Molecular phylogeny, biogeography and insights into the origin of parthenogenesis in the Neotropical genus *Leposoma* (Squamata: Gymnophthalmidae): Ancient links between the Atlantic Forest and Amazonia

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**A B S T R A C T**

*Leposoma* is a conspicuous component of leaf litter herpetofauna of South and Central American rainforests. The 15 bisexual and one parthenogenetic species are allocated to the *parietale* and *scincoides* groups based on morphology. Phylogenetic analyses of 1830 bp (mtDNA + nuclear) were performed on 63 specimens of four species from Amazonian and Panamanian rainforests, and six species and one undescribed form from the Atlantic Forest. Different methods of tree reconstruction were explored, with *Anotosaura vanzolinia* and *Colobosauroidea cearensis* as outgroups. The monophyly of the *parietale* and *scincoides* groups is strongly supported. Contrary to previous hypotheses suggesting a recent contact between Atlantic and Amazon forests, our estimates point to an initial split in Miocene. The position of *Leposoma baturitensis*, endemic to relictual forests in the semiarid Caatingas northeastern Brazil, and its divergence from the remaining species of the Atlantic Forest, suggests an ancient isolation with no indication of a secondary contact with forests of the eastern coast. Our data do not permit unambiguous assignment of parental species of the unisexual *Leposoma percarinatum* or the mechanism involved in the origin of parthenogenesis, but revealed two highly divergent diploid and triploid lineages within *L. percarinatum*, indicating that the unisexuals represent a species complex.

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1. Introduction

The small lizards of the genus *Leposoma* (Gymnophthalmidae) represent a conspicuous component of the leaf litter herpetofauna of tropical rainforests from Costa Rica throughout Amazonia to the Atlantic Forest of eastern Brazil (Rodrigues, 1997; Rodrigues and Borges, 1997; Rodrigues et al., 2002a,b; Rodrigues and Ávila-Pires, 2005). Ruibal (1952) was the first to review the genus, and recognized two distinct species complexes on the basis of external morphology: the *parietale* and *scincoides* groups. Currently 16 species are recognized in the genus (Uzzell and Barry, 1971; Ayala and Harris, 1982; Ávila-Pires, 1995; Rodrigues, 1997; Rodrigues and Borges, 1997; Rodrigues et al., 2002a,b; Esqueda, 2005; Rodrigues and Ávila-Pires, 2005).

The *parietale* group ranges from Amazonia to Central America and includes 10 bisexual species (*hexalepis*, *ioanna*, *rugiceps*, *southeri*, *parietale*, *guianense*, *osvaldi*, *snelthageae*, *caparensis* and *ferreirai*), and the unisexual *Leposoma percarinatum* (*Uzzell and Barry, 1971; Hoogmoed, 1973*). The most obvious diagnostic features for this group include the presence of short and wide dorsal and ventral scales arranged in regular longitudinal rows. The *scincoides* group includes five bisexual species, four of which (*Leposoma scincoides*, *Leposoma annectans*, *Leposoma nanodactylus*, and *Leposoma puk*) are restricted to the formerly continuous strip of Atlantic Forests of eastern Brazil, and *Leposoma baturitensis*, which is endemic to an isolated forested mountain range in the semiarid Caatingas of the state of Ceará, northeastern Brazil (Rodrigues, 1997; Rodrigues and Borges, 1997; Rodrigues et al., 2002a,b). This group is diagnosed by narrower, elongated dorsal scales and lanceolate ventrals that are arranged in diagonal rather than longitudinal rows.
Species of Leposoma were first genetically surveyed on the basis of chromosome studies carried out on Leposoma guianense and Leposoma osvaldoi from the Brazilian Amazon, and on L. scincoides from the Atlantic Forest (Pellegrino et al., 1999). This study revealed highly distinctive genomes; a \(2n = 44\) karyotype, with a clear-cut distinction between macro- and microchromosomes (20M + 24m), in the two Amazon species, whereas a \(2n = 52\) karyotype with gradual decrease in chromosome size characterizes L. scincoides. This conspicuous karyotypic difference, coincident with Ruibal’s (1952) morphological groupings, was inferred to be the result of Robertsonian rearrangements and pericentric inversions (Pellegrino et al., 1999). Later, Pellegrino et al. (2003) found a \(3n = 66\) (30M + 36m) karyotype in the unisexual *L. percarinatum* from Vila Rica (Brazilian state of Mato Grosso), and hypothesized that the triploid genome originated from a hybridization event between a bisexual species with a *L. guianense*/L. osvaldoi-like karyotype (\(n = 22, 10M + 12m\)), and an as-yet undiscovered unisexual diploid cryptic form of *L. percarinatum*.

Rodrigues and Ávila-Pires (2005) reported the occurrence of three sympatric species of the parietale group in the Arquipelago das Anavilhanas, State of Amazonas, in the lower Rio Negro area, Brazil: *L. percarinatum*, *L. guianense*, and a new bisexual species *Leposoma ferreirai*, this latter species was indicated as a putative parental species of the unisexual *L. percarinatum*. Based on the coexistence of *L. ferreirai*, *L. guianense* and *L. percarinatum* in the lower Rio Negro area, the authors suggested that one possible origin for the diploid form of *L. percarinatum* would be the hybridization between *L. guianense* and *L. ferreirai*, whereas the triploid (Pellegrino et al., 2003) would represent a further hybridization between the unisexual diploid form and *L. osvaldoi*, since *L. guianense* does not occur that far south.

