

## RESEARCH ARTICLE

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# Isolation by environment and recurrent gene flow shaped the evolutionary history of a continentally distributed Neotropical treefrog

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## Abstract

**Aim:** Phylogeographic studies show how historical and current changes in landscapes shape the geographic distribution of genetic diversity in species of animals and plants. In particular, for the species of the Diagonal of Open Formations (DOF), the compartmentalization of the Central Brazilian Plateau (CBP) during the Tertiary and climatic oscillations during the Quaternary have often been invoked to explain the origin and current patterns of biodiversity. We investigated how landscape changes and climatic oscillations shaped the distribution and diversification history of a widespread South American treefrog.

**Location:** South American Diagonal of Open Formations (DOF) including Caatinga, Cerrado, and Chaco biomes.

**Taxon:** Treefrog *Boana raniceps*.

**Methods:** We used a multi-locus dataset from 288 individual frogs collected at 115 localities throughout most of the species' distribution. We used population assignment analysis, species distribution models, historical demography models, approximate Bayesian computation and landscape genetic analyses to test alternative hypotheses of diversification.

**Results:** We found two genetic lineages that diverged during the mid-Pleistocene with continued gene flow. Approximate Bayesian computation supported a scenario of isolation with migration until the Last Glacial Maximum, followed by more recent population expansion in north-eastern Brazil and stability at the southwest in South America. Isolation by environment was the best predictor of genetic distance between populations, which is in accordance with their different environmental niches. As *Boana raniceps* is a lowland species, steep slopes in the CBP likely restrained gene flow enough to sustain population divergence. We found evidence for major range contraction during the Last Glacial Maximum, raising the possibility of synergic action of climate change and the CBP compartmentalization in regulating migration.

**Main conclusions:** Our findings highlight how landscape and climatic changes can shape the diversification of DOF biota. Past climatic fluctuations and environmental

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resistance due to topography acted in concert, forming a semipermeable barrier to gene flow, promoting intraspecific differentiation in a continentally distributed species.

#### KEYWORDS

Anura, approximate Bayesian computation, *Boana raniceps*, isolation by environment, landscape genetics, lowland species, Quaternary climatic fluctuation, riverine effects, South America, topography

## 1 | INTRODUCTION

The origin of the high Neotropical biodiversity has been related to a complex and continuous history of geological events and climatic dynamics (Rull, 2008). In particular, the Andean uplift, marine incursions, completion of the Panamanian Isthmus during the Tertiary and climatic oscillations during the Quaternary are invoked to explain current patterns of biodiversity in the Neotropical region (Hoorn et al., 2010; Montes et al., 2015; Rull, 2011). Historical landscape changes affect the spatial and genetic structure of populations by decreasing or facilitating population connectivity and gene flow (Cooke et al., 2012; Wang & Bradburd, 2014).

The South American Diagonal of Open Formations (DOF) is a large and well-connected belt of seasonally dry biomes between Amazonia and the Atlantic Forest (Werneck, 2011). The DOF is composed of three biomes, seasonally stressed by drought and stretching from north-eastern to south-western South America: the Caatinga (seasonally dry tropical forest), the Cerrado (tropical savanna) and the Gran Chaco (semi-arid forests and woodlands) (Collevatti et al., 2020; Pennington et al., 2006). The main promoters for the diversification of the DOF biota are the compartmentalization of the Central Brazilian Plateau (CBP) in the Tertiary (final uplift, ~5 Ma) and climatic oscillations of the Quaternary (after ~2.6 Ma) (Colli, 2005; Werneck, 2011). However, there are several open questions on how these promoters influenced the tempo and geography of speciation of the DOF biota. For instance, recent studies revealed a complex biogeographic history of the DOF and resulted in inferences of speciation with gene flow, founder effect, niche divergence, and vicariance due to rainforest expansion (Fonseca et al., 2018; Oliveira et al., 2015; Thomé et al., 2016).

Epeirogenic movements related to the convergence of the Nazca and South American plates, as well as the Andean orogeny, caused the geomorphological compartmentalization of the CBP through intense erosion and sedimentation, resulting in the creation of ample depressions (valleys) among ancient plateaus (Assine et al., 2015; Cogné et al., 2012; Ross, 2016). The compartmentalization of the CBP, created plateaus ranging elevations between 500 and 1,700 m and peripheral depressions from 100 to 500 m (Silva, 1997), which promoted the diversification of several groups by acting as soft or hard barriers to gene flow, depending on species' requirements (Faria et al., 2013; Fonseca et al., 2018; Oliveira, Gehara, et al., 2018; Prado et al., 2012).

Quaternary palaeoclimatic oscillations impacted DOF species by influencing their historical demography, structuring populations through habitat fragmentation and persistence of refugia. Distribution models for South American biomes predict contraction and fragmentation of savannas and seasonally dry forests during the last glacial maximum – LGM (Costa et al., 2018). These oscillations promoted conspicuous demographic responses in several DOF species (Brusquetti et al., 2019; Gehara et al., 2017; Vasconcellos et al., 2019). Areas of climatic instability in the DOF also reduced gene flow among climatically stable areas (isolation by instability), promoting population divergence (Vasconcellos et al., 2019). Finally, climate oscillations structured DOF populations by affecting other landscape features, such as rivers (Oliveira, Martinez, et al., 2018). Because the permeability of the barrier is taxon dependent, given that species with different physiologies and natural history can respond differently to the current and past environment complexity (Zamudio et al., 2016), drivers of diversification among different studies on the DOF frequently do not match.

Life history traits can affect species dispersal ability and gene flow; consequently, they can determine how landscape changes will affect the distribution of individuals and genes (Paz et al., 2015). Landscape resistance attributed to elevation, hydrology and habitat suitability is one of the main drivers of genetic differentiation in amphibians (Barratt et al., 2018; McCartney-Melstad & Shaffer, 2015). Furthermore, because of low individual dispersal ability and tendency towards philopatry, anurans are expected to show marked phylogeographic structure (Beebe, 2005; Gehara et al., 2014; Reading et al., 1991). Among DOF anurans, the treefrog *Boana raniceps* (Cope, 1862) is an ideal target for testing the effects of past and contemporary landscape features on spatiotemporal patterns of genetic diversity at a continental scale. *Boana raniceps* has a widespread distribution in South America, mainly associated with the DOF and Amazonian savannas, generally occurring in lowlands, below 800 m of elevation, in almost all major river basins of South America (except those that drain to the Pacific). Despite being broadly distributed, which often indicates the potential for hidden genetic diversity, previous studies found low levels of genetic divergence between widely separated populations (Fouquet et al., 2007). As a lowland species, we expect that the compartmentalization of the CBP played a major role on the evolution of *B. raniceps*. Furthermore, hydrology may also have affected its evolutionary history because both adults and larvae can be carried by water bodies. Finally, palaeoclimatic changes in

the DOF also potentially impacted its distribution and demography, once its biomes historically responded to climatic oscillations.

