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Quaternary climatic fluctuations influence the demographic history of two species of sky-island endemic amphibians in the Neotropics



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ABSTRACT

We evaluated the role of Quaternary climatic fluctuations on the demographic history and population structure of amphibian species endemic to the 'campo rupestre' in the Neotropics, evaluating their distributional shifts, demographic changes, and lineage formation from the end of Pleistocene to present. We chose two anurans endemic to the high-elevation 'campo rupestre' in the Espinhaço Range (ER) in northeastern and southeastern Brazil (Bokermannohyla alvarengai and Bokermannohyla oxente), as models to test the role of Quaternary climatic fluctuations over their distribution range in this region. We collected tissue samples throughout their distribution range and used statistical phylogeography to examine processes of divergence and population demography. We generated spatial-temporal reconstructions using Bayesian inference in a coalescent framework in combination with hind-cast projections of species distribution models (SDMs). We also used the results and literature information to test alternative diversification scenarios via approximate Bayesian computation (ABC). Our results show that Quaternary climatic fluctuations influenced the geographic ranges of both species showing population expansion during the last glacial maximum (LGM) and range contraction during interglacial periods, as inferred from selected ABC models and from past projections of SDMs. We recovered Pleistocene diversification for both species occuring in distinctly unique periods for each taxon. An older and range-restricted lineage was recovered in a geographically isolated geological massif, deserving conservation and further taxonomic study. The diversification and distribution of these amphibian species endemic to the Neotropical 'campo rupestre' were influenced by Quaternary climatic fluctuations. The expansion of cold adapted species restricted to higher elevations during glacial periods and their concomitant retraction during interglacial periods may have been crucial for producing patterns of species richness and endemism along elevation gradients in tropical and subtropical domains. Such processes may influence the evolution of the biota distributed in heterogeneous landscapes with varied topography.

1. Introduction

One of the main goals in biogeography is to determine the historical

factors influencing lineage diversification that generated the observed patterns of endemism, range limits and species richness (Wollenberg et al., 2008). Such processes are important for understanding the

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Received 22 January 2020; Received in revised form 13 January 2021; Accepted 8 February 2021 Available online 19 February 2021 1055-7903/© 2021 Elsevier Inc. All rights reserved. evolution of species endemic to high-elevation areas that are spatially distributed in "islands" isolated by a "sea" of lowland assemblages (Warshall, 1995), referred to as sky-island systems (sensu Heald, 1951). In these systems, patterns of diversity may be determined by vertical distributional shifts in response to climatic and geological processes, adaptations to environmental constraints, and biotic exchanges with the surrounding lowlands (Graham et al., 2014; Hoorn et al., 2018).

The elevation shift model (sensu Galbreath et al., 2009) states that populations of cold adapted species inhabiting high elevations expanded to lowland areas during periods of climate cooling, increasing spatial connectivity and gene flow (DeChaine and Martin, 2005; Galbreath et al., 2009; Newman and Austin, 2015), while the present highland distribution may be relictual (Brown and Knowles, 2012; Galbreath et al., 2009; McCormack et al., 2008). Many studies support this hypothesis for tropical and subtropical domains (Antonelli et al., 2010; Bonatelli et al., 2014; Collevatti et al., 2009; Robin et al., 2010; Zhang et al., 2008), while others support population stability (Amaro et al., 2012; Batalha-Filho et al., 2012; Firkowski et al., 2016; Wilmé et al., 2006), indicating the existence of different diversification processes in high-elevation areas. In this context, the evolutionary history of each taxa defined by distinct life history traits should generate different demographic responses to past climate change.

The Espinhaço Range (hereafter ER) is an understudied highelevation area in tropical South America. These mountains encompass a significant portion of the remaining 'campo rupestre' (i.e., rupestrian grasslands) ecosystem in the continent (Rapini et al., 2008), which are savanna like formations generally distributed above 1000 m above sea level where oligotrophic soils formed over quartzitic rocky outcrops (Bonatelli et al., 2014). This high-elevation ecosystem occupies less than 1% of the Brazilian territory, yet it is recognized by its high diversity and endemism of angiosperms, birds, and amphibians (Magalhães et al., 2017; Rapini et al., 2008; Silveira et al., 2016). The 'campo rupestre' is threatened due to human impact (Rapini et al., 2008) and by the inefficiency of the actual network of protected areas in preserving endemic biota, particularly for amphibians (Magalhães et al., 2017; Oliveira et al., 2017; Ramos et al., 2017). Furthermore, future climatic models suggest reduction of up to 95% of the distribution of habitat in the 'campo rupestre' in less than 100 years (Fernandes et al., 2014). For a finer prediction of the role of climate change on high-elevation areas (Bonatelli et al., 2014; Fernandes et al., 2014), it is essential to understand the evolution of endemic species and the influence of past climatic cycles on the demographic history of threatened biota occurring in the 'campo rupestre' (Prates et al., 2016). Studies of high-elevation angio-sperm taxa endemic to the 'campo rupestre' support a sky-island system influenced by climatic fluctuations, where populations expanded during glacial periods (Antonelli et al., 2010; Bonatelli et al., 2014; Collevatti et al., 2009). Nevertheless, it is unknown if the fauna endemic to these environments show a similar demographic history.

