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Original Article

Lineage diversification of the Sky Island treefrog *Scinax curicica* (Anura, Hylidae) in the Espinhaço Mountain Range

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ABSTRACT

Sky Islands present unique landscapes for organismal evolution because they comprise high mountain peaks separated by low valleys with vastly different environmental conditions. The Espinhaço Mountain Range in eastern Brazil is formed of groups of Sky Islands in the states of Minas Gerais and Bahia. Several phylogeographic studies have discovered strong genetic structure among populations of organisms occupying the *campo rupestre* (rupestrian fields) in the mountains of the Espinhaço. In this study, we aimed to test the hypothesis of spatio-temporal structuring of populations of *Scinax curicica* in the *campo rupestre* of the Espinhaço. We recovered three lineages of *S. curicica*: one lineage occurs in the north portion of the Espinhaço (North Lineage) in an area called Chapada Diamantina, and other two occur in the south portion of the Espinhaço (South 1 and South 2 Lineages) in Minas Gerais. All three lineages showed stable population sizes through time, probably due to the climatic stability of mountaintop areas, which was supported by ecological niche modelling.

Keywords: campos rupestres; phylogeography; speciation; treefrog; climatic oscillations

INTRODUCTION

Montane areas in tropical regions are often called 'cradles of biodiversity' and 'species pumps' because of their high species richness (García-Rodríguez *et al.* 2021). The ecological and evolutionary processes generating and maintaining high biodiversity in montane regions is an active area of research (Graham *et al.* 2014, Rahbek *et al.* 2019). Some mountain ranges referred to as Sky Islands present unique landscapes for organismal evolution because they comprise high mountain peaks separated by low valleys with vastly different environmental conditions (e.g. temperature, humidity, vegetation). These valleys act as barriers to the dispersal between populations of organisms adapted to environmental conditions at higher elevations, leading to isolation and divergence of populations on different mountains (McCormack *et al.* 2011, Sekar and Karanth 2013). Within high-altitude areas, usually above 1000 m a.s.l., elevational variation in environmental conditions can also result in barriers to dispersal and gene flow in some species or dispersal corridors for others, depending on their ecology (Haffer and Prance 2002, Salerno *et al.* 2012). Furthermore, these are dynamic systems in which environmental changes may result in alternating periods of isolation and connection of populations. Such histories are often manifest in the distributions and population genetic structure of organisms, and even with the diversification of species, as has been shown in species of lizards (Sinervo *et al.* 2010), mammals (Beever *et al.* 2011), and amphibians (Shepard and Burbrink 2008, 2009, Salerno *et al.* 2012).

The Espinhaço Mountain Range (hereafter Espinhaço) in eastern Brazil is formed of groups of smaller ranges (Sky Islands) in the states of Minas Gerais and Bahia, extending approximately

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1200 km from north to south, with altitudes varying from 800 to 2000 m a.s.l. The Espinhaço form a border between the Atlantic Forest to the east and the open, savanna landscapes of the Cerrado and Caatinga bioregions to the north and west (Guedes *et al.* 2020). The Espinhaço is divided into two main portions (from north to south): the north portion of the Espinhaço in Bahia State, known as Chapada Diamantina and the south portion, which extends from south-western border of Chapada Diamantina to the Quadrilátero Ferrífero region representing its southernmost limit, in Minas Gerais State.

Along the length of the Espinhaço, different altitudes rise to a variety of climatic conditions and habitats, with the *campo rupestre* (rupestrian fields) being the predominant formation at altitudes above 1000 m a.s.l. (Schaefer *et al.* 2016, Miola *et al.* 2021). The *campo rupestre* is characterized as a high-altitude mosaic of grassy-shrubby vegetation, interspersed with rocky outcrops, and encompassing grasslands with patches of transitional vegetation from Caatinga, Cerrado, and Atlantic Forest (Silveira *et al.* 2016). In terms of its ecological significance, *campo rupestre* has been identified as microrefugia during the interglacial periods of the Pleistocene, providing shelter for species adapted to cold and drought in tropical regions (Collevatti *et al.* 2012, Bonatelli *et al.* 2021). These rocky outcrops acted as refuges for species during climatic fluctuations, helping to maintain local biodiversity. Furthermore, research suggests that the cyclic expansion and contraction of species ranges during Quaternary climate changes have played a significant role in shaping the current levels of population genetic structure for *campo rupestre* species (Barres *et al.* 2019, Oliveira *et al.* 2021). These historical range dynamics have contributed to the genetic differentiation and isolation of populations, leading to unique genetic signatures within *campo rupestre* species.

