Universidade Federal de Goiás
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Genética e Biologia Molecular

# A HISTÓRIA EVOLUTIVA DE UMA PERERECA SUL-AMERICANA

# *Scinax squalirostris* (LUTZ, 1925) (ANURA, HYLIDAE):

# UM RESGATE DO PASSADO E CONSEQUÊNCIAS FUTURAS

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**Orientadora:** Prof.ª Dr.ª Rosane Garcia Collevatti
**Co-orientador:** Prof. Dr. Natan Medeiros Maciel

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**ATA DA SESSÃO PÚBLICA DE DEFESA DE TESE DE Nº 015**

Aos trinta e um dias do mês de outubro do ano de dois mil e dezoito (31/10/2018), às 08hs30min, no auditório do ICB V/UFG, reuniram-se os componentes da banca examinadora: Prof. Dr. Natan Medeiros Maciel, co-orientador; Profa. Dra. Daniela de Melo e Silva; Profa. Dra. Natácia Evangelista de Lima; Profa. Dra. Luciana Signorelli Faria Lima e Prof. Dr. Iberê Farina Machado, para, em sessão pública presidida pelo primeiro examinador citado, procederem à avaliação da defesa de tese intitulada: **"A HISTÓRIA EVOLUTIVA DE UMA PERERECA SUL-AMERICANA *Scinax squalirostris* (LUTZ, 1925) (ANURA, HYLIDAE): UM RESGATE DO PASSADO E CONSEQUÊNCIAS FUTURAS"**, em nível de doutorado, área de concentração em **Genética e Biologia Molecular**, de autoria de **TATIANNE PIZA FERRARI ABREU JARDIM**, discente do Programa de Pós-Graduação em Genética e Biologia Molecular da Universidade Federal de Goiás. Em virtude da orientadora Profa. Dra. Rosane Garcia Colevatti ter sido convocada para missão no CNPq, assumiu a presidência da banca o co-orientador Prof. Dr. Natan Medeiros Maciel, que fez a apresentação formal dos membros da banca. A palavra, a seguir, foi concedida à autora da tese que, em cerca de 45 minutos, procedeu à apresentação de seu trabalho. Terminada a apresentação, cada membro da banca arguiu a examinada, tendo-se adotado o sistema de diálogo sequencial. Terminada a fase de arguição, procedeu-se a avaliação da tese. Tendo-se em vista o que consta na Resolução nº 1294 de 06 de Junho de 2014 do Conselho de Ensino, Pesquisa, Extensão e Cultura (CEPEC), que regulamenta o Programa de Pós-Graduação em Genética e Biologia Molecular, a tese foi APROVADA, considerando-se integralmente cumprido este requisito para fins de obtenção do título de Doutor (a) em Genética e Biologia Molecular pela Universidade Federal de Goiás. A conclusão

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do curso dar-se-á quando da entrega da versão definitiva da tese na secretaria do programa, com as devidas correções sugeridas pela banca examinadora, no prazo de trinta dias a contar da data da defesa. Cumpridas as formalidades de pauta, às 12 horas e 17 minutos, encerrou-se a sessão de defesa e, para constar, o presidente da Banca lavrou a presente ata que, após lida e aprovada, será assinada pelos membros da banca examinadora em três vias de igual teor.

Prof. Dr. Natan Medeiros Maciel
**Presidente da Banca**
**UFG/GO**

Profa. Dra. Daniela de Melo e Silva
**UFG/GO**

Profa. Dra. Natácia Evangelista de Lima
**UFG/GO**

Profa. Dra. Luciana Signorelli Faria Lima
**UFG/GO**

Prof. Dr. Iberê Farina Machado
**UFG/GO**

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# SUMÁRIO

## RESUMO GERAL

Diferentes eventos, como geológicos do Neógeno e climáticos do Quaternário, tiveram um papel importante com alterações da paisagem e do clima na América do Sul, influenciando diretamente a história evolutiva dos organismos da região nos últimos milhões de anos. Essas mudanças levaram à alternância entre períodos quentes e úmidos com frios e secos, e essa alternância iniciou a dinâmica de retração e expansão de paisagens abertas e florestais. Espécies associadas a esses ambientes evoluíram seguindo essa dinâmica, levando a alteração na conformação genética, diferenciação de linhagens e até a especiação. Assim como no passado, mudanças climáticas futuras podem alterar a paisagem causando mudanças na distribuição geográfica das espécies. Além disso, o aquecimento global previsto pode levar a diminuição da diversidade genética e ocasionar a extinção devido à baixa capacidade das espécies de se adaptarem as mudanças drásticas tão rapidamente. Nesta tese utilizou-se duas regiões do DNA mitocondrial (Cytb e 12S) e uma nuclear (RAG-1) juntamente com simulações coalescentes, e de Modelagem de Nicho Ecológico para acessar a história evolutiva de uma espécie de perereca *Scinax squalirostris* (Lutz, 1925) associada aos Campos (*grasslands*) Sul-Americanos. No primeiro capítulo buscou-se entender como eventos geológicos do Neógeno e climáticos do Quaternário podem ter moldado a atual distribuição disjunta e o padrão de diversidade genética de *S. squalirostris*. Encontrou-se que as populações de *S. squalirostris* possuem alta diversidade genética, com nenhum sinal de fluxo gênico atual, uma alta diferenciação genética e história demográfica estável ao longo do tempo com origem de dispersão no Sul do Brasil. Eventos de coalescência dataram do Plioceno-Pleistoceno, com compartilhamento de haplótipos entre as populações geograficamente distantes, indicando um arranjo incompleto de linhagens. A modelagem de paleodistribuição sugere que as linhagens de *S. squalirostris* tinham uma ocorrência de ampla distribuição no último máximo glacial (LGM) com contração e mudança de área no período pós-LGM. Tais resultados indicam que a atual distribuição geográfica e diversidade genética de *S. squalirostris* é devido a contração de uma área amplamente distribuída no passado, gerada pela dinâmica de retração de *grasslands* nos períodos mais quentes devido à perda de áreas adequadas para sua ocorrência. No segundo capítulo testou-se a hipótese de que as populações atuais de *S. squalirostris* poderiam representar linhagens distintas, com potencial (is) espécie(s) candidata(s) não descrita(s), devido a atual distribuição disjunta. Com a utilização de dados moleculares e dados morfométricos resgatou-se a formação de dois grupos, sendo um destes com uma espécie a ser descrita, um grupo restrito a região Centro-Oeste do Brasil, e outro grupo abrangendo populações do Sul e Sudeste do Brasil, Paraguai, Uruguai e Argentina. No terceiro capítulo utilizou-se a modelagem de nicho ecológico, juntamente com as análises moleculares e as simulações de agrupamentos genéticos para verificar o quanto as mudanças climáticas futuras poderão alterar a diversidade genética e a distribuição de *S. squalirostris*. Através de dois cenários climáticos com diferentes alterações na temperatura para 2100 (cenário 4.5 RCP aumenta 1.8ºC e estabiliza, e o cenário 8.5 aumenta 3.7°C e continua aumentando), a análise de modelagem de nicho

indicou uma diminuição de áreas adequadas na região Centro-Oeste e Sudeste, com um deslocamento em direção ao Sul do Brasil adentrando até a região central da Argentina em direção a áreas mais antropizadas. A maioria das populações do Centro-Oeste e norte da região Sudeste poderão ser extintas devido à ausência de áreas climáticas adequadas para a sua ocorrência e sua baixa diversidade genética. Além disso, foi observado que as Unidades de Conservação (UCs) detêm, atualmente, grande parte da diversidade genética de *S. squalirostris*. Com as mudanças climáticas previstas, as UCs em áreas que serão ideais para a ocorrência de *S. squalirostris* conseguirão manter altos níveis de diversidade genética, porém com perdas de diversidade na região Centro-Oeste e Sudeste. Este trabalho indica que as mudanças climáticas futuras afetarão negativamente essa espécie, pois as áreas adequadas para sua ocorrência serão reduzidas e deslocadas. A perda e as alterações nos agrupamentos genéticos, podem levar a uma possível perda do potencial evolutivo das populações de *S. squalirostris* em responder às mudanças climáticas futuras, o que poderia resultar na extinção de algumas populações.

**Palavras-chave:** conservação; delimitação; diversidade genética; simulação coalescente; filogeografia; mudança climática.

## GENERAL ABSTRACT

Geological events of the Neogene and the climatic fluctuations of the Quaternary played an important role in shaping the landscape and climate of South America therefore directly influencing the evolutionary history of the organisms of this area over the last million years. These changes led to the alternation between warm and humid, cold and dry periods. Such alternation dictated the dynamics of retraction and expansion of open and forest landscapes. Species associated to these environments evolved following this dynamic, which lead to alteration in genetic conformation, lineage differentiation and even speciation. As in the past, future changes in climate can modify the landscape causing changes in the geographical distribution of species. In addition, predicted global warming may lead to a decline in genetic diversity as well as lead to extinction due to species' low ability to adapt to drastic and quick changes. In this thesis two regions of mitochondrial DNA (Cytb and 12S) and one nuclear (RAG-1) were used together with coalescing simulations, and ecological niche modelling to access the evolutionary history of a *Scinax squalirostris* (Lutz, 1925), a species associated to the South American grasslands. In the first chapter, we sought to understand how Neogene and Quaternary geological or climatic events, respectively, may have shaped the current disjunct distribution and the genetic diversity pattern of *S. squalirostris*. The populations of *S. squalirostris* were found to have high genetic diversity, with no sign of current gene flow, a high genetic differentiation, and a stable demographic history over time with scattered origin in southern Brazil. Coalescence events date from Pliocene-Pleistocene, with haplotype sharing among geographically distant populations, which indicates incomplete lineage sorting. The paleodistribution models suggests that *S. squalirostris* lineages were widely distributed during the last glacial maximum (LGM) but afterwards contracting and changing their area of occurrence. These results indicate that the current geographic distribution and genetic diversity of *S. squalirostris* is due to the contraction of an area widely distributed in the past, generated by the dynamics of retraction of grasslands in warmer periods due to the loss of areas suitable for their occurrence. In the second chapter, we tested the hypothesis that the current populations of *S. squalirostris* could represent distinct lineages with candidate species not previously described, due to the current disjunct distribution. Using molecular and morphometric data the formation of two groups was rescued. One of them consists in a candidate species to be described, which is a lineage restricted to the Central-West region of Brazil. The other one comprises of populations from the South and Southeast Brazil, Paraguay, Uruguay and Argentina. In the third chapter, ecological niche modelling, molecular techniques and simulations of genetic groups were used to verify how future climate changes could alter the genetic diversity and distribution of *S. squalirostris*. Through two climatic scenarios with different temperature changes to 2100 (scenario 4.5 RCP increases 1.8 ° C and stabilizes, and scenario 8.5 increases 3.7 ° C and continues to increase), ecological niche modelling analysis indicated a decrease of suitable areas in the Central-West and Southeast regions, with a displacement towards the South of Brazil entering the central region of Argentina towards more anthropized areas. Most of

the Central West and Northern Southeast populations may be extinct due to the absence of climatic suitable areas for their occurrence and low genetic diversity. In addition, it was observed that Protections Areas (PAs) currently harbors a large part of the genetic diversity of *S. squalirostris*. Thus, PAs in areas that will be ideal for the occurrence of *S. squalirostris* will be able to maintain their high levels of genetic diversity, but with losses of genetic diversity in the Midwest and Southeast regions. This work indicates that future climate changes will negatively affect this species, since the appropriate areas for its occurrence will be reduced and displaced. The loss and changes in genetic clusters may lead to a possible loss of the evolutionary potential of *S. squalirostris* populations in responding to future climate changes, which could result in the extinction of some populations.

**Keywords:** coalescent simulations; conservation; climate change; delimitation; genetic diversity; phylogeography.

# INTRODUÇÃO GERAL

Os eventos geológicos do Neógeno e climáticos do Quaternário tiveram um papel importante nesse processo, com alterações da paisagem e do clima (Burnham & Graham, 1999) na América do Sul, influenciando diretamente a história evolutiva dos organismos da região nos últimos milhões de anos (Behling 2002, Ortiz-Jaureguizar & Cladera, 2006, Tuomisto, 2007). Eventos como retração e transgressão marinha, soerguimento e formações costeiras e flutuações climáticas levaram a expansão e a retração de florestas e áreas abertas (Behling, 2002, Ortiz-Jaureguizar & Cladera, 2006; Overbeck, 2007) moldando a distribuição da biota encontrada atualmente. Assim, entender e reconstruir a história dessas biotas pode nos ajudar a entender os padrões de organização espacial dos organismos e os processos que resultaram nesses padrões (Riddle et al., 2008) e termos um melhor conhecimento sobre como os eventos geológicos e climáticos do Neógeno e Quaternário podem ter afetado os organismos.

