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COMO EVENTOS PALEOAMBIENTAIS E ECOLÓGICOS MOLDARAM A DIVERSIDADE DE LAGARTOS NEOTROPICAIS (IGUANIA: LEIOSAURIDAE)

How paleoenvironmental and ecological events shaped the diversity of Neotropical lizards (Iguania: Leiosauridae)

MARINA MONJARDIM

Vitória, 17 de dezembro de 2021

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Tese apresentada ao Programa de Pós-graduação em Ciências Biológicas (Biologia Animal) da Universidade Federal do Espírito Santo, como requisito parcial à obtenção do título de Doutor em Biologia Animal.

Orientadora: Dra. Sarah Maria Vargas

Coorientadora: Dra. Fernanda de Pinho Werneck

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“*Afortunado é aquele que não se perde nos labirintos da filosofia, mas vai direto à fonte onde tudo surge.*”

Sri Ramana Maharshi

“*Ouça não a mim, mas ao Logos, é sábio reconhecer que todas as coisas são uma.*”

Heráclito

Ao Tao, que por Ele tudo é feito através de nós.

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“O Caminho não escolhido”

Robert Frost, 1916

Num bosque amarelo dois caminhos se separavam,

E lamentando não poder seguir os dois

E sendo apenas um viajante, fiquei muito tempo parado

E olhei para um deles tão distante quanto pude

Até onde se perdia na mata;

Então segui o outro, como sendo mais merecedor,

E tendo talvez melhor direito,

Porque coberto de mato e querendo uso

Embora os que por lá passaram

Os tenham realmente percorrido de igual forma,

E ambos ficaram essa manhã

Com folhas que passo nenhum pisou.

Oh, guardei o primeiro para outro dia!

Embora sabendo como um caminho leva para longe,

Duvidasse que algum dia voltasse novamente.

Direi isto suspirando

Em algum lugar, daqui a muito e muito tempo:

Dois caminhos se separaram em um bosque e eu…

Eu escolhi o menos percorrido

E isso fez toda a diferença.

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RESUMO

A Região Neotropical é uma das regiões mais biodiversas da Terra e para os répteis é onde ocorre o maior número de espécies e famílias. Vários eventos moldaram a biodiversidade desta região ao longo de sua história evolutiva como, por exemplo, a formação do Istmo do Panamá, a elevação dos Andes, as mudanças climáticas durante o período Plio-Pleistoceno. Além disso, várias hipóteses foram propostas para explicar tal diversidade. Leiosauridae é uma família de lagartos (Squamata: Iguania) distribuída exclusivamente nas subregiões chilena e brasileira da região Neotropical, e está intimamente relacionada com Opluridae, distribuída em Madagascar. Para trazer uma maior compreensão da biodiversidade na região Neotropical e melhorar o entendimento da história evolutiva de Leiosauridae, testamos hipóteses relacionadas à toxonomia, biogeografia e taxas de diversificação e tempo, usando dados moleculares robustos de elementos ultraconservados (UCEs). Estimamos a filogenia mais completa de Leiosauridae. Com uma árvore de espécies datada, discutimos sobre a taxonomia desta família, e o status taxonômico de suas subfamílias, gêneros e espécies, e a relação entre eles. Embora uma maior diversidade seja esperada em regiões tropicais, não encontramos diferenças entre as taxas de diversificação entre os clados da subregião Chilena e Brasileira. Descobrimos que para os clados mais profundos (subfamílias, gêneros, clados A e B de Enyalius, E. leechii e D. darwinii e outras espécies de Diplolaemus) os eventos relacionados aos períodos Oligoceno/Mioceno e à elevação dos Andes tiveram mais influência, enquanto para a coroa (espécies) foram os eventos de mudanças climáticas dos períodos Plioceno/Pleistoceno. Os eventos desencadeados, possivelmente, pela elevação dos Andes, também parecem ter influenciado a distribuição desses grupos. Em relação aos Leiosaurinae, por exemplo, após sua origem no extremo sul da Patagônia, eles se dispersaram para o norte possivelmente devido a mudanças ambientais. Além disso, tais eventos também influenciaram a distribuição atual dos Enyaliinae, pois contribuíram para a formação da Diagonal seca, assim como para a separação da Mata Atlântica em norte e sul durante o Mioceno, evento mais antigo do que o proposto na literatura até o momento. Esses achados trazem um novo ponto de vista para melhorar o entendimento da história evolutiva dessa família e dos biomas Neotropicais.

Palavras-chave: Neotropical, Filogenômica, Biodiversidade, Mata Atlântica, Patagônia.

ABSTRACT

The Neotropical Region is one of the most biodiverse regions on Earth and for reptiles is where the largest number of species and families occur. Several events shaped the biodiversity of this region throughout its evolutionary history, for instance, the formation of the Panama Isthmus, the Andes uplift, climate changes during the Plio-Pleistocene period. Moreover, several hypotheses were proposed to explain such diversity. Leiosauridae is a family of lizards (Squamata: Iguania) exclusively distributed in the Chilean and Brazilian subregions in the Neotropical region, and is closely related to Opluridae, distributed in Madagascar. To bring a greater understanding of the biodiversity in Neotropics and improve the understanding of the evolutionary history of Leiosauridae, we test hypotheses related to taxonomy, biogeography, and diversification rates and time, using a robust data from ultra-conserved elements (UCEs). We estimate the most complete phylogeny for Leiosauridae. With a dated species tree, we discuss about the taxonomy of this family, and the taxonomic status of its subfamilies, genera and species, and the relationship among them. Although a greater diversity is expected in tropical regions, we did not find differences between diversification rates among Chilean and Brazilian subregion clades. We found that for the deeper clades (subfamilies, genera, clades A and B of *Enyalius*, *E. leechii*, and *D. darwinii* and other species of *Diplolaemus*) those events related to the Oligocene/Miocene periods and the Andes uplift had more influence, while for the crown (species) it was the events of Plio-Pleistocene climate changes. The events triggered possibly by the Andes uplift, also seems to have had an influence on the distribution of these groups. Regarding Leiosaurinae, for example, after its origin in the extreme south of Patagonia, it seems that they dispersed towards the north possibly due to environmental changes. Furthermore, such events also influenced the current distribution of the Enyaliinae, as they contributed to the formation of the dry Diagonal, as the separation of the Atlantic Forest in north and south during the Miocene, an event older than that proposed in the literature. These findings bring a new point of view to improve the understanding of the evolutionary history of this family and of the Neotropical biomes.

keywords: Neotropics, Phylogenomics, Biodiversity, Atlantic Forest, Patagonia

Introduction

Neotropical Biodiversity

The Neotropical region is the most species-rich realm on Earth, and since the 19th century, acclaimed naturalists such as Alexander Von Humboldt (1820), Maximilian von Weid-Neuwied (1820; Vanzolini and Myers, 2015), Alfred Russel Wallace (1876) and Charles Darwin (1909) have described and attempted to understand the drivers of its incredible biodiversity. According to Wallace (1876), richness and geographic isolation are the main features of Neotropical fauna, and no other biogeographic region comes close in terms of richness of endemic families and genera (Wallace 1876). In the same book Wallace, based on the peculiarities of the local fauna, proposed four well-marked sub-regions in the Neotropical region: two of which are in South America, namely: Tropical South America / Brazilian subregion and Temperate South America / Chilean subregion; in addition, he proposed more two subregions: the North America Tropical / Mexican subregion and West Indies / Antilles subregion. Recent authors also observed similar subregionalizations of the Neotropical Region as Harrington (1962), Holt *et al.* (2013) and Morrone (2014).

The Neotropical region is extremely biodiverse for many groups of organisms, but for reptiles this diversity is relatively striking compared to the rest of the world; the largest number of species and families of reptiles occurs there (Moura and Jetz 2021, Guedes *et al*. 2018, Roll *et al*. 2017, Gaston & Spicer 2004). Also, regarding reptile diversity, this is the most remarkable group in terms of global biodiversity, being the second largest tetrapod group in number of species, only behind the birds (Pincheira-Donoso *et al*., 2013). But there is still a lot to discover, for instance, a quarter of potential discoveries is estimated just for Brazil in Neotropical region (Moura and Jetz 2021).

Antonelli and Sanmartin (2011) assign the biodiversity of the Neotropical region to several factors, including biotic and abiotic. Besides of a rich biotic variety that allows more interactions, adaptations, etc, the region also has a great abiotic variety (Antonelli and Sanmartin, 2011). From ice in Patagonia, to arid regions in the Dry Diagonal, and places with highest humidity and pluviosity with highest biodiversity as Amazonia (AM) and Atlantic Forest (AF). Being the AF one of the most important hotspots of biodiversity in the world, due its high biodiversity, high endemism and its intense degradation (Mittermeier et al., 2005). Great variations in altitude and latitude are also found in the region, and the Andes uplift is considered an important event that promoted the biodiversity in South America, generated species turnover, and a wider climatic gradient (Hoorn et al., 2010, Gan et al., 2004; Zhou and Lau, 1998, Lau and Zhou, 2000). This rich biotic and abiotic variety makes the Neotropical region the most biodiverse region in the world (Ledo and Colli, 2017, Antonelli and Sanmartin, 2011, Hoorn et al., 2010, Mittermeier et al., 2005), making the evolutionary process intricate with an extremely complex scenario.

The naturalist Alexander von Humboldt was one of the first to understand, during his journey in the Neotropics, that the interaction between the biotic and abiotic factors have influence in the biodiversity (Humboldt and Bonpland, 1805). After him, several other hypotheses have been proposed to explain the diversity of the Neotropical region, such as: River barriers (Wallace, 1889), Pleistocene Refuges (Haffer, 1969), Museum hypothesis (Stebbins, 1974), Phylogenetic niche conservatism (Wiens and Donoghue 2004), Out of the Tropics (Jablonski *et al.,* 2006), among others (Antonelli *et al*., 2018). The geological history of this region is also extremely complex involving the South American, Caribbean and North Americna tectonic plates (Antonelli and Sanmartin, 2011). During the periods

Oligocene/Miocene, there were intense changes around the world, both climatic and geological. The increase in the speed of deposition of the ocean floor, for example, promoted an acceleration in the movement of tectonic plates And for the neotropical region, these movements promoted the Andes uplift with the subduction of the Nasca plate and the elevation of the South American plate (Potter and Szatmari 2009). These events were extremely important for the Neotropical region, influencing climate and temperature, and the entire dynamics of biomes causing in the Brazilian subregion, for example, the formation of the Diagonal of Dry biomes and having a great influence in the Amazon basin (Morley 2000, Antonelli and Sanmartin, 2011, Werneck 2011, Werneck *et al*., 2011, Sobral-Souza *et al.,* 2015, Bacon et al. 2015). While in the Chilean subregion, for example, it made the Patagonia region more arid, and promoting the origin of the Monte biome (Morando *et al*., 2015, Folfuera *et a*l., 2011). The Andes uplift and consequently the formation of Dry Diagonal, separated the tropical forest into Amazonia (AM) and Atlantic Forest (AF; Ledo and Colli, 2017), and these forests are considered the most biodiverse in the world (Hoorn *et al*., 2010; Mittermeier *et al*., 2005). Over time, the dynamics of these forests were altered, influenced by cyclic climatic changes, allowing the reconnection between these forests at different times, with the NorthWest-SouthEeast (NW-SE) route being established from the uplift Andes and the most recent NorthEast (NE) route in the Pleistocene (Ledo and Colli, 2017). Several studies with fauna and flora taxon have shown that the specimens from northeastern region of the AM is closer to specimens from the eastern region of the AM than to the specimens from southern AM itself (Prates *et al*., 2016, Santos *et al*., 2007). While the specimens from southern AF are more closely related to the specimens from western AM (Batalha-Filho *et al*., 2013, Costa, 2003). The Pliocene and Pleistocene climatic dynamics were also important processes shaping biodiversity around the globe, including the Neotropics, when intense climate change occurred (Burbrink *et al*., 2019, Oliveira *et al*., 2015, Carstens and Knowles, 2007, Klicka and Zink, 1999, Avise *et al*., 1998).

Historical Systematics and Distribution of Leiosauridae

Leiosauridae is a family of lizards (Squamata: Iguania) exclusively distributed in the extreme biodiverse region Neotropical and have been proposed to share a MRCA with Opluridae, restricted to Madagascar (Frost *et al.,* 2001, Münchenberg et al., 2008, Wiens *et al*., 2012, Pyron *et al*., 2013, Reeder *et al*., 2015, Burbrink *et al*. 2020). With few exceptions, as Opluridae, Plerodonta (Iguania) is mainly distributed in America continent (Burbrink *et al*., 2020, Münchenberg *et al*., 2008, Noonan and Chippindale, 2006).

Leiosauridae is subdivided in two subfamilies with three genera each. The subfamily Leiosaurinae occurs in the Chilean subregion, while the Enyaliinae subfamily is distributed in the Brazilian subregion (Frost 2001, Münchenberg et al., 2008, Wiens *et al*., 2012, Pyron *et al*., 2013, Rodrigues *et al*., 2014, Reeder *et al*., 2015, Morando *et al*., 2015, Burbrink *et al*. 2020) (Figure 1). Leiosaurinae species are relatively stout compared to Enyaliinae and occur mainly in xeric habitats in southern South America, in the Patagonia and Monte biomes (Figure 1). Some species of this group are threatened, probably due their restricted distributions to small areas in Argentina and Chile (Abdala *et al*., 2012, Morando *et al.,* 2015). *Leiosaurus* contains four species distributed in north and northwestern Argentina (Morando *et al*. 2015). *Diplolaemus* includes four species, often indistinct with conserved morphology that occurs mainly in Patagonia biome (Cei, 1986, Frost 2001, Scolaro *et al*., 2003, Victoriano *et al*., 2010, Morando *et al*. 2015). *Pristidactylus* is the most diverse genus of Leiosaurinae with four species in Chile and six species in Argentina (Frost 2001, Morando *et al*. 2015).

Within Enyaliinae, the genera *Anisolepis* (three species) and *Urostrophus* (two species) are distributed from northern Argentina to southeastern Brazil, restricted to cooler and high altitudinal environments. These genera have a complex historical taxonomy with the most recent complete revision proposed by Etheridge and Williams (1991). In contrast, *Enyalius* is the most diverse genus of subfamily with 11 species, and exclusively distributed in Brazil (Figure 1), covering the biomes: Atlantic Forest, Cerrado, Caatinga and Amazon (Rodrigues *et al*., 2014). It is worth mentioning that the Brazilian AF is one of the most important hotspots of biodiversity in the world world (Hoorn et al., 2010; Mittermeier et al., 2005) and it is home of most species of Enyaliinae, being: 8 species of *Enyalius*, two species of *Anisolepis* and one species of *Urostrophus*. Unfortunately, it is categorized as one of the most severely threatened biomes (Galindo-Leal and Câmara 2003).

In this work we used genome-scale data to generate the complete phylogeny of Leiosauridae to date, covering the two subfamilies, all six genera, and 73.5% of the species of the family. Since our data is the most complete for the group so far, we contrast our results in relation to those previously published to bring a greater understanding and a revision of the evolution of this exclusively Neotropical family. We tested several hypotheses concerning about the evolution, systematic and biogeography of the family. We performed biogeographic analyzes to understand the dynamics and geographic distribution of Leiosauridae in Neotropical biomes. We also tested the hypothesis of connections between AF and AM, and which route, SE—NW or NE, was used by *Enyalius* to reach AM from AF. We estimate the diversification rates and compare among branches, to observe if there is difference between the rates of the groups of Chilean and Brazilian subregions, as greater diversity is expected in the tropical region. We estimate whether there was a change in the group's diversification rate at some time in its evolutionary history and compared it with the literature, and we tested if the hypothesis of Plio-Pleistocene climatic changes events influenced its diversification. We also hypothesize the influence of the changes of Oligocene/Miocene and Andes uplift in the Neotropical biomes dynamics and in the Leiosauridae diversification.