Several recent phylogeographic studies have been published on endemic anuran species in the *campo rupestre* (Carvalho *et al.* 2020, Oliveira *et al.* 2021, Oswald *et al.* 2022); however, these focused on species restricted to the southern or northern portion of the Espinhaço. Nonetheless, these studies have demonstrated that, even at a smaller spatial scale, the mountains comprising the southern Espinhaço act as Sky Islands, generating strong genetic structure among populations of organisms occupying these rocky meadows. The leaf frog *Pithecopus megacephalus* was found to comprise three phylogeographic lineages, with one exclusively from a separate area in north Minas Gerais (Magalhães *et al.* 2017). The treefrog *Bokermannohyla saxicola*, another species endemic to the southern portion of the Espinhaço, was recently found to consist of four lineages that diverged from the Pliocene to Early Pleistocene (Oswald *et al.* 2022). A species from the same genus, *Bokermannohyla alvarengai*, which also occurs in the southern Espinhaço, was shown to comprise two geographically distinct lineages (Oliveira *et al.* 2021). Despite the number of lineages differing among these taxa, the genetic breaks appear concordant geographically, with the mountains in northern Minas Gerais separated from the others. One of the few studies with frogs from the northern portion of the Espinhaço in Bahia (Chapada Diamantina), was conducted with *Bokermannohyla oxente*, which is structured in two lineages even within its small distribution (Oliveira *et al.* 2021).

Among amphibians, treefrogs of the genus *Scinax* Wagler, 1830 form the second most diverse genus in Hylidae, and are represented by a wide range of species occupying different environmental conditions in the Neotropics (Faivovich *et al.* 2005). For some species of this genus, occurrence in the *campo rupestre* of the Espinhaço has been an important factor in speciation. Examples include the narrow endemic *Scinax cabralensis* found exclusively in Serra do Cabral, Minas Gerais, on the western slopes of the southern portion of the Espinhaço (Drummond *et al.* 2007), *Scinax machadoi*, which is endemic to the southern portion of the range (Leite *et al.* 2008), and *Scinax montivagus*, which is found only in Chapada Diamantina, Bahia, in the north portion of the Espinhaço (Juncá *et al.* 2015). In the Espinhaço, *Scinax curicica* occupies *campo rupestre* areas (Pugliese *et al.* 2004) with a discontinuous distribution along the whole Espinhaço, exclusively at altitudes above 850 m a.s.l. (Leite *et al.* 2006, 2008). The species reproduces mainly in permanent lentic environments, but also in small slow-flowing streams (Eterovick 2003, Pugliese *et al.* 2004).

Our study focuses on the spatio-temporal structuring of *S. curicica* populations in the *campo rupestre* of the Espinhaço. We propose that Pleistocene climate changes and the unique landscape of altitude islands played a key role in the species' diversification. These climate fluctuations prompted habitat shifts and speciation in various organisms. The EMR, with isolated high-altitude areas, created a fragmented landscape enabling geographic isolation and limited gene flow among *S. curicica* populations. These geographic barriers, combined with Pleistocene climatic variability, likely led to distinct genetic lineages. Accordingly, we predict genetic differentiation and unique adaptations in *S. curicica* populations across the *campo rupestre* of the EMR, reflecting specific local environments. We used phylogenetic analysis of mitochondrial and nuclear DNA, divergence dating, historical demography analysis, and ecological niche modelling (ENM) to examine the population history of this species. Specifically, we evaluated the evolutionary history and the effects of Quaternary climate change on the population demography of *S. curicica*, predicting that mountaintops act as Sky Islands, driving the diversification of genetic lineages within this endemic Espinhaço treefrog.

MATERIAL AND METHODS

Taxon sampling

DNA was sampled from 51 specimens from 13 sites along the Espinhaço (Fig. 1; Supporting Information, Table S1). Tissue was removed from either the thigh muscle or the liver. All samples were preserved in absolute (100%) alcohol and stored in a freezer at -20 °C until the time of DNA extraction. Voucher specimens are housed in the Amphibian Collection of the Universidade Federal de Minas Gerais (UFMG-AMP) (Supporting Information, Table S1). We conducted this study under Sistema de Autorização e Informação em Biodiversidade (SISBIO) license number ICMBio 21185-1.

DNA extraction, amplification, and sequencing

We extracted genomic DNA using either the CTAB technique or a Qiagen DNeasy Blood and Tissue® Kit (QIAGEN GmbH, Hilden, Germany). We performed PCR using specific primers for amplifying the mitochondrial 16S rRNA gene: 16sAR (CGCCTGTTTATCAAAAACAT) and 16sBR

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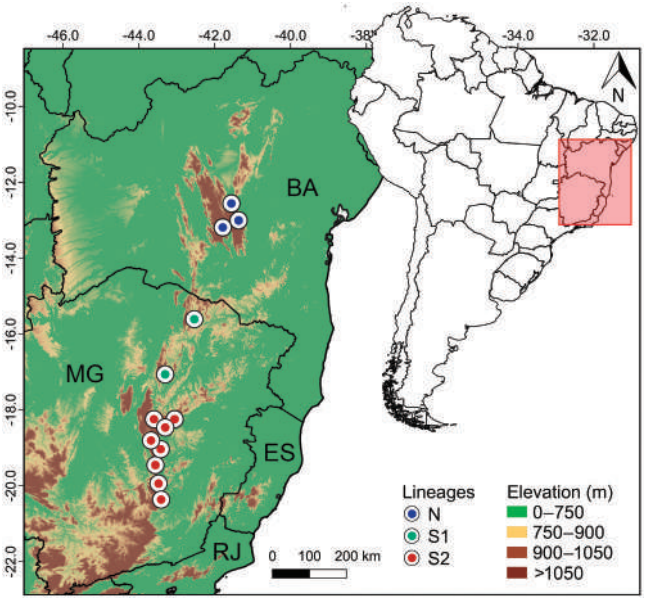