In this study we investigate the evolutionary history of two amphibian species endemic to the 'campo rupestre' in the ER: *Bokermannohyla alvarengai* (Bokermann, 1956, Fig. 1) and *Bokermannohyla oxente* (Lugli and Haddad, 2006, Fig. 2). Considering their ecological specificity to these high-elevation environments, such species are adequate models to infer the influence of past climatic fluctuations in the dynamics of these sky-island systems.

Our study aims to answer the following question: will the target species show a historical pattern of diversification and demography associated with the elevation shift model (hereafter ESM)? Accordingly, these amphibians should present structured populations in highelevation areas separated by valleys, and signatures of population expansion during the Last Glacial Maximum (LGM) with contraction in the Holocene. Alternatively, these populations may show an unchanging demographic history consistent with a glacial stability model (hereafter GSM). To answer these questions, we combined molecular techniques with species distribution models (hereafter SDMs), paleoclimatic projections and tested demographic scenarios using a simulation-based approach.

2. Material and methods

2.1. Population sampling, DNA extraction, amplification, and sequencing

We collected samples for both target species through their distribution range in the ER, tissues were further extracted, and DNA sequenced



Fig. 1. (a) Geographic distribution of sampled localities for *Bokermannohyla alvarengai* in the northern, central, and southern portions of the Espinhaço Range (black dots), and its location in South America, between the Cerrado (Ce), Caatinga (Ca), and Atlantic Forest (AF) domains. (b) Elevation map depicting the two delineated mitochondrial lineages (right), together with assignment results from GENELAND (left). (c) Mitochondrial coalescent tree from COI gene showing the main divergence events among the two delineated lineages, with results from population assignment methods. Black dots show posterior probabilities > 0.90.



Fig. 2. (a) Geographic distribution of sampled localities for *Bokermannohyla oxente* in the Chapada Diamantina region (black dots) and the location of the Espinhaço Range in South America, between the Cerrado (Ce), Caatinga (Ca), and Atlantic Forest (AF) domains. (b) Elevation map depicting the three delineated mitochondrial lineages (right), with results from GENELAND (left), which assembled together the Southern and Sincorá lineages. (c) Mitochondrial coalescent tree from COI gene showing the main divergence events among the three delineated lineages, with results from population assignment methods. Black dots show posterior probabilities > 0.90.

(for a more complete description of the study system and target species, see Appendix S1 in Supplementary Material, Table S1, and Fig. S1). Vouchered specimens were deposited in the zoological collections of the Universidade Estadual de Santa Cruz (UESC), in the Universidade Federal de Minas Gerais (UFMG), and in the Pontifícia Universidade Católica de Minas Gerais (PUC Minas). We sampled muscle, toe clip, and liver, preserved them in 100% ethanol, and sequenced five distinct gene fragments: One mitochondrial fragment of the cytochrome *c* oxidase subunit I gene (COI), and four nuclear loci: β -fibrinogen intron 7 gene (β -fibrin7), a segment of the exon 1 of tyrosinase gene (TYR), a segment with exon 2, and intron 2 of the cellular myelocytomatosis gene (C-myc2), and a segment of the exon 3 of tensine gene (TNS3). Details and specifications of DNA extraction, amplification and sequencing techiques are given in the supplementary material (Appendix S2 in Supplementary Material and Table S2).

We edited sequences using GENEIOUS v 7.1.3. (Kearse et al., 2012). For nuclear genes, we coded polymorphic nucleotide positions of heterozygous individuals with the IUPAC ambiguity codes. For the heterozygous genotypes, we inferred phased haplotypes with the Bayesian algorithm implemented in PHASE v 2.1.1. (Stephens et al., 2001). We included sequences with probabilities of 80% or above for heterozygosity in each pair of alleles in all subsequent analyses. We aligned sequences in MEGA v 6.1 (Tamura et al., 2013) using the Muscle algorithm (Edgar, 2004). All sequences are available in GenBank (Table S1 in Supplementary Material).

2.2. Population structure and assignment

We used three different methods to evaluate patterns of population structure including single locus and multi-loci approaches. First, we delimited mitochondrial lineages using the Bayesian implementation of the Generalized mixed Yule-coalescent (bGMYC) (Fujisawa and Barraclough, 2013; Reid and Carstens, 2012) using R 3.1.1 (R Core Team, 2017). We used 100 mitochondrial gene trees obtained from BEAST 1.8 (Drummond et al., 2012) as input for this analysis (see below).

