Diversification history of clown tree frogs in Neotropical rainforests (Anura, Hylidae, *Dendropsophus leucophyllatus* group)

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ARTICLE INFO

ABSTRACT

General consensus emphasizes that no single biological process can explain the patterns of species' distributions and diversification in the Neotropics. Instead, the interplay of several processes across space and time must be taken into account. Here we investigated the phylogenetic relationships and biogeographic history of tree frogs in the *Dendropsophus leucophyllatus* species group (Amphibia: Hylidae), which is distributed across Amazonia and the Atlantic rainforests. Using Next Generation Sequencing (NGS) and double digest restriction-site associated DNA (ddRADseq), we inferred phylogenetic relationships, species limits, and temporal and geographic patterns of diversification relative to the history of these biomes. Our results indicate that the *D. leucophyllatus* species group includes at least 14 independent lineages, which are currently arranged into ten described species. Therefore, a significant portion of species in the group are still unnamed. Different processes were associated to the group diversification history. For instance, the Andes uplift likely caused allopatric speciation for Cis-Andean species, whereas it may also be responsible for changes in the Amazonian landscape triggering parapatric speciation by local adaptation to ecological factors. Meanwhile, Atlantic Forest ancestors unable to cross the dry diagonal biomes after rainforest's retraction, evolved in isolation into different species. Diversification in the group began in the early Miocene, when connections between Atlantic Forest and the Andes (Pacific Dominion) by way of a south corridor were possible. The historical scenario in Amazonia, characterized by several speciation events and habitat heterogeneity, helped promoting diversification, resulting in the highest species diversity for the group. This marked species diversification did not happen in Atlantic Forest, where speciation is very recent (late Pliocene and Pleistocene), despite its remarkable climatic heterogeneity.

1. Introduction

The Neotropical region harbors the highest biodiversityin the world. However, this biogeographic region historically received less scientific attention than temperate regions with respect to investigations of phylogenetic relationships and diversification processes over evolutionary timescales (Beheregaray, 2008; Hickerson et al., 2010).

Fortunately, this unbalanced scenario is gradually changing, and the Neotropical region has now been the subject of several recent investigations and syntheses on biota diversification, many of them considering big data and modern analytical approaches (Smith et al., 2014; Antonelli et al., 2018a; Antonelli et al., 2018b; Rangel et al., 2018; Pirani et al., 2019; Rull and Carnaval, 2020; Thom et al., 2020).

The biodiversity of taxa associated with South American rainforests

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is still underestimated, and the processes responsible for generating current diversity patterns are poorly understood (Leite and Rogers, 2013; Rangel et al., 2018). Most hypotheses proposed to explain the processes responsible for species origin and accumulation in South America focus on the breaking down of population/species geographic distributions, often associated with allopatric speciation scenarios (e.g., the refuge hypothesis – Haffer, 1969; and the river barrier hypothesis – Wallace, 1852). These hypotheses tend to underestimate the potential of alternative mechanisms of speciation across complex environments, such as the role of local ecological adaptation (Sheu et al, 2020), and the effects of dispersal capacities and taxa persistence time in the landscape (Smith et al., 2014).

Alternative explanations, such as the gradient hypothesis that advocates parapatric speciation among populations along geographical or environmental clines, often facilitated by ecological adaptation (Endler, 1973; Nosil et al., 2009), could also be important to uncover the biodiversity organization and species assemblages in the Neotropics. Further, geomorphological processes, such as the Andes uplift, drastically changed the landscape and climate during the Neogene (Hoorn et al., 2010). Combined with these changes, the final uplift of the Brazilian Shield and expansion of savannas resulted in the establishment of a drier area known as the South American dry diagonal that includes different biomes that physically and ecologically separate the Amazonia and Atlantic rainforests (Hoorn et al., 2010; Werneck, 2011). Biotic interchange between these main bigeographic regions has been shown to be pervasive, with frequent transitions from forest to open biomes, and vice-versa (Antonelli et al., 2018b). Yet, some groups have failed to colonize the dry diagonal and incorporation of such information into historical biogeography studies in Amazonia is still infrequent (see reviews Moritz et al., 2000; Haffer, 2008; Leite and Rogers, 2013).

While the dry diagonal biomes currently act as an important barrier between Amazonia and the Atlantic rainforests, there is substantial evidence to support past contact between these forest biomes across many animal groups (Antonelli et al., 2018; Batalha-Filho et al., 2013; Oliveira et al., 1999; Prates et al., 2016, 2017, 2018, 2020; Costa, 2003), including frogs (Fouquet et al., 2012a,b; Ledo and Colli, 2017; de Sá et al., 2019). Analyses of hydroclimate variability in Amazonia at different time scales show a more humid portion in the west and a drier portion in the east (Cheng et al., 2013). This climatic gradient could have facilitated different historical events, making possible Amazonia-Atlantic Forest connections at different times: older routes (mid-late Miocene) through southern Brazil, and more recent routes (Pliocene and Pleistocene) through northern Cerrado and Caatinga biomes in northeastern Brazil (Batalha-Filho et al., 2013; Cheng et al., 2013; Ledo and Colli, 2017). Each of these routes have profound consequences for understanding speciation processes and relationships between disjunct Amazonian and Atlantic Forest taxa. So far, empirical evidence showed that rainforest corridors during different times (e.g., recent connections during Plio-Pleistocene; and old connections during Oligocene-Miocene) allowed dispersal of several Amazonian vertebrates into the Atlantic Forest, including frogs (Santos et al., 2009; Fouquet et al., 2012a,b; de Sá et al., 2019), squamate reptiles (Pellegrino et al., 2011; Rodrigues et al., 2014; Prates et al., 2016; Dal Vechio et al., 2018, 2019), small mammals (Costa, 2003), and birds (Batalha-Filho et al., 2013) — for an overview, see also Ledo and Colli (2017).

Herein, we aim to infer the processes involved in the diversification of a charismatic group of Neotropical frogs – the clown tree frogs from the *Dendropsophus leucophyllatus* species group (Hylidae, Amphibia), whose distributions span the South American rainforests and recognized species diversity has varied from eight (Faivovich et al., 2005) to fourteen (Caminer et al., 2017). We take a phylogenetic approach based on genomic data to evaluate the spatial and temporal patterns of diversification in the group, mainly focusing on speciation across Amazonia and the Atlantic Forest, prior to the northward spread of the only clade that occupies tropical Mexico (Duellman et al., 2016).

1.1. Diversity of clown tree frogs

The *Dendropsophus leucophyllatus* species group currently includes 14 species (but see discussion about *D. anceps* below), 11 of which are distributed in the Amazonia: *D. arndti* Caminer et al. (2017); *D. bifurcus* (Andersson, 1945); *D. leucophyllatus* (Beireis, 1783); *D. manonegra* Rivera-Correa and Orrico (2013); *D. mapinguari* Peloso et al. (2016); *D. reticulatus* (Jiménez de la Espada, 1870); *D. rossalleni* (Goin, 1959); *D. salli* Jungfer et al. (2010); *D. sarayacuensis* (Shreve, 1935); *D. triangulum* (Gunther, 1869 "1868"), and *D. vraemi* Caminer et al. (2017); one distributed in Central America and Chocó: *D. ebraccatus* (Cope, 1874); two species from the Atlantic Forest: the broadly distributed *D. elegans* (Wied, 1824), and the recently described and restricted distributed *D. nekronastes* Dias et al. (2017).

The position of *D. anceps* (Lutz, 1929) is controversial in relation to other members of *Dendropsophus*. Faivovich et al. (2005) included *D. anceps* in the *D. leucophyllatus* group based on its placement in a phylogenetic tree estimated from molecular data (four mitochondrial and five nuclear markers), despite this species not sharing the presence of pectoral glands (in both sexes) with all other species of this group. Following Faivovich et al. (2005), some authors subsequently recognized *D. anceps* as part of the *D. leucophyllatus* species group (Rivera-Correa and Orrico, 2013), whereas others did not (Jungfer et al., 2010; Wiens et al., 2010; Pyron and Wiens, 2011; Pyron et al., 2014; Peloso et al., 2016).