Figure 1. Topographic map showing the known geographic distribution of *S. curicica* in white circles. All genetic samples used in this study are illustrated by coloured circles within white circles coded by lineage. Abbreviations are as follows: MG—Minas Gerais, BA—Bahia, ES—Espírito Santo, RJ—Rio de Janeiro.

(CTCCGGTTTGAACTCAGATCA) (Palumbi *et al.* 1991). We also amplified a portion of the nuclear-encoded *RHOD* (rhodopsin) gene using the following primers: Rhod1A (ACCATGAACGGAACAGAAGGYCC) and Rhod1C (CCAAGGGTAGCGAAGAARCCTTC) (Bossuyt and Milinkovitch 2000). Reactions contained 0.5 U of GoTaq G2 Hotstart DNA polymerase (Promega), 1 × PCR buffer, 0.25 mM of each dNTP, 2 mM of $MgCl_2$, and 0.25 mM of each primer. PCR conditions followed Costa *et al.* (2016) and Mângia *et al.* (2020). We purified PCR products using the Acetate-Ammonium technique (Irwin *et al.* 2003) and we verified the quality of the purified DNA using a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, USA). Purified PCR products were then sequenced on an ABI 3500 Genetic Analyzer (Applied Biosystems, USA). We aligned sequences with Geneious® R9 9.1.3 (Biomatters Ltd, Auckland, New Zeland).

Mitochondrial phylogeny and molecular dating

We estimated a Bayesian gene tree for the 16S rRNA locus using BEAST 2.6 (Bouckaert *et al.* 2019). The most appropriate substitution model was GTR+I+G, which was determined using jModelTest (Darriba *et al.* 2012). We used a Yule speciation prior, implemented a strict-clock rate of 0.00735 (minimum of 0.0061; maximum of 0.0087) substitutions per site per million years (Gehara *et al.* 2014) with a uniform distribution prior, and ran the analysis for 50 million generations sampling every 5000 generations. We used Tracer 1.7.1 (Rambaut *et al.* 2018) to assess effective sample sizes (ESS) of estimated parameters and stationarity, ensuring that ESSs of all parameters were > 200 (Rambaut *et al.* 2018).

Population analysis assignment and genetic diversity

We performed analysis of population structure in GENELAND 4.0.3 (Guillot *et al.* 2005) as implemented in R 3.1.1 (R Core Team 2015). GENELAND analysis is a spatial genetic analysis tool that applies Bayesian methods to genetic data. It assumes that genetic information shows spatial patterns and aims to identify the number and locations of distinct populations. GENELAND deals with spatial genetic structure and population boundaries within a specific geographic region (Guillot *et al.* 2005). For this analysis, we used 16S rRNA sequences. We determined *K* by the Markov chain Monte Carlo method (MCMC) with six repetitions of *K* from 1 to 5. This range was chosen based on the main clades found in the mtDNA tree. We also performed species delimitation analysis using the generalized mixed Yule coalescence (GMYC) and multi-rate Poisson tree process (mPTP) methods. Both methods aim to identify species boundaries based on patterns observed in molecular phylogenies, given that GMYC employs the Yule process to model speciation and coalescence events (Fujisawa and Barraclough 2013), whereas mPTP determines the transition from a between- to a within-species process, incorporating different levels of intraspecific diversity deriving from differences in either the evolutionary history or sampling of each species (Kapli *et al.* 2017). In addition, mPTP has demonstrated superiority over PTP and other widely-used distance-based approaches, consistently providing more accurate delimitations in accordance with taxonomy (Blair and Bryson 2017, Kapli *et al.* 2017). For both analyses, we used the ultrametric 16S rRNA phylogenetic tree generated in BEAST. The GMYC approach was carried out in R 4.2.3 (R Core Team 2023) using the *splits* (Ezard *et al.* 2010) and *ape* (Paradis *et al.* 2004) packages.

Finally, we built haplotype networks for each of the genes using the median-joining method (Bandelt *et al.* 1999) implemented in Population Analysis with Reticulate Trees (PopART), using the standard configurations (Leigh and Bryant 2015). We calculated the number of haplotypes (h), haplotype diversity (Hd), and nucleotide diversity (π) for each mitochondrial and nuclear locus for each of the lineages identified by the GMYC and mPTP analysis using DnaSP (Rozas *et al.* 2017). We quantified genetic differentiation among and within lineages through Analysis of Molecular Variance (AMOVA) as implemented in PopART (Leigh and Bryant 2015).