We also investigated population structure through multi-loci approaches. First, we used the software STRUCTURE 2.3.4 (Pritchard et al., 2000) to determine population structure with the four nuclear loci

using the linkage model. We built a genotype matrix from phased alignments (Falush et al., 2003), and explored a range of values from 1 to 8 populations (k), considering the maximum number of geological massifs in the ER that may harbor distinct populations (Fig. S1) in 10 replicates. We implemented each independent run in 10×10^6 generations following a burn-in of 10×10^4 generations and assuming uncorrelated allele frequencies with admixture. We chose the best value of k using the Δk according to Evanno et al. (2005) implemented in STRUCTURE HARVESTER v0.6.9 (Earl and vonHoldt, 2012).

To further evaluate population structure in the spatial context, we used GENELAND 4.0.3 (Guillot et al., 2005a; Guillot et al., 2005b) implemented in R using all five loci, together with respective georeferenced data (Table S1). We determined the number of populations (k) using the Markov Chain Monte Carlo (MCMC) algorithm in two distinct runs. We ran this for 1×10^6 iterations with a thinning interval of 100 along ten replicates, employing uncorrelated allele frequencies, checking for a range of values from 1 to 8 populations (k), and including the option "uncertainty on the coordinates" in all runs. We assessed convergence by comparing k values in each replicate. We estimated population membership in the second run with a fixed k value (the one with highest posterior probability obtained in the first run), also over 1×10^6 iterations.

2.3. Parameters and tree estimates

We ran BEAST to estimate clock rates for all partitions and relative substitution rates for nuclear loci, as well as to build mitochondrial gene trees for both species. We also built species trees in order to obtain divergence time estimates (in million years) between each assigned population. Prior to this, we determined substitution models for each partition in the software JMODELTEST (Posada, 2008) using the Bayesian information criterion (Table S3).

For each run, we employed different tree priors according to the parameter or tree being estimated, which also differed given their respective MCMC chain lengths (Table S3). We performed three independent runs using random starting seed numbers to check for convergence of parameters. In all runs, we used a burn-in of 25%, and used the software TRACER v.1.6 (Rambaut et al., 2014) to check for effective

sample sizes (ESS values \geq 200), and for convergence. We built consensus trees in TREEANNOTATOR 1.8 (Drummond et al., 2012). We built consensus trees using the option: "maximum clade credibility tree".

We performed three independent runs using different seed numbers to check for convergence of parameters (for a complete description of each run, parameters, tree priors and tree estimates, see Appendix S3 in Supplementary Material).

2.4. Species distribution modeling and projection into past scenarios

We estimated changes in habitat suitability during specific periods in the Pleistocene and Holocene using past projections of contemporary SDMs. These were used to generate hypotheses of past population expansions or contractions in both species. Therefore, these results were used as additional evidence to develop models to test diversification scenarios (see below).

We built contemporary SDMs for both species using the Maximum Entropy algorithm in MAXENT 3.3.3 k (Elith et al., 2006; Phillips et al., 2006; Wisz et al., 2008). We projected the species ranges into two Pleistocene and one Holocene timeframes: The Last Interglacial (LIG, 120 kyr), the Last Glacial Maximum (LGM, 21 kyr), and the Mid-Holocene (MH, 6 kyr). We downloaded the bioclimatic variables from Worldclim v 2.0 (Fick and Hijmans, 2017) at 2.5 min resolution and used them to produce different models for the present (1960 to 1990), as well as for the aforementioned past scenarios. We used downscaled data derived from two Global Climate Models (GCMs): CCSM4 and MIROC-ESM models for LGM and Mid-Holocene, and one additional GCM for LIG (Otto-Bliesner et al., 2008). We inferred changes in habitat availability from respective changes in habitat suitability in each period. For additional information on modeling procedures (occurrence records, variable selection and model procedures and evaluation) see Appendix S4 in Supplementary Material.

2.5. Tests of diversification scenarios and biogeographical hypotheses

We used a two-stage approach to test diversification scenarios from proposed biogeographical hypotheses. First, we used PHRAPL (Jackson et al., 2017) to obtain the best demographic model related to the diversification of the target species through the comparison of approximate likelihoods of simulated models. PHRAPL executes model selection with demographic parameters such as migration and population sizes, taking into account tree distance for approximate likelihood calculations. It selects the best model using log-likelihood scores of the data in relation to each evaluated model. Gene trees for nuclear loci generated from BEAST runs were used as inputs in these analyses. For both species, we tested all 10 possible models for a two-population data set according to the results of assignment tests (assuming K = 2 for both species, see results). We took a total of 200 subsamples of five individuals per population for each locus and simulated 20,000 trees on each run.

Next, we took the selected PHRAPL models in each species and used them with results from projections of SDMs and with literature evidence (see below) to build the diversification scenarios representing our two competing hypotheses. These were tested using Approximate Bayesian Computation (ABC). This is a likelihood-free method that evaluates distinct scenarios via simulation (Bertorelle et al., 2010; Csilléry et al., 2010) and was used to test the following hypotheses: i) The elevation shift model (ESM), where populations expansions took place during the LGM and contracted in the Holocene, as shown for other vertebrate species endemic to tropical and subtropical high-elevation habitats (Robin et al., 2010; Zhang et al., 2008), as well as from SDMs results and from literature supporting the expansion of angiosperms endemic to the 'campo rupestre' in central and eastern Brazil during LGM (Antonelli et al., 2010; Bonatelli et al., 2014; Collevatti et al., 2009); ii) The glacial stability model (GSM), where population sizes remained stable during LGM according to trends from vertebrates endemic to high elevations in southeastern Brazil (Amaro et al., 2012; Batalha-filho et al., 2012; Firkowski et al., 2016), and from other tropical regions (Wilmé et al., 2006).

