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Temporal and spatial diversification along the Amazonia-Cerrado transition in Neotropical treefrogs of the *Boana albopunctata* species group

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ABSTRACT

Despite extensive research on biodiversity in Neotropical forests, biodiversity in seasonally dry, open biomes in South America has been underestimated until recently. We leverage a widespread group, *Boana albopunctata*, to uncover cryptic lineages and investigate the timing of diversification in Neotropical anurans with a focus on dry diagonal biomes (Cerrado, Caatinga and Chaco) and the ecotone between Amazonia and the Cerrado. We inferred a multilocus phylogeny of the *B. albopunctata* species group that includes 15 of 18 described species, recovered two cryptic species, and reconstructed the timing of diversification among species distributed across multiple South American biomes. One new potential species (*B.* aff. *steinbachi*), sampled in the Amazonian state of Acre, clustered within the *B. calcara-fasciata* species complex and is close to *B. steinbachi*. A second putative new species (B. aff. *multifasciata*), sampled in the Amazonia-Cerrado ecotone, is closely related to *B. multifasciata*. Lastly, we place a recently identified Cerrado lineage (*B.* aff. *albopuncata*) into the *B. albopunctata* species group phylogeny for the first time. Our ancestral range reconstruction showed that species in the *B. albopuctata* group likely dispersed from Amazonia-Cerrado into the dry-diagonal and Atlantic Forest. Intraspecies demography showed, for both *B. raniceps* and *B. albopunctata*, signs of rapid expansion across the dry diagonal. Similarly, for one clade of *B. multifasciata*, our analyses support an invasion of the Cerrado from Amazonia, followed by a rapid expansion across the open diagonal biomes. Thus, our study recovers several recent divergences along the Amazonia-Cerrado ecotone in northern Brazil. Tectonic uplift and erosion in the late Miocene and climate oscillations in the Pleistocene corresponded with estimated divergence times in the dry diagonal and Amazonia-Cerrado ecotone. Our study highlights the importance of these threatened open formations in the generation of biodiversity in the Neotropics.

1. Introduction

Diversification in South America is often attributed to the large number of climatically-diverse biomes including Amazonia, Atlantic Forest, Caatinga, Cerrado, and Chaco, and biotic interchanges between them (Antonelli et al., 2010; Ledo and Colli, 2017; Thomé et al., 2016; Turchetto-Zolet et al., 2013). Each biome has distinct climatic and geological features: Amazonia and Atlantic Forest are humid, while the Caatinga (seasonally dry tropical forest), Chaco (temperate dry forest), and Cerrado (tropical savanna) comprise the "dry diagonal", a more arid, seasonal, and open vegetation area that spans northeastern Brazil to Paraguay. Until recently, few studies had examined taxa distributed across multiple biomes, particularly those that span rainforests and the dry diagonal (but see Wüster et al., 2005; Werneck et al., 2009; Pavan

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and Marroig, 2017, Vasconcellos et al., 2020). Although historically considered an area of low biodiversity with faunal compositions derived from nearby biomes, more recent phylogeographic studies reveal high levels of endemism and cryptic lineages in the dry diagonal (Bartoleti et al., 2017; Domingos et al., 2017; Lanna et al., 2018; Prado et al., 2012), indicating that it may play an unappreciated role in the origin of South American diversity. Because climate change and widespread agricultural and urban development threaten large portions of these biomes, our understanding of their contributions to the origins and maintenance of biodiversity is increasingly important.

Traditionally, vicariance has been considered the principal driver of diversification in the Neotropics (Haffer, 2008; Hoorn et al. 2010, Smith et al., 2014). Within the dry diagonal, geomorphological compartmentalization in the Cerrado seems to have promoted lineage diversification and speciation in response to tectonic activities since the early Miocene (Guarnizo et. al, 2016). Erosion following the uplift of the Brazilian Shield molded the landscape into ancient plateaus, such as the Chapada dos Guimarães, which formed approximately 5 –7 mya (Camurugi et al., 2021; Prado et al., 2012; Silva, 1997). These plateaus are dominated by savanna-like vegetation, while younger valleys are dominated by more heterogeneous forest assemblages (Werneck, 2011). Although a possible scenario of reciprocal monophyly of lineages in plateaus and valleys has been proposed (Werneck, 2011), two other general patterns of diversification linked to this uplift have been recovered: a northwest-southeast directionally exhibited by some Cerrado endemic species (Guarnizo et al., 2016; Prado et al., 2012, Santos et al., 2014) and a southwest-northeast directionality (common in groups widespread in the dry diagonal) (i.e., Werneck et al., 2012; Fig. 1 arrows). Diversification is largely consistent among Cerrado endemics, likely reflecting the effects of these ubiquitous geomorphological events in the diversification of its fauna (Guarnizo et al., 2016).

However, despite the focus on vicariance-driven diversification in the Neotropics, biotic exchanges between older, more humid biomes such as Amazonia into the novel biomes of the dry diagonal may have promoted new adaptations and increased phylogenetic diversity (Simon et al., 2009). Indeed, dispersal between Amazonia and other Neotropical regions was identified as the "primary source" for Neotropical diversity in a recent *meta*-analysis (Antonelli et al., 2018). Similarly, a study on

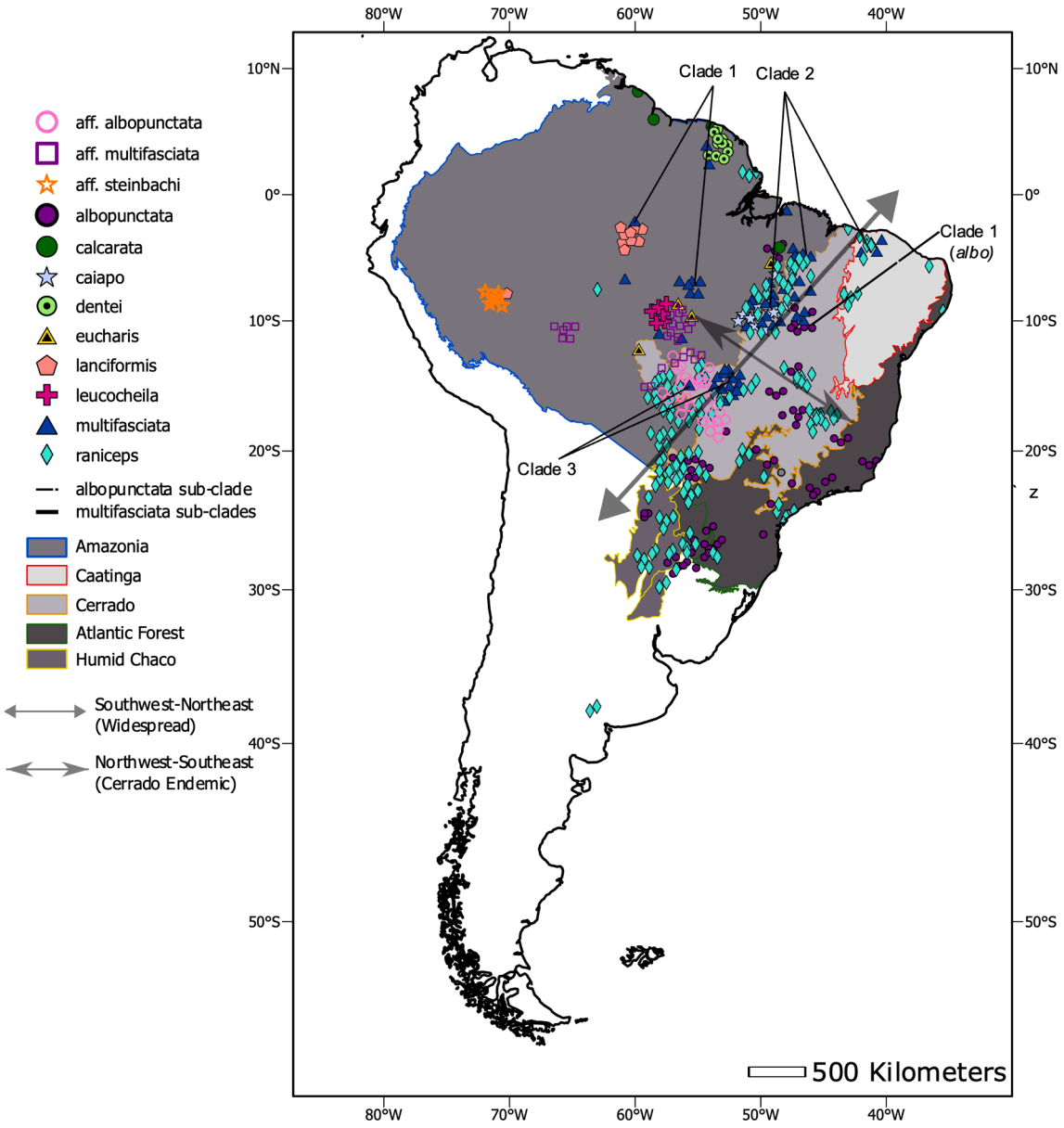

Fig. 1. Map of sampling localities for the *Boana albopunctata* species group. Potential new species are represented by open symbols. Biomes are highlighted in different colors as indicated in the legend. Points are lightly offset to remove overlap, see Supp. Table 1 for precise coordinates.

Neotropical birds recovered spatially and temporally discordant diversification across lineages, which is inconsistent with large-scale geomorphological events as the primary drivers of diversification. This highlights the role of dispersal, rather than only vicariance in generating Neotropical biodiversity (Smith et al., 2014).

Climatic shifts during the Quaternary (~2.6 mya) (Carnaval et al., 2009; Da Silva et al., 2014; van der Hammen and Hooghiemstra, 2000) may have promoted such exchanges between biomes. For instance, isolated patches of savanna-like vegetation within Amazonia may have harbored distinct lineages, which later came into contact with the Cerrado during climatic fluctuations, although the contribution of Amazonian Savannas to Cerrado biodiversity remains unclear (Resende-Moreira et al., 2019). Pleistocene climatic oscillations have also driven diversification within the dry diagonal (Vasconcellos et al., 2019) with lineages from the Cerrado colonizing and diversifying withing the Caatinga (Oliveira et al. 2018), as evidenced by population expansions in lowland (Gehara et al., 2017) and highland (de Oliveira et al., 2021) Caatinga species. Thus, a complete understanding of evolutionary roots of the incredible Neotropical diversity requires an understanding of the complex relationships among biotas of humid tropical forests and the dry diagonal, as well as the idiosyncratic relationships among lineages of dry diagonal biomes. To achieve such understanding the study of species groups distributed across these biomes is necessary.

