

Relationships of Scincid Lizards (*Mabuya* spp; Reptilia: Scincidae) from the Cape Verde Islands Based on Mitochondrial and Nuclear DNA Sequences

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Partial DNA sequences from two mitochondrial (mt) and one nuclear gene (cytochrome *b*, 12S rRNA, and *C-mos*) were used to estimate the phylogenetic relationships among the six extant species of skinks endemic to the Cape Verde Archipelago. The species form a monophyletic unit, indicating a single colonization of the islands, probably from West Africa. *Mabuya vaillanti* and *M. delalandii* are sister taxa, as indicated by morphological characters. *Mabuya fogensis* and *M. stangeri* are closely related, but the former is probably paraphyletic. *Mabuya spinalis* and *M. salensis* are also probably paraphyletic. Within species, samples from separate islands always form monophyletic groups. Some colonization events can be hypothesized, which are in line with the age of the islands. *C-mos* variation is concordant with the topology derived from mtDNA. © 2001 Academic Press

Key Words: *Mabuya*; Cape Verde; cytochrome *b*; 12S rRNA; *C-mos* phylogeny; colonization.

INTRODUCTION

Studies of among-island variation have been fundamental in evolutionary theory. Nonvolant tetrapod groups, such as lizards, have become model organisms for the study of colonization and dispersal patterns, especially in the mid-Atlantic islands which have well-known geological histories (e.g., Thorpe *et al.*, 1994; Brown and Pestano, 1998; Carranza *et al.*, 2000). Initial colonization of these archipelagos has often been followed by invasion of nearby islands and rapid speciation (Carranza *et al.*, 2000). Reconstruction of such events can be assessed with DNA sequence data. Given the limited range of these island endemic species and that island populations have a higher risk of extinction

(Frankham, 1997), assessment of variation is also critical for conservation efforts.

The Cape Verde Archipelago lies 450 km from the West African Coast and comprises 10 islands and 3 islets of volcanic origin which have never been connected to the mainland (Mitchell-Thomé, 1983). The set configuration is of an arch (Fig. 1), the oldest islands being Maio, Sal, and Boavista and the youngest being Santo Antão and Brava, the tips of the “arch.” Because the sea depth between islands varies from a few meters to more than 3 km it is probable that some of them were linked due to sea level fluctuations during the Pleistocene or even more recently. During these periods some of the northwestern group of islands were most likely linked—São Vicente, Santa Lucia, Branco, and Raso, and possibly also Boavista and Maio from the “central” group of islands. Apart from these cases the water channels are very deep (>1000 m) so it is highly improbable that these islands were ever linked by land connections.

The genus *Mabuya* comprises species of skinks of moderate to large size, with cylindrical bodies (Hoogmoed, 1974). They have a worldwide distribution in tropical and subtropical areas with approximately 101 described species. In the Cape Verde islands the genus is represented by 6 recognized species, the only diurnal lizards present, all of which are endemic to the archipelago (Table 1). The giant scincid *Macrosclincus coctei* was another endemic species but it is believed to have been extinct from the beginning of the twentieth century (Schleich, 1982). The taxonomic status of some of these 6 species remains controversial especially at the subspecific level (e.g., Mertens, 1955; Schleich, 1987; Joger, 1993). Based on morphological characters, the two sympatric species *M. delalandii* and *M. vaillanti* are considered to form a closely related group (Greer, 1976; Pinheiro, 1989; Joger, 1993). They possess fused interparietals and parietals (unique in skinks) and retain 26 presacral vertebrae, which is typical in *Mabuya* (Greer *et al.*, 2000). The remaining species in the Cape Verde islands usually have 27 presacral vertebrae. *M.*

