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Molecular dating and phylogenetic relationships among Teiidae (Squamata) inferred by molecular and morphological data

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Abstract

We present a phylogenetic analysis of teiid lizards based on partitioned and combined analyses of 12S and 16S mitochondrial DNA sequences, and morphological and ultrastructural characters. There were some divergences between 12S and 16S cladograms, but phylogenetic analyses of the combined molecular data corroborated the monophyly of Tupinambinae, Teiinae, and ''cnemidophorines'', with high support values. The total combined analysis (molecules + morphology) produced similar results, with well-supported Teiinae and ''cnemidophorines''. We present an evolutionary scenario for the evolution of Teiidae, based on molecular dating of evolutionary events using Bayesian methods, ancestral areas analysis, the fossil record, the geographic distribution of genera, and environmental and geologic changes during the Tertiary. According to this scenario, (1) all current teiid genera, except *Aspidoscelis*, originated in isolation in South America; (2) most teiid genera originated during the Eocene, a period characterized by savanna expansion in South America; and (3) *Cnemidophorus* originated in South America, after which some populations dispersed to Central America during the Late Miocene.

Keywords: Teiidae; mtDNA; Phylogeny; Molecular dating; Evolutionary scenario

1. Introduction

Living Teiidae (sensu Presch, 1983) comprise 10 genera: *Ameiva*, *Aspidoscelis*, *Cnemidophorus*, *Callopistes*, *Crocodilurus*, *Dicrodon*, *Dracaena*, *Kentropyx*, *Teius*, and *Tupinambis*. The group is restricted to the New World, from Argentina to northeastern United States (Krause, 1985; Pough et al., 1998). *Callopistes*, *Crocodilurus*, *Dicrodon*, *Dracaena*, *Kentropyx*, *Teius*, and *Tupinambis* are restricted to South-America (Krause, 1985), whereas *Cnemidophorus* is also found in West Indies and *Ameiva* reaches Central and North America. *Aspidoscelis* ranges from the United States to extreme northwestern Costa Rica (Reeder et al., 2002). The name *Aspidoscelis* was recently resurrected for the North American clade of *Cnemidophorus* (*sensu lato*), since mtDNA sequences, allozymes, and morphologic data indicated that *Cnemidophorus* is paraphyletic (Reeder et al., 2002), with South American species being more closely related to *Kentropyx* and *Ameiva* than to North American taxa (see also Giugliano et al., 2006; Reeder et al., 2002).

Boulenger (1885) recognized four groups of Teiidae, based on external morphological characters. Group I was characterized by the lack of frontonasals separating the anterior nasals, well-developed limbs, and medium to large body size. This group became known as macroteiids and is currently the sole member of Teiidae (sensu Presch, 1983). Boulenger's groups II, III, and IV are currently recognized as a separate family, Gymnophthalmidae (Presch, 1983), based on osteology (Presch, 1974), external morphology (Vanzolini and Valencia, 1965), karyotype (Gorman, 1970), jaw adductor musculature (Rieppel, 1980), and brain morphology (Northcutt, 1978). Although widely accepted, this division of Boulenger's Teiidae is still debated (Harris, 1985; Myers and Donnelly, 2001).

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The phylogenetic relationships within Teiidae are unclear. Osteological (Presch, 1974, 1983; Veronese and Krause, 1997), external morphological (Vanzolini and Valencia, 1965), and karyotype (Gorman, 1970) data support two monophyletic subfamilies: Teiinae, comprising *Ameiva*, *Aspidoscelis*, *Cnemidophorus*, *Dicrodon*, *Kentropyx*, and *Teius*; and Tupinambinae, comprising *Callopistes*, *Crocodilurus*, *Dracaena*, and *Tupinambis* (Presch, 1983). An analysis of the cranial musculature of teiids (Moro and Abdala, 2000) found little support for the monophyly of Teiinae and Tupinambinae and indicated that Teiidae is monophyletic only after the inclusion of *Pantodactylus* (= *Cercosaura*, sensu Doan, 2003), a gymnophthalmid. A combined analysis of osteological, external morphological, hemipenial, and tongue and sperm ultrastructural data corroborated the monophyly of Teiinae and Tupinambinae if *Callopistes* is transferred to the former (Teixeira, 2003).

Phylogenetic relationships among teiid genera are mostly discordant in previous studies. Chromosomal (Gorman, 1970) and external morphological data (Vanzolini and Valencia, 1965) indicated that *Tupinambis* and *Dracaena* are sister groups. However, osteological data (Presch, 1974) support a close relationship between *Tupinambis* and *Crocodilurus*. In addition, a combined analysis of traditional morphological with sperm ultrastructural data favored a *Crocodilurus* and *Dracaena* grouping (Teixeira, 2003). Within Teiinae, previous works supported one monophyletic group, formed by *Ameiva*, *Cnemidophorus* (and, therefore, *Aspidoscelis*), and *Kentropyx*, the ''cnemidophorines'' (Reeder et al., 2002), whereas relationships among ''cnemidophorines'', *Dicrodon*, and *Teius* are unclear (Gorman, 1970; Presch, 1974; Reeder et al., 2002; Teixeira, 2003; Vanzolini and Valencia, 1965).

Around 16 fossil teiid genera are known from the Cretaceous of North America (Denton and O'Neill, 1995; Estes, 1964; Nydam, 2002; Nydam and Cifelli, 2005; Winkler et al., 1990). Estes (1983) placed three of these genera in extant subfamilies, *Leptochamops* and *Meniscognathus* in Teiinae, and *Chamops* in Tupinambinae, suggesting an old divergence between these groups. However, a phylogenetic analysis including several fossil groups concluded that these three genera form a sister group of the living subfamilies, and should be placed in another subfamily, Chamopsiinae (Denton and O'Neill, 1995). Thus, the oldest fossil of the extant subfamilies is an unnamed teiid from the Paleocene of South America (Denton and O'Neill, 1995; Estes and Báez, 1985). Cretaceous fossils of another group, Polyglyphanodontinae, are known from the Mongolian desert (Gao and Norell, 2000; Sulimski, 1972, 1978) and Romania (Folie and Codrea, 2005), indicating a larger distribution of teiids during this period. Therefore, teiids probably colonized South America close to the K-T transition (Presch, 1974). The oldest fossils of living teiid genera date from the Miocene, including South American *Tupinambis* and *Dracaena* (Estes, 1961; Estes and Báez, 1985; Krause, 1985), and the reappearance of Teiidae in North America, after a long hiatus since the Cretaceous (Estes, 1964; Estes and Báez, 1985). Savage (1966, 1982) advanced that the separation of South and North America between the Late Cretaceous and early Paleocene split the range of the common ancestor of *Ameiva* and *Cnemidophorus*, leading to the differentiation of *Cnemidophorus* in the north and *Ameiva* in the south. The closure of the Panamanian portal during the Pliocene allowed the southward migration of *Cnemidophorus*, and the northward migration of *Ameiva* (Savage, 1966, 1982). A phylogenetic analysis of osteological data indicated that *Cnemidophorus* and *Ameiva* are sister-taxa, corroborating Savage's proposal (Presch, 1974). In summary, previous studies suggest that living teiid genera originated between the Paleocene and the Miocene, when South America was still isolated from Central and North America (Presch, 1974).