The most recent cytogenetic study that included specimens of *L. percarinatum* and *L. ferreirai* from Anavilhanas, Amazon, corroborated some of those previous hypotheses (Laguna et al., 2010). A very similar diploid karyotype of \(2n = 44\) (20M + 24m) was found for the sympatric *L. percarinatum* and *L. ferreirai* in Anavilhanas, revealing the existence of the previous hypothesized diploid cryptic form of the unisexual *L. percarinatum*, and its contribution to the origin of the triploid lineage (\(3n = 66, 30M + 36m\)). Although there are no data on karyotypes for the type locality of *L. percarinatum* (Peixe-Boi, state of Para, Brazil) and the geographic distributions of the diploid and triploid parthenoforms are poorly known, the authors assumed that the unisexual *L. percarinatum* represent a complex of species and argued the need of further taxonomic work to resolve the nomenclature of this complex (Laguna et al., 2010).

Beyond the question of the origin of parthenogenesis in the *percarinatum* complex, species of *Leposoma* present interesting evolutionary questions related to their ecological fidelity to the Atlantic and Amazonian rainforests. Contrary to several other lizards sympatric with *Leposoma* in both forests, but that also occur in the drier Central Brazilian Cerrados (e.g. the sphaeoactydil *Coleodactylus*, gymnophthalmid *Cercosaura*, and the leioosaurid *Enyalus*), *Leposoma* has never been recorded from the Cerrados, despite intensive field sampling (Costa et al., 2007) and the presence of putatively favourable habitats in the region (extensive gallery forests). However, *L. baturitensis* occurs in the relicual forests of Serra de Baturité, Ceará, northeastern Brazil, in the core of the semiarid Caatingas. These areas, locally referred to as “brejos”, are geographically intermediate between Amazonia and Atlantic forests and harbour species either with Amazonian or Atlantic Forest relationships (Vanzolini, 1981; Rodrigues, 1990). Considering that *Leposoma* cannot cross the Caatingas, its presence in the isolated Serra do Baturité has been explained by the existence of a putative Quaternary corridor linking Amazonian and Atlantic Forests across the Caatingas (Ab’Saber, 1969, 1974; Jackson, 1978; Haffer, 1979; Vanzolini, 1981; Andrade-Lima, 1982; Vanzolini and Williams, 1970, 1981; Rodrigues and Borges, 1997; Rodrigues et al., 2002b).

Alternatively, either an eastern extension of the Amazon forest or an expansion of the Atlantic Forest to the west followed by retraction and differentiation, could explain this pattern without recourse to a complete forest bridge. In Amazonia, an east–west pattern of differentiation has been also observed in several biogeographic studies based on species distributions (Ávila-Pires, 1995; da Silva and Oren, 1996; Bates et al., 1998; Ron, 2000; Hall and Harvey, 2002). These same patterns were also identified in recent molecular phylogenetic studies (Glor et al., 2001; Chek et al., 2001; Symula et al., 2003; Kronauer et al., 2005; Funk et al., 2007; Gamble et al., 2008).

This classical and popular refuge hypothesis was also used in a broader scale to explain diversification within the Amazonian and Atlantic rainforests (Haffer, 1969; Vanzolini and Williams, 1970; Moritz et al., 2000; Noonan and Wray, 2006; Cabanne et al., 2008). However, further palæoecological evidence has generated an ongoing debate about its veracity, leading to alternative hypotheses to explain Neotropical diversification including those based on palaeoecographical reorganizations (e.g. continental drift and Andean orogeny; Rull, 2008), or gradient models (see review in Moritz et al., 2000). Although originally proposed for Amazonia, the refuge hypothesis has recently been also tested for the Atlantic coastal forest on the basis of combined paleoclimatic modelling of species distributions and comparative phylogeographic inferences of Pleistocene refugia from genetic data (Carnaval and Moritz, 2008; Carnaval et al., 2009; Thomé et al., 2010).

The Pleistocene refuge hypothesis suggests that during dry and cold periods forested areas contracted, whereas in presumably moist and warm climatic phases these areas expanded to their present positions (Müller, 1973; Jackson, 1978; Haffer, 2001). These habitat shifts were the drivers of speciation processes both for South American open and forest taxa (Vanzolini and Williams, 1981; Haffer and Prance, 2001). Although originally the timing of most speciation events associated with the refuge theory was credited to the Quaternary, recent advances in molecular dating techniques have shown that most speciation events occurred earlier, generally from the Oligocene to the Miocene (e.g. Geurgias et al., 2008; Gamble et al., 2008; Werneck et al., 2009). In a similar vein, the timing of differentiation for the Atlantic Forest lizards *Gymnodactylus darwini* was associated with the Miocene, but hypothesized to be driven by a river barrier (Pellegrino et al., 2005). Further, the major vicariance events including shifts in the course of the São Francisco river (Bahia, Brazil) in the tropidurid genus *Eurolophosaurus*, seemed to have occurred also during the Pliocene and the Miocene (Passoni et al., 2008).