A Filogeografia (Avise et al., 1987) é um campo que trata tanto das relações filogenéticas entre os táxons de interesse bem como dos componentes históricos responsáveis pela distribuição espacial das linhagens (Avise et al., 1987; Freeland, 2005). Essa área tem por objetivo entender como os eventos históricos influenciaram na atual distribuição geográfica da diversidade genética, de populações e de espécies (Freeland, 2005). A obtenção dessa história demográfica através da análise filogeográfica é de grande importância para melhorar a compreensão de processos microevolutivos na escala espaço/temporal genética (Knowles e Maddison, 2002). A modelagem de paleodistribuição projetada em cenários climáticos passados podem (i) fornecer um contexto espacial da história demográfica da espécie para as análises filogeográficas, (ii) os prováveis precursores dos padrões de divergência e (iii) a distribuição observada atualmente nas espécies (e.g. Lima et al., 2014; Collevatti et al., 2015; Vitorino et al., 2017).

Além de entender a influência dos eventos históricos, é importante também predizer o efeito das mudanças climáticas futuras na distribuição e diversidade genética dos organismos, e podemos acessar esses efeitos através da utilização da modelagem de nicho ecológico (ENM) (e.g. Lima et al., 2017; Vasconcelos *et al*., 2018). A diversidade genética intraespecífica representa o potencial evolutivo das espécies, nas quais a

seleção natural atua para promover a evolução dos organismos e sua adaptação às mudanças ambientais (Urban et al., 2012, 2013). Altos níveis de diversidade genética podem favorecer uma rápida resposta evolutiva, mas populações com baixa diversidade genética têm seu potencial evolutivo reduzido e tornando-as propensas à extinção local (Spielman et al., 2004; Frankham, 2005). Como é esperado que até 2100 a temperatura aumente em aproximadamente 2ºC a 4ºC (New *et al*., 2011; Fischer *et al*., 2018) devido a emissão de gases de efeito estufa (IPCC, 2014), se faz necessário entender como essas mudanças podem afetar as espécies e se terão capacidade de responder de forma tão rápida quanto a intensidade que tais alterações ocorrem. Além disso, é importante compreender o quanto a diversidade genética dessas espécies está representada em Unidades de Conservação e avaliar se, devido às mudanças climáticas essa diversidade será mantida.

Estudos filogeográficos da biota sul-americana têm mostrado uma história complexa de diversificação, na qual a combinação dos eventos orogênicos do Neógeno e as oscilações climáticas do Quaternário contribuíram para moldar a presente diversidade e distribuição das linhagens do continente, inclusive para anuros (Rull, 2011; Turchetto-Zolet et al., 2013). Além disso, estudos elucidam que os anuros são os grupos que mais podem sofrer com mudanças climáticas futuras (Loyola *et al*., 2014; Vilela *et al*., 2018; Vasconcelos *et al*., 2018). Tendo em vista ao apresentado até aqui, nesta tese, nós buscamos resgatar a história evolutiva passada e predizer os efeitos das mudanças climáticas, em um complexo de espécie de uma perereca sul-americana *Scinax squalirostris*.

*Scinax squalirostris* foi descrita por Adolpho Lutz em 1925, tendo como localidade tipo a Fazenda Bonito na Serra da Bocaina, fronteira entre os estados brasileiros de São Paulo e Rio de Janeiro. O nome específico se refere ao formato do focinho parecido com tubarão, *shark-like snout* (Lutz, 1925). *Scinax squalirostris* pertence ao clado *Scinax ruber* (Faivovich et al., 2005; Wiens et al., 2010). O clado *S. ruber* é bem diversificado e possui uma taxonomia complexa, sendo que algumas espécies podem representar um complexo de espécies (Fouquet et al., 2007). Duellman e colaboradores (2016) realizaram um estudo filogenético e biogeográfico com hilídeos a fim de tentar compreender melhor a relação e a história evolutiva dessa linhagem. Utilizando 44% espécies de hilídeos formalmente descritas obteve um clado bem suportado onde *S. squalirostris* se encontra. Foi sugerida então a criação de uma

subfamília chamada Scinaxinae para abrigar este clado. Em análises para essa subfamília Duellman et al. (2016) obtiveram a formação de clados com suportes baixos, indicando que o clado *Scinax ruber* é uma das linhagens que necessitam de estudos adicionais com o intuito de compreender com maior refinamento suas relações de parentesco.

Esta espécie de perereca possui uma ampla distribuição geográfica, mas de forma disjunta. Ocorre na região meridional do continente sul-americano, incluindo o Centro-Oeste, Sudeste e Sul do Brasil, Sul do Paraguai, Uruguai, Norte da Argentina e região Leste da Bolívia (Brandão et al., 1997; Uetanabaro et al., 2007). Possui como habitat áreas abertas, ocorrendo em áreas denominada de Campos (Eterovick & Sazima, 2004; Cruz et al., 2009). *Scinax squalirostris* é considerada uma espécie fora de perigo de extinção (IUCN, 2018). No meio científico esta espécie é considerada um complexo de espécies crípticas (Eterovick & Sazima, 2004), possuindo variação acústica (Faria et al., 2013) e variações em relação ao tamanho e coloração (observação pessoal).

Esta tese será apresentada e composta em capítulos, sendo composta por 3 capítulos. O primeiro capítulo abrange a filogeografia e os processos associados à distribuição de *S. squalirostris*. Neste capítulo nós utilizamos uma abordagem filogeográfica, com simulações coalescentes e modelagem de paleodistribuição, para compreender a atual distribuição geográfica e a diversidade genética das populações de *Scinax squalirostris*, bem como quais os eventos geológicos e/ou climáticos podem ter moldado e afetado a distribuição geográfica e diversidade genética dessa espécie.

O segundo capítulo é um trabalho de delimitação de espécie, em que testamos a existência de mais de uma linhagem evolutiva sob o mesmo nome de *S. squalirostris*. Como a sua distribuição é dada de forma disjunta e dada a sua baixa capacidade de dispersão, é esperado que haja uma alta estruturação genética populacional, devido à restrição do fluxo gênico. Isso poderia ter levado as populações a se diversificarem, e apresentarem linhagens evolutivas distintas. Assim, nós utilizamos uma abordagem de taxonomia integrativa, utilizando dados moleculares juntamente com dados morfométricos para testar a existência de linhagens candidatas a espécie.

No terceiro capítulo, verificamos como as mudanças climáticas previstas para 2100 poderão afetar a distribuição das populações de *S. squalirostris* e se essa espécie estaria ameaçada sob esses eventos. Para isso, nós utilizamos simulações genéticas e

modelagem de nicho ecológico para tentar compreender como as mudanças climáticas futuras poderão afetar a diversidade genética e a distribuição das populações de *S. squalirostris*. Além disso, avaliamos se a diversidade genética atual é bem representada em Unidades de Conservação e se, devido às mudanças climáticas futuras, essa diversidade genética ainda será preservada.

## OBJETIVO GERAL

O objetivo desta tese foi investigar a origem da distribuição geográfica e da variação genética, história evolutiva e as relações filogenéticas de *Scinax squalirostris*.

## OBJETIVOS ESPECÍFICOS

1. Investigar a história demográfica e origem da distribuição disjunta de *Scinax squalirostris* e avaliar a dinâmica de dispersão ao longo do tempo.

2. Verificar a existência de mais de uma linhagem evolutivamente distinta nas populações analisadas de *Scinax squalirostris*.

3. Compreender se e como as mudanças climáticas futuras podem apresentar risco para a distribuição e diversidade genética de *Scinax squalirostris*.

4. Verificar como a riqueza genética atual está representada em Unidades de Conservação e predizer o quanto essa diversidade genética ainda será representada no futuro.

# Capítulo 1

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Capítulo elaborado conforme as normas da revista e para submissão:
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RESEARCH PAPER

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# Inferring responses to Neogene-Quaternary climate dynamics from historical demography in a South American grassland treefrog

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**Short running title:** Phylogeography of the anura *Scinax squalirostris* (Anura, Hylidae)

**Number of words:** 7.149

**Data accessibility:** It will be deposited to GenBank and TreeBASE

**Abstract**

**Aim:** Our goal was to reconstruct the demographic history of lineages of *Scinax squalirostris* and to understand the dispersal dynamics through the Neogene-Quaternary periods. Additionally, we aimed to investigate how the climatic cycles of these periods influenced its current geographical distribution and genetic diversity patterns.

**Location:** grasslands of Meridional South America

**Methods:** We sampled 219 individuals of *Scinax squalirostris* from 26 localities in Southern South America and applied a statistical phylogeography framework, integrating coalescent simulations, ecological niche modelling and reconstruction of spatio-temporal dynamics. The genetic data was based on polymorphisms with different mutation rates from two mitochondrial and one nuclear regions.

**Results:** *Scinax squalirostris* populations were genetically structured and are characterized by high genetic diversity. Coalescent tree and Bayesian clusters showed incomplete lineage sorting with geographically distant populations sharing haplotypes

and even ancestral. The ancestral location reconstruction placed the root in South of Brazil, and the dispersal events were mediated by the suitable climatic conditions occurring during Pliocene-Pleistocene transition. Paleodistribution modelling showed a large area of occurrence during the LGM, with contraction in the Holocene until present.

**Main conclusions:** Incomplete lineage sorting and common ancestry among *Scinax squalirostris* populations from Pampas, Chaco, Atlantic Forest and Cerrado biome are evidences of the past connection among these biomes, indicating that the current disjunct distribution is most likely due to the range contraction of a previous ancient and wider distribution in response to climatic dynamics in Quaternary.

**Keywords:** anuran, coalescent analysis, climatic changes, discrete phylogeography, divergence times, genetic diversity, Scinaxinae, *Scinax squalirostris*.

## 1 | INTRODUCTION

A mosaic of vegetations comprising forests, open vegetations (e.g. Cerrado, Caatinga and Pampas) and grasslands (Behling, 2002) cover the Southeastern of South America. South America grasslands are patchy and discontinuous vegetation (Overback *et al*., 2007) composed by a mixture of wood, shrubland and herbaceous plants, occurring in areas of strong environmental seasonality, ranging their annual precipitation from 600 mm to 1.500 mm and average annual temperatures from -5ºC to 20ºC (Woodward *et al*., 2004). Grasslands lie on tropical, subtropical and temperate regions where they may be exemplified, respectively, by *campo limpo*, *campo sujo*, and *campos rupestres* in Cerrado biome, *campos de altitude* in Atlantic Forest and *campos sulinos* (temperate grassland) in Pampas biome of Argentina, Uruguay and Southern of Brazil (Leite &

Klein, 1990; Overback *et al*., 2007). Additionally, grasslands could be also finde associated with forests resulting a mosaic of vegetation, such as Araucaria Forest mosaic in Southern Brazilian Atlantic Forest (Leite & Klein, 1990; Overback *et al*., 2007).

Grasslands have been shaped along time by an ancient and complex history since Miocene geologic events (Behling 2002, Ortiz-Jaureguizar & Cladera, 2006). In Miocene-Late and Pliocene periods, the widely distributed "Paranean Sea" regressed allowing grasslands to occupy plains extending from North/Northern Patagonia to central and Northern Argentina and Uruguay (Ortiz-Jaureguizar & Cladera, 2006). Thereafter, during Late Pliocene, the climate became drier and cooler promoting further expansion of the open vegetations (Ortiz-Jaureguizar & Cladera, 2006). Also, during Pleistocene glacial period, temperature decreased from 5º to 7ºC, allowing grassland distribution to expand toward North of South America (Behling, 2002; Ortiz-Jaureguizar & Cladera, 2006). In this period of grassland expansions, Atlantic Forest retracted its distribution and became restricted to coastal areas (Behling, 2002). However, as temperature increased and climate became humid during the interglacial period, grasslands retracted their distributions allowing forests to expand again over South America (Ortiz-Jaureguizar & Cladera, 2006; Overbeck, 2007). Consequently, grasslands have been fragmented and lost suitable areas since the end of the dry and cold climatic conditions of Pliocene and Pleistocene, resulting their current disjuncted distribution (Behling 2002, Ortiz-Jaureguizar & Cladera, 2006).

As grasslands distribution have been shaped by cycles of expansion and contraction, and the species inhabiting at these habitats may have been evolutionarily pressured it to adapt or to track grasslands dynamics. There are still few phylogeographic studies concerning species inhabiting open vegetations and grasslands of South America (Turchetto-Zolet *et al*., 2013), most of them restricted to South and

Southeastern Brazil (e.g. Langone *et al*., 2015; Silva *et al*., 2017). Species do not respond equally to climatic cycles, such as expanding their distribution ranges or losing genetic diversity (see Turchetto-Zolet *et al*., 2013). For instance, *Calibrachoa heterophylla*, which occurs in Southern Brazil, had a retraction of its distribution during the colder periods, but expanded along interglacial periods, due to formation of new suitable areas in response to warmer temperatures (Mäder *et al*., 2013). Also, Langone *et al*. (2015) showed that the amphibian species *Pseudopaludicola falcipes*, from Southern temperate grassland, also expanded its distribution along interglacial periods favouring gene flow among its populations. For other species, colder and drier periods favoured expansion and stability of their geographic ranges, but warmer periods caused range retraction (Cristiano *et al*., 2016), resulting in different demographic and genetic effects, such as divergence and genetic structuration (e.g. Collevatti, *et al*., 2009; Lorenz-Lenke *et al*., 2010, Maia *et al*., 2017 and Peçanha *et al*., 2017). In addition, some geomorphological events in the Quaternary, exemplified by marine transgressions or coastal plain development, drove species diversification (Mäder *et al*., 2013, Silva *et al*., 2017).