Historical demography

We evaluated past changes in effective population size (N_e) of each lineage using the Bayesian Skyline Plot (BSP) method implemented in BEAST 2.6 (Bouckaert *et al.* 2019). For the BSPs, we used an average substitution rate of 0.00735 (with a minimum of 0.0061 and a maximum of 0.0087) substitutions per site per million years (Gehara *et al.* 2014) and the GTR+I+G substitution model. Each BSP analysis was evaluated with a piecewise-linear population function, five groups, 50 million generations, and 10% burn-in. We checked for stationarity by visually inspecting trace plots and ensuring that all values for effective sample sizes (ESS) were above 200 in Tracer 1.7.1 (Rambaut *et al.* 2018). We copied the curve values from Tracer 1.7.1 and built the BSP curve in Microsoft Excel.

Ecological niche modelling

In order to elucidate the effect of Quaternary climate change on the population structure of *S. curicica*, we used an ENM approach to model the species' potential distribution under past climate change scenarios. Current and historical (6 kya, 21 kya,

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and 120 kya) climatic data consisted of the 19 standard Bioclim variables (30 arc-seconds resolution) (Fick and Hijmans 2017). This period was selected because it represents the climatic extremes during the past 120 kya and other authors suggest that important climatic changes occurred in the *campo rupestre* during this period (Carvalho *et al.* 2020, Magalhães *et al.* 2021). We analysed a total of 26 occurrence records prior to filtering from the databases of the Mapinguari Laboratory at the Federal University of Mato Grosso do Sul, and the Sagarana Laboratory at the Federal University of Viçosa - Campus Florestal, Brazil. To avoid overprediction and low specificity, we cropped the environmental layers to span from -25° to -10° latitude and -48° to -38° longitude. Locality data were spatially filtered at 2 km^2 using the R package *spThin* (Aiello-Lammens *et al.* 2015) to eliminate spatial clusters of localities, which resulted in 23 spatially independent occurrence locations for modelling. To avoid bias related to multicollinearity of environmental explanatory variables, we calculated the Variance Inflation Factor (VIF) values for variables. All values that were highly correlated (VIF > 5) were removed through a stepwise procedure, using the *usdm* R package v.1.1-18 (Naimi 2017). Thus, we retained five of the 19 bioclimatic variables that were used throughout this study (Bio1—Annual Mean Temperature, Bio2—Mean Diurnal Range, Bio3—Isothermality, Bio14—Precipitation of Driest Month, Bio18—Precipitation of Warmest Quarter).

We performed species distribution modelling on *S. curicica* using nine different algorithms implemented in the '*biomod2*' package (Thuiller *et al.* 2016) in R 4.1 (R Core Team 2021) including the following: three regression methods [GAM: general additive model (Hastie and Tibshirani 1990), GLM: general linear model (McCullagh and Nelder 1989), MARS: multivariate adaptive regression splines (Friedman 1991)]; three machine learning methods [GBM: generalized boosting model (Ridgeway 1999), MAXENT: Maximum Entropy (Phillips *et al.* 2006), RF: random forest (Breiman 2001)], two classification methods [CTA: classification tree analysis (Breiman 1984), FDA: flexible discriminant analysis (Hastie *et al.* 1994)], and one envelope model [SRE: Surface Range Envelop (Booth *et al.* 2014)]. To meet the criteria of having absence (or pseudo-absence) data for most of these models (except SRE), we generated two equal-sized (to the true presence records) sets of random pseudo-absence (PA) points across the model background (500 PA points in each set). The models were calibrated using 70% of randomly selected data. The other 30% of the data were used for intrinsic model evaluation.

Individual model performance was evaluated using two metrics—true skill statistic (TSS) and the area under the curve of receiver operating characteristics (ROC) implemented in the '*biomod2*' package. TSS is calculated as 'sensitivity + specificity -1' and ranges from -1 to +1, where +1 indicates perfect agreement, a value of 0 implies agreement expected by chance, and a value of less than 0 indicates agreement lower than expected by chance. Models with high predictive accuracy (TSS > 0.8) were used for the projection of anuran distribution. We constructed ensemble maps based on the median of two runs of all the selected models in which individual accuracy had a TSS value equal to or greater than 0.8. Regions of habitat stability through time were identified by stacking and averaging the current and three projected-palaeoclimate ENMs. Regions highlighted in these stacked projects were inferred to be regions of climate refugia through time for *S. curicica*.

RESULTS

Mitochondrial phylogeny and molecular dating

The 16S rRNA gene tree topology recovered three main clades in *S. curicica* (Fig. 2). One lineage occurs in the north portion of the Espinhaço (North Lineage), in the Chapada Diamantina region, precisely in the Serra da Almas, Serra da Tromba, and Serra do Sincorá, and the other two lineages occur in the South Espinhaço (South 1 and South 2 Lineages) in Minas Gerais (Fig. 1). South 1 Lineage occurs from the Quadrilátero Ferrífero region, along the Serra do Cipó. South 2 Lineage occurs in the Serra de Itacambira and Serra Nova (Supporting Information, Table S1).