Another long-standing question in the phylogeny of *D. leucophyllatus* species group is the paraphyly of *D. leucophyllatus* with *D. triangulum* (Chek et al., 2001; Lougheed et al., 2006; Peloso et al., 2016), and the possible existence of additional putative unnamed species in the group (Peloso et al., 2016; Caminer et al., 2017). Species in the group are usually characterized by strong and vibrant colors, and some have strikingly different color morphs (Duellman, 1974; Caminer et al., 2017). The fact that very similar color morphs occurs in distantly related species boosts the taxonomic confusion in the group (Duellman, 1974; Jungfer et al., 2010; Caminer et al., 2017). Confusion regarding what actually constitutes polymorphism and what represents variation across independent evolutionary lineages (i.e., species) began to be solved by the study of Caminer et al. (2017). Using phylogenetic methods based on DNA sequence data, the authors revealed several unnamed species in the group—some of which were believed to be color morphs of either *D. leucophyllatus* or *D. triangulum*. Despite its charisma and conspicuousness, little precise information exists about most of the species' ecology, range limits, and their biogeographic history. Particularly, no study has investigated these contentious topics on the *D. leucophyllatus* species group diversification using genomic-level data.

Duellman et al., (2016) inferred a large phylogeny of Arboranae and included eight species of the *D. leucophyllatus* species group in their dataset. Their work suggests that the diversification of the *D. leucophyllatus* species group started in the early Miocene approximately 18.7 million years ago (Mya) (15.7–21.6; Duellman et al., 2016), with pairs of taxa distributed respectively in the Amazonia and Atlantic Forest (*D. salli* and *D. elegans*) diverging as early as 13.3 Mya (15.5–8.0). These data suggest that older connections between the Amazonia and the Atlantic Forest through a southern corridor might have facilitated species divergence.

Herein, by analyzing genome-wide Single Nucleotide Polymorphism (SNP) data from most species in the *D. leucophyllatus* species group (10 out of 14 species; Fig. 1) we intend to: (i) review species boundaries within the group by delimiting species lineages in a model-based approach and accessing their phylogenetic relationships; and (ii) investigate spatial and temporal aspects of the group biogeography by evaluating the correspondence of speciation events with geological events that took place over millions of years of the dynamic geologic and environmental landscape history of South America.

Fig. 1. Diversity of the *Dendropsophus leucophyllatus* species group. **A)** *D. bifurcus*, **B)** *D. ebraccatus*, **C)** *D. elegans*, **D)** *D. leucophyllatus*, **E)** *D. mapinguari*, **F)** *D. reticulatus*, **G)** *D. rossalleni*, **H)** *D. sarayacuensis*, and **I)** *D. triangulum*. Photos: Morley Read (A), Santiago Ron (B, F, I), and Pedro Peloso (C, D, E, G, H).

2. Methods

2.1. Sampling and data generation

We collected genomic data from 196 specimens representing ten of the 14 currently recognized species of the *Dendropsophus leucophyllatus* group (we failed to sample *D. manonegra*, *D. salli*, *D. vraemi*, and *D. nekronastes*), sampled from 100 localities distributed in the Neotropical rainforests (see Appendix A, Table A1), plus three outgroup taxa: *D. minutus*, *D. anceps* and *D. marmoratus*. All species were represented by at least two individuals from each locality; except for *D. leucophyllatus* and *D. triangulum* for which at least six individuals were collected from each locality in order to better infer the lineages limits and relationships within these taxa. The complete list of samples used in this study is provided in Appendix A (Table A1).

Genomic DNA was extracted from muscle or liver samples of each individual using a Qiagen DNeasy Blood and Tissue Kit and following manufacturer's protocol. Two reduced representation libraries were constructed using the Double Digest Restriction Associated DNA Sequencing approach (ddRADseq) following the protocol from Peterson et al. (2012). DNA was double digested with restriction enzymes *EcoR1* and *MseI*. Unique barcodes (10 bp) and Illumina adaptors were ligated to the digested fragments. Individuals were pooled together for each library and DNA fragments between 350 and 450 bp were size selected using Pippin Prep (Sage Science). Fragments were amplified by PCR, with 8 cycles. After each step, we performed a cleanup using AMPure beads (1.6x, except after Pippin Prep) and quantified the material with Qubit high sensitivity assay. Libraries were sequenced in an Illumina 2500 platform at the Center for Applied Genomics (Toronto, Canada) to generate 150 bp single-end reads.

2.2. Processing Illumina data

Raw data was processed using the ipyRAD v.0.7.17 pipeline (Eaton, 2014; Eaton and Overcast, 2020). All samples were processed together with the following parameter specifications. First, sequences were de-multiplexed and the restriction sites and barcodes were trimmed.

Approximately 287 million reads were generated across all 196 individuals sequenced. All sequence reads with greater than 5 low-quality bases (quality score < 20) were removed. Because the number of loci stabilizes above 600,000 reads, we eliminated 19 samples that were below this threshold before conducting the next steps in the remaining 177 samples – the number of reads per individual ranged between 679,227 and 3,062,914 (Appendix A, Table A2). After that, the reads for each sample were clustered into putative loci using a 90% similarity threshold and a maximum number of indels per cluster of 6. Heterozygosity and error-rate were estimated from the base counts in each site across all clusters, and the average values were used to establish consensus sequences. We excluded clusters with coverage less than a minimum depth of 6 in order to ensure accurate base calls. Consensus sequences from all samples were clustered by sequence similarity, with their input order randomized, using the same similarity threshold as the within-sample clustering (90%). Note that the selected values are in line with other recent studies focusing on within-genus divergences (e.g., Huang, 2016). The minimum length of the loci was trimmed off, so that all loci had the same final alignment length (110 bp). Any locus appearing heterozygous at the same site across more than 5 samples were discarded. For the output, we excluded a minimum of 60 samples per locus and maximum of 20 SNPs per locus. The remaining clusters are treated as RAD loci, i.e., multiple alignments of putatively orthologous sequences, which were assembled into phylogenetic data matrices. All the ipyRAD steps were run in parallel execution with eight threads on the University of Michigan flux computing cluster.

To set the best parameters for the dataset, we first ran an ipyRAD test with broader parameters and checked all the sequences and alignments using a custom script to visualize the filtering and clustering results (available on github.com/airbugs/Dynastes_introgression, Huang, 2016). After rerunning the program using the ipyRAD Branching workflow, we excluded excessive variation and new assembly errors arising from the clustering of loci from the final dataset (Fig. A1). The final dataset for *D. leucophyllatus* species group has a total of 916,866 prefiltered loci, 4,048 putative unlinked SNPs for 177 individuals, and 50% of missing data. See Appendix A, Table A2 for additional information on ipyRAD steps and processing summary

statistics.

2.3. Reconstruction of individuals tree and interspecific diversity

The unlinked SNP dataset was used to infer the phylogeny of the group and the overall genealogical patterns of divergence between and within species by considering the complete dataset. To do so, we manually edited the output from ipyRAD, and performed a species' tree reconstruction using the coalescent-based program SVDquartets (Chifman and Kubatko, 2014), implemented in PAUP (version 4a164; Swofford, 2002). Each SNP was treated as an independent locus even though the input dataset was in a concatenate format. We evaluate all quartets, selecting the consensus tree using the QFM quartet assembly, and we also performed bootstrapping with 100 replicates to calculate branch support. The tree was rooted *a posteriori* (after tree topology search) on the species *D. marmoratus* based on a previous study (Peloso et al., 2016). We also used a concatenated maximum likelihood analysis on RAxML v. 8.2.8 (Stamatakis, 2014) to access phylogenetic relationships for our complete sampling and infer branch lengths for all the loci with missing data (total of 527,962 loci of the full dataset), using the GTRCAT model and a bootstrapping of 100 replicates. All the analyses above were run under parallel execution with 16 threads on the University of Michigan flux computing cluster.

2.4. Species delimitation

To infer and delimit the main lineages within the *D. leucophyllatus* species group, we used the coalescent-based program SNAPP v.1.3.0 (Bryant et al., 2012) implemented by BEAST v. 2.4.8 (Drummond et al., 2012). A reduced dataset was used for this analysis as this program is computationally intensive and does not allow missing data among terminal taxa (*i.e.*, a locus must be sequenced in at least one representative of each population/species) (see Leaché and Bouckaert, 2018).

Based on the results of the above phylogenetic analyzes that considered all available sampling, we subsampled 47 individuals from the complete dataset considering the following caveats: significant divergent lineages at the individuals' trees considering all individuals, geographic concordance, high node support, substantial branch lengths, and also highest number of reads (see Appendix A, Table A1 for details of the samples selected). Different species combinations were considered for seven lineage delimitation models based on the current taxonomy and the strongly supported clustered clades that resulted from the SVDQuartets and RAxML analyses (see Fig. 2 and Appendix A, Fig. A2, respectively). To test alternative hypotheses of main lineages within *Dendropsophus elegans*, which is broadly distributed in the Atlantic Forest, we combined our phylogenetic analyses based on individuals with the topology proposed by Tonini et al. (2013) to select subsamples across main clades.