Herein, we present a phylogenetic analysis of Teiidae based on 12S and 16S mitochondrial DNA sequences, with molecular dating by Bayesian methods (Kishino et al., 2001; Thorne and Kishino, 2002). We also conducted combined phylogenetic analyses by the addition of morphological and ultrastructural characters from Teixeira (2003). In addition, we propose a new biogeographical scenario for the evolution of Teiidae, based on ancestral areas analyses.

2. Materials and methods

2.1. Samples and DNA sequencing

Eleven teiid species, representing all genera, were selected and *Cercosaura ocellata* (Gymnophthalmidae) was used as outgroup. Analyses were based on 12S and 16S mitochondrial DNA sequences previously published (GenBank—NCBI www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=pubmed) or obtained by us (Table 1). Whole genomic DNA was extracted from liver using DNeasy™ tissue kits (QIAGEN). A fragment of nearly 350 bp of the 12S ribosomal gene and a fragment of nearly 500 bp of the 16S gene were amplified with 12Sa, 12Sb, 16SaR, and 16Sd primers using the same PCR conditions described in Reeder (1995). PCR products were sequenced on an ABI Prism 377 automated DNA sequencer (Applied Biosystems, CA) using DYEnamic™ ET terminator cycle sequencing kit (Amersham Pharmacia Biotech, Sweden), according to manufacturer's instructions. When possible, two individuals of the same species and from the same locality were sequenced to control sequencing contamination and other laboratory errors.

2.2. Sequence alignment and phylogenetic analysis

Sequences were analyzed and edited using BioEdit 5.09 (Hall, 1999). A multiple alignment, based on an optimally criterion (parsimony) of minimal cost of phylogenetic tree, was obtained with MALIGN 2.7 (Wheeler and Gladstein, 1994). Gap costs were assigned for internal gaps (2) and

Table 1
Species, locality, collection, collection number and GenBank accession number

Species	Locality	Collection	Tag	GenBank Accession No.
Ameiva ameiva 1	Peru: Cuzco Amazônico	SBH	267103	12S—AY359473, 16S—AY359493
Ameiva ameiva 2	Peru: Cuzco Amazônico	KU	205000	12S—AY046423, 16S—AY046465
Aspidoscelis gularis	USA: Texas	TNHC	53222	12S—AY046433, 16S—AY046475
Callopistes flavipunctatus	Peru	MHNSM	Not cataloged	12S—EF029873, 16S—EF029880
Callopistes maculatus 1	Chile	MNHN	Not cataloged	12S—EF029874, 16S—EF029881
Callopistes maculatus 2	Chile	MNHN	Not cataloged	12S—EF029875, 16S—EF029882
Cercosaura ocellata	Brazil: Aripuanã-MT	MZUSP	MRT 977406	12S—AF420677, 16S—AF420731
Cnemidophorus ocellifer 1	Brazil: Barra do Garças-MT	MZUSP	MZ 78779	12S—AY218041, 16S—AY217992
Cnemidophorus ocellifer 2	Brazil: Barra do Garças-MT	MZUSP	MRT 946089	12S—AF420706, 16S—AF420759
Crocodilurus amazonicus 1	Brazil: Humaitá-AM	CHUNB	32582	12S—EF029877, 16S—EF029883
Crocodilurus amazonicus 2	Brazil: Humaitá-AM	CHUNB	32614	12S—EF029876, 16S—EF029884
Dicrodon guttulatum	No data	SDSU	3906	12S—AY046453, 16S—AY046495
Dracaena guianensis 1	Brazil: Amapá-AP	CHUNB	15197	12S—EF029879, 16S—EF029886
Dracaena guianensis 2	Brazil: Amapá-AP	CHUNB	15199	12S—EF029878, 16S—EF029885
Kentropyx altamazonica 1	Peru: Loreto;	KU	205015	12S—AY046456, 16S—AY046498
Kentropyx altamazonica 2	Venezuela: Amazonas; Neblina Base Camp on River Mawarinuma	AMNH	R-134175	12S—AY046455, 16S—AY046497
Teius teyou	Argentina: Córdoba	REE	150	12S—AY046461, 16S—AY046503
Tupinambis teguixin	Peru: Madre de Dios	KU	205023	12S—AY046422, 16S—AY046464

SBH—Tissue collection of S. Blair Hedges, Pennsylvania State University; KU—Natural History Museum, University of Kansas; TNHC—Texas Natural History Collection of the Texas Memorial Museum, University of Texas in Austin; MHNSM—Museo de Historia Natural de San Marcos; MNHN—Museo Nacional de Historia Natural (Chile); MZUSP—Museu de Zoologia da Universidade de São Paulo; CHUNB—Coleção Herpetológica da Universidade de Brasília; SDSU—San Diego State University; AMNH—American Museum of Natural History; REE—Private collection of Robert Espinoza; eventually to be deposited at California State University, Northridge.

leading and trailing gaps (1), but equal weight was assigned for transitions and transversions. For maximum parsimony (MP) analyses, characters were equally weighted. Phylogenetic analyses were performed independently for each gene sequence and for combined sequences, using MP with PAUP* v.4.0b10 (Swofford, 1999) and Bayesian methods with MrBayes v.3.0b4 (Huelsenbeck and Ronquist, 2001). For MP analysis, branch-and-bound searches were used with gaps coded as a fifth state (Giribet and Wheeler, 1999). Reliability of MP results was assessed by bootstrap, with 1000 replications (Felsenstein, 1985), and Bremer support (Bremer, 1994), using MacClade 4.0 (Maddison and Maddison, 1999) and PAUP. For both 12S and 16S mitochondrial DNA sequences, the model of sequence evolution was chosen by hierarchical likelihood ratio tests (HLRTs) implemented in Modeltest 3.7 (Posada and Crandall, 1998). For the combined data (12S + 16S), each sequence had its own independent model of evolution and model parameters. As none of the models available in Modeltest 3.7 consider gaps as character states, they were excluded from sequence evolution model analyses and from Bayesian-based phylogenetic analyses. Bayesian analyses started with randomly generated trees and ran for 2.0×10^6 generations. Tree sampling occurred at intervals of 100 generations producing 20,000 trees. We plotted the log-likelihood scores of the 20,000 trees against generation time to detect stationarity. All sample points before stationarity were considered burn-in samples that contained no useful information about parameters. For each analysis, we conducted two independent runs. The frequency of any particular clade in the majority-rule consensus tree of the stationary stage, from the two independent runs, represented the posterior probability of that node (Huelsenbeck and Ronquist, 2001).