Material and Methods

Sampling

Our ingroup comprised 49 terminal taxa representing 25 species (73,5%) and 6 genera (100%) distributed among Leiosauridae. Outgroup taxa included 6 species (85%) and 2 genera (100%) from the sister family Opluridae and 5 species of Iguania: *Iguana iguana* (Iguanidae), *Physignathus leseueuri* (Agamidae), *Furcifer pardalis* (Camaleonidae), *Callisaurus draconoides* (Phrynosomatidae) and *Urosaurus nigrucaudus* (Phrynosomatidae). First, we sequenced six loci for Enyallinae for a total of 230 samples, covering the most range of the geographic ranges and deep genetic divergences within species. We used the same six genes from 35 individuals of Leiosaurinae extracted from Morando et al., (2015) deposited in Genbank. After that we selected a subset of 60 individuals representing different clades of Leiosauridae (putative cryptic species), and covering most of the geographic distribution, including outgroups, for UCE's sequencing (Appendix 1, Figure 1). These taxa were selected in an attempt to maximize the sampling of divergent clades and to cover the known ranges of the nominal species, while also accounting for underscribed cryptic diversity within large species complexes.

Sanger sequencing

DNA extraction, amplification and sequencing

The DNA was extracted from ethanol preserved muscle or liver samples with Bruford *et al.* (1992) protocol and quantified using NanoDrop ND1000 (Thermo Scientific). Six gene regions were amplified (mitochondrial: 12S and CytB; nuclear: C-MOSS, DMLX, RBMX and NKTR), using previously described primers Appendix 2). The DNA was sequenced at 'Núcleo de Genética Aplicada à Conservação da Biodiversidade' (NGACB) at Federal University of Espirito Santo using an automatic sequencer 3500 Genetic Analyzer (Applied Biosystems, Waltham, MA, U.S.A.).

Alignment and analyses

Sequences were checked for the correct loci amplification and taxonomy using Blast (Altschul *et al.*, 1990) from the GenBank database. Subsequently, the sequences were aligned using the Geneious 7.1.3 software (Kearse *et al.*, 2012) using the Clustal W algorithm (Thompson *et al.*, 1994) with default parameters. The model GTR+I+G was chosen as the best-fit model for all genes (each used as a single partition) by jmodeltest 0.1 (Posada, 2008) before the phylogenetic reconstruction. We also ran each gene separately and the same model GTR+I+G was chosen by Jmodeltest 0.1 for each one. We infer the phylogenetic relations of each gene and concatenated genes was inferred using Bayesian inference (BI) and maximum likelihood (ML). BI trees were generated using the software Mrbayes 3.2.2 (Ronquist *et al.*, 2012), and ML trees were generated using ML RaxML (Stamatakis, 2014), both on the CIPRES Portal (Miller *et al.*, 2010). The species tree using coalescent estimates was generated using the software ASTRALL-III (Zhang *et al.* 2018).

Genomics: UCE data

DNA extraction, amplification and sequencing

Whole genomic DNA was extracted from ethanol preserved muscle or liver samples with the DNeasy Tissue Kit (Qiagen) and quantified using the qubit dsDNA broad range (BR) Assay Kit (Invitrogen, Life Technologies) following manufacturer's instructions. Sequencing and library preparation were performed at Rapid-Genomics (Florida). The ultra-conserved elements of DNA (UCE's) were sequenced using the "Tetrapods 5k" probes described by Faircloth *et al.* (2012).

Alignment and analyses

Quality control, assembly and alignment of the sequences were carried out with Phyluce (Faircloth, 2016). After sequencing, adapter contamination and low-quality bases were trimmed using the Illumiprocessor (Bolger *et al*., 2014; https://github.com/ faircloth-lab/illumiprocessor). After trimming, we assembled Illumina reads into contigs using Velvet (Zerbino and Birney, 2008) on Phyluce using "phyluce_assembly_assemblo_velvet" code. After sequence assembly, we looked for the UCE loci in our contigs using the uce 5k probes set (https://raw.githubusercontent.com/faircloth-lab/uce-probe-sets/master/uce-5k-probe-set/uce-5k-probes.fasta) on Phyluce using "*phyluce_assembly_match_contigs_to_probes*" code. After that we extracted UCE loci using *"phyluce_assembly_get_match_counts"* code and extracted fasta data that correspond to the loci with "*phyluce_assembly_get_fastas_from_match_counts*" code both in Phyluce. To align the UCE data we ran the edge-trimming, as suggested by Faircloth

(2016) for taxa closely related (<30-50 MYA), with MAFFT alignment algorithm (Nakamura *et al.*, 2018) using "*phyluce_align_seqcap_align*" code on Phyluce. We kept 75%, 65% and 60% of UCEs missing data to explore its effects in phylogenetic inference (Hosner *et al.*, 2016; Streicher *et al.*, 2016).

We performed ML analyses on the concatenated matrices under each filtering scheme using RAxML version 8.1.3 and BI was performed in ExaBayes version 1.5 (Aberer *et al.,* 2014), both on the CIPRES Portal. The maximum likelihood was inferred with the concatenated data to estimate the gene trees in the IQ-TREE II (Minh *et al.*, 2020). Here, we partitioned each locus, assessed which among 279 models of substitution best fit each partition using Bayesian information criterion (BIC), and estimated tree support using the ultrafast bootstrap approximation, UFBoot2 (*n* = 1000; Hoang et al., 2018). Optimal gene trees for each UCEs under maximum likelihood were generated and the unrooted gene trees were used to estimate the true species tree in ASTRAL-III, under the multi-species coalescent model with default hyperparameter inputs (Yule prior for branch lengths and the species tree set to 0.5; Zhang et al. 2018).

To date the species tree generated by ASTRAL-III using penalized likelihood, first we estimated the size of the branches and bootstrap (rapid bootstrap function – UFBoot2) values on IQ-TREE. To measure the underlying agreements or disagreement among sites and genes for supporting any topological arrangement we ran the gCF ("gene concordance factor"-% of genes that support the branch) and sCF ("site concordance factor"-% of sites supporting the branch) also on IQTREE (Naser-Khdour *et al.*, 2019). The dating (with error) using the penalized-likelihood approach was performed in treePL (Smith and O'Meara, 2012) and calibration using the data extracted from Burbrink *et al.* (2020) (Appendix 3). We date the node of the most recent common ancestor (mrca) with Iguania (min =149 mya and max=158 mya), with Pleurodonta (min = 81 mya and max = 100 mya), and with Opluridae+Leiosauridae (min

= 67 mya, max = 84 mya; Appendix 3, Figure 4). We estimated dating error around those dates using the bootstrapped tree of concatenated datasets fitted for Astral III generated on IQ-TREE on Tree Anotator (Drummond and Rambaut (2007).

We estimated ancestral areas using BioGeoBEARS (Matzke 2013, 2014) on R 3.3.0 (R Core Team 2016). We used the three models of BioGeoBEARS (Matzke 2013): Dispersal–Extinction–Cladogenesis (DEC – Ree and Smith 2008); DIVALIKE, a likelihood implementation on the parsimony DIVA program (Ronquist 1997); and BAYAREALIKE, a likelihood implementation on the BayArea program (Landis *et al.*, 2013). These models were run with and without the parameter weighting jump dispersal (+J) at cladogenesis (Matzke 2014). Terminal species were coded as occurring in one or multiple areas that included Patagonia, Monte, Chaco, Pampa, Atlantic Forest (Central-South and North), Chapada Diamantina, Cerrado, Caatinga and Amazonia, and Madagascar. The areas were selected according to the distribution pattern of the species. We estimated ancestral area and colonization times using the biogeographic model with the lowest AICc.

The diversification analyzes in Leiosauridae were estimated with the MCMCTress performed in BAMM (Rabosky, 2014), and the results were visualized with the "BAMMTools" package (Rabosky et al., 2014) in R. The results produced by BAMM were compared to the results generated with the "RPANDA" package (Morlon et al., 2016). To analyze distinct patterns of diversification within the phylogeny of Leiosauridae and compare diversification rates among clades we ran the R package "RPANDA" (Morlon et al. 2016) using the spectR and BICompare functions. To determine if speciation and extinction rates changed over time and if Bayes factors support any mass extinction events, we used rjMCMC CoMET (May *et al.*, 2016) in the R package "TESS" (Höhna *et al.*, 2015).

Results

Data and Phylogenies

We successfully sequenced 230 individuals for cytb (761 bp), 195 individuals for 12S (975 bp), 155 individuals for CMOS (543 bp), 117 individuals for DMLX (989 bp), 159 individuals for RBMX (606 bp) and 67 individuals for NKTR (904 bp), totaling two mitochondrial genes and four nuclear genes, and 4778 bp. For UCE's, we sequenced a subset from sanger sequencing of 60 samples of Leiosauridae (100% of genera and 75% of species), Opluridae (100% of genera and 72% of species) and one from *Iguana iguana* (Iguanidae), *Physignathus leseueuri* (Agamidae), *Furcifer pardalis* (Camaleonidae), *Callisaurus draconoides* (Phrynosomatidae) and *Urosaurus nigrucaudus* (Phrynosomatidae). After processing the UCE's data, our 75% matrix size comprised 379 loci, totaling 452.597 bp.

The major inferred clades were all strongly supported (Bayesian posterior probability = 1, Bootstrap values = 100) by all approaches. However, for internodes, while the concatenated analyses of ExaBayes, RAxML, IQ-TREE, recovered an identical topology (Appendix 4), for the ASTRAL-III species tree analyses retrieved a slightly different topology for the ingroup species (Figure 2, Appendix 5). We also recovered some differences in topology between sanger sequences and UCE's results (Figure 2, Figure 3).

Phylogenetic relationships in Leiosauridae

Leiosauridae was recovered as the sister group of Opluridae, both monophyletic with strong support (Figure 2). Within Leiosauridae the subfamilies Enyaliinae and Leiosaurinae were also recovered as monophyletic sister clades with strong support (Figure 2). The three genera of Leiosaurinae were recovered as monophyletic, with *Diplolaemus* as the sister group of the other genera of the Leiosaurinae (*Leiosaurus* and *Pristidactylus*), but weakly supported (Figure 2). Two main clades were recovered for the genus *Diplolaemus*: one highly supported containing samples from the well delimited species *Diplolaemus darwinii,* and another with all other species of *Diplolaemus* analyzed with poorly supported for interspecific relations (Figure 2). We found *Leiosaurus belli* as a sister species of the other species of the genus in all our analyses: *Leiosaurus belli* + (*Leiosaurus paronae* + (*Leiosaurus jaguaris* + *Leiosaurus catamarcensis*)). Within the *Pristidactylus* clade, the only well supported relationship was among *Pristidactylus achalensis* and *Pristidactylus scapulatus* (Figure 2).

In all our inferences, *Anisolepis* and *Urostrophus* were recovered as sisters within a monophyletic clade. As previously reported in the literature, *Enyalius* diverged early into two clades: Group A, more distributed in southern of Brazil and Group B has a more northern distribution. Based on UCE data these clades were recovered as sisters (Figure 2), while with sanger sequencing the genus *Enyalius* was not recovered as monophyletic, being *Anisolepis* + *Urostrophus* sister of *Enyalius* clade A (Figure 2, Figure 3).

Our data recovered different composition and relationships in the clades A and B from previous inferences proposed in literature (Rodrigues *et al*., 2014, Breitman *et al.,* 2018). *Enyalius leechii*, the only Amazonian species, was recovered in the clade A, not B, as sister group to the other species from this group (Figure 2). We recovered with high support *E. boulengeri* as species sister to the others *(E.leechii + (E.boulengueri + (E.ihenrigii + (E.perditus + E.brasiliensis))))* (Figure 2). Our results with a subsample using UCE data indicate *E. perditus* as paraphyletic in relation to *E. brasiliensis* (Figure 2, Appendix 4, Appendix 5). With a much larger sample using sanger data, our results also showed that the species are not reciprocally monophyletic (Appendix 6). *E. iheringii* was recovered in two main clades: one distributed more south in Rio Grande do Sul to Santa Catarina states and other distributed in Sao Paulo state.

The Clade B was recovered with species that occur further north, in the Atlantic Forest, Cerrado and Catinga. *Enyalius bilineatus* occurs mainly in the central Atlantic Forest and was recovered as a sister to *E. capetinga*, the species from Cerrado. *Enyalius bilineatus and E. capetinga* were recovered in a clade and as sisters of the other species of the clade B: *E. pictus, E. catenatus* and *E. bibronii*. With sanger data, we inferred *E. catenatus* as polyphyletic, the same of the literature. Individuals from Bahia, *Enyalius cat1*, were recovered with *E. pictus*, and individuals from Pernanbuco, *Enyalius cat2,* were recovered with E*. bibronii*. However, all our analyzes with UCE data recovered *E. catenatus* as paraphyletic in relation to *E. pictus*, including the same samples that was recovered with *E. bibronii* in sanger data analysis. We observed that the mitochondrial markers show that *E. catenatus* is polyphyletic; on the other hand, nuclear markers and UCE's recovered *E. catenatus* paraphyletic in relation to *E. pictus*. (Figure 2, Appendix 6)

Biogeography

The biogeographic model that best fitted our data was Bayareal + J (Appendix 7), where estimating the jump ("J" parameter) is consistent with a single over-ocean colonization between Madagascar and South America clades estimated to have occurred during the Paleocene (~60Ma). Our results support that Opluridae, and its two genera, diversified in Madagascar during the Miocene (~16 Ma; Figure 4, Figure 5). Meanwhile, Leiosauridae diversification was mosthly centered in the southern and South America continent, Neotropical region, and started during the Late Eocene (~37Ma), between the Patagonia and Monte biomes, with subsequent clade dispersions northwards on the same continent. The northernmost distribution of this family is in Amazon biome, which was reached during Miocene (~21mya; Figure 4, Figure 5).

The ancestor of Leiosaurinae probably originated between the Patagonia and Monte biomes and most of the early speciation events occurred in those areas during the Oligocene (~27 Ma; Figure 4, Figure 5). All species of *Diplolaemus* occurs in Patagonia, and the first speciation of *Diplolaemus* occurs between *D. darwinii* and the other *Diplolaemus species*, with the other species, during the Miocene (~20Ma; Figure 4, Figure 5). Meanwhile, speciation events whithin *Diplolaemus*, without *D. darwinii*, occurred much more recently during the Pliocene (~5Ma; Figure 4, Figure 5). *Leiosaurus* speciation events occurred between the Monte and Patagonia during the Pliocene (~14Ma; Figure 4, Figure 5) and the species distributions followed a from south to north direction. *Leiosaurus belli* is distributed in the two regions and the other species of the group only in the Monte. We only sequenced the Argentinean group of *Pristidactylus*, and it appears to have a more recent origin than the other genera, dating back to approximately 7Ma at the late Miocene-early Pliocene transition. The species *Pristidactylus nigroigulus* and *Pristidactylus fasciatus* occurs between the regions Patagonia and Monte, and the species *Pristidactylus scapulatus* and *Pristidactylus achalensis* occurs one in the Patagonia and the other in the Pampas, respectively (Figure 4, Figure 5).