The three main clades, which corresponded to the three lineages, were recovered with strong support of posterior probability (pp ≥ 0.99; Fig. 2). The divergence between the North Lineage and Southern Lineages occurred around 2.51 Mya (95% HPD: 1.73–3.35 Mya), and the separation between the two South lineages dated to about 1.68 Mya (95% HPD [Highest Posterior Density]: 1.11–2.31 Mya; Fig. 2).

Population analysis assignment and genetic diversity

Using both mtDNA and nuDNA loci, GENELAND detected three populations corresponding to the three mitochondrial lineages ($K = 3$; Fig. 3; Supporting Information, Fig. S1), and revealed a clear geographical population structure concordant with the different regions of the Espinhaço. The GMYC and mPTP (likelihood ratio: 9.364408; Null-model score: -47.635644) species delimitation methods yielded identical results, recovering the same three evolutionary entities (Fig. 2).

The 16S rRNA haplotype network revealed high haplotype diversity with no shared haplotypes among the three lineages (Fig. 2). Despite high diversity in mtDNA, our haplotype network of the nuclear-encoded *RHOD* gene showed only two haplotypes with no congruence with geographic distributions. In our AMOVA analysis, we found a high index of fixation for the mitochondrial 16S rRNA (Fst = 0.93924, $P < 0.001$), whereas the index of fixation was low for *RHOD* (Fst = 0.41322, $P < 0.001$), indicating most of the variation in *RHOD* occurs among localities rather than among populations.

Historical demography and ecological niche modelling

The North population of *S. curicica* had the most recent coalescent point, approximately 47 kya. On the other hand, both South 1 and South 2 populations had their coalescent points around 300 kya. Despite the different coalescent times, all three populations show stable effective population sizes (N_e) through time (Fig. 4).

The resulting ensemble models based on nine SDM algorithms yielded average values of TSS = 0.97 and ROC = 0.99. The variable Bio1 (annual mean temperature) was the most important variable (63% of explication) to *S. curicica* distribution, followed by precipitation of driest month (Bio14, 36% of explanation) and precipitation of warmest quarter (Bio18, 33% of explication). The potential distribution for *S. curicica* (Fig. 5) generated by ENMs for the present coincides well with

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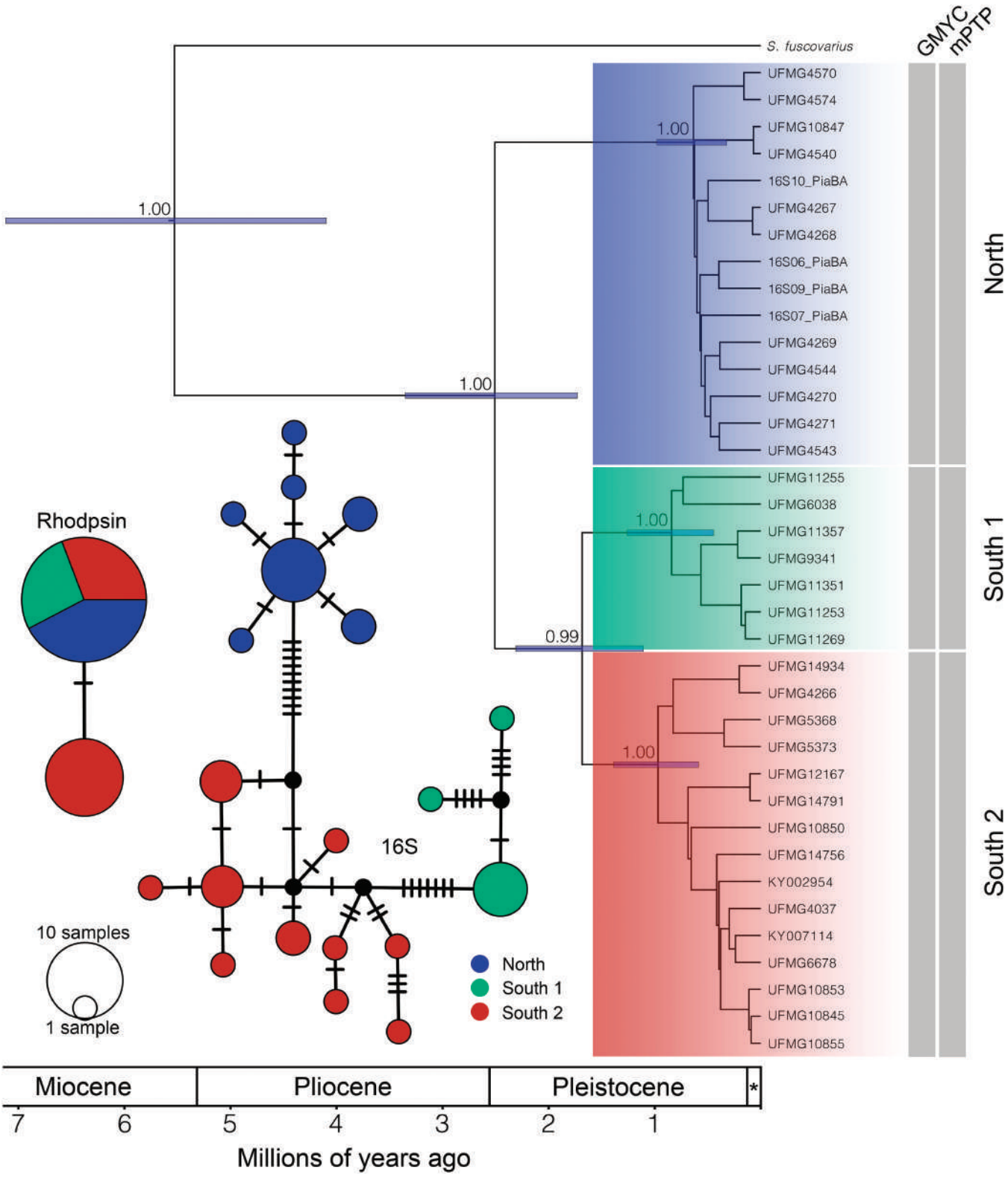