In this context, DNA based phylogenies represent a promising tool to investigate the historical reconstruction of highly diverse biomes and the timing of diversification of extant taxa, along with data on palaeoecology and landscape history (Moritz et al., 2000; Rull, 2006, 2008). The genus *Leposoma*, characterized by relatively high species diversity, clear morphological and karyotypic gaps between the *parietale* and *scincoides* groups, presence of unisexual species with diploid and triploid lineages, and species with relictual distributions indicative of a complex biogeographic history, is a perfect group for these kinds of studies. In this study we used DNA sequences from species and populations of *Leposoma* to: (1) test the monophyly of the two species groups originally recognized by Ruibal (1952) and present a phylogenetic hypothesis for the genus; (2) determine the affinity of *L. baturitensis*; and (3) determine the phylogenetic position of the unisexual *L. percarinatum*, and thereby gain some insight into its origin. Ultimately, this work may contribute to the understanding of the historical biogeography of South American rainforests.
2. Materials and methods

2.1. Taxon sampling

Sixty-three lizards assigned to five species from the *parietale* species group and six (including one not formally described) species from the *scincoides* group were sampled. Table 1 summarizes the information about number of specimens and populations from Panamanian, Amazonian and Atlantic rainforests, their respective localities (Fig. 1), and the gene regions successfully sequenced for each individual. The gymnophthalmids *Anotosaura vanzolinia* and *Colobosauroides cearenis* were selected as outgroups, because both were recovered as closely related taxa to *Leposoma* within the tribe Ecleopodini (*sensu Pellegrino et al., 2001*; see Rodrigues et al., 2009 for the correction of the spelling “Ecleopini”).

2.2. Collection of sequences and alignment

The molecular data set included 1830 bp from regions of three mitochondrial genes (the ribosomal 12S, the protein-coding cytochrome b [cyt-b] and ND4), and one nuclear gene (the protein-coding oocyte maturation factor Mos – c-mos). Most of the 63 focal specimens were successfully sequenced for all three mtDNA regions with few missing data for some specimens. For the nuclear c-mos region, sequences were collected for 39 focal lizards, with three specimens of *L. percarnatum* (-MT4, -MT5, -MT6) being sequenced exclusively for this nuclear region (Table 1).

Total genomic DNA was extracted from frozen tissues (liver or tail) or tissues preserved in 95% ethanol (Fetzner, 1999), and used in PCR reactions for the four different gene regions with the primers and protocols listed in Table 2. The size of the target region was estimated by electrophoresis on a 2% agarose gel, followed by the direct purification of the PCR products with the GeneClean III Kit (BIO 101, INC., Vista, CA). Double stranded DNA was sequenced using the Perkin Elmer ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction (PE Applied Biosystems, Foster City, CA). Excess PCR products were removed with the CentriSep spin columns (Princeton Separations, Inc., Adelphia, NJ), with sequences being run on a ABI PRISM 377 automated DNA sequencer (PE Applied Biosystems, Foster City, CA), at the DNA Sequencing Center at Brigham Young University.

Sequences were edited and initially aligned using the program Sequencher 3.1.1 (Gene Codes Corp., Inc., 1995) and the protein coding regions cyt-b, ND4 and c-mos were also translated into amino acids to confirm alignments. Few questionable bases were coded as missing. The alignment of the 12S sequences was conducted on Clustal X (Thompson et al., 1997) using default parameters for gap and mismatch penalties, with some subsequent manual adjustments. Sequences were deposited in GenBank under the accession numbers JN588621-JN588748.

2.3. Phylogenetic analyses

In order to increase accuracy with the traditional tree-based phylogenetic methods that require reasonably large numbers of variable sites to reconstruct relationships (Huelsenbeck and Hillis, 1993), we selected only non-redundant haplotypes for the cyt-b region for the subsequent analyses. We used this gene region because it presented the highest number of variable sites among all regions amplified for this study. This selection was performed using the software package TCS version 1.0 (Clement et al., 2000; available at http://bioag.byu.edu/zoolgy/crandall_lab/programs.htm), which implements statistical parsimony haplotype network estimation on the basis of the method described in Templeton et al. (1992). Haplotypes related at a probability \( > 95\% \) are represented in the same network. After network estimations, we selected only the non-redundant haplotypes within each network to be used in the subsequent phylogenetic analyses, which included the other gene regions.

All phylogenetic analyses using maximum parsimony (MP) and maximum likelihood (ML) optimality criteria were implemented in PAUP+ 4.0b10 (Swofford, 2001). Separate and combined analyses were performed under maximum parsimony (MP) using heuristic searches implemented with 1000 replicates of random taxon addition, tree-bisection-reconnection branch-swapping (TBR), characters equally weighted and gaps coded as missing data. Prior to the combined analysis under the MP and other tree-based phylogenetic methods, conflict between topologies recovered from individual partitions under MP was assessed using a qualitative evaluation suggested by Wiens (1998). In the absence of strong conflict among individual partitions, concatenated analyses of the mitochondrial and nuclear data sets were implemented, because this kind of procedure is known to increase the support indexes for nodes by improving the congruence of independent characters (e.g. Flores-Villela et al., 2000; Pellegrino et al., 2001; Whiting et al., 2006).

ML analyses were conducted only on the concatenated dataset using heuristic searches with 10 replicates of random stepwise addition and TBR branch-swapping. The GTR + I + F model (Yang, 1994) was selected as the best-fit model of sequence evolution for the combined matrix through hierarchical likelihood ratio tests in Modeltest version 3.4 (Posada and Crandall, 1998).