The ancestral area of Enyalinae was the Central+South Atlantic Forest and the events of speciation occurred from there. The divergence between *Urostrophus* and *Anisolepis* from *Enyalius* occurred during the Oligocene (~26Ma; Figure 4, Figure 5). Our results suggested that different from the general movement of the family to north, *Urostrophus* and *Anisolepis* made the opposite biogeographic movement, dispersing from the south Atlantic Forest to south in Chaco and Pampas biomes. The main cladogenesis of *Enyalius* happened during the Oligocene (~25 Ma; Figure 4, Figure 5), with group A formed by species with more southern distributions and group B formed by species with more northern distributions. Within clade A: *Enyalius* *leechii* is the species of the family that reach the northernmost distribution at the Amazonia rainforest through dispersion from the Atlantic Forest during the Miocene (~20 Ma; Figure 4, Figure 5). All the other four species of clade A remained in the Atlantic Forest, *E. iheringii* with the southernmost distribution in Atlantic Forest and *E. boulengeri* with the northernmost distribution, at central Atlantic Forest. Clade B also originated in Central+South Atlantic Forest during the Miocene (~16 Ma; Figure 4, Figure 5) being *Enyalius bilineatus* the only species remaining in Atlantic Forest, with an common ancestor with *E. capetinga* the species that colonized Cerrado biome during the Pliocene (~5 Ma; Figure 4, Figure 5). The ancestor of the clade with *E. pictus*, *E. catenatus* and *E. bibronii* probably originated in the north Atlantic Forest and subsequently dispersed into the Caatinga biome and Diamantine plateau (*E. bibronii* and *E. erythroceneus*) and the Cerrado (*E. pictus*) during the Pliocene (~5 Ma; Figure 4, Figure 5).

Diversification

Our analyzes in RPANDA demonstrated that the diversification model that best fit with our data was Model 5 (Morlon *et al*., 2012), representing constant speciation rates with no estimate for extinction rate. We analyzed, using BAMM, whether there were differences in diversification rates between the subfamilies and genera, and the ANOVA results showed that they are not significant (Figure 6). The diversification over time was analyzed using rjMCMC CoMET, showed some support for shifts in speciation rates between Pliocene and Pleistocene (Figure 4). These results are consistent with the period of emergence of all current species of Leiosauridae, which originated close to 5 million years ago between the Pliocene and the Pleistocene.

Discussion

Phylogenetic relationships and Taxonomy of Leiosauridae

We estimated the most complete phylogeny for Leiosauridae, bringing the relationships between genera and species with greater support than had already been proposed in the literature. We found the family and the subfamilies Leiosaurinae and Enyalinae well suported and we will discuss the relationships within each subfamily below.

For Leiosaurinae, the genus *Diplolaemus* have been well studied along the time, despite that, its species taxonomy remains uncertain. While *D. darwinii* is a well-defined species sister to the others, the limits and the relationship among the other species of the group still unclear (Figure 2), the same were found by Morando *et al*., (2015) and Femenias *et al*., (2019) using few loci, and by Victoriano *et al*., (2010) with morphological data. In the present work we sampled the lineages 1, 3 and 4 founded by Femenias *et al.* (2019) and recovered a different composition for this clade that proposed in the literature. *D. leopardinus* and *D. sexcinctus* had repeated changes in its taxonomy, sometimes individuals from *D. leopardinus* being reallocated in *D. sexcinctus* and vice-versa (Werner 1898, Donoso-Barros 1996, Victoriano et al., 2010, Femenias et al., 2019). Victoriano *et al*., (2010) limited the distribution of *D. leopardinus* to Mendoza assigning all the other populations as *D. sexcinctus*. Femenias *et al.,* (2019) agreed with that author and proposed more 6 distinct lineages to *D. sexcinctus*. Our results showed *D.s. Lineage* 1 related to *D. leopardinus* with strong support. These results allow us to assign *D.s. Lineage 1* as *D. leopardinus* increasing the distribution of the species at least to Mendoza and Neuquén. Our findings are contrary to that proposed by Femenias *et al.*, (2019) and Victoriano *et al.*, (2010), and supports, at least in part, with Donoso-Barros (1966) that included the individuals from Neuquén *in D. leopardinus*. Since the *D.s.* Lineage 4 was strong related to *D.*

sexcinctus we concluded that this lineage remains as *D. sexcinctus* (Figure 2). We did not sample the *D.s.* Lineage 5, however, as Femenias *et al.,* (2019) do not found differences between *D. sexcinctus* and *D.s.* Lineage 5, this lineage remains as *D. sexcinctus* as well. Our results recovered *D.s*. Lineage 3 poorly supported with *D. bibronii*, so its position in phylogeny remains unclear (Figure 2). About the relationships among the species of *Leiosaurus*, our finds reiterates to that found by Morando *et al. ,*2015. On the other hand, differently that found by literature (Femenias *et al*., 2019, Morando *et al.* ,2015), our findings did not support the relationship among the *Pristidactylus* species, except for *Pristidactylus achalensis* and *Pristidactylus scapulatus* (Figure 2). Even using a large and robust UCE dataset, we also could not recover the relationship among the genera of Leiosaurinae. Therefore, more studies are necessary to clarify the relationships among these genera. As the findings in relationship among species of *Pristictylus* was completely discrepant among our data and the literature, and the relationship in *Diplolaemus* and the taxonomic status of *D.s* Lineage 3 and the *Ds.* Lineage 2 remains open, more studies are necessary to understand these relationships.

While for Enyaliinae the genera *Anisolepis* was find as sister of *Urostrophus*, followed by *Enyalius*. *Enyalius* was recovered in two main clades (A and B) with differences in composition and phylogenetic relationships of those proposed in the literature (Rodrigues *et al*., 2014 and Breitman *et al*., 2018). *Enyalius leechii,* the only species from Amazonia, was recovered with high support in clade A as sister to all other species of this clade, different of that found in the literature, that was recovered in the clade B with poor support (Rodrigues *et al*., 2014). We recovered *E. boulengeri* as a species in the clade A as sister to the others *(E.leechii + (**E.boulengueri** + (E.ihenrigii + (E.perditus + E.brasiliensis))))* with high support (Figure 2), These results reiterates its specific status, since Jackson (1978) changed the status of *E. boulengeri* described by Etheridge (1969) to a subspecies of *E. brasiliensis*, however Rodrigues *et al*. (2014) returned *E. boulengeri* with the full specific status, but its position remained unclear until this present work, since it was recovered with poor support as sister of *E. ihenrigii*.

In the same article, Jackson (1978) described the species *E. perditus*, as a "lost species" (Perditus= Lost from Latin) within *E. catenatus*. Limiting *E. catenatus* a more north distribution and *E. perditus* distributed in the southern Atlantic Forest (AF) around Rio de Janeiro and Sao Paulo. Our results with a subsample using UCE data indicate *E. perditus* as paraphyletic in relation to *E. brasiliensis*, as found in the literatue (Rodrigues *et al*., 2014 and Breitman *et al*., 2018). However, with a much larger sample using sanger data, our results showed that these species are indistinguishable, they aren't recirpocally monophyletic (Figure 2, Appendix 6). Given our results, *E. perditus* seems to correspond to specimens of *E. brasiliensis*.

Clade B was recovered with the species *E. bilineatus* and *E. capetinga*, as sister of *E. Catenatus*, *E. pictus* and *E. bibronii* (Figure 2). Jackson (1978) synonymized the species *E. pictus* and *E. bibronii* with *E. catenatus*. However, Rodrigues *et al*. (2006) resurrected *bibronii*, *catenatus* and *pictus* to full species status. We recovered these three species in the same clade with differences on the phylogenetic relationships comparing sanger and UCE's results (Figure 2,Figure 3). With sanger data, we inferred *E. catenatus* as polyphyletic, as was recovered by Rodrigues et al. (2014). Individuals from Bahia, *Enyalius cat1*, were recovered with *E. pictus*, and individuals from Pernanbuco, *Enyalius cat2,* were recovered with E*. bibronii*. However, all our analyzes with UCE data recovered *E. catenatus* as paraphyletic in relation to *E. pictus*, including that samples that clustered with *E. bibronii* in sanger data analysis. We observed that the mitochondrial markers show that *E. catenatus* is polyphyletic; on the other hand, nuclear markers and UCE's recovered *E. catenatus* paraphyletic in relation to *E. pictus*. Our results indicate a complex evolutionary scenario associated with *E. catenatus, E. pictus* and *E. bibronii*. Therefore, further analysis is important and necessary to understand if these differences between data analyzed are due, for instance, to hybridization, and if *E. catenatus* is a distinct species from *E. pictus*. We recovered *E. bibronii* in a monophyletic clade agreeing with Rodrigues *et al*., (2006) that *E. bibronii* is a valid species. We sampled one specimen identified as *E. bibronii* from Chapada Diamantina (Morro do Chapeu – BA) as sister group of other specimens sampled of *E. bibronii*. This specimen could be an exemplar of *E. erythroceneus* corroborating the relationship among this species proposed by Rodrigues *et al.* (2006, 2014).

Is Enyalius a single and monophyletic genus?

Our results demonstrated that *Enyalius* appears in two well delimited and statically supported clades. The deep divergence and time of diversification of clades A and B of *Enyalius* can be comparable to the other genera of the family. As mentioned above, a similar structure was recovered also by literature (Rodrigues *et al*., 2014, and Breitman *et al*., 2018) showing the strong structuration of these clades. In our analyses with UCE data the clades A and B of *Enyalius* were sisters, but just to emphasize the profound differences, our results with sanger data were not able to recover *Enyalius* as a monophyletic,, and *Anisolepis* + *Urostrophus* were shown to be the sister group of *Enyalius* clade A (Figure 3). This indicates a possibility to the clades A and B of Enyalius could be two distinct subgenera or even genera. As *E. catenatus* Weid 1821 is the oldest species of the genus and the type of the genus, and it is part of clade B, we proposed that the clade B must be the genus *Enyalius* and the Clade A represents an undescribed genus awaiting for a proper taxonomic and systematic account.

Biogeography in Neotropics

Leiosauridae is an interesting family of lizards (Iguania: Plerodonta) that originated, diversified, and is distributed exclusively in the Neotropical region, specifically in South

America at the Chilean and Brazilian subregions. These regions have differences that can be traced back to the Precambrian, as observed geologically (Kearey, 2001, Almeida *et al*., 1976, Cordani and Neves, 1982). For some old faunal groups, such as the mayflies (Leptofhlebiidae), the distribution pattern evokes the Gondwanan breaks and movements. For example, mayflies from the Brazilian subregion are more related to animals distributed in Africa than to the group distributed in the Chilean region, which shares fauna with Australia (Monjardim *et al*., 2020). For lizards, the observed patterns are different from those observed for mayflies, probably due the more recent origin of the group. For instance, Plerodonta (Iguania) is mainly distributed in America continent, with few exceptions as Opluridae that occurs exclusively in Madagascar (Burbrink *et al*., 2020, Münchenberg *et al*., 2008, Noon and Chippindale, 2006). Opluridae is sister of Leiosauridae, and according to our analysis, they diverged from each other approximately 63 Ma. We didn't sample all other iguanids families, because our focus is on Leiosauridae diversification in the Neotropics. However, as the other families of Pleurodonta, also occur mainly in the Neotropics, it is more likely that the common ancestor of Leiosauridae and Opluridae also has its origins in the Neotropical region. Noon and Chippindale (2006) proposed that Opluridae used the "Gunnerus Ridge" route passing through the south of South America, via Antarctica, arriving in Madagascar, since the Gondwana division predates the group's origin. However, Ali & Krause (2011) ruled out this hypothesis of a "Gunnerus Ridge" for oplurids because the formation of the connection is older than the origin of Opluridae. Therefore, the separation of the families occurred possibly by over-sea dispersion, not via continental bridge (Samonds *et al*., 2013).

As demonstrated, the distribution of Leiosauridae is in markedly distinct environments, and interestingly, the family is divided into two subfamilies, each of which occurs mostly in one of these subregions with the MRCA dated from ~38 Ma in the Eocene (Paleogene; Figure 1, Figure 4, Figure 5). Our ancestral area reconstruction demonstrated that the family originated further south on the continent, in Patagonia, where the climate is drier and with lower temperatures. Leiosaurinae originated in the southern Chilean subregion, in Patagonia. In general, our data showed that both the subfamily and the genera had a similar pattern, with its origin further south and later dispersing further north. This pattern can possibly be explained by the influence of the Andes uplift in the region, as such events drastically changed its geology, climate and biology, by making the southern region more inhospitable influenced the march of these animals to the north, possibly in search of environments that best fits for its survival. (Morando *et al*., 2015, Folguera *et al*., 2011, Hernández *et al*., 2005, Cei , 1986)

While Enyaliinae originated and diversified northeast in the continent, mainly in the heart of the Atlantic Forest (AF), in the Brazilian subregion. For this group, mainly two periods were important to explain its current distribution. During the Oligocene/Miocene, it was when the subfamily originated in the southern portion of the AF and three important events happened, being them: southern dispersal of *Anisolepis* and *Urostrophus*, colonization of Amazonia via SE-NW route by *E. Leechi*, and dispersal to north of *Enyalius* clade B. And three others during the Pliocene/Pleistocene periods, namely: dispersion of clade A of *Enyalius* in the southern portion of MA, Dispersion of clade *E. bilineatus* + *E. capetinga* through the southern portion of AF and Cerrado, and dispersion of *E. bibronii*, *E. catenatus* and *E. pictus* through the northern portion of AF, and colonization of Caatinga, Cerrado and Chapada diamantina.

Chilean subregion

The ancestral area reconstruction showed that Leiosaurinae originated in the extreme south of South America, in Patagonia, with subsequent dispersion mostly towards the north. The common ancestor that gave rise to the genera *Diplolaemus*, *Leiosaurus* and *Pristidactylus* originated approximately 27 Ma at the end of the Oligocene (Figure 4, Figure 5). The first major lineages of the subfamily, which gave rise to the living genera, diversified among themselves during the Miocene, possibly influenced by the uplift of Andean cordillera, which provided intense climatic and biotic changes in the Patagonian region (Morando *et al*., 2015, Folguera *et al*., 2011).

The genus *Diplolaemus* was recovered into two main clades that split approximately 20 Ma during the Miocene: one originating *D. darwinii* species and the other a lineage with all the other species, all exclusively distributed in the Patagonia region (Figure 4, Figure 5). The origin of the genus *Leiosaurus* was reconstructed for the Miocene, approximately 14 Ma (Figure 4, Figure 5). This period is consistent with the end of the last major Andean uplift (Morando *et al*., 2015, Hernández *et al*., 2005), and is congruent to that found for species that have similar distribution, as *Liolaemus montanus* (Olave *et al*., 2015). This genus is distributed in the Patagonia and Monte biomes, with *L. belli* being the most widely distributed species, occurring in both biomes, and all other species with more restricted distribution in northwestern Argentina, occurring exclusively in the Monte biome (Morando *et al.*, 2015). Our ancestral area reconstruction demonstrated that the genus possibly originated in Patagonia with further diversification following south-to-north direction (Figure 5). Femenias *et al*., (2019) recovered *L. belli* geographically structured, and proposed each lineage as a potential species. However, when a species is widely distributed, in different landscapes, it is common to find greater diversity within the group. Therefore, more analyzes should be carried out aiming to understand the level of gene flow and isolation of these populations to establish if it is a single species or not.

The genus *Pristidactylus* occurs on both sides of the Andes and is recognized in the literature for having two main groups: a Chilean group with characteristics for arboreal lifestyle distributed west of the Andes and an Argentinean group east of the Andes (Morando *et al*., 2015). The arboreal characteristics found in the genus allowed Cei (1986) to suggest that the genus arose before the uplift of the Andes in forested areas. Unfortunately, we were unable to amplify the only sample from the Chilean group that we had from *P. torquatus*, and our sample consisted of five species of six from the Argentinean group. Therefore, we could not date the separation between these two groups. It is worth noting that Morando *et al*., (2015) did not recover *P. torquatus*, the species endemic to temperate Chilean beech forests, with the other species of the Argentinian *Pristidactylus* group, but as a sister group of *Leiosaurus* with high support. These results demonstrate that more studies must be carried out to shed light on the biogeography and taxonomy of this genus, and to understand which factors influenced the diversification of the groups. If the Chilean and Argentinean groups stay separate, after new analyses, including species from both groups, it could be an indication that possibly they could be differents genera. Different of the Chilean group, the species of Argentinean group have adaptations to drier climates (Morando *et al*., 2015), and diversified approximately 6 Ma in the late Pliocene period, with the origin of MRCA reconstructed to Patagonia, reaching from there the Monte and Pampa biomes, indicating a south-to-north direction distribution, as demonstrated in other species of Leiosaurinae.

