

Comparative landscape genetics in two amphibians demonstrates the influence of habitat specialization in a mountain range in Northeastern Brazil

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Abstract

Context Within the same geographical area, geographical distance is the most important landscape feature limiting dispersal in some species, whereas for others environmental variation, such as climate and vegetation patterns, may curtail dispersal. Such landscape effects are usually determined by the ecological traits of the species. Although very intuitive, landscape hypotheses remain poorly investigated in tropical regions. **Objectives** We developed a comparative landscape genetic study evaluating the association and importance of geographic and environmental landscape features on the genetic differentiation in two ecologically differing anurans, the treefrog *Dendropsophus oliveirai* (a reproductive habitat specialist) and the chorus frog *Physalaemus cuvieri* (a reproductive habitat generalist), in highland forests inserted in a dry vegetation matrix in Northeastern Brazil. **Methods** We applied Mantel tests and Structural Equation Modeling to assess associations between geographic distance and environmental factors on mitochondrial genetic variation of the two study species. **Results** We found lower genetic differentiation in *P. cuvieri*, mainly associated with geographic distance along a latitudinal gradient and weakly influenced by environmental variation among highland forests. Conversely, the higher genetic differentiation in *D. oliveirai* was associated with a combination of geographic distance along a longitudinal gradient, environmental variation, and past climatic variation. **Conclusions** Idiosyncrasies in the genetic structure and associations with landscape features in the two species may have resulted from different abilities to cross the dry lowland among the mesic highland forests. Our findings also suggest selective and neutral evolutionary mechanisms conducive to intraspecific genetic variation in the study area.

Introduction

Dispersal is an eco-evolutionary process of movement of individuals across space, influencing gene flow and genetic differentiation between populations (Travis et al. 2012). If the rate of dispersal is low, gene flow will be low and genetic distances will increase with time. Space alone can affect dispersal and gene flow, given that it decreases the likelihood of successfully dispersing through distant locations. This neutral effect of space in isolating populations as a result of their dispersal ability is known as Isolation by Distance (IBD) (Wright 1947; Jenkins et al. 2010), and is observed as a positive relationship between geographic distances and genetic distances. Yet, space is rarely homogeneous and distinct landscape features, such as climatic variation and vegetation, may impede or reduce dispersal, consequently affecting gene flow. In such cases, the environment plays an important role in isolating populations and influencing genetic differentiation, generating a pattern of Isolation by Environment (IBE) (Wang and Summers 2010; Wang and Bradburd 2014), where the environmental distance is a better predictor of genetic distances. These two processes are not mutually exclusive, although one can have a predominant influence relative to the other (Sexton et al. 2013; Wang et al. 2013).

Species are constantly adapting and, as a consequence of each unique eco-evolutionary history, each species has its own peculiarities when interacting with the environment. Specifically, while some species tolerate a wider range of environmental conditions, others have more specific requirements. Thus, within the same geographical area, we may find some species to which environmental variation poses no friction to dispersal, and species to which it represents a limiting factor curtailing dispersal (Goldberg and Waits 2010;

Richardson 2012; Wang et al. 2013; Wollenberg Valero 2015; Garcia et al. 2017; Lourenço et al. 2019). Additionally, different comparative studies with plants (Hamrick and Godt 1996; Duminil et al. 2007; Alvarez et al. 2009) and animals (Nevo and Beiles 1991; Luiz et al. 2012; Pabijan et al. 2012; Paz et al. 2015; Rodríguez et al. 2015) indicate that life history traits such as mating system, life form, seed dispersal, vagility, reproductive behavior, and body size are related to genetic divergence among populations, explaining idiosyncrasies in genetic distances of co-occurring species. Although intrinsic factors, such as body size, have been suggested as important ecological predictors for the distribution of genetic variation in amphibians (Pabijan et al. 2012; Paz et al. 2015), in many tropical anuran species, topographic complexity and macrohabitat type have also been demonstrated as key ecological constraints (Rodríguez et al. 2015).

Habitat generalist anuran species usually present reduced intraspecific genetic divergence due to their capacity to breed in different sites along their distribution (even in dry landscapes), maintaining gene flow and low genetic differentiation between populations. Conversely, habitat-specific anurans restricted to forests tend to show higher genetic differentiation because of their lower dispersal ability (plus lower vagility in some species) in dry landscapes among forest patches (Rodríguez et al. 2015). These divergent responses to landscape configuration were also observed in other contemporary studies of genetic differentiation in amphibians in different systems across the planet (e.g. Keyghobadi et al. 2005; Blair et al. 2013; Coster et al. 2015; Firkowski et al. 2016; Sandberger-Loua et al. 2017; García-Rodríguez et al. 2021; Antunes et al. 2022). Thus, it would be reasonable to assume that specialist species lie at one end of a continuum, where dispersal decreases as environmental distance increases, leading to IBE (Wang and Bradburd 2014) that is not necessarily associated with geographic distance. At the other end of this continuum lie generalist species with broader habitat tolerance, where dispersal is only or mostly affected by geographic distances, leading to IBD (Jenkins et al. 2010; Batalha-Filho et al. 2023). Although this assumption makes sense from a theoretical perspective, it still has a lot of room for further investigation and comparative studies, especially in tropical regions (Storfer et al. 2010; Manel and Holderegger 2013; Torres-Florez et al. 2018; Monteiro et al. 2019; Covarrubias et al. 2021; Carvalho and Côrtes, 2023).

Complex landscape systems, such as mountain ranges, have higher habitat heterogeneity which leads to increased intraspecific genetic differentiation (Guarnizo and Cannatella 2013; Rodríguez et al. 2015). The Borborema Plateau is a geomorphologic unit distributed north of the São Francisco River in north-eastern Brazil, originally covered by Atlantic Forest and Caatinga (seasonally dry forest and shrub) vegetation. This mountain range is environmentally heterogeneous, with different types of Caatinga vegetation covering lowlands which surround sparsely distributed mountains and highlands 500–1100 m a.s.l, where remnants of tropical Atlantic Forest predominate (Andrade-Lima 1982; Velloso et al. 2002; Tabarelli and Santos 2004; Ribeiro et al. 2009; Silveira et al. 2019). It has been suggested that local landscapes became more arid after the Pleistocene, resulting in the extirpation of taxa adapted to wet climates and the expansion of dry-adapted ones (Gehara et al. 2017), particularly in lowland areas. Thus, highland forests represent current climatic and environmental microrefugia for plants and animals adapted to mesic conditions (Santos et al. 2007; Carnaval et al. 2009; Porto et al. 2013; Carnaval et al. 2014; Costa et al. 2017; Silveira et al. 2019; Castro et al. 2019).

