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Phylogeography and phylogenetic relationships of Malagasy tree and ground boas

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Three species of boid snakes are recognized in Madagascar, namely the genus *Sanzinia* (one species and two subspecies) and the genus *Acrantophis* (two species). In the present study, we studied the patterns of genetic variation of these species across Madagascar using a fragment of the mitochondrial 16S rRNA gene in 77 specimens. To support the phylogenetic relationships of the lineages identified, three further gene fragments (cytochrome *b*, 12S rRNA and *c-mos*) were analyzed in a reduced but representative set of samples. The results obtained corroborate that the genus *Sanzinia* includes two highly divergent mitochondrial lineages that evolved independently from each other on the east versus the west side of Madagascar. Each of these lineages presents a further subdivision that separates northern from southern groups. The nuclear marker showed no variation among the Malagasy boas, indicating either very low substitution rates in this gene or relatively recent speciation events coupled with high mitochondrial substitution rates. Because the broad geographic sampling detected no admixture among haplotypic lineages within *Sanzinia*, it is hypothesized that these may represent distinct species. Deviant haplotypes of snakes morphologically similar to *Acrantophis dumerili* indicate that this taxon may be a complex of two species as well.

ADDITIONAL KEYWORDS: evolution – phylogeography – speciation – Squamata: Boidae: *Sanzinia*, *Acrantophis*.

INTRODUCTION

Madagascar is one of the world's biodiversity hotspots (Myers *et al.*, 2000; Brown & Gurevitch, 2004; Mittermeier *et al.*, 2004). The island's high degree of endemicity at deeper phylogenetic levels (Myers *et al.*, 2000; Mittermeier *et al.*, 2004) has been explained by Madagascar's long geographical isolation from Africa and India (approximately 160 Mya and 90 Mya, respectively) (Briggs, 2003). It also has been hypothesized that the island's great biodiversity could have arisen by speciation in watersheds that remained isolated during climatic shifts (Wilmé, Goodman & Ganzhorn, 2006) and the presence of riverine barriers (Yoder *et al.*, 2005). Another factor influencing Madagascar's species distributions is the presence of a mountainous range running from the north to the south of the island, which could have led to vicariant effects (Yoder & Heckman, 2006) and is a determining

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factor for the distribution of the island's humid rain forests (Wells, 2003). These geographic occurrences have determined species distribution patterns in: (1) an east–west biogeographic constraint (Nussbaum & Raxworthy, 1994; Pastorini, Forstner & Martin, 2001; Andreone *et al.*, 2002) and (2) a north–south pattern of differentiation (Nussbaum & Raxworthy, 1994; Raxworthy & Nussbaum, 1995; Pastorini, Forstner & Martin, 2000; Yoder *et al.*, 2000; Pastorini *et al.*, 2001; Pastorini, Thalmann & Martin, 2003; Vences & Glaw, 2003; Boumans *et al.*, 2007).

One of the most enigmatic groups of vertebrates in Madagascar is that of the giant snakes. Three species of Malagasy boids are known (Duméril & Bibron, 1844): *Sanzinia madagascariensis*, *Acrantophis dumerili*, and *Acrantophis madagascariensis*. Like other large reptiles, they are highly attractive for the pet trade (Foekema, 1975; Branch & Erasmus, 1976; Anonymous, 1991; Wengler, 1996) causing these snakes to be listed in the Vulnerable category of International Union for Conservation of Nature's Red List (IUCN, 2006) although they are not particularly rare in the wild (Glaw & Vences, 1994; Raxworthy & Nussbaum, 2000). To avoid uncontrolled collecting of these snakes in the wild, they are further listed on Appendix I of the Convention on the International Trade in Endangered Species (http://www.cites.org/eng/resources/species.html).

The Malagasy giant snakes are classified within the family Boidae (Underwood, 1976; Kluge, 1991; Vences *et al.*, 2001), but their phylogenetic relationships to the other members of the family have been strongly debated. Although morphological data were unanimous in defining the Malagasy boids as a monophyletic group related to the Neotropical *Boa constrictor* (Kluge, 1991), cytochrome (*cyt*) *b* DNA sequences indicated that *Sanzinia* and *Acrantophis* were no close relatives of *Boa* (Campbell, 1997; Austin, 2000). Inclusion of partial sequences of the 12S and 16S genes obtained further support a basal position of the Malagasy taxa among the Boidae (Vences *et al.*, 2001). Burbrink (2005), without referring to these previous data sources, reanalyzed the *cyt b* data set of Campbell (1997) and also failed to obtain a convincing hypothesis on the phylogenetic position of *Sanzinia* and *Acrantophis* with respect to other boids. Recently, Noonan & Chippindale (2006a, b) analyzed DNA sequences from multiple nuclear genes and, surprisingly, found that *Sanzinia* and *Acrantophis* were not directly related to other boas from the Neotropics and the Pacific area but, instead, they formed a clade together with the African mainland genus *Calabaria*. The phylogeny and origin via dispersal over an Antarctic land bridge of Malagasy boas has been hypothesized (Noonan & Chippindale, 2006a, b), but population-level data are still lacking, except for some preliminary information provided by Vences & Glaw (2003).

In the present study, we analyzed the molecular differentiation of *Sanzinia* and *Acrantophis* based on a broad geographic sampling, and test whether the phylogeographic distribution of *Sanzinia* follows rather an east–west and/or a north–south divergence pattern.

MATERIAL AND METHODS

Taxon sampling

Blood or tissue samples of *Sanzinia* ($N = 44$) and *Acrantophis* ($N = 14$) were obtained during independent expeditions between 2000 and 2006 in Madagascar. These were combined with samples included in the preliminary study by Vences & Glaw (2003) (Genbank accession numbers: AF215272, AF215276, and AY336060-AY336074), yielding a total of 54 samples of *S. madagascariensis*, five samples of *A. dumerili*, two samples of unclear taxonomic status and named *A.* sp. cf. *dumerili*, and 14 samples of *A. madagascariensis*. Sampling localities (Fig. 1, Table 1) covered almost the whole distribution range of both genera (Glaw & Vences, 2007). For the phylogenetic analyses, samples of *Candoia* sp., *Eunectes* sp., *Boa constrictor*, and *Calabaria reinhardtii* were used as outgroup (Table 2).

Laboratory protocols

Total genomic DNA was extracted from blood and tail tissues according to a standard phenol-chloroform protocol (Sambrook, Fritsch & Maniatis, 1989). For the phylogeographic analyses, a fragment of the mitochondrial 16S rRNA gene (491 bp) was amplified in the 58 new samples and combined with the available 16S rRNA fragments of *Sanzinia* and *Acrantophis* from Vences & Glaw (2003) (Genbank accession numbers of newly determined 16s sequences: EU419777–EU419787, EU419790, EU419791, EU419793, EU419798, EU419801–EU419807, EU419810, EU419811, EU419813–EU419822, EU419824–EU419826, EU419828–EU419832, EU419834–EU419847 and EU419849). The primers used to amplify the 16S fragment were 16Sar-L/16Srb-H (Palumbi *et al.*, 1991). This genetic marker was chosen because it was the locus for which sequences were available for several key samples of western localities from which tissue to isolate DNA was not longer available and because, in a previous study using a reduced sample size (Vences & Glaw, 2003), the 16S rRNA presented sufficient variability allowing to differentiate between two possible groups of *Sanzinia*.