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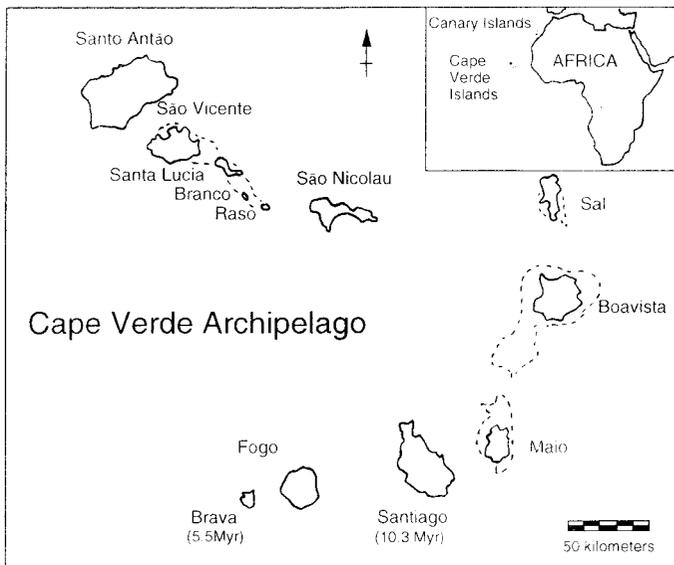


FIG. 1. Map of the Cape Verde islands. Localities of specimens used in this study are given in Table 1. Shallow water areas, which were probably above sea level in the geologically recent past, are marked with dashed lines.

delalandii and *M. vaillanti* are restricted to the leeward islands of the Archipelago; Brava, Fogo, and Santiago. *M. fogoensis* is distributed on the windward islands of Santo Antão and São Nicolau and is divided into two endemic subspecies, *M. f. antaoensis* and *M. f. nicolauensis* (Schleich, 1987). The remaining 3 species, *M. stangeri*, *M. salensis*, and *M. spinalis*, share several morphological characteristics and have been the center of most of the taxonomic controversy (Angel, 1935; Mertens, 1955; Schleich, 1987; Joger, 1993). Based on morphological characters Joger (1993) proposed that all Scincidae from the Cape Verde islands, including the extinct giant skink *M. coctei*, are a monophyletic group presumably from the nearby West African coast. However, recent molecular analyses of the Cape Verde island geckos *Tarentola* (Carranza *et al.*, 2000) and *Hemidactylus* (Jesus *et al.*, 2001) infer multiple colonizations in *Hemidactylus* and a direct colonization from the more distant Canary Islands in the case of *Tarentola*. Mitochondrial DNA evidence suggests that *Mabuya* may have colonized Madagascar more than once (Mausfield *et al.*, 2000). Therefore, the monophyletic status of *Mabuya* in the islands and its probable origin require further investigation.

Here we examine *Mabuya* from the Cape Verde Archipelago, covering almost the entire distribution of the extant species. On the basis of mitochondrial and nuclear DNA sequences, we elucidate the phylogenetic relationships among species and address historical dispersal processes that may have shaped their present distribution pattern.