A BEAST .xml file was generated with BEAUti v2.4.8 with the following parameters: an independent theta ($\theta = 4\mu Ne$) was estimated for each branch under (1) a gamma (1.0, 3.0) prior distribution, and (2) a gamma (2, 18) prior distribution using a custom script to ensure that the inferred topology was robust for this dataset (all SNPs). The backward and forward mutation rates, u and v, were coestimated, using initial values based on the stationary frequencies. SNAPP analysis does not require defining outgroups, as the program samples the root position along with the other nodes of the tree. Each model was run implementing 100,000 MCMC generations, sampling every 1,000 steps, and a 10% burn-in. After running all replicates, we selected the best-fit species delimitation model based on Bayes factor delimitation (BFD) following Leache et al. (2014). We calculate the Marginal likelihood estimates (MLE) and Bayes Factors across each competing species tree as implemented by Grummer et al. (2014), to estimate the best species delimitation model based on the best model likelihood directly from our dataset (following Leache et al., 2014).

Following results for the lineages supported by our delimitation approach, we inferred a species tree using the coalescent-based program SVDquartets. As suggested by Schmidt-Lebuhn et al. (2017), SVDquartets analysis inferred more stable topologies, even when a large amount of missing data is present, compared to SNAPP inferences. For this analysis, we used the complete dataset (4,048 unlinked SNPs, 177 individuals) to evaluate all quartets, selecting consensus tree using the QFM quartet assembly, and also performed bootstrapping with 500 replicates to calculate branch support. The tree was rooted on the same clade as the complete individuals' species tree (see methods above). The resulting topology was visualized in FigTree v1.4.4 (available from https://github.com/rambaut/figtree/releases). All analyses were run under parallel execution with eight threads on the University of Michigan flux computing cluster.

2.5. Divergence times and ancestral range estimates

We inferred divergence times for the *D. leucophyllatus* species group using StarBEAST2 v0.15.5 (Ogilvie et al., 2017), running under BEAST2 v2.6.1 (Bouckaert et al., 2014) on the complete dataset (4,048 unlinked SNPs for 177 individuals in a concatenated matrix). For this approach, we constrained clades whose support values were 90 or above in the tree topology provided by SVDQuartets species tree, allowing branches within low supported clades to have their position estimated. For time calibration, we used two secondary points estimated by Duellman et al. (2016) based on fossil data for the unranked Arboranae: (1) the divergence time between *D. elegans* from the Atlantic Forest and the other species of the group distributed in Amazonia to the mid-Miocene geologic period at approximately 18.7 Mya (15.7–21.6); (2) the split between *D. reticulatus* and its sister group (*D. leucophyllatus* and *D. triangulum* complex species) for late Miocene at approximately 6.8 Mya (9.5 – 4.6). Secondary points were selected based on the high support for these species' relationships based on previous literature (see Jungfer et al., 2010; Rivera-Correa and Orrico, 2013; Peloso et al., 2016; Caminer et al., 2017).

We used a lognormal relaxed clock-model with a GTR model of substitution and rate heterogeneity estimated with ten discrete categories of a gamma distribution. We used an exponential prior for the diversification rate applied to the species tree. The Markov chain Monte Carlo was set for 100 million generations with sampling every 10,000 generations. Convergence statistics were examined using Tracer v1.7.1 (Rambaut et al., 2014) and the time tree was obtained with TreeAnnotator v2.6.1 (Drummond et al., 2012) using the maximum clade credibility (MCC) tree from all trees after discarding 10% as burn-in, and without posterior limit for each node.

In order to reconstruct possible historical biogeography scenarios for the *Dendropsophus leucophyllatus* species group, we performed an ancestral area reconstruction under a maximum likelihood framework using the R package BioGeoBEARS (BioGeography with Bayesian and likelihood Evolutionary Analysis in R Scripts) (Matzke, 2013a,b; Matzke and Sidje, 2013; R Core Team, 2016), combined with the ultrametric tree obtained from *BEAST2. We performed comparisons among three alternative models using the Akaike Information Criterion (AICc), including Dispersal-Extinction Cladogenesis (DEC), Dispersal-Vicariance Analysis (DIVA), and Bayesian Inference of historical biogeography for discrete areas (BayArea) to test whether biogeographic processes of dispersal, extinction and cladogenesis have shaped species distributions. We did not test models considering the jump dispersal parameter (+J) following the cautionary advice of Ree and Sanmartín (2018) that such parameters ignore the probability factor with respect to time and tends to artificially inflate the contribution of cladogenetic events to the likelihood in detriment to anagenetic and time-dependent range evolution. Therefore, the use of the + J parameter is usually not justifiable beyond island systems (Ree and Sanmartín, 2018), which is not the case of our biological system, which is present in the continuous continental setting of South America.
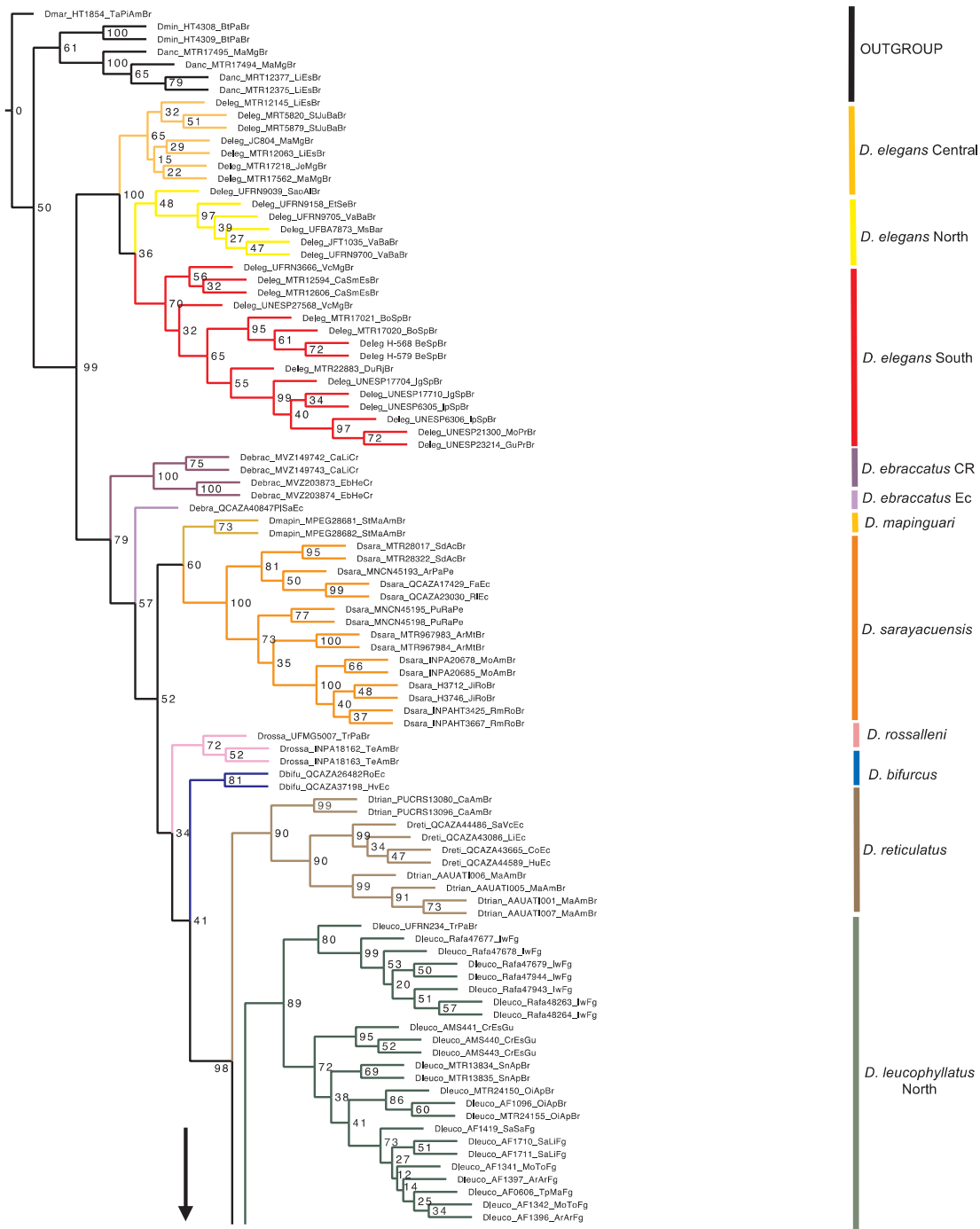
Fig. 2. Molecular phylogeny of sampled individuals from the *Dendropsophus leucophyllatus* species group based on SVDQuartets analysis from 4,048 unlinked SNPs. Clade colors correspond to the topology used for defining the lineages tree at SNAPP analysis. Scale bar represents estimated substitution per site. Posterior probability values for all nodes are based on 100 bootstrap pseudoreplicates.