For the phylogenetic analyses, fragments of the nuclear gene *c-mos*, and of the mitochondrial *cyt b*

Species	Localities	Localities on Map
Sanzinia madagascariensis madagascariensis	Andasibe	1
	Betampona	2
	Mantadia	3
	Vatovavy	4
	Vohidrazana	5
	Zahamena	6
	Andranofotsy	7
	Mananara Nord	8
	Manombo	9
	Mantadia	3
	Marojejy	10
	Masoala	11
	Andasibe	1
	Mantadia	3
	Maromizaha	12
	Ambohitsara	13
	Andohahela	14
	Ifanadiana	15
	Kianjavato	16
	Manantantely	17
	Manombo	9
	Nahampoana	18
	Ranomafana (Village)	19
	Tolongoina	20
	Vatovavy	4
Sanzinia madagascariensis volontany	Ankarafantsika	21
	Berara	22
	Beroboka	23
	Isalo	24
	Kirindy	25
	Ankarana	26
	Manongarivo	27
	Nosy Be	28
Acrantophis madagascariensis	Anjiamangirana	29
	Ankarafantsika	21
	Beroboka	23
	Ankarana	26
	Mahamasina	30
	Nosy Be	28
	Montagne des Français	31
	Ankarafantsika	21
Acrantophis dumerili	Sakaraha	32
	Isalo	24
	Ifaty	24
A. sp. cf. *dumerili*	Tolagnaro	33
	Berenty	34

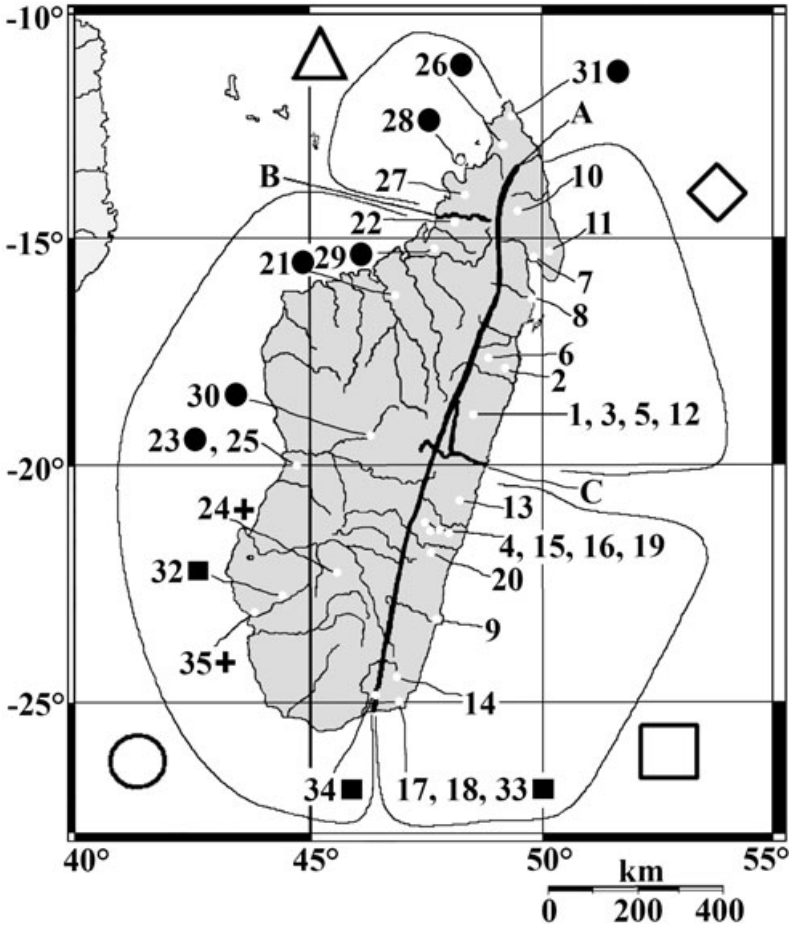

Figure 1. Sampling localities of *Sanzinia* and *Acrantophis*. Sampling localities are coded with numbers. A list of the number to locality correspondence is shown on a table next to the map. *Sanzinia* samples are defined within biogeographic regions following Boumans *et al.* (2007): □, South East and South Central East; ◇, North East and North Central East; △, North-West, and Sambirano region; O, Central, South, West and North-West. *Acrantophis* sampling locations are coded with symbols: ✚, *Acrantophis dumerili*; ■, *Acrantophis* sp. cf. *dumerili*; ●, *Acrantophis madagascariensis.* Symbols for both genera correspond to the clusters found by BAPS. Thick black lines correspond to Madagascar's mountainous central range (A), the Maevarano river (B), and Mangoro river (C).

and 12S rRNA genes were also amplified from the Malagasy taxa and jointly used with the 16S dataset (2594 bp total aligned length). The primers S77/S78 (Lawson *et al.*, 2005), L14910/H16064 (de Queiroz, Lawson & Lemos-Espinal, 2002), and L25195/H2916 (Vences *et al.*, 2000) were used to amplify the *c-mos*, *cyt b*, and 12S fragments respectively.

Cycle sequencing reactions were performed using ABI PRISM BigDye Terminator, version 1.1, Cycle Sequencing Kits following manufacturers' recommendations, and analyzed on an ABI PRISM 3100 DNA Sequencer (Applied Biosystems) at the Center for Conservation and Research, Henry Doorly Zoo (Omaha, NE, USA) and at the University of Amsterdam. All 16S and 12S sequences were aligned with CLUSTALX (Thompson *et al.*, 1997) and confirmed visually, whereas the sequences of the other fragments were aligned manually.

Phylogenetic analyses

We assessed the phylogenetic relationships between Malagasy boas based on two different datasets due to the availability of sample material. The first dataset was composed of the 16S rRNA DNA fragment sequences amplified for all individuals included in this research, and the second dataset was composed of fewer individuals due to the scarcity of sample material, but extended regarding the number of loci considered (i.e. 16S rRNA, 12S rRNA, *cyt-b*, and

Table 1. List of haplotypes for the sequenced fragment of the 16S rRNA gene

Species	Haplotype	Localities	Coordinates	*N*	Positioning
Sanzinia madagascariensis madagascariensis	Smm 1	Andasibe	18°55′S 48°24′E	5	North Central East
		Betampona	19°49′S 48°24′E	1	North Central East
		Mantadia	18°50′S 48°26′E	5	North Central East
		Vatovavy	21°24′S 47°56′E	1	South Central East
		Vohidrazana	18°58′S 48°31′E	1	North Central East
		Zahamena	17°40′S 48°50′E	1	North Central East
	Smm 2	Andranofotsy	15°26′S 49°48′E	1	North East
		Mananara Nord	16°22′S 49°47′E	1	North Central East
		Manombo	23°02′S 47°44′E	1	South Central East
		Mantadia	18°50′S 48°26′E	1	North Central East
	Smm 3	Marojejy	14°26′S 49°46′E	1	North East
	Smm 4	Masoala	15°17′S 50°01′E	1	North East
	Smm 5	Andasibe	18°55′S 48°24′E	1	North Central East
		Mantadia	18°50′S 48°26′E	2	North Central East
		Maromizaha	18°59′S 48°28′E	1	North Central East
	Smm 6	Ambohitsara	21°21′S 47°49′E	1	South Central East
		Andohahela	24°44′S 46°50′E	2	South East
		Ifanadiana	21°21′S 47°37′E	1	South Central East
		Kianjavato	21°22′S 47°52′E	1	South Central East
		Manantantely	24°59′S 46°55′E	1	South East
		Manombo	23°02′S 47°44′E	1	South East
		Nahampoana	24°58′S 46°58′E	1	South East
		Ranomafana (Village)	21°16′S 47°28′E	4	South Central East
		Tolongoina	21°33′S 47°31′E	2	South Central East
		Vatovavy	21°24′S 47°56′E	2	South Central East
Sanzinia madagascariensis volontany	Smv 1	Ankarafantsika	16°18′S 46°49′E	2	Central (West)
	Smv 2	Berara	14°19′S 47°55′E	1	Sambirano
		Beroboka	19°59′S 44°41′E	2	Central (West)
		Isalo	22°26′S 45°17′E	3	South
		Kirindy	20°05′S 44°40′E	1	Central (West)
	Smv 3	Ankarana	12°57′S 49°8′E	1	North
		Manongarivo	13°59′S 48°23′E	2	Sambirano
		Nosy Be	13°24′S 48°16′E	2	Sambirano
Acrantophis madagascariensis	Am 1	Anjiamangirana	15°09′S 47°43′E	1	Central (West)
		Ankarafantsika	16°18′S 46°49′E	1	Central (West)
		Beroboka	19°59′S 44°41′E	5	Central (West)
	Am 2	Ankarana	12°57′S 49°8′E	3	North
		Mahamasina	19°22′S 46°16′E	1	Central (West)
		Nosy Be	13°24′S 48°16′E	1	Sambirano
	Am 3	Montagne des Français	12°20′S 49°21′E	1	North
	Am 4	Ankarafantsika	16°18′S 46°49′E	1	Central
A. dumerili	Ad 1	Isalo	22°26′S 45°17′E	1	South
	Ad 2	South of Ambositra*	No coordinates	1	
		No Locality*	No coordinates	1	
	Ad 3	Ifaty	23°10′S 43°35′E	1	South
A. sp. cf. *dumerili*	Acfd 1	Sakaraha	22°53′S 44°27′E	1	South
		Tolagnaro	25°02′S 46°57′E	1	South East
	Acfd 2	Berenty	24°58′S 46°16′E	1	South

