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JOSÉ HENRIQUE DE ANDRADE LIMA

DISTRIBUIÇÃO DA VARIABILIDADE GENÉTICA E ACÚSTICA E DEMOGRAFIA HISTÓRICA DE DUAS ESPÉCIES DE ANUROS NO PLANALTO DA BORBOREMA, NORDESTE DO BRASIL

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2025

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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de doutor em Biologia Animal.

Orientador: Pedro Ivo Simões

Coorientador: Marco Katzenberger

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A todos aqueles que acreditaram em mim, especialmente minha família.

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RESUMO

As montanhas desempenham um papel fundamental na evolução de diversos táxons, influenciando a diversidade biológica em seus diferentes níveis, padrões de distribuição e endemismos. No Nordeste do Brasil, ao norte do rio São Francisco, enclaves de florestas úmidas situam-se no topo de cadeias montanhosas, cercados por uma matriz de vegetação seca da Caatinga. Esses enclaves resultam de múltiplos eventos de expansão e retração das florestas úmidas ao longo do Quaternário e devido sua relativa estabilidade climática ao longo daquele período, acredita-se que tenham funcionado como refúgios climáticos no Pleistoceno. No entanto, ainda há incertezas sobre como populações de diferentes espécies responderam às variações climáticas e como a paisagem atual influencia a sua variação fenotípica e genética. Nesta tese, utilizei dados acústicos, morfológicos e genéticos de duas espécies de anuros coocorrentes nos enclaves florestais da região que diferem em relação a seus habitats de reprodução: *Dendropsophus oliveirai*, um habitat-especialista, e *Physalaemus cuvieri*, um habitat-generalista. Investiguei como fatores geográficos e ambientais ao longo do Planalto da Borborema influenciam a variação fenotípica e genética dessas espécies, e como ciclos climáticos e vegetacionais influenciaram processos populacionais, como divergência, migração e variação no tamanho populacional. Para isso, analisei características biológicas como canto de anúncio, tamanho corporal e variação genética (marcador mitocondrial 16S e dados genômicos), em relação a elementos da paisagem (distância geográfica, latitude, longitude, variáveis bioclimáticas, vegetação e estabilidade climática) e fatores históricos (ciclos climáticos e vegetacionais da região). Usei métodos estatísticos para analisar as associações entre caracteres biológicos e variáveis da paisagem, por fim, apliquei filogeografia estatística combinada à approximate Bayesian Computation (ABC) e Supervised Machine-Learning (SML) para investigar a influência de fatores históricos na dinâmica demográfica populacional das espécies. No primeiro capítulo, demonstrei que a variação acústica em *P. cuvieri* é maior e fortemente influenciada pelo ambiente, mas também pela distância geográfica entre populações. Em *D. oliveirai*, o ambiente foi o principal fator determinante da variação acústica, seguido pela influência genética e morfológica, havendo uma possível influência indireta da distância geográfica. No segundo capítulo, observei menor diferenciação genética em *P. cuvieri*, principalmente associada à distância geográfica latitudinalmente e pouca influência da variação ambiental. Em *D. oliveirai*, a diferenciação genética foi maior e associada à distância geográfica longitudinalmente, variação ambiental e estabilidade climática. No terceiro capítulo, estimei o tempo de divergência populacional nas duas espécies, a qual data do Pleistoceno Médio, com aumento da migração e do tamanho populacional após o Último Máximo Glacial, seguidos de possíveis reduções no Holoceno. Considerados em conjunto, os resultados demonstram como caracteres distintos evoluem de forma desacoplada e como traços ecológicos espécie-específicos influenciam a história evolutiva das espécies. Além disso, ressaltam o papel dos ciclos climáticos do Pleistoceno e da complexidade topográfica na estruturação genética da biota de uma região. Minha tese contribui para uma melhor compreensão da diversidade e estruturação genética, assim como da dinâmica demográfica das espécies do Planalto da Borborema, destacando a importância dos enclaves florestais do norte da Mata Atlântica para a conservação da biodiversidade do Nordeste brasileiro.

Palavras-chave: características ecológicas; influencia da paisagem; montanhas; mudanças demográficas; variação genética; variação fenotípica.

ABSTRACT

Mountains play a fundamental role in the evolution of various taxa, influencing biological diversity at different levels, distribution patterns, and endemism. In northeastern Brazil, north of the São Francisco River, enclaves of humid forests are found atop mountain chains, surrounded by a matrix of dry Caatinga vegetation. These enclaves result from multiple events of expansion and contraction of humid forests throughout the Quaternary and, due to their relative climatic stability during that period, are believed to have served as climatic refugia in the Pleistocene. However, there are still uncertainties about how populations of different species responded to climatic variations and how the current landscape influences their phenotypic and genetic variation. In this dissertation, I used acoustic, morphological, and genetic data from two anuran species co-occurring in the forest enclaves of the region which differ in their reproductive habitats: *Dendropsophus oliveirai*, a habitat specialist, and *Physalaemus cuvieri*, a habitat generalist. I investigated how geographical and environmental factors across the Borborema Plateau influence the phenotypic and genetic variation of these species, and how climatic and vegetation cycles influenced population processes such as divergence, migration, and changes in population size. To achieve this, I analyzed biological traits such as advertisement call, body size, and genetic variation (mitochondrial 16S marker and genomic data) in relation to landscape elements (geographical distance, latitude, longitude, bioclimatic variables, vegetation, and climatic stability) and historical factors (climatic and vegetation cycles of the region). I used statistical methods to analyze the associations between biological characters and landscape features and, finally, applied statistical phylogeography combined with Approximate Bayesian Computation (ABC) and Supervised Machine-Learning (SML) to investigate the influence of historical factors on the demographic dynamics of the species. In the first chapter, I demonstrated that acoustic variation in *P. cuvieri* is greater and strongly influenced by the environment, but also by the geographical distance between populations. In *D. oliveirai*, the environment was the main determinant of acoustic variation, followed by genetic and morphological associations, with a possible indirect effect of geographical distance. In the second chapter, I observed lower genetic differentiation in *P. cuvieri*, mainly associated with geographical distance in a latitudinal direction and little influence from environmental variation. In *D. oliveirai*, genetic differentiation was higher and associated with geographic distance longitudinally, environmental variation, and climatic stability. In the third chapter, I identified population divergence in both species during the Middle Pleistocene, with increased migration and population size following the Last Glacial Maximum, followed by possible reductions in the Holocene. When considered together, results demonstrate how distinct traits evolve in a decoupled manner and how species-specific ecological traits influence the evolutionary history of species. Additionally, they highlight the role of Pleistocene climatic cycles and topographic complexity in shaping the genetic structure of the biota in a region. My thesis contributes to a better understanding of diversity and genetic structuring, as well as the demographic dynamics of species in the Borborema Plateau, emphasizing the importance of the northern Atlantic Forest enclaves for the conservation of biodiversity in northeastern Brazil.

Keywords: demographic changes; ecological traits; phenotypic variation; genetic variation; landscape influence; mountains.

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1 INTRODUÇÃO

Entender quais são os mecanismos responsáveis pela distribuição espacial e temporal da biodiversidade e o papel dos diferentes ecossistemas nesses processos tem sido o foco de estudos ecológicos e evolutivos por muito tempo (Maestre *et al.* 2014; Hu *et al.* 2020). Nesta busca pela compressão de processos evolutivos geradores de diversidade, as montanhas passaram a ser consideradas ambientes-chave em muitos estudos (Lomolino, 2001; Sanders e Rahbek, 2012). Tal reconhecimento resulta da sua singular capacidade de gerar e manter elevados níveis de diversidade biológica e endemismo devido a suas condições ambientais diferenciadas das terras baixas que as rodeiam (Hughes e Atchison, 2015; Guedes *et al.* 2020). Assim, as montanhas tiveram um importante papel na origem e manutenção da biodiversidade atual (Körner *et al.* 2017), seja de maneira direta, promovendo barreiras físicas à dispersão, ou de maneira indireta, gerando barreiras ecológicas devido à variação nas condições ambientais e composição biótica ao longo de suas vertentes (ver Hu *et al.* 2020).

A Região Neotropical abriga a maior biodiversidade do mundo, a qual resulta de sua complexa história geológica, climática e heterogeneidade ambiental (Antonelli e Sanmartín, 2011). Assim como em outras regiões do mundo, as montanhas neotropicais tiveram papeis importantes como refúgios climáticos e florestais durante períodos de grande instabilidade climática do Quaternário (Lagomarsino *et al.* 2016; Vargas e Simpson, 2019), o que explica parcialmente os padrões de distribuição de espécies, endemismos (Marquet *et al.* 2004; Rull, 2005; Guedes *et al.* 2020) e a estrutura genética em muitos táxons, a depender de sua tolerância ecológica (Wiens 2004). Entre os ambientes montanhosos neotropicais, a Cordilheira dos Andes foi o mais estudado, certamente devido sua enorme dimensão e importância para a biota do continente americano (Guedes *et al.* 2020). Contudo, outras cadeias de montanhas da América do Sul, embora pouco exploradas, já demonstraram sua importância histórica e contemporânea para a biota sul-americana, como aquelas localizadas a nordeste, centro e sul do Brasil (e.g. Carnaval, 2002; Carnaval e Bates, 2007; Pie *et al.* 2018; Oliveira *et al.* 2021; Batalha-Filho *et al.* 2023).

No Nordeste do Brasil, ao norte do rio São Francisco, localiza-se o Planalto da Borborema, uma cadeia de montanhas que percorre os estados do Rio Grande do Norte, Paraíba, Pernambuco e Alagoas, com variação altitudinal entre 600 e 1200 m nos seus picos (Agra *et al.* 2004). Nas suas partes mais altas, encontram-se os Brejos de Altitude, “ilhas” de florestas com elementos de Mata Atlântica, marcadas por suas condições mésicas, diferenciadas da matriz de caatinga das terras baixas circundantes (Andrade-Lima, 1960; Sales *et al.* 1998; Silveira *et al.* 2019). A origem da vegetação destas florestas serranas está relacionada às mudanças climáticas do Pleistoceno, advindas de ciclos glaciais (últimos 2 milhões – 10 mil anos), que permitiram a entrada da Mata Atlântica no domínio das Caatingas e sua permanência em elevadas altitudes (Tabarelli e Santos, 2004).

Estudos filogeográficos demonstraram que as mudanças climáticas do Quaternário foram responsáveis por eventos de isolamento geográfico e mudanças demográficas em espécies de anuros da Mata Atlântica nestes refúgios florestais (Carnaval, 2002; Carnaval e Bates, 2007). Outros estudos apontam tais áreas como locais com moderado a alto endemismo filogeográfico devido sua maior estabilidade climática nos últimos 120.000 anos (Carnaval *et al.* 2014). Estes estudos revelaram o importante papel das florestas serranas na manutenção de populações de espécies menos tolerantes às condições semiáridas da caatinga circundante, mas não abordaram de forma comparativa como espécies com diferentes níveis de vagilidade responderam aos mesmos eventos climáticos do passado. Estudos dessa natureza elucidariam o papel da ecologia das espécies em padrões de estruturação genética, assim como permitiriam uma previsão de resposta das espécies à futuras mudanças climáticas.

Além de sua complexidade histórica, o Planalto da Borborema apresenta também uma elevada heterogeneidade ambiental. Nesta cadeia de montanhas existe um gradiente climático no sentido leste-oeste, com maior precipitação nas florestas serranas mais próximas da costa (1.000-1.600 mm) e redução da precipitação nas áreas interioranas (400-600 mm) (Velloso *et al.* 2002; Rodal *et al.* 2008; Silva *et al.* 2018). Esse gradiente climático criou um gradiente de composição vegetal e estrutura florestal que varia de floresta serrana ombrófila densa a floresta serrana estacional semidecidual (Velloso *et al.* 2002; Santos *et al.* 2007; Rodal *et al.* 2008). Tendo em vista que a variação em fatores bióticos e abióticos ao longo da distribuição geográfica de uma espécie pode levar a diferenciação populacional (Endler, 1977; Schluter, 2009), seria intuitivo se perguntar quanto dessa variação climática-ambiental explica a diferenciação genética e fenotípica das espécies com ocorrência nessa região, e se existe congruência entre as espécies na magnitude do efeito dos diferentes fatores da paisagem sobre a diferenciação desses caracteres biológicos. Dessa forma, as florestas serranas do Planalto da Borborema fornecem um cenário singular para estudos comparativos que investiguem hipóteses filogeográficas, assim como a influência de fatores geográficos e ambientais da paisagem sobre a variação de diferentes caracteres biológicos em espécies ecologicamente diferentes.

Os anfíbios são comumente usados em estudos filogeográficos integrativos (e.g., Kaefer *et al.* 2013; Maia *et al.* 2017; Fernandes *et al.* 2021) por possuírem pronunciada filopatria e características anatômicas e fisiológicas que os tornam menos capazes de se dispersarem em longas distâncias e mais vulneráveis às mudanças climáticas (Pounds *et al.* 1999). Além disso, suas vocalizações carregam forte sinal filogenético (Erdtmann e Amézquita, 2009; Escalona-Sulbarán *et al.* 2019) e estão geralmente relacionadas ao tamanho corporal (Hoskin et al. 2009; Gingras *et al.* 2013), podendo elucidar reciprocamente a história da população e os mecanismos responsáveis pela divergência evolutiva. Portanto, esse táxon permite uma abordagem comparativa mais completa que investigue diferenciação populacional e suas causas.

Nesta tese, investiguei, de forma comparativa, a diferenciação populacional em duas espécies de anuros com nichos ecológicos e ambientes de reprodução distintos, distribuídas no Planalto da Borborema: *Dendropsophus oliveirai* (Bokermann, 1963) e *Physalaemus cuvieri* Fitzinger, 1826. Para isto, utilizei a análise de caracteres acústicos, morfológicos e genéticos, testando associações entre a variação nestes caracteres biológicos e fatores geográficos e ambientais da paisagem. Também avaliei sinais de mudanças demográficas históricas nestas populações e suas possíveis causas. Esta tese foi organizada em três capítulos. No Capítulo 1, investigo a variação acústica entre populações e sua relação com as distâncias geográfica, ambiental, genética e no tamanho corporal. No Capítulo 2, descrevo padrões de estruturação genética entre as populações das duas espécies e testo as hipóteses de isolamento por distância geográfica (IBD) e isolamento por distância ambiental (IBE). Por fim, no Capítulo 3, avalio variações demográficas espaço-temporais e a influência de eventos climáticos do passado sobre estas variações por meio da análise de dados genômicos.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar, de forma comparativa, a diversidade genética e fenotípica de populações de duas espécies de anuros em florestas serranas no extremo nordeste do Brasil, descrevendo o padrão de distribuição desta diversidade, e testando como este padrão é influenciado pela ecologia das espécies, por fatores históricos e pelo ambiente atual.

2.2 Objetivos específicos

a) Investigar a variação acústica interpopulacional em *Dendropsophus oliveirai* e *Physalaemus cuvieri* e testar a influência de fatores abióticos (distância geográfica e ambiental) e cofatores bióticos (distância genética e tamanho corporal) sobre esta variação;

b) Descrever a estrutura genética de populações das duas espécies ao longo do Planalto da Borborema e testar suas associações com a distância geográfica entre as populações, com a variação ambiental atual e com a estabilidade climática (a partir do Pleistoceno) sobre a variabilidade genética destas espécies tendo em vista suas diferenças ecológicas;

c) Avaliar congruências ou divergências das relações entre as variações genética e fenotípica nas duas espécies e sua relação com fatores espaço-temporais da paisagem;

d) Investigar mudanças demográficas nas populações de cada espécie buscando relação com eventos climáticos do passado;

e) Propor mecanismos evolutivos potencialmente responsáveis pela variação fenotípica e genética de populações de anuros de florestas serranas no Nordeste do Brasil.

3 ESTRUTURA DA TESE E HIPÓTESES

No primeiro capítulo, investiguei a variação acústica no canto de anúncio das duas espécies ao longo de florestas serranas, mais conhecidas como "Brejos de Altitude" localizadas ao norte, centro e sul do Planalto da Borborema. Neste estudo, busquei entender como a variação acústica nas duas espécies está associada com fatores geográficos (distância geográfica) e ambientais (variação climática e vegetação) da paisagem, assim como com fatores biológicos (tamanho corporal e genética) à luz de suas diferenças ecológicas. Nossas hipóteses foram que a variação ambiental estaria associada à variação acústica em ambas as espécies como resultado de seleção local, relacionada à hipótese de adaptação acústica (Morton, 1975); a distância geográfica afeta mais fortemente a variação acústica na espécie menos generalista (*Dendropsophus oliveirai*) por ser uma espécie menos vágil na matriz de vegetação seca; e, também na espécie menos generalista, a genética está correlacionada com a variação acústica devido isolamento por adaptação (Rundle e Nosil, 2005; Wang e Bradburd, 2014). Este estudo foi publicado no periódico *Biotropica* (Andrade Lima *et al.* 2024).

No segundo capítulo, utilizando os mesmos dados genéticos do estudo anterior (marcador mitocondrial 16S) desenvolvi uma abordagem de genética da paisagem guiando minha investigação baseado nas hipóteses de isolamento por distância (IBD) (Wright, 1947) e isolamento por ambiente (IBE) (Wang e Summers, 2010; Wang e Bradburd, 2014). Nesta investigação, testamos associação da variação genética com a distância geográfica, latitude, longitude, variação ambiental atual (clima e vegetação), e variação climática do passado (instabilidade/estabilidade climática), buscando entender como o fluxo gênico em espécies com tolerâncias ecológicas diferentes é influenciado pelos diferentes fatores da paisagem. Nossas expectativas foram de que, devido sua maior especificidade de habitat, a espécie menos generalista apresenta maior estruturação genética, mais fortemente influenciada por IBE e secundariamente por IBD (Pertoldi *et al.* 2014; Wang e Bradburd, 2014), já que o ambiente varia geograficamente especialmente em regiões topograficamente complexas, como a região do estudo. Por outro lado, a outra espécie possui menor ou ausência de estrutura genética como resultado de sua maior tolerância a variação ambiental no espaço e no tempo ao longo da área de estudo, como previsto para espécies habitat-generalista (Jenkins *et al.* 2010). Este estudo foi submetido no periódico *Landscape Ecology* em julho de 2024.

No terceiro capítulo, usando dados genômicos, minha investigação se aprofunda no passado das mesmas populações do estudo, mais especificamente durante o Pleistoceno.

Inicialmente, fiz a delimitação das populações, investiguei suas diversidades genéticas e tempo de divergência. Em seguida, através de modelagem coalescente usando *Approximate Bayesian Computation* (ABC) e *Supervised Machine Learning* (SML), testei diferentes cenários de migração e mudanças no tamanho populacional ao longo tempo, focando no último Máximo Glacial (LGM). Essa abordagem foi especialmente guiada pelos achados e hipóteses do estudo mais recente para a região (Silveira *et al.* 2019). Aqui, minha principal hipótese é a de que as espécies responderam de maneira diferente aos eventos climáticos do passado, também como resultado de suas diferentes tolerâncias ambientais.

4 CAPÍTULO 1

Advertisement call variation is related to environmental and geographic distances in two anuran species inhabiting highland forests in Northeastern Brazil*

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Advertisement call variation is related to environmental and geographic distances in two anuran species inhabiting highland forests in Northeastern Brazil

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Abstract

Highlands are of paramount importance to the study of evolution as they are frequently implicated in historical and ecological processes that generate and maintain biological diversity. In northeastern Brazil, sparse rainforest remnants located in highlands north of the São Francisco River are surrounded by the dry and open landscape of the Caatinga biome. Earlier studies suggested that these forests were historical refuges to the rainforest fauna and flora during Pleistocene's climatic cycles. However, it is still unclear whether populations in distinct highlands experienced phenotypic differentiation as a result of adaptation to environmental conditions of each forest remnant. Herein, we used two frog species widely distributed and ecologically different, *Dendropsophus oliveirai*, a habitat specialist, and *Physalaemus cuvieri*, a habitat generalist, to investigate the relationships between environmental variation, geographic, genetic, and body size distance with advertisement call variation among populations inhabiting different highlands. Our results indicated that acoustic variation among *P. cuvieri* populations is strongly influenced by environmental variation, but also by the geographic distance among populations. In *D. oliveirai*, environment is also the most influent factor on acoustic variation, followed by a lower influence of genetic and morphological variation. Associations between environmental and geographic factors suggest indirect effects of geographic distance on acoustic variation in both species through an environmental gradient. We believe that selective processes and isolation by distance possibly act together in driving interpopulational acoustic variation with habitat-specific species being more affected by geographic isolation in suitable habitats.

Keywords

Atlantic Forest, Caatinga, environmental variation, forest enclaves, phenotypic variation

1. INTRODUCTION

Mountain ranges are remarkable systems for the study of evolution due to their role in generating and maintaining biodiversity (Hughes & Atchison, 2015; Pie et al. 2018; Guedes et al. 2020). In northeastern Brazil, humid Atlantic Forest enclaves are found in isolated highlands scattered amongst lowlands covered by xerophilous Caatinga vegetation (Andrade-Lima, 1960; Sales et al. 1998; Silveira et al. 2019). These forest remnants originated from cycles of rainforest expansion and contraction into the Caatinga during late Pliocene and Pleistocene climatic cycles (Andrade-Lima, 1982; Santos et al. 2007). Contemporary studies on population genetics and species composition suggest that, because of their stability, these forest remnants have played an important role as climatic refuges for the Atlantic Forest biota, including angiosperms and many terrestrial vertebrates (Prance, 1982; Carnaval et al. 2009; Porto et al. 2013; Costa et al. 2017; Gehara et al. 2017; Silveira et al. 2019; Menezes et al. 2020).

Amphibians have been particularly well-studied in some of these highlands, and the evaluation of molecular data across different anuran species uncovered strong population structure, high genetic diversity and evidence of demographic expansions during pluvial maxima in the Neogene (Carnaval, 2002; Carnaval & Bates, 2007; Carnaval et al. 2009). They also suggested that differences in natural history among species, specifically their degree of association with canopy cover, could be related to differences in spatial patterns in genetic structure among populations. However, no study to date evaluated whether anuran populations inhabiting different highland forests experienced phenotypic differentiation as a result of adaptation to local environmental conditions.

Climate in the northeastern highlands is more stable than that in the surrounding lowlands, but an east-west precipitation gradient exists from areas near the Atlantic coast (where annual precipitation ranges between 1,000–1,600 mm) to the hinterlands within the Caatinga biome (400–600 mm) (Velloso et al. 2002; Rodal et al. 2008; Silva et al. 2018). This variation in precipitation creates notable gradients in plant composition and forest structure (Velloso et al. 2002; Santos et al. 2007; Rodal et al. 2008), which could affect the phenotypic expression in populations distributed in this region. Given that environmental heterogeneity often generates phenotypic diversity (Calsbeek et al. 2007), differences in biotic and abiotic factors along a species' geographic distribution may potentially lead to population differentiation (Endler, 1977; Schluter, 2009).

Anurans are good models for investigating environmental influence on phenotypic variation because of their sensitivity to climate and habitat variation across the landscape, which commonly influences their physiology and phenotype (Titon Junior & Gomes, 2015; Mitchell & Bergmann, 2016; Fernandes et al. 2021). Additionally, male anurans rely on advertisement calls to attract females and repel conspecific males (Wells, 2007; Köhler et al. 2017). Such calls are species-specific and genetically inherited, and their acoustic properties are often under environmental or sexual selection (Gerhardt & Huber, 2002; Wells, 2007; Rosa et al. 2023), although they may also vary due to geographic isolation, genetic drift or selection on body size (Amézquita et al. 2009; Gingras et al. 2013; Kaefer et al. 2013; Toledo et al. 2015). Because of the role of calls in reproduction, interpopulation call differentiation is often associated with reproductive isolation in anurans, with different calls acting as prezygotic reproductive barriers (Armansin et al. 2019). In some cases, the inability of females to recognize calls from another population may reinforce pre-existing genetic differentiation, leading to speciation (Hoskin et al. 2005; Wilkins et al. 2013). Hence, detecting and mapping call differentiation among allopatric populations may be a first step in uncovering the drivers of phenotypic variation and locating areas where biological diversity can be generated and maintained.

In this study, we investigated the geographic variation in acoustic traits of the advertisement calls of two species of frogs not phylogenetically closely related (Jetz & Pyron, 2018), which have different body sizes, habits and reproductive modes, and which are widespread in highland forests in northeastern Brazil. We tested potential drivers of acoustic variation, assessing the relative roles of environment and geographic distances, while accounting for potential effects of genetic distance and body size variation among populations on call traits. Additionally, we evaluated population differentiation in call traits, in order to detect geographic boundaries and highland forest sites where incipient evolutionary divergence might be taking place. In view of the climatic and vegetational gradients that predominate in the region, our hypotheses were: (a) environmental variation is strongly related to variation in call traits, because of local selection on sound signals imposed by different forest environments; (b) geographic distance among populations affects more strongly the calls of the least habitat-generalist species, for which the open vegetation matrix is potentially less permeable; and (c) acoustic and genetic distances are correlated in the least habitat-generalist species as a consequence of isolation by adaptation, which is part of isolation by environment (Rundle & Nosil, 2005; Wang & Bradburd 2014).

2. METHODS

2.1 Study area

We conducted fieldwork between May 2021 and June 2022 in seven sampling locations distributed in highlands of northeastern Brazil, within the states of Alagoas, Paraíba and Pernambuco (Figure 1; Table 1). All locations comprise mosaics of altered landscapes and one type of native highland forest (Coimbra Filho & Câmara, 1996; Theulen, 2004; Ribeiro et al. 2009) (Table 1). Geographic distance between sampling locations varied between 40 and 354 km (Table S2). Sampling locations were distributed on highlands of the same major geomorphological unit, the Borborema Plateau. Environmental conditions across this plateau vary longitudinally, due to the orographic barrier created by high altitudes along its eastern border, resulting in dry and warm climate across the plateau's western stretches, where rainfall does not exceed 650 mm and open Caatinga vegetation predominates (Velloso et al. 2002), whereas in eastern areas of the Plateau precipitation ranges between 1,000-1,600 mm and Atlantic rainforests predominate (Silva et al. 2018).

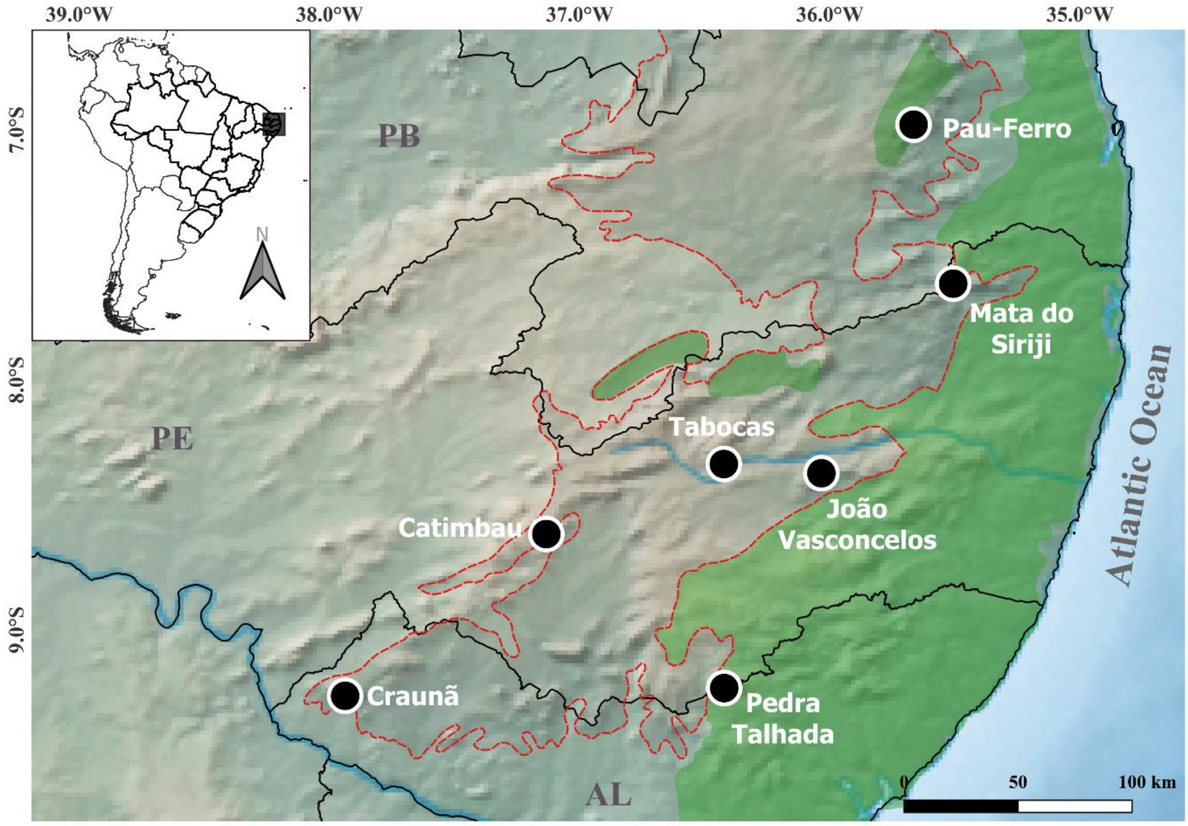

Figure 1. Geographic distribution of the sampling locations in the Borborema Plateau (dashed red line) in northeastern Brazil, north of the São Francisco River (below PE and AL states). Atlantic Forest biome in light green; Caatinga biome in green shades; PB = Paraíba; PE = Pernambuco; AL = Alagoas. Pau-Ferro = Parque Estadual Mata do Pau-Ferro, Areia-PB; Mata do Siriji = Refúgio da Vida Silvestre Matas do Sirigi, São Vicente Férrer-PE; João Vasconcelos = Parque Natural Municipal Professor João Vasconcelos Sobrinho, Caruaru-PE; Tabocas = Fazenda Vale Tabocas, Belo Jardim-PE; Catimbau = Parque Nacional do Catimbau, Buíque-PE; Pedra Talhada = Reserva Biológica de Pedra Talhada, Quebrangulo-AL; Craunã = Refúgio de Vida Silvestre Morros do Craunã e do Padre, Água Branca-AL.

Table 1. Geographic coordinates, elevation, forest type present in each sampling locations, and sample size of *Physalaemus cuvieri* and *Dendropsophus oliveirai* males from which we obtained recordings, morphological measurements and DNA sequences (number of 16S rRNA sequences in parentheses). Forest types according to Tabarelli & Santos (2004) and Studer (2015). Sampling locations abbreviations: Pau-Ferro = Parque Estadual Mata do Pau-Ferro, Areia-PB; Mata do Siriji = Refúgio da Vida Silvestre Matas do Sirigi, São Vicente Férrer-PE; João Vasconcelos = Parque Natural Municipal Professor João Vasconcelos Sobrinho, Caruaru-

PE; Tabocas = Fazenda Vale Tabocas, Belo Jardim-PE; Catimbau = Parque Nacional do Catimbau, Buíque-PE; Pedra Talhada = Reserva Biológica de Pedra Talhada, Quebrangulo-AL; Craunã = Refúgio de Vida Silvestre Morros do Craunã e do Padre, Água Branca-AL.

Sampling locations	**Latitude**	**Longitude**	**Elevation (m a.s.l)**	**Forest Type**	**Sample size**	
					P. cuvieri	***D. oliveirai***
Pau-Ferro	06°58’12.53” S	35°43’04.27” W	550	Atlantic Forest-Caatinga ecotone	10 (8)	7 (7)
Mata do Siriji	07°37’01.79” S	35°30’27.84” W	537	Atlantic Forest-Caatinga ecotone	9 (8)	6 (3)
João Vasconcelos	08°21’8.80” S	36°01’51.60” W	803	Atlantic Forest-Caatinga ecotone	8 (8)	7 (7)
Tabocas	08°14’47.5” S	36°22’46.00” W	677	semideciduous seasonal forests	12 (8)	12 (12)
Catimbau	08°34’20.60” S	37°14’12.30” W	823	semideciduous seasonal forests	7 (8)	7 (10)
Pedra Talhada	09°15’40.94” S	36°26’20.22” W	536	open ombrophylous forests	12 (8)	12 (6)
Craunã	09°20’47.90” S	37°52’60.00” W	338	semideciduous seasonal forests	12 (8)	6 (8)
Total					**70 (56)**	**57 (53)**

2.2 Study species

Physalaemus cuvieri Fitzinger, 1826 (Leptodactylidae) is a small foam-nesting frog with a wide distribution in South America, from Argentina to Venezuela (Frost, 2022). It breeds in lentic, mainly temporary water bodies, within open or forested areas (Pombal & Haddad, 2007, Aguiar et al. 2014). Their eggs are deposited in foam nests and tadpoles hatch within a few days, shortly completing their development (Barreto & Andrade, 1995; Haddad & Prado, 2005). Along their geographic distribution in northeastern Brazil, populations of *P. cuvieri* experience distinct environmental conditions, ranging from mesic to semi-arid microclimatic conditions in the Caatinga biome, and in patches of Atlantic rainforest, suggesting the species has high environmental plasticity (Barreto & Andrade, 1995; Oliveira et al. 2019). Its advertisement call has one note with complex harmonic structure, usually forming one upward-downward segment. Duration of notes range between 0.257─0.311 s and dominant frequency varies between 0.7─0.8 kHz (Hepp & Pombal Jr., 2020; Figure 2).

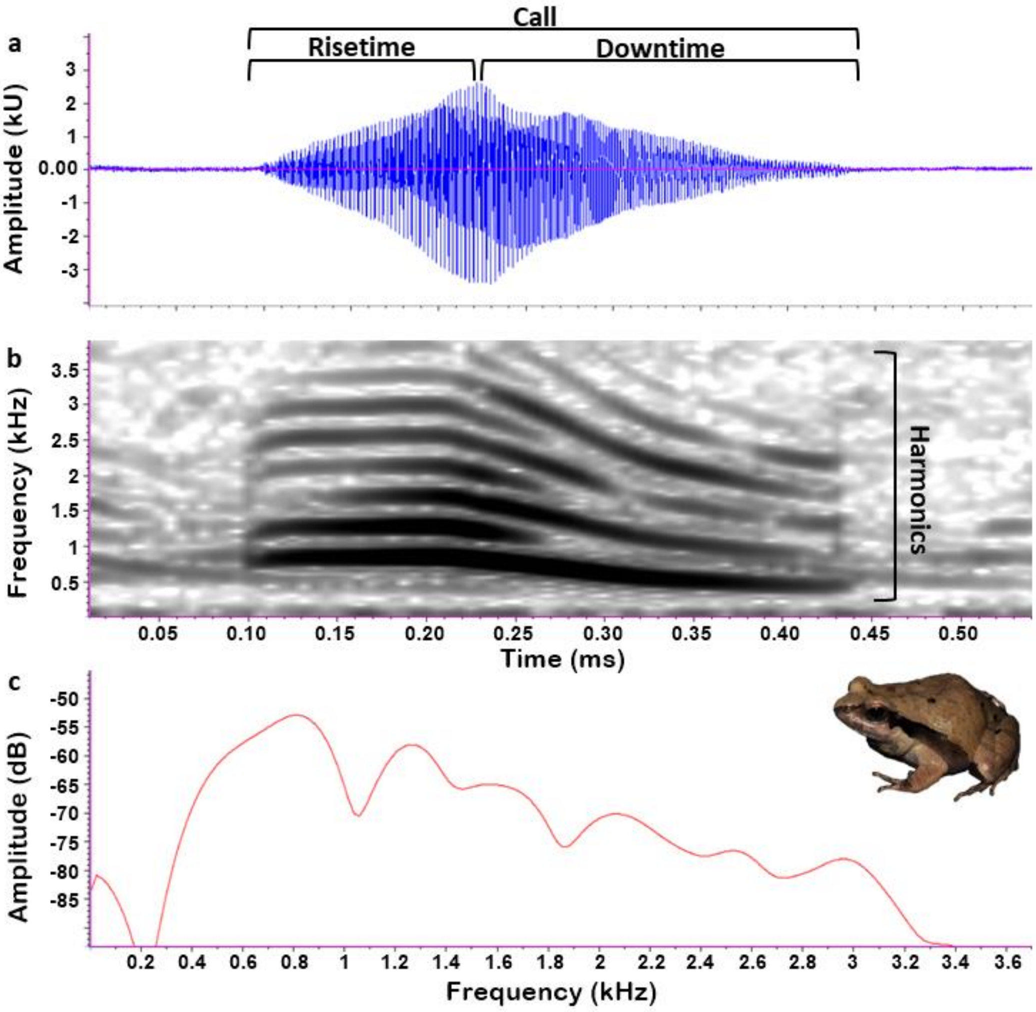

Figure 2. Oscillogram (a), spectrogram (b) and power spectrum (c) of a sample advertisement call of *Physalaemus cuvieri*.

Dendropsophus oliveirai (Bokermann, 1963) (Hylidae) is a small treefrog endemic to northeastern Brazil, found among vegetation alongside seasonal or perennial ponds near forest edges or in adjacent low vegetation (Menezes et al. 2020). Females deposit their eggs on leaves hanging over the water, where exotrophic tadpoles dive after hatching and complete their development (Haddad & Prado, 2005; Silva et al. 2019). The species is not usually found in rain pools or in shallow water bodies with short hydroperiod, but is common in lakes, ponds and other long-lived lentic water bodies that form under mesic or wet conditions in the Caatinga and Atlantic Forest biomes. The advertisement call of male *D. oliveirai* has a short (0.06─0.15 s) pulsed note (5─14 pulses per note) emitted at a high dominant frequency (5.7─6.9 kHz) (Santana et al. 2011; Figure 3).

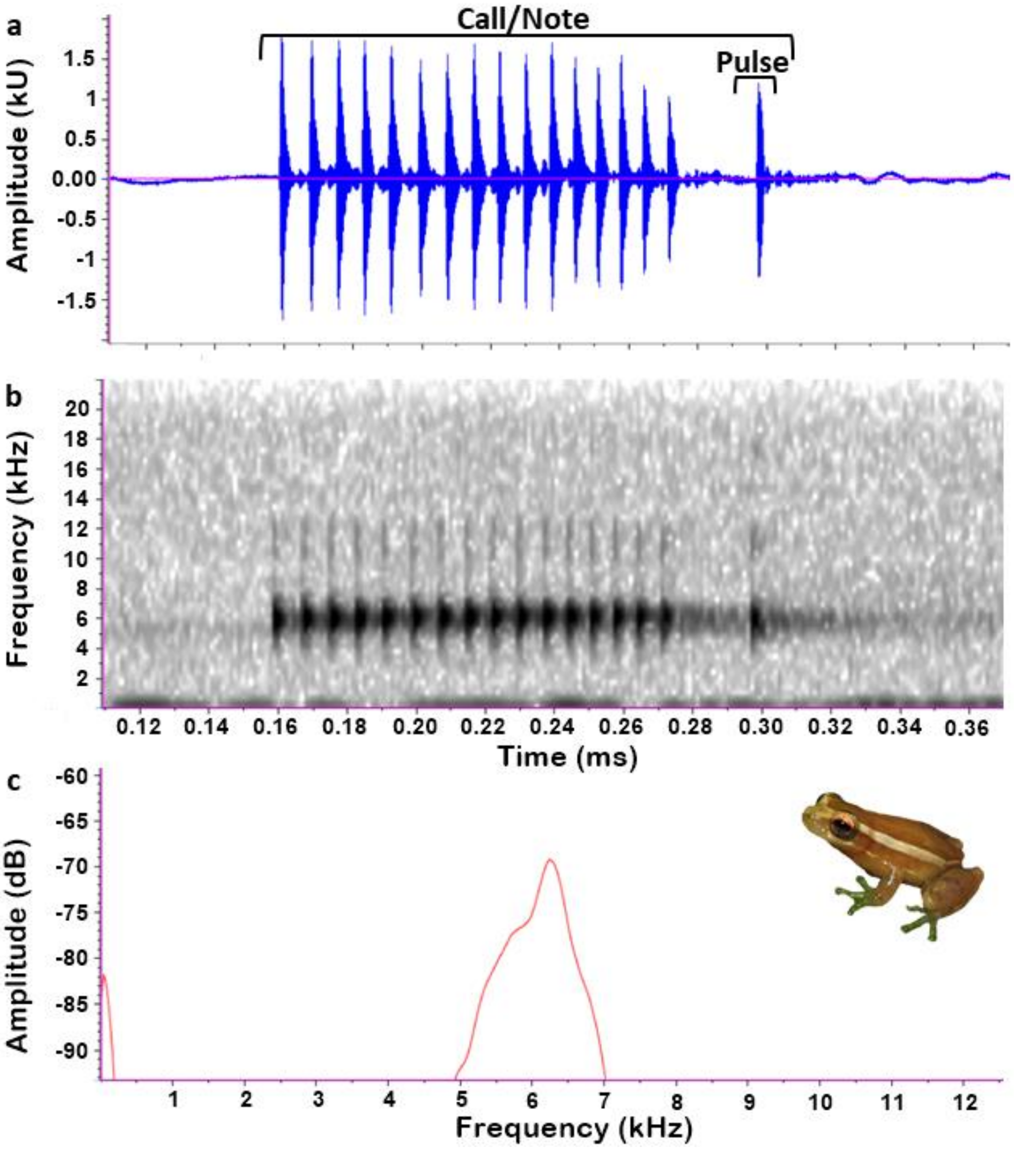

Figure 3. Oscillogram (a), spectrogram (b) and power spectrum (c) of a sample advertisement call of *Dendropsophus oliveirai*.

2.3 Acoustic data

At each sampling location we recorded advertisement calls of males from 1700 to 2300 h using digital recorders (Tascam Dr-05x, Zoom H1n or Marantz PMD620) coupled to a AKG D7 directional microphone positioned 0.5–1.0 m from the calling male. Recording settings were the same for all recorders: 44.1 kHz sampling rate, 16-bit resolution, and .WAV file format. Air temperature at the time of recording ranged between 19.5–27.9°C (*P. cuvieri*) and 19.1–26.3°C (*D. oliveirai*). In order to avoid potential variation in acoustic parameters caused by differences in behavioral context among recorded males, for recordings of both species, we selected males which were engaged in the continuous emission of advertisement calls, and were sufficiently apart from neighbour conspecifics to prevent visual contact. Males engaged in close contact interactions with other conspecifics (e.g., courtship, aggressive interactions) were not recorded. We captured each recorded male manually and transported them to a field laboratory at the nearest lodge. Specimens were anesthetized/euthanized with topical benzocaine solution (50 mg/g) and their snout-to-vent length (SVL) measured with a caliper. Then they were fixed in 10% formalin solution and preserved in 70% ethanol. We dissected a small sample of thigh muscle from each specimen before fixation and preserved it in 98% ethanol. We deposited all specimens and samples at Coleção Herpetológica da Universidade Federal de Pernambuco (CHUFPE), in Recife, Pernambuco (Supplementary Material S1).

We analyzed advertisement calls in Raven Pro 1.6 (K. Lisa Yang Center for Conservation Bioacoustics, 2023). Spectrogram parameters were: Blackman window function, window size of 10 ms (*P. cuvieri*) or 5 ms (*D. oliveirai*), overlap of 80% and DFT size of 2048 samples. Due to structural differences between advertisement calls of the two species, we measured different parameters from their calls (Table S3). For *D. oliveirai*: note duration, number of pulses per note, rate of pulse emission (pulses/second), lower frequency of the call, upper frequency of the call, frequency bandwidth of call and peak frequency of the call. For *P. cuvieri*: call duration, rise time (the time elapsed from the beginning of the call until it reached maximum amplitude), lower frequency of rise time, upper frequency of rise time, bandwidth of rise time, dominant frequency of rise time, down time (the time elapsed from the peak of amplitude until the end of the call), lower frequency of down time, upper frequency of down time, bandwidth of down time and peak frequency of down time. We measured spectral variables 20 dB below the peak frequency to avoid overlap with background noise (Erdtmann & Amézquita, 2009; Kaefer & Lima, 2012). We analysed an average of 15 calls in each recording of *D. oliveirai* (range 5–18 calls/recording) and 10 calls in each recording of *P. cuvieri* (range 6–10 calls/recording).

2.4 Molecular data

We extracted genomic DNA from preserved tissue samples using chelex-100 chelating resin (Walsh et al. 1991). We amplified a fragment of the 16S rDNA mitochondrial gene using primers 16sarL and 16sbrH of Palumbi et al. (2002). For each sample, we used 12.5 µL GoTaq DNA Master Mix (Promega, USA), 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 minutes. We purified PCR products using Wizard SV GEL and PCR Clean-up System (Promega, USA), following the manufacturer's protocol. We sequenced PCR products in both directions using the BigDye Terminator v3.1 kit (ThermoFisher) in a Genetic Analyzer 3500 automated sequencer (Applied Biosystems). We verified the resulting sequences in BioEdit 7.2 (Hall, 1999) and MEGA 11.0.13 (Tamura et al. 2021), and assembled consensus sequences using softwares Pregap4 v 1.6-r and Gap4 (Staden, 1996). The resulting sequences varied in length between 549–590 base-pairs. We aligned the sequence set of each species in MAFFT v7.017 (Katoh, 2002) using the standardized parameters. Finally, we produced a matrix of pairwise genetic distance-based fixation indexes (F_{st}; Wright, 1951) (Table S2), using Arlequin 3.5.2.2 (Excoffier & Lischer, 2010). All sequences were deposited in GenBank (accession numbers OR491066–OR491121 and OR491200–OR491252).

2.5 Environmental data

For each location, we obtained data on 19 bioclimatic variables (corresponding to the period between 1970–2000) and elevation represented in rasters with cell resolution of 30 arcseconds (~1.0 km^2) from the WorldClim 2.1 database (Fick & Hijmans, 2017). We downloaded data in R (R Core Team, 2019) using the *raster* (Hijmans et al. 2017), *sp* (Pebesma & Bivand, 2005), and *rgeos* (Bivand & Rundel, 2023) packages and the *extend* and *crop* functions. We gathered values of each variable for each sampling location using the *extract* function (Hijmans et al. 2017).

We also obtained values of EVI (Enhanced Vegetation Index) provided by the MODIS VI satellite in a 250 m resolution (MOD13Q1.061 Terra Vegetation Indices 16-Day) in the time interval between 2000 and 2022, using Google Earth Engine (https://earthengine.google.com/) (Gorelick et al. 2017). The EVI was developed to reduce the atmospheric elements and canopy background effects (Huete et al. 1997) and capture with more accuracy the green vegetation signal at higher green biomass levels (Huete et al. 2002). Finally, we calculated the maximum and minimum average of EVI using respectively highest and lowest annual values of EVI for each locality. These averages represented the vegetation density in each locality in the rainy and dry seasons, respectively.

2.6 Statistical analyses

We calculated individual averages of each acoustic parameter. Using these averages, we conducted simple linear regression analyses between call parameters and air temperature at time of recording in order to detect and remove potential effects of temperature on call variation (Gerhardt & Huber, 2002).

To test for associations and relative effect sizes of environment, geographic, genetic, and body size distances among populations on the acoustic differentiation in both species, we used two statistical approaches, the Mantel/partial Mantel tests (Mantel, 1967) and multiple matrix regression with randomization (MMRR; Wang, 2013). Mantel tests evaluate correlations between matrix pairs, whereas MMRR uses regression models involving two or more matrices and tests which class of data has the greatest influence on the matrix representing the response variable. MMRR demands the use of independent predictor variables (Manly, 1986; Legendre et al. 1994; Wang, 2013), so Mantel tests also served as a preliminary analysis to detect and exclude strongly correlated predictor variables.

From individual averages we calculated the mean of each acoustic parameter for each population (Table S3). Then, we produced acoustic and body size distance matrices among populations using the average values for each trait in each population based on Euclidean distances. We also produced an environmental distance matrix using Euclidian distances among sampling locations, combining all environmental variables (Worldclim and EVI) for each locality. We created a geographic distance matrix (Table S2) using the geodesic geographic distance among locations based on GPS coordinates, using the *rdist.earth* function of *fields* 14.1 R package (Nychka et al. 2021). We tested correlations between acoustic, body size, genetic, environmental, and geographic distance matrices using Mantel tests with 1,000 permutations, via Pearson method (Mantel, 1967) in the *vegan* 2.6.4 R package (Oksanen et al. 2022). We conducted the MMRR with 10,000 permutations using the *MMRR* function of the *PopGenReport* 3.0.7 package (Adamack & Gruber, 2015), with acoustic distance as the response matrix and body size, genetic, environmental, and geographic distances as explanatory matrices. In MMRR the set of different distances created from explanatory variables is known as least-cost distance. We assigned body size and genetic distance matrices as covariates because they are often associated with variation of acoustic parameters in anurans (Gerhardt & Huber, 2002; Smith et al. 2003; Amézquita et al. 2009).

To evaluate call differentiation among populations of both species, we applied a principal component analysis (PCA) on the mean values of acoustic variables for each population (Tables S4–S5). We used the resulting scores of the first and second principal components (PC1 and PC2) to graphically evaluate the distribution of population averages along the acoustic space. We applied another PCA on individual means of acoustic variables and used the scores of the two first components in a Kruskal-Wallis and *post hoc* (Wilcoxon-Mann-Whitney) tests to verify differences among populations. Finally, we tested for differences in body size among populations using ANOVA. All statistical analyses were conducted in R (R Core Team, 2019) at a significance level of $\alpha=0.05$.

3. RESULTS

Linear regression analyses indicated no high significant relationships between air temperature and acoustic parameters in both species (Table S6). Also, an additional Mantel test between average differences in air temperature and geographic distances showed no significant association between these variables for sampling locations of both species (*P. cuvieri*: $r = -0.05; p = 0.52$; *D. oliveirai*: $r = -0.37; p = 0.93$). Despite that, we present Mantel/partial Mantel and MMRR results for both not adjusted and temperature-adjusted acoustic data.

Mantel tests revealed that acoustic distances among highland populations of *Physalaemus cuvieri* correlated more strongly with environmental distances among sampling locations (Table 2). A weaker but significant correlation was also detected between acoustic distances and geographic distances among populations. Correlation between acoustic distances and environmental distances were still significant after controlling for the effect of geographic distance on environmental distances on a partial Mantel test. Genetic and body size distances among populations were not associated with acoustic distances in *P. cuvieri*. Statistically significant correlation among association matrices based on predictor variables and covariates was only detected between genetic distances and geographic distances among sampling locations (Table 2).

In *Dendropsophus oliveirai*, acoustic distances among populations also correlated more strongly with environmental distances among sampling locations. The correlation remained significant after controlling for potential effects of geographic distance on environmental distances among locations (Table 2). Genetic and body size distances among populations were weakly, but significantly correlated with acoustic distances. There was no correlation between geographic and acoustic distances among sampling locations, although geographic and genetic distances were moderately correlated. Statistically significant correlation was detected between genetic, geographic, and environmental distances among populations (Table 2).

Table 2. Correlation coefficients based on Mantel (x) and partial Mantel (-) tests on acoustic (CALL), environmental (ENV), geographic (GEO), body size (SVL), and genetic (GEN) distances for seven populations of the anurans *Physalaemus cuvieri* and *Dendropsophus oliveirai* inhabiting highland forests in northeastern Brazil. Values in bold highlight statistically significant correlations ($p \leq 0.05$).

Correlations	***P. cuvieri***	***D. oliveirai***
GEO x CALL	**$r = 0.57; p = 0.02$**	$r = 0.21; p = 0.20$
ENV x CALL	**$r = 0.71; p = 0.03$**	**$r = 0.59; p < 0.01$**
GEN x CALL	$r = 0.05; p = 0.41$	**$r = 0.42; p = 0.03$**
SVL x CALL	$r = -0.01; p = 0.48$	**$r = 0.40; p = 0.05$**
GEO x GEN	**$r = 0.62; p < 0.01$**	**$r = 0.57; p < 0.01$**
ENV x GEN	$r = -0.03; p = 0.46$	**$r = 0.51; p < 0.01$**
GEO x SVL	$r = -0.04; p = 0.50$	$r = 0.13; p = 0.25$
ENV x SVL	$r = -0.09; p = 0.64$	$r = 0.32; p = 0.07$
GEN x SVL	$r = -0.20; p = 0.86$	$r = 0.18; p = 0.22$
GEO x ENV	**$r = 0.51; p = 0.03$**	**$r = 0.45; p = 0.05$**
ENV x CALL - GEO	**$r = 0.60; p = 0.05$**	**$r = 0.56; p = 0.03$**

As collinearity was not strong (< 0.63) among distance matrices of explanatory variables and covariates, we used all matrices in the MMRR for both species. The overall fit of the MMRR model for *P. cuvieri* was relatively high ($R^2 = 0.61; F = 6.24; p = 0.04;$ Figure S7a). Among the parameters in the least-cost distance the geographic distance was the best predictor of acoustic distance among populations (Table 3). A second potential predictor, environmental distance was moderately correlated with acoustic distance among populations, but the association was not statistically significant ($\beta = 0.42$; $p = 0.11$). In *D. oliveirai*, the overall fit of the MMRR model was marginally significant ($R^2 = 0.42; F = 2.89; p = 0.07;$ Figure S7b), with only environmental distance potentially acting as a moderate, yet not statistically significant ($\beta = 0.48$; $p = 0.11$), predictor of acoustic distance among populations (Table 3).

Table 3. Results of the multiple matrix regression with randomization test on acoustic (CALL), environmental (ENV), geographic (GEO), body size (SVL), and genetic (GEN) distances for seven populations of the anurans *Physalaemus cuvieri* and *Dendropsophus oliveirai* inhabiting highland forests in northeastern Brazil. Values in bold highlight statistically significant correlations ($p \leq 0.05$).

Regressions	***P. cuvieri***	***D. oliveirai***
GEO x CALL	**$\beta = 0.55; p = 0.04$**	$\beta = -0.13; p = 0.62$
ENV x CALL	$\beta = 0.42; p = 0.11$	$\beta = 0.48; p = 0.11$
GEN x CALL	$\beta = -0.32; p = 0.28$	$\beta = 0.18; p = 0.46$
SVL x CALL	$\beta = -0.02; p = 0.91$	$\beta = 0.25; p = 0.25$

Replacing acoustic variables weakly correlated with environmental temperature (Table S6) with their residuals in Mantel/partial Mantel and MMRR did not change the observed relationships between acoustic and environmental and geographic distances for *P. cuvieri* (Table S8). However, for *D. oliveirai*, the only significant relationship observed was between acoustic distance and environmental distance, suggesting that associations between acoustic distance and geographic, body-size, and genetic distances in this species may be partly due to differences in temperature at time of recording among sampling localities (Table S8).

The first two principal components from the *P. cuvieri's* PCA explained together 80.5% of the acoustic variation among populations. The first component (PC1) explained 55.2% of the total acoustic variation, with spectral parameters (upper frequency of the risetime and bandwidth of risetime) presenting high loadings (Figure 4b; Table S4). The second component (PC2) explained 25.3% of the total acoustic variation among populations, with temporal parameters (call duration and risetime) presenting high loadings (Figure 4b; Table S4). The distribution of populations of *P. cuvieri* along PC1 indicated the population of Craunã as highly different from the other highland populations based on spectral acoustic parameters (Figure 4b; Table S5). Along PC2, which mostly represented temporal call traits, Pedra Talhada was highly divergent from other populations (Figure 4b; Table S5). Remaining populations were grouped relatively close to each other on the acoustic space.

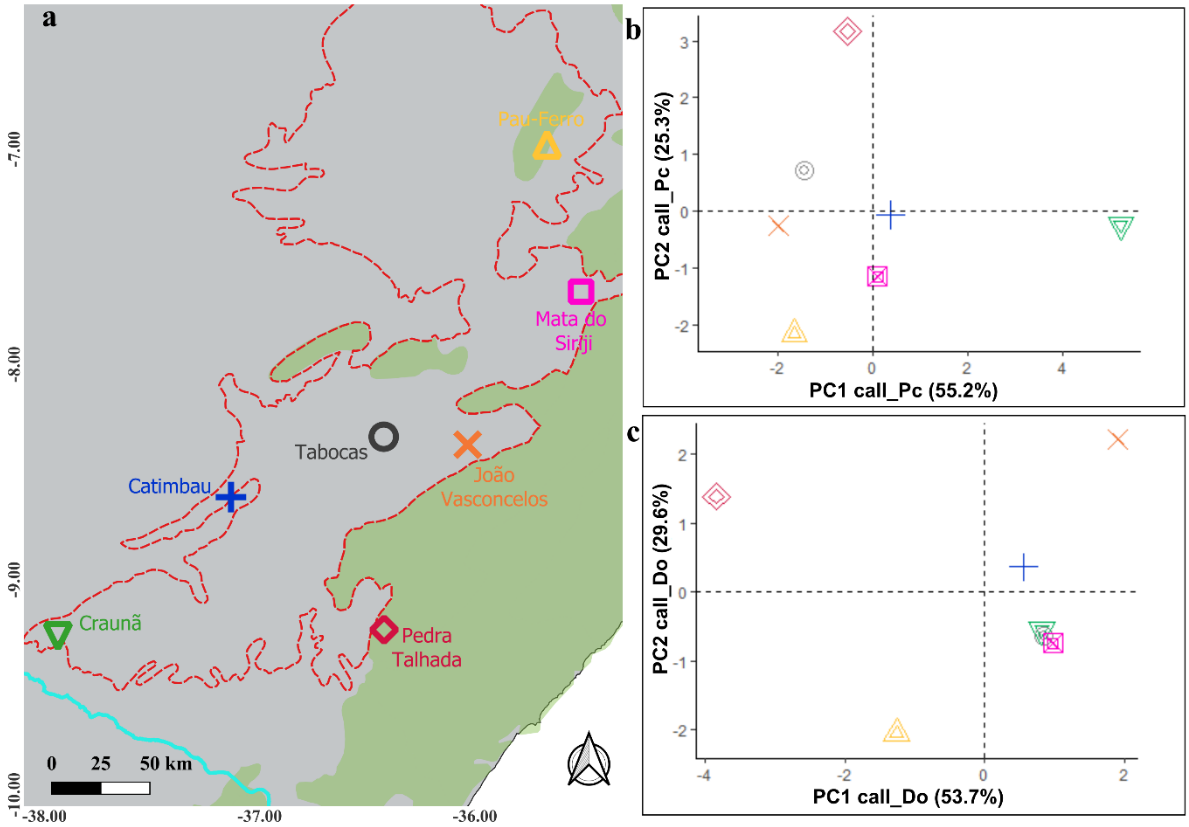

Figure 4. Geographic distribution of sampling locations across the Borborema Plateau (dashed red line) in northeastern Brazil (a). Distribution of populations of *P. cuvieri* (b) and *D. oliveirai* (c) along the first two principal components based on mean values of acoustic parameters of their advertisement calls. Atlantic Forest biome in light green; Caatinga biome in gray; São Francisco River below Borborema Plateau.

The first two principal components from PCA of *D. oliveirai* accounted for 83.3% of the total acoustic variation among populations. PC1 explained 53.7% of the total acoustic variation, with spectral parameters (peak frequency and upper frequency) presenting high loadings (Figure 4c; Table S4). PC2 explained 29.6% of the total acoustic variation, with structural parameters (pulse number and pulse rate) presenting high loadings (Figure 4c; Table S4). The distribution of *D. oliveirai* populations along PC1, which account mostly for spectral parameters, indicated Pedra Talhada as highly differentiated from the other populations (Figure 4c; Table S5), which grouped relatively close along the two principal components. Populations from Pau-Ferro and João Vasconcelos diverged slightly from the others, although in opposite directions in the acoustic space (Figure 4c; Table S5).

Based on the PCA scores from the raw (individual) data of acoustic parameters, *P. cuvieri* populations were significantly different in the first ($H = 36.96; p < 0.0001$) and second

(*H = 17.24; p = 0.008*) components (Figure S9a,b). *Post hoc* tests showed that Craunã was significantly different from Tabocas (*p = 0.003*), Pau-Ferro (*p < 0.0001*), Catimbau (*p = 0.0008*), João Vasconcelos (*p = 0.006*), Pedra Talhada (*p < 0.0001*), and Mata do Siriji (*p = 0.001*) in the first component. The localities Craunã and Pedra Talhada were also significantly different from each other in the second principal component (*p = 0.003*).

Dendropsophus oliveirai populations were significantly different in the first acoustic component (*H = 15.73; p = 0.01*) but not in the second (*H = 5.27; p = 0.51*) (Figure S9c,d). Only João Vasconcelos and Pedra Talhada were different from each other in the first component (*p = 0.05*). There was a significant difference in body size among *P. cuvieri* populations (*F = 3.89; p = 0.002*), but not among *D. oliveirai* populations (*F = 0.66; p = 0.68*) (Figure S10).

4. DISCUSSION

Our results demonstrate that acoustic variation among populations of *P. cuvieri* and *D. oliveirai* inhabiting highland forests in northeastern Brazil is more strongly influenced by environmental factors and isolation by distance than by other common predictors of call variation in anurans. Genetic distance and body size have weak or inexistent influence on acoustic variation of those populations. These relationships, however, are idiosyncratic, varying in strength and relevance between the two study species, with mixed contributions of putative causal factors and covariates in explaining acoustic differentiation in each taxon. These are partly in agreement with our main hypotheses, and with some predictions raised in relation to potential effects of habitat variation and isolation by distance on call variation among populations of species more or less associated with forest habitats. Our study shows that islands of moist environments in high altitudes across northeastern Brazil can also be related to microevolutionary processes generating phenotypic divergence.