MATERIALS AND METHODS

The geographic locations of the specimens used in this study, voucher codes, and number of specimens used are given in Table 1. All specimens belong to the collections of the University of Madeira. Total genomic DNA was extracted from small pieces of tail by use of standard methods. Polymerase chain reaction (PCR) primers used in both amplification and sequencing were cytochrome *b*1 and 2 and 12Sa and 12Sb from Kocher *et al.* (1989) and G73 and G74 for *C-mos* from Saint *et al.* (1998). Amplification conditions were the same as those described by Harris *et al.* (1998, 1999). Amplified fragments were sequenced from both strands on a 373 Applied Biosystem DNA Sequencing Apparatus. Sequences from the cytochrome *b* and 12S rRNA belonging to the same individual were merged in the subsequent analysis. These and the *C-mos* sequences were aligned with Clustal W (Thompson *et al.*, 1994). Cytochrome *b*, 12SrRNA, and *C-mos* sequences were, respectively, 280, 333, and 375 bp long. Three species of *Mabuya* from the Africa/Madagascar group, *M. affinis*, *M. elegans*, and *M. quinquetaeniata* (Mausfield *et al.*, 2000), and *Chalcides ocellatus* and *C. viridis* from the Canary Islands (Brown and Pestano, 1998) were included in the analysis of the mtDNA sequences. The mtDNA sequences were aligned with Clustal W (Thompson *et al.*, 1994). The cytochrome *b* sequences contained no indels. The data were then imported into PAUP* 4.0b3a (Swofford, 2000) for phylogenetic analysis. When estimating phylogenetic relationships among sequences, one assumes a model of evolution. Determining which model is most appropriate for a given data set is a statistical problem (Goldman, 1993). We used the approach outlined by Huelsenbeck and Crandall (1997) to test 56 alternative models of evolution, employing PAUP* 4.0b3a and Modeltest (Posada and Crandall, 1998). A starting tree was obtained with neighbor-joining. With this tree, likelihood scores were calculated for various models of evolution and then compared statistically with a χ^2 test with degrees of freedom equal to the difference in free parameters between the models being tested. The null hypotheses tested in this way included (1) nucleotide frequencies are equal, (2) transition rates are equal to transversion rates, (3) there is only one transition rate, (4) there are only two transversion rates, (5) there is rate homogeneity among sites, and (6) there is no significant proportion of invariable sites. Once a model of evolution was chosen, it was used to estimate a tree with the minimum-evolution (ME) optimality criteria (Rzhetsky and Nei, 1992). Support for nodes was estimated using the bootstrap (Felsenstein, 1985) technique, with 1000 replicates. A maximum-parsimony (MP) analysis was also carried out (100 replicate heuristic search), and support for nodes was estimated with decay analysis (Bremner, 1988) and bootstrapping with 1000 repli-

TABLE 1
List of Specimens and Locations Used in This Study

Species (number included for mtDNA)	Voucher code	Island locality
<i>M. fogoensis</i> (O'Shaughnessy, 1874) ¹ (6)	R34, 35, 36, 80, 100, cv133	Santo Antão
<i>M. fogoensis</i> (O'Shaughnessy, 1874) ¹ (3)	R01, 72, 93	São Nicolau
<i>M. stangeri</i> (Gray, 1845) ² (1)	R78	São Vicente
<i>M. stangeri</i> (Gray, 1845) ² (2)	R76, 86	Ilhéu Raso
<i>M. s. maioensis</i> Mertens, 1955 ³ (2)	R66, 67, 69	Maio
<i>M. spinalis</i> Boulenger, 1906 ⁴ (2)	R61, 62	Fogo
<i>M. spinalis</i> Boulenger, 1906 ⁴ (2)	R60, cv58	Santiago
<i>M. salensis</i> Angel 1935 ⁵ (1)	R03	Boavista
<i>M. salensis</i> Angel, 1935 ⁵ (2)	R79, 82	Sal
<i>M. vaillanti</i> Boulenger, 1887 (2)	R92, 94, 96	Fogo
<i>M. vaillanti</i> Boulenger, 1887 (2)	R98, 00, rvst999	Santiago
<i>M. delalandii</i> (Dumeril & Bibron, 1839) ⁶ (1)	R55	Brava
<i>M. delalandii</i> (Dumeril & Bibron, 1839) ⁶ (3)	R43, 49, 51	Santiago
<i>M. delalandii</i> (Dumeril & Bibron, 1839) ⁶ (3)	R52, 98, 99	Fogo

¹ Formerly attributed to the genus *Euprepes* but renamed by Boulenger, 1887 as *Mabuya fogoensis*; Schleich (1987) created two new subspecies for Santo Antão and São Nicolau, respectively, *M. f. antaoensis* and *M. f. nicolauensis*.

² Formerly attributed to the genus *Euprepes* but renamed by Boulenger, 1887 as *Mabuya stangeri*.