Although all highland forests of the Borborema Plateau share similar mesic conditions, they differ in terms of vegetation structure, plant species composition, and climate because of their geographic position along a steep longitudinal gradient in annual rainfall. Forest enclaves closer to the coast experience higher annual precipitation (1,000–1,600 mm) and support denser forests, whereas those located further west are subject to lower annual rainfall (400–600 mm), resulting in increased presence of Caatinga vegetation (Velloso et al. 2002; Rodal et al. 2008; Silva et al. 2018; Silveira et al. 2019). Such environmental differences could preclude connections among highland populations depending on the ecology of the species (Funk et al. 2005; Pease et al. 2009; Ortego et al. 2012; Lawson 2013; Santana et al. 2024). Thus, highland forests of the Borborema Plateau provide an ideal system to investigate different hypotheses of landscape genetics in a tropical region and to test the relative influence of selective and neutral processes in the patterns of intraspecific genetic variation (Hendry et al. 2002; Nosil and Crespi 2004; Wang and Summers 2010).

In this study we carried out a comparative landscape genetics analysis using data from the same localities to evaluate the association and importance of landscape features on the genetic variation among populations of two amphibian species with different ecologies, *Dendropsophus oliveirai* (Bokermann, 1963) (Hylidae) and *Physalaemus cuvieri* Fitzinger, 1826 (Leptodactylidae). *Dendropsophus oliveirai* is a small treefrog distributed from Central to Northern Atlantic Forest of Brazil, occurring mainly along lowland coastal rainforests but also in ecotonal areas, and reaching moist habitats within the Caatinga biome (Frost 2023; Magalhães et al. 2013; Menezes et al. 2020). It depends on permanent or long-lived ponds located on forest edges or adjacent dry areas to breed. Their eggs are deposited on the herbaceous vegetation hanging over such ponds, where tadpoles dive as soon as they hatch to complete their development (Haddad and Prado 2005; Haddad et al. 2013; Silva et al. 2019). Conversely, *P. cuvieri* is a small chorus frog widely distributed in different biomes of South America, from Argentina to Venezuela (Frost 2023). It breeds in a great variety of lentic water bodies, ranging from small rain puddles with very short hydroperiods to the margins of lakes and ponds located in dry areas or within forest habitats (Pombal and Haddad 2007; Aguiar et al. 2014), suggesting broad environmental tolerance (Barreto and Andrade 1995; Oliveira et al. 2019). Mating pairs of *P. cuvieri* deposit their eggs in foam nests built on the margins of water bodies, where tadpoles complete their development (Barreto and Andrade 1995; Haddad and Prado 2005; Grosso et al. 2019). Here, we considered historic climatic instability and contemporary environment as proxies of IBE, and geographic distance as proxies of IBD. We developed this comparative genetic landscape approach with the following hypotheses: (I) since habitat specialist species are more affected by landscape configuration, geographic populations of *D. oliveirai* should present higher genetic structure and IBE, with a secondary influence of IBD, as the environment varies geographically across the Borborema Plateau (Pertoldi et al. 2014; Wang and Bradburd 2014); (II) the habitat generalist species, populations of *P. cuvieri* should have lower or no genetic structure across the plateau, as predicted by IBD, since environmental variation potentially represents less friction to its dispersal in the landscape (Jenkins et al. 2010).

Materials and methods

Study design and collection of tissue samples

From November 2020 to April 2023, we collected thigh muscle tissue from 53 *Dendropsophus oliveirai* and 56 *Physalaemus cuvieri* specimens from natural ponds and puddles in seven highland forests of the Borborema Plateau in Northeastern Brazil (Fig. 1; Table 1). Previously to tissue dissection, all specimens were anesthetized and euthanized using topical benzocaine (50 mg/g). Each tissue sample was preserved in 98% alcohol and stored under – 20°C until DNA extraction. All samples and specimens were deposited at Coleção Herpetológica da Universidade Federal de Pernambuco (CHUFPE; Supplementary Information S1), in Recife, Pernambuco, Brazil.

Table 1

Geographical coordinates and elevation of sampling localities in Northeastern Brazil, and number of samples of *Dendropsophus oliveirai* and *Physalaemus cuvieri* obtained in each locality of the Borborema Plateau

Sampling locations	Subregion	Latitude	Longitude	Elevation (m a.s.l)	Sample size	
					D. oliveirai	P. cuvieri
1 – Pau Ferro	Northeast	06°58'12.53" S	35°43'04.27" W	550	7	8
2 – Mata do Siriji	Northeast	07°37'01.79" S	35°30'27.84" W	537	3	8
3 – João Vasconcelos	Central	08°21'8.80" S	36°01'51.60" W	803	7	8
4 - Tabocas	Central	08°14'47.5" S	36°22'46.00" W	677	12	8
5 – Catimbau	Central	08°34'20.60" S	37°14'12.30" W	823	10	8
6 – Pedra Talhada	Southeast	09°15'40.94" S	36°26'20.22" W	536	6	8
7 - Craunã	Southwest	09°20'47.90" S	37°52'60.00" W	338	8	8
Total					53	56

DNA extraction, amplification, and sequencing

We extracted genomic DNA from tissue samples using Chelex-100 chelating resin (Walsh et al. 1991). We immersed an aliquot of each tissue sample in 200 µl 10% Chelex and incubated at 58°C for 160 min. Then we transferred the sample to a thermocycler and incubated them at 94 °C for 30 min. After that, we centrifuged them for 10 min at 13,000 rpm. Lastly, we collected the resulting supernatant and permanently stored it at -20°C. From these DNA samples, we amplified a fragment of the 16S rDNA mitochondrial gene using primers 16sarL (5– *CGCTG* † *TATC* ∇ ∇ *ACAT* – 3) and 16sbrH (5 – *CGGTCTG* ∇ *CTCAGATCACGT* – 3) (Palumbi et al. 2002). For each sample we used 12.5 µL GoTaq DNA polimerase (Master Mix; Promega), 8.5 µL nuclease-free water, 1.0 µL of each primer, and 2.0 µL DNA. Polymerase Chain Reactions (PCR) consisted of an initial denaturation step at 94°C for 30 s followed

by 35 cycles of 94°C for 10 s, 50°C for 35 s and 72°C for 90 s and final extension at 72°C for 10 min. We purified PCR products with the Wizard SV GEL/PCR Clean-up System and ReliaPrep DNA Clean-Up and Concentration System (Promega), following the manufacturer's protocol. Amplicons had a final average concentration of 73.5 ng/μL (20.1–133.5 ng/μL) and were diluted to 30 ng/μL for sequencing. We sequenced PCR products in both directions using the BigDye Terminator v.3.1 kit (ThermoFisher) and a Genetic Analyzer 3500 automated sequencer (Applied Biosystems). We produced consensus sequences using the software Pregap4 v.1.6-r and Gap4 of the Staden Package (Staden 1996). Consensus sequences ranged between 607 and 629 bp after editing in MEGA v.11.0.13 (Tamura et al. 2021). After alignment using MAFFT v7.017 (standard parameters; Katoh 2002) and trimming using MEGA, we obtained a sequence matrix with 551 bp for *D. oliveirai* and with 599 bp for *P. cuvieri*. All sequences used are available in GenBank (*P. cuvieri*: OR491066–OR491121; *D. oliveirai*: OR491200–OR491252).

