



Systematics and biogeography of the Neotropical genus *Mabuya*, with special emphasis on the Amazonian skink *Mabuya nigropunctata* (Reptilia, Scincidae)

A. Miralles^{a,b}, S. Carranza^{c,*}

^a Département d'Ecologie et de Gestion de la Biodiversité, FRE 2696 – Adaptation et évolution des systèmes ostéomusculaires, 55 rue Buffon, Muséum National d'Histoire Naturelle, 75005 Paris, France

^b Laboratoire Populations, Génétique & Evolution, UPR 9034, CNRS, 91198 Gif-sur-Yvette Cedex, France

^c Institute of Evolutionary Biology (CSIC-UPF), Passeig Marítim de la Barceloneta 39-47, 08003 Barcelona, Spain

ARTICLE INFO

Article history:

Received 3 May 2009

Revised 24 September 2009

Accepted 9 October 2009

Available online 27 October 2009

Keywords:

Phylogeny

Biogeography

Mabuya

Diversification

Amazonia

Orinoco

DNA contamination

ABSTRACT

Phylogenetic analyses using up to 1532 base pairs (bp) of mitochondrial DNA from 106 specimens of Neotropical *Mabuya*, including 18 of the 19 recognized South American and Mesoamerican species, indicate that most species of the genus are monophyletic, including *M. nigropunctata* that had previously been reported to be paraphyletic. The present results shows that this species includes three highly divergent and largely allopatric lineages restricted to occidental, meridional, and oriental Amazonia. Our dataset demonstrates that previous claims regarding the paraphyletic status of *M. nigropunctata* and the phylogenetic relationships within this species complex based on the analysis of three mitochondrial and four nuclear genes (approx. 5000 bp) were erroneous and resulted from two contaminated cytochrome *b* sequences.

The phylogenetic results indicate that diversification in the Neotropical genus *Mabuya* started approximately in the Middle Miocene (15.5–13.4 Ma). The divergence dates estimated for the *Mabuya nigropunctata* species complex suggest that the major cladogenetic events that produced the three main groups (occidental (oriental + meridional)) occurred during the Late Miocene. These estimations show that diversification within the *M. nigropunctata* species complex was not triggered by the climatic changes that occurred during the Pleistocene, as has been suggested by several authors. Rather, our data support the hypothesis that the late tertiary (essentially Miocene epoch) was a period that played a very important role in the generation of biological diversity in the Amazonian forests.

Speciation between *Mabuya carvalhoi*, endemic to the coastal mountain range of Venezuela, and *M. croizati*, restricted to the Guiana Shield, occurred during the Middle Miocene and may have been as the result of a vicariant event produced by the formation of the present day Orinoco river drainage basin and the consequent appearance of the Llanos del Orinoco, which acted as a barrier to dispersal between these two species. The split between *M. bistrata* and *M. altamazonica* and between the occidental and (meridional + oriental) clades of *M. nigropunctata* fits very well with the biogeographic split between the eastern and western Amazon basins reported for several other taxa.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

The genus *Mabuya* was one of the largest genera of the family Scincidae, and the only skink genus with a circumtropical distribution (Greer and Broadley, 2000; Greer and Nussbaum, 2000), until it was divided into four units, according to a molecular phylogenetic analysis using partial sequences of the mitochondrial 12S and 16S rRNA genes and the geographic distribution of each unit (Mausfeld et al., 2002). These authors placed the species of the Cape Verde archipelago in the genus *Chioninia*, the Asian species

in the genus *Eutropis*, the African and Malagasy species in *Euprepis* (subsequently *Trachylepis*; see Bauer, 2003), and all the South American, Central American, and Caribbean species, except *Trachylepis atlantica*, which is endemic to Fernando de Noronha, Brazil and the enigmatic *Trachylepis tschudii*, described from the Peruvian Amazonia (see Miralles et al., 2009a), in the nominal genus *Mabuya*. Such a taxonomic splitting is nevertheless controversial, Jesus et al. (2005) and Whiting et al. (2006) considering that the division of *Mabuya* into four genera is premature, since a fifth and still unnamed distinct genetic lineage can be identified. Although the taxonomy of the supergroup *Mabuya* sensu lato is still not totally resolved (see Carranza and Arnold, 2003; Jesus et al., 2005; Whiting et al., 2006; Mausfeld and Schmitz, 2003), the Neotropical genus *Mabuya*, which is the only representative

* Corresponding author. Fax: +34 932309555.

E-mail addresses: miralles.skink@gmail.com (A. Miralles), salvador.carranza@ibe.upf-csic.es (S. Carranza).

of the family Scincidae in South America, is supported in all the molecular phylogenetic analyses carried out to date (Carranza and Arnold, 2003; Mausfeld et al., 2002; Jesus et al., 2005; Whiting et al., 2006; Miralles et al., 2009b) and by several morphological synapomorphies, such as the second (or rarely, the first) supraocular scale in contact with the frontal scale, absence of pterygoid teeth, elevated number of presacral vertebrae (average of 29 or more), and the production of fully formed young from very small eggs that are almost entirely nourished by placentation (Blackburn and Vitt, 1992; Mausfeld et al., 2002; Miralles et al., 2009a). For all these reasons, we consider this clade endemic to the Neotropics sufficiently differentiated from the other lineages, to follow the taxonomy previously proposed by Mausfeld et al. (2002), and to recognize it as a distinct genus, namely *Mabuya* sensu stricto. The ancestor of the genus *Mabuya* arrived to South America from Africa during the Mid-Miocene by means of long-distance transmarine colonization, most probably following the South Equatorial Current. This journey, which involves a transatlantic crossing of more than 3000 km, was repeated on at least one other occasion by the ancestor of *Trachylepis atlantica*, endemic to the island of Fernando de Noronha (Mausfeld et al., 2002; Carranza and Arnold, 2003).

The approximately 26 recognized species of the genus *Mabuya* are widespread across much of the continent, as well as on many offshore islands, and constitute an important component of South American lizard communities (Miralles, 2006). One of the species of *Mabuya* with the largest distribution range is *M. nigropunctata*, which is widespread across the whole Amazonian basin (approximately 7,050,000 km²; Lundberg et al., 1998), the Guiana shield, the Caribbean coast of Venezuela, the western part of the Brazilian shield, the northern part of the Atlantic forest, Trinidad (but not Tobago), and the Grenada and St. Vincent islands (Vanzolini, 1981; Ávila-Pires, 1995; Massary et al., 2001; Miralles et al., 2005; present study). This species was included in a recent phylogenetic analysis by Whiting et al. (2006), together with eight other species of *Mabuya*, for which three mitochondrial and four nuclear genes were sequenced. The authors of that work, however, erroneously referred to *M. nigropunctata* as *M. bistrata* and *M. bistrata* sensu stricto as *M. ficta* (see Miralles et al., 2005 for nomenclatorial explanations). One of the most intriguing results of Whiting et al. (2006) was that the Amazonian species *Mabuya carvalhoi* branched inside *Mabuya nigropunctata* with very high support. This result was totally unexpected as these two species are very distinct from a morphological point of view, each one of them being easily diagnosable from the other, and having their own distinctive characters, allegedly to be derived (Miralles et al., 2009b, see also the result section). Apart from the strange position of *M. carvalhoi*, the phylogenetic tree produced by Whiting et al. (2006) also revealed the presence of multiple independent monophyletic groups within the *M. nigropunctata* complex, thereby highlighting the need for further molecular work and extensive sampling to clarify the phylogeography and taxonomy of the different entities.

Although *M. nigropunctata* is the most widely distributed Amazonian species of the genus *Mabuya*, five other such species live in this region (Ávila-Pires, 1995; Miralles et al., 2006, 2009a). The phylogenetic relationships and the timing of diversification between these species can be used to check if cladogenesis in this group coincided with a burst of diversification in other studied Amazonian (or “Peri-amazonian”) animal groups including amphibians (Chek et al., 2001; Symula et al., 2003; Noonan and Wray, 2006), reptiles (Glor et al., 2001; Gamble et al., 2008; Zamudio and Green, 1997; Vidal et al., 2005; Wüster et al., 2005; Quijada-Mascareñas et al., 2007), mammals (Patton and Da Silva, 1997; Da Silva and Patton, 1998; Patton and Pires Costa, 2003; Steiner and Catzeflis, 2004), birds (Cracraft and Prum, 1988; Aleixo, 2004; Pereira and Baker, 2004; Ribas et al., 2006), insects (Hall and Harvey, 2002), or mollusks (Wesselingh and Salo, 2006).

The Oligocene and early Miocene periods were dominated by dramatic climatic change and Andean orogeny, therefore, it has been suggested that these factors might have played an important role in the origin of diversity found in tropical rainforests (Gamble et al., 2008). For instance, a very common pattern observed in Amazonian taxa is a split between lineages that correspond to the eastern and western Amazon basin, although it has been shown that this split might not be the result of a common vicariant event in all taxa (Gamble et al., 2008). Reptiles and amphibians are excellent model organisms for the investigation of historical patterns in Amazonia because they present low dispersal capabilities in comparison to other vertebrate groups such as birds and mammals, are relatively abundant and easy to capture, and seem to be affected by both geographic and climatic events (Gamble et al., 2008; Graham et al., 2006).

In the present work, we have assembled a new molecular data set that includes most species of Neotropical *Mabuya* in order to further investigate the phylogenetic position of *M. carvalhoi*, the phylogenetic relationships of the *M. nigropunctata* species complex, and to unravel the geographic and climatic factors that produced cladogenesis in the Amazonian *Mabuya* species and to see if this pattern agrees with the findings for other Amazonian taxa.

2. Materials and methods

2.1. Specimens

2.1.1. Morphological study

Three-hundred and twenty-five specimens of the genus *Mabuya* were examined morphologically (see Appendix I). Of these, 220 correspond to specimens belonging to the *Mabuya nigropunctata* species complex. We included the maximum number of specimens in our morphological comparisons in order to (1) emphasize the remarkable morphological divergence existing between *M. carvalhoi* and *M. nigropunctata*, and (2) determine if the different lineages of the *M. nigropunctata* complex had diverged sufficiently to be morphologically diagnosable. Specimens examined for the present study (all preserved in 70% ethanol, most of them having been fixed in formalin) are deposited at the American Museum of Natural History, New York (AMNH), Coleção Herpetológica da Universidade de Brasília (CHUNB), Carnegie Museum, Pittsburgh (CM), Field Museum of Natural History, Chicago (FMNH), Los Angeles County Museum, Los Angeles (LACM), Museum of Comparative Zoology, Cambridge (MCZ), Museo de Historia Natural La Salle, Caracas (MHNLS), Museum National d’Histoire Naturelle, Paris (MNHN), Museu Paraense Emilio Goeldi, Belém (MPEG), Museu de Zoologia, Universidade de São Paulo (MZUSP), Sam Noble Oklahoma Museum of Natural History, Norman (OMNH), Nationaal Natuurhistorisch Museum Naturalis, Leiden (RMNH), University of Michigan Museum of Zoology, Ann Arbor (UMMZ).

Characters examined here are routinely used in taxonomy of Scincidae, such as scale counts, presence or absence of homologous scale fusions or the variability in color patterns. Scale nomenclature, scale counts, and measurements used in the morphological analyses follow Ávila-Pires (1995) and Miralles (2006). Despite the fact that some of these characters might be hypothesized as putative apomorphies supporting the monophyly of both *M. carvalhoi* and *M. nigropunctata*, we did not have enough characters to perform a phylogenetic analysis using morphological data.

2.1.2. Molecular study

A total of 106 specimens were included in the present study. Of these 103 were representatives of the Neotropical genus *Mabuya* and included 18 members of the 19 South American and Mesoamerican species and 2 out of the 7 Caribbean species recognized

to date (Miralles, 2006; Miralles et al., 2009b). Information on the locality, voucher availability and GenBank accession numbers for all the sequences used is shown in Table 1.