Nodal support for MP and ML analyses was assessed by non-parametric bootstrap (BS) analyses (Felsenstein, 1985) in heuristic searches with 1000 (MP) and 100 (ML) random stepwise additions per pseudo-replicate and TBR branch-swapping. Bootstrap values \( > 70\% \) were interpreted as significant support for a node (Hillis and Bull, 1993, with caveats). Total (BV) and partitioned Bremer support (PBS) values (Baker and DeSalle, 1997), the latter representing the contribution of each specified data partition to each node, were calculated for all nodes of the combined MP topology using the program TreeRot v. 2.0 (Soreson, 1999).

A partitioned Bayesian analysis (BA) using a combined matrix composed of the four partitions was carried out using Mr. Bayes 3.1.2. (Ronquist and Huelsenbeck, 2003) under the evolutionary models for each individual gene region as selected in Modeltest [12S (TrN + I + F), ND4 (TVM + I + F), cyt-b (GTR + I + F) and c-mos (HKY + I)]. We used three independent runs, with four Markov chains and for 6,000,000 generations to prevent analyses to be stuck in local optimal (Huelsenbeck and Bollback, 2001). Markov chains were sampled at intervals of 100 generations. Convergence diagnostic for independent runs was implemented in Tracer v. 1.5 (Rambaut and Drummond, 2007), with the average standard of the split frequencies and ESS (effective sample sizes) values being monitored. A standard deviation below 0.01 was indicative of convergence. A conservative burn-in of 25% was sufficient enough for parameters to reach stationary, with ESS values above all 550. Trees prior to stationary were discarded and a 50% majority rule consensus tree was obtained from 45,001 data points. Nodes on consensus trees from the independent runs with posterior probability (PP values) \( > 95\% \) were considered as evidence of significant support for clades (Huelsenbeck and Ronquist, 2001).

2.4. Estimation of divergence times

We used a relaxed molecular clock approach implemented in the program BEAST 1.5.3 (Drummond and Rambaut, 2007) to estimate the timing of major events of diversification within *Leposoma*. A mutation rate of 2% for cytchrome b was used, given the lack of alternative calibration points, and that this rate
Table 1
List of all ingroup and outgroup taxa used in this study, with their respective localities (coordinates), voucher/field numbers and identification label for terminals shown in Fig. 2. Locality numbers (in parentheses) match those in Fig. 1. Political units (under “locality”) are the following Brazilian states: AP – Amapá, AM – Amazonas, BA – Bahia, CE – Ceará, ES – Espírito Santo, MT – Mato Grosso, MA – Maranhão, PA – Pará, PB – Paraíba, RO – Rondônia, and RR – Roraima.

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<th>Species/specimens</th>
<th>Locality and coordinates</th>
<th>Voucher/field no.</th>
<th>Paper ID</th>
<th>mtDNA</th>
<th>nuclear</th>
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MZUSP = Museu de Zoologia da Universidade de São Paulo, SP, Brazil; MPEG = Museu Paraense Emílio Goeldi, PA, Brazil; LSUMZ = Louisiana State University Museum of Natural Science; USNM = United States National Museum (The Smithsonian); UFC = Universidade Federal do Ceará; INPA = Instituto Nacional de Pesquisas da Amazônia; HNCI = Museu de História Natural Capão de Imbuia, PR.

* Field numbers: MRT/MTR: Miguel Trefaut Rodrigues (IBUSP, São Paulo, Brazil); LG: Laboratório de Citogenética de Vertebrados, (IBUSP, São Paulo, Brazil), MD: Marianna Dixo (IBUSP, São Paulo, Brazil), FJ: Flora Juncá, Universidade Estadual de Feira de Santana (BA).

* Outgroup taxa.

a L. spn = undescribed species.

b 3n specimens.
c 2n specimens.
has been calibrated and used in other related reptiles (e.g. Paulo et al., 2008; Siedchlag et al., 2010). We performed the Bayesian dating analyses using the four data partitions under the parameters of each individual model as selected in Modeltest, and ran the analysis for 20 $\times$ 10^6 generations, sampling every 1000 generations. Convergence was assessed in Tracer v. 1.5 (Rambaut and Drummond, 2007). The first 2 $\times$ 10^6 samples were discarded as burn-in.

### 3. Results

#### 3.1. Phylogenetic analyses

The molecular dataset of Leposoma included 1830 bp of aligned sequences collected across regions of the mtDNA (12S: 447 bp, cyt-b: 350 bp, ND4: 633 bp) and one nuclear gene (c-mos: 400 bp) for 11 species from Panamanian, Amazonian, and Atlantic rain forests,
and for two outgroup taxa (Table 1). Separate datasets of all individual gene regions, obtained for 35 specimens representing non-redundant haplotypes (selected on the basis of 55 specimens for the cyt-b region; Table 1) were first analyzed under maximum parsimony (MP) and neighbor-joining distance methods. Because conflict among topologies derived from individual partitions was not detected after a qualitative evaluation, all the subsequent phylogenetic analyses were performed on a combined dataset of mtDNA and nuclear regions.