Thus, species strictly associated to grasslands requires more studies to understand the evolutionary history of South America diversity, especially amphibians, one of the least studied groups (see Turchetto-Zolet *et al*., 2013). *Scinax squalirostris* Lutz, 1925, is a small treefrog (23 to 28 mm) (Cei, 1980) widely distributed throughout of the Meridional South America region, from central, Southeastern and Southern Brazil, Northern Bolivia, Southern Paraguay, Uruguay to Northeastern Argentina (Brandão *et al*., 1997; De la Riva *et al*., 2000; Leite *et al*., 2008; Frost, 2018). Although its geographic distribution is continuous over Pampas and humid Chaco, it is spatially disjoint in the Atlantic Forest and the Cerrado region, occurring in areas of high altitudes

(sky-islands), being found in grasslands, shrublands and swamps (Brandão *et al*., 1997; Leite *et al*., 2008; Cruz *et al*., 2009).

In order to investigate the demographic history and the origin of the disjunct geographical distribution of *S. squalirostris*, a useful approach is coupling coalescent simulations and paleoclimatic simulations using ecological niche modelling (ENM) in a multi-model inference framework (e.g., Carstens & Richards, 2007; Knowles & Carstens, 2007; Collevatti *et al.*, 2013; 2015a). In addition, spatio-temporal lineage dispersal inferences (Lemey *et al*., 2009; 2010) may also give clues on the climatic footprint on demographic history and spatial pattern of genetic diversity of current populations (see also Collevatti *et al*., 2015b). Here, we reconstruct the demographic history and the dispersal dynamics of treefrog *S. squalirostris*, using an extensive sampling and DNA regions with different mutation rates to investigate how the Neogene and Quaternary events shaped the current spatial pattern of genetic diversity and geographical distribution. We hypothesized that the disjunct distribution of *S. squalirostris* is due to range contraction of a previously more distributed species, caused by changes in climatic conditions that affected suitable habitat distribution. In this scenario, we expect a larger distribution area of *S. squalirostris* in the colder periods and range contractions in the hotter periods, causing habitat fragmentation. As consequence, current populations would have high genetic differentiation, low or absence of gene flow and an old time of their most recent common ancestor (TRMCA).

## 2 | MATERIAL AND METHODS

### 2.1 | Population sampling and genetic data

We sampled in field and gathered from museum collection 228 individuals of *Scinax squalirostris* from 26 localities, throughout its geographical distributions in Brazil,

Uruguay and Paraguay (Figure 1; Appendix S1: Table S1.1 in Supporting Information). We amplified and sequenced two mitochondrial (mtDNA) fragments: 12S and cytochrome B (Cytb), and nuclear DNA (nDNA) recombination activation gene (RAG1). For details of primers, amplifications and sequencing conditions see Appendix S1: Tables S1.2-S1.4. Information about alignment, saturation test (see Supporting Information: Appendix S3: Figure S3.1) and recombination tests and treatment of heterozygous individuals (see Appendix S2 in Supporting Information).

### 2.2 | Genetic diversity and population structure

To investigate genetic diversity and population structure, mtDNA and nDNA were analysed separately. We estimated the overall and population nucleotide ($\pi$) and haplotype ($h$) diversities using ARLEQUIN 3.11 (Excoffier *et al*., 2005a). We performed a Bayesian clustering analysis to verify the genetic admixture among populations using BAPS 5.0 (Corander *et al*., 2008). We also tested the hypothesis of population genetic differentiation using an analysis of molecular variance (AMOVA) implemented in ARLEQUIN 3.11. We tested for isolation-by-distance (IBD) and isolation-by-environment (IBE) using a Multiple Regression on Distance Matrices (MRM) (Lichstein, 2007). For the genetic distance matrix, we used pairwise genetic differentiation (Slatkin's linearized pairwise FST). The geographic distance matrix was obtained from the logarithm of the geodesic distances between pair of populations. For environment analysis, we selected the five variables that explained the most of the variance among populations of *S. squalirostris* from 19 bioclimatic variables. We tested regression significance 10,000 permutations using the function 'mrm' implemented in the package 'ecodist' (Goslee & Urban, 2007) in R 3.3.1 (for details on the generation of environmental matrices and data processing see Appendix S2).

## 2. 3 | Population demographic history

We performed all coalescent analysis with concatenated partitions of mitochondrial and nuclear DNA, but assigning different priors for each DNA fragments. The best-fit evolutionary model for each gene and their maximum likelihood parameters were selected using the corrected Akaike Information Criterion (AICc) implemented in JMODELTEST 2 (Darriba *et al*., 2012) (Appendix S1: Table S1.5). For molecular dating we used mutation rates previously estimated for mitochondrial and nuclear fragments for molecular dating to anura amphibians (Appendix S1: Table S1.6) because of the lack of Scinaxinae fossils.

We estimated the population effective size (*Ne*) using the demographic parameter $\theta$, calculated by Bayesian estimation implemented in LAMARC 2.1.10 (Kuhner, 2006). Analyses were run with 20 initial chains of 15,000 steps and three final chains of 80,000 steps. The initial and final chains were sampled every 100 steps. We performed two independent runs to assess convergence. Results for each region were combined with LOGCOMBINER and checked convergence and stationarity (Effective Sample Size, ESS $\geq$ 200) using TRACER 16 (Rambaut *et al*., 2013). The effective population size was obtained using a generation time of one year, based on data for the related (Kluge, 1981) treefrog *Boana rosenbergi* (Boulenger, 1898). To understand changes in effective population size throughout time we performed an Extended Bayesian Skyline Plot (EBSP) analysis (Drummond *et al*., 2005) using BEAST (Drummond *et al*., 2013). We used the substitution models and mutation rates reported above (see Appendix S1: Table S1.5 and S1.6) and the relaxed molecular clock model (uncorrelated lognormal). Two independent analyses were run for 100 million generations, sampled every 2,300 generations. Convergence and stationarity (ESS $\geq$

200) were checked using TRACER 16. We combined runs and trees after removing a 20% burn-in with LOGCOMBINER (Drummond *et al*., 2013).

### 2.4 | Coalescent tree and Time of Most Recent Common Ancestor

We run a Bayesian coalescent analysis implemented in BEAST (Drummond *et al*., 2013) to infer a coalescent tree and estimate the time of the most recent common ancestor (TMRCA). For this analysis, we used 139 haplotypes obtained from concatenated mtDNA and nDNA regions (see results below). We defined *Scinax fuscomarginatus* as outgroup based on Duellman *et al*. (2016). We used an uncorrelated lognormal molecular clock and the same priors of EBSP analysis (see above). For tree prior we used Coalescent Constant model based on EBPS results (see below). MCMC conditions and number of runs were the same as EBSP analysis. Convergence and stationarity were assessed (ESS were ≥ 200) using TRACER 1.6. We combined runs and trees after removing a 20% burn-in with LOGCOMBINER (Drummond *et al*., 2013) and the Maximum Clade Credibility (MCC) tree was obtained with TREEANNOTATOR (Drummond *et al*., 2013). We also ran an empty alignment (sampling only from priors) to verify the sensitivity of results to the assumed priors. The analysis showed that parameters posterior distribution was different from those obtained from the empty alignment, indicating that our data is informative. BEAST analyses were performed in Cyberinfrastructure for Phylogenetic Research CIPRES 3.3 (Miller *et al.,* 2010).

### 2.5 | Lineage Dispersal

To reconstruct the spatio-temporal history of lineage dispersal we used the relaxed random walk model (RRW, Lemey *et al*., 2009, 2010) implemented in the software BEAST (Drummond *et al*., 2013). We performed the analyses for both concatenated

mitochondrial and nuclear partitions with unlinked priors. The diffusion process parameters were searched by a Bayesian Stochastic Search Variable Selection (BSSVS). We set the same priors for sequence evolution as TMRCA analysis, Coalescent GMRF Bayesian Skyride Model (Minin *et al*., 2008) for tree prior and CTMC rate reference (Ferreira & Suchard, 2008) for location state rate prior. We performed two independent runs of 80 million generations, sampled at every 8,000 steps. We checked the convergence and stationarity (ESS ≥ 200) using TRACER 1.6. We combined the runs and trees with LOGCOMBINER (Drummond *et al*., 2013) considering a burn-in of 10% and obtained the MCC tree using TREEANNOTATOR (Drummond *et al*., 2013). After that, we reconstructed the spatio-temporal diffusion using SPREAD 1.0.6 (Bielejec *et al*., 2011). Transitions rates between localities were considered only for strong support, Bayes factors > 8.0.

### 2.6 | Paleodistribution modelling

To model the present and past potential geographic distributions of *S. squalirostris* we obtained 257 occurrence records (Appendix S1: Table S1.7) from the online databases VerNet (Vertebrate Network, available at http://portal.vertnet.org), GBIF (Global Biodiversity Information Facility, available at http://www.gbif.org/) and SpeciesLink (http://splink.cria.org.br/). All records were examined for errors and we excluded duplicate or imprecise occurrences. We mapped the occurrences records in a grid of cells of 0.5 x 0.5° (longitude x latitude) encompassing the Neotropics. The environmental climatic layers were derived from five Atmosphere-Ocean General Circulation Models (AOGCMs) (see Appendix S1: Table S1.8; and Appendix S2 for analysis details). We used five bioclimatic variables (annual mean temperature, mean diurnal range, isothermality (mean diurnal range/temperature annual range),

precipitation of wettest three months and precipitation of driest three months) selected by factorial analysis using Varimax rotation from the 19 bioclimatic variables (see Appendix S2 for details). The distribution of *S. squalirostris* was inferred for pre-industrial (representing current climate conditions), middle Holocene (6Ka) and Last Glacial Maximum (LGM, 21ka) using 12 algorithms (Appendix S1: Table S1.9). We obtain the consensus map for each time period estimating the average of frequencies for each cell across the 60 models taking into account the TSS values as weights. The historical refugium map was the combination of the predictive maps from all time periods and considered all grid cells with suitability values $\geq 0.5$ in the three time periods as refugium (more details in Appendix S2).

### 2.7 | Demographical scenarios and simulations

To set the demographic hypothesis, we classified the 60 predictive ENM maps, following Collevatti *et al*. (2013b; 2015) and classified the predictive maps according to three general demographic scenarios (i) 'Range Retraction' (77 % of the maps), range size was larger at LGM than in present-day; (ii) 'Range Expansion' (8 % of the maps), range size was smaller at LGM than in present-day, and (iii) 'Range Stability' (15 % of the maps), constant range size through time. Furthermore, we also set *a priori* biogeographic hypothesis of 'Multiple Refugia' based on Ab'Saber (2000). This model predicts an expansion-retraction in vegetation, during glacial-interglacial periods, with heterogeneous vegetation responses, creating many refuges of different effective sizes.

We simulated the demographic scenarios using coalescent models implemented in DIYABC 2.0 (Cornuet *et al*., 2014). The relative fits of these models were calculated using the Approximate Bayesian Computation (ABC) method (Excoffier *et al*.; 2005b). Each scenario simulation (Figure 2) included seven populations according to the clades

(A, B, C, D, E, F and G) recovered in coalescent tree estimates (see results below) and the coalescent events (td, t5, t4, t3, t2). Population admixture was also simulated and changes of effective population size (*Ne*) through time, N0 (present effective population size) and N1 (past effective population size), following the demographic scenarios derived from the ENMs. To model calibration, we used the demographic parameters estimated in LAMARC and the mutation rates and evolutionary models used in coalescent analyses (see above).

For each scenario, we considered the change of effective population size according to ENMs. For instance, for scenarios with range expansion we attributed effective population size increase. All seven groups had the same population size N0 and the N1 shift according to our theoretical expectation for each scenario (see Figure 2 for a visual representation of these scenarios). For the "Range Expansion" hypothesis (scenario 1 and 5), we considered N0 = 10,000 and N1 = 1,000. The "Range Retraction" hypothesis (scenario 2 and 6) was simulated with N0 = 10,000 and N1= 100,000 for all populations. In "Stability Range" hypothesis (scenario 3 and 7) we simulated the effective population size as unchanged over the time (N0 = 10,000 and N1 = 10,000). For the "Multiple Refuges" hypothesis (scenario 4 and 8) we set N0 = 10,000 for each population, then we draw random N1 values from a set compound by 0; 10; 100; 1,000; 10,000; 100,000 *Ne,* which represents extinction, bottleneck, stable and expansion processes (Figure 2). Scenarios 5, 6, 7 and 8 (Figure 2) comprised simulations with lineage admixture event between clades E, F and G. We performed 600,000 simulations (for mtDNA and nDNA separately), and all scenarios were compared using direct approach, which is the relative proportion of each scenario in the simulated dataset closest to the observed data set, and with logistic approach, which is the logistic

regression of each scenario probability being the deviation between simulated summary statistics and observed (Cornuet, *et al*. 2008).