We tested these two hypotheses in three different scenarios, a) diversification without migration, b) diversification with migration into the northern population, and c) diversification with a founder effect, for a total of six models (a.i, a.ii, b.i, b.ii, c.i, and c.ii; see Fig. 5). To estimate the probability of each model we simulated summary statistics under the coalescent model using the PipeMaster r-package (Gehara et al., 2016) available in github (github/gehara/PipeMaster), and used them to perform a supervised machine learning (SML) classification. For each of the six models, we simulated 10,000 independent datasets sampling model parameters from prior distributions (see Appendix S5 in Supplementary Material). We then used 75% of this data to train an Artificial Neural Network classification algorithm with one hidden layer using "nnet" and "caret" r-packages (Kuhn, 2016). The remaining 25% was used to test the classification. We performed 10 bootstrap replicates to tune the parameters of the algorithm (e.g., number of nodes and decay) using the trainControl and train functions and tested the accuracy using the *postResample* function after prediction of the testing data. To reduce the number of models per classification, we compared the models hierarchically. First, we compared all four Glacial Stability models against each other, and all four Elevation Shift models also against each other. These two comparisons identify the best models in each category. In the final analysis we took the best models selected in each category and compared the Glacial Stability and Elevation Shift models against each other to infer which hypotheses best fit our data (for further details on simulation procedures such as prior distributions, summary statistics, model-fit evaluation and parameter estimation see Appendix S5 in Supplemetary Material).

3. Results

We extracted and sequenced a total of 63 samples for *Bokermannohyla alvarengai* and 46 for *B. oxente* throughout their geographic range (Table S1, Fig. S1), obtaining ~4400 total base pairs (~2300 and 2100 base pairs, respectively), and 700 sequences (after the exclusion of unresolved phased sequences) summing across all five loci (Tables S1 and S3).

3.1. Population structure, assignment and mitochondrial lineages

For *Bokermannohyla alvarengai*, bGMYC delineated two mitochondrial lineages distributed through the north–south axis of its geographical range (k = 2, Fig. S2a). These two populations were also recovered in STRUCTURE using only nuclear loci (Fig. S3), and in the GENELAND spatial model with all five loci (Fig. 1b). These groups are named hereafter northern and southern populations, and the phylogeographic break which divides them is located in the central ER (Fig. 1b). The main discrepancy among these three methods was related to the assignment of two sampled localities in this region: Itacambira (n = six individuals) and Botumirim (n = four individuals), which are geographically adjacent (separated by a low altitude valley of about 20 km wide, Figs. 1b, 1c and S1).

For *B. oxente*, bGMYC supported three mitochondrial lineages (k = 3, Fig. 2c): One older spatially isolated lineage (hereafter named the Jacobina lineage), one from localities only within Serra do Sincorá (hereafter named the Sincorá lineage), and a third geographically broad lineage encompassing samples from distinct regions along the southern portion of the Chapada Diamantina (hereafter named Southern lineage). These latter two mitochondrial lineages showed a contact zone with possible admixture/introgression in the localities of Mucugê and Palmeiras (Figs. 2b, 2c, and S6). When evaluated under the spatial model using five loci in GENELAND, the Southern and Sincorá lineages were grouped together (k = 2, hereafter named the Southern-Sincorá population, Fig. 2b). STRUCTURE results from the four nuclear loci were

intermediate between both previous methods, determining three populations (k = 3, Fig. S4), but with high levels of admixture between the Southern and Sincorá lineages (Figs. 2c and S4), corroborating GENE-LAND and bGMYC results (Figs. 2c and S6). The Jacobina population was clearly assigned in all three methods and is located in a small and isolated geological massif in the northeastern most portion of the ER (the Serra da Jacobina, Fig. 2b, 2c).

3.2. Divergence times between populations

Divergence times estimated from the species trees recovered diversification during late Pleistocene in *Bokermannohyla alvarengai*, which diverged about 0.340 Ma (95% HPD = 0.153-0.597 Ma), and an older divergence in *B. oxente*, where the Southern-Sincorá population split from the Jacobina population in the Plio-Pleistocene transition, at 2.207 Ma (95% HPD = 0.816-4.377 Ma).

3.3. Species distribution modeling and projection into past scenarios

Both current models accurately predicted the distributions of the evaluated species, with AUC values of 0.986 (\pm 0.001) and 0.985 (\pm 0.002), and TSS values of 0.906 (\pm 0.006) and 0.902 (\pm 0.022), for *Bokermannohyla alvarengai* and *B. oxente*, respectively (Figs. 3b and 4b).