All tree-based phylogenetic methods recovered almost identical trees with the reciprocal monophyly for the parietale and scincoides groups highly supported by different indexes. ML analyses yielded a single tree (−ln = 13781.49587) under the GTR + I + Ψ model of sequence evolution (estimated base frequencies A = 0.3095, C = 0.3014, G = 0.1425, T = 0.2466, Ψ = 0.4891 and Ψ = 0.9875; Fig. 2), and the MP searches recovered six most parsimonious reconstructions, of which the strict consensus tree (L = 2649; CI = 0.44, RI = 0.72) is similar to that produced by ML. Bayesian

Fig. 2. Maximum likelihood (ML) tree based on 1830 bp of combined mtDNA and nuclear datasets under the GTR + I + Ψ model (−ln = 13781.49587). The nodes identified by numbers (in circles) correspond to the support indexes for the maximum parsimony (MP) consensus topology listed in Table 3. Number above branches represent ML/MP bootstrap proportions (>50%), and numbers below branches correspond to Bayesian posterior probabilities. The asterisk (node 13) and solid circle (node 28) indicate divergent topologies between ML and MP, and ML and Bayesian methods, respectively.
inference (BA) recovered a consensus tree very similar to that obtained under ML, but differed by the placement of a single taxon within the parietale group. We present only the ML tree here, but all recovered phylogenetic relationships are similar to those depicted in Fig. 2. Exceptions related to alternative relationships or differences in support for nodes across the three methods are described.

The parietale group was recovered with strong support (node 17: BS = 100; PP = 1.0; BV = 52; Fig. 2, Table 3). The PBS analyses revealed a major contribution of the c-mos gene (PBS = 21.8), followed by the 12S region (PBS = 12.2), to the total Bremer support for node 17 (BV = 52; Table 3). Within the parietale group, taxa sequenced for several specimens (Table 1), were also recovered as sister to all other Amazonian species but with weak support (PP = 0.82). The clade represented by node 27 is resolved as the sister group of the unisexual L. percarinatum with weak support (node 22: BS < 50%, BV = 1, PP = 0.52), whereas L. guianense (node 18) is basal to all other species of the parietale group studied (Fig. 2). Although the above nodes were only weakly supported by conventional indexes (bootstrap proportions and Bremer values), all three mtDNA partitions contributed to their support (see PBS analyses in Table 3).

The monophyly of the Atlantic Forest group was also strongly supported (node 2: BS = 99 in ML and 94 in MP; BV = 12; PP = 1.0; Fig. 2). The PBS analyses revealed that the contribution to the total Bremer support for node 2 derived only from the mtDNA regions (Table 3), although separate analyses of the c-mos partition consistently recovered the monophyly of Atlantic Forest group under all tree-reconstructions methods used here.

Two well-supported sister clades were recovered for L. scincoides: node 11 (BS = 100; PP = 1.0; BV = 19) and node 14 (BS = 100 in ML and 96 in MP; PP = 1.0; BV = 8; Fig. 2, Table 3). Node 14 recovers two strongly supported clades that reunited three specimens from Una, Bahia (node 16: BS = 100; BV = 33; PP = 1.0; Table 3) and two individuals from localities in the state of Espírito Santo (node 15: BS = 96 in ML and 97 in MP; PP = 1.0; BV = 11; Table 3). Within the group represented by node 11, one clade (node 12) groups the remaining specimens from Una as sister to a specimen from Serra do Teimoso, both localities in southern Bahia (Table 1). Thus the sample of L. scincoides from Una (Fig. 2) is not monophyletic. Although relationships within node 12 differed between ML and MP criteria (node 13:
scincoides-BA6 + scincoides-BA11 in ML/BA, and scincoides-BA4 + scincoides-BA11 in MP), both alternative topologies received low support across the three methods (BS = 52 in ML; BS ≤ 50 and BV = 1, in MP, and PP = 0.58 in BA; Fig. 2).

*L. scincoides* is resolved (node 5: BS = 99 in ML and 96 in MP; BV = 13; PP = 1.0) as sister taxon to a clade composed of *L. annectans* (node 7: BS = 100; PP = 1.0; BV = 35), and an undescribed species related to the latter from the state of Bahia (*Leposoma spn*; Table 1; node 8: BS = 100; PP = 1.0; BV = 21). The support for the sister relationship between *L. annectans* and *L. spn* (node 6; Fig. 2) was higher under ML and BA (BS = 79 and PP = 0.95; Fig. 2) than that under MP (node 6: BS = 63; BV = 3 Table 3). *L. puk* was recovered basal to this major grouping (node 5), with strong support (node 4: BS = 100 in ML and BS = 92 in MP; PP = 1.0; BV = 9). Finally, clade 3 recovered a sister relationship between *L. nanodactylus* and *L. baturitensis* placed basal within the Atlantic Forest radiation, but with low to marginally strong support: BS = 62 in ML, BS = 78 and BV = 5 in MP, and PP = 0.94 in BA (Fig. 2; Table 3).

3.2. Divergence timing analyses

Under a Bayesian framework, we estimated divergence times for selected clades of the Amazon/Central American and Atlantic Forest groups of the *Leposoma*, with 95% confidence intervals (Fig. 2, Table 4). These rates range from 22.1 Mya for the age of the genus (node 1) to 4.2 Mya for the diversification of the unisexual *L. percarinatum* (node 23). The diversification of the Atlantic Forest group is older (16.3 Mya, node 2) than that of the Amazon/Central American sister clade (13.4 Mya, node 17). The isolation of the ancestral lineage of the relictual *L. baturitensis* is also

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Finally, regarding the analyses of the nuclear marker c-mos within the unisexual *L. percarinatum*, we did not observe heterozygous individuals (n = 12; Table 1) at any nucleotide positions within the 330 bp fragment analyzed.