### 2.8 | Patterns in genetic diversity

We used quantile regressions (Cade & Noon, 2003) to analyse the relationships of climatic suitability and stability through time with haplotype ($h$) and nucleotide ($\pi$) diversities. We defined climate stability as the differences in ensembled suitabilities between: LGM to present-day, Holocene to present-day and LGM to Holocene. Besides that, we analysed whether historical changes in the geographical range of *S. squalirostris* generated a spatial pattern in haplotype ($h$) and nucleotide ($\pi$) diversities. Therefore, we obtained the distance between each analysed population in relation to centroid and edge of the historical refuge, and then we performed quantile regressions of haplotype ($h$) and nucleotide ($\pi$) diversities against this spatial distance. We also analysed the genetic diversity against the altitude. Quantile regression was run using the function 'rq' implemented in package 'quantreg' (Koenker, 2017) in R.

## 3 | RESULTS

### 3.1 | Genetic diversity and population structure

Populations of *Scinax squalirostris* had high haplotype ($h_{\text{mtDNA}}$ = 0.97071 and $h_{\text{nDNA}}$ = 0.93695) and nucleotide diversities ($\pi_{\text{mtDNA}}$ = 0.021735, SD = 0.010878 and $\pi_{\text{nDNA}}$ = 0.016684, SD = 0.008720) for both mtDNA and nDNA (Table 1; Appendix S3: Figure 3.2). We found 63 haplotypes in 589 bp of mtDNA (12S and CytB) and 73 haplotypes in 413 bp of nDNA (RAG-1) (Figure 1; Appendix S1: Table S1.10). Overall, the majority of mitochondrial and nuclear haplotypes were unique haplotypes, but 16 populations shared mitochondrial haplotypes and 15 shared nuclear haplotypes (Figure

1; Appendix S1: Table S1.10). The Bayesian clustering (BAPS) indicated an optimal partition of eight clusters for mtDNA and six clusters for nDNA (Figure 3). Populations of Central-Western Brazil (CGO and BDF) were grouped in the same clusters (Figure 3a: green clusters, 4 and 5 for mtDNA, and Figure 3b: light pink cluster, 5 for nDNA). Populations of Southeast Brazil were grouped in the same clusters (Figure 3a: blue clusters mtDNA and Figure 3b for nDNA: 1, 2, 3), populations of South Brazil were grouped for mtDNA in clusters 7 and 8 (Figure 3a: light and dark pink clusters), and South/Southeast for nDNA in clusters 6 (Figure 3b: dark pink cluster). Populations of *S. squalirostris* showed high genetic differentiation for both mtDNA and nDNA ($F_{STmtDNA}$ = 0.937; $p < 0.001$ and $F_{STnDNA}$ = 0.651; $p < 0.001$) (Appendix S1: Table S1.11). In general, there were not strong patterns of IBD and IBE on genetic differentiation in MRM analysis ($R^2$: mitochondrial 0.0536 (p= 0.18) and nuclear 0.0746 (p=0.06); Appendix S1: Table S1.12).

**3.2 | Demographic population history and Time of Most Recent Common Ancestor**

Coalescent analysis showed low values of mutation parameter $\theta$ for all populations and overall population ($\theta$ = 0.0447, Table 1). Overall effective population size *Ne* was 6,114.06 (95% CI = 5,026.30-7,259.35), but most populations had low *Ne* (Table 1). The EBSP showed constant population size through time (Appendix S3: Figure S3.3). The TMRCA for *S. squalirostris* lineages dated from the Miocene ~7.6 Ma (95% CI = 1.1-23.2), with the coalescence of two major lineages comprising the clades ((A, B), (C), D) and (E, (F, G)) (Figure 4). Lineages of *S. squalirostris* started to diverge ~5.0 Ma (95% CI = 0.8-15Ma). Major divergence events occurred in the Pliocene/Pleistocene transition (~2.9 Ma; 95% CI = 0.4-9Ma) (Figure 4a, b) and resulted in incomplete lineage sorting, with geographically distant populations sharing haplotypes and

common ancestors. Haplotypes of populations from Cerrado and Atlantic Forest and Pampas, composed the strongly supported Northern clade, dated from ~5.0 Ma (Figure 4a, b). The Southern clade, dated from ~4.7 Ma, was formed by haplotypes of Atlantic Forest and Pampas populations (SJRS, BRS, SFRS, ISC and URU) also, Humid Chaco and Cerrado (APY, EPY, BMS) (Figure 4a, b). The large overlap of the 95% HPD in these estimates suggests that the Northern and Southern clades may have diversified during the same period.

### 3.3 | Lineage Dispersal

The phylogeographic reconstruction of lineage dispersal showed the most probable ancestral location for *S. squalirostris* lineages in the South Brazil (BSC) (Figure 5a; AppendixS3: Figure S3.4). Lineages dispersed during the Pliocene-Pleistocene transition (~4.5-0.6 Ma), and no dispersal event was observed during the LGM. Lineages started spreading at ~4.5 Ma (Figure 5a; Appendix S3: Figure S3.4) in three main routes: from population BSC towards populations in South (ISC, PPR, PGPR, ASC), West (BMS) and Southwest Brazil (CGO, ISP, SMG, SVMG, SRMG) (Figure 5a, b; Appendix S3: Figure S3.4). The most of dispersal events occurred in cooler periods (Figure 5c, d; Appendix S3: Figure S3.4). After ~2.9 Ma, lineages dispersed to multiple directions (Figure 5c-d; Appendix S3: Figure S3.4). The last dispersal events, which occurred during Middle Pleistocene (~0.6 Ma), revealed connections among populations in different clades (Figure 4 and 5; Appendix S3: Figure S3.4).

### 3.4 | Paleodistribution modelling

The ENM modelling were well evaluated by *True Skill* statistics (TSS) and the Area under the Curve (AUC) (Appendix S1: Table S1.13). The hierarchical ANOVA showed

higher variance for time component (see Appendix S1: Table S1.14 and Appendix S3: Figure S3.5), meaning that the ENMs were able to detect the effects of climatic changes through time on the distribution dynamics of *S. squalirostris*. The ENM ensembles predicted that *S. squalirostris* had a wider potential distribution at the LGM (Figure 6b) than at the Holocene or present day (Figure 6c, d). The highest levels of suitability were restricted to South, East and Southeast Brazil and Southern Paraguay and North Argentina in LGM. During the mid-Holocene (Figure 6c) climatic suitability decreased in the Central-Western Brazil and increased in the Southeast, East and South Brazil and Uruguay, which was maintained until the present day (Figure 6d). The geographical range size decreased over time, being higher at LGM than present-day. The range shifts were higher between present-day and LGM than Holocene and present-day, indicating quasi-stability between the Holocene and present-day (Appendix S3: Figure S3.6). The climatic historical refugium extended from the East and the Southeast Atlantic Forest towards South Brazil, Uruguay and Argentina (Figure 6e).

### 3.5 | Demographical scenarios and simulations

The direct approach showed a small variation among the different demographic scenarios for both partitions (Appendix S1: Table S1.15; Appendix S3: Figure S3.7). However, the logistic approach showed that the scenario "Range retraction" (scenario 6) with admixture among clades E, F and G, were the most likely hypothesis to predict the observed genetic parameters of *S. squalirostris* for mtDNA and nDNA.

### 3.6 | Spatial patterns in genetic diversity

Overall, quantile regression did not showed effects of climate change on the genetic diversity of mitochondrial and nuclear data (see Appendix S3: Figures S3.8 - S312).

Only for populations at greater distances from the historic refugium had lower nuclear and mitochondrial nucleotide diversity (Figure S3.12f and S3.13f).

## 4 | DISCUSSION

Our findings from paleodistribution modelling, phylogeographic analysis and coalescent simulations supported the hypothesis that the disjunct distribution of *S. squalirostris* is most likely due to of an ancient wider distribution. The high genetic differentiation and diversity currently found in *S. squalirostris* together with results of stable demography and incomplete lineage sorting reinforces that a more continuous population have once occurred through South/Southeast of South America in the past.

Our results of lineage dispersal reconstruction showed an origin of dispersion in the Southern Brazil with multiple dispersions in many directions over time toward Central-West and Southeastern Brazil. The initial dispersal events occurred in Pliocene, when the climate was warm and the forests were still widespread. Which may have restricted the dispersions to geographically nearest areas. Thus, with precipitation decrease and the beginning of climatic cycles in the Late Pliocene, the forest environments began to retract (Ortiz-Jaureguizar & Cladera, 2006), and dispersion of these lineages become more frequent from Southern Brazil towards many directions. The last dispersions routes dated ~ 600.000 ka, in a colder Pleistocene period. We also observed that in the colder periods the dispersal events reached areas more distant (Central-West and Southeastern) from the center of dispersal (Southern Brazil). Such dispersal routes have most likely occurred because suitable climatic conditions were available across a wide region during cooler phases, allowing spatial displacements, acting as main factor for the uninterrupted dispersion of the lineages. For other Pampean grassland amphibian species, *Pseudopaludicola falcipes,* showed an opposite pattern of

dispersal, which had its initial dispersion in ca. 1.46 Ma and was continuous along interglacial periods (Langone *et al*., 2015), being favored by warm climate. However, this contrast dispersal pattern between *P. falcipes* and *S. squalirostris*, despite their similar distributions in the South region, suggests that the effects of climate change also depend on the biological characteristics of each species, as well as geographic characteristics and climate changes at each site (Hewitt, 2000). For *S. squalirostris*, the preference for colder climate was also corroborated by paleodistribution models. Our results showed a larger distribution area of *S. squalirostris* during the LGM, in agreement with the greater distribution of grasslands during the LGM, when the climate was cold and dry (Behling, 2002; 2007). The paleodistribution models also showed a decrease of suitable areas in West areas of predicted distribution and increase of suitable areas in East, from LGM to Holocene, which has been maintained until today. Indeed, simulations of demographic hypotheses showed "Range Retraction" (range size was larger at LGM than in present-day) as the most likely scenario among alternative hypotheses, and paleodistribution modelling has, in fact, more changes in suitability over time and geographic decline, especially in West areas, than gains of new areas. Several studies have shown that species associated with grasslands were favored by the colder and drier climate and had larger suitable areas during LGM (Lorenz-Lenke *et al*., 2010; Maäder *et al*., 2013 and Maia *et al*., 2017).

Nucleotide and haplotype diversities found for *S. squalirostris* were high and similar to found for other South American species, 12S: *Pseudopaludicola falcipes*, $\pi$ = 0.2119 (Langone *et al*., 2015); RAG-1: *Scinax eurydice*, $h$ = 0.81 and $\pi$ = 0.0057 (Menezes *et al*., 2016); CytB: *Pithecopus megacephalu*s, $h$ = 0.94 and $\pi$ = 0.0054 (Ramos *et al*., 2017). High levels of genetic diversity within populations have been associated with high effective population sizes over long periods of time (Pabijan *et al*.,

2012). Like this, the high genetic diversity and low mutation parameter may be the consequence of long-term stability in the geographical range of *S. squalirostris*. In fact, coalescent analysis (EBSP) showed historical constant population size. Other studies with species associated with the open vegetations and grasslands of South America also found low effect of glacial dynamics on the demography and genetic diversity (e.g. Langone *et al*., 2015; Cristiano *et al*., 2016; Vitorino *et al*., 2016). In addition, the spatial dynamics of climatically suitable areas through time did not affect or caused differences in genetic diversity. However, areas farthest from the predicted historical refugium showed lower genetic diversity, likely due to cycles of range expansion and retraction in glaciation and interglacial periods, what has been shown to cause loss of genetic diversity in peripheral areas (Excoffier, *et al*., 2009). Moreover, the wide historical refugium may have maintained the most of genetic diversity through time.