2.3. Combined analysis: DNA and morphology

A total evidence phylogeny was produced by the combination of molecular data with 163 morphologic characters assembled by Teixeira (2003). Morphological characters included sperm ultrastructure, osteology, pholidosis, and tongue and hemipenis morphology. Qualitative morphological characters (133) were not ordered in analyses, whereas quantitative characters (30) were gap coded (Thiele, 1993), using 0.5 standard deviation as cut-point, and ordered. Phylogenetic analyses of the combined data were performed as described above, except that, for Bayesian analysis, we chose different models of evolution for morphological character. For qualitative characters, we used a "standard model", which considers equal probability for all character state changes, whereas for quantitative characters we used the "ordered model".

2.4. Molecular dating

We estimated divergence times based on a Bayesian relaxed molecular clock approach, using the MULTIDISTRIBUTE package (Kishino et al., 2001; Thorne and Kishino, 2002; Thorne et al., 1998). This approach allows the incorporation of multiple time constraints, and takes into account both molecular and palaeontological uncertainties to estimate the variance of divergence times. Additionally,

the calibration time is a minimum estimate of divergence time between two clades and an "*a priori* expected number of time units between tip and root" is also required. Hence, divergence times depicted in the resulting chronogram may be higher than those used for calibration. In this analysis, we used the consensus topology found by Bayesian analysis of 12S and 16S mitochondrial DNA sequences. We used the fossil record to provide minimum time constraints at three points in our phylogenetic hypothesis: (1) origin of living lineages of Teiidae at 65 MYA (Estes and Báez, 1985); (2) divergence of *Tupinambis* from other Tupinambinae at 24 MYA (Estes, 1961; Estes and Báez, 1985); (3) origin of "cnemidophorines" (*Ameiva* + *Cnemidophorus* + *Kentropyx*) at 5 MYA (Estes, 1964). We used the oldest *Cnemidophorus* fossil to calibrate "cnemidophorines" because there are no derived osteological characters to distinguish among the three genera (Vanzolini and Heyer, 1985). In addition, Estes (1964) recognized that it is not possible to conclude, based on morphology, whether this fossil belongs to *Ameiva* or *Cnemidophorus*, placing the fossil in *Cnemidophorus* only due to the current absence of *Ameiva* in the region were the fossil was found.

2.5. Dispersal-vicariance analysis

We inferred ancestral areas based on parsimony, using DIVA 1.1 (Ronquist, 1997). This method searches for optimal ancestral areas that minimize dispersal and extinction events (Ronquist, 1997). We used in the analysis four large areas, separated by major geographic barriers (Andes and the Caribbean Sea) during the Tertiary, which played fundamental roles in the diversification of the South American herpetofauna (Colli, 2005; Vanzolini and Heyer, 1985): (1) trans-Andean South America, (2) cis-Andean South America, (3) West Indies, and (4) North and Central America.

3. Results

3.1. Molecular phylogeny

Three equally parsimonious alignments were found for 12S, with slight differences among them and only one most parsimonious alignment was found for the 16S region (TreeBase accession number 15446). Phylogenetic analyses were carried out for the three 12S region alignments and for the three possible combinations with 16S. Nevertheless, all trees presented the same topology with small differences in bootstrap indices and Bremer support (results not shown). Thus, only one 12S alignment was chosen in the following analyses (Appendix I). The likelihood ratio test implemented in Modeltest favored the TrN + *I* + *G* (Tamura–Nei model (Tamura and Nei, 1993), *I*, invariable sites; *G*, gamma distribution) model of sequence evolution for both 12S and 16S. The inferred base frequencies, the ratio of invariable sites and the gamma distribution parameter are shown in Table 2.

Table 2
Parameters of molecular substitution model found by ModelTest to 12S and 16S regions

DNA region	Base frequencies	Substitution frequency	Gamma distribution (*G*)	Ratio of invariable sites (*I*)
12S	A = 0.3694 C = 0.2584 G = 0.1352 T = 0.2370	A-C = 1.0000 A-G = 4.3311 A-T = 1.0000 C-G = 1.0000 C-T = 8.5475 G-T = 1.0000	1.2436	0.5199
16S	A = 0.3706 C = 0.2663 G = 0.1422 T = 0.2209	A-C = 1.0000 A-G = 2.6354 A-T = 1.0000 C-G = 1.0000 C-T = 7.0218 G-T = 1.0000	0.4259	0.4126

The multiple alignments for 12S generated a fragment of 349 bp with 125 informative characters. The unweighted branch-and-bound search resulted in one most parsimonious tree (Fig. 1a) with 408 steps (CI = 0.600, RI = 0.702). Three well-supported monophyletic groups were found: (1) *Callopistes* as the basal group; (2) a clade formed by *Crocodilurus*, *Tupinambis*, and *Dracaena*; (3) a clade formed by *Dicrodon*, *Teius*, *Kentropyx*, *Ameiva*, *Aspidoscelis*, and *Cnemidophorus* (Teiinae). The consensus tree obtained by Bayesian analysis under the TrN + *I* + *G* model of evolution was consistent with the MP tree, containing the same three well-supported groups but with less resolution (Fig. 1b).

For the 16S gene, a 458 bp fragment was obtained with 136 informative characters. One most parsimonious tree with 434 steps was found (CI = 0.590, RI = 0.732, Fig. 2a), with much incongruence relative to the 12S results (Fig. 1). Two well-supported groups, representing Teiinae and Tupinambinae, were obtained. In the MP analysis based on 12S, *Callopistes* formed a more basal group, but the 16S results placed the genus in a well-supported clade with *Crocodilurus*, *Tupinambis*, and *Dracaena*, corroborating the monophyly of Tupinambinae. The majority-rule consensus of Bayesian analysis under the TrN + *I* + *G* model had a similar topology (Fig. 2b), the only difference being the relationship among *Ameiva*, *Cnemidophorus*, *Aspidoscelis*, and *Kentropyx*, which were weakly supported with both methods.