Genetic diversity and phylogeographic analyses

In order to assess patterns of genetic diversity and genetic structure, we calculated the number of segregating sites (S), nucleotide diversity (Pi), haplotype number (h), and haplotype diversity (Hd) in samples of each locality in DNAsp v. 6.12 (Rozas et al. 2017). To evaluate genetic differentiation among localities we estimated pairwise genetic differentiation between and within localities using uncorrected fixation indexes (Fst and Fis) and estimated the relative partitioning of genetic variation through standard molecular analysis of variance (AMOVA), under $p < 0.05$ using 10,000 permutations, in Arlequin v.3.5.2.2 (Excoffier and Lischer 2010). We performed a Bayesian Analysis of Population Structure (BAPS) using the *hierBAPS* function from the package *rhierbaps* (Cheng et al. 2013) in R (R Core Team, 2022) to infer the most likely number of genetic clusters and their distribution across sampling localities based on nucleotide frequencies. In BAPS, those individuals assigned to the same cluster are considered genetically more similar (Corander and Tang 2007; Corander et al. 2008). BAPS analyses were set to a maximum number of 10 populations and three levels of hierarchical search in both species. We used the same 16SrDNA alignment to build haplotype networks using the median-joining method (Bandelt et al. 1999) in PopArt v.1.7 (Leigh et al. 2016) in order to describe the spatial structure and relationships among the haplotypes identified in DNAsp v.6.12.

Environmental data: climate, altitude and forest cover

To represent current landscape features, we gathered rasters of 19 bioclimatic variables (spanning the years 1970–2000) and elevation data from WorldClim v.2.1 at 30 arc-sec resolution ($\sim 1 \text{ km}^2$) (Fick and Hijmans 2017) using the *raster* (Hijmans et al. 2017), *sp* (Pebesma and Bivand 2005), and *rgeos* (Bivand and Rundel 2023) packages and the *extend* and *crop* functions in R. We then extracted values to each of the seven sampling localities using the *extract* function (Hijmans et al. 2017) (Supplementary Information S2). We also gathered EVI (Enhanced Vegetation Index) values to each locality through MODIS VI satellite images, in 8 arc-sec resolution (250 m) (MOD13Q1.061 Terra Vegetation Indices 16-Day, years 2000–2022) using Google Earth Engine (<https://earthengine.google.com/>) (Gorelick et al. 2017). EVI reduces the effects of atmospheric elements while increasing the range of vegetation signals (Huete et al. 1997). Finally, we calculated the major and minor average of EVI using, respectively, maximum and minimum EVI annual values to each location (Supplementary Information S2). We used these averages as proxies to forest vegetation coverage during the rainy and dry seasons. The higher layer resolution for EVI calculations allows

for a more precise sample-point estimation. Although the bioclimatic variables and elevation data are the same for both species, EVI data varied slightly between species depending on the exact pond where they were collected within each locality. Hence, we used two separate environmental datasets for current environmental variation, one for each species. These datasets compile adequate descriptors of the environmental conditions observed in the sampling sites, with potential effects on amphibian dispersal, as they have been described to be associated with amphibian water balance, locomotor performance, and susceptibility to infections (Raffel et al. 2006; Titon Junior and Gomes 2015; Mitchell and Bergmann 2016).

Past climatic variation

To represent past climatic variation among sampling localities, using the same methods and packages described above, we gathered rasters of the same 19 bioclimatic variables from WorldClim v.1.4 for the Mid Holocene (Mid-Hol; 30" arc-seconds = $\sim 1 \text{ km}^2$), Last Glacial Maximum (LGM; 2.5' arc-minutes = $\sim 5 \text{ km}^2$) (Fick and Hijmans 2017) and Last Interglacial Maximum (LIG; 30" arc-seconds) (Otto-Bliesner et al. 2006). We used the most precise resolution available in Worldclim for each period. We extracted values from three different models (CCSM4, MIROC-ESM, and MPI-ESM-P) for Mid-Hol and LGM periods (LIG has only one model available). Then, we calculated the average of each variable for Mid-Hol and LGM using those three different values (Supplementary Information S3). Finally, we calculated the variance of each bioclimatic variable between LIG and present (1970–2000) using values from each period: present (1970–2000), Mid-Hol (average), LGM (average), and LIG. These variance values (Supplementary Information S4) were used to represent the past climatic stability of each locality.

Statistical analyses: associations among different data sets

We used two different and complementary approaches to test associations and effect sizes of current environment, past climatic variation, and geographic distance on the genetic variation among geographic populations of the two study species. To test correlations between genetic distance matrices and matrices representing environmental or geographic distances, we applied Mantel and partial Mantel tests (Mantel 1967). We produced an environmental distance matrix among localities represented by Euclidian distances calculated from the values of bioclimatic variables (Worldclim; 1970–2000) and EVI (maximum and minimum) for each locality. Similarly, calculated a past climate variation distance matrix among localities using variance values of each bioclimatic variable obtained previously. We also created a geodesic geographic distance matrix (Supplementary Information S5) from GPS coordinates using the *rdist.earth* function from the *fields* v.14.1 R package (Nyckka et al. 2021). We conducted Mantel and partial Mantel tests with 1,000 permutations via the *Pearson* method in the *vegan* v.2.6.4 R package (Oksanen et al. 2022) using the pairwise genetic differentiation (*Fst*) matrix between localities, environmental distance, past climatic variation distance, and geographical distance matrices. Geographic distance was used as a covariate of environmental and past climatic variation distances in the partial Mantel tests. Considering a possible influence of the past climate on the current climate, we included past climate variation distance as covariate in the partial Mantel tests when necessary. Finally, we conducted a Structural Equation Modeling (SEM) analysis using the *lavaan* v.0.6.16 R package (Rosseel 2012; Fan et al. 2016) to verify the direct and indirect relationships among all variables simultaneously through regression models and to assess the degree of contribution that each predictor variable had on genetic variation. To apply SEM, we used as a

response variable the first principal component (PC1: Supplementary Information S6) from a principal component analysis (PCA) conducted on the 16rDNA alignment using the *adeigenet* R package (Jombart 2008). As predictor variables, we used the environmental PC1 (Supplementary Information S6), the past climatic variation PC1 (Supplementary Information S6) – both obtained from PCA conducted on the environmental variables in the *FactoMineR* v.2.8 R package (Lê et al. 2008) – and the geographic coordinates (latitude and longitude). Due to different magnitudes of each predictor, we standardized them before applying SEM.