Treefrogs of the family Hylidae are the most diverse Neotropical amphibians (Frost, 2021). The *Boana albopunctata* species group, a clade of hylid treefrogs, includes 18 species widely distributed in South America, and one in the Caribbean (Frost, 2021). Their widespread distribution provides the opportunity to examine patterns of Neotropical diversity across South American biomes. Indeed, this group has been targeted in recent studies of phylogeography (Prado et al., 2012; Camurugi et al., 2021), phenotypic diversification (Rainha et al., 2021), and systematics (Fouquet et al. 2021). In Amazonia vicariance drove an initial east–west divergence followed by transriverine dispersal over the last 5 my, which marked more recent diversification in the group (Fouquet et al. 2021). Within the Cerrado, endemic species of the group show the classic northwest-southeast directionality (Prado et al., 2012; Guarnizo et al., 2016) and both vicariance and isolation by environment have contributed to population divergence in more widespread species, such as *B. raniceps* (Camurugi et al., 2021). Mapping transitions from Amazonia to the dry diagonal in a phylogenetic context, and inferring the timing of such events will allow us to investigate how such ecological opportunities may promote diversification (Losos, 2010; Schluter, 2000).

Recent phylogenetic studies in the *Boana albopunctata* species group have focused on Amazonia using mitochondrial data to reconstruct group phylogenies (Pinheiro et al., 2018; Fouquet et al. 2021). In the present study we investigate cryptic diversity and the timing of diversification in this group using both mitochondrial and nuclear loci with emphasis on the Amanzonia-Cerrado ecotone and the open dry diagonal. At the interspecific level we used a time-calibrated species tree to infer divergence times and reconstruct ancestral ranges for each lineage. In addition to species-level analyses, we examined phylogeographic trees to examine intraspecific population structure and demographic processes within *B. albopunctata*, *B. raniceps*, and *B. multifasciata*, three species with multi-biome ranges distributed across the Amazonia-Cerrado ecotone, the Caatinga, the Cerrado, and the Atlantic Forest (Fig. 1).

Our goals were three-fold: 1) Sequence B. *albopunctata* group species across the Amazonia-Cerrado ecotone to detect undescribed lineages and place these within the group's phylogeny; 2) quantify recent diversification within the dry diagonal indicated by speciation events occurring within the Cerrado, and the presence of population structure within *B. albopunctata*, *B. raniceps*, and *B. multfasciata* across dry biomes; and lastly, 3) examine the concordance of diversification events with biotic exchange between Amazonia and the Cerrado (which would be represented by dispersal from Amazonia to the dry diagonal in ancestral range reconstructions), Pleistocene climate oscillations (~3 mya; van der Hammen and Hooghiemstra, 2000) and tectonic events such as the uplift of the Chapada does Guimarães plateau (5–7 mya; Silva, 1997), and tectonic activity in western Amazonia (~10 mya; Hoorn et al., 2010). Examination of both species level and intra-species diversification expands the current knowledge of biodiversity in this group of treefrogs and has the potential to elucidate the relative importance of biotic exchanges among open and forested biomes in promoting diversification in the Neotropics.

2. Materials and methods

2.1. Study system

The *Boana albopunctata* species group, found in the Caribbean and throughout South America currently contains 18 species: *B. albopunctata* (Spix, 1824), *B. alfaroi* (Caminer and Ron, 2014), *B. almendarizae* (Caminer and Ron, 2014), *B. caiapo* (Pinheiro et al., 2018), *B. calcarata* (Troschel, 1848), *B. courtoisae* (Fouquet et al., 2021), *B. dentei* (Bokermann, 1967), *B. eucharis* (Fouquet et al., 2021), *B. heilprini* (Noble, 1923), *B. fasciata* (Günther, 1859), *B. lanciformis* (Cope, 1871), *B. leucocheila* (Caramaschi and Niemeyer, 2003), *B. maculateralis* (Caminer and Ron, 2014), *B. multifasciata* (Günther, 1858), *B. paranaiba* (Carvalho et al., 2010), *B. raniceps* (Cope, 1862), *B. steinbachi* (Boulenger, 1905), and *B. tetete* (Caminer and Ron, 2014). Previous phylogenetic analyses indicate that the earliest divergence in this group is between *B. heilprini*, from Hispaniola, and the remaining species, with the more derived lineages (*B. albopunctata* and *B. multifasciata*) occurring in dry habitats of Brazil (Pyron and Wiens, 2011). *Boana raniceps* and the newly described *B. caiapo* also occur in the dry diagonal, and all other named species have ranges within Amazonia or in the Cerrado-Amazonia ecotone.

2.2. Taxonomic sampling

Fifteen of the 18 species in the *B. albopuntata* group are represented in our analyses. Our dataset included multiple specimens of ten named species: *B. albopunctata* (N = 117), *B. calcarata* (N = 8), *B. dentei* (N = 11), *B. heilprini* (N = 6), *B. lanciformis* (N = 10), *B. leucocheila* (N = 6), *B. multifasciata* (N = 33), *B. raniceps* (N = 156), *Boana caiapo* (N = 11), and *B. eucharis* (N = 4) (Fig. 1; Supp. Table 1). We also obtained 16S mitochondrial sequences for five additional named species: *B. alfaroi*, *B. almendarizae, B. fasciata, B. maculateralis*, and *B. tetete,* as well as *B. caiapo,* from GenBank (Supp. Table 2) to place them within the larger species complex. We lacked samples of *B. paranaiba*, *B. steinbachi*, and *B. courtoisae*, the only three described species missing in our dataset. *Boana albomarginata* (N = 1) and *B. faber (N = 1)* were included as outgroups in our analyses. Following filtering, several samples were eliminated from the dataset, resulting in the following sample sizes: *B. albopunctata* (N = 70), *B. calcarata* (N = 50).

Lastly, we sampled three putative new species. First, a deeply divergent mitochondrial cryptic lineage within *B. albopunctata* from the plateau of Chapada dos Guimarães in the Cerrado, previously recovered by Prado et al. (2012) (hereafter, "*B.* aff. *albopunctata*", N = 29). Next, we identified two cryptic lineages during sequencing: a putative species from the Amazonian state of Acre in northern Brazil (*B.* aff. *steinbachi*, N = 6), and specimens collected in Amazonia and within the Amazonia-Cerrado ectone (*B.* aff. *multifasciata*, N = 27). All samples were included in the final dataset.

2.3. Laboratory protocols

DNA extraction was performed following methods outlined in Prado et al. (2012). We amplified one mitochondrial gene fragment including NADH dehydrogenase subunit 1, the adjacent $tRNA^{Leu}$ and $tRNA^{Ile}$, the flanking $tRNA^{Gln}$ and partial sequence for the flanking 16S region (1836

bp). We also sequenced two nuclear genes, β-fibrinogen (partial exons 1 and 2, and intron 1; 509 bp) and rhodopsin (exon 1; 309 bp). Sequencing primers are listed in Supp. Table 3. PCR followed protocols in Prado et al. (2012), with ND1 annealing temperature at 54.7 °C, β-fibrinogen at 49 °C, and rhodopsin at 46 °C. Cycle sequencing reactions in both directions were performed using Big Dye terminator sequencing chemistry (Applied Biosystems, Foster City, CA, USA). We used SEQUENCHER v4.1 (GeneCodes, Ann Arbor, MI, USA) to assemble contigs for each gene fragment.

2.4. Alignment/Annotation

We aligned sequences using MAFFT (Katoh and Toh, 2010) and visually inspected and annotated sequences in AliView (Larsson, 2014). We used PHASE to resolve nuclear haplotypes (Garrick et al., 2010). Individuals with more than 50% missing data were removed from each gene matrix. To retain the greatest number of individuals with more than 50% coverage, we trimmed the ND1 sequence to include only the 16S portion (base pairs 1–734). All *B. eucharis* samples had more than 50% missing data for this 16S fragment, however; we included these samples to place these populations within our species tree. All samples and their localities are shown in Fig. 1, and samples with their biome assignment are listed in Supp. Table 1.

We used PartitionFinder v1.1.1 to determine the appropriate substitution model for each partition (Lanfear et al., 2012). Settings for PartitionFinder were: branch lengths unlinked, "all" models of evolution searched, search scheme set to "greedy," and BIC scores used for model selection. We tested models that partitioned by genes and by codons within independent nuclear genes. The JC model was the best for Rhodopsin, HKY + G model for β-fibrinogen, and HKY + G for 16S. Models were implemented in BEAST without invariant site (I) estimation because there is some indication that the separation of I and G is biologically irrelevant and may bias results of Bayesian phylogeny estimation methods (Jia et al. 2014).

2.5. Dated species tree with all genes and all samples

We created a species tree with ten named species and three putative new species (*Boana* aff. *albopuncta, B.* aff. *multifasciata,* and *B.* aff. *steinbachi*) for which we had multiple samples and all three gene sequences. We used *BEAST implemented in BEAST2 and prepared our data for input into BEAST2 using BEAUTi (v 2.4.7) (Drummond et al., 2012) with the following settings: Yule model prior (Bell et al., 2015), strict clock, and site models and trees unlinked between partitions. Clocks were linked between the nuclear genes and we used published substitution rate of 0.00753 substitutions/million years for 16S to date the phylogeny (Gehara et al., 2014). BEAST was run twice for 450,000,000 generations. Outputs from both runs were combined in Tracer (Drummond et al., 2012) to check for convergence, and to ensure that the ESS for the posterior was above 200.

Using the species trees created by *BEAST, we constructed a Maximum Clade Credibility tree in TreeAnnotator v2.4.1 (Drummond et al., 2012), with the first 10% of trees discarded. As part of the construction of the species tree, *BEAST also creates individual gene trees. The three individual gene trees were also checked for ESS scores and convergence, and we generated Maximum Clade Credibility trees for each gene independently.