MP analysis of the combined data (12S + 16S) resulted in one most parsimonious tree (Fig. 3a) with 859 steps (CI = 0.583, RI = 0.704). The MP tree corroborated the monophyly of Tupinambinae, as in the 16S analyses. The topology of this tree is consistent with the MP tree showing the same two well-supported groups. The Bayesian analyses resulted in a similar topology (Fig. 3b). The only difference concerns the relationship among *Tupinambis*, *Crocodilurus*, and *Dracaena*, which were poorly resolved in both methods. As the Bayesian-based phylogeny did not consider gaps as character states, we performed a

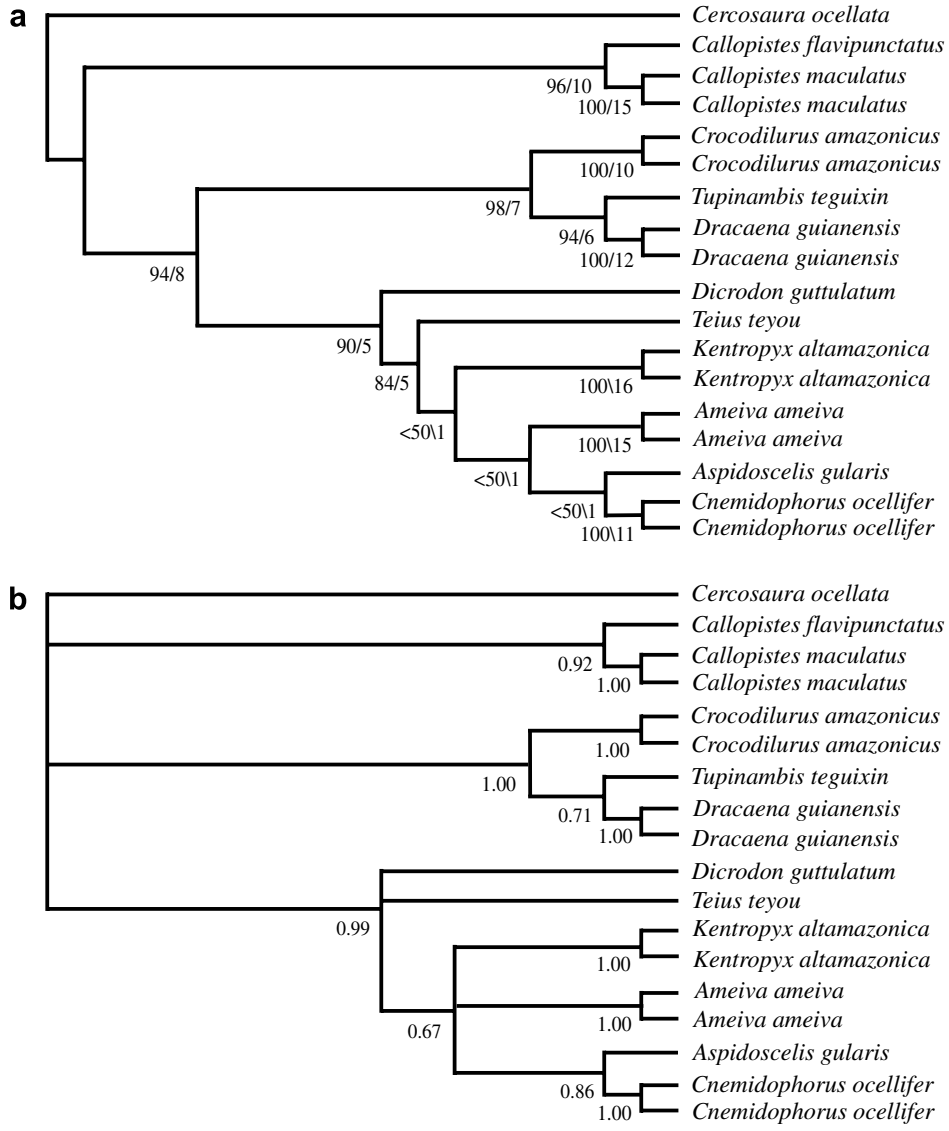

Fig. 1. Teiid phylogeny inferred from 12S sequences. (a) Most parsimonious tree, with bootstrap and Bremer support values, respectively. (b) Tree inferred by Bayesian analysis using the TrN + I + G model, with posterior probability values.

parsimony-based analysis excluding gaps. The results were fully congruent with the parsimony-based analysis considering gaps (results not shown).

3.2. Total evidence: DNA and morphology

The combined data included 807 molecular and 163 morphological characters, with 332 informative characters. The MP analysis produced a single most parsimonious tree with 1162 steps (CI = 0.633, RI = 0.475, Fig. 4a), whereas the Bayesian analysis produced pretty much the same topology (Fig. 4b). Both the MP and the Bayesian trees presented two major clades, corresponding to Teiinae (strongly supported) and Tupinambinae (weakly supported). The only incongruence between the MP and the Bayesian trees regarded the relationships within a group containing *Crocodilurus*, *Dracaena*, and *Tupinambis*, even though its monophyly was strongly supported (Fig. 4).

3.3. Molecular dating

The molecular dating analysis indicated (1) an early divergence of *Callopistes* during the Paleocene; (2) the divergence of most living genera, including *Dicrodon*, *Kentropyx*, *Ameiva*, *Tupinambis*, *Crocodilurus*, and *Dracaena*, during the Eocene/Oligocene; (3) an early divergence of the two species of *Callopistes* during Oligocene; (4) the divergence of the clade *Cnemidophorus* + *Aspidoscelis* at the late Oligocene; and (5) the divergence of *Cnemidophorus* and *Aspidoscelis* at the Miocene (Fig. 5).

3.4. Dispersal-vicariance analysis

Two equally most parsimonious reconstructions, with six ingroup dispersals, were implied by Diva (Fig. 6). The only difference between the two scenarios is the area of the common ancestor of *Cnemidophorus* and *Aspidoscelis*.