Results

Genetic diversity and differentiation

Summary genetic diversity parameters for the seven geographic populations of *Dendropsophus oliveirai* and *Physalaemus cuvieri* sampled are presented in Table 2. In both species, the number of polymorphic sites in the 16S rDNA sequence samples was the same (8). Nucleotide diversity was slightly higher in *P. cuvieri*, whereas haplotype diversity and the number of haplotypes were higher in *D. oliveirai* (Table 2).

Table 2

Summary statistics of genetic diversity for *Dendropsophus oliveirai* and *Physalaemus cuvieri* from seven highland forests in northeastern Brazil based on 16S rDNA mitochondrial gene. *N* = Sample size, *S* = number of segregating/polymorphic sites, *Pi* = nucleotide diversity, *Hd* = haplotype diversity, *h* = number of haplotypes

	<i>Dendropsophus oliveirai</i>					<i>Physalaemus cuvieri</i>				
Locality	N	S	Pi	Hd	h	N	S	Pi	Hd	H
1-Pau-Ferro	7	1	0.0005	0.290	2	8	0	0.0000	0.000	1
2-Mata do Siriji	3	0	0.0000	0.000	1	8	8	0.0057	0.429	2
3-João Vasconcelos	7	0	0.0000	0.000	1	8	1	0.0007	0.429	2
4-Tabocas	12	0	0.0000	0.000	1	8	8	0.0033	0.250	2
5-Catimbau	10	3	0.0017	0.644	3	8	6	0.0025	0.250	2
6-Pedra Talhada	6	2	0.0015	0.733	3	8	0	0.0000	0.000	1
7-Craunã	8	0	0.0000	0.000	1	8	0	0.0000	0.000	1
Total	53	8	0.0041	0.750	8	56	8	0.0055	0.470	4

Overall genetic differentiation among geographic populations of *D. oliveirai* was relatively high ($F = 88\%$; $df = 6$; $p < 0.001$) whereas genetic variation within each locality was low ($F = 12\%$; $df = 46$; $p < 0.001$). Pairwise *Fst* values in *D. oliveirai* were generally high among most of localities, especially between those which were geographically distant, with statistically significant comparisons ranging between 0.36–1.00 (Table 3). High *Fis* values were observed in Pau-Ferro, Catimbau, and Pedra Talhada (Table 3). In *P. cuvieri*, overall genetic differentiation was lower than that observed in *D. oliveirai* ($F = 71\%$; $df = 6$; $p < 0.001$), but genetic variation within localities was generally high ($F = 29\%$; $df = 49$; $p < 0.001$). Pairwise *Fst* in *P. cuvieri* revealed high and

significant genetic differentiation only between the localities Pau-Ferro and Mata do Siriji and the remaining localities, ranging between 0.51–1.00 (Table 3). *Fst* values among samples of the remaining localities were relatively low, especially between those which were geographically close, ranging between 0.12–0.14. *Fis* in one northeastern locality (Mata do Siriji) and three central localities (João Vasconcelos, Tabocas and Catimbau) were moderate to high.

Table 3

Fst values between (upper and lower matrices) and *Fis* values within (central diagonal, in bold) seven populations of the amphibians *Dendropsophus oliveirai* (upper matrix; after slash in the diagonal) and *Physalaemus cuvieri* (lower matrix; before slash in the diagonal) distributed in highland forests in Northeastern Brazil. *Fst* and *Fis* were based on a fragment of the 16S rDNA mitochondrial gene. Asterisks indicate statistically significant distances

	1	2	3	4	5	6	7
1-Pau Ferro	0.00/0.29	0.00	0.96*	0.97*	0.82*	0.88*	0.97*
2-Mata do Siriji	0.14	1.00/0.00	1.00*	1.00*	0.80*	0.86*	1.00*
3-João Vasconcelos	0.97*	0.68*	0.43/0.00	0.00	0.36*	0.84*	1.00*
4-Tabocas	0.86*	0.51*	0.00	1.00/0.00	0.45*	0.87*	1.00*
5-Catimbau	0.89*	0.56*	0.00	0.00	1.00/0.93	0.52*	0.77*
6-Pedra Talhada	1.00*	0.71*	0.14	0.00	0.00	0.00/0.87	1.00*
7-Craunã	1.00*	0.71*	0.14	0.00	0.00	0.00	0.00/0.00

Genetic structure

BAPS indicated (Fig. 2a; Supplementary Information S9) that samples of *D. oliveirai* most probably correspond to four genetic clusters: K1, encompassing samples from the northeastern localities of Pau-Ferro and Mata do Siriji, K2, encompassing samples from the central localities of João Vasconcelos, Tabocas and Catimbau, K3, with samples from central and southeastern localities of Catimbau and Pedra Talhada, and K4, with samples from central and southwestern localities Catimbau and Craunã. The neighbour joining haplotype network revealed that the northeastern populations of Pau-Ferro and Mata do Siriji are distinguished from the remaining populations of *D. oliveirai* in the region for at least four mutation steps. Exclusive haplotypes were found in Pau-Ferro and also in the southeastern locality of Pedra Talhada and in the central locality of Catimbau (Fig. 2c).

For *P. cuvieri*, BAPS (Fig. 2b) indicated that samples most probably correspond to two genetic clusters: K1, encompassing samples from the northeastern and central localities of Pau-Ferro, Mata do Siriji, Tabocas and Catimbau, and K2, encompassing samples from the northeastern, central and southern localities of Mata do Siriji, João Vasconcelos, Tabocas, Catimbau, Pedra Talhada and Craunã. One haplotype (Hap 1) was found in all localities except the northern locality of Pau-Ferro (Fig. 2d). Exclusive haplotypes were found in João Vasconcelos and Catimbau.

Geographic and environmental correlates of genetic variation

Mantel tests uncovered that geographic distance was positively and weakly correlated with environmental distances in *P. cuvieri* (Table 4). In *D. oliveirai* this correlation was weaker and only marginally significant. Past climatic variation was not correlated with either geographic, environmental or genetic distances among sampling locations of both species. Geographic and environmental distances were positively and moderately correlated with genetic distances among populations of *D. oliveirai*. Correlations between environmental distance and genetic distance among populations of *D. oliveirai* remained statistically significant, but decreased in strength after accounting for the influence of geographic distance in a partial Mantel test (Table 4). After accounting for any influence of past climatic variation distance on the environmental distance, the relationship between environmental distance and genetic distance in *D. oliveirai* became weaker but still significant. Correlation between geographic distance and genetic distance remained significant, but weaker, after accounting for the effect of the environmental distance in *D. oliveirai* (Table 4). Geographic distance among sampling locations was the only parameter positively correlated with genetic distances among populations of *P. cuvieri*. Correlations between genetic distances and environment or past climatic variation were not revealed in *P. cuvieri* even after accounting for their relationships with geographic distance. The correlation between geographic distance and genetic distance increased after remove the effect of the environmental distance (Table 4).

