

First Data on the Molecular Phylogeography of Scincid Lizards of the Genus *Mabuya*

Patrick Mausfeld,^{*1} Miguel Vences,^{*} Andreas Schmitz,^{*} and Michael Veith[†]

^{*}Zoologisches Forschungsinstitut und Museum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany; and

[†]Zoologisches Institut der Universität Mainz, Saarstrasse 21, 55099 Mainz, Germany

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A 487-bp fragment of the mitochondrial 16S rRNA gene was sequenced in 26 species of the circumtropical lizard genus *Mabuya* and used to analyze phylogenetic relationships within the genus. The species from Africa and Madagascar formed a monophyletic group relative to the included Asian and South American taxa. The Malagasy species included (*M. elegans*, *M. cf. dumasi*, and *M. comorensis*) did not appear as a monophylum. Combined and separate analysis of the 16S data and additional sequences of the mitochondrial 12S rRNA, ND4, and cytochrome *b* genes (a total of 2255 bp) in one Asian, two Malagasy, and two African species also did not result consistently in a monophyletic grouping of the Malagasy taxa. However, a monophylum containing African and Malagasy taxa was strongly supported by the combined analysis. These preliminary results indicate that *Mabuya* probably colonized Madagascar from Africa through the Mozambique Channel. © 2000 Academic Press

Key Words: Squamata; Scincidae; *Mabuya*; Madagascar; Africa; Asia; molecular phylogeny; biogeography.

INTRODUCTION

The family Scincidae currently contains more than 1300 species grouped in over 85 genera (Bauer, 1992). About 100 of these are grouped in the lygosomine genus *Mabuya* (sensu Greer, 1977), which is the only lizard genus with a circumtropical distribution. *Mabuya* contains about 15 neotropical (Blackburn and Vitt, 1992) and about 30 Asian species. The largest number of *Mabuya* species (ca. 60) occur in Africa (Greer, 1977). Eleven described species are known from Madagascar (Nussbaum and Raxworthy, 1998; Köhler *et al.*, 1998). In the Indian Ocean, two endemic species are found on the Seychelles, one species on the Comoros, and one species on the small Europa island (Brygoo, 1981).

In the present paper we present preliminary molec-

ular data on the phylogeny of *Mabuya*, focusing on the relationships between Malagasy and African species.

MATERIALS AND METHODS

Tissue samples (liver or muscle; either fresh or preserved in 98% ethanol) were available from 26 *Mabuya* and one outgroup species. Voucher specimens are deposited in the Naturhistorisches Museum Wien, NMW, and the Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn, ZFMK (see appendix). All specimens which were not collected were reliably identified by photographs.

DNA was extracted using QIAmp tissue extraction kits (Qiagen). We used the primers (of Palumbi *et al.*, 1991) 16SA-L (light chain; 5' - CGC CTG TTT ATC AAA AAC AT - 3') and 16SB-H (heavy chain; 5' - CCG GTC TGA ACT CAG ATC ACG T - 3') to amplify a section of the mitochondrial 16S ribosomal RNA gene, and the primers 12SA-L (light chain; 5' - AAA CTG GGA TTA GAT ACC CCA CTA T - 3') and 12SB-H (heavy chain; 5' - GAG GGT GAC GGG CGG TGT GT - 3') to amplify a section of the mitochondrial 12S ribosomal RNA gene. PCR conditions followed Vences *et al.* (2000). Additionally we used the primers L14841 (light chain; 5' - CTC CCA GCC CCA TCC AAC ATC TCA GCA TGA TGA AAC TTC G - 3'), modified from Kocher *et al.* (1989), and CB3-H (heavy chain; 5' - GGC AAA TAG GAA GTA TCA TTC TG - 3'), modified from Palumbi *et al.* (1991), to amplify a section of the mitochondrial cytochrome *b* (*cyt b*) gene. The PCR cycling procedure was performed as follows: 34 cycles of denaturation for 90 s at 95°C, primer annealing for 60 s at 50°C, and extension for 90 s at 72°C. Furthermore, we used the primers ND4 (light chain; 5' - CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC - 3') and Leu (heavy chain; 5' - TTT TAC TTG GAK TTG CAC CA - 3'), modified from Arevalo *et al.* (1994) to amplify a section of the mitochondrial ND4 gene. The PCR cycling procedure was performed as follows: 35 cycles of denaturation 45 s at 94°C, primer annealing for 60 s at 50°C, and extension for 120 s at 74°C. PCR products