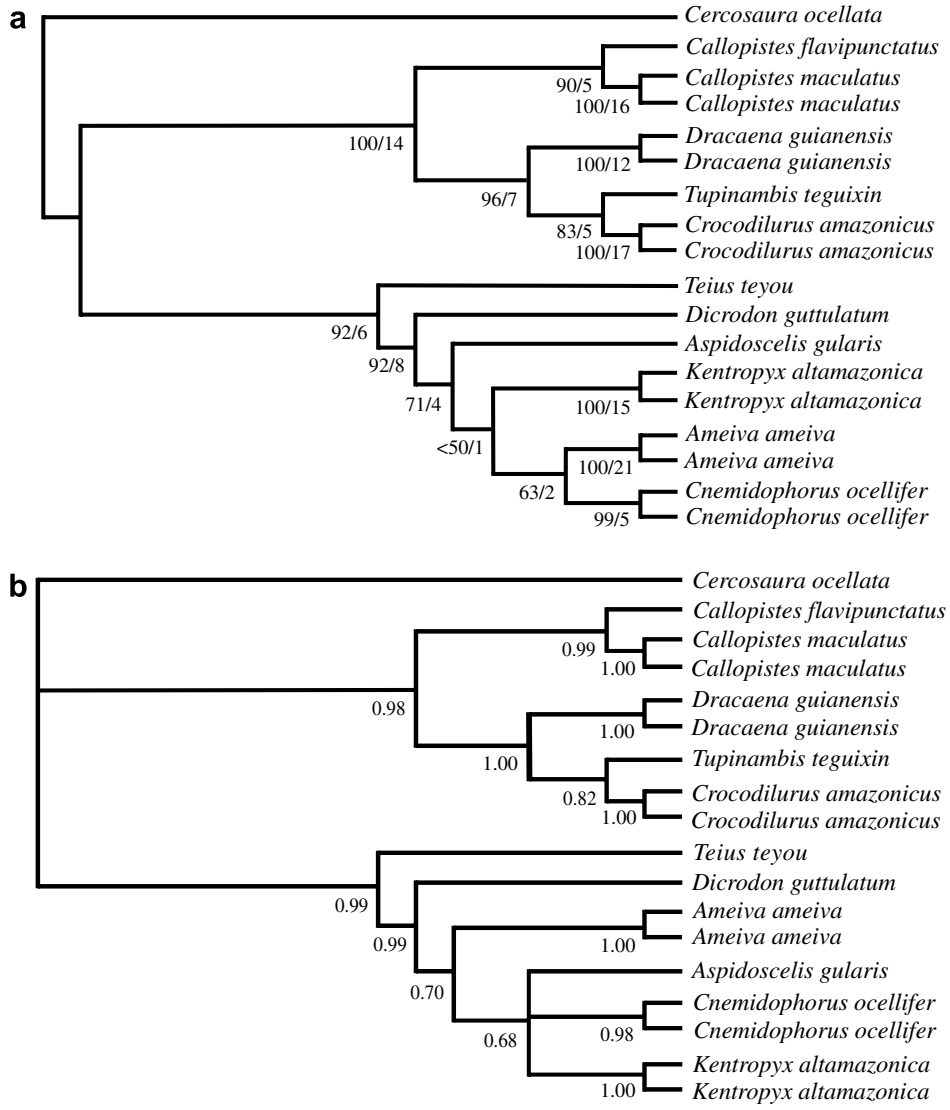

Fig. 2. Teiid phylogeny inferred from 16S sequences. (a) Most parsimonious tree, with bootstrap and Bremer support values, respectively. (b) Tree inferred by Bayesian analysis using the TrN + I + G model, with posterior probability values.

In the first reconstruction (Fig. 6a), this ancestral area includes North and Central America and cis-Andean South America, whereas in the second reconstruction this ancestral area also includes the West Indies (Fig. 6b). In both scenarios, cis-Andean South America is the ancestral area of *Aspidoscelis* + *Cnemidophorus* and the "cnemidophorines" (Fig. 6). Both reconstructions also indicate that *Callopistes* and *Dicrodon* (trans-Andean genera) originated by vicariance.

4. Discussion

4.1. Phylogenetic relationships among teiids

The total evidence phylogeny, based on morphological and molecular data, supported the monophyly of Teiinae, in both the MP or Bayesian analyses (Fig. 4). Conversely, Tupinambinae was weakly supported and incongruent with the 12S analysis. This incongruence and low support apparently resulted from the different placements of *Callopistes* in analyses based on 12S, 16S, and morphology (Teixeira, 2003) (Figs. 1 and 2). *Dracaena*, *Tupinambis*, and *Crocodilurus* formed a well-supported monophyletic group in all analyses.

The monophyly of Tupinambinae and Teiinae was supported by the combined 12S and 16S data (Fig. 3) and the combined morphological and molecular data (Fig. 5), with Teiinae presenting high branch support in all analyses. These results are in agreement with many previous studies (Gorman, 1970; Presch, 1974, 1983; Rieppel, 1980; Vanzolini and Valencia, 1965; Veronese and Krause, 1997). The phylogenetic tree obtained by Teixeira (2003), based on morphological data, differed from the results of our total evidence analysis using Bayesian methods only in the position of *Callopistes* (Fig. 5b). Teixeira (2003) concluded that *Callopistes* is closely related to Teiinae and should be