To reconstruct possible ancestral ranges, we associate the distribution of each species to nine different geographic areas following Dominion units proposed by Morrone (2014) for the Neotropical region and areas of endemism/biogeographical regions in the Amazonia proposed for amphibians by Godinho and Silva (2018), namely: (1) Pacific Dominion (PcD), (2) Paraná Dominion (PaD), (3) Biogeographical region 1 (BR1), (4) Biogeographical region 2 (BR2), (5) Biogeographical region 3 (BR3), (6) Biogeographical region 4 (BR4), (7) Biogeographical region 5 (BR5), (8) Biogeographical region 6 (BR6), (9) Biogeographical region 7 (BR7). For all three BioGeoBEARS analyses, we allowed for a maximum of four reconstructed areas per node, which corresponds to

the highest observed number of areas occupied by sampled taxa distributed in Amazonia. The complete list of *D. leucophyllatus* species group distribution is provided in the online Appendix A, Table A1.

3. Results

3.1. Interspecific diversity, species/lineages delimitation and trees

All phylogenetic analyses for the entire *Dendropsophus leucophyllatus* species group (SVDQuartets, RAxML and SNAPP) recovered the monophyly of most species currently recognized (*sensu* Peloso et al.,
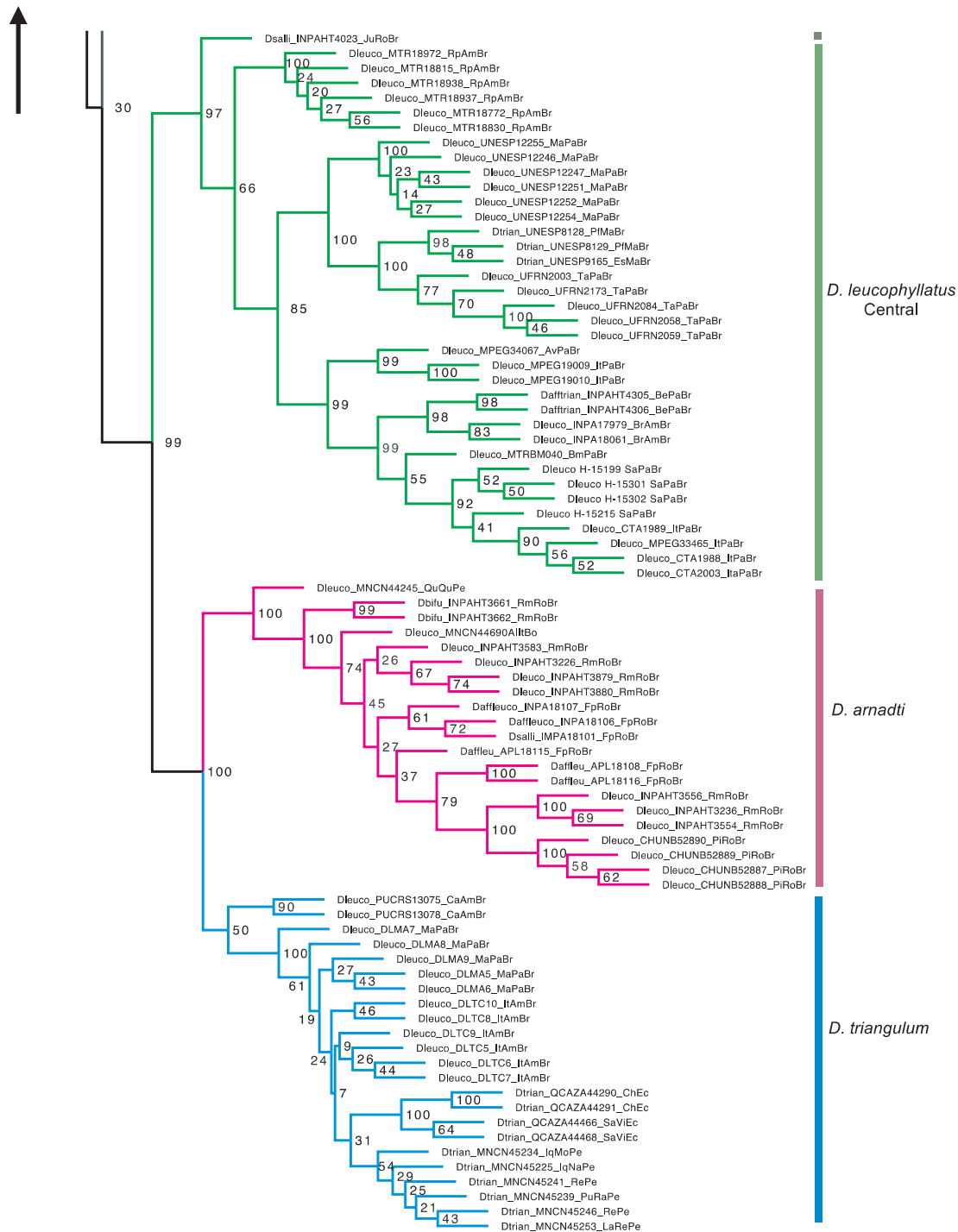
Fig. 2. (*continued*)

2016; Caminer et al., 2017), with different levels of bootstrap support. Two exceptions are *D. ebraccatus* and *D. leucophyllatus*, which were recovered as paraphyletic, with two different lineages each (see Fig. 2, Fig. 3A and Appendix A, Fig. A2).

The coalescent consensus tree inferred from the unlinked SNPs dataset retrieved a monophyletic *D. leucophyllatus* species group with moderate support (50%; Fig. 3A). *Dendropsophus anceps* was not recovered within the D. *leucophyllatus* species group, instead it grouped with *D. minutus* as an outgroup (see Fig. 2, and Appendix A, Fig. A2). The first split within the *D. leucophyllatus* species group is represented by an Atlantic Forest clade assembling three distinct lineages of *D. elegans* (North, Central and South) with different supports. The South

lineage, limited in the north by the Doce river, has high support (100%) and is sister to Central + North lineages with low support (48.4%; Fig. 3A). The Central lineage has its southern distribution limited by the Doce river and its northern limit reaches the southern bank of Paraguaçu river; *D. elegans* North lineage is restricted to the northern portion of the Paraguaçu river (Fig. 3B).