As a result of the wide distribution range of *M. nigropunctata*, 45 specimens from 25 widespread localities were included in the phylogenetic analyses. Of these, sequences were newly produced for 22 specimens, sequences for five specimens were obtained from Carranza and Arnold (2003), Vrcibradic et al. (2006), Miralles et al. (2006, 2009a,b) and sequences for the remaining 18 specimens were from Whiting et al. (2006). Additionally, 58 individuals including representative of the remaining 19 Neotropical species were added in order to test the monophyletic status of *M. nigropunctata* and to infer the diversification patterns of the Amazonian species. Of these, two sequences for a specimen of *M. altamazonica* were new.

Eumeces egregius, *Trachylepis quinquetaeniata* and “*Mabuya*” vittata were used as outgroups. The latter two species were chosen based on previous phylogenetic studies of the genus *Mabuya* sensu lato, which indicated that “*Mabuya*” vittata (a Mediterranean species) and *Trachylepis quinquetaeniata* were closely related to the Neotropical *Mabuya* (Mausfeld et al., 2002; Carranza and Arnold, 2003; Whiting et al., 2006).

2.2. Molecular analyses

2.2.1. DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from 95% ethanol-preserved tissues (muscles, skin or liver) using a CTAB protocol (Winnepenninckx et al., 1993). The primers 12SA-L (5'-AAA CTG GGA TTA GAT ACC CCA CTA T-3') and 12SB-H (5'-GAG GGT GAC GGG CGG TGT GT-3') of Kocher et al. (1989) were used to amplify approximately 380 base pairs (bp) of the mitochondrial 12S rRNA gene, with the following PCR cycling procedure: 94 °C (3:00); 94 °C (0:30), 58 °C (0:40), 72 °C (0:50) for 30 cycles; 72 °C (1:00). The complete mitochondrial cytochrome *b* (*cytb*) gene (approximately 1150 bp) was amplified in two fragments, using (1) the primers L15146 (5'-CAT GAG GAC AAA TAT CAT TCT GAG-3') and H15915sh (5'-TTC ATC TCT CCG GTT TAC AAG AC-3') of Irwin et al. (1991) [(94 °C (3:00); 94 °C (0:40), 53 °C (0:30), 72 °C (1:00) for 33 cycles; 72 °C (1:00)], and (2) a pair of primers specifically designed for this work, MAB1 (5'-AGA ACC ACC GTT GTA TTC AAC TAC-3') and MAB2 (5'-GRG TYA RGG TTG CRT TGT CTA CTG-3') [(94 °C (3:00); 94 °C (0:30), 55 °C (0:40), 72 °C (0:50) for 30 cycles; 72 °C (1:00)]. The successful PCR products were purified and sequenced both strands with an automated DNA sequencer (CEQ 2000 DNA Analysis System, Beckman Coulter Inc.).

2.2.2. Phylogenetic analyses

DNA sequences were aligned using ClustalX (Thompson et al., 1997) with default parameters (gap opening = 10; gap extension = 0.2). For the mitochondrial cytochrome *b* sequences no gaps were postulated. These sequences were translated into amino acids using the vertebrate mitochondrial code and no stop codons were observed, suggesting that were probably all functional. Although some gaps were postulated in order to resolve length differences in the 12S rRNA gene fragment, all positions could be unambiguously aligned and were, therefore, included in the analyses.

Phylogenetic analyses were carried out using maximum-likelihood (ML) and Bayesian methods. The computer program JModel-Test (Posada, 2008) was used to select the most appropriate model of sequence evolution using the Akaike information criterion. This was the General Time Reversible (GTR) model, taking into account the shape of the gamma distribution (G) and the number of invariant sites (I) for the data set containing the *cytb* + 12S rRNA genes and also for the two independent partitions (*cytb* and 12S). ML

analyses were performed with PHYML v. 2.4.4 (Guindon and Gascuel, 2003) with model parameters fitted to the data by likelihood maximization. Reliability of the ML trees was assessed by bootstrap analysis (Felsenstein, 1985), involving 1000 replications.

Bayesian analyses were performed on MRBAYES v. 3.1.2 (Huelsenbeck and Ronquist, 2001). For the combined analysis each partition had its own model of sequence evolution and model parameters (see above). Four incrementally heated Markov chains with the default heating values were used. All analyses started with randomly generated trees and ran for 2.5×10^6 generations in two independent runs with samplings at intervals of 100 generations that produced 25,000 trees. After verifying that stationarity had been reached, both in terms of likelihood scores and parameters estimation, the first 6200 trees were discarded in both independent runs and the combined analyses and a majority rule consensus tree was generated from the remaining 18,800 (post-burnin) trees. The frequency of any particular clade among the individual trees contributing to the consensus tree represents the posterior probability of that clade (Huelsenbeck and Ronquist, 2001).

Topological incongruence among partitions was tested using the incongruence length difference (ILD) test (Michkevich and Farris, 1981; Farris et al., 1994). In this test, 10,000 heuristic searches were carried out after removing all invariable characters from the data set (Cunningham, 1997). To test for incongruence among data sets, we also used a reciprocal 70% bootstrap proportion or a 95% posterior probability threshold (Mason-Gamer and Kellogg, 1996). Topological conflicts were considered significant if two different relationships for the same set of taxa where both supported.

2.3. Estimation of divergence times

Unfortunately, there are no internal calibration points available for the Neotropical genus *Mabuya* or for any of the other three genera of the supergroup *Mabuya* sensu lato (*Chioninia*, *Eutropis*, or *Trachylepis*). As a result, and in order to have an idea of the approximate time of the different cladogenetic events of our phylogeny, we had to apply the substitution rates calculated for other lizard groups using exactly the same mitochondrial region as in the present work. These rates range from 1.15% per lineage per million years in the geckos of the genus *Hemidactylus* (Arnold et al., 2008) to 1.35% per lineage per million years in the lacertid lizards of the tribe Lacertini (Carranza et al., 2004; Arnold et al., 2007) and the skinks of the genus *Chalcides*, *Scincus*, and *Eumeces* (Carranza et al., 2008).

The evolutionary rates calculated previously were applied to a linearized tree using the nonparametric rate smoothing (NPRS) algorithm implemented in r8s v1.6.4 (Sanderson, 1997, 2002) with the ML tree estimated from the concatenated data set (*cytb* + 12S) and the GTR + I + G model of sequence evolution (reference tree), assigning an arbitrary value of 1 to the root node. This transformed the reference tree into a linearized tree with arbitrary scale. To re-establish the genetic distance scale we calculated the *K* scaling factor that approximates the linearized tree to the reference tree as much as possible, using the method developed by Soria-Carrasco et al. (2007) and implemented in the computer program Ktreedist (available at <http://molevol.cmima.csic.es/castresana/Ktreedist.html>). In our case, *K* = 0.357. Upon scaling the NPRS tree with an arbitrary scale with this factor, we obtained a linearized tree with the most appropriate genetic distance scale (NPRS tree with genetic distance scale). The calculated evolutionary rates for other lizard groups (1.15% and 1.35% per million years) were applied to the NPRS tree with genetic distance scale using TreeEdit v 1.0 (available at: <http://tree.bio.ed.ac.uk/software/treededit>).

Table 1
List of specimens, collection and accession numbers of the sequences with their references, and localities. Genbank accession numbers of new sequences obtained for this study are in bold; all the rest are from Kumazawa and Nishida (1999), Honda et al. (2000), Mausfeld and Lötters (2001), Carranza and Arnold (2003), Vrcibradic et al. (2006), Whiting et al. (2006), Miralles et al. (2009b); Dashes represent missing data.

Species	Country	No voucher/sample (ID in Whiting et al., 2005)	Locality	Cytb	12S	
<i>Ingroup:</i>						
<i>M. agilis</i>	Br., Bahia	LG 464	Jacobina	DQ239175	DQ239256	
	Br., Espírito Santo	MRT 1206	UHE Rosal	DQ239152	DQ239233	
	Br., Pernambuco	E11108 ^a	Exu	EU443102	AY151428	
	Br., Piauí	SC 21	Serra das Confusões	DQ239170	DQ239251	
	Br., Tocantins	MRT 3951	Peixe	DQ239126	DQ239207	
<i>M. agmosticha</i>	Br., Alagoas	LG 902	Xingó (a)	DQ239134	DQ239215	
	Br., Alagoas	LG 901	Xingó (b)	DQ239133	DQ239214	
<i>M. altamazonica</i>	Br., Acre	MBS 001 (=ficta-AC)	Estirão do Painha, PNSD	DQ239129	DQ239210	
	Br., Amazonas	OMNH 37191 (LSUMZ h14114)	Rio Ituxí, Madeirera Scheffer	GQ982545	GQ982525	
	Pe, San Martín	MNHN 2006.0291 ^b	km 34 of the road « Tarapoto-Yurimaguas » (a)	EU443103	DQ368663	
<i>M. bistriata</i>	Pe, San Martín	MHNC 6703	PN. Rio Abiseo (07°21' 44.9"S; 76°50' 13.8"W) (b)	EU515212	EU515210	
	Br., Amazonas	OMNH 37183 (LSUMZ h14104 = ficta-AM)	Rio Ituxi, Madeira Scheffer (8°20'47,0"S; 65°42'57,9"W)	EU443104	EU477258	
<i>M. carvalhoi</i>	Fr. Guiana	Not collected	Matoury	EU443105	DQ368664	
	Br., Roraima	OMNH 36332 (LSUMZ H-12420)	jonction BR-174 / BR-210	EU443106	EU477259	
<i>M. cochabambae</i>	Bo., Santa Cruz	ZFMK 72151	Vicinity of Pampagrande (18°05'S ; 64°06'W)	–	AF202625	
<i>M. croizati</i>	Ve., Antzoátegui	MNHN 17670	Cerro El Guamal, Turimiquire massif	EU443107	EU477260	
<i>M. dorsivittata</i>	Br., D. F.	(E 11106)	Brasília	EU443108	AY151426	
	Br., São Paulo	(LG 1089)	São Paulo	DQ239176	DQ239257	
<i>M. falconensis</i>	Ar., Cordoba	(LAV-5000)	Rio Cuarto city	DQ239149	DQ239230	
	Ve., Falcón	MHNLS 17095	Península de Paraguaná (a)	EU443109	EU477261	
	Ve., Falcón	not collected	Península de Paraguaná (b)	EU443110	EU477262	
<i>M. frenata</i>	Trin. & Tobago	ZFMK 62603	Tobago island	–	AY070339	
	Br., Goiás	LG 861	Santa Rita do Araguaia	DQ239128	DQ239209	
	Br., M. G. do Sul	E 11107	?	EU443111	AY151427	
	Br., Piauí	SC 28	Serra das Confusões	DQ239173	DQ239254	
<i>M. guaporicola</i>	Br., Tocantins	PNA 77	Parque Nacional do Araguaia	DQ239165	DQ239246	
	Br., M. G. do Sul	E 11101 ^a	?	EU443112	AY151434	
	Br., Mato Grosso	LG 1574	UHE Manso	DQ239250	DQ239169	
	Br., Tocantins	PNA 185	Parque Nacional do Araguaia	DQ239141	DQ239222	
	Bo., Beni	UTA 55700 (MBH 5870)	El Refugio	EU443113	EU477263	
<i>M. mabouya</i>	Lesser Antilles	MNHN 2003.0838	Dominica island (a)	EU443114	EU477264	
	Lesser Antilles	Not collected	Dominica island (b)	EU443115	EU477265	
<i>M. macrorhyncha</i>	Br., Sao Paulo	LG 1102	Ilha da Queimada Grande (a)	DQ239162	DQ239243	
		LG 1103	Ilha da Queimada Grande (b)	DQ239132	DQ239213	
<i>M. meridensis</i>	Ve., Mérida	Not collected	Mérida (a)	EU443116	EU477266	
		MHNLS 17081	Mont Zerpa, near Mérida (b)	EU443117	EU477267	
<i>M. nebulosylvestris</i>	Ve., Aragua	MHNLS 17088	Colonia Tovar (a)	EU443134	EU477280	
		MNHN 2007.0272	Colonia Tovar (b)	EU443135	EU477281	
	Ve., Lara	MHNLS 17106	Cubiro (9°48'29.0"N; 69°33'25.7"W)	EU443136	EU477282	
	Ve., Miranda	MHNLS 17330	Carrizal, Los Teques	EU443137	EU477283	
	Ve., Trujillo	Not collected	Trujillo (a)	EU443138	EU477284	
		MHNLS 16649	Trujillo, near the Laguna Negra (b)	EU443139	EU477285	
	Ve., Vargas	MHNLS 17093 ^b	Pico Codazzi (a)	EU443140	EU477286	
		MHNLS 17103	Pico Codazzi (b)	EU443141	EU477287	
	<i>M. nigropalmata</i>	Pe, Madre de Dios	MHNC 5718	Manu national park	EU515213	EU515211
	<i>M. nigropunctata</i>	Br., Acre	(LSUMZ H-13610) (= bistriata AC1)	5 km N. Porto Walter, inland from the Rio Juruá (8°15'31.2"S ; 72°46'37"W) (a)	DQ239188	DQ239269
Br., Acre		(LSUMZ H-13900) (=bistriata AC2)	5 km N. Porto Walter, inland from the Rio Juruá (8°15'31.2"S ; 72°46'37"W) (b)	DQ239109	DQ239290	
<i>M. nigropunctata</i>	Br., Amapá	MRT 6300 (= bistriata AP1)	Igarapé Camaipi (a)	DQ239130	DQ239211	
	Br., Amapá	MRT 6303 (= bistriata AP2)	Igarapé Camaipi (b)	DQ239131	DQ239213	
	Br., Amazonas	(LSUMZ H-16446)	Castanho, S. Manaus (03°30,9'S ; 59°54,2'W) (a)	GQ982546	GQ982526	
	Br., Amazonas	OMNH 37687 (LSUMZ h16441)	Castanho, S. Manaus (03°30,9'S ; 59°54,2'W) (b)	GQ982547	GQ982527	
	Br., Amazonas	(LSUMZ h16489) (= bistriata AM1)	Castanho, S. Manaus (03°30,9'S ; 59°54,2'W) (d)	DQ239111	DQ239192	
	Br., Amazonas	LSUMZ h16490 (= bistriata AM2)	Castanho, S. Manaus (03°30,9'S ; 59°54,2'W) (e)	DQ239112	DQ239193	
	Br., Amazonas	OMNH 37186 (LSUMZ h14107)	Rio Ituxi, Madeira Scheffer (8°20'47,0"S; 65°42'57,9"W) (c)	GQ982548	GQ982528	
	Br., DF	CHUNB 9624	Brasília	–	AF548783	
	Br., Ceara	MRT 154 (= bistriata CE1)	Mulungú (a)	DQ239159	DQ239240	
	Br., Ceara	MRT 097 (= bistriata CE2)	Pacoti (b)	DQ239157	DQ239238	
	Br., Goiás	LG 1085 (= bistriata GO)	Niquelandia	DQ239168	DQ239249	
	Br., Mato Grosso	967956 (=bistriata MT1)	Aripuanã (a)	DQ239174	DQ239255	
	Br., Mato Grosso	967904 (=bistriata MT2)	Aripuanã (b)	DQ239180	DQ239261	
	Br., Mato Grosso	LG 1558 (=bistriata MT3)	UHE Manso (c)	DQ239171	DQ239252	
	Br., Mato Grosso	LG 1561 (=bistriata MT4)	UHE Manso (d)	DQ239172	DQ239253	
	Br., Pará	(LSUMZ H-14223)	Agropecuaria Treviso LTDA (3°8'56,7"S ; 54°50'26,8"W) (a)	EU443118	DQ368667	
	Braz., Pará	OMNH 36830 (LSUMZ H-14238)	Agropecuaria Treviso LTDA (3°8'56,7"S ; 54°50'26,8"W) (b)	GQ982549	GQ982529	
	Br., Pará	MRT 916872 (=bistriata PA1)	Alter do Chão (c)	DQ239177	DQ239258	
	Br., Pará	LG 756 (=bistriata PA2)	Vai-Quem-Quer (d)	DQ239158	DQ239239	
	Br., Piauí	MRT 2502 (=bistriata PI)	Uruçuí-uma	DQ239167	DQ239248	
Br., Rondônia	OMNH 37414 (LSUMZ h17860)	Parque Estadual Guajara-Mirim (10°19'S ; 64°33'W) (a)	GQ982550	GQ982530		