Table 4

Results of Mantel and partial Mantel tests between geographic (Geo), current environmental (Env), and past climatic variation (PastCliVar) distances for seven sampling localities of the amphibians *Dendropsophus oliveirai* and *Physalaemus cuvieri* in highland forests in Northeastern Brazil, and their correlations with average genetic distances (Gen) of populations of those two species. Different r values between species in the tests Geo X Env and PastCliVar X Env result from different EVI_{max} and EVI_{min} between species (see Supplementary Information S2)

Distance matrix comparisons	<i>Dendropsophus oliveirai</i>	<i>Physalaemus cuvieri</i>
Geo X Gen	$r = 0.566; p = 0.006^*$	$r = 0.620; p = 0.004^*$
Env X Gen	$r = 0.482; p = 0.012^*$	$r = -0.027; p = 0.492$
PastCliVar X Gen	$r = 0.340; p = 0.062$	$r = 0.178; p = 0.211$
Geo X Env	$r = 0.447; p = 0.062$	$r = 0.512; p = 0.029^*$
Geo X PastCliVar	$r = 0.235; p = 0.161$	$r = 0.235; p = 0.177$
PastCliVar X Env	$r = 0.226; p = 0.214$	$r = 0.262; p = 0.160$
Partial Mantel		
Env X Gen – Geo	$r = 0.310; p = 0.046^*$	$r = -0.512; p = 0.941$
PastCliVar X Gen – Geo	$r = 0.259; p = 0.129$	$r = 0.042; p = 0.427$
Env X Gen – PastCliVar	$r = 0.442; p = 0.019^*$	$r = -0.078; p = 0.523$
Geo X Gen – Env	$r = 0.447; p = 0.046^*$	$r = 0.74; p = 0.001^*$

The first two components of the PCA based on genetic data explained, respectively, 80% and 9% of genetic variation among populations in *D. oliveirai*, and 75% and 25% in *P. cuvieri* (Supplementary Information S10). In PCA for environmental variation among localities, the first two components explained, respectively, 48% and 31% in *D. oliveirai* and 47% and 31% in *P. cuvieri* (Supplementary Information S7). In both species 12 variables contributed highly and equally to the PC1 (Supplementary Information S8). In PCA for past climatic variation among localities, the first two components explained, respectively, 49% and 23% in both species (Supplementary Information S7) and nine variables contributed highly and equally to the PC1 (Supplementary Information S8).

SEM model evaluation indices did not indicate rejection (comparative fit index; CFI = 1; RMSEA = 0, SRMR = 0), and the model modification did not suggest any change in the model structure for any of the two species, indicating that the models tested were appropriate. The SEM for *D. oliveirai* (Fig. 3a) demonstrated that environmental variation has the largest and positive effect on genetic variation, followed by the positive effect of longitude, and negative effect of the past climatic variation. The SEM for *P. cuvieri* (Fig. 3b) revealed a strong and positive relationship between latitude and genetic variation, followed by a weak and positive influence of the environmental variation on the genetic variation. Longitude and latitude had no significant influence on the environmental variation or past climatic variation in both species.

Discussion

Our results uncovered different patterns of genetic structure and geographic distribution of genetic diversity in the two study species. Using a combination of different methods, we found support for our hypotheses that the two species interact differently with the same landscape factors. Jointly, our results demonstrated the individual influence of geographic distance and past and current environmental variation on the genetic structure of the study species, suggesting that genetic differentiation in *D. oliveirai* has been driven mainly by IBE, whereas IBD has driven the genetic differentiation in *P. cuvieri*. Such findings are another demonstration that selective and neutral evolutionary mechanisms act simultaneously in the same region, but with different strengths for each species. It also supports the idea that Northeastern Brazil is a mosaic of organisms with adaptations to contrasting environments such as mesic forest and xeric habitats (Gehara et al. 2017). Considering that we collected data from the same localities at the same time for both species, our investigation disentangles any effects of different current and historical landscape features which could confound our analyses and interpretations of different results between species.

The relationships between genetic variation and geographic distance, past climatic variation, and current environment in *D. oliveirai* demonstrate that IBD and IBE explain together the current distribution of genetic variation in this species, probably with IBE being weightier. Current and historical barriers to gene flow, as well as environmental heterogeneity and climatic changes, can cause genetic drift, increasing genetic distances among previously connected populations, while local adaptation occurs when local environmental features favor different alleles in each locality through selection pressures (Nosil et al. 2005; Balkenhol et al. 2019). Given that neutral and selective mechanisms can act together resulting in IBE (Wang and Bradburd 2014), we believe that both genetic drift and local adaptation conduce the genetic differentiation in *D. oliveirai*. Although our study is based on presumed neutral genetic markers, an adaptive process can still be

indirectly inferred from the data (Andrew et al. 2012; Manel and Holderegger 2013). Additionally, the effect of the past climatic variation only on the genetic variability of *D. oliveirai* confirmed that past climatic instability had an important role in the past gene flow and genetic diversity of species adapted to mesic conditions. Such influence of historical climatic variation on genetic differentiation of tropical amphibian species is already known (e.g. Carnaval et al. 2009; Gehara et al. 2017; Brusquetti et al. 2019), especially for those adapted to mesic climates and/or living in highland forests (e.g. Carnaval and Bates 2007; Amaro et al. 2012; Guarnizo and Cannatella 2013; Oliveira et al. 2021). Our findings indicate that *D. oliveirai* is better adapted to mesic habitats and that climatic fluctuations, probably aridification, during the Pleistocene caused the isolation of populations in separated forest microrefugia. Today, the connectivity between these populations seems to be mainly influenced by the east-west climatic/environmental gradient, which results in genetic differentiation longitudinally.

Genetic variability in *P. cuvieri* was mostly explained by IBD with a very small contribution from the environmental variation, which is also expected for wide-range species (see Jenkins et al. 2010). Being widely distributed from the south to the north of South America (Haddad et al. 2008; Frost 2023), *P. cuvieri* experiences a variety of habitat conditions, which could shape its genetic variation according to a latitudinal gradient. A possible important factor is the latitudinal asynchrony of the rainy season (see Bates et al. 2008; Silva et al. 2019), which is important to explosive breeding amphibians (Aichinger 1987; Duellman 1995; Prado et al. 2005; Ulloa et al. 2019). Such asynchrony could lead to asynchrony in breeding season among populations (mate mismatch) in different latitudinal localities, which in turn could result in genetic differentiation, even among short geographic distances, especially in regions of high topographic relief (Martin et al. 2009). In other words, it is possible that the strong association between geographic distance and genetic variation in *P. cuvieri* includes the effect of asynchrony in the rainy season along this latitudinal gradient (see Maes et al. 2006; Thomé et al. 2021). Similar patterns were previously described for a widely distributed anuran endemic to the Caatinga, *Rhinella granulosa* (Thomé et al. 2021), and for *Amietia wittei*, a tropical Afrotropical frog (Zancolli et al. 2014).