Dendropsophus ebraccatus was recovered as paraphyletic in both the individuals tree (Fig. 2) and the species tree (Fig. 3A), with two poorly supported lineages, one from Costa Rica and another from Cis-Andean Ecuador (Fig. 3B). Most other species, *D. rossalleni, D. bifurcus, D. mapinguari, D. sarayacuensis, D. arndti, D. triangulum,* and *D. reticulatus* were recovered as monophyletic lineages in all analyses. *Dendropsophus*
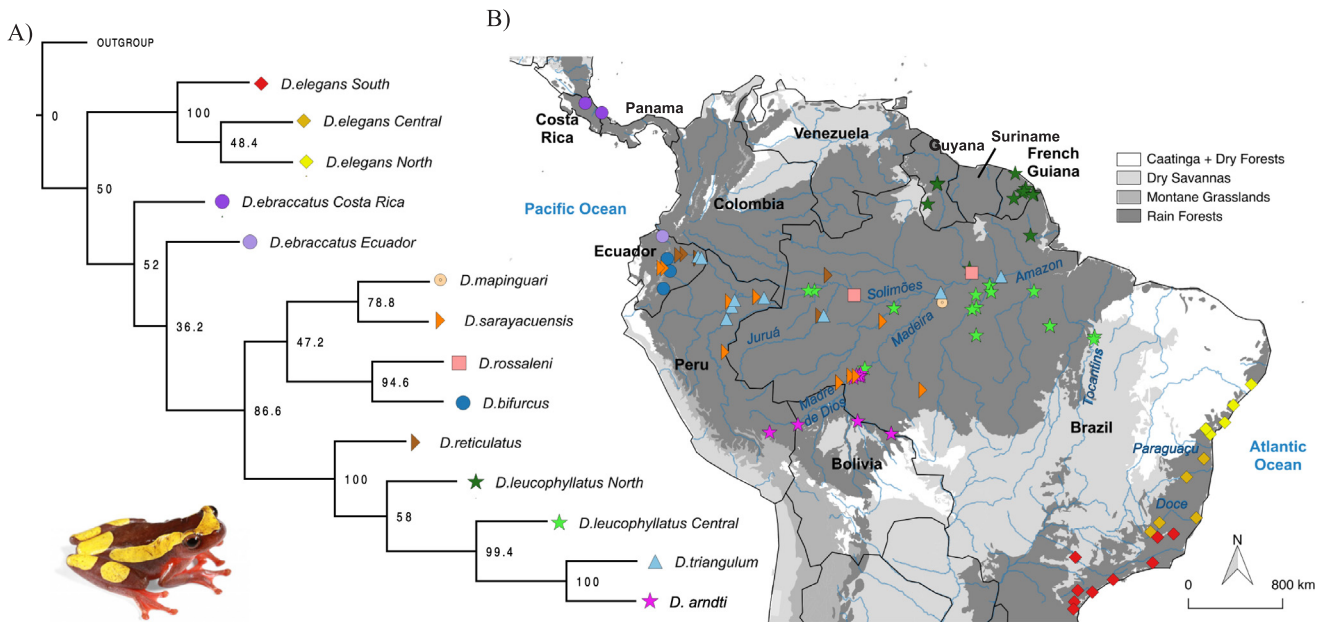
Fig. 3. A) Topology inferred from SVDquartets species/lineages tree analysis showing the phylogenetic relationships of all the 14 *Dendropsophus leucophyllatus* species and lineages addressed in our study based on 4,048 unlinked SNPs for 177 individuals. Numbers at the inter nodes represent the bootstrap support values. Outgroup includes *D. anceps*, *D. marmoratus* and *D. minutus*. **B)** Samples distribution of *D. leucophyllatus* species group included in this study from the Neotropical rainforests at South America. Photo: Santiago Ron.

rossalleni is sister of *D. bifurcus* (94.6% support), and *D. mapinguari* is sister of *D. sarayacuensis* (78.8%), and these two clades are sister but with a low support for this relationship (47.2%; Fig. 3A). Finally, *D. reticulatus* is sister of the *D. leucophyllatus-triangulum* complex (100%; Fig. 3A).

As expected, *Dendropsophus leucophyllatus* was recovered as paraphyletic, with a monophyletic *D. triangulum* and *D. arndti* nested within it and two well supported lineages of *D. leucophyllatus* (North and Central; Fig. 3A). *D. leucophyllatus* North lineage is restricted to the Guiana Shield, north of the Amazon River, spanning Brazil, Guiana and French Guiana (Fig. 3B); and *D. leucophyllatus* Central lineage is restricted to the south of the Solimões and Amazon rivers and to the west of the Tocantins river, with a large distribution in the Brazilian Shield. *Dendropsophus arndti* is distributed in southwestern Amazonia to the right side of the Madre de Dios river, Bolivia, and on both sides of Madeira river in Brazil, including Peru and a sympatric distribution with the *D. leucophyllatus* Central lineage in the Madeira river region (Fig. 3B). Finally, *D. triangulum* is composed of one lineage with a large distribution spanning the Amazonian lowland drainage system (named *várzea*) in Brazil, Peru and Ecuador (Fig. 3A and B).

Despite such overall congruence, we also found some differences in the topology between phylogenetic analysis based on tree lineages and individuals using SVDQuartets. For example, *D. rossalleni* species was recovered as a sister clade of *D. bifurcus*, *D. reticulatus* and the *D. leucophyllatus-triangulum* complex with low support for this relationship (Fig. 2). As for *D. elegans* lineages relationships, the Central clade appears with high support (100%) as sister to the clade South + North that groups with low support (36%; Fig. 2). The RAxML individuals tree recovered the same topology as the lineages tree with an overall stronger node support, the only exception was the relationship between *D. elegans* lineages (Appendix A, Fig. A2).

The species delimitation method implemented with SNAPP selected, based on the highest value of Marginal Likelihood Estimate (MLE), the model number 7 (-1884; Table 1), which splits *D. leucophyllatus*, *D. ebraccatus* and *D. elegans* species with the highest number of lineages (Table 1). Model 7 also demonstrated the highest value of Bayes Factor (BF = 3,734), a model selection criteria that is simple and well suited for the purposes of comparing species delimitation models (Leache and

Table 1

Results for the seven species delimitation models based on the current taxonomy (following Peloso et al., 2016; Caminer et al., 2017) and the SVDQuartets (Fig. 2) and RAxML results (Appendix A, Fig. A2). Positive BF values indicate support for the alternative model instead of the current taxonomy. MLE = Marginal likelihood estimate, BF = Bayes factor.

Model	Lineages	MLE	Steps	BF
Model 1, current taxonomy	10	−3,751	32	–
Model 2, split *D. elegans*	12	−3,284	32	934
Model 3, split *D. ebraccatus*	11	−2,466	40	2,570
Model 4, split *D. leucophyllatus*	11	−2,902	24	1,698
Model 5, split *D. leucophyllatus* and *D. elegans*	13	−2,575	30	2,352
Model 6, split *D. leucophyllatus* and *D. ebraccatus*	12	−2,080	32	3,342
Model 7, split *D. leucophyllatus*, *D. ebraccatus* and *D. elegans*	14	−1,884	32	3,734

Bouckaert, 2018). The lowest value of MLE (-3,751; Table 1) was found for model number 1, which represents the current phylogenetic relationships (Peloso et al., 2016; Caminer et al., 2017).

We found four localities where species and/or lineages were syntopic: *D. leucophyllatus* Central overlaps with *D. arndti* and *D. sarayacuensis* in Morrinho area at the Madeira river; *D. leucophyllatus* North overlaps with *D. rossalleni* at the Trombetas locality in Pará, Brazil, close to the Amazon river; and *D. triangulum* overlaps with *D. reticulatus* in San Vicente, Ecuador, and Carauari, Brazil. For localities details see Appendix A, Table A1.