*S. squalirostris* had high values of genetic differentiation, comparable to other anurans species such as *Leptodactylus fuscus* (Camargo *et al*., 2006) and *Physalaemus cuvieri* (Conte *et al*., 2014). Amphibians generally have low dispersion capacity, and consequently, amphibians' populations are expected to be genetically structured and show limited gene flow, which facilitates isolation (Vences and Wake, 2007). High genetic differentiation indicates low or lack of gene flow among current populations of *S. squalirostris*. In fact, there was no correlation between genetic differentiation and geographical distance or environmental isolation, which may result from a rupture of the gene flow, likely due to the range contraction in warmer periods, when climatically suitable habitats became less available. This range contraction may have led to disjunct formation of a widely distributed area. Other species of plants, rodents and lizards from grasslands and open areas of South America, also had their ranges conformed disjunctly due to climatic oscillations and leading to vicariance events, for instance *Lychnophora*

*ericoides* (Collevatti *et al*., 2009), Micrablepharus *atticolus* (Santos *et al*., 2014), *Tabebuia serratifolia* (Vitorino *et al*., 2016), *Oxymycterus nasutus* (Peçanha *et al*., 2017) and *Cereus hildmannianus* (Silva *et al*, 2017). Furthermore, the absence of gene flow found for *S. squalirostris* may indicate that the incongruence between the coalescent tree of haplotypes and geography may be a consequence of incomplete lineage sorting, with populations geographically distant sharing a common ancestral. Populations from Pampas and Chaco, Atlantic Forest and Cerrado biomes were connected in the past as showed by their shared haplotypes and Bayesian clustering of populations. In fact, the areas currently designated by these biomes were occupied by grassland up to the Quaternary (Behling & Hooghiemstra, 2001; Behling, 2003; Carnaval & Moritz, 2008), facilitating the wide and continuous distribution of *S. squalirostris* ancestral during cold and dry Quaternary climate.

Our results also indicate an ancient origin of *S. squalirostris*, from ~ 7.6 Ma, with subsequent divergence into two large clades, Northern and Southern. Northern clade was formed by populations from Pampas, Atlantic Forest and Cerrado grasslands. Southern clades were composed mainly by populations from Pampas and Chaco. Nonetheless, most of *S. squalirostris* divergences occurred in the Pliocene-Pleistocene transition. This period is also associated to divergences between South and Southeast Brazil clades in a co-generic species *Scinax eurydice* (Menezes *et al*., 2016) and *Cereus hildmannianus* (Silva *et al*., 2017), what is explained by primary events of Quaternary climate fluctuations, and there is no relationship to LGM effects. In fact, most coalescence events of *S. squalirostris* occurred before the LGM (ca. 2.8- 1.2 Ma). We hypothesized that the climatic conditions for *S. squalirostris* were spatially restricted during these periods, causing a compartmentalization of regional grasslands. Besides, times of coalescent events are directly related to effective population size (Kingman,

1982), so populations with higher *Ne* values tend to present older coalescent times, which is in according to the results found for *S. squalirostris*. Thus, despite the expansion during glacial periods, indicated by the paleodistribution modelling, the coalescent tree indicates a regional ancient process of differentiation without latter secondary contact. This suggest an ancient process of incomplete lineage sorting, probably mediated by warmer cycles, shaping the population structure of *Scinax squalirostris*.

In conclusion, our results support that the current disjunct distribution of *S. squalirostris* is most likely due to the range contraction of an ancient wider distribution due to climatic dynamics in Quaternary indicated by high haplotype and nucleotide diversity, older coalescent times and an incomplete lineage sorting. Moreover, our results showed that the Southern Brazil was the region of routes of the lines of *S. squalirostris* and this distribution was more widely in cold and dry climate until LGM. These results reinforce that Pliocene-Pleistocene played a fundamental through dynamics of expansion and retraction of South America grasslands, affecting the species associated with these habitats. In addition, our study provides further evidence that more studies should be done on grasslands-associated species, since these habitats show a different and complex evolutionary history.

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## BIOSKETCH

The authors work with bridging macroecology and population genetics, with particular emphasis on phylogeography and geographical patterns of genetic diversity in Neotropical species.

**Author contributions**: R.G.C., N. M. M., R. F. M., M. S. L. R., G. R. C., C. F. B. H. and T. P. F. A. J. conceived the overall study; R. F. M and T. P. F. A. J. generated the genetic data; T. P. F. A. J. performed the phylogeographical analyses; M. S. L. R. and T. P. F. A. J. performed niche modelling analyses. T. P. F. A. J., R.G.C. and N. M. M wrote the manuscript.

## SUPPORTING INFORMATION

Additional Supporting information may be found in the online version of this article:

**Appendix S1** Supplementary tables (Tables S1.1 - S1.15) with sampling locations, details of genetic sampling, mutation rates, haplotype information, details of ecological niche modelling, AMOVA and demography simulations probabilities.

**Appendix S2** Supplementary additional information of details of Material and Methods.

**Appendix S3** Supplementary figures (Figures S3.1 - S3.13) with details of saturation analysis, details of sampled, Bayesian Analysis of Population Structure, EBSP, location diffusion tree, details of ecological niche modelling, details of demography simulations and quantile regressions.

**Conflict of Interest Statement** The authors declare no conflict of interest.

**TABLE 1** Genetic diversity and demographic parameters of *Scinax squalirostris* populations. N – sample size, *k* - number of haplotypes, *h* – haplotype diversity, $\pi$ - nucleotide diversity and standard deviation (s.d.), $\theta$ – coalescent parameter and 95% CI, *Ne* – effective population size and 95% CI. See Table S1 for population names.

| **Population** | **mtDNA** | | | | **nDNA** | | | | **mtDNA+nDNA** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Code** | **N** | ***h*** | **π (s.d.)** | ***nh*** | **N** | ***h*** | **π (s.d.)** | ***nh*** | ***θ*** | ***θ*-95% CIi-CIs** | ***Ne*** | ***Ne*-95% CIi-CIs** |
| ISP | 5 | 0.4000 | 0.000000 ± 0.000000 | 2 | 5 | 0.4000 | 0.009685 ± 0.006791 | 2 | 0.000677 | 0.00002 - 0.00331 | 92.50282 | 2.76005 - 452.53963 |
| BSP | 12 | 0.9091 | 0.000540 ± 0.000663 | 8 | 11 | 0.7091 | 0.009509 ± 0.005820 | 4 | 0.000194 | 0.00009 - 0.00092 | 26.50745 | 12.61153 - 125.84209 |
| SRMG | 11 | 0.7455 | 0.003118 ± 0.002184 | 4 | 12 | 0.3182 | 0.000000 ± 0.000000 | 1 | 0.000394 | 0.00005 - 0.00099 | 53.83473 | 6.94112 - 135.81654 |
| OMG | 16 | 0.6833 | 0.002221 ± 0.001637 | 5 | 16 | 0.1250 | 0.002421 ± 0.001926 | 2 | 0.000694 | 0.00009 - 0.00164 | 94.82564 | 13.51197 - 224.35690 |
| CMG | 3 | 0.0000 | 0.000000 ± 0.000000 | 1 | 3 | 1.0000 | 0.006457 ± 0.005795 | 3 | - | - | - | - |
| PMG | 1 | 0.0000 | 0.000000 ± 0.000000 | 1 | - | - | - | - | - | - | - | - |
| SMG | 16 | 0.8167 | 0.001042 ± 0.000975 | 7 | 16 | 0.6667 | 0.001957 ± 0.001663 | 5 | 0.001309 | 0.00054 - 0.00307 | 178.85700 | 74.87672 - 419.74690 |
| SVMG | 22 | 0.5628 | 0.000154 ± 0.000319 | 5 | 21 | 0.7333 | 0.003517 ± 0.002483 | 8 | 0.000838 | 0.00040 - 0.00154 | 114.50127 | 55.74763 - 210.69327 |
| SCMG | 13 | 0.4615 | 0.000000 ± 0.000000 | 2 | 12 | 0.5758 | 0.000734 ± 0.000919 | 3 | 0.000315 | 0.00001- 0.00077 | 43.04045 | 2.66440 - 106.30309 |
| CGO | 24 | 0.5833 | 0.001556 ± 0.001243 | 5 | 23 | 0.2451 | 0.000613 ± 0.000794 | 3 | 0.000387 | 0.00011 - 0.0010 | 52.87827 | 15.43990 - 146.20090 |
| BDF | 19 | 0.6433 | 0.000318 ± 0.000474 | 4 | 18 | 0.1111 | 0.000000 ± 0.000000 | 1 | 0.000157 | 0.00002 - 0.00055 | 21.45191 | 2.82837 - 75.96981 |
| BMS | 7 | 0.8571 | 0.003881 ± 0.002762 | 3 | 7 | 0.9048 | 0.011991 ± 0.007602 | 5 | 0.006335 | 0.00239 - 0.02047 | 865.59136 | 327.51736 - 2797.49290 |
| ISC | 26 | 0.8492 | 0.000846 ± 0.000831 | 12 | 24 | 0.9565 | 0.008861 ± 0.005180 | 18 | 0.004665 | 0.00430 - 0.00920 | 637.40864 | 588.08290 - 1257.7377 |
| SJRS | 10 | 0.4667 | 0.000000 ± 0.000000 | 2 | 10 | 1.0000 | 0.007802 ± 0.004968 | 10 | 0.007237 | 0.00505 - 0.00814 | 988.83736 | 690.97009 - 1112.62990 |
| SFRS | 2 | 1.0000 | 0.052632 ± 0.053474 | 2 | 2 | 1.0000 | 0.019370 ± 0.02054 | 2 | - | - | - | - |
| BRS | 1 | 0.0000 | 0.000000 ± 0.000000 | 1 | 1 | 0.0000 | 0.000000 ± 0.000000 | 1 | - | - | - | - |
| PPR | 2 | 1.0000 | 0.000000 ± 0.000000 | 2 | 2 | 1.0000 | 0.014528 ± 0.015692 | 2 | - | - | - | - |
| PGPR | 8 | 0.6071 | 0.000000 ± 0.000000 | 3 | 8 | 0.8929 | 0.010896 ± 0.006829 | 6 | 0.001567 | 0.000673 - 0.00326 | 214.10918 | 91.95627 - 445.84445 |
| CBSC | 1 | 0.0000 | 0.000000 ± 0.000000 | 1 | 1 | 0.0000 | 0.000000 ± 0.000000 | 1 | - | - | - | - |
| CNSC | 2 | 0.0000 | 0.000000 ± 0.000000 | 1 | 2 | 1.0000 | 0.009685 ± 0.010828 | 2 | - | - | - | - |
| BSC | 5 | 0.4000 | 0.000000 ± 0.000000 | 2 | 5 | 1.0000 | 0.013075 ± 0.008861 | 5 | 0.000525 | 0.001482 - 0.21940 | 71.74775 | 202.49509 - 29978.56472 |
| CSC | 3 | 0.0000 | 0.000000 ± 0.000000 | 2 | 2 | 1.0000 | 0.014528 ± 0.015692 | 2 | - | - | - | - |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ASC | 1 | 0.0000 | 0.000000 ± 0.000000 | 2 | 2 | 1.0000 | 0.016949 ± 0.018119 | 2 | - | - | - | - |
| EPY | 1 | 0.0000 | 0.000000 ± 0.000000 | 1 | 1 | 0.0000 | 0.000000 ± 0.000000 | 1 | - | - | - | - |
| APY | 7 | 0.2857 | 0.000000 ± 0.000000 | 2 | 4 | 1.0000 | 0.008071 ± 0.006213 | 3 | 0.002072 | 0.000397 - 0.00880 | 283.11055 | 54.24463 - 1202.400000 |
| URU | 1 | 0.0000 | 0.000000 ± 0.000000 | 1 | 1 | 0.0000 | 0.000000 ± 0.000000 | 1 | - | - | - | - |
| Overall | 219 | 0.9686 | 0.017793 ± 0.009009 | 69 | 209 | 0.9333 | 0.013087 ± 0.007007 | 73 | 0.044747 | 0.036786 - 0.053129 | 6114.06736 | 5026.30527 - 7259.35336 |

**Figures Labels**

**FIGURE 1.** Geographical distribution of the 26 *Scinax squalirostris* populations sampled and haplotypes for (a) concatenated mitochondrial and (b) nuclear sequence data. Different colours were assigned for each haplotype following the legends. The size of the circle represents the sample size in each population and the circle sections represent the haplotype frequency in each sampled population.

**FIGURE 2.** Demographical and biogeographical scenarios considered in ABC analyses for *Scinax squalirostris* populations. Each scenario simulation included seven populations according to the clades (1: clade A, 2: clade B, 3: clade C, 4: clade D, 5: clade E, 6: clade F and 7 clades: G) and the coalescent events (td, t5, t4, t3, t2) recovered in coalescent tree estimates (Figure 4). The admixture of populations was simulated between populations of Pampas/Chaco and Atlantic Forest. Changes of effective population size (*Ne*) were simulated through time (N0 present-day and N1-Pleistocene period), following the demographic scenarios derived from the ENMs.

**FIGURE 3.** Bayesian clustering of populations of *Scinax squalirostris* obtained from the (a) concatenated mitochondrial and (b) nuclear sequence data. Different colours were assigned to different clusters following the figure legends. The bar plot represents the clusters populations (k= 8 for mitochondrial data and k= 6 for nuclear). For details on population codes and localities see Table S1.1 in Appendix S1.

**FIGURE 4.** Time for the most recent common ancestor of *Scinax squalirostris* haplotypes. (a) Coalescent tree. The blue bars correspond to the credibility interval at 95% of the average time for the common ancestor. The numbers below the branches correspond to the support of the node (posteriori probabilities) and the numbers above the branches represent the node age. The scale is in millions of years (Ma) before present. For details on population codes and localities see Table S1.1 in Appendix S1. (b) Geographical distribution of seven clades showed in coalescent tree across Humid Chaco, Pampas, Atlantic Forest and Cerrado. Colors represent each clade according legend.