2.6. Expanded species phylogeny with GenBank sequences

In addition to the species tree, we constructed a tree using a portion of the 16S sequence, hereafter referred to as the "expanded species phylogeny", to include species within the *Boana albopunctata* group obtained from GenBank: *B. alfaroi*, *B. almendarizae*, *B. caiapo*, *B. maculateralis*, and *B. tetete* (Supp. Table 2). The goal of this analysis was to create a more complete phylogeny of the *B. albopuncata* group, and to check the similarity of putative new species to all described species for which we could obtain sequence data. We chose a 499 bp section of 16S that was sufficiently sequenced in both our samples and samples obtained in GenBank. We did not include *B. eucharis* in this tree, as we had only a small portion of the 16S sequence for this species. We used GBLOCKS to align the sequences, and visually inspected sequences for errors (Talavera and Castresana, 2007). We used BEAST2 and BEAUTi (v 2.4.7) (Drummond et al., 2012) with an uncorrelated relaxed clock and a GTR + G substitution model to infer the phylogeny. We used the same fossil-calibrated 16S Hylid substitution rate as in the species tree (Gehara et al., 2014). BEAST was run twice for 50,000,000 generations. Outputs from both runs were combined in Tracer (Drummond et al., 2012) to check for convergence and to ensure that the ESS for the posterior was above 200.

2.7. Intraspecies population structure and demographic statistics

The 16S gene tree produced by species tree analysis was used to visualize intraspecies population structure because 16S was the gene with the most complete sampling and had the most phylogenetic signal. We also used this 16S sequence to detect patterns of demographic expansion in *Boana albopunctata*, *B.* aff. *albopunctata*, *B. multifasciata*, and *B. raniceps*. We used DNAsp (v6) (Rozas et al., 2017), to calculate Fu's F (Fu, 1997), Tajima's D (Tajima, 1989), and Ramos-Onsins and Rozas's R2 (Ramos-Onsins and Rozas, 2002). Negative values of Fu's F and Tajima's D, and significant values of R2 are indicative of departures from neutrality. Deviations in these three statistics can indicate population growth, which we might expect when species expand into new biomes. We calculated each index separately for each clade: one clade for *B. raniceps*, one for *B. albopunctata*, one for *B.* aff. *albopunctata*, and three clades for *B. multifasciata* (see Fig. 4 for clades, and Supp. Table 6 for sample sizes). For each statistic, we used 1000 coalescent simulations of the data to produce p-values for each estimate.

2.8. Ancestral range reconstruction

We re-constructed the biogeographic history of the *Boana albopunctata* species group to detect speciation in the Cerrado (Goal 2) and to examine the concordance of diversification with range expansion from Amazonia to the Cerrado (Goal 3). Specifically, we aimed to quantify the number of speciation events occurring within the Cerrado and the number of dispersal events between Amazonia and Cerrado across the phylogenetic tree. We used Amphibian Species of the World (Frost, 2021), IUCN databases, and primary literature (Carvalho et al., 2010; Pinheiro et al., 2018; Zina and Prado, 2010) to determine the distribution of each species and assign them to one or more of the following biomes: Amazonia, Cerrado, Caatinga, and Atlantic Forest. Using the wrapper program, RASP (Yu et al., 2015), we then tested the six biogeographic models used by BioGeoBEARS (Matzke, 2014, 2013a, 2013b) to determine the best model for our data.

We chose the best model based on AICc score, and then re-ran RASP with the suggested model, Dispersal-Extinction-Cladogenesis (DEC) without founder event speciation (J), as recommended in the RASP documentation. The DEC model describes scenarios in which range changes occur during cladogenesis (Ree and Smith 2008). The model includes speciation via vicariance, where daughter species occupy complementary subsets of the ancestor's full range; "sympatric subset" speciation, where one daughter inherits one area of the ancestral range, with the other inheriting the remaining regions; and lastly, sympatric "range copying" speciation, where the ancestor has only one ancestral area (e.g. – "Amazonia"), which is then inherited by both daughters (Matzke, 2014). DEC differs from the other models tested (DIVA, BayArea, BBM) in including "sympatric subset" speciation, and in disallowing both widespread sympatry (daughters inherit identical, multi-region ranges), and widespread vicariance (daughters occupy distinct, multi-region ranges). Thus, the DEC model would describe the scenario

of interest, in which the ancestor disperses from Amazonia to the Cerrado, resulting in a sister species in each biome.

In the re-run, we included both the maximum clade credibility tree, as well as a set of 1000 randomly selected trees (S-DEC method) from our BEAST output. This "S" option in RASP implements the DEC model, but also allows for uncertainty by incorporating multiple trees. We ran the S-DEC model twice to confirm the stability of our results (Beaulieu et al., 2013; Ree and Smith 2008).

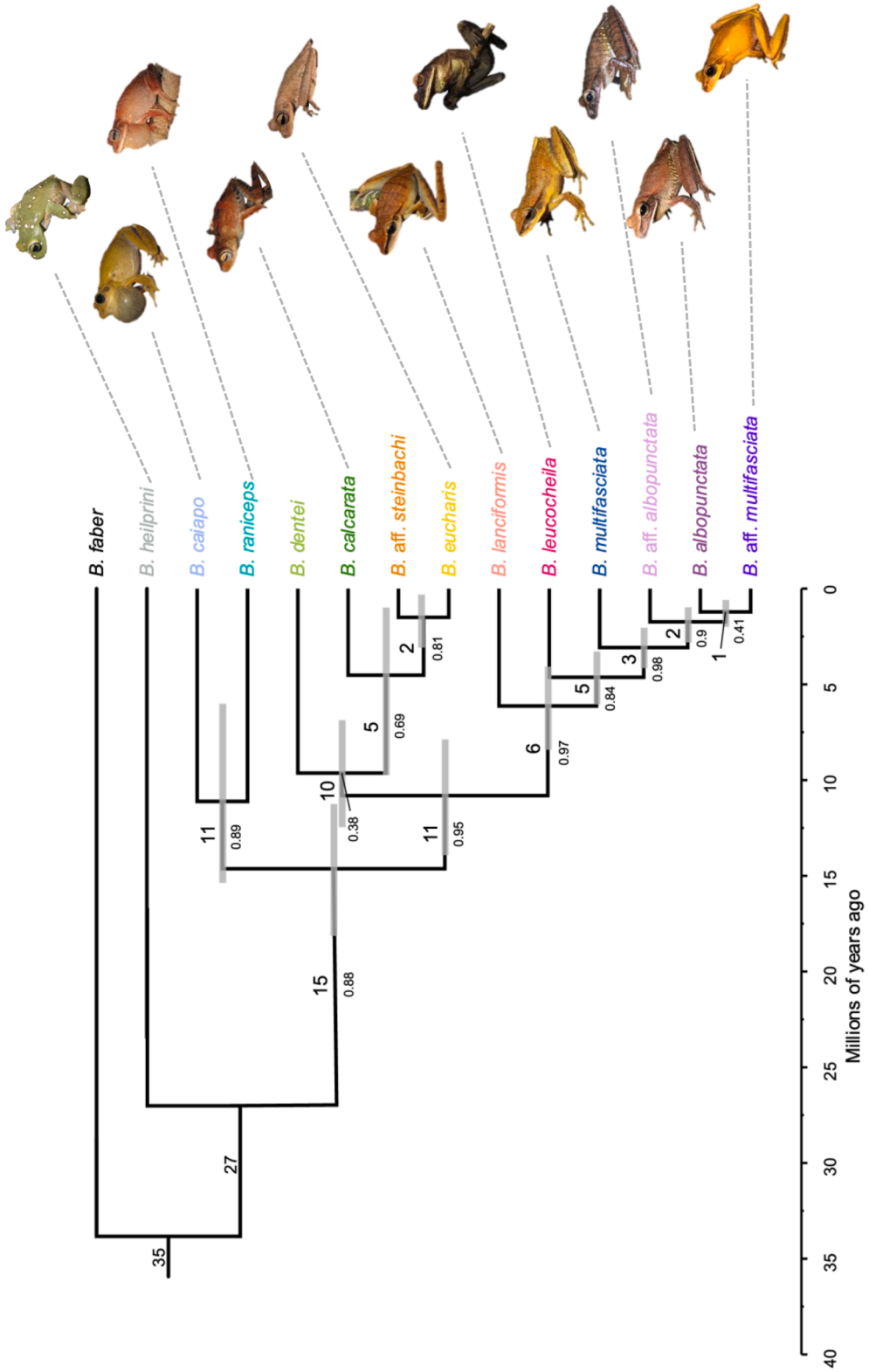

Fig. 2. Dated species tree using 16S, β-fibrinogen, and rhodopsin gene sequences. Numbers above branch are node age, bars represent 95% confidence intervals. Posterior probability values are listed below each branch. Photo credits (from top): H.W. Greene, L. Alves, D.J. Rodrigues, D.J. Rodrigues, M. Gordo, M. Gordo, L. Alves, C.P.A Prado, C.F.B. Haddad, A. Pansonato.

3. Results

3.1. Species tree topology

The earliest divergence in the *Boana albopunctata* group is *B. heilprini*, which diverged from the rest of the group approximately 27 mya (Fig. 2). The next divergence is the clade containing *B. raniceps* and *B. caiapo*, which split from the remaining species approximately 15 mya (posterior = 0.88, 95% CI for divergence: 6–15). The remaining species comprise two clades that diverged approximately 11 mya (posterior = 0.95, 95% CI for divergence: 8–14). The first clade contains the Amazonian species of the *B. calcarata-fasciata* complex: *B. dentei*, *B. calarata*, and lastly, *B.* aff. *steinbachi* as sister to *B. eucharis* (posterior = 0.81; 95% CI for divergence 0.3–3.1 mya).

The second clade contains both Amazonian and dry diagonal species. *Boana lanciformis* split from the remaining species ~6 mya (posterior = 0.97, 95% CI for divergence: 4–8 mya), followed by *B. leucocheila* (posterior = 0.84, 95% CI for divergence: 4–6 mya). Lastly, we recover *B.* aff. *albopunctata* as sister to *B. albopunctata* and *B.* aff. *multifasciata,* splitting approximately 2 mya (posterior = 0.90, 95% CI for divergence: 1–2.8 mya) and *B.* aff. *multifasciata* as sister to *B. albopunctata*, but with low support (posterior = 0.41).

3.2. Expanded species phylogeny with GenBank sequences

Boana aff. *steinbachi* is related to *B. alfaroi* and *B. tetete*, which form a well-supported clade with *B. dentei* (Fig. 3, posterior = 1). Like the species tree, *B.* aff. *albopunctata*, *B.* aff. *multifasciata,B. albopunctata* and *B. multifasciata* fall within the same clade with low support for relationships within the clade. *Boana leucochelia* also falls within this clade, but with low support. All potentially new species (*Boana* aff. *steinbachi*, *B.* aff. *albopunctata*, and *B.* aff. *multifasciata*) are monophyletic, and distinct from other named species.