For each species (column 1), its corresponding haplotypes are presented (column 2). Haplotypes are subdivided in the localities where they were found (column 3), and locality coordinates are presented in column 4. Sample size per haplotype and locality are given in column 5. Sample's regional provenance is following Boumans *et al.* (2007) (column 6).
*Samples without geographical coordinates.

Table 2. Samples and Genbank accession numbers used for the phylogenetic analysis

	c-mos	*cyt b*	12S	16S
Ad1	EU403581	EU403574	EU403569	EU419793
Am2	EU403578	–	EU403566	EU419787
Acfd1	EU403582	EU403575	EU403570	EU419791
Acfd1	EU403584	EU403577	EU403572	EU419790
Smv3	EU403579	EU403573	EU403567	EU419805
Smm6	EU403580	–	EU403568	EU419845
Smm6	EU403583	EU403576	EU403571	EU419834
Boa constrictor	AF471115	U69740	AB177354	AY188080
Calabaria reinhardtii	AY611924	AY612015	Z46464	AY611832
Candoia carinata	AY099961	AY099984	AF544741	EU419850
Eunectes sp.	AY099964	U69810	AF368057	AF215274

Ad, *Acrantophis dumerili*; Am, *Acrantophis madagascariensis*; Acfd, *Acrantophis* sp. cf. *dumerili*; *Smv*, *Sanzinia madagascariensis volontany*; *Smm*, *Sanzinia m. madagascariensis.* Numbers after the abbreviation correspond to the haplotype names according to the 16S dataset, as in Table 1.

c-mos). The 16S rRNA dataset was analyzed with four different methodologies to avoid any bias arising from the tree building methods. *Candoia carinata* was used as outgroup. Bayesian inference, maximum likelihood (ML), Neighbor-joining clustering (NJ), and parsimony (MP) were applied on a haplotype matrix to ease calculations. Modeltest, version 3.7 (Posada & Crandall, 1998) and MrModeltest, version 2.2 (Nylander, 2004) were used to determine the appropriate nucleotide substitution model for the likelihood and the Bayesian analyses, respectively. The Bayesian analysis of phylogenetic inference was carried out using MrBayes, version 3.1.2 (Ronquist & Huelsenbeck, 2003) running three cold and one hot Markov chains for ten million generations and with sample retention every 100th generation. The analysis was repeated ten times without specifying priors. The initial 25% of the trees were discarded as 'burn in' following visual examination of the stabilization of likelihood values. ML analysis was performed with the web based service of PHYML (Guindon *et al.*, 2005; http://atgc.lirmm.fr/phyml). NJ and MP analyses were performed using PAUP* 4b10 (Swofford, 2002). Support for the branching of the trees was assessed with nonparametric bootstrapping (npb; Felsenstein, 1985). The number of bootstrap replicates was 500 for ML and 2000 for NJ and MP. For the second dataset, although fewer samples were available for all loci, representatives of all Malagasy taxa were included, as well as four other boids, *C. carinata*, *Eunectes* sp., *B. constrictor* and *C. reinhardtii*. These taxa were chosen based on the results of Noonan & Chippindale (2006a). This dataset was analyzed with parsimony, and support for the branching of the tree was assessed with 2000 npb using PAUP* 4b10 (Swofford, 2002).

Phylogeographic analyses

The number of parsimony-informative sites of the 16S rRNA dataset was calculated based on the number of segregating sites in DNAsp 4.10.9 (Rozas *et al.*, 2003). A Bayesian analysis of structure with BAPS, version 4.14 (Corander & Martinen, 2006) was performed independently for the *Sanzinia* and the *Acrantophis* samples. This analysis determines the most probable number of clusters present in a sample set and which samples correspond to each cluster found, independently of *ad hoc* hypothesis regarding the sample structure. Each analysis was performed three times for several values of number of possible clusters present in the sample to ensure reliability of the results. A median joining network with the NETWORK, version 4.2.0.0 (Bandelt, Forster & Rohl, 1999) using the 16S sequences was constructed to assess patterns of divergence between the observed haplotypes. In addition, an analysis of molecular variance (AMOVA) was performed with the ARLEQUIN, version 3.1 (Excoffier, Laval & Schneider, 2005) to characterize levels of differentiation between the clusters of boas defined by BAPS. This software was also used to calculate Nei's average number of pairwise differences between the referred clusters (Nei & Li, 1979). The presence of isolation by distance was analyzed with Mantel tests between the matrix of pairwise genetic distances between samples and pairwise geographic distances between sampling localities, and performing 10 000 permutations to assess significance. A final analysis regarding the estimation of the geographical position of possible barriers for gene flow between pairs of samples was performed with Monmonier's maximum difference algorithm (Monmonier, 1973). Briefly, a Delauny network (Watson, 1992) is

built between pairs of sampled localities and related to a matrix of pairwise mismatch genetic distances between sequences. The connections between pairs of geographical localities in the network are termed edges. In a first step, the method searches for the largest pairwise genetic distance between all pairs of samples and then locates on the network the edge connecting the two most differentiated samples. The edge connecting those two samples is the first fragment of a possible barrier. In the following step, the algorithm searches among the edges connecting to the first fragment of the barrier for the one with the next largest genetic difference and marks it as the second edge of the possible barrier. The algorithm moves in this way interconnecting edges until it reaches an edge linking two localities on the periphery of the network or an internal edge already defined as part of the possible barrier (Miller, 2005). The network was overlapped manually on a map of Madagascar to determine the geographical location of the estimated barrier. The last two analyses were carried out in AIS (Miller, 2005).