Here, we used a multi-locus approach to investigate the role of historical and contemporary processes of landscape changes in the diversification of *Boana raniceps*. We tested four non-mutually exclusive hypotheses: (a) genetic divergence follows an isolation by distance model (IBD); (b) the CBP compartmentalization resulted in the isolation of lineages (at least two) either associated with valleys or plateaus; (c) genetic divergence is associated with lack of connectivity among major river basins, according to an isolation by environment resistance model (IBE) and (d) Quaternary climatic fluctuations affected demographic history by promoting population expansion and contraction through time and genetic differentiation among climatically stable areas (refugia). These hypotheses plus the null hypothesis of panmixia, along with their predictions, are summarized in Table 1.

## 2 | MATERIALS AND METHODS

### 2.1 | Data collection and sample sequencing

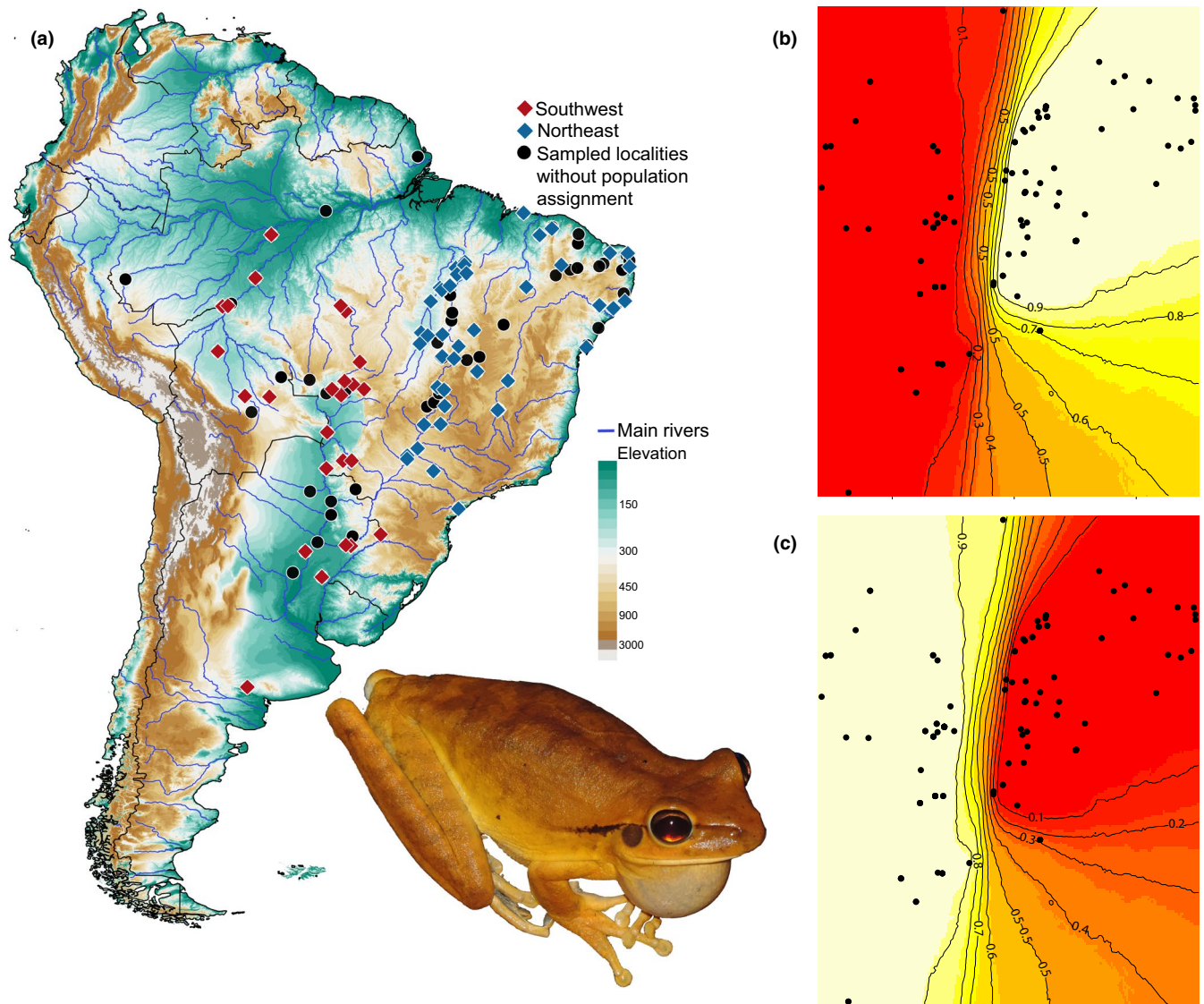
We obtained 288 tissue samples of *B. raniceps* from 115 localities (Figure 1), covering most of its geographic distribution, and one sample of the congener *B. albopunctata* (Spix 1824) as outgroup. Samples were collected by the authors and through loans from herpetological collections (see Table S1 in Appendix 1 in Supporting Information).

We extracted total genomic DNA from liver or muscle using a standard salt extraction protocol (Bruford et al., 1992). Using polymerase chain reaction, we amplified fragments of two mitochondrial genes: 16S rRNA (16S, 478 aligned bp) and NADH dehydrogenase subunit 1 (ND1, 848 bp). Additionally, we amplified four nuclear genes:  $\beta$ -fibrinogen intron 7 (Fib, 444 bp), proopiomelanocortin (POMC, 447 bp), ribosomal protein L3 intron 5 (RPL3, 521 bp) and tyrosinase (Tyr, 426 bp). Detailed information about amplification and sequencing protocols is in Table S2 (see Appendix 1 in Supporting Information). Sequencing was performed at the Cornell Genomics Facility or by Macrogen Inc., Seoul, Republic of Korea. First, we sequenced 283 individuals for 16S, from which we selected a subset comprising 123 samples from 75 localities. From this subset, we obtained sequences of the remaining genes. All sequences are available in GenBank (accession numbers in Table S1).