Figure 2. Phylogeny and divergence times of *S. curicica* lineages estimated in BEAST, with results from GMYC and mPTP analysis. Haplotype networks from neighbour-joining analysis for the two genes (16S rRNA and Rhodopsin). Crossed traits indicate additional mutational steps for branches with more than one mutation. Different colours indicate species-level units. The black dots are median vectors (hypothesized sequences).

the species known distribution throughout the Espinhaço, although it also predicts suitable conditions exist in other mountainous areas in south-eastern Brazil, including part of the Serra da Mantiqueira Mountain Range, and other highlands in the Cerrado ecoregion. The predicted distribution for the Holocene (6 kya) climate indicates minor changes for *S. curicica*. However,

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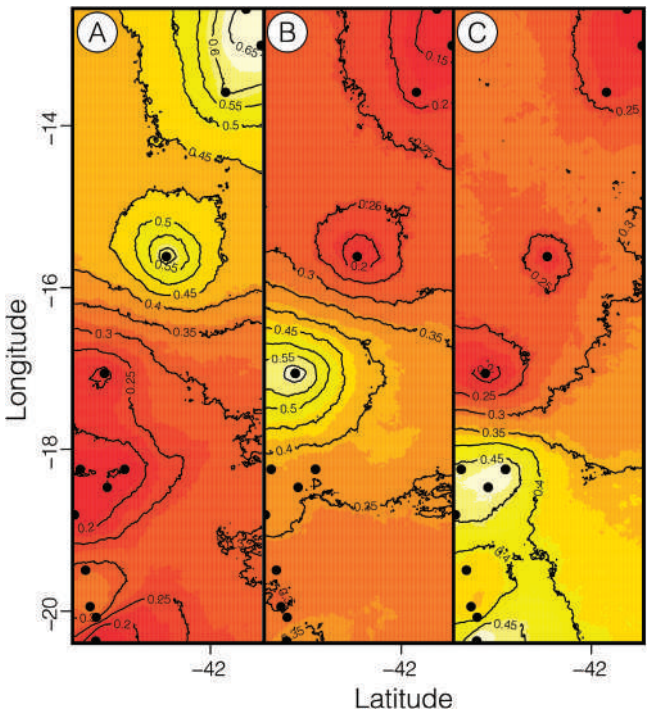

Figure 3. GENELAND analysis of *S. curicica* with posterior probability isoclines, indicating extensions of the genetic populations found (black lines with inclusion probabilities). Light colour zones in maps indicate the groups of localities with greater probabilities of belonging to the same genetic unit. Black dots indicate locations of the 13 analysed localities. (A) North population, (B) South 1 population, and (C) South 2 population.