RESULTS

In the 16S dataset, a total of 18 haplotypes were observed (Table 1). Nine haplotypes corresponded to *Sanzinia*, four to *A. madagascariensis*, three to *A. dumerili*, and two to the *A.* sp. cf. *dumerili* samples. We determined with a likelihood ratio test implemented in TREE-PUZZLE (Schmidt *et al.*, 2002) that a non-clock like pattern of evolution fits significantly better for the 16S rRNA gene data set than a clock-like pattern of evolution. Thus, the presence of a similar amount of haplotypes in each genus despite of the different sample sizes could be the outcome of a higher substitution rate for this locus in *Acrantophis*. The 16S *Sanzinia* and *Acrantophis* dataset included a total of 36 segregating sites, five of them singletons and 31 parsimony-informative sites. Among the 371 bp amplified from the 12S locus, ten positions were singletons and 29 parsimony-informative sites. Almost with twice as many variable sites, the *cyt b* presented 100 positions corresponding to singletons and 111 to parsimony-informative sites among the 1117 bp amplified. In contrast to the mitochondrial loci, the nuclear gene *c-mos* presented no polymorphism in the 570 bp amplified from samples of *Sanzinia* and *Acrantophis*.

Phylogenetic analysis

In the 16S haplotype dataset of *Sanzinia* and *Acrantophis*, five phylogenetically relevant clades were identified with the methodologies implemented. The most important observations are a split of the *Sanzinia* specimens into an eastern and a western clade, and the differentiation of three clades of *Acrantophis* (Fig. 2). Within each *Sanzinia* clade, a further split could be detected. For the western *Sanzinia* clade, the split presented high support values, whereas, for the eastern clade, the split was only found with the ML analysis and a npb support value of 29.8. The node relating the *Acrantophis* of uncertain taxonomic status and *A. madagascariensis* was not found by the NJ and the MP analyses, probably due to a lack of sufficient phylogenetic signal in the 16S dataset. This is sustained by the little support that the other three methodologies give to the branch leading to *A. madagascariensis* and the samples of uncertain taxonomic status (Fig. 2).

The division between the two *Sanzinia* clades and between the *Acrantophis* samples was also recovered with high support values using additional markers (Fig. 3). Although the *A. madagascariensis* sample and the *Sanzinia* from Ranomafana could not be sequenced for the *cyt b*, the partitions and support values for the clades were not affected by the shorter dataset (total aligned length excluding *cyt b*: 1477 bp). Although the main clades identified in the analysis based on 16S rRNA also showed strong divergences in the other two mitochondrial genes (12S rRNA, *cyt b*), the nuclear gene *c-mos* showed no variability in both genera of Malagasy boas.

Phylogeographic analysis

The 16S dataset split the *Sanzinia* samples in four clusters according to the Bayesian analysis of structure with a posterior probability of 1. Using the biogeographic scheme of Madagascar by Boumans *et al.* (2007), these clusters occur in the South-East and South-Central-East regions (cluster 1), in the North-East and North-Central-East regions (cluster 2), in the North and Sambirano regions (cluster 3), and in the North-West, Central and South regions (cluster 4). Only two individuals from the Southern Central East, sampled in Vatovavy and Manombo, had haplotypes Smm1 and Smm2, respectively, and thus did not follow this pattern of haplotype distribution. The *Acrantophis* samples were split in three clusters with a posterior probability of 0.923, representing (1) the *A. madagascariensis* samples, (2) the *A. dumerili* samples, and (3) the three *A.* sp. cf. *dumerili* samples all from southern Madagascar.

AMOVA between the four clusters of *Sanzinia* showed that most of the genetic variation lies between these clusters (93.11%), whereas only 6.89% of the genetic variation occurred within clusters. A similar pattern was observed between the three clusters of *Acrantophis* where 85.54% of the genetic

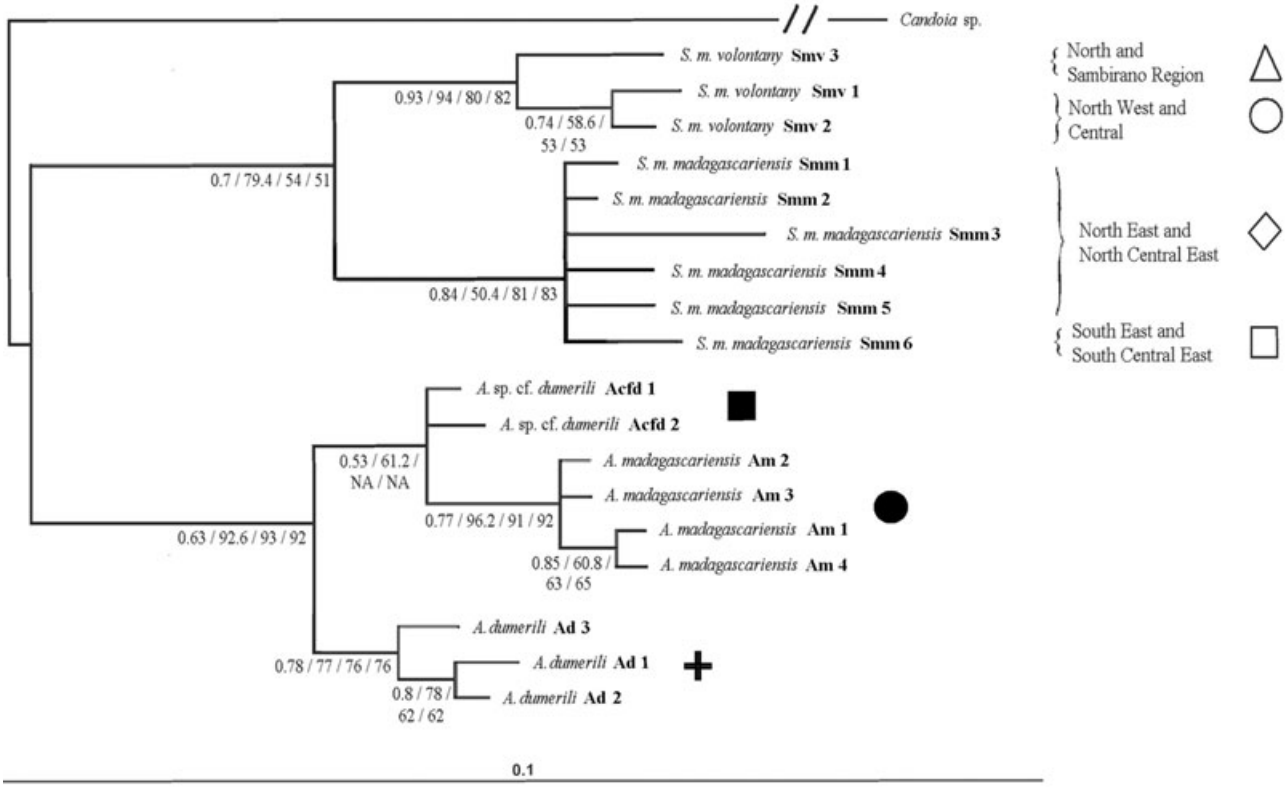

Figure 2. Bayesian tree of Malagasy boa haplotypes based on 16S rRNA sequences. *Candoia* sp. was used as outgroup. Below branches in respective order: posterior probabilities, nonparametric bootstrap support values (npb) in percentage for maximum likelihood (500 replications), npb for Neighbor-joining (2000 replicates) and npb for maximum parsimony (2000 replicates). NA, not available. Haplotype names correspond to those in Table 1. Biogeographical regions are named *sensu* Boumans *et al.* (2007). Symbols correspond to the sampling localites shown in Fig. 1.