Acoustic adaptation to open or forested environments has been investigated in many animal groups that rely on sound signals for long-range communication, including anurans (e.g., Ey & Fisher, 2009; Wilkins et al. 2013; Erdtmann & Lima, 2013; Velásquez et al. 2018; Gomes et al. 2022; Rosa et al. 2023), but conclusions have been contentious. According to the acoustic adaptation hypothesis (AAH - Morton, 1975), habitat vegetation affects the transmission of sound through reverberation, degradation, and differential attenuation of particular frequencies, dense forest environments selecting calls with lower dominant frequencies, narrower frequency bandwidths, and longer duration (Morton, 1975; Wilkins et al.

2013). Across our study area, highland forests vary in tree coverage according to a longitudinal precipitation gradient, resulting in distinct selective pressures on call transmission at each sampling location. This could explain the associations found between acoustic and environmental variation in our study species. Craunã has the most open forest habitat among sampling locations. There, *P. cuvieri* males produce calls with the highest observed frequencies and the shortest duration (Figure S9a,b), suggesting that calls might be adapted to its Caatinga-influenced environment. On the other hand, in the densely vegetated forests east of Craunã, *P. cuvieri* produce calls with relatively low frequencies and long duration (Table S9a,b).

Associations between the surrounding vegetation and acoustic parameters were also investigated in calls of the treefrog *Pithecopus gonzagai* in different environments of the Caatinga and Atlantic Forest biomes (Röhr et al. 2020; Gomes et al. 2022). The first study demonstrated partial support to the AAH based on differences in acoustic parameters among populations and in the efficiency of sound propagation in different environments. Both approaches showed that temporal and structural call parameters correlated with vegetation density. Gomes et al. (2022) reported an inverse relationship between spectral parameters and vegetation coverage. Although not focused on highland forest populations, these studies reinforce our finding that the AAH has a mixed role in explaining acoustic variation in anuran species and might be dependent on the taxa and populations studied.

Among populations of *D. oliveirai,* acoustic variation was also more strongly influenced by environmental distance among sampling locations, and weakly by genetic and body size distances. In comparison with *P. cuvieri*, *D. oliveirai* breeds in lentic water bodies with longer hydroperiods, normally available in more humid climates (Menezes et al. 2020), suggesting that these populations could be potentially isolated from each other. We hypothesize that calls of highland populations of *D. oliveirai* may vary along the environmental gradient mostly as a result of geographic isolation in more suitable environments, as suggested by the relatively higher genetic distances between them (Table S2) and its correlation with geographic and environmental distances. Associations between geographic and environmental distances and their relationships with genetic and acoustic variation potentially indicate an accumulation of neutral and selective processes (Gould & Johnston, 1972), which may have led to greater differentiation among populations of *D. oliveirai*.

Although largely sympatric in our study area, our study species are not closely related phylogenetically and have distinct ecological requirements. *Physalaemus cuvieri* inhabits both forested and open vegetation areas, which is suggestive of increased phenotypic plasticity or adaptive response to different environmental conditions. This is supported by higher acoustic differentiation among populations (Figure S9), even under apparently high gene flow, and by acoustic distance being correlated with environmental distance, but not with genetic distance among populations, hinting at a scenario of adaptative divergence (Zamudio et al. 2016). Uncoupled evolution among phenotypic and neutral genetic traits is not uncommon among Neotropical anuran populations, especially among those which experienced demographic expansions during Neogene climatic cycles (Nosil, 2008; Amézquita et al. 2009; Pinho & Hey, 2010; Twomey et al. 2013).

Despite some degree of acoustic variation among populations of *D. oliveirai*, acoustic conservatism among them is apparently strong. Call frequency varied significantly only among two populations (Figure S9c). Their limitation to lentic reproductive habitats, with longer hydroperiods, could be related to stabilising selection on calls, as a result of similar habitat structure. Alternatively, convergence or stability of call properties among populations could be maintained by stabilizing selection by female choice (Tobias et al. 2011; 2014; Toledo et al. 2015). These non-exclusive hypotheses deserve future investigation based on call propagation across reproductive environments and on playback experiments addressing mate choice. Additionally, since adaptative phenotypic variation and genetic divergence are direct or indirectly correlated, isolation by adaptation (Andersson, 1994; Boughman, 2001; Hoekstra et al. 2004; Boul et al. 2007) could be one evolutionary mechanism operating on *D. oliveirai* populations in this region.

Although there was no significant difference in body size among *D. oliveirai* populations (Figure S10), we found a possible low association between acoustic and body size distances. In each location, different natural or sexual selection regimes can affect variation in average body size of males (Robertson & Robertson, 2008) which, in turn, can result in variation of acoustic parameters through allometric relationships with the sound producing apparatus (Ryan & Brenowitz, 1986; Ryan, 1988; Gerhardt & Huber, 2002). An inverse relationship between body size and call frequencies is well known in anurans, with larger males producing calls with lower frequencies (Gingras et al. 2013).

When populations are weakly differentiated from each other, MMRR has more difficulties detecting the influence of landscape features (Wang, 2013). Hence, low acoustic differentiation among populations of *D. oliveirai* could partly explain the marginally significant associations between acoustic distance and the explanatory variables in the MMRR model. Moreover, MMRR can slightly underestimate the relative strength of the explanatory variables as a result of statistical noise caused by the stochastic variation of the response variable (Wang, 2013). This may have led to some relationships being recovered as not statistically significant in MMRR analyses, while statistically significant when addressed in Mantel tests.

Our results demonstrate that the acoustic evolution of co-occurring anuran populations of different species in highland forests of northeastern Brazil followed different patterns. However, environmental variation is possibly the most important factor influencing acoustic variation in both species, with a secondary role played by isolation by distance in the more generalist species. Although call differentiation is probably not a pre-zygotic reproductive barrier among highland populations of *D. oliveirai* in the present, in evolutionary time, post-zygotic barriers may develop due to genetic differentiation among them. Regardless of the acting evolutionary process, our perspective is that the growing fragmentation among highland forests will probably make them increasingly isolated, resulting in greater genetic disparity among populations. On the other hand, acoustic adaptation in response to geographic variation in reproductive environments seems to be the main driver of call differentiation in *P. cuvieri*, with low genetic distances among populations indicating that any barriers to gene flow among populations are currently pervasive, or have been in the recent past.

Our findings highlight that geographic variation in climate and vegetation of highland forests potentially affect advertisement call differentiation, which is probably intensified by isolation by distance among populations. Thus, in the heterogeneous landscape system of highland forests of northeastern Brazil, adaptation to the local environment and genetic drift probably act together in determining spatial patterns of acoustic signal variation in anurans. Our findings reinforce the idea that environmental variation and dispersion limits imposed by geographic distance can act together, affecting phenotypic differentiation in small vertebrates (Sun et al. 2013; Engen & Saether, 2016).

In Brazil, conservation action planning and environmental legislation generally overlooks biodiversity below the taxonomic level of species, jeopardizing phenotypic and genetic diversity which are key to the adaptive response of any species in the face of imminent climate change (Chevin & Lande, 2010; Kelly, 2019). Our work reinforces the role of Brazilian northeastern highland forests as environments prone to generating and maintaining biological diversity. Hence, their conservation, whether by means of delimitation of conservation units, or by scientifically informed management of these areas, is of paramount importance.

AUTHOR CONTRIBUTIONS

JHAL and PIS conceived the manuscript, designed sampling strategies, and collected the data. JHAL analyzed the data. JHAL, PIS and MK wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

We declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.5x69p8d95 (Andrade Lima, 2023).

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REFERENCES

Adamack, A. T., & Gruber, B. (2015). “Landgenreport: a new R function to simplify landscape genetic analysis using resistance surface layers.” Molecular Ecology Resources 15(5), 1172 – 1178. https://doi.org/10.1111/1755-0998.12381

Andersson, M. (1994). *Sexual selection*. Princeton University Press. https://doi.org/10.1515/9780691207278

Aguiar, A., Toledo, G. M., Anjos, L. A., & Silva, R. J. (2014). Helminth parasite communities of two *Physalaemus cuvieri* Fitzinger, 1826 (Anura: Leiuperidae) populations under different conditions of habitat integrity in the Atlantic Rain Forest of Brazil. Brazilian Journal of Biology 75(4), 963–968. https://doi.org/10.1590/1519-6984.03614

Amézquita, A., Lima, A. P., Jehle, R., Castellanos, L., Ramos, O., Crawford, A. J., Gasser, H., & Höld, W. (2009). Calls, colours, shape, and genes: a multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. Biological Journal of the Linnean Society 98, 826–838. https://doi.org/10.1111/j.1095-8312.2009.01324.x

Andrade-Lima, D. (Ed.). (1960). Estudos fitogeográficos de Pernambuco. *Arquivo do Instituto de Pesquisa Agronômica* (vol 5, pp. 305–341). Secretaria da Agricultura, Indústria e Comércio, Pernambuco. Brasil.

Andrade-Lima, D. (1982). Present day forest refuges in Northeastern Brazil. In G.T. Prance (ed.), *Biological Diversification in the Tropics* (pp. 245–254). Columbia University Press, New York.

Armansin, N. C., Stow, A.J., Cantor, M., Leu, S. T., Klarrevas-Irby, J. A., Chariton, A. A., & Farine, D. R. (2019). Social Barriers in Ecological Landscapes: The Social Resistance Hypothesis. Trends in Ecology and Evolution 35(2), 137–148. https://doi.org/10.1016/j.tree.2019.10.001

Atkinson, D. (1994). Temperature and organism size — a biological law for ectotherms? Advances in Ecological Research 25, 1–58. https://doi.org/10.1016/S0065-2504(08)60212-3

Barreto, L., & Andrade, G. V. (1995). Aspects of the reproductive biology of *Physalaemus cuvieri* (Anura: Leptodactylidae) in northeastern Brazil. Amphibia-Reptilia, Leiden 16(1), 67–76. https://doi.org/10.1163/156853895X00208

Bivand. R, & Rundel, C. (2023). *rgeos: Interface to Geometry Engine - Open Source ('GEOS')*. https://r-forge.r-project.org/projects/rgeos/

Boughman, J. W. (2001). Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411(6840), 944–948. https://doi.org/10.1038/35082064

Boul, K. E., Funk, W. C., Darst, C. R., Cannatella, D. C., & Ryan, M. J. (2007). Sexual selection drives speciation in an Amazonian frog. Proceedings of the Royal Society B: Biological Sciences 274(1608), 399–406. https://doi.org/10.1098/rspb.2006.3736

Calsbeek, R., Smith, T. B., Bardeleben, C. (2007). Intraspecific variation in *Anolis sagrei* mirrors the adaptive radiation of Greater Antillean anoles. Biological Journal of the Linnean Society 90, 189–199. https://doi.org/10.1111/j.1095-8312.2007.00700.x

Carnaval, A. C. O. Q. (2002). Phylogeography of Four Frog Species in Forest Fragments of Northeastern Brazil—A Preliminary Study. Integrative and Comparative Biology 42, 013–921. https://doi.org/10.1093/icb/42.5.913

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

Carnaval, A. C., Hickerson, M. J., Haddad, C. F. B., Rodrigues, M. T., & Moritz, C. (2009). Stability Predicts Genetic Diversity in the Brazilian Atlantic Forest Hotspot. Science 323, 785–789. https://doi.org/10.1126/science.1166955

Charif, R. A., Strickman, L. M., & Waack, A. M. (2010). Raven Pro 1.4 User's Manual. The Cornell Lab of Ornithology, Ithaca, NY.

Chevin, L-M., Lande, R. (2010) When do adaptive plasticity and genetic evolution prevent extinction of a density-regulated population? Evolution 64, 1143 – 1150. https://doi.org/10.1111/j.1558-5646.2009.00875.x

Coimbra Filho, A. F., & Câmara, I. G. (Eds.). (1996). *Os limites originais do bioma Mata Atlântica na região Nordeste do Brasil*. Fundação Brasileira para a Conservação da Natureza (FBCN), Rio de Janeiro.

Costa, G. C., Hampe, A., Ledru, M-P., Martinez, P. A., Mazzochini, G. G., Shepard, D. B., Werneck, F. P., Moritz, C., & Carnaval, A. C. (2017). Biome stability in South America over the last 30 kyr: Inferences from long-term vegetation dynamics and habitat modelling. Global Ecology and Biogeography 00, 1–13. https://doi.org/10.1111/geb.12694

Endler, J. A. (1977). *Geographic variation, speciation and clines*. Princeton University Press, Princeton.

Engen, S., & Sæther, B. (2016). Phenotypic evolution by distance in fluctuating environments: the contribution of dispersal, selection and random genetic drift. Theoretical Population Biology 109, 16–27. https://doi.org/10.1016/j.tpb.2016.01.003

Erdtmann, L., & Amézquita, A. (2009). Differential evolution of advertisement call traits in dart-poison frogs (Anura: Dendrobatidae). Ethology 115(9), 801–811. https://doi.org/10.1111/j.1439-0310.2009.01673.x

Erdtmann, L. K., & Lima, A. P. (2013). Environmental effects on anuran call design: what we know and what we need to know. Ethology Ecology & Evolution 25(1), 1–11. https://doi.org/10.1080/03949370.2012.744356

Ey, E., & Fischer, J. (2009). The "acoustic adaptation hypothesis"–a review of the evidence from birds, anurans and mammals. Bioacoustics 19(1–2), 21–48. https://doi.org/10.1080/09524622.2009.9753613

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Fernandes, I. Y., Moraes, L. J. C. L., Menin, M., Farias, I. P., & Lima, A. P. (2021). Unlinking the Speciation Steps: Geographical Factors Drive Changes in Sexual Signals of an Amazonian Nurse-Frog Through Body Size Variation. Evolutionary Biology 48(22), 1–13. https://doi.org/10.1007/s11692-020-09525-7

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37(12), 4302–4315. https://doi.org/10.1002/joc.5086

Frost, D. R. (2022). [viewed 18 January 2022]. Amphibian species of the world: an online reference. Version 6.1. Electronic database [online]. New York: American Museum of Natural History. Available from: https://amphibiansoftheworld.amnh.org/index.php.

Gehara, M., Garda, A. A., Werneck, F. P., Oliveira, E. F., Fonseca, E. M., Camurugi, F., Magalhães, F. M., Lanna, F. M., Sites Jr., J. W., Marques, R., Silveira-Filho, R., São Pedro, V. A., Colli, G. R., Costa, G. C., & Burbrink, F. T. (2017). Estimating synchronous demographic changes across populations using hABC and its application for a herpetological community from northeastern Brazil. Molecular Ecology 26, 4756–4771. https://doi.org/10.1111/mec.14239

Gerhardt, H. C., & Huber, F. (Eds.). (2002). *Acoustic communication in insects and anurans: Common problems and diverse solutions*. University of Chicago Press, Chicago, Illinois.

Gingras, B., Boeckle, M., Herbst, C. T., & Fitch, W. T. (2013). Call acoustics reflect body size across four clades of anurans. Journal of Zoology 289(2), 143–150. https://doi.org/10.1111/j.1469-7998.2012.00973.x

Gomes, L., Röhr, D. L., Sousa-Lima, R. S., Garda, A. A., & Juncá, F. A. (2022). Environmental factors influencing call propagation in *Pithecopus nordestinus*: testing the acoustic adaptation hypothesis. Bioacoustics 31(6), 696–709. https://doi.org/10.1080/09524622.2022.2042855

Gorelick, N., Hancher, M., Dixon, M., Ilyushchenko, S., Thau, D., & Moore, R. (2017). Google Earth Engine: Planetary-scale geospatial analysis for everyone. Remote Sensing Environment 202, 18–27. https://doi.org/10.1016/j.rse.2017.06.031

Gould, S. J., Johnston, R. F. (1972). Geographic variation. Annual Review of Ecology, Evolution, and Systematics 3, 457–498. https://doi.org/10.1146/annurev.es.03.110172.002325

Guedes, T. B., Azevedo, J. A. R., Bacon, C. D., Provete, D. B., & Antonelli, A. (2020). Diversity, endemism, and evolutionary history of montane biotas outside the Andean region. In: V. Rull, & A. C. Carnaval (Eds.). *Neotropical Diversification: patterns and process* (pp. 299–328). Springer. https://doi.org/10.1007/978-3-030-31167-4_13

Haddad, C. F. B., & Prado, C. P. A. (2005). Reproductive Modes in Frogs and Their Unexpected Diversity in the Atlantic Forest of Brazil. BioScience 55(3), 207–217. https://doi.org/10.1641/0006-3568(2005)055[0207:RMIFAT]2.0.CO;2

Hall, T. A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95–98.

Hepp, F., & Pombal Jr., J. P. (2020). Review of bioacoustical traits in the genus *Physalaemus* Fitzinger, 1826 (Anura: Leptodactylidae: Leiuperinae). Monograph 4725(1), 001–106. https://doi.org/10.11646/zootaxa.4725.1.1

Hijmans, R. J. (2017). *raster: geographic data analysis and modeling*. R package version 2.6–7. Available from: https://CRAN.R-project.org/package=raster

Hoekstra, H. E., Drumm, K. E., & Nachman, M. W. (2004). Ecological genetics of adaptive color polymorphism in pocket mice: Geographic variation in selected and neutral genes. Evolution 58(6), 1329–1341. https://doi.org/10.1111/j.0014-3820.2004.tb01711.x

Hoskin, C. J., Higgie, H., McDonald, K. R., & Moritz, C. 2005. Reinforcement drives rapid allopatric speciation. Nature 437, 1353–1356. http://dx.doi.org/10.1038/nature04004

Huete, A., Liu, H. Q., Batchily, K., & Van Leeuwen, W. (1997). A comparison of vegetation indices over a global set of Tm images for EOS-MODIS. Remote Sensing of Environment 59, 440–451. https://doi.org/10.1016/S0034-4257(96)00112-5

Huete, A., Didan, K., Miura, T., Rodriguez, E. P., Gao, X., & Ferreira, L. G. (2002). Overview of the radiometric and biophysical performance of the MODIS vegetation indices. Remote Sensing of Environment 83, 195–213. https://doi.org/10.1016/S0034-4257(02)00096-2

Hughes, C. E., & Atchison, G. W. (2015). The ubiquity of alpine plant radiations: from the Andes to the Hengduan Mountains. The New Phytologist 207, 275–282. https://doi.org/10.1111/nph.13230

Jetz, W., & Pyron, R. A. (2018). The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. Nature Ecology & Evolution 2, 850–858. https://doi.org/10.1038/s41559-018-0515-5

Kaefer, I. L., & Lima, A. P. (2012). Sexual signals of the Amazonian frog *Allobates paleovarzensis*: geographic variation and stereotypy of acoustic traits. Behaviour 149, 15–33. https://doi.org/10.1163/156853912X623757

Kaefer, I. L., Tsuji-Nishikido, B. M., Mota, E. P., Farias, I. P., & Lima, A. P. (2013). The Early Stages of Speciation in Amazonian Forest Frogs: Phenotypic Conservatism Despite Strong Genetic Structure. Evolutionary Biology 40, 228–245. https://doi.org/10.1007/s11692-012-9205-4

Katoh, K. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research, 30, 3059–3066. https://doi.org/10.1093/nar/gkf436

Kelly, M. (2019). Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. Philosophical Transactions Royal Society B 374, 20180176. http://dx.doi.org/10.1098/rstb.2018.0176

K. Lisa Yang Center for Conservation Bioacoustics. (2023). Revan Pro: Interactive Sound Analysis Software (Version 1.6.4). Ithaca, NY: The Cornell Lab of Ornithology. Available from: http://ravensoundsoftware.com/

Köhler, J., Jansen, M., Rodríguez, A., Kok, P. J. R., Toledo, L. F., Emmrich, M., Glaw, F., Haddad, C. F. B., Rödel, M-O., & Vences, M. (2017). The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. Zootaxa 4251(1), 001–124.

Legendre, P., Lapointe, F.-J., & Casgrain, P. (1994). Modeling brain evolution from behavior: a permutational regression approach. Evolution 48,1487–1499. https://doi.org/10.2307/2410243

Manly, B. F. J. (1986). Randomization and regression methods for testing for associations with geographical, environmental and biological distances between populations. Researches on Population Ecology. Ecol. 28,201–218. https://doi.org/10.1007/BF02515450

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. Cancer Research 27, 209–220.

Menezes, L., Batalha-Filho, H., Garda, A. A., & Napoli, M. F. (2020). Tiny treefrogs in the Pleistocene: Phylogeography of *Dendropsophus oliveirai* in the Atlantic Forest and associated enclaves in northeastern Brazil. Journal of Zoological Systematics and Evolutionary Research 00, 1–16. https://doi.org/10.1111/jzs.12422

Mitchell, A., & Bergmann, P. J. (2016). Thermal and moisture habitat preferences do not maximize jumping performance in frogs. Functional Ecology 30(5), 733–742. https://doi.org/10.1111/1365-2435.12535

Morton, E. S. (1975). Ecological sources of selection on avian sounds. The American Naturalist 109, 17–34. https://doi.org/10.1086/282971

Nosil, P. (2008). Speciation with gene flow could be common. Molecular Ecology 17, 2103–2106.

Nychka, D., Furrer, R., Paige, J., & Sain, S. (2021). *"fields: Tools for spatial data."* R package version 14.1. https://github.com/dnychka/fieldsRPackage

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'hara, R. B., Simpson, G.L., Solymos, P., & Stevens, M. H. H. (2022). *Vegan: community ecology package* (version 2.6-4). The Comprehensive R Archive Network.

Oliveira, C.R., Ávila, L.W., & Morais, D.H. (2019). Helminths Associated with Three Physalaemus Species (Anura: Leptodactylidae) from Caatinga Biome, Brazil. Acta Parasitologica 64, 205–212. https://doi.org/10.2478/s11686-018-00022-8

Palumbi, S., Martin, A., Romano, S., McMillan, W. O., Stice, L., & Grabowski, G. (2002). the Simple fool's guide to PCR. Version 2. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu, 1–45.

Paz, A., Ibáñez, R., Lips, K. R., & Crawford, A. J. (2015). Testing the role of ecology and life history in structuring genetic variation across a landscape: a trait-based phylogeographic approach. Molecular Ecology 24, 3723–3737. https://doi.org/10.1111/mec.13275

Pebesma, E., & Bivand, R. (2005). *"Classes and methods for spatial data in R"*.R News, 5(2), 9–13. https://CRAN.R-project.org/doc/Rnews/

Pie, M. R., Faircloth, B. C., Ribeiro, L. F., Bornschein, R., & McCormack, J. E. (2018). Phylogenomics of montane frogs of the Brazilian Atlantic Forest is consistent with isolation in sky islands followed by climatic stability. Biological Journal of the Linnean Society 125, 72–82. https://doi.org/10.1093/biolinnean/bly093

Pinho, C., & Hey, J. (2010). Divergence with gene flow: models and data. Annual Review Ecology, Evolution, and Systematics 41, 215–230.

Pombal, J. P., & Haddad, C. F. B. (2007). Estratégias e modos reprodutivos em anuros. In: .B. Nascimento, P. M. E. Oliveira (Eds.). *Herpetologia no Brasil II* (pp. 101–116). Sociedade Brasileira de Herpetologia, São Paulo.

Porto, T. J., Carnaval, A. C., & Rocha, P. L. B. (2013). Evaluating forest refugial models using species distribution models, model filling and inclusion: a case study with 14 Brazilian species. Diversity and Distributions 19, 330 – 340. https://doi.org/10.1111/j.1472-4642.2012.00944.x

Prance, G. T. (1982). Forest refuges: evidence from woody angiosperms. In :G.T. Prance (Ed.), *Biological diversification in the tropics* (pp. 137–158). Columbia University Press, New York.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing. http://www.Rproject.org/

Ribeiro, M. C., Metzger J. P., Martensen, A. C., Ponzoni, F. J., & Hirota, M. M. (2009). The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biological Conservation 142, 1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021

Robertson, J. M., & Robertson, A. D. (2008). Spatial and temporal patterns of phenotypic variation in a Neotropical frog. Journal of Biogeography 35, 830–843. https://doi.org/10.1111/j.1365-2699.2007.01824.x

Rodal, M. J. N., Barbosa, M. R. V., & Thomas, W. W. (2008). Do the seasonal forests in northeastern Brazil represent a single floristic unit? Brazilian Journal of Biology 68(3), 467–475. https://doi.org/10.1590/S1519-69842008000300003

Röhr D.L., Camurugi F., Martinez P.A., Sousa-Lima R.S., Junca F.A., Garda A.A. (2020). Habitat-dependent advertisement call variation in the monkey frog *Phyllomedusa nordestina*. Ethology 126(6), 651–659. https://doi.org/10.1111/eth.13017

Rosa, M. V., Ferrão, M., Pequeno, P. A. C. L., & Lima, A. P. (2023). How do tree density and body size influence acoustic signals in Amazonian nurse frogs? Bioacoustics 30(1), 1–15. https://doi.org/10.1080/09524622.2023.2204313

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. Ecology Letters 8(3), 336–352. https://doi.org/10.1111/j.1461-0248.2004.00715.x

Ryan, M. J. (1988). Constraints and patterns in the evolution of anuran acoustic communication. In: B. Fritzch, M. J. Ryan, W. Wilczynski, T. E. Heherington, & W. Walkowiak (Eds). *The evolution of the amphibian auditory system* (pp. 637–677). Wiley, New York.

Ryan, M. J., & Brenowitz, E. A. (1986). The role of the body size, phylogeny, and ambient noise in the evolution of bird song. The American Naturalist 126(1), 87 – 100. https://doi.org/10.1086/284398

Sales, M. F., Mayo, S. J., & Rodal, M. J. (Eds.). (1998). *Plantas vasculares das florestas serranas de Pernambuco: um checklist da flora ameaçada dos brejos de altitude, Pernambuco, Brasil*. Imprensa Universitária, Universidade Federal Rural de Pernambuco, Recife.

Santana, D. J., Mesquita, D. O., & Garda, A. A. (2011). Advertisement call of *Dendropsophus oliveirai* (Anura, Hylidae). Zootaxa 2997, 67–68. https://doi.org/10.11646/zootaxa.2997.1.5

Santos, A. M. M., & Tabarelli, M. (2004). Integridade, esforço e diretrizes para conservação dos Brejos de Altitude da Paraíba e Pernambuco. In: K. C. Pôrto, J. J. P. Cabral, & M. Tabarelli (Eds.). *Brejos de Altitude em Pernambuco e Paraíba: História natural, ecologia e conservação* (pp. 309–318). Ministério do Meio Ambiente, Brasília.

Santos, A. M. M., Cavalcanti, D. R., Silva, J. M. C., & Tabarelli, M. (2007). Biogeographical relationships among tropical forests in north-eastern Brazil. Journal of Biogeography 34, 437–446. https://doi.org/10.1111/j.1365-2699.2006.01604.x

Schluter, D. (2009). Evidence for ecological speciation and its alternative. Science 323, 737–741. https://doi.org/10.1126/science.1160006

Silva, J. M. C., Leal, I. R., & Tabarelli, M. (Eds.). (2018). *Caatinga: the largest tropical dry forest region in South America*. Springer.

Silva, N. R., Neto, J. A., Prado, C. P. A., & Mott, T. (2019). Reproductive biology of *Dendropsophus haddadi* (Bastos and Pombal, 1994), a small treefrog of the Atlantic Forest. Herpetology Notes 12, 319–325.

Silveira, M. H. R., Mascarenhas, R., Cardoso, D., & Batalha-Filho, H. (2019). Pleistocene climatic instability drove the historical distribution of forest islands in the northeastern Brazilian Atlantic Forest. Palaeogeography, Palaeoclimatology, Palaeoecology 527, 67–76. https://doi.org/10.1016/j.palaeo.2019.04.028

Smith, M. J., Osborne, W., & Hunter, D. (2003). Geographic variation in the advertisement call structure of *Litoria verreauxii* (Anura: Hylidae). Copeia 4:750–758. https://doi.org/10.1643/HA02-133.1

Staden, R. (1996). The Staden sequence analysis package. Molecular Biotechnology, 5: 233–241. https://staden.sourceforge.net/.

Studer, A. (2015). Aves. In: A. Studer, L. Nusbaumer, & R. Spichiger (Eds.). *Biodiversidade da Reserva Biológica de Pedra Talhada* (pp. 377–405). Alagoas / Pernambuco, Brasil.

Sun, K., Luo, L., Kimball, R. T., Wei, X., Jin, L., Jiang, T., Li, G., & Feng, J. (2013). Geographic variation in the acoustic traits of greater horseshoe bats: testing the importance of drift and ecological selection in evolutionary processes. PLoS ONE 8, 1–11. https://doi.org/10.1371/journal.pone.0070368

Tabarelli, M., & Santos, A. M. M. (2004). Uma breve descrição sobre a história natural dos Brejos Nordestinos. In: K. C. Pôrto, J. J. P. Cabral, & M. Tabarelli (Eds.). *Brejos de Altitude em Pernambuco e Paraíba: História natural, ecologia e conservação* (pp. 99–110). Ministério do Meio Ambiente, Brasília.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution, 38(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Theulen, V. (2004). Conservação dos Brejos de Altitude no estado de Pernambuco. In: K. C. Pôrto, J. J. P. Cabral, & M. Tabarelli (Eds.). *Brejos de Altitude em Pernambuco e Paraíba história natural, ecologia e conservação* (pp. 299–302). Ministério do Meio Ambiente, Brasília.

Titon Junior, B., & Gomes, F. R. (2015). Relation between Water Balance and Climatic Variables Associated with the Geographical Distribution of Anurans. PLoS ONE 10(10), e0140761. https://doi.org/10.1371/journal.pone.0140761

Tobias, M. L., Evans, B. J., & Kelley, D. B. (2011). Evolution of advertisement calls in African clawed frogs. Behaviour 148, 519 – 549. https://doi.org/10.1163/000579511X569435

Tobias, M. L., Planqué, R., Cram, D. L., & Seddon, N. (2014). Species interactions and the structure of complex communication networks. Proceedings of the National Academy of Sciences 111(3), 10120 – 1025. https://doi.org/10.1073/pnas.1314337111

Toledo, L. F., Llusia, D., Vieira, C. A., Corbo, M., & Márquez, R. (2015). Neither convergence nor divergence in the advertisement call of sympatric congeneric neotropical treefrogs. Bioacoustics 24(1), 31– 47. https://doi.org/10.1080/09524622.2014.926831

Twomey, E., Yeager, J., Brow, J. L., Morales, V., Cummings, M., & Summers, K. (2013). Phenotypic and Genetic Divergence among Poison Frog Populations in a Mimetic Radiation. PLoS ONE 8(2), e55443. https://doi.org/10.1371/journal.pone.0055443

Velásquez, N. A., Moreno-Gómez, F. N., Brunetti, E., & Penna, M. (2018). The acoustic adaptation hypothesis in a widely distributed South American frog: Southernmost signals propagate better. Scientific reports 8(6990), 1–12. https://doi.org/10.1038/s41598-018-25359-y

Velloso, A. L., Sampaio, E. V. S. B., & Pareyn, F. G. C. (Eds.). (2002). *Ecorregiões propostas para o bioma Caatinga*. Associação Plantas do Nordeste, Instituto de Conservação Ambiental The Nature Conservancy do Brasil, Recife.

Walsh, P. S., Metzger, D. A., & Higuchi, R. (1991). Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques, 10, 506−513.

Wang, P. S. (2013). Examining the full effects of landscape heterogeneity on spatial genetic variation: a multiple matrix regression approach for quantifying geographic and ecological isolation. Evolution 67(12), 3403 – 3411. https://doi.org/10.1111/evo.12134

Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. Molecular Ecology 23(23), 5649–5662. https://doi.org/10.1111/mec.12938

Wells, K. D. (2007). The Ecology and Behavior of Amphibians. Chicago: University of Chicago Press. https://doi.org/10.7208/chicago/9780226893334.001.0001

Wilkins, M. R., Seddon, N., & Safran, R. J. (2013). Evolutionary divergence in acoustic signals: causes and consequences. Trends in Ecology and Evolution 28(3), 156–166. https://doi.org/10.1016/j.tree.2012.10.002

Wright, S. (1951). The genetical structure of populations. Annals of Human Genetics 15, 323–354. https://doi.org/10.1111/j.1469-1809.1949.tb02451.x

Zamudio, K. R., Bell, R. C., & Mason, N. A. (2016). Phenotypes in phylogeography: Species' traits, environmental variation, and vertebrate diversification. PNAS 113(29), 8041–8048. https://doi.org/10.1073/pnas.1602237113

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

5 CAPÍTULO 2

Comparative landscape genetics in two amphibians demonstrates the influence of habitat specialization in highlands in Northeastern Brazil*

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Comparative landscape genetics in two amphibians demonstrates the influence of habitat specialization in highlands 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.

Keywords: Atlantic Forest, Caatinga, dispersal, genetic differentiation, highland forests, local selection.

Introduction

Dispersal is an eco-evolutionary process involving the movement of individuals across space, influencing gene flow and genetic differentiation among populations (Travis et al. 2012). When dispersal rates are low, gene flow is reduced, and genetic distances increase over time. Spatial distance alone can impact dispersal and gene flow by decreasing the likelihood of successful dispersal across distant locations. This neutral effect of space in isolating populations based on their dispersal ability is known as Isolation by Distance (IBD) (Wright 1947; Jenkins et al. 2010), which manifests as a positive correlation between geographic and genetic distances. However, space is rarely homogeneous, and landscape features such as climatic variation and vegetation can impede or reduce dispersal, thereby influencing gene flow. In such cases, the environment plays a key role in isolating populations and shaping genetic differentiation, leading to a pattern known as Isolation by Environment (IBE) (Wang and Summers 2010; Wang and Bradburd 2014), where environmental distance better predicts genetic distance. These two processes are not mutually exclusive, though one may exert a stronger influence than the other depending on the system (Sexton et al. 2013; Wang et al. 2013).

Due to their unique eco-evolutionary histories, species exhibit distinct interactions with the environment. While some tolerate a wide range of environmental conditions, others have more specific requirements. Within the same geographical area, certain species may experience little resistance to dispersal, whereas for others, environmental variation acts as a limiting factor (Goldberg and Waits 2010; Richardson 2012; Wang et al. 2013; Wollenberg Valero 2015; Garcia et al. 2017; Lourenço et al. 2019). Comparative studies on 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) suggest that life history traits—such as mating system, life form, seed dispersal, vagility, reproductive behavior, and body size—are linked to genetic divergence among populations, explaining variations in genetic distances among co-occurring species. While intrinsic factors like body size have been proposed as key ecological predictors of genetic variation in amphibians (Pabijan et al. 2012; Paz et al. 2015), topographic complexity and macrohabitat type have also been identified as significant constraints in many tropical anuran species (Rodríguez et al. 2015).

Divergent responses to landscape configuration have been observed in numerous contemporary studies on genetic differentiation in amphibians across various systems globally (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). Consequently, it is reasonable to hypothesize that species with more restrictive life history traits and ecological constraints (e. g. small body size, low dispersal capability, and dependent on vegetation to reproduce) occupy one end of a continuum, where dispersal decreases as environmental distance increases, resulting in IBE (Wang and Bradburd 2014), which is not necessarily correlated with geographic distance. At the other end of the continuum, species with broader habitat tolerance are predominantly influenced by geographic distance, leading to IBD (Jenkins et al. 2010; Batalha-Filho et al. 2023). Although this assumption is theoretically plausible, it requires further exploration and comparative studies, particularly in tropical regions, where such studies are scarce (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, exhibit higher habitat heterogeneity, which leads to increased intraspecific genetic differentiation (Guarnizo and Cannatella 2013; Rodríguez et al. 2015). The Borborema Plateau (BP) is a geomorphological unit located north of the São Francisco River in northeastern Brazil, historically covered by both Atlantic Forest and Caatinga (seasonally dry forest and shrub) vegetation. This plateau is environmentally diverse, with various types of Caatinga vegetation in the lowlands surrounding scattered mountains and highlands (500–1100 m a.s.l.), where remnants of tropical Atlantic Forest prevail (Andrade-Lima 1982; Velloso et al. 2002; Tabarelli and Santos 2004; Ribeiro et al. 2009; Silveira et al. 2019). It has been proposed that local landscapes became more arid after the Pleistocene, leading to the extirpation of taxa adapted to wet climates and the expansion of those adapted to dry conditions, especially in the lowland areas (Gehara et al. 2017). Consequently, the highland forests currently serve as climatic and environmental microrefugia for plants and animals adapted to mesic conditions, and are also suitable habitats for species with broad habitat tolerance (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 BP share similar mesic conditions, they exhibit notable differences in vegetation structure, plant species composition, and climate due to their geographic position along a steep longitudinal gradient in annual rainfall. Forest enclaves closer to the coast receive higher annual precipitation (1,000–1,600 mm) and support denser forests, whereas those further west experience lower rainfall (400–600 mm), leading to an increased presence of Caatinga vegetation (Velloso et al. 2002; Rodal et al. 2008; Silva et al. 2018; Silveira et al. 2019). These environmental differences may hinder connections among highland populations, depending on the species' ecological requirements (Funk et al. 2005; Pease et al. 2009; Ortego et al. 2012; Lawson 2013; Santana et al. 2024). Therefore, the highland forests of the BP present an ideal system to explore various landscape genetics hypotheses in a tropical region and to assess the relative influence of selective and neutral processes on patterns of intraspecific genetic variation whitin a local context.

In this study, we conducted a comparative landscape genetics analysis using data from the same localities to evaluate the association and significance of landscape features in shaping genetic variation among localities of two amphibian species with different ecological requirements: *Dendropsophus oliveirai* (Bokermann, 1963) (Hylidae) and *Physalaemus cuvieri* Fitzinger, 1826 (Leptodactylidae). *Dendropsophus oliveirai* is a small treefrog found in the Central to Northern Atlantic Forest of Brazil, primarily along lowland coastal rainforests but also in ecotonal areas, extending into moist habitats within the Caatinga biome (Frost 2023; Magalhães et al. 2013; Menezes et al. 2020). This species depends on permanent or long-lived ponds located at forest edges or in adjacent dry areas for breeding. Its eggs are laid on herbaceous vegetation overhanging these ponds, where tadpoles dive immediately after hatching to complete their development (Haddad and Prado 2005; Haddad et al. 2013; Silva et al. 2019). In contrast, *P. cuvieri* is a small chorus frog widely distributed across South American biomes, from Argentina to Venezuela (Frost 2023). It breeds in a wide range of lentic water bodies, from small rain puddles with very short hydroperiods to the margins of lakes and ponds in both dry and forested areas (Pombal and Haddad 2007; Aguiar et al. 2014), suggesting a broad environmental tolerance (Barreto and Andrade 1995; Oliveira et al. 2019). Mating pairs of *P. cuvieri* deposit their eggs in foam nests 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 environmental factors as proxies for Isolation by Environment (IBE), and geographic distance as a proxy for Isolation by Distance (IBD). Although this is a landscape genetics study, we consider it important to analyze and present the phylogeographic structure of the species in the study region to demonstrate how IBD and IBE have influenced connectivity between localities. We developed this comparative genetic landscape approach based on the following hypotheses: (I) Being a small, less-vagile treefrog that deposits eggs with prolonged development in vegetation over water ponds, *D. oliveirai* is likely more sensitive to landscape configuration. Therefore, we expect higher genetic structure among its geographic populations driven by greater IBE, with a secondary influence of IBD, as environmental variation occurs geographically across the BP (Pertoldi et al. 2014; Wang and Bradburd 2014); (II) As a terrestrial, medium-size species with foam nests and higher vagility, *P. cuvieri* exhibits lower or no genetic structure across the plateau, resulting from IBD, since environmental variation is likely to present less of a barrier 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 the same seven highland forests of the BP (Fig. 1; Table 1), covering the different parts of this geomorphological unit. Considering that *D. oliveirai* is not found in the lowlands between the forest enclaves and our financial constraints, we decided not to sample *P. cuvieri* in these areas, prioritizing the sample both species in forest enclaves across different parts of the study region. We used this sampling design trying to better explore the influence of the climatic and environmental heterogeneity of the region on the genetic variation of both species.

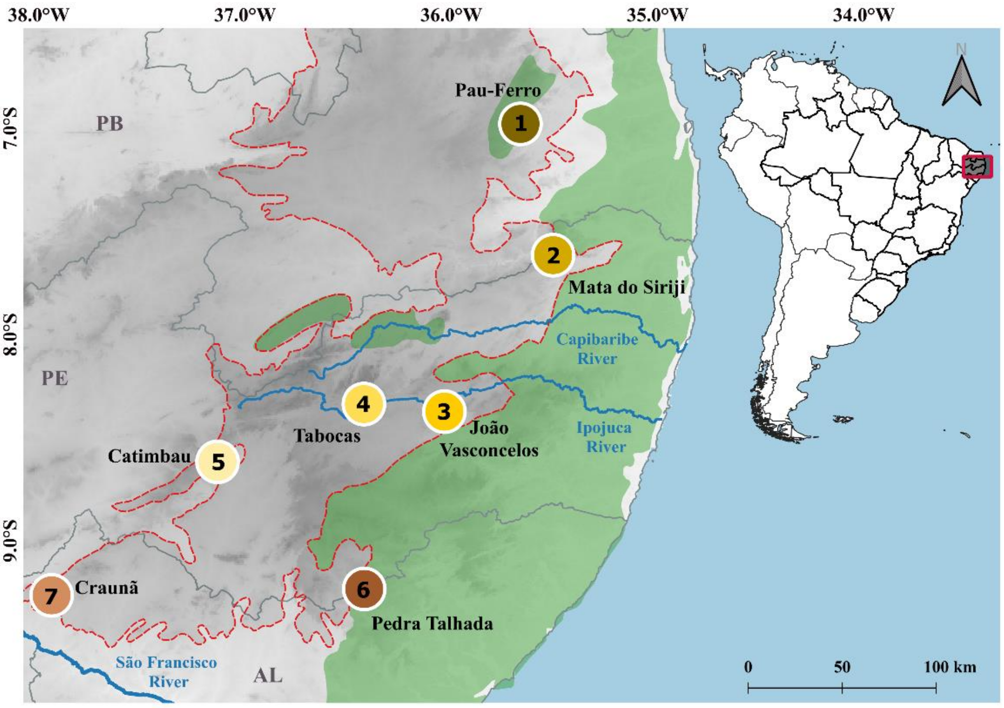

Fig. 1 Geographic position of sampling locations on the Borborema Plateau (dashed red line) in Northeastern Brazil. The Atlantic Forest domain is highlighted in light green. Blue lines correspond to large rivers in the region: São Francisco (bottom blue line), Capibaribe (top blue line) and Ipojuca (middle blue line). Grey lines correspond to state limits. Each locality is represented by a unique color to match the following figure (in Results)

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**

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.

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 ºC 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`-CGCCTGTTTATCAAAAACAT-3`) and 16sbrH (5`-CCGGTCTGAACTCAGATCACGT-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*. We used BLAST from NCBI to confirm the species identification. 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 whitin 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 (~1 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 = ~1 km²), Last Glacial Maximum (LGM; 2.5' arc-minutes = ~5 km²) (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 *lavan 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 *adegenet* 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

	Dendropsophus oliveirai					***Physalaemus cuvieri***				
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).

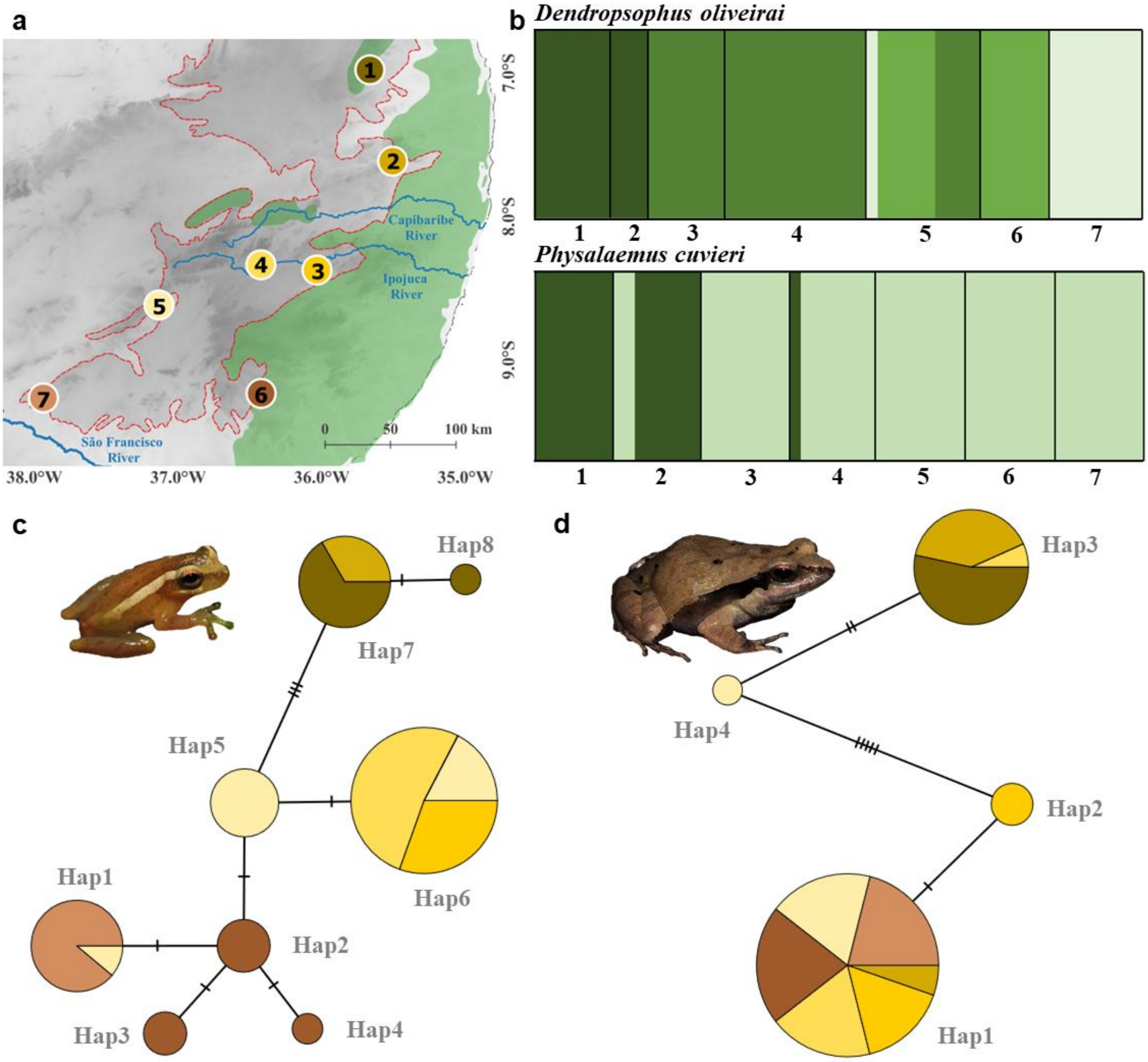

Fig. 2 (a) Sampling localities of the frogs *Dendropsophus oliveirai* and *P. cuvieri* across the Borborema Plateau in northeastern Brazil. Dashed red line = geographic limits of the Borborema Plateau. Blue lines = Capibaribe, Ipojuca, and São Francisco rivers from top to bottom. (b) Bayesian Analysis 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

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 EVImax and EVImin between species (see Supplementary Information S2)

Distance matrix comparisons	***Dendropsophus oliveirai***	***Physalaemus cuvieri***
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.

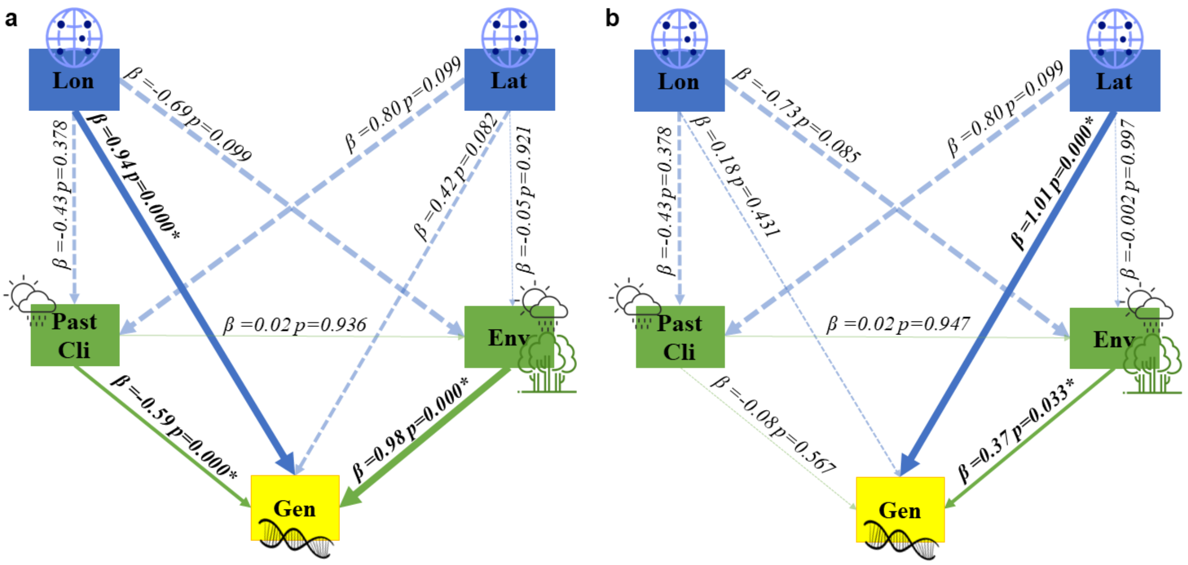

Fig. 3 Structural Equation Model (SEM) summarization of landscape feature effects on the genetic variation of *Dendropsophus oliveirai* (a) and *Physalaemus cuvieri* (b) from highland forests in Northeastern Brazil. Lat = latitude, Lon = longitude, Env = environment (first component from PCA of environmental 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

Discussion

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. Although we are aware of the biases that the lack of sampling in the lowland areas of the study region may introduce to the results for *P. cuvieri*, we also believe that our sampling design and analyses were sufficient to answer our questions. 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. We also

The relationship between genetic variation and geographic distance, past climatic changes, and the current environment in *D. oliveirai* suggests that both IBD and IBE shape the species' genetic structure, with IBE likely playing a stronger role. Current and historical barriers to gene flow, combined with environmental heterogeneity and climatic fluctuations, can drive genetic drift, increasing genetic differentiation among previously connected populations. At the same time, local adaptation occurs when environmental conditions favor different alleles in distinct localities through selection pressures (Nosil et al. 2005; Balkenhol et al. 2019). Since neutral and selective mechanisms can act together to produce IBE (Wang and Bradburd 2014), we infer that both genetic drift and local adaptation contribute to the genetic differentiation of *D. oliveirai*. Although our study relies on presumed neutral genetic markers, the data still suggest a potential adaptive process (Andrew et al. 2012; Manel and Holderegger 2013). Additionally, past climatic variation had a significant impact on the genetic diversity of *D. oliveirai*, indicating that historical climatic instability played a key role in shaping gene flow and genetic variation, particularly in species with restrictive life history traits and narrow habitat tolerance. The influence of historical climatic variation on genetic differentiation in tropical amphibians is well-documented (e.g., Carnaval et al. 2009; Gehara et al. 2017; Brusquetti et al. 2019), especially in populations inhabiting highland forests (e.g., Carnaval and Bates 2007; Amaro et al. 2012; Guarnizo and Cannatella 2013; Oliveira et al. 2021). Our findings suggest that climatic and forest fluctuations—likely including aridification and forest contractions during the Pleistocene (Silveira et al., 2019)—led to the isolation of *D. oliveirai* populations in distinct forest enclaves. However, this isolation was not due to strict forest dependence, but rather to the species' reliance on specific ponds typically found in or near forests. Today, the connectivity between populations is primarily shaped by an east-west climatic and environmental gradient, driving longitudinal genetic differentiation.

Genetic variability in *P. cuvieri* was primarily explained by IBD, with only a minimal contribution from environmental variation—an expected pattern for species with wide geographic ranges (Jenkins et al. 2010). As a broadly distributed species spanning from southern to northern South America (Haddad et al. 2008; Frost 2023), *P. cuvieri* encounters diverse habitat conditions, which may shape its genetic variation along a latitudinal gradient. One potentially significant factor is the latitudinal asynchrony of the rainy season (Bates et al. 2008; Silva et al. 2019), a critical period for amphibians whose reproduction is closely tied to rainfall cycles (Aichinger 1987; Duellman 1995; Prado et al. 2005; Ulloa et al. 2019). This asynchrony could lead to temporal mismatches in breeding seasons across populations, reducing gene flow even over short geographic distances, particularly in regions with pronounced topographic relief (Martin et al. 2009). In other words, the strong correlation between geographic distance and genetic variation in *P. cuvieri* may, in part, reflect the influence of rainy season asynchrony along this latitudinal gradient (Maes et al. 2006; Thomé et al. 2021). Similar patterns have been observed in other widely distributed anurans, such as *Rhinella granulosa*, endemic to the Caatinga (Thomé et al. 2021), and *Amietia wittei*, a tropical Afromontane frog (Zancolli et al. 2014).

Our findings indicate that geographic distance is the primary landscape factor influencing genetic differentiation in a species with broader habitat tolerance, whereas in a species with more restricted habitat preferences, genetic differentiation is shaped by a combination of past climatic variation, current environmental conditions, and geographic distance. Unless driven by unknown historical factors, the contrasting levels of genetic structure observed in *D. oliveirai* and *P. cuvieri* likely stem from their differing abilities to traverse the dry lowlands between highland forests. This study contributes to the growing body of landscape genetics research in tropical regions, reinforcing the idea that landscape features influence genetic variation in a species-specific manner (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 commonly highlight elevation, forest cover, rivers, and geographic distance as key factors regulating gene flow, though their relative importance varies across species. In our previous work on acoustic variation within these same populations, we found that geographic and environmental distances were moderately and strongly associated, respectively, with acoustic variation in *P. cuvieri*, while in *D. oliveirai*, environmental distance showed a moderate correlation with acoustic variation, with body size and genetic differentiation as covariates (Andrade Lima et al. 2024). Given that IBD and IBE are linked to genetic drift and divergent natural selection, respectively (Dobzhansky 1937; Crispo et al. 2006; Lee & Mitchell-Olds 2011; Wang 2013; Sexton et al. 2014), our combined findings suggest that both selective and neutral evolutionary mechanisms drive intraspecific genetic variation in this Neotropical region, with their relative importance varying according to species ecology.

Genetic structure among localities reflects the differing levels of landscape permeability for each species. Life-history traits such as body size and reproductive mode play a crucial role in shaping habitat preferences, dispersal potential, and, consequently, genetic structure (Wollenberg et al. 2011; Paz et al. 2015; Lourenço et al. 2019). Given that *D. oliveirai* and *P. cuvieri* have distinct reproductive strategies and ecological niches, these traits may contribute to the differences in their population genetic structure. Although *D. oliveirai* is not strictly forest-dependent, this small treefrog relies on breeding sites with long hydroperiods and dense herbaceous vegetation—habitats commonly found in montane forests of the study region but scarce in the surrounding lowlands. This likely led to geographic isolation or reduced gene flow between populations. Similar patterns of limited dispersal and high genetic structure due to the absence of suitable breeding environments have been observed 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). Conversely, we found lower phylogeographic structure in *P. cuvieri*, as expected for species with broader ecological tolerance (Miller et al. 2015; Kort et al. 2021), particularly those adapted to breeding in temporary water bodies in dry environments (Ortiz et al. 2018). Despite its association with arid habitats, this species reproduces in ephemeral ponds, has large clutch sizes, and exhibits rapid larval development (Barreto and Andrade 1995; Haddad and Prado 2005; Aguiar et al. 2014)—traits that enhance dispersal ability in amphibians (Van Bocxlaer et al. 2010; Streicher et al. 2012). Thus, the genetic structure observed in *P. cuvieri* in our study is likely a consequence of its dispersal capacity rather than environmental barriers.

Despite differences in genetic structure between species, we identified a potential shared phylogeographic break near 8° latitude, between northeastern and central localities, where the Capibaribe and Ipojuca rivers are located. Although subtle, similar phylogeographic patterns have been documented in other anurans, *Proceratophrys renalis* and *Pristimantis ramagii*, in the same region (Carnaval 2002; Carnaval and Bates 2007). Since rivers can act as complete or partial barriers depending on a species' dispersal ability (Coelho et al. 2022), this pattern suggests that the Capibaribe and Ipojuca rivers may play a vicariant role, to varying degrees, in shaping genetic differentiation among species. These rivers are the only prominent landscape features near this latitude and have never been proposed or investigated as potential barriers before. Our findings suggest that even smaller rivers could contribute to genetic structuring in northeastern Brazil. A broader comparative study including multiple taxa or a more extensive genetic dataset for the species examined here could further clarify this hypothesis. Regardless of whether these rivers influence genetic differentiation in our study species, our analyses clearly demonstrate the strong role of geographic distance in shaping genetic variation. Thus, any phylogeographic breaks between northeastern and central localities, potentially driven by local barriers, do not fully explain the observed relationship between geographic and genetic distances.

Conclusions

Understanding how gene flow between populations is shaped by geographic and environmental variation provides insight into the early stages of biological divergence (Schluter 2001; Wang et al. 2013) and offers valuable information for managing and predicting biodiversity loss amid rapid environmental change (Sexton et al. 2013; Manel and Holderegger 2013). Here, we examined the genetic variability of two ecologically distinct species in the highland forests of northeastern Brazil, demonstrating how landscape features impose different constraints depending on species-specific ecological traits. Our findings contribute to a better understanding of the evolutionary dynamics of Neotropical animal populations in mountain regions. Through a comparative approach, we corroborate Rodríguez et al. (2015), showing that species less sensitive to dry habitat conditions exhibit lower genetic structure, as landscape features—aside from geographic distance—pose minimal resistance to their dispersal. In contrast, species with more specific habitat requirements experience stronger effects from spatiotemporal changes in the landscape matrix, leading to a more structured genetic pattern. Given that habitat loss is the primary threat to amphibians (Luedtke et al. 2023), our study underscores the importance of conserving remaining forest fragments and maintaining their connectivity to facilitate gene flow in species with limited dispersal ability. These species are particularly vulnerable to stochastic events and inbreeding, which can increase the risk of local extirpation. We advocate for future multi-taxon studies in landscape genetics, focusing on species with varying ecological requirements but overlapping distributions, to further assess how geographic distance, rivers, and environmental variation influence genetic structure. Such research will improve our ability to predict the responses of different species to climate change and habitat loss in this region.

References

Aguiar A, Toledo GM, Anjos LA, Silva RJ (2014) Helminth parasite communities of two *Physalaemus cuvieri* Fitzinger, 1826 (Anura: Leiuperidae) populations under different conditions of habitat integrity in the Atlantic Rain Forest of Brazil. Braz J Biol 75(4): 963–968 https://doiorg/101590/1519–698403614

Aichinger M (1987) Annual activity patterns of anurans in a seasonal neotropical environment. Oecologia 71: 583–92 https://doiorg/101007/BF00379302

Alvarez N, Thiel–Egenter C, Tribsch A, Holderegger R, Manel S, Schonswetter P, Taberlet P, Brodbeck S, Gaudeul M, Gielly L, Kupfer P, Mansion G, Negrini R, Paun O, Pellecchia M, Rioux D, Schupfer F, Loo MV, Winkler M, Gugerli F, Consortium I (2009) History or ecology? Substrate type as a major driver of spatial genetic structure in Alpine plants. Ecol Lett 12:632–640. https://doi.org/10.1111/j.1461-0248.2009.01312.x

Amaro, RC, Rodrigues, MT, Yonenaga–Yassuda, Y, Carnaval, AC (2012) Demographic processes in the montane Atlantic rainforest: molecular and cytogenetic evidence from the endemic frog *Proceratophrys boiei*. Mol Phylogenet Evol 62(3): 880–888 https://doiorg/101016/jympev201111004

Andrade–Lima D (1982) Present day forest refuges in Northeastern Brazil. In: Prance GT (ed) Biological Diversification in the Tropics. Columbia University Press, New York, pp 245–254.