3.3. Intraspecies demography

At the population level in the 16S gene tree (Fig. 4), *B. albopunctata* is shallowly diverged (Fig. 4A). One nested, well supported clade includes individuals from Tocantins state, Brazil, in the northeast corner of the Cerrado. The remaining *B. albopunctata* samples form a polytomy with individuals from the Atlantic Forest, the Cerrado, and the Chaco in the southernmost extent of the dry diagonal. Similarly, *B. raniceps* shows little phylogeographic structuring (Fig. 4B), with a shallow yet well supported clade from Tocantins.

In contrast, *B. multifasciata* is comprised of three deeply divergent

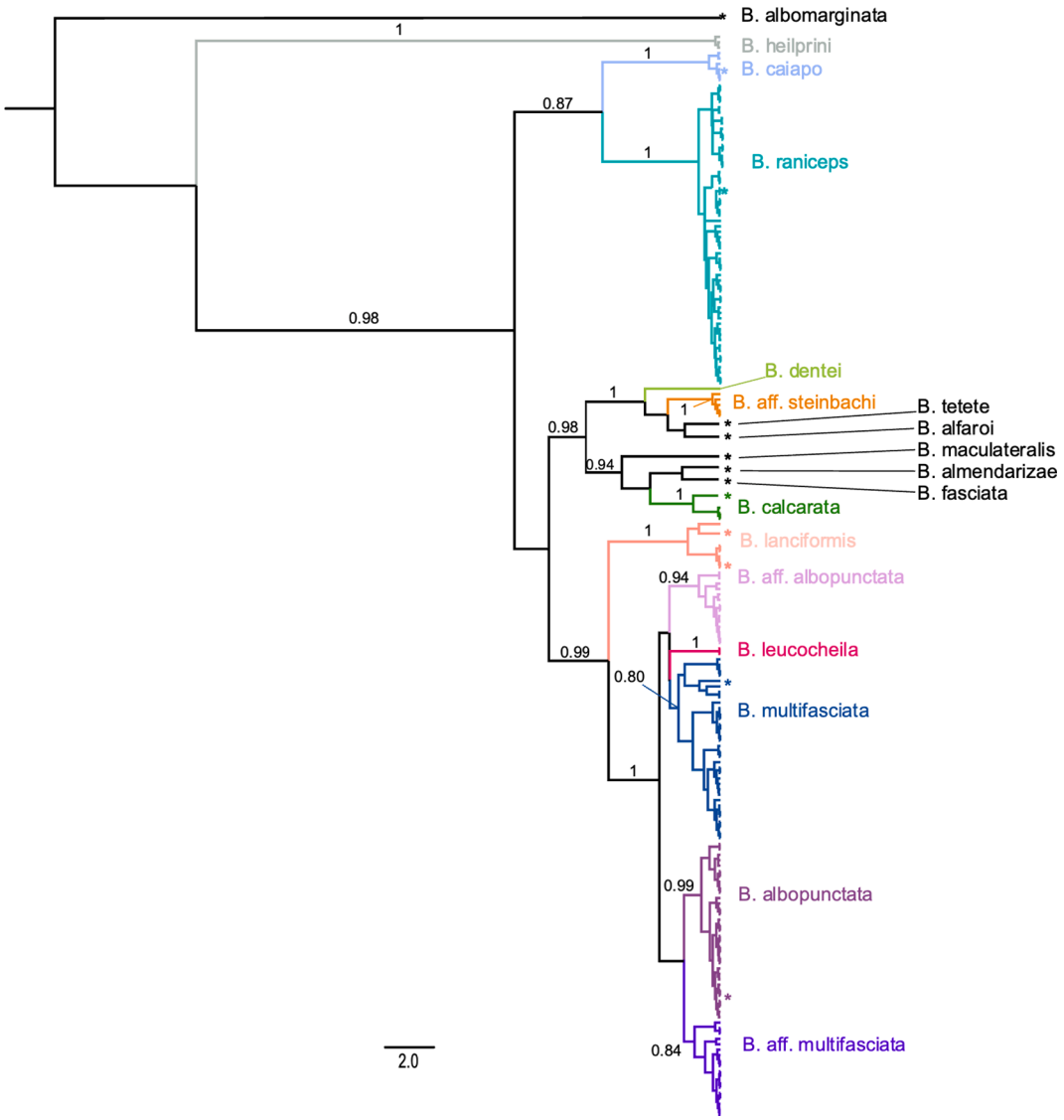

Fig. 3. Expanded species phylogeny with additional 16S sequences from the *Boana albopunctata* species group. Node support greater than 0.75 are indicated and colors are as in Fig. 2. Asterisks represent samples from GenBank. See Supp. Table 1 for sample numbers.

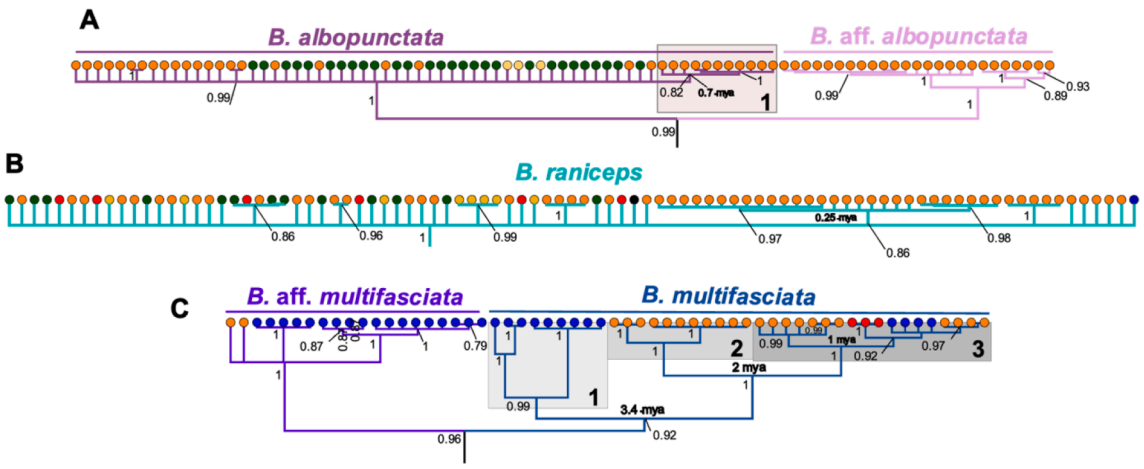

Fig. 4. Subclades of the 16S gene tree (*BEAST analysis) for the *Boana albopunctata* species group showing intraspecies sampling. A) *B. albopunctata* and *B. aff. albopunctata* B) *B. raniceps* C) *B. multifasciata* and *B. aff. multifasciata*. Clades within *B. albopunctata* and *B. multifasciata* are highlighted and numbered as in the text and map (Fig. 1). Branch colors and support values as in Fig. 2. Support values<0.75 are collapsed into polytomies. Sample names are colored by biome as in Fig. 1.

clades (Fig. 4C). The earliest diverging clade (~3.4 mya) contains individuals collected from French Guiana and Brazilian Amazonia. The remaining *B. multifasciata* split into two groups approximately 1.9 mya. The second clade contains individuals from the Cerrado region of Mato Grosso to the west, in central Brazil. The third clade contains individuals from the states of Tocantins, Maranhão, and Ceará, which traverse the Amazonia, Cerrado and Caatinga biomes, from central to northeast Brazil.

Negative values of both Fu's F and Tajima's D indicate higher than expected numbers of alleles, and an excess of low frequency polymorphisms, respectively. Similarly, low, significant values of R2 indicate an excess of singleton mutations relative to average nucleotide differences, consistent with rapid population growth (Ramírez-Soriano et al. 2008). *Boana raniceps* and *B. albopunctata* showed significant values indicative of population expansion. *Boana raniceps* had a significantly negative Fu's F (−12.50, $p < 0.001$) and Tajima's D (−1.78, $p < 0.05$), and a significant, low R2 (0.04, $p < 0.05$). In *B. albopunctata* Fu's F was significant and negative (−6.90, $p < 0.001$), as was Tajima's D (−1.63, $p < 0.05$), while R2 was also significant (0.04, $p < 0.05$). Clade three of *B. multifasciata* showed a significantly negative Fu's F (−3.292, $p < 0.05$). All other results were statistically insignificant (Supp. Table 6).

3.4. Ancestral range reconstruction (BioGeoBEARS)

The ancestor of the *Boana albopunctata* group likely inhabited Amazonia, the Cerrado, and perhaps the Caatinga (Fig. 5). The best model for our data was DEC (Dispersal-Extinction-Cladogenesis) without founder-event speciation. This model is consistent with speciation following dispersal to a new biome (e.g. – Amazonia to the Cerrado). The highest dispersal rates occurred from Amazonia to the Cerrado, which was four times higher than dispersal between other biomes (Fig. 5). Our model also showed that within-biome speciation occurred eight times within Amazonia and four times within the Cerrado. Lastly, we found evidence (probability > 0.5, Supp. Table 5) of one vicariance event between Amazonia (A) and the Cerrado-Atlantic Forest (CD), leading to divergence between *B.* aff. *multifasciata* (A) and *B. albopunctata* (CD) (Fig. 5, see arrow).

4. Discussion

4.1. Overall phylogenetic structure of Boana albopuncata species group

The first goal of this work was to construct a multi-locus species group phylogeny, and identify cryptic lineages in the Amazonia-Cerrado ectone and dry diagonal. We include *B.* aff. *albopunctata* (Cerrado) and *B.* aff. *multifasciata* (Amazonia, Cerrado, and Caatinga) for the first time, and improve upon previous phylogenies by using both mitochondrial and nuclear loci. Our species tree (Fig. 2) and expanded species phylogeny (Fig. 3) are largely consistent with recent phylogenies for the named species in the group (Faivovich et al., 2005; Fouquet et al., 2021; Pinheiro et al., 2018; Pyron and Wiens, 2011). In the species tree, we recover *B. leucocheila* as an outgroup to the clade containing *B. multifasciata* and *B. albopunctata* with moderate support (Fig. 2, posterior = 0.84), a relationship recovered with low support in the phylogeny of Fouquet et al. (2021) based on mtDNA. Thus, additional nuclear data and sampling in this study allowed us to confirm this placement of *B. leucocheila*.