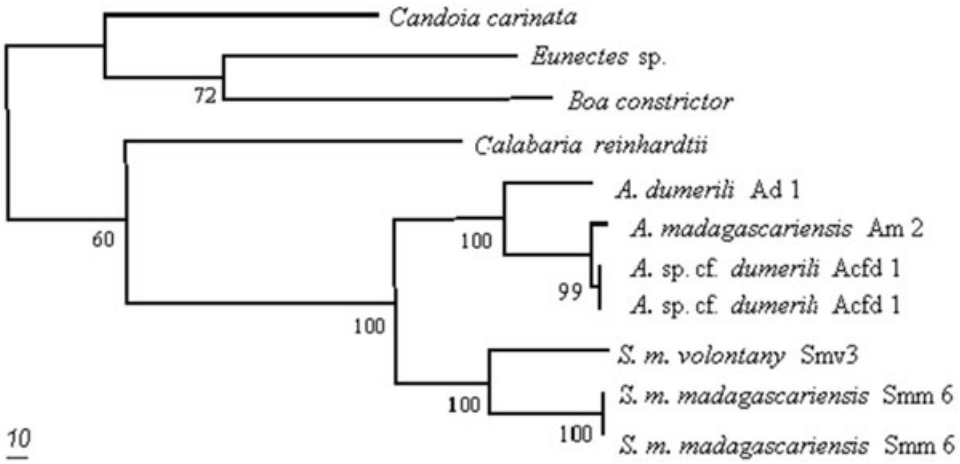

Figure 3. Phylogram inferred under the maximum parsimony optimality criterion based on DNA sequences of the 16S rRNA, 12S rRNA, *c-mos*, and *cyt b* genes. Nonparametric bootstrap support values (npb) are presented below branches.

Table 3. Uncorrected population average pairwise differences between Malagasy boas: *Sanzinia* clusters

	Smv South West	*Smv* North West	*Smm* North East	*Smm* South East
Smv South West		1.11	3.02	3.05
Smv North West	5.44		3.22	3.26
Smm North East	14.82	15.82		0.62
Smm South East	15.00	16.00	3.05	

Below the diagonal, Nei's average population pairwise differences and, above the diagonal, the same results presented as a percentage, estimated from the 16S dataset. *Smv*, *Sanzinia madagascariensis volontany*; *Smm*, *Sanzinia m. madagascariensis*.

variation was found between clusters and only 14.46% of the genetic variation is found within clusters. We also nested the four *Sanzinia* groups within a single category and the three *Acrantophis* groups into another category and analyzed both categories simultaneously with the AMOVA. Interestingly, we found that approximately the same amount of variation was explained by the between categories component of variation (i.e. between genera) as by the within category component of variation (i.e. within genus), namely 50.95% and 44.91%, respectively (the remaining 4.14% of the genetic variation occurred within clusters). The last comparison shows that, on average, the amount of variation between the clusters of *Sanzinia* and between the clusters of *Acrantophis* is almost as much as the variation between any *Acrantophis* and *Sanzinia* clusters.

The observed differentiation between clusters of boas in the previous analyses was defined in terms of the number of average pairwise differences between clusters and median joining networks. The number of differences between the *Sanzinia* clusters in the East or in the West is much smaller than the differences between any of the eastern and western clusters (Table 3). This pattern of dissimilarity is reflected in the *Sanzinia* median joining network, where the eastern and western clades are joined by 12 substitutional steps, whereas one to five substitutions are sufficient to relate haplotypes within the east or the west of Madagascar (Fig. 4A). The *Acrantophis* of uncertain taxonomic status show a similar average pairwise number of differences with respect to any of the other two *Acrantophis* (Table 4). This also becomes evident in the median joining network, where the *A.* sp. cf. *dumerili* are separated by at least four substitutions from any of the other taxa, and are placed in the middle of the branch connecting the other two species of *Acrantophis* (Fig. 4B). As observed in the AMOVA, the *Acrantophis* network shows that the number of substitutional differences between haplotypes within the clusters defined by BAPS is much smaller than between clusters.

Table 4. Uncorrected population average pairwise differences between Malagasy boas: *Acrantophis* clusters

	Am	*Ad*	Acfd
Am		2.13	1.03
Ad	10.46		1.24
Acfd	5.05	6.08	

Below the diagonal, Nei's average population pairwise differences and, above the diagonal, the same results presented as a percentage, estimated from the 16S dataset. Ad, *Acrantophis dumerili*; Am, *Acrantophis madagascariensis*; Acfd, *Acrantophis* sp. cf. *dumerili*.

Overall and within the eastern and the western clusters of *Sanzinia*, some level of isolation by distance was found. The geographic distance to genetic distance correlation with the Mantel tests for all *Sanzinia* samples was $r = 0.489$ ($P < 0.001$), within the eastern *Sanzinia* $r = 0.508$ ($P < 0.001$) and within the western *Sanzinia* $r = 0.504$ ($P < 0.005$). Due to the presence of correlation between genetic and geographic distances, the Monmonier maximum difference analysis was performed on the residual genetic distances instead of on the raw genetic distance (Manni, Guerard & Heyer, 2004). This was conducted because, in the presence of isolation by distance, the raw pairwise genetic distance will tend to place the edge of maximum difference in the midpoint between the most divergent samples, which, under isolation by distance, are the two most distant samples on the geographical landscape. The Monmonier maximum difference analysis showed the presence of two barriers that fit to the Malagasy landscape. The first barrier runs from the north to the south of Madagascar and, when manually overlapped on a map of Madagascar, it corresponds to the central highland range that splits the island into the eastern rain forest and the dry western region. The second barrier, if extrapolated on the Malagasy landscape, corresponds to the Maevarano river that separates the Sambirano region from the rest of western Madagascar.