Areas of higher suitability in the current model for *B. alvarengai* encompassed most of its current geographic range, with less suitable areas also occurring in the eastern portion of the ER and in the Chapada Diamantina (Fig. 3b). The current model for *B. oxente* predicted a higher suitability in the Serra do Sincorá and to the southin the Serra das Almas and Serra dos Barbados region near the Contas river headwater (Fig. 4b). Areas of lower suitability were also predicted in the northern range of the Chapada Diamantina, as well as in the northern, central and eastern portions of the ER. Isolated patches of low suitable areas were also predicted outside the ER, in the semi-arid Caatinga domain to the north, and an isolated patch to the east, in the Atlantic Forest domain (Fig. 4b).

As a general trend, both species showed suitability increases during the LGM (Figs. 3e, 3f and 4e, 4f) as compared to the other evaluated past periods (Mid-Holocene and LIG). During LGM, *Bokermannohyla alvarengai* was predicted to occupy most of the ER (Fig. 3e, 3f), whereas *B. oxente* was predicted to expand its range to northern areas outside its current range in the Chapada Diamantina, within the Caatinga (Fig. 4e, 4f). As a general trend, suitability areas were similar during both interglacial periods (current and LIG) for both species (Figs. 3b, 3g and 4b, 4g), with *B. oxente* showing higher values of suitability during the LIG inside the range of the ER (Fig. 4g), whereas suitability areas for *B. alvarengai* were similar in geographic range between both periods, with an increase in the southern ER in the current model (Fig. 3b).

3.4. Tests of diversification scenarios and biogeographical hypotheses

The PHRAPL results supported a model with divergence and unidirectional migration from south to north as the best model in Bokermannohyla alvarengai and a model with divergence and no migration in B. oxente (Table 1). Following this, the selected diversification models in ABC with highest values of posterior probabilities were those which corroborate the ESM with a widespread ancestor for both species, where the model with unidirectional migration (Fig. 5b1) was selected for B. alvarengai (p = 0.902, Table 2), and the model without migration (Fig. 5a1) was selected for *B. oxente* (p = 0.892, Table 3), corroborating PHRAPL results. Both models showed adequate fit to the observed data, as seen by the first two axis of the PCA (Figs. S7 and S8). They recovered a Pleistocene divergence between populations for B. alvarengai (mean of 0.49 million years, Table S5), similarly to BEAST estimates. However, the estimated divergence times differed from BEAST in B. oxente, with an estimated divergence in the first half of Pleistocene (mean of 1.46 million years, Table S6).

4. Discussion

4.1. The role of Quaternary climatic fluctuations in the diversification and demographic history of the 'campo rupestre' endemic biota

We found support for the existence of a dynamic demographic system in the high-elevation areas of the 'campo rupestre' in the ER suggesting recurrent range shifts through the elevation gradient. This pattern underscores the influence of Quaternary climatic fluctuations in the diversification and distribution of the studied amphibian species



Fig. 3. (a) Occurrence points used for building contemporary SDMs for *Bokermannohyla alvarengai* (n = 44). Scale bar depicts altitude values. (b) to (g): Species distribution models (SDMs) for *B. alvarengai* during current (b), Mid-Holocene (c, d), LGM (e, f) and LIG (g) periods. Scale bars depict suitability values. Areas inside the black dotted line indicate regions in the Espinhaço Range which are higher than 500 m above sea level.



Fig. 4. (a) Occurrence points used for building contemporary SDMs for *Bokermannohyla oxente* (n = 41). Scale bar depicts altitude values. (b) to (g): Species distribution models (SDMs) for *B. oxente* during current (b), Mid-Holocene (c, d), LGM (e, f) and LIG (g) periods. Scale bars depict suitability values. Areas inside the black dotted line indicate regions in the Espinhaço Range which are higher than 500 m above sea level.

Table 1

Results of model selection by PHRAPL for *Bokermannohyla alvarengai* and *Bokermannohyla oxente*, showing the two best models (those with AIC delta values <2). Parameters included on each models: θ : Effective population sizes, M: Migration and D: Divergence.

B. alvarengai - Model	AIC	lnL	Delta AIC	wAIC
θ (Southern) > θ (Northern); M _{Southern} \rightarrow _{Northern} ; D(present)	97.24	-45.620	0	0.277
θ (Southern) > θ (Northern); D(present)	97.979	-46.989	0.739	0.191
B. oxente - Model	AIC	lnL	Delta AIC	wAIC
θ (Southern-Sincorá) > θ (Jacobina); D (present)	92.222	-44.111	0	0.428
θ (Southern-Sincorá) > θ (Jacobina); M _{Southern-Sincorá → Jacobina} ; D(present)	93.976	-43.988	1.754	0.178

Table 2

Probabilities, using the artificial neural network algorithm, for the two proposed diversification models (GSM and ESM) tested using ABC for *Bokermannohyla alvarengai*, and the three distinct parameterizations employed in each of them regarding divergence and migration: FE (founder effect), I (isolation) and IM (isolation with migration). The first run compares models only within a given category (GSM and ESM), using each of the three parameterizations. Models and parameters on run #2 were the ones with highest probabilities in run #1. For more details in each model, see Fig. 5.