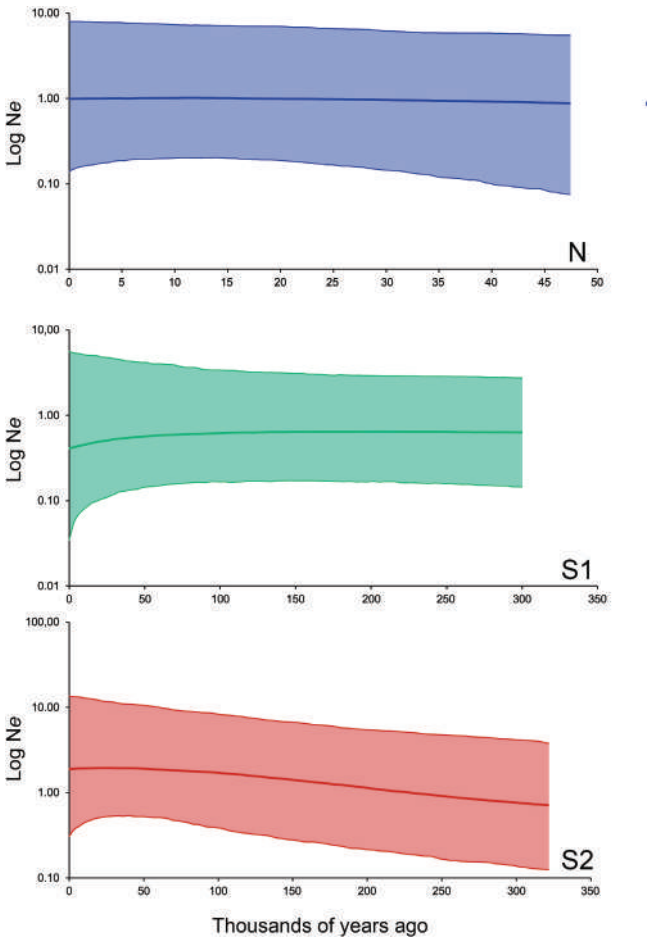

Figure 4. Bayesian skyline plots illustrating effective population sizes (N_e) through time for the three *S. curicica* lineages: North (N), South 1 (S1), and South 2 (S2). The darker inner line represents median population size, and the shaded area represents 95% HPD.

during the Last Glacial Maximum (21 kya) the potential distribution was immensely expanded throughout surrounding regions. The predicted distribution of *S. curicica* during the Last Interglacial (120 kya) was similar to the current and Holocene periods but slightly more expansive. The stability map generated with all four distribution models suggests suitable regions with a similar distribution to the current known distribution for this species, predicting mainly the highlands of the Espinhaço.

DISCUSSION

We found that *S. curicica* comprises three geographically distinct lineages that originated at the Pliocene–Pleistocene boundary (~2.51 Mya) and in the Early Pleistocene (~1.68 Mya). The three lineages, which were supported by the GENELAND analysis and in both species' delimitation methods, have remained distinct throughout much of the Pleistocene. Our ENMs suggested climatically stable regions very similar to the current species' distribution, which coincides with the Sky Islands of the Espinhaço Range (elevations > 1000 m a.s.l.). Our models indicate potentially suitable habitat for this taxon has not shifted greatly through time, despite the great expansion over 21 kya, which supports a strong relationship with a *campo rupestre* mountaintop habitat for this species. The climatic scenario in Sky Islands is known for promoting diversification in several amphibians throughout the globe (Shepard and Burbrink 2008, 2009, Salerno *et al.* 2012, Pan *et al.* 2019), including in other mountain complexes in southern Brazil (Pie *et al.* 2018) and even for other hylids in the Espinhaço (Carvalho *et al.* 2020, Magalhães *et al.* 2021, Oliveira *et al.* 2021, Oswald *et al.* 2022). Our findings for *S. curicica* corroborate the same climatic scenario for the other hylids from Espinhaço, in which mountaintops acted as refugia during the climatic oscillations of the Pleistocene, promoting allopatric divergence (Nascimento *et al.* 2018, Oliveira *et al.* 2021, Oswald *et al.* 2022). The stable population sizes for the three lineages we recovered also supports the stability of climate and habitat on mountaintops along the Espinhaço.

Consistent with the Espinhaço being Sky Islands, climatic breaks are evident along the range, with a marked break between the northern (Chapada Diamantina) and southern portions, and other discrete breaks in the southern portion (Fig. 5). These breaks coincide with the distributions of the three allopatric lineages of *S. curicica*, reinforcing their geographic isolation. Despite the climatic stability through time on the mountaintops, divergences among the three *S. curicica* lineages are older than the modelled climatic scenarios, but still within the Pleistocene, a period characterized by global climatic oscillations (Peçanha *et al.* 2017, Cassino *et al.* 2020, Rocha *et al.* 2020). Divergence times among *B. saxicola* lineages within the Southern Espinhaço and for *B. oxente* within the north portion of the Espinhaço, also occurred during the Pleistocene, with well-structured geographic distributions on Sky Islands (Oliveira *et al.* 2021, Oswald *et al.*