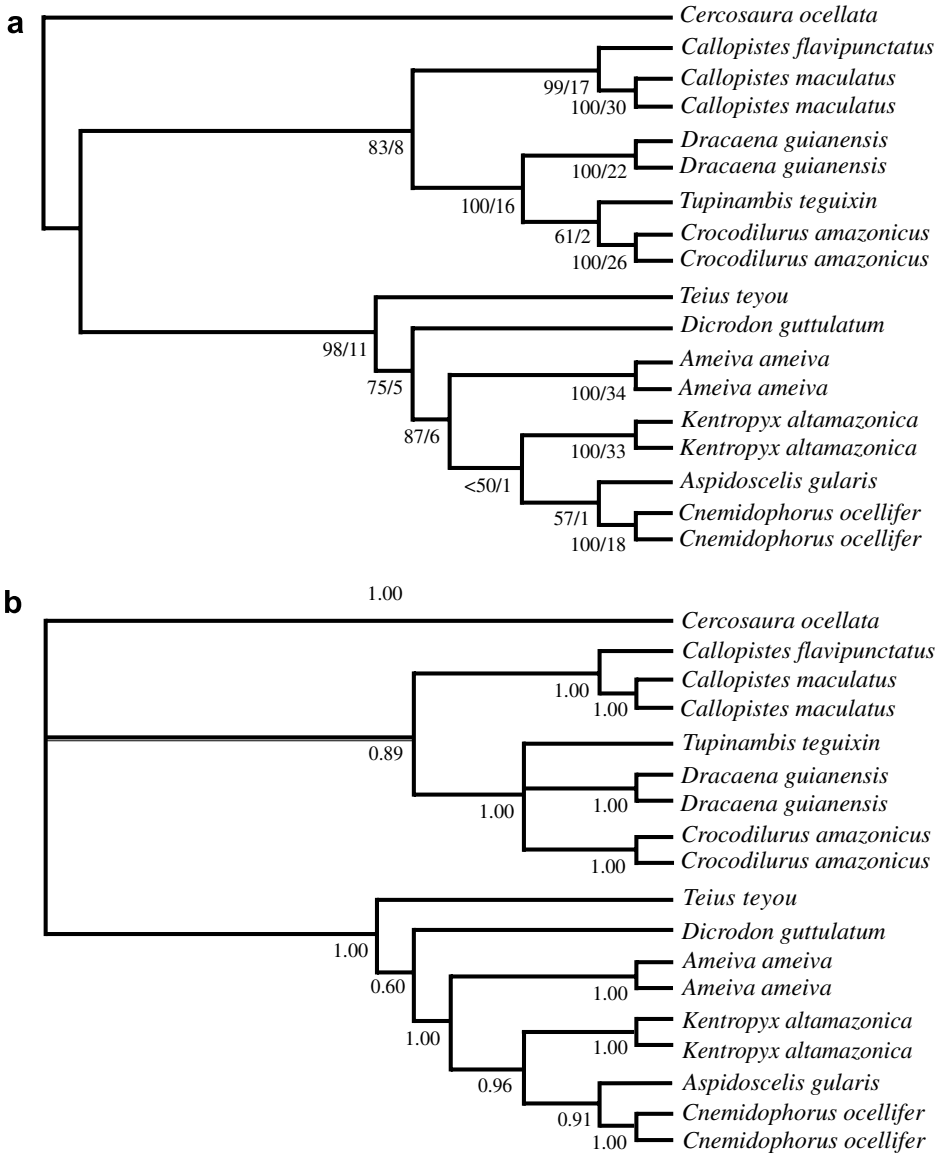

Fig. 3. Teiid phylogeny inferred from combined 12S and16S sequences. (a) Most parsimonious tree, with bootstrap and Bremer support values, respectively. (b) Tree inferred by Bayesian analysis using the TrN + I + G model, with posterior probability values.

reallocated. However, the placement of the *Callopistes* in her analysis was weakly supported, with a bootstrap value of 61%. Our results do not support this reallocation.

Within Tupinambinae, both the combined 12S + 16S, and the combined morphological and molecular analyses based on MP (Figs. 3, 4a) indicated the configuration (*Callopistes* (*Dracaena* (*Tupinambis*, *Crocodilurus*))). However, the combined 12S + 16S Bayesian analysis resulted in a polytomy (Fig. 3b), whereas the total evidence Bayesian analysis indicated a closer relationship between *Crocodilurus* and *Dracaena* (Fig. 4b). Our results support a sister position of *Callopistes* to other genera within Tupinambinae, in agreement with Presch (1974). Nevertheless, the relationships among *Dracaena*, *Tupinambis*, and *Crocodilurus* could not be satisfactorily resolved. The close relationship between *Crocodilurus* and *Dracaena* supported by the morphological data can possibly be due to convergence, since two character states that support this clade are related to their semi-aquatic life-style (Mesquita et al., 2006): presence of laterally compressed tail and dorsal tail cristae (Teixeira, 2003). The addition of more data, either molecular or morphological, should assist in resolving the relationships among *Crocodilurus*, *Dracaena*, and *Tupinambis*.

Within Teiinae, the combined 12S + 16S data (Fig. 3) and the total evidence data set (Fig. 5) resulted in the following configuration (*Teius* (*Dicrodon* (*Ameiva* (*Kentropyx* (*Aspidoscelis*, *Cnemidophorus*))))), corroborating the close relationship among *Ameiva*, *Kentropyx*, and *Cnemidophorus* (including *Aspidoscelis*) suggested in previous works (Gorman, 1970; Presch, 1974, 1983; Teixeira, 2003; Vanzolini and Valencia, 1965). It should be noted that *Ameiva* and *Cnemidophorus* are probably paraphyletic (Giugliano

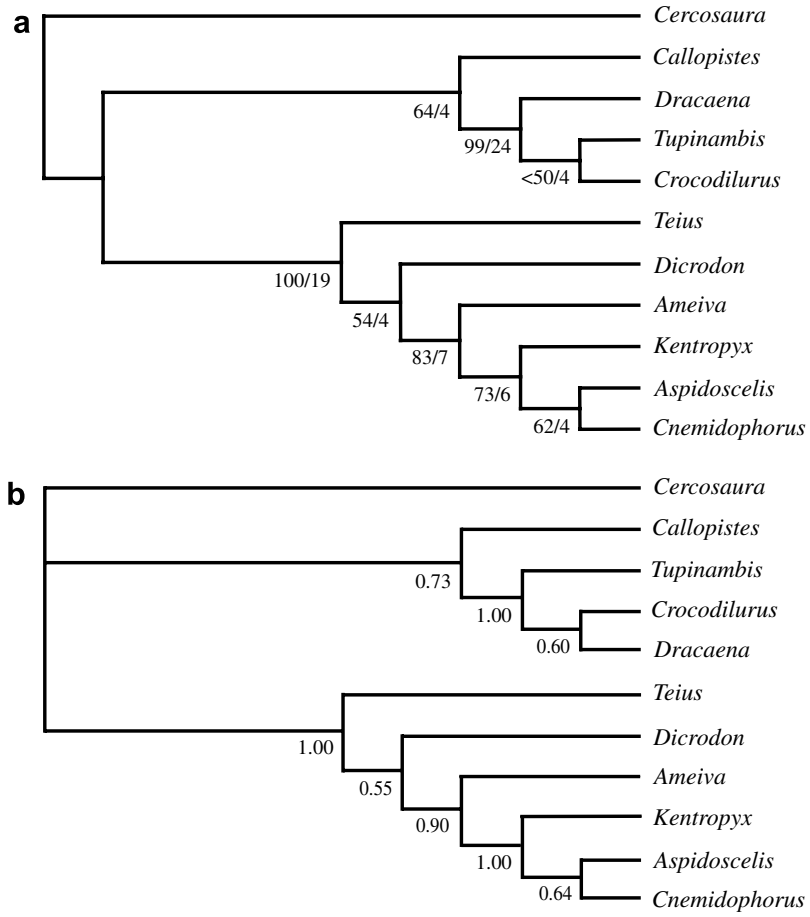

Fig. 4. Teiid phylogeny inferred from combined molecular (12S + 16S) and morphological data. (a) Most parsimonious tree, with bootstrap and Bremer support values, respectively. (b) Tree inferred by Bayesian analysis using the TrN + I + G model, with posterior probability values.

