biogeographical distribution of genetic variation within *Podarcis* wall lizards across the Strait of Gibraltar.– Journal of Biogeography, Oxford; 29 (9): 1257–1262. HUELSENBECK, J. P. &. CRANDALL, K. A. (1997): Phylogeny estimation and hypothesis testing using maximum likelihood.– Annual Review of Ecology and Systematics, Palo Alto; 28: 437-466. KOCHER, T. D. & THOMAS, R. 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. – Proc. National Academic Sciences USA, Washington; 86: 6196–6200. Nagy, Z. T. & Joger, U. & WINK, M. & GLAW, F. & VENCES, M. (2003): Multiple colonization of Madagascar and Socotra by colubrid snakes: evidence from nuclear and mitochondrial gene phylogenies.– Proc. Royal Society London, Biological Sciences, London; 270: 2613-2621. PAULO,

©Österreichische Gesellschaft für Herpetologie e.V., Wien, Austria, download unter www.biologiezentrum.at

O. & PINTO, I. & BRUFORD, M. W. & JORDAN, W. C. & NICHOLS, R. A. (2002): The double origin of Iberian peninsular chameleons.- Biological Journal of the Linnean Society, London; 75: 1-7. PINHO, C. & FERRAND, N. & HARRIS, D. J. (2006): Reexamination of the Iberian and North African *Podarcis* phylogeny indicates unusual relative rates of mitochondrial gene evolution in reptiles.– Molecular Phylogenetics and Evolution, Chicago; 38: 266-273. POSADA, D. &. CRANDALL, K. A. (1998): Modeltest: testing the model of DNA substitution.– Bioinformatics, Oxford; 14: 817–818. SWOFFORD, D. L. (2002): PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0. Sinauer Associates, Uderland, Massachusetts. WADE, E. (2001): Review of the False Smooth snake genus *Macroprotodon* (Serpentes, Colubridae) in Algeria with a description of a new species.– Bulletin National History Museum London (Zoology), London; 67 (1): 85-107.

KEYWORDS: mitochondrial DNA, cytochrome b, *Macroprotodon*, evolution, systematics, Iberian Peninsula, North Africa

SUBMITTED: April 1, 2005

AUTHORS: Raquel VASCONCELOS, Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO\UP), ICETA, Campus Agrário de Vairão, 4445-661 Vila do Conde, Portugal. D. James HARRIS, Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO\UP), ICETA, Campus Agrário de Vairão, 4445-661 Vila do Conde, Portugal and Faculdade de Ciências da Universidade do Porto, Praça Gomes Teixeira 4099-002 Porto, Portugal < james@mail.icav.up.pt >

Death adders (*Acanthophis laevis* complex) from the island of Ambon (Maluku, Indonesia)

Death adders of the genus *Acanthophis* DAUDIN, 1803 are widely distributed in most of Australia, New Guinea and adjacent islands, and the islands of Maluku, Indonesia. In Maluku, death adders have been reported from the Tanimbar islands (Timor Laut of the older literature), the Aru islands, Kai Kecil, Kai Dulah, and Kai Besar, Obi, Seram, Haruku, and Saparua (DE ROOIJ 1917; DE HAAS 1950; KLEMMER 1963; SUPRIATNA 1995; HOW & KITCHENER 1997; ISKANDAR & COLIJN 2001; HOSER 2002). Surprisingly, no published record of *Acanthophis* existed from Ambon, in spite of the fact that this island of 771 km^2 served as the principal regional basis for European colonialism, travel, commerce, and research throughout the centuries. Since the 1990s, numerous death adders have been shipped from Ambon to exporters' facilities in Java and Bali by the live animal trade. As a consequence, it has become accepted that these snakes do occur on the island (WÜSTER et al. 2005). However, most if not all death adders shipped from Ambon had actually been collected on different Maluku islands, namely Seram, but also Obi, Yamdena, and others (BAADILLA, pers. comm.), and the presence of these elapids on Ambon itself remained unsubstantiated. In this communication we report on a death adder from Ambon, which local collectors found in the vicinity of the village Negeri Lima in April 1998. Negeri Lima is situated in the northeast of Ambon's hilly and less densely populated Hitu peninsula (Central Maluku regency, Maluku province, Indonesia). The snake was photographed (fig. 1), and color slides were deposited in the herpetological slide collection of the Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main, Germany (SMF-F 235). Villagers of Hitu peninsula recognized this snake as belonging to a local species that was however said to be secretive and only very rarely encountered.


Fig. 1: Adult death adder (*Acanthophis laevis* complex) from Negeri Lima, Ambon (Central Maluku regency, Maluku province, Indonesia). Photograph by U. KUCH.

The viper-like elapid snakes of the genus *Acanthophis* are characterized by considerable taxonomic and nomenclatural confusion. While the prevailing opinion through many decades was that this genus contains between two and four species or subspecies (e.g., KLEMMER 1963; STORR et al. 1986), there is currently a trend to more fully appreciate the species diversity of death adders. This has resulted in the resurrection of old names from synonymy and

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: Herpetozoa

Jahr/Year: 2006

Band/Volume: 19_1_2

Autor(en)/Author(s): Vasconcelos Raquel, Harris David James

Artikel/Article: Phylogeography of Macroprotodon: mt DNA sequences from Portugal confirm European populations arrived recently from NW Africa 77-81