Table 1 (continued)

Species	Country	No voucher/sample (ID in Whiting et al., 2005)	Locality	Cytb	12S
	Br., Rondônia	OMNH 37417 (LSUMZ h17865) (= bistriata RO2)	Parque Estadual Guajara-Mirim (10°19'S; 64°33'W) (b)	GQ982551	GQ982531
	Br., Rondônia	OMNH 37416 (LSUMZ h17863)	Parque Estadual Guajara-Mirim (10°19'S; 64°33'W) (c)	GQ982552	GQ982532
	Br., Rondônia	OMNH 37413 (LSUMZ h17859)	Parque Estadual Guajara-Mirim (10°19'S; 64°33'W) (d)	GQ982553	GQ982533
	Br., Rondônia	LSUMZ h17864 (= bistriata RO1)	Parque Estadual Guajara-Mirim (10°19'S; 64°33'W) (e)	DQ239113	DQ239194
	Br., Roraima	OMNH 36316 (LSUMZ h12332)	Fazenda Nova Esperanca (BR-210, 44 km W BR-174) (a)	GQ982554	GQ982534
	Br., Roraima	OMNH 36318 (LSUMZ h12369) (= bistriata RR1)	Fazenda Nova Esperanca (BR-210, 41 km W BR-174) (b)	EU443119	DQ368668
	Br., Roraima	OMNH 36317 (LSUMZ h12365)	Fazenda Nova Esperanca (BR-210, 44 km W BR-174) (c)	GQ982555	GQ982535
	Br., Roraima	LSUMZ h12311 (= bistriata RR2)	Fazenda Nova Esperanca (BR-210, 41 km W BR-174) (d)	DQ239187	DQ239268
	Co., Guainia	E111016	Puerto Inirida	EU443120	AY151438
	Guyana	BPN 160	(5°37,8'N; 60°14,7'W)	GQ982556	GQ982536
	Fr. Guiana	not collected	Mitaraka (a)	GQ982557	GQ982537
	Fr. Guiana	not collected	Mitaraka (b)	GQ982558	GQ982538
	Fr. Guiana	MNHN 2005-9719	St Eugène (04,8500°N; 53,0613°W) (c)	GQ982559	DQ368666
	Fr. Guiana	MNHN 2005.9721	St Eugène (04,8500°N; 53,0613°W) (d)	GQ982560	GQ982539
	Fr. Guiana	MNHN 2005.9717	St Eugène (04,8500°N; 53,0613°W) (e)	GQ982561	GQ982540
	Fr. Guiana	MNHN 2005.9720	St Eugène (04,8500°N; 53,0613°W) (f)	GQ982562	GQ982541
	Fr. Guiana	Michel Blanc	Summit of the Pic Coudreau (g)	GQ982563	GQ982542
	Fr. Guiana	MNHN 2004.0103	Foot of the Pic Coudreau (h)	GQ982564	GQ982543
	Trin. & Tobago	E 11103	Trinidad, Talparo (a) ^a	GQ982565	AY151436
	Trin. & Tobago	E 11104	Trinidad, Talparo (b) ^a	GQ982566	AY151437
	Ve., Aragua	MHNLS 17080	Turiamo	EU443121	EU477268
	Ve., Sucre	WES 636	Península de Paria.	GQ982567	GQ982544
<i>M. sloanii</i>	Lesser Antilles	MNHN 2003.0844	St Barthélémy island (a)	EU443122	EU477269
	Lesser Antilles	MNHN 2003.0843	St Barthélémy island (b)	EU443123	—
	Lesser Antilles	YPM 15082	British Virgin Islands, Guana Island	EU443124	EU477270
<i>M. unimarginata</i>	Costa Rica	not collected	Tortugueros	EU443125	EU477271
	Guat., Zacapa	UTA 41513	Zacapa	EU443126	EU477272
	Ho., I. de la Bahía	SMF 79851	Isla de Útila	—	AB057378
	Ho., Olancho	UTA 41227	Las Trojas, San Esteban	EU443127	EU477273
	Mex., Guerrero	Not collected	Chichihualco (17°39'32"N; 99°42'07"W)	EU443128	EU477274
	Mex., Oaxaca	Not collected	On road « El Camaron-Tehuantepec »	EU443129	EU477275
<i>M. zuliae</i>	Ve., Zulia	MHNLS 16676	Cerro el Mirador (08°36'45"; T2°31'34"W) (a)	EU443130	EU477276
	Ve., Zulia	MHNLS 16677	Cerro el Mirador (08°36'45"; 72°31'34"W) (b)	EU443131	EU477277
	Ve., Zulia	MHNLS 16647 ^b	Rio Escalante, Secteur El Cañon, Catatumbo (c)	EU443132	EU477278
	Ve., Zulia	MNHN 2007.0273	La Orchila, S. de Perijá (10°48'44"N; 72°21'13"W) (d) [*]	EU443133	EU477279
Outgroup:					
<i>Eumeces egregius</i>	North America	MVZ 150128	Florida	AB016606 ^d	
<i>Trachylepis quinquetaeniata</i> ^c	Africa	MNHN 2004-0102/BEV 7202	Unkown locality and Egypte, Assouan	EU443143	—
				—	EU477289
<i>"Mabuya" vittata</i>	Middle East	BEV 1446	Turkey, Osmandere	EU443142	U477288

Ar.: Argentina, Bo.: Bolivia, Br.: Brazil, Co. Colombia, Guat.: Guatemala, Ho.: Honduras, Mex.: Mexico, Pe.: Peru, Trin.: Trinidad, Ve.: Venezuela.

^a Four samples sequenced by Carranza and Arnold (2003) have been here reidentified: *Mabuya agilis* (synonymous of *M. haethi*, E 11108); *M. guaporicola* (instead of *M. agilis*, E11101); *M. nigropunctata* (instead of *M. bistriata*, E11103-04).

^b Holotype specimens.

^c Composite samples, based on Cytb and 12S sequences from two different specimens.

^d Complete mitochondrial genome (Kumazawa and Nishida, 1999).

3. Results

3.1. Molecular results

A preliminary analysis including all the sequences of the genus *Mabuya* from Whiting et al. (2006) revealed that two of their sequences were the product of contaminations; they were therefore excluded from the present data set. A detailed analysis of these contaminations is presented in Table 2. The conclusions are that the cytochrome *b* sequence DQ239186 allegedly corresponding to sample LSUMZ H-12420, from specimen OMNH 36332 of *Mabuya carvalhoi*, was in fact a contamination from specimen LSUMZ H-13610 of *M. nigropunctata* from Acre state, Brazil (the cytochrome *b* sequences of these two specimens are identical while the 12S sequences differ by 6.41%; see Table 2). In order to obtain the correct

cytochrome *b* sequence of *M. carvalhoi* and to further proof that it was a contamination, we resequenced exactly the same sample as Whiting et al. (2006) (LSUMZ H-12420) for both mitochondrial genes (cytochrome *b* and 12S). As shown in Table 2, our cytochrome *b* sequence of sample LSUMZ H-12420 differed from Whiting et al. (2006) cytochrome *b* sequence by 15.77%, while the two 12S sequences independently obtained from the same specimen were identical.