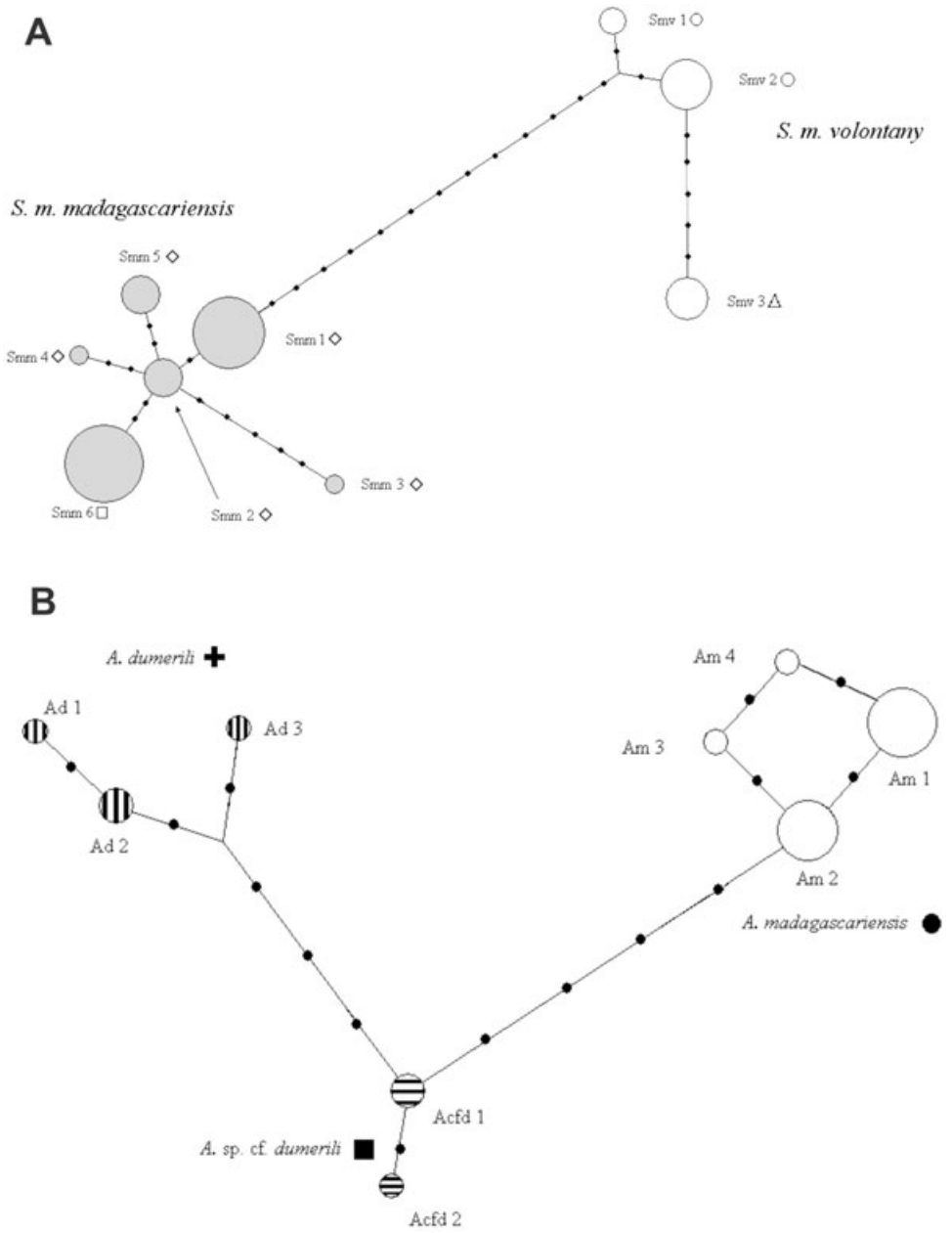

Figure 4. Median-joining networks (MJN) of 16S rRNA sequences of (A) *Sanzinia* and (B) *Acrantophis.* A, light grey circles correspond to haplotypes of *Sanzinia madagascariensis madagascariensis* and white circles correspond to haplotypes of *Sanzinia madagascariensis volontany*. *Sanzinia madagascariensis madagascariensis* haplotype Smm 2 is indicated with an arrow. B, White circles correspond to haplotypes of *Acrantophis madagascariensis*, circles with horizontal lines correspond to haplotypes of *Acrantophis* sp. cf. *dumerili*, and circles with vertical lines correspond to haplotypes of *A. dumerili*. Symbol coding corresponds to that shown in Fig. 1. Small black circles indicate substitutions on a branch connecting two haplotypes. Haplotype names are those in Table 1. Symbols next to the haplotype names, or next to species names, correspond to the coding given to the clustering solution found by BAPS, and are the same symbols used in Fig. 1.

DISCUSSION

PHYLOGEOGRAPHY OF *SANZINIA*

Our analyses provide robust support to the partitioning of *S. madagascariensis* into an eastern and a western lineage, corresponding to the two subspecies *Sanzinia madagascariensis madagascariensis* and *Sanzinia madagascariensis volontany*, *sensu* Vences & Glaw (2003), based on mitochondrial data. The eastern haplotypes follow strictly the eastern band of

rainforest, and show a differentiation between two clusters, a widespread south-eastern and south-central-eastern haplotype (Smm 6), and five other eastern haplotypes (Smm 1 to Smm 5) found mostly north of Mangoro river. A similar subdivision into two geographically well defined clusters is found among the western haplotypes, where a single haplotype (Smv 3) is restricted to a northern most location, whereas the remaining two western haplotypes (Smv 1 and Smv 2) are widespread in the remaining area of western of Madagascar.

Although our sampling is much denser than in the preliminary analysis of Vences & Glaw (2003), we did not identify any contact zone of the two main mitochondrial lineages (i.e., *S. m. madagascariensis* and *S. m. volontany*). A number of localities would be of particular interest in this respect and need to be surveyed in the future:

1. Montagne d'Ambre in the far north, which is known to harbour relict populations of rainforest species not present in dryer northern areas such as Ankarana or Montagne des Français [e.g. the frogs *Aglyptodactylus madagascariensis* (Nussbaum, Raxworthy & Cadle, 2004) and *Guibemantis liber* (Glaw & Vences, 2007)]. Here, a relict *Sanzinia* population with haplotypes belonging to the eastern group might be found.
2. The Manongarivo/Tsaratanana complex in the Sambirano region, where specimens with western haplotypes occur but the existence of specimens with eastern haplotypes (e.g. at higher elevations) may be suspected.
3. The area between Ambalavao-Ambositra, close to eastern rainforests, where *Acrantophis dumerili* is known to occur east of the central mountain chain and also *Sanzinia* populations of the western haplotype group could occur in close proximity to those of the eastern group (Glaw & Vences, 2007).
4. The Andohahela area in the far south, where clines between extremely dry and rainforest habitats occur over very short distances (Ramanamanjato, Mcintyre & Nussbaum, 2002) and, so far, only specimens of the eastern haplotype group are known

The two *Sanzinia* subspecies are highly divergent as seen in the median-joining haplotype network (Fig. 4). The apparent lack of haplotypic introgression among these two subspecies indicates the presence of geographical, ecological, and/or taxonomic barriers to gene flow. Although the two subspecies are largely separated by the central Malagasy high plateau, a connection between western and eastern forests occurs in northern regions of the island where suitable habitat for *Sanzinia* connects the North-East and Sambirano regions. Nonetheless, no admixture between the two major haplotype lineages of *Sanzinia* has been observed in this area.

Within the geographic ranges of the two subspecies, the presence of barriers for dispersal is also evident by the patterns of isolation by distance observed with the Mantel tests. We could not determine an edge of maximum differentiation separating the two eastern clusters with the residual genetic distances but exploratory analysis using the raw genetic distances found a possible barrier that, if overlapped on the Malagasy map, approximately corresponds to the Mangoro river. This river is also known to constitute a barrier for the dispersal of other taxa (Goodman & Ganzhorn, 2004; Louis *et al.*, 2006; Boumans *et al.*, 2007). The observations also fit with a scenario of southwards dispersal of *Sanzinia* out of refuges north of the Mangoro, with a bottleneck preceding the colonization of the South Central East and South East of Madagascar. As we found this possible barrier with the raw genetic distance, the eastern barrier may also reflect isolation by distance rather than a real genetic barrier. Consequently, there is no certainty on the existence of a barrier separating the two eastern haplotype clusters. By contrast to what is observed in *S. m. madagascariensis*, an edge of maximum differentiation separating the *S. m. volontany* clusters was found. This edge corresponds most likely to the Maevarano river that acts as the natural margin separating the Sambirano region from the North-Western region of Madagascar (Boumans *et al.*, 2007). The Maevarano river has previously been shown to be a major barrier delimiting lemurs distribution ranges at the species and subspecies level (Pastorini *et al.*, 2003; Guschanski *et al.*, 2007); thus, it may likely be the barrier to dispersal of the western haplotypic clusters of *S. m. volontay.*

Species-level taxonomy of Malagasy boas

The genus *Sanzinia* has been traditionally considered a monotypic genus represented by *S. madagascariensis*. Lately, two subspecies, *S. m. madagascariensis* and *S. m. volontany*, have been recognized based on a small sample set of 16s rRNA DNA sequences (Vences & Glaw, 2003). In the present study, we have shown, employing a larger dataset covering the whole distribution rage of the genus, that the differentiation between the two subspecies is well supported. This conclusion is apparently contradicted by the absence of divergence for the *c-mos* gene among all *Sanzinia* samples. The *c-mos* gene has been successfully applied to phylogenetic analysis of bird orders (Cooper & Penny, 1997) and reptiles (Saint *et al.*, 1998; Harris, Marshal & Crandall, 2001; Noonan & Chippindale, 2006a), and to determine the branching order of the major groups of reptiles, birds, and mammals (Hedges & Poling, 1999), but, due to its high conservation, it

has been suggested that for comparisons of less divergent taxa (e.g. closely related genera), *c-mos* may present a general lack of substitution differences (Lovette & Bermingham, 2000), possibly related to its important cellular role (Sagata *et al.*, 1988; Gebauer & Richter, 1997). Further studies should consider longer fragments of *c-mos* that may harbor variation not yet observed (Godinho *et al.*, 2006; Willis *et al.*, 2007) or more variable alternatives to *c-mos*, such as the brain-derived neurotrophic factor precursor, the recombination activating gene 1, neurotrophin-3, or the ornithine decarboxylase (Noonan & Chippindale, 2006b). In any case, the lack of variability in the *c-mos* gene among the clades of *Sanzinia* cannot serve as evidence against their taxonomic distinctness because, as mentioned previously, *Sanzinia* and *Acrantophis* (which undoubtedly represent distinct biological species of partly syntopic occurrence with different morphologies, ecologies, and which do not interbreed) have identical *c-mos* sequences.