**FIGURE 5.** Spatio-temporal dynamics of lineage diffusion among the 26 populations of *Scinax squalirostris*. Arrows between locations represent branches in the tree along which the relevant location transition occurs. Grey area represents the historical refugium for *S. squalirostris*, predicted using palaeodistribution models. The $\delta^{18}$ curve corresponds to the composite benthic stable oxygen isotope ratios (Lisiecki & Raymo, 2005). For details on population codes and localities see Table S1.1 in Appendix S1.

**FIGURE 6.** Maps of consensus expressing the ensemble climatic suitability for *Scinax squalirostris*, based on paleodistribuion modelling. (a) Occurrence records of *Scinax squalirostris*, used to generate the paleodistribution models. Potential distribution across the Neotropics during the (b) Last Glacial Maximum (LGM – 21ka), (c) mid-Holocene (6 ka), (d) present-day and (e) Historical refugium predicted in ecological niche modeling analysis.

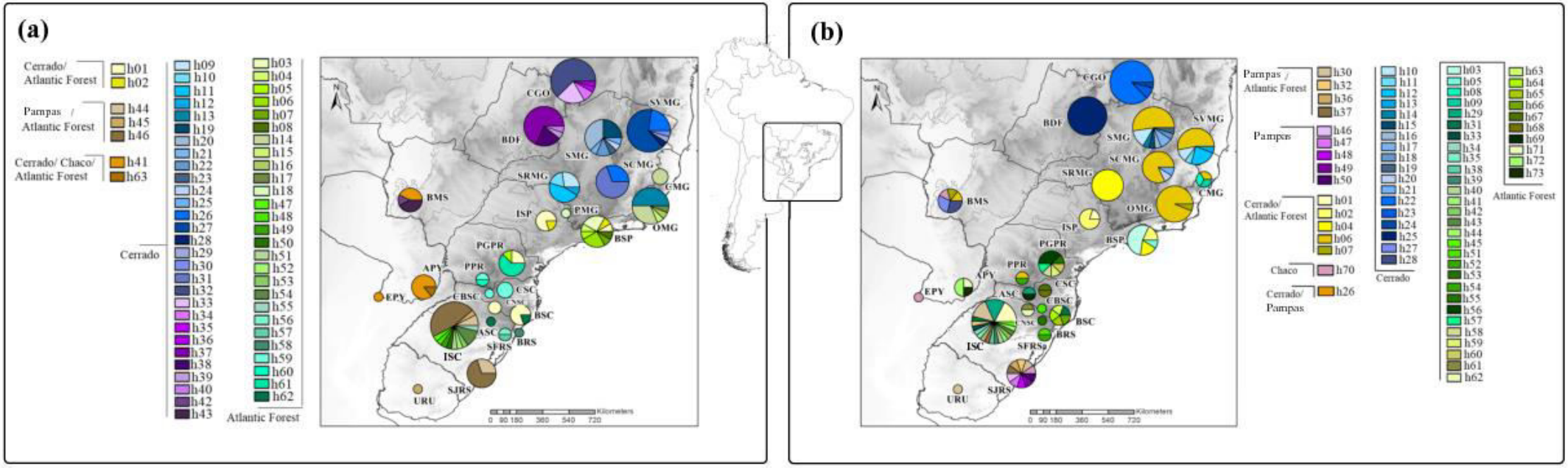

(a)
Cerrado/ Atlantic Forest
h01
h02
Pampas / Atlantic Forest
h44
h45
h46
Cerrado/ Chaco/ Atlantic Forest
h41
h63
Cerrado
h09
h10
h11
h12
h13
h19
h20
h21
h22
h23
h24
h25
h26
h27
h28
h29
h30
h31
h32
h33
h34
h35
h36
h37
h38
h39
h40
h42
h43
h03
h04
h05
h06
h07
h08
h14
h15
h16
h17
h18
h47
h48
h49
h50
h51
h52
h53
h54
h55
h56
h57
h58
h59
h60
h61
h62
Atlantic Forest
CGO
SVMG
BDF
SMG
SCMG
SRMG
GMG
BMS
ISP
PMG
OMG
BSP
PGPR
APY
PPR
EPY
CBSC
CSC
BSC
ASC
BRS
ISC
SFRS
SJRS
URU
0 90 180 360 540 720
(b)
Pampas / Atlantic Forest
h30
h32
h36
h37
Pampas
h46
h47
h48
h49
h50
Cerrado/ Atlantic Forest
h01
h02
h04
h06
h07
Chaco
h70
Cerrado/ Pampas
h26
h10
h11
h12
h13
h14
h15
h16
h17
h18
h19
h20
h21
h22
h23
h24
h25
h27
h28
Cerrado
h03
h05
h08
h09
h29
h31
h33
h34
h35
h38
h39
h40
h41
h42
h43
h44
h45
h51
h52
h53
h54
h55
h56
h57
h58
h59
h60
h61
h62
h63
h64
h65
h66
h67
h68
h69
h71
h72
h73
Atlantic Forest

**Figure 1**

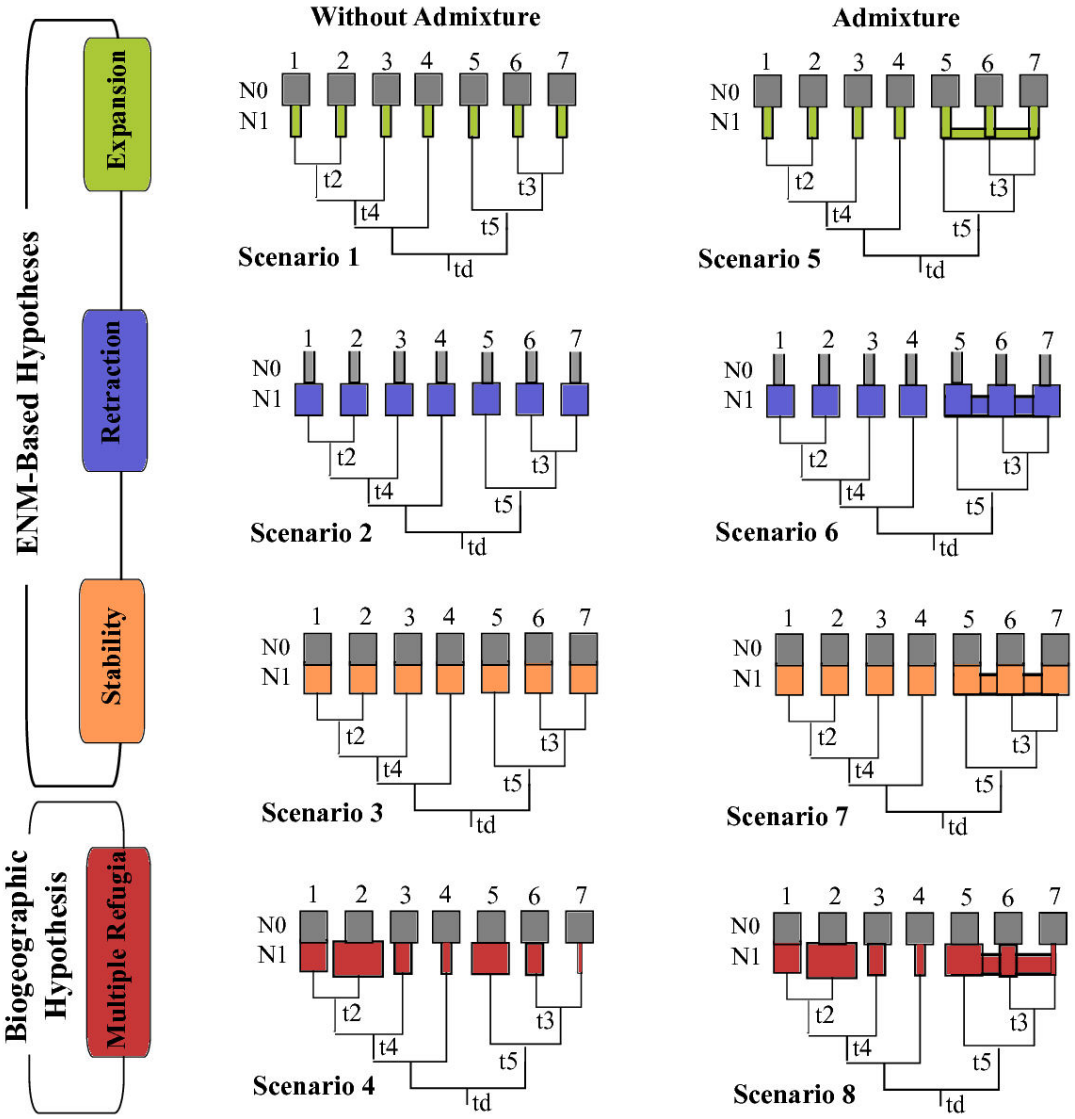

ENM-Based Hypotheses
Expansion
Retraction
Stability
Biogeographic Hypothesis
Multiple Refugia
Without Admixture
Admixture
N0
N1
1 2 3 4 5 6 7
t2
t3
t4
t5
td
Scenario 1
Scenario 2
Scenario 3
Scenario 4
Scenario 5
Scenario 6
Scenario 7
Scenario 8