Boana aff. *steinbachi* likely represents the undescribed lineage *B.* aff. *steinbachi* 1 recovered by Fouquet et al. (2021) based on the position in the expanded species phylogeny (Fig. 3), collection locality, and morphology (T.R. Carvalho and P. Marinho, *pers comm*). Although we sampled this species from a single locality, previous work outlines the probable extent of this lineage, which does not overlap with *B. steinbachi* in our sampling area (Fouquet et al. 2021). The phylogenetic placement of *B. eucharis* in the expanded phylogeny (Fig. 3) agrees with previous studies (Fouquet et al. 2021, T.R. Carvalho, *pers comm*), however, we include sampling from the Brazilian state of Pará, thus expanding the known range of *B. eucharis*, originally described from Mato Grosso and Rondônia states, a region of Cerrado-Amazonia transition in northern Brazil. With additional sampling, it is possible that the range may be even further expanded.

4.2. Potential new species

Boana aff. *albopunctata* was previously identified by Prado et al. (2012) from Chapada dos Guimarães in the Cerrado of central Brazil. This putative species is separated from B. albopunctata, B. multifasciata, and B. aff. multifasciata sampled below the Chapada dos Guimarães plateau. Our species tree indicates that the split between *B.* aff. *albopunctata* and the clade containing *B. albopunctata* and *B.* aff. *multifasciata* occurred approximately 1.7 mya (95% confidence interval: 1.0–2.8 mya), however, *B.* aff. *albopunctata* and *B. albopunctata* are not recovered as sister species (Fig. 2). The 16S gene tree shows the same relationship as Prado et al. (2012), with *B.* aff. *albopunctata* as sister to *B. albopunctata* with high support (Fig. 4).

Boana aff. *multifasciata* is distinct from the other species and its position in the species tree is unresolved (posterior = 0.41). However, it is

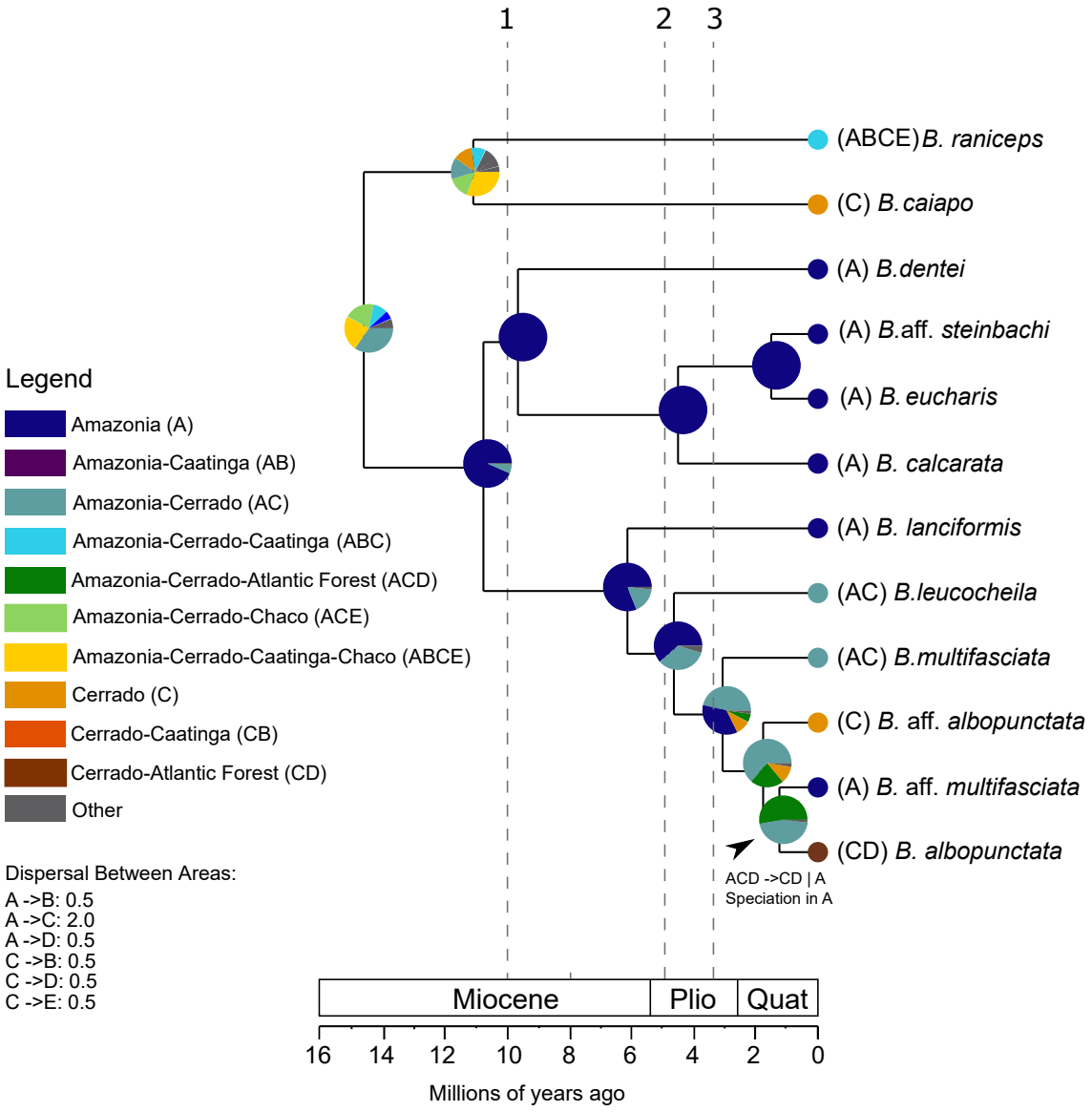

Fig. 5. Dated species tree of the *Boana albopunctata* species group with ancestral ranges estimated in BioGeoBEARS. The significant vicariance event is indicated by an arrow at the corresponding node. Dashed lines indicate significant geologic and climatic events: 1. Formation of the Amazon basin (~10 mya). 2. Final uplift the the Cerrado Plateau (~5 mya). 3. Pleistocene climatic oscillations (~2.6 mya).

sister to *B. multifasciata* in the 16S gene tree with high support (posterior = 0.96) (Fig. 4). *Boana* aff. *multifasciata* is paraphyletic and clustered with both *B. albopunctata* and *B. multifasciata* in the β-fibrinogen and rhodopsin trees (Supp. Figs. 1 and 2), likely due to low phylogenetic signal. This putative species was collected in northern Mato Grosso and Rondônia states, in the Cerrado-Amazonia transition. There is distance between our sampling localities, as the area between *B. multifasciata* clades 2 and 3 has been severely deforested for agriculture (Aldrich et al., 2012). It is possible that the distribution of *B. multifasciata* and *B.* aff. *multifasciata* were once more continuous, and sampling across the region would have shown a signature of isolation by distance. However, despite gaps in sampling areas of *B.* aff. *multifasciata* (Fig. 1, open purple squares), these populations are still more closely related to each other than to *B. multifasciata* (Fig. 1, solid blue triangles) which was sampled nearby. These *B.* aff. *multifasciata* individuals could be related to *B. paranaiba*; however, *B. paranaiba* is known only from its type locality in Minas Gerais state, in the Cerrado of southeast Brazil (Carvalho et al., 2010). Thus, this lineage is likely part of a species complex with *B. multifasciata* and *B. albopunctata*.

4.3. Diversification within the dry diagonal

The second goal of the study was to identify speciation events within the Amazonia-Cerrado ecotone and dry diagonal, and to examine population structure within species with multi-biome distributions. Our ancestral range reconstruction detected four speciation events within the Cerrado (Fig. 5), and we find several species pairs across the Amazonia-Cerrado ectone. *Boana caiapo* and *B. raniceps* are an Amazonia-Cerrado pair, with *B. caiapo* occurring in Amazonia-Cerrado ecotone, and *B. raniceps* occurring mainly in open habitats of the dry diagonal (Cerrado-Chaco-Caatinga) and subsequently expanding (Figs. 4 and 5). This split occurred approximately 11 mya, when the modern character of the Cerrado was starting to form (Silva, 1997). This pattern was also recovered in the Amazonia-Cerrado ecotone species *B. leucocheila* which split from an Amazonian ancestor~ 6 mya, and in Amazonian and dry-diagonal clades of *B. multifasciata* which split ~ 3.4 mya (Fig. 4). Lastly, both *B. albopunctata* and *Boana* aff. *albopunctata* were found in the Cerrado, having descended from a more widely distributed ancestor (Fig. 5).

Within species, we see varying levels of population structure. Although widely distributed, *Boana raniceps* shows little biogeographic structuring. Shallow divergences between populations (Fig. 4) and demographic statistics (Supp. Table 6) indicate that *B. raniceps* spread rapidly across the dry diagonal (Cerrado-Chaco-Caatinga). This finding of little structuring and rapid expansion is consistent with a recent biogeographical study focused on *B. raniceps*, which recovered a northeastern lineage with little population structure, and recent (100 Ka) demographic expansion (Camurugi et al., 2021).

Similarly, *Boana albopunctata* shows little population structure as well as genetic signatures of expansion. For both *B. raniceps* and *B. albopunctata*, an origin in the Cerrado, or near the Amazonia-Cerrado ecotone, seems to have facilitated rapid expansion across the dry diagonal. The pattern of shallow divergence between Cerrado populations, even across large areas, has been uncovered in other taxa (Capurucho et al., 2018). Intuitively, one could attribute this result to the more open landscape of the Cerrado and its apparent lower complexity. However, because many species of lizards and frogs show significant structuring and cryptic diversity in the Cerrado (Nali et al., 2020; Prado et al., 2012), it is more likely that generalist habitat requirements and greater dispersal capacity facilitating gene flow account for the shallow population structure in *B. albopunctata* and *B. raniceps* (Camurugi et al., 2021; Rodríguez et al., 2015; this study).

In contrast, *B. multifasciata* exhibits deeper intraspecific phylogenetic structure (Fig. 4C), and we recovered three distinct lineages within *B. multifasciata*. Clade 1 (Fig. 4C) individuals were collected within the known range of *B. multifasciata,* mostly located in the Amazon (Frost, 2021). Clades 2 and 3 both contain individuals outside the currently described range of *B. multifasciata*. Clade 2 (Fig. 4C) consists of individuals from the Cerrado, and may represent expansion from Amazonia. Clade 3 contains individuals from Amazonia, but bordering the Cerrado, as well as Cerrado and Caatinga individuals (collected in Amazon-like habitat; Thomé et al., 2016). Clade 3 showed signatures of a recent population expansion, similar to *B. raniceps*, and other frog species found in the dry diagonal and Caatinga in particular (Gehara et al., 2017).