¹ E-mail: uzse22@uni-bonn.de.

were purified using QIAquick purification kits (Qiagen). We sequenced single-stranded fragments using an automatic sequencer (ABI 377). The obtained sequences (lengths referring to the aligned sequences including gaps) comprised 524 bp (16S), 411 bp (12S), 676 bp (cyt *b*), and 681 bp (ND4) homologous to bp positions 4005–4566 (16S), 2569–2917 (12S), 16384–16877 (cyt *b*), and 12931–13618 (ND4) of the *Xenopus laevis* mitochondrial genome (Roe *et al.*, 1985). Sequences have been submitted to GenBank (Accession Numbers AF153554–AF153593, AF228553–AF228558).

Sequences were aligned using the computer program Sequence Navigator (Applied Biosystems). Alignments were subsequently adjusted manually. We omitted four short sections (together 37 bp) from the original 16S data set which were too variable to be reliably aligned. The complete alignment is available from the authors on request.

Sequences were analyzed using PAUP*, version 4 beta (Swofford, 1998). We calculated maximum parsimony (MP) trees with gaps treated as a fifth character, and neighbor-joining (NJ) trees based on the Jukes-Cantor distance correction (Jukes and Cantor, 1969), with gaps treated as missing data. For the MP analysis a heuristic search was conducted with initial trees obtained by simple stepwise addition, followed by branch swapping using the TBR (tree bisection-reconnection) routine implemented in PAUP*. Only minimal length trees were saved and zero length branches were collapsed. Multistate characters within taxa were interpreted as uncertainties and an accelerated transformation procedure (ACCTRAN in PAUP*) was used for character optimization. Exhaustive searches were performed in the analyses of the reduced set of taxa. Following Hedges (1992), 2000 bootstrap replicates (Felsenstein, 1985) were run in all analyses.

Eumeces egregius (GenBank Accession No. AB016606; Kumazawa and Nishida, 1999) and *Scelotes mirus*, both belonging to the subfamily Scincinae, were used as outgroups. No cyt *b* sequence could be obtained from *Scelotes*; the cyt *b* tree was therefore only rooted with *Eumeces*, and in the combined analysis, the *Scelotes* cyt *b* characters were entered as missing data.

RESULTS AND DISCUSSION

In the 16S data set, 140 of 487 characters were variable; 97 (=69%) of these were phylogenetically informative. Transition-transversion ratios were below 2:1 in most pairwise comparisons. Levels of sequence differentiation corroborated the specific status of a number of taxa which in the past were often regarded as subspecies, namely *M. margaritifera* (divergence to *M. quinquetaeniata* 6.2%), *M. occidentalis* (divergence to *M. capensis* 4.1%), *M. spilogaster* (divergence to *M. striata* 3.7–4.3%). In contrast, the differentiation found between *M. comorensis* and *M. maculilabris* (1.6%) was