In the second contamination of Whiting et al. (2006), the cytochrome *b* sequence DQ239110, allegedly corresponding to sample LSUMZ H-14358 from a specimen of *M. nigropunctata* from Pará State, Brazil, was in fact a contamination from specimen LSUMZ H-13900 of *M. nigropunctata* from Acre State, Brazil. In Table 2 it is shown that both specimens were identical in the cytochrome *b* gene while they differed by 5.61% in the 12S. Moreover, two

Table 2

Two contaminations have been detected in the data-set published by Whiting et al. (2006) (uncorrected *p*-distances calculated on the basis of 12S rRNA and cytochrome *b*): (A) the alleged cytochrome *b* of the single sample of *M. carvalhoi* (LSUMZ H-12420), which is in fact a sequence of a *Mabuya nigropunctata* from the Acre state of Brazil (LSUMZ H-13610); and (B) the alleged cytochrome *b* of a specimen of *Mabuya nigropunctata* from the Pará state (LSUMZ H-14358), which was contaminated by an other specimen from Acre state (LSUMZ H-13900).

		<i>M. carvalhoi</i> Roraima state LSUMZ H-12420 (resequenced in the present study)		<i>M. nigropunctata</i> Acre state LSUMZ H-13610 (Whiting et al.)		<i>M. nigropunctata</i> Acre state LSUMZ H-13900 (Whiting et al.)	
A							
<i>M. carvalhoi</i> Roraima state LSUMZ H-12420 (Whiting et al.)	cytb 12S	15.77% Identical		Identical 6.41%		0.28% 6.41%	
		<i>M. nigropunctata</i> Pará state LSUMZ H-14223 (present study)	<i>M. nigropunctata</i> Pará state LSUMZ H-14238 (present study)	<i>M. nigropunctata</i> Acre state LSUMZ H-13610 (Whiting et al.)		<i>M. nigropunctata</i> Acre state LSUMZ H-13900 (Whiting et al.)	
B							
<i>M. nigropunctata</i> Pará state LSUMZ H-14358 (Whiting et al.)	cytb 12S	10.00% Identical	10.70% Identical	0.28% 5.61%		Identical 5.61%	

individuals of *M. nigropunctata* from Pará state were newly sequenced for this study (LSUMZ H-14223 and LSUMZ H-14238) and, as shown in Table 2, they differed from LSUMZ H-14358 by 10 and 10.7% in the cytochrome *b* gene, while were identical to this specimen in the 12S.

These two contaminations perfectly explain the strange position of both *M. carvalhoi* (branching inside *M. nigropunctata*) and *M. nigropunctata* (LSUMZ H-14358) from Pará State, Brazil (branching on a different clade than the other samples from the same locality) in the phylogenetic tree of Whiting et al. (2006).

After eliminating these two sequences, the aligned data set contained 106 specimens and 1532 bp, 1154 of which corresponded to the *cytb* gene (563 variable and 484 parsimony-informative) and 378 to the 12S rRNA (149 variable and 112 parsimony-informative). The results of the phylogenetic analyses are summarized in Fig. 1. Both ML and Bayesian analyses gave very similar results and show that *M. carvalhoi* and *M. croizati* form a clade that is basal to all the remaining species of *Mabuya* included in the analysis. All 20 species of *Mabuya* analyzed are monophyletic with high bootstrap and posterior probability support, with the exceptions of *M. dorsivittata* and *M. cochabambae*, which are part of the same clade and do not form reciprocally monophyletic units. The phylogenetic relationships between the different species of *Mabuya* included in the analysis are not very well resolved, which suggests that speciation may have occurred relatively fast. The monophyly of the *M. nigropunctata* species complex is very well supported. Relationships within this species complex are very well resolved and show three clades with high bootstrap and posterior probability support that present allopatric geographic ranges with few overlapping localities (Fig. 2A). These clades are: (1) the occidental clade, composed by western Amazonian samples, ranging from the Venezuelan coast and Trinidad island to the Acre states in Brazil; (2) the oriental clade, composed by eastern Guyano-amazonian samples widespread from the Guyanan shield to the Brazilian shield; and (3) the meridional clade, restricted to the southern peripheral part of Amazonas, from the Rondônia to the Goiás states in Brazil. According to the phylogenetic tree shown in Fig. 1, the occidental clade is basal to the other two clades. The phylogenetic relationship between the oriental and meridional clades is supported by a bootstrap value of 75% and a posterior probability of 99%. Genetic divergence (uncorrected *p*-distances) values both within and between the occidental, meridional, and oriental clades are shown in Table 3. Divergence values within clades ranged between 4.21–7.81% for the *cytb* gene and 1.32–2.06% for the 12S rRNA, whereas divergence values between clades ranged between 10.28–11.62% for the *cytb* gene and 3.21–4.69% for the 12S rRNA.

3.2. Morphological results

Mabuya carvalhoi and *M. nigropunctata* are two very distinct species both being easily and reliably diagnosable from a morphological point of view: *Mabuya carvalhoi* is smaller than *M. nigropunctata* and it has a more slender head, a very different color pattern, with two well-contrasted dark stripes on the back (which are absent in *M. nigropunctata*), light colored palms and soles (dark in *M. nigropunctata*), a bright blue tail (nearly always grey/black in *M. nigropunctata*), and a very characteristic cephalic scalation, which differs from *M. nigropunctata* in the number and disposition of the prefrontals, frontoparietals, and nuchal scales (Fig. 3, Table 4; see also Rebouças-Spieker and Vanzolini, 1990; Ávila-Pires, 1995; Miralles, 2006; Miralles et al., 2005, 2009a). Two of these characters are highly probably autapomorphies supporting the monophyly of each one of these two species. Indeed, the fusion of the prefrontals is exclusively associated to *M. carvalhoi*, whereas the interparietal scale separating the two parietals is exclusively associated to *M. nigropunctata*, none of these characteristics being present in the other species of the genus *Mabuya*.

Additionally, some specimens of *M. nigropunctata* from the Guajará-Mirim park (Rio Formoso, Parque Estadual Guajará-Mirim, approx. 90 km North of Nova Mamoré [= 10°19'17.2"S ; 64°33'47.94"W], Rondônia state, Brasil) have been accurately examined, as the phylogenetic tree from Fig. 1 showed that two distinct lineages live in sympatry in this locality (specimens Br-Rondônia A and D belong to the oriental clade and specimens Br-Rondônia B, E and C to the meridional clade; see Fig. 1). Two phenotypes (I and II) have been distinguished on the basis of both scalation and coloration characters (all were collected by L.J. Vitt, between February and April, 1998). Characteristics allowing their distinction are presented on Table 5 for each examined specimen, with information on their sex and their phylogenetic placement (when molecular data was available). These results suggest that morphological differences between the two clades (oriental and meridional) exist in this contact zone and that these are not the result of sexual dimorphism.

4. Discussion

The present study indicates that the phylogenetic relationships between Neotropical *Mabuya* published by Whiting et al. (2006) were incorrect as a result of contamination problems. Although these contaminations only affected two *cytb* sequences from two specimens, their overall effect on the phylogeny was dramatic

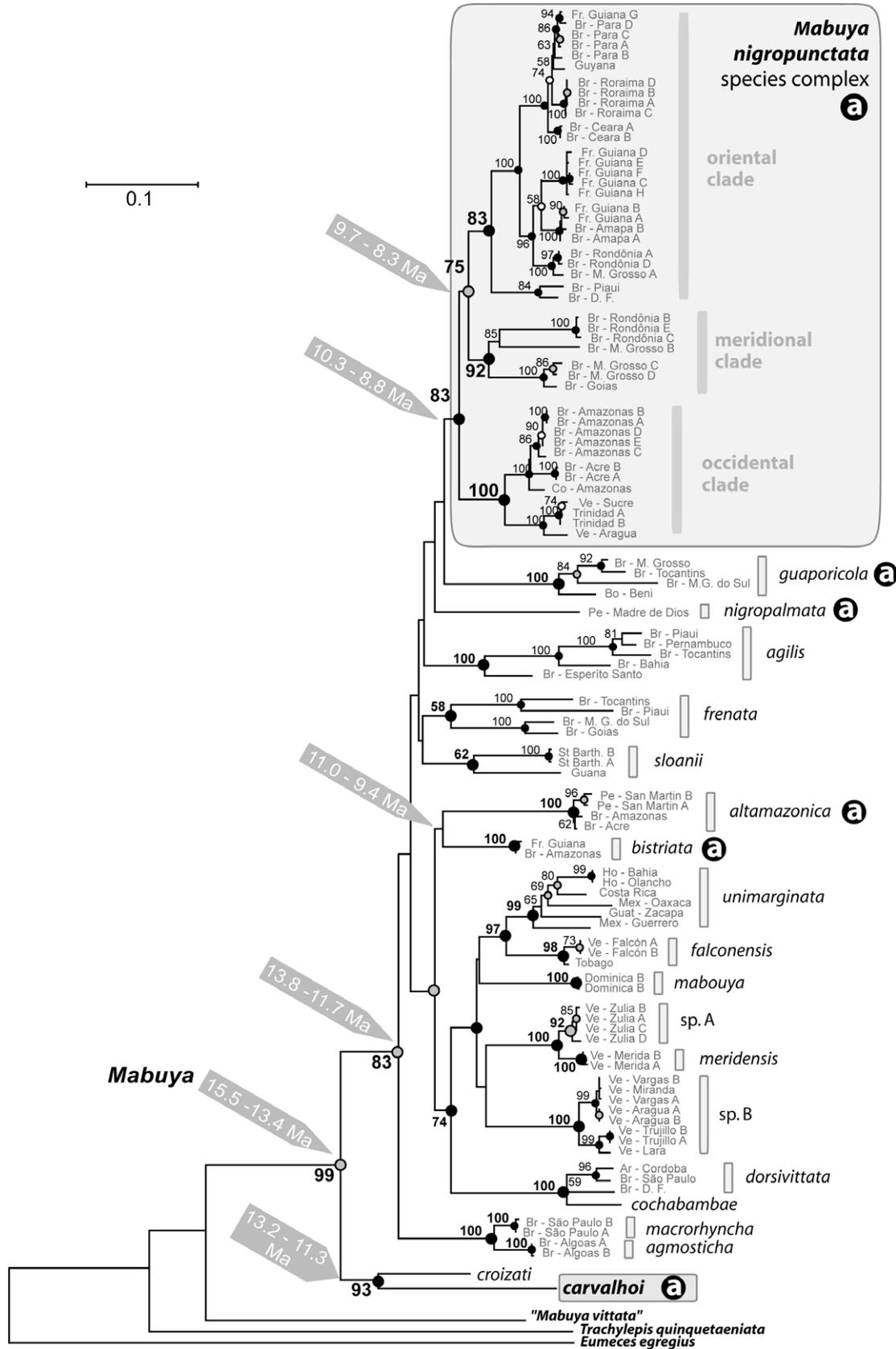


Fig. 1. Maximum Likelihood phylogenetic tree of the Neotropical genus *Mabuya* inferred from 12S and *cytb* sequences. Bootstrap values above 70% are indicated by the nodes. Nodes with a posterior probability support above 0.90, are highlighted with black (posterior probability = 1), grey (0.95 < posterior probability < 0.99) or white circles (0.90 < posterior probability < 0.94). Amazonian species are highlighted with an “a”. Grey arrows indicate the estimated time of divergence for some relevant nodes (in million of years).