4.4. Timing and patterns of diversification: Geomorphological events

Our third goal was to examine the concordance diversification with geomorphological events, Pleistocene climate fluctuations, and biotic exchange between Amazonia and the Cerrado. Within the Amazonian clade, which includes species of the *B. calcarata-fasciata* complex (*B. dentei*, *B.* aff. *steinbachi*, *B. eucharis*, and *B. calcarata*), diversification occurs after the formation of the Amazon basin 10 mya (Fig. 5). One possibility is that tectonic activity in western Amazonia changed the hydrology across the region, creating barriers during the Miocene at the time of these speciation events. In fact, the development of the Amazonian Basin was found to have driven divergence within the *calcarata-fasciata* species complex, via repeated dispersal across the newly formed basin (Fouquet et al., 2021). The timing of diversification in our phylogeny does not align with the river barrier hypothesis of vicariance caused by the development of large rivers. We did not find sister species across the Amazon or Juruá rivers as found in other Amazonian frogs and birds (Capurucho et al., 2018; Gascon et al., 2000), nor across other major rivers, such as the São Francisco in the Caatinga. Previous studies found little evidence for river barriers in these frogs (Camurugi et al., 2021; Fouquet et al., 2021), although Pirani et al. (2019) found that the Amazon may serve as a secondary barrier to gene flow in another hylid species, enforcing pre-existing divergences between populations.

Within the Cerrado, tectonic uplift and erosion likely contributed to diversification in central Brazil, specifically in the Chapada dos Guimarães plateau in Mato Grosso state, 5–7 mya (Silva, 1997), which created the high plateaus that now harbor high endemism, particularly for lizards and amphibians (Azevedo et al., 2016; Pinto et al., 2006; Valdujo et al., 2012). The combination of tectonic uplift and erosion generates altitudinal gradients, creating distinct habitats which shift in response to climate cycles, such as during the Pleistocene (Carnaval and Bates, 2007; Azevedo et al., 2016; Camurugi et al., 2021). Camurugi et al. (2021) found that the central Brazilian plateau in the Cerrado acted as a soft, and perhaps indirect barrier to gene flow among populations of *B. raniceps,* with isolation by environment facilitating divergence between lineages. These processes likely impacted diversification also within *B. albopunctata*, as the closely related new species *B.* aff. *albopunctata* lives at higher elevations in this region (Prado et al. 2012). Clade 2 of *B. multifasciata* is also from this higher altitude region of Mato Grosso and split from its sister clade 1.9 mya (Fig. 5). The presence of this pattern in both *B. albopunctata* and *B. multifasciata* in high elevation areas indicate that tectonic uplift and the subsequent establishment of altitudinal gradients have broadly affected diversification across the *B. albopunctata* species group.

4.5. Timing and patterns of diversification: Biotic exchange across Amazonia and the Cerrado

We found diversification across the Amazonia-Cerrado border for multiple species at different time points, and population structure in some, but not all species occurring in both Amazonia and the dry diagonal (Figs. 4 and 5). These incongruent patterns are more consistent with dispersal and diversification over time than with large-scale vicariance. Repeated movement and diversification within Amazonia and the dispersal to the Cerrado was recovered in the ancestral range reconstruction, and aligns with Pleistocene climatic fluctuations for *B. albopunctata, B.* aff. *albopunctata*, *B.* aff. *multifasciata*, and divergence between clades in *B. multifasciata* (Figs. 4 and 5). During this time period, pockets of stable Cerrado habitats were formed near the Amazonia-Cerrado ecotone (Vasconcellos et al. 2019). These areas could have harbored distinct lineages associated with open formations, leading to speciation within regions such as Tocantins, where *B. raniceps*, *B. caiapo*, and supported clades within both *B. raniceps* and *B. albopunctata* are found (Figs. 1 and 4).

This region in Tocantins state was also recognized by Azevedo et al. (2016) as an area of endemism for herpetofauna, and Vasconcellos et al. (2019) showed that genetic structure of the Cerrado endemic *Boana lundii* is correlated with areas of historical climate stability, promoting diversification over similar timescales as in the *B. albopunctata* group. Although not directly addressed in their study, Fouquet et al. (2021) hypothesized that more recently diverged lineages in the *B. albopunctata* group may have originated along the transition between Amazonia and the Cerrado. Our study corroborates this hypothesis, recovering several cryptic lineages and recent divergences along this ecotone with corresponding shifts in biome occupancy (Fig. 5).

5. Conclusions

We placed two new species (*Boana* aff. *albopunctata*, and *B.* aff. *multifasciata*) in the *Boana albopunctata* species group phylogeny. We also identified several cryptic lineages within the Amazonia-Cerrado ecotone and dry diagonal. The timing of diversification in this group aligns with uplift events in the Cerrado and Pleistocene climate fluctuations. Repeated expansion from Amazonia to the Cerrado may have been facilitated by these climate oscillations, resulting in rich biodiversity at the Amazonia-Cerrado ecotone, and within the dry diagonal. Dry biomes in South America are highly endangered (Strassburg et al., 2017). In particular, the Brazilian Cerrado is threatened by anthropogenic development and climate change (Collevatti et al., 2013) and only 2.2% of the Cerrado land area is under any conservation protection (Machado et al., 2015). Our study adds to growing research documenting endemic reptile, amphibian, plant, and mammal species associated with the Cerrado (Guarnizo et al., 2016). Continued loss of habitat in this region will undoubtedly lead to the loss of biodiversity both known, and yet to be described.

CRediT authorship contribution statement

Cinnamon S. Mittan: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Project administration. **Kelly R. Zamudio:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition. **M. Tereza C. Thomé:** Resources, Writing – review & editing, Supervision, Funding acquisition. **Felipe Camurugi:** Methodology, Formal analysis, Resources, Writing – review & editing, Funding acquisition. **Guarino R. Colli:** Investigation, Writing – review & editing, Supervision, Funding acquisition. **Adrian A. Garda:** Investigation, Writing – review & editing, Supervision, Funding acquisition. **Célio F.B. Haddad:** Resources, Supervision, Funding acquisition. **Cynthia P.A. Prado:** Conceptualization, Investigation, Resources, Data curation, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availablity

All sequences generated by this research have been deposited in GenBank (Accession numbers: ON513036 - ON513377; ON246353 - ON246903; OP037446 - OP037724) and will be released following paper acceptance.

Biosketch

The authors share a common interest in understanding the mechanisms of amphibian diversification in the Neotropics. This collaboration was part of CPAP's postdoctoral research on phylogeography in Cerrado frogs, and CM's doctoral research on drivers of diversification and adaptation in anurans. CPAP, KZ and CM conceived the study. CPAP, KZ, TT, CFBH, GC, and AG contributed with samples. CPAP conducted laboratory work. CM and FC performed data analysis, and created figures. CM led the writing of the manuscript. All authors contributed to the manuscript, and reviewed and approved the final paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2022.107579.

References

Aldrich, S., Walker, R., Simmons, C., Caldas, M., Perz, S., 2012. Contentious land change in the Amazon's arc of deforestation. Ann. Assoc. Am. Geogr. 102 (1), 103–128.

Antonelli, A., Quijada-Mascareñas, A., Crawford, A.J., Bates, J.M., Velazco, P.M., Wüster, W., 2009. Molecular studies and phylogeography of Amazonian tetrapods and their relation to geological and climatic models. In: Hoorn, C., Wesselingh, F.P. (Eds.), Amazonia: Landscape and Species Evolution: A look into the past. Wiley-Blackwell Publishing Ltd., Oxford, UK, pp. 386–404. https://doi.org/10.1002/9781444306408.ch24.

Antonelli, A., Zizka, A., Carvalho, F.A., Scharn, R., Bacon, C.D., Silvestro, D., Condamine, F.L., 2018. Amazonia is the primary source of Neotropical biodiversity. Proc. Natl. Acad. Sci. 115 (23), 6034–6039. https://doi.org/10.1073/pnas.1713819115.

Azevedo, J.A.R., Valdujo, P.H., de C. Nogueira, C., 2016. Biogeography of anurans and squamates in the Cerrado hotspot: coincident endemism patterns in the richest and most impacted savanna on the globe. J. Biogeography 43, 2454–2464. https://doi.org/10.1111/jbi.12803.

Bartoleti, L.F.d.M., Peres, E.A., Sobral-Souza, T., Fontes, F.V.H.M., Silva, M.J.d., Solferini, V.N., 2017. Phylogeography of the dry vegetation endemic species Nephila sexpunctata (Araneae: Araneidae) suggests recent expansion of the Neotropical Dry Diagonal. J. Biogeogr. 44 (9), 2007–2020. https://doi.org/10.1111/jbi.12998.

Beaulieu, J.M., Tank, D.C., Donoghue, M.J., 2013. A Southern Hemisphere origin for campanulid angiosperms, with traces of the break-up of Gondwana. BMC Evol. Biol. 13 (1), 80. https://doi.org/10.1186/1471-2148-13-80.

Bell, R.C., Drewes, R.C., Channing, A., Gvoždík, V., Kielgast, J., Lötters, S., Stuart, B.L., Zamudio, K.R., Emerson, B., 2015. Overseas dispersal of *Hyperolius* reed frogs from Central Africa to the oceanic islands of São Tomé and Príncipe. J. Biogeogr. 42 (1), 65–75. https://doi.org/10.1111/jbi.12412.

Bokermann, W.C.A., 1967. Nova especie de Hyla do Amapa (Amphibia, Hylidae). Rev. Bras. Biol. 27, 109–112.

Boulenger, G.A., 1905. Descriptions of new taillesss batrachians in the collection of the British Museum. Annals Mag. Natural History 16, 180–184.

Caminer, M., Ron, S., 2014. Systematics of treefrogs of the *Hypsiboas calcaratus* and *Hypsiboas fasciatus* species complex (Anura, Hylidae) with the description of four new species. ZooKeys 370, 1–68. https://doi.org/10.3897/zookeys.370.6291.

Camurugi, F., Gehara, M., Fonseca, E.M., Zamudio, K.R., Haddad, C.F.B., Colli, G.R., Thomé, M.T.C., Prado, C.P.A., Napoli, M.F., Garda, A.A., 2021. Isolation by environment and recurrent gene flow shaped the evolutionary history of a continentally distributed Neotropical treefrog. J. Biogeogr. 48 (4), 760–772. https://doi.org/10.1111/jbi.14035.