Andrade Lima JH, Novo MJKB, Simões PI (2024) Advertisement call variation is related to environmental and geographic distances in two anuran species inhabiting highland forests in northeastern Brazil. Biotropica 56(2):e13329. https://doi.org/10.1111/btp.13329

Andrew RL, Ostevik KL, Ebert DP, Rieseberg LH (2013) Adaptation with gene flow across the landscape in a dune sunflower Molecular Ecology, 21(9): 2078–2091 https://doiorg/101111/j1365–294X201205454x

Antunes B, Figueiredo–Vázquez C, Dudek K, Liana M, Pabijan M, Zieliński P, Babik W (2022) Landscape genetics reveals contrasting patterns of connectivity in two newt species (*Lissotriton montandoni* and *L vulgaris*). Mol Ecol 32: 4515–4530. https://doi.org/10.1111/mec.16543

Bakenhol N, Dudaniec R, Krutovisky KV, Johnson JS, Cairns DM, Segelbacher G, Selkoe KA, Heyden SVD, Wang IJ, Selmoni O, Joost S (2019) Landscape Genomics: Understanding Relationships Between Environmental Heterogeneity and Genomic Characteristics of Populations Pp 261–322. In: Rajora, OP (ed) Population Genomics: Concepts, Approaches and Applications. Springer International Publishing AG https://doiorg/101007/13836_2017_2

Bandelt HJ, Forster P, Röhl A (1999) Median–joining networks for inferring intraspecific phylogenies Molecular Biology and Evolution, 16: 37–48 https://doiorg/101093/oxfordjournalsmolbeva026036

Barreto L, Andrade GV (1995) Aspects of the reproductive biology of *Physalaemus cuvieri* (Anura: Leptodactylidae) in northeastern Brazil. Amphib Reptil Leiden 16(1):67–76 https://doiorg/101163/156853895X00208

Batalha-Filho H, Barreto SB, Silveira MHB, Miyaki CY, Afonso S, Ferrand N, Carneiro M, Sequeira F (2023) Disentangling the contemporary and historical effects of landscape on the population genomic variation of two bird species restricted to the highland forest enclaves of northeastern Brazil. Hered 132:77–88 https://doiorg/101038/s41437–023–00662–1

Bates BC, Kundzewicz ZW, Wu S, Palutikof JP (2008) Climate change and water Technical paper of the intergovernmental panel on climate change, IPCC Secretariat, Geneva.

Bivand R, Keitt T, Rowlingson B (2023) *rgdal: Bindings for the 'Geospatial' Data Abstraction Library*. https://r–forger–projectorg/projects/rgdal/

Blair C, Jiménez Arcos VF, Mendez de la Cruz FR, Murphy RW (2013) Landscape Genetics of Leaf–Toed Geckos in the Tropical Dry Forest of Northern Mexico. PLoS ONE 8(2):e57433 https://doiorg/101371/journalpone0057433

Bonatelli IAS, Gehara M, Carstens B, Colli GR, Moraes EM (2021) Comparative and predictive phylogeography in the South American diagonal of open formations: Unravelling the biological and environmental influences on multitaxon demography. Mol Ecol 31: 331–342 https://doiorg/101111/mec16210

Brusquetti F, Netto F, Baldo D, Haddad CFB (2019) The influence of Pleistocene glaciations on Chacoan fauna: genetic structure and historical demography of an endemic frog of the South

American Gran Chaco. Biol J Linn Soc 126:404–416. https://doi.org/10.1093/biolinnean/bly203

Camurugi F, Gehara M, Fonseca EM, Zamudio KR, Haddad CFB, Colli GR, Thomé MTC, Prado CPA, Napoli MF, Garda AA (2020) Isolation by environment and recurrent gene flow shaped the evolutionary history of a continentally distributed neotropical treefrog. J Biogeogr 48(11):760–772 https://doiorg/101111/jbi14035

Carnaval ACOQ (2002) Phylogeography of Four Frog Species in Forest Fragments of Northeastern Brazil—A Preliminary Study. Integr Comp Biol 42:013–921 https://doiorg/101093/icb/425913

Carnaval AC, Bates JM (2007) Amphibian DNA shows marked genetic structure and tracks Pleistocene climate change in Northeastern Brazil. Evol 61(12): 2942–2957 https://doiorg/101111/j1558–5646200700241x

Carnaval AC, Hickerson MJ, Haddad CFB, Rodrigues MT, Moritz C (2009) Stability Predicts Genetic Diversity in the Brazilian Atlantic Forest Hotspot. Science 323:785–789 https://doiorg/101126/science1166955

Carnaval AC, Moritz C (2008) Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic Forest. J Biogeogr 35:1187–1201 https://doiorg/101111/j1365–2699200701870x

Carnaval A C, Waltari E, Rodrigues M T, Rosauer D, VanDerWal J, Damasceno R, Prates I, Strangas M, Spanos Z, Rivera D, Pie MR, Firkowski CR, Bornschein MR, Ribeiro LF, Moritz C (2014) Prediction of phylogeographic endemism in an environmentally complex biome. Proc. R. Soc. B 281:14–61 https://doiorg/101098/rspb20141461

Carvalho CS, Côrtes MC (2023) Landscape Genetics in the Neotropics In: Galetti Jr. PM (ed) Conservation genetics in the Neotropics, Springer, 1 ed., São Carlos, Brazil, pp 145–165.

Castro DP, Rodrigues JFM, Borges-Leite MJ, Lima DC, Borges-Nojosa DM (2019) Anuran diversity indicates that Caatinga relictual Neotropical forests are more related to the Atlantic Forest than to the Amazon. PeerJ 6:e6208 https://doiorg/107717/peerj6208

Cheng L, Connor TR, Siren J, Aanensen DM, Corander J (2013) “Hierarchical and spatially explicit clustering of DNA sequences with BAPS software”. Mol Biol Evol 30(5):1224–1228 http://dxdoiorg/101093/molbev/mst028

Coelho FEA, Guillory WX, Gehara M (2022) Coalescent simulations indicate that the São Francisco River is a biogeographic barrier for six vertebrates in a seasonally dry South American forest. Front Ecol Evol 10: 983134. https://doi.org/10.3389/fevo.2022.983134

Corander J, Tang J (2007) Bayesian analysis of population structure based on linked molecular information. Mathematical Biosciences 205:19–31. https://doi.org/10.1016/j.mbs.2006.09.015

Corander J, Marttinen P, Sirén J, Tang J (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinformatics 9:539. https://doi.org/10.1186/1471-2105-9-539

Costa GC, Hampe A, Ledru M-P, Martinez PA, Mazzochini GG, Shepard DB, Werneck FP, Moritz C, Carnaval AC (2017) Biome stability in South America over the last 30 kyr: Inferences from long–term vegetation dynamics and habitat modelling. Glob Ecol Biogeogr 00:1–13 https://doiorg/101111/geb12694

Coster SS, Babbitt KJ, Cooper A, Kovach AI (2015) Limited influence of local and landscape factors on finescale gene flow in two pond–breeding amphibians. Mol Ecol 24:742–758 https://doiorg/101111/mec13062

Covarrubias S, González C, Gutiérrez-Rodríguez C (2021) Effects of natural and anthropogenic features on functional connectivity of anurans: a review of landscape genetics studies in temperate, subtropical and tropical species. J Zool 313:159–171 https://doiorg/101111/jzo12851

Crispo E, Bentzen P, Reznick DN, Kinnison MT, Hendry AP (2006) The relative influence of natural selection and geography on gene flow in guppies. Mol Ecol 15:49–62 https://doiorg/101111/j1365–294X200502764x

Devitt TJ, Devitt SEC, Hollingsworth BD, Mcguire JA, Moritz C (2013) Montane refugia predict population genetic structure inthe Large-blotched *Ensatina* salamander. Mol Ecol 22:1650-1665. https://doi.org/10.1111/mec.12196

Dobzhansky TG (1937) Genetics and the Origin of Species Columbia University Press, New York.

Duellman WE (1995) Temporal fluctuations in abundances of anuran amphibians in a seasonal amazonian rainforest. J Herpetol 29:13 https://doiorg/102307/1565080

Duminil J, Fineschi S, Hampe A, Jordano P, Salvini D, Vendramin GG, Petit RJ (2007) Can population genetic structure be predicted from life-history traits? Am Nat 169:662–672. https://doi.org/10.1086/513490

Engler JO, Balkenhol N, Filz KJ, Habel JC, Rödder D (2014) Related Sympatric Hesperid Butterflies with Diverging Ecological Traits. PLoS ONE 9(9):e106526 https://doiorg/101371/journalpone0106526

Excoffier L, Lischer HEL (2010) Arlequin suite v 35: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567 https://doiorg/101111/j1755–0998201002847x

Fan Y, Chen J, Shirkey G, John R, Wu SR, Park H, Shao C (2016) Applications of structural equation modeling (SEM) in ecological studies: an updated review. Ecol Process 5(19):1–12 https://doiorg/101186/s13717–016–0063–3

Fick SE, Hijmans RJ (2017) WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. Int. J Climatol 37(12):4302–4315 https://doiorg/101002/joc5086

Firkowski CR, Bornschein MR, Ribeiro LF, Pie MR (2016) Species delimitation, phylogeny and evolutionary demography of co-distributed, montane frogs in the southern Brazilian Atlantic Forest. Mol Phylogenet Evol 100: 345-360. https://doi.org/10.1016/j.ympev.2016.04.023

Frost D R (2023) Amphibian Species of the World: an Online Reference Version 61 (24 April 2023). Electronic Database accessible at https://amphibiansoftheworldamnhorg/indexphp. American Museum of Natural History, New York, USA.

Funk WC, Blouin MS, Corn PS, Maxell BA, Pilliod DS, Amish S, Allendorf FW (2005) Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. Mol Ecol 14(2):483–496 https://doiorg/101111/j1365–294X200502426x

García-Rodríguez A, Guarnizo CE, Crawford AJ, Garda AA, Costa, GC (2021) Idiosyncratic responses to drivers of genetic differentiation in the complex landscapes of Isthmian Central America. Hered (Edinb) 126:251–265. https://doi.org/10.1038/s41437-020- 00376-8

Garcia VOS, Ivy C, Fu J (2017) Syntopic frogs reveal different patterns of interaction with the landscape: A comparative landscape genetic study of *Pelophylax nigromaculatus* and *Fejervarya limnocharis* from central China. Ecol Evol 7:9294–9306 https://doiorg/101002/ece33459

Goldberg CS, Waits LP (2010) Comparative landscape genetics of two pond–breeding amphibian species in a highly modified agricultural landscape. Mol Ecol 19:3650–3663 https://doiorg/101111/j1365–294X201004673x

Gonzales-Voyer A, Padial J, Castroviejo-Fisher S (2011) Correlates of species richness in the largest Neotropical amphibian radiation. J Evol Biol 24:931–942 https://doiorg/101111/j1420–9101201102243x

Gorelick N, Hancher M, Dixon M, Ilyushchenko S, Thau D, Moore R (2017) Google Earth Engine: Planetary-scale geospatial analysis for everyone. Remote Sens. Environ 202:18–27 https://doiorg/101016/jrse201706031

Grosso J, Baldo D, Cardozo D, Kolenc F, Borteiro C, Oliveira MIR, Bonino MF, Barrasso DA, Candioti FV (2019) Early ontogeny and sequence heterochronies in Leiuperinae frogs (Anura: Leptodactylidae). PLoS ONE 14(6): e0218733 https://doiorg/101371/journalpone0218733

Guarnizo CE, Cannatella DC (2013) Genetic divergence within frog species is greater in topographically more complex regions. J Zool Syst Evol Res 51:333–340 https://doiorg/101111/jzs12027

Gugger PF, Ikegami M, Sork VL (2013) Influence of late Quaternary climate change on present patterns of genetic variation in valley oak, *Quercus lobata* Née. Mol Ecol 22(13):3598-3612. https://doi.org/10.1111/mec.12317

Haddad CFB, Prado CPA (2005) Reproductive Modes in Frogs and Their Unexpected Diversity in the Atlantic Forest of Brazil. Biosci 55(3):207–217 https://doiorg/101641/0006–3568(2005)055[0207:RMIFAT]20CO,2

Haddad CFB, Toledo LF, Prado CPA (2008) Anfíbios da Mata Atlântica São Paulo, Editora Neotropica, São Paulo, Brazil.

Haddad C F B, Toledo L F, Prado C P A, Loebmann D, Gasparini J L, Sazima I (2013) Guia dos Anfíbios da Mata Atlântica: Diversidade e Biologia São Paulo–SP, Anolis Books, São Paulo, Brazil.

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species Philos Trans R Soc Lond B Biol Sci 351:1291–1298. https://doi.org/10.1098/rstb.1996.0112

Hijmans RJ (2017) *raster: geographic data analysis and modeling*. R package version 26-7. https://CRANR–projectorg/package=raster

Huete A, Liu HQ, Batchily K, Van Leeuwen W (1997) A comparison of vegetation indices over a global set of Tm images for EOS–MODIS. Remote Sens Environ 59:440–451. https://doiorg/101016/S0034–4257(96)00112–5

Jenkins DG, Carey M, Czerniewska J, Fletcher J, Hether T, Jones A, Knight S, Knox J, Long T, Mannino M, McGuire M, Riffle A, Segelsky S, Shappell L, Sterner A, Strickler T, Tursi R (2010) A meta-analysis of isolation by distance: relic or reference standard for landscape genetics? Ecography 33(2):315–320. https://doiorg/101111/j1600–0587201006285x

Jombart T (2008) *adegenet: A R package for the multivariate analysis of genetic markers*. Bioinform 24(11): 1403–1405. https://doiorg/101093/bioinformatics/btn129

Katoh K (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066. https://doiorg/101093/nar/gkf436

Keyghobadi N (2007) The genetic implications of habitat fragmentation for animals. Can J Zool 85:1049–64. https://doiorg/101139/Z07–095

Keyghobadi N, Roland J, Matter SF, Strobeck C (2005) Among-and within-patch components of genetic diversity respond at different rates to habitat fragmentation: an empirical demonstration. Proc R Soc Lond B Biol Sci 272: 553–560. https://doi.org/10.1098/rspb.2004.2976

Kort HD, Prunier JG, Ducatez S, Honnay O, Baguette M, Stevens VM, Blanchet S (2021) Life history, climate and biogeography interactively affect worldwide genetic diversity of plant and animal populations. Nat Commun 12:516. https://doiorg/101038/s41467–021–20958–2

Lawson LP (2013) Diversification in a biodiversity hot spot: landscape correlates of phylogeographic patterns in the African spotted reed frog. Mol Ecol 22(7):1947–1960. https://doiorg/101111/mec12229

Lê S, Josse J, Husson F (2008) *“FactoMineR: A Package for Multivariate Analysis”*. J Stat Softw 25(1):1–18. doi:10.18637/jss.v025.i01

Lee C-R, Mitchell-Olds T (2011) Quantifying effects of environmental and geographical factors on patterns of genetic differentiation. Mol Ecol 20:4631–4642. https://doiorg/101111/j1365–294X201105310x

Leigh J, Bryant D, Steel M (2016) PopART: Population analysis with reticulate trees. http://popart.otago.ac.nz/index.shtml

Lourenço A, Gonçalves J, Carvalho F, Wang IJ, Velo-Antón G (2019) Comparative landscape genetics reveals the evolution of viviparity reduces genetic connectivity in fire salamanders. Mol Ecol 28:4573–4591. https://doiorg/101111/mec15249

Luedtke JA, Chanson J, Kelsey N, Hobin L, Maciel AO, Catenazzi A, Borzée A, Hamidy A, Aowphol A, Jean A, et al. (2023) Ongoing declines for the world’s amphibians in the face of emerging threats. Nature 622:308–314. https://doi.org/10.1038/s41586-023-06578-4

Luiz OJ, Madin JS, Robertson DR, Rocha LA, Wirtz P, Floeter SR (2012) Ecological traits influencing range expansion across large oceanic dispersal barriers: insights from tropical Atlantic reef fishes. P Roy Soc B-Biol Sci 279:1033–1040. https://doi.org/10.1098/rspb.2011.1525

Maes GE, Pujolar JM, Hellemans B, Volckaert FAM (2006) Evidence for isolation by time in the European eel (*Anguilla anguilla* L). Mol Ecol 15:2095–2107. https://doiorg/101111/j1365–294X200602925x

Magalhães FM, Dantas, AKBP, Brito, MRM, Medeiros, PHS, Oliveira, AF, Pereira, TCSO, Queiroz, MHC, Santana DJ, Silva WP, Garda AA (2013) Anurans from an Atlantic Forest-Caatinga ecotone in Rio Grande do Norte State, Brazil. Herpetol Notes 6:1–10.

Manel S, Holderegger R (2013) Ten years of landscape genetics. Trends Ecol Evol 28(10):614–621. https://doiorg/101016/jtree201305012

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220.

Martin P, Bonier F, Moore I, Tweksbury J (2009) Latitudinal variation in the asynchrony of seasons: implications for higher rates of population differentiation and speciation in the tropics. Ideas Ecol Evol 2:9–17. https://doiorg/104033/iee200923n

Menezes L, Canedo C, Batalha-Filho H, Garda AA, Gehara M, Napoli MF (2016) Multilocus Phylogeography of the Treefrog *Scinax eurydice* (Anura, Hylidae) Reveals a Plio-Pleistocene Diversification in the Atlantic Forest. Plos One 11(6):e0154626. https://doiorg/101371/journalpone0154626

Miller JED, Damschen EI, Harrison SP, Grace JB (2015) Landscape structure affects specialists but not generalists in naturally fragmented grasslands. Ecol 96:3323–3331. https://doiorg/101890/15–02451

Mitchell A, Bergmann PJ (2016) Thermal and moisture habitat preferences do not maximize jumping performance in frogs Functional. Ecol 30(5):733–742. https://doiorg/101111/1365–243512535

Monteiro WP, Veiga JC, Silva AR, Carvalho CS, Lanes ECM, Rico Y, Jaffe R (2019) Everything you always wanted to know about gene flow in tropical landscapes (but were afraid to ask). PeerJ 7:e6446. http://doiorg/107717/peerj6446

Nagaraju SK, Gudasalamani R, Barve N, Ghazoul J, Narayanagowda GK, Ramanan US (2013) Do ecological niche model predictions reflect the adaptive landscape of species?: a test using *Myristica malabarica* Lam, an endemic tree in the Western Ghats, India. PLoS ONE 8(11):e82066. https://doiorg/101371/journalpone0082066

Nali RC, Becker CG, Zamudio KR, Prado CPA (2020) Topography, more than land cover, explains genetic diversity in a Neotropical savanna tree frog. Divers distrib 26:1798–1812. https://doiorg/101111/ddi13154

Nevo E, Beiles A (1991) Genetic diversity and ecological heterogeneity in amphibian evolution. Copeia 1991:565–592. https://doi.org/10.2307/1446386

Nosil P, Vines TH, Funk DJ (20050 Reproductive isolation caused by natural selection against immigrants from divergent habitats. Evol 59:705–19. https://doiorg/101111/j0014–38202005tb01747x

Nowakowski AJ, Dewoody JA, Fagan ME, Willoughby JR, Donnelly MA (2015) Mechanistic insights into landscape genetic structure of two tropical amphibians using field-derived resistance surfaces. Mol Ecol 24:580–595. https://doiorg/101111/mec13052

Nychka D, Furrer R, Paige J, Sain S (2021) *"fields: Tools for spatial data"*. R package version 14.1. https://githubcom/dnychka/fieldsRPackage

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MHH (2022) *Vegan: community ecology package*. R package version 2.6-4. https://github.com/vegandevs/vegan

Oliveira CR, Ávila LW, Morais DH (2019) Helminths Associated with Three *Physalaemus* Species (Anura: Leptodactylidae) from Caatinga Biome. Acta Parasitol 64:205–212. https://doiorg/102478/s11686–018–00022–8

Oliveira FFR, Gehara M, Solé M, Lyra M, Haddad CFB, Silva DP, Magalhães RF, Leite FSF, Burbrink FT (2021) Quaternary climatic fluctuations influence the demographic history of two species of sky-island endemic amphibians in the Neotropics. Mol Phylogenet Evol 160:107113. https://doiorg/101016/jympev2021107113

Ortego J, Riordan EC, Gugger PF, Sork VL (2012) Influence of environmental heterogeneity on genetic diversity and structure in an endemic southern Californian oak. Mol Ecol 21(13):3210–3223. https://doiorg/101111/j1365–294X201205591x

Ortiz DA, Lima AP, Werneck FP (2018) Environmental transition zone and rivers shape intraspecific population structure and genetic diversity of an Amazonian rain forest tree frog. Evol Ecol 32:359–378. https://doiorg/101007/s10682–018–9939–2

Otto-Bliesner BL, Marshall SJ, Overpeck JT, Miller GH, Hu A (2006) Simulating Arctic Climate Warmth and Icefield Retreat in the Last Interglaciation. Science 311(5768):1751–1753. https://doiorg/101126/science1120808

Pabijan M, Wollenberg KC, Vences M (2012) Small body size increases the regional differentiation of populations of tropical mantellid frogs (Anura: Mantellidae). J Evol Biol 25:2310–2324. https://doiorg/101111/j1420–9101201202613x

Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (2002) the Simple fool's guide to PCR. Version 2. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu.

Paz A, Ibáñez R, Lips KR, Crawford AJ (2015) Testing the role of ecology and life history in structuring genetic variation across a landscape: A trait–based phylogeographic approach. Mol Ecol 24(14):3723–3737. https://doiorg/101111/mec13275

Pease KM, Freedman AH, Pollinger JP, McCormack JE, Buermann W, Rodzen J, Banks J, Meredith E, Bleich VC, Schaefer RJ, Jones K, Wayne RK (2009) Landscape genetics of California mule deer (*Odocoileus hemionus*): the roles of ecological and historical factors in generating differentiation. Mol Ecol 18(9):1848–1862. https://doiorg/101111/j1365–294X200904112x

Pebesma EJ, Bivand RS (2005) *Classes and methods for spatial data in R: the sp package*. R News 5(2). http://cranr–projectorg/doc/Rnews/

Pertoldi C, Bundgaard J, Loeschcke V, Barker JSF (2014) The phenotypic variance gradient - a novel concept. Ecol Evol 4(22):4230–4236. https://doiorg/101002/ece31298

Pie MR, Faircloth BC, Ribeiro LF, Bornschein MR, Mccormack JE (2018) Phylogenomics of montane frogs of the Brazilian Atlantic Forest is consistent with isolation in sky islands followed by climatic stability. Biol J Linn Soc 125: 72-82. https://doi.org/10.1093/biolinnean/bly093

Pombal JP, Haddad CFB (2007) Estratégias e modos reprodutivos em anuros In: Nascimento LB, Oliveira PME (eds) Herpetologia no Brasil II São Paulo: Sociedade Brasileira de Herpetologia, pp 101-116.

Porto TJ, Carnaval AC, Rocha PLB (2013) Evaluating forest refugial models using species distribution models, model filling and inclusion: a case study with 14 Brazilian species. Divers distrib 19:330-340. https://doiorg/101111/j1472–4642201200944x

Prado C, Uetanabaro M, Haddad C (2005) Breeding activity patterns, reproductive modes, and habitat use by anurans (Amphibia) in a seasonal environment in the Pantanal, Brazil. Amphib-Reptil 26:211–21. https://doiorg/101163/1568538054253375

Raffel TR, Rohr JR, Kiesecker JM, Hudson PJ (2006) Negative effects of changing temperature on amphibian immunity under field conditions. Funct Ecol 20(5):819–828. https://doiorg/101111/j1365–2435200601159x

R Core Team (2022) R: A language and environment for statistical computing R Foundation for Statistical Computing. Vienna, Austria.

Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM (2009) The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biol Conserv 142:1141–1153. https://doiorg/101016/jbiocon200902021

Richardson JL (2012) Divergent landscape effects on population connectivity in two co-occurring amphibian species. Mol Ecol 21:4437–4451. https://doiorg/101111/j1365–294X201205708x

Rodal MJN, Barbosa MRV, Thomas WW (2008) Do the seasonal forests in northeastern Brazil represent a single floristic unit? Braz J Biol 68(3):467–475. https://doiorg/101590/S1519–69842008000300003

Rodríguez A, Börner, M, Pabijan, M, Gehara, M, Haddad CFB, Vences, M (2015) Genetic divergence in tropical anurans: deeper phylogeographic structure in forest specialists and in topographically complex regions. Evol Ecol 29:765–785. https://doiorg/101007/s10682–015–9774–7

Rosseel Y (2012) *lavaan: An R Package for Structural Equation Modeling*. J Stat Softw 48(2):1–36. https://doiorg/1018637/jssv048i02

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado. P, Ramos-Onsins SE, Sánchez–-Gracia A (2017) DnaSP v6: DNA Sequence Polymorphism Analysis of Large Datasets Mol Biol Evol 34:3299–3302. https://doiorg/101093/molbev/msx248

Sandberger-Loua L, Rodel M-O, Feldhaar H (2018) Gene–flow in the clouds: landscape genetics of a viviparous, montane grassland toad in the tropics. Conserv Genet 19:169–180. https://doiorg/101007/s10592–017–1029–4

Santana DJ, Ragalzi E, Koroiva R, Mângia S, Ceron K, Leite FSF, Shepard DB (2024) Lineage diversification of the Sky Island treefrog *Scinax curicica* (Anura, Hylidae) in the Espinhaço Mountain Range. Biol. J. Linn. Soc. XX: 1–10. https://doi.org/10.1093/biolinnean/blad125

Santos, AMM, Tabarelli, M (2004) Integridade, esforço e diretrizes para conservação dos Brejos de Altitude da Paraíba e Pernambuco In: Pôrto, KC, Cabral, JJP, Tabarelli, M (eds) Brejos de Altitude em Pernambuco e Paraíba: História natural, ecologia e conservação Ministério do Meio Ambiente, Brasília, Brazil, pp 309–318.

Santos AMM, Cavalcanti D R, Silva JMC, Tabarelli M (2007) Biogeographical relationships among tropical forests in north-eastern Brazil. J Biogeogr 34:437–446. https://doi.org/10.1111/j.1365-2699.2006.01604.x

Schluter D (2001) Ecology and the origin of species. Trends Ecol Evol 16:372–380. https://doiorg/101016/S0169–5347(01)02198–X

Sexton JP, Hangartner SB, Hoffmann AA (2014) Genetic isolation by environment or distance: which pattern of gene flow is most common? Evol 68:1–15. https://doiorg/101111/evo12258

Silva JMC, Leal I R, Tabarelli M (2018) Caatinga: the largest tropical dry forest region in South America. Springer.

Silva NR, Neto JA, Prado CPA, Mott T (2019) Reproductive biology of *Dendropsophus haddadi* (Bastos and Pombal, 1994), a small treefrog of the Atlantic Forest. Herpetol Notes 12:319–325.

Silveira MHR, Mascarenhas R, Cardoso D, Batalha-Filho H (2019) Pleistocene climatic instability drove the historical distribution of forest islands in the northeastern Brazilian Atlantic Forest. Palaeogeogr Palaeoclimatol Palaeoecol 527:67–76. https://doiorg/101016/jpalaeo201904028

Staden R (1996) The Staden sequence analysis package. Mol Biotechnol 5:233–241. https://stadensourceforgenet/

Storfer A, Murphy MA, Spear SF, Holderegger R, Waits L (2010) Landscape genetics: where are we now? Mol Ecol 19:3496–3514. https://doiorg/101111/j1365–294X201004691x

Streicher JW, Cox CL, Campbell JA, Smith EN, de Sá RO (2012) Rapid range expansion in the Great Plains narrow–mouthed toad (*Gastrophryne olivacea*) and a revised taxonomy for North American microhylids. Mol Phylogenet Evol 64:645–653. http://dxdoiorg/101016/jympev201205020

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38(7):3022–3027. https://doiorg/101093/molbev/msab120

Titon Junior B, Gomes FR (2015) Relation between Water Balance and Climatic Variables Associated with the Geographical Distribution of Anurans. PLoS ONE 10(10):e0140761. https://doiorg/101371/journalpone0140761

Thomé MT, Carstens BC, Rodrigues MT, Galetti PM, Alexandrino J, Haddad CFBH (2021) A role of asynchrony of seasons in explaining genetic differentiation in a Neotropical toad. Hered 127:363–372. https://doiorg/101038/s41437–021–00460–7

Torres-Florez JP, Johnson WE, Nery MF, Eizirik E, Oliveira-Miranda MA, Galetti Jr PM (2018) The coming of age of conservation genetics in Latin America: what has been achieved and what needs to be done. Conserv Genet 19:1–15. https://doiorg/101007/s10592–017–1006–y

Travis JMJ, Mustin K, Barton KA, Benton TG, Clobert J, Delgado MM, Dytham C, Hovestadt T, Palmer SCF, Dyck HV, Bonte D (2012) Modelling dispersal: an eco–evolutionary framework incorporating emigration, movement, settlement behaviour and the multiple costs involved. Methods ecol evol 3(4):628–641. https://doiorg/101111/j2041–210X201200193x

Ulloa JS, Aubin T, Llusia D, Courtois EA, Fouquet A, Gaucher P, Pavoine S, Sueur J (2019) Explosive breeding in tropical anurans: environmental triggers, community composition and acoustic structure. BMC Ecol 19(28):1–17. https://doiorg/101186/s12898–019–0243–y

Van Bocxlaer I, Loader SP, Roelants K, Biju SD, Menegon M, Bossuyt F (2010) Gradual adaptation toward a range-expansion phenotype initiated the global radiation of toads. Science 327:679–682. http://dxdoiorg/101126/science1181707

Velloso AL, Sampaio EVSB, Pareyn FGC (2002) Ecorregiões propostas para o bioma Caatinga Associação Plantas do Nordeste, Instituto de Conservação Ambiental The Nature Conservancy do Brasil, Recife, Pernambuco.

Verdade VK, Rodrigues MT (2007) Taxonomic Review of *Allobates* (Anura, Aromobatidae) from the Atlantic Forest Brazil. J Herpetol 41:566–580. https://doiorg/101670/06–0941

Walsh PS, Metzger DA, Higuchi R (1991) Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506–513.

Wang IJ, Bradburd GS (2014) Isolation by environment. Mol Ecol 23:5649–62. https://doiorg/101111/mec12938

Wang IL, Glor RE, Losos JB (2013) Quantifying the roles of ecology and geography in spatial genetic divergence. Ecol Lett 16:175–182. https://doiorg/101111/ele12025

Wang I J, Summers K (2010) Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. Mol Ecol 19:447–458. https://doiorg/101111/j1365–294X200904465x

Wang Y-H, Yang KC, Bridgman CL, Lin LK (2008) Habitat suitability modelling to correlate gene flow with landscape connectivity. Landsc Ecol 23:989–1000. https://doiorg/101007/s10980–008–9262–3

Wollenberg KC, Vieites DR, Glaw F, Vences M (2011) Speciation in little: The role of range and body size in the diversification of Malagasy mantellid frogs. BMC Evol Biol 11:1–16. https://doiorg/101186/1471–2148–11–217

Wollenberg Valero KC (2015) Evidence for an intrinsic factor promoting landscape genetic divergence in Madagascar leaflitter frogs. Front genet 6:155. https://doi.org/10.3389/fgene.2015.00155

Wright S (1943) Isolation by distance. Genet 28:114–38. https://doiorg/101093/genetics/282114

Zancolli G, Rodel M-O, Stefan-Dewenter I, Storfer A (2014) Comparative landscape genetics of two river frog species occurring at different elevations on Mount Kilimanjaro. Mol Ecol 23:4989–5002. https://doiorg/101111/mec12921

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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).

6 CAPÍTULO 3

Demographic dynamics in two co-occurring amphibians reveals idiosyncratic responses to past climate changes in highlands in Northeastern Brazil*

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Demographic dynamics in two co-occurring amphibians reveals idiosyncratic responses to past climate changes in highlands in Northeastern Brazil

Running title: Different species and idiosyncratic demographic responses

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Abstract

Aim: Pleistocene climatic cycles played a paramount role in diversification and demographic dynamics of the Neotropical biota, especially in topographically complex regions such as mountain ranges. Despite this, their importance remains poorly understood in some areas, such as forest enclaves in northeastern Brazil. Here, we develop a comparative phylogeographic framework using two amphibian species inhabiting forest enclaves north of the São Francisco River to investigate how ecologically distinct species responded to climatic and forest extension changes during the Pleistocene.

Location: Northeastern Brazil.

Taxa: two amphibian species.

Methods: We used genomic data, statistical phylogeography, and coalescent-based model inference.

Results: We found that the species that rely on permanent water bodies for reproduction (*Dendropsophus oliveirai*) exhibits greater geographic structure than the reproductive habitat-generalist species (*Physalaemus cuvieri*). Populations of the two species diverged during the Mid-Pleistocene, but not simultaneously. Migration rates and changes in population size were positively influenced by the Last Glacial Maximum in both species. The reproductive habitat-specialist species initiated secondary contact coinciding with population expansion during this period, whereas the reproductive habitat-generalist species increased preexisting migration along with population growth.

Main conclusions: Our findings support previous hypotheses regarding the expansion and contraction of the northernmost portion of the Atlantic Forest. Moreover, they demonstrate how spatiotemporal climatic and vegetational shifts shape the demographic history of different

species within the same region, albeit with varying intensities depending on their specific ecological traits.

Keywords: Borborema plateau, comparative phylogeography, dry forests, ecological traits, highland forests, Neotropical region, Pleistocene

Introduction

The current knowledge on neotropical biota indicates that Quaternary climatic oscillations and their consequent habitat shifts had an important role in the diversification and demographic processes of different species in this biodiversity hotspot (Rull 202; Baker et al. 2020; Ledo et al. 2020; Meseguer et al. 2022). In this region, mountains are often implicated in many diversification processes, phylogeographic patterns, and in the spatial distribution of endemisms (Rull 2005; Guedes et al. 2020; Huber et al. 2018), as those environments present high spatial and temporal habitat heterogeneity along their range as a result of their geological and climate history (Costa et al. 2017; Silveira et al. 2019; Guedes et al. 2020). Pleistocene climatic shifts were firstly explored by Haffer (1969), who intended to explain biogeographical patterns of Amazonian avifauna based on present-day distribution of lowland Amazonian rainforests (Rull 2020). Haffer's research gave rise to the "refugia" hypothesis, which proposes that forests were probably fragmented into isolated patches surrounded by open vegetation during Pleistocene oscillations, favoring speciation by vicariance. Today, such hypothesis is the most used to explain patterns of phylogeographic diversity and structure as well as demographic patterns and process in mountain ranges or "sky-island" systems in neotropics (Pie et al. 2018; Oliveira et al. 2021; Dantas-Queiroz et al. 2023; Batalha-Filho et al. 2024). Therefore, the rugged relief and fluctuation between contraction and expansion of forest habitats induced by climate instability during the Pleistocene seems to be the most important factors driving the evolutionary history in neotropical mountains (Amaral et al. 2021).

Studies have shown that the biomes in South America share a complex and ancient ecological and evolutionary relationship that has shaped current patterns of species diversity, phylogeographic structure, and past demographic changes in plant and animal populations (Carnaval and Bates 2007; Santos et al. 2007; Rodrigues et al. 2014; Dal Vechio et al. 2018; Silveira et al. 2019; Castro et al. 2019; Ledo et al. 2020; Oliveira et al. 2021; Guillory et al. 2024). This is particularly evident in forest enclaves, such as those found in Northeastern Brazil. More specifically, north of the São Francisco River, the largest river in the region, is located the Borborema Plateau (BP), a geomorphological unit encompassing almost the whole northeastern Brazilian Shield, arranged in an approximately NNE-SSW axis, and surrounded by the marginal lowlands of the Depressão Sertaneja (to the north and northwest), the São Francisco Sand Dunes (to the south and southwest), and the costal cuestas (to the northeast and southeast of BP) (Morais Neto et al. 2009). This plateau presents relatively high altitudes ranging between 500–1200 m a.s.l and a very heterogeneous landscape, with seasonal dry forests (Caatinga) in the lowlands surrounding scattered remnants of highland mesic forests (Andrade-Lima 1982; Velloso et al. 2002; Tabarelli and Santos 2004). This pattern of vegetation distribution resulted from multiple events of expansion and retraction of the Atlantic rainforest into the Caatinga over the Pleistocene climatic cycles (Wang et al. 2004; Porto et al. 2013; Costa et al. 2017; Silveira et al. 2019).

Based on palaeoindicators and niche modeling it was suggested that much of the Pleistocene was marked by rainfall peaks (Oliveira et al. 1999; Behling et al. 2000; Auler et al. 2004; Wang et al. 2004; Silveira et al. 2019), and it is thought that increased rainfall and humidity allowed the establishment of connections between the Amazon and the Atlantic rainforests, replacing stretches of the otherwise semiarid Caatinga (Batalha-Filho et al. 2013; Ledo and Colli 2017). In comparison with the lowlands, where climatic variation led to unstable rainforest pathways, highland forest enclaves remained more climatically stable (Porto et al. 2013; Costa et al. 2017; Silveira et al. 2019), providing suitable habitats (refuges) especially for species adapted to mesic conditions (Carnaval 2002; Carnaval and Bates 2007; Dal Vechio et al. 2018; Castro et al. 2019; Ledo et al. 2020; Oliveira et al. 2021; Batalha-Filho et al. 2023), with gene flow among isolated populations possibly occurring sporadically (Pinaya et al. 2019). Similar to other sky-island systems in more central and southern ranges of the Brazilian Atlantic rainforest (Collevatti et al. 2009; Antonelli et al. 2010; Bonatelli et al. 2014), the most recent ecological niche modeling study involving forest enclaves across Northeastern Brazil suggested that during the Last Glacial Maximum (LGM; 21 kyr) the Atlantic rainforest was more widespread, connecting forest enclaves and, possibly, animal populations (Silveira et al. 2019). The same study proposed specifically that forest enclaves in the BP were generally connected with each other and with the coastal Atlantic rainforests from 120 kyr (Last Interglacial Maximum; LIG) until present, with higher connectivity during the LGM and lower connectivity before and after this moment. Also, modeling maps produced by this study indicated an earlier isolation of stable forest enclaves in the north portion of the BP. Then, populations inhabiting those enclaves probably diverged in different times until the LIG, expanded jointly around the

LGM, and experienced distribution retraction and genetic bottlenecks from the Mid-Holocene (MH), when the Caatinga started to expand again.

Although climatic cycles have affected gene flow and demographic dynamics in many montane species around the world, their effects on genetic structure of populations are very unpredictable because of specie-specifics constraints (Avise 2000; Helmstetter et al. 2020; Ericson and Irestedt 2022). In a gradient of habitat specialization, forest-dependent species are normally the most affected by climate change, environmental variation, and geographic distance, while species adapted to open vegetation are usually more stable in changing scenarios (Paz et al. 2015; Rodríguez et al. 2015). Recent comparative landscape genetics studies with bird (Batalha-Filho et al. 2023) and amphibian (Oliveira et al. 2021) populations from forest enclaves in Northeastern Brazil demonstrated how species ecologically different can differ in their responses to geographic distance and past and current climatic/environmental variation. Two previous studies (Andrade Lima et al. 2024; Andrade Lima et al., submitted) demonstrated that the associations between acoustic and genetic variation and their abiotic correlates in two amphibian species with different reproductive strategies and habitat requirements are idiosyncratic. These findings show that even non-forest-dependent species respond differently to spatiotemporal climatic and environmental variations, depending on their species-specific traits.

Although vegetation cover of rainforest enclaves in the BP was favored during the LGM (Silveira et al. 2019), we still lack evidence on how ecological niches and reproductive modes in different animal species may have influenced their responses to forest expansion and retraction during that period. Here, we used genomic information on populations of two anuran species, *Dendropsophus oliveirai* (Bokermann, 1963) (Hylidae) and *Physalaemus cuvieri* Fitzinger, (1826) (Leptodactylidae), and coalescent-based model inference to investigate the spatiotemporal population dynamics of amphibians across rainforest enclaves in the BP. *D. oliveirai* is a small reproductive habitat-specialist, with low vagility, herbaceous habitat, and prolonged reproduction in permanent or long-duration ponds (Haddad and Prado 2005; Silva et al. 2019). *P. cuvieri* is a medium-size reproductive habitat-generalist, with terrestrial habitat, opportunist reproduction (during rainfall), and using foam nests to protect nourish its eggs (Pombal and Haddad 2007, Aguiar et al. 2014). Although differences in habitat use and reproductive modes between these species, both have wide geographic distributions. *Dendropsophus oliveirai* occurs from Central to the Northern Atlantic Forest along the coast of Brazil, with disjoint distribution in highland forests within the Caatinga biome (Frost 2025;

Magalhães et al. 2013; Menezes et al. 2020); *P. cuvieri* occurs in different biomes throughout South America, from Argentina to Venezuela (Frost 2025), with a possible center of origin in south-central Amazonia, from where it dispersed to south and central Cerrado, then the Caatinga (Miranda et al. 2019). *Dendropsophus oliveirai* lineages distributed north of the São Francisco River, which includes populations in the present study, are thought to have diverged for the southern populations of this species around 810 kyr ago, with no evidence of gene flow in recent times (Menezes et al. 2020). In *P. cuvieri*, despite its wide distribution, strong regional population structure its seen, with the northeastern Brazil population diverging around 5 Myr ago and presenting wider distribution in all lineages during the LGM compared to the Holocene and present-day (Miranda et al. 2019).

We strategically sampled the same localities for both species to (1) estimate divergence time and demographic changes through time possibly associated with past climatic and vegetational shifts; and (2) search for congruent or divergent demographic responses to past climate changes between those two ecologically different species. More specifically, we tested alternative diversification scenarios using coalescent simulations as follows: first we tested if the diversification of the different populations of the two species was associated with earlier habitat fragmentation occurred between the Mid-Pleistocene and Late-Pleistocene, around 1 Ma and 120 kyr (LIG) (e. g. Werneck et al. 2011), or if it was associated with post-LIG landscape changes. After testing for alternative divergence times, we evaluated if the two species were able to move between forest fragments by testing if the populations remained completely isolated or exchanged migrants after divergence induced by forest expansions during the LGM (e. g. Silveira et al. 2019). Finally, we estimated simultaneously the probability of demographic expansion and changes in migration rate after the LGM to evaluate if our data supports the proposed post-LGM climatic changes and forest expansion. Although we do not expect synchronous population divergence between species, we expect that their populations diverged before the LIG. On the other hand, we expect synchronous responses to the LGM conditions but idiosyncratically, with the reproductive habitat-specialist species showing earlier and more dramatically demographic changes.

Methods

Sampling, GBS library preparation, and sequencing

We conducted our sampling in seven localities across north, central and south parts of the BP, in Northeastern Brazil, north of the São Francisco River (Figure 1; Table S1). The geographic distance between those localities varied between 40 and 354 km. We collected 66 males of *Dendropsophus oliveirai* (7–12 per locality) and 78 males of *Physalaemus cuvieri* (9–14 per locality) along available water bodies during the rainy season between 2020 and 2023. We anesthetized and euthanized each individual using topical benzocaine (50 mg/g) and dissected a sample of thigh muscle tissue, which was preserved in 98% alcohol and stored under -20 °C until DNA extraction. We deposited all specimens and tissue samples at Coleção Herpetológica da Universidade Federal de Pernambuco (CHUFPE; List S2), in Recife, Pernambuco, Brazil. All samples were collected, transported and preserved under permits provided by SISBIO-ICMBio (RAN-SISBIO 69767-3 and RAN-SISBIO 76544-1).

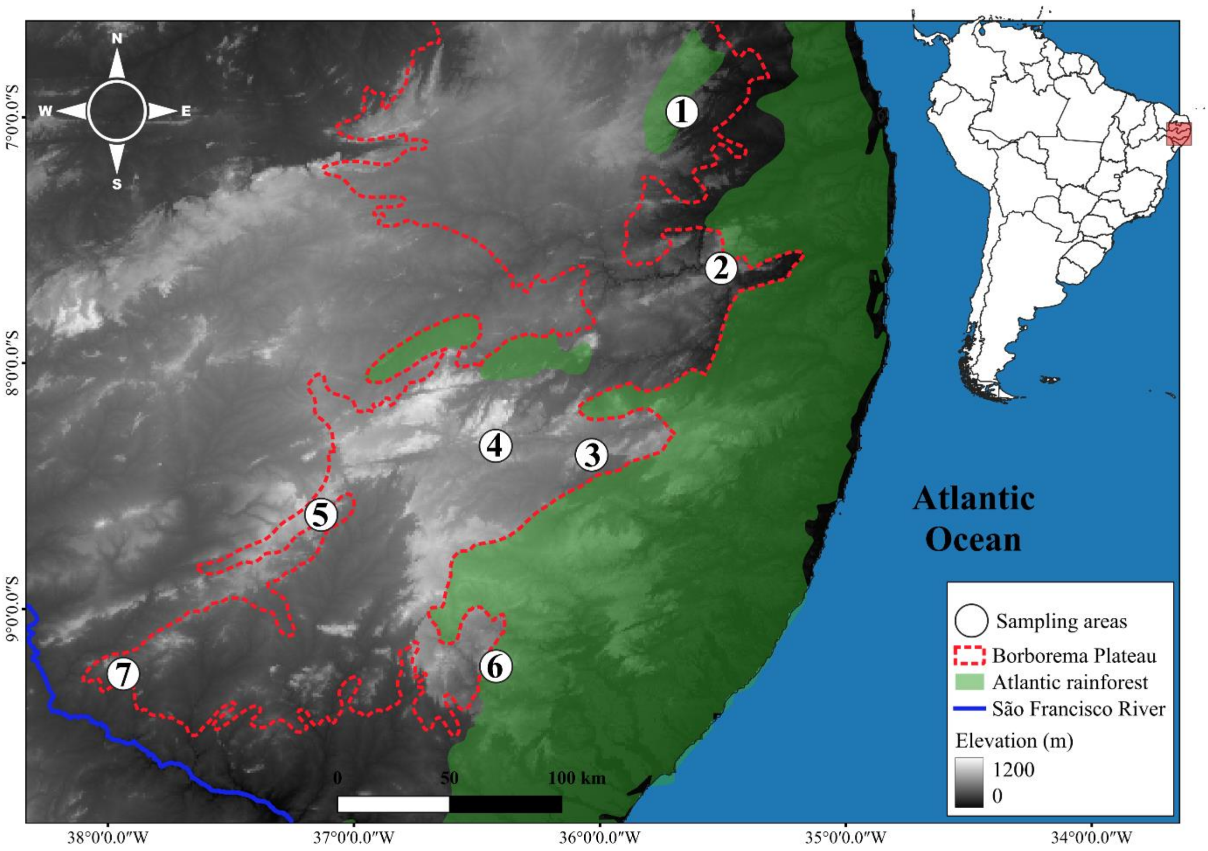


Figure 1. Geographic location of the sampling localities of *Dendropsophus oliveirai* and *Physalaemus cuvieri* in the Borborema Plateau in the north of the São Francisco River, Northeastern Brazil.

We hired the Biotechnology Center (Madison, Wisconsin, USA) to conduct sample processing steps from whole genomic DNA extraction to genotyping by sequencing (GBS). GBS was conducted using Illumina sequencer (NovaSeq platform; Flow Cell: 10B Half Lane [450M reads]; Read Length: 2x150 [Paired-End Sequencing]; Enzyme: Nsil-Mspl [*D. oliveirai*] and ApeKI [*P. cuvieri*]). GBS can provide a large number of high-quality loci and SNPs without a reference genome (Stone and Wolfe, 2021). The libraries were prepared using 100 ng of DNA from each sample. All samples were shipped from Brazil to the USA under SisGen-MMA permit Nº A6F1555.

Filtering GBS reads

We demultiplexed, filtered and clustered the GBS raw sequence reads using iPyRAD 0.9.96 (Eaton and Overcast 2020). We did not allow base mismatches in barcodes during demultiplexing and clustering threshold was set to 95%. We set a minimum of four individuals to validate a locus and 50% of maximum proportion for shared polymorphic sites within a locus. Some individuals were discarded according to the locus count in all individuals, leading to 60 and 76 individuals of *D. oliveirai* and *P. cuvieri*, respectively (Tables S3 and S4). After that, we filtered the .vcf file from iPyRAD using VCFTools 0.1.16 (Danecek et al. 2011), keeping only bi-allelic loci with a maximum of 30% and 50% missing data at a locus for *D. oliveirai* and *P. cuvieri* respectively based on the quality of the data (Table S5). In VCFTools filtering, we excluded individuals with more than 50% of missing data. In the next step, we retained one SNP (bi-allelic loci) per locus, so our data comprised only potentially unlinked SNPs for genetic structure analyses (Dataset 1, Figure S23). In this initial filtering, we focused on including as many individuals from all localities as possible. Our primary goal was to retain at least 1,000 SNPs (one per locus), each present in at least 50% of the individuals. Based on the structure analyses, we selected individuals from each population with a high number of loci shared by the majority of individuals within the species (41 in *D. oliveirai* and 39 in *P. cuvieri*) for a second filtering allowing only 10% of missing data (Table S6). This new dataset was used in genetic diversity analyses and divergence-time estimation (Dataset 2, Figure S23). To select individuals, we employed ‘Matrix Condenser’ version 1.1 from the package ‘shiny’ (de Medeiros and Farrell 2018; de Medeiros 2024) in R (R Core Team 2022) to visualize missing data percentage, loci number and loci sharing (Table S7).

We also filtered reads at the population level for cluster-based demographic analyses (one .vcf file for each population). Based on the assignment values of the structure analysis (only *q*-value ≥ 0.75) we selected representative individuals from each population to avoid potential biases in demographic inference caused by mixed-ancestry individuals (e.g., Heller et al. 2013). We filtered per population (selecting the 8–10 best individuals) while allowing only 10% missing data. However, this time, we considered more than one SNP per locus (Dataset 3, Figure S23), as the first demographic approach we used allows for the use of multiple SNPs per locus while controlling their dependencies, thereby improving inference (Liu and Fu 2020). We conducted one last filtering to produce the 'testing divergent scenarios' dataset (Dataset 4, Figure S23) using the .alleles file from iPyRAD filtering. To do that, we first segregated loci information in different .fasta files using the function 'iPyrad.alleles.loci2fasta' from the R package 'PipeMaster' version 0.0.9 (Gehara et al. 2017b) and performed loci trimming using Python commands to eliminate individuals with insufficient data, remove missing data, and any character that is not a nucleotide base. All bioinformatic analyses were run under parallel execution at the Rutgers University flux computing cluster, in Newark, New Jersey, USA.

Population structure

We performed two population structure analyses to identify the most probable number of populations (clusters; *K*) and genetic discontinuities in each species. All analyses were performed testing $K = 10$ as the maximum number of clusters. We first ran Sparse Non-negative Matrix Factorization (sNMF) using the function 'snmf' from the package 'LEA' version 3.17.2 (Frichot et al. 2015) in R. This analysis is similar to STRUCTURE (Pritchard et al. 2000; Hubisz et al. 2009) but it does not use Bayesian models to estimate ancestry proportions. For sNMF, we ran 10 iterations for each *K* and chose the best *K* based on the cross-entropy values. To apply 'smnf' function, we used alpha = 3 and alpha = 1 for *D. oliveirai* and *P. cuvieri*, respectively, according to previous cross-validation for cross-entropy variation, using the function 'cross.entropy'. We plotted the sNMF clusters following the same order of the resulting output. As second evidence on the *K* number and assignments, we performed a Discriminant Analysis of Principal Components (DAPC) applying the function 'dapc' from the *R* package 'adegenet' version 1.3-1 (Jombart 2008). This is an unsupervised multivariate method which is based on Principal Component Analysis (PCA) followed by Discriminant Analysis (DA) to identify and discriminate clusters with no assumption about allelic frequencies or gene flow. To perform the 'dapc' function, we selected the number of clusters using K-means and the Bayesian information criterion (BIC*)* score applying function ‘find.clusters’ from the package ‘adegenet’. sNMF considers admixture, while DAPC maximizes the separation between groups. Thus, differences in individual cluster assignment between these analyses are expected, especially when population structure occurs in a gradient (without well-defined boundaries; Isolation by Distance) (Oleksiak, 2018; Cullingham et al., 2022) – our previous study with the same populations as the present study, using mitochondrial DNA, demonstrated the contribution of Isolation by Distance in both species (Andrade Lima et al. submitted). When any individual showed different cluster assignment between sNMF and DAPC, we prioritized the DAPC results since it showed higher assignment values.

To obtain more evidence on the number of populations and their range, we also performed the Estimated Effective Migration Surfaces (EEMS) method to evaluate gene flow patterns among localities without inferring the direction of migrations (Petkova et al. 2016). Considering a model of isolation-by-distance, this method provides an intuitive visual representation of spatial patterns in genetic variation and highlights regions of higher-than-average and lower-than-average historical gene flow (Petkova et al. 2016). As inputs to each species, we used a polygon that includes the entire geographic range of our sampling (from -38.94° to -34.96° of longitude and -10.46° to -05.91° of latitude), coordinates of sampling localities, and a genetic distance matrix calculated using the packages ‘adegenet’ and ‘stringr’ version 1.4.0 (Wickham, 2019). We set as parameters nDemes = 600, numMCMCIter = 10000000, numBurnIter = 2000000, and numThinIter = 9999 and performed five independent runs until reaching convergence among them. We assessed convergence among runs and produced effective migration surface plots using the *R* packages ‘reemsplots2’ (Petkova et al. 2016), ‘ggplot2’ version 3.4.4 (Wickham et al. 2023), ‘sf’ (Pebesma 2018), ‘maps’ *version*3.4.1 (Becker et al. 2023), and ‘tidyverse’ version 2.0.0 (Wickham et al. 2023).

Genetic diversity and divergence-time estimation

In order to investigate the distribution of genetic variability among geographic populations of the two species, we performed genetic diversity analyses on genetic clusters defined in the previous population structure analyses using the *R* packages ‘diveRsity’ version 1.9.90 (Keenan et al. 2013), ‘PopGenKit’ version 1.0 (Pequette 2012), and ‘hierfstat’ version 0.5-11 (Goudet 2005) applying respectively the functions ‘divBasic’, ‘bootstrapHet_byloc’, and ‘basic.stats’. These functions provide summary statistics as allelic richness (*AR*), proportion of alleles (*A%*), observed heterozygosity (*Ho*), expected heterozygosity (*He*, *Ht*, *Htp*), inbreeding coefficient (*Fis*), and genetic differentiation (*Dst*, *Dstp*, *Fst*, *Fstp*, *Dest*).

We inferred a population tree and divergence-time estimation using SNAPP (Bryant et al. 2012), a Bayesian approach implemented in BEAST2 (Bouckaert et al. 2014) which uses integration over all possible gene trees on the basis of the multispecies coalescent (MSC) model. As SNAPP implements the coalescent model, which does not need rooting to polarize the topology, we did not include any outgroup on the dataset. We ran 1,000,000 MCMC steps, sampling every 500 steps, with lognormal prior distribution (*D. oliveirai*: mean = 0.81 and SD = 0.441; *P. cuvieri*: mean = 5.30 and SD = 1.25) on the root of the topology. These parameter values of prior distribution were based on divergence times of lineages occurring in the same region, which were previously studied by Menezes et al. (2020) and Miranda et al. (2019). We used default priors on mutation rates (mutation rate U = 1.0, mutation rate V = 1.0). After running *SNAPP*, we discarded the first 10% runs as burn-in and checked the convergence of the analysis (ESS>200) using Tracer version 1.7 (Rambaut et al. 2018) and used Densitree and FigTree, also from the BEAST2 package, to visualize respectively the maximum clade credibility tree with posterior probability and the divergence-time (95% of Highest Posterior Density Interval).

Demographic inference

We used two approaches to investigate the demographic history and their possible climatic drivers in both species. First, we used the Stairway plot version 2.1.1 (Liu and Fu 2020) approach, a method based on the site frequency spectrum (SFS). We calculated the SFS of each population using the Python script easySFS (http://github.com/isaacovercast/easySFS) (Coffman et al. 2015). Values of SFS for singletons were discarded since they carry little demographic signal from deeper events and can represent sequencing errors, increasing noise in analyses (Keinan and Clark 2012; Mazet et al. 2016). We calculated the parameter “L” (total number of nucleotides from which SNPs were sampled) for the Stairway plot parameter file by multiplying the total number of shared loci by all individuals in each population by the mean length of loci in each individual in each population (Table S7). We used 2/3 (0.67) of sites for training and estimated the median and respective 95% confidence intervals with 500 input files at each estimation point. We considered a mutation rate of 2.1 10^{-9} per generation (Gottscho et al. 2014; Thomé et al. 2021) and a one-year generation time.

Coalescent demographic modeling

To better investigate and explain the demographic dynamic in each species, our second approach was testing alternative scenarios for diversification, migration and effective population size changes using the R package ‘PipeMaster’ to construct models using ‘msABC’, which simulate genetic data and summary statistic (Hudson 2002; Pavlidis et al. 2010). To develop this coalescent approach, we delimited populations according to the previous population structure analyses, tested the divergence time according to SNAPP results, and allowed for change in N_e using values close to Stairway plot median estimations (Table S8). Priors for mutation rate were based on the literature (Gehara et al. 2017a). For each model we ran 10,000 simulations, verifying the adequacy of the simulated data according to the summary statistics of the observed data using histograms and PCAs.

We used a hierarchical approach with four general scenarios and five variations of migration tests (Figure 2): 1) ***Divergence.*** This was the most basic scenario, in which we only tested divergence time among populations, not accounting for changes in population size or migration. This scenario tested two models, divergence between LGM and LIG (20 kyr and 120 kyr) (A-LIG) and between LIG and 1 Myr (B-LIG). Respectively, these models test possible LIG effects and the effects of even more ancient climatic/environmental change, as longer glacial and interglacial cycles during the Mid-Pleistocene (Sun et al. 2019). We set only one parameter in each model: the condition of early divergence in population 3 and subsequent divergence between populations 1 and 2 in both species, according to our previous analyses (this condition was adopted in all scenarios). This test was basically applied to confirm SNAPP results and to find a good prior of divergence for additional models (scenarios of migration and population size change); 2- ***Migration.*** In this scenario, the first model assumed no migration among any populations at any time (IS; it corresponds to the B-LIG model with changes in *Ne* priors), the second model considered migration among all populations throughout their history (MIG), the third model considered migration until the beginning of the LGM (~30 kyr) and isolation thereafter (MIG-T1), the fourth model assumed isolation until the GLM and migration thereafter (i.e. a possible secondary contact) (MIG-T2), and the fifth model considered migration throughout the entire period with any change (increase or decrease) occurring from the LGM (MIG-CT2). All remaining scenarios included these previous variation of migration models, with any changes in population size; 3- ***Migration and expansion*** (ISE, MIGE, MIGE-T1, MIGE-T2, MIGE-CT2). Expansion in all populations from the LGM, regardless of whether migration occurred; 4- ***Migration and differential expansion*** (ISDE, MIGDE, MIGDE-T1, MIGDE-T2, MIGDE-CT2). Greater expansion in population 1, followed by population 2 and 3 from the LGM, regardless of whether migration occurred. We tested this scenario in order to test whether the plateau where our populations are located remained more climatically stable from north to south along this period, as seemed to had been according to modeling maps from Silveira et al. (2019). We did not incorporate any bottleneck scenario because our previous tests showed signs of expansion in all populations (Figure S9). In total, we tested 17 models, two models (one scenario) exclusively for divergence, five models (one scenario) exclusively for migration and 10 models (two scenarios) for migration and population size change.

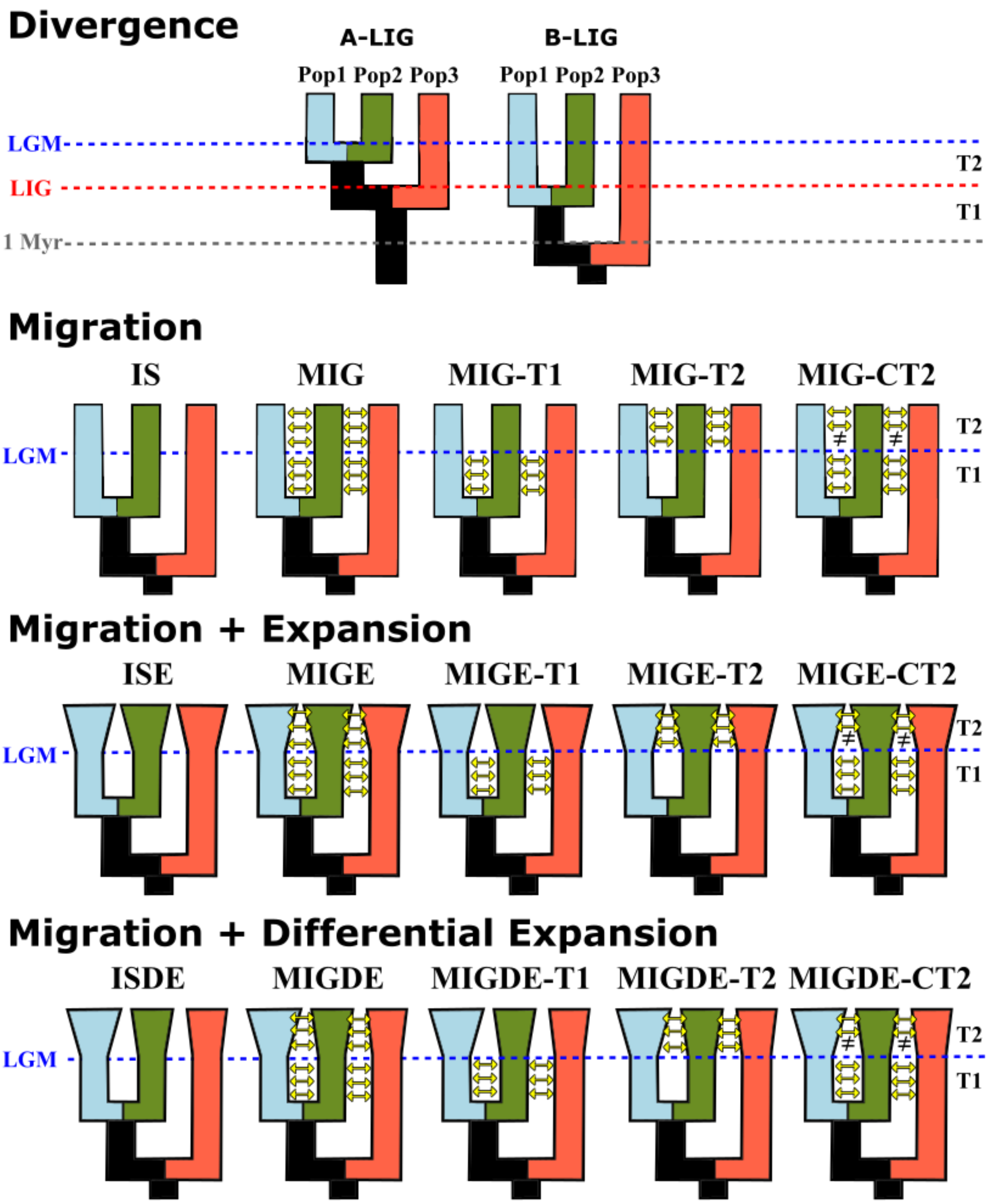


Figure 2. Hierarchical models for alternative scenarios of diversification, migration, and population size change teste with Approximate Bayesian Computation (ABC) and Supervised Machine Learning (SML) for *Dendropsophus oliveirai* and *Physalaemus cuvieri* in highland forests of the Borborema Plateau, in Northeastern Brazil. LGM = Last Glacial Maximum, LIG = Last Interglacial Maximum, T1 = time before any change, T2 = time from any change until now. Posterior probabilities of all models are available in Table 1. Prior information for each parameter and model is available in Supplementary Information Table S8. Description of the models in Methods.