Hence, from a mitochondrial perspective, *S. madagascariensis* is composed of two well differentiated genetic lineages that show a large amount of divergence from each other. These two clades show a very limited amount of shared variability as result of their shared ancestry and historical genetic isolation. The morphological differences between the lineages alone are too faint to warrant a distinction at the species level (Vences & Glaw, 2003). However, despite contiguous potential habitat in the northern regions of Madagascar, the data provided in the present study show that, apparently, no broad admixture of these mitochondrial lineages occurs. Future studies are required to test whether the high differentiation between the two major clades of *Sanzinia* persists when sampling potential contact zones among the two taxa, and when analyzing nuclear markers of sufficient resolution. If such analyses confirm the lack of broad admixture among the two taxa, in mitochondrial as well as nuclear markers, a taxonomic conclusion might be that these taxa correspond to different species according to the evolutionary species concept (Wiley, 1978) and possibly also according to the biological species concept (Mayr, 1942).

Within *Acrantophis*, the haplotypes Aspd 1 and Aspd 2 in *A.* sp. cf. *dumerili* correspond to a form of southern Madagascar that morphologically resembles *A. dumerili* (Vences & Glaw, 2003) but genetically is more closely related to *A. madagascariensis*. Our data confirm that this taxon occurs in southernmost Madagascar, but also provide evidence for its occurrence at Sakaraha, much further north. Phylogenetic analysis of *cyt b* and 12S sequences (Fig. 3) corroborates the paraphyletic arrangement of the two main haplotype lineages of *A. dumerili* relative to *A. madagascariensis*. Further analysis of nuclear markers are needed to test whether this situation reflects the existence of three *Acrantophis* species (and, hence, an undescribed species of ground boa in southern Madagascar) or an ancient introgression of ancestral *A. madagascariensis* haplotypes into southern populations of *A. dumerili*.

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REFERENCES

Andreone F, Vences M, Guarino FM, Glaw F, Randrianirina JE. 2002. Natural history and larval morphology of *Boophis occidentalis* (Anura: Mantellidae: Boophinae) provide new insights into the phylogeny and adaptative radiation of endemic Malagasy frogs. *Journal of Zoology (London)* **257:** 425–438.

Anonymous. 1991. Riesenschlangen: Schmuggelgut im Wäschesack. *WWF Journal* **2:** 40.

Austin C. 2000. Molecular phylogeny and historical biogeography of Pacific island boas (Candoia). *Copeia* **2:** 341–352.

Bandelt HJ, Forster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16:** 37–48.

Boumans L, Vieites DR, Glaw F, Vences M. 2007. Geographical patterns of deep mitochondrial differentiation in widespread Malagasy reptiles. *Molecular Phylogenetics and Evolution* **76:** 5269–5673.

Branch W, Erasmus H. 1976. Reproduction in Madagascar ground and tree boas *Acrantophis madagascarienisis* and

Sanzinia madagascariensis. *International Zoo Year Book* **16:** 77–80.

Briggs JC. 2003. The biogeographic history of India. *Journal of Biogeography* **30:** 381–388.

Brown KA, Gurevitch J. 2004. Long-term impacts of logging on forest diversity in Madagascar. *Proceedings of the National Academy of Sciences of the United States of America* **101:** 6045–6049.

Burbrink F. 2005. Inferring the phylogenetic position of *Boa constrictor* among the Boidae. *Molecular Phylogenetics and Evolution* **31:** 167–180.

Campbell BN. 1997. Hic sunt serpentes: molecular phylogenetics and the Boidae (Serpentes: Booidea). Unpublished PhD Thesis, Queens University.

Cooper A, Penny D. 1997. Mass survival of birds across the Cretaceous-Tertiary boundary: molecular evidence. *Science* **275:** 1109–1113.

Corander J, Martinen P. 2006. Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology* **10:** 2833–2843.

Duméril AMC, Bibron G. 1844. *Erpetologie Générale ou Histoire Naturelle Complete des Reptiles*. Paris: FRA.

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioninformatics Online* **1:** 47–50.

Felsenstein J. 1985. Confidence limits on phylogenetics: an approach using the bootstrap. *Journal of Organic Evolution* **39:** 783–791.

Foekema G. 1975. Ontwikkeling en voortplanting van *Sanzinia madagascariensies* in een huiskamerterrarium. *Lacerta* **33:** 71–82.

Gebauer F, Richter J. 1997. Synthesis and function of Mos: the control switch of vertebrate oocyte meiosis. *Bioessays* **19:** 23–28.

Glaw F, Vences M. 1994. *A field guide to the amphibians and reptiles of Madagascar*. Cologne: Vences & Glaw Verlag.

Glaw F, Vences M. 2007. *A field guide to the amphibians and reptiles of Madagascar*. Cologne: Vences & Glaw Verlag.

Godinho R, Domingues V, Crespo E, Ferrand N. 2006. Extensive intraspecific polymorphism detected by SSCP at the nuclear C-mos gene int he endemic Iberian lizard Lacerta schreiberi. *Molecular Ecology* **15:** 731–738.

Goodman SM, Ganzhorn JU. 2004. Biogeography of lemurs in the humid forests of Madagascar: the role of elevational distribution and rivers. *Journal of Biogeography* **31:** 47–55.

Guindon S, Lethiec F, Duroux P, Gascuel O. 2005. PHYML online – a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Research* **33:** W557–W559.

Guschanski K, Olivieri G, Funk SM, Radespiel U. 2007. MtDNA reveals strong genetic differentiation among geographically isolated populations of the golden brown mouse lemur, *Microcebus ravelobensis*. *Conservation Genetics* **8:** 809–821.

Harris D, Marshal J, Crandall KA. 2001. Squamate relationships based on C-mos nuclear DNA sequences: increased taxon sampling improves bootstrap support. *Amphibia-Reptilia* **22:** 235–242.

Hedges SB, Poling LL. 1999. A molecular phylogeny of reptiles. *Science* **283:** 998–1001.

IUCN. 2006. *IUCN red list of threatened species*. International Union for Conservation of Nature and Natural Resources. Available at http://www.iucn.org

Kluge AG. 1991. Boine snake phylogeny and research cycles. *Miscellaneous Publications of the Museum of Zoology University of Michigan* **178:** 1–58.

Lawson R, Slowinski JB, Crother BI, Burbrink FT. 2005. Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* **37:** 581–601.

Louis EE, Coles ME, Andrintompohavana R, Sommer JA, Engberg SE, Zaonarivelo JR, Mayor MI, Brenneman RA. 2006. Revision of the mouse lemurs (*Microcebus*) of Eastern Madagascar. *International Journal of Primatology* **27:** 347–389.

Lovette IJ, Bermingham E. 2000. c-mos variation in songbirds: molecular evolution, phylogenetic implications, and comparisons with mitochondrial differentiation. *Molecular Biology and Evolution* **17:** 1569–1577.

Manni F, Guerard E, Heyer E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Human Biology* **76:** 173–190.