![Fig. 3. Phylogenetic relationships, karyotypes, and distributions of the two lineages within the parthenogenetic species *Leposoma percarinatum*.](image-url)
ancient (13.9 Mya, node 3). These estimates indicate that the Leposoma diversification may have begun in early Miocene.

4. Discussion

4.1. Phylogenetic relationships and taxonomic implications

The combined analyses of multiple gene regions strongly support the monophyly of the scincoides and parietale groups of the gymnophthalmid lizard Leposoma. This indicates that subsequent to an initial split, Atlantic and Amazon/Central American forest radiations of the genus evolved independently of each other. This basal dichotomy is also supported by conspicuous morphological and karyotypic differences (Ruihälä, 1952; Pellegrino et al., 1999, 2003; Laguna et al., 2010), and the clades are sufficiently distinct that they could be allocated to different genera. However, as we lack molecular data on the problematic Amapasaurus tetradactylus, a monotypic Amazonian genus possibly related to the parietale species group of Leposoma (Pellegrino et al., 2001), we prefer to defer this decision until molecular data are available for this species.

Although the largest diversity of the genus is associated with the parietale group and we sampled only 5 of its 11 species, we detected undescribed diversity. Our analyses indicate that the paraphenetic L. percarinatum includes at least two different species that can be diagnosed on the basis of molecular and karyotypic data. A more geographically representative study including specimens from the type locality will be necessary to morphologically characterize and taxonomically diagnose these parthenoforms. Further, genetic divergence levels between the populations of L. osvaldoi suggest that the samples from state of Amazonas, currently assigned to this species might represent another undescribed taxon.

Most of the species of the Atlantic Forest Leposoma radiation were described after 1997, and our results provide some additional insights into its diversity in the area. For example, we detected one strongly supported haplotype (node 8; Fig. 2) sister to L. annectans from the state of Bahia. Uncorrected mtDNA divergence levels (almost 12%) and morphological differences (MTR; unpubl. data) reveal species-level diagnostic attributes. This discovery will render the tropical rainforests of this area the richest in terms of species diversity for this genus. Second, the populations of the widespread L. scincoides from southern regions of state of Bahia were not recovered as monophyletic. We cannot address the possible causal mechanisms for this pattern, but note that the same phylogeographic pattern exists in populations of the co-distributed lizards Enyalius catenatus, Coleodactylus meridionalis, and the frog Proceratophrys renalis (our unpublished data).

4.2. Historical biogeography of the South and Central American genus Leposoma

Contrary to previous hypotheses suggesting recent (Pleistocene) contact between the Atlantic Forest and Amazonia (Vanzolini, 1981; Vanzolini and Williams, 1981; Ab’Saber, 1974; Andrade-Lima, 1982), our data suggest that contact between the two largest South-American forest blocks occurred earlier. Estimates of divergence times indicate that the initial split of an ancestral lineage of the parietale and scincoides radiations may have taken place during early Miocene at about 22 Mya (node 1; Fig. 2, Table 4). The diversification of the Atlantic Forest radiation is older (16.3 Mya) than of its sister group in Amazon/Central American (13.8 Mya, Table 4). As the monophyly of both groups is strongly supported and their ranges remain allopatric, either no further contact between these forests occurred or no signature of subsequent contact is evident in the history of Leposoma. L. batu-ritensis, the species that geographically most closely approaches its congener from Amazon/Central American radiation (locality 21; Fig. 1), is recovered as the sister species of L. nanodactylus, in the Atlantic Forest radiation.

Evidences of older connections (Pliocene) between Amazonian and Atlantic forests sister clades were also found in studies of small mammals (Costa, 2003) and birds (Cabanne et al., 2008). By contrast, the low genetic differentiation found between the subspecies of the bushmaster Lachesis muta from Amazonian basin and Atlantic Forest was attributed to episodic gene flow during the Pleistocene, but the authors indicated the need for more extensive study within and between its subspecies, which probably would reveal an older diversification of these lineages (Zamudio and Greene, 1997).

Dating estimates of the relictually isolated L. batu-ritensis and the remaining species of the Atlantic Forest group is also ancient. The placement for L. batu-ritensis and its divergence from L. nanodactylus (node 3; Fig. 2) suggest that this event occurred soon after the separation of Atlantic and Amazon/Central American radiations at about 13.9 Mya (Fig. 2; Table 4). Leposoma is absent from the Atlantic lowland forests north to the Rio São Francisco, but reappears at similar latitudes only in the relictual forests of Serra de Baturité. The Baturité mountains reach 1.025m elevation and are characterized by lower temperatures; thus it is possible that L. batu-ritensis prefers more mesic climates than those present nowadays at coastal Atlantic Forest north of the Rio São Francisco. All of this suggests an old isolation for the mountains of Baturité with no indication of a subsequent contact between its forests and those in the eastern coast at similar latitudes. Conversely, in the bird Xiphorhynchus fuscus atlanticus, which also inhabits (but is not endemic to) the brejos de Serra de Baturité, evidence for differentiation and isolation from other lineages from the central Atlantic Forest was found, as well as occurrence of recent gene flow. Divergence times between northern and central Atlantic Forest lineages of X. fuscus were estimated to be ~0.5 Mya, and related to a mid-Pleistocene expansion of Caatingas of northeastern Brazil (Cabanne et al., 2008).