Brazilian Subregion

The ancestral area reconstruction showed that Enyalinae originated in the Atlantic Forest, in the Brazilian sub-region, and from there it dispersed into two main periods, first during the Oligocene/Miocene and the other during the Pliocene/Pleistocene periods. Despite having its vegetation cover reduced to approximately 7%, mainly due to anthropic actions, the Atlantic Forest remains extremely rich, being one of the main biodiversity hotspots in the world (Mittermeier *et al*., 2005). Enyalinae is majority distributed at the AF, except for *A. undulatus*, *E. leechii* and *U. gallardoi*, all the other 11 species occurs there. Approximately 26 Ma the genera *Urostrophus* and *Anisolepis* separated from *Enyalius* in the Atlantic Forest during the Oligocene (Paleocene). These two genera are poorly studied, with the last revision made by Etheridge and Williams (1991). The genus *Urostrophus* has two species being *U. vautieri* mainly distributed in Atlantic Forest and *U. gallardoi* mainly distributed in Chaco biome (Etheridge and Williams, 1991, Santosi *et al.,* 2009, Gasparini *et al*., 2010). According to our ancestral area reconstruction, it has dispersed further south into the Atlantic Forest and from there possibly reached the Chaco. Our results showed that representatives of the genus *Anisolepis*, with three species, followed a similar route, marching south, reaching the Pampa and Chaco biomes (Etheridge and Williams, 1991).

Our ancestral area reconstruction showed that the genus originated and diversified in the AF occurring approximately 25 Ma during the Oligocene (Figure 4, Figure 5), date congruent to the last major Andes uplift (Morando *et al*., 2015). Apparently, events triggered by the Andes uplift, such as intense climate change, gave rise to the formation of Dry Diagonal, separating AM from AF, and also separated AF into two poles, one formed by the central and south region of AF, and another by the north region of AF. Our data showed that species from the central and south AF are more connected to each other and with AM, while the north AF is more connected to the Caatinga and the Cerrado. Being the clade A more distributed in the central and south regions of AF and in Amazonia. While clade B mainly distributed in the north AF, caatinga and Cerrado, with one species in the central region of the AF. The clade with the species distributed in the central region of AF of clade B, diverged from the other species from north region of AF, caatinga and Cerrado, approximately 16 Ma, being the oldest separation of clade B (Figure 4). Date close to the events mentioned above about the separation of the AF. It is important to emphasize that many other works on the Atlantic Forest fauna have also found the same pattern of separation in "north and south" clades (Menezes et al., 2016, Thomé *et al*., 2010, Carnaval, Costa *et al*., 2000, Costa 2003), however most cases investigated are related to the intraspecific level, and consequently have been associated to more recents events as Plio-Pleistocene refuges and its climate changes. Here, working with supra-specific groups, at the generic level, we associate this division with older periods between the Oligocene and Miocene periods. We indicated for the first time with a faunal group this north and south division in an earlier period, this pattern can also be observed for the plant genus *Bertolonia* (Melastomataceae), being reported for the first time by Bacci *et al*., 2021. During the Miocene, the entire globe underwent intense changes (Potter e Szatmari, 2009), which will be discussed better in the next section, including the acceleration of the uplift of the Andes, which triggered several changes in the neotropical region, both in Chilean and Brazilian sub-regions, and we propose for the first time that these events also had an influence on the Atlantic Forest, influencing its division into north and south. Thus, the oldest division, at the supraspecific level, is associated with Oligocene/Miocene events, while for the intraspecific level, the more recent Pliocene/Pleistocene events.

The clade A origin and actual distribution is mainly in the central-south Atlantic Forest, reaching the Amazon probably via SE-NW bridge (Bigarella *et al*., 1975, Ledo and Colli, 2017) with *E. leechii*. The Atlantic Forest has been previously connected to the Amazon, but the uplift of the Andes triggered the formation of the Dry Diagonal, which comprises the more xeric biomes as Cerrado, Caatinga and Chaco, separating the tropical forest into two poles, the AF and the AM (AM; Ledo and Colli, 2017, Werneck, 2011). Despite that, throughout evolutionary history, several paths have emerged at different time periods, reconnecting these biomes through corridors across the Dry Diagonal and/or along the coast (Ledo and Colli, 2017).

Rodrigues *et al*. (2014) recovered *E. lechii* with clade B, which has a more northerly distribution, and therefore proposed that *E. leechii* reached Amazon via route NE. However, as proposed by Ledo and Colli (2017) using environmental niche modelling, Palaeobiological and molecular data the SE-NW route is more suitable for the group in question, corroborating our findings.

Our ancestral area reconstruction demonstrated that clade B also originated in the central-south AF approximately 16 Ma, and dispersed to north AF approximately 11 Ma, and from north AF dispersed more recently to other biomes (Figure 4, Figure 5). The events related to Plio-Pleistocene climate change seems to be more associated with species diversification in this clade. For instance, the species *E. bilineatus* and *E. capetinga* diversified from each other approximately 7 Ma. This clade is distributed more in the central AF and central Brazil, reaching the Cerrado biome. *E. bilineatus* has a more eastern distribution in the Atlantic Forest, reaching further inland to regions bordering the Cerrado, while *E. capetinga* is more distributed in Cerrado, dwelling in gallery forests (Rodrigues *et al*., 2014, Breitman *et al*., 2018). As previously presented, the climatic fluctuations that occurred during this period allowed incursions by the AF biota towards the Cerrado and Caatinga (Ledo et al., 2020, Ledo and Colli 2017). This can justify the presence of these species in the Cerrado gallery forests and on the borders between the Cerrado and AF, showing that these species have adaptations to the forest and that in a period they were allowed to disperse into these areas by forest connections that no longer exist, as the connections cited by Ledo and Colli (2017). The same events appear to be related to the clade composed by *E. bibronii, E catenatus* and *E. pictus*, that started to differentiate approximately 11 Ma, with *E. bibronii* having a great diversification with a wide distribution approximately 4.5 Ma (Figure 4, Figure 5). This species can be found in the Atlantic Forest and Caatinga biomes, but also in the Diamantina plateau. While *E. catenatus* and *E. pictus* in the central AF and Cerrado between the Doce and Jequitinhonha rivers (Rodrigues *et al*., 2014).

Diversification

No differences in diversification rates were found between the main clades, nor between Leiosaurinae and Enyaliinae (Figure 6). The lack of differences in speciation rates between clades in the Chilean subregion and the Brasilian subregion was surprising, as greater biodiversity is expected in tropical regions and in lower latitudes (Mannion *et al*., 2014, Jenkins *et al*., 2013, Condamine *et al*., 2012, Mittelbach *et al*., 2007, Wiens *et al*., 2006, Gaston and Spicer, 2004), despites that remained the same.

We observed some support for a speciation shift between Pliocene and Pleistocene (Figure 6). These results are consistent with the period of emergence of all current species of Leiosauridae, which originated close to 5 Ma between the late Pliocene and the early Pleistocene. During this period, the planet experienced major changes such as climatic, tectonic, and marine intrusion changes, which influenced habitats changes and consequently, their associated biota, generating new species by isolation and divergence across the globe (Burbrink *et al*., 2019, Oliveira *et al*., 2015, Rull 2015, Ruane *et al*., 2014, Moyle *et al*., 2009, Carstens and Knowles, 2007, Weir and Schluter, 2004, Knowles, 2001, Klicka and Zink, 1999, Avise *et al*., 1998). At the Neotropical region, we can observe several cases of “genetic breaks” in lineages from both, the Brazilian subregion (De Mello Martins, 2011, Thomé *et al*., 2010, Grazziotin *et al*., 2006) and the Chilean subregion (Morando *et al*., 2015, Markgraf *et al*., 1995, Baéz and Scillato Yané, 1979), are reported, which have been associated with paleoenvironmental changes during the Plio-Pleistocene period. This period is important to understand the diversification of Leiosauridae. In *Diplolaemus*, for instance, apparently between the end of the Miocene and the beginning of the Pliocene, approximately 5 Ma there was a boom of cladogenesis in this genus giving rise to the main lineages that we know in the present. And in

Enyalius, during the Plio-Pleistocene the incursion of Atlantic Forest biotas across the Cerrado and Caatinga was possible, due the climates changes (Ledo et al., 2020).

Despite not finding significant shifts in other times periods, we observe that important events happened for the diversification of the group between approximately 20-30 Ma, during the Oligocene (Paleogene) and the Miocene (Neogeno), when the subfamilies and genera of Leiosauridae given rise. At the same period, in Enyaliinae, there were a split between the genera *Anisolepis*, *Urostrophus* and *Enyalius*, and between clades A and B of *Enyalius*, and *E. leechii* in *Enyalius*. Towards Leiosaurinae, in this same period occours the split between the genera *Leiosaurus*, *Diplolaemus* and *Pristidactylus*, and between the clades *D. darwinii* and the other especies of *Diplolaemus*. Such "simultaneous" events demonstrate that apparently the same general processes shaped the diversification in the entire family across this broad scale.

Between the Oligocene and the Miocene, changes influenced by the Earth's interior promoted an intensification of the movement of tectonic plates around the world, which generated changes across the planet, changing the sedimentation pattern of the ocean floor, altering the courses of the oceans, with the closing of the central circulation, decrease in global temperature, desertification of central Asia, North Africa, Australia and southern South America, expansion of the ice in Antarctica, and intensification of the processes of the Andes uplift (Potter and Szatmari, 2009) . On a global scale, it was a period where much of today's geography and biotic composition was formed (Hoorn et al., 2020). In relation to the Neotropical region, the Andes uplift, appears to be one of the main factors that promoted this diversification and division of clades. During this entire period, this geological activity has brought both, structural and climatic, changes to the region, but as mentioned above, it intensification approximately 20 million years ago, promoting the most drastic changes (Ledo and Colli, 2017, Morando *et al*., 2015, Werneck, 2011, Antonelli and Sanmartin, 2011, Potter e Szatmari, 2009, Blisniuk *et al*., 2006). These events influenciated the formation of the Dry

Diagonal, forming a drier region separating AF from AM, as influenced the formation of the Amazon basin, as well as drying the Patagonia region and originating the Monte biome (Ledo and Colli, 2017, Morando *et al*., 2015, Antonelli and Sanmartin, 2011). Here we are proposing that these same events also influenced the separation of AF into north and south. Because it has such an influence on the dynamics of the Neotropical biomes, and because the species are associated with the biomes that are found, the uplifting of the Andes had an expressive contribution modeling the diversity that we find today in Neotropical region and in Leiosauridae, with species from Patagonia, passing through several biomes and climatic types, reaching the Amazon. Another interesting point to be highlighted is the separation of the Amazonian species *E. leechii* from the others, which occurred approximately 20 Ma, a period close to the separation of the genera. This division can also be explained by the formation of the Andes and the Dry Diagonal formation that separates the AF from the AM, with possible reconnections formed in this period and where the dispersion of the species possibly occurred, as discussed in the previous section.

Conclusions

The diversification history of Neotropical region, and consequently of Leiosauridae, seems to be directly related to the events of Oligocene/Miocene periods and the Andes uplift, and to the climate changes of Plio-Pleistocene. For the deeper clades of the studied Family (subfamilies, genera, clades A and B of *Enyalius*, *E. leechii*, and *D. darwinii* and others species of *Diplolaemus*) those related to Oligocene/Miocene and the Andes uplift seems to be associated, while for the crown (the species) it was the Plio-Pleistocene climate changes. The Andes uplift and global events during the Oligocene/Miocene periods, in addition to having caused the division of the mentioned groups above, also seems to have had an influence on the distribution of these groups. Regarding Leiosaurinae, for instance, after its origin in the extreme south of Patagonia, it seems that they dispersed towards the north, possibly due to environmental changes caused by the mentioned event, that make the southernmost regions, more inhospitable for survival. While for the events triggered by the Andes uplift, in addition to having promoted the formation of Dry Diagonal, separating the AF from the AM, apparently also triggered changes that separated the regions of central + south of AF from north of AF, separating *Enyalius* in clade A and B, with the species from the central and southern regions of the AF being more connected to each other (clade A), while the northernmost AF is more connected with the Caatinga and the Cerrado (Clade B).

This work brings a new point of view of the understanding of the diversification of Leiosauridae in such a complex scenario as is the case of the Neotropical region and represents an effort to improve the understanding of biodiversity Neotropical. Many questions were answered, but others emerged. Further studies must be carried out to understand the real taxonomic status of genera such as *Enyalius*, *Pristidactylus* and *Diplolaemus*. As well as further studies should be directed to understand the relationship between *E. catenatus, E. bibronii* and

E. pictus, and their possible hybridization. We believe that new effort to sample a larger variety of *Anisolepis* and *Urostrophus*, comprising the group distribution, and representants of *Pristdactylus* chilean group, is interesting to improve the understanding of these groups.

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Figures

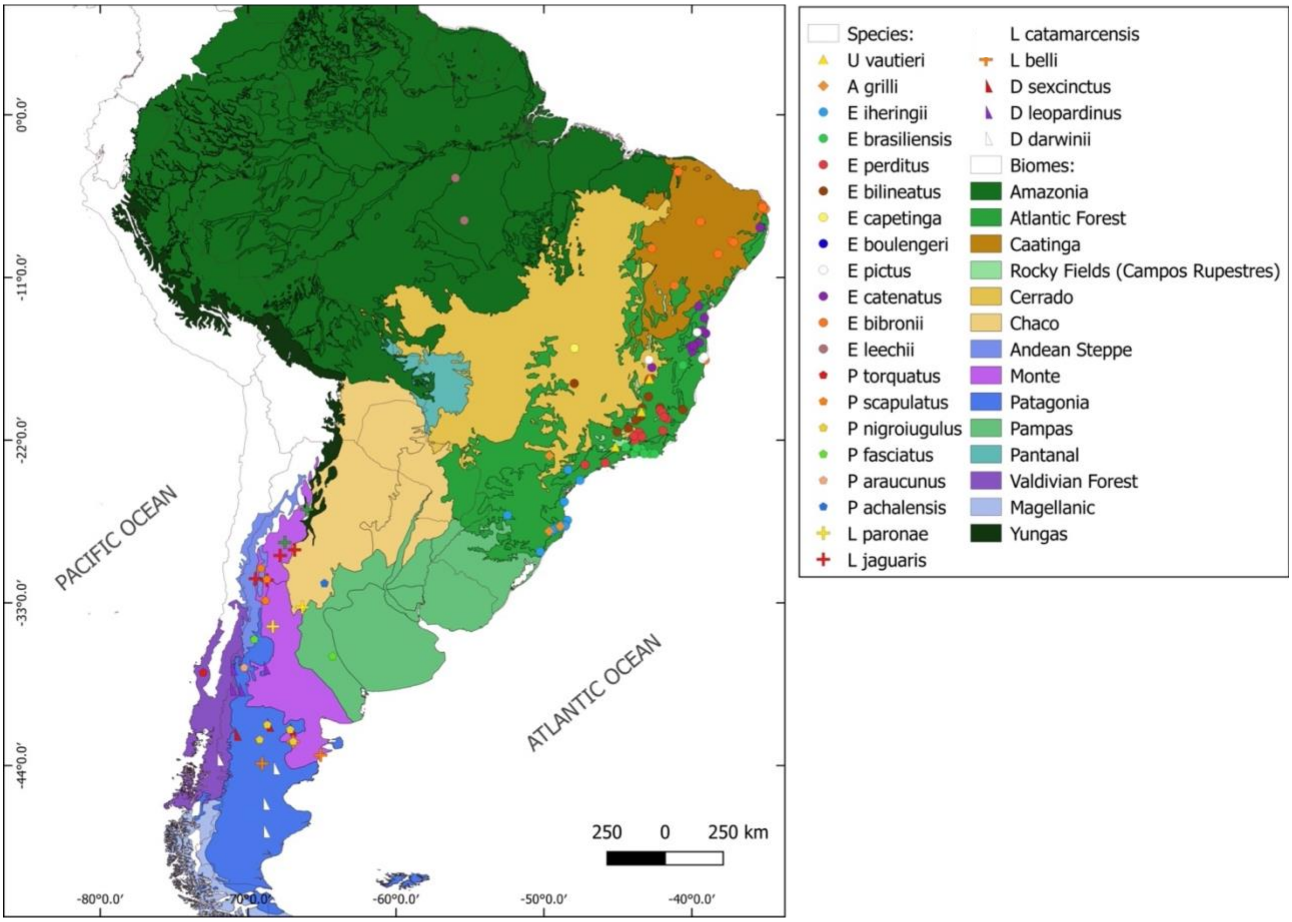


Figure 1. Map with the biomes of the neotropical region with the distribution of species of the Leiosauridae family and specimens that were sampled in this study.