We edited chromatograms, assembled and aligned sequences in GENEIOUS 9.1.6 (<https://www.geneious.com>), using the MUSCLE algorithm (Edgar, 2004). Gaps found in 16S, Fib and RPL3 genes were removed using GBLOCKS 0.91b (Castresana, 2000), using default options. We defined the most probable allele pairs for each nuclear gene sequence with the Phase algorithm (Stephens et al., 2001) in DNASP 5.10 (Librado & Rozas, 2009), using default options and keeping allele pairs with reconstruction probabilities higher than 60%. Lastly, we estimated the best substitution model for each gene fragment using Bayesian information criterion (BIC) in JMODELTEST 2.1.7 (Darriba et al., 2012).

**TABLE 1** Hypotheses of genetic differentiation in the treefrog *Boana raniceps*. For each mechanism involved in the processes of species diversification, considering the effects of landscape and natural history, a prediction stating the expected pattern in the data and the hypothesis acceptance is presented.

| Hypothesis                                           | Mechanism                                                                                                       | Prediction                                                                                                                                                       | Acceptance |
|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Isolation by distance (IBD)                          | Dispersal limitation restricts gene flow among distant populations                                              | Positive correlation between spatial distance and genetic distance                                                                                               | No         |
| Central Brazilian Plateau (CBP) compartmentalization | For lowland species the CBP was a strong vicariant barrier segregating at least two lineages                    | Deep phylogeographic structure and absence of gene flow between populations. Divergence time between lineages should match with the final uplift period (7–5 Ma) | No         |
|                                                      | For large and highly dispersive species, the CBP can be a soft vicariant barrier to gene flow                   | Shallow genetic structure with gene flow between populations. Permeability of CBP drives to isolation with migration model                                       | Partially  |
| River connectivity                                   | For amphibians highly dependent on water bodies, rivers should favour connectivity among populations            | Negative correlation between presence of rivers and genetic distance                                                                                             | No         |
| Quaternary climatic fluctuations                     | Climatic shifts during Quaternary promoted population expansion and contraction through time                    | Changes of effective population sizes through time                                                                                                               | Yes        |
|                                                      | Climatic instability areas cause resistance to gene flow among populations from climatic stable areas (refugia) | Positive correlation between presence of climatically unsuitable areas and genetic differentiation. Isolation of populations in refugia promoted diversification | No         |
| Panmixia (= null hypothesis)                         | Species with high dispersal ability and tolerance to habitat fragmentation                                      | A single and widely distributed lineage                                                                                                                          | No         |



**FIGURE 1** (a) Geographic distribution of samples of *Boana raniceps* used in this study. Results of GENELAND population assignment analysis, depicting (b) north-eastern and (c) south-western lineages; colours and isoclines indicate posterior probabilities of assignment to each lineage

## 2.2 | Population assignment and genetic diversity

Our second, third and fourth hypotheses posit that landscape features led to population structuring through restrictions in gene flow (Table 1). To assess predictions on genetic structure, we estimated number of populations and their boundaries with the R package GENELAND 4.0.8 (Guillot et al., 2005). GENELAND allows incorporation of both haploid and diploid data in a spatial model, linking genetic variation to the geographic origin of each sample. We performed the analysis with 15 repetitions,  $5 \times 10^6$  iterations each, sampling at every  $5 \times 10^3$  iterations. We used a range of the number of populations from 1 to 6. We refer to the populations/clusters delimited in the GENELAND analysis as lineages. For each lineage, we estimated summary statistics and performed a hierarchical analysis of molecular variance (AMOVA) to assess the genetic differentiation between populations (see Appendix 2 in Supporting Information).

## 2.3 | Historical suitable areas

Species distribution models (SDM) are commonly used in phylogeographic studies because it is assumed that part of the genetic variation can be explained by both past and present-day environments (Alvarado-Serrano & Knowles, 2014). Thus, we used SDMs to assess suitable areas, changes in the *B. raniceps* potential distribution ranges during the Late Quaternary climatic fluctuations, and predict climatically stable areas (refugia) during the Late Quaternary (last 130 thousand years). We also used these models to generate a matrix of environmental resistances to test if part of the species genetic differentiation was predicted by current and historical habitat suitability (see below).

To build SDMs, we downloaded environmental data from Worldclim (19 bioclimatic variables; available at <http://www.worldclim.org>) at a spatial resolution of 2.5 arc-minutes (Hijmans



et al., 2005). After excluding highly correlated variables, we retained nine bioclimatic variables (Pearson correlation coefficient  $<0.8$ ), as follows: mean diurnal range (BIO2), temperature seasonality (BIO4), max. temperature of warmest month (BIO5), min. temperature of coldest month (BIO6), precipitation seasonality (BIO15), precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17), precipitation of warmest quarter (BIO18) and precipitation of coldest quarter (BIO19). We built models with the maximum entropy algorithm MAXENT (Phillips et al., 2006). To tune and evaluate MAXENT models, we used the ENMEVAL package (Muscarella et al., 2014) with 10,000 background points and six feature classes (FC) combinations (L, H, LQ, LQH, LQHP and LQHPT). We used the area under the curve (AUC) to assess model performance. To produce binary maps for each period (current, Holocene, LGM and LIG), we used a threshold obtained from “10th percentile presenceEqual training sensitivity and specificity logistic threshold” value. Afterwards, we superimposed and summed the maps, to reveal putatively stable areas over time. We repeated these procedures for each lineage (see Results).

To identify stable areas, we constructed four SDMs for the following periods: current, Holocene (past 6 ka), Last Glacial Maximum (LGM; 21 ka) and Last Interglacial (LIG; 120 ka). We used 354 geographic coordinates of the species obtained from our sampling plus occurrence data available in digital databases (SpeciesLink, <http://splink.cria.org.br/>; Global Biodiversity Information Facility, <http://www.gbif.org/>; July 2016). To avoid sampling bias, we filtered geographic points at a spatial distance of 30 km with *spThin* package (Aiello-Lammens et al., 2015). We repeated this procedure for each recovered lineage. See Appendix S2 in Supporting Information (Table S5) for details.