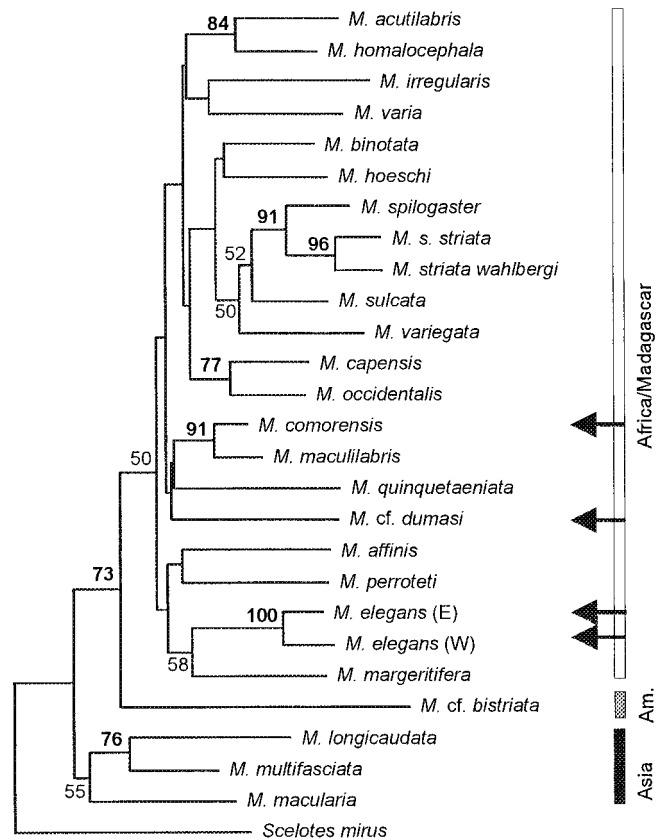


FIG. 1. Neighbor-joining tree using the Jukes-Cantor distance correction, based on an analysis of 487 bp of the mitochondrial 16S gene; gaps were treated as missing data; numbers above branches are bootstrap values in percentages (2000 replicates). Bootstrap supports of 70% and higher are printed in bold type. *Scelotes mirus* was used as an outgroup. Vertical bars indicate the geographic distribution of species: Asia, South America (Am.), and Africa/Madagascar. Malagasy species are indicated by black arrows.

clearly below the values found between other *Mabuya* species.

In the MP analysis, three equally most-parsimonious trees were found (tree length 453 steps; consistency index 0.479). The general topology of the MP tree (not shown) and the NJ tree (Fig. 1) was rather similar.

The asiatic species were grouped as a monophylum which formed the sister group to the remaining *Mabuya*. The American *M. cf. bistrinata* was the sister group to a clade containing the African and Malagasy species, but bootstrap support for these and several other basal splits in the tree was very low. The two included species from the mainland of Madagascar, *M. cf. dumasii* and *M. elegans*, did not form a monophyletic group. However, once again, bootstrap analyses showed that these relationships were not well resolved. The third *Mabuya* species from Madagascar, *Mabuya comorensis* from the small Malagasy offshore island Nosy Tanikely, appeared to be most related to *M. maculilabris* from eastern Africa.

To further assess the phylogenetic position of the Malagasy species relative to the Asian and African taxa, we sequenced additional gene fragments (12S, *cyt b*, ND4) from a reduced set of taxa: *M. elegans*, *M. cf. dumasi*, *M. affinis*, *M. quinquetaeniata*, and *M. macularia* (including *Scelotes* and *Eumeces* as outgroups). The monophyletic group of the Malagasy and African taxa was supported by the 16S analyses (MP, 97%; NJ, 93%), 12S analyses (MP, 67%; NJ, 76%), the MP analysis of ND4 (41%) and the combined analysis of the total of 2255 bp of all four fragments (MP, 97%; NJ, 92%). The NJ analysis of ND4 grouped the African *M. quinquetaeniata* with the Asian *M. macularia*, and the *cyt b* analyses grouped *M. macularia* with the two Malagasy taxa; however, none of these groupings was supported by bootstrap values >55%. Regarding the questionable monophyly of the two Malagasy species, the results were contradictory. Their nonmonophyly was indicated by the MP and NJ analyses of the 16S data (bootstrap support of the *elegans*-*quinquetaeniata* clade: 85 and 94%), the MP and NJ analysis of the ND4 data (bootstrap support of the *dumasi*-*affinis* clade: 43 and 66%), and the combined MP analysis (bootstrap support of the *elegans*-*quinquetaeniata* clade: 62%). Their monophyly was indicated by MP and NJ analyses of the 12S data (61 and 60%), the NJ analysis of the *cyt b* data (48%), and the NJ analysis of the combined data (44%).

The monophyly of the Afro-Malagasy species of *Mabuya*, as indicated by the 16S data and confirmed by the combined analysis, is in accordance with the hypothesis of Greer (1977) who supposed that the genus originated in tropical Asia and colonized Africa at a later stage, giving rise to an extensive African radiation.