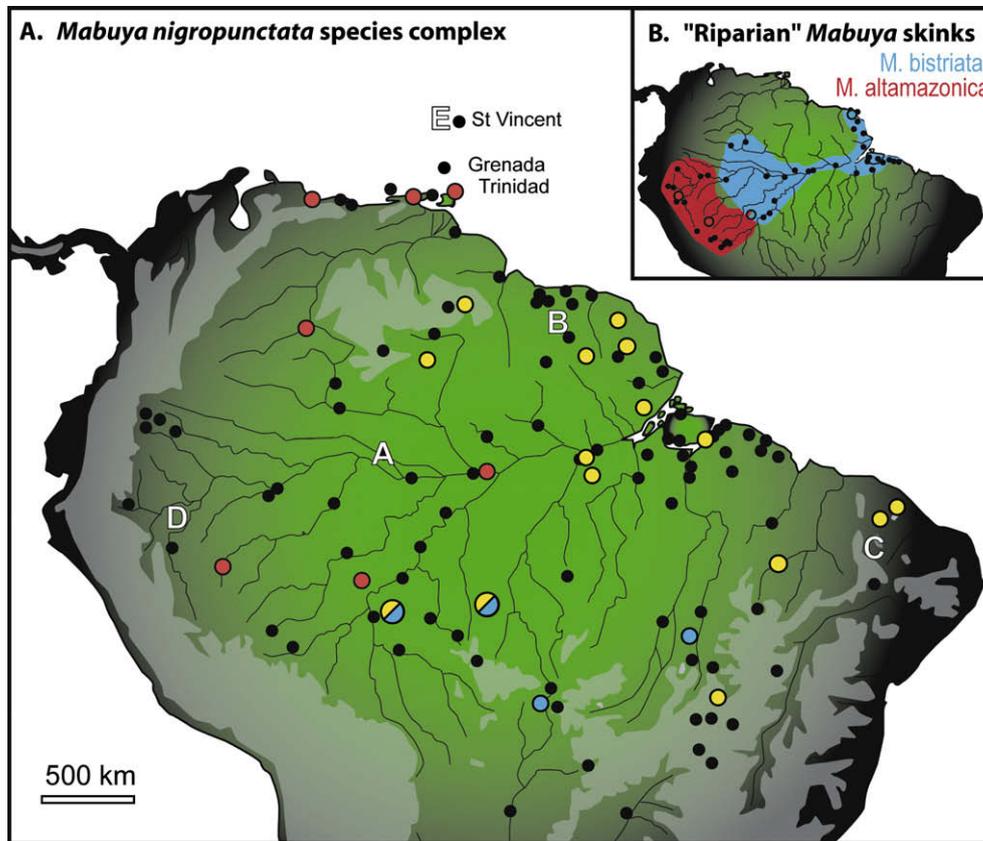


Fig. 2. Distribution maps of the *Mabuya nigropunctata* species complex (A), and of the two Amazonian species of riparian *Mabuya* (B), namely *M. altamazonica* and *M. bistriata*. Red, yellow and blue circles represent localities of specimens of *M. nigropunctata* from the occidental, oriental and meridional clades, respectively, included in the present phylogenetic analyses. Locality names for these specimens are given in Table 1. Black dots represent specimens that have not been included in the molecular phylogenies (see appendix for the exact localities), but that have been used for the morphological study of *Mabuya nigropunctata* (see Table 4). Two-colored circles (yellow-blue) represent localities where the meridional and the oriental clades of *M. nigropunctata* are sympatric. Capital letters in white indicate: A, exact neotype's locality of *Mabuya nigropunctata* (Spix, 1825); B, approximate type locality of *M. surinamensis* (Hallowell, 1857); C, exact type locality of *M. arajara* Rebouças-Spieker, 1981; D, approximate type locality of *Copeoglossum cinctum* Tschudi 1845, and E, exact type locality of *Tiliqua aenae* Gray, 1931. The delimitation range between *M. bistriata* and *M. altamazonica* (Fig. 2B) is based on both molecular and morphological evidence, the two species being easily distinguishable (see Miralles et al. 2006).

Table 3
Summary of the cytochrome *b* and 12S genetic divergences (uncorrected *p*-distances) estimated within and between the three main clades of *Mabuya nigropunctata* (oriental, meridional and occidental clades). The mean is followed by the standard deviation and the range of genetic distances is given between parentheses; "n" indicates the number of pairwise comparisons. All distance values are expressed in %.

	Intra-clade distances				Inter-clades distances			
	Oriental	Meridional	Occidental	Mean	Oriental/meridional	Oriental/occidental	Meridional/occidental	Mean
Cytb	5.16 ± 2.56 (0–11.71) n = 300	7.81 ± 4.36 (0.14–11.20) n = 21	4.21 ± 2.57 (0–7.70) n = 55	5.73 ± 1.87	10.40 ± 0.71 (8.83–12.65) n = 176	10.28 ± 0.94 (8.68–13.72) n = 275	11.62 ± 0.74 (10.38–13.73) n = 77	10.77 ± 0.74
12S	1.32 ± 0.86 (0–2.95) n = 300	2.06 ± 1.33 (0–3.75) n = 21	1.58 ± 1.16 (0–3.75) n = 66	1.65 ± 0.38	3.21 ± 1.60 (1.60–4.83) n = 175	4.69 ± 0.57 (3.49–5.93) n = 300	3.94 ± 0.85 (2.95–6.17) n = 84	3.95 ± 0.74

(see Fig. 4): these two contaminated sequences completely distorted the topology of the *M. nigropunctata* species complex, with *M. carvalhoi* branching inside it and with some populations of *M. nigropunctata* from eastern Amazonia (Pará) appearing more closely related to populations from western Amazonia than to other specimens from exactly the same locality.

These results highlight the importance of thoroughly checking the results of each independent data set before proceeding with the concatenate analysis in order to make sure that the partitions are not incongruent. Topological incongruence among partitions can easily be tested using the ILD test (Michkevich and Farris, 1981) or the reciprocal 70% bootstrap proportion or a 95% posterior probability threshold (Mason-Gamer and Kellogg, 1996). In the present case, the results of the ILD test showed that the two data-

sets (cytb and 12S) containing the contaminated sequences were highly incongruent ($P = 0.004$). When the two contaminated sequences were removed from the dataset, however, the ILD test did not detect any incongruence ($P = 0.60$). Moreover, the two datasets containing the contaminated sequences were also completely incongruent according to Mason-Gamer and Kellogg (1996) (100% bootstrap and posterior probability values; data not shown).

Another relevant point is that despite the fact that eight genes and approximately 5000 bp were used in the phylogenetic analyses by Whiting et al. (2006), the single contaminated cytb sequence of *M. carvalhoi* caused this species to cluster within the *M. nigropunctata* species complex with a posterior probability support of 100%. Moreover, the support for all the main nodes within the *M.*

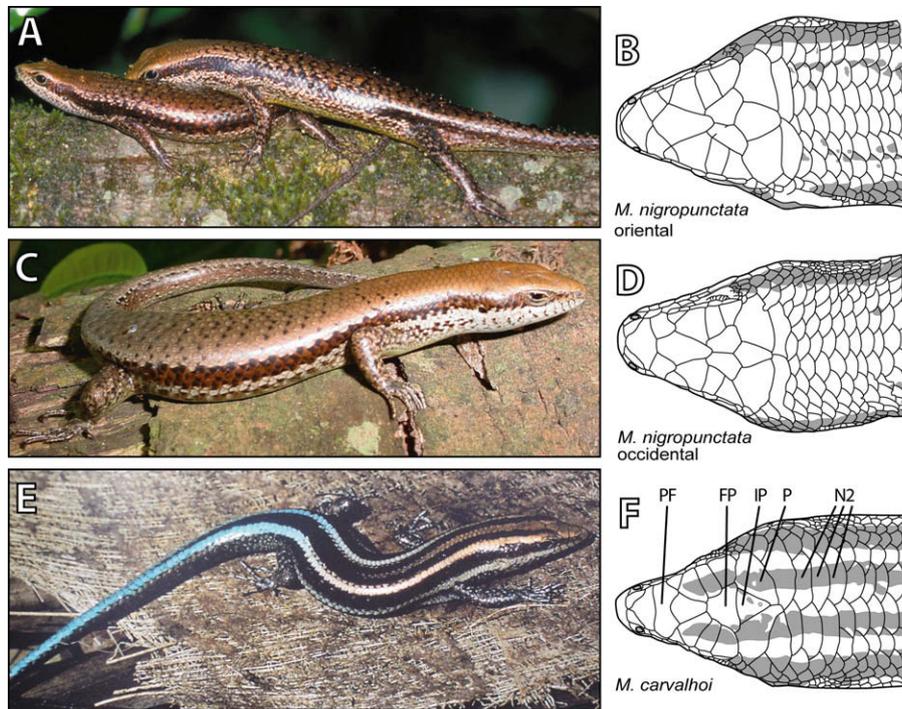


Fig. 3. Morphological comparison between *Mabuya nigropunctata* from the oriental (A and B) and occidental (C and D) clades with *M. carvalhoi* (E and F), highlighting the differences between both taxa and the external similarity between the two divergent lineages of the *M. nigropunctata* species complex (A–D). In addition to the very distinct color pattern, body size and head shape of *M. carvalhoi*, this species can be further distinguished from *M. nigropunctata* by the presence of secondary nuchal scales (N2) (absent in *M. nigropunctata*; B and D); prefrontals fused into a single median scale (FP) (separated in *M. nigropunctata*; B and D), prefrontals fused into a single median scale (PF) (separated in *M. nigropunctata*; B and D), and by the broad contact of the parietal scales (P) behind the interparietal (IP) (not in contact in 91% of the 220 specimens of *M. nigropunctata* analyzed in the present study). Photographs: (A) a couple of *M. nigropunctata* of the oriental clade, St Eugène, French Guiana; (C) *M. nigropunctata* of the occidental clade, Puerto Ayacucho, Colombo-Venezuelan frontier; (E) *M. carvalhoi*, Roraima state, Brazil (courtesy of J.C. de Massary, M.A.N. Mumaw, and L.J. Vitt, respectively). Drawings: (B) *M. nigropunctata* (OMNH 36834) from the oriental clade, Para state, Brazil; (D) *M. nigropunctata* (MHNSL 16203), from the occidental clade, Sucre state, Venezuela; *M. carvalhoi* (AMNH 137372), from Roraima state, Brazil.

Table 4

Comparisons of the most remarkable characters distinguishing *Mabuya carvalhoi* from *M. nigropunctata*. Morphological data for *M. carvalhoi* was obtained partly from Rebouças-Spieker and Vanzolini (1990) and Ávila-Pires (1995), whereas data for *M. nigropunctata* was obtained by examining 220 specimens collected over its whole distribution range (see Fig. 2 for an overview of the distribution of the specimens examined and Annex 1 for data on their exact localities).

	<i>M. carvalhoi</i>	<i>M. nigropunctata</i>
Frontoparietals fused into a single scale	100% (19)	0% (210)
Prefrontals fused into a single scale	100% (19)	0% (207)
Parietal scales in broad contact behind the interparietal scale.	100% (19)	9.3% (194)
Number of pairs of secondary nuchal scales		
N = 0	0%	99.5%
N = 1	0%	0.5%
N = 2	25%	0%
N = 3	75% (4)	0% (203)
Presence of two well defined dark dorsal stripes extending up to the eyes.	100% (19)	0% (220)
Maximum snout-vent length	63 mm (3)	106 mm (201)

Table 5

Phenotype comparisons of *M. nigropunctata* in a contact zone. Specimens of *M. nigropunctata* from the Guajará-Mirim park (Rio Formoso, Parque Estadual Guajará-Mirim, approx. 90 km North of Nova Mamoré [=10°19'17.2"S ; 64°33'47.94"W], Rondônia state, Brasil) were separated into two distinct phenotypes (I and II) on the basis of both scalation and coloration characters (all were collected by L. J. Vitt, between February and April, 1998). Characteristics allowing their distinction are presented for each examined specimens, with information on their sex and their phylogenetic placement (when molecular data were available). Comparisons suggest that morphological differences between the two clades (oriental and meridional) exist in this contact zone and that these are not the result of sexual dimorphism.

Specimens (OMNH collection number)	Phenotype I				Phenotype II		
	37411	37412*	37413	37414	37415	37416	37417
Phylogenetic position within the <i>M. nigropunctata</i> complex: oriental clade (O)/meridional clade (M)	?	?	O	O	?	M	M
Dark ventrolateral stripes: present (X)/absent (–)	X	X	X	X	–	–	–
White dots on the back: present (X)/absent (–)	X	–	X	X	–	–	–
Supranasals: in broad contact (+++)/in point contact (+)/separated (–)	+++	+++	+++	+++	+	–	–
Dorsal cycloid scales: strongly tricrenated (+++)/slightly tricrenated (+)/smooth (–)	+++	+	+++	+++	+	–	–
Sex: male (♂)/female (♀)	?	?	♂	♀	♀	♂	♂

* Specimen OMNH 37412 presents faded characteristics, what is probably due to the fact that this specimen is a juvenile.