Run # 1	FE	Ι	IM	Accuracy	Карра
Glacial stability model (GSM)	5.99e- 06	0.369	0.63	0.717	0.576
Elevation shift model (ESM)	2.72e- 11	0.091	0.908	0.901	0.579
Run # 2					
GSM - IM	ESM - IM	Accuracy	Карра		
0.097	0.902	0.912	0.106		

endemic to the "campo rupeste habitat." Similar to studies on angiosperm endemics in this ecosystem (Antonelli et al., 2010; Bonatelli et al., 2014; Collevatti et al., 2009), our analyses support a sky-island system in the ER where populations expanded their sizes and ranges during periods of glacial maxima, with concomitant retractions during interglacial periods.

The evolution of species in sky-island systems is subject to the influence of demographic processes that may promote recurrent connections and isolation of populations (Graham et al., 2014), such as shifts in distribution ranges through the elevation gradient during periods of climate change. Thus, montane ecosystems may act as centers for speciation by providing suitable habitats during climatic oscillations in which older species persist and new lineages are generated (Fjeldsä and Lovett, 1997; Tzedakis et al., 2002). The relative climatic stability of tropical and subtropical montane ecosystems in relation totemperate counterparts may be responsible for the long-term persistence of old and endemic lineages (Fjeldsä and Lovett, 1997; Rangel et al., 2018; Wollenberg et al., 2008). The results in the present study corroborates this pattern, where a range restricted and older lineage was recovered in one of the studied species located in a small and geographically isolated massif (the Jacobina lineage in Bokermannohyla oxente). Contrary to our findings for the ER, some studies suggest demographic stability during the late Pleistocene for some vertebrate taxa in the highlands of the Atlantic Forest (AF) domain (Amaro et al., 2012; Batalha-Filho et al., 2012; Firkowski et al., 2016). A more detailed hypothesis test employing genomic-scale data is needed to better understand patterns of diversification and adaptation of the high-elevation endemic biota. Such information is crucial for planning conservation actions in these environments, ensuring the viability of range restricted populations and lineages which are imperiled by global climate change and habitat retraction.

4.2. Biogeographic and phylogeographic patterns in the Espinhaço range

The ER (Fig. S1) is distributed across two different lowland environments: its northernmost portion (the Chapada Diamantina) is composed of xeric Caatinga habitat, and the massifs to the south constitute an ecotone between the Cerrado and the AF domains. A low



Fig. 5. Models used for testing the diversification scenarios with approximate Bayesian computation for *Bokermannohyla alvarengai* and *Bokermannohyla oxente* through sky-islands in the Espinhaço Range given the Elevation Shift Model (1) and the Glacial Stability Model (2). (a) Models without migration. Both populations originate from a widespread ancestral population in the past, expanding during the last glacial maximum (a.1), or remaining at a constant size until the present (a.2). (b) Models with migration. Both populations originate from a widespread ancestral population. Both populations originate from a widespread ancestral population. Both populations originate from a widespread ancestral population in the past, expanding during the last glacial maximum (a.1), or remaining at a constant size until the present (b.2). (c) Founder effect models: one current population originated from the other through past dispersal events (according to PHRAPL results) expanding during the last glacial maximum (c.1), or remaining at a constant size until the present (c.2). Td = Time of divergence. Legends for populations: In *B. alvarengai*: Pop. 1 = Southern, and Pop. 2 = Northern. In *B. oxente*: Pop. 1 = Southern-Sincorá, and Pop. 2 = Jacobina.

altitude plateau separates the Chapada Diamantina from the other southern massifs of the ER (sensu Chaves and Brandão, 2004, Fig. S1). This plateau is characterized by the predominance of Caatinga vegetation and coincides with the geographic limit in the ranges of both studied species (Fig. S1). Other anurans endemic to sky islands in the ER have similar distribution limits, such as *Pithecopus megacephalus*, whose northernmost record is similar to that of *Bokermannohyla alvarengai*, immediately south of this low elevation plateau (Brandão et al., 2012).

Table 3

Probabilities, using the artificial neural network algorithm, for the two proposed diversification models (GSM and ESM) tested with ABC for *Bokermannohyla oxente*, and the three distinct parameterizations employed in each of them regarding divergence and migration: FE (founder effect), I (isolation) and IM (isolation with migration). The first run compares models only within a given category (GSM and ESM), using each of the three parameterizations. Models and parameters on run #2 were the ones with highest probabilities in run #1. For more details in each model, see Fig. 5.