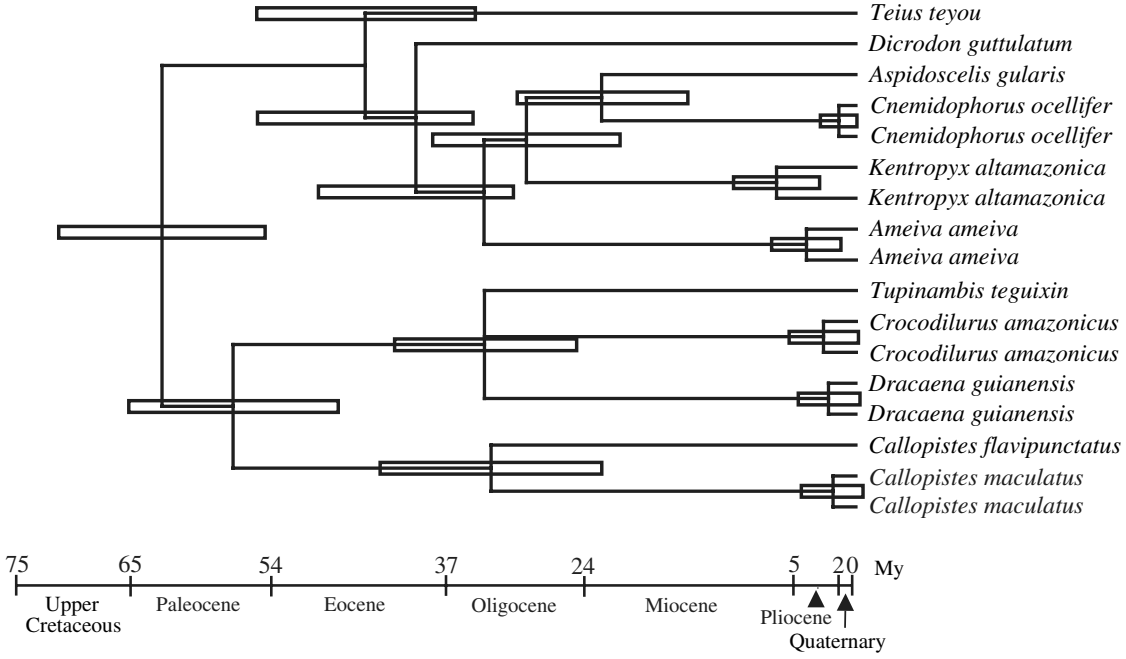

Fig. 5. Cronogram of teiid evolution based on the combined molecular data (12S + 16S), with divergence times estimated from a Bayesian relaxed molecular clock approach. Boxes indicate mean divergence time ± one standard deviation.

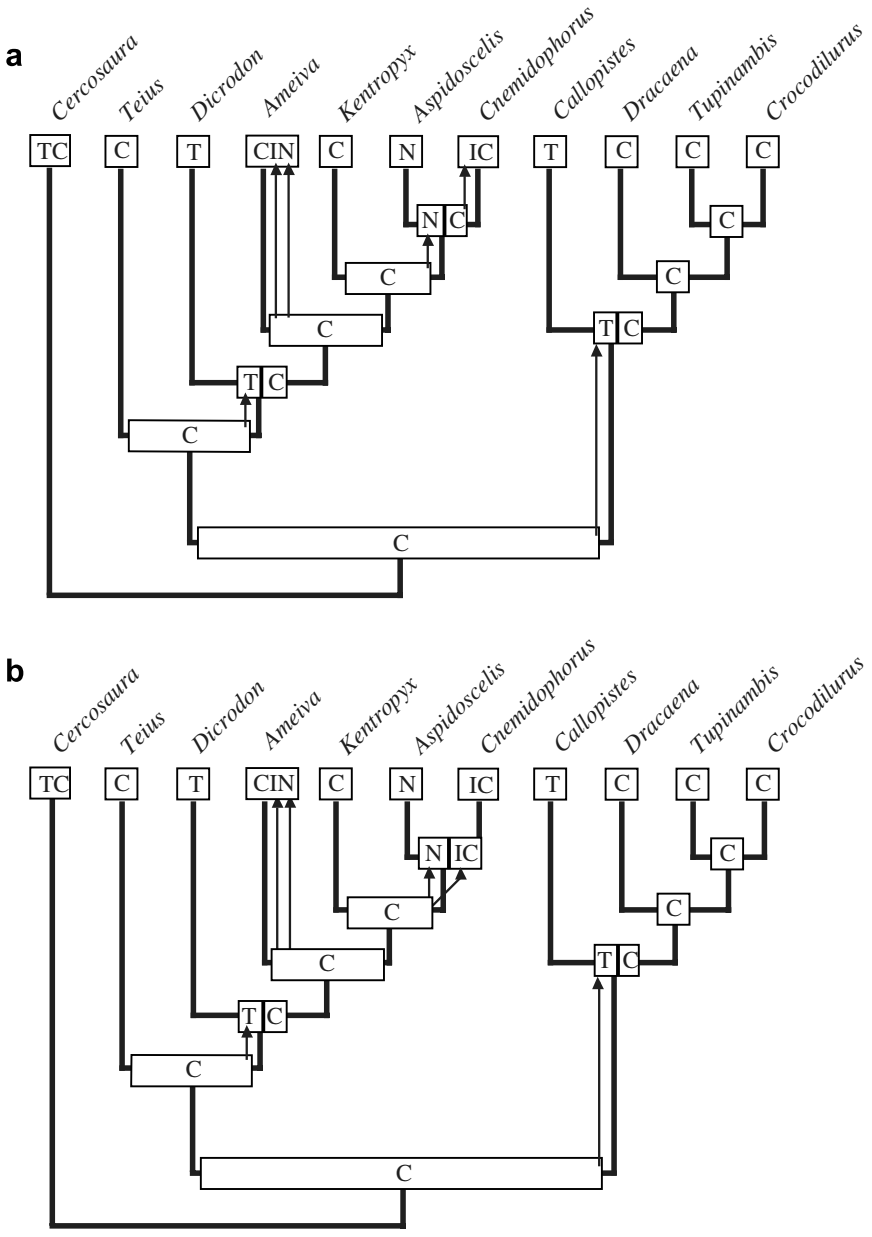

Fig. 6. Four major geographic areas used in the dispersal-vicariance analysis and reconstructed ancestral distributions for each node on the two most parsimonious solution obtained (a and b), based on the total evidence (molecules + morphology) analysis of teiids. Arrows indicate dispersals and vertical bars indicate vicariance events. C: cis-Andean South America, I: West Indies, N: Central and North America, T: trans-Andean South America.

et al., 2006; Reeder et al., 2002) and, therefore, differences in phylogenetic relationship among different studies can be influenced by the choice of taxa.