After running all general scenarios (17 models), we performed two different methods to calculate and compare the posterior probabilities (PP) and accuracy (ACC) of each model: Approximate Bayesian Computation using the function ‘postpr’ (tolerance 0.001) and the rejection (REJ) function ‘cv4postpr’ (tolerance 0.001) from the R package ‘abc’ (Csilléry et al. 2010); and neural networks of Supervised Machine Learning (SML)/Deep Learning (DL), from a combination of the R packages ‘tensorflow’ version 2.15.0 (Allaire et al. 2024) and ‘keras’ version 2.13.0 (Kalinowski et al. 2023), which are interfaces to TensorFlow (Weber et al. 2021) and Keras (Chicho and Sallow 2021). These methods helped us to select the best model in each scenario. After that, we ran 50,000 simulations for each selected model in migration and population size change scenarios and compared them. In those scenarios where different models were selected by different methods, we selected the one with the highest posterior probability. Also, if different models were selected by different methods but with similar PP and ACC, we chose the one indicated by SML/DL, as the method usually has stronger predictive power (see Gehara et al. 2020). After finding the model with the greatest support we estimated its summary statistics from an additional simulation with 100,000 runs.

Results

We obtained total 607,019,717 raw reads for 60 individuals of *D. oliveirai* (295,538–17,496,427 raw reads per specimen) and 397,792,892 raw reads for 76 individuals of *P. cuvieri* (729–8,741,655 raw reads per specimen). After iPyRAD filtering steps, we retained 11,870,515 (829–333,430) consensus reads for *D. oliveirai* and 12,086,361 (66,916–212,854) consensus reads for *P. cuvieri* (Tables S3 and S4). Our alignment matrix considering a minimum depth coverage of 6 for consensus base calling and clustering for *D. oliveirai* recovered a total of 1,002,310 loci and 2,632,164 SNPs with 77.07% of missing data. For *P. cuvieri,* we recovered a total of 832,686 loci and 2,720,030 SNPs with 76.82% of missing data (Table S23). After 30% VCFTools filtering per locus, we retained 3,820 loci (30% missing data) for *D. oliveirai* and 72,254 loci (50% missing data) for *P.cuvieri* (Table S5). These VCFTools filtering excluded individuals with 50% of missing data, keeping 44 individuals of *D. oliveirai* and 76 individuals of *P. cuvieri* and generated .vcf files (one SNP per locus) with 1,339 SNPs for *D. oliveirai* and 14,099 SNPs for *P. cuvieri* (Dataset 1, Figure S23). The 10% VCFTools filtering per locus recovered 85,682 loci with 25,174 SNPs (one per locus) in *D. oliveirai* (41 individuals) and 200,077 loci with 38,944 SNPs (one per locus) in *P. cuvieri* (39 individuals) (Table S6; Dataset 2, Figure S23). The filtering by population retained between 33,618 – 34,665 shared loci with 106,136 – 218,378 SNPs in *D. oliveirai* (27 individuals) and between 23,954 – 45,939 shared loci with 125,633 – 374,712 SNPs in *P. cuvieri* (28 individual) (Table S7; Dataset 3, Figure S23). Our loci filtering for testing divergence scenarios retained 4,603 shared loci (between 120 – 150 bp) for five individuals per population in *D. oliveirai* and 23,220 shared loci (we used 6,000) (between 120 – 139 bp) for eight individuals per population in *P. cuvieri* (Dataset 4, Figure S23).

Population structure

The individual assignment in SNMF and DAPC were not completely congruent in both species (Tables S10 and S11). In *D. oliveirai*, the sNMF and DAPC results indicated $K = 3$ (Figure 3a,b; Table S10), with *K1* having the highest contribution in the southern regions of the BP (localities 6 and 7), high to moderate contribution in central localities (localities 5,4, and 3), and very low to no contribution in northern localities (localities 1 and 2). *K3* showed the opposite pattern, with decreasing contribution from north to south. *K2* had high contribution in one southern locality (locality 6) and very low contribution in central and north localities. In a central locality (locality 4) there was strong admixture among the three clusters. Corroborating sNMF and DAPC results, EEMS indicated gene flow among localities in three regions: north (localities 1 and 2), central (3 and 4), and south-central (localities 5, 6, and 7) with a very low migration between central and south-central locations (Figure 3c; Figure S12).

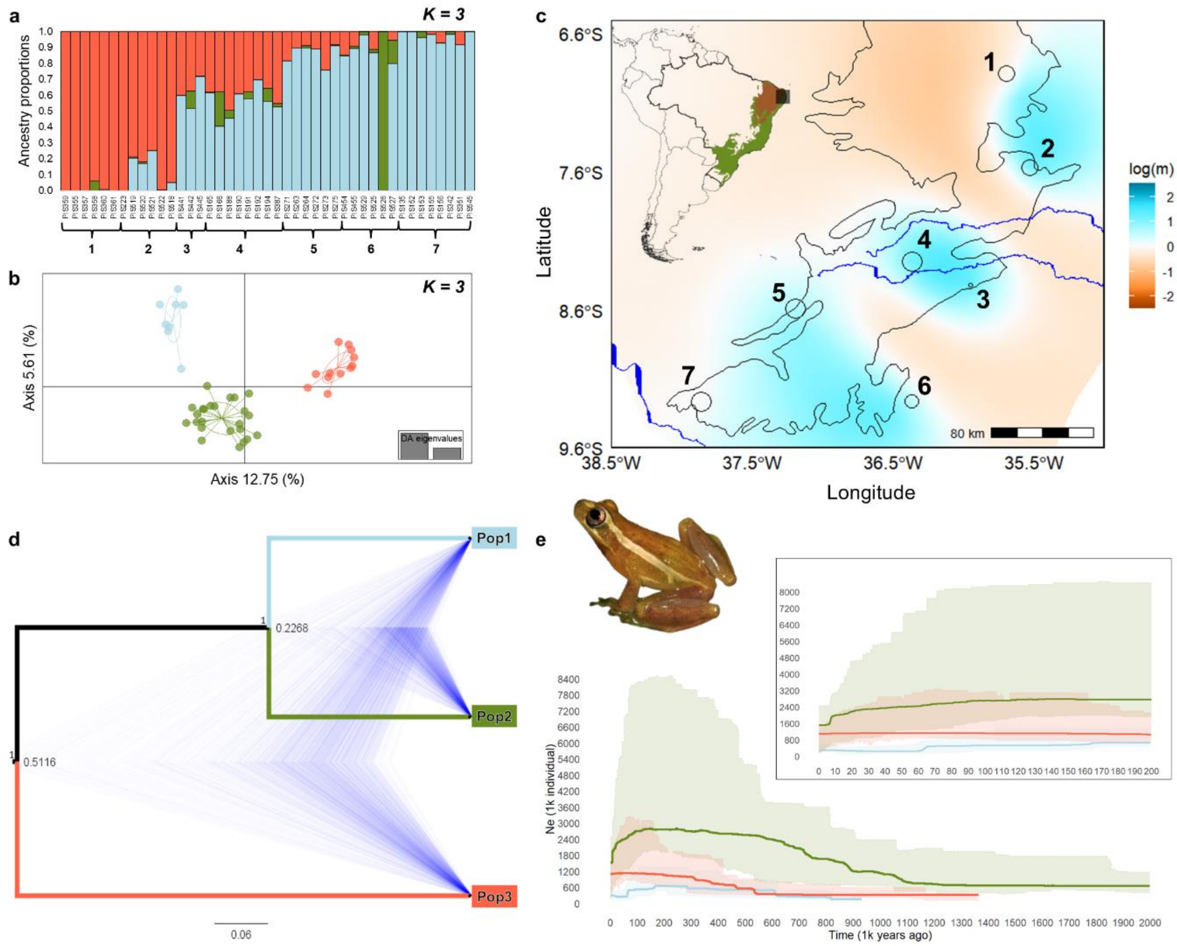


Figure 3. Distribution of genetic variation, patterns of gene flow, phylogenetic relationship, divergence time, and population sizes through time of *Dendropsophus oliveirai* from seven highland forests (1–7) in the Borborema Plateau, Northeastern Brazil. a) Sparse Non-negative Matrix Factorization (sNMF) barplots representing ancestry proportions of each cluster (*K*; each color) for each individual (each bar). b) Discriminant Analysis of Principal Components (DAPC) suggested clusters and their dissimilarities in two axes. c) Estimated effective migration surface (EEMS) showing the posterior mean migration rates *m* (on the log10 scale) in blue and orange shades indicating respectively higher and lower migration rates than the overall average rate (in white). Black line in the map = geographic limits of the Borborema Plateau. Blue lines = Capibaribe, Ipojuca, and São Francisco rivers from top to bottom. Caatinga in brown and Atlantic Forest remnants in green. d) Maximum clade credibility tree with posterior probability (left) and the divergence-time (right; in decimal) inferred by SNAPP. e) Effective population size (N_e) changes over the last two million years (last 200 kyr in detail) estimated in Stairway plot. Thick lines indicate the median of 500 inferences and shaded areas the 95% confidence interval. Blue = K1 (in a and b) and Pop1 (in d and e), green = K2 (in a and b) and Pop2 (in d and e), salmon = K3 (in a and b) and Pop3 (in d and e).

In *P. cuvieri*, sNMF and DAPC results suggested *K* = 3 (Figure 4a,b; Table S11) with evident admixture between *K1* and *K3* in two central localities (3 and 5) and one southern locality (locality 6). In the latter, one individual showed admixture among the three clusters. *K1* was predominant in southern localities, with moderate to no contribution in central localities. *K3* was predominant in northern localities and had high to low contribution in central and southern localities. *K2* had high contribution in the northern and central regions and lower contribution in the southern region of the BP. The EEMS results indicate low to moderate migration among all localities, corroborating the occurrence of those three ancestral populations in almost all localities, as suggested by sNMF and DAPC results, and indicating a pattern of gene flow across the BP (Figure 4c; Figure S13).

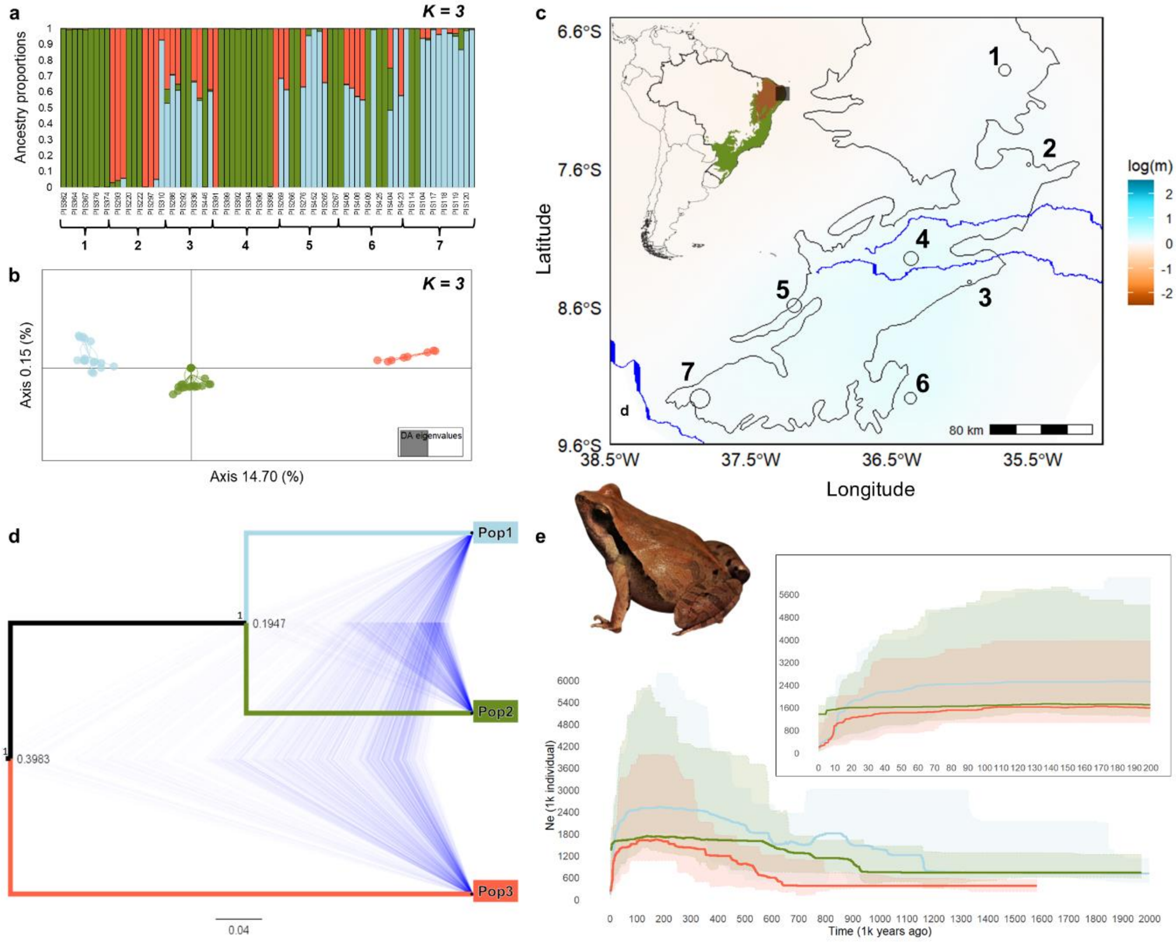


Figure 4. Distribution of genetic variation, patterns of gene flow, phylogenetic relationship, divergence time, and population sizes through time of *Physalaemus cuvieri* from seven highland forests (1–7) in the Borborema Plateau, Northeastern Brazil. a) Sparse Non-negative Matrix Factorization (sNMF) barplots representing ancestry proportions of each cluster (*K*; each color) for each individual (each bar). b) Discriminant Analysis of Principal Components (DAPC) suggested clusters and their dissimilarities in two axes. c) Estimated effective migration surface (EEMS) showing the posterior mean migration rates *m* (on the log10 scale) in blue and orange shades indicating respectively higher and lower migration rates than the

overall average rate (in white). Black line in the map = geographic limits of the Borborema Plateau. Blue lines = Capibaribe, Ipojuca, and São Francisco rivers from top to bottom. Caatinga in brown and Atlantic Forest remnants in green. d) Maximum clade credibility tree with posterior probability (left) and the divergence-time (right; in decimal) inferred by SNAPP. e) Effective population size (N_e) changes over the last two million years (last 200 kyr in detail) estimated in Stairway plot. Thick lines indicate the median of 500 inferences and shaded areas the 95% confidence interval. Blue = K1 (in a and b) and Pop1 (in d and e), green = K2 (in a and b) and Pop2 (in d and e), salmon = K3 (in a and b) and Pop3 (in d and e).

Genetic diversity and divergence-time

Hereafter, we refer to *K1* as *Pop1*, *K2* as *Pop2*, and *K3* as *Pop3* in both species. We delimited populations combining results from the three structure analyses previously described. Genetic diversity statistics (Table S14) indicated higher heterozygosity in *P. cuvieri* than in *D. oliveirai*, with the former showing also higher inbreeding and lower genetic differentiation. In *D. oliveirai*, *Pop1* showed lower heterozygosity and inbreeding, and *Pop2* and *Pop3* had similar values, with *Pop3* presenting the highest inbreeding value. In *P. cuvieri*, *Pop1* and *Pop3* demonstrated the lowest values of heterozygosity and the highest values of inbreeding, with *Pop3* showing the highest inbreeding index. *Pop2*, showed the highest values of heterozygosity and the lowest inbreeding coefficient.

The SNAPP topology was well supported in both species, with posterior probabilities equal to 1.0 at all nodes (Figures 3 and 4). For both species, two divergence events happened in different times. In *D. oliveirai*, the analysis indicated *Pop3* as diverging the earliest, with divergence time around 512 kyr ago. Divergence between *Pop1* and *Pop2* was suggested to having occurred around 227 kyr ago (Figure 3d). In *P. cuvieri*, we found a similar topology but more recent divergence times, with the *Pop3* diverging around 398 kyr ago and *Pop1* and *Pop2* around 195 kyr ago (Figure 4d).

Historical demography

The inferred demographic histories suggested a general pattern of ancient expansion in all populations and recent bottleneck in some populations of both species. In *D. oliveirai*, we observed a gradual and synchronic expansion of effective population size (N_e) between 600 – 200 kyr ago, which started around 1,100 kyr in *Pop2* (Figure 3e). *Pop1* and *Pop2* had very gradual expansion in that period. From 200 kyr, *Pop3* had constant population size, as in *Pop1* and *Pop2*, with a small bottleneck between 60–70 kyr in *Pop1* and an evident bottleneck in *Pop2* between 5–10 kyr, followed by a period of stability in both populations. In general, all *D.*

oliveirai populations remained stable most of the time from 200 kyr ago until now, including during the LIG (~ 116–129 kyr) and LGM (~ 19–27 kyr).

In *P. cuvieri,* the effective population size also expanded between 600–200 kyr, with more dramatic changes compared with those in *D. oliveirai* (Figure 4e). This expansion started earlier in *Pop1* and *Pop2* (respectively, 1,200 and 900k years). From 200 kyr on, population size in all populations stabilized, beginning to decline at about 20 kyr (LGM) (*Pop1*) and after 10 kyr (Mid-Holocene) (*Pop 2* and *Pop3*), with *Pop2* with more stable population size compared with *Pop1* and *Pop3,* which showed a greater and abrupt bottleneck.

Demographic models

In both species, the divergence time estimations corroborated SNAPP results, indicating that populations of the two species diverged before LIG (120 kyr – 1 Myr; ABC: PP = 1.000; REJ ≥ 0.9850; ACC ≥ 0.9900; SML: PP = 1.000; ACC > 0.9982; loss ≥ 0.0044) (Table 1; Figure 2). Regarding to population size changes, the demographic models suggested as the most adequate for both species were different from Stairway plot results. In *D. oliveirai,* two models were indicated by the different methods, MIG-T2 (isolation before LGM and migration from there with no change in population size; ABC PP = 0.9867, REJ = 0.9676; ACC = 0.9867; Figure S15 for confusion matrix plot) and MIGE-T2 (isolation before LGM and migration from there with expansion; SML PP = 0.9993, ACC = 0.9981, loss = 0.0142) (Table 1; Figure 2). These models are not completely exclusive, as possible population expansion after the LGM is the only difference between them. We selected MIGE-T2 over MIG-T2, because our previous tests indicated expansion in all populations (Figure S9) and SML is recognized as a more powerful method compared to ABC. Then, *D. oliveirai* was favored by the LGM conditions, initiating contact between populations and increasing population size following the expansion of the forest enclaves.

In *P. cuvieri*, the model with the highest probability was MIGE-CT2 (constant migration with any difference from LGM and expansion; ABC PP = 0.7333, REJ = 0.8022; ACC = 0.9000; Figure S16 for confusion matrix plot; SML PP = 1.0000, ACC = 0.9722, loss = 0.0790). The simulated datasets also produced summary statistics in the range of our observed data for both species (Figures S17, S18, S19, S20, S21, S22). In this case, *P. cuvieri* was also favored by the LGM conditions increasing an existent migration and the population size.

Table 1. Results from Approximate Bayesian Computation (ABC) and Supervised Machine Learning (SML)/Deep Learning (DL) hierarchical modeling assuming five general scenarios, one for diversification and four demographic scenarios involving migration, expansion, and bottleneck (see Figure 2) among three populations of *Dendropsophus oliveirai* and *Physalaemus cuvieri* from forest enclaves within the Caatinga biome, in Northeastern Brazil. Values represent posterior probabilities among different models in each general scenario and among the best models selected from each general scenario. Best models are highlighted in bold. ABC tolerance = 0.001, LIG = last interglacial maximum, LGM = last glacial maximum. * = Model with the highest support.

Models	***D. oliveirai***		***P. cuvieri***	
	ABC	**SML/DL**	**ABC**	**SML/DL**
Divergence				
A-LIG	0.0000	0.0000	0.1000	0.0000
B-LIG	**1.0000**	**1.0000**	**0.9000**	**1.0000**
Migration				
IS	0.0000	0.0006	0.1600	0.0000
MIG	0.0000	0.0000	0.0600	0.0000
MIG-T1	0.0000	0.0000	**0.4000**	0.0000
MIG-T2	**1.0000**	**0.9983**	0.3600	**0.9996**
MIG-CT2	0.0000	0.0011	0.0200	0.0004
Migration+Expansion after LGM				
ISE	0.1200	0.0000	**0.4600**	0.2086
MIGE	0.0000	0.0000	0.0000	0.0000
MIGE-T1	0.1000	0.0000	0.1000	0.0112
MIGE-T2	0.3600	**0.9990**	0.1000	0.0283
MIGE-CT2	**0.4200**	0.0009	0.3400	**0.7518**
Migration+Diferencial Expansion after LGM				
ISDE	0.1400	0.0056	0.3200	**0.5220**
MIGDE	0.0000	0.0000	0.0000	0.0000
MIGDE-T1	**0.4800**	**0.9235**	0.3200	0.4626
MIGDE-T2	0.0000	0.0000	0.0000	0.0000
MIGDE-CT2	0.3800	0.0709	**0.4600**	0.0154
Best models				
MIG-T2	**0.9867**	0.0000	0.2533	0.0000
MIGE-T2*	0.0067	**0.9993**		
MIGE-CT2*			**0.7333**	**1.0000**
ISDE			0.0133	0.0000
MIGDE-T1	0.0067	0.0007		

Discussion

Using a combination of different methods, our study demonstrated the potential role of past climate change in shaping the demographic history of anurans inhabiting Atlantic rainforest enclaves in highlands of the Borborema Plateau, in Northeastern Brazil. It also uncovered that divergence among populations and historical demographic dynamics varied between species, possibly as a result of their distinct niches and reproductive ecologies. Specifically, we found that (1) the LGM and associated vegetational shifts are probably not related to divergence events in the two studied species, and divergence-time among populations varied between them, with ancestral populations of *D. oliveirai* diverging earlier than those of *P. cuvieri*, suggesting that different climatic events and specie-specific traits are potentially related to population divergence in each species; (2) ancestral populations across northern BP diverged first in both species, indicating that distinct climatic and environmental zones existed along this plateau, encompassing more than one highland forest remnant; (3) Migration rates, population size changes, gene flow and genetic diversity of populations were probably influenced by the LGM and by Holocene climate conditions, however in different levels between the two species. Below, we discuss how our findings relate to current knowledge on historical landscape dynamics in Northeastern Brazil, the possible causes of different demographic patterns among the study species and the importance of our findings for devising informed conservation strategies for anurans distributed in the northern Atlantic Forest of Brazil.

Fauna divergence in the Borborema Plateau predates LGM and varies among taxa

The divergence-time of populations of both study species dates between 120 kyr – 1 Myr, before Late Pleistocene (~11–126 kyr). This pattern of diversification predating the LGM is in line with previous studies of forest anurans and birds in the same area (Carnaval and Bates 2007; Bocalini et al. 2021) and other studies along the Atlantic rainforest with a wide array of taxa (e.g., Cabanne et al. 2008; Àlvarez-Presas et al. 2011; D'horta et al. 2011; Ribeiro et al. 2011; Thomé et al. 2014; Batalha-Filho and Miyaki 2016; Bartoleti et al. 2018; Batalha-Filho et al. 2019; Mascarenhas et al. 2019), including species in other systems of forest enclaves (Oliveira et al. 2021). Such patterns of divergence time indicate that earlier climatic and environmental events potentially explain the emergence of different evolutionary lineages in this region. Quaternary landscapes were generally complex and spatially heterogeneous, also varying in relatively short evolutionary times (Bush and Silman 2004; Werneck et al. 2011). The period was marked by changes in global ocean temperature, fluctuating sea levels, variation in monsoonal climate, and shifts in vegetation cover around the world (Huntley et al. 2013; Sun et al. 2019; Herbert 2023; Huntley et al. 2023). Most studies addressing environmental variation in this period in Northeastern Brazil evaluated landscapes up to 210 kyr ago (Behling et al. 2000; Auler et al. 2004; Wang et al. 2004), making it difficult to directly associate any evolutionary process with a specific earlier event. The most likely explanation for our findings regarding divergence-times is potentially the long glacial and interglacial stages (which lasted from 41 kyr to 100 kyr) during Mid-Pleistocene (~126 kyr – 700 Myr) (Runddiman et al. 1989; Raymo et al. 1997; Sun et al. 2019). For instance, these have been previously suggested to explain divergence times in bird populations distributed in the BP (Bocalini et al. 2021).

In both species, populations occurring in the northern stretches of the BP diverged first. We interpret such congruence as a result of differential environmental stability along this plateau, influenced by a combination of geology and climate that caused isolation of forest enclaves in this portion earlier. The BP comprises a series of crustal blocks bounded by thrust- and strike-slip shear zones (Van Schmus et al. 1995; Santos and Caxito 2021) creating distinct climatic zones that affects soil profiles, distribution of rainfall, and vegetation cover in space and time (Soares et al. 2010; Almeida et al. 2015; Castelo Branco et al. 2021). Historical distribution ranges of woody plants inhabiting forest enclaves in the northern Atlantic Forest of Brazil suggest different degrees of forest stability across the region from the LIG until present, with some areas in the northern part of the BP apparently stable but have been isolated first (Silveira et al. 2019). Since strong genetic divergence in other anuran species was related to stable climatic regions during the Pleistocene (Vasconcellos et al. 2019), it is possible that earlier isolation combined with stability in north areas of the BP created opportunities for the ancestral population to diverge first. In this scenario, current higher genetic diversity is expected in those populations (Carnaval et al. 2009; Carnaval et al. 2014), something that we found in the northern population of *D. oliveirai*, our reproductive habitat-specialist species. Nevertheless, future model-based approaches testing direction of historical gene flow in different taxa are necessary to confirm that these areas were in fact more climatically/environmentally stable than other regions of the Plateau.

Synchronous but idiosyncratic responses to Pleistocene climatic and forest changes

Our study favors the hypothesis that a wider extension of forest enclaves existed in the BP during the LGM and that forest cover was probably reduced before and after this period (during LIG and along the Holocene, respectively - Silveira et al. 2019). According to our model-based approach, both species showed signs of change in migration and population size after the onset of the LGM, with *D. oliveirai* initiating expansion and secondary contact among all populations, while *P. cuvieri* increased preexisting migration among populations, along with population expansion. Since our focus was to investigate specific demographic changes induced by the LGM, our models may hide population changes after the LGM, for instance during the Holocene. Our model-based results provide a good approximation of the demographic history of the species immediately before and after the LGM, with Stairway plot analyses reflecting the most recent (Holocene) demographic changes, generally characterized by population bottlenecks (Cabanne et al. 2008; Bocalini et al. 2021; Oliveira et al. 2021; Batalha-Filho et al. 2023), possibly as a consequence of retraction of the forest enclaves and of drier climatic conditions (Silveira et al. 2019). These findings suggest that, in a greater or lesser degree, populations occurring in forest enclaves in the north Atlantic rainforest probably have a similar history to those species in other sky-island system in central Atlantic rainforest (e. g. Oliveira et al. 2021; Dantas-Queiroz et al. 2023). That is, ancestral populations from forest enclaves expanded into areas currently covered by Seasonally Dry Tropical Forests, such as Caatinga, during the LGM (Cabanne et al. 2016; Mascarenhas et al. 2019; Bocalini et al. 2021).

Differences in ecological attributes may drive responses to climatic and paleogeographical changes (Batalha-Filho et al. 2012; Cabanne et al. 2016; Bocalli et al. 2021). Although the scenario of migration and expansion during glacial periods and possible isolation events and bottlenecks fits exactly with our expectations for *D. oliveirai*, as it is a reproductive habitat-specialist dependent of ponds with long hydroperiod (Haddad and Prado 2005; Haddad et al. 2013; Silva et al. 2019), the possible population bottleneck in *P. cuvieri* during the Holocene, surprising. *Physalaemus cuvieri* is a generalist species, well-adapted to several types of habitats, including Seasonally Dry Tropical Forests (Pombal and Haddad 2007; Aguiar et al. 2014), which are thought to had expanded during the Holocene conditions (Oliveira et al. 1999; Auler et al. 2004; Wang et al. 2004; Pinaya et al. 2019; Piacsek et al. 2021). Despite its ecological traits that may result in colonizing advantages, this species has a mating season strongly associated with the beginning of rainy season, being an explosive breeder (Prado et al. 2005; Ulloa et al. 2019). Between 10 kyr and 210 kyr the number and duration of pluvial maxima phases reduced in northeastern Brazil, with the last one occurring between 14,800 – 15,900 yrs (Auler et al. 2004; Wang et al. 2004), right before populations of *P. cuvieri* started to decline. It is possible that the subsequent lower and irregularly distributed rainy seasons affected the demographic dynamic in this species, as was suggested in a previous study to explain part of its association between genetic distance and geographic distance and latitude (Andrade Lima et al. submitted). Although none of the species in the present is stenoic to moist rainforests, our results, combined with previous studies on the same populations, demonstrate the importance of species-specific ecological traits in shaping the evolutionary history of a taxon, reflecting different responses even between less-specialized species.

As expected, our reproductive habitat-specialist species, *D. oliveirai*, presented evidence of secondary contact associated with expansion from the LGM until the beginning of the Holocene. Alternatively, in the same period, our reproductive habitat-generalist, *P. cuvieri*, experienced an increase in migration rates associated with population expansion. The patterns of genetic structure and diversity in both species found here is in accordance with our previous work using mitochondrial genetic information (Andrade Lima et al. submitted). *D. oliveirai* shows higher genetic structure and less heterozygosity and genetic diversity, as a consequence of genetic drift and/or local adaptation; while *P. cuvieri*, presents soft genetic structure, and higher levels of endogamy, heterozygosity, and genetic diversity, as a consequence of small to moderate constant gene flow among populations, explaining not too different populations sharing common areas, which collectively indicates possible zones of admixture. Under the Pleistocene climatic change scenario, populations are expected to have experienced periods of connectivity and segregation (Carnaval et al. 2009), leaving signatures of genetic drift, local adaptation, secondary contact, and hybridization events in their genetic makeup (Rull, 2005). Especially in a context of highly topographic complex regions, as mountain ranges, patterns of genetic structure are usually different between specialist and generalist amphibian species (Rodríguez et al. 2015). Our findings are evidence that probably all species in forest enclaves synchronously change their demographic patterns, yet in idiosyncratic ways, with habitat specialists showing more earlier and pronounced responses to shifts in temperature, moisture, and vegetation cover.

The biogeographic history of the species (see Menezes et al. 2020 and Miranda et al. 2019) may also reflect the divergence and demographic patterns observed here. Although population divergence in both species began in the northern BP, the colonization of this plateau by *D. oliveirai* may have occurred through two east-west routes connecting the coastal Atlantic Forest to the BP enclaves via the northern and central parts (see Silveira et al. 2019), since the most ancient lineage of this taxon occurs in central Atlantic Forest (Menezes et al. 2020). In contrast, for *P. cuvieri*, colonization may have started from the north of the BP, with the São

Francisco River potentially acting as a barrier in the southern part, as demonstrated for other animal species (Coelho et al. 2022).

Relevance of the present study to the conservation of the north Atlantic Forest

Our study presents significant and instrumental findings for future conservation actions in the northern portion of the Atlantic Forest in light of present and future threats to the local biota. Although the species analyzed here are not endemic to the investigated area, they serve as proxies for species with different ecological limits and consequent different responses to spatial and temporal environmental pressures and changes. Therefore, they reflect the evolutionary history of various vertebrate species co-occurring in this region and facing the same threats.

We showed that spatiotemporal climatic and environmental changes affect the demographic patterns and processes independently of the species. These findings provide us enough information to predict possible future scenarios for the populations in this region, especially because populations inhabiting high elevations may decline in the next few decades as a result of reduction in suitable habitats (Bonatelli et al. 2014). This is even more concerning in the north region of the Atlantic Forest. Although this region still comprises many areas of extreme biological importance (Pôrto et al. 2005), it is the most fragmented, threatened (Ribeiro et al. 2009) and less studied (Romanelli et al. 2024). In this context, this region exhorts immediate and more efficient conservation strategies based in science.

According to our population structure analyses and gene flow surface in *D. oliveirai*, all populations are present in more than one enclave, meaning that no population occurs exclusively in a single locality. This finding suggests that, instead of each mountaintop harboring a distinct refuge (e.g., Firkowski et al. 2016; Oliveira et al. 2021), different adjacent enclaves may share environmental conditions, forming unique refuges that shelter local populations, which behave as single populations over evolutionary time. Thus, conservation strategies could be more effective and powerful if they focus their efforts on forest groups to maintain the resilience (adaptive potential) of populations.

Conclusions

The evolutionary history of the biota in any region is always more complex than any model we build trying to describe it. This high complexity results from a combination of numerous geological and climatic cycles with species-specific ecological attributes that provide to populations different opportunities to isolate and connect repeatedly (Burney and Brumfield 2009; Smith et al. 2014; Bocalini et al. 2021). The evolutionary patterns and processes of the Neotropical biota in non-Andean mountain ranges are still under reasonably understood, especially due to their vast environmental heterogeneity and biodiversity (not only taxonomically, but also phylogenetically and ecologically). This study provides a better understanding of how populations of different ecological species of the fauna from forest enclaves in the northernmost portion of the Atlantic Forest faced the pressures imposed by the Pleistocene, demonstrating how dynamically these species responded to past climatic and environmental changes during that period.

Previous studies with the same species and populations well investigated the influence of contemporary landscape factors driving their phenotypic (Andrade Lima et al. 2024) and genetic differentiation (Andrade Lima et al. submitted). The last one also explored the influence of climatic stability among the areas. Here, we delved into the history of those populations, attempting to explore historical factors that, combined with previous studies, better explain the current patterns of genetic structure in those populations. We found that probably prolonged climatic events during the Mid-Pleistocene were responsible by divergence in both species. While the LGM and the Holocene promoted opposite responses in both species, with their migration rate and population size being favored during the LGM, however, in different levels according to their specific ecological traits. With all this information, we can confidently predict future demographic responses of these populations in the context of increasing global warming and habitat loss, and even extrapolate to other vertebrate species in the same region—an aspect that must impact conservation actions.

References

Aguiar, A., Toledo, G. M., Anjos, L. A., & Silva, R. J. (2014). Helminth parasite communities of two *Physalaemus cuvieri* Fitzinger, 1826 (Anura: Leiuperidae) populations under different conditions of habitat integrity in the Atlantic Rain Forest of Brazil. *Brazilian Journal of Biology*, *75*(4), 963–968. https://doi.org/10.1590/1519-6984.03614

Allaire, J. J., Kalinowski, T., Falbel, D., Eddelbuettel, D., Tang, Y., Golding, N., Google Inc., & Posit, PBC. (2024). *tensorflow: R Interface to 'TensorFlow'* (Version 2.15.0). [R package]. Disponível em https://CRAN.R-project.org/package=tensorflow

Almeida, Y. B., Julià, J., & Frassetto, A. (2015). Crustal architecture of the Borborema Province, NE Brazil, from receiver function CCP stacks: Implications for Mesozoic stretching and Cenozoic uplift. *Tectonophysics, 649*, 68–80. https://doi.org/10.1016/j.tecto.2015.03.001

Alvarez-Presas, M., Carbayo, F., Rozas, J., & Riutort, M. (2011). Land planarians (Platyhelminthes) as a model organism for fine-scale phylogeographic studies: Understanding patterns of biodiversity in the Brazilian Atlantic Forest hotspot. *Journal of Evolutionary Biology*, *24*, 887–896. https://doi.org/10.1111/j.1420-9101.2010.02220.x

Amaral, F. R., Thom, G., Lima-Ribeiro, M. S., Alvarado-Serrano, D. F., Montesanti, J. A. C., Pellegrino, K. C. M., Miyaki, C. Y., Hickerson, M. J., & Maldonado-Coelho, M. (2021). Rugged relief and climate promote isolation and divergence between two neotropical cold-associated birds. *Evolution*, *75*(10), 2371–2387. https://doi.org/10.1111/evo.14318

Andrade-Lima, D. (1982). Present-day forest refuges in Northeastern Brazil. In G. T. Prance (Ed.), *Biological Diversification in the Tropics* (pp. 245–254). Columbia University Press.

Andrade Lima, J. H., Novo, M. K. B., & Simões, P. I. (2024). Advertisement call variation is related to environmental and geographic distances in two anuran species inhabiting highland forests in northeastern Brazil. *Biotropica*, *56*(4), e13329. https://doi.org/10.1111/btp.13329

Andrade Lima, J. H., Novo, M. K. B., Gehara, M., & Simões, P. I. (2025). (No prelo). Comparative landscape genetics in two amphibians demonstrates the influence of habitat specialization in highlands in Northeastern Brazil.

Antonelli, A., Verola, C. F., Parisod, C., & Gustafsson, A. L. S. (2010). Climate cooling promoted the expansion and radiation of a threatened group of South American orchids (Epidendroideae: Laeliinae). *Biological Journal of the Linnean Society, 100*, 597–607. https://doi.org/10.1111/j.1095-8312.2010.01438.x

Auler, A. S., Wang, X., Edwards, R. L., Cheng, H., Cristalli, P. S., Smart, P. L., & Richards, D. A. (2004). Quaternary ecological and geomorphic changes associated with rainfall events in present semi-arid northeastern Brazil. *Journal of Quaternary Science, 19*, 693–701. https://doi.org/10.1002/jqs.876

Avise, J. C. (2000). *Phylogeography: The history and formation of species*. Harvard University Press. https://doi.org/10.2307/j.ctv1nzfgj7

Baker, P. A., Fritz, S. C., Battisti, D. S., Dick, C. W., Vargas, O. M., Asner, G. P., Martin, R. E., Wheatley, A., & Prates, I. (2020). Beyond refugia: New insights on Quaternary climate variation and the evolution of biotic diversity in tropical South America. In V. Rull & A. C. Carnaval (Eds.), *Neotropical diversification: Patterns and processes* (pp. 51–70). Springer Nature Switzerland. https://doi.org/10.1007/978-3-030-31167-4_3

Castelo Branco, R. M. G., Castro, N. A., Oliveira, K. M. L., Santos, F. A. M., Almeida, E. P., Silva, F. M., & Castelo Branco, J. L. (2021). Mapping the basement architecture using magnetotelluric data across a coastal part of the Borborema structural province, Ceará – Brazil. *Journal of South American Earth Sciences, 112*, 103525. https://doi.org/10.1016/j.jsames.2021.103525

Coelho, F. E. A., Guillory, W. X., & Gehara, M. (2022) Coalescent simulations indicate that the São Francisco River is a biogeographic barrier for six vertebrates in a seasonally dry South American forest. *Frontiers in Ecology and Evolution*, 10, 983134. https://doi.org/10.3389/fevo.2022.983134

Bartoleti, L. F. M., Peres, E. A., Fontesvon, F. H. M., da Silva, M. J., & Solferini, V. N. (2018). Phylogeography of the widespread spider *Nephila clavipes* (Araneae: Araneidae) in South America indicates geologically and climatically driven lineage diversification. *Journal of Biogeography*, *45*(6), 1246–1260. https://doi.org/10.1111/jbi.13217

Batalha-Filho, H., Barreto, S. B., Silveira, M. H. B., Miyaki, C. Y., Afonso, S., Ferrand, N., Carneiro, M., & Sequeira, F. (2024). Disentangling the contemporary and historical effects of landscape on the population genomic variation of two bird species restricted to the highland forest enclaves of northeastern Brazil. *Heredity*, *132*(1), 77–88. https://doi.org/10.1038/s41437-023-00662-1

Batalha-Filho, H., Cabanne, G. S., & Miyaki, C. Y. (2012). Phylogeography of an Atlantic forest passerine reveals demographic stability through the last glacial maximum. *Molecular Phylogenetics and Evolution*, *65*(3), 892–902. https://doi.org/10.1016/j.ympev.2012.08.010

Batalha-Filho, H., Fjeldså, J., Fabre, P.-H., & Miyaki, C. Y. (2013). Connections between the Atlantic and the Amazonian forest avifaunas represent distinct historical events. *Journal of Ornithology*, *154*(1), 41–50. https://doi.org/10.1007/s10336-012-0866-7

Batalha-Filho, H., Maldonado-Coelho, M., & Miyaki, C. Y. (2019). Historical climate changes and hybridization shaped the evolution of Atlantic Forest spinetails (*Aves: Furnariidae*). *Heredity*, *123*(6), 675–693. https://doi.org/10.1038/s41437-019-0234-y

Batalha-Filho, H., & Miyaki, C. Y. (2016). Late Pleistocene divergence and postglacial expansion in the Brazilian Atlantic Forest: Multilocus phylogeography of *Rhopias gularis* (*Aves: Passeriformes*). *Journal of Zoological Systematics and Evolutionary Research*, *54*(2), 137–147. https://doi.org/10.1111/jzs.12118

Becker, R. A., Wilks, A. R., Brownrigg, R., Minka, T. P., & Deckmyn, A. (2023). *maps: Draw geographical maps* (R package version 3.4.1). Comprehensive R Archive Network (CRAN). https://CRAN.R-project.org/package=maps

Behling, H., Arz, H. W., Pätzold, J., & Wefer, G. (2000). Late Quaternary vegetational and climate dynamics in northeastern Brazil, inferences from marine core GeoB 3104-1. *Quaternary Science Reviews*, *19*(10), 981–994. https://doi.org/10.1016/S0277-3791(99)00046-3

Bocalini, F., Bolívar-Leguizamón, S. D., Silveira, L. F., & Bravo, G. A. (2021). Comparative phylogeographic and demographic analyses reveal a congruent pattern of sister relationships between bird populations of the northern and south-central Atlantic Forest. *Molecular Phylogenetics and Evolution*, *154*, 106973. https://doi.org/10.1016/j.ympev.2020.106973

Bonatelli, I. A. S., Perez, M. F., Peterson, A. T., Taylor, N. P., Zappi, D. C., Machado, M. C., & Moraes, E. M. (2014). Interglacial microrefugia and diversification of a cactus species complex: Phylogeography and palaeodistributional reconstructions for *Pilosocereus aurisetus* and allies. *Molecular Ecology*, *23*(12), 3044–3063. https://doi.org/10.1111/mec.12780

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M. A., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLOS Computational Biology, 10*(4), e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Bryant, D., Bouckaert, R., Felsenstein, J., Rosenberg, N. A., & RoyChoudhury, A. (2012). Inferring species trees directly from biallelic genetic markers: Bypassing gene trees in a full coalescent analysis. *Molecular Biology and Evolution, 29,* 1917–1932. https://doi.org/10.1093/molbev/mss086

Burney, C. W., & Brumfield, R. T. (2009). Ecology predicts levels of genetic differentiation in neotropical birds. *The American Naturalist, 174,* 358–368. https://doi.org/10.1086/603613

Bush, M., & Silman, M. R. (2004). Observations on Late Pleistocene cooling and precipitation in the lowland Neotropics. *Journal of Quaternary Science, 19,* 677–684. https://doi.org/10.1002/jqs.883

Cabanne, G. S., Calderón, L., Trujillo-Arias, N., Flores, P., Pessoa, R. O., D'Horta, F., & Miyaki, C. Y. (2016). Effects of Pleistocene climate changes on species ranges and evolutionary processes in the Neotropical Atlantic Forest. *Biological Journal of the Linnean Society, 119,* 856–872. https://doi.org/10.1111/bij.12844

Cabanne, G. S., D'Horta, F. M., Sari, E. H. R., Santos, F. R., & Miyaki, C. Y. (2008). Nuclear and mitochondrial phylogeography of the Atlantic Forest endemic *Xiphorhynchus fuscus* (Aves: Dendrocolaptidae): Biogeography and systematics implications. *Molecular Phylogenetics and Evolution, 49*, 760–773. https://doi.org/10.1016/j.ympev.2008.09.013

Carnaval, A. C. O. Q. (2002). Phylogeography of four frog species in forest fragments of Northeastern Brazil—A preliminary study. *Integrative and Comparative Biology, 42,* 913–921. https://doi.org/10.1093/icb/42.5.913

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

Carnaval, A. C., Hickerson, M. J., Haddad, C. F. B., Rodrigues, M. T., & Moritz, C. (2009). Stability predicts genetic diversity in the Brazilian Atlantic Forest hotspot. *Science, 323*, 785–789. https://doi.org/10.1126/science.1166955

Carnaval, A. C., Waltari, E., Rodrigues, M. T., Rosauer, D., VanDerWal, J., Damasceno, R., Prates, I., Strangas, M., Spanos, Z., Rivera, D., Pie, M. R., Firkowski, C. R., Bornschein, M. R., Ribeiro, L. F., & Moritz, C. (2014). Prediction of phylogeographic endemism in an environmentally complex biome. *Proceedings of the Royal Society B, 281*, 20141461. https://doi.org/10.1098/rspb.2014.1461

Castro, D. P., Rodrigues, J. F. M., Borges-Leite, M. J., Lima, D. C., & Borges-Nojosa, D. M. (2019). Anuran diversity indicates that Caatinga relictual Neotropical forests are more related to the Atlantic Forest than to the Amazon. *PeerJ, 6*, e6208. https://doi.org/10.7717/peerj.6208

Chicho, B. T., & Sallow, A. B. (2021). A comprehensive survey of deep learning models based on Keras framework. *Journal of Soft Computing and Data Mining, 2*(2), 49–62. https://doi.org/10.30880/jscdm.2021.02.02.005

Coffman, A. J., Hsieh, P. H., Gravel, S., & Gutenkunst, R. N. (2015). Computationally efficient composite likelihood statistics for demographic inference. *Molecular Biology and Evolution, 33*(2), 591–593. https://doi.org/10.1093/molbev/msv255

Collevatti, R. G., Rabelo, S. G., & Vieira, R. F. (2009). Phylogeography and disjunct distribution in *Lychnophora ericoides* (Asteraceae), an endangered Cerrado shrub species. *Annals of Botany, 104*, 655–664. https://doi.org/10.1093/aob/mcp157

Costa, G. C., Hampe, A., Ledru, M.-P., Martinez, P. A., Mazzochini, G. G., Shepard, D. B., Werneck, F. P., Moritz, C., & Carnaval, A. C. (2017). Biome stability in South America over the last 30 kyr: Inferences from long-term vegetation dynamics and habitat modelling. *Global Ecology and Biogeography, 27*(3), 1–13. https://doi.org/10.1111/geb.12694

Csilléry, K., Blum, M. G., Gaggiotti, O. E., & François, O. (2010). Approximate Bayesian computation (ABC) in practice. *Trends in Ecology & Evolution, 25*, 410–418. https://doi.org/10.1016/j.tree.2010.04.001

Cullingham, C., Peery, R. M., & Miller, J. M. (2023). A roadmap to robust discriminant analysis of principal components. *Molecular Ecology Resources*, 23(3), 519–522. http://dx.doi.org/10.1111/1755-0998.13724

D'Horta, F. M., Cabanne, G. S., Meyer, D., & Miyaki, C. Y. (2011). The genetic effects of Late Quaternary climatic changes over a tropical latitudinal gradient: Diversification of an Atlantic Forest passerine. *Molecular Ecology, 20,* 1932–1935. https://doi.org/10.1111/j.1365-294X.2011.05063.x

Dal Vechio, F., Prates, I., Grazziotin, F. G., Zaher, H., & Rodrigues, M. T. (2018). Phylogeography and historical demography of the arboreal pitviper *Bothrops bilineatus* (Serpentes, Crotalinae) reveal multiple connections between Amazonian and Atlantic rain forests. *Journal of Biogeography, 45*, 2415–2426. https://doi.org/10.1111/jbi.13421

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics,* *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

de Medeiros, B. A. S., & Farrell, B. D. (2018). Whole-genome amplification in double-digest RADseq results in adequate libraries but fewer sequenced loci. *PeerJ, 6,* e5089. https://doi.org/10.7717/peerj.5089

de Medeiros, B. A. (2024). Matrix Condenser v.1.1. Available at: https://github.com/brunoasm/matrix_condenser/

Dantas-Queiroz, M. V., Hurbath, F., Godoy, F. M. R., Lanna, F. M., Versieux, L. M., & Palma-Silva, C. (2023). Comparative phylogeography reveals the demographic patterns of neotropical ancient mountain species. *Molecular Ecology, 32,* 3165–3181. https://doi.org/10.1111/mec.16929

Eaton, D. A. R., & Overcast, I. (2020). ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics*. https://doi.org/10.1093/bioinformatics/btz966

Ericson, P. G. P., & Irestedt, M. (2022). Comparative population genomics reveals glacial cycles to drive diversifications in tropical montane birds (Aves, Timaliidae). *Avian Research, 13*, 100063. https://doi.org/10.1016/j.avrs.2022.100063

Firkowski, C. R., Bornschein, M. R., Ribeiro, L. F., & Pie, M. R. (2016). Species delimitation, phylogeny and evolutionary demography of co-distributed, montane frogs in the southern Brazilian Atlantic forest. *Molecular Phylogenetics and Evolution, 100*, 345–360. https://doi.org/10.1016/j.ympev.2016.04.023

Frichot, E., Schoville, S. D., Bouchard, G., & François, O. (2015). Testing for admixture in genomic data. *Molecular Ecology Resources, 15*(1), 10–20.

Frost, D. R. (2025). *Amphibian species of the world: An online reference version 61 (11 January 2025).* Electronic database accessible at https://amphibiansoftheworldamnhorg/indexphp. American Museum of Natural History, New York, USA.

Gehara, M., Barth, A., Oliveira, E. F., Costa, M. A., Haddad, C. F. B., & Vences, M. (2017a). Model-based analyses reveal insular population diversification and cryptic frog species in the *Ischnocnema parva* complex in the Atlantic forest of Brazil. *Molecular Phylogenetics and Evolution, 112*, 68–78. https://doi.org/10.1016/j.ympev.2017.04.007

Gehara, M., Garda, A. A., Werneck, F. P., Oliveira, E. F., da Fonseca, E. M., Camurugi, F., Magalhães, F. M., Lanna, F. M., Sites, J. W., Jr., Marques, R., Silveira-Filho, R., São Pedro, V. A., Colli, G. R., Costa, G. C., & Burbrink, F. T. (2017b). Estimating synchronous demographic changes across populations using hABC and its application for a herpetological community from northeastern Brazil. *Molecular Ecology, 26*, 4756–4771. https://doi.org/10.1111/mec.14239

Gehara, M., Mazzochinni, G. G., & Burbrink, F. (2020). PipeMaster: Inferring population divergence and demographic history with approximate Bayesian computation and supervised machine-learning in R. *bioRxiv: The Preprint Server for Biology*. https://doi.org/10.1101/2020.12.04.410670

Gottscho, A. D., Marks, S. B., & Jennings, W. B. (2014). Speciation, population structure, and demographic history of the Mojave Fringe-toed Lizard (*Uma scoparia*), a species of conservation concern. *Ecology and Evolution, 4*(12), 2546–2562. https://doi.org/10.1002/ece3.1111

Goudet, J. (2005). hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes, 5*, 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x

Guedes, T. B., Azevedo, J. A. R., Bacon, C. D., Provete, D. B., & Antonelli, A. (2020). Diversity, endemism, and evolutionary history of montane biotas outside the Andean region. In V. Rull & A. C. Carnaval (Eds.), *Neotropical diversification: Patterns and processes* (pp. 299–328). Springer Nature Switzerland AG. https://doi.org/10.1007/978-3-030-31167-4_13

Guillory, W. X., Magalhães, F. M., Coelho, F. E. A., Bonatelli, I. A. S., Palma-Silva, C., Moraes, E. M., Garda, A. A., Burbrink, F. T., & Gehara, M. (2024). Geoclimatic drivers of diversification in the largest arid and semi-arid environment of the Neotropics: Perspectives from phylogeography. *Molecular Ecology, 33*, e17431. https://doi.org/10.1111/mec.17431

Haddad, C. F. B., & Prado, C. P. A. (2005). Reproductive modes in frogs and their unexpected diversity in the Atlantic forest of Brazil. *Bioscience, 55*(3), 207–217. https://doi.org/10.1641/0006-3568(2005)055[0207:RMIFAT]20CO,2

Haddad, C. F. B., Toledo, L. F., Prado, C. P. A., Loebmann, D., Gasparini, J. L., & Sazima, I. (2013). *Guia dos anfíbios da Mata Atlântica: Diversidade e biologia*. São Paulo–SP, Anolis Books, São Paulo, Brazil.

Haffer, J. (1969). Speciation in Amazonian forest birds. *Science, 165*, 131–137. https://doi.org/10.1126/science.165.3889.131

Heller, H., Chikhi, L., & Siegismund, H. R. (2013). The confounding effect of population structure on Bayesian skyline plot inferences of demographic history. *PLoS ONE, 8*, e62992. https://doi.org/10.1371/journal.pone.0062992

Helmstetter, A. J., Béthune, K., Kamdem, N. G., Sonké, B., & Couvreur, T. L. P. (2020). Individualistic evolutionary responses of Central African rain forest plants to Pleistocene climatic fluctuations. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(51), 32509–32518. https://doi.org/10.1073/pnas.2001018117

Herbert, T. D. (2023). The Mid-Pleistocene Climate Transition. *Annual Review of Earth and Planetary Sciences*, *51*, 389–418. https://doi.org/10.1146/annurev-earth-032320-104209

Huber, O., Prance, G. T., Kroonenberg, S. B., & Antonelli, A. (2018). The Tepuis of the Guiana Highlands. In C. Hoorn, A. Perrigo, & A. Antonelli (Eds.), *Mountains, climate and biodiversity* (pp. 339–353). Wiley-Blackwell.

Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, *9*(5), 1322–1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x

Hudson, R. R. (2002). Generating samples under a Wright-Fisher neutral model of genetic variation. *Bioinformatics*, *18*, 337–338. https://doi.org/10.1093/bioinformatics/18.2.337

Huntley, B., Allen, J. R. M., Collingham, Y. C., Hickler, T., Lister, A. M., Singarayer, J., Stuart, A. J., Sykes, M. T., & Valdes, P. J. (2013). Millennial climatic fluctuations are key to the structure of Last Glacial ecosystems. *PLoS ONE*, *8*(4), e61963. https://doi.org/10.1371/journal.pone.0061963

Huntley, B., Allen, J. R. M., Forrest, M., Hickler, T., Ohlemüller, R., Singarayer, J. S., & Valdes, P. J. (2023). Global biome patterns of the Middle and Late Pleistocene. *Journal of Biogeography*, *50*, 1352–1372. https://doi.org/10.1111/jbi.14619

Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, *27*(21), 3070–3071. https://doi.org/10.1093/bioinformatics/btr521

Kalinowski, T., Falbel, D., Allaire, J. J., Chollet, F., RStudio, Google, Tang, Y., Van Der Bijl, W., Studer, M., & Keydana, S. (2023). keras: R Interface to 'Keras'. *R package version 2.13.0*. Available at https://CRAN.R-project.org/package=keras

Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution, 4*(8), 782–788. https://doi.org/10.1111/2041-210X.12067

Keinan, A., & Clark, A. G. (2012). Recent explosive human population growth has resulted in an excess of rare genetic variants. *Science, 336*, 740–743. https://doi.org/10.1126/science.1217283

Ledo, R. M. D., & Colli, G. R. (2017). The historical connections between the Amazon and the Atlantic Forest revisited. *Journal of Biogeography, 44*, 2551–2560. https://doi.org/10.1111/jbi.13049

Ledo, R. M. D., Domingos, F. M. C. B., Giugliano, L. G., Sites Jr., J. W., Werneck, F. P., & Colli, G. R. (2020). Pleistocene expansion and connectivity of mesic forests inside the South American Dry Diagonal supported by the phylogeography of a small lizard. *Evolution, 74*(9), 1988–2004. https://doi.org/10.1111/evo.13978

Liu, X., & Fu, Y. X. (2020). Stairway plot 2: Demographic history inference with folded SNP frequency spectra. *Genome Biology, 21*, 280. https://doi.org/10.1186/s13059-020-02196-9

Magalhães, F. M., Dantas, A. K. B. P., Brito, M. R. M., Medeiros, P. H. S., Oliveira, A. F., Pereira, T. C. S. O., Queiroz, M. H. C., Santana, D. J., Silva, W. P., & Garda, A. A. (2013). Anurans from an Atlantic Forest-Caatinga ecotone in Rio Grande do Norte State, Brazil. *Herpetology Notes, 6*, 1–10.

Mascarenhas, R., Myiaki, C., Ricardo, M., Henrique, D., & Filho, B. (2019). Late Pleistocene climate change shapes population divergence of an Atlantic Forest passerine: A model-based phylogeography hypothesis test. *Journal of Ornithology, 55*. https://doi.org/10.1007/s10336-019-01650-1

Mazet, O., Rodriguez, W., Grusea, S., Boitard, S., & Chikhi, L. (2016). On the importance of being structured: Instantaneous coalescence rates and human evolution—lessons for ancestral population size inference. *Heredity, 116*(4), 362–371. https://doi.org/10.1038/hdy.2015.104

Menezes, L., Batalha-Filho, H., Garda, A. A., & Napoli, M. F. (2020). Tiny treefrogs in the Pleistocene: Phylogeography of Dendropsophus oliveirai in the Atlantic Forest and associated enclaves in northeastern Brazil. *Journal of Zoological Systematics and Evolutionary Research, 00*, 1–16. https://doi.org/10.1111/jzs.12422

Meseguer, A. S., Michel, A., Fabre, P-H., Escobar, O. A. P., Chomicki, G., Riina, R., Antonelli, A., Antoine, P-O., Delsuc, F., & Condamine, F. L. (2022). Diversification dynamics in the Neotropics through time, clades, and biogeographic regions. *eLife, 11*, e74503. https://doi.org/10.7554/eLife.74503

Miranda, N. E. O., Maciel, N. M., Lima-Ribeiro, M. S., Colli, G. R., Haddad, C. F. B., & Collevatti, R. G. (2019). Diversification of the widespread neotropical frog *Physalaemus cuvieri* in response to Neogene-Quaternary geological events and climate dynamics. *Molecular Phylogenetics and Evolution, 132*, 67–80. https://doi.org/10.1016/j.ympev.2018.11.003

Morais Neto, J. M., Hegarty, K. A., Karner, G. D., & Alkmim, F. F. (2009). Timing and mechanisms for the generation and modification of the anomalous topography of the Borborema Province, northeastern Brazil. *Marine and Petroleum Geology, 26*, 1070–1086. https://doi.org/10.1016/j.marpetgeo.2008.07.002

Oleksiak, M. F. (2018). Adaptation Without Boundaries: Population Genomics in Marine Systems. In O. P. Rajora (Ed.), *Population Genomics* (pp. 587–612). Springer International Publishing AG, Springer Nature. https://doi.org/10.1007/13836_2018_32

Oliveira, F. F. R., Gehara, M., Solé, M., Lyra, M., Haddad, C. F. B., Silva, D. P., Magalhães, R. F., Leite, F. S. F., & Burbrink, F. T. (2021). Quaternary climatic fluctuations influence the demographic history of two species of sky-Island endemic amphibians in the neotropics. *Molecular Phylogenetics and Evolution, 160*, 107113. https://doi.org/10.1016/j.ympev.2021.107113

Oliveira, P. E., Barreto, A. M. F., & Suguio, K. (1999). Late Pleistocene/Holocene climatic and vegetational history of the Brazilian caatinga: The fossil dunes of the middle São Francisco River. *Palaeogeography, Palaeoclimatology, Palaeoecology, 152*, 319–337. https://doi.org/10.1016/S0031-0182(99)00061-9

Pavlidis, P., Laurent, S., & Stephan, W. (2010). msABC: A modification of Hudson's ms to facilitate multi-locus ABC analysis. *Molecular Ecology Resources*, *10*, 723–727. https://doi.org/10.1111/j.1755-0998.2010.02832.x

Paz, A., Ibáñez, R., Lips, K. R., & Crawford, A. J. (2015). Testing the role of ecology and life history in structuring genetic variation across a landscape: A trait-based phylogeographic approach. *Molecular Ecology*, *24*(14), 3723–3737. https://doi.org/10.1111/mec.13275

Pebesma, E. (2018). Simple Features for R: Standardized Support for Spatial Vector Data. *The R Journal*, *10*(1), 439–446. https://doi.org/10.32614/RJ-2018-009

Pequette, R. S. (2012). PopGenKit: Useful functions for (batch) file conversion and data resampling in microsatellite datasets (R package version 1.0). https://rdocumentation.org/packages/PopGenKit/versions/1.0

Petkova, D., Novembre, J., & Stephens, M. (2016). Visualizing spatial population structure with estimated effective migration surfaces. *Nature Genetics*, *48*, 94–100. https://doi.org/10.1038/ng.3464

Piacsek, P., Behling, H., Ballalai, J. M., Nogueira, J., Venancio, I. M., & Albuquerque, A. L. S. (2021). Reconstruction of vegetation and low latitude ocean-atmosphere dynamics of the past 130 kyr, based on South American montane pollen types. *Global and Planetary Change*, *201*, 103477. https://doi.org/10.1016/j.gloplacha.2021.103477

Pie, M. R., Faircloth, B. C., Ribeiro, L. F., Bornschein, M. R., & McCormack, J. E. (2018). Phylogenomics of montane frogs of the Brazilian Atlantic Forest is consistent with isolation in sky islands followed by climatic stability. *Biological Journal of the Linnean Society*, *125*, 72–82. https://doi.org/10.1093/biolinnean/bly093

Pinaya, J. L. D., Cruz, F. W., Ceccantini, G. C. T., Corrêa, P. L. P., Pitman, N., Vemado, F., et al. (2019). Brazilian montane rainforest expansion induced by Heinrich Stadial 1 event. *Scientific Reports*, *9*, 17912. https://doi.org/10.1038/s41598-019-53036-1

Pombal, J. P., & Haddad, C. F. B. (2007). Estratégias e modos reprodutivos em anuros. In L. B. Nascimento & P. M. E. Oliveira (Eds.), *Herpetologia no Brasil II* (pp. 101–116). Sociedade Brasileira de Herpetologia.