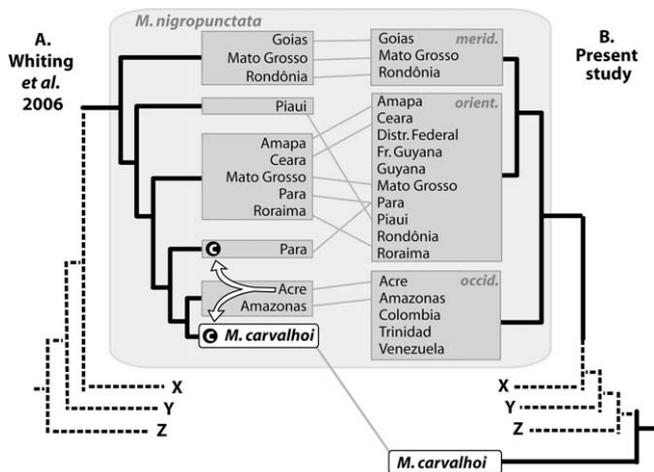


Fig. 4. (A) Simplified phylogeny of the *Mabuya nigropunctata* species complex obtained by Whiting et al. 2006; (B) new results of the present study. Both topologies are remarkably distinct regarding the position of *M. carvalhoi*, as well as the number of distinct lineages within the *M. nigropunctata* species complex and their phylogenetic relationships. Letters X, Y and Z represent the remaining *Mabuya* species, emphasizing the basal position of *M. croizati* within the genus obtained in the present study. The two sequences from Whiting et al. (2006) that were contaminated by a specimen of *M. nigropunctata* from Acre state have been highlighted with a «C». The white arrows indicate the direction of the contamination event.

nigropunctata species complex ranged between 95% and 100% in all cases, despite the fact that the topology obtained was completely altered by the presence of two contaminated sequences (Fig. 4).

4.1. Molecular taxonomy of the Amazonian *Mabuya*

Due to both nomenclatural and taxonomic difficulties, the systematics of the genus *Mabuya* sensu stricto was highly confusing for a long time (Taylor, 1956; Ávila-Pires, 1995; Mausfeld and Lötters, 2001; Mausfeld and Vrcibradic, 2002; Miralles, 2005; Miralles et al., 2005, 2009b), and no revision of the whole Neotropical *Mabuya* lineage has been undertaken since Emmet R. Dunn published his “Notes on American *mabuyas*” in 1936. This is especially true for the Amazonian species group, even though Ávila-Pires (1995) has clarified some aspects of its systematics. Six species of *Mabuya* are currently recognized in the Amazonian basin: *Mabuya altamazonica* Miralles et al., 2006; *M. bistrata* (Spix, 1825); *M. carvalhoi* Rebouças-Spieker and Vanzolini, 1990; *M. guaporicola* Dunn, 1936; *M. nigropalmata* Andersson, 1918; and *M. nigropunctata* (Spix, 1825); the latter is by far the commonest and most widely distributed of these species in Amazonia. Despite the very low resolution of the intermediate nodes of the phylogenetic tree shown in Fig. 1, the results presented herein suggest that the Amazonian *Mabuya* are divided into at least five distinct lineages: (1) the riparian *Mabuya* clade (*M. altamazonica* + *M. bistrata*); (2) the clade of the *M. nigropunctata* species complex; (3) *M. carvalhoi*, sister to *M. croizati*, a relationship previously hypothesized on the basis of shared morphological characters such as the acute muzzle, the presence of secondary nuchal scales, the fusion of frontoparietals, and the presence of two wide dark dorsal stripes (Rebouças-Spieker and Vanzolini, 1990; Miralles et al., 2005); (4) the *M. guaporicola* lineage; and (5) the *M. nigropalmata* lineage. Although the present phylogeny is not very conclusive regarding the evolutionary relationships between these latter two lineages, they are very distinct from a morphological point of view (they do not share any clear morphological synapomorphies) and therefore most probably do not form a clade.

Mabuya altamazonica and *M. bistrata* are two Amazonian species, which predominantly inhabit the borders of large rivers and

várzea forests (in contrast to *M. nigropunctata*, which is essentially restricted to terra firme lowland). *Mabuya altamazonica*, described from western Amazonia (Miralles et al., 2006), was for a long time considered to be part of the *M. nigropunctata* complex due to both taxa being morphologically very similar and living in sympatry. Specimen MBS 001 (see Table 1), classified as *M. bistrata* by Whiting et al. (2006) (under the synonym *M. ficta*; see Introduction), was included in the present paper. The phylogenetic results summarized in Fig. 1 show that this specimen is actually *M. altamazonica* (Br-Acre; the present sampling includes the holotype of *M. altamazonica*). In both the present study and the paper published by Whiting et al. (2006), *M. altamazonica* is sister to *M. bistrata* sensu Ávila-Pires, thereby confirming that, despite their overall morphological similarity, *M. altamazonica* is not closely related to *M. nigropunctata* (Miralles et al., 2006). Unfortunately, the bootstrap and posterior probability values that support the monophyly of the clade formed by *M. altamazonica* + *M. bistrata* are very low in both the present study and in Whiting et al. (2006). It is important to note, however, that this clade was one of the few nodes that showed a low support in the phylogenetic tree of Whiting et al. (2006), which included a very large data set of eight genes and approximately 5000 bp. Nevertheless, the relatively similar ecology of both species (they are the only two riparian species of *Mabuya* present in the whole Amazonian basin), and their parapatric and remarkably complementary distribution ranges (see Fig. 2), together with the phylogenetic results, suggest that these species might have been affected by a past vicariant event.

The results of the phylogenetic tree presented in Fig. 1 and the genetic distances from Table 3 suggest that *M. nigropunctata* is a complex that includes several cryptic species. Taken together, the phylogenetic, morphological, and biogeographic data all argue in favor of the hypothesis that each of the three main lineages of *M. nigropunctata* could be regarded as different species. The meridional and oriental clades are sympatric in at least two localities—Rondônia and Mato Grosso (see Fig. 2). In the Guajara-Mirim Park (Rondônia), seven specimens from both clades were collected over the same period by the same collector (Table 5). Examination of their external morphology (lepidosis and coloration) revealed that these specimens could be divided into two distinct phenotypic groups, which coincided with the meridional and oriental clades recovered in the phylogenetic analyses based on mitochondrial genes and presented in Fig. 1. The clear morphological differentiation between specimens genetically assigned to the oriental and meridional clades that occur in sympatry (see Table 5), together with the high genetic divergence between these two lineages (*p*-distances ranging between 10.6% and 11.2% for the *cytb* gene, and 3.2% for the 12S rRNA), argue in favor of the specific distinctiveness of the oriental and meridional clades. Consequently, if, according to the principle of phylogenetic species, both the meridional and oriental clades represent two distinct species, the occidental clade, which is basal to the other two clades, should also be considered as a third distinct species (Cracraft, 1983; Mishler and Theriot, 2000; Wheeler and Platnick, 2000). The results of this deductive process are also supported by an examination of the genetic divergence values estimated between the three main clades (divergences range between 10.28% and 11.62% for the *cytb* gene and between 3.21% and 4.69% for the 12S rRNA). These values are higher than those usually observed for the *cytb* gene between distinct species of reptiles (Harris, 2002). Moreover, the interspecific genetic distances calculated for the Neotropical genus *Mabuya* range between 3.45% and 8.20% (mean: 5.95%) for the 12S rRNA gene (Miralles et al., 2006).

The splitting of the *M. nigropunctata* complex into three distinct species would involve taxonomic and nomenclatural changes. Since these three species are largely allopatric, it is possible to propose a list of available names on the basis of the type locality of the

synonyms described in the past (Fig. 2A). However, it is clear that a thorough morphological study will be needed in the future to propose a definitive classification for this complex. This taxonomic revision should include diagnosis for each of the three taxa, which is beyond the main objectives of the present work. The available names for the three units are as follows: (1) Occidental clade: the binomen *Mabuya nigropunctata* (Spix, 1825) must be restricted to the species of this clade. The neotype's locality (Locality A in Fig. 2A) is unambiguously placed within the distribution area of the occidental clade ["Santa Rita, Município de Maraã, left margin of Rio Japurá (Lago Paricá), Amazonas, Brasil"; Ávila-Pires, 1995]. As a result, of their type localities, two other nomen can be regarded as junior subjective synonyms of this species: *Tiliqua aenae* [Gray, 1831; "St. Vincent, West Indies" (Gray, 1839: 292)] (locality E in Fig. 2A) and *Copeoglossum cinctum* [Tschudi, 1845; "Republica Peruana (. . .) Waldregion" = Peruvian Amazonian forest] (locality D Fig. 2A); (2) Oriental clade: the binomen *Euprepis surinamensis* (Hallowell, 1857; formerly regarded as a synonym of *Mabuya nigropunctata* sensu lato, Miralles et al., 2005) is here considered as the oldest available name referring to the species of the oriental clade, its type locality being "Surinam". This locality can be unambiguously placed within the distribution area of the oriental clade (locality B in Fig. 2A). This nomen should, therefore, be resurected and applied to the eastern species under the name *Mabuya surinamensis* (Hallowell, 1857). Additionally, *Mabuya arajara* Rebouças-Spieker, 1981 might be regarded as a junior synonym of *Mabuya surinamensis*. Indeed, despite a lighter coloration, this poorly known species clearly belongs to the *M. nigropunctata* complex and its range (only known from its type locality) is unambiguously placed within the distribution range of the oriental clade (locality C in Fig. 2A). (3) Meridional clade: to the best of our knowledge, there is no available name applicable to the species of the meridional clade. Further morphological studies will therefore, be needed in order to find reliable diagnostic morphological characters.

4.2. Divergence date estimates and diversification in the Amazonia

The phylogenetic results shown in Fig. 1 suggest that diversification in the Neotropical genus *Mabuya* started approximately 15.5–13.4 Ma. This figure suggests that the ancestor of the genus made the 3000-km crossing of the Atlantic Ocean from Africa into America during the Mid-Miocene. This date is older than the previously estimated date for this crossing (9–7 Ma; Carranza and Arnold, 2003). This underestimation was probably due to the deficient sampling of Neotropical *Mabuya* by Carranza and Arnold (2003), which did not include the basalmost *M. carvalhoi* and *M. croizati* and the different methodology employed to calculate the time of divergence (NPRS tree with genetic distance scale in the present work versus Kimura-2-parameter distances in Carranza and Arnold, 2003).

According to our estimations, the split between *M. carvalhoi* and *M. croizati* occurred approximately 13.3–11.3 Ma (Fig 1), thereby suggesting that a connection may have occurred between the montane habitats of the coastal mountain range of Venezuela (especially the Turimiquire massif from where *M. croizati* is endemic) and the Guiana Shield (to which *M. carvalhoi* is restricted) during the Middle Miocene (15.97 ± 0.05 – 11.608 ± 0.05 Ma). These regions are currently separated by the lower section of the Orinoco river drainage basin (Llanos del Orinoco), a vast area of grassland devoid of any *Mabuya* species (Miralles et al., 2005). These results agree with Steyermark (1966, 1974, 1979), who hypothesized from phytogeographic data that these two forest regions' biota had been connected in the past. More interestingly, analyses of paleogeographical data indicate that the Orinoco river changed its lower course, an event which has been dated very precisely to have oc-

curred at the end of the Middle Miocene. The proto-Orinoco river previously ran in a south-north direction, flowing into the Caribbean sea. The deformation and uplift of the Eastern Cordillera of Colombia and the southwestern end of the Mérida Andes subsequently caused the distal course of the river to be deflected towards a west-east direction, flowing between the Coastal mountain range of Venezuela and the Guiana Shield into the Atlantic Ocean (Díaz de Gamero, 1996). This suggests that speciation between *M. carvalhoi* and *M. croizati* may have occurred as a result of a vicariant event produced by the formation of the present day Orinoco river drainage basin and the consequent appearance of the Llanos del Orinoco, which act as a barrier to dispersal between these two species.