Capurucho, J.M.G., Ashley, M.V., Ribas, C.C., Bates, J.M., 2018. Connecting Amazonian, Cerrado, and Atlantic forest histories: Paraphyly, old divergences, and modern population dynamics in tyrant-manakins (Neopelma/Tyranneutes, Aves: Pipridae). Mol. Phylogenet. Evol. 127, 696–705. https://doi.org/10.1016/j.ympev.2018.06.015.

Caramaschi, U., Niemeyer, H., 2003. New species of the *Hyla albopunctata* group from central Brazil (Amphibia, Anura, Hylidae). Boletim do Museu Nacional Nova Série 1–8.

Carnaval, A.C., Bates, J.M., 2007. Amphibian DNA shows marked genetic structure and tracks Pleistocene climate change in northeastern Brazil. Evolution 61 (12), 2942–2957. https://doi.org/10.1111/j.1558-5646.2007.00241.x.

Carnaval, A.C., Hickerson, M.J., Haddad, C.F.B., Rodrigues, M.T., Moritz, C., 2009. Stability Predicts Genetic Diversity in the Brazilian Atlantic Forest Hotspot. Science 323 (5915), 785–789.

Carvalho, T.R., Giaretta, A.A., Facure, K.G., 2010. A new species of *Hypsiboas* Wagler (Anura : Hylidae) closely related to *H. multifasciatus* Günther from southeastern Brazil. Zootaxa 52, 37–52. https://doi.org/10.5281/zenodo.196269.

Collevatti, R.G., Lima-ribeiro, M.S., Diniz-filho, J.A.F., Oliveira, G., Dobrovolski, R., Terribile, L.C., 2013. Stability of Brazilian Seasonally Dry Forests under Climate Change : Inferences for Long-Term Conservation. Am. J. Plant Sci. 792–805. https://doi.org/10.4236/ajps.2013.44098.

Cope, E.D., 1862. Catalogues of the reptiles obtained during the explorations of the Parana, Paraguay, Vermejo and Uraguay Rivers, by Capt. Thos. J. Page, U.S.N.; and of those procured by Lieut. N. Michler, U.S. Top. Eng., Commander of the Expedition conducting the survey. Proc. Nat. Acad. Sci. Philadelphia 12, 346–359.

Cope, E.D., 1871. Eighth contribution to the herpetology of tropical America. Proc. Am. Philos. Soc. 11, 553–559.

da Silva, F.R., Almeida-Neto, M., Arena, M.V.N., Machado, R.B., 2014. Amphibian beta diversity in the Brazilian Atlantic Forest: Contrasting the roles of historical events and contemporary conditions at different spatial scales. PLoS ONE 9 (10), e109642. https://doi.org/10.1371/journal.pone.0109642.

de Oliveira, F.F.R., Gehara, M., Solé, M., Lyra, M., Haddad, C.F.B., Silva, D.P., de Magalhães, R.F., Leite, F.S.F., Burbrink, F.T., 2021. Quaternary climatic fluctuations influence the demographic history of two species of sky-island endemic amphibians in the Neotropics. Mol. Phylogenet. Evol. 160, 107–113. https://doi.org/10.1016/j.ympev.2021.107113.

Domingos, F.M.C.B., Colli, G.R., Lemmon, A., Lemmon, E.M., Beheregaray, L.B., 2017. In the shadows: Phylogenomics and coalescent species delimitation unveil cryptic

diversity in a Cerrado endemic lizard (Squamata: Tropidurus). Mol. Phylogenet. Evol. 107, 455–465. https://doi.org/10.1016/j.ympev.2016.12.009.
Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29 (8), 1969–1973. https://doi.org/10.1093/molbev/mss075.
Faivovich, J., Haddad, C.F.B., Garcia, P.C.A., Darrel, R., Campbell, J.A., Wheeler, W.C., 2005. Systematic Review of the Frog Family Hylidae, With Special Reference To Hylinae : Phylogenetic Analysis and Taxonomic Revision Systematic Review of the Frog Family Hylidae, With Special Reference To Hylinae : Phylogenetic Analysis and. Bull. Am. Museum Natural History 11, 10–12. https://doi.org/10.1206/0003-0090(2005)294.
Fouquet, A., Marinho, P., Réjaud, A., Carvalho, T.R., Caminer, M.A., Jansen, M., Rainha, R.N., Rodrigues, M.T., Werneck, F.P., Lima, A.P., Hrbek, T., Giaretta, A.A., Venegas, P.J., Chávez, G., Ron, S., 2021. Systematics and biogeography of the *Boana albopunctata* species group (Anura, Hylidae), with the description of two new species from Amazonia. Syst. Biodivers. 19 (4), 375–399. https://doi.org/10.1080/14772000.2021.1873869.
Frost, D.R., 2021. Amphibian Species of the World: an Online Reference. Version 6.1. https://amphibiansoftheworld.amnh.org.
Fu, Y.X., 1997. Statistical tests of neutrality against population growth, hitchhiking and background selection. Genetics 147. https://doi.org/10.1093/genetics/147.2.915.
Garrick, R.C., Sunnucks, P., Dyer, R.J., 2010. Nuclear gene phylogeography using PHASE: dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. BMC Evol. Biol. 10, 1–17. https://doi.org/10.1186/1471-2148-10-118.
Gascon, C., Malcolm, J.R., Patton, J.L., da Silva, M.N.F., Bogart, J.P., Lougheed, S.C., Peres, C.A., Neckel, S., Boag, P.T., 2000. Riverine barriers and the geographic distribution of Amazonian species. PNAS 97 (25), 13672–13677. https://doi.org/10.1073/pnas.230136397.
Gehara, M., Crawford, A.J., Orrico, V.G.D., Rodríguez, A., Lötters, S., Fouquet, A., Barrientos, L.S., Brusquetti, F., De la Riva, I., Ernst, R., Urrutia, G.G., Glaw, F., Guayasamin, J.M., Hölting, M., Jansen, M., Kok, P.J.R., Kwet, A., Lingnau, R., Lyra, M., Moravec, J., Pombal, J.P., Rojas-Runjaic, F.J.M., Schulze, A., Señaris, J.C., Solé, M., Rodrigues, M.T., Twomey, E., Haddad, C.F.B., Vences, M., Köhler, J., Colgan, D.J., 2014. High levels of diversity uncovered in a widespread nominal taxon: continental phylogeography of the Neotropical tree frog *Dendropsophus minutus*. PLoS ONE 9 (9), e103958. https://doi.org/10.1371/journal.pone.0103958.
Gehara, M., Barth, A., de Oliveira, E.F., Costa, M.A., Haddad, C.F.B., Vences, M., 2017. Model-based analyses reveal insular population diversification and cryptic frog species in the *Ischnocnema parva* complex in the Atlantic Forest of Brazil. Mol. Phylogenet. Evol. 112, 68–78. https://doi.org/10.1016/j.ympev.2017.04.007.
Guarnizo, C.E., Werneck, F.P., Giugliano, L.G., Santos, M.G., Fenker, J., Sousa, L., D'Angiolella, A.B., dos Santos, A.R., Strüssmann, C., Rodrigues, M.T., Dorado-Rodrigues, T.F., Gamble, T., Colli, G.R., 2016. Cryptic lineages and diversification of an endemic anole lizard (Squamata, Dactyloidae) of the Cerrado hotspot. Mol. Phylogenet. Evol. 94, 279–289. https://doi.org/10.1016/j.ympev.2015.09.005.
Günther, A.C.L.G., 1859. Neue Batrachier in der Sammlung des britischen Museums. Archiv fur Naturgeschichte 24, 319–328.
Günther, A.C.L.G., 1858. Catalogue of the Batrachia Salientia in the collection of the British Museum. Taylor and Francis, London. https://doi.org/10.5962/bhl.title.8326.
Haffer, J., 2008. Hypotheses to explain the origin of species in Amazonia. Brazilian J. Biol. = Revista brasleira de biologia 68 (4 suppl), 917–947. https://doi.org/10.1590/S1519-69842008000500003.
Hoorn, C., Wesselingh, P., ter Steege, H., Bermudez, M.A., Mora, A., Sevink, J., Sanmartín, I., Sanchez-Meseguer, A., Anderson, C.L., Figueiredo, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Särkinen, T., Antonelli, A., 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Science 330, 927–931. https://doi.org/10.1126/science.os-1.14.154.
Jia, F., Lo, N., Ho, S.Y.W., Moreau, C.S., 2014. The impact of modelling rate heterogeneity among sites on phylogenetic estimates of intraspecific evolutionary rates and timescales. PLoS ONE 9 (5), e95722. https://doi.org/10.1371/journal.pone.0095722.
Katoh, K., Toh, H., 2010. Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics 26, 1899–1900. https://doi.org/10.1093/bioinformatics/btq224.
Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29 (6), 1695–1701. https://doi.org/10.1093/molbev/mss020.
Lanna, F.M., Werneck, F.P., Gehara, M., Fonseca, E.M., Colli, G.R., Sites, J.W., Rodrigues, M.T., Garda, A.A., 2018. The evolutionary history of Lygodactylus lizards in the South American open diagonal. Mol. Phylogenet. Evol. 127, 638–645. https://doi.org/10.1016/j.ympev.2018.06.010.
Larsson, A., 2014. AliView: A fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30, 3276–3278. https://doi.org/10.1093/bioinformatics/btu531.
Ledo, R.M.D., Colli, G.R., 2017. The historical connections between the Amazon and the Atlantic Forest revisited. J. Biogeogr. 44 (11), 2551–2563. https://doi.org/10.1111/jbi.13049.
Losos, J., 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. Am. Nat. 175 (6), 623–639. https://doi.org/10.1086/652433.
Machado, R., Klink, C.A., Machado, R.B., 2015. Conservation of the Brazilian Cerrado 19, 707–713. https://doi.org/10.1111/j.1523-1739.2005.00702.x.
Matzke, N.J., 2013b. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. Front. Biogeogr. 5 https://doi.org/10.21425/f5fbg19694.
Matzke, N.J., 2014. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. Syst. Biol. 63, 951–970. https://doi.org/10.1093/sysbio/syu056.
Matzke, N.J., 2013a. BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R Scripts. R package, version 0.2.
Nali, R.C., Becker, C.G., Zamudio, K.R., Prado, C.P.A., Hou, Z., 2020. Topography, more than land cover, explains genetic diversity in a Neotropical savanna tree frog. Divers. Distrib. 26 (12), 1798–1812. https://doi.org/10.1111/ddi.13154.
Noble, G.K., 1923. Six new batrachians from the Dominican Republic. Am. Mus. Novit. 61, 1–6.
Oliveira, E.F., Gehara, M., São-Pedro, V.A., Costa, G.C., Burbrink, F.T., Colli, G.R., Rodrigues, M.T., Garda, A.A., 2018. Phylogeography of Muller's termite frog suggests the vicariant role of the Central Brazilian Plateau. J. Biogeogr. 45 (11), 2508–2519. https://doi.org/10.1111/jbi.13427.
Pavan, A.C., Marroig, G., 2017. Timing and patterns of diversification in the Neotropical bat genus Pteronotus (Mormoopidae). Mol. Phylogenet. Evol. 108, 61–69. https://doi.org/10.1016/j.ympev.2017.01.017.
Pinheiro, P.D.P., Cintra, C.E.D., Valdujo, P.H., Silva, H.L.R., Martins, I.A., da Silva, N.J., Garcia, P.C.A., 2018. A New Species of the *Boana albopunctata* Group (Anura: Hylidae) from the Cerrado of Brazil. South Am. J. Herpetol. 13 (2), 170–182. https://doi.org/10.2994/SAJH-D-17-00040.1.
Pinto, J.R.R., Oliveira-filho, A.T., Hay, J.D.V., 2006. Influence of soil and topography on the composition of a tree community in a central Brazilian valley forest. Edinburgh J. Botany 62 (1-2), 69–90. https://doi.org/10.1017/S0960428606000035.
Pirani, R.M., Werneck, F.P., Thomaz, A.T., Kenney, M.L., Sturaro, M.J., Ávila-Pires, T.C. S., Peloso, P.L.V., Rodrigues, M.T., Knowles, L.L., 2019. Testing main Amazonian rivers as barriers across time and space within widespread taxa. J. Biogeogr. 46 (11), 2444–2456. https://doi.org/10.1111/jbi.13676.
Prado, C.P.A., Haddad, C.F.B., Zamudio, K.R., 2012. Cryptic lineages and Pleistocene population expansion in a Brazilian Cerrado frog. Mol. Ecol. 21, 921–941. https://doi.org/10.1111/j.1365-294X.2011.05409.x.
Pyron, R.A., Wiens, J.J., 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Mol. Phylogenet. Evol. 61, 543–583. https://doi.org/10.1016/j.ympev.2011.06.012.
Rainha, R.N., Martinez, P.A., Moraes, L.J., Castro, K.M., Réjaud, A., Fouquet, A., Leite, R. N., Rodrigues, M.T., Werneck, F.P., 2021. Subtle environmental variation affects phenotypic differentiation of shallow divergent treefrog lineages in Amazonia. Biol. J. Linn. Soc. 134, 177–197. https://doi.org/10.1080/14772000.2021.1873869.
Ramírez-Soriano, A., Ramos-Onsins, S.E., Rozas, J., Calafell, F., Navarro, A., 2008. Statistical power analysis of neutrality tests under demographic expansions, contractions and bottlenecks with recombination. Genetics 179, 555–567. https://doi.org/10.1534/genetics 10.1534.107.083006.
Ramos-Onsins, S.E., Rozas, J., 2002. Statistical properties of new neutrality tests against population growth. Mol. Biol. Evol. 19, 2092–2100. https://doi.org/10.1093/oxfordjournals.molbev.a004034.
Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Systematic Biol. 57, 4–14. https://doi.org/10.1080/10635150701883881.
Resende-Moreira, L.C., Knowles, L.L., Thomaz, A.T., Prado, J., Souto, A.P., Lemos-Filho, J.P., Lovato, M.B., 2019. Evolving in isolation: Genetic tests reject recent connections of Amazonian savannas with the central Cerrado. J. Biogeogr. 46 (1), 196–211. https://doi.org/10.1111/jbi.13468.
Rodríguez, A., Börner, M., Pabijan, M., Gehara, M., Haddad, C.F.B., Vences, M., 2015. Genetic divergence in tropical anurans: deeper phylogeographic structure in forest specialists and in topographically complex regions. Evol. Ecol. 29 (5), 765–785. https://doi.org/10.1007/s10682-015-9774-7.
Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA Sequence Polymorphism analysis of large datasets. Mol. Biol. Evol. 34, 3299–3302. https://doi.org/10.1093/molbev/msx248.
Santos, M.G., Nogueira, C., Giugliano, L.G., Colli, G.R., Riddle, B., 2014. Landscape evolution and phylogeography of *Micrablepharus atticolus* (Squamata, Gymnophthalmidae), an endemic lizard of the Brazilian Cerrado. J. Biogeogr. 41 (8), 1506–1519. https://doi.org/10.1111/jbi.12291.
Schluter, D., 2000. The ecology of adaptive radiation. Oxford University Press, New York, USA.
Silva, J.M., 1997. Endemic bird species and conservation in the Cerrado Region, South America. Biodivers. Conserv. 6, 435–450. https://doi.org/10.1023/A:1018368809116.
Simon, M.F., Grether, R., de Queiroz, L.P., Skema, C., Pennington, R.T., Hughes, C.E., 2009. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. Proc. Natl. Acad. Sci. 106 (48), 20359–20364. https://doi.org/10.1073/pnas.0903410106.
Smith, B.T., McCormack, J.E., Cuervo, A.M., Hickerson, M.J., Aleixo, A., Cadena, C.D., Pérez-Emán, J., Burney, C.W., Xie, X., Harvey, M.G., Faircloth, B.C., Glenn, T.C., Derryberry, E.P., Prejean, J., Fields, S., Brumfield, R.T., 2014. The drivers of tropical speciation. Nature 515 (7527), 406–409. https://doi.org/10.1038/nature13687.
Spix, J.B., 1824. Animalia nova sive species novae Testudinum et Ranarum, quas in itinere per Brasiliam annis MDCCCXVII–MDCCCXX jussu et auspiciis Maximiliani Josephi I Bavariae Regis/suscepto, collegit et descripsit Dr. J. B. de Spix. Typis Franc. Seraph. Hübschmanni, Munich.
Strassburg, B.B.N., Brooks, T., Feltran-Barbieri, R., Iribarrem, A., Crouzeilles, R., Loyola, R., Latawiec, A.E., Oliveira Filho, F.J.B., De Scaramuzza, C.A.M., Scarano, F.