Prado, C., Uetanabaro, M., & Haddad, C. (2005). Breeding activity patterns, reproductive modes, and habitat use by anurans (Amphibia) in a seasonal environment in the Pantanal, Brazil. *Amphibia-Reptilia*, *26*, 211–221. https://doi.org/10.1163/1568538054253375

Porto, T. J., Carnaval, A. C., & Rocha, P. L. B. (2013). Evaluating forest refugial models using species distribution models, model filling, and inclusion: A case study with 14 Brazilian species. *Diversity and Distributions*, *19*, 330–340. https://doi.org/10.1111/j.1472-4642.2012.00944.x

Pôrto, K. C., Almeida-Cortez, J. S., & Tabarelli, M. (2005). *Diversidade Biológica e Conservação da Floresta Atlântica ao Norte do Rio São Francisco*. Ministério do Meio Ambiente.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032

Raymo, M. E., Oppo, D. W., & Curry, W. B. (1997). The mid-Pleistocene climate transition: A deep sea carbon perspective. *Paleoceanography*, *12*, 546–559. https://doi.org/10.1029/2021GL097121

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J., & Hirota, M. M. (2009). The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation*, *142*, 1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021

Ribeiro, R. A., Lemos-Filho, J. P., Ramos, A. C. S., & Lovato, M. B. (2011). Phylogeography of the endangered rosewood Dalbergia nigra (Fabaceae): Insights into the evolutionary history and conservation of the Brazilian Atlantic Forest. *Heredity (Edinb)*, *106*, 46–57. https://doi.org/10.1038/hdy.2010.64

Rodríguez, A., Börner, M., Pabijan, M., Gehara, M., Haddad, C. F. B., & Vences, M. (2015). Genetic divergence in tropical anurans: Deeper phylogeographic structure in forest specialists and in topographically complex regions. *Evolutionary Ecology*, *29*, 765–785. https://doi.org/10.1007/s10682-015-9774-7

Rodrigues, M. T., Bertolotto, C. E. V., Amaro, R. C., Yonenaga-Yassuda, Y., Freire, E. M. X., & Pellegrino, K. C. M. (2014). Molecular phylogeny, species limits, and biogeography of the Brazilian endemic lizard genus Enyalius (Squamata: Leiosauridae): An example of the historical relationship between Atlantic Forests and Amazonia. *Molecular Phylogenetics and Evolution*, *81*, 137–146. https://doi.org/10.1016/j.ympev.2014.07.019

Romanelli, J. P., Kroc, E., Assad, M. L. L., Santos, J. P. B., Boschi, R. S., & Rodrigues, R. R. (2024). Uncovering data gaps in biodiversity research within Brazilian Atlantic Forest restoration. *Restoration Ecology*, *e14316*. https://doi.org/10.1111/rec.14316

Ruddiman, W., Raymo, M., Martinson, D., Clement, B., & Backman, J. (1989). Pleistocene evolution: Northern Hemisphere ice sheets and North Atlantic Ocean. *Paleoceanography*, *4*, 353–412. https://doi.org/10.1016/j.epsl.2010.10.013

Rull, V. (2005). Biotic diversification in the Guayana Highlands: A proposal. *Journal of Biogeography*, *32*, 921–927. https://doi.org/10.1111/j.1365-2699.2005.01252.x

Rull, V. (2020). Neotropical diversification: Historical overview and conceptual insights. In V. Rull & A. C. Carnaval (Eds.), *Neotropical diversification: Patterns and processes* (pp. 13–49). Springer Nature Switzerland AG. https://doi.org/10.1007/978-3-030-31167-4

Santos, A. M. M., Cavalcanti, D. R., Silva, J. M. C., & Tabarelli, M. (2007). Biogeographical relationships among tropical forests in north-eastern Brazil. *Journal of Biogeography*, *34*, 437–446. https://doi.org/10.1111/j.1365-2699.2006.01604.x

Santos, L. C. M., & Caxito, F. (2021). Accretionary models for the Neoproterozoic evolution of the Borborema Province: Advances and open questions. *Brazilian Journal of Geology*, *51*(2), e20200104. https://doi.org/10.1590/2317-4889202120200104

Silva, N. R., Neto, J. A., Prado, C. P. A., & Mott, T. (2019). Reproductive biology of Dendropsophus haddadi (Bastos and Pombal, 1994), a small treefrog of the Atlantic Forest. *Herpetological Notes*, *12*, 319–325.

Silveira, M. H. B., Mascarenhas, R., Cardoso, D., & Batalha-Filho, H. (2019). Pleistocene climatic instability drove the historical distribution of forest islands in the northeastern Brazilian Atlantic Forest. *Palaeogeography, Palaeoclimatology, Palaeoecology, 527*, 67–76. https://doi.org/10.1016/j.palaeo.2019.04.028

Smith, B. T., McCormack, J. E., Cuervo, A. M., Hickerson, M. J., Aleixo, A., Cadena, C. D., Pérez-Emán, J., Burney, C. W., Xie, X., Harvey, M. G., Faircloth, B. C., Glenn, T. C., Derryberry, E. P., Prejean, J., Fields, S., & Brumfield, R. T. (2014). The drivers of tropical speciation. *Nature*, *515*, 406–409. https://doi.org/10.1038/nature13687

Soares, J. E. P., Lima, M. V. A. G., Fuck, R. A., & Berrocal, J. (2010). Características sísmicas da litosfera da província Borborema: Resultados parciais do experimento de refração sísmica profunda. *IV Simpósio Brasileiro de Geofísica*, Brasília.

Stone, B. W., & Wolfe, A. D. (2021). Phylogeographic analysis of shrubby beardtongues reveals range expansions during the Last Glacial Maximum and implicates the Klamath Mountains as a hotspot for hybridization. *Molecular Ecology*, *30*, 3826–3839. https://doi.org/10.1111/mec.15992

Sun, Y., Yin, Q., Crucifix, M., Clemens, S. C., et al. (2019). Diverse manifestations of the mid-Pleistocene climate transition. *Nature Communications*, *10*, 352. https://doi.org/10.1038/s41467-018-08257-9

Tabarelli, M., & Santos, A. M. M. (2004). Uma breve descrição sobre a história natural dos Brejos Nordestinos. In K. C. Pôrto, J. J. P. Cabral, & M. Tabarelli (Eds.), *Brejos de Altitude em Pernambuco e Paraíba: História natural, ecologia e conservação* (pp. 99–110). Ministério do Meio Ambiente.

Thomé, M. T., Carstens, B. C., Rodrigues, M. T., Alexandrino, J., & Haddad, C. F. B. (2021). Genomic data from the Brazilian sibilator frog reveal contrasting Pleistocene dynamics and regionalism in two South American dry biomes. *Journal of Biogeography, 48*, 1112–1123. https://doi.org/10.1111/jbi.14064

Thomé, M. T. C., Zamudio, K. R., Haddad, C. F. B., & Alexandrino, J. (2014). Barriers, rather than refugia, underlie the origin of diversity in toads endemic to the Brazilian Atlantic Forest. *Molecular Ecology, 23*, 6152–6164. https://doi.org/10.1111/mec.12986

Ulloa, J. S., Aubin, T., Llusia, D., Courtois, E. A., Fouquet, A., Gaucher, P., Pavoine, S., & Sueur, J. (2019). Explosive breeding in tropical anurans: Environmental triggers, community composition and acoustic structure. *BMC Ecology, 19*(28), 1–17. https://doi.org/10.1186/s12898-019-0243-y

Van Schmus, W. R., Brito Neves, B. B., Hackspacher, P., & Baninski, M. (1995). U/Pb and Sm/Nd geochronologic studies of eastern Borborema Province, northeastern Brazil: Initial conclusions. *Journal of South American Earth Sciences, 8*(3-4), 276–288. https://doi.org/10.1016/0895-9811(95)00013-6

Vasconcelos, M. M., Colli, G. R., Weber, J. N., Ortiz, E. M., Rodrigues, M. T., & Cannatella, D. C. (2019). Isolation by instability: Historical climate change shapes population structure and genomic divergence of treefrogs in the Neotropical Cerrado savanna. *Molecular Ecology, 28*(7), 1748–1764. https://doi.org/10.1111/mec.15045

Velloso, A. L., Sampaio, E. V. S. B., & Pareyn, F. G. C. (2002). *Ecorregiões propostas para o bioma Caatinga*. Associação Plantas do Nordeste, Instituto de Conservação Ambiental The Nature Conservancy do Brasil.

Wang, X., Auler, A. S., Edwards, R. L., Cheng, H., Cristalli, P. S., Smart, P. L., Richards, D. A., & Shen, C. C. (2004). Wet periods in northeastern Brazil over the past 210 kyr linked to distant climate anomalies. *Nature, 432*, 740–743. https://doi.org/10.1038/nature03067

Weber, M., Wang, H., Qiao, S., et al. (2021). DeepLab2: A TensorFlow library for deep labeling. *arXiv Preprint*. http://arxiv.org/abs/2106.09748

Wickham, H. (2019). *stringr: Simple, consistent wrappers for common string operations* (R package version 1.4.0). https://CRAN.R-project.org/package=stringr

Wickham, H., Bryan, J., Kalicinski, M., Valery, K., & Vaughan, D. (2023). *tidyverse: Easily install and load the 'Tidyverse'* (R package version 2.0.0). https://CRAN.R-project.org/package=tidyverse

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., Woo, K., & Yutani, H. (2023). *ggplot2: Create elegant data visualizations using the grammar of graphics* (R package version 3.4.3). https://CRAN.R-project.org/package=ggplot2

Statements and Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

JHAN, PIS, and MG conceived the manuscript. JHAN and PIS designed sampling strategies and collected the data. JHAL and RS analyzed the data. JHAL, RS, PIS, and MG wrote the manuscript. JHAL, RS, PIS, MK and MG revised the manuscript.

Data availability

The dataset analyzed is available as supplementary information in the online version ****.

Licenses

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

7 CONSIDERAÇÕES FINAIS

A história e atual heterogeneidade ambiental do Planalto da Borborema possui alta contribuição para a história evolutiva da biota do Nordeste do Brasil

O presente estudo somado à literatura demonstra que, assim como outras cadeias de montanhas da região Neotropical, o Planalto da Borborema possui uma complexidade climática e ambiental histórica e contemporânea com impactos significativos na história evolutiva da biodiversidade do Nordeste brasileiro, nos biomas Caatinga e Mata Atlântica. Em sua paisagem atual, tanto fatores geográficos, como distância geográfica, quanto variação ambiental, como na temperatura, precipitação e cobertura vegetal, conduzem a diferenciação intraespecífica, seja através de variação fenotípica ou genética. Já em um contexto histórico, mudanças climáticas e as consequentes mudanças na distribuição da vegetação são os condutores de processos demográficos. No entanto, a intensidade de respostas das espécies a tais fatores atuais ou passados é dependente de suas limitações ecológicas.

O fenótipo e o genótipo das espécies evoluem independentemente, sendo guiados pela combinação de fatores da paisagem e traços espécie-específicos

Variações ambientais são o principal responsável pela variação em caracteres fenotípicos, como o canto de anuncio, em populações de anuros de florestas serranas, havendo maior diferenciação em espécies habitat-generalistas devido sua maior capacidade de se dispersar e colonizar diferentes ambientes. Isso foi evidenciado pela maior associação entre variação acústica e variação ambiental em ambas as espécies. O que indica uma maior atuação de forças seletivas locais guiando a diferenciação acústica de anuros no Planalto da Borborema, e um papel secundário de mecanismos evolutivos neutros causados pelo isolamento geográfico.

No que diz respeito a diferenciação genética, nossos achados indicam que populações de espécies habitat-especialistas se encontram mais estruturas como resultado do isolamento por ambiente combinado ao isolamento por distância geográfica e influência da estabilidade climática desde o LIG. Por outro lado, espécies habitat-generalistas apresentam um padrão de diferenciação genética exclusivamente guiado pela distância geográfica, a qual pode incluir o efeito da assincronia na precipitação latitudinalmente. Tais achados destacam novamente a atuação conjunta de mecanismos evolutivos seletivos e neutros ao longo da região, promovendo variação entre populações de acordo com a capacidade dispersiva das espécies na matriz de vegetação seca/aberta de Caatinga entre os enclaves florestais.

Assim como em outros sistemas "sky-island" no Nordeste do Brasil, no Planalto da Borborema as condições climáticas e ambientais do LGM afetaram a demografia das espécies

Historicamente, mudanças climáticas e na cobertura da vegetação durante o LGM favoreceram a migração e expansão populacional em ambas as espécies e, possivelmente durante o Holoceno houve redução nestes parâmetros também em ambas as espécies. Estes resultados confirmam hipóteses anteriores para períodos de conexão e isolamento dos enclaves florestais e demonstram que os enclaves florestais do Planalto da Borborema possuem uma história semelhante a outros sistemas "sky-island" no Nordeste do Brasil. Contudo, apesar da sincronia temporal entre as espécies e de ambas terem sido favorecidas pelas condições do LGM, a espécie habitat-especialista apresentou um padrão demográfico mais intensamente transformado a partir daquele período, evidenciando mais uma vez a maior sensibilidade destas espécies a mudanças espaço-temporais.

Fornecemos informações de base para conservação da porção norte da Mata Atlântica

Na minha tese, realizei uma série de três investigações diferentes e metodologicamente comparativas explorando o presente e passado de populações coocorrentes de anuros com tolerâncias ecológicas e histórias biogeográficas diferentes para fornecer uma melhor compreensão da história evolutiva da fauna em florestas serranas na região norte da Mata Atlântica. Nesta trilogia, consegui demonstrar até que ponto as características de uma região e sua história determinam respostas semelhantes em espécies diferentes e até que ponto traços espécie-específicos determinam respostas diferentes entre as espécies. Contudo, mais do que isso, deixo retratos do passado e do presente de populações animais desta região de extrema importância biológica para biodiversidade neotropical - mas altamente ameaçada pela perda de habitat - esperando que eles sirvam de base nos próximos planos de gestão e conservação dessa região.

REFERÊNCIAS

AGRA, Maria de Fátima.; BARBOSA, Maria Regina Vasconcellos; STEVENS, Warren Douglas. Levantamento florístico preliminar do Pico do Jabre, Paraíba, Brasil. Em: PÔRTO, Cavalcanti Kátia; CABRAL, Jaime Joaquim da Silva Pereira; TABARELLI, Marcelo. (Org.). **Brejos de Altitude em Pernambuco e Paraíba: História natural, ecologia e conservação.** Ministério do Meio Ambiente, 2004. p. 123-138.

ANDRADE-LIMA, Dárdamo. Estudos fitogeográficos de Pernambuco. Em: **Arquivo do Instituto de Pesquisa Agronômica**. V. 5. Secretaria da Agricultura, Indústria e Comércio, 1960. p. 305-341.

ANTONELLI, Alexandre; SANMARTÍN, Isabel. Why are there so many plant species in the Neotropics? **Taxon**, v. 60, 403-414, 2011.

BATALHA-FILHO, Henrique. et al. Disentangling the contemporary and historical effects of landscape on the population genomic variation of two bird species restricted to the highland forest enclaves of northeastern Brazil. **Heredity**, v. 132, 77-88, 2023.

CARNAVAL, Ana Carolina; BATES, John Marshall. Amphibian DNA shows marked genetic structure and tracks Pleistocene climate change in northeastern Brazil. **Evolution**, v. 61, n. 12, 2942-2957, 2007.

CARNAVAL, Ana Carolina O. Q. Phylogeography of four frog species in forest fragments of northeastern Brazil—A preliminary study. **Integrative and Comparative Biology**, v. 42, 13-921, 2002.

CARNAVAL, Ana Carolina. et al. Prediction of phylogeographic endemism in an environmentally complex biome. **Proceedings of the Royal Society**, v. 281, n. 1792, 20141461, 2014.

ENDLER, John Arthur. **Geographic variation, speciation and clines**. Princeton University Press, 1977.

ERDTMANN, Luciana; AMÉZQUITA, Adolfo. Differential evolution of advertisement call traits in dart-poison frogs (Anura: Dendrobatidae). **Ethology**, v. 115, 801-811, 2009.

ESCALONA SULBARÁN, Moisés David. et al. Neotropical frogs and mating songs: The evolution of advertisement calls in glassfrogs. **Journal of Evolutionary Biology**, v. 32, 163-176, 2019.

FERNANDES, Igor Yuri et al. Unlinking the speciation steps: Geographical factors drive changes in sexual signals of an Amazonian nurse-frog through body size variation. **Evolutionary Biology**, v. 48, n. 22, 1-13, 2021.

GINGRAS, Bruno. et al. Call acoustics reflect body size across four clades of anurans. **Journal of Zoology**, v. 289, n. 2, 143-150, 2013.

GUEDES, Thaís Barreto et al. Diversity, endemism, and evolutionary history of montane biotas outside the Andean region. Em: RULL, V.; CARNAVAL, A. C. (Org.). **Neotropical diversification: Patterns and process**. Springer, 2020. p. 299-328.

HOSKIN, Conrad J; JAMES, S.; GRIGG, Gordon C. Ecology and taxonomy- driven deviations in the frog call–body size relationship across the diverse Australian frog fauna. **Journal of Zoology**, v. 278, n. 1, 36-41, 2009.

HU, Ang. et al. Mountain biodiversity and ecosystem functions: interplay between geology and contemporary environments. **The ISME Journal**, v. 14, 931-944, 2020.

HUGHES, Colin E.; ATCHISON, Guy W. The ubiquity of alpine plant radiations: from the Andes to the Hengduan Mountains. **New Phytologist**, v. 207, 275-282, 2015.

KAEFER, Igor Luis. et al. The early stages of speciation in Amazonian forest frogs: phenotypic conservatism despite strong genetic structure. **Evolutionary Biology**, v. 40, 228-245, 2013.

KÖRNER, Christian. et al. A global inventory of mountains for bio-geographical applications. **Alpine Botany**, v. 127, 1-15, 2017.

LAGOMARSINO, Laura P. et al. The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). **New Phytologist**, v. 210, 1430-1442, 2016.

LOMOLINO, Mark V. Elevation gradients of species-density: historical and prospective views. **Global Ecology and Biogeography**, v. 10, 3-13, 2001.

MAESTRE, Fernando T. et al. Plant species richness and ecosystem multifunctionality in global drylands. **Science**, v. 335, 214-18, 2012.

MAIA, Gabriela Farias.; LIMA, Albertina Pimentel; KAEFER, Igor Luis. Not just the river: genes, shapes, and sounds reveal population-structured diversification in the Amazonian frog *Allobates tapajos* (Dendrobatoidea). **Biological Journal of the Linnean Society**, v. 20, 1-14, 2017.

OLIVEIRA, Francisco Fonseca Ribeiro. et al. Quaternary climatic fluctuations influence the demographic history of two species of sky-island endemic amphibians in the Neotropics. **Molecular Phylogenetics and Evolution**, v. 160, 107113, 2021.

PIE, Marcio R. et al. Phylogenomics of montane frogs of the Brazilian Atlantic Forest is consistent with isolation in sky islands followed by climatic stability. **Biological Journal of the Linnean Society**, v. 125, 72-82, 2018.

POUNDS, J Alan; FOGDEN, Michael P.L.; CAMPBELL, John H. Biological response to climate change on a tropical mountain. **Nature**, v. 398, 611-615, 1999.

RODAL, Maria Jesus Nogueira; BARBOSA, Maria Regina Vasconcellos; THOMAS, William Wayt. Do the seasonal forests in northeastern Brazil represent a single floristic unit? **Brazilian Journal of Biology**, v. 68, n. 3, 467-475, 2008.

RULL, Valentí. Biotic diversification in the Guayana Highlands: a proposal. **Journal of Biogeography**, v. 32, 921-927, 2005.

SALES, Margareth Ferreira; MAYO, Simon Joseph; RODAL, Maria Jesus Nogueira. **Plantas vasculares das florestas serranas de Pernambuco: um checklist da flora ameaçada dos brejos de altitude, Pernambuco, Brasil**. Recife: Imprensa Universitária, Universidade Federal Rural de Pernambuco, 1998.

SANDERS, Nathan J.; RAHBEK, Carsten. The patterns and causes of elevational diversity gradients. **Ecography**, v. 35, 1-3, 2012.

SANTOS, André Maurício Melo. et al. Biogeographical relationships among tropical forests in North-eastern Brazil. **Journal of Biogeography**, v. 34, 437-446, 2007.

SCHLUTER, Dolph. Evidence for ecological speciation and its alternative. **Science**, v. 323, 737-741, 2009.

SILVA, José Maria Cardoso; LEAL, Inara. R.; TABARELLI, Marcelo. **Caatinga: The largest tropical dry forest region in South America.** Springer, 2018, 482 p.

SILVEIRA, Mario Henrique Barros. et al. Pleistocene climatic instability drove the historical distribution of forest islands in the northeastern Brazilian Atlantic Forest. **Palaeogeography, Palaeoclimatology, Palaeoecology**, v. 527, 67-76, 2019.

TABARELLI, Marcelo.; SANTOS, André Maurício Melo. Uma breve descrição sobre a história natural dos Brejos Nordestinos. Em: PÔRTO, C. Kátia; CABRAL, Jaime J. P.; TABARELLI, Marcelo. (Org.). **Brejos de Altitude em Pernambuco e Paraíba: História natural, ecologia e conservação.** Ministério do Meio Ambiente, 2004. p. 17-24.

VARGAS, Oscar M.; SIMPSON, Beryl B. 2019. Allopatric speciation is more prevalent than parapatric ecological divergence in tropical montane systems (Asteraceae: Piofontia). **BioRxiv**:10.1101/ 868216

VELLOSO, Agnes L.; SAMPAIO, Everaldo Valadares de Sá Barretto; PAREYN, Frans Germain Corneel. **Ecorregiões propostas para o bioma Caatinga**. Associação Plantas do Nordeste, Instituto de Conservação Ambiental The Nature Conservancy do Brasil, 2002.

WERNECK, Fernanda de Pinho. et al. Revisiting the historical distribution of Seasonally Dry Tropical Forests: new insights based on palaeodistribution modelling and palynological evidence. **Global Ecology and Biogeography**, v. 20, 272-288, 2011.

WIENS, John J. 2004. Speciation and ecology revisited: phylogenetic niche conservatism and the origin of species. **Evolution**, v. 58, 193-197, 2004.

APÊNDICE A - MATERIAL SUPLEMNETAR DO CAPÍTULO 1

Supplementary information

S1. *Physalaemus cuvieri*: CHUFPE1321,CHUFPE1322,CHUFPE1323,CHUFPE1324,CHUFPE1325,CHUFPE1326,CHUFPE1327, CHUFPE1328,CHUFPE1329,CHUFPE1330,CHUFPE1331,CHUFPE1332,CHUFPE1603,CHUFPE1602,CHUFPE1598,CHUFPE1599, CHUFPE1597,CHUFPE1601,CHUFPE1600,CHUFPE1596,CHUFPE1813,CHUFPE1812,CHUFPE1811,CHUFPE1522,CHUFPE1523,CHUFPE1524,CHUFPE1675,CHUFPE1672,CHUFPE1670,CHUFPE1671,CHUFPE1674,CHUFPE1673,CHUFPE1676,CHUFPE1619,CHUFPE1618,CHUFPE1620,CHUFPE1695,CHUFPE1694,CHUFPE1696,CHUFPE1795,CHUFPE1796,CHUFPE1729,CHUFPE1732,CHUFPE1730,CHUFPE1726,CHUFPE1723,CHUFPE1731,CHUFPE1725,CHUFPE1727,CHUFPE1728,CHUFPE1724,CHUFPE1755,CHUFPE1758,CHUFPE1753,CHUFPE1750,CHUFPE1751,CHUFPE1759,CHUFPE1756,CHUFPE1752,CHUFPE1754,CHUFPE1760,CHUFPE1757,CHUFPE1761,CHUFPE1766,CHUFPE1773,CHUFPE1770,CHUFPE1763,CHUFPE1765,CHUFPE1772,CHUFPE1762,CHUFPE1767,CHUFPE1771,CHUFPE1768,CHUFPE1764,CHUFPE1769; *Dendropsophus oliveirai*: CHUFPE1370,CHUFPE1378,CHUFPE1372,CHUFPE1376,CHUFPE1374, CHUFPE1377,CHUFPE1369,CHUFPE1371,CHUFPE1375,CHUFPE1373,CHUFPE1748,CHUFPE1749,CHUFPE1364,CHUFPE1366, CHUFPE1703,CHUFPE1704,CHUFPE 1700,CHUFPE1808,CHUFPE1807,CHUFPE1805,CHUFPE1804,CHUFPE1801,CHUFPE1803, CHUFPE1802,CHUFPE1518,CHUFPE1677,CHUFPE1608,CHUFPE1609,CHUFPE1607,CHUFPE1606,CHUFPE1610,CHUFPE1604,CHUFPE1605,CHUFPE1816,CHUFPE1814,CHUFPE1815,CHUFPE1499,CHUFPE1498,CHUFPE1785,CHUFPE1790,CHUFPE1781,CHUFPE1783, CHUFPE1784,CHUFPE1787,CHUFPE1786,CHUFPE1791,CHUFPE1788,CHUFPE1780,CHUFPE1782,CHUFPE1789,CHUFPE1733,CHUFPE1738,CHUFPE1734,CHUFPE1736,CHUFPE1735,CHUFPE1737,CHUFPE1739,CHUFPE2049,CHUFPE2050,CHUFPE2051, CHUFPE2052,CHUFPE2055,CHUFPE2056,CHUFPE2057,CHUFPE2058,CHUFPE2059,CHUFPE2060,CHUFPE2061,CHUFPE2062, CHUFPE2063,CHUFPE2064,CHUFPE2070,CHUFPE2071.

S2. Matrix of geographic (km; below diagonal) and genetic distance (Fst 16S; above diagonal) distances among *Dendropsophus oliveirai* (before forward slash) and *Physalaemus cuvieri* (after forward slash) populations. Parque Estadual Mata do Pau-Ferro = Pau-Ferro, Refúgio de Vida Silvestre Matas do Siriji = Mata do Siriji, Parque Natural Municipal Professor João Vasconcelos Sobrinho = João Vasconcelos, Fazenda Vale do Tabocas = Tabocas, Parque Nacional do Catimbau = Catimbau, Refúgio de Vida Silvestre Morros do Craunã e do Padre = Craunã, Reserva Biológica de Pedra Talhada = Pedra Talhada.

	Catimbau	**Mata do Siriji**	**Tabocas**	**João Vasconcelos**	**Craunã**	**Pedra Talhada**	**Pau-Ferro**
Catimbau	—	0.80 / 0.56	0.45 / -0.12	0.36 / -0.02	0.77 / 0.00	0.52 / 0.00	0.82 / 0.89
Mata do Siriji	218.56	—	1.00 / 0.51	1.00 / 0.68	1.00 / 0.71	0.86 / 0.71	-0.17 / 0.14
Tabocas	101.13	118.85	—	0.00 / -0.02	1.00 / 0.00	0.87 / 0.00	0.97 / 0.86
João Vasconcelos	135.06	99.96	40.12	—	1.00 / 0.14	0.84 / 0.14	1.00 / 0.97
Craunã	111.42	325.12	206.75	233.72	—	0.75 / 0.00	0.97 / 1.00
Pedra Talhada	116.17	208.00	112.43	109.36	164.05	—	0.88 / 1.00
Pau-Ferro	244.28	75.00	159.01	156.75	354.37	264.96	—

S3. Table with temporal (in s) and spectral (in Hz) variables measured in the *Dendropsophus oliveirai* and *Physalaemus cuvieri* advertisement calls. The values are provided as average ± standard deviation by population. Parque Estadual Mata do Pau-Ferro = Pau-Ferro, Refúgio de Vida Silvestre Matas do Siriji = Mata do Siriji, Parque Natural Municipal Professor João Vasconcelos Sobrinho = João Vasconcelos, Fazenda Vale do Tabocas = Tabocas, Parque Nacional do Catimbau = Catimbau, Refúgio de Vida Silvestre Morros do Craunã e do Padre = Craunã, Reserva Biológica de Pedra Talhada = Pedra Talhada.

Dendropsophus oliveirai	**Pau-Ferro (n=7)**	**Mata do Siriji (n=6)**	**João Vasconcelos (n=7)**	**Tabocas (n=12)**	**Catimbau (n=7)**	**Craunã (n=6)**	**Pedra Talhada (n=12)**
Note duration	0.1076±0.01	0.1230±0.01	0.1258±0.02	0.1206±0.03	0.1091±0.02	0.1060±0.03	0.1053.00±0.01
Pulse number per note	14.96±2.79	14.69±2.15	12.99±3.74	15.50±4.30	12.97±2.74	13.73±4.69	13.15±2.73
Pulse rate (p/s)	141.61±26.01	120.71±17.48	105.71±30.33	129.56±24.28	119.99±21.27	128.74±20.12	125.17±21.91
Lower frequency	5352.17±377.98	5371.98±326.37	5065.87±233.31	5005.70±262.51	5084.64±322.48	4795.64±195.29	5438.31±302.52
Upper frequency	7206.02±293.07	7379.46±476.91	7036.44±185.00	7040.92±334.65	7039.53±349.41	7101.89±205.87	7587.71±462.83
Bandwidth	1853.84±289.18	2007.47±404.23	1970.57±142.56	2035.22±296.64	1954.89±471.26	2306.25±295.65	2149.40±303.27
Peak frequency	6195.52±251.11	6327.61±340.86	6016.85±140.18	6072.31±200.72	6057.87±168.75	6087.78±165.48	6469.30±349.57

Physalaemus cuvieri	**Pau-Ferro (n=10)**	**Mata do Siriji (n=9)**	**João Vasconcelos (n=8)**	**Tabocas (n=12)**	**Catimbau (n=7)**	**Craunã (n=12)**	**Pedra Talhada (n=12)**
Call duration	0.2838±0.03	0.3015±0.04	0.2879.00±0.06	0.3157±0.03	0.3026±0.02	0.3620±0.01	0.3607±0.03
Risetime	0.1099±0.01	0.1051±0.01	0.1069.00±0.02	0.1252±0.03	0.1190±0.01	0.1320±0.01	0.1360±0.01
Lower frequency of risetime	814.97±215.66	703.40±189.74	587.59±39.24	662.57±164.54	614.29±39.85	767.59±24.31	588.25±32.00
Upper frequency of risetime	1483.64±142.84	1313.16±360.02	1490.04±364.42	1460.79±304.66	1220.86±163.55	922.04±22.28	1326.17±211.14
Bandwidth of risetime	668.67±192.77	609.76±400.12	902.45±390.41	798.21±356.73	606.57±166.40	154.46±6.34	737.91±230.51
Peak frequency of risetime	1101.21±200.22	925.165±182.65	840.33±71.66	983.89±196.18	829.64±36.49	843.30±24.06	815.73±91.01
Downtime	0.1756±0.02	0.1964±0.03	0.1810±0.04	0.1905±0.02	0.1836.00±0.03	0.2301±0.02	0.2247±0.03
Lower frequency of downtime	344.51±28.31	363.42±40.57	357.31±46.78	342.03±47.11	402.96±45.05	429.28±18.44	332.74±23.45
Upper frequency of downtime	1337.80±544.45	1241.76±652.37	1408.50±708.87	1817.43±738.40	1259.78±397.18	863.13±33.22	1651.67±531.76
Bandwidth of downtime	993.29±564.86	878.33±673.15	1051.19±679.80	1475.41±728.78	856.81±387.92	433.85±29.03	1318.92±545.39
Peak frequency of downtime	679.16±56.58	706.03±30.05	657.03±53.61	763.89±333.65	710.39±52.71	745.76±40.11	669.64±42.27

S4. Loadings of acoustic Principal Component Analysis from average of acoustic parameters of each *Dendropsophus oliveirai* and *Physalaemus cuvieri* population.

	Dendropsophus oliveirai			***Physalaemus cuvieri***	
Acoustic parameters	**PC1**	**PC2**	**Acoustic parameters**	**PC1**	**PC2**
ND	14.95922	6.028552	**CD**	7.4503	16.8378
LF	22.89755	0.007195	**RT**	4.3043	19.6692
HF	24.5078	2.972285	**LFRT**	3.1135	16.1470
BW	6.072969	17.66565	**HFRT**	16.1858	0.1298
DF	25.65981	1.152104	**BRT**	15.6055	1.0452
PN	0.008799	35.61303	**DFRT**	2.3236	13.9485
PR	5.89385	36.56118	**DT**	8.5622	12.4119
			LFDT	11.9009	2.4246
			HFDT	19.3785	9.3377
			BDT	11.7440	7.5191
			DFDT	8.4310	0.5285

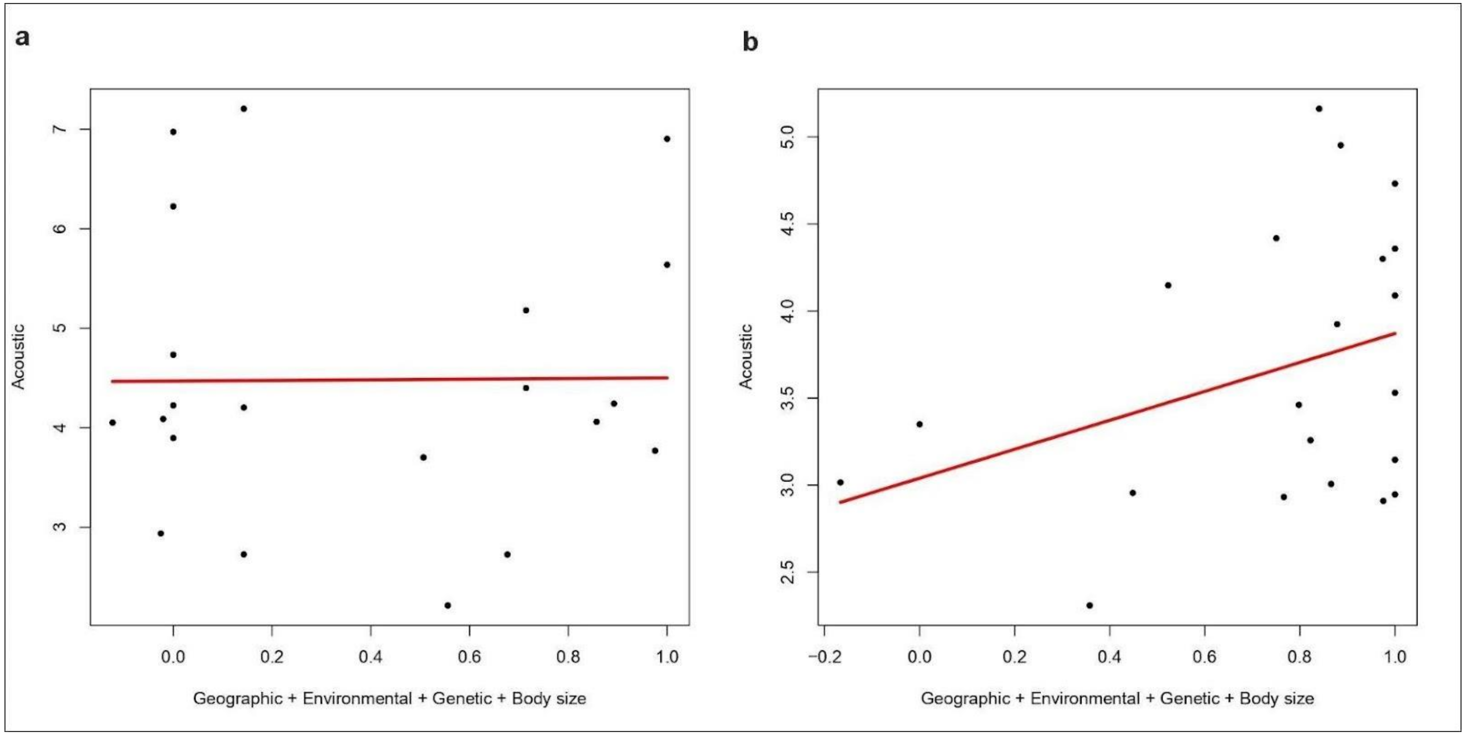
S5. Scores of acoustic Principal Component Analysis from average of acoustic parameters of each *Dendropsophus oliveirai* and *Physalaemus cuvieri* population.

	Dendropsophus oliveirai		***Physalaemus cuvieri***	
Population	**PC1**	**PC2**	**PC1**	**PC2**
Catimbau	1.382126031	1.071275263	0.37217682	0.023279603
Mata do Siriji	4.184074884	4.43578412	0.022534308	7.995023115
Tabocas	3.175741052	3.248010911	5.826484356	3.000798358
João Vasconcelos	16.07802637	39.68309527	10.98678324	0.394960822
Craunã	2.930714065	2.371738237	74.38176494	0.338736702
Pedra Talhada	65.23814807	15.41201528	0.808303133	60.1710864
Pau-Ferro	7.011169536	33.77808092	7.601953205	28.076115

S6. Linear regression results between air temperature and acoustic parameters of the *Physalaemus cuvieri* and *Dendropsophus oliveirai* advertisement call. Values in bold highlight statistically significant regressions ($p \leq 0.05$).

Dendropsophus oliveirai	
Note duration	**$R^2 = 0.07$; $F = 4.26$; $p = 0.04$**
Lower frequency of the call	**$R^2 = 0.10$; $F = 6.50$; $p = 0.01$**
Upper frequency of the call	**$R^2 = 0.15$; $F = 9.94$; $p < 0.01$**
Frequency bandwidth of call	$R^2 = 0.01$; $F = 0.73$; $p = 0.39$
Peak frequency of the call	**$R^2 = 0.13$; $F = 7.98$; $p < 0.01$**
Number of pulses per note	$R^2 = 0.05$; $F = 3.45$; $p = 0.07$
Rate of pulse emission	$R^2 = 0.01$; $F = 0.28$; $p = 0.60$
Physalaemus cuvieri	
Call duration	**$R^2 = 0.09$; $F = 6.46$; $p = 0.01$**
Rise time	$R^2 < 0.01$; $F = 0.30$; $p = 0.59$
Lower frequency along rise time	$R^2 < 0.01$; $F = 0.02$; $p = 0.90$
Upper frequency along rise time	$R^2 = 0.02$; $F = 1.80$; $p = 0.18$
Bandwidth of rise time	$R^2 = 0.02$; $F = 1.48$; $p = 0.23$
Dominant frequency along rise time	$R^2 = 0.01$; $F = 0.71$; $p = 0.40$
Down time	**$R^2 = 0.12$; $F = 9.54$; $p < 0.01$**
Lower frequency along down time	$R^2 < 0.01$; $F = 0.09$; $p = 0.77$
Upper frequency along down time	$R^2 < 0.01$; $F = 0.38$; $p = 0.54$
Bandwidth of down time	$R^2 < 0.01$; $F = 0.33$; $p = 0.57$
Peak frequency along down time	$R^2 < 0.01$; $F = 0.10$; $p = 0.75$

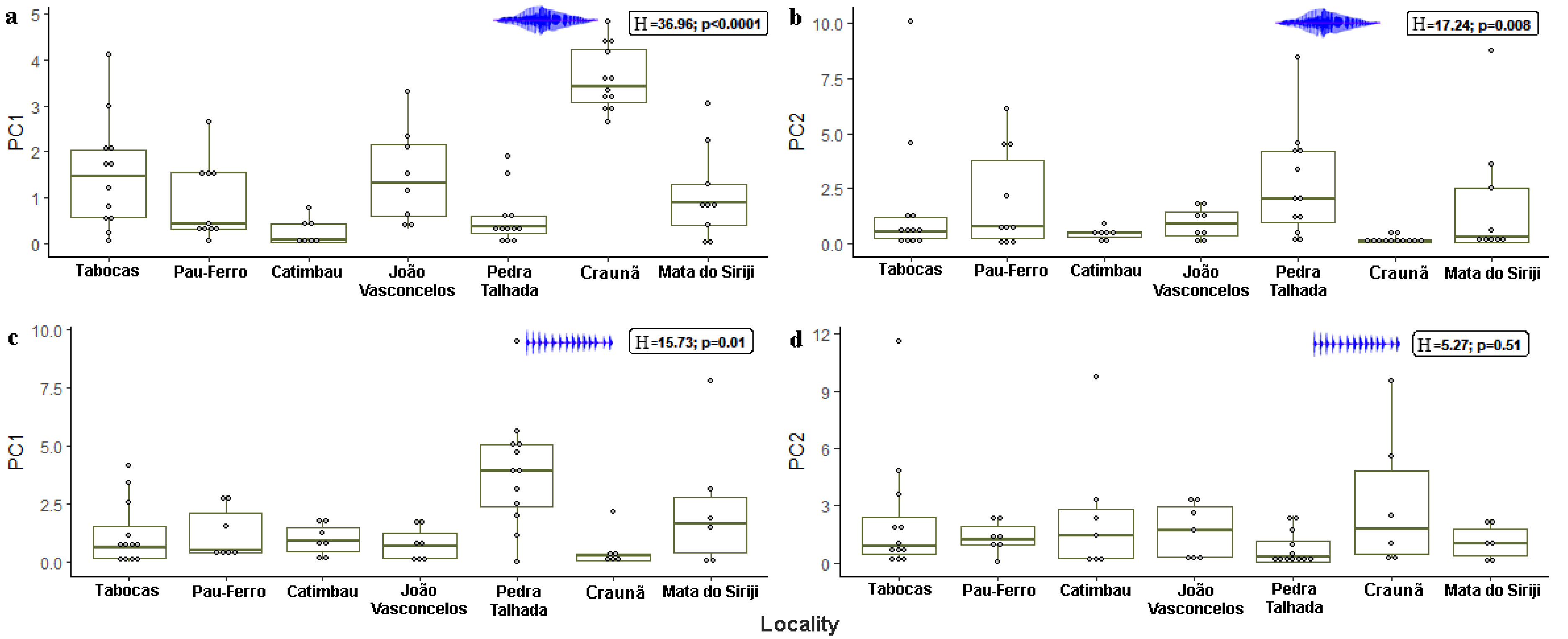
S7. MMRR analysis plot performed to test the combined effect of geographic, environmental, genetic and body size distances on the acoustic distance among *Physalaemus cuvieri* (a) and *Dendropsophus oliveirai* (b) populations from highland forests above the São Francisco River.



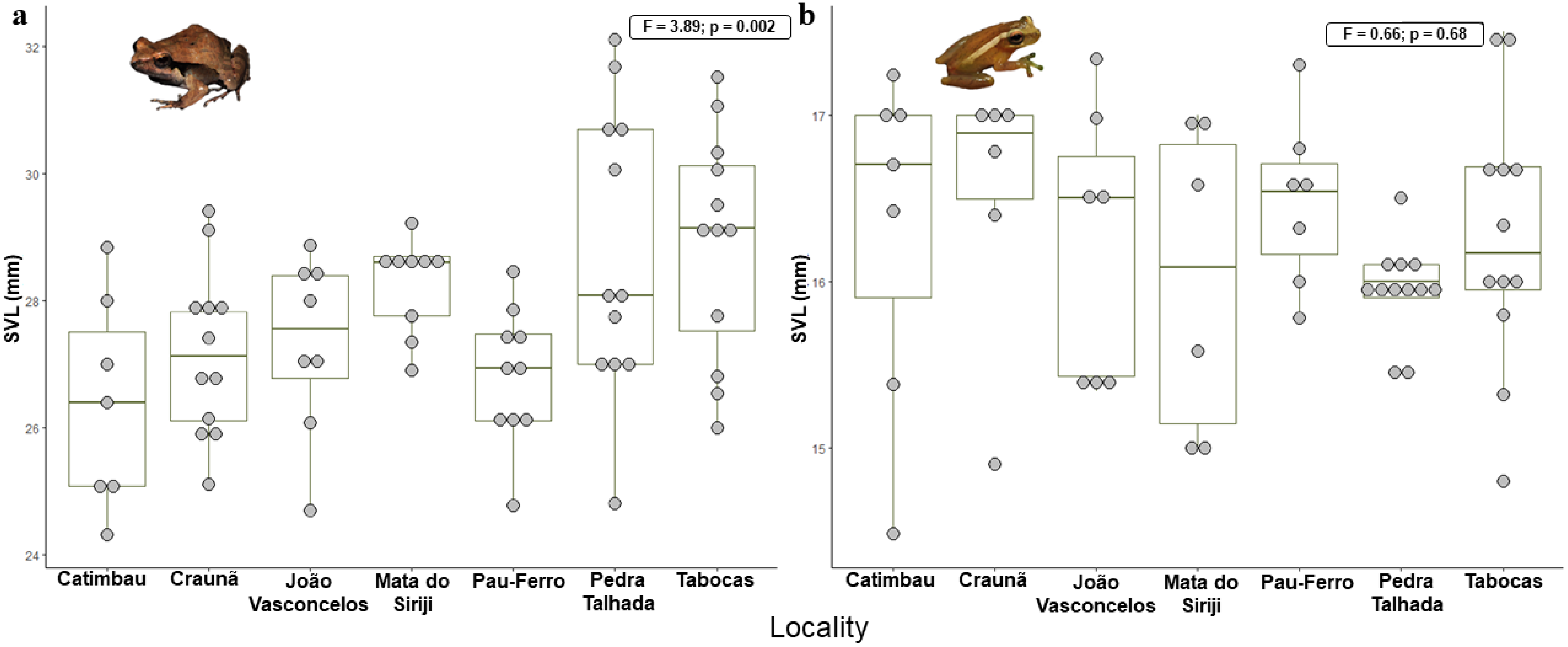
S8. Correlation coefficients based on Mantel (x)/partial Mantel (-) tests and regression coefficients based on MMRR (x) tests on acoustic (CALL), environmental (ENV), geographic (GEO), body size (SVL), and genetic (GEN) distances for seven populations of the anurans *Physalaemus cuvieri* and *Dendropsophus oliveirai* inhabiting highland forests in northeastern Brazil. Values in bold highlight statistically significant ($p \leq 0.05$) or almost significant (between p = 0.06 and p = 0.07) correlations and regressions.

Correlations (Mantel/partial Mantel)	***P. cuvieri***	***D. oliveirai***
GEO x CALL	**r = 0.45; p = 0.05**	r = 0.23; p = 0.18
ENV x CALL	**r = 0.59; p = 0.04**	**r = 0.51; p = 0.01**
GEN x CALL	r = -0.15; p = 0.75	r = 0.26; p = 0.11
SVL x CALL	r = 0.28; p = 0.10	r = 0.03; p = 0.45
ENV x CALL - GEO	**r = 0.47; p = 0.07**	**r = 0.47; p = 0.06**
Regressions (MMRR)		
Overall	**R^2 = 0.58; F = 5.49; p = 0.05**	R^2 = 0.28; F = 1.59; p = 0.26
GEO x CALL	**β = 0.58; p = 0.06**	β = -0.42; p = 0.99
ENV x CALL	β = 0.30; p = 0.25	**β = 0.57; p = 0.07**
GEN x CALL	β = -0.45; p = 0.14	β = -0.49; p = 0.98
SVL x CALL	β = 0.24; p = 0.15	β = -0.15; p = 0.57

S9. Acoustic variation among seven populations of *Physalaemus cuvieri* (a and b) and *Dendropsophus oliveirai* (c and d) from highland forests above the São Francisco River. The Y axis values represent the scores of two principal components. The values into the boxes are results of the Kruskal-Wallis test. Fazenda Vale do Tabocas = Tabocas, Parque Estadual Mata do Pau-Ferro = Pau-Ferro, Parque Nacional do Catimbau = Catimbau, Parque Natural Municipal Professor João Vasconcelos Sobrinho = João Vasconcelos, Reserva Biológica de Pedra Talhada = Pedra Talhada, Refúgio de Vida Silvestre Morros do Craunã e do Padre = Craunã, Refúgio de Vida Silvestre Matas do Siriji = Mata do Siriji.



S10. Body size variation among seven populations of *Physalaemus cuvieri* (a) and *Dendropsophus oliveirai* (b) from highland forests above the São Francisco River. Values into the boxes are results of the ANOVA test. Fazenda Vale do Tabocas = Tabocas, Parque Estadual Mata do Pau-Ferro = Pau-Ferro, Parque Nacional do Catimbau = Catimbau, Parque Natural Municipal Professor João Vasconcelos Sobrinho = João Vasconcelos, Reserva Biológica de Pedra Talhada = Pedra Talhada, Refúgio de Vida Silvestre Morros do Craunã e do Padre = Craunã, Refúgio de Vida Silvestre Matas do Siriji = Mata do Siriji.



APÊNDICE B - MATERIAL SUPLEMNETAR DO CAPÍTULO 2

S1. *Physalaemus cuvieri*:
CHUFPE1321,CHUFPE1322,CHUFPE1323,CHUFPE1324,CHUFPE1325,CHUFPE1326,CHUFPE1327,CHUFPE1328,CHUFPE1329,CHUFPE1330,CHUFPE1331,CHUFPE1332,CHUFPE1603,CHUFPE1602,CHUFPE1598,CHUFPE1599,CHUFPE1597,CHUFPE1601,CHUFPE1600,CHUFPE1596,CHUFPE1813,CHUFPE1812,CHUFPE1811,CHUFPE1522,CHUFPE1523,CHUFPE1524,CHUFPE1675,CHUFPE1672,CHUFPE1670,CHUFPE1671,CHUFPE1674,CHUFPE1673,CHUFPE1676,CHUFPE1619,CHUFPE1618,CHUFPE1620,CHUFPE1695,CHUFPE1694,CHUFPE1696,CHUFPE1795,CHUFPE1796,CHUFPE1729,CHUFPE1732,CHUFPE1730,CHUFPE1726,CHUFPE1723,CHUFPE1731,CHUFPE1725,CHUFPE1727,CHUFPE1728,CHUFPE1724,CHUFPE1755,CHUFPE1758,CHUFPE1753,CHUFPE1750,CHUFPE1751,CHUFPE1759,CHUFPE1756,CHUFPE1752,CHUFPE1754,CHUFPE1760,CHUFPE1757,CHUFPE1761,CHUFPE1766,CHUFPE1773,CHUFPE1770,CHUFPE1763,CHUFPE1765,CHUFPE1772,CHUFPE1762,CHUFPE1767,CHUFPE1771,CHUFPE1768,CHUFPE1764,CHUFPE1769;
Dendropsophus oliveirai:
CHUFPE1370,CHUFPE1378,CHUFPE1372,CHUFPE1376,CHUFPE1374,CHUFPE1377,CHUFPE1369,CHUFPE1371,CHUFPE1375,CHUFPE1373,CHUFPE1748,CHUFPE1749,CHUFPE1364,CHUFPE1366,CHUFPE1703,CHUFPE1704,CHUFPE1700,CHUFPE1808,CHUFPE1807,CHUFPE1805,CHUFPE1804,CHUFPE1801,CHUFPE1803,CHUFPE1802,CHUFPE1518,CHUFPE1677,CHUFPE1608,CHUFPE1609,CHUFPE1607,CHUFPE1606,CHUFPE1610,CHUFPE1604,CHUFPE1605,CHUFPE1816,CHUFPE1814,CHUFPE1815,CHUFPE1499,CHUFPE1498,CHUFPE1785,CHUFPE1790,CHUFPE1781,CHUFPE1783,CHUFPE1784,CHUFPE1787,CHUFPE1786,CHUFPE1791,CHUFPE1788,CHUFPE1780,CHUFPE1782,CHUFPE1789,CHUFPE1733,CHUFPE1738,CHUFPE1734,CHUFPE1736,CHUFPE1735,CHUFPE1737,CHUFPE1739,CHUFPE2049,CHUFPE2050,CHUFPE2051,CHUFPE2052,CHUFPE2055,CHUFPE2056,CHUFPE2057,CHUFPE2058,CHUFPE2059,CHUFPE2060,CHUFPE2061,CHUFPE2062,CHUFPE2063,CHUFPE2064,CHUFPE2070,CHUFPE2071.

S2. Values of 19 bioclimatic variables (1970 – 2000; ~1km²), elevation (WorldClim 2.1), and Enhanced Vegetation Index (EVI; MODIS VI satellite; Google Earth Engine; ~250m; 2000 – 2022) obtained from *Physalaemus cuvieri* and *Dendropsophus oliveirai* coordinates/localities. Bio1 = annual mean temperature (°C), Bio2 = mean diurnal range (°C), Bio3 = isothermality (%), Bio4 = temperature seasonality (SD), Bio5 = max temperature of warmest month (°C), Bio6 = min temperature of coldest month (°C), Bio7 = temperature annual range (°C), Bio8 = mean temperature of wettest quarter (°C), Bio9 = mean temperature of driest quarter (°C), Bio10 = mean temperature of warmest quarter (°C), Bio11 = mean temperature of coldest quarter (°C), Bio12 = annual precipitation (mm), Bio13 = precipitation of wettest month (mm), Bio14 = precipitation of driest month (mm), Bio15 = precipitation seasonality (%), Bio16 = precipitation of wettest quarter (mm), Bio17 = precipitation of driest quarter (mm), Bio18 = precipitation of warmest quarter (mm), Bio19 = precipitation of coldest quarter (mm), Elev = elevação (m), Evimax = EVI maximum, Evimin = EVI minimum.

Locality	**Bio1**	**Bio2**	**Bio3**	**Bio4**	**Bio5**	**Bio6**	**Bio7**	**Bio8**	**Bio9**	**Bio10**	**Bio11**	**Bio12**	**Bio13**	**Bio14**	**Bio15**	**Bio16**	**Bio17**	**Bio18**	**Bio19**	**Elev**	**EVImax (P.cuvieri)**	**EVImin (P.cuvieri)**	**EVImax (D.oliveirai)**	**EVImin (D.oliveirai)**
1-Pau-Ferro	21.60	8.85	70.80	136.93	28.00	15.50	12.50	21.35	22.25	22.98	19.72	1184.00	171.00	25.00	56.73	470.00	89.00	298.00	421.00	550	0.464543	0.22373	0.464543	0.22373
2-Mata do Siriji	21.82	8.57	69.72	125.47	28.10	15.80	12.30	20.72	22.33	23.17	20.12	1065.00	193.00	18.00	65.32	487.00	71.00	216.00	442.00	537	0.603861	0.183643	0.593687	0.190704
3-João Vasconcelos	20.29	8.71	69.11	150.06	26.90	14.30	12.60	20.83	20.38	21.77	18.18	708.00	105.00	11.00	60.22	284.00	55.00	189.00	251.00	803	0.544013	0.233778	0.547513	0.207117
4-Tabocas	21.30	8.88	69.34	152.37	28.10	15.30	12.80	21.82	21.50	22.75	19.13	718.00	136.00	13.00	66.99	341.00	51.00	238.00	192.00	677	0.542143	0.253261	0.535817	0.25887
5-Catimbau	21.23	9.67	70.05	159.69	28.60	14.80	13.80	21.53	21.45	22.88	19.12	815.00	131.00	19.00	54.99	338.00	69.00	127.00	221.00	823	0.626787	0.220504	0.581939	0.209561
6-Pedra Talhada	21.85	8.11	68.14	146.45	28.20	16.30	11.90	20.50	22.73	23.33	19.92	1434.00	241.00	26.00	68.07	708.00	110.00	154.00	636.00	536	0.595548	0.292122	0.615043	0.285139
7-Craunã	24.24	9.69	67.30	195.04	31.70	17.30	14.40	22.12	24.62	26.08	21.50	637.00	83.00	12.00	48.93	246.00	56.00	148.00	197.00	338	0.6109	0.1502	0.556717	0.272385

S3. Values of 19 bioclimatic variables (WorldClim 2.1) of three different periods in the past obtained for each locality. Values for Mid Holocene and Last Glacial Maximum are averages obtained from values of three models (CCSM4, MIROC-ESM, and MPI-ESM-P).