Mayr E. 1942. *Systematics and the origin of species*. New York, NY: Columbia University Press.

Miller MP. 2005. Alleles in space (AIS): computer software for the joint analysis of interindividual spatial and genetic information. *Journal of Heredity* **96:** 722–724.

Mittermeier RA, Langrand O, Lowry P II, Schatz G, Gerlach J, Goodman SM, Steininger M, Hawkins F, Raminosoa N, Ramilijaona O, Andriamaro O, Randrianasolo H, Rakotobe Z. 2004. Madagascar and the Indian Ocean islands. In: Mittermeier RA, Robles P, Hoffmann M, Pilgrim J, Brooks T, Goettsch M, Lamoreux J, da Fonseca GA, eds. *Hotspots revisited: earth's biologically richest and most endangered terrestrial ecoregions*. Chicago, IL: University of Chicago Press.

Monmonier M. 1973. Maximum-difference barriers: and alternative numerical regionalization method. *Geographical Analysis* **5:** 245–261.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403:** 853–858.

Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences* **76:** 5269–5673.

Noonan BP, Chippindale PT. 2006a. Dispersal and vicariance: the complex evolutionary history of boid snakes. *Molecular Phylogenetics and Evolution* **40:** 347–358.

Noonan BP, Chippindale PT. 2006b. Vicariant origin of Malagasy reptiles supports late cretaceous Antarctic land bridge. *American Naturalist* **168:** 730–741.

Nussbaum RA, Raxworthy CJ. 1994. A new species of *Uroplatus* Duméril (Reptilia: Squamata: Gekkonidae) from southern Madagascar. *Herpetologica* **50:** 319–325.

Nussbaum RA, Raxworthy CJ, Cadle J. 2004. *Aglyptodac-*

tylus madagascariensis *2007 IUCN Red List of Threatened Species*: IUCN 2007.

Nylander JA. 2004. *MrModeltest*. 2nd ed. Uppsala, SWE: Evolutionary Biology Centre, Uppsala University. Program distributed by the author.

Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991. *The simple fool's guide to PCR,* Version 2.0. Honolulu, HI: University of Hawaii.

Pastorini J, Forstner MR, Martin RD. 2000. Relationships among brown lemurs (*Eulemur fulvus*) based on mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **16:** 418–429.

Pastorini J, Forstner MR, Martin RD. 2001. Phylogenetic history of sifakas (*Propithecus*: Lemuriformes) derived from mtDNA sequences. *American Journal of Primatology* **53:** 1–17.

Pastorini J, Thalmann U, Martin RD. 2003. A molecular approach to comparative phylogeography of extant Malagasy lemurs. *Proceedings of the National Academy of Sciences of the United States of America* **100:** 5879–5884.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

de Queiroz A, Lawson R, Lemos-Espinal JA. 2002. Phylogenetic relationships of North American garter snakes (Thamnophis) based on four mitochondrial genes: how much DNA sequence is enough? *Molecular Phylogenetics and Evolution* **22:** 315–329.

Ramanamanjato JB, Mcintyre P, Nussbaum RA. 2002. Reptile, amphibian, and lemur diversity of the Malahelo Forest, a biogeographical transition zone in southeastern Madagascar. *Biodiversity and Conservation* **11:** 1791–1807.

Raxworthy CJ, Nussbaum RA. 1995. Systematics, speciation and biogeography of the dwarf chamaleons (*Brookesia*; Reptilia, Squamata, Chamaeleontidae) of northern Madagascar. *Journal of Zoology (London)* **234:** 525–558.

Raxworthy CJ, Nussbaum RA. 2000. Extinction and extinction vulnerability of amphibians and reptiles in Madagascar. *Amphibian and Reptile Conservation* **2:** 15–23.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19:** 2496–2497.

Sagata M, Oskarsson M, Copeland T, Brumbaugh J, van de Woude G. 1988. Function of c-mos proto-oncogene product in meiotic maturation in *Xenopus* oocytes. *Nature* **335:** 519–525.

Saint KM, Austin CC, Donnellan SC, Hutchinson MN. 1998. C-mos, a nuclear marker useful for squamate phylogenetic analysis. *Molecular Phylogenetics and Evolution* **10:** 259–263.

Sambrook J, Fritsch E, Maniatis T. 1989. *Molecular cloning: a laboratory manual*. New York: Cold Spring Harbor Library Press.

Schmidt HA, Strimmer K, Vingron M, von Haesler A. 2002. TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics* **18:** 502–504.

Swofford D. 2002. *PAUP*. phylogenetic analysis using parsimony* (and other methods) v4.10b*. 4.10b ed. Sunderland, MA: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25:** 4876–4882.

Underwood G. 1976. A systematic analysis of boid snakes. In: d'Abeallairs A, Cox CB, eds. *Morphology and biology of reptiles, Linnean Society symposium series*. London: Academic Press, 151–175.

Vences M, Glaw F. 2003. Phylogeography, systematics and conservation status of boid snakes from Madagascar (*Sanzinia* and *Acrantophis*). *Salamandra* **39:** 181–206.

Vences M, Glaw F, Kosuch J, Böhme W, Veith M. 2001. Phylogeny of South American and Malagasy boinae snakes: molecular evidence for the validity of *Sanzinia* and *Acrantophis* and biogeographic implications. *Copeia* **4:** 1151–1154.

Vences M, Kosuch J, Lotters S, Widmer A, Jungfer KH, Kohler J, Veith M. 2000. Phylogeny and classification of poison frogs (Amphibia: dendrobatidae), based on mitochondrial 16S and 12S ribosomal RNA gene sequences. *Molecular Phylogenetics and Evolution* **15:** 34–40.

Watson D. 1992. *Contouring: a guide to the analysis and display of spatial data*. New York, NY: Pergamon Press.

Wells N. 2003. Some hypothesis on the Mesozoic and Cenozoic paleoenvironmental history of Madagascar. In: Goodman SM, Benstead J, eds. *The natural history of Madagascar*. Chicago, IL: The University of Chicago Press.

Wengler W. 1996. Erfahrungen mit der Haltung und Vermehrung von *Acrantophis dumerili*. *Reptilia* **1:** 46–48.

Wiley EO. 1978. The evolutionary species concept reconsidered. *Systematic Zoology* **27:** 17–26.

Willis R, McAliley L, Neeley E, Densmore L III. 2007. Evidence for placing the false gharial (*Tomistoma schlegelii*) into the family Gavialidae: inferences from nuclear gene sequences. *Molecular Phylogenetics and Evolution* **43:** 787–794.

Wilmé L, Goodman SM, Ganzhorn JU. 2006. Biogeographic evolution of Madagascar's microendemic biota. *Science* **312:** 1063–1065.

Yoder AD, Heckman KL. 2006. Mouse lemur phylogeography revises a model of ecogeography constraint in Madagascar. In: Fleagle J, Lehman S, eds. *Primate biogeography: progress and prospects*. New York, NY: Springer Verlag Inc.

Yoder AD, Olson LE, Hanley C, Heckman KL, Rasoloarison R, Russell AL, Ranivo J, Soarimalala V, Karanth KP, Raselimanana AP, Goodman SM. 2005. A multidimensional approach for detecting species patterns in Malagasy vertebrates. *Proceedings of the National Academy of Sciences of the United States of America* **102** (Suppl. 1)**:** 6587–6594.

Yoder AD, Rasoloarison RM, Goodman SM, Irwin JA, Atsalis S, Ravosa MJ, Ganzhorn JU. 2000. Remarkable species diversity in Malagasy mouse lemurs (Primates, *Microcebus*). *Proceedings of the National Academy of Sciences of the United States of America* **97:** 11325–11330.