The divergence dates estimated for the *Mabuya nigropunctata* species complex (Fig 1) suggest that the major cladogenetic events that produced the three main groups (occidental (oriental + meridional)) occurred during the Late Miocene (11.608 ± 0.005 – 5.332 ± 0.005 Ma). These estimations suggest that diversification within the *M. nigropunctata* species complex was not triggered by the climatic changes that occurred during the Pleistocene, as has been suggested by several authors (Haffer, 1969; see also Colinvaux, 1998). Our data support the hypothesis that the late tertiary period (essentially the Miocene epoch) played a very important role in the generation of biological diversity in the Amazonian forests (Bush, 1994; Nores, 1999; Glor et al., 2001; Da Silva and Patton, 1998; Moritz et al., 2000; Patton and Pires Costa, 2003; Pereira and Baker, 2004; Vidal et al., 2005; Noonan and Wray, 2006). The east-west division observed between the occidental and (oriental + meridional) clades has frequently been inferred from numerous biogeographic and/or molecular phylogenetic studies of butterflies (Hall and Harvey, 2002), lizards (Ávila-Pires, 1995; Glor et al., 2001; Kronauer et al., 2005; Gamble et al., 2008), frogs (Ron, 2000; Symula et al., 2003), birds (Bates et al., 1998; Eberhard and Bermingham, 2005), and mammals (Da Silva and Patton, 1993; Da Silva and Oren, 1996; Steiner and Catzefflis, 2004). However, Gamble et al. (2008) have stressed the fact that there are large differences in the timing of the divergence between east-west taxon pairs, which suggests that these divergences are not the result of a common vicariant event.

The split between *M. bistrata* and *M. altamazonica* might represent another east-west vicariant event (see Figs. 1 and 2), although the support for these two species being sister taxa in the present analysis and in the analysis by Whiting et al. (2006) is very low. This cladogenetic event (11.0–9.4 Ma) perfectly matches the east-west vicariance observed within the *M. nigropunctata* species complex (10.3–8.8 Ma). However, their respective spatial localizations do not match very well (Fig. 2). The approximate boundary between the western (occidental) and eastern (oriental + meridional) clades of *M. nigropunctata* can be inferred at approximately the centre of the Amazonian basin (Rio Madeira and Rio Negro), whereas the boundary between *M. bistrata* and *M. altamazonica* is located significantly further west (coinciding with the watershed that separates the Marañón-Ucayali and the Madre de Dios sub-basins from the Acre sub-basin). In the case of *M. nigropunctata*, the Rio Madeira and Rio Negro cannot be considered the main cause of the vicariance event that produced both sub-clades. As an example, the Amazon river, which is by far the largest river in South America, crosses right through the distribution area of both clades and does not seem to have a barrier effect on this taxon (at least four distinct crossings of the Amazon river can be inferred within the oriental clade from the phylogenetic results shown in Fig. 1, plus a fifth one within the occidental clade). These rivers most probably simply constitute secondary boundaries between the distribution areas of both clades after their differentiation. It is, therefore, possible that the boundaries between the east-west clades of *M. nigropunctata* and between *M. bistrata* and *M. altamazonica* may

have changed since their occurrence and therefore that both east–west splits were caused by the same, as yet unknown, vicariant event.

Acknowledgments

We are very grateful to all people that donated samples for this study, provided access to specimens or photographs, or helped in the field: E. Åhlander, P. Arntzen, T.C.S. Ávila-Pires, C.L. Barrio-Amoros, J.-C. Chaparro-Auza, D. Dittmann, A. Estep, R. Feeny, J.-P. Gasc, L.S. Ford, E. Gasso Miracle, Ph. Gaucher, I. Ineich, J.-C. de Massary, M.A.N. Mumaw, A. Resetar, G. Rivas, S.P. Rogers, W. Schargel, G.E. Schneider, J.C. Señaris, L.J. Vitt, and M.G. Zatz. Many thanks to the Service Commun de Systématique Moléculaire (CNRS IFR 1541, MNHN) for supporting molecular work and to A. Ohler for welcoming one of us (AM) in the UMS 602 Taxinomie et Collections—Reptiles et Amphibiens, of the Département Systematique et Evolution (MNHN). We wish also to thank two anonymous referees for their helpful comments. This work is based in part upon work supported by SYNTHESYS to AM (GB-TAF-3373). SC was supported by Grant CGL2008-00827/BOS from the Ministerio de Educación y Ciencia, Spain.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.10.016.

References

- Arnold, E.N., Vasconcelos, R., Harris, D.J., Mateo, J., Carranza, S., 2008. Systematics, biogeography and evolution of the endemic *Hemidactylus* geckos (Reptilia, Squamata, Gekkonidae) of the Cape Verde Islands: based on morphology and mitochondrial and nuclear DNA sequences. *Zool. Scr.* 37, 619–636.
- Arnold, E.N., Arribas, O., Carranza, S., 2007. Systematics of the Palaearctic and Oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae) with descriptions of eight new genera. *Zootaxa* 1430, 1–86.
- Aleixo, A., 2004. Historical diversification of a Terra-firme forest bird superspecies: A phylogeographic perspective on the role of different hypotheses of Amazonian diversification. *Evolution* 58 (6), 1303–1317.
- Andersson, L.G., 1918. New Lizards from South America. *Arkiv för Zoologi* 11 (16), 1–9.
- Ávila-Pires, T.C.S., 1995. Lizards of Brazilian Amazonia (Reptilia: Squamata). *Zool. Verh., Leiden* 599, 1–706.
- Bates, J.M., Hackett, S.J., Cracraft, J., 1998. Area-relationships in the Neotropical lowlands: an hypothesis based on raw distributions of Passerine birds. *J. Biogeogr.* 25, 783–793.
- Bauer, A.M., 2003. On the identity of *Lacerta punctata* Linnaeus 1758, the type species of the genus *Euprepis* Wagler 1830, and the generic assignment of Afro-Malagasy skinks. *Afr. J. Herp.* 52, 1–7.
- Blackburn, D.G., Vitt, L.J., 1992. Reproduction in viviparous South American lizards of the genus *Mabuya*. In: Hamlett, W.C. (Ed.), *Reproductive Biology of South American Vertebrates*. Springer-Verlag, New York, pp. 150–163.
- Bush, M.B., 1994. Amazonian speciation: a necessarily complex model. *J. Biogeogr.* 21, 5–17.
- Carranza, S., Arnold, E.N., 2003. Investigating the origin of transoceanic distributions: mtDNA shows *Mabuya* lizards (Reptilia, Scincidae) crossed the Atlantic twice. *Syst. Biodiv.* 1, 275–282.
- Carranza, S., Arnold, E.N., Amat, F., 2004. DNA phylogeny of *Lacerta* (*Iberolacerta*) and other lacertine lizards (Reptilia: Lacertidae): did competition cause long-term mountain restriction? *Syst. Biodiv.* 2, 57–77.
- Carranza, S., Arnold, E.N., Geniez, Ph., Roca, J.L., Mateo, J.A., 2008. Radiation, multiple dispersal and parallelism in Moroccan skinks, *Chalcides* and *Sphenops* (Squamata: Scincidae), with comments on *Scincus* and *Scincopus* and the age of the Sahara Desert. *Mol. Phylogenet. Evol.* 46, 1071–1094.
- Chek, A.A., Loughheed, S.C., Bogard, J.P., Boag, P.T., 2001. Perception and History: molecular phylogeny of a diverse group of Neotropical frogs, the 20-chromosome *Hyla* (Anura: Hylidae). *Mol. Phylogenet. Evol.* 8, 370–385.
- Colinvaux, P.A., 1998. A new vicariance model for Amazonian endemics. *Global Ecol. Biogeogr. Lett.* 7, 95–96.
- Cracraft, J., 1983. Species concepts and speciation analysis. *Curr. Ornithol.* 1, 159–187.
- Cracraft, J., Prum, R.O., 1988. Patterns and Processes of Diversification: Speciation and Historical Congruence in some Neotropical Birds. *Evolution* 42 (3), 603–620.
- Cunningham, C.W., 1997. Is congruence between data partitions a predictor of phylogenetic accuracy? Empirically testing an iterative procedure for choosing among phylogenetic methods. *Syst. Biol.* 46, 464–478.
- Da Silva, J.M.C., Oren, D.C., 1996. Application of parsimony analysis of endemism in Amazonian biogeography, an example with primates. *Biol. J. Linn. Soc.* 59, 427–437.
- Da Silva, M.N.F., Patton, J.L., 1993. Amazonian phylogeography: mtDNA sequence variation in arboreal echimyid rodents (Caviomorpha). *Mol. Phylogenet. Evol.* 2, 243–255.
- Da Silva, M.N.F., Patton, J.L., 1998. Molecular phylogeography and the evolution and conservation of Amazonian mammals. *Mol. Ecol.* 7, 475–486.
- Díaz de Gámero, M.L., 1996. The changing course of the Orinoco River during the Neogene: a review. *Palaeogeogr. Palaeoclimatol.* 123, 385–402.
- Dunn, E.R., 1936. Notes on American *mabuyas*. *Proc. Acad. Nat. Sci. Phila.* 87, 533–557.
- Eberhard, J.R., Bermingham, E., 2005. Phylogeny and comparative biogeography of *Pionopsitta* parrots and *Pteroglossus* toucans. *Mol. Phylogenet. Evol.* 36, 288–304.
- Farris, J.S., Källersjö, A.G., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. *Cladistics* 10, 315–319.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 738–791.
- Gamble, T., Simons, A.M., Colli, G.R., Vitt, L.J., 2008. Tertiary climate change and the diversification of the Amazonian gecko genus *Gonatodes* (Sphaerodactylidae, Squamata). *Mol. Phylogenet. Evol.* 46, 269–277.
- Glor, R.E., Vitt, L.J., Larson, A., 2001. A molecular phylogenetic analysis of diversification in Amazonian *Anolis* lizards. *Mol. Ecol.* 10, 2661–2668.
- Graham, C.H., Moritz, C., Williams, S.E., 2006. Habitat history improves prediction of biodiversity in rainforest fauna. *Proc. Natl. Acad. Sci. USA* 103 (3), 632–636.
- Gray, J.E., 1831. A Synopsis of the species of the class Reptilia. In: Griffith, E., Pidgeon, E. (Eds.), *The Class Reptilia Arranged by the Baron Cuvier, with Specific Descriptions*. Whittaker, Treacher & Co., London, pp. 1–110 (appendix).
- Gray, J.E., 1839. Catalogue of the Slender-tongued Saurians, with Descriptions of many new genera and Species. *Ann. Nat. Hist.* 2, 287–293.
- Greer, A.E., Broadley, D.G., 2000. Six characters of systematic importance in the scincid lizard genus *Mabuya*. *Hamadryad* 25, 1–12.
- Greer, A.E., Nussbaum, R.A., 2000. New character useful in the systematics of the scincid lizard genus *Mabuya*. *Copeia* 2000, 615–618.
- Guindon, S., Gascuel, O., 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52, 696–704.
- Haffer, J., 1969. Speciation in Amazonian forest birds. *Science* 165, 131–137.
- Hall, J.P.W., Harvey, D.J., 2002. The phylogeography of Amazonia revisited: new evidence from Rionid Butterflies. *Evolution* 56 (7), 1489–1497.
- Hallowell, E., 1857. On several new species of reptiles in the collection of the Academy of Natural Sciences. *Proc. Acad. Nat. Sci. Phila.* 8, 153–156.
- Harris, D.J., 2002. Reassessment of comparative genetic distance in reptiles from the mitochondrial cytochrome *b* gene. *Herpetol. J.* 12, 85–86.
- Honda, M., Ota, H., Kobayashi, M., Nabhitabhata, J., Yong, H.-S., Hikida, T., 2000. Phylogenetic relationships, character evolution, and biogeography of the subfamily Lygosominae (Reptilia: Scincidae) inferred from mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* 15, 452–461.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17, 754–755.
- Irwin, D.M., Kocher, T.D., Wilson, A.C., 1991. Evolution of the cytochrome *b* gene of Mammals. *J. Mol. Evol.* 32, 128–144.
- Jesus, J., Brehm, A., Harris, D.J., 2005. Relationships of scincid lizards (*Mabuya* spp.) from the islands of the Gulf of Guinea based on mtDNA sequence data. *Amphibia-Reptilia* 26, 467–473.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* 86, 6196–6200.
- Kronauer, D.J.C., Bergmann, P.J., Mercer, J.M., Russell, A.P., 2005. A phylogeographically distinct and deep divergence in the widespread Neotropical turnip-tailed gecko, *Thecadactylus rapicauda*. *Mol. Phylogenet. Evol.* 34, 431–437.
- Kumazawa, Y., Nishida, M., 1999. Complete mitochondrial DNA sequences of the green turtle and blue-tailed mole skink: Statistical evidence for archosaurian affinity of turtles. *Mol. Biol. Evol.* 16, 784–792.
- Lundberg, J.G., Marshall, L.G., Guerrero, J., Horton, B., Malabarba, M.C.S.L., Wesselingh, F., 1998. The stage for neotropical fish diversification: a history of tropical South American rivers. In: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M.S., Lucena, C.A.S. (Eds.), *Phylogeny and Classification of Neotropical Fishes*. EDIPUCRS, Porto Alegre, pp. 13–48.
- Mason-Gamer, R.J., Kellogg, E.A., 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae Gramineae. *Syst. Biol.* 45, 524–545.
- de Massary, J.-C., Gasc, J.-P., Blanc, M., 2001. Occurrence of *Mabuya bistriata* (Spix, 1825) (Sauria, Scincidae) in French Guiana. *Brit. Herp. Soc. Bull.* 74, 19–25.
- Mausfeld, P., Schmitz, A., Böhme, W., Misof, B., Vrcibradic, D., Rocha, C.F.D., 2002. Phylogenetic affinities of *Mabuya atlantica* Schmidt, 1945, endemic to the Atlantic Ocean Archipelago of Fernando de Noronha (Brazil): Necessity of partitioning the genus *Mabuya* Fitzinger, 1826 (Scincidae: Lygosominae). *Zool. Anz.* 241, 281–293.
- Mausfeld, P., Lötters, S., 2001. Systematics of *Mabuya frenata cochabambae* Dunn, 1935 (Reptilia, Sauria, Scincidae) from Inner-Andean Dry Valleys of Bolivia. *Stud. Neotrop. Fauna Environ.* 36 (1), 49–55.