Mid Holocene (~1km²)																			
Locality	**Bio1**	**Bio2**	**Bio3**	**Bio4**	**Bio5**	**Bio6**	**Bio7**	**Bio8**	**Bio9**	**Bio10**	**Bio11**	**Bio12**	**Bio13**	**Bio14**	**Bio15**	**Bio16**	**Bio17**	**Bio18**	**Bio19**
1-Pau-Ferro	21.10	9.17	69.33	130.53	28.13	14.97	13.17	196.33	22.27	22.47	19.17	1413.00	216.67	25.33	54.67	581.67	116.00	124.67	507.00
2-Mata do Siriji	22.23	9.27	67.33	138.70	29.50	15.83	13.67	203.67	23.40	23.60	20.17	1159.00	202.33	19.33	60.00	525.33	94.00	99.00	451.00
3-João Vasconcelos	19.90	9.23	66.33	150.40	27.40	13.57	13.83	178.33	21.20	21.50	17.67	689.67	116.33	10.33	60.67	305.00	50.33	57.67	254.67
4-Tabocas	21.03	9.57	65.33	166.43	28.87	14.27	14.60	208.00	22.47	22.77	18.60	781.67	142.67	11.67	62.67	367.00	54.67	83.33	200.67
5-Catimbau	20.40	9.47	63.67	178.20	28.43	13.67	14.77	199.67	22.07	22.33	17.87	942.67	138.00	18.00	51.67	384.33	75.33	91.33	272.67
6-Pedra Talhada	21.37	9.10	63.67	161.87	29.30	15.03	14.27	193.33	23.03	23.17	19.03	1559.67	286.67	34.33	65.67	782.33	140.33	149.33	674.00
7-Craunã	23.87	10.10	62.33	191.50	32.37	16.30	16.07	222.00	24.73	25.83	20.97	620.33	91.00	14.33	45.67	243.67	62.33	108.67	183.00
Last Glacial Maximum (~5km²)																			
Locality	**Bio1**	**Bio2**	**Bio3**	**Bio4**	**Bio5**	**Bio6**	**Bio7**	**Bio8**	**Bio9**	**Bio10**	**Bio11**	**Bio12**	**Bio13**	**Bio14**	**Bio15**	**Bio16**	**Bio17**	**Bio18**	**Bio19**

1-Pau-Ferro	18.87	9.57	73.67	122.57	25.63	12.77	12.87	17.10	19.50	20.07	16.97	1323.00	230.67	27.67	53.67	553.67	121.67	304.33	541.33
2-Mata do Siriji	19.53	9.47	72.00	131.43	26.33	13.33	13.00	18.03	20.17	20.90	17.53	1047.00	211.00	20.33	61.00	475.00	92.00	203.00	459.67
3-João Vasconcelos	17.53	9.40	72.33	135.17	24.43	11.53	12.90	15.87	18.10	18.90	15.47	611.00	107.67	12.33	58.00	266.67	57.00	141.33	242.00
4-Tabocas	16.73	9.27	70.67	141.33	23.63	10.63	13.00	16.57	17.23	18.07	14.50	691.67	120.00	12.00	57.67	293.67	60.33	206.67	228.00
5-Catimbau	17.43	9.10	69.67	149.03	24.33	11.37	12.97	17.17	17.60	18.80	15.10	912.00	142.67	22.33	50.33	371.67	90.00	272.67	283.00
6-Pedra Talhada	19.00	9.23	69.67	147.17	26.10	12.93	13.17	17.30	20.13	20.43	16.73	1491.67	280.00	41.67	63.67	736.00	149.00	180.67	668.33
7-Craunã	21.17	9.60	66.33	178.37	28.73	14.33	14.40	19.10	20.53	22.97	18.47	613.33	97.33	17.33	43.67	234.67	72.33	163.00	183.67

Last Interglacial Maximum (~1km²)

Locality	**Bio1**	**Bio2**	**Bio3**	**Bio4**	**Bio5**	**Bio6**	**Bio7**	**Bio8**	**Bio9**	**Bio10**	**Bio11**	**Bio12**	**Bio13**	**Bio14**	**Bio15**	**Bio16**	**Bio17**	**Bio18**	**Bio19**
1-Pau-Ferro	20.30	8.30	61.00	127.00	28.40	14.80	13.60	20.50	18.70	22.00	18.60	1151.00	180.00	25.00	54.00	463.00	103.00	372.00	165.00
2-Mata do Siriji	21.40	8.40	63.00	125.20	29.10	15.90	13.20	21.50	19.80	23.10	19.70	943.00	169.00	19.00	58.00	407.00	90.00	277.00	147.00

3-João Vasconcelos	19.00	8.40	62.00	126.70	26.80	13.30	13.50	19.50	18.20	20.70	17.30	558.00	100.00	10.00	57.00	229.00	46.00	154.00	105.00
4-Tabocas	20.20	8.80	63.00	126.60	28.20	14.40	13.80	21.50	19.30	21.90	18.60	631.00	117.00	11.00	58.00	255.00	46.00	250.00	125.00
5-Catimbau	19.70	8.80	66.00	110.30	27.30	14.10	13.20	21.20	19.00	21.20	18.30	806.00	116.00	17.00	49.00	297.00	63.00	297.00	95.00
6-Pedra Talhada	20.60	8.30	64.00	114.60	27.80	15.00	12.80	20.90	19.10	22.10	19.10	1383.00	235.00	27.00	59.00	656.00	134.00	413.00	169.00
7-Craunã	23.40	9.60	72.00	110.00	30.90	17.60	13.30	23.40	22.60	25.00	22.20	556.00	73.00	12.00	43.00	199.00	50.00	180.00	91.00

S4. Variance values of 19 bioclimatic variables between Last Interglacial Maximum and current days obtained for each locality from values of the same variables in each period (Last Interglacial Maximum, Last Glacial Maximum, Mid Holocene, and current days).

Locality	**Bio1**	**Bio2**	**Bio3**	**Bio4**	**Bio5**	**Bio6**	**Bio7**	**Bio8**	**Bio9**	**Bio10**	**Bio11**	**Bio12**	**Bio13**	**Bio14**	**Bio15**	**Bio16**	**Bio17**	**Bio18**	**Bio19**
1-Pau-Ferro	2.66	0.15	9.35	821.42	3.90	2.21	0.64	3.94	4.38	3.28	3.04	4720.25	135.58	5.36	6.60	1526.99	134.44	10596.96	7450.47
2-Mata do Siriji	1.42	0.27	14.80	40.38	1.99	1.58	0.32	2.22	2.98	1.48	1.56	7838.33	328.11	0.93	9.53	2431.14	112.92	5462.92	23139.14
3-João Vasconcelos	4.44	0.13	12.55	284.78	5.77	4.26	0.68	5.95	5.47	5.02	4.64	3899.73	153.58	0.69	19.45	2477.44	36.52	5826.19	1911.81
4-Tabocas	1.50	0.21	19.19	136.10	1.76	1.38	0.31	4.61	2.44	1.67	1.40	4881.56	46.77	1.06	3.09	1035.89	24.25	3098.85	5228.33
5-Catimbau	1.90	0.06	15.77	1584.30	2.49	2.18	1.30	3.36	3.93	2.00	2.64	1239.48	110.47	6.40	7.09	474.26	91.15	927.14	2388.44
6-Pedra Talhada	1.56	0.32	8.97	396.95	1.76	1.94	0.96	2.61	3.75	1.78	1.87	5780.18	698.44	53.21	14.82	2793.36	279.78	16024.55	60414.11
7-Craunã	1.42	0.29	29.59	36.79	1.65	1.44	0.22	3.37	3.43	1.62	1.41	14931.58	819.51	1.66	1.89	3550.40	211.81	11132.77	28931.81

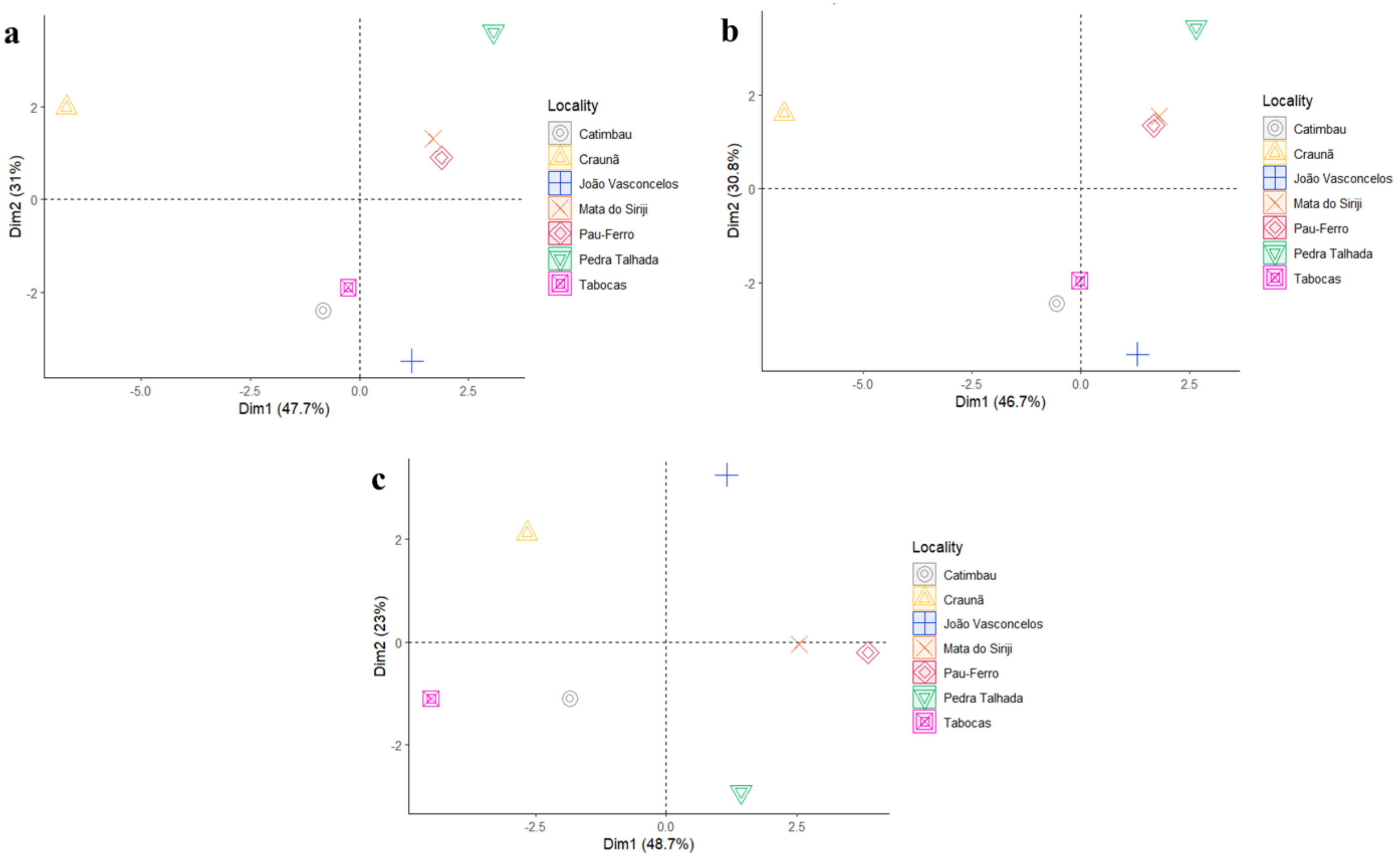
S5. Geodesic geographic distance (km) between seven highland forests where individuals of *Physalaemus cuvieri* and *Dendropsophus oliveirai* were collected.

Locality	Pau-Ferro	Mata do Siriji	João Vasconcelos	Tabocas	Catimbau	Pedra Talhada	Craunã
1-Pau-Ferro	–						
2-Mata do Siriji	75.00	–					
3-João Vasconcelos	156.75	99.96	–				
4-Tabocas	159.01	118.85	40.12	–			
5-Catimbau	244.28	218.56	135.06	101.13	–		
6-Pedra Talhada	264.96	208.00	109.36	112.43	116.17	–	
7-Craunã	354.37	325.12	233.72	206.75	111.42	164.05	–

S6. Scores of principal component analysis for current environmental (EnvPC1), past climatic (PastCliVarPC1), and genetic (GenPC1) variation of each *Physalaemus cuvieri* and *Dendropsophus oliveirai* locality. Data used in Generalized Linear Model (GLM) and Structural Equation Modeling (SEM) analyses.

Locality	***Physalaemus cuvieri***			***Dendropsophus oliveirai***		
	EnvPC1	**PastCliVarPC1**	**GenPC1**	**EnvPC1**	**PastCliVarPC1**	**GenPC1**
1-Pau-Ferro	4.5120	26.8561	-3.02032	5.5564	26.8561	-11.4894
2-Mata do Siriji	5.2100	11.6687	-7.86868	4.4823	11.6687	-5.0823
3-João Vasconcelos	2.7548	2.4084	-21.8808	2.2884	2.4084	-19.1544
4-Tabocas	0.0013	36.4955	-19.9896	0.1088	36.4955	-32.8362
5-Catimbau	0.5070	6.0868	-20.6308	1.1177	6.0868	-26.5269
6-Pedra Talhada	11.4041	3.7254	-22.4138	14.891	3.7254	-13.6353
7-Craunã	75.6108	12.7590	-22.4138	71.5549	12.7590	-17.4561

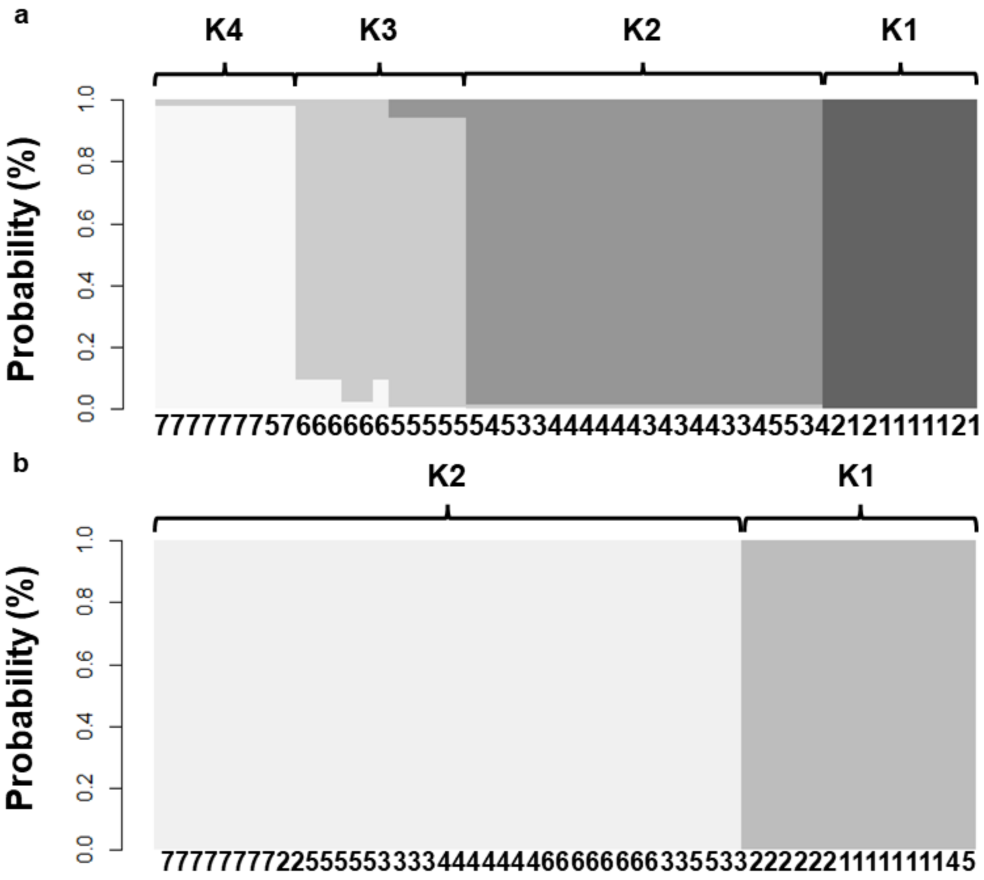
S7. Distribution of seven localities along the first two principal components of three different principal component analysis based on environmental data. Current environmental similarity between *D. oliveirai* (a) localities, current environmental similarity among *P. cuvieri* (b) localities, and similarity in environmental variance in both species (c).



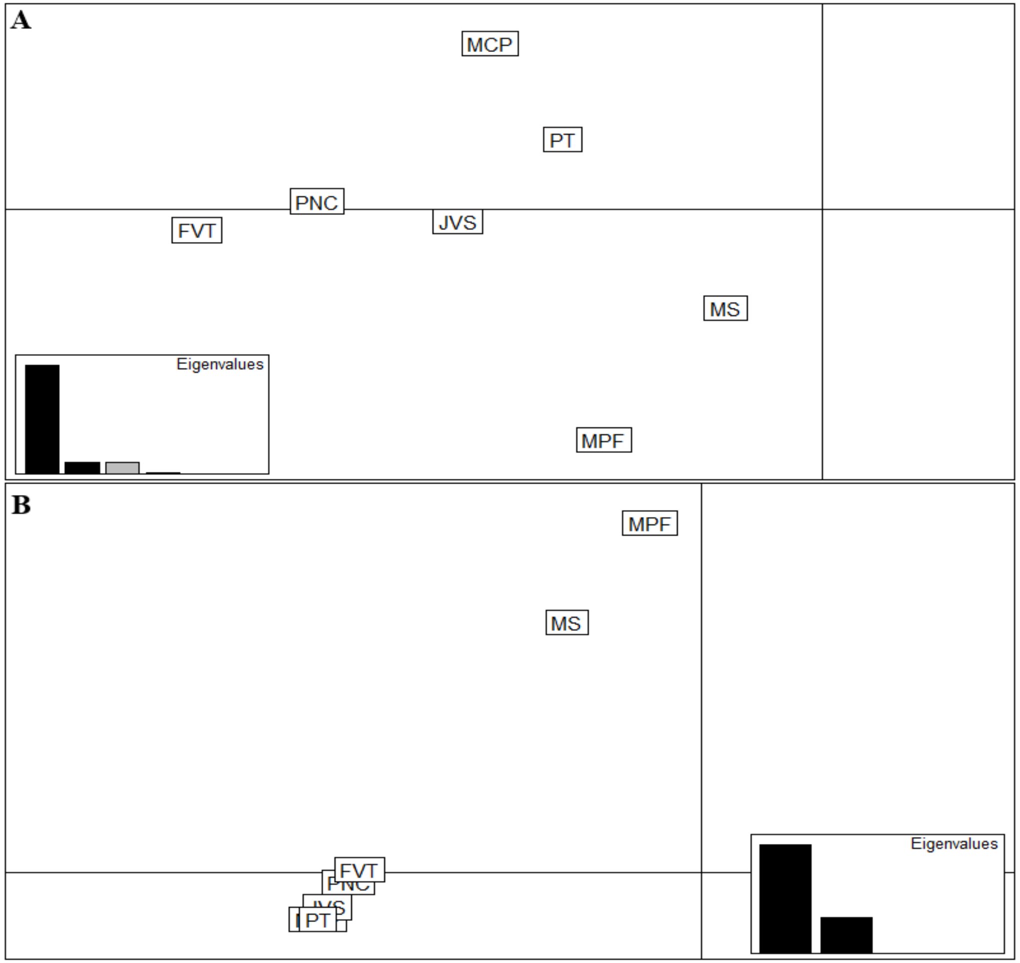
S8. Loadings per environmental variable for the first two dimensions of each principal component analysis. Loadings in bold indicate variables that are strongly related to the respective principal component.

	Present – *D. oliveirai*		**Present - *P. cuvieri***		**Past – Both species**	
	PCA1 (47.7%)	**PCA2 (31.0%)**	**PCA1 (46.7%)**	**PCA2 (30.8%)**	**PCA1 (49.0%)**	**PCA2 (23.0%)**
Bio1	**0.737**	0.669	**0.775**	0.626	**0.878962**	0.268895
Bio2	**0.823**	-0.338	**0.794**	-0.367	**-0.79417**	0.402358
Bio3	-0.550	-0.409	-0.585	-0.318	-0.48365	-0.57361
Bio4	**0.884**	-0.030	**0.893**	-0.113	**0.754923**	0.021845
Bio5	**0.883**	0.448	**0.907**	0.392	**0.880584**	0.241863
Bio6	0.497	**0.856**	0.549	**0.817**	**0.874931**	0.376165
Bio7	**0.926**	-0.219	**0.908**	-0.270	0.695361	0.221361
Bio8	**0.825**	-0.208	**0.807**	-0.240	0.641783	-0.23473
Bio9	0.588	**0.803**	0.636	**0.769**	**0.740742**	0.567581
Bio10	**0.787**	0.614	**0.823**	0.564	**0.851393**	0.308582
Bio11	0.572	**0.791**	0.614	**0.765**	**0.938755**	0.297741
Bio12	**-0.741**	0.620	-0.699	0.672	**-0.7598**	0.355605
Bio13	**-0.820**	0.481	**-0.797**	0.531	-0.54456	**0.711885**
Bio14	-0.578	0.554	-0.542	0.606	-0.04135	0.469634
Bio15	**-0.784**	0.054	**-0.790**	0.078	0.574989	0.54583
Bio16	**-0.767**	0.598	**-0.731**	0.640	-0.45329	**0.711192**
Bio17	-0.581	0.661	-0.530	0.697	-0.44911	0.646481
Bio18	-0.406	-0.026	-0.406	0.051	-0.38971	**0.803907**
Bio19	**-0.718**	0.663	-0.671	**0.708**	-0.8292	0.517775
Elev	-0.542	**-0.788**	-0.593	**-0.761**	**-**	**-**
EVImax	-0.127	**0.788**	0.131	**0.766**	**-**	**-**
EVImin	-0.492	0.145	0.012	-0.138	-	-

S9. Bayesian Analysis of Population Structure (BAPS) based on a fragment of the 16S rDNA mitochondrial gene of *Dendropsophus oliveirai* (a) and *Physalaemus cuvieri* (b) from seven highland forests in Northeastern Brazil. The graphic demonstrates K clusters inferred for each species, the probability of each individual to belong to a cluster, and sings of admixture. Numbers at the bottom of each graphic represents the locality where each individual were collected. 1 = Pau-Ferro, 2 = Mata do Siriji, 3 = João Vasconcelos, 4 = Tabocas, 5 = Catimbau, 6 = Pedra Talhada, 7 = Craunã.



S10. Distribution of seven populations of *D. oliveirai* (A) and *P. cuvieri* (B) along the first two principal components of a principal component analysis based on 16S rDNA mitochondrial gene. Axis x = PC1 and axis y = PC2. PC1 = 80% and PC2 = 9% of genetic variation in *D. oliveirai* and PC1 = 75% and PC2 = 25% of genetic variation in *P. cuvieri*). MPF = Pau-Ferro, MS = Mata do Siriji, JVS = João Vasconcelos, FVT = Tabocas, PNC = Catimbau, PT = Pedra Talhada, MCP = Craunã.



APÊNDICE C - MATERIAL SUPLEMNETAR DO CAPÍTULO 3

Supplementary Material

Table S1. Sampling locations in Northeastern Brazil and their respective localization in the Borborema Plateau, geographic coordinates, elevation, and number of samples for *Dendropsophus oliveirai* and *Physalaemus cuvieri* obtained in each locality.

Sampling locations	City-State	Subregion	Latitude	Longitude	Elevation (m a.s.l)	Field voucher of *Dendropsophus oliveirai*	Field voucher of *Physalaemus cuvieri*
1-Parque Estadual Mata do Pau-Ferro	Areia-Paraíba	Northeast	06°58’12.53” S	35°43’04.27” W	550	7=PIS00355,PIS00360,PIS00356,PIS00358,PIS00357,PIS00359,PIS00361	10=PIS00373,PIS00376,PIS00374,PIS00365,PIS00362,PIS00375,PIS00364,PIS00366,PIS00367,PIS00363
2-Refúgio de Vida Silvestre Matas do Siriji	São Vicente Férrer-Pernambuco	Northeast	07°37’01.79” S	35°30’27.84” W	537	8=PIS00223,PIS00320,PIS00517,PIS00518,PIS00519,PIS00520,PIS00521,PIS00522	10=PIS00220,PIS00221,PIS00222,PIS00298,PIS00295,PIS00293,PIS00294,PIS00297,PIS00296,PIS00310
3-Parque Natural Municipal Professor	Caruaru-Pernambuco	Central	08°21’8.80” S	36°01’51.60” W	803	7=PIS00450,PIS00442,PIS00443,PIS00439,PIS00445,PIS00441,PIS00440	9=PIS00286,PIS00285,PIS00292,PIS00336,PIS00321

João Vasconcelos Sobrinho							,PIS00337,PIS00447,PIS00448,PIS00446
4-Fazenda Vale Tabocas	Belo Jardim-Pernambuco	Central	08°14’47.5” S	36°22’46.00” W	677	12=PIS00166,PIS00194,PIS00188,PIS00192,PIS00190,PIS00193,PIS00165,PIS00187,PIS00191,PIS00189,PIS00386,PIS00387	12=PIS00393,PIS00396,PIS00391,PIS00384,PIS00385,PIS00397,PIS00394,PIS00390,PIS00392,PIS00398,PIS00395,PIS00399
5-Parque Nacional do Catimbau	Buíque-Pernambuco	Central	08°34’20.60” S	37°14’12.30” W	823	10=PIS00273,PIS00274,PIS00272,PIS00271,PIS00275,PIS00263,PIS00264,PIS00456,PIS00454,PIS00455	11=PIS00277,PIS00276,PIS00267,PIS00268,PIS00266,PIS00270,PIS00269,PIS00265,PIS00453,PIS00452,PIS00451
6-Reserva Biológica de Pedra Talhada	Quebrangulo-Alagoas	Southeast	09°15’40.94” S	36°26’20.22” W	536	12=PIS00198,PIS00197,PIS00523,PIS00524,PIS00525,PIS00526,PIS00527,PIS00528,PIS00529,PIS00530,PIS00531,PIS00532	12=PIS00408,PIS00427,PIS00424,PIS00405,PIS00407,PIS00426,PIS00404,PIS00409,PIS00425,PIS00410,PIS00406,PIS00423
7-Refúgio de Vida Silvestre Morros do Craunã e do Padre	Água Branca-Alagoas	Southwest	09°20’47.90” S	37°52’60.00” W	338	10=PIS00152,PIS00135,PIS00155,PIS00153,PIS00154,PIS0	14=PIS00103,PIS00104,PIS00109,PIS00110,PIS00111,PIS00112,PIS00113,PIS

	0156,PIS00342,PIS00351,PIS00544,PIS00545	00114,PIS00115,PIS00116,PIS00117,PIS00118,PIS00119,PIS00120
Total	**66**	**78**

List S2. Individual vouchers at the Coleção Herpetológica da Universidade Federal de Pernambuco, in Recife, Pernambuco, Brazil (CHUFPE).

Physalaemus cuvieri: CHUFPE1319,CHUFPE1320,CHUFPE1321,CHUFPE1322,CHUFPE1323,CHUFPE1324,CHUFPE1325,CHUFPE1326,CHUFPE1327,CHUFPE1328,CHUFPE1329,CHUFPE1330,CHUFPE1331,CHUFPE1332,CHUFPE1603,CHUFPE1602,CHUFPE1598,CHUFPE1599,CHUFPE1597,CHUFPE1601,CHUFPE1600,CHUFPE1596,CHUFPE1813,CHUFPE1812,CHUFPE1811,CHUFPE1522,CHUFPE1523,CHUFPE1524,CHUFPE1675,CHUFPE1672,CHUFPE1670,CHUFPE1671,CHUFPE1674,CHUFPE1673,CHUFPE1676,CHUFPE1619,CHUFPE1618,CHUFPE1620,CHUFPE1695,CHUFPE1694,CHUFPE1696,CHUFPE1795,CHUFPE1796,CHUFPE1794,CHUFPE1729,CHUFPE1732,CHUFPE1730,CHUFPE1726,CHUFPE1723,CHUFPE1731,CHUFPE1725,CHUFPE1727,CHUFPE1728,CHUFPE1724,CHUFPE1755,CHUFPE1758,CHUFPE1753,CHUFPE1750,CHUFPE1751,CHUFPE1759,CHUFPE1756,CHUFPE1752,CHUFPE1754,CHUFPE1760,CHUFPE1757,CHUFPE1761,CHUFPE1766,CHUFPE1773,CHUFPE1770,CHUFPE1763,CHUFPE1765,CHUFPE1772,CHUFPE1762,CHUFPE1767,CHUFPE1771,CHUFPE1768,CHUFPE1764, CHUFPE1769

Dendropsophus oliveirai: CHUFPE1363,CHUFPE1364,CHUFPE1365,CHUFPE1367,CHUFPE1368,CHUFPE1369,CHUFPE1370,CHUFPE1371,CHUFPE1372,CHUFPE1373,CHUFPE1374,CHUFPE1375,CHUFPE1376,CHUFPE1377,CHUFPE1378,CHUFPE1498,CHUFPE1499,CHUFPE1518,CHUFPE1604,CHUFPE1605,CHUFPE1606,CHUFPE1607,CHUFPE1608,CHUFPE1609,CHUFPE1610,CHUFPE1677,CHUFPE1703,CHUFPE1704,CHUFPE1733,CHUFPE1734,CHUFPE1735,CHUFPE1736,CHUFPE1737,CHUFPE1738,CHUFPE1739,CHUFPE1748,CHUFPE1749,CHUFPE1801,CHUFPE1802,CHUFPE1803,CHUFPE1804,CHUFPE1806,CHUFPE1807,CHUFPE1808,CHUFPE1814,CHUFPE1815,CHUFPE1816,CHUFPE2047,CHUFPE2048,CHUFPE2049,CHUFPE2050,CHUFPE2051,CHUFPE2052,CHUFPE2055,CHUFPE2056,CHUFPE2057,CHUFPE2058,CHUFPE2059,CHUFPE2060,CHUFPE2061,CHUFPE2062,CHUFPE2063,CHUFPE2064,CHUFPE2070,CHUFPE2071

Table S3. Summary information from iPyRAD filtering steps on *Dendropsophus oliveirai* GBS reads.

Individuals	state	reads_raw	reads_passed_filter	clusters_total	clusters_hidepth	hetero_est	error_est	reads_consens	loci_in_assembly
A1PIS00135	7	7942034	7880645	1734092	251231	0.009921	0.004664	228448	172040
A2PIS00152	7	11905273	11758718	1657860	382403	0.009356	0.003636	357899	277343
A3PIS00153	7	8927493	8840503	1606448	289139	0.011014	0.004125	266674	208397
A4PIS00155	7	9624069	9518766	1800707	323800	0.010391	0.004467	298026	222495
A4PIS320	7	12533662	12444037	1994969	342266	0.011244	0.004927	309737	141951
A5PIS00156	7	9944000	9852969	1739544	337370	0.010197	0.003558	312510	245168
A5PIS274	7	12412436	12318033	1934787	344802	0.010824	0.004697	313542	154514
A6PIS00165	7	9767336	9667626	1640870	317620	0.010689	0.003848	294015	230734
A6PIS528	7	11556349	11452161	1910945	305391	0.010044	0.005037	275302	143955
A7PIS00166	7	9309567	9207535	1730473	287079	0.01188	0.004626	262033	194883
A8PIS00187	7	295538	272968	106028	3251	0.019147	0.007257	2549	829
A9PIS00188	7	6770673	6692487	1524992	211893	0.010717	0.004518	194043	150066
B10PIS00271	7	10291170	10209474	1663812	339692	0.011631	0.003975	313609	248958
B1PIS00190	7	9462429	9372070	1611730	304871	0.010985	0.00395	281741	223199
B2PIS00191	7	7991323	7934920	1618933	255044	0.011063	0.00419	233173	182630
B2PIS386	7	11230101	11168567	1932426	301785	0.010101	0.005032	271748	138769
B3PIS00192	7	9331413	9259200	1544859	304590	0.010532	0.003787	282001	223293
B4PIS00193	7	2419281	2396462	764177	49361	0.017995	0.006614	41889	25580
B4PIS517	7	2327224	2315720	671872	25119	0.019357	0.007245	19056	6947
B5PIS00194	7	7690630	7644658	1697870	244836	0.012056	0.004589	221571	166550
B7PIS00223	7	9932791	9848736	1693416	324075	0.011246	0.00378	298449	232711
B8PIS00263	7	11399297	11313500	1946956	399333	0.010878	0.003816	369427	281602
B9PIS00264	7	10732358	10615414	1654576	345377	0.011039	0.003834	319007	253295
C10PIS00359	7	10795307	10723513	1793816	370961	0.011493	0.003935	340479	251060
C1PIS00272	7	9958943	9877168	1561117	322017	0.011677	0.003914	297313	232914
C2PIS00273	7	11025677	10928457	1954481	395580	0.010567	0.00406	365136	274954
C3PIS00275	7	11421150	11324763	1718734	371662	0.011612	0.00364	343830	270925
C3PIS443	7	8253842	8120546	1811781	146316	0.012255	0.005583	124518	44640
C5PIS00342	7	13112189	13026908	2092329	449928	0.010254	0.004278	412270	308531
C5PIS456	7	11862823	11783269	2247966	321952	0.009805	0.005172	287571	125385

C6PIS00351	7	14824717	14705299	2129667	483869	0.010829	0.004314	444132	333430
C6PIS530	7	13027247	12939293	2123998	283807	0.01047	0.005399	249124	113705
C7PIS00355	7	10668374	10603568	1855991	358492	0.011719	0.003951	326378	245030
C8PIS00357	7	10208630	10147786	1736026	352627	0.010438	0.003857	323813	238767
C9PIS00358	7	7215812	7183973	1633188	235427	0.011093	0.004304	212892	154366
D10PIS00518	7	8535124	8486500	1712887	287158	0.010945	0.004262	260963	192151
D1PIS00360	7	13583660	13493591	1968135	447299	0.011545	0.003733	410530	307477
D1PIS189	7	14198431	14130900	2193347	388581	0.010625	0.004647	352477	156390
D2PIS00361	7	14189869	14063962	1865729	451907	0.011402	0.003566	416525	312217
D4PIS00387	7	8400625	8333456	1515809	274512	0.01091	0.004217	253087	199546
D5PIS00441	7	13900733	13785664	1963492	450209	0.012107	0.003753	413219	312868
D5PIS197	7	17496427	17411496	2270515	515440	0.009129	0.004229	475314	189781
D6PIS00442	7	11987772	11857874	1739352	376533	0.012188	0.003758	349045	266315
D6PIS531	7	9613650	9448640	1417001	103911	0.015096	0.006296	84167	34862
D7PIS00445	7	12949737	12865085	2034946	429762	0.011349	0.004106	391731	296557
D8PIS00454	7	13046280	12953874	1993196	437413	0.011237	0.00382	401232	304228
D9PIS00455	7	12905222	12803433	1874527	426475	0.010534	0.003781	393054	298811
E10PIS00529	7	10973992	10869820	1576481	336055	0.011932	0.003904	309980	243308
E1PIS00519	7	9004568	8896679	1644010	299564	0.011829	0.004184	275756	205486
E2PIS00520	7	8228664	8154784	1378598	270297	0.010849	0.003905	250862	196115
E3PIS00521	7	7666884	7610039	1616547	238439	0.009963	0.004591	217736	163443
E3PIS450	7	9836612	9765500	2145638	227672	0.011535	0.005628	197828	101244
E4PIS00522	7	10105047	10004018	1542717	321371	0.011643	0.003794	297598	233741
E5PIS00523	7	8559502	8499358	1556838	282033	0.011244	0.003988	259733	202760
E5PIS198	7	9200944	9033946	1838819	190677	0.012607	0.005366	166145	70935
E6PIS00525	7	10679501	10596041	1806613	347474	0.011545	0.004243	319228	248730
E6PIS532	7	11174159	11106157	1973294	213158	0.017136	0.006099	173766	79623
E7PIS00526	7	5220655	5189476	1355278	137931	0.011649	0.005434	122343	80100
E8PIS00527	7	8171224	8084678	1542646	255263	0.011107	0.004432	233661	181028
F4PIS00545	7	11219277	11116347	1816942	397199	0.010877	0.003842	368773	273183

Table S4. Summary information from iPyRAD filtering steps on *Physalaemus cuvieri* GBS reads.

Individuals	**state**	**reads_raw**	**reads_passed_filter**	**clusters_total**	**clusters_hidepth**	**hetero_est**	**error_est**	**reads_consens**	**loci_in_assembly**
A1PIS00103	7	3158011	3156853	432292	130010	0.006009	0.003575	123767	97081
A1PIS285	7	5138598	5126762	614562	245606	0.00644	0.004382	232174	184219
A2PIS269	7	7645279	7598706	646087	273163	0.008339	0.003799	259140	207951
A3PIS406	7	7843198	7791413	718961	270860	0.008374	0.004345	256159	204961
A4PIS293	7	5193016	5170755	644243	261860	0.007395	0.004044	248317	195134
A5PIS104	7	7498203	7463280	670338	255495	0.009137	0.00397	242475	195683
A6PIS116	7	7350353	7327874	682778	269003	0.009168	0.004398	254866	205041
A7PIS391	7	6497382	6469451	647000	267263	0.007535	0.004196	253391	197617
A8PIS00114	7	4002105	3994298	529148	174751	0.007362	0.003745	166559	135012
B1PIS286	7	7950052	7904363	642237	268151	0.009106	0.003732	254815	204598
B2PIS270	7	5978100	5959998	624913	267606	0.007294	0.004027	253966	203577
B3PIS407	7	6348054	6324489	625579	266775	0.008087	0.003962	253379	203308
B4PIS294	7	8741655	8678465	659370	263554	0.008744	0.003828	250751	195333
B5PIS00220	7	5597021	5580997	636563	226665	0.008776	0.003786	215817	164339
B5PIS109	7	5582736	5561689	608617	245384	0.007012	0.003665	233433	187959
B6PIS00221	7	4848406	4841342	584616	203223	0.008939	0.003558	193498	150804
B6PIS117	7	7914077	7879881	650891	264136	0.009055	0.004022	250711	201282
B7PIS00222	7	3044973	3043014	484915	141020	0.006383	0.003609	134057	101715
B8PIS00266	7	3407187	3406105	450817	152315	0.005952	0.003461	145497	113379
B9PIS00268	7	4330938	4325170	532990	184018	0.007012	0.003665	175396	140499
C10PIS00362	7	3394111	3391503	435238	131954	0.007591	0.003372	125521	97483
C1PIS321	7	5292101	5249539	624084	251653	0.007033	0.00413	238524	191708
C2PIS276	7	7917624	7870628	630525	271611	0.007441	0.003609	258120	207162
C3PIS408	7	5440366	5418937	587951	236483	0.007573	0.003587	225217	180253
C4PIS00292	7	4014534	4011199	526763	171749	0.006932	0.003247	163579	130005
C4PIS295	7	5867996	5851316	609451	259299	0.007017	0.004052	246421	194129
C5PIS110	7	6621607	6589637	620455	258154	0.007199	0.003756	245671	197833
C6PIS118	7	5952543	5927305	618073	241240	0.007756	0.004003	228845	182987
C9PIS00337	7	4661083	4649185	570785	188023	0.008676	0.00364	178795	143291
D10PIS00385	7	44154	42610	15211	1357	0.003633	0.003522	1294	754

D1PIS00363	7	6101095	6075639	626658	227760	0.010816	0.003669	217043	163763
D1PIS336	7	5668894	5646295	612760	253871	0.007959	0.003914	241136	194283
D2PIS00364	7	5354453	5337552	620317	218973	0.009796	0.003605	208806	160247
D2PIS277	7	5390444	5374268	604469	253909	0.006542	0.003794	241376	195571
D3PIS00366	7	2413653	2412915	232256	105228	0.004116	0.003419	101558	66916
D3PIS410	7	7067206	7033469	644961	280322	0.007729	0.003865	266105	212341
D4PIS00367	7	4664240	4656823	586008	185673	0.009114	0.003652	175089	138783
D4PIS296	7	5484549	5462965	644327	257940	0.007188	0.004232	244059	191015
D5PIS111	7	6821996	6792645	616562	272788	0.007951	0.003848	259483	208584
D6PIS00374	7	3802850	3798487	527497	169520	0.008016	0.003415	161275	127262
D6PIS119	7	6007413	5987872	636929	266516	0.007319	0.004212	252381	201180
D7PIS00375	7	3285313	3280501	510437	150843	0.007994	0.003577	143189	114042
D8PIS00376	7	4102370	4097151	545680	177919	0.008074	0.003602	168960	133002
D8PIS365	7	737	380	98	14	0.002876	0.002921	13	0
E10PIS00399	7	4265594	4256413	538900	179942	0.008436	0.003509	171387	138703
E1PIS00390	7	3874542	3872839	486356	161040	0.006534	0.003487	153504	121735
E1PIS447	7	5714103	5701116	630038	263461	0.00681	0.004176	249907	200441
E2PIS452	7	6276145	6258559	648829	260819	0.00753	0.004674	247086	200700
E3PIS00392	7	4127121	4119020	561334	187167	0.007609	0.003539	178375	142106
E3PIS423	7	7776942	7719608	657052	258201	0.008753	0.003746	245280	195455
E4PIS00393	7	3641304	3637906	482386	159170	0.006188	0.003384	151741	116883
E4PIS297	7	6407259	6374324	629974	265511	0.006498	0.004153	252448	197693
E5PIS00394	7	4024169	4018045	551334	177036	0.007753	0.003558	168472	135963
E5PIS112	7	5140707	5122604	560920	201352	0.007136	0.003973	190761	146254
E6PIS00395	7	3229556	3228123	473618	142566	0.006821	0.003542	135554	106473
E6PIS120	7	6922452	6894647	664761	264230	0.008069	0.004518	249587	199895
E7PIS00396	7	4977372	4958498	676554	204296	0.009563	0.005378	191537	149655
E8PIS00397	7	3517918	3514396	486279	152207	0.006954	0.003427	144773	115798
E9PIS00398	7	4534380	4526294	544269	183370	0.009112	0.003407	174606	140990
F10PIS00446	7	6371403	6339191	601906	221521	0.010906	0.00348	211357	160878
F1PIS448	7	5676190	5656961	637869	264191	0.007178	0.004116	250477	200844
F2PIS453	7	5389768	5374041	585714	242450	0.005851	0.003813	231313	186499

F3PIS00409	7	3170527	3168717	463802	138531	0.006139	0.003576	131666	98949
F3PIS424	7	8107048	8050042	686224	278677	0.008847	0.004111	264544	212854
F4PIS298	7	6330326	6298008	624448	263285	0.007997	0.003859	250554	197974
F5PIS113	7	8259206	8192341	663259	259493	0.009252	0.003811	246607	197136
F6PIS384	7	7700481	7642324	658702	267676	0.008798	0.004141	253875	197847
F7PIS00425	7	4967357	4955095	567915	197578	0.010302	0.003367	188455	147951
F8PIS00426	7	4589432	4586235	560911	194475	0.007374	0.003472	185444	144514
G1PIS265	7	6454224	6423981	633339	262046	0.007437	0.003908	249065	199645
G2PIS00451	7	5005174	4996946	606638	212957	0.008955	0.003728	203307	162376
G2PIS404	7	3756938	3748838	517789	190266	0.005994	0.003961	180799	138139
G3PIS427	7	7469047	7419913	706585	272432	0.008404	0.004507	258271	208256
G4PIS310	7	6663867	6633291	659072	263729	0.007522	0.004044	250624	201626
H1PIS267	7	729	708	469	2	0.003384	0.003116	2	1
H2PIS405	7	10935	10888	5807	122	0.014821	0.007181	89	42

Table S5. Summary information from the first *VCFTools* filtering on *Dendropsophus oliveirai* and *Physalaemus cuvieri* data after *iPyRAD* filtering steps.

		Dendropsophus oliveirai			***Physalaemus cuvieri***				
		keeping 30% missing data					**keeping 50% missing data**		
Individuals	**N_DATA**	**N_GENOTYPES_FILTERED**	**N_MISS**	**F_MISS**	**Individuals**	**N_DATA**	**N_GENOTYPES_FILTERED**	**N_MISS**	**F_MISS**
A1PIS00135	3820	0	107	0.028011	A1PIS00103	72254	0	71993	0.996388
A2PIS00152	3820	0	33	0.008639	A1PIS285	72254	0	9303	0.128754
A3PIS00153	3820	0	96	0.025131	A2PIS269	72254	0	1481	0.020497
A4PIS00155	3820	0	174	0.04555	A3PIS406	72254	0	1311	0.018144
A4PIS320	3820	0	3705	0.969895	A4PIS293	72254	0	5845	0.080895
A5PIS00156	3820	0	20	0.005236	A5PIS104	72254	0	1482	0.020511
A5PIS274	3820	0	3712	0.971728	A6PIS116	72254	0	1422	0.019681
A6PIS00165	3820	0	15	0.003927	A7PIS391	72254	0	2601	0.035998
A6PIS528	3820	0	3732	0.976963	A8PIS00114	72254	0	71795	0.993647
A7PIS00166	3820	0	550	0.143979	B1PIS286	72254	0	1335	0.018477
A8PIS00187	3820	0	3749	0.981414	B2PIS270	72254	0	2103	0.029106
A9PIS00188	3820	0	119	0.031152	B3PIS407	72254	0	1998	0.027652
B10PIS00271	3820	0	27	0.007068	B4PIS294	72254	0	1714	0.023722
B1PIS00190	3820	0	23	0.006021	B5PIS00220	72254	0	71727	0.992706
B2PIS00191	3820	0	55	0.014398	B5PIS109	72254	0	2721	0.037659
B2PIS386	3820	0	3733	0.977225	B6PIS00221	72254	0	71779	0.993426
B3PIS00192	3820	0	37	0.009686	B6PIS117	72254	0	1752	0.024248
B4PIS00193	3820	0	3606	0.943979	B7PIS00222	72254	0	71988	0.996319
B4PIS517	3820	0	3820	1	B8PIS00266	72254	0	71917	0.995336
B5PIS00194	3820	0	400	0.104712	B9PIS00268	72254	0	71857	0.994505
B7PIS00223	3820	0	24	0.006283	C10PIS00362	72254	0	72001	0.996498
B8PIS00263	3820	0	35	0.009162	C1PIS321	72254	0	4423	0.061215
B9PIS00264	3820	0	29	0.007592	C2PIS276	72254	0	1302	0.01802
C10PIS00359	3820	0	97	0.025393	C3PIS408	72254	0	2907	0.040233
C1PIS00272	3820	0	152	0.039791	C4PIS00292	72254	0	71855	0.994478
C2PIS00273	3820	0	83	0.021728	C4PIS295	72254	0	4026	0.05572
C3PIS00275	3820	0	34	0.008901	C5PIS110	72254	0	1482	0.020511
C3PIS443	3820	0	3806	0.996335	C6PIS118	72254	0	2082	0.028815

C5PIS00342	3820	0	55	0.014398	C9PIS00337	72254	0	71833	0.994173
C5PIS456	3820	0	3768	0.986387	D10PIS00385	72254	0	72253	0.999986
C6PIS00351	3820	0	41	0.010733	D1PIS00363	72254	0	71692	0.992222
C6PIS530	3820	0	3770	0.986911	D1PIS336	72254	0	2475	0.034254
C7PIS00355	3820	0	82	0.021466	D2PIS00364	72254	0	71743	0.992928
C8PIS00357	3820	0	60	0.015707	D2PIS277	72254	0	4819	0.066695
C9PIS00358	3820	0	420	0.109948	D3PIS00366	72254	0	72046	0.997121
D10PIS00518	3820	0	164	0.042932	D3PIS410	72254	0	1403	0.019418
D1PIS00360	3820	0	37	0.009686	D4PIS00367	72254	0	71867	0.994644
D1PIS189	3820	0	3683	0.964136	D4PIS296	72254	0	5094	0.070501
D2PIS00361	3820	0	51	0.013351	D5PIS111	72254	0	1945	0.026919
D4PIS00387	3820	0	27	0.007068	D6PIS00374	72254	0	71928	0.995488
D5PIS00441	3820	0	57	0.014922	D6PIS119	72254	0	3079	0.042614
D5PIS197	3820	0	3668	0.960209	D7PIS00375	72254	0	71958	0.995903
D6PIS00442	3820	0	230	0.060209	D8PIS00376	72254	0	71872	0.994713
D6PIS531	3820	0	3818	0.999476	D8PIS365	72254	0	72254	1
D7PIS00445	3820	0	40	0.010471	E10PIS00399	72254	0	71866	0.99463
D8PIS00454	3820	0	31	0.008115	E1PIS00390	72254	0	71883	0.994865
D9PIS00455	3820	0	52	0.013613	E1PIS447	72254	0	3324	0.046004
E10PIS00529	3820	0	61	0.015969	E2PIS452	72254	0	1729	0.02393
E1PIS00519	3820	0	142	0.037173	E3PIS00392	72254	0	71888	0.994935
E2PIS00520	3820	0	44	0.011518	E3PIS423	72254	0	1444	0.019985
E3PIS00521	3820	0	137	0.035864	E4PIS00393	72254	0	72000	0.996485
E3PIS450	3820	0	3764	0.98534	E4PIS297	72254	0	2727	0.037742
E4PIS00522	3820	0	20	0.005236	E5PIS00394	72254	0	71887	0.994921
E5PIS00523	3820	0	52	0.013613	E5PIS112	72254	0	11803	0.163354
E5PIS198	3820	0	3816	0.998953	E6PIS00395	72254	0	72007	0.996582
E6PIS00525	3820	0	47	0.012304	E6PIS120	72254	0	2236	0.030946
E6PIS532	3820	0	3783	0.990314	E7PIS00396	72254	0	71773	0.993343
E7PIS00526	3820	0	1815	0.475131	E8PIS00397	72254	0	72002	0.996512
E8PIS00527	3820	0	355	0.092932	E9PIS00398	72254	0	71854	0.994464
F4PIS00545	3820	0	130	0.034031	F10PIS00446	72254	0	71738	0.992859

	F1PIS448	72254	0	3307	0.045769
	F2PIS453	72254	0	4108	0.056855
	F3PIS00409	72254	0	72024	0.996817
	F3PIS424	72254	0	1793	0.024815
	F4PIS298	72254	0	2286	0.031638
	F5PIS113	72254	0	1839	0.025452
	F6PIS384	72254	0	2107	0.029161
	F7PIS00425	72254	0	71815	0.993924
	F8PIS00426	72254	0	71850	0.994409
	G1PIS265	72254	0	1560	0.021591
	G2PIS00451	72254	0	71766	0.993246
	G2PIS404	72254	0	19703	0.272691
	G3PIS427	72254	0	1207	0.016705
	G4PIS310	72254	0	1426	0.019736
	H1PIS267	72254	0	72254	1
	H2PIS405	72254	0	72254	1

Table S6. Summary information from the second *VCFTools* filtering on *Dendropsophus oliveirai* and *Physalaemus cuvieri* data after *iPyRAD* filtering steps.

		Dendropsophus oliveirai					***Physalaemus cuvieri***		
		keeping 10% missing data					**keeping 10% missing data**		
Individuals	**N_DATA**	**N_GENOTYPES_FILTERED**	**N_MISS**	**F_MISS**	**Individuals**	**N_DATA**	**N_GENOTYPES_FILTERED**	**N_MISS**	**F_MISS**
A1PIS00135	25174	0	2784	0.11059	A1PIS285	38944	0	4360	0.111956
A2PIS00152	25174	0	354	0.014062	A2PIS269	38944	0	977	0.025087
A3PIS00153	25174	0	1560	0.061969	A3PIS406	38944	0	1002	0.025729
A4PIS00155	25174	0	2139	0.084969	A4PIS293	38944	0	2472	0.063476
A5PIS00156	25174	0	656	0.026059	A5PIS104	38944	0	1235	0.031712
A6PIS00165	25174	0	595	0.023636	A6PIS116	38944	0	1207	0.030993
A7PIS00166	25174	0	3591	0.142647	A7PIS391	38944	0	1542	0.039595
B10PIS00271	25174	0	651	0.02586	B1PIS286	38944	0	1012	0.025986
B1PIS00190	25174	0	655	0.026019	B2PIS270	38944	0	1314	0.033741
B2PIS00191	25174	0	1662	0.066021	B3PIS407	38944	0	1071	0.027501
B3PIS00192	25174	0	704	0.027965	B4PIS294	38944	0	1558	0.040006
B5PIS00194	25174	0	4614	0.183284	B5PIS109	38944	0	2024	0.051972
B7PIS00223	25174	0	753	0.029912	B6PIS117	38944	0	1161	0.029812
B8PIS00263	25174	0	687	0.02729	C1PIS321	38944	0	2183	0.056055
B9PIS00264	25174	0	642	0.025503	C2PIS276	38944	0	834	0.021415
C10PIS00359	25174	0	2052	0.081513	C3PIS408	38944	0	2494	0.064041
C1PIS00272	25174	0	1401	0.055653	C4PIS295	38944	0	2229	0.057236
C2PIS00273	25174	0	1045	0.041511	C5PIS110	38944	0	1372	0.03523
C3PIS00275	25174	0	637	0.025304	C6PIS118	38944	0	1739	0.044654
C5PIS00342	25174	0	790	0.031382	D1PIS336	38944	0	1471	0.037772
C6PIS00351	25174	0	597	0.023715	D2PIS277	38944	0	2429	0.062372
C7PIS00355	25174	0	1735	0.06892	D3PIS410	38944	0	838	0.021518
C8PIS00357	25174	0	1992	0.079129	D4PIS296	38944	0	2475	0.063553
D10PIS00518	25174	0	4179	0.166005	D5PIS111	38944	0	1015	0.026063
D1PIS00360	25174	0	759	0.03015	D6PIS119	38944	0	1583	0.040648
D2PIS00361	25174	0	651	0.02586	E1PIS447	38944	0	1902	0.048839
D4PIS00387	25174	0	1022	0.040597	E2PIS452	38944	0	1156	0.029684
D5PIS00441	25174	0	715	0.028402	E3PIS423	38944	0	1179	0.030274

D6PIS00442	25174	0	1716	0.068166	E4PIS297	38944	0	1703	0.04373
D7PIS00445	25174	0	859	0.034123	E6PIS120	38944	0	1260	0.032354
D8PIS00454	25174	0	697	0.027687	F1PIS448	38944	0	1775	0.045578
D9PIS00455	25174	0	643	0.025542	F2PIS453	38944	0	3063	0.078651
E10PIS00529	25174	0	911	0.036188	F3PIS424	38944	0	1084	0.027835
E1PIS00519	25174	0	2591	0.102924	F4PIS298	38944	0	1278	0.032816
E2PIS00520	25174	0	1133	0.045007	F5PIS113	38944	0	1354	0.034768
E3PIS00521	25174	0	2900	0.115198	F6PIS384	38944	0	1393	0.035769
E4PIS00522	25174	0	625	0.024827	G1PIS265	38944	0	1149	0.029504
E5PIS00523	25174	0	1203	0.047787	G3PIS427	38944	0	895	0.022982
E6PIS00525	25174	0	802	0.031858	G4PIS310	38944	0	1184	0.030403
E8PIS00527	25174	0	2671	0.106102					
F4PIS00545	25174	0	1606	0.063796					

Table S7. Dataset summary after VCFTools filtering by population (cluster-based) of *Dendropsophus oliveirai* and *Physalaemus cuvieri* from northeastern Brazil. Pop/pop = population. K1, K2 and K3 based on sNMF and DAPC analyses.

		Dendropsophus oliveirai			***Physalaemus cuvieri***	
K1	**Individual**	**Loci_mean_length**	**Missing_data**	**Individual**	**Loci_mean_length**	**Missing_data**
	A1PIS00135	94.23	82.83	**A6PIS116**	119.30	75.37
	A2PIS00152	94.62	72.33	**B6PIS117**	117.50	75.83
	A3PIS00153	91.68	79.21	**C5PIS110**	120.41	76.24
	A4PIS00155	90.12	77.8	**D5PIS111**	119.51	74.95
	A5PIS00156	91.81	75.54	**E2PIS452**	120.54	75.9
	C5PIS00342	91.52	69.22	**E6PIS120**	119.46	75.99
	C6PIS00351	93.11	66.73	**F3PIS424**	118.14	74.43
	F4PIS00545	86.91	72.74	**F5PIS113**	116.39	76.32
				G3PIS427	119.43	74.99
				G4PIS310	120.42	75.79
Pop_mean_length		91.75			119.11	
Total_loci		620,488			439,209	
Shared_loci		34,607			44,978	
Shared_loci*pop_mean_length		3,175,096			5,357,354	
SNPs		106,136			374,712	
K2	**Individual**	**Loci_mean_length**	**Missing_data**	**Individual**	**Loci_mean_length**	**Missing_data**
	A6PIS00165	93.50	76.98	**D1PIS00363**	116.13	80.33
	B8PIS00263	93.15	71.9	**D2PIS00364**	116.88	80.75
	C2PIS00273	89.75	72.57	**B5PIS00220**	117.49	80.26
	C3PIS00275	91.33	72.97	**B6PIS00221**	118.21	81.89
	D5PIS00441	92.59	68.78	**F10PIS00446**	114.03	80.68
	D6PIS00442	89.58	73.43	**E7PIS00396**	115.82	82.03
	D7PIS00445	92.31	70.41	**E9PIS00398**	120.44	83.07
	D8PIS00454	93.01	69.65	**G2PIS00451**	119.47	80.5
	D9PIS00455	94.75	70.19	**F7PIS00425**	117.51	82.23
	E6PIS00525	90.13	75.18	**F8PIS00426**	123.37	82.64
Pop_mean_length		92.01090903			117.94	

Total_loci		686,799			323,588	
Shared_loci		34,665			23,954	
Shared_loci*pop_mean_length		3,189,558			2,825,029	
SNPs		218,378			125,633	
K3	**Individual**	**Loci_mean_length**	**Missing_data**	**Individual**	**Loci_mean_length**	**Missing_data**
	B7PIS00223	93.69	76.78	**A4PIS293**	123.86	76.57
	C10PIS00359	97.05	74.95	**A7PIS391**	120.01	76.27
	C7PIS00355	94.40	75.55	**B4PIS294**	118.40	76.54
	C8PIS00357	99.25	76.18	**C4PIS295**	121.64	76.69
	D10PIS00518	101.69	80.83	**D4PIS296**	123.14	77.06
	D1PIS00360	93.58	69.32	**E4PIS297**	120.35	76.26
	D2PIS00361	92.58	68.85	**F4PIS298**	118.79	76.22
	E2PIS00520	95.67	80.43	**F6PIS384**	117.28	76.24
	E4PIS00522	93.94	76.68			
Pop_mean_length		95.76			120.43	
Total_loci		590,555			400,934	
Shared_loci		33,618			45,939	
Shared_loci*pop_mean_length		3,219,292			5,532,613	
SNPs		114,571			223,242	

Table S8. Parameters and prior distributions used to test alternative scenarios for diversification, migration, and effective population size changes (N_e) in populations of *Dendropsophus oliveirai* and *Physalaemus cuvieri* forest enclaves northeastern Brazil using Approximate Bayesian Computation and Supervised Machine Learning. Ne1 and mig1 = N_e and migration, Ne0 and mig0 = current N_e and migration, LIG = last interglacial maximum, LGM = last glacial maximum. See Figure 1 for a schematic representation of the hierarchical models.

		D. oliveirai		***P. cuvieri***			
	Loci	4,603		6,000			
Models		**Min**	**Max**	**Min**	**Max**	**Condition**	**Max**
	Divergence						
A-LIG, B-LIG		10,000	3,000,000	10,000	3,000,0000		
IS, MIG, MIG-T1, MIG-T2, MIG-CT2		10,000 – 1,000,000 (no change)		10,000 – 1,000,000 (no change)			
ISE, MIGE, MIGE-T1, MIGE-T2, MIGE-CT2	**Ne1**	10,000	100,000	10,000	500,000		
ISDE, MIGDE, MIGDE-T1, MIGDE-T2, MIGDE-CT2(Pop1)		10,000	50,000	10,000	100,000		
ISDE, MIGDE, MIGDE-T1, MIGDE-T2, MIGDE (Pop2)		10,000	500,000	10,000	700,000		
ISDE, MIGDE, MIGDE-T1, MIGDE-T2, MIGDE (Pop3)		10,000	1,000,000	10,000	900,000		
	Ne0	**Min**	**Max**	**Min**	**Max**		
ISE, MIGE, MIGE-T1, MIGE-T2, MIGE-CT2, ISDE, MIGDE, MIGDE-T1, MIGDE-T2, MIGDE-CT2 (Pop3)		50,000	1,300,000	50,000	1,000,000		
ISDE, MIGDE, MIGDE-T1, MIGDE-T2, MIGDE-CT2 (Pop1)		50,000	500,000	50,000	500,000		
ISDE, MIGDE, MIGDE-T1, MIGDE-T2, MIGDE-CT2 (Pop2)		50,000	800,000	50,000	900,000		
	Migration	**Min**	**Max**	**Min**	**Max**		
MIG, MIGE, MIGDE		0.1 – 6.0 (no change)		0.1 – 10.0 (no change)			
IS, ISE, ISDE		Absent		Absent			
MIG-T1, MIGE-T1, MIGDE-T1	**mig1**	0.1	6.0	0.1	10.0		
	mig0	0.0	0.0	0.0	0.0		
MIG-T2, MIGE-T2, MIGDE-T2	**mig1**	0.0	0.0	0.0	0.0		
	mig0	0.1	6.0	0.1	10.0		

MIG-CT2, MIGE-CT2, MIGDE-CT2	**mig1**	0.1	6.0	0.1	10.0	In MIGE-CT2 and MIGDE-CT2 mig0 > mig1
	mig0	0.1	6.0	0.1	10.0	
	Divergence time	**Min**	**Max**	**Min**	**Max**	
After LIG		20,000	120,000	20,000	120,000	Always join1_2 < join2_3 (pop3 diverge first), based on previous analyses (*SNAPP*)
All the other models		120,000	1,000,000	120,000	1,000,000	Always join1_2 < join2_3 (pop3 diverge first), based on previous analyses (*SNAPP*)
	Migration time					
MIG, MIGE, MIGDE		No change		No change		
MIG-T1, MIGE-T1, MIGDE-T1		Until 30,000 years ago (LGM)		Until 30,000 years ago (LGM)		
MIG-T2, MIGE-T2, MIGDE-T2		Isolation until 30,000 years ago, migration from there until now		Isolation until 30,000 years ago, migration from there until now		
MIG-CT2, MIGE-CT2, MIGDE-CT2		Any change from 30,000 years ago until now		Any change from 30,000 years ago until now		
	Ne change time					
ISE, MIGE, MIGE-T1, MIGE-T2, MIGE-CT2, ISDE, MIGDE, MIGDE-T1, MIGDE-T2, MIGDE-CT2,	**Ne1**	Until 30,000 years ago (LGM)		Until 30,000 years ago (LGM)		
	Ne0	From 30,000 years ago until now		From 30,000 years ago until now		
		Min-Max		**Min-Max**		

	Mutation rate (Gehara et al. 2017)	$5\text{x}10^{-10} – 5 \times 10^{-9}$	$5\text{x}10^{-10} – 5 \times 10^{-9}$

Figure S9. Histograms of simulated and observed Tajima's D for each population of *Dendropsophus oliveirai* (a) and *Physalaemus cuvieri* (b). Observe the negative in s_average. 1 = Pop1, 2 = Pop2; 3 = Pop3.