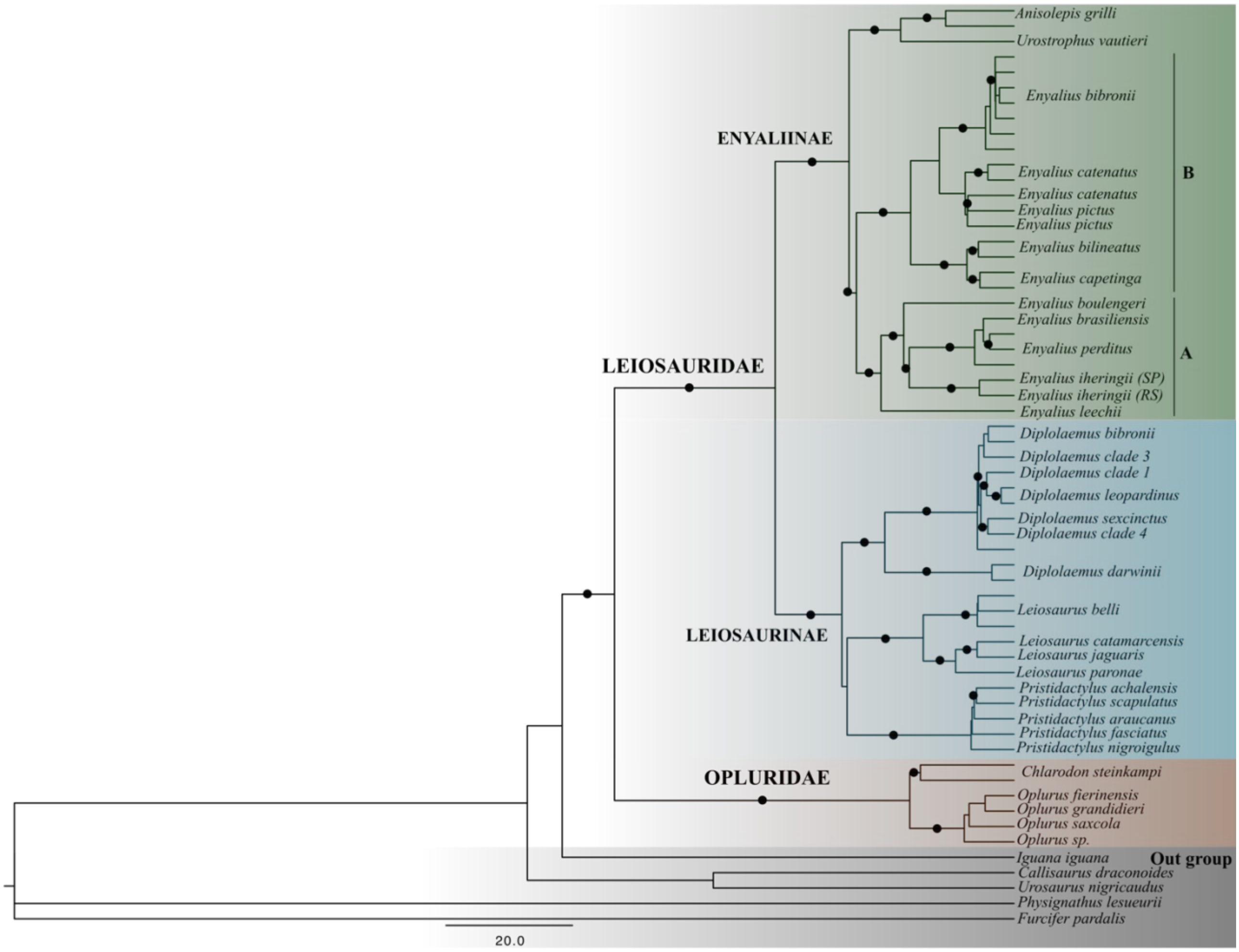


Figure 2. Species tree generated using 1000 posterior probability gene tree in the ASTRAL-III program with UCE data. Opluridae is colored in brown, while Enyaliinae in green and Leiosaurinae in blue (both belonging to the Leiosauridae family). Black dots on the branches represent statistically well supported clades.

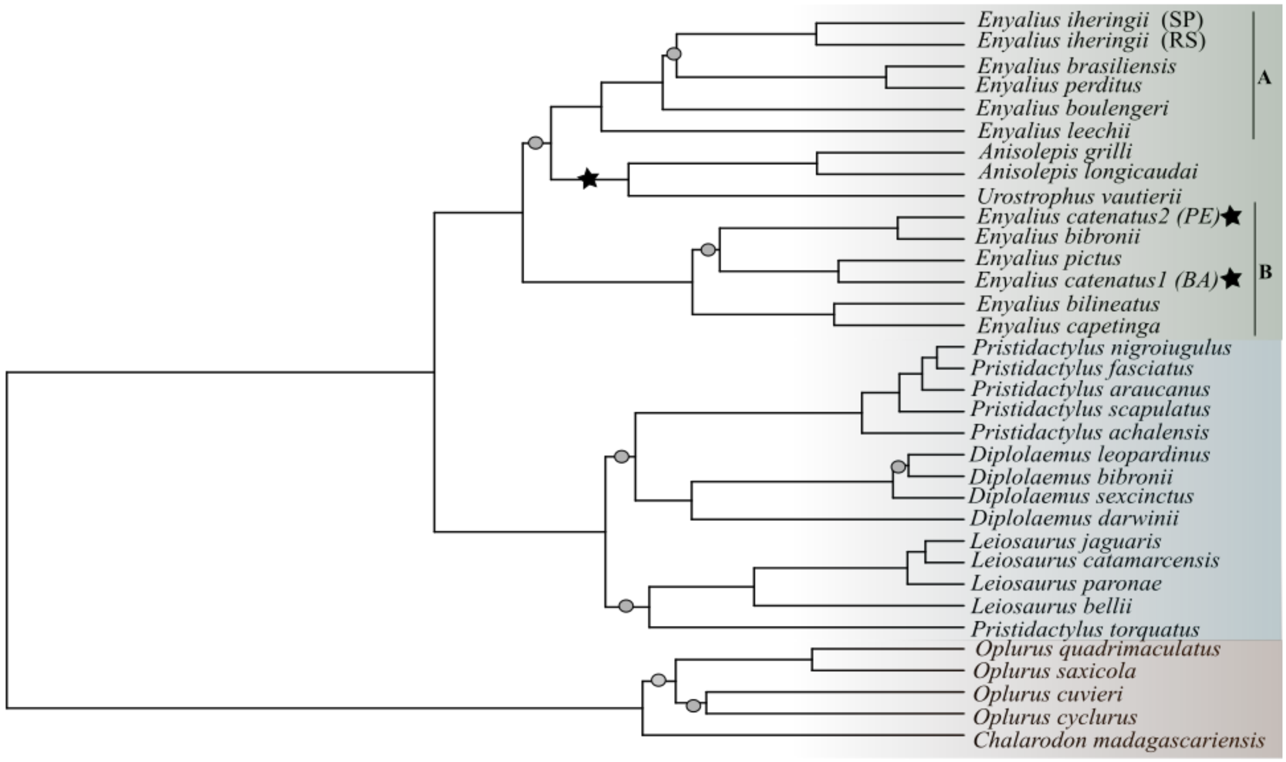


Figure 3. Species tree generated using 1000 posterior probability gene tree in the ASTRAL-III program with sanger data. Opluridae is colored in brown, while Enyaliinae in green and Leiosaurinae in blue (both belonging to the Leiosauridae family). Grey dots on the branches represent poor supported clades, and differences found between the sanger and UCE data are highlighted with black stars.

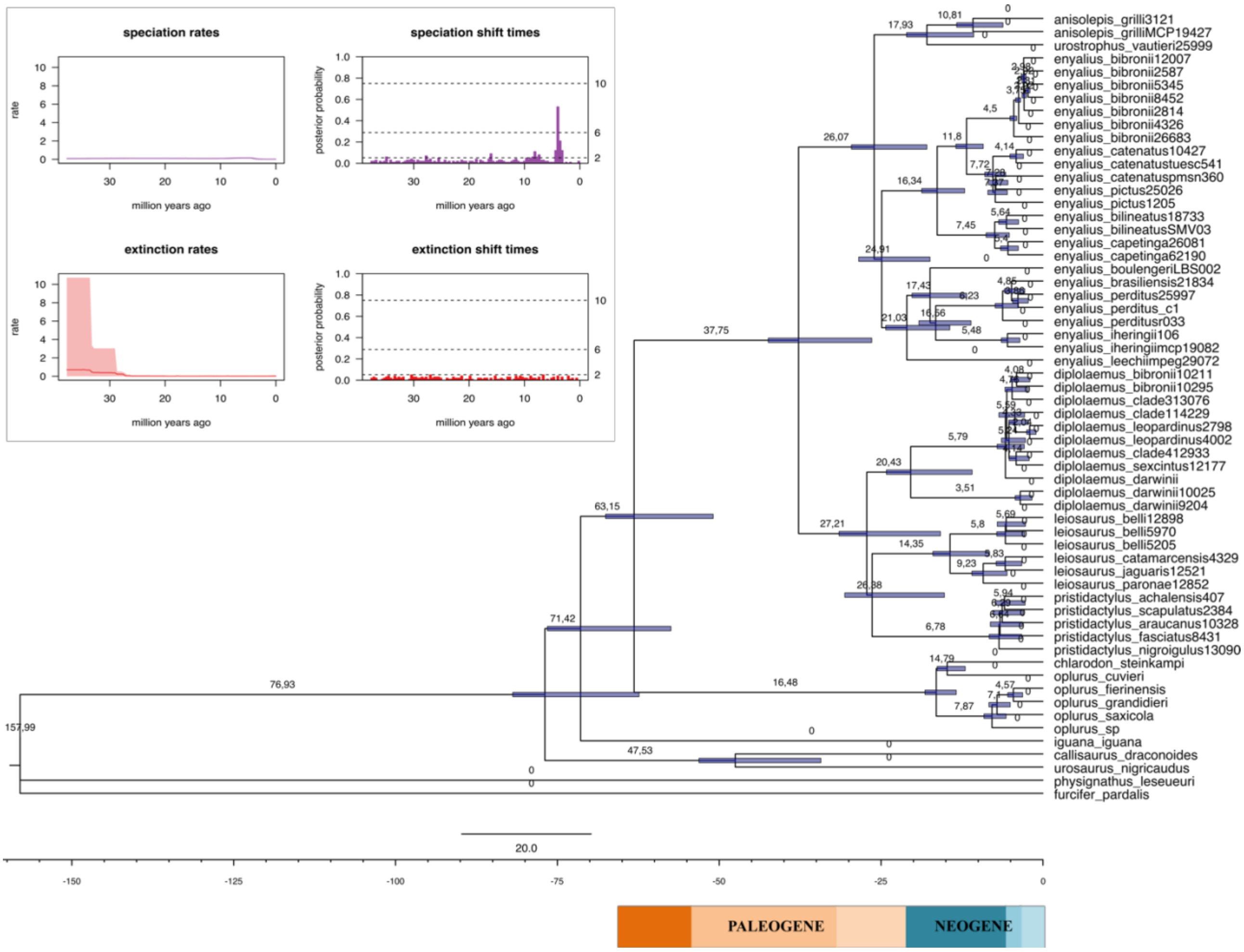


Figure 4. Dated phylogeny for all species sampled of Leiosauridae + Opluridae using ASTRAL III and TreePL. The numbers above the branches indicate the average age of formation of clades in millions of years. Estimates of speciation and extinction rates over time using CoMET (TESS package) with probabilities of these rates changing indicated by Bayes factors in bootstrap with 1000 phylogenies.

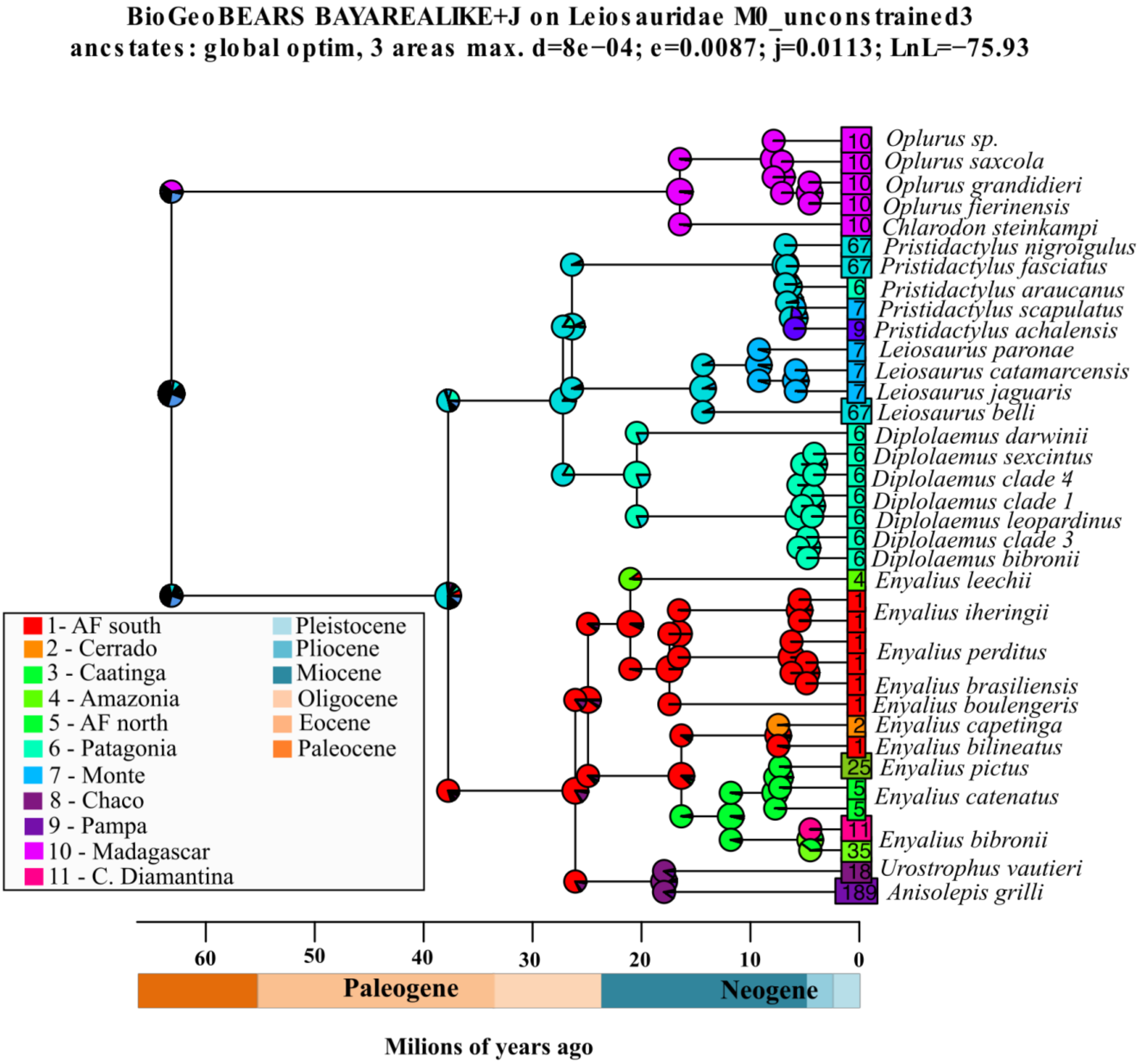


Figure 5. Ancestral Area Reconstruction for Leiosauridae using the BayArea+J model.