3.2. Divergence times and ancestral range estimates

The divergence time analysis estimated the origin of *D. leucophyllatus* species group for the early Miocene, approximately 18.25 Mya (95% HPD: 21–15; Fig. 4, and Appendix A, Fig. A3). The first split separating the Atlantic Forest species (*D. elegans*) and all the remaining Amazonia species was estimated at approximately 16.57 Mya. Most of the subsequent speciation events occurred during middle-late Miocene, between 13 and 5 Mya (see Fig. 4). The only Cis-Andean species, *D. ebraccatus*, diversified around 12 Mya (BP; 95% HPD: 13.9–7.3)
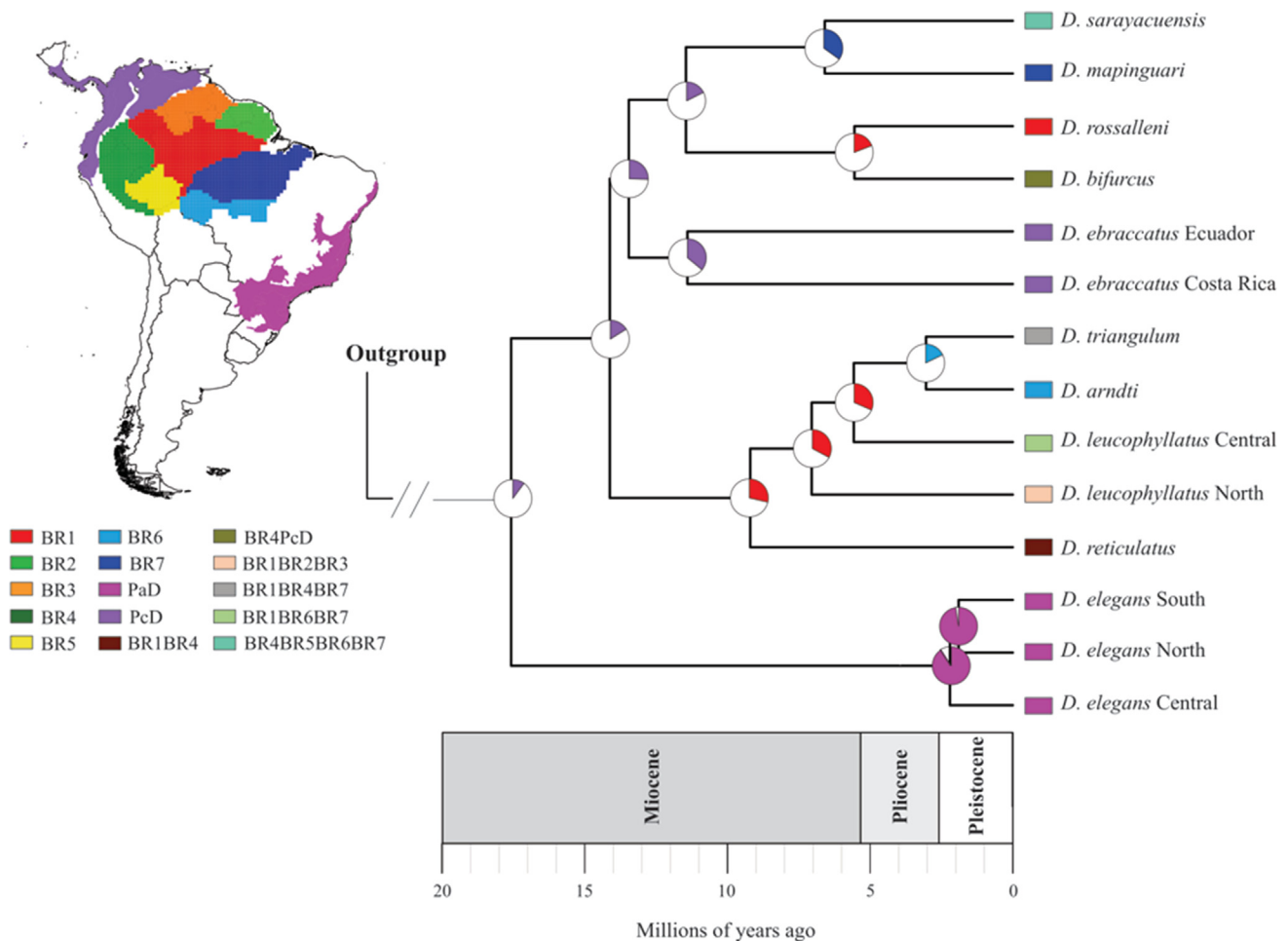
Fig. 4. Biogeographic reconstruction of *Dendropsophus leucophyllatus* species group estimated by the best-fit DEC model and geographic range estimates with the highest marginal probabilities for ancestral nodes. Pie charts at nodes show the probability of the most likely ancestral estimated areas in colors and less likely estimations in white. Regions that species are known to occupy: PaD = Paraná dominion, PcD = Pacific dominion, BR1 = Biogeographical region 1, BR2 = Biogeographical region 2, BR3 = Biogeographical region 3, BR4 = Biogeographical region 4, BR5 = Biogeographical region 5, BR6 = Biogeographical region 6, BR7 = Biogeographical region 7.

followed by the split between Costa Rica and Ecuador lineages at 8 Mya (BP; 95% HPD: 13.7–1.3; Fig. 4). However, confidence intervals are large and node support for the *D. ebraccatus* lineage clade received a very low posterior probability (0.35), and it was not recovered by the species/lineage tree (see Fig. 3A), which is due to the fact that we only fixed two secondary points for the *BEAST2 calibration (see methods). The most recent diversification events occurred among the three Atlantic Forest *D. elegans* lineages during late Pliocene and Pleistocene: the Central lineage first diverged around 2.1 Mya (BP; 95% HPD: 3.1–1.1) followed by North and South lineages, at 1.6 Mya (BP; 95% HPD: 2.4–0.7) (Appendix A, Fig. A3).

After comparing log-likelihood values of the three biogeographic models with BioGeoBEARS, the Dispersal-Extinction Cladogenesis (DEC) had the best fit for the data as supported by AICc (see Table 2). The model estimated that the most recent common ancestor of

Dendropsophus leucophyllatus species group was likely distributed at the Pacific Dominion (PcD) area and diverged by dispersal to the Paraná Dominion (PaD)/Atlantic Forest (Fig. 4). The ancestral area recovered for the Amazonian clade was also the Pacific Dominion (PcD; Fig. 4). For the clade including *D. leucophyllatus* (both clades North and Central), *D. arndti*, *D. reticulatus*, and *D. triangulum,* the ancestral area inferred was central Amazonia, or the Biogeographic Region 1 (BR1, Fig. 4). Finally, the most recent common ancestor of *D. bifurcus/D. rossalleni*, *D. mapinguari/D. sarayacuensis* clades and *D. ebraccatus* species were recovered from the Pacific Dominion (PcD; Fig. 4).

4. Discussion

Our genome-wide SNP data suggests that the *Dendropsophus leucophyllatus* species group includes at least 14 independent lineages, which

Table 2
Biogeographic model performance and log-likelihood values of the three models compared with BioGeoBEARS (Matzke, 2013). LnL = ln(likelihood); d = dispersal; e = extinction; j = founder-event speciation; AIC = Akaike Information Criterion; AICc = corrected Akaike Information Criterion; AICc_wt = weight of the models based on the AICc. Bold values are from the best-fit model (DEC).

Model	LnL	d	e	j	AIC	AICc	AICc_wt
DEC	**−58.44754**	**0.035909958**	**0.31097357**	**0**	**120.89509**	**121.75**	**0.9942**
DIVA	−63.73774	0.01	0.01	0	131.47549	132.33	0.0050
BAYAREA	−65.59041	0.011627501	0.10263042	0	135.18089	136.03	0.0007

are currently arranged into ten species, uncovering a significant underestimated diversity for this frog complex (see Fig. 3). Considering that our study did not include samples of four known species, *D. vraemi*, *D. manonegra*, *D. nekronastes* and *D. salli*, and that some of the inter-clade relations received different support at different analyses (see Figs. 2, 3 and Appendix A, Fig. A2), cryptic diversity of the group can be potentially higher. Also, divergence dating and biogeographic reconstruction propose that the species of *D. leucophyllatus* group began to diverge around early Miocene from ancestors distributed in the Pacific Dominion region. At this time, a southern corridor was probably connecting western Amazonia and the south portion of the Atlantic Forest (see Batalha-Filho et al., 2013; Fig. 4), and has been proposed as an important diversification driver of several other vertebrates (e.g., Fouquet et al., 2012a,b; Prates et al., 2016, 2019). Our results suggest that after eastwards dispersal events, speciation followed in the Amazonia, while in the Atlantic Forest a more recent Pleistocene pulse of diversification occurred within *D. elegans* (Fig. 4).

4.1. Phylogeny and species boundaries

Our phylogenetic analyses based on genomic data further corroborate the species group monophyly and interspecific relationships suggested by previous work (see Chek et al., 2001; Faivovich et al., 2005; Jungfer et al., 2010; Wiens et al., 2010; Rivera-Correa and Orrico, 2013; Pyron et al., 2014; Duellman et al., 2016; Peloso et al., 2016; Caminer et al., 2017), with some small but noteworthy differences. For instance, per Caminer et al. (2017), *D. reticulatus* appears in the same clade as *D. leucophyllatus* and *D. arndti,* and in a different clade from *D. triangulum*. Meanwhile, based on RADseq data, *D. reticulatus* is sister to the *D. leucophyllatus-triangulum* complex, which include different lineages of *D. leucophyllatus*, *D. arndti* and *D. triangulum*—with deep genetic structure (Fig. 3A). Differences in the datasets and data analyses employed between both studies probably account for the distinct relations inferred.

Despite those minor differences, our results corroborate the position of *D. elegans*, as the sister species to the remaining *D. leucophyllatus* group (Fig. 3A), more distantly related to *D. leucophyllatus* (i.e., the two main clades split during early Miocene, Fig. 4). Moreover, *D. elegans* assembles three spatially structured genomic lineages with different values of nodal support (Figs. 2, 3A, and Appendix A, Fig. A2), in accordance with the relations proposed by Tonini et al. (2013) based on mitochondrial data. Despite a study have shown that differences in advertisement calls (an important taxonomic characteristic for anurans) among populations are not correlated with phylogeographic clades (Forti et al., 2017), *D. elegans* is unequivocally composed by three lineages that can eventually represent unconfirmed candidate species (sensu Padial et al., 2010) with highly conserved phenotypic features. Further analyses of phenotype (e.g., tadpoles, behavior, internal anatomy) associated with these lineages are needed to search for congruent differences that can be used to diagnose these lineages and therefore recognize them as full species.