³ Renamed *M. spinalis maioensis* by Joger (1993).

⁴ Considered a synonym of *M. stangeri* by Schleich (1987), but Joger (1993) reassigned it as *M. spinalis* subspecies *spinalis*.

⁵ Initially designated *M. salensis* by Angel (1935) and then changed to *M. stangeri* (Dekeyser and Villiers, 1951). Mertens (1955) and Schleich (1982) created a subspecies *M. stangeri salensis* with the *terra typica* in the island of Sal. Joger (1993) refers *M. spinalis salensis* Angel, 1935 to the specimens from Boavista and Sal islands and *M. spinalis maioensis* Mertens, 1955 to the specimens from Maio island.

⁶ First attributed to the genus *Euprepes* but renamed by Boulenger, 1887.

cates. Assessment of saturation in the third positions of the cytochrome *b* by the plotting of numbers of transitions and transversions against uncorrected distances indicated that these position were not saturated (not shown). Therefore, all positions were included in the analysis.

RESULTS

We obtained 613 bp of aligned sequences from the combined 12S rRNA and cytochrome *b* genes for 32 taxa. GenBank Accession Nos. are AF335016–AF335088. With the included published data this gave 35 representatives of *Mabuya* and 2 *Chalcides*, which were designated outgroups. Using Modeltest (Posada and Crandall, 1998) under the Akaike information criteria, we concluded that the GTR model (Rodríguez *et al.*, 1990), with a gamma-distributed rate heterogeneity model (four rate categories, $\Gamma = 0.85$; Yang, 1994) and an estimated proportion of invariant sites (0.56) was the most appropriate model of evolution for this data. A 10-replicate heuristic search with this model produced a single tree with a minimum-evolution score of 1.4 (Fig. 2). Maximum-parsimony analysis found two trees of 515 steps which were similar to the ME tree (Fig. 2); 158 characters were parsimony informative. For the nuclear gene *C-mos*, nine sequences of 375 bp were analyzed. Eleven positions were variable, and 6 positions were parsimony informative. There were no insertions or deletions. Since there was no homoplasy in the data set, the sequences were joined in a most

parsimonious network. As all the synapomorphies were in agreement with the topology derived from mtDNA data, they were simply mapped onto the ME tree (Fig. 2). Inclusion in the analysis of *C-mos* sequences (from Saint *et al.*, 1998) of other skinks from the same subfamily Lygosominae (Greer, 1970; Honda *et al.*, 2000) did not alter this topology.

DISCUSSION

With the available outgroups, the mitochondrial DNA sequences suggest that the *Mabuya* from the Cape Verde islands are a monophyletic clade. Within the islands, they can be separated into two groups. One is constituted by *M. vaillanti* from Fogo and Santiago and the sympatric *M. delalandii*, which also occurs in the island of Brava. The separation of these species from the others is in accordance with morphological characters. Both of these species are monophyletic, with 99–100% bootstrap support from the mtDNA analysis and a single synapomorphy in the *C-mos* sequence data. In *M. delalandii* low variation was found in individuals from different islands, and no variation was found within islands. In the distance analysis *M. vaillanti* samples are separated according to their island of origin, but with MP the two individuals from Santiago were successive branches to the two from Fogo.

The other main branch comprises all remaining species studied. This branch can also be subdivided into two groups. One contains *M. fogoensis* from Santo