4.2. Evolution of teiids

Based on our molecular dating (Fig. 4), the dispersal-vicariance analysis (Fig. 6), the fossil record (Estes and Báez, 1985; Gao and Fox, 1996; Krause, 1985), the distribution of living genera (Krause, 1985), and environmental and geological changes during the Tertiary (Colli, 2005; Iturralde-Vinent and MacPhee, 1999; Ortiz-Jaureguizar and Cladera, 2006), we propose the following scenario for the evolution of Teiidae. The oldest teiid fossil dates back to the early Cretaceous of North America (Nydam, 2002; Winkler et al., 1990), whereas the oldest fossil representing living lineages is from the Paleocene of South America (Albino, 1996; Denton and O'Neill, 1995; Estes and Báez, 1985). This suggests a southward dispersal of the group during the late Cretaceous (Denton and O'Neill, 1995; Nydam, 2002; Presch, 1974), probably through the Cretaceous Volcanic Arc, which briefly connected North and South America during the late Campanian and early Maastrichtian (Iturralde-Vinent and MacPhee, 1999).

Our molecular dating indicates an early origin of *Callopistes* during the Paleocene, which presumably is associated

with the incongruence between our results (12S in relation to the others sets) and those of Teixeira (2003) in the placement of this genus. Our dispersal-vicariance analysis also indicates that *Callopistes* diverged by vicariance, because the ancestral area of Tupinambinae includes trans- and cis-Andean South America, whereas *Callopistes* is restricted to trans-Andean South America (Fig. 6). This vicariance event may be related with the ''Salamancan Sea'', a Paleocene marine transgression that covered a great portion of South America, from Patagonia to Bolivia and Peru, isolating the southwestern and northeastern parts of the continent (Ortiz-Jaureguizar and Cladera, 2006; Sylwan, 2001).

According to our analyses, most living genera of Teiidae originated during the Eocene/Oligocene (Fig. 5), epochs characterized by a global cooling and desiccation of the climate, with the expansion of savanna formations in South America (Ortiz-Jaureguizar and Cladera, 2006; Romero, 1993). The divergence between the two species of *Callopistes* (*C. maculatus* from Chile and *C. flavipunctatus* from Peru) occurred in the Oligocene. During this epoch, the present configuration of the Andes of central Chile began to develop and a new marine transgression (''Patagonian Sea'') occurred in the same region covered by the ''Salamancan Sea'' (Ortiz-Jaureguizar and Cladera, 2006). This old divergence may explain the large morphological differences between the two species.

Our molecular dating (Fig. 4) indicates that *Cnemidophorus* and *Aspidoscelis* diverged during the Miocene. However, since *Cnemidophorus* is paraphyletic, these results should be interpreted with caution. Previous studies suggest that the common ancestor of *Ameiva* and *Cnemidophorus* became isolated during the Cenozoic due to the separation between North and South America, with *Ameiva* originating in the south and *Cnemidophorus* in the north (Presch, 1974; Savage, 1966, 1982). This proposal was mainly based on (1) the distribution of living genera, which lead to the assumption that the center of origin of *Cnemidophorus* is North or Central America (Savage, 1966, 1982), (2) phylogenetic analyses where *Ameiva* and *Cnemidophorus* appear as sister groups (Presch, 1974), and (3) the presence of fossil *Cnemidophorus* in Miocene–Pliocene deposits of North America (Estes, 1964). This proposal implies that the ancestor of *Cnemidophorus* and *Aspidoscelis* migrated to North America during the Late Cretaceous (Presch, 1974), differentiated, and returned to South America after the re-establishment of the Panamanian Isthmus (Presch, 1974; Savage, 1966). However, our evolutionary scenario indicates (1) a much later origin of *Cnemidophorus* + *Aspidoscelis*, after the separation of North and South America (Fig. 5); (2) a divergence between *Cnemidophorus* and *Aspidoscelis* before the reconnection of the Panamanian Isthmus, during the Pliocene (Fig. 5); (3) that the sister group of *Cnemidophorus* + *Aspidoscelis* is *Kentropyx* (strict South-American group), and not *Ameiva* (Figs. 3 and 4); and (4) that the ancestral area of the lineage *Cnemidophorus* + *Aspidoscelis* is restricted to South America (Fig. 6). Thus, our results indicate a South American origin of *Cnemidophorus* + *Aspidoscelis*, after which some lineage (possibly close to *C. longicaudus*) migrated to North America during the early Miocene. The presence of a Miocene–Pliocene fossil of *Cnemidophorus* (or *Aspidoscelis*) from Nebraska (USA) (Estes, 1964) agrees with this assertive. It should be stressed that this single specimen was lost and its classification was solely based on an illustration and previous notes (Estes, 1964). Except for this single specimen, there are no other fossils of Teiidae from the Tertiary of North America.

The assumption of a *Cnemidophorus* + *Aspidoscelis* center of origin in North America is flawed, because it exclusively relies on the large number of species currently living in this region, ignoring the phylogenetic relationship of the group (Bremer, 1992; Humphries and Parenti, 1999; Ronquist, 1997). Given that *Cnemidophorus* and *Ameiva* are paraphyletic (Giugliano et al., 2006; Reeder et al., 2002), further work on the phylogenetic relationship among ''cnemidophorines'' is necessary to clarify the evolution and historical biogeography of the group.

Therefore, the published evidence and our results (Figs. 5 and 6) indicate an early dispersal of the ancestor of *Aspidoscelis* to North America, before the reconnection of the Panamanian Isthmus. This could have been accomplished by two means: island hopping or over water dispersal. GAARlandia, a structure formed by the Greater Antilles and the Aves Ridge, which connected to South America during the Eocene–Oligocene transition (Iturralde-Vinent and MacPhee, 1999), could have facilitated the northward dispersal of South American elements. However, our analyses indicate an early Miocene divergence of *Aspidoscelis*, after the subsidence of GAARlandia. Hedges (1996) suggested that most nonvolant vertebrate fauna arrived in the West Indies by over water dispersal from South America, based on molecular estimates of divergence times between island taxa and their mainland counterparts. In addition, the divergence between *Ameiva ameiva* and its island congeners was estimated around 27 MYA, after the subsidence of GAARlandia, thus favoring the hypothesis of over water dispersal (Hower and Hedges, 2003).

Our results showed that Tupinambinae and Teiinae are monophyletic, in agreement with Presch (1983); and that *Callopistes* was the first living tupinambine to diverge, probably during the Paleocene. Additionally, within Teiinae, ''cnemidophorines'' are monophyletic, in agreement with most previous studies (Gorman, 1970; Presch, 1974, 1983; Teixeira, 2003; Vanzolini and Valencia, 1965) and *Teius* was the first member to diverge. Molecular dating and biogeography analysis indicate that all living genera, except *Aspidoscelis*, originated in isolation in South America and most living genera diversified during the Eocene, coinciding with the expansion of savannas in South America. *Cnemidophorus* may have originated in South America, and some of its populations (ancestor of *Aspidoscelis*) may have migrated northward between the Oligocene and Miocene.

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