**Figure 2**

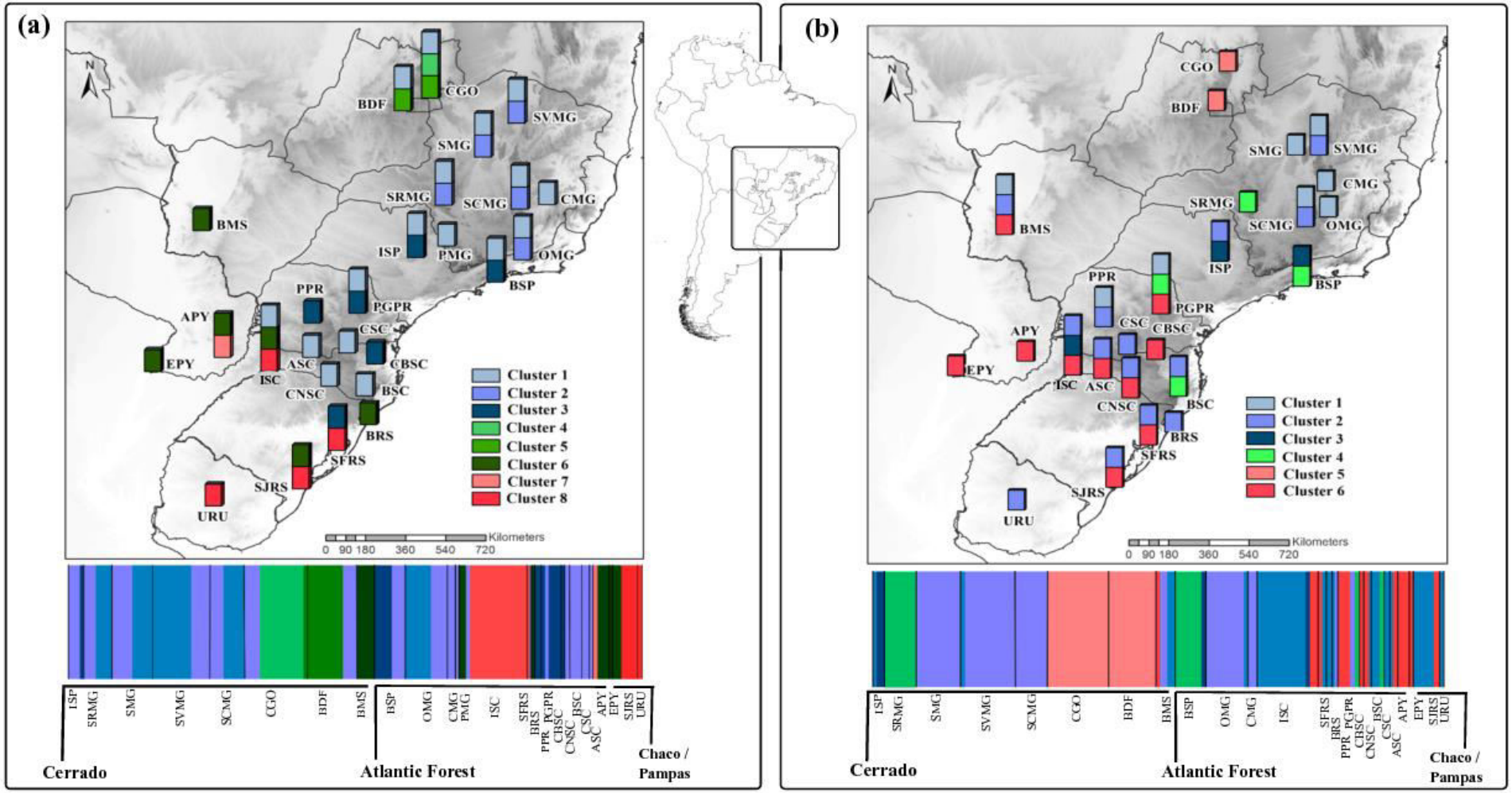
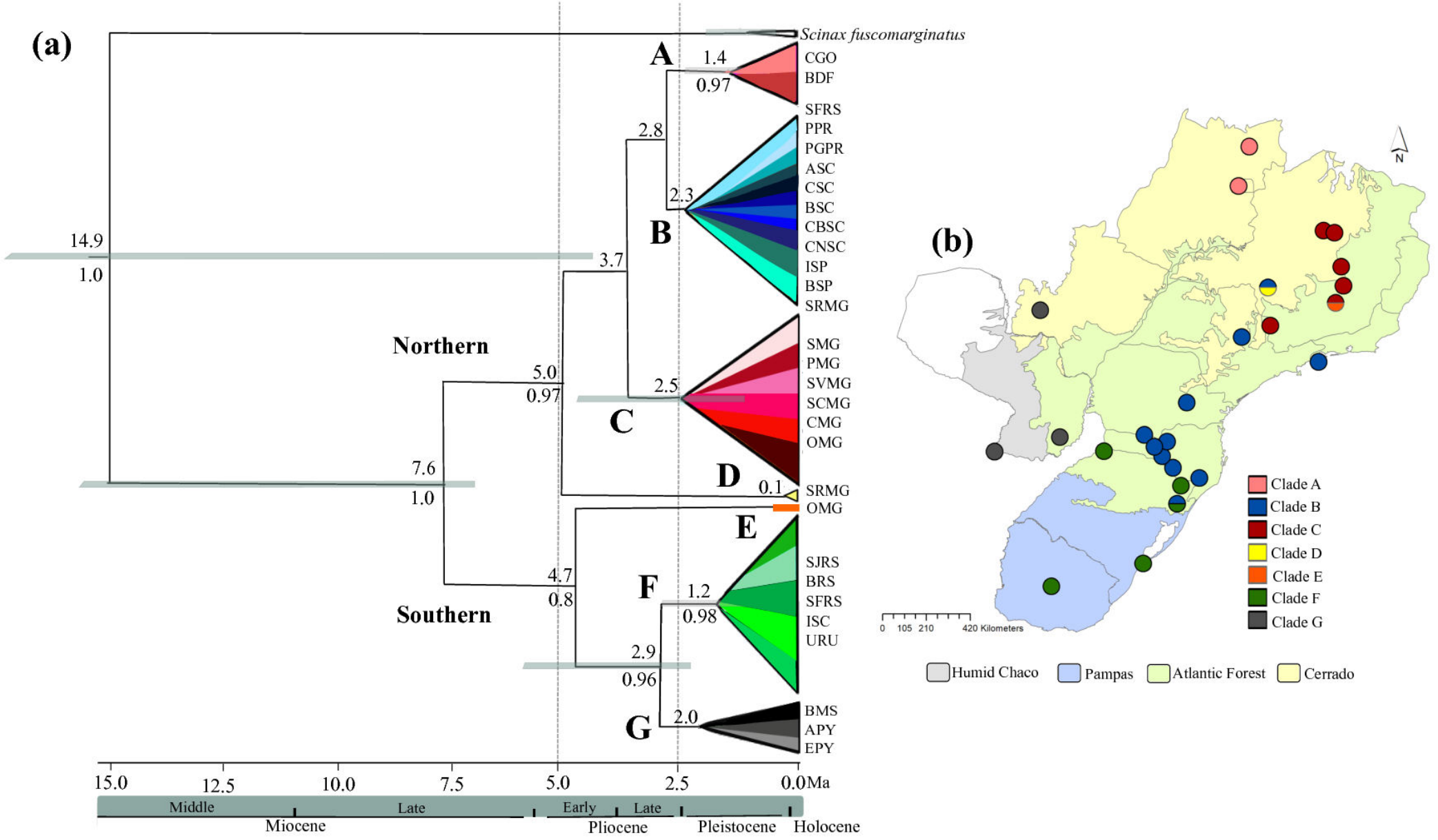
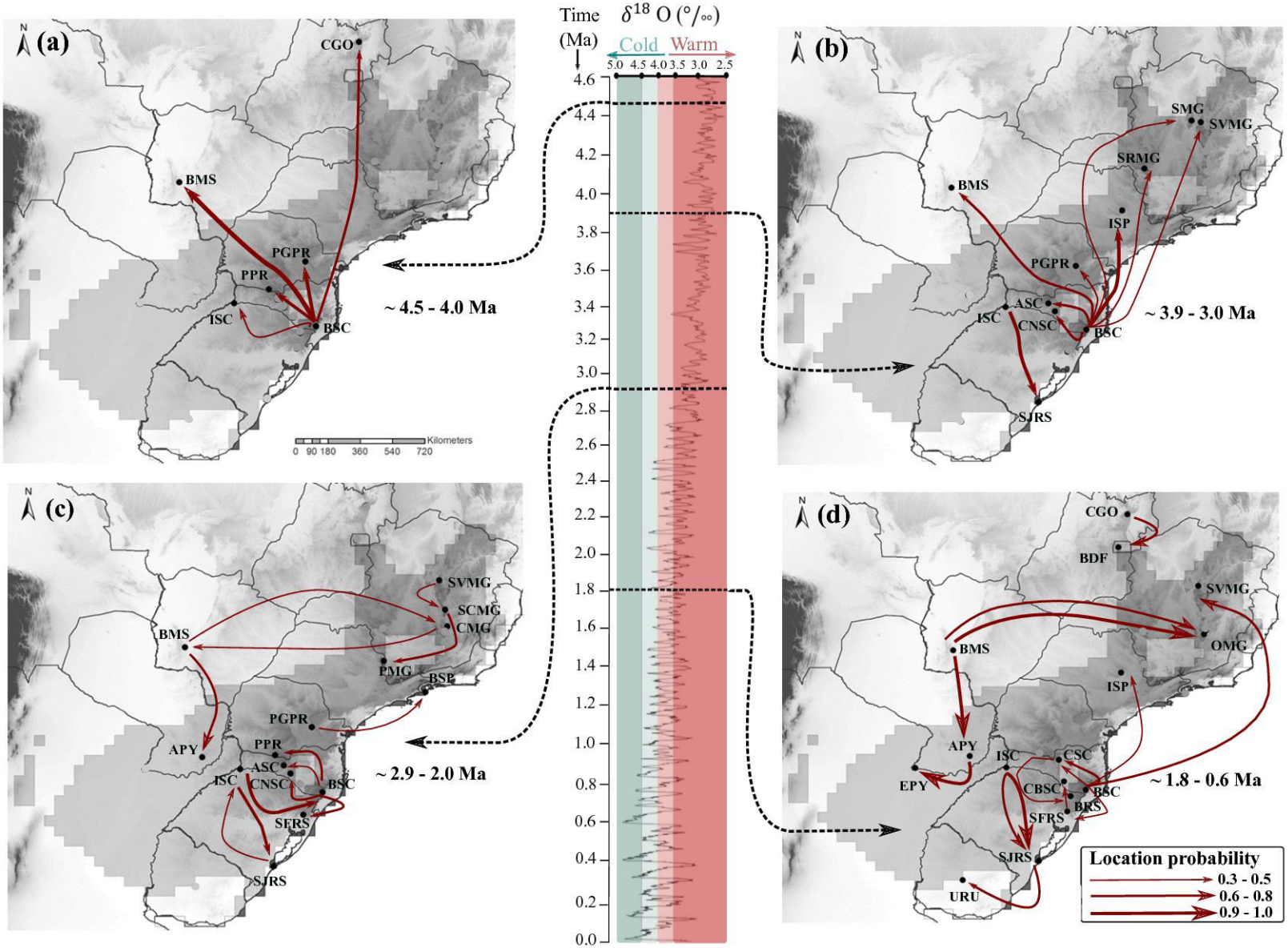
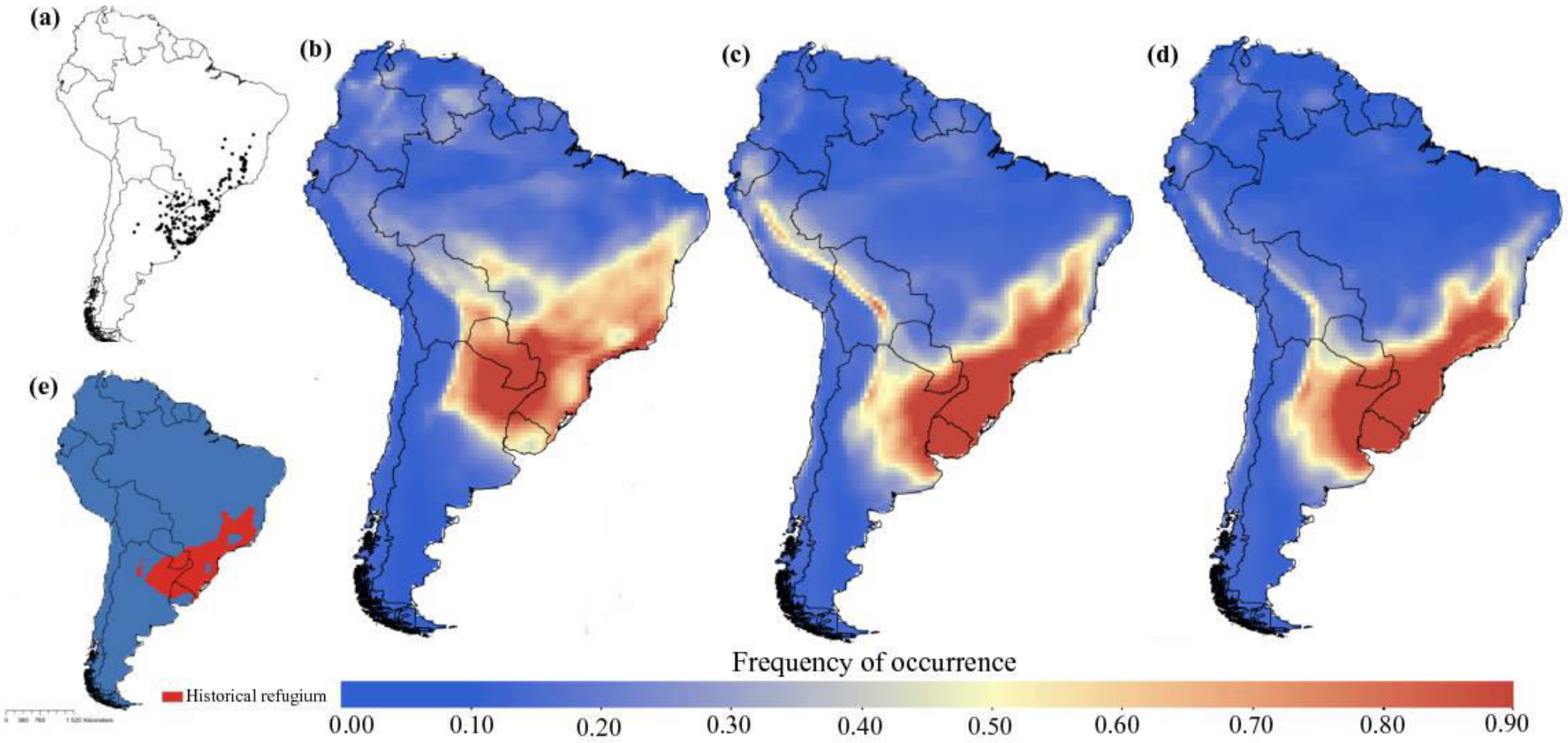

(a)
N
BDF
CGO
SVMG
SMG
SRMG
SCMG
CMG
BMS
ISP
PMG
OMG
BSP
PPR
PGPR
APY
CSC
CBSC
EPY
ASC
ISC
CNSC
BSC
BRS
SFRS
SJRS
URU
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
Kilometers
0 90 180 360 540 720
ISP SRMG SMG SVMG SCMG CGO BDF BMS
BSP OMG CMG PMG ISC SFRS BRS PPR PGPR CBSC CNSC BSC CSC ASC APY EPY SJRS URU
Cerrado
Atlantic Forest
Chaco / Pampas
(b)
N
CGO
BDF
SMG
SVMG
CMG
SRMG
SCMG
OMG
BMS
ISP
BSP
PPR
PGPR
CSC
CBSC
APY
EPY
ISC
ASC
CNSC
BSC
BRS
SFRS
SJRS
URU
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Kilometers
0 90 180 360 540 720
ISP SRMG SMG SVMG SCMG CGO BDF BMS
BSP OMG CMG ISC SFRS BRS PPR PGPR CBSC CNSC BSC CSC ASC APY EPY SJRS URU
Cerrado
Atlantic Forest
Chaco / Pampas