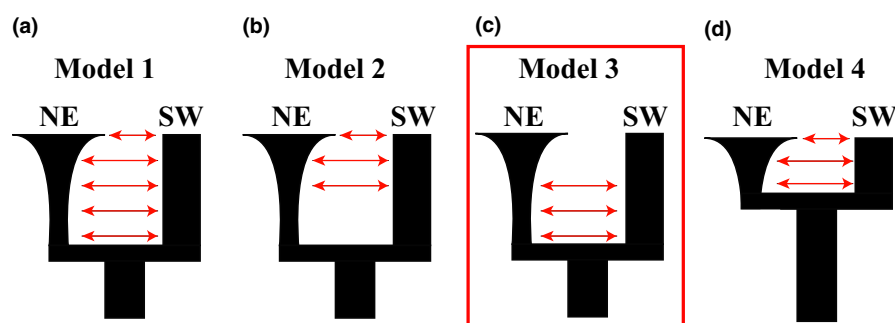
## 2.4 | Testing scenarios of diversification with ABC

To test our hypothesis that Quaternary climatic fluctuations affected the diversification and/or demography of *Boana raniceps*, we used approximate Bayesian computation approach (ABC). The preceding analyses recovered two geographically structured

lineages, one in the northeast and another in the southwest portion of the range. Before implementing ABC, we took migration and demographic parameters as prior information. Therefore, we used a coalescent-based analysis that uses an isolation-with-migration model (IM) to estimate migration rate, divergence times between lineages and population sizes ( $N_e$ ) implemented in IMA2 (Hey, 2010; Hey & Nielsen, 2007). We assessed changes in population size over time for the two lineages (northeast and southwest) using Bayesian Skyline Plots (BSP) in BEAST 1.8.4 (Drummond et al., 2012). IMA2 and Bayesian skyline analyses and ABC priors are detailed in Appendix 2 in Supporting Information.

With this information, we ranked four alternative diversification scenarios (Figure 2). The scenarios differ in the timing of divergence between the two lineages (LGM or earlier), and in the timing of gene flow relative to the divergence event (continuous or during certain periods), as follows: (a) ancient (pre LGM) divergence with constant gene flow and recent (post-LGM) population growth of the northeast lineage – Model 1; (b) ancient divergence in isolation followed by recent gene flow and expansion in northeast lineage – Model 2; (c) ancient divergence with gene flow followed by recent isolation and expansion in the northeast lineage – Model 3; and (d) recent divergence with gene flow from LGM and expansion in the northeast lineage – Model 4. For the first three diversification scenarios, we incorporated divergence time recovered in IMA2.

We used the R package PIPEMASTER (Gehara et al., in review; [www.github.com/gehara/PipeMaster](http://www.github.com/gehara/PipeMaster)) to simulate 100,000 data points under each model. Our simulated datasets mimicked our observed dataset in number of genetic markers, individuals per lineage, genetic inheritance and sequence length. This information is presented in Table S6 (Appendix 2 in Supporting Information). We assumed a uniform prior distribution with minimum and maximum values extracted from 95% confidence intervals obtained in previous analyses (BEAST, IMA and Skyline plots). From each simulation we calculated six summary statistics for each lineage and for the whole species (totalling 18 summary statistics): number of polymorphic sites, nucleotide diversity, haplotype diversity, Tajima's *D*, and Fu and Li's *D* and *F*. We estimated posterior probabilities and model support for each model using the *postpr* function in R package ABC (Csilléry



**FIGURE 2** Divergence scenarios tested using ABC for north-eastern (NE) and south-western (SW) lineages of *Boana raniceps*. All scenarios consider recent population expansion in the north-eastern (NE) lineage. Scenarios assume (a) constant gene flow through time, (b) gene flow after the LGM, (c) gene flow until the LGM or (d) constant gene flow from the LGM. Divergence time of lineages in models 1, 2 and 3 was estimated with IMA2 (during middle Pleistocene) and in model 4 was set at the Last Glacial Maximum (LGM). The best model is highlighted by a red box

et al., 2012). We set the tolerance value to 0.01 and used the multinomial logistic regression method to compare models. To evaluate model accuracy, we used cross-validation and built misclassification bar plots with the *cv4abc* function of the ABC package.

## 2.5 | Niche overlap

If environmental preferences were relevant and associated with the diversification processes of *B. raniceps*, we expect that lineages will exhibit low niche overlap. Otherwise, other landscape features (e.g. vicariant barriers) should explain the observed phylogeographic structure. To address whether the climatic niches of lineages are divergent, we used a PCA-env approach (Broennimann et al., 2012). We used binary maps derived from SDMs as background areas for each lineage (Figure S1 in Appendix S2) with the ECOSPAT package (Di Cola et al., 2017), and used Schoener's *D* to estimate niche overlap. Schoener's *D* varies from 0 to 1, indicating no to complete overlap. The observed overlap was compared with a null distribution obtained from 100 random points at the background. Statistical significance of the niche overlap ( $p \leq 0.05$ ) was obtained with niche equivalency and similarity tests, with the following settings: alternative = "lower" and rand.type = 1 (detailed information in Appendix 2).

## 2.6 | Isolation by distance and isolation by environmental resistance

Our first hypothesis of IBD postulates a positive correlation between spatial distance and genetic distance. To estimate the pairwise distance between localities, we used Geographic distance Matrix Generator 1.2.3 (Ersts, 2011) and obtained a matrix of geographic distances.

Our second, third and fourth hypotheses posit that landscape heterogeneity can shape patterns of genetic differentiation in *B. raniceps*. Therefore, we tested hypotheses of genetic differentiation driven by isolation by environmental resistance (IBE). We built IBE models based on six predictors: climate suitability (current, LGM and refugia), rivers and tributaries, and topographic complexity (elevation and slope). We used circuit theory to predict spatial resistance among pairs of population for each of the six predictors of the IBE hypotheses. To calculate pairwise resistance, we built environmental resistance surfaces with CIRCUITSCAPE 5.0 package (Anantharaman et al., 2019). For scenarios of genetic differentiation related to climate, we used current habitat suitability, LGM and stability SDM models. For the stability map, we scaled values to range from 0 to 1 (i.e. 0.25 for presence in one period, 0.50 in two periods, 0.75 in three periods and 1 for presence in all four periods). Because lower values of suitability correspond to higher costs to population connectivity, we calculated the resistance due to current and historical unsuitability habitats (LGM and refugia), inverting the SDMs' raster values (1-suitability). In the same way, we used main perennial rivers as drivers of spatial connectivity while its absence was considered

as barriers to dispersion. Our river network raster contains the presence of main rivers from South America (stream lines at 1:10m scale; <https://www.natureearthdata.com/>). We obtained the elevation raster from NASA Jet Propulsion Laboratory (<https://landscape.jpl.nasa.gov/>). We used the elevation map to derive a slope raster in ARCMAP v.10.3 (ESRI). We assumed that higher elevations or steeper slopes meant a higher cost for gene flow. All rasters were at a spatial resolution of 2.5 arc-minutes. CIRCUITSCAPE reads zeros as hard barriers and because of that we changed all 0 by 0.0001.