R., Soares-Filho, B., Balmford, A., 2017. Moment of truth for the Cerrado hotspot. Nat. Ecol. Evol. 1, 1–3. https://doi.org/10.1038/s41559-017-0099.
Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595. https://doi.org/10.1093/genetics/123.3.585.
Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56, 564–577. https://doi.org/10.1080/10635150701472164.
Thomé, M.T.C., Sequeira, F., Brusquetti, F., Carstens, B., Haddad, C.F.B., Rodrigues, M. T., Alexandrino, J., 2016. Recurrent connections between Amazon and Atlantic forests shaped diversity in Caatinga four-eyed frogs. J. Biogeogr. 43 (5), 1045–1056. https://doi.org/10.1111/jbi.12685.
Troschel, F.H., 1848. Theil 3. Versuch einer Zusammenstellung der Fauna und Flora von Britisch-Guiana. In: Schomburgk, R. (Ed) Reisen in Britisch-Guiana in den Jahren 1840 - 1844. Im Auftrage Sr. Majestät des Königs von Preussen ausgeführt. J.J. Weber, Leipzig, Germany.
Turchetto-Zolet, A.C., Pinheiro, F., Salgueiro, F., Palma-Silva, C., 2013. Phylogeographical patterns shed light on evolutionary process in South America. Mol. Ecol. 22 (5), 1193–1213. https://doi.org/10.1111/mec.12164.
Valdujo, P.H., Silvano, D.L., Colli, G., Martins, M., 2012. Anuran species composition and distribution patterns in Brazilian Cerrado, a Neotropical hotspot. South Am. J. Herpetol. 7 (2), 63–78. https://doi.org/10.2994/057.007.0209.
van der Hammen, T., Hooghiemstra, H., 2000. Neogene and Quaternary history of vegetation, climate, and plant diversity in Amazonia. Quart. Sci. Rev. 19, 725–742. https://doi.org/10.1016/S0277-3791(99)00024-4.
Vasconcellos, M.M., Colli, G.R., Weber, J.N., Ortiz, E.M., Rodrigues, M.T., Cannatella, D. C., 2019. Isolation by instability: Historical climate change shapes population structure and genomic divergence of treefrogs in the Neotropical Cerrado savanna. Mol. Ecol. 28 (7), 1748–1764. https://doi.org/10.1111/mec.15045.
Vasconcellos, M.M., Colli, G.R., Cannatella, D.C., 2020. Paleotemperatures and recurrent habitat shifts drive diversification of treefrogs across distinct biodiversity hotspots in sub-Amazonian South America. J. Biogeogr. 48 (2), 305–320. https://doi.org/10.1111/jbi.13997.
Werneck, F.P., 2011. The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. Quat. Sci. Rev. 30 (13-14), 1630–1648. https://doi.org/10.1016/j.quascirev.2011.03.009.
Werneck, F.P., Gamble, T., Colli, G.R., Rodrigues, M.T., Sites Jr, J.W., 2012. Deep diversification and long-term persistence in the South American 'dry diagonal': Integrating continent-wide phylogeography and distribution modeling of geckos. Evolution: International Journal of Organic. Evolution 66, 3014–3034. https://doi.org/10.1111/j.1558-5646.2012.01682.x.
Werneck, F.D., Giugliano, L.G., Collevatti, R.G., Colli, G.R., 2009. Phylogeny, biogeography and evolution of clutch size in South American lizards of the genus Kentropyx (Squamata: Teiidae). Mol. Ecol. 18, 262–278. https://doi.org/10.1111/j.1365-294X.2008.03999.x.
Wüster, W., Ferguson, J.E., Quijada-Mascareñas, J.A., Pook, C.E., Salomão, M.D.G., Thorpe, R.S., 2005. Tracing an invasion: Landbridges, refugia, and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). Mol. Ecol. 14, 1095–1108. https://doi.org/10.1111/j.1365-294X.2005.02471.x.
Yu, Y., Harris, A.J., Blair, C., He, X., 2015. RASP (Reconstruct Ancestral State in Phylogenies): A tool for historical biogeography. Mol. Phylogenet. Evol. 87, 46–49. https://doi.org/10.1016/j.ympev.2015.03.008.
Zina, J., Prado, C.A.P., 2010. 1862: Distribution extension. Conserv. Biol. 6, 230–231. https://doi.org/10.15560/6.2.230.