In a previous study Rodrigues (1997) hypothesized that the some morphological characters shared by L. nanodactylus and L. batu-ritensis, in particular the ventral black spots and the slight longitudinal striations in head scales, would suggest a close relationship between these species; this is confirmed herein by our data. Further, L. nanodactylus is apparently a rare relictual species restricted to the highest part of isolated mountain forests in the state of Bahia, subject to lower temperatures than those present in adjacent lowland forests. These habitat preferences similar to those of L. batu-ritensis provide additional ecological evidence for their probable close relationship. In this context, we suggest as a working hypothesis that the ancestor of the Atlantic Forest Leposoma was adapted to lower temperatures than the current species, and that this climatic envelope extended over a larger area than at present. A further split of these climatic conditions promoted diversification between L. nanodactylus and L. batu-ritensis. The successive basal position of L. puk in the phylogeny (node 4) and its restriction to the mountains of southern Bahia reinforces this hypothesis.

The more derived (nested) positions of L. scincoides and L. annectans indicate that both have more recent origins (7.4 Mya; node 5; Fig. 2, Table 4). As both are also habitat generalists, this further suggests that expansion into lowland rainforest habitats is a derived distribution. However, we do not feel confident to comment further on these species before completion of a more detailed taxonomic revision. The paralogy for populations of L. scincoides from southern Bahia might be due to ancestral polymorphism, relatively recent divergence of northern populations on the periphery of the range, or to in situ differentiation followed by secondary contact and mtDNA introgression (Funk and Omland, 2003; Alves
et al., 2006). If the latter hypothesis is the case, divergence in iso-
lated refugia during dry episodes in the Quaternary, followed by
secondary contact, could account for this pattern. As mentioned
above, the same pattern is recovered in other taxa (lizards, frogs,
and Drosophila [Franco et al., unpubl. data]) from the same re-
gion. Resolution must therefore wait for denser geographic sam-
ping and phylogeographic analyses based on larger data sets, but
this is the nature of phylogeographic research (Buckley, 2009).

Although our sampling of the Amazonian and Central American
species comprises only five of the 11 recognized species in the
parietale clade, some preliminary conclusions are possible. The
phylogeny indicates that L. guianense, a species restricted to the
Guyana Shield, is the sister group to the remaining species, and
that diversification in Amazonia occurred more recently in the his-
tory of the genus. Likewise, although the position of L. southi is
not definitively established, its position is compatible with a Pliocene
colonization of Central America during the rise of the Isthmus of
Panama. We cannot comment further on L. parietale or L. osvaldoi
until a more detailed taxonomic study is completed.

A final puzzling point regarding the geographic distribution of
Leposoma is their absence from the gallery forests of the Central
Brazilian Cerrados, the intermediate area between Amazonian and
Atlantic Forest regions. We have no explanation for this pattern, gi-
ven that several other lizard genera (Coleodactylus, Enyalius), with
similar forested habitat requirements and Amazon–Atlantic Forest
distributions matching that of Leposoma, do occur in the Cerrado
gallery forests.

Although we are aware of the uncertainty and potential errors
of using molecular clock estimates, especially in the absence of
external data for calibration (Graur and Martin, 2004), our esti-
mates for the major events of diversification of Leposoma pre-date
the Quaternary climatic fluctuations, being placed in the Tertiary.
Ancient temporal diversification (pre-Pleistocene) of Neotropical
fauna has been frequently reported for other lizards and snakes
(Glor et al., 2001; Pellegrino et al., 2005; Graziotin et al., 2006;
Geurgas et al., 2008; Werneck et al., 2009), frogs (Fitzpatrick
et al., 2009; Thomé et al., 2010; Brunes et al., 2010), birds (Amala-
er et al., 2009; Ribas et al., 2009; Patel et al., 2011) and small mam-
mals (Lara and Patton, 2000; Patton et al., 2000; Costa, 2003; Vilela
et al., 2009). These patterns of diversification may be related to dif-
ferent major climatic variations associated with marine transgres-
sions, geotectonic processes and river dynamics, and a single
model of vicariance or climatic changes will not reflect the com-
plexity of the speciation in the Neotropics.

4.3. Insights into the origin of the parthenogenesis in L. percarinatum

The most conspicuous finding in our analyses regarding the uni-
sexuality issue is the high level of molecular divergence of the two
lineages within the L. percarinatum in both mtDNA and nuclear
markers. Pairwise corrected cyt-b sequences comparisons between
the two unisexual lineages revealed 12.8% level of divergence
(nodes 24 and 25 in Fig. 2, and Fig. 3), and they also differ in 10
nucleotide positions (3%) for a short fragment of the nuclear c-
mos. These results strongly indicate that the unisexual L. percarin-
atum is a species complex including at least two different species.