D. ebraccatus is composed of two lineages, and was recovered as paraphyletic in the RAxML analysis, and monophyletic in the SVDquartets and BEAST analyses. One lineage includes samples from Costa Rica whereas the other includes samples from Ecuador. The divergence time recovered as monophyletic with divergence between the two lineages estimated for around 12 Mya—*D. ebraccatus* lineages are subsequent sister taxa to all remaining species (Fig. 3A). The long branch lengths that separate samples of *D. ebraccatus* (Fig. 4 and Appendix A, Fig. A2) suggest that these lineages might represent two different species, and here we treat them as unconfirmed candidate species. We argue that further work including more samples covering a larger geographic area and finer resolution of biogeographic reconstructions is necessary to clarify species limits and the potential impact of the Panama Isthmus formation (Bacon et al., 2013) on *D. ebraccatus*. Moreover, detailed comparative phenotypic analysis might

be useful to elucidate this mystery.

4.2. The *Dendropsophus leucophyllatus-triangulum* complex

Previous studies recognized the conflicts regarding boundaries between *D. leucophyllatus* and *D. triangulum* (that is, *D. triangulum* is sister of some lineages of *D. leucophyllatus* but not others) (Chek et al., 2001; Jungfer et al., 2010; Peloso et al., 2016). The confusion over the systematic status of both species date back to their original descriptions: *D. triangulum* was described by Günther (1869 "1868") having Brazil as type locality; *D. leucophyllatus* was named in 1783 by Beireis from an unknown locality (possibly Suriname) and the holotype was lost (Frost, 2019). Caminer et al. (2017) designated a neotype for *D. leucophyllatus* (from Sinnamary, French Guiana), described new characters to diagnose *D. triangulum* and *D. leucophyllatus*, resurrected *D. reticulatus* and, described two previously unnamed species (*D. vraemi* and *D. arndti*). They also suggested that four lineages should each be considered candidate species (named Clades D—G in their study).

We recovered four main clades within the *D. leucophyllatus-triangulum* complex (Fig. 3A). Each of these clades has smaller subclades nested within them (Fig. 2 and Appendix A, Fig. A2) that would be in line with the recognition of additional candidate species. However, we assume a conservative view and recognize only these four major clades with one candidate species, *D. leucophyllatus* (Central Clade). Three of these clades can be promptly assigned to named taxa based on the sample's origin and comparison with the taxonomy proposed by Caminer et al. (2017): *Dendropsophus leucophyllatus* (North Clade)*, D. arndti* and *D. triangulum.* For the other clade*,* which we label *D. leucophyllatus* (Central Clade) assigning it to named taxa or to unnamed candidate species of Caminer et al., (2017) is not straightforward.

According to Caminer et al.'s (2017) neotype designation for *D. leucophyllatus*, our North lineage corresponds to *D. leucophyllatus* sensu stricto (type locality at French Guiana and distributed in the Guiana Shield). Although *D. leucophyllatus-triangulum* complex exhibits a high level of color patterns polymorphism (Chek et al., 2001; Peloso et al., 2016) and its phylogeny shows deep genetic structure (see Lougheed et al., 2006; Fig. 3A), phenotypic polymorphism and genetic structure do not seem to follow congruent patterns in the complex. Caminer et al. (2017) suggested that some species are polymorphic, with multiple color patterns present in some populations, rejecting the possibility that each color morph may represent a distinct lineage. Inasmuch as we did not perform morphological analyses, our results corroborate the findings of Caminer et al (2017). Mismatches between phenotypes and genomic structure can be related to different evolutionary processes, such as ancestral polymorphism, parallel adaptation to locally variable conditions or phenotypic plasticity (Zamudio et al., 2016). Further work needs to be done to evaluate these possibilities within the *D. leucophyllatus-triangulum* complex.

4.3. Historical biogeography and rainforest connections

Our analyses suggest that the most recent common ancestor of the *D. leucophyllatus* species group had a Pacific Dominion distribution and originated during early Miocene (about 18.7 Mya, Fig. 4; see also Duellman et al., 2016). Per our ancestral geographic area reconstructions and dating estimates, the geographic distributions of most ancestors were concentrated in northwestern Amazonia, where the large basin was mostly flooded during early-middle Miocene forming the Pebas System (Hoorn et al., 2010). At the large Pebas system area, ancestors could have inhabited and diverged *in situ* in exposed lands and grassy vegetations until the system later (~10–14 mya) gradually transitioned into the modern Amazon watershed simultaneously with the expansion of *terra firme* habitats (Hoorn et al., 2010; Shephard et al., 2010). Flooded environments meet the amphibians' general biological preferences (Duellman and Trueb, 1994), and certainly with that of many members of the *D. leucophyllatus* group, and could have facilitated

the origin of the ancestor of all Amazonia species and lineages, agreeing with the high amphibian richness found in western Amazonia soils that developed on Neogene (Andean) sediments (see Hoorn et al., 2010). This pattern of older lineages in the wetter western Amazonia and subsequent occupation of eastern regions is also recovered for other taxonomic groups, such as birds (Silva et al., 2019).

We found that a biogeographic scenario incorporating dispersal, extinction, and cladogenesis events (DEC) is the best fit for our data. Although little is known about the *D. leucophyllatus* species group dispersal abilities, we suspect the group may have a good ability to diffuse in space — e.g., some species commonly colonize anthropically disturbed habitats (Tonini et al., 2013; our personal observations). Thus, we hypothesize that during the early/mid Miocene, when connections between rainforests were possible (e.g., Costa, 2003; Batalha-Filho et al., 2013; Ledo and Colli, 2017), the ancestor of the *D. leucophyllatus* species group was most likely distributed throughout the Pacific Dominion region and extended into the Atlantic Forest, where *D. elegans* would later diverge, and into central and eastern Amazonia through sequential eastward dispersal events (Sheu et al., 2020).

The expansion and retraction of these biomes and the formation of a dry corridor comprising savanna-like biomes (Zachos et al., 2001) represent an early impact on the species group distribution and likely made speciation possible across the Neotropical landscape (Batalha-Filho et al., 2012), which seem to be congruent across many other animal groups (e.g., Costa, 2003; Fouquet et al., 2012a,b; Castroviejo-Fisher et al., 2014; Fouquet et al., 2014; Rodrigues et al., 2014; Prates et al., 2016; Capurucho et al., 2018). This scenario is compatible with the divergence time proposed by Duellman et al., (2016) for *D. elegans* and *D. salli* (which we, unfortunately, were unable to sample) for the mid-Miocene (around 13 Mya). *Dendropsophus salli* is known with certainty from southwestern Amazonia (Bolivia, Brazil and Peru), i.e., Biogeographic Region 5, which supports the hypothesis that *D. elegans* colonized the Atlantic Forest from a southern route during the Miocene.

The role of the Andes as a major determinant of Neotropical speciation patterns emerges from our results (Rangel et al., 2018). Besides the landscape changes on the Amazonian lowlands, the final uplift of northern Andes took place at approximately 10 to 7 Mya (Hoorn et al., 2010) and could be responsible for the allopatric speciation of *D. ebraccatus*, the only species and lineages of the group with Cis-Andean (Chocó) and Central America distributions (Fig. 3B). Per our results, a species ancestral diversification happened around 12 Mya (see Fig. 4), when connections between east Andes and not only Amazonia but also Atlantic Forest biome were possible (e.g., sister basal taxon between Andes and Atlantic Forest are well reported also by other studies on Neotropical species, Percequillo, et al., 2011; Batalha-Filho, et al., 2013; Cabanne, et al., 2019). The Andes uplift closing those connections, could have restricted the gene flow between populations, causing *D. ebraccatus* speciation.

The fact that we did not sample all of the known species of the *D. leucophyllatus* species group likely does not affect the main patterns recovered by our biogeographic reconstruction. Missing species mostly overlap in distribution with species sampled and with which they are hypothesized to be closely related to by previous studies (i.e., *D. manonegra* is distributed in the Pacific Dominion and sister of *D. bifurcus* (Dias et al., 2017); *D. salli* is distributed in Biogeographic Region 5 and sister of *D. elegans* (Dias et al., 2017)—see also comments above; and *D. vraemi* occurs in Biogeographic Region 4 and sister of *D. salli* (Caminer et al., 2017). Given these points, the main ancestral area estimates would likely be little affected or not be affected at all.