Here, we found that geographic distance is the most important landscape feature influencing genetic differentiation in the habitat generalist (*P. cuvieri*), whereas the genetic differentiation of the habitat specialist (*D. oliveirai*) was highly influenced by a combined effect of past climatic variation, current environment, and geographic distance. Barring any particular unknown historical reasons, the contrasting levels of genetic structure that we found between *D. oliveirai* and *P. cuvieri* result from their different abilities to cross the dry lowlands between highland forests. Our findings join the literature of landscape genetics in tropical regions suggesting or demonstrating that landscape features act on genetic variation depending on species-specific traits (e.g. Blair et al. 2013; Engler et al. 2014; Zancolli et al. 2014; Nowakowski et al. 2015; Sandberger-Loua et al. 2017; Monteiro et al. 2019; Nali et al. 2020). Such studies usually indicate elevation, forest cover, rivers, and geographic distance as the main factors responsible for resistance or permeability to gene flow, but always at different degrees of importance for different species. In our previous work investigating acoustic variation and its drivers in the same populations we demonstrated that geographic and environmental distance have, respectively, moderate and higher association with acoustic distance among *P. cuvieri* populations, while environmental distance is moderately related to acoustic distance among *D. oliveirai* populations, with body size and genetic differentiation as covariates (Andrade Lima et al.

2024). Since IBD and IBE imply, respectively, genetic drift and divergent natural selection (Dobzhansky 1937; Crispo et al. 2006; Lee e Mitchell-Olds 2011; Wang 2013, Sexton et al. 2014), combining our current and past findings, we suggest that selective and neutral evolutionary mechanisms conduct intraspecific genetic variation in this Neotropical region, with varied relative importance depending on the ecology of the species. Future investigations involving spatiotemporal demographic analyses using genomic data from these populations will make it possible to confirm local selective processes on the habitat specialist species.

Genetic structure and diversity among localities suggest different levels of landscape permeability for each species. Body size and reproductive mode are life-history traits that can determine habitat preferences, dispersal potential and, consequently, genetic structure (Wollenberg et al. 2011; Paz et al. 2015; Lourenço et al. 2019). Considering that *D. oliveirai* and *P. cuvieri* have distinct reproduction habits and ecological niches, perhaps these traits are contributing to the differentiation in their population genetic structure. Although *D. oliveirai* is not a strict forest dependent species, this tiny treefrog has a reproductive mode strongly related to ponds with long hydroperiod and herbaceous vegetation, which are common places in montane forests in the study region but usually rare in the surrounding lowlands. This potentially led to geographic isolation or lower gene flow between populations. Patterns of limited dispersal and high genetic structure resulting from the absence of suitable environments for reproduction have been seen in other montane amphibians, such as *Scinax* treefrogs in the central Atlantic Forest (Santana et al. 2024), *Melanophryniscus* and *Brachycephalus* toads in the southern Atlantic Forest (Pie et al. 2018), and *Ensatina* salamanders in the western United States (Devitt et al. 2013). On the other hand, we found less phylogeographic structure in *P. cuvieri*, as expected for habitat generalist species (Miller et al. 2015; Kort et al. 2021), especially those adapted to breeding in low hydroperiod water bodies in dry areas (Ortiz et al. 2018). Besides its association with dry habitats, this species uses temporary ponds to reproduce, with large clutch sizes and fast larval development (Barreto and Andrade 1995; Haddad and Prado 2005; Aguiar et al. 2014). Such life-history traits may increase dispersal ability in amphibians (Van Bocxlaer et al. 2010; Streicher et al. 2012). As such, the genetic structure found in *P. cuvieri* by our study is probably a result of its dispersal capability and not of environmental barriers.

Despite differences in the patterns of genetic structure between *D. oliveirai* and *P. cuvieri*, we found a putative congruent phylogeographic break close to 8° latitude, between northeastern and central localities, where the Capibaribe and Ipojuca rivers are located. Although very subtle, similar phylogeographic patterns were previously documented in other anurans, *Proceratophrys renalis* and *Pristimantis ramagii*, in the same region (Carnaval 2002; Carnaval and Bates, 2007). Since rivers could represent complete or partial barriers depending on the dispersal ability of each species (Coelho et al. 2022), such congruences could suggest a vicariant role (more or less pervasive) of the Capibaribe and Ipojuca rivers for different species. These rivers are the only physical landscape elements near that latitude, and have never been suggested to be or investigated as a barrier before. Thus, we highlight that even those smaller rivers could also account for the current genetic structure of different species in northeastern Brazil. Another comparative study involving more animal species from different groups, or even a most comprehensive genetic dataset of the present study species, could investigate this question more confidently. Regardless of the rivers possibly affecting the genetic differentiation of the present study species, the influence of geographic distance was clearly demonstrated to both species in our analyses. Therefore, the phylogeographic breaks resulting from any

local barrier between the northeastern and central localities do not account for the full relationship between geographic and genetic distances.

Conclusions

Understanding how gene flow between populations is modulated by geographic or environmental variation may offer a glimpse of the earliest stages of biological divergence (Schluter 2001; Wang et al. 2013), and suggest how to manage and predict biodiversity loss under current rapid environmental changes (Sexton et al. 2013; Manel and Holderegger 2013). Here, we demonstrated how the genetic variability of two ecologically different species is distributed in highland forests in Northeastern Brazil and how the landscape imposes different challenges on each species, depending on their ecological constraints. Our results contribute to a better understanding on the evolutionary dynamics of Neotropical animal populations from mountain regions. Using a comparative investigation, we corroborate Rodríguez et al. (2015), demonstrating that habitat generalist species (less sensitive to dry habitat conditions) are genetically less structured than habitat specialists, since the landscape factors (except geographic distance) impose little to no resistance to their dispersion. On the other hand, we saw that spatiotemporal changes in the landscape matrix have strong consequences for habitat specialist species, which results in a more structured genetic pattern. Since habitat loss is the main threat to amphibians (Luedtke et al. 2023), our work recalls the importance of conserving the remaining forest fragments and their connectivity in this region, maintaining gene flow among populations of low-vagility species, which are usually subject to stochasticity and inbreeding, factors that could lead to extirpation. We suggest future multi-taxa investigations in landscape genetics involving species with different ecological constraints that are widely distributed in the same region for confirmation that geographic distance, rivers and environmental variation affect genetic variation in generalists and specialists differently. This approach will allow us to predict the future of species with different ecological requirements co-occurring in the same region in the face of climatic changes and habitat loss.

