

Relationships of scincid lizards (*Mabuya* spp.) from the islands of the Gulf of Guinea based on mtDNA sequence data

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Abstract. Relationships of *Mabuya* lizards from the islands of the Gulf of Guinea were estimated using partial 12S rRNA, 16S rRNA and cytochrome *b* mitochondrial gene sequences. *Mabuya maculilabris* from São Tomé and Príncipe are discreet monophyletic units, highly divergent from each other and from mainland populations, indicating *M. maculilabris* may be a species complex. *Mabuya affinis* from Príncipe is similarly distinct from mainland populations of this species. The relationships of *Mabuya ozorii* from Annobon are unclear, but the three species in the Gulf of Guinea islands are not closely related, indicating multiple independent colonization events. The recent proposal to partition *Mabuya* into four genera is premature, since at least five distinct genetic lineages can be identified.

Introduction

The genus *Mabuya* (sensu Greer, 1977) is the only lizard genus with a circumtropical distribution. More than 100 species are known from Asia, Africa and the Neotropics (Greer et al., 2000). The genus is also widespread on islands, including the Seychelles, Comores, the Cape Verde islands, Fernando de Noronha, and the islands of the Gulf of Guinea. While colonisation patterns of the Cape Verde islands (Brehm et al., 2001; Carranza et al., 2001) and across the Atlantic (Carranza and Arnold, 2003; Mausfeld et al., 2002) have been well studied, little information is known regarding the species from the islands of the Gulf of Guinea, despite the fact that these islands are part of one of the worlds biodiversity hotspots (Myers et al., 2000).

The oldest island in the Gulf of Guinea, and the closest to Africa, is Bioko (fig. 1). More geographically isolated are São Tomé and Príncipe (1001 km² combined) that include a number of small islets, and 160 km southwest of São Tomé, Annobon (17 km²). While São Tomé,

Príncipe and Annobon have never been connected, Bioko (formerly Fernando Poo) was connected to Africa during sea level fluctuations and thus has an essentially continental herpetofauna, including representatives of families such as Lacertidae and Chamaeleonidae that are not present on the other islands. Oldest geological dates for Príncipe, São Tomé and Annobon are 31 my, 14my and 4.8 my respectively (Lee et al., 1994). Three species have been reported from these islands — the endemic *Mabuya ozorii* from Annobon (Bocage, 1893) and two widespread species, *Mabuya maculilabris* from São Tomé and Príncipe, and *Mabuya affinis* on Príncipe. Our analysis of *Hemidactylus* geckos from these islands showed that, in this group, widespread species were anthropogenically introduced (Jesus et al., 2005). Our aim was to use mitochondrial DNA sequence data to determine the relationships of the species from the islands relative to the many published species (Carranza and Arnold, 2003; Carranza et al., 2001; Mausfeld et al., 2000, 2002). This should answer many questions regarding how the islands were colonised, and if the current taxonomy accurately reflects the evolutionary history of the species. At the same time we use the data to critically assess the recent proposal to partition the genus *Mabuya* into four genera (Mausfeld et al., 2002).

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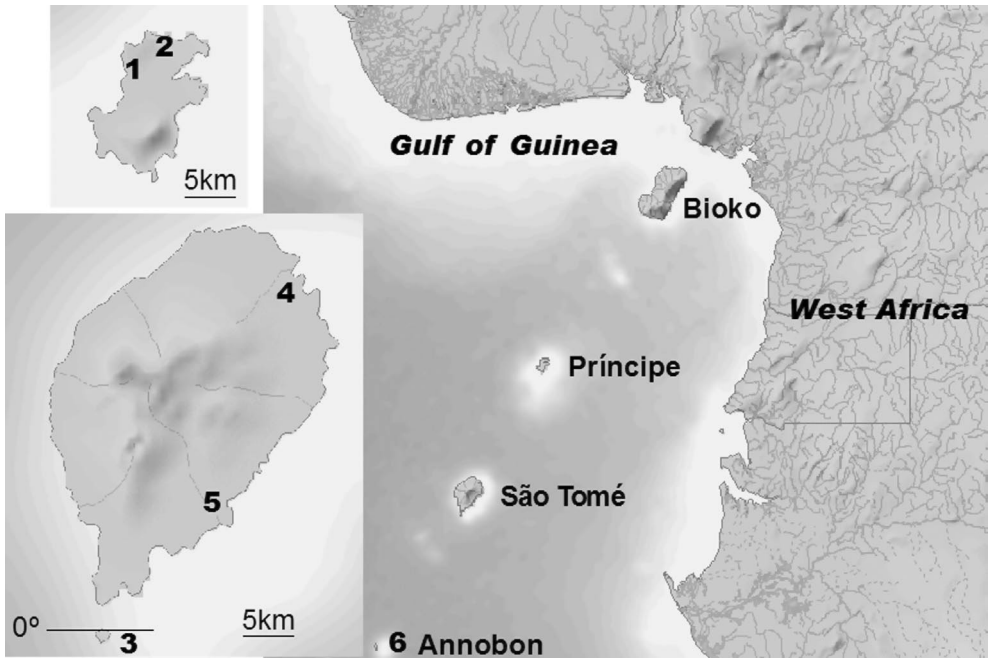