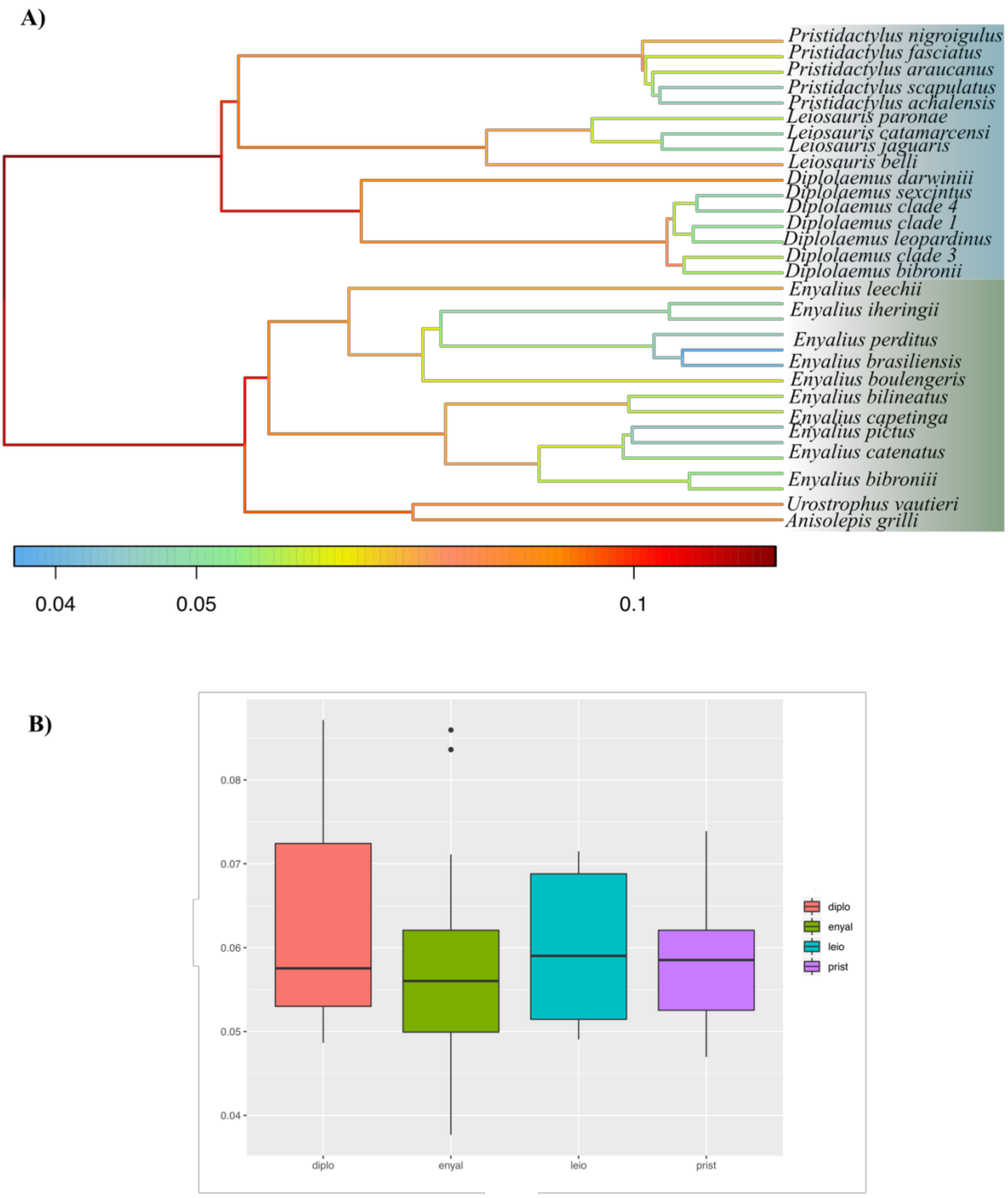


Figure 6. A) Diversification analysis performed with RPANDA was similar to BAMM results. B) The ANOVA showed that there was not significant differences in the rate of diversification between clades, neither between subfamilies, nor between genera.

Appendixes

Appendix 1. Table with samples used for UCE data.

Collection	Voucher	I5 Barcode	I7 Barcode	Family	Subfamily	Genus	Species	Locality
BYU	10295	ACGTCCTG	GTGTTCTA	Leiosauridae	Leiosaurinae	Diplolaemus	bibronii	Chubut Escalante Prov. Rte. 25, 3 km SO junction Prov. Rte. 27, 13 km NE junction with way to Sierra Chaira
BYU	10211	ACGTCCTG	AGTCACTA	Leiosauridae	Leiosaurinae	Diplolaemus	bibronii	Chubut Escalante Prov. Rte. 26, 21.1 km NO junction Prov. Rte. 37, 29.1 km NO junction Natl. Rte.26, 3.9 km SO Estancia Rancho Grande
BYU	14229	ACGTCCTG	ATTGGCTC	Leiosauridae	Leiosaurinae	Diplolaemus	clade 1	Neuquén Pehuenches Auca Mahuida, way to the antennas
BYU	13076	ACGTCCTG	CAGATCTG	Leiosauridae	Leiosaurinae	Diplolaemus	clade 3	Chubut Río Senguer Natl. Rte.40, 2.5 km N junction Natl. Rte.26
BYU	12933	ACGTCCTG	CCGTGAGA	Leiosauridae	Leiosaurinae	Diplolaemus	clade 4	Río Negro Bariloche Prov. Rte. 80, way to Cerro Bernal, 19.5 km S junction access to Bariloche Airport
BYU	9204	ACGTCCTG	ACACGACC	Leiosauridae	Leiosaurinae	Diplolaemus	darwinii	Chubut Escalante Holdich Station
BYU	10025	ACGTCCTG	AGCACCTC	Leiosauridae	Leiosaurinae	Diplolaemus	darwinii	Santa Cruz Magallanes Prov. Rte. 77, 66.6 km NO junction Prov. Rte. 25, 9.1 km SE Estancia Vega Grande
BYU	4002	ACGTCCTG	AAGACGGA	Leiosauridae	Leiosaurinae	Diplolaemus	leopardinus	Mendoza Malargüe Prov. Rte. 180, 90 Km S El Nihuil
BYU	2798	ACGTCCTG	CCAGTTCA	Leiosauridae	Leiosaurinae	Diplolaemus	leopardinus	Río Negro 25 de Mayo Prov. Rte. 6, 18 km N Colan Conhué
BYU	12177	ACGTCCTG	GGAGAACA	Leiosauridae	Leiosaurinae	Diplolaemus	sexcinctus	Río Negro Valcheta 18 km W Cona Niyeu, route to El Cain

BYU	5970	ACGTCCTG	AGATCGCA	Leiosauridae	Leiosaurinae	Leiosaurus	belli	Neuquén Chos Malal Natl. Rte.40. 14,5 Km N Chos Malal
BYU	5205	ACGTCCTG	TCTTCACA	Leiosauridae	Leiosaurinae	Leiosaurus	belli	Mendoza San Rafael Prov. Rte. 179, 21 km S El Nihuil
BYU	12898	ACGTCCTG	ACTATGCA	Leiosauridae	Leiosaurinae	Leiosaurus	belli	Catamarca Santa Maria Ruta Nacional 40, 37 Km W Punta de Balasto
BYU	4329	ACGTCCTG	CAAGACTA	Leiosauridae	Leiosaurinae	Leiosaurus	catamarcensis	San Juan Calingasta Provinc. Rte. 412, junction Provinc. Rte. 425, 26 km N Villa Nueva
BYU	12521	ACGTCCTG	CCTCCTGA	Leiosauridae	Leiosaurinae	Leiosaurus	jaguaris	Mendoza San Rafael Provinc. Rte. 153, 38.3 km S Aristides Villanueva, 7 km N Monte Coman, 1 km N junction Ruta Nacional 146
BYU	12852	ACGTCCTG	AAACATCG	Leiosauridae	Leiosaurinae	Leiosaurus	paronae	Cordoba San Alberto Posta de Pampa de Achala, Quebrada del Condorito National Park
BYU	407	ACGTCCTG	ACCTCCAA	Leiosauridae	Leiosaurinae	Pristidactylus	achalensis	Neuquén Chos Malal Provinc. Rte. 37, 24,6 km N junction Ruta Nacional 40, Paraje Los Ranchos
BYU	10328	ACGTCCTG	CGGATTGC	Leiosauridae	Leiosaurinae	Pristidactylus	araucanus	La Pampa Puelén Provinc. Rte. 23, 6,6 km S to Provinc. Rte. 26
BYU	8431	ACGTCCTG	ACATTGGC	Leiosauridae	Leiosaurinae	Pristidactylus	fasciatus	Chubut Languiñeo Establecimiento 6 Hermanos, 10 km N NE junction Natl. Rte.25, way to Prov. Rte. 62, Pocitos de Quichaura
BYU	13090	ACGTCCTG	ATTGAGGA	Leiosauridae	Leiosaurinae	Pristidactylus	nigroigulus	San Juan Iglesia Alto del Colorado
BYU	2384	ACGTCCTG	GTCGTAGA	Leiosauridae	Leiosaurinae	Pristidactylus	scapulatus	Región del Bío Bío (Chile) Provincia de Biobío Nahuelbuta Mountains
PUCRS	MCP 19427	ACGTCCTG	GGTGCGAA	Leiosauridae	Enyaliinae	Anisolepis	grilli	Águas Mornas/ Parque Estadual da Serra do Tabuleiro,SC
UFSC	3121	ACGTCCTG	ACCACTGT	Leiosauridae	Enyaliinae	Anisolepis	grilli (cf)	Urubici/RPPN Portal das Nascentes/SC
MNRJ	26683	ACGTCCTG	ACAGATTC	Leiosauridae	Enyaliinae	Enyalius	bibronii	Município Morro do Chapéu, BA
UFRN	4326	ACGTCCTG	TGGAACAA	Leiosauridae	Enyaliinae	Enyalius	bibronii	Paulo Afonso,BA

INMA	SMV 03	ACGTCCTG	TGGTGGTA	Leiosauridae	Enyaliinae	Enyalius	bilineatus	Santa Teresa,ES
MNRJ	18733	ACGTCCTG	CGAACTTA	Leiosauridae	Enyaliinae	Enyalius	bilineatus	Serra do Caraça, MG
UFES	LBS002	ACGTCCTG	ACAGCAGA	Leiosauridae	Enyaliinae	Enyalius	boulengeri	EBSL,ES
MNRJ	21834	ACGTCCTG	ATCATTCC	Leiosauridae	Enyaliinae	Enyalius	brasiliensis	REBIO do Tinguá, Nova Iguaçu, RJ
CHUNB	26081	ACGTCCTG	ATGCCTAA	Leiosauridae	Enyaliinae	Enyalius	capetinga	Brasilia, DF
CHUNB	62190	ACGTCCTG	TCCGTCTA	Leiosauridae	Enyaliinae	Enyalius	capetinga	Catalão, GO
UFRN	10427	ACGTCCTG	AAGGTACA	Leiosauridae	Enyaliinae	Enyalius	catenatus	Coaraci,BA
UFPE	PMSN 360	ACGTCCTG	GACAGTGC	Leiosauridae	Enyaliinae	Enyalius	catenatus	Serra de Jundiá, Vicência,PE
UESC	TUESC 541	ACGTCCTG	GACTAGTA	Leiosauridae	Enyaliinae	Enyalius	catenatus(cf)	
PUCRS	MCP 19082	ACGTCCTG	AAGAGATC	Leiosauridae	Enyaliinae	Enyalius	iheringii	Maquiné/Barra do Ouro,RS
UFRN	106	ACGTCCTG	CTCAATGA	Leiosauridae	Enyaliinae	Enyalius	iheringii	Capão Bonito,SP
MPEG	MPEG 29072	ACGTCCTG	GCTAACGA	Leiosauridae	Enyaliinae	Enyalius	leechii	Itaituba,PA
UFJF	R 033	ACGTCCTG	AATCCGTC	Leiosauridae	Enyaliinae	Enyalius	perditus	Reserva Particular do Patrimônio Natural Chapadão da Serra Negra, Serra Negra da Mantiqueira, Santa Bárbara do Monte Verde,MG
CELSO	1	ACGTCCTG	CAACCACA	Leiosauridae	Enyaliinae	Enyalius	perditus	Faz. Nova Sama, Ibiúna,SP
MNRJ	25997	ACGTCCTG	CGCTGATC	Leiosauridae	Enyaliinae	Enyalius	perditus	Estação Biológica de Boracéia, Salesópolis, SP.
CEUNES	1205	ACGTCCTG	CTAAGGTC	Leiosauridae	Enyaliinae	Enyalius	pictus	RPPN Estação Veracel,BA
MNRJ	25026	ACGTCCTG	GAGCTGAA	Leiosauridae	Enyaliinae	Enyalius	pictus	Grão Mogol, MG
MNRJ	25999	ACGTCCTG	AAGGACAC	Leiosauridae	Enyaliinae	Urostrophus	vautieri	Trilha da Bocaina, RPPN Santuário do Caraça, Catas Altas, MG.
UFRN	8452	ACGTCCTG	GCCACATA	Leiosauridae	Enyaliinae	Enyalius	bibronii	Buique, PE
UFRN	12007	ACGTCCTG	CCGACAAC	Leiosauridae	Enyaliinae	Enyalius	bibronii	Ubajara, CE
UFRN	5345	ACGTCCTG	ATAGCGAC	Leiosauridae	Enyaliinae	Enyalius	bibronii	São Raimundo Nonato, PI
UFRN	2814	ACGTCCTG	TGAAGAGA	Leiosauridae	Enyaliinae	Enyalius	bibronii	Goianinha, RN
UFRN	2587	ACGTCCTG	CATCAAGT	Leiosauridae	Enyaliinae	*Enyalius*	*bibronii*	Canguaretama, RN
AMNH		GTCAGTAC	AGCAGGAA	Opluridae		*Chalarodon*	*steinkampi*	
AMNH		GTCAGTAC	CCGAAGTA	Opluridae		*Oplurus*	*cuvieri*	

AMNH		GTCAGTAC	CAGCGTTA	Opluridae		*Oplurus*	*fierinensis*	
AMNH		GTCAGTAC	AGATCGCA	Opluridae		*Oplurus*	*grandidieri*	
AMNH		GTCAGTAC	ACCACTGT	Opluridae		*Oplurus*	*quadrimaculatus*	
AMNH		GTCAGTAC	AAGAGATC	Opluridae		*Oplurus*	*saxicola*	
AMNH		GTCAGTAC	GTCTGTCA	Opluridae		*Oplurus*	*sp.*	
AMNH		GTCAGTAC	CGACTGGA	Phrynosomatidae		*Callisaurus*	*Draconoides*	
AMNH		GTCAGTAC	GGTGCGAA	Phrynosomatidae		*Urosaurus*	*Nigricaudus*	
AMNH		GTCAGTAC	TATCAGCA	Iguanidae		*Iguana*	*iguana*	
AMNH		GTCAGTAC	GCGAGTAA	Agamidae		*Physignathus*	*Leseueuri*	
AMNH		GTCAGTAC	GATGAATC	Chamaleonidae		*Furcifer*	*Pardalis*	

Appendix 2. List of primers used in sanger sequencing.