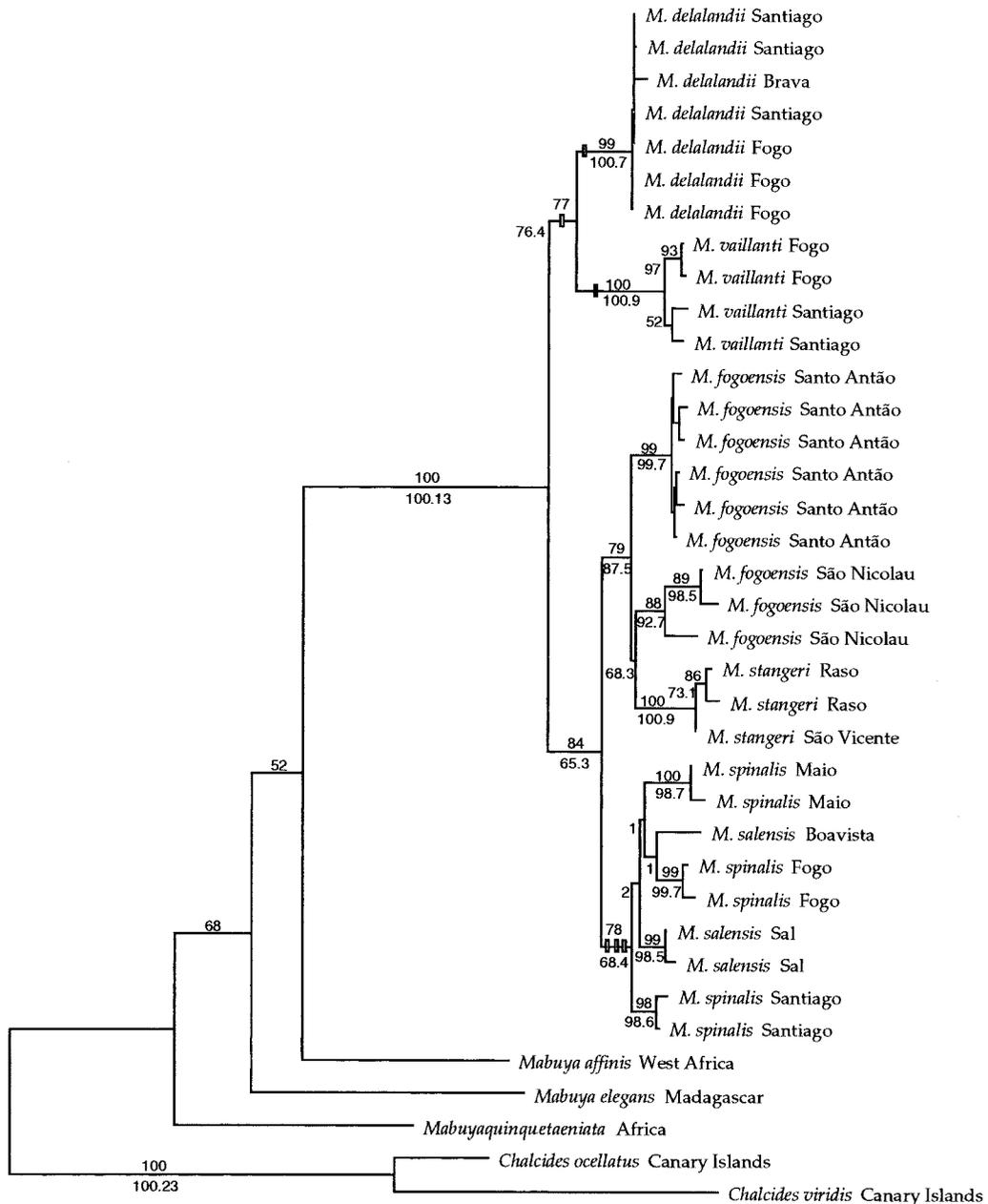


FIG. 2. Tree derived from a minimum-evolution (ME) search using the model described in the text. Maximum-parsimony (MP) trees differed in that *M. vaillanti* from Santiago was not a monophyletic group and that the three *Mabuya* outgroup taxa were joined as a clade (61% bootstrap support). Bootstrap values (>50%) for ME and MP are given above and below nodes, respectively. Decay indices for the MP analysis are given below the nodes. The tree was rooted with the *Chalcides* sequences. Six synapomorphies derived from *C-mos* sequences are mapped onto the tree (open rectangles).