Run # 1	FE	Ι	IM	Accuracy	Карра
Glacial stability model (GSM)	1.24e- 6	0.504	0.495	0.844	0.767
Elevation shift model (ESM)	4.41e- 8	0.95	0.049	0.851	0.776
Run # 2					
GSM - IM	ESM - I	Accuracy	Карра		
0.107	0.892	0.721	0.443		

This plateau is also thought to be a potential driver of diversification in birds of the genus *Augastes* (Vasconcelos et al., 2012) and angiosperms endemic to the ER 'campo rupestre' (Bünger et al., 2014). Past projections of SDMs suggest increases in habitat suitability for both studied species during LGM (Figs. 3e, 3f and 4e, 4f), and in the case of *Bokermannohyla alvarengai*, this low altitude plateau was less effective during cooler periods in the past. Although we found that the demographic histories of both species were influenced by historical climate change, differences were found regarding their divergence time estimates and diversification.

The phylogeographic break within *Bokermannohyla alvarengai* is located in another low altitude plateau of only 20 km wide (Fig. 1b) in the region of the Grão Mogol municipality. This break is spatially and temporally concordant with another phylogeographic break in an angiosperm species (*Pilosocereus aurisetus*, Cactaceae; Bonattelli et al., 2014). Nascimento et al. (2018) also found this same discontinuity for another anuran species endemic to the ER (*Bokermannohyla saxicola*) separating two clades in this species. This may indicate similar evolutionary processes possibly influenced by climatic fluctuations for the fauna and flora associated to the 'campo rupestre' in the central portion of the ER.

Unidirectional migration was recovered in *Bokermannohyla alvarengai* from southern to northern regions through the ER (Tables 1 and S5, Fig. 5b1). This south-north pattern is also observed in the diversification of the orchid genus *Hoffmannseggella* endemic to the 'campo rupestre' (Antonelli et al., 2010), supporting the existence of historical corridors for the dispersion of high-elevation taxa during periods of climate cooling (Antonelli et al., 2010). Nascimento et al. (2018) also found a directional pattern of diversification in the southern Espinhaço for *B. saxicola*, but in the opposite direction (a north–south pattern), and during a much older period (from late Miocene to late Pliocene). The authors suggest the role of past climatic fluctuations (during the Tertiary) influencing the diversification in this species.

In the present study, diversification estimates for major clades varied between the studied species. *Bokermannohyla alvarengai* showed more recent diversification (during the Pleistocene) as compared to its northern counterpart (*Bokermannohyla oxente*), which its two major clades diverged between the Pliocene-Pleistocene transition and the first half of Pleistocene. The more recent divergence time recovered in *B. alvarengai* (Table S5), together with the presence of migration (Table 1) is expected given life history traits such as large body size and basking behavior (Tattersall et al., 2006), which are likely to increase dispersal rates. Basking behavior in frogs enhances their ability to disperse during the day, allowing them to move greater distances when compared to strictly nocturnal species.

The Plio-Pleistocene divergence estimated in *Bokermannohyla oxente* between the Southern-Sincorá and Jacobina populations (according to BEAST results) agree with estimates for other high-elevation endemic

anuran of the ER (*Bokermannohyla saxicola*), in which diversification among major clades occurred from late Miocene (about 6.1 Mya) to the Pliocene-Pleistocene transition (about 2.56 Mya; Nascimento et al., 2018). As found here for *B. oxente*, intra lineage diversification for other species of the Neotropical herpetofauna also occurred mainly during Pleistocene (Gehara et al., 2017; Machado et al., 2014; Nascimento et al., 2018; Thomé et al., 2010; Tonini et al., 2013). Considering the estimated divergence times and the selected model in the ABC analysis, the Jacobina population may be a relict of a once widespread ancestral population.

Few studies have determined divergence times for amphibians in the Neotropical 'campo rupestre'; however Magalhães et al. (2017) found a recent Pleistocene diversification in the frog Pithecopus ayeaye, a skyisland endemic anuran from the 'campos rupestres' of southeastern and central Brazil, which is similar to results found for Bokermannohyla alvarengai in the present study. Ramos et al. (2017) recovered evolutionarily independent lineages and spatially-isolated populations in the frog P. megacephalus endemic to the ER. This study proposed that relictual distributions during interglacial periods triggered by Quaternary climatic fluctuations may explain the observed patterns of population structure and lineage diversification in this species. In addition, some speciation events in endemic birds from high elevations in eastern and southern Brazil are alos estimateds to have occurred during the late Pleistocene (Chaves et al., 2014); this may indicate a common role for historical processes in the evolution of co-distributed vertebrate species in the Neotropical 'campo rupestre'.