Figure 1. Map showing sampling localities of *Mabuya* sequenced in this study. Codes for samples are given in table 1.

Table 1. Specimens sequenced for this analysis with locality and voucher code.

Species	Locality	Island	Map locality and Code
<i>Mabuya affinis</i>	Montalegre	Príncipe	1 602
<i>Mabuya affinis</i>	Montalegre	Príncipe	1 603
<i>Mabuya affinis</i>	Terra Velha	Príncipe	2 635
<i>Mabuya maculilabris</i>	Rolas	São Tomé	3 502
<i>Mabuya maculilabris</i>	São Tomé	São Tomé	4 503
<i>Mabuya maculilabris</i>	S.J. Angolares	São Tomé	5 505
<i>Mabuya maculilabris</i>	Terra Velha	Príncipe	2 595
<i>Mabuya maculilabris</i>	Terra Velha	Príncipe	2 599
<i>Mabuya maculilabris</i>	Montalegre	Príncipe	1 749
<i>Mabuya ozorii</i>	Annobon	Annobon	6 623
<i>Mabuya ozorii</i>	Annobon	Annobon	6 625
<i>Mabuya ozorii</i>	Annobon	Annobon	6 627

Methods

Specimens were identified in the field from Príncipe, São Tomé and Annobon, and tail tips were collected (table 1). Voucher specimens are housed in the collection of the University of Madeira. Total genomic DNA was extracted from these small pieces of tail using standard methods, following Harris et al. (1998). Polymerase Chain Reaction primers used in both amplification and sequencing were 12Sa and 12Sb, 16SL and 16SH, and cytochrome *b1* and cytochrome *b3* from Kocher et al. (1989), Simon et al. (1990) and Palumbi et al. (1991). These were the most commonly sequenced regions in earlier studies, and were chosen so that this published data could be combined with

the new sequences. Amplified fragments were sequenced on a 310 Applied Biosystem DNA Sequencing Apparatus.

Mitochondrial DNA sequences were aligned using Clustal W (Thompson et al., 1994). Previously published sequences of *Mabuya* included all species available for all three genes were included in the analysis, with three out-group species, *Amphiglossus igneo-caudatus*, *Pamelaescincus gardineri* and *Tiliqua gigas*. A total of 40 taxa were analysed. Aligned sequences for 12S, 16S and cytochrome *b* were 355, 519 and 400 base pairs long (1274 in total), although several of the sequences from GenBank were slightly shorter than the others. Thirty-three base pairs of 16S rRNA sequence were excluded due to ambiguous align-

ment. The alignment is available on request from the corresponding author.

The data were imported into PAUP* 4.0b10 (Swofford, 2002) for phylogenetic analysis. For the phylogenetic analysis of the mtDNA data we used maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference. We followed the approach outlined by Huelsenbeck and Crandall (1997) to test 56 alternative models of evolution, employing PAUP* 4.0b10 and Modeltest (Posada and Crandall, 1998). Once a model of evolution was chosen, it was used to estimate a tree using ML, and support for nodes estimated by bootstrapping with 500 replicates (Felsenstein, 1985) using the “fast” option. A MP analysis was carried out (100 replicate heuristic search, TBR branch-swapping) with gaps treated as missing data, and support for nodes estimated by bootstrapping with 1000 replicates. The Bayesian analysis was implemented using MrBayes (Huelsenbeck and Ronquist, 2001). Bayesian analyses were conducted with random starting trees, run 0.5×10^6 generations, and sampled every 1000 generations using a general-time-reversible model of evolution with a gamma model of among site rate variation. In both searches stationarity of the 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 were discarded. All data collected at stationarity were used to estimate posterior nodal probabilities and a summary phylogeny. Two independent replicates were conducted and inspected for consistency to check for local optima (Huelsenbeck and Bollback, 2001).