We obtained our pairwise matrix of genetic differentiation among localities ( $\phi_{st}$ ) with an analysis of molecular variance (AMOVA) in ARLEQUIN 3.5.2 (Excoffier & Lischer, 2010). The significance of pairwise comparisons was assessed by 10,000 permutations. Because the number of missing data in nuDNA fragments and the different heritage of markers, we used solely the 16S fragment from 237 individuals from 61 sites. We replaced negative  $\phi_{st}$  values ( $n = 74$ ) with 0.0001 because negative values of  $\phi_{st}$  are stated as 0.

To test whether different landscape features promoted genetic differentiation in *B. raniceps*, we conducted a generalized dissimilarity modelling (GDM). This analysis is a matrix regression tool that takes into account non-linear rather than linear relationships with the use of I-spline basis functions (Ferrier et al., 2007). I-splines can model turnover of the response variable (i.e. genetic data) in response to each environmental predictor (Fitzpatrick & Keller, 2015). For each predictor, GDM calculates the dissimilarity between site pairs and fits the coefficients to the I-spline basis functions. The maximum height of the I-splines indicates the amount of genetic differentiation, for example, along the gradient of each predictor of the landscape. Thus, we assessed the relationship between genetic differentiation (response variable) and seven environmental distances (predictors): geographic distance, current habitat suitability, LGM, refugia, rivers, slope and elevation. We performed GDM with the GDM R package (Manion et al., 2018). First, we assessed the importance of each predictor with non-zero coefficients by summing the three I-spline coefficients of the I-splines functions. Then, to find the best predictors of  $\phi_{st}$  turnover, we applied a stepwise matrix permutation (Ferrier et al., 2007). Thereby, we used the *gdm.varImp* function in GDM R package with 1,000 random permutations with backward elimination, removing at each step the predictor with the least significant contribution.

## 3 | RESULTS

### 3.1 | Population assignment and sequence information

After excluding gaps, we obtained a final alignment of 1,306 bp for mtDNA and 1838 bp for nuDNA. Highest levels of genetic diversity were found for ND1 and RPL3. GENELAND recovered two geographically structured populations (see Figure 1; Figure S3 in Appendix S2): one ranging from the Caatinga to northern Cerrado (northeast lineage), the other in southern Cerrado, Chaco and Amazonian savannas

(southwest lineage). Lineages also showed shared haplotypes (see haplotype networks in Figure S4 in Appendix S2).

The north-eastern lineage had in general higher nucleotide diversity than southwest lineage, except for Bfib and Tyr. Tajima's  $D$  suggested a rapid and significant population expansion only for 16S in the north-eastern lineage (Table S3 in Appendix 2). AMOVA showed that most of the genetic variation was observed within populations for all genes (74%–97%), when compared with variation between lineages level (3%–25%), with low but significant  $F_{ST}$  values between lineages (from 3% to 25%; see Table S4 in Appendix 2).

### 3.2 | Historical suitable areas

Species distribution model performed well in predicting the current occurrence of *B. raniceps* ( $AUC = 0.93$ ). The model predicted areas of suitability along most of the DOF and in Amazonian savannas, with some over-prediction in Venezuela and Colombia, a region with disjunct savannas on the Guyana shield. We inferred complex distribution dynamics during the Pleistocene climatic oscillations. Current and LGM habitat suitability showed a marked discontinuity in central Brazil that closely matches the boundaries between the

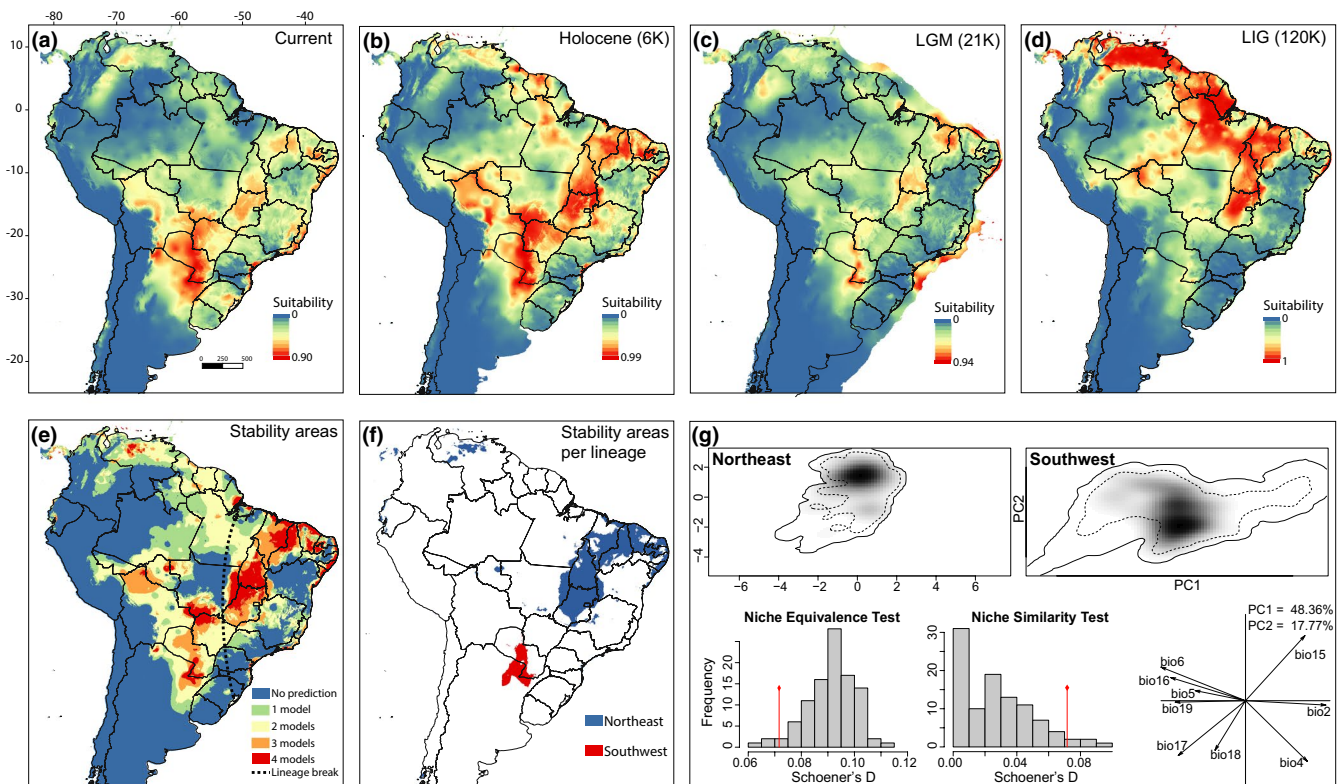
two genetic lineages, while this discontinuity is not apparent during the Holocene and Last Interglacial – LIG; a major contraction in climatically suitable areas occurred during the LGM (Figure 3). During the periods modelled, we also identified areas of climatic stability for both lineages: north Cerrado and Caatinga for the north-eastern lineage and the Chaco region for the south-western lineage.