The absence of *D. nekronastes* in our sampling is, however, noteworthy. Given its phylogenetic affinities, as proposed in Dias et al., 2017 (sister to all species in the group except *D. elegans* and *D. salli*) the taxon most likely diverged early in the group history. Until this species is included in a dated phylogeny, its origin and biogeographic history will remain speculative. Nonetheless, the species is distributed in the Atlantic forest and sister of a clade composed exclusively of Amazonian/Pacific Domain taxa, which further corroborate an ancient link between Atlantic Forest and Amazonia.

Regarding *D. elegans*, the only Atlantic forest taxon included in the analyses, our result support the notion that the species only recently (Pleistocene) expanded its range and dispersed through the Atlantic Forest and, nowadays is a widespread species in the biome, with strong population-level divergence and potential unconfirmed candidate species along its latitudinal gradient. Our results recovered *D. elegans* as being composed of three distinct lineages that split at approximately 2 Mya. These results generally agree with the model proposed for *D. elegans* (Tonini et al., 2013), and a scenario of Late Quaternary climatic stability within the Atlantic Forest proposed by Carnaval and Moritz (2008) and detailed in Carnaval et al. (2009).

Most of the speciation events in the group occurred *in situ* within the Amazonian region during middle Miocene. Noteworthy are the splits involving the species pairs *D. bifurcus/D. rossalleni* and *D. sarayacuensis/ D. mapinguari* taxon pairs, and the events that happened within the *D. reticulatus*, *D. leucophyllatus-triangulum* complex (Fig. 4). Such events coincide with, and could have been driven by, major landscape and climatic changes affecting rivers formation, forest distribution and dispersal of ancestral species (e.g., Antonelli et al., 2010; Hoorn et al., 2010; Häggi et al., 2017; Rangel et al., 2018; Pirani et al., 2019). Within the *D. leucophyllatus* species group, we believe that Amazonian environmental changes, caused mostly by the final Andes uplift and transition of the Pebas system into the modern Amazon river drainage, enabled the ancestral species dispersal along newly formed phytophysiognomies (e.g., the mega wetland disappeared and *terra firme* upland forest rainforests expanded; see Hoorn et al., 2010). Emergence of new habitats could also be responsible for species diversification caused by ecological factors (e.g., Ortiz et al., 2018).

Although several of the species in the group overlap their ranges in the Amazonia (Fig. 3B), they usually occupy environmentally distinct regions, suggesting restrictions imposed by the landscape on dispersal abilities. It is possible that a parapatric speciation may have occurred even in the absence of clear barriers, influenced by ecological adaptation to local environments (de Aguiar et al., 2009). For example, *D. mapinguari* is restricted to *várzea* and *igapó* (floodplain lowland forests), and permanent ponds, whereas *D. sarayacuensis* mainly occurs in temporary ponds in *terra-firme* (upland forest) and swamps (Peloso et al., 2016; Read and Ron, 2018). Similarly, ecological factors may have played a role in the divergence between *D. bifurcus* and *D. rossalleni*. *Dendropsophus bifurcus* is larger (males 23–28 mm, females 29–35 mm) and inhabits shallow temporary and permanent ponds in open habitats (Rodriguez and Duellman, 1994), whereas *D. rossalleni* is smaller (males 19.0–22.3, females 28.2–28.7) and inhabits *várzea* habitats (Ramalho et al., 2018; PLVP and MJS personal observations).

Ancestors of the *D. leucophyllatus-triangulum* complex may have dispersed all the way to northern and eastern Amazonia (e.g., *D. leucophyllatus* North and Central lineages, Fig. 3), suggesting that other more recent physical changes on the landscape and climatic gradients could have also facilitated west-eastern species dispersal around the Pliocene (e.g., Ruokolainen et al., 2018; Silva et al., 2019; Sheu et al, 2020). For instance, favored by the change in the Amazon and Solimões rivers course, *terra firme* species could have gradually, over tens of thousands of years, accessed new areas and dispersed all the way to central and eastern Amazonia (e.g., *D. leucophyllatus* North inhabits the *terra firme* landscapes; Caminer et al., 2017). Although for other taxa central Amazonian rivers can act as dispersal barriers (Hayes and Sewlal, 2004), most of the species herein demonstrate no strong distribution restrictions caused by main rivers (e.g., *D. leucophyllatus* Central lineage and *D. arndti* overlap distribution at the Madeira River, Fig. 3B). This leads to the conclusion that rivers formation can act more like secondary dispersal barriers to gene flow at varying degrees according to species habitat preferences and dispersal capacities, than as vicariant barriers separating previously widespread ancestors (Pirani et al., 2019).

5. Concluding remarks

Previous work suggested that the *Dendropsophus leucophyllatus* species group comprises a high genetic diversity and includes a number of new potential species to be described (e.g., Caminer et al., 2017; Dias et al., 2017). However, previous studies did not investigate support for these patterns based on large-scale genomic datasets, including the group's complete distribution (Central America, Amazonia and Atlantic Forest). Here we firmly corroborate the cryptic diversity within the group, where more than one species demonstrated different independent evolutionary lineages as estimated by genomic-level data. We also confirm the paraphyly of *D. leucophyllatus-triangulum* complex by recovering four main clades that are genetically and geographically structured. We also agree that further work incorporating other types of data (e.g., morphological and ecological data) should be included for the candidate species to be recognized as new species.

Our work concludes that a large fraction of the group's diversity results from Miocene *in situ* diversification within the Amazon basin, following a western-eastern biogeographic directionality. Given that scenario, we infer that changes in Neotropical landscapes caused by the final Andes and Brazilian Shield uplifts, drainage rearrangements, and temperature oscillation could be responsible for most of the group biodiversity (Hoorn et al., 2010; Antonelli et al., 2018b; Rangel et al., 2018). The results support that dispersal led the diversification process from southwest Amazonia, where Atlantic Forest and Andes (Pacific Dominion; Fig. 4) were interconnected through the south corridor (Batalha-Filho et al., 2013). Also, in Amazonia, where most species are distributed, local adaptation to ecological factors may have reduced populations gene flow during the divergence history of *D. leucophyllatus* species group causing parapatric speciation. The historical scenario in Amazonia characterized by high habitat heterogeneity (Rangel et al., 2018) and large-scale environmental gradients (e.g., a moisture gradient) could have promoted speciation processes, resulting in the highest species diversity for the group. Meanwhile, in the Atlantic Forest, diversification events are much more recent and the overall species diversity for the group is lower.

CRediT authorship contribution statement

Renata M. Pirani: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing. **Pedro L.V. Peloso:** Conceptualization, Writing - review & editing. **Joyce R. Prado:** Methodology, Software, Validation, Writing - review & editing. **Érico M. Polo:** Formal analysis, Methodology, Software, Validation, Writing - review & editing. **L. Lacey Knowles:** Funding acquisition, Resources, Writing - review & editing. **Santiago R. Ron:** Resources, Writing - review & editing. **Miguel T. Rodrigues:** Resources, Writing - review & editing. **Marcelo J. Sturaro:** Funding acquisition, Resources, Writing - review & editing. **Fernanda P. Werneck:** Conceptualization, Funding acquisition, Resources, Supervision, Writing - original draft, Writing - review & editing.

Appendix A. Supplementary data

Supplementary data (Appendix A. Supplementary Material. Samples of *Dendropsophus leucophyllatus* species group and outgroup species used in this study with the next-generation sequencing, voucher number and locality of origin (Table A1). Processing information and ipyRAD summary statistics for specimens sequenced (Table A2). Results from the original ipyRAD output file (Fig. A1). Maximum likelihood tree based on a concatenated matrix of a total of 527,962 loci with missing data analyzed in RAXML (Fig. A2). Time calibrated phylogeny for *Dendropsophus leucophyllatus* species group and outgroup derived under a coalescent model with StarBEAST2 (Fig. A3). Supplementary data to this article can be found online at Dryad Input files for all analyses have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.f7m0cfxsj). Title: Diversification History of Clown Tree Frogs in Neotropical Rainforests (Anura, Hylidae, *Dendropsophus leucophyllatus* group). Journal: Molecular Phylogenetics and Evolution manuscript number: YMPE 106877.) This article can be found online at https://doi.org/10.1016/j.ympev.2020.106877.

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