Results and discussion

Including outgroups, 40 combined mtDNA sequences were analyzed. We concluded that the GTR model (with a gamma distributed rate heterogeneity model (4 rate categories, $G = 0.698$) and an estimated proportion of invariable sites (0.53) was the most appropriate model of evolution for these data. A ten replicate heuristic search incorporating this model found one tree of $-\ln 10671$. Maximum parsimony analysis of 392 informative characters found 8 trees of 2205 steps, the 50% bootstrap consensus of which was identical to the ML analysis, but less well resolved (fig. 2). The estimate of phylogeny obtained using Bayesian analyses was similar to the ML tree, although with much higher levels of support (fig. 2).

Genetic variation within Mabuya maculilabris

Mabuya maculilabris as presently accepted has a huge range across sub-saharan Africa, includ-

ing islands off both the East and West coasts. Our analysis suggests extremely high genetic divergence between populations from São Tomé and Príncipe — 10.8% based on cytochrome *b* sequences, which is in the range typically found between species (Harris, 2002). Several more sequences were available from GenBank for the 12S gene region of *Mabuya maculilabris*. Therefore we also produced an estimate of relationships within this species based only on 12S sequences (fig. 3). Again, using these sequences genetic diversity is very high — up to 8.5%. Five distinct groups are supported; samples from Cameroon, from São Tomé, from Príncipe, *M. m. casuarine* from Mozambique with *M. comorensis*, and *T. m. maculilabris* from Mozambique. Given that *M. comorensis* clusters within *M. maculilabris*, and given the high genetic distances observed *M. maculilabris* should be considered a species complex. However greater sampling across the range will be needed to delimit the different genetic groups within this complex.

Genetic variation within Mabuya affinis

Mabuya affinis is widespread in West Africa, from Angola to Senegal. The specimens from Príncipe show a relatively high degree of differentiation from the single sample included from GenBank, from Guinea-Bissau (15%). Again further investigations will be needed to determine if this is also a species complex.

Genetic affinities of Mabuya ozorii

Mabuya ozorii is a poorly-studied species endemic to the tiny island of Annobon. Our analysis suggests that it is unrelated to the other species on the islands of the Gulf of Guinea, and thus probably colonised Annobon directly from the Continent, rather than “island-hopping” via São Tomé and Príncipe. The degree of divergence from the closest other species sampled is very high (10.8% for cytochrome *b*), especially given that Annobon is only 4.8 million years old. This confirms the distinctive-