Antão and São Nicolau and *M. stangeri* from São Vicente and the islet of Raso. The population from São Nicolau has been regarded by some as a separate subspecies from *M. fogoensis* of Santo Antão with the name *M. fogoensis nicolauensis* (e.g., Schleich, 1987). In our ME analysis *M. fogoensis* is paraphyletic, with *M. stangeri* more closely related to *M. fogoensis* from Santo Antão. When we constrained *M. fogoensis* to be monophyletic and compared this against the ME tree

with the same model of evolution with the likelihood variance (KH) test of Kishino and Hasegawa (1989), the hypothesis could not be refuted (differences in $-\ln l = 0.35$, $P = 0.7$). Whereas the use of the KH test has recently been questioned, in this case, since we reject the null hypothesis with a $P/2 > 0.05$, our results would be the same using the alternative Shimodaira and Hasegawa test (see Goldman *et al.*, 2000). Therefore, we cannot reject the possibility that *M. fogoensis*

is monophyletic. However, the samples from the two islands are clearly morphologically and genetically distinct. *M. stangeri* from São Vicente and Raso are closely related (100% bootstrap support), which is not surprising if one takes into consideration that the islands were probably united during recent glacial periods. This scenario agrees with Greer's (1976) hypothesis of evolution of the extinct giant skink *M. coctei*, which was known from the islands of Raso and Branco and regarded by this author as derived from either *M. fogoensis* or *M. stangeri*.

The third group of species includes *M. salensis* (from Sal and Boavista islands), *M. spinalis* from Santiago and Fogo, and samples from Maio that have been referred to either *M. stangeri* or *M. spinalis*. Both mtDNA (78% bootstrap) and three synapomorphies from the *C-mos* sequences support this clade. Within the group all the islands that were sampled form monophyletic units (98–100% support from mtDNA), but relationships between the islands are very poorly supported. In the ME tree the two species do not form monophyletic units, but this possibility cannot be significantly rejected using the KH test. Based upon our analysis, the samples from Maio should be considered *M. spinalis maioensis* rather than *M. stangeri*, supporting Joger (1993).

Due to the lack of support for relationships within the *M. spinalis/M. salensis* clade, little can be hypothesized about colonization patterns for these species. However, the possible radiation in other species can be predicted. *M. delalandii* and *M. vaillanti* may have originated and speciated on Santiago, which is the oldest of the islands on which they are found and the closest to Africa. Separate colonizations of Fogo, and in the case of *M. delalandii*, Brava, would then have occurred. If one assumes a molecular clock of 1.9% per million years (after Carranza *et al.*, 2000), the two speciated approximately 3 mya, with *M. vaillanti* colonizing Fogo 1 mya and *M. delalandii* even more recently. These dates are much more recent than the origin of the islands. In the *M. fogoensis/M. stangeri* clade, higher genetic variation is found within *M. f. nicolauensis*, on the older more eastern island of São Nicolau, and from here the lizards could have successively colonized the newer more western islands of São Vicente and Santo Antão.

CONCLUSIONS

Analysis of mtDNA sequence variation resolves many of the relationships within *Mabuya* from the Cape Verde islands, and the limited *C-mos* variation is in concordance with these. Variation within *Mabuya* is lower than that found among geckos on the islands (Carranza *et al.*, 2000; Jesus *et al.*, 2001), implying a more recent colonization. Intraisland colonization events also appear to be fewer. This is not surprising,

as geckos generally are more successful transmarine colonizers, as indicated by their presence on more oceanic islands, than skinks. Further, some geckos may have been moved between islands anthropogenically (Jesus *et al.*, 2001).

Mitochondrial DNA still reveals considerable variation between islands, which helps the understanding of the systematics of the group and is essential knowledge for their future conservation. Many taxa have very restricted ranges, and, given that *M. coctei* has probably already gone extinct in the last century, more work will be needed to ensure their continuing existence.

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