- Mausfeld, P., Vrcibradic, D., 2002. On the Nomenclature of the Skink (*Mabuya*) Endemic to the Western Atlantic Archipelago of Fernando de Noronha, Brazil. *J. Herpetol.* 36 (2), 292–295.
- Mausfeld, P., Schmitz, A., 2003. Molecular phylogeography, intraspecific variation and speciation of the Asian scincid lizard genus *Eutropis*, 1843 (Squamata: Reptilia: Scincidae): taxonomic and biogeographic implications. *Org. Divers. Evol.* 3, 161–171.
- Michkevich, M.F., Farris, J.S., 1981. The implications of congruence in *Menidia*. *Syst. Zool.* 30, 351–370.
- Miralles, A., 2005. The Identity of *Lacertus mabouya* Lacepède, 1788, with Description of a neotype: an approach toward the taxonomy of new world *Mabuya*. *Herpetologica* 61, 46–53.
- Miralles, A., Rivas, G., Barrio-Amorós, C.L., 2005. Taxonomy of the genus *Mabuya* (Reptilia, Squamata, Scincidae) in Venezuela. *Zoosystema* 27 (4), 825–837.
- Miralles, A., 2006. Taxinomie, Systématique moléculaire et Biogéographie du genre *Mabuya* Fitzinger 1826 (Squamata, Scincidae) dans la région néotropicale. Ph.D. dissertation, Muséum national d'Histoire naturelle, Paris, p. 329.
- Miralles, A., Barrio-Amorós, C.L., Rivas, G., Chaparro-Auza, J.C., 2006. Speciation in the "Varzea" flooded forest: a new *Mabuya* (Squamata: Scincidae) from Western Amazonia. *Zootaxa* 1188, 1–22.
- Miralles, A., Chaparro, J.C., Harvey, M.B., 2009a. Three rare and enigmatic South American Skinks. *Zootaxa* 2012, 47–68.
- Miralles, A., Rivas Fuenmayor, G., Bonillo, C., Schargel, W.E., Barros, T., García-Perez, J.E., Barrio-Amorós, C.L., 2009b. Molecular systematics of Caribbean skinks of the genus *Mabuya* (Reptilia, Scincidae), with descriptions of two new species from Venezuela. *Zool. J. Linn. Soc.* 156, 598–616.
- Mishler, B.D., Theriot, E.C., 2000. The phylogenetic species concept (sensu Mishler and Theriot): monophyly, apomorphy, and phylogenetic species concepts. In: Wheeler, Q.D., Meier, R. (Eds.), *Species Concepts and Phylogenetic Theory*. Columbia University Press, New York, pp. 44–54.
- Moritz, C., Patton, J.L., Schneider, C.J., Smith, T.B., 2000. Diversification of rainforest faunas: an integrated molecular approach. *Annu. Rev. Ecol. Syst.* 31, 533–563.
- Noonan, B.P., Wray, K.P., 2006. Neotropical diversification: the effects of a complex history on diversity within the poison frog genus *Dendrobates*. *J. Biogeogr.* 33, 1007–1020.
- Nores, M., 1999. An alternative hypothesis for the origin of Amazonian bird diversity. *J. Biogeogr.* 26, 475–485.
- Patton, J.L., da Silva, M.N.F., 1997. Definition of species of pouched four-eyed opossums (Didelphidae, Philander). *J. Mammal.* 78, 90–102.
- Patton, J.L., Pires Costa, L., 2003. Molecular phylogeography and species limits in rainforest didelphid marsupials of South America. In: Jones, M., Dickman, C., Archer, M. (Eds.), *Predators with Pouches*. CISRO Publishing, Collingwood, pp. 63–81.
- Pereira, S.L., Baker, A.J., 2004. Vicariant speciation of curassows (Aves, Cracidae): A hypothesis based on mitochondrial DNA phylogeny. *Auk* 121, 682–694.
- Posada, D., 2008. JModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* 25, 1253–1256.
- Quijada-Mascareñas, J.A., Ferguson, J.E., Pook, C.E., Da Graça Salomão, M., Thorpe, R.S., Wüster, W., 2007. Phylogeographic patterns of trans-Amazonian vicariants and Amazonian biogeography: the Neotropical rattlesnake (*Crotalus durissus* complex) as an example. *J. Biogeogr.* 34, 1296–1312.
- Rebouças-Spieker, R., 1981. Sobre uma nova espécie de *Mabuya* do nordeste do Brasil (Sauria, Scincidae). *Pap. Avulsos Zool. (São Paulo)* 34 (9), 121–123.
- Rebouças-Spieker, R., Vanzolini, P.E., 1990. *Mabuya carvalhoi*, espécie nova do estado de Roraima, Brasil (Sauria, Scincidae). *Rev. Bras. Biol.* 50 (2), 377–386.
- Ribas, C.C., Joseph, L., Miyaki, C.Y., 2006. Molecular systematics and patterns of diversification in *Pyrrhura* (Psittacidae), with special reference to the *picta-leucotis* complex. *Auk* 123 (3), 660–680.
- Ron, S.R., 2000. Biogeographic area relationships of lowland Neotropical rainforest based on raw distributions of vertebrate groups. *Biol. J. Linn. Soc.* 71, 379–402.
- Sanderson, M.J., 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Mol. Biol. Evol.* 14, 1218–1231.
- Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol. Biol. Evol.* 19, 101–109.
- Soria-Carrasco, V., Talavera, G., Igea, J., Castresana, J., 2007. The K tree score: quantification of differences in the relative branch length and topology of phylogenetic trees. *Bioinformatics* 23, 2954–2956.
- Spix, J.B., 1825. *Animalia Nova sive species novae lacertarum, quas in itinere per Brasiliam annis MDCCCXVII-MDCCCXX jussu et auspiciis Maximiliani Josephi I. Bavariae regis, München: F. S. Hübschmann.*
- Steiner, C., Catzeflis, F.M., 2004. Genetic variation and geographical structure of five mouse-sized opossums (Marsupiala, Didelphidae) throughout the Guiana Region. *J. Biogeogr.* 31, 959–973.
- Steyermark, J.A., 1966. El Cerro Turimiquire y la región oriental adyacente. *Acta Botánica Venezolana* 1, 154–157.
- Steyermark, J.A., 1974. Relación florística entre la Cordillera de la Costa y la zona de Guyana y Amazonas. *Acta Botánica Venezolana* 9, 245–252.
- Steyermark, J.A., 1979. Plant Refuge and Dispersal centres in Venezuela: Their Relict and Endemic Element. In: Larsen, K., Holm-Nielsen, L.B. (Eds.), *Tropical Botany*. Academic Press, London, pp. 185–221.
- Symula, R., Schulte, R., Summers, K., 2003. Molecular systematics and Phylogeography of Amazonian poison frogs of the genus *Dendrobates*. *Mol. Phylogenet. Evol.* 26, 452–475.
- Taylor, E.H., 1956. A Review of the Lizards of Costa Rica. *Kans. Univ. Sci. Bull.* 38, 1–322.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequences alignment aided by quality analysis tools. *Nucleic Acids Res.* 24, 4876–4882.
- Tschudi, J.J., 1845. *Reptilium conspectus quae in Republica Peruana reperiunter et plaqueraque observata vel collecta sunt in itinere a Dr. J.J. Tschudi*. *Archiv für Naturgeschichte* 11 (1), 150–170.
- Vanzolini, P.E., 1981. A quasi-historical approach to the natural history of the differentiation of reptiles in tropical geographic isolates. *Pap. Avulsos Zool. (Sao Paulo)* 34 (19), 189–204.
- Vidal, N., Henderson, R.W., Delmas, A., Hedges, B., 2005. A Phylogenetic Study of the Emerald Treeboa (*Corallus caninus*). *J. Herpetol.* 39 (3), 500–503.
- Vrcibradic, D., Mausfeld-Lafdihiya, P., Rocha, C.F.D., 2006. Molecular phylogeny of Brazilian *Mabuya* (Reptilia, Squamata, Scincidae) of the *Agilis/Caissara/Heathi* complex. *Herpetol. J.* 16 (1), 83–91.
- Wesselingh, F.P., Salo, J.A., 2006. A miocene perspective on the evolution of the Amazon basin. *Scripta Geol.* 133, 439–458.
- Wheeler, Q.D., Platnick, N.I., 2000. The Phylogenetic Species Concept (sensu Wheeler and Platnick). In: Wheeler, Q.D., Meier, R. (Eds.), *Species Concepts and Phylogenetic Theory*. Columbia University Press, New York, pp. 55–69.
- Whiting, A.S., Sites Jr., J.W., Pellegrino, K.C.M., Rodrigues, M.T., 2006. Comparing alignment methods for inferring the history of the new world lizard genus *Mabuya* (Squamata: Scincidae). *Mol. Phylogenet. Evol.* 38, 719–730.
- Winnepenninckx, B., Backeljau, T., Dewachter, R., 1993. Extraction of high molecular weight DNA from molluscs. *Trends Genet.* 9, 407.
- Wüster, W., Ferguson, J.E., Quijada-Mascareñas, J.A., Pook, C.E., Da Graça Salomão, M., Thorpe, R.S., 2005. Tracing an invasion: landbridges, refugia, and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). *Mol. Ecol.* 14, 1095–1108.
- Zamudio, K.R., Green, H.W., 1997. Phylogeography of the Bushmaster (*Lachesis muta*: Viperidae): Implications for Neotropical biogeography, systematics, and conservation. *Biol. J. Linn. Soc.* 62, 421–442.