According to our data, *M. comorensis* is closely related to *M. maculilabris* from eastern Africa, and it was formerly seen as a subspecies of the latter (e.g., Brygoo, 1981). As the Comoros are a rather young and isolated volcanic archipelago, *M. comorensis* almost certainly evolved from ancestors rafted from the eastern African coast. The remaining *Mabuya* species from Madagascar are classified in two species groups, the *M. aureopunctata* group and the *M. elegans* group (Brygoo, 1983b; Nussbaum and Raxworthy, 1998). These authors discuss the possibility of a polyphyletic origin of Malagasy *Mabuya* species. Our sample includes a representative of the *M. aureopunctata* group (*M. cf. dumasi*) and a representative of the *M. elegans* group (*M. elegans*). Although the molecular results did not provide unambiguous evidence for the nonmonophyly of these two taxa, they did not contradict a scenario in which Madagascar has been subject to at least two colonization events by *Mabuya*.

According to Barron *et al.* (1981), the breaking up of the southern supercontinent Gondwana into three land masses, Australia–Antarctica, South America–Africa,

and Madagascar–India, was initiated about 140 my ago. Current fossil evidence (Estes, 1983) does not indicate evolution of lygosomine lizards at that stage. Greer (1977) hypothesized that *Mabuya* species reached Madagascar from Africa by passing the Mozambique channel as has been suggested for Malagasy lemurs (Yoder *et al.*, 1996). The low differentiation of Malagasy *Mabuya* to their African relatives makes their origin posterior to Gondwana fragmentation very likely. An origin by ancestors rafting from Africa through the Mozambique channel would also be in accordance with other dispersal patterns of scincid lizards in the Indian Ocean (Brygoo, 1983a, 1986).

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APPENDIX: SPECIMENS EXAMINED

Scelotes mirus (voucher not collected; Malolotja Reserve, Swaziland); *Mabuya occidentalis* (NMW 35105; Aus, Namibia); *Mabuya variegata* (NMW 35097.1; Otjimbingwe, Namibia); *Mabuya capensis* (voucher in Windhoek collection; Windhoek, Namibia); *Mabuya spilogaster* (NMW 35099.2; Aranos, Namibia); *Mabuya hoeschi* (NMW 35102.1; Swakop, Namibia); *Mabuya perroteti* (ZFMK 51844; Gambia); *Mabuya varia* (ZFMK 68413; Waterberg, Namibia); *M. sulcata* (ZFMK 66424; Ongongo waterfall, Kaokoland, Namibia); *M. striata striata* (voucher not collected; Mtunzini, KwaZulu-Natal, South Africa); *M. striata wahlbergi* (ZFMK 66432; Grootfontein, Namibia); *M. acutilabris* (ZFMK 66429; 10 km N Vis Myn, Namibia); *M. binotata* (ZFMK 66426; Ruacana waterfall, Namibia); *M. homalocephala* (voucher not collected; Rine's Nature Reserve, W Mossel Bay, Eastern Cape, South Africa); *M. quinquetaeniata* (ZFMK 68646; no locality, obtained through the pet trade); *M. margaritifera* (ZFMK 68647; probably Tanzania, obtained through the pet trade); *M. irregularis* (ZFMK 66631; Mt. Elgon, Uganda); *M. elegans* (voucher not collected; Kirindy near Morondava, western Madagascar); *M. elegans* (ZFMK 66681; juvenile from Antalaha, eastern Madagascar); *M. cf. dumasi* (voucher not collected; Kirindy near Morondava, Madagascar); *M. macularia* (ZFMK 66753; probably Thailand; obtained through the ani-

mal trade); *M. comorensis* (ZFMK 62192; Nosy Tanikely, Madagascar); *M. maculilabris* (voucher kept alive in ZFMK; obtained through the animal trade); *M. cf. bistrinata* (ZFMK uncataloged; Trinidad, Beni, Bolivia); *M. longicaudata* (voucher kept alive in ZFMK; obtained through the animal trade); *M. multifasciata* (voucher kept alive in ZFMK; obtained through the animal trade).

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