**Figure 3**


(a)
Scinax fuscomarginatus
A
1.4
0.97
CGO
BDF
SFRS
2.8
PPR
PGPR
ASC
2.3
CSC
BSC
B
CBSC
CNSC
14.9
1.0
3.7
ISP
BSP
SRMG
Northern
SMG
PMG
5.0
0.97
SVMG
2.5
SCMG
C
CMG
OMG
7.6
1.0
D
0.1
SRMG
OMG
E
SJRS
4.7
0.8
F
1.2
0.98
BRS
SFRS
ISC
Southern
URU
2.9
0.96
G
2.0
BMS
APY
EPY
15.0
12.5
10.0
7.5
5.0
2.5
0.0Ma
Middle
Late
Early
Late
Miocene
Pliocene
Pleistocene
Holocene
(b)
N
Clade A
Clade B
Clade C
Clade D
Clade E
Clade F
Clade G
0 105 210 420 Kilometers
Humid Chaco
Pampas
Atlantic Forest
Cerrado


(a)
CGO
BMS
PGPR
PPR
ISC
BSC
~ 4.5 - 4.0 Ma
Kilometers
0 90 180 360 540 720
Time (Ma)
δ18 O (°/∞)
Cold
Warm
5.0 4.5 4.0 3.5 3.0 2.5
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
(b)
SMG
SVMG
SRMG
BMS
ISP
PGPR
ASC
ISC
CNSC
BSC
~ 3.9 - 3.0 Ma
SJRS
(c)
SVMG
SCMG
CMG
BMS
PMG
BSP
PGPR
APY
PPR
ASC
ISC
CNSC
BSC
SFRS
~ 2.9 - 2.0 Ma
SJRS
(d)
CGO
BDF
SVMG
OMG
BMS
ISP
APY
ISC
CSC
EPY
CBSC
BSC
BRS
SFRS
~ 1.8 - 0.6 Ma
SJRS
URU
Location probability
0.3 - 0.5
0.6 - 0.8
0.9 - 1.0


(a)
(b)
(c)
(d)
(e)
Historical refugium
Frequency of occurrence
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90

*Journal of Biogeography*

**SUPPORTING INFORMATION**

# Inferring responses to Neogene-Quaternary climate dynamics from historical demography in a South American grassland treefrog

Tatianne P. F. Abreu-Jardim | Rafael F. Magalhães | Natan M. Maciel | Matheus Souza Lima-Ribeiro | Guarino R. Colli | Célio F. B. Haddad and Rosane G. Collevatti.

**Appendix S1** Supplementary tables (Tables S1.1- S1.15) analyses details: with sampling locations, details of genetic sampling, mutation rates, haplotype information, details of ecological niche modelling, AMOVA and demography simulations probabilities.

**Table S1.1** Sampling locations of the 26 populations of *Scinax squalirostris*. Name of localities in the Population column we presented the abbreviation of Brazilian states and a Paraguayan department. Collection column we presented the abbreviation of donor institutions. (Amphibian Collection Célio F. B. Haddad (CFBH), Collection of the Universidade Federal de Minas Gerais (UFMG), Zoological Collection of the Universidade Federal de Goiás (ZUFG), Collection Elaine Maria Lucas Gonsales (EMLG), Museum of Ciências e Tecnologia da PUCRS (MCT), Collection of the Universidade Federal do Rio Grande do Sul (UFRGS), Herpetology Collection of the Instituto de Investigación Biológica del Paraguay (IIBPH).

| Population | Population Code | Collection | Latitude | Longitude |
|---|---|---|---|---|
| Itirapina-SP | ISP | CFBH | -22.2744 | -47.7958 |
| PARNA Bocaina-SP | BSP | CFBH | -23.3458 | -44.4825 |
| São Roque de Minas-MG | SRMG | CFBH | -20.1461 | -46.6628 |
| Ouro Preto-MG | OMG | CFBH | -20.2875 | -43.5081 |
| Catas Altas-MG | CMG | UFMG | -20.0747 | -43.4075 |
| Poços de Caldas-MG | PMG | UFMG | -21.7878 | -46.5614 |
| Serra do Cabral-MG | SMG | LOD | -17.6992 | -44.2714 |
| PARNA Sempre Vivas-MG | SVMG | ZUFG | -17.7883 | -43.7977 |
| PARNA Serra do Cipó-MG | SCMG | ZUFG | -19.2469 | -43.5100 |
| PARNA Chapada dos Veadeiros-GO | CGO | ZUFG | -14.0833 | -47.4667 |
| Brasília-DF | BDF | ZUFG | -15.7797 | -47.9297 |
| Bonito-MS | BMS | ZUFG | -21.1211 | -56.4819 |
| Itaparinga-SC | ISC | CFBH | -27.1833 | -53.7333 |
| São José do Norte-RS | SJRS | UFRGS | -32.0147 | -52.0417 |
| São Francisco de Paula-RS | SFRS | MCP | -29.4481 | -50.5836 |
| Bom Jesus-RS | BRS | CFBH | -28.6678 | -50.4167 |
| Palmas-PR | PPR | ZUFG | -26.4842 | -51.9906 |
| Ponta Grossa-PR | PGPR | ZUFG | -25.095 | -50.1619 |
| Campo Belo do Sul-SC | CBSC | MCP | -27.8992 | -50.7608 |
| Campos Novos-SC | CNSC | CFBH | -27.4017 | -51.2250 |
| Bom Jardim da Serra-SC | BSC | CFBH | -28.3369 | -49.6247 |
| Caçador-SC | CSC | EMLG | -26.7753 | -51.0150 |
| Água Doce-SC | ASC | EMLG | -26.9978 | -51.5561 |
| Estancia San José-Ñeembucú-Py | EPY | IIBPH | -27.2016 | -58.4486 |
| Alto Vera, Yataí-Itapuá-Py | APY | IIBPH | -26.5833 | -55.6333 |
| Uruguai | URU | - | -33.0000 | -56.0000 |

**Table S1.2** PCR Primers used to sequence CytB, 12S and RAG-1 genes used in this study. Nucleotide sequence based primers: 12Sa-12Sb; Reeder, 1995, MVZ15L - Moritz *et al*., 1992 and H15149 – Kocher *et al*., 1989, Heinicke *et al*., 2007.

| Gene region | Primer | Direction | Nucleotide Sequence (5'-3') |
|---|---|---|---|
| CytB | MVZ15 | forward | GAACTAATGGCCCACACWWTACGNAA |
| | H1415 (cyt-b2) (H15149) | reverse | AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA |
| 12S | 12S | forward | AAACTGGGATTAGATACCCCACTAT |
| | 12S | reverse | GAGGGTGACGGGCGGTGTGT |
| RAG | RAG1 | forward | ATGCATCRAAAATTCARCAAT |
| | RAG1 | reverse | CCYCCTTTRTTGATAKGGWCATA |

**Table S1.3** PCR mix components and quantities for each of the amplified fragments used in this study.

| **Reagent** | **Cytb, 12S and RAG-1** |
|---|---|
| Deionized water | 8.5 µL |
| DNA | 2.0 µL |
| Forward primer (2mM) | 3.0 µL |
| Reverse primer (2mM) | 3.0 µL |
| Buffer 1X* | 2.0 µL |
| DNTPs (2,5 mM) | 1.2 µL |
| Taq polymerase (5u/µL) | 0.3 µL |
| Total volume | 20 µL |

*Buffer 1X (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2)

**Table S1.4** PCR thermal program for each of the amplified fragments used in this study.

| **Gene region** | **Initial heating** | **Desnaturation** | **Annealing** | **Extension** | **Final extension** |
|---|---|---|---|---|---|
| | | | **35 cycles** | | |
| CytB | 2 min at 94°C | 60 sec at 94°C | 60 sec at 56°C | 90 sec at 72°C | 6 min at 72°C |
| 12S | 2 min at 94°C | 60 sec at 94°C | 60 sec at 54°C | 90 sec at 72°C | 6 min at 72°C |
| RAG | 2 min at 94°C | 60 sec at 94°C | 60 sec at 58°C | 90 sec at 72°C | 6 min at 72°C |

**Table S1.5** Parameters of selected models for fragments used in this study.

| **Datasets** | **Cytb** | **12S** | **RAG-1** |
|---|---|---|---|
| **Model** | K80+I | K80+G | JC |
| **p-inv** | 0.8790 | - | - |
| **Kappa** | 17.7938 | 5.4465 | |
| **gamma shape** | - | 0.1200 | - |
| **Nst** | 2 | 2 | 1 |
| **Ncat** | - | 4 | - |

**Table S1.6** Mutation rates for each DNA region used in coalescent analyses.

| | Mutation rate region (subs./millions of years) Mean -SD | Reference |
|---|---|---|
| 12S | 0.0026 - 0.0001 | Evans *et al*., 2004 |
| CytB | 0.0069 - 0.0010 | Macey *et al*., 1998 |
| RAG | 0.0015 - 0.0001 | Heinicke *et al*., 2007 |

**Table S1.7** Geographical coordinates of 257 occurrence records of *Scinax squalirostris* used in ecological niche modelling.

| longitude | latitude | longitude | latitude | longitude | latitude |
|---|---|---|---|---|---|
| -54.80 | -34.70 | -55.67 | -26.83 | -50.42 | -28.67 |
| -56.46 | -34.75 | -56.17 | -27.23 | -51.23 | -27.40 |
| -54.92 | -34.80 | -49.63 | -28.33 | -53.57 | -30.16 |
| -54.10 | -31.92 | -56.77 | -27.14 | -43.31 | -17.06 |
| -54.28 | -34.03 | -57.02 | -26.01 | -44.27 | -18.11 |
| -56.69 | -34.71 | -58.40 | -33.87 | -46.01 | -17.01 |
| -53.77 | -34.35 | -58.58 | -34.33 | -50.58 | -29.45 |
| -57.54 | -30.90 | -58.23 | -28.05 | -51.18 | -29.17 |
| -55.67 | -26.76 | -64.87 | -31.10 | -52.53 | -26.56 |
| -56.74 | -27.36 | -54.42 | -25.72 | -43.68 | -18.81 |
| -56.50 | -27.42 | -65.63 | -32.65 | -43.69 | -20.52 |
| -56.17 | -27.23 | -56.68 | -36.75 | -42.54 | -15.61 |
| -55.94 | -25.07 | -58.54 | -34.63 | -44.18 | -17.87 |

| | | | | | |
|---|---|---|---|---|---|
| -56.76 | -27.14 | -58.05 | -29.79 | -41.77 | -13.15 |
| -57.23 | -24.69 | -59.26 | -29.14 | -43.80 | -20.25 |
| -55.73 | -27.18 | -61.11 | -32.16 | -55.06 | -34.83 |
| -56.83 | -25.68 | -58.60 | -33.78 | -58.52 | -33.01 |
| -57.27 | -27.40 | -58.88 | -32.37 | -60.08 | -31.10 |
| -57.54 | -25.46 | -58.54 | -34.58 | -46.37 | -20.25 |
| -55.67 | -26.83 | -58.52 | -33.02 | -46.62 | -21.13 |
| -46.52 | -23.67 | -60.44 | -26.79 | -48.35 | -23.40 |
| -47.35 | -21.92 | -60.64 | -32.06 | -56.33 | -34.86 |
| -44.75 | -22.57 | -60.57 | -31.71 | -44.57 | -22.64 |
| -49.55 | -28.40 | -59.02 | -33.69 | -58.99 | -27.47 |
| -46.32 | -23.78 | -60.30 | -31.73 | -46.56 | -21.79 |
| -35.73 | -84.83 | -58.44 | -32.35 | -51.32 | -264.28 |
| -48.43 | -22.87 | -59.22 | -31.48 | -49.20 | -25.93 |
| -59.50 | -32.67 | -60.52 | -31.73 | -51.32 | -26.43 |
| -58.73 | -34.38 | -57.85 | -35.02 | -51.63 | -26.52 |
| -58.84 | -33.69 | -57.33 | -37.62 | -46.62 | -21.78 |
| -58.87 | -32.37 | -54.91 | -34.80 | -44.58 | -22.65 |
| -58.96 | -31.99 | -54.28 | -34.04 | -51.74 | -27.01 |
| -59.24 | -31.54 | -54.13 | -34.58 | -46.37 | -20.25 |
| -58.88 | -33.91 | -57.53 | -30.95 | -51.02 | -30.08 |
| -57.17 | -28.54 | -56.45 | -34.74 | -50.23 | -30.25 |
| -58.70 | -34.58 | -54.17 | -31.87 | -50.21 | -30.18 |
| -58.10 | -27.92 | -55.06 | -34.83 | -52.10 | -32.04 |
| -58.64 | -34.36 | -56.30 | -34.87 | -51.23 | -30.03 |
| -60.38 | -29.42 | -55.60 | -30.87 | -50.07 | -28.75 |
| -58.06 | -29.79 | -53.80 | -34.39 | -51.93 | -29.83 |
| -58.87 | -33.89 | -57.88 | -30.53 | -51.76 | -29.91 |
| -57.93 | -35.78 | -57.87 | -30.58 | -53.67 | -31.56 |
| -58.35 | -34.61 | -57.73 | -30.78 | -56.00 | -28.66