Declarations

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Author contributions All authors conceived the manuscript. JHAN and PIS designed sampling strategies and collected the data. JHAL analyzed the data. JHAL, MG, PIS, and MK wrote the manuscript.

Data availability Mitochondrial DNA sequences used in this work are available on GenBank (accession numbers informed in Materials and methods). The dataset analyzed is available as supplementary information. Data is provided within the manuscript or supplementary information files.

Conflict of interest The authors declare no conflict of interest.

Licenses All data was collected, transported and preserved under the permits from SISBIO-ICMBio (RAN-SISBIO 69767-3 and RAN-SISBIO 76544-1).

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Figures

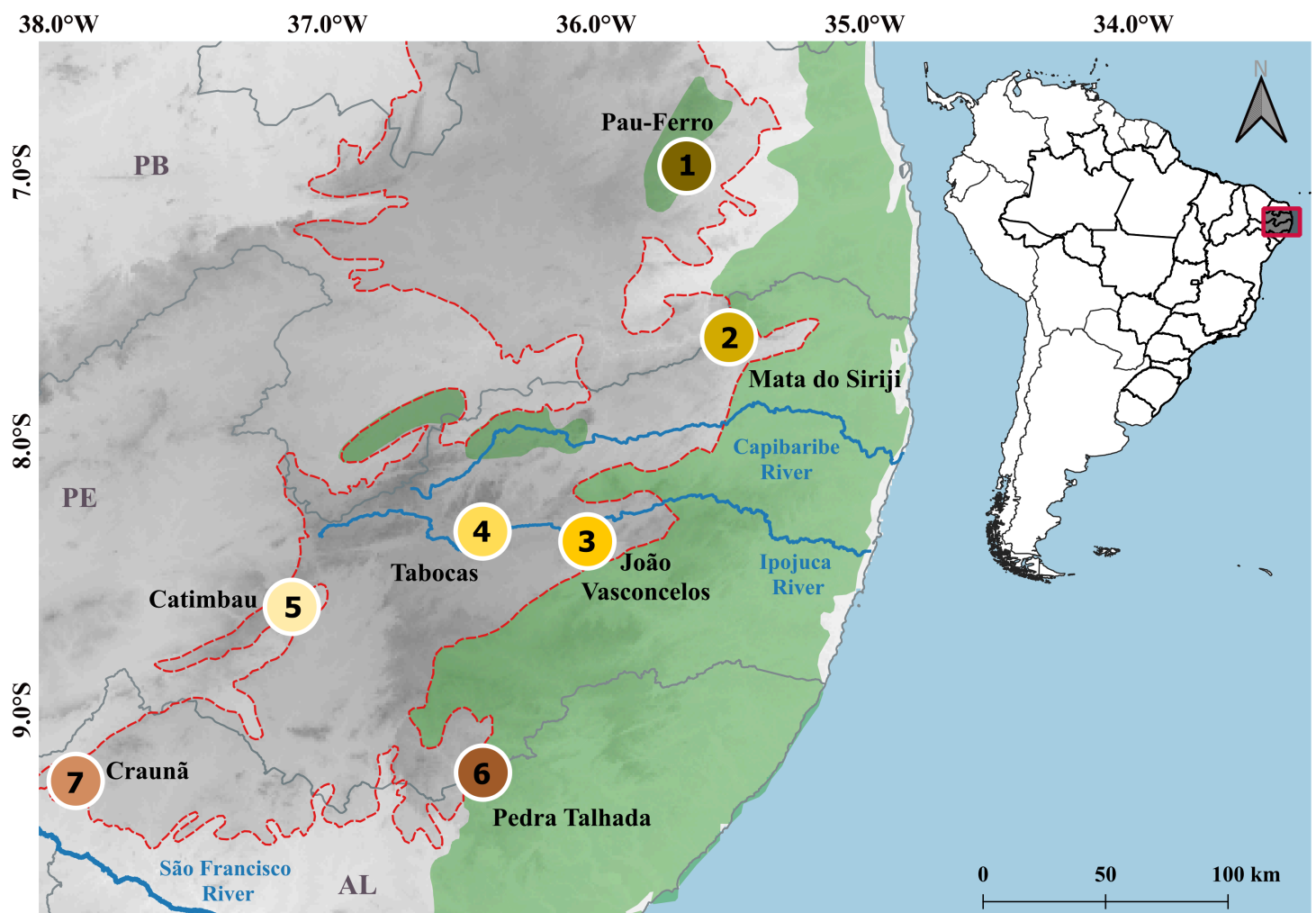


Figure 1

Geographic position of sampling locations on the Borborema Plateau (dashed red line) in Northeastern Brazil. The Atlantic Forest domain is highlighted in light green. Blue lines correspond to large rivers in the region: São Francisco (bottom blue line), Capibaribe (top blue line) and Ipojuca (middle blue line). Grey lines correspond to state limits