Gene	Primers sequences (5' - 3')	References
CytB	IguaCytob_F2: CCACCGTTGTTATTCAACTAC	Werneck *et al.*, 2012
	PhyCytob_R2: GCTGCGGTAKTTTGAGGTGT	
12S	tPhe2 AAAGCACRGCACTGAAGATGC	Wiens *et al.*, 1999
	12e GTRCGCTTACCWTGTTACGACT	
CMOS	F: CTCTGGKGGCTTTGGKKCTGTSTACAA	Werneck et al., 2012
	R: GGGGTGATGGCAAANGAGTAGATGTCTGC	
NKTR	F: AGTAAATGGGAYTCKGARTCAAA	Werneck et al., 2012
	R: AGTAAATGGGAYTCKGARTCAAA	
DMLX	F2: GTCTAGGGAGGATGGTTCACATA	Werneck et al., 2012
	R2: GAATGAAGCAAGTGACSAGAAAGA	
RBMX	F1: TCCTCTTACAGTGAYCGTGATG	Gamble *et al.*, 2011
	R1: TCCCGTAATCATCATAGCGACT	

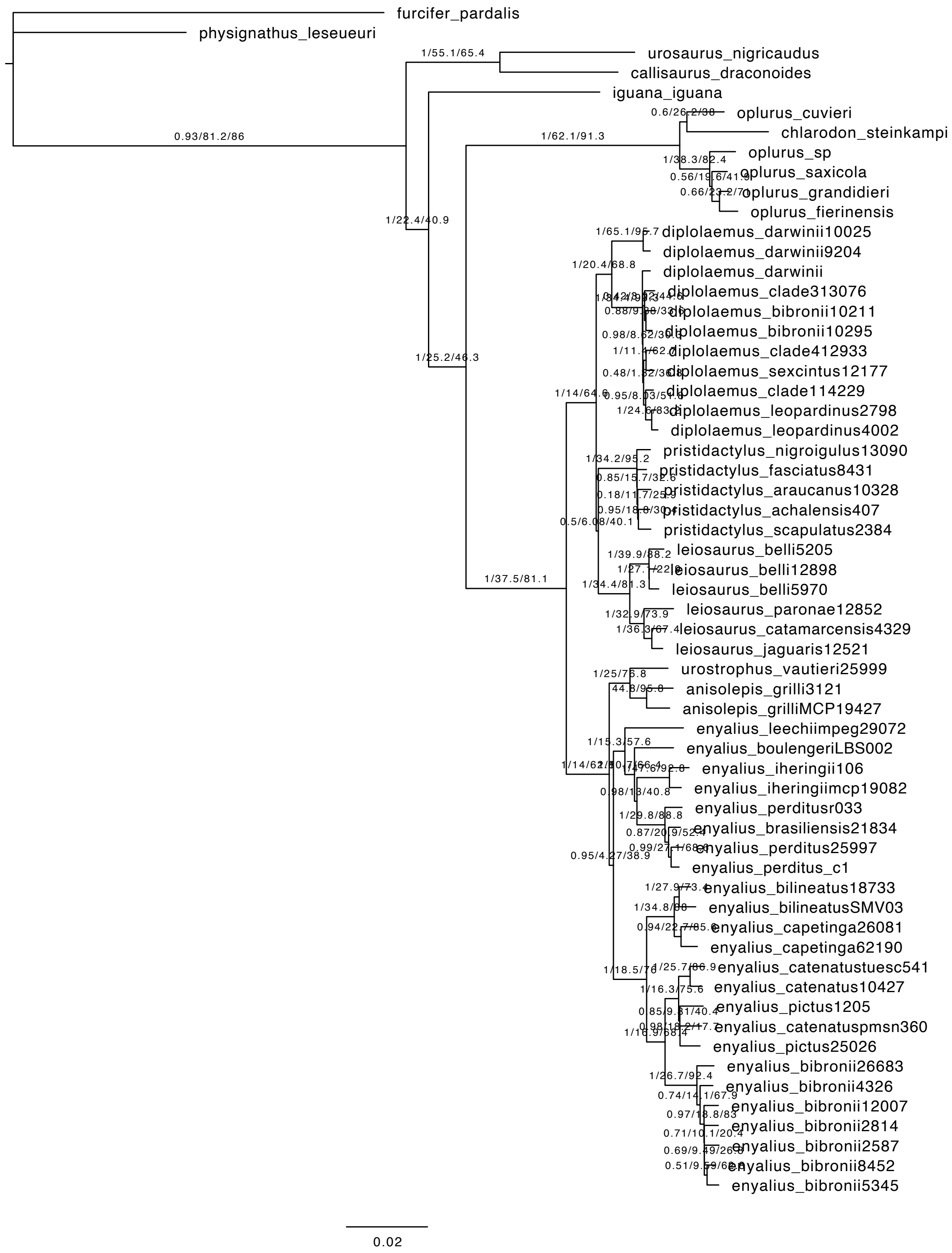
Appendix 3. Control file used to date (with error) using treePL (Smith and O'Meara, 2012) and calibration data extracted from Burbrink *et al.* (2020)

```
treefile = astral2.boottrees
smooth = 1
numsites = 452597
# Iguania (Pleurodonta+Acrodonta)--95% quantile obtained from Burbrink et al. 2020
mrca = Iguania leiosaurus_belli5205 physignathus_leseueuri
min = Iguania 149
max = Iguania 158
# 2 Pleurodonta--95% quantile obtained from Burbrink et al. 2020
mrca = Pleuro leiosaurus_belli5205 callisaurus_draconoides
min pleuro 81
max pleuro 100
#Oplurid-Leiosaurid - 95% quantile obtained from Burbrink et al. 2020
mrca = OpLe leiosaurus_belli5205 oplurus_fierinensis
min OpLe 67
max OpLe 84
outfile = outPL_lei
cv
randomcv
[verbose]
[thorough]
[prime]
cviter = 3
cvstart = 10000
cvstop = 0.0000001
randomcviter = 14
randomcv
pliter = 3
opt = 4
moredetail
optad = 2
moredetailad
optcvad = 4
log_pen
nthreads = 4
```

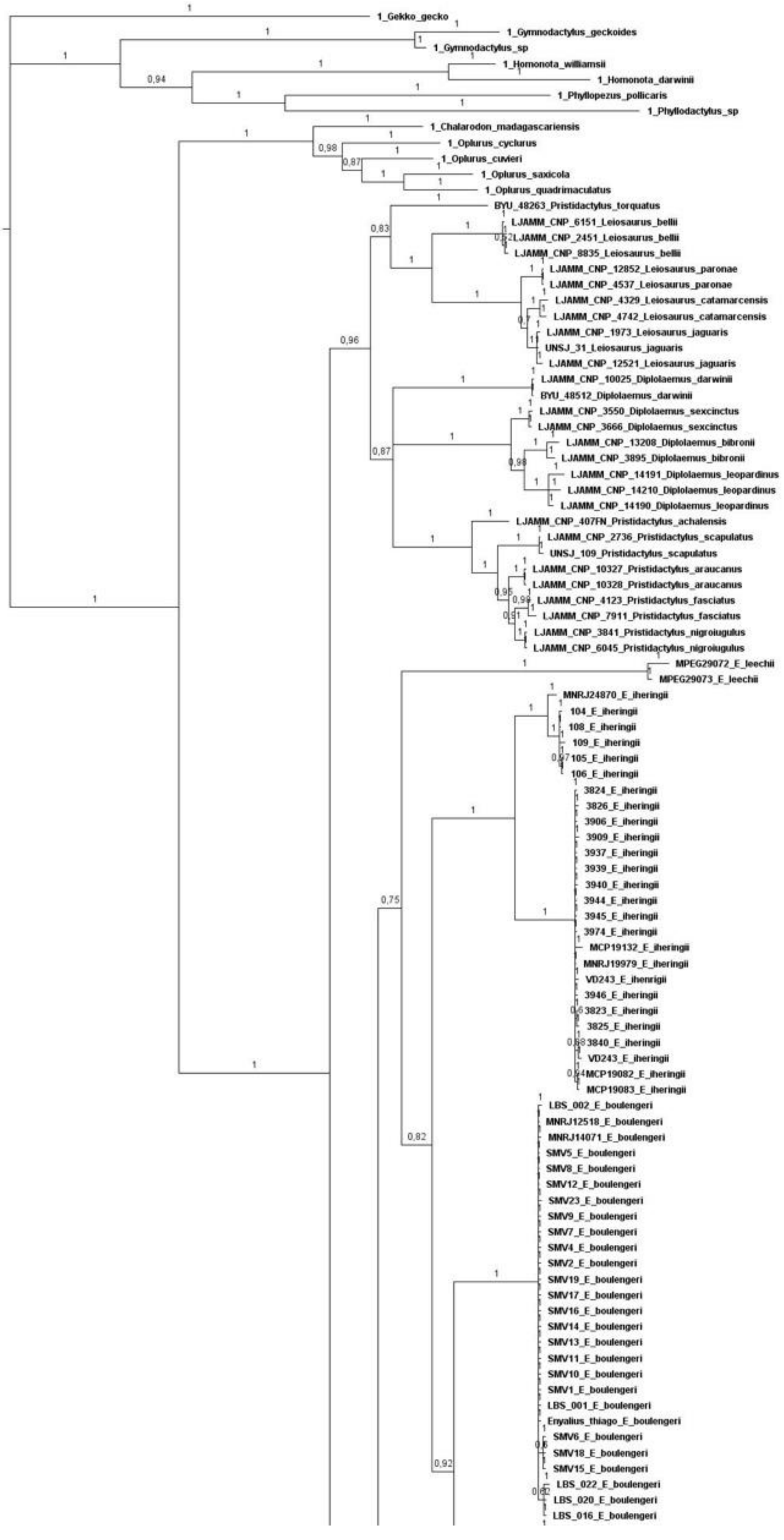
```
x<-list.files()
x<-x[-grep("control",x)]
x<-x[-grep("treePL",x)]
for(i in 1:length(x)){
  y<-readLines("control.txt")
  y[1]<-paste("treefile =",x[i])
  y[16]<-paste("outfile = outPL_",x[i],sep="")
  write(y,"control.txt")
  system("treePL control.txt",ignore.stdout = T)
  print(i)
}
```

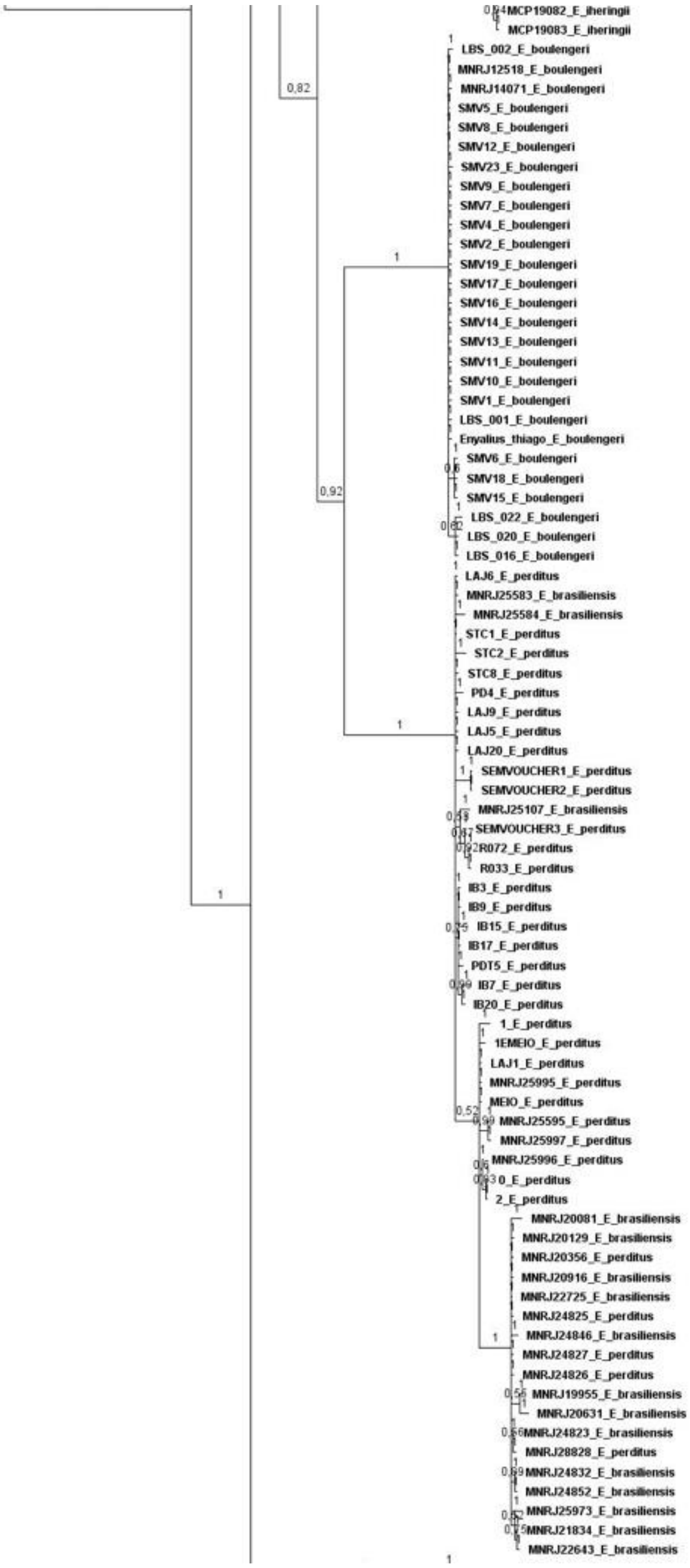
Appendix 4. Gene tree estimated in the IQ-TREE II. The values above the branches are the bootstrap, gCF and sCF factors.

Appendix 5. Astral III tree. The values above the branches are the bootstrap (estimated in the IQ-TREE II), gCF and sCF factors.



Appendix 6. Tree of mitochondrial Cocatenated genes (CitB+12S).