### 3.3 | Testing scenarios of diversification with ABC

ABC analysis recovered the highest posterior probability (0.93, see Figure S5 in Appendix S2) for model 3, supporting the scenario of divergence with gene flow until the LGM and recent population expansion in the north-eastern lineage as the LGM (Figure 2).

### 3.4 | Niche overlap

*Boana raniceps* lineages showed very limited niche overlap in the PCA-env analysis (Schoener's  $D = 0.07$ ). Furthermore, their niches were not equivalent ( $p < 0.05$ ) and background similarity was not significant ( $p > 0.05$ ; Figure 3), suggesting niche divergence with



**FIGURE 3** Species distribution models and niche comparison of northeast and southwest lineages of the treefrog *Boana raniceps*. Projection of potential suitable areas during the Late Quaternary is shown in panels a–d. Warmer colours indicate higher probabilities of occurrence based solely on environmental variables, and do not account for barriers or biotic interactions. The map of stability areas represents the sum of maps in the four periods for *B. raniceps* (panel e) and for each lineage separately (panel f). In the panel g, upper graphs represent the environmental space occupied by both lineages, with darker cells showing the highest density of occurrences and available background in lines. In the lower left corner, histograms of niche equivalence ( $p = 0.04$ ) and niche similarity tests ( $p = 0.95$ ), with observed overlap in red ( $D = 0.07$ ); in the lower right corner, contribution of each variable in the environmental space

lineages occupying different environmental niches. The first two PCA axes accounted for 63.13% of the total variance of the nine climatic variables (Figure 3g). The low niche overlap between lineages can be viewed by the distribution of highest density of occurrence of each lineage in the environmental space, mainly in PC2 axis (Figure 3g).

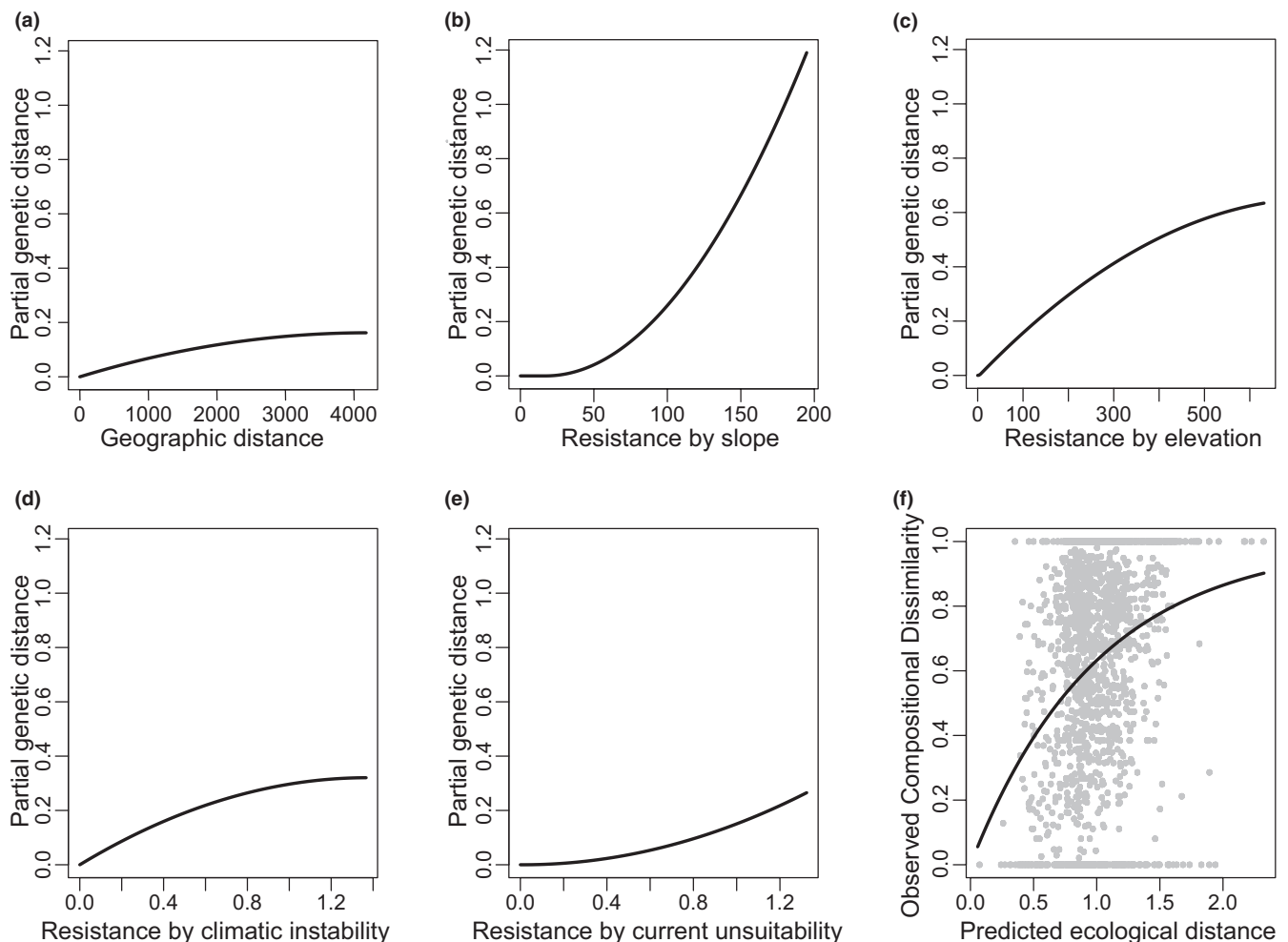
### 3.5 | Isolation by distance and isolation by environmental resistance

The full GDM model explained 6.8% of the total observed genetic variation, considering all seven variables. Of the variables used, five predictors contributed to explain the total deviance (Figure 4). The main predictors for genetic differentiation were associated with environmental resistance due to: topographic complexity through differences in slope of the terrain (summing of the coefficient of the *I*-spline basis function = 1.20) and higher elevations (0.64); followed by resistance from unsuitable climate through instability areas (0.32)

and current climate (0.27); and then geographic distance (0.16). Only resistance of slope had a significant contribution ( $p \leq 0.05$ ) for the observed genetic differentiation, and explained 4.7% of the total observed variation.