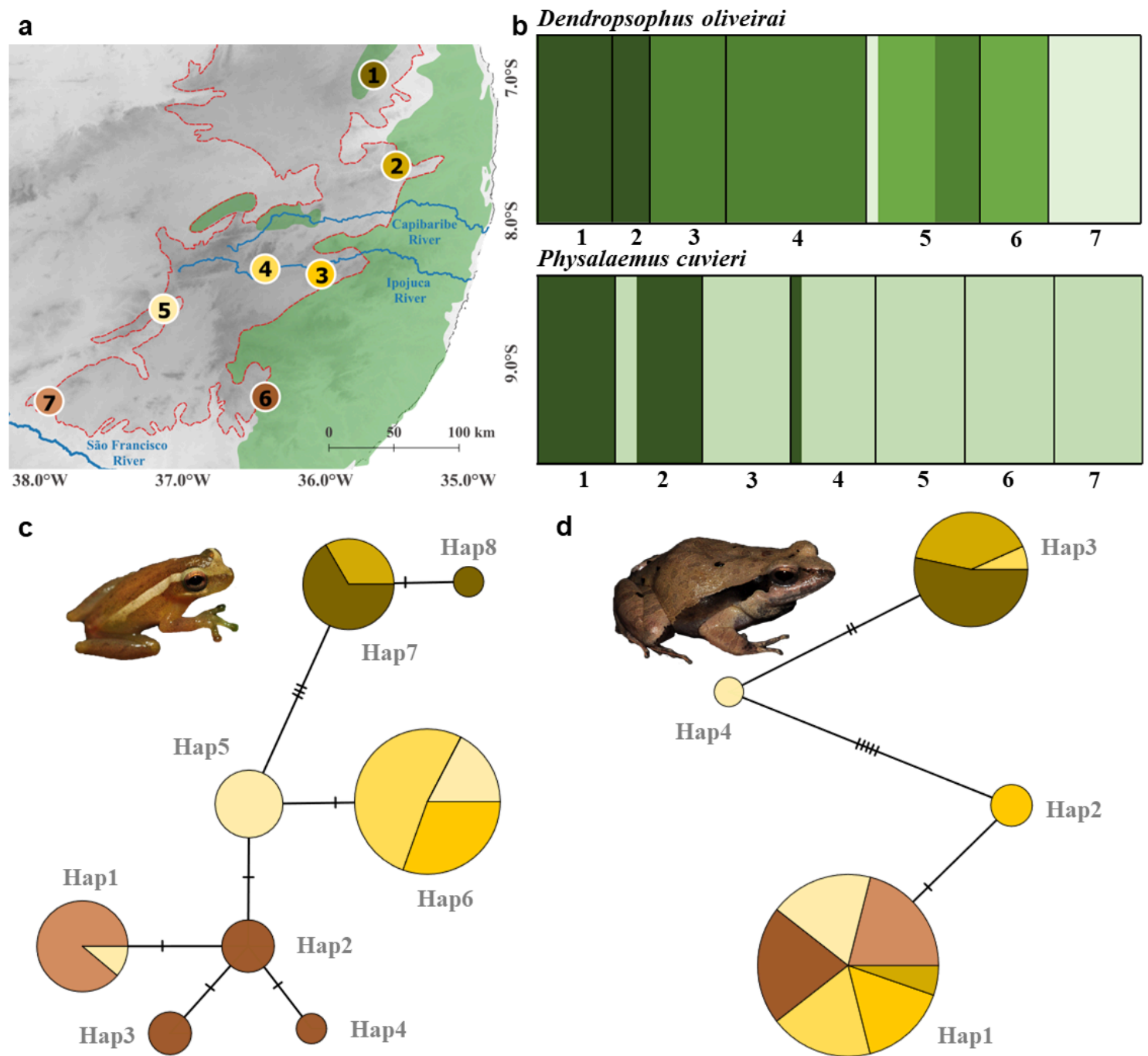


Figure 2

(a) Sampling localities of the frogs *Dendropsophus oliveirai* and *P. cuvieri* across the Borborema Plateau in northeastern Brazil. Dashed red line = geographic limits of the Borborema Plateau. Blue lines = Capibaribe, Ipojuca, and São Francisco rivers from top to bottom. (b) Bayesian Analysis of Population Structure (BAPS)

based on a fragment of the 16S rDNA mitochondrial gene of *D. oliveirai* and *P. cuvieri*. Numbers on the X-axis represent sampling locations depicted in (a), green shades represent different genetic clusters recovered in BAPS. (c) and (d) Neighbour-joining haplotype networks based on the same 16SrDNA alignments for *D. oliveirai* and *P. cuvieri*, respectively. Colors in the haplotype networks correspond to those indicating sampling localities in (a). Circle sizes in the haplotype networks are proportional to haplotype frequency among samples

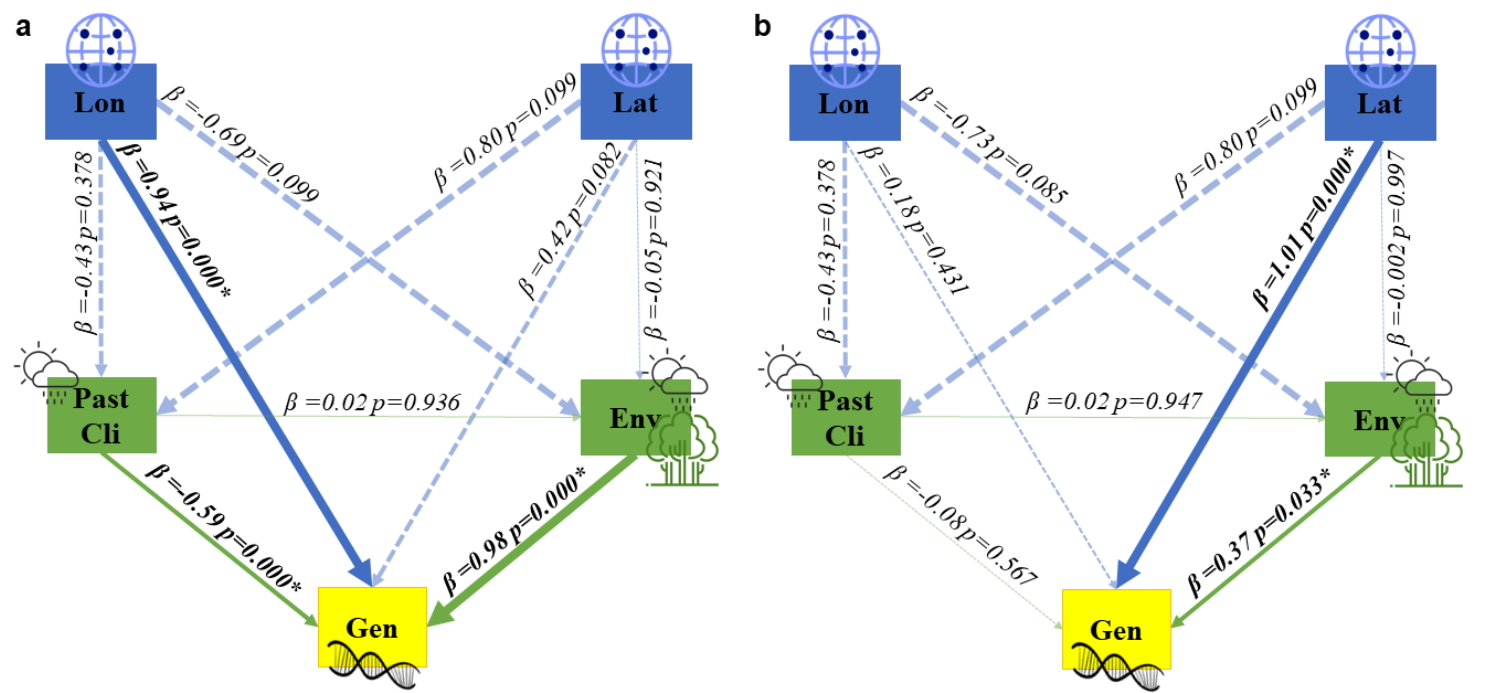


Figure 3

Structural Equation Model (SEM) summarization of landscape feature effects on the genetic variation of *Dendropsophus oliveirai* (a) and *Physalaemus cuvieri* (b) from highland forests in Northeastern Brazil. Lat = latitude, Lon = longitude, Env = environment (first component from PCA of environmental variation), Past Cli = past climatic variation, Gen = genetic (first component from PCA of genetic variation). Arrow thickness represents the effect size of predictor variables. Solid arrows represent significant effects (values highlighted in bold). Dotted arrows represent no significant effect. β = standardized regression coefficients

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