0,94
MCP19082_E_iheringii
MCP19083_E_iheringii
0,82
1
LBS_002_E_boulengeri
MNRJ12518_E_boulengeri
MNRJ14071_E_boulengeri
SMV5_E_boulengeri
SMV8_E_boulengeri
SMV12_E_boulengeri
SMV23_E_boulengeri
SMV9_E_boulengeri
SMV7_E_boulengeri
SMV4_E_boulengeri
SMV2_E_boulengeri
1
SMV19_E_boulengeri
SMV17_E_boulengeri
SMV16_E_boulengeri
SMV14_E_boulengeri
SMV13_E_boulengeri
SMV11_E_boulengeri
SMV10_E_boulengeri
SMV1_E_boulengeri
LBS_001_E_boulengeri
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SMV6_E_boulengeri
SMV18_E_boulengeri
SMV15_E_boulengeri
0,92
0,62
LBS_022_E_boulengeri
LBS_020_E_boulengeri
LBS_016_E_boulengeri
LAJ6_E_perditus
MNRJ25583_E_brasiliensis
MNRJ25584_E_brasiliensis
STC1_E_perditus
STC2_E_perditus
STC8_E_perditus
PD4_E_perditus
LAJ9_E_perditus
1
LAJ5_E_perditus
LAJ20_E_perditus
SEMVOUCHER1_E_perditus
SEMVOUCHER2_E_perditus
0,59
MNRJ25107_E_brasiliensis
0,87
SEMVOUCHER3_E_perditus
0,92
R072_E_perditus
R033_E_perditus
IB3_E_perditus
1
IB9_E_perditus
0,75
IB15_E_perditus
IB17_E_perditus
PDT5_E_perditus
0,99
IB7_E_perditus
IB20_E_perditus
1_E_perditus
1EMEIO_E_perditus
LAJ1_E_perditus
MNRJ25995_E_perditus
MEIO_E_perditus
0,52
0,99
MNRJ25595_E_perditus
MNRJ25997_E_perditus
0,6
MNRJ25996_E_perditus
0,93
0_E_perditus
2_E_perditus
MNRJ20081_E_brasiliensis
MNRJ20129_E_brasiliensis
MNRJ20356_E_perditus
MNRJ20916_E_brasiliensis
MNRJ22725_E_brasiliensis
MNRJ24825_E_perditus
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MNRJ24846_E_brasiliensis
MNRJ24827_E_perditus
MNRJ24826_E_perditus
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MNRJ19955_E_brasiliensis
MNRJ20631_E_brasiliensis
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MNRJ24823_E_brasiliensis
MNRJ28828_E_perditus
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MNRJ24852_E_brasiliensis
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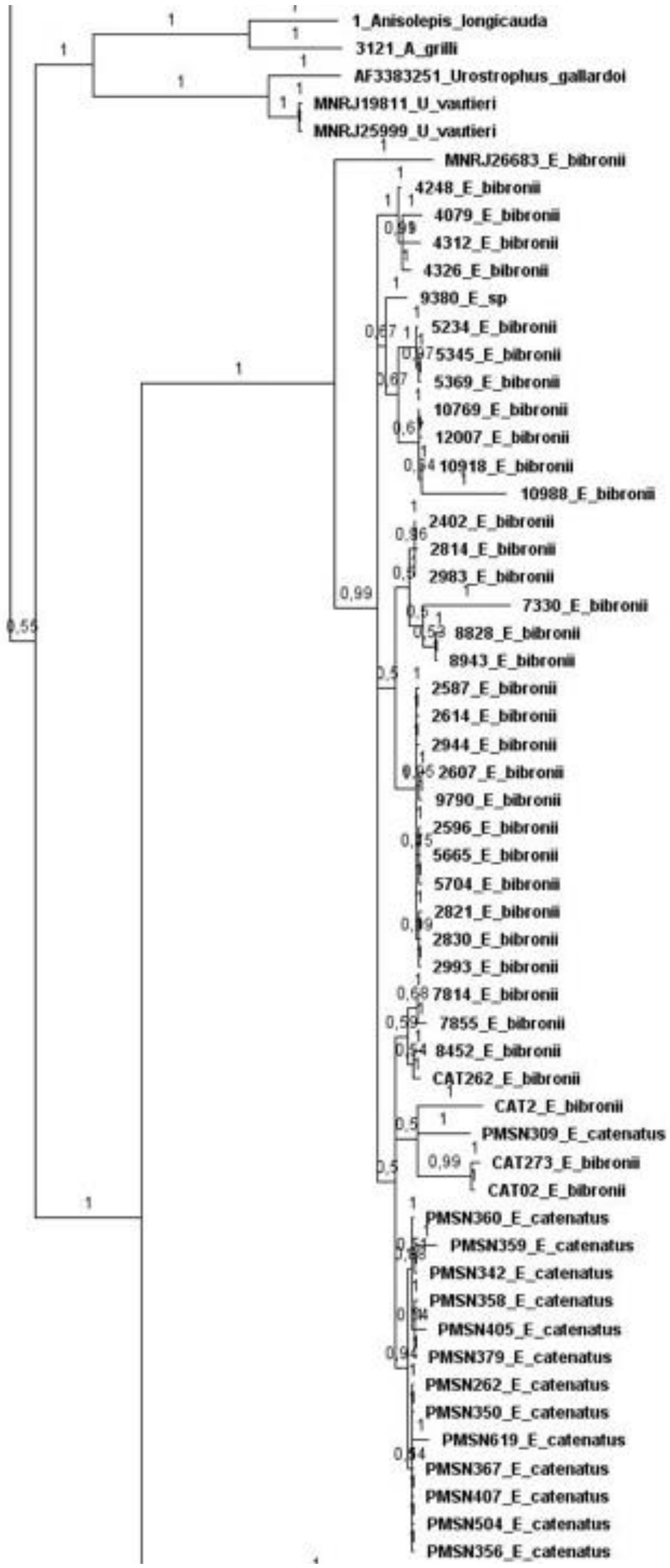
MNRJ25973_E_brasiliensis
0,75
MNRJ21834_E_brasiliensis
MNRJ22643_E_brasiliensis
1


1_Anisolepis_longicauda
3121_A_grilli
AF3383251_Urostrophus_gallardoi
MNRJ19811_U_vautieri
MNRJ25999_U_vautieri
MNRJ26683_E_bibronii
4248_E_bibronii
4079_E_bibronii
4312_E_bibronii
4326_E_bibronii
9380_E_sp
5234_E_bibronii
5345_E_bibronii
5369_E_bibronii
10769_E_bibronii
12007_E_bibronii
10918_E_bibronii
10988_E_bibronii
2402_E_bibronii
2814_E_bibronii
2983_E_bibronii
7330_E_bibronii
8828_E_bibronii
8943_E_bibronii
2587_E_bibronii
2614_E_bibronii
2944_E_bibronii
2607_E_bibronii
9790_E_bibronii
2596_E_bibronii
5665_E_bibronii
5704_E_bibronii
2821_E_bibronii
2830_E_bibronii
2993_E_bibronii
7814_E_bibronii
7855_E_bibronii
8452_E_bibronii
CAT262_E_bibronii
CAT2_E_bibronii
PMSN309_E_catenatus
CAT273_E_bibronii
CAT02_E_bibronii
PMSN360_E_catenatus
PMSN359_E_catenatus
PMSN342_E_catenatus
PMSN358_E_catenatus
PMSN405_E_catenatus
PMSN379_E_catenatus
PMSN262_E_catenatus
PMSN350_E_catenatus
PMSN619_E_catenatus
PMSN367_E_catenatus
PMSN407_E_catenatus
PMSN504_E_catenatus
PMSN356_E_catenatus
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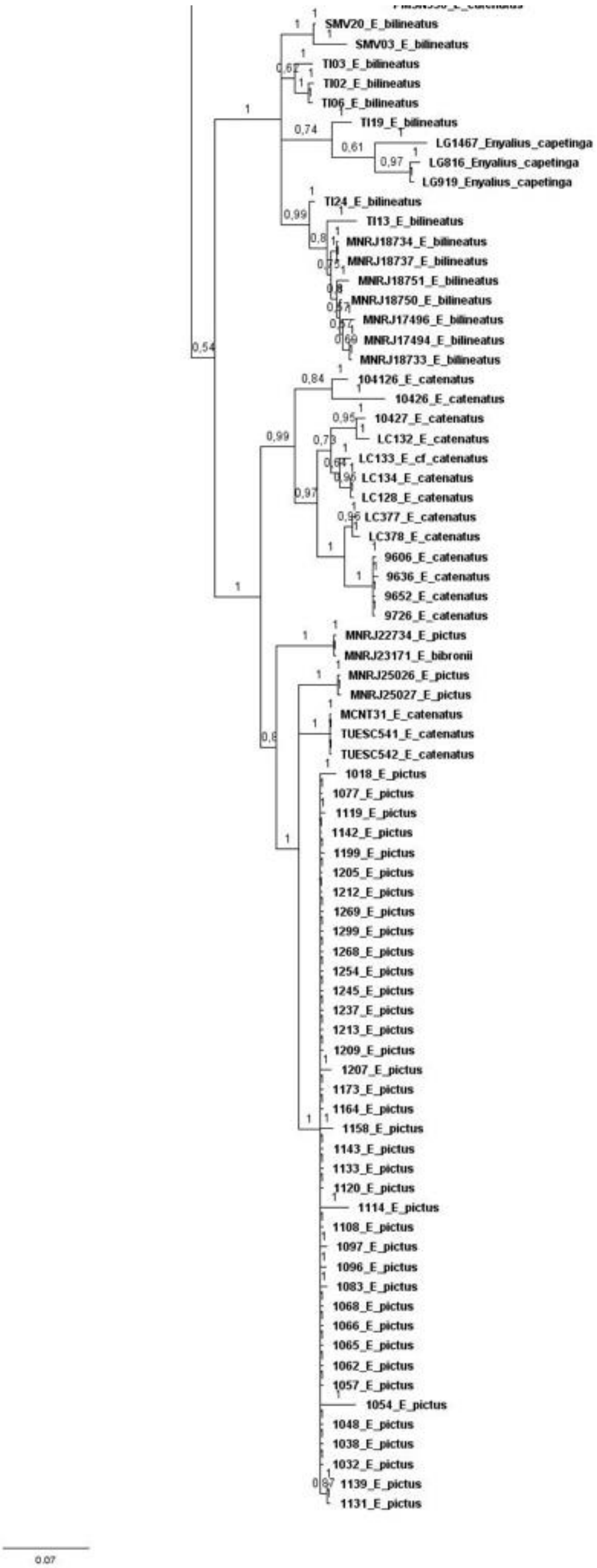
0,99


SMV20_E_bilineatus
SMV03_E_bilineatus
TI03_E_bilineatus
TI02_E_bilineatus
TI06_E_bilineatus
TI19_E_bilineatus
LG1467_Enyalius_capetinga
LG816_Enyalius_capetinga
LG919_Enyalius_capetinga
TI24_E_bilineatus
TI13_E_bilineatus
MNRJ18734_E_bilineatus
MNRJ18737_E_bilineatus
MNRJ18751_E_bilineatus
MNRJ18750_E_bilineatus
MNRJ17496_E_bilineatus
MNRJ17494_E_bilineatus
MNRJ18733_E_bilineatus
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10426_E_catenatus
10427_E_catenatus
LC132_E_catenatus
LC133_E_cf_catenatus
LC134_E_catenatus
LC128_E_catenatus
LC377_E_catenatus
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9606_E_catenatus
9636_E_catenatus
9652_E_catenatus
9726_E_catenatus
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MNRJ23171_E_bibronii
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MNRJ25027_E_pictus
MCNT31_E_catenatus
TUESC541_E_catenatus
TUESC542_E_catenatus
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1077_E_pictus
1119_E_pictus
1142_E_pictus
1199_E_pictus
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1131_E_pictus
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0,95
0,73
0,64
0,96
0,97
0,96
0,8
0,87
0.07

Appendix 7. Table results of BioGeoBEARS best model

	LnL	numparams	d	e	j
	Rbind (restable,res2,res1)	Rbind (restable, res2, res1)	Rbind (restable, res2, res1)	Rbind (restable, res2, res1)	Rbind (restable, res2, res1)
DEC	-81.02478	2	0.00246279	1.00E-12	0
DEC+J	-76.05099	3	0.001592849	1.00E-12	0.009809026
DIVALIKE	-80.3616	2	0.00263357	1.00E-12	0
DIVALIKE+J	-76.17303	3	0.001898196	1.00E-12	0.007958705
BAYAREALIKE	-87.25716	2	0.002209214	0.027529919	0
BAYAREALIKE+J	-75.92837	3	0.000810158	0.008705235	0.01125943

alt	null	LnLalt	LnLnull	pval	AIC1
		Rbind (teststable,tmp_tests)	Rbind (teststable, tmp_tests)	Rbind (teststable, tmp_tests)	Rbind (teststable,tmp_tests)
DEC+J	DEC	-76.05098579	-81.02478286	0.001610597	158.102
DIVALIKE+J	DIVALIKE	-76.17302823	-80.36160465	0.003799672	158.3461
BAYAREALIKE+J	BAYAREALIKE	-75.92837343	87.25715668	1.90E-06	157.8567

PANORAMA GERAL E PRÓXIMOS PASSOS

Nesta tese, foi investigada a história evolutiva da família Leiosauridae (Iguania), perpassando pelas relações filogenéticas, distribuição geográfica e diversificação, avaliando como os eventos biogeográficos, paleoclimáticos e ecológicos impactaram sua história, distribuição e diversidade. Este trabalho trouxe a matriz mais completa, tanto em representantes quanto em dados moleculares, para o grupo até o momento, permitindo avançar no entendimento sobre o grupo em questão e sobre a dinâmica na região Neotropical. Nossos resultados sugerem que mudanças que ocorreram entre os períodos Oligoceno e Mioceno impactaram profundamente a história dos biomas na região Neotropical como: tornando a Patagônia e Monte mais áridos, a formação da Diagonal Seca, consequentemente a separação entre a Amazônia e a Mata Atlântica. Propomos que essas mesmas mudanças também impactaram na dinâmica da Mata Atlântica, separando-a em polos Norte e Sul, sendo o Sul incluindo o corredor central somando a ela a porção sul. Possivelmente essas alterações nos biomas foram desencadeadas como resultado do soerguimento dos Andes, processo esse que se iniciou há aproximadamente 100 Ma, teve uma intensificação há 20 Ma (período citado), e que continua até hoje de forma mais branda. Propomos que tais eventos podem ser evocados para explicar as relações filogenéticas e atual distribuição das subfamílias, gêneros e alguns clados específicos, como *D. darwinii* e demais espécies dos gêneros, grupos chileno e argentino de *Pristidactylus*, clados A e B de *Enyalius*, assim com o *E. Leechii* e *E. bilineatus*. Ademais, concluímos que os eventos de mudanças climáticas dos períodos Plio-Pleistoceno parecem ter vital importância na diversificação do grupo em se tratando das espécies que existem atualmente. Sobre o gênero *Enyalius*, vale a pena ressaltar, que diferentemente do encontrado na literatura, a única espécie amazônica da família, foi recuperada junto ao clado A (Figura 2).

Tais resultados indicaram que além da rota utilizada pela espécie para chegar da Mata Atlântica na Amazônia ter sido outra da proposta previamente na literatura, o período é similar àquele proposto para os dos eventos ocorridos durante os períodos Oligoceno/Mioceno e aqueles desencadeados pelo soerguimento dos Andes, corroborando sua importância para o entendimento dos processos de diversificação na Mata Atlântica.

Esta tese trouxe grandes avanços em relação à sistemática do grupo, e novos questionamentos surgiram. Acredito que novos esforços devam ser direcionados para se compreender o real status taxonômico dos gêneros as *Pristidactylus*, *Diplolaemus*. Assim como uma investigação para entender a relação entre as espécies *E. bibronii, E. catenatus* e *E. pictus* e o possível caso de hibridização que está ocorrendo entre elas. Novos estudos com os gêneros *Anisolepis* e *Urostrophus* também são indicados, visto que são pouco estudados e têm muito a contribuir para o entendimento dessa dinâmica dos biomas da região Neotropical.

As próximas etapas deste trabalho, serão a investigação do caso de possível hibridização entre as espécies *E. catenatus*, *E. picuts* e *E. bibronii*. Bem como novas análises envolvendo diversificação, adaptação às variáveis climáticas, adaptação relacionada à distribuição geográfica, serão realizadas com os dados gerados nesta tese, a fim de melhor compreender as possíveis adaptações dos representantes desta família em resposta aos eventos que moldaram sua diversidade.

Em conclusão, esta tese contribuiu para o vasto conhecimento sobre a biodiversidade da região Neotropical trazendo novo olhar aos eventos envolvidos. Ajudou a aumentar o entendimento da taxonomia de lagartos da família Leiosauridae, trazendo a filogenia mais completa e robusta para o grupo. Trouxe novas ideias para a dinâmica das conexões entre o AF-AM para Leiosauridae, bem como trouxe um novo olhar para o conhecido caso da divisão norte e sul da Mata Atlântica. Com isso evidenciando a importância de eventos durante os períodos Oligoceno/Mioceno como o soerguimento dos Andes, para relações mais antigas, e as mudanças climáticas do Plio-Pleistoceno, para relações mais recentes, na formação da biodiversidade na região Neotropical.

"Come forth into the light of things, let nature be your teacher."
William Wordsworth
“Aproxime-se da luz que emana de todas as coisas, permita que
a Natureza seja sua professora.”