## 4 | DISCUSSION

*Boana raniceps* is spatially structured in two lineages: a northern Cerrado and Caatinga lineage (north-eastern) and a southern Cerrado, Chaco and Amazonian savannas lineage (south-western). We found support for the isolation by environment hypothesis, where steeper slopes in the CBP act as a soft vicariant barrier, reducing gene flow between lineages significantly but not completely. Genetic differentiation is shallow (Figure S4 in Appendix S2), and resulted from a scenario of isolation with migration from the middle Pleistocene until the LGM. Recent population expansion was inferred for the north-eastern lineage, starting in the LGM. The genetic break between these lineages matches geographically a



**FIGURE 4** Generalized dissimilarity model-fitted I-splines (partial regression fit) of geographic distance (a) and environmental resistance by: slope (b), elevation (c), historical climatic instability (d) and current climatic unsuitability (e), as predictors of genetic differentiation in *Boana raniceps*. In panel (f) the relationship between observed pairwise genetic dissimilarity and the linear predictor of the GDM (predicted pairwise between-cell ecological distance)





highly complex topographic region in the CBP within the Cerrado, which is coincident with the break between the central plateau and the western lowlands of the Amazonia and Pantanal (Assine et al., 2015; Ross, 2016). However, this break partially rejected our hypothesis that population structures correspond to plateaus and valleys (Table 1), whereas CBP compartmentalization apparently is related to a small fraction of the genetic differentiation due to topographic complexity. Moreover, different environmental niches occupied by lineages and reduction of gene flow from LGM suggest that current and historical climates are possibly the main drivers of genetic diversification, with secondary effects of regional geomorphology.

The north-eastern lineage is distributed along most of the Brazilian shield, in a highly complex topographic region, while the southwest lineage is mainly associated with lowlands of Chaco-Paraná and Amazon basins (see Figure 1). According to our ABC results, lineages diverged during the middle Pleistocene under bidirectional gene flow. Genetic differentiation can be partially explained by isolation by environmental resistance through topographic complexity, but periods of DOF biomes expansions during Quaternary climatic oscillations seem to have contributed to gene flow between lineages across time. This result is congruent with the scenario of gene flow breaks and reconnection through time, in which current and LGM climates could reduce migration of populations. Furthermore, north-eastern and south-western lineages occupy different environmental niches that, along with topography, could potentially reduce gene flow between them. Our findings diverge from phylogeographic studies on widely distributed lineages from the South American DOF that showed deeply structured lineages with ancient divergences (Fonseca et al., 2018; Lanna et al., 2018; Oliveira, Gehara, et al., 2018; Recoder et al., 2014; Werneck et al., 2012). However, similar to our findings, diversification processes associated with Pleistocene climatic shifts apparently are pervasive for several plants (Bonatelli et al., 2014; Correa Ribeiro et al., 2016; Diniz-Filho et al., 2016) and animal species (Bartoletti et al., 2017; Prado et al., 2012).

Landscape features may limit or promote dispersal, causing genetic differentiation to be more related to environmental resistance/connectivity than to geographic distances (Lawson, 2013; McRae, 2006). Fouquet et al. (2007) suggested that *B. raniceps* could be a single, widespread anuran species by comparing mtDNA samples from extreme locations across its latitudinal distribution (French Guiana and Argentina). Indeed, although this species seems to fit the Unified Species Concept (de Queiroz, 2007), the two individuals used by Fouquet et al. (2007) likely belong to the same lineage (south-western), what could have masked the total within species diversity recovered with our dataset. Thus, it is important to consider both geographic distances and landscape complexity when evaluating the intraspecific genetic differentiation. Southwest lineage, for example, occurs in a region with smoother slopes favouring migration. However, the GDM showed that the IBE hypothesis explains only 6.8% of the total observed genetic differentiation, with 4.7% significantly explained by resistance by topography.

During the Late Miocene, the CBP compartmentalization promoted the diversification of different organisms along the DOF. However, the strength of this factor as a driver of diversification varies according to each species' biology. For lowland species, high plateaus could have acted as a topographic barrier, promoting isolation between lineages occurring in the valleys (Oliveira, Gehara, et al., 2018; Werneck et al., 2012). For the gecko *Phyllopezus pollicaris* (Spix, 1825) and the frogs *Dermatonotus muelleri* (Boettger, 1885) and *Physalaemus cuvieri* Fitzinger, 1826, the CBP acted as the primary source of genetic structure (Miranda et al., 2019; Oliveira, Gehara, et al., 2018; Werneck et al., 2012). Our analysis testing IBE identified slope as a secondary predictor of environmental resistance to gene flow for *B. raniceps*. The erosion of plateau surfaces during the Quaternary promoted the expansion of vast depressions between plateaus, increasing fragmentation and landscape complexity in the Cerrado (Ab'Sáber, 1998; Colli, 2005). Hence, although diversification in *B. raniceps* was not influenced by the uplift itself, part of the genetic diversity and differentiation within the species seem to have been influenced by the topography resulting from topographic compartmentalization (see plateaus of central Brazil in Figure S6 in Appendix S2). However, topography likely exerted a greater influence on species genetic differentiation during climatic periods that favoured the shrinkage of DOF biomes (e.g. LGM period). Conversely, because lineages occur in different climatic envelopes, in which the break of lineages matches with the CBP, it is possible that topographic compartmentalization exerted a very limited or no direct effect on the species diversification, given that less than 5% of the observed genetic differentiation was explained by slope. In a rapidly changing landscape, mtDNA may not reflect genetic differentiation due to its slow mutation rate (Hall & Beissinger, 2017) compared with other markers such as microsatellite loci and Single Nucleotide Polymorphisms (SNPs). This limits its power to predict genetic differentiation turnover at finer temporal and spatial scales (Bohonak & Vandergast, 2011; Storfer, Murphy, Spear, Holderegger, & Waits, 2010). Using SNP data, for example, Vasconcellos et al. (2019) found that the genetic structure of *Boana lundii* (Burmeister, 1856) was associated with climatic stable areas, and climatic unstable areas halted gene flow among populations (isolation by instability). In the present study, GDM I-splines detected a minor and non-significant association between genetic distances and resistance by climatic instability and current climatic unsuitability (Figure 4). Because *B. raniceps* presents high migration rates and recent genetic divergences (see Table S6, Appendix S2 in Supporting Information), possibly due to rapid landscape changes, our data (mtDNA) might have not detected the association between genetic differentiation and resistance by climatic instability. Anurans have a tendency to show higher phylogeographic structure in topographically complex habitats, but open-area species usually have lower intraspecific genetic divergences (Rodríguez et al., 2015). Accordingly, *Boana albopunctata*, a species closely related to *B. raniceps* and also associated with open areas, occurs in habitats with more complex topography, at elevations from 0 to 2,000 m (Aquino et al., 2010).