The two unisexual lineages can be easily diagnosed not only on
the basis of molecular but also karyotypic data. Karyological data,
a although fragmentary, strongly suggests that two different species
are involved, one diploid and one triploid. The triploid clade (Line-
age I, Fig. 3A and B) occurs in eastern Amazonia along a 2000 km
long SE–NW diagonal from Vila Rica (state of Mato Grosso) to the
northern part of state of Roraima (Fig. 3C). At first glance, the tri-
ployd lineage seems more associated with transitional areas situated
in the periphery of Amazonia or in areas close to open habitats,
whereas the diploid one seems to be more associated with primary

forest. The diploid clade (Lineage I; Fig. 3A and B) also has a large
distribution but it is predominantly associated with the central-
western part of Amazonia, extending from Guajará-Mirim (state of
Rondonia) to Vai-Quem-Quer (state of Pará; Fig. 3C). Although
largely allopatric, the distributions of the two unisexual lineages
approach each other in the area of Rio Tapajós/ Rio Trombetas
(localities 3 and 7; Fig. 3C) in Central Amazonia. This western/east-
ern pattern of distribution involving related taxa differs from those
reported, which usually show subdivisions between northern and
southern parts of Amazonia, but this is not a new finding. A recent
phylogeographic study with the sphaerodactylid gecko Coleodacty-
lus amazonicus recovered a similar western/eastern pattern (Geur-
gas et al., 2008). Nevertheless, although there are some overlap in
the areas involved between the lineages of L. percarinatum and the
haploclades of C. amazonicus, divergence times are strikingly differ-
ent. Whereas the eastern/western Coleodactylus clades have been
separated since the early Miocene (Geurgas et al., 2008; Geurgas
and Rodrigues, 2010), differentiation is much more recent in the
case of L. percarinatum (between 2.8 and 5.7 Ma, Table 4).

The available data assembled here still do not permit either pre-
cisely determine the mechanism involved in the origin of parthe-
nogenesis or unambiguously assign the parental species of the L. per-
carinatum haploclades, if hybridization events occurred. Unfor-
natunately, only four of 10 recognized bisexual species of the parie-
tale group were included in our sample despite our tireless effort
to obtain tissues of the other species. Also, considering that the
morphological differences among them are so slight, we cannot
even infer their putative position in the phylogeny of the group.
However, based on the real difficulty in obtaining tissues for Lepo-
soma species, we think it is worth to advance here some hypothe-
ses regarding the origin of parthenogenesis in the genus in order to
stimulate further research.

Uzzell and Barry (1971) suggested, on the basis only of geo-
graphic distribution, that parthenogenesis in Leposoma probably
originated through hybridization between closely related species,
and identified L. guianense and L. parietale as putative candidates
for parental species.

Our mtDNA data reveal a conspicuous level of divergence be-
 tween L. percarinatum and the parietale group bisexual species
sampled for this study, which may suggest two possibilities: (1)
either the species most closely related to the ancestral maternal
parent of the L. percarinatum haplodge was not sampled, and/or
(2) the hybridization event(s) was ancient. For instance, L. percar-
atum and L. parietale differ at 19.8% in 259 bp of cyt-b sequences,
and the difference between the parthenoform and the bisexual L. guianense/L. osvaldoi is about 15% in the same fragment. Diversifi-
cation of the diploid and triploid lineages of L. percarinatum was
estimated to have begun between 5.7 and 2.8 Ma, which contrasts
with a more recent origin (Pleistocene) for parthenogenetic lizards
(review in Fujita and Moritz, 2009; Kearney, 2005). Based on these
ancient estimates, if parthenogenesis in Leposoma has in fact a hy-
brid origin, we cannot discard the hypothesis that the maternal
ancestor is extinct. Besides, the analyses of a short fragment of the
nuclear c-mos for both 2n and 3n lineages of L. percarinatum
did not reveal presence of heterozygous sites. This result might
bring support to the idea of ancient events of hybridization in Lepo-
soma, but it is necessary to include a more extensive set of multiple
nuclear markers in order to confirm this possibility.

Although parthenogenetic lizards (40 species in eight families
out of 9247 squamate reptiles; Uetz et al., 2007) are almost always
of hybrid origin, some are suggested to have a spontaneous or non-
hybrid origin (Kearney et al., 2009; Fujita and Moritz, 2009). In the
xantusuids Lepidophyma reticulatum and Lepidophyma flavimacula-
tum extensive DNA sequence and microsatellite analyses did not
support a hybrid origin (Sinclair et al., 2010), as well as in the
gymnophthalmid Gymnophthalmus underwoodi from Roraima.
Recently, a possible non-hybrid origin for parthenogenesis in *L. percarinatum* was proposed, based only on the fact that the triploid karyotype found in Mato Grosso, Brazil (Pellegrino et al., 2003) present identical genomic complements instead of fixed pair of different genomes inherited from divergent sexual relatives (Fujita and Moritz, 2009). Considering that the karyotypes described so far for species of the *parietale* group (*L. guianense*, *L. osovaldi* and *L. ferreirai*, and the diploid cryptic form of *L. percarinatum*; Pellegrino et al., 1999; Laguna et al., 2010) are all similar (2n = 44, 20M + 24m), and if hybridization involved species with this 2n = 44-like karyotype, we would expect this homogeneous pattern in the triploid, at least for analyses based on conventionally-stained specimens. Apart from this, we do not have the data needed to really account for a non-hybrid hypothesis in *Leposoma* at this time, but this possibility clearly deserves further investigation.

In conclusion, although the present data have provided some insights into the better comprehension of the origin of the unisexuality in *L. percarinatum*, additional molecular data that include multiple diagnostic nuclear markers and samples from all or almost all the recognized species in the *parietale* radiation are essential to allow that a complete scenario about the origin of parthenogenesis in *Leposoma* be hypothesized.

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