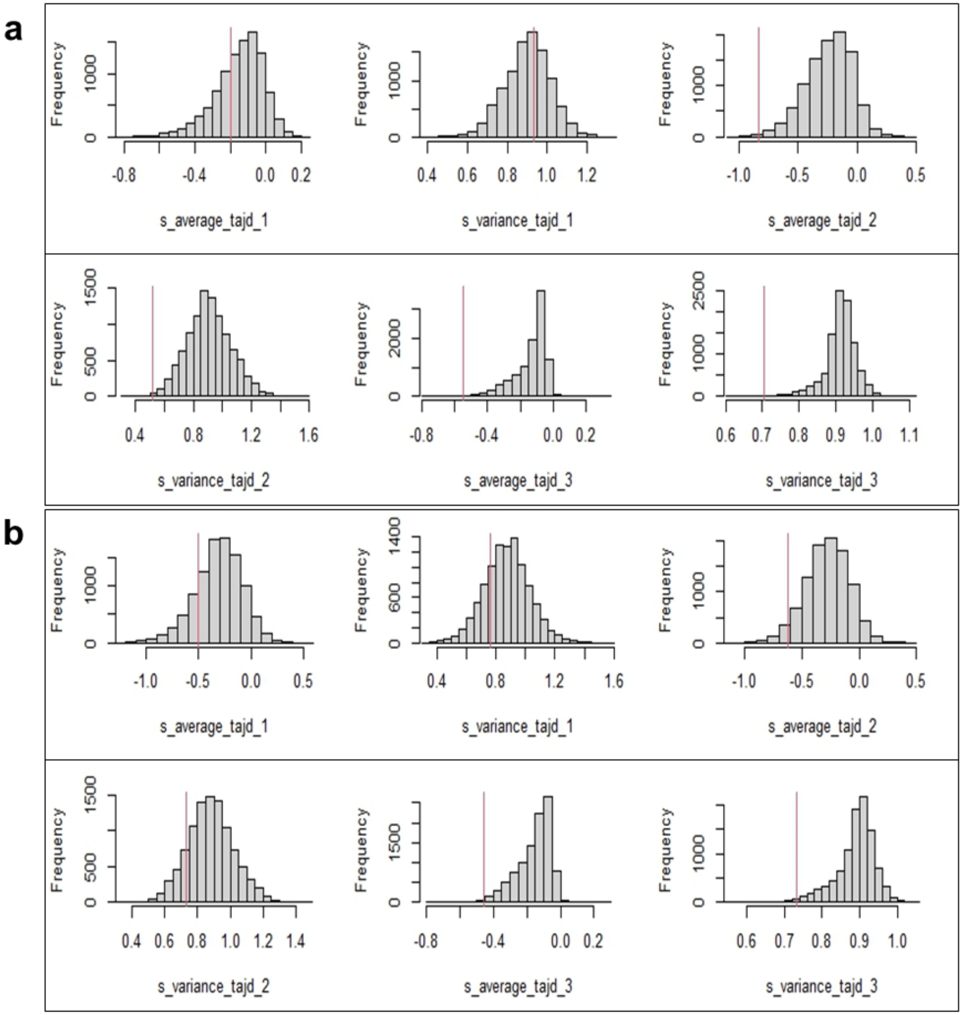


Table S10. Individual population assignment in *Dendropsophus oliveirai* from northeastern Brazil estimated by sNMF (Sparse Non-Negative Matrix Factorization) and DAPC (Discriminant Analysis of Principal Components) using GBS data with one SNP per locus.

		sNMF			**DAPC**		
Individual	**Locality**	**K1**	**K2**	**K3**	**K1**	**K2**	**K3**
PIS359	1	1.00E-04	1.00E-04	0.9998	7.59E-18	2.73E-12	1
PIS355	1	1.00E-04	1.00E-04	0.9998	5.42E-21	1.40E-13	1
PIS357	1	1.00E-04	1.00E-04	0.9998	4.31E-20	1.44E-13	1
PIS358	1	1.00E-04	0.063362	0.936538	2.91E-20	1.97E-10	1
PIS360	1	0.007993	1.00E-04	0.991907	4.79E-22	1.74E-13	1
PIS361	1	1.00E-04	1.00E-04	0.9998	4.89E-22	2.55E-12	1
PIS519	2	0.203865	0.006457	0.789678	2.97E-15	7.42E-07	0.999999
PIS520	2	0.17021	0.00952	0.82027	5.92E-18	2.07E-08	1
PIS521	2	0.254164	1.00E-04	0.745736	1.92E-14	9.49E-05	0.999905
PIS522	2	0.001268	0.002644	0.996088	4.24E-18	6.21E-09	1
PIS518	2	0.051916	1.00E-04	0.947984	5.45E-19	2.87E-07	1
PIS441	3	0.600892	1.00E-04	0.399008	1.00E-11	1	4.65E-09
PIS442	3	0.516954	0.109819	0.373228	1.83E-10	0.99999931	6.88E-07
PIS445	3	0.719358	0.003353	0.277289	6.10E-11	0.99999999	7.39E-09
PIS165	4	0.615736	0.005797	0.378467	1.33E-09	0.9999997	2.99E-07
PIS166	4	0.40603	0.21867	0.375299	2.55E-11	1	1.29E-10
PIS188	4	0.456079	0.053594	0.490327	1.47E-13	0.99999958	4.17E-07
PIS190	4	0.610537	1.00E-04	0.389363	3.26E-07	0.99999958	9.21E-08
PIS191	4	0.578146	0.046403	0.375451	1.17E-13	1	1.80E-09
PIS192	4	0.69719	1.00E-04	0.30271	4.76E-14	1	2.20E-10
PIS194	4	0.56406	0.080112	0.355828	1.73E-13	0.99999999	9.34E-09
PIS387	4	0.528456	0.019893	0.451651	1.33E-07	0.99998094	1.89E-05
PIS271	4	0.817176	1.00E-04	0.182724	7.28E-07	0.99999927	1.05E-11
PIS263	5	0.898714	1.00E-04	0.101186	7.27E-11	1	2.42E-15
PIS264	5	0.898391	0.015102	0.086507	6.28E-07	0.99999937	6.17E-13
PIS272	5	0.891955	1.00E-04	0.107945	8.84E-07	0.99999912	4.85E-13
PIS273	5	0.759765	1.00E-04	0.240135	3.78E-07	0.99999962	2.99E-11
PIS275	5	0.914222	0.003958	0.08182	1.29E-07	0.99999987	2.40E-16

PIS454	5	0.848594	0.007097	0.144309	2.13E-08	0.99999998	5.21E-14
PIS455	5	0.894803	0.014984	0.090213	3.21E-09	1	1.63E-14
PIS223	2	1.00E-04	1.00E-04	0.9998	3.60E-22	1.21E-12	1
PIS529	6	0.981664	0.018236	1.00E-04	4.20E-07	0.99999958	2.97E-14
PIS523	6	0.829556	0.030681	0.139763	3.93E-08	0.99999996	8.40E-15
PIS525	6	0.867204	0.022493	0.110303	5.46E-06	0.99999454	1.69E-14
PIS526	6	1.00E-04	0.9998	1.00E-04	5.22E-07	0.99999945	2.37E-08
PIS527	6	0.801651	0.145527	0.052822	0.000473	0.99952728	7.07E-11
PIS135	7	0.9998	1.00E-04	1.00E-04	1	2.18E-07	5.18E-19
PIS152	7	0.9998	1.00E-04	1.00E-04	1	5.94E-08	6.33E-20
PIS153	7	0.964286	0.035614	1.00E-04	0.999999	6.60E-07	5.36E-19
PIS155	7	0.983132	1.00E-04	0.016768	1	8.00E-12	1.23E-19
PIS156	7	0.92901	1.00E-04	0.07089	1	1.98E-09	1.68E-17
PIS342	7	0.983009	0.016891	1.00E-04	1	6.51E-11	2.08E-22
PIS351	7	0.919579	1.00E-04	0.080321	0.758358	0.24164239	4.14E-14
PIS545	7	0.9998	1.00E-04	1.00E-04	1	5.65E-10	3.61E-19

Table S11. Individual population assignment in *Physalaemus cuvieri* from northeastern Brazil estimated by sNMF (Sparse Non-Negative Matrix Factorization) and DAPC (Discriminant Analysis of Principal Components) using GBS data with one SNP per locus. Some individuals were excluded from DAPC analyses because of high number of missing data.

			sNMF			**DAPC**	
Individual	**Locality**	**K1**	**K2**	**K3**	**K1**	**K2**	**K3**
PIS362	1	0.000604	0.999296	1.00E-04	2.91E-24	1	1.55E-105
PIS363	1	1.00E-04	0.994868	0.005032	4.33E-24	1	6.62E-106
PIS364	1	1.00E-04	0.99841	0.00149	3.57E-24	1	9.92E-106
PIS367	1	1.00E-04	0.995441	0.004459	3.51E-24	1	9.91E-106
PIS296	1	1.00E-04	0.031474	0.968426	7.71E-224	2.64E-104	1
PIS374	1	0.003187	0.996713	1.00E-04	3.87E-24	1	8.17E-106
PIS375	1	0.001445	0.998455	1.00E-04	5.14E-24	1	4.49E-106
PIS376	1	0.002883	0.997017	1.00E-04	4.14E-24	1	6.95E-106
PIS293	2	1.00E-04	0.044499	0.955401	1.82E-181	3.93E-75	1
PIS294	2	0.058212	1.00E-04	0.941688	5.01E-196	6.07E-85	1
PIS220	2	1.00E-04	0.9998	1.00E-04	3.86E-24	1	8.31E-106
PIS221	2	0.001545	0.998355	1.00E-04	4.97E-24	1	4.81E-106
PIS222	2	1.00E-04	0.9998	1.00E-04	4.39E-24	1	6.37E-106
PIS295	2	1.00E-04	0.004933	0.994967	2.71E-218	1.82E-100	1
PIS297	2	1.00E-04	1.00E-04	0.9998	5.61E-252	7.57E-124	1
PIS298	2	0.052152	1.00E-04	0.947748	6.81E-201	2.14E-88	1
PIS310	2	0.92809	1.00E-04	0.07181	1	1.46E-17	1.03E-208
PIS285	3	0.530743	0.090377	0.37888	6.49E-22	1	9.12E-115
PIS286	3	0.709787	1.00E-04	0.290113	2.99E-18	1	3.00E-125
PIS321	3	0.611641	0.038358	0.350001	1.39E-25	1	1.52E-107
PIS292	3	1.00E-04	0.9998	1.00E-04	5.40E-24	1	3.91E-106
PIS337	3	1.00E-04	0.9998	1.00E-04	3.13E-24	1	1.29E-105
PIS336	3	0.66535	0.006623	0.328028	4.86E-22	1	1.71E-115
PIS447	3	0.54821	0.013527	0.438264	5.86E-27	1	2.62E-104
PIS446	3	0.00369	0.99621	1.00E-04	4.31E-24	1	6.43E-106
PIS448	3	0.605615	0.012206	0.382179	5.50E-23	1	2.39E-113

PIS391	4	1.00E-04	1.00E-04	0.9998	5.41E-263	1.69E-131	1
PIS399	4	0.000451	0.999449	1.00E-04	4.38E-24	1	6.19E-106
PIS390	4	0.001504	0.998396	1.00E-04	4.30E-24	1	6.60E-106
PIS392	4	1.00E-04	0.99739	0.00251	3.32E-24	1	1.14E-105
PIS393	4	1.00E-04	0.9998	1.00E-04	4.64E-24	1	5.65E-106
PIS394	4	1.00E-04	0.997007	0.002893	3.90E-24	1	8.08E-106
PIS395	4	1.00E-04	0.9998	1.00E-04	3.64E-24	1	9.32E-106
PIS396	4	0.001326	0.998574	1.00E-04	4.11E-24	1	7.15E-106
PIS397	4	1.00E-04	0.9998	1.00E-04	4.01E-24	1	7.56E-106
PIS398	4	0.003508	0.996392	1.00E-04	4.79E-24	1	5.24E-106
PIS384	4	1.00E-04	1.00E-04	0.9998	2.99E-260	1.22E-129	1
PIS269	5	0.68735	1.00E-04	0.31255	3.24E-20	1	3.73E-120
PIS270	5	0.615314	1.00E-04	0.384586	4.14E-25	1	1.33E-108
PIS266	5	1.00E-04	0.9998	1.00E-04	3.74E-24	1	8.92E-106
PIS268	5	1.00E-04	0.9998	1.00E-04	3.65E-24	1	9.31E-106
PIS276	5	0.632233	1.00E-04	0.367667	4.73E-20	1	1.67E-119
PIS277	5	0.957602	0.042298	1.00E-04	1	2.55E-28	2.10E-234
PIS452	5	0.9998	1.00E-04	1.00E-04	1	1.15E-29	8.40E-239
PIS453	5	0.983649	0.016251	1.00E-04	1	2.75E-30	2.42E-240
PIS265	5	0.659481	1.00E-04	0.340419	3.80E-21	1	1.37E-116
PIS451	5	1.00E-04	0.9998	1.00E-04	4.03E-24	1	7.57E-106
PIS406	6	0.647757	0.002132	0.350111	2.69E-27	1	5.97E-104
PIS407	6	0.625261	1.00E-04	0.374639	1.86E-28	1	2.22E-101
PIS408	6	0.573736	1.00E-04	0.426164	1.19E-33	1	1.16E-89
PIS410	6	0.552192	1.00E-04	0.447708	2.92E-34	1	2.35E-88
PIS423	6	0.577227	0.000117	0.422656	1.32E-30	1	1.32E-96
PIS409	6	1.00E-04	0.999421	0.000479	3.21E-24	1	1.25E-105
PIS424	6	0.995615	0.004285	1.00E-04	1	1.53E-24	9.13E-224
PIS425	6	0.000873	0.999027	1.00E-04	3.43E-24	1	1.07E-105
PIS426	6	1.00E-04	0.9998	1.00E-04	3.05E-24	1	1.41E-105
PIS404	6	0.485645	0.267996	0.246359	2.40E-31	1	4.08E-93
PIS427	6	0.9998	1.00E-04	1.00E-04	1	2.50E-27	7.88E-232

PIS103	7	1.00E-04	0.9998	1.00E-04	2.54E-24	1	2.06E-105
PIS104	7	0.940739	1.00E-04	0.059161	1	1.20E-20	7.68E-220
PIS116	7	0.9998	1.00E-04	1.00E-04	1	9.50E-26	4.98E-232
PIS114	7	1.00E-04	0.9998	1.00E-04	2.75E-24	1	1.77E-105
PIS109	7	0.928557	0.011556	0.059887	1	5.22E-12	5.03E-191
PIS117	7	0.992792	0.007108	1.00E-04	1	2.43E-25	2.34E-231
PIS110	7	0.96236	1.00E-04	0.03754	1	1.35E-21	1.96E-222
PIS118	7	0.999108	0.000792	1.00E-04	1	1.11E-25	2.36E-232
PIS111	7	0.971006	0.004042	0.024951	1	5.56E-21	7.38E-218
PIS119	7	0.954636	0.014156	0.031208	1	2.54E-21	3.88E-219
PIS112	7	0.868166	0.131734	1.00E-04	1	5.45E-18	7.80E-209
PIS120	7	0.988138	0.011762	1.00E-04	1	1.13E-28	1.02E-241
PIS113	7	0.994744	0.005156	1.00E-04	1	8.58E-26	1.24E-232
PIS385	4	1.00E-04	0.9998	1.00E-04	3.31E-24	1	1.16E-105
PIS366	1	1.00E-04	0.99659	0.00331	3.44E-24	1	1.08E-105
PIS365	1	1.00E-04	0.9998	1.00E-04	1.82E-181	3.93E-75	1
PIS267	1	1.00E-04	0.9998	1.00E-04			
PIS405	5	1.00E-04	0.9998	1.00E-04			

Figure S12. EEMS (Estimated Effective Migration Surfaces) outputs based on GBS data (one SNP per locus) of *Dendropsophus oliveirai* from northeastern Brazil. mrates02 = posterior probability contours P(log(m) > 0) = p and P(log(m) < 0) = p for the given probability level p, emphasizes regions with effective migration that is significantly higher/lower than the overall average (a); qrates01 = effective diversity surface on log 10 scale after mean centering (b); qrates02 = posterior probability contours P(log(q) > 0) = p and P(log(q) < 0) = p, applied to the effective diversity rates (c); rdist01 = scatter plot of the observed vs the fitted between-deme component of genetic dissimilarity (d); rdist02 = scatter plot of the observed vs the fitted within-deme component of genetic dissimilarity (e); rdist03 = scatter plot of observed genetic dissimilarities between demes vs observed geographic distances between demes (f); pilog101 = posterior probability trace (g).

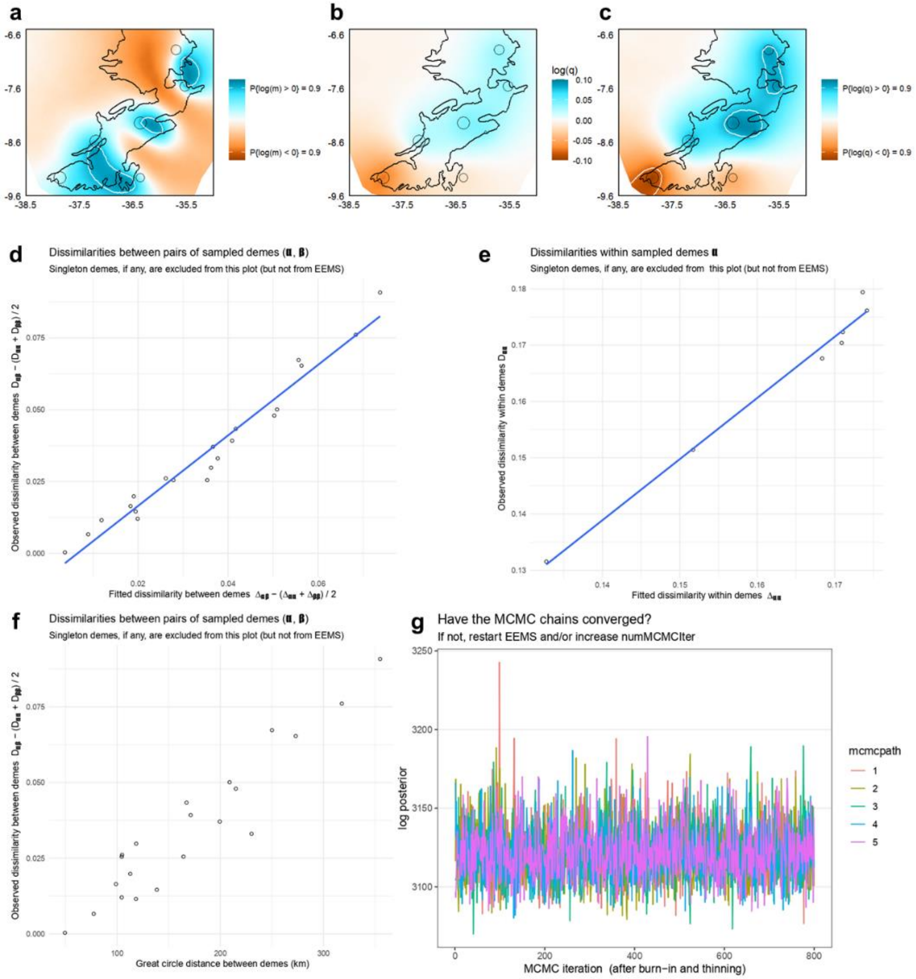


Figure S13. EEMS (Estimated Effective Migration Surfaces) outputs based on GBS data (one SNP per locus) of *Physalaemus cuvieri* from northeastern Brazil. mrates02 = posterior probability contours P(log(m) > 0) = p and P(log(m) < 0) = p for the given probability level p, emphasizes regions with effective migration that is significantly higher/lower than the overall average (a); qrates01 = effective diversity surface on log 10 scale after mean centering (b); qrates02 = posterior probability contours P(log(q) > 0) = p and P(log(q) < 0) = p, applied to the effective diversity rates (c); rdist01 = scatter plot of the observed vs the fitted between-deme component of genetic dissimilarity (d); rdist02 = scatter plot of the observed vs the fitted within-deme component of genetic dissimilarity (e); rdist03 = scatter plot of observed genetic dissimilarities between demes vs observed geographic distances between demes (f); pilog101 = posterior probability trace (g).

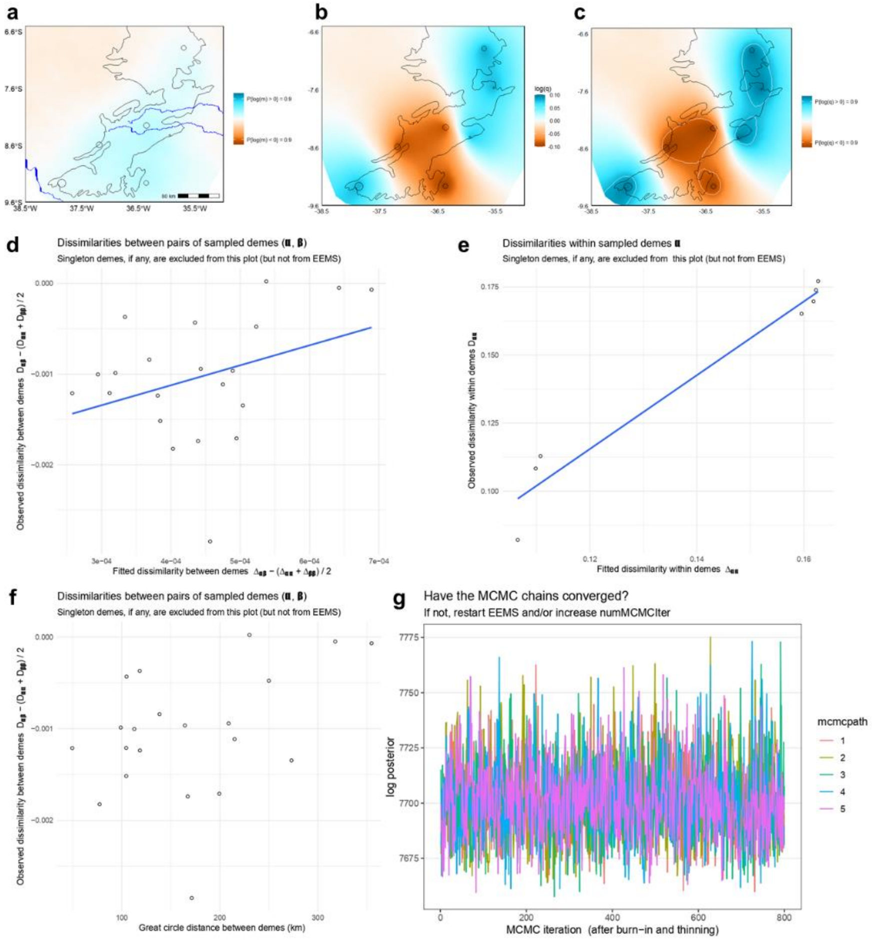


Table S14. Summary statistics based on GBS data (one SNP per locus; 10% missing data) of each population in *Dendropsophus oliveirai* and *Physalaemus cuvieri* from northeastern Brazil. N = number of samples; AR = allelic richness; A% = proportion of alleles; Ho = observed heterozygosity; He = expected heterozygosity; Fis = inbreeding coefficient; Ht = total expected heterozygosity; Dst = genetic differentiation between populations; Htp = corrected total heterozygosity; Dstp = corrected genetic differentiation; Fst = fixation index; Fstp = corrected fixation index; Dest = Jost's population differentiation measure (focuses on the allelic diversity rather than heterozygosity).

Species/ Population (Locality)	**N**	**AR**	**A%**	**Ho**	**He**	**Fis**	**Ht**	**Dst**	**Htp**	**Dstp**	**Fst**	**Fstp**	**Dest**
Dendropsophus oliveirai	41						0.106	0.010	0.111	0.015	0.097	0.138	0.017
Pop1 (7)	8	1.22	64.89	0.073	0.073	0.0581							
Pop2 (3,4,5,6)	22	1.28	85.56	0.089	0.099	0.0795							
Pop3 (1,2)	11	1.34	75.65	0.089	0.098	0.0947							
Physalaemus cuvieri	39						0.114	0.008	0.118	0.012	0.072	0.104	0.014
Pop1 (2,5,6,7)	16	1.34	76.97	0.084	0.097	0.1204							
Pop2 (1,2,3,4,5,6,7)	15	1.37	79.86	0.088	0.101	0.1093							
Pop3 (1,2,4)	8	1.33	72.03	0.084	0.100	0.1590							

Figure S15. Confusion matrix measuring the accuracy of the approximate Bayesian computation (ABC) for the final test (50,000 simulations) in *D. oliveirai*. Numbers (Y axis) represent percentages, which were calculated based on 100 images for each model.

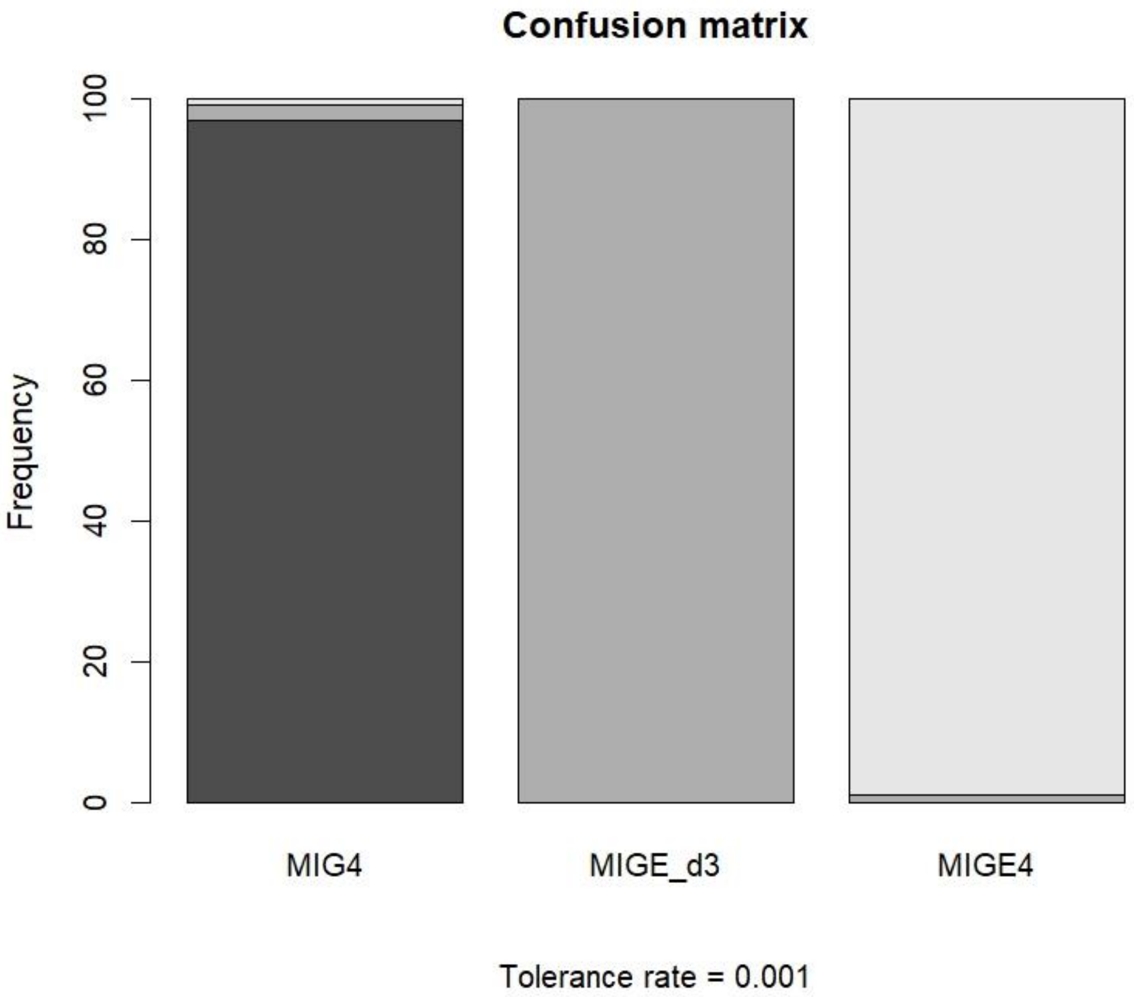


Figure S16. Confusion matrix measuring the accuracy of the approximate Bayesian computation (ABC) for the final test (50,000 simulations) in *P. cuvieri*. Numbers (Y axis) represent percentages, which were calculated based on 100 images for each model.

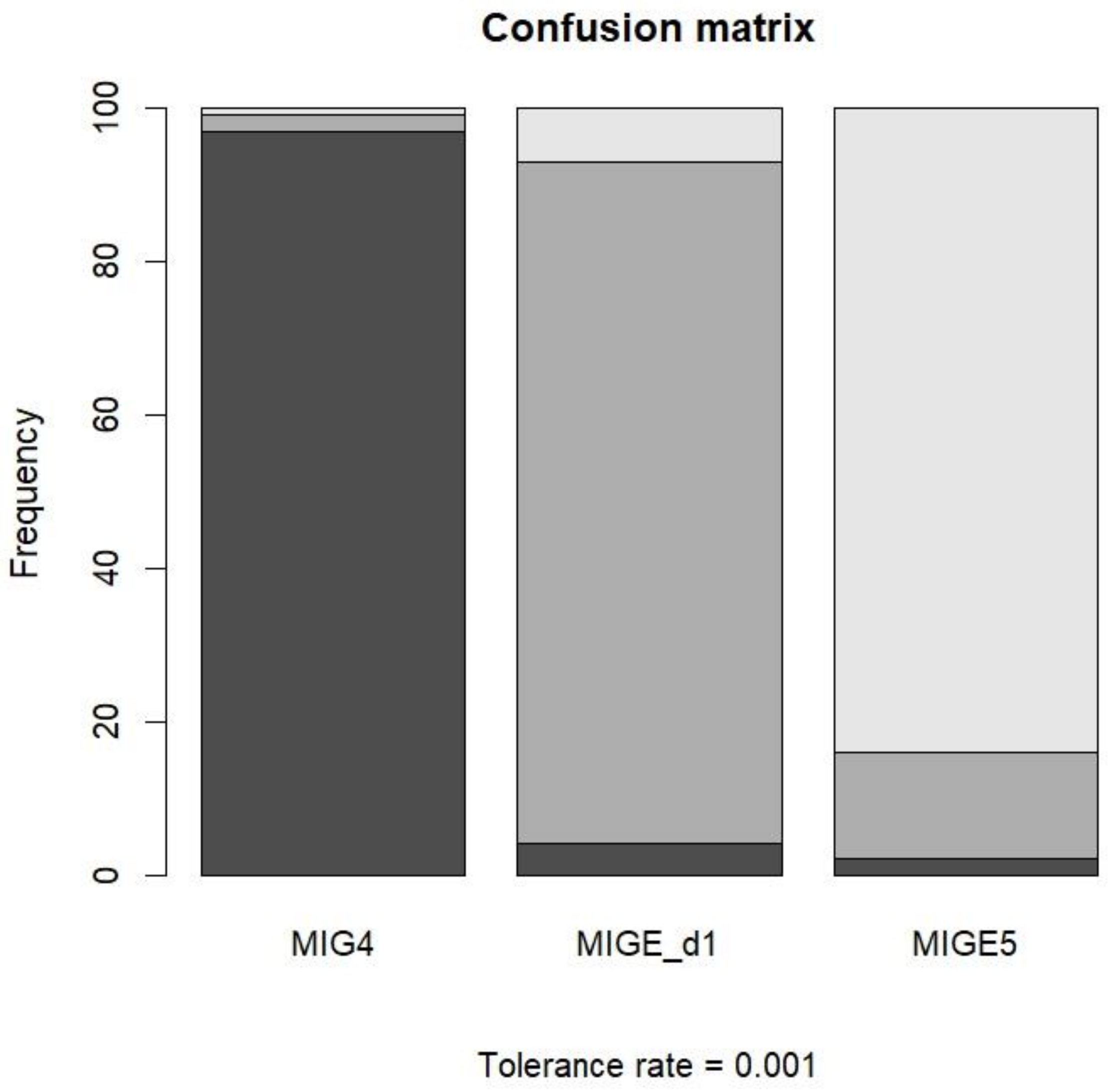


Figure S17. Principal Components Analysis plots of the final test in *D. oliveirai* derived from the 50,000 simulated distributions of summary statistics (Models MIG-T2, MIGE-T2, and MIGDE-T1) and observed dataset (asterisk).

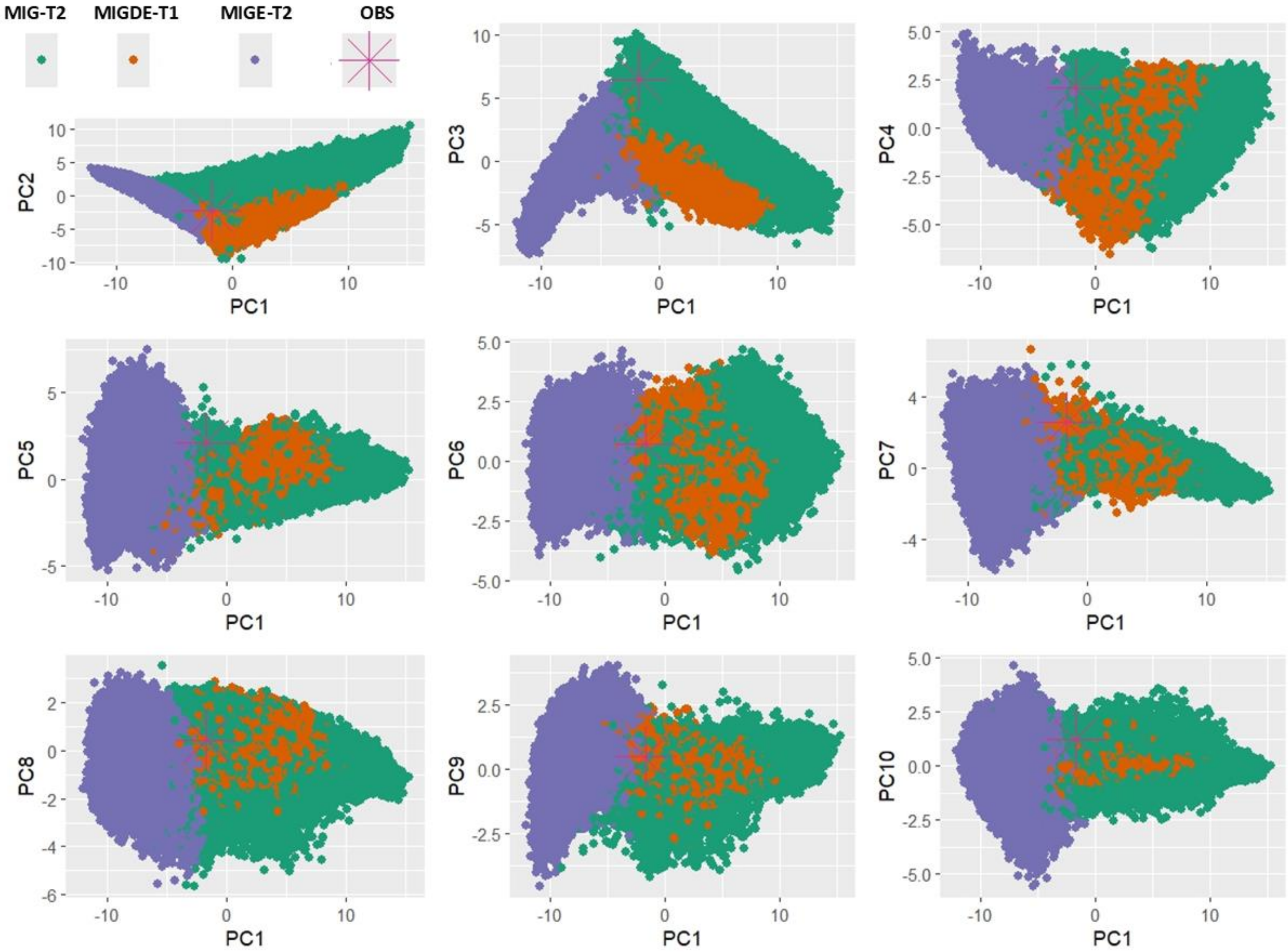


Figure S18. Approximate Bayesian Computation (ABC) parameters estimative from 100,000 simulations of the best supported model (MIG-T2) in *D. oliveirai*.

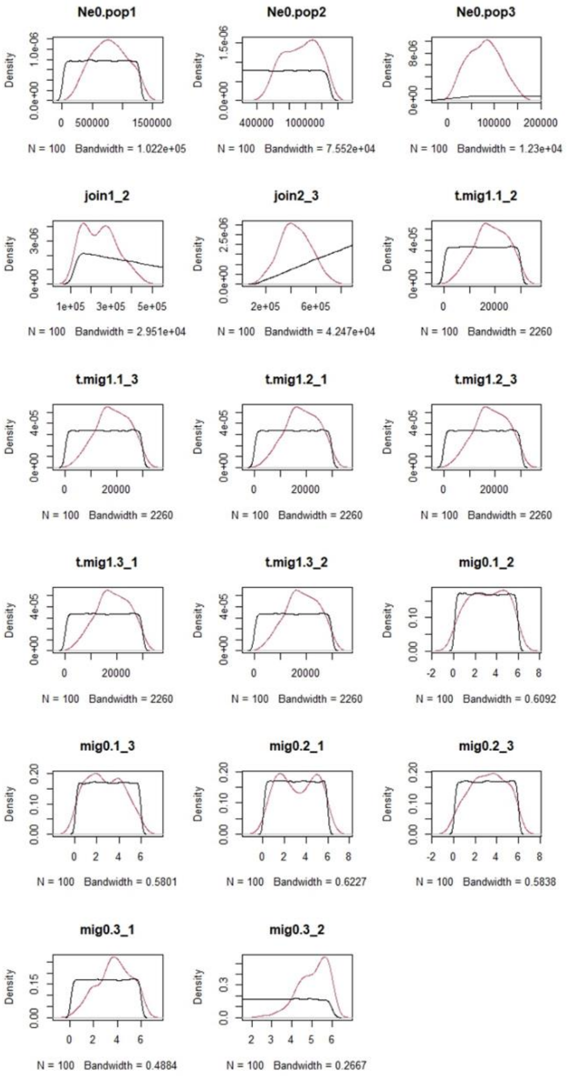


Figure S19. Supervised Machine Learning (SML) parameters estimative from 100,000 simulations of the best supported model (MIGE-T2) in *D. oliveirai*.

Figure S20. Principal Components Analysis plots of the final test in *P. cuvieri* derived from the 50,000 simulated distributions of summary statistics (Models MIG-T2, MIGE-CT2, and MIGDE) and observed dataset (asterisk).

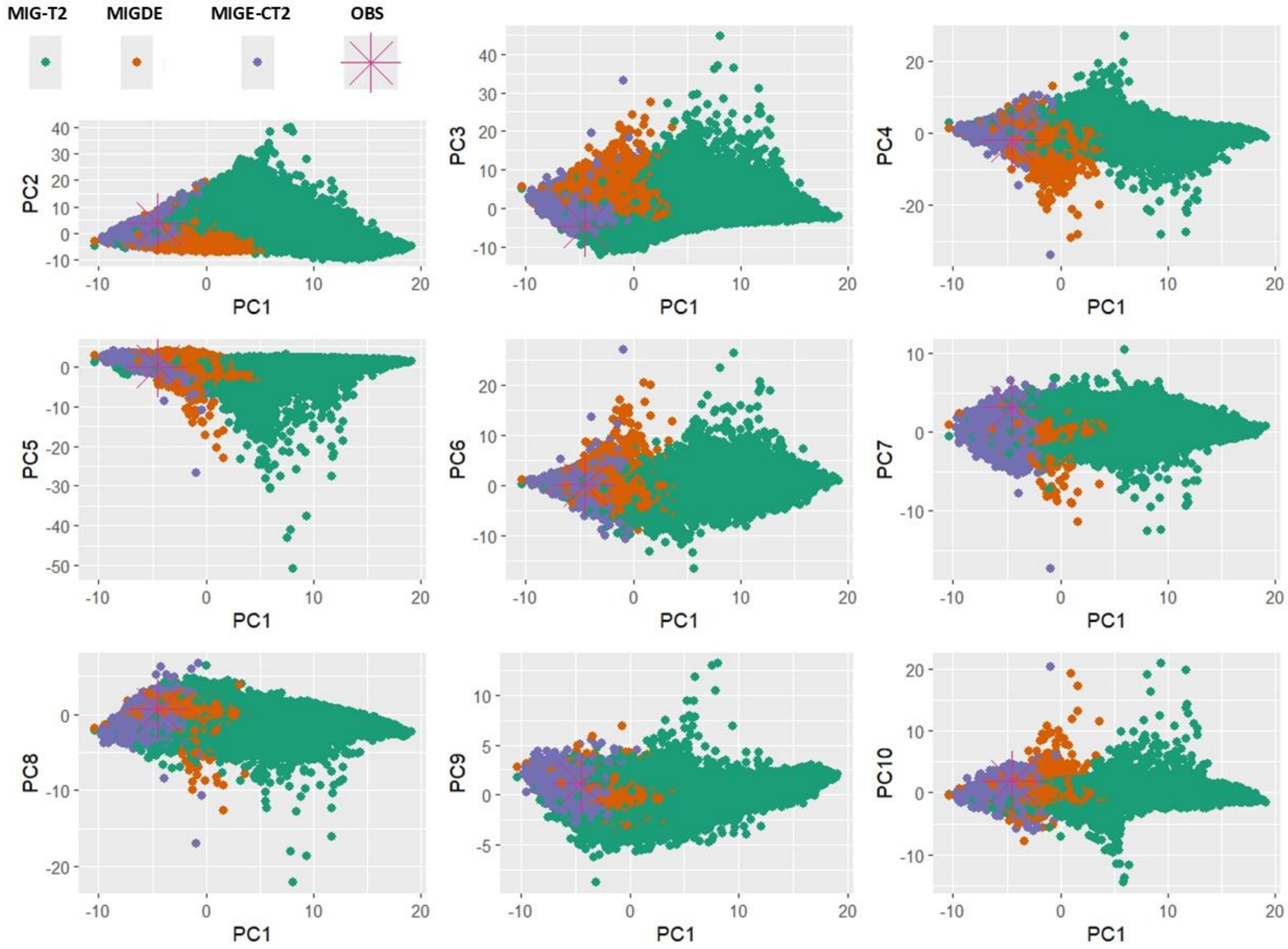


Figure S21. Approximate Bayesian Computation (ABC) parameters estimative from 100,000 simulations of the best supported model (MIGE-T2) in *P.cuvieri*.

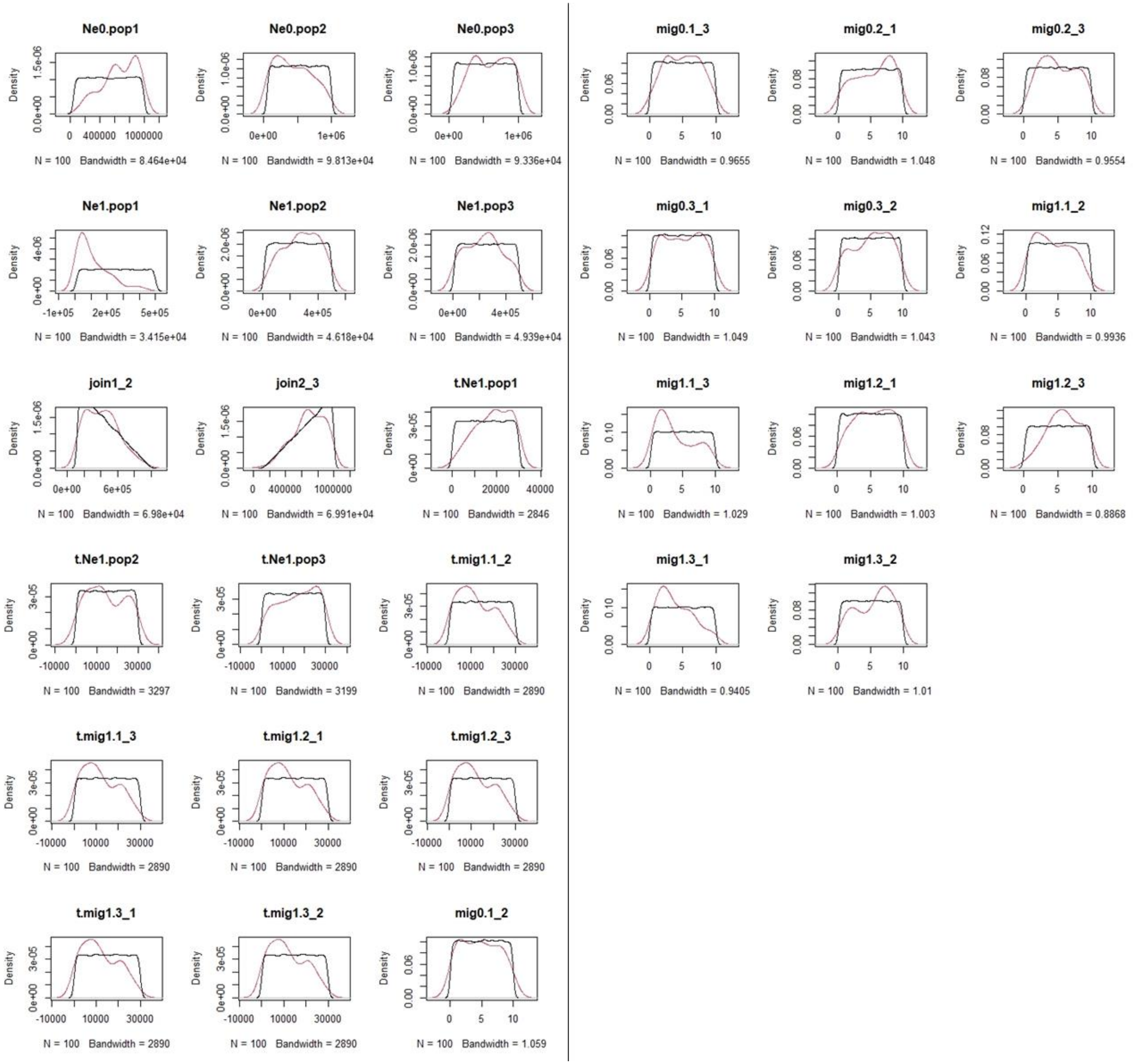


Figure S22. Supervised Machine Learning (SML) parameters estimative from 100,000 simulations of the best supported model (MIGE-T2) in *P.cuvieri*.

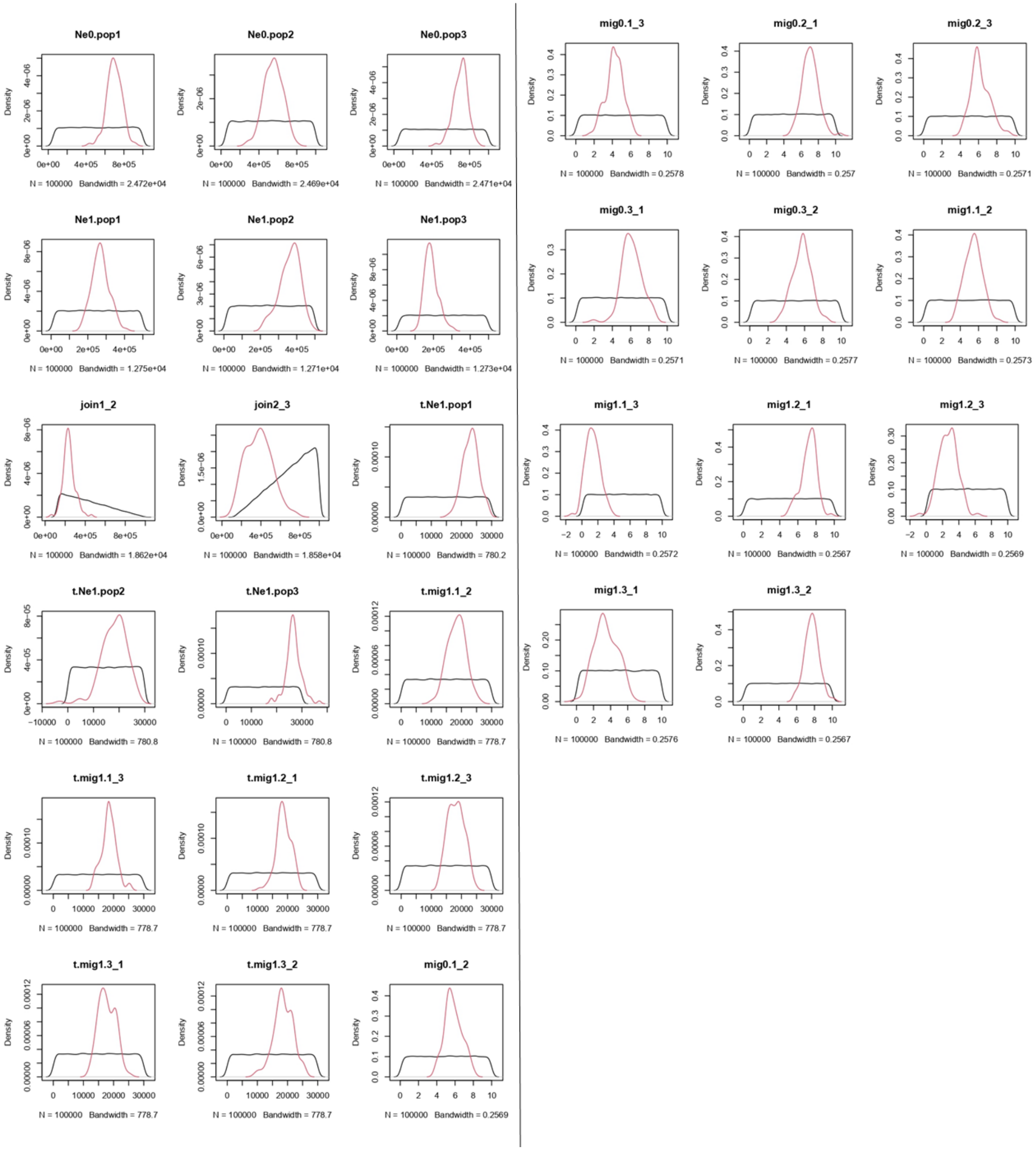
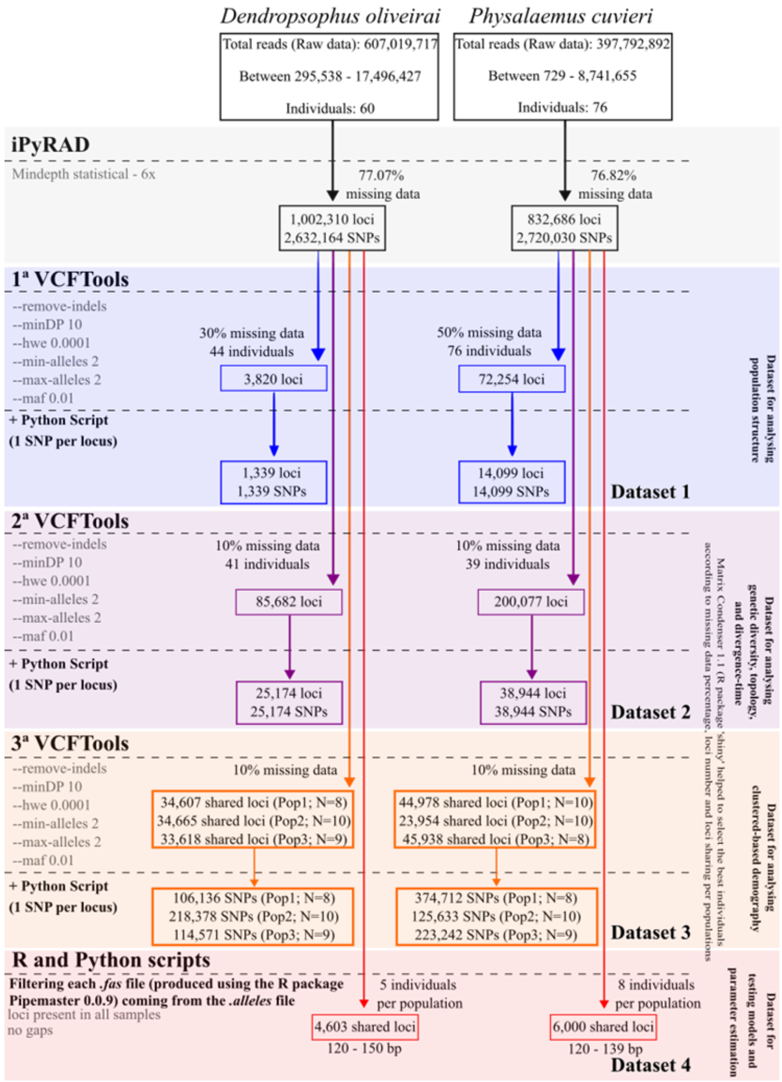


Figure S23. Filtering pipeline diagram used to generate the dataset for each analysis using genomic data from *D. oliveirai* and *P. cuvieri* populations from Northeastern Brazil.



APÊNDICE D – Advertisement call variation is related to environmental and geographic distances in two anuran species inhabiting highland forests in northeastern Brazil

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ORIGINAL ARTICLE

Advertisement call variation is related to environmental and geographic distances in two anuran species inhabiting highland forests in northeastern Brazil

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Abstract

Highlands are of paramount importance to the study of evolution as they are frequently implicated in historical and ecological processes that generate and maintain biological diversity. In northeastern Brazil, sparse rainforest remnants located in highlands north of the São Francisco River are surrounded by the dry and open landscape of the Caatinga biome. Earlier studies suggested that these forests were historical refuges to the rainforest fauna and flora during Pleistocene's climatic cycles. However, it is still unclear whether populations in distinct highlands experienced phenotypic differentiation as a result of adaptation to environmental conditions of each forest remnant. Herein, we used two frog species widely distributed and ecologically different, *Dendropsophus oliveirai*, a habitat specialist, and *Physalaemus cuvieri*, a habitat generalist, to investigate the relationships between environmental variation, geographic, genetic, and body size distance with advertisement call variation among populations inhabiting different highlands. Our results indicated that acoustic variation among *P. cuvieri* populations is strongly influenced by environmental variation, but also by the geographic distance among populations. In *D. oliveirai*, environment is also the most influent factor on acoustic variation, followed by a lower influence of genetic and morphological variation. Associations between environmental and geographic factors suggest indirect effects of geographic distance on acoustic variation in both species through an environmental gradient. We believe that selective processes and isolation by distance possibly act together in driving interpopulational acoustic variation with habitat-specific species being more affected by geographic isolation in suitable habitats.

Abstract in Portuguese is available with online material.

KEYWORDS
Atlantic Forest, Caatinga, environmental variation, forest enclaves, phenotypic variation

1 | INTRODUCTION

Mountain ranges are remarkable systems for the study of evolution due to their role in generating and maintaining biodiversity (Guedes et al., 2020; Hughes & Atchison, 2015; Pie et al., 2018). In northeastern Brazil, humid Atlantic Forest enclaves are found in isolated highlands scattered among lowlands covered by xerophilous Caatinga vegetation (Andrade-Lima, 1960; Sales et al., 1998; Silveira et al., 2019). These forest remnants originated from cycles of rainforest expansion and contraction into the Caatinga during late Pliocene and Pleistocene climatic cycles (Andrade-Lima, 1982; Santos et al., 2007). Contemporary studies on population genetics and species composition suggest that, because of their stability, these forest remnants have played an important role as climatic refuges for the Atlantic Forest biota, including angiosperms and many terrestrial vertebrates (Carnaval et al., 2009; Costa et al., 2017; Gehara et al., 2017; Menezes et al., 2020; Porto et al., 2013; Prance, 1982; Silveira et al., 2019).

Amphibians have been particularly well-studied in some of these highlands, and the evaluation of molecular data across different anuran species uncovered strong population structure, high genetic diversity and evidence of demographic expansions during pluvial maxima in the Neogene (Carnaval, 2002; Carnaval et al., 2009; Carnaval & Bates, 2007). They also suggested that differences in natural history among species, specifically their degree of association with canopy cover, could be related to differences in spatial patterns in genetic structure among populations. However, no study to date evaluated whether anuran populations inhabiting different highland forests experienced phenotypic differentiation as a result of adaptation to local environmental conditions.

Climate in the northeastern highlands is more stable than that in the surrounding lowlands, but an east–west precipitation gradient exists from areas near the Atlantic coast (where annual precipitation ranges between 1000 and 1600 mm) to the hinterlands within the Caatinga biome (400–600 mm) (Rodal et al., 2008; Silva et al., 2018; Velloso et al., 2002). This variation in precipitation creates notable gradients in plant composition and forest structure (Rodal et al., 2008; Santos et al., 2007; Velloso et al., 2002), which could affect the phenotypic expression in populations distributed in this region. Given that environmental heterogeneity often generates phenotypic diversity (Calsbeek et al., 2007), differences in biotic and abiotic factors along a species' geographic distribution may potentially lead to population differentiation (Endler, 1977; Schluter, 2009).

Anurans are good models for investigating environmental influence on phenotypic variation because of their sensitivity to climate and habitat variation across the landscape, which commonly influences their physiology and phenotype (Fernandes et al., 2021; Mitchell & Bergmann, 2016; Titon Junior & Gomes, 2015). Additionally, male anurans rely on advertisement calls to attract females and repel conspecific males (Köhler et al., 2017; Wells, 2007). Such calls are species-specific and genetically inherited, and their acoustic properties are often under environmental or sexual selection (Gerhardt & Huber, 2002; Rosa et al., 2023; Wells, 2007), although they may also vary due to geographic isolation, genetic drift or selection on body size (Amézquita et al., 2009; Gingras et al., 2013; Kaefer et al., 2013; Toledo et al., 2015). Because of the role of calls in reproduction, interpopulation call differentiation is often associated with reproductive isolation in anurans, with different calls acting as prezygotic reproductive barriers (Armansin et al., 2019). In some cases, the inability of females to recognize calls from another population may reinforce pre-existing genetic differentiation, leading to speciation (Hoskin et al., 2005; Wilkins et al., 2013). Hence, detecting and mapping call differentiation among allopatric populations may be a first step in uncovering the drivers of phenotypic variation and locating areas where biological diversity can be generated and maintained.

In this study, we investigated the geographic variation in acoustic traits of the advertisement calls of two species of frogs not phylogenetically closely related (Jetz & Pyron, 2018), which have different body sizes, habits and reproductive modes, and which are widespread in highland forests in northeastern Brazil. We tested potential drivers of acoustic variation, assessing the relative roles of environment and geographic distances, while accounting for potential effects of genetic distance and body size variation among populations on call traits. Additionally, we evaluated population differentiation in call traits, in order to detect geographic boundaries and highland forest sites where incipient evolutionary divergence might be taking place. In view of the climatic and vegetational gradients that predominate in the region, our hypotheses were: (a) environmental variation is strongly related to variation in call traits, because of local selection on sound signals imposed by different forest environments; (b) geographic distance among populations affects more strongly the calls of the least habitat-generalist species, for which the open vegetation matrix is potentially less permeable; and (c) acoustic and genetic distances are correlated in the least habitat-generalist species as a consequence of isolation by adaptation, which is part of isolation by environment (Rundle & Nosil, 2005; Wang & Bradburd, 2014).