Most amphibians rely upon water bodies for reproduction, and in the present study population expansions during past periods in the Quaternary may be as well related to increased rainfall in addition to climate cooling. Such pattern is proposed for Bokermannohyla alvarengai considering the complex dynamics of climatic fluctuations registered in the past 60 kyr along its present distribution range, where a mesic climate predominated from the end of Pleistocene to mid Holocene in the southern and central portions of the ER, with a marked decrease in temperatures and increased rainfall. Therefore, population expansions for this species during the LGM may have been influenced also in response to increased rainfall, which may have triggered a concomitant increase in the availability temporary pools used for reproduction. Also, distinct sources indicate the predominance of a mesic climate in the semi-arid Caatinga, from the end of Pleistocene to the beginning of Holocene (Auler and Smart, 2001; De Oliveira et al., 1999). In fact, De Oliveira et al. (1999) and Wang et al. (2004) suggest the existence of a wide Pleistocene corridor of mesic vegetation encompassing a significant portion of the Chapada Diamantina region, which may have influenced in the diversification of the amphibian fauna during late Pleistocene. Also, Sobral et al. (2015) using past projections of ENMs postulated the expansion of the AF over this region in the period corresponding to the LGM. Such biogeographic processes may be additional factors which determined the observed pattern of increase in habitat suitability for Bokermannohyla oxente during the LGM, with a concomitant decrease from this period to present (Fig. 4b-f). Therefore, this species may present a historical demographic response which may follow past expansions of forested environments in this semi-arid domain, a pattern already suggested for another anuran species endemic to mesic enclaves in high elevations in the Brazilian semi-arid (Carnaval and Bates, 2007).

4.3. Relevance of the present study to the conservation of high-elevation endemic amphibians

Our study has important implications for the conservation of the biota endemic to the South American 'campo rupestre'. The population structure and diversification patterns found in both studied species can provide guidelines for effective conservation measures for endemic amphibians from these ecosystems, which may be negatively impacted by the current human induced climate change.

In such contexts, the Jacobina population should be treated as a distinct species of Bokermannohyla, deserving adequate conservation measures given the higher probability of extinction risk as observed for small-ranged vertebrates in montane habitats (Fjeldsä et al., 2012). Further taxonomic studies should reveal if this lineage is likely a unique microendemic species (Ochoa-Ochoa et al., 2011). Deep lineage divergence between spatially isolated populations with the possible existence of cryptic species is observed in other studies of vertebrate taxa endemic to tropical and subtropical high elevations (Gehara et al., 2013; Koscinski et al., 2008; Robin et al., 2010). In these regions, phylopatric species such as small bodied vertebrates may show distinct genetic structure between populations at finer spatial scales (Hollycross and Douglas, 2007). Therefore, conservation measures for sky-island endemics should take into account patterns of genetic structure and the existence of independent evolving lineages between distinct mountain tops.

Species inhabiting high elevations (i.e., montane endemics) may be particularly vulnerable to the effects of rapid climate change (Parmesan, 2006) given that a considerable amount of montane habitats may be lost in the near future due to climate warming (Chen et al., 2011; Moritz et al., 2008). In this context, ensuring biodiversity conservation strategies that preserve uniquely evolving lineages and the adaptive potential of species to respond to climate change is a key component for maintaining ongoing evolutionary processes (Byrne, 2008). Due to the effects of climate change in the next few decades, some populations of highelevation species may undertake several events of declines and local extinctions due to the reduction of suitable habitats (Bonatelli et al., 2014; Fernandes et al., 2014). Our study suggest a relationship between amphibian distribution range and historical climatic fluctuations in the Neotropical 'campo rupestre' of the ER, where the studied species will be negatively impacted by the increase of atmospheric temperatures. In such context, future projections on the distribution of anurans endemic to the 'campos rupestres' are still scarce and controversial. One study suggests that as much as 95% of suitable areas of the 'campo rupestre' ecosystem will be lost by the end of the century in central Brazil and throughout the ER (Fernandes et al., 2014). However, Magalhães et al. (2017) studying Pithecopus ayeaye obtained results indicating trends of future increases in its distribution ranges. These contrasting results reinforce the need for additional phylogeographic approaches with codistributed species endemic to these ecosystems and from other high elevation regions.

5. Conclusions

Our study shows the possible role of historical climatic cycles in the evolution and diversification of two amphibian species endemic to high elevations of a poorly studied geological formation in southeastern and northeastern Brazil (the ER), which harbors a significant proportion of the remaining 'campos rupestres' in South America. The main divergence events for the species of *Bokermannohyla* examined here date back to the Plio-Pleistocene transition to the end of Pleistocene and provides evidence for the role of Quaternary climatic oscillations for lineage diversification. Previous studies with co-distributed angiosperms corroborate this pattern, and a few studies with other amphibians in these ecosystems also propose the role of climate in their diversification. It is imperative that genomic-scale data should be applied to future studies together with hypothesis testing through model-based approaches to better understand the patterns of evolution and diversification.

Also, empirical evidence from distinct regions through the globe strongly suggests that the high-elevation biota is going through severe habitat decrease due to the rise of global temperatures. Therefore, it is crucial that effective measures should be taken by governments and decision makers from the most industrialized and polluter countries to drastically decrease global warming in the next few decades (such as the massive and widespread adoption of clean energy sources), in order to preserve species that are actually restricted to high elevations.

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CRediT authorship contribution statement

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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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Appendix A. Supplementary material

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