Although it occupies a smaller geographic area, *B. albopunctata* has three known lineages, representing significantly more complex spatial population genetic structure than *B. raniceps* (Prado et al., 2012). At lower elevations, *B. raniceps* seems to replace *B. albopunctata*, and both species rarely occur syntopically (Prado et al., 2012), suggesting that different habitat requirements and biotic interactions between these closely related species can also influence population structure. Because *B. albopunctata* occurs throughout a wider altitudinal range (Aquino et al., 2010) than *B. raniceps*, the observed differences in geographic structure of the species might be expected.

The middle Pleistocene witnessed the split among several South American plant, vertebrate and invertebrate lineages (Collevatti et al., 2020; Turchetto-Zolet et al., 2013). Similarly, divergences between lineages of *B. raniceps* dated from 0.26 to 0.52 Ma (Table S6, Appendix 2). Likewise, South American treefrogs from open habitats like *Boana albopunctata* and *B. lundii* also show population genetic clusters formed during middle Pleistocene (Prado et al., 2012; Vasconcellos et al., 2019). Climatic changes during the Pleistocene altered the extension of biomes (Costa et al., 2018) and demographic history of populations and communities. In the Caatinga biome, for example, climatic changes promoted synchronous demographic responses of squamates and frogs in which most populations expanded during the late Pleistocene (Gehara et al., 2017). We also detected a recent demographic expansion for the north-eastern lineage, albeit more recently (100 ka). Because we detected a substantial expansion of suitable areas for the south-western lineage after the LGM, we expected to find a concordant population expansion, but we did not observe expansion during that time period.

Novel published climatic models suggest that Neotropical forests expanded during the LGM, promoting the fragmentation and contraction of open and dry biomes (Costa et al., 2018; Ledo & Colli, 2017). Indeed, our data indicate that during LGM gene flow between *B. raniceps* lineages halted and SDMs accordingly indicate a contraction of climatically suitable areas for *B. raniceps* during this period. The Amazon and Atlantic forests limit the distribution of *B. raniceps* to the west and to the east respectively. Additionally, as a lowland species, a complex topographic gradient in central Brazil also reduces the chances of panmixia. Thus, it is possible that different current and historical landscape features have affected genetic structure of *B. raniceps* in opposite ways. Genetic structure in *B. raniceps* is related to topographic resistance in central Brazil, but palaeoclimatic changes either reinforced this pattern (e.g. LGM) or maximized gene flow during warmer periods (e.g. LIG and Holocene). We recovered historically stable climatic areas for the occurrence of the two lineages. These areas largely match refugial areas previously identified for Cerrado and seasonally dry forest biomes as the LGM (Werneck, Costa, Colli, Prado, & Sites, 2011; Werneck et al., 2012). Because seasonally dry forests (i.e. Caatinga and the Chaco region) were highly unstable (Costa et al., 2018), the smaller stability area for the southeast lineage was expected. These historical refugial areas might also be related to the environmental niche divergence of lineages. Adaptations to distinct environments with increased

niche divergence of lineages can ultimately lead to ecological speciation (Hua & Wiens, 2010; Schluter, 2009). For the lizard *Polychrus acutirostris* Spix, 1825, for example, niche divergence rather than geomorphology was related to species evolutionary history (Fonseca et al., 2018). Using multiple approaches, we revealed a complex evolutionary scenario where recurrent gene flow mediated by climatic oscillation and a soft geomorphological barrier, coupled with niche divergence, drove the diversification of lineages of *B. raniceps*.

## 5 | CONCLUSIONS

Our results show a complex population history and a mid-Pleistocene genetic divergence within a continentally distributed anuran species in South America. *Boana raniceps* corresponds to a single and widespread species, composed of two geographically structured lineages that occupy different niches in the environmental space. North-eastern and south-western lineages divergence was mediated by niche divergence and a semipermeable barrier formed by an environmental constraint related to topography and climate. Climatic changes throughout the Late Pleistocene apparently affected the north-eastern lineage as expansion started during this period. Our results reinforce the need for more studies in this region as other species may reveal distinct patterns and processes of diversification. However, we hypothesize that the history of instability in south-western DOF areas may have impacted lowland communities in a similar fashion.

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## DATA AVAILABILITY STATEMENT

All sequences were deposited in GenBank (accession numbers: MW197780–MW198051; MW199839–MW199995; MW206801–MW207167). Supporting data are available in Tables S1 to S7

(Appendix S1 and S2). Appendix S3 (pairwise matrices; Tables S8–S15) has been uploaded to DRYAD database (<https://doi.org/10.5061/dryad.4qrfj6q8f>).

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## BIOSKETCH

**Felipe Camurugi** is broadly interested in patterns and processes of diversification of Neotropical anuran species. This study is part of his PhD work at Universidade Federal da Paraíba, in Brazil, on the biogeography, phylogeography and evolution of acoustic signals of Neotropical anurans.

Author contributions: F.C, M.G., and A.A.G. designed the study; F.C., K.R.Z, C.F.B.H, G.R.C, C.P.A.P, M.F.N, and A.A.G collected samples; F.C and M.T.C.T conducted laboratory work; F.C. and E.M.F. analyzed the data; F.C. wrote the manuscript with substantial input from all authors.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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