2 | METHODS

2.1 | Study area

We conducted fieldwork between May 2021 and June 2022 in seven sampling locations distributed in highlands of northeastern Brazil, within the states of Alagoas, Paraíba, and Pernambuco (Figure 1; Table 1). All locations comprise mosaics of altered landscapes and one type of native highland forest (Coimbra Filho & Câmara, 1996; Ribeiro et al., 2009; Theulen, 2004) (Table 1). Geographic distance between sampling locations varied between 40 and 354 km (Table S2). Sampling locations were distributed on highlands of the same major geomorphological unit, the Borborema Plateau. Environmental conditions across this plateau vary longitudinally, due to the orographic barrier created by high altitudes along its eastern border, resulting in dry and warm climate across the plateau's western stretches, where rainfall does not exceed 650 mm and open Caatinga vegetation predominates (Velloso et al., 2002), whereas in

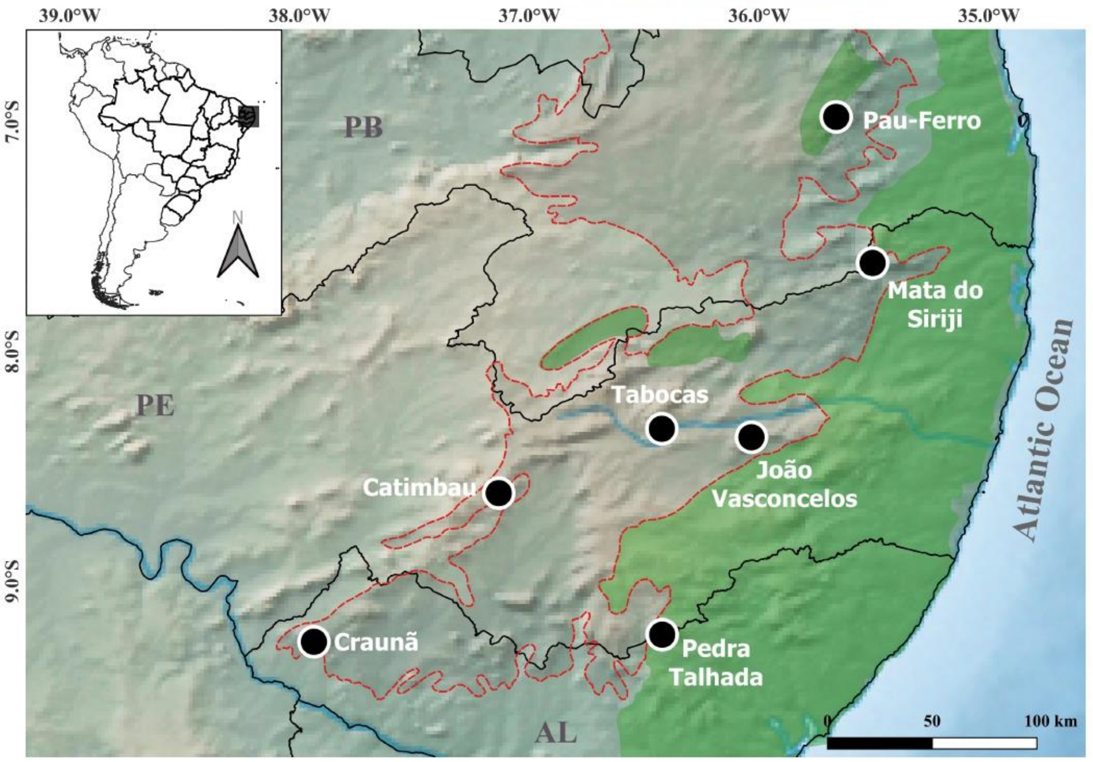

FIGURE 1 Geographic distribution of the sampling locations in the Borborema Plateau (dashed red line) in northeastern Brazil, north of the São Francisco River (below PE and AL states). Atlantic Forest biome in light green; Caatinga biome in green shades; PB = Paraíba; PE = Pernambuco; AL = Alagoas. Pau-Ferro = Parque Estadual Mata do Pau-Ferro, Areia-PB; Mata do Siriji = Refúgio da Vida Silvestre Matas do Sirigi, São Vicente Férrer-PE; João Vasconcelos = Parque Natural Municipal Professor João Vasconcelos Sobrinho, Caruaru-PE; Tabocas = Fazenda Vale Tabocas, Belo Jardim-PE; Catimbau = Parque Nacional do Catimbau, Buíque-PE; Pedra Talhada = Reserva Biológica de Pedra Talhada, Quebrangulo-AL; Craunã = Refúgio de Vida Silvestre Morros do Craunã e do Padre, Água Branca-AL.

TABLE 1 Geographic coordinates, elevation, forest type present in each sampling locations, and sample size of *Physalaemus cuvieri* and *Dendropsophus oliveirai* males from which we obtained recordings, morphological measurements and DNA sequences (number of 16S rRNA sequences in parentheses). Forest types according to Tabarelli and Santos (2004) and Studer (2015).

Sampling locations	Latitude	Longitude	Elevation (m a.s.l)	Forest type	Sample size	
					P. cuvieri	*D. oliveirai*
Pau-Ferro	06°58′12.53″ S	35°43′04.27″ W	550	Atlantic Forest-Caatinga ecotone	10 (8)	7 (7)
Mata do Siriji	07°37′01.79″ S	35°30′27.84″ W	537	Atlantic Forest-Caatinga ecotone	9 (8)	6 (3)
João Vasconcelos	08°21′8.80″ S	36°01′51.60″ W	803	Atlantic Forest-Caatinga ecotone	8 (8)	7 (7)
Tabocas	08°14′47.5″ S	36°22′46.00″ W	677	Semideciduous seasonal forests	12 (8)	12 (12)
Catimbau	08°34′20.60″ S	37°14′12.30″ W	823	Semideciduous seasonal forests	7 (8)	7 (10)
Pedra Talhada	09°15′40.94″ S	36°26′20.22″ W	536	Open ombrophylous forests	12 (8)	12 (6)
Craunã	09°20′47.90″ S	37°52′60.00″ W	338	Semideciduous seasonal forests	12 (8)	6 (8)
Total					70 (56)	57 (53)

Note: Sampling locations abbreviations: Pau-Ferro, Parque Estadual Mata do Pau-Ferro, Areia-PB; Mata do Siriji, Refúgio da Vida Silvestre Matas do Sirigi, São Vicente Férrer-PE; João Vasconcelos, Parque Natural Municipal Professor João Vasconcelos Sobrinho, Caruaru-PE; Tabocas, Fazenda Vale Tabocas, Belo Jardim-PE; Catimbau, Parque Nacional do Catimbau, Buíque-PE; Pedra Talhada, Reserva Biológica de Pedra Talhada, Quebrangulo-AL; Craunã, Refúgio de Vida Silvestre Morros do Craunã e do Padre, Água Branca-AL.

eastern areas of the Plateau precipitation ranges between 1000 and 1600 mm and Atlantic rainforests predominate (Silva et al., 2018).

2.2 | Study species

Physalaemus cuvieri Fitzinger, (1826) (Leptodactylidae) is a small foam-nesting frog with a wide distribution in South America, from Argentina to Venezuela (Frost, 2022). It breeds in lentic, mainly temporary water bodies, within open or forested areas (Aguiar et al., 2014; Pombal & Haddad, 2007). Their eggs are deposited in foam nests and tadpoles hatch within a few days, shortly completing their development (Barreto & Andrade, 1995; Haddad & Prado, 2005). Along their geographic distribution in northeastern Brazil, populations of *P. cuvieri* experience distinct environmental conditions, ranging from mesic to semi-arid microclimatic conditions in the Caatinga biome, and in patches of Atlantic rainforest, suggesting the species has high environmental plasticity (Barreto & Andrade, 1995; Oliveira et al., 2019). Its advertisement call has one note with complex harmonic structure, usually forming one upward-downward segment. Duration of notes range between 0.257 and 0.311 s and dominant frequency varies between 0.7 and 0.8 kHz (Hepp & Pombal Jr., 2020; Figure 2).

Dendropsophus oliveirai (Bokermann, 1963) (Hylidae) is a small treefrog endemic to northeastern Brazil, found among vegetation alongside seasonal or perennial ponds near forest edges or in adjacent low vegetation (Menezes et al., 2020). Females deposit their eggs on leaves hanging over the water, where exotrophic tadpoles dive after hatching and complete their development (Haddad & Prado, 2005; Silva et al., 2019). The species is not usually found in rain pools or in shallow water bodies with short hydroperiod, but is common in lakes, ponds and other long-lived lentic water bodies that form under mesic or wet conditions in the Caatinga and Atlantic Forest biomes. The advertisement call of male *D. oliveirai* has a short (0.06–0.15 s) pulsed note (5–14 pulses/note) emitted at a high dominant frequency (5.7–6.9 kHz) (Santana et al., 2011; Figure 3).

2.3 | Acoustic data

At each sampling location we recorded advertisement calls of males from 1700 to 2300 h using digital recorders (Tascam Dr-05x, Zoom H1n or Marantz PMD620) coupled to a AKG D7 directional microphone positioned 0.5–1.0 m from the calling male. Recording settings were the same for all recorders: 44.1 kHz sampling rate, 16-bit resolution, and WAV file format. Air temperature at the time of recording ranged between 19.5–27.9°C (*P. cuvieri*) and 19.1–26.3°C (*D. oliveirai*). In order to avoid potential variation in acoustic parameters caused by differences in behavioral context among recorded males, for recordings of both species, we selected males which were engaged in the continuous emission of advertisement calls, and were sufficiently apart from neighbor conspecifics to prevent visual

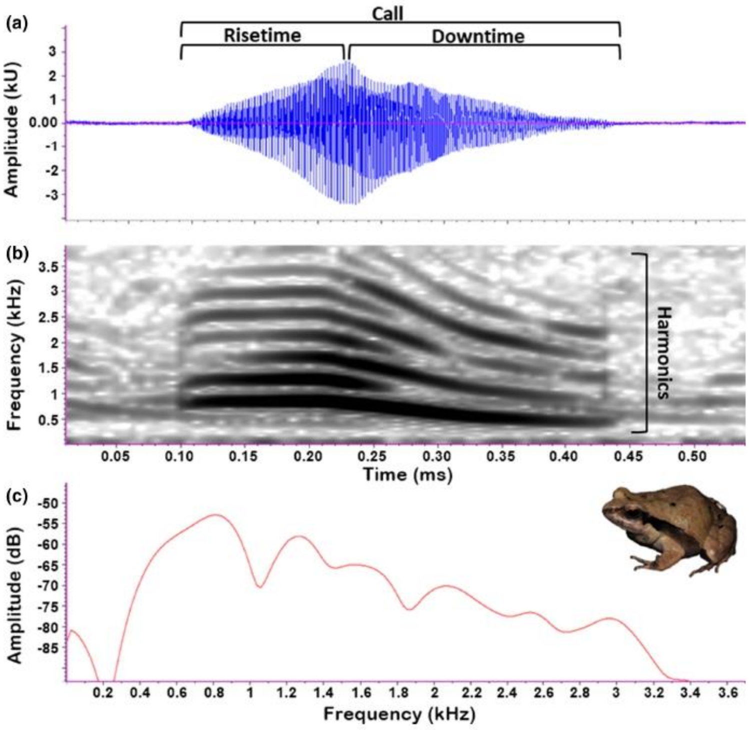

FIGURE 2 Oscillogram (a), spectrogram (b) and power spectrum (c) of a sample advertisement call of *Physalaemus cuvieri*.

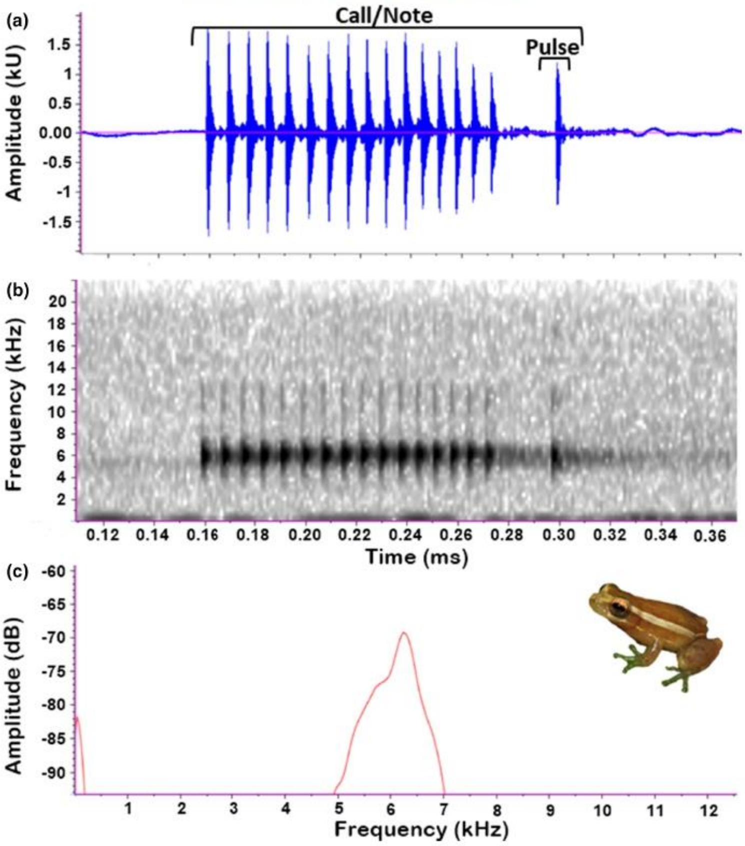

FIGURE 3 Oscillogram (a), spectrogram (b) and power spectrum (c) of a sample advertisement call of *Dendropsophus oliveirai*.

contact. Males engaged in close contact interactions with other conspecifics (e.g., courtship, aggressive interactions) were not recorded. We captured each recorded male manually and transported them to a field laboratory at the nearest lodge. Specimens were anesthetized/euthanized with topical benzocaine solution (50 mg/g) and their snout-to-vent length (SVL) measured with a caliper. Then they were fixed in 10% formalin solution and preserved in 70% ethanol. We dissected a small sample of thigh muscle from each specimen before fixation and preserved it in 98% ethanol. We deposited all specimens and samples at Coleção Herpetológica da Universidade Federal de Pernambuco (CHUFPE), in Recife, Pernambuco, Brazil (Appendix S1).

We analyzed advertisement calls in Raven Pro 1.6 (K. Lisa Yang Center for Conservation Bioacoustics, 2023). Spectrogram parameters were: Blackman window function, window size of 10 ms (*P. cuvieri*) or 5 ms (*D. oliveirai*), overlap of 80% and DFT size of 2048 samples. Due to structural differences between advertisement calls of the two species, we measured different parameters from their calls (Table S3). For *D. oliveirai*: note duration, number of pulses per note, rate of pulse emission (pulses/second), lower frequency of the call, upper frequency of the call, frequency bandwidth of call and peak frequency of the call. For *P. cuvieri*: call duration, rise time (the time elapsed from the beginning of the call until it reached maximum amplitude), lower frequency of rise time, upper frequency of rise time, bandwidth of rise time, dominant frequency of rise time, down time (the time elapsed from the peak of amplitude until the end of the call), lower frequency of down time, upper frequency of down time, bandwidth of down time and peak frequency of down time. We measured spectral variables 20 dB below the peak frequency to avoid overlap with background noise (Erdtmann & Amézquita, 2009; Kaefer & Lima, 2012). We analyzed an average of 15 calls in each recording of *D. oliveirai* (range 5–18 calls/recording) and 10 calls in each recording of *P. cuvieri* (range 6–10 calls/recording).

2.4 | Molecular data

We extracted genomic DNA from preserved tissue samples using chelex-100 chelating resin (Walsh et al., 1991). We amplified a fragment of the 16S rDNA mitochondrial gene using primers 16sarL and 16sbrH of Palumbi et al. (2002). For each sample, we used 12.5 μL GoTaq DNA Master Mix (Promega, USA), 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 using

Wizard SV GEL and PCR Clean-up System (Promega, USA), following the manufacturer's protocol. We sequenced PCR products in both directions using the BigDye Terminator v3.1 kit (ThermoFisher) in a Genetic Analyzer 3500 automated sequencer (Applied Biosystems). We verified the resulting sequences in BioEdit 7.2 (Hall, 1999) and MEGA 11.0.13 (Tamura et al., 2021), and assembled consensus sequences using softwares Pregap4 v 1.6-r and Gap4 (Staden, 1996). The resulting sequences varied in length between 549 and 590 basepairs. We aligned the sequence set of each species in MAFFT v7.017 (Katoh, 2002) using the standardized parameters. Finally, we produced a matrix of pairwise genetic distance-based fixation indexes (F_{st}; Wright, 1951) (Table S2), using Arlequin 3.5.2.2 (Excoffier & Lischer, 2010). All sequences were deposited in GenBank (accession numbers OR491066–OR491121 and OR491200–OR491252).

2.5 | Environmental data

For each location, we obtained data on 19 bioclimatic variables (corresponding to the period between 1970 and 2000) and elevation represented in rasters with cell resolution of 30 arcseconds (~1.0 km^2) from the WorldClim 2.1 database (Fick & Hijmans, 2017). We downloaded data in R (R Core Team, 2019) using the *raster* (Hijmans, 2017), *sp* (Pebesma & Bivand, 2005), and *rgeos* (Bivand & Rundel, 2023) packages and the *extend* and *crop* functions. We gathered values of each variable for each sampling location using the *extract* function (Hijmans, 2017).

We also obtained values of EVI (Enhanced Vegetation Index) provided by the MODIS VI satellite in a 250 m resolution (MOD13Q1.061 Terra Vegetation Indices 16-Day) in the time interval between 2000 and 2022, using Google Earth Engine (https://earthengine.google.com/) (Gorelick et al., 2017). The EVI was developed to reduce the atmospheric elements and canopy background effects (Huete et al., 1997) and capture with more accuracy the green vegetation signal at higher green biomass levels (Huete et al., 2002). Finally, we calculated the maximum and minimum average of EVI using respectively highest and lowest annual values of EVI for each locality. These averages represented the vegetation density in each locality in the rainy and dry seasons, respectively.

2.6 | Statistical analyses

We calculated individual averages of each acoustic parameter. Using these averages, we conducted simple linear regression analyses between call parameters and air temperature at time of recording in order to detect and remove potential effects of temperature on call variation (Gerhardt & Huber, 2002).

To test for associations and relative effect sizes of environment, geographic, genetic, and body size distances among populations on the acoustic differentiation in both species, we used two statistical approaches, the Mantel/partial Mantel tests (Mantel, 1967) and multiple matrix regression with randomization (MMRR; Wang, 2013). Mantel tests evaluate correlations between matrix pairs, whereas MMRR uses regression models involving two or more matrices and tests which class of data has the greatest influence on the matrix representing the response variable. MMRR demands the use of independent predictor variables (Legendre et al., 1994; Manly, 1986; Wang, 2013), so Mantel tests also served as a preliminary analysis to detect and exclude strongly correlated predictor variables.

From individual averages we calculated the mean of each acoustic parameter for each population (Table S3). Then, we produced acoustic and body size distance matrices among populations using the average values for each trait in each population based on Euclidean distances. We also produced an environmental distance matrix using Euclidian distances among sampling locations, combining all environmental variables (Worldclim and EVI) for each locality. We created a geographic distance matrix (Table S2) using the geodesic geographic distance among locations based on GPS coordinates, using the *rdist.earth* function of *fields* 14.1 R package (Nychka et al., 2021). We tested correlations between acoustic, body size, genetic, environmental, and geographic distance matrices using Mantel tests with 1000 permutations, via Pearson method (Mantel, 1967) in the *vegan* 2.6.4 R package (Oksanen et al., 2022). We conducted the MMRR with 10,000 permutations using the *MMRR* function of the *PopGenReport* 3.0.7 package (Adamack & Gruber, 2015), with acoustic distance as the response matrix and body size, genetic, environmental, and geographic distances as explanatory matrices. In MMRR the set of different distances created from explanatory variables is known as least-cost distance. We assigned body size and genetic distance matrices as covariates because they are often associated with variation of acoustic parameters in anurans (Amézquita et al., 2009; Gerhardt & Huber, 2002; Smith et al., 2003).

To evaluate call differentiation among populations of both species, we applied a principal component analysis (PCA) on the mean values of acoustic variables for each population (Tables S4 and S5). We used the resulting scores of the first and second principal components (PC1 and PC2) to graphically evaluate the distribution of population averages along the acoustic space. We applied another PCA on individual means of acoustic variables and used the scores of the two first components in a Kruskal–Wallis and post hoc (Wilcoxon–Mann–Whitney) tests to verify differences among populations. Finally, we tested for differences in body size among populations using ANOVA. All statistical analyses were conducted in R (R Core Team, 2019) at a significance level of $\alpha = .05$.

3 | RESULTS

Linear regression analyses indicated no high significant relationships between air temperature and acoustic parameters in both species (Table S6). Also, an additional Mantel test between average differences in air temperature and geographic distances showed no significant association between these variables for sampling locations of both species (*P. cuvieri*: $r = -.05$; $p = .52$; *D. oliveirai*: $r = -.37$; $p = .93$). Despite that, we present Mantel/partial Mantel and MMRR results for both not adjusted and temperature-adjusted acoustic data, respectively.

Mantel tests revealed that acoustic distances among highland populations of *P. cuvieri* correlated more strongly with environmental distances among sampling locations (Table 2). A weaker but significant correlation was also detected between acoustic distances and geographic distances among populations. Correlation between acoustic distances and environmental distances were still significant after controlling for the effect of geographic distance on environmental distances on a partial Mantel test. Genetic and body size distances among populations were not associated with acoustic distances in *P. cuvieri*. Statistically significant correlation among association matrices based on predictor variables and covariates was only detected between genetic distances and geographic distances among sampling locations (Table 2).

In *D. oliveirai*, acoustic distances among populations also correlated more strongly with environmental distances among sampling locations. The correlation remained significant after controlling for potential effects of geographic distance on environmental distances among locations (Table 2). Genetic and body size distances among populations were weakly, but significantly correlated with acoustic distances. There was no correlation between geographic and acoustic distances among sampling locations, although geographic and genetic distances were moderately correlated. Statistically significant correlation was detected between genetic, geographic, and environmental distances among populations (Table 2).

As collinearity was not strong (<0.63) among distance matrices of explanatory variables and covariates in Mantel, we used all matrices in the MMRR for both species. The overall fit of the MMRR model for *P. cuvieri* was relatively high ($R^2=0.61$; $F=6.24$; $p=.04$; Figure S7a). Among the parameters in the least-cost distance the geographic distance was the best predictor of acoustic distance among populations (Table 3). A second potential predictor, environmental distance was moderately correlated with acoustic distance among populations, but the association was not statistically significant ($\beta=.42$; $p=.11$). In *D. oliveirai*, the overall fit of the MMRR model was marginally significant ($R^2=0.42$; $F=2.89$; $p=.07$; Figure S7b), with only environmental distance potentially acting as a moderate, yet not statistically significant ($\beta=0.48$; $p=.11$), predictor of acoustic distance among populations (Table 3).

TABLE 2 Correlation coefficients based on Mantel (×) and partial Mantel (−) tests on acoustic (CALL), environmental (ENV), geographic (GEO), body size (SVL), and genetic (GEN) distances for seven populations of the anurans *Physalaemus cuvieri* and *Dendropsophus oliveirai* inhabiting highland forests in northeastern Brazil.

Correlations	*P. cuvieri*	*D. oliveirai*
GEO × CALL	**$r=.57$; $p=.02$**	$r=.21$; $p=.20$
ENV × CALL	**$r=.71$; $p=.03$**	**$r=.59$; $p<.01$**
GEN × CALL	$r=.05$; $p=.41$	**$r=.42$; $p=.03$**
SVL × CALL	$r=-.01$; $p=.48$	**$r=.40$; $p=.05$**
GEO × GEN	**$r=.62$; $p<.01$**	**$r=.57$; $p<.01$**
ENV × GEN	$r=-.03$; $p=.46$	**$r=.51$; $p<.01$**
GEO × SVL	$r=-.04$; $p=.50$	$r=.13$; $p=.25$
ENV × SVL	$r=-.09$; $p=.64$	$r=.32$; $p=.07$
GEN × SVL	$r=-.20$; $p=.86$	$r=.18$; $p=.22$
GEO × ENV	**$r=.51$; $p=.03$**	**$r=.45$; $p=.05$**
ENV × CALL − GEO	**$r=.60$; $p=.05$**	**$r=.56$; $p=.03$**

Note: Values in bold highlight statistically significant correlations ($p\leq 0.05$).

Replacing acoustic variables weakly correlated with environmental temperature (Table S6) with their residuals in Mantel/partial Mantel and MMRR did not change the observed relationships between acoustic and environmental and geographic distances for *P. cuvieri* (Table S8). However, for *D. oliveirai*, the only significant relationship observed was between acoustic distance and environmental distance, suggesting that associations between acoustic distance and geographic, body-size, and genetic distances in this species may be partly due to differences in temperature at time of recording among sampling localities (Table S8).

The first two principal components from the *P. cuvieri*'s PCA explained together 80.5% of the acoustic variation among populations. The first component (PC1) explained 55.2% of the total acoustic variation, with spectral parameters (upper frequency of the risetime and bandwidth of risetime) presenting high loadings (Figure 4b; Table S4). The second component (PC2) explained 25.3% of the total acoustic variation among populations, with temporal parameters (call duration and risetime) presenting high loadings (Figure 4b; Table S4). The distribution of populations of *P. cuvieri* along PC1 indicated the population of Craunã as highly different from the other highland populations based on spectral acoustic parameters (Figure 4b; Table S5). Along PC2, which mostly represented temporal call traits, Pedra Talhada was highly divergent from other populations (Figure 4b; Table S5). Remaining populations were grouped relatively close to each other on the acoustic space.

The first two principal components from PCA of *D. oliveirai* accounted for 83.3% of the total acoustic variation among populations. PC1 explained 53.7% of the total acoustic variation, with spectral parameters (peak frequency and upper frequency) presenting high

TABLE 3 Results of the multiple matrix regression with randomization test on acoustic (CALL), environmental (ENV), geographic (GEO), body size (SVL), and genetic (GEN) distances for seven populations of the anurans *Physalaemus cuvieri* and *Dendropsophus oliveirai* inhabiting highland forests in northeastern Brazil.

Regressions	*P. cuvieri*	*D. oliveirai*
GEO × CALL	**$\beta=.55$; $p=.04$**	$\beta=-.13$; $p=.62$
ENV × CALL	$\beta=.42$; $p=.11$	$\beta=.48$; $p=.11$
GEN × CALL	$\beta=-.32$; $p=.28$	$\beta=.18$; $p=.46$
SVL × CALL	$\beta=-.02$; $p=.91$	$\beta=.25$; $p=.25$

Note: Values in bold highlight statistically significant correlations ($p\leq .05$).

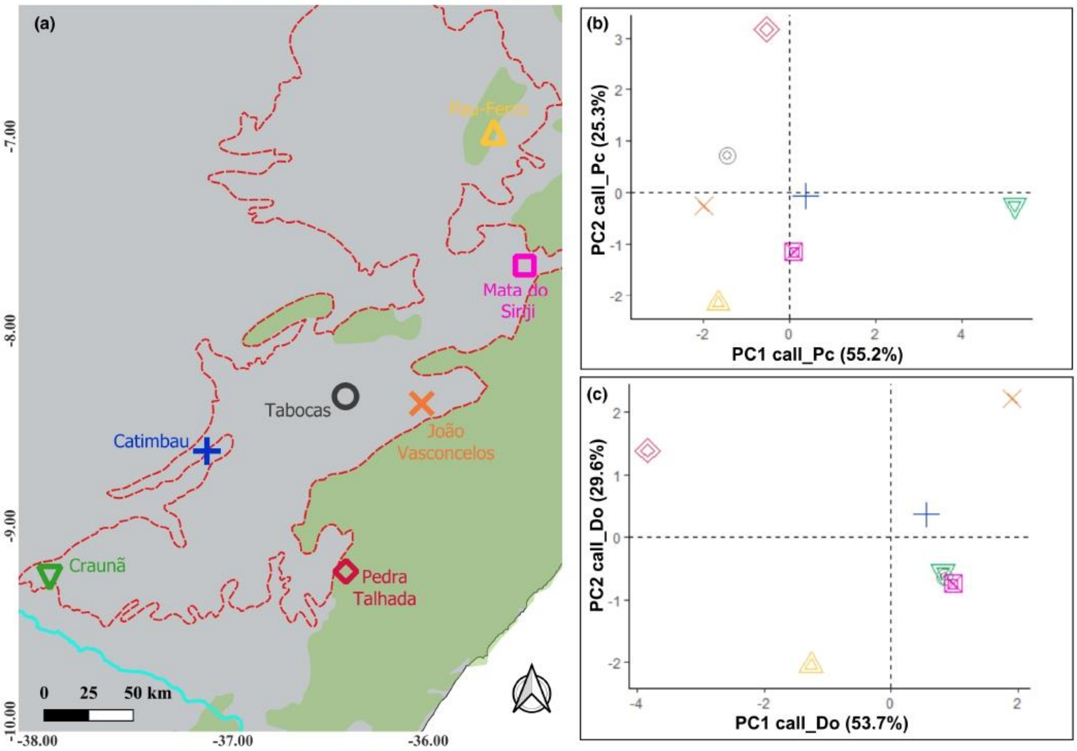

FIGURE 4 Geographic distribution of sampling locations across the Borborema Plateau (dashed red line) in northeastern Brazil (a). Distribution of populations of *P. cuvieri* (b) and *D. oliveirai* (c) along the first two principal components based on mean values of acoustic parameters of their advertisement calls. Atlantic Forest biome in light green; Caatinga biome in gray; São Francisco River below Borborema Plateau.

loadings (Figure 4c; Table S4). PC2 explained 29.6% of the total acoustic variation, with structural parameters (pulse number and pulse rate) presenting high loadings (Figure 4c; Table S4). The distribution of *D. oliveirai* populations along PC1, which account mostly for spectral parameters, indicated Pedra Talhada as highly differentiated from the other populations (Figure 4c; Table S5), which grouped relatively close along the two principal components. Populations from Pau-Ferro and João Vasconcelos diverged slightly from the others, although in opposite directions in the acoustic space (Figure 4c; Table S5).

Based on the PCA scores from the raw (individual) data of acoustic parameters, *P. cuvieri* populations were significantly different in the first ($H=36.96$; $p<.0001$) and second ($H=17.24$; $p=.008$) components (Figure S9a,b). Post hoc tests showed that Craunã was significantly different from Tabocas ($p=.003$), Pau-Ferro ($p<.0001$), Catimbau ($p=.0008$), João Vasconcelos ($p=.006$), Pedra Talhada ($p<.0001$), and Mata do Siriji ($p=.001$) in the first component. The localities Craunã and Pedra Talhada were also significantly different from each other in the second principal component ($p=.003$).

D. oliveirai populations were significantly different in the first acoustic component ($H=15.73$; $p=.01$) but not in the second ($H=5.27$; $p=.51$) (Figure S9c,d). Only João Vasconcelos and Pedra Talhada were different from each other in the first component ($p=.05$). There was a significant difference in body size among *P. cuvieri* populations ($F=3.89$; $p=.002$), but not among *D. oliveirai* populations ($F=.66$; $p=.68$) (Figure S10).

4 | DISCUSSION

Our results demonstrate that acoustic variation among populations of *P. cuvieri* and *D. oliveirai* inhabiting highland forests in northeastern Brazil is more strongly influenced by environmental factors and isolation by distance than by other common predictors of call variation in anurans. Genetic distance and body size have weak or inexistent influence on acoustic variation of those populations. These relationships, however, are idiosyncratic, varying in strength and relevance between the two study species, with mixed contributions of putative causal factors and covariates in explaining acoustic differentiation in each taxon. These are partly in agreement with our main hypotheses, and with some predictions raised in relation to potential effects of habitat variation and isolation by distance on call variation among populations of species more or less associated with forest habitats. Our study shows that islands of moist environments in high altitudes across northeastern Brazil can also be related to microevolutionary processes generating phenotypic divergence.

Acoustic adaptation to open or forested environments has been investigated in many animal groups that rely on sound signals for long-range communication, including anurans (e.g., Erdtmann & Lima, 2013; Ey & Fischer, 2009; Gomes et al., 2022; Rosa et al., 2023; Velásquez et al., 2018; Wilkins et al., 2013), but conclusions have been contentious. According to the acoustic adaptation hypothesis (AAH—Morton, 1975), habitat vegetation affects the transmission of sound through reverberation, degradation, and differential attenuation of particular frequencies, dense forest environments selecting calls with lower dominant frequencies, narrower frequency bandwidths, and longer duration (Morton, 1975; Wilkins et al., 2013). Across our study area, highland forests vary in tree coverage according to a longitudinal precipitation gradient, resulting in distinct selective pressures on call transmission at each sampling location. This could explain the associations found between acoustic and environmental variation in our study species. Craunã has the most open forest habitat among sampling locations. There, *P. cuvieri* males produce calls with the highest observed frequencies and the shortest duration (Figure S9a,b), suggesting that calls might be adapted to its Caatinga-influenced environment. On the other hand, in the densely vegetated forests east of Craunã, *P. cuvieri* produce calls with relatively low frequencies and long duration (Table S9a,b).

Associations between the surrounding vegetation and acoustic parameters were also investigated in calls of the treefrog *Pithecopus gonzagai* in different environments of the Caatinga and Atlantic Forest biomes (Gomes et al., 2022; Röhr et al., 2020). The first study demonstrated partial support to the AAH based on differences in acoustic parameters among populations and in the efficiency of sound propagation in different environments. Both approaches showed that temporal and structural call parameters correlated with vegetation density. Gomes et al. (2022) reported an inverse relationship between spectral parameters and vegetation coverage. Although not focused on highland forest populations, these studies reinforce our finding that the AAH has a mixed role in explaining acoustic variation in anuran species and might be dependent on the taxa and populations studied.

Among populations of *D. oliveirai*, acoustic variation was also more strongly influenced by environmental distance among sampling locations, and weakly by genetic and body size distances. In comparison with *P. cuvieri*, *D. oliveirai* breeds in lentic water bodies with longer hydroperiods, normally available in more humid climates (Menezes et al., 2020), suggesting that these populations could be potentially isolated from each other. We hypothesize that calls of highland populations of *D. oliveirai* may vary along the environmental gradient mostly as a result of geographic isolation in more suitable environments, as suggested by the relatively higher genetic distances between them (Table S2) and its correlation with geographic and environmental distances. Associations between geographic and environmental distances and their relationships with genetic and acoustic variation potentially indicate an accumulation of neutral and selective processes (Gould & Johnston, 1972), which may have led to greater differentiation among populations of *D. oliveirai*.

Although largely sympatric in our study area, our study species are not closely related phylogenetically and have distinct ecological requirements. *P. cuvieri* inhabits both forested and open vegetation areas, which is suggestive of increased phenotypic plasticity or adaptive response to different environmental conditions. This is supported by higher acoustic differentiation among populations (Figure S9), even under apparently high gene flow, and by acoustic distance being correlated with environmental distance, but not with genetic distance among populations, hinting at a scenario of adaptative divergence (Zamudio et al., 2016). Uncoupled evolution among phenotypic and neutral genetic traits is not uncommon among Neotropical anuran populations, especially among those which experienced demographic expansions during Neogene climatic cycles (Amézquita et al., 2009; Nosil, 2008; Pinho & Hey, 2010; Twomey et al., 2013).

Despite some degree of acoustic variation among populations of *D. oliveirai*, acoustic conservatism among them is apparently strong. Call frequency varied significantly only among two populations (Figure S9c). Their limitation to lentic reproductive habitats, with longer hydroperiods, could be related to stabilizing selection on calls, as a result of similar habitat structure. Alternatively, convergence or stability of call properties among populations could be maintained by stabilizing selection by female choice (Tobias et al., 2011, 2014; Toledo et al., 2015). These non-exclusive hypotheses deserve future investigation based on call propagation across reproductive environments and on playback experiments addressing mate choice. Additionally, since adaptative phenotypic variation and genetic divergence are direct or indirectly correlated, isolation by adaptation (Andersson, 1994; Boughman, 2001; Boul et al., 2007; Hoekstra et al., 2004) could be one evolutionary mechanism operating on *D. oliveirai* populations in this region.

Although there was no significant difference in body size among *D. oliveirai* populations (Figure S10), we found a possible low association between acoustic and body size distances. In each location, different natural or sexual selection regimes can affect variation in average body size of males (Robertson & Robertson, 2008) which, in turn, can result in variation of acoustic parameters through allometric relationships with the sound producing apparatus (Gerhardt & Huber, 2002; Ryan, 1988; Ryan & Brenowitz, 1986). An inverse relationship between body size and call frequencies is well known in anurans, with larger males producing calls with lower frequencies (Gingras et al., 2013).

When populations are weakly differentiated from each other, MMRR has more difficulties detecting the influence of landscape features (Wang, 2013). Hence, low acoustic differentiation among populations of *D. oliveirai* could partly explain the marginally significant associations between acoustic distance and the explanatory variables in the MMRR model. Moreover, MMRR can slightly underestimate the relative strength of the explanatory variables as a result of statistical noise caused by the stochastic variation of the response variable (Wang, 2013). This may have led to some relationships being recovered as not statistically significant in MMRR analyses, while statistically significant when addressed in Mantel tests.

Our results demonstrate that the acoustic evolution of co-occurring anuran populations of different species in highland forests of northeastern Brazil followed different patterns. However, environmental variation is possibly the most important factor influencing acoustic variation in both species, with a secondary role played by isolation by distance in the more generalist species. Although call differentiation is probably not a pre-zygotic reproductive barrier among highland populations of *D. oliveirai* in the present, in evolutionary time, post-zygotic barriers may develop due to genetic differentiation among them. Regardless of the acting evolutionary process, our perspective is that the growing fragmentation among highland forests will probably make them increasingly isolated, resulting in greater genetic disparity among populations. On the other hand, acoustic adaptation in response to geographic variation in reproductive environments seems to be the main driver of call differentiation in *P. cuvieri*, with low genetic distances among populations indicating that any barriers to gene flow among populations are currently pervasive, or have been in the recent past.

Our findings highlight that geographic variation in climate and vegetation of highland forests potentially affect advertisement call differentiation, which is probably intensified by isolation by distance among populations. Thus, in the heterogeneous landscape system of highland forests of northeastern Brazil, adaptation to the local environment and genetic drift probably act together in determining spatial patterns of acoustic signal variation in anurans. Our findings reinforce the idea that environmental variation and dispersion limits imposed by geographic distance can act together, affecting phenotypic differentiation in small vertebrates (Engen & Saether, 2016; Sun et al., 2013).

In Brazil, conservation action planning and environmental legislation generally overlooks biodiversity below the taxonomic level of species, jeopardizing phenotypic and genetic diversity which are key to the adaptive response of any species in the face of imminent climate change (Chevin & Lande, 2010; Kelly, 2019). Our work reinforces the role of Brazilian northeastern highland forests as environments prone to generating and maintaining biological diversity. Hence, their conservation, whether by means of delimitation of conservation units, or by scientifically informed management of these areas, is of paramount importance.

AUTHOR CONTRIBUTIONS

JHAL and PIS conceived the manuscript, designed sampling strategies, and collected the data. JHAL analyzed the data. JHAL, PIS and MJKBN wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

We declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.5x69p8d95.

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REFERENCES

Adamack, A. T., & Gruber, B. (2015). Landgenreport: A new R function to simplify landscape genetic analysis using resistance surface layers. *Molecular Ecology Resources*, *15*(5), 1172–1178. https://doi.org/10.1111/1755-0998.12381

Aguiar, A., Toledo, G. M., Anjos, L. A., & Silva, R. J. (2014). Helminth parasite communities of two *Physalaemus cuvieri* Fitzinger, 1826 (Anura: Leiuperidae) populations under different conditions of habitat integrity in the Atlantic rain forest of Brazil. *Brazilian Journal of Biology*, *75*(4), 963–968. https://doi.org/10.1590/1519-6984.03614

Amézquita, A., Lima, A. P., Jehle, R., Castellanos, L., Ramos, O., Crawford, A. J., Gasser, H., & Höld, W. (2009). Calls, colours, shape, and genes: A multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. *Biological Journal of the Linnean Society*, *98*, 826–838. https://doi.org/10.1111/j.1095-8312.2009.01324.x

Andersson, M. (1994). *Sexual selection* (Vol. *72*). Princeton University Press. https://doi.org/10.1515/9780691207278

Andrade-Lima, D. (Ed.). (1960). Estudos fitogeográficos de Pernambuco. In *Arquivo do Instituto de Pesquisa Agronômica* (Vol. *5*, pp. 305–341). Secretaria da Agricultura, Indústria e Comércio.

Andrade-Lima, D. (1982). Present day forest refuges in northeastern Brazil. In G. T. Prance (Ed.), *Biological diversification in the tropics* (pp. 245–254). Columbia University Press.

Armansin, N. C., Stow, A. J., Cantor, M., Leu, S. T., Klarrevas-Irby, J. A., Chariton, A. A., & Farine, D. R. (2019). Social barriers in ecological landscapes: The social resistance hypothesis. *Trends in Ecology & Evolution*, *35*(2), 137–148. https://doi.org/10.1016/j.tree.2019.10.001

Barreto, L., & Andrade, G. V. (1995). Aspects of the reproductive biology of *Physalaemus cuvieri* (Anura: Leptodactylidae) in northeastern Brazil. *Amphibia-Reptilia, Leiden*, *16*(1), 67–76. https://doi.org/10.1163/156853895X00208

Bivand, R., & Rundel, C. (2023). Rgeos: Interface to geometry engine—Open source ('GEOS'). https://r-forge.r-project.org/projects/rgeos/

Boughman, J. W. (2001). Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, *411*(6840), 944–948. https://doi.org/10.1038/35082064

Boul, K. E., Funk, W. C., Darst, C. R., Cannatella, D. C., & Ryan, M. J. (2007). Sexual selection drives speciation in an Amazonian frog. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1608), 399–406. https://doi.org/10.1098/rspb.2006.3736

Calsbeek, R., Smith, T. B., & Bardeleben, C. (2007). Intraspecific variation in *Anolis sagrei* mirrors the adaptive radiation of greater Antillean anoles. *Biological Journal of the Linnean Society*, *90*, 189–199. https://doi.org/10.1111/j.1095-8312.2007.00700.x

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

Carnaval, A. C., Hickerson, M. J., Haddad, C. F. B., Rodrigues, M. T., & Moritz, C. (2009). Stability predicts genetic diversity in the Brazilian Atlantic Forest hotspot. *Science*, *323*, 785–789. https://doi.org/10.1126/science.1166955

Carnaval, A. C. O. Q. (2002). Phylogeography of four frog species in forest fragments of northeastern Brazil—A preliminary study. *Integrative and Comparative Biology*, *42*, 13–921. https://doi.org/10.1093/icb/42.5.913

Chevin, L.-M., & Lande, R. (2010). When do adaptive plasticity and genetic evolution prevent extinction of a density-regulated population? *Evolution*, *64*, 1143–1150. https://doi.org/10.1111/j.1558-5646.2009.00875.x

Coimbra Filho, A. F., & Câmara, I. G. (Eds.). (1996). *Os limites originais do bioma Mata Atlântica na região Nordeste do Brasil*. Fundação Brasileira para a Conservação da Natureza (FBCN).

Costa, G. C., Hampe, A., Ledru, M.-P., Martinez, P. A., Mazzochini, G. G., Shepard, D. B., Werneck, F. P., Moritz, C., & Carnaval, A. C. (2017). Biome stability in South America over the last 30 kyr: Inferences from long-term vegetation dynamics and habitat modelling. *Global Ecology and Biogeography*, *00*, 1–13. https://doi.org/10.1111/geb.12694

Endler, J. A. (1977). *Geographic variation, speciation and clines*. Princeton University Press.

Engen, S., & Sæther, B. (2016). Phenotypic evolution by distance in fluctuating environments: The contribution of dispersal, selection and random genetic drift. *Theoretical Population Biology*, *109*, 16–27. https://doi.org/10.1016/j.tpb.2016.01.003

Erdtmann, L., & Amézquita, A. (2009). Differential evolution of advertisement call traits in dart-poison frogs (Anura: Dendrobatidae). *Ethology*, *115*(9), 801–811. https://doi.org/10.1111/j.1439-0310.2009.01673.x

Erdtmann, L. K., & Lima, A. P. (2013). Environmental effects on anuran call design: What we know and what we need to know. *Ethology Ecology & Evolution*, *25*(1), 1–11. https://doi.org/10.1080/03949370.2012.744356

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Ey, E., & Fischer, J. (2009). The "acoustic adaptation hypothesis"–A review of the evidence from birds, anurans and mammals. *Bioacoustics*, *19*(1–2), 21–48. https://doi.org/10.1080/09524622.2009.9753613

Fernandes, I. Y., Moraes, L. J. C. L., Menin, M., Farias, I. P., & Lima, A. P. (2021). Unlinking the speciation steps: Geographical factors drive changes in sexual signals of an Amazonian nurse-frog through body size variation. *Evolutionary Biology*, *48*(22), 1–13. https://doi.org/10.1007/s11692-020-09525-7

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315. https://doi.org/10.1002/joc.5086

Frost, D. R. (2022). *Amphibian species of the world: An online reference*. American Museum of Natural History. Retrieved January 18, 2022, from https://amphibiansoftheworld.amnh.org/index.php

Gehara, M., Garda, A. A., Werneck, F. P., Oliveira, E. F., Fonseca, E. M., Camurugi, F., Magalhães, F. M., Lanna, F. M., Sites, J. W., Jr., Marques, R., Silveira-Filho, R., São Pedro, V. A., Colli, G. R., Costa, G. C., & Burbrink, F. T. (2017). Estimating synchronous demographic changes across populations using hABC and its application for a herpetological community from northeastern Brazil. *Molecular Ecology*, *26*, 4756–4771. https://doi.org/10.1111/mec.14239

Gerhardt, H. C., & Huber, F. (Eds.). (2002). *Acoustic communication in insects and anurans: Common problems and diverse solutions*. University of Chicago Press.

Gingras, B., Boeckle, M., Herbst, C. T., & Fitch, W. T. (2013). Call acoustics reflect body size across four clades of anurans. *Journal of Zoology*, *289*(2), 143–150. https://doi.org/10.1111/j.1469-7998.2012.00973.x

Gomes, L., Röhr, D. L., Sousa-Lima, R. S., Garda, A. A., & Juncá, F. A. (2022). Environmental factors influencing call propagation in *Pithecopus nordestinus*: Testing the acoustic adaptation hypothesis. *Bioacoustics*, *31*(6), 696–709. https://doi.org/10.1080/09524622.2022.2042855

Gorelick, N., Hancher, M., Dixon, M., Ilyushchenko, S., Thau, D., & Moore, R. (2017). Google Earth Engine: Planetary-scale geospatial analysis for everyone. *Remote Sensing of Environment*, *202*, 18–27. https://doi.org/10.1016/j.rse.2017.06.031

Gould, S. J., & Johnston, R. F. (1972). Geographic variation. *Annual Review of Ecology, Evolution, and Systematics*, *3*, 457–498. https://doi.org/10.1146/annurev.es.03.110172.002325

Guedes, T. B., Azevedo, J. A. R., Bacon, C. D., Provete, D. B., & Antonelli, A. (2020). Diversity, endemism, and evolutionary history of montane biotas outside the Andean region. In V. Rull & A. C. Carnaval (Eds.), *Neotropical diversification: Patterns and process* (pp. 299–328). Springer. https://doi.org/10.1007/978-3-030-31167-4_13

Haddad, C. F. B., & Prado, C. P. A. (2005). Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. *Bioscience*, *55*(3), 207–217. https://doi.org/10.1641/0006-3568(2005)055[0207:RMIFAT]2.0.CO;2

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

Hepp, F., & Pombal, J. P., Jr. (2020). Review of bioacoustical traits in the genus *Physalaemus* Fitzinger, 1826 (Anura: Leptodactylidae: Leiuperinae). *Monograph*, *4725*(1), 1–106. https://doi.org/10.11646/zootaxa.4725.1.1

Hijmans, R. J. (2017). raster: Geographic data analysis and modeling. R package version (Vol. *2*, pp. 6–7). Retrieved from https://CRAN.R-project.org/package=raster

Hoekstra, H. E., Drumm, K. E., & Nachman, M. W. (2004). Ecological genetics of adaptive color polymorphism in pocket mice: Geographic variation in selected and neutral genes. *Evolution*, *58*(6), 1329–1341. https://doi.org/10.1111/j.0014-3820.2004.tb01711.x

Hoskin, C. J., Higgie, H., McDonald, K. R., & Moritz, C. (2005). Reinforcement drives rapid allopatric speciation. *Nature*, *437*, 1353–1356. https://doi.org/10.1038/nature04004

Huete, A., Didan, K., Miura, T., Rodriguez, E. P., Gao, X., & Ferreira, L. G. (2002). Overview of the radiometric and biophysical performance of the MODIS vegetation indices. *Remote Sensing of Environment*, *83*, 195–213. https://doi.org/10.1016/S0034-4257(02)00096-2

Huete, A., Liu, H. Q., Batchily, K., & Van Leeuwen, W. (1997). A comparison of vegetation indices over a global set of Tm images for EOS-MODIS. *Remote Sensing of Environment*, *59*, 440–451. https://doi.org/10.1016/S0034-4257(96)00112-5

Hughes, C. E., & Atchison, G. W. (2015). The ubiquity of alpine plant radiations: From the Andes to the Hengduan Mountains. *The New Phytologist*, *207*, 275–282. https://doi.org/10.1111/nph.13230

Jetz, W., & Pyron, R. A. (2018). The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nature Ecology & Evolution*, *2*, 850–858. https://doi.org/10.1038/s41559-018-0515-5

K. Lisa Yang Center for Conservation Bioacoustics. (2023). *Revan pro: Interactive sound analysis software (version 1.6.4)*. The Cornell Lab of Ornithology. Retrieved from http://ravensoundsoftware.com/

Kaefer, I. L., & Lima, A. P. (2012). Sexual signals of the Amazonian frog *Allobates paleovarzensis*: Geographic variation and stereotypy of acoustic traits. *Behaviour*, *149*, 15–33. https://doi.org/10.1163/156853912X623757

Kaefer, I. L., Tsuji-Nishikido, B. M., Mota, E. P., Farias, I. P., & Lima, A. P. (2013). The early stages of speciation in Amazonian forest frogs: Phenotypic conservatism despite strong genetic structure. *Evolutionary Biology*, *40*, 228–245. https://doi.org/10.1007/s11692-012-9205-4

Katoh, K. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, *30*, 3059–3066. https://doi.org/10.1093/nar/gkf436

Kelly, M. (2019). Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philosophical Transactions of the Royal Society B*, *374*, 20180176. https://doi.org/10.1098/rstb.2018.0176

Köhler, J., Jansen, M., Rodríguez, A., Kok, P. J. R., Toledo, L. F., Emmrich, M., Glaw, F., Haddad, C. F. B., Rödel, M.-O., & Vences, M. (2017). The use of bioacoustics in anuran taxonomy: Theory, terminology, methods and recommendations for best practice. *Zootaxa*, *4251*(1), 1–124.

Legendre, P., Lapointe, F.-J., & Casgrain, P. (1994). Modeling brain evolution from behavior: A permutational regression approach. *Evolution*, *48*, 1487–1499. https://doi.org/10.2307/2410243

Manly, B. F. J. (1986). Randomization and regression methods for testing for associations with geographical, environmental and biological distances between populations. *Researches on Population Ecology*, *28*, 201–218. https://doi.org/10.1007/BF02515450

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research*, *27*, 209–220.

Menezes, L., Batalha-Filho, H., Garda, A. A., & Napoli, M. F. (2020). Tiny treefrogs in the Pleistocene: Phylogeography of *Dendropsophus oliveirai* in the Atlantic Forest and associated enclaves in northeastern Brazil. *Journal of Zoological Systematics and Evolutionary Research*, *00*, 1–16. https://doi.org/10.1111/jzs.12422

Mitchell, A., & Bergmann, P. J. (2016). Thermal and moisture habitat preferences do not maximize jumping performance in frogs. *Functional Ecology*, *30*(5), 733–742. https://doi.org/10.1111/1365-2435.12535

Morton, E. S. (1975). Ecological sources of selection on avian sounds. *The American Naturalist*, *109*, 17–34. https://doi.org/10.1086/282971

Nosil, P. (2008). Speciation with gene flow could be common. *Molecular Ecology*, *17*, 2103–2106.

Nychka, D., Furrer, R., Paige, J., & Sain, S. (2021). fields: Tools for spatial data. R package version (Vol. *14*, p. 1). https://github.com/dnychka/fieldsRPackage

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'hara, R. B., Simpson, G. L., Solymos, P., & Stevens, M. H. H. (2022). *Vegan: Community ecology package (version 2.6-4)*. The Comprehensive R Archive Network.

Oliveira, C. R., Ávila, L. W., & Morais, D. H. (2019). Helminths associated with three *Physalaemus* species (*Anura: Leptodactylidae*) from Caatinga biome, Brazil. *Acta Parasitologica*, *64*, 205–212. https://doi.org/10.2478/s11686-018-00022-8

Palumbi, S., Martin, A., Romano, S., McMillan, W. O., Stice, L., & Grabowski, G. (2002). *The simple fool's guide to PCR. Version 2*. (pp. 1–45). Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.

Pebesma, E., & Bivand, R. (2005). Classes and methods for spatial data in R. *R News*, *5*(2), 9–13. https://CRAN.R-project.org/doc/Rnews/

Pie, M. R., Faircloth, B. C., Ribeiro, L. F., Bornschein, R., & McCormack, J. E. (2018). Phylogenomics of montane frogs of the Brazilian Atlantic Forest is consistent with isolation in sky islands followed by climatic stability. *Biological Journal of the Linnean Society*, *125*, 72–82. https://doi.org/10.1093/biolinnean/bly093

Pinho, C., & Hey, J. (2010). Divergence with gene flow: Models and data. *Annual Review of Ecology, Evolution, and Systematics*, *41*, 215–230.

Pombal, J. P., & Haddad, C. F. B. (2007). Estratégias e modos reprodutivos em anuros. In B. Nascimento & P. M. E. Oliveira (Eds.), *Herpetologia no Brasil II* (pp. 101–116). Sociedade Brasileira de Herpetologia.

Porto, T. J., Carnaval, A. C., & Rocha, P. L. B. (2013). Evaluating forest refugial models using species distribution models, model filling and inclusion: A case study with 14 Brazilian species. *Diversity and Distributions*, *19*, 330–340. https://doi.org/10.1111/j.1472-4642.2012.00944.x

Prance, G. T. (1982). Forest refuges: Evidence from woody angiosperms. In G. T. Prance (Ed.), *Biological diversification in the tropics* (pp. 137–158). Columbia University Press.

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. http://www.Rproject.org/

Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J., & Hirota, M. M. (2009). The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation*, *142*, 1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021

Robertson, J. M., & Robertson, A. D. (2008). Spatial and temporal patterns of phenotypic variation in a neotropical frog. *Journal of Biogeography*, *35*, 830–843. https://doi.org/10.1111/j.1365-2699.2007.01824.x

Rodal, M. J. N., Barbosa, M. R. V., & Thomas, W. W. (2008). Do the seasonal forests in northeastern Brazil represent a single floristic unit? *Brazilian Journal of Biology*, *68*(3), 467–475. https://doi.org/10.1590/S1519-69842008000300003

Röhr, D. L., Camurugi, F., Martinez, P. A., Sousa-Lima, R. S., Junca, F. A., & Garda, A. A. (2020). Habitat-dependent advertisement call variation in the monkey frog *Phyllomedusa nordestina*. *Ethology*, *126*(6), 651–659. https://doi.org/10.1111/eth.13017

Rosa, M. V., Ferrão, M., Pequeno, P. A. C. L., & Lima, A. P. (2023). How do tree density and body size influence acoustic signals in Amazonian nurse frogs? *Bioacoustics*, *30*(1), 1–15. https://doi.org/10.1080/09524622.2023.2204313

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, *8*(3), 336–352. https://doi.org/10.1111/j.1461-0248.2004.00715.x

Ryan, M. J. (1988). Constraints and patterns in the evolution of anuran acoustic communication. In B. Fritzch, M. J. Ryan, W. Wilczynski, T. E. Heherington, & W. Walkowiak (Eds.), *The evolution of the amphibian auditory system* (pp. 637–677). Wiley.

Ryan, M. J., & Brenowitz, E. A. (1986). The role of the body size, phylogeny, and ambient noise in the evolution of bird song. *The American Naturalist*, *126*(1), 87–100. https://doi.org/10.1086/284398

Sales, M. F., Mayo, S. J., & Rodal, M. J. (Eds.). (1998). *Plantas vasculares das florestas serranas de Pernambuco: um checklist da flora ameaçada dos brejos de altitude, Pernambuco, Brasil*. Imprensa Universitária, Universidade Federal Rural de Pernambuco, Recife.

Santana, D. J., Mesquita, D. O., & Garda, A. A. (2011). Advertisement call of *Dendropsophus oliveirai* (Anura, Hylidae). *Zootaxa*, *2997*, 67–68. https://doi.org/10.11646/zootaxa.2997.1.5

Santos, A. M. M., Cavalcanti, D. R., Silva, J. M. C., & Tabarelli, M. (2007). Biogeographical relationships among tropical forests in Northeastern Brazil. *Journal of Biogeography*, *34*, 437–446. https://doi.org/10.1111/j.1365-2699.2006.01604.x

Schluter, D. (2009). Evidence for ecological speciation and its alternative. *Science*, *323*, 737–741. https://doi.org/10.1126/science.1160006

Silva, J. M. C., Leal, I. R., & Tabarelli, M. (Eds.). (2018). *Caatinga: The largest tropical dry forest region in South America*. Springer.

Silva, N. R., Neto, J. A., Prado, C. P. A., & Mott, T. (2019). Reproductive biology of *Dendropsophus haddadi* (Bastos and Pombal, 1994), a small treefrog of the Atlantic Forest. *Herpetology Notes*, *12*, 319–325.

Silveira, M. H. R., Mascarenhas, R., Cardoso, D., & Batalha-Filho, H. (2019). Pleistocene climatic instability drove the historical distribution of forest islands in the northeastern Brazilian Atlantic Forest. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *527*, 67–76. https://doi.org/10.1016/j.palaeo.2019.04.028

Smith, M. J., Osborne, W., & Hunter, D. (2003). Geographic variation in the advertisement call structure of *Litoria verreauxii* (*Anura: Hylidae*). *Copeia*, *4*, 750–758. https://doi.org/10.1643/HA02-133.1

Staden, R. (1996). The Staden sequence analysis package. *Molecular Biotechnology*, *5*, 233–241. https://staden.sourceforge.net/

Studer, A. (2015). Aves. In A. Studer, L. Nusbaumer, & R. Spichiger (Eds.), *Biodiversidade da Reserva Biológica de Pedra Talhada* (pp. 377–405). Alagoas / Pernambuco.

Sun, K., Luo, L., Kimball, R. T., Wei, X., Jin, L., Jiang, T., Li, G., & Feng, J. (2013). Geographic variation in the acoustic traits of greater horseshoe bats: Testing the importance of drift and ecological selection in evolutionary processes. *PLoS One*, *8*, 1–11. https://doi.org/10.1371/journal.pone.0070368

Tabarelli, M., & Santos, A. M. M. (2004). Uma breve descrição sobre a história natural dos Brejos Nordestinos. In K. C. Pôrto, J. J. P. Cabral, & M. Tabarelli (Eds.), *Brejos de Altitude em Pernambuco e Paraíba: História natural, ecologia e conservação* (pp. 99–110). Ministério do Meio Ambiente.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Theulen, V. (2004). Conservação dos Brejos de Altitude no estado de Pernambuco. In K. C. Pôrto, J. J. P. Cabral, & M. Tabarelli (Eds.), *Brejos de Altitude em Pernambuco e Paraíba história natural, ecologia e conservação* (pp. 299–302). Ministério do Meio Ambiente.

Titon Junior, B., & Gomes, F. R. (2015). Relation between water balance and climatic variables associated with the geographical distribution of anurans. *PLoS One*, *10*(10), e0140761. https://doi.org/10.1371/journal.pone.0140761

Tobias, M. L., Evans, B. J., & Kelley, D. B. (2011). Evolution of advertisement calls in African clawed frogs. *Behaviour*, *148*, 519–549. https://doi.org/10.1163/000579511X569435

Tobias, M. L., Planqué, R., Cram, D. L., & Seddon, N. (2014). Species interactions and the structure of complex communication networks. *Proceedings of the National Academy of Sciences*, *111*(3), 10120–11025. https://doi.org/10.1073/pnas.1314337111

Toledo, L. F., Llusia, D., Vieira, C. A., Corbo, M., & Márquez, R. (2015). Neither convergence nor divergence in the advertisement call of sympatric congeneric neotropical treefrogs. *Bioacoustics*, *24*(1), 31–47. https://doi.org/10.1080/09524622.2014.926831

Twomey, E., Yeager, J., Brow, J. L., Morales, V., Cummings, M., & Summers, K. (2013). Phenotypic and genetic divergence among poison frog populations in a mimetic radiation. *PLoS One*, *8*(2), e55443. https://doi.org/10.1371/journal.pone.0055443

Velásquez, N. A., Moreno-Gómez, F. N., Brunetti, E., & Penna, M. (2018). The acoustic adaptation hypothesis in a widely distributed south American frog: Southernmost signals propagate better. *Scientific Reports*, *8*(6990), 1–12. https://doi.org/10.1038/s41598-018-25359-y

Velloso, A. L., Sampaio, E. V. S. B., & Pareyn, F. G. C. (Eds.). (2002). *Ecorregiões propostas para o bioma Caatinga*. Associação Plantas do Nordeste, Instituto de Conservação Ambiental The Nature Conservancy do Brasil.

Walsh, P. S., Metzger, D. A., & Higuchi, R. (1991). Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, *10*, 506–513.

Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology*, *23*(23), 5649–5662. https://doi.org/10.1111/mec.12938

Wang, P. S. (2013). Examining the full effects of landscape heterogeneity on spatial genetic variation: A multiple matrix regression approach for quantifying geographic and ecological isolation. *Evolution*, *67*(12), 3403–3411. https://doi.org/10.1111/evo.12134

Wells, K. D. (2007). *The ecology and behavior of amphibians*. University of Chicago Press. https://doi.org/10.7208/chicago/9780226893334.001.0001

Wilkins, M. R., Seddon, N., & Safran, R. J. (2013). Evolutionary divergence in acoustic signals: Causes and consequences. *Trends in Ecology & Evolution*, *28*(3), 156–166. https://doi.org/10.1016/j.tree.2012.10.002

Wright, S. (1951). The genetical structure of populations. *Annals of Human Genetics*, *15*, 323–354. https://doi.org/10.1111/j.1469-1809.1949.tb02451.x

Zamudio, K. R., Bell, R. C., & Mason, N. A. (2016). Phenotypes in phylogeography: Species' traits, environmental variation, and vertebrate diversification. *PNAS*, *113*(29), 8041–8048. https://doi.org/10.1073/pnas.1602237113

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