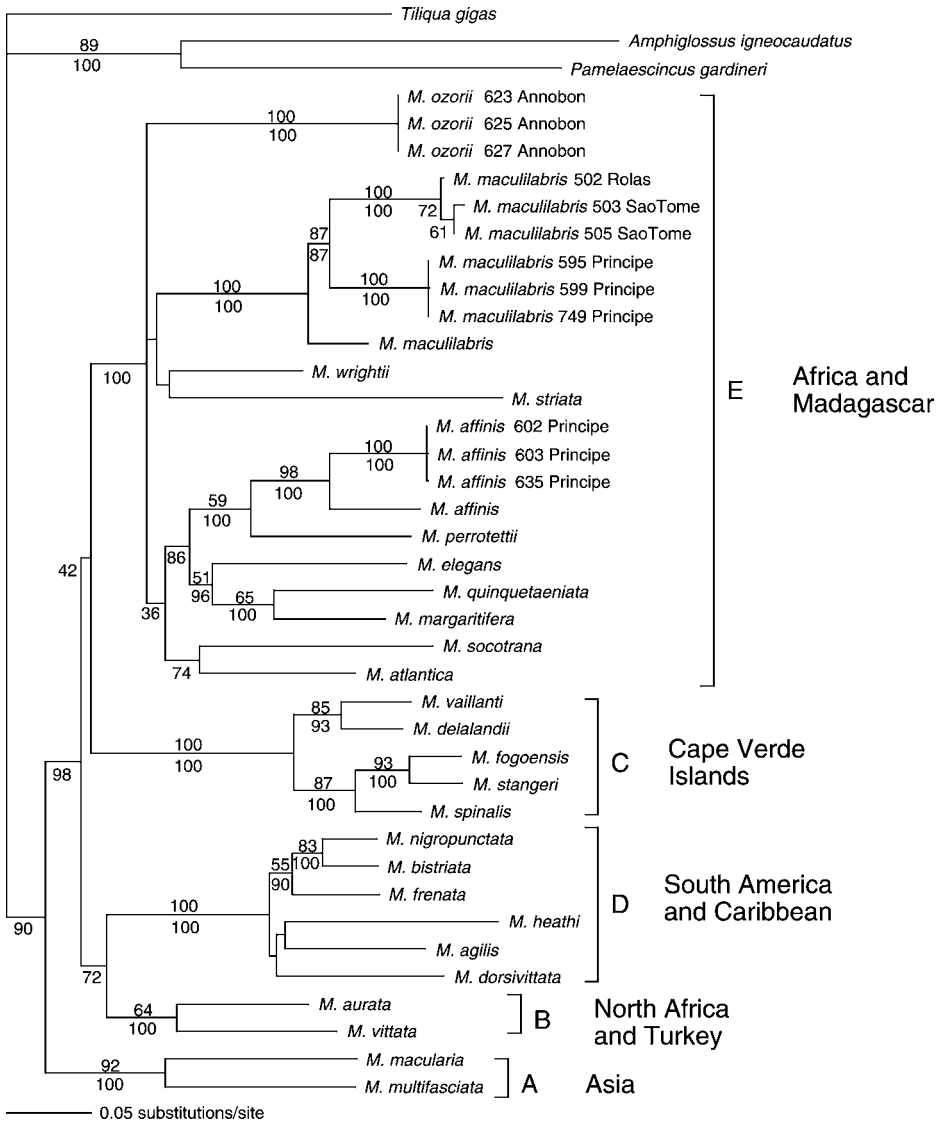


Figure 2. Single tree derived from a ML analysis using the model described in the text. All analyses produced similar estimates of relationships to the one shown. Posterior node probabilities from the Bayesian analyses are indicated below nodes and bootstrap values for MP are given above nodes.

ness of *M. ozorii*, and indicates Annobon may have been colonized soon after its emergence. As expected, *M. ozorii* belongs to the African-Malagasy group of *Mabuya*.

Phylogenetic divisions within *Mabuya*

Five major lineages within *Mabuya* that have some biogeographical coherency can be ascertained using our data. The species from tropical

Asia, *Mabuya macularia* and *Mabuya multifasciata* are sister taxa to the remaining *Mabuya* and are well supported as a clade (clade A, fig. 2). The other major groups are species from North Africa and Turkey (clade B), those from the Cape Verde islands (C), South America and the Caribbean (D), and Africa and Madagascar (E). Bootstrap support for these groups varies from quite weak (clade E) to strong (clades C and D — 100%), although all clades have high