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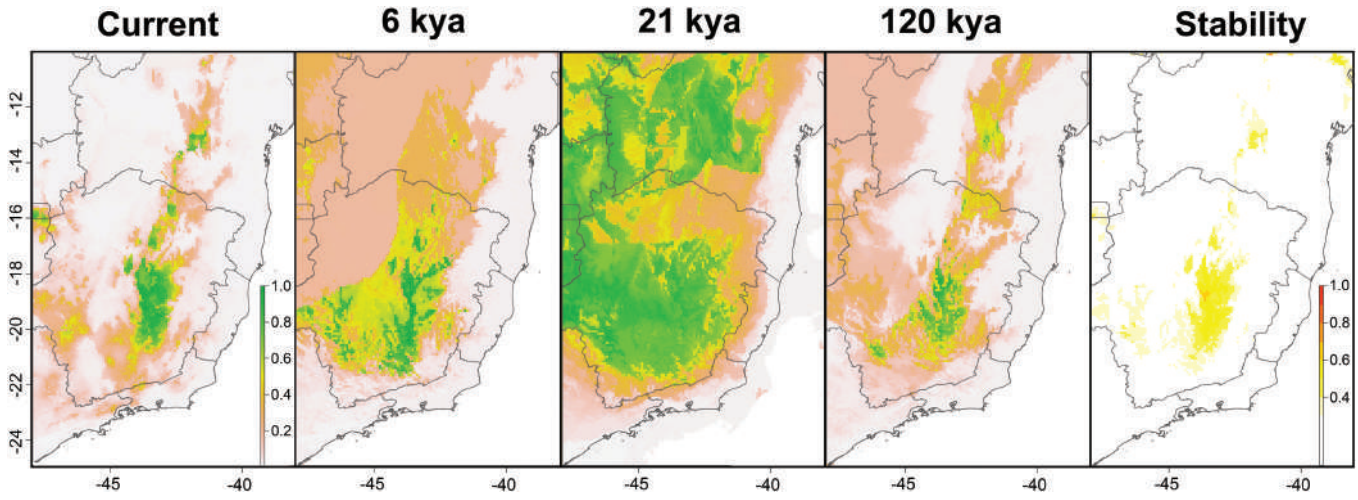

Figure 5. ENMs for *S. curicica*. From left to right, the ENM under current climate conditions, Mid-Holocene climate conditions (~6 kya), Last Glacial Maximum (LGM) climate conditions (~21 kya), Last Interglacial (LIG, ~120 kya), and regions of climate suitability through time based on all four projections.

2022). Persistence and allopatric divergence of *S. curicica* on the Sky Islands of the Espinhaço throughout the Pleistocene are consistent with results for many other amphibians in different Sky Island systems (e.g. Shepard and Burbrink 2008, Salerno *et al.* 2012, Magalhães *et al.* 2021, Ortiz *et al.* 2022). Our results provide additional support for the importance of Pleistocene climatic oscillations in promoting lineage divergence and speciation in montane anurans.

The major portions of the Espinhaço share similar geomorphological features with many mountaintops and intervening valleys within the major portions. Even these smaller valleys, which are not as low in elevation or as wide as the valleys between the major portions, can act as barriers to gene flow and promote diversification. For example, the paratelmatobiin frog, *Rupirana cardosoi*, occurs only in the northern portion of the Espinhaço (Chapada Diamantina), but two populations occur within this portion, which were recently shown to be two allopatric lineages (Santos *et al.* 2020). Many other anurans, and perhaps other dispersal-limited organisms, will probably share similar patterns of population genetic structure. The same microregional scenario could occur with other frog genera such as dwarf odontophrynids of the genus *Proceratophrys* (Teixeira Junior *et al.* 2012), the direct-developing strabomantid *Pristimantis* (Trevisan *et al.* 2020), and other treefrogs of the genus *Bokermannohyla* (Oliveira *et al.* 2021). Climatic heterogeneity in Sky Island landscapes can result in both broad- and fine-scale patterns of genetic differentiation and thus, the ecology of each species must be considered in order to understand how climatic variation affects different species (Shepard and Burbrink 2008, Gehara *et al.* 2017).

Few anuran species (or species complex) endemic to the Espinhaço are well distributed throughout the range, occurring in both its northern and southern portions. Although *Pleurodema alium* (see Maciel and Nunes 2010: fig. 4) and *Leptodactylus avivoca* (see Carvalho *et al.* 2020: fig. 1) occur in the south portion of the Espinhaço and in the Espinhaço of the Bahia State, neither of these species reach the Chapada Diamantina in fact, as they occur in an extension of the southern Espinhaço throughout Bahia. To date, *S. curicica* (Leite *et al.* 2008, present work) and the *Odontophrynus juquinha* complex (Rocha *et al.* 2017, Moroti *et al.* 2022) are the only anurans with this distribution, occurring from southernmost Espinhaço to southern Chapada Diamantina, and revealed a similar allopatric scenario of lineage diversification. A recent evaluation of *O. juquinha* even suggested that populations from north Espinhaço (Chapada Diamantina) are a putative new species (Moroti *et al.* 2022).

Here we showed *S. curicica* is composed of three genetically and geographically distinct lineages across the Espinhaço. Following these results, we advocate for an integrative taxonomic approach including a thorough study of the acoustics, and adult and larval morphology to determine if these lineages should be considered distinct species (Padial *et al.* 2010). Until this is determined, we suggest the North and South 1 Lineages warrant special attention for conservation purposes because they occur in a small area, restricted to a few Sky Islands above 1000 m a.s.l.

SUPPLEMENTARY DATA

Supplementary data is available at *Biological Journal of the Linnean Society* online.

Table S1. Collecting localities, GenBank accession numbers, corresponding lineage for each individual sample sequenced, and voucher accession information for all samples included in the molecular analyses.

Figure S1. Plot of the number of populations simulated from the posterior distribution obtained with GENELAND for *Scinax curicica*.

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DATA AVAILABILITY

The data and script supporting this study are available at: https://github.com/Rhinella85/Scinax-curicica-phylogeography

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