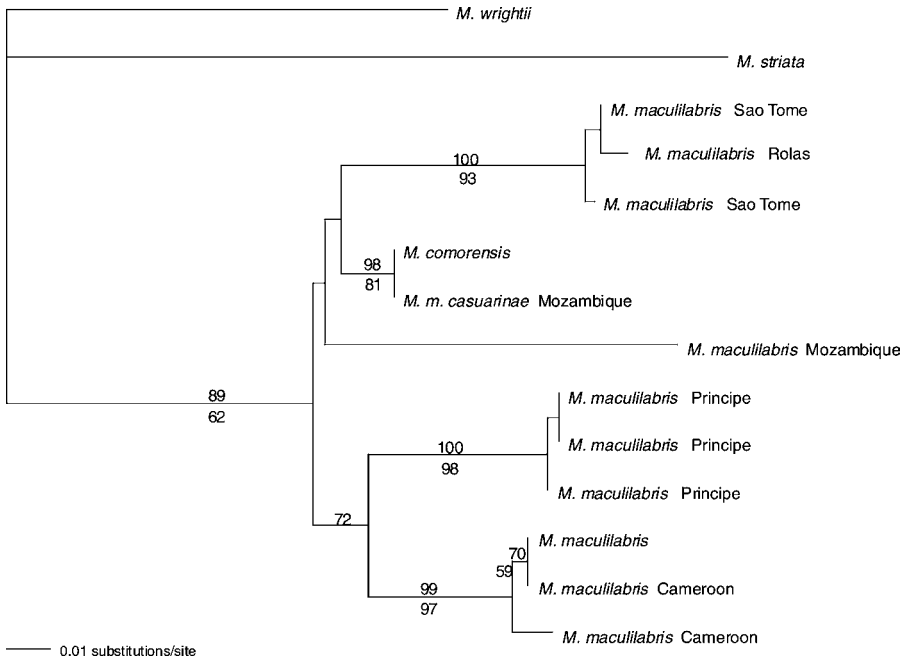


Figure 3. Single tree derived from a ML analysis based only on 12S rRNA sequences, following the same procedure used in fig. 2. *Mabuya wrightii* and *M. striata* were used to root the tree, following fig. 2. Bootstrap values for MP and ML and given above and below the nodes respectively.

Bayesian posterior node probabilities. Relationships between clades B-E are all weakly supported. The same major clades were found by Carranza and Arnold (2003), although the estimate of relationships between clades B-E differed in this study. Similarly Mausfeld et al. (2002) recovered four of these clades, the fifth (B) not having been sampled. They also obtained different estimates of relationships between the major clades. Our sampling overall is too limited to draw many conclusions concerning relationships within these clades. However, for the species from the Cape Verde islands we recover the same estimate of relationships as previous studies with more extensive sampling (Brehm et al., 2001; Carranza et al., 2001).

Taxonomic revision of *Mabuya*

Mausfeld et al. (2002), on the basis of sampling 21 species (circa 20% of known *Mabuya*) recommended a taxonomic partitioning of *Mabuya* into four new genera, *Eutropis*, *Chioninia*, *Mabuya* and *Euprepis* for clades A, C, D and

E respectively. Bauer (2003) has since reported that *Euprepis* is a junior synonym of *Mabuya*, and that *Trachylepis* is the appropriate name for the African-Malagasy group (Clade E). Our data indicates that this original separation was premature, since species from North Africa and Turkey clearly do not belong to any of the four new genera. Furthermore, bootstrap support that these groups are monophyletic has decreased with increased species sampling. Thus these clades may well need to be split further when more species are added. We suggest taxonomic instability can be avoided by referring to these clades as subgenera, pending further studies. Clade B would then need to be formally described as an additional subgenus. Use of subgenera to avoid taxonomic instability has been suggested before (Arnold, 2000; Harris and Carretero, 2003). It has also been criticised, as genera are considered “simply as operational units and is contra productive to modern efforts for a new less arbitrary generic concept” (Böhme and Köhler in press). However, Mausfeld et al.

(2002) recommend splitting *Mabuya* based on high genetic distances found between groups. This is equally arbitrary since the genetic distance chosen as a cut-off point at which new taxonomic units are named has nothing to do with biological reality. More important is the need for extensive sampling before taxonomic changes are made. Since *Mabuya* is probably paraphyletic (Honda et al., 1999) taxonomic changes are needed. However, with a high degree of uncertainty regarding taxonomic relationships, and with few species sequenced, we suggest further work is required before these changes are made.

Conclusions

Four genetically distinct entities of *Mabuya* are reported from the islands of the Gulf of Guinea — *Mabuya ozorii* from Annobon, *M. maculilabris* from São Tomé, *M. maculilabris* from Príncipe and *M. affinis* from Príncipe. *Mabuya maculilabris* appears to be a species complex. Since none of these four entities are sister taxa, each is the product of a separate colonization event. Thus while *Mabuya* colonised the Cape Verde islands only once (Brehm et al., 2001; Carranza et al., 2001), the islands of the Gulf of Guinea were colonized at least four times. The recently proposed taxonomic split of *Mabuya* into four genera is inappropriate, since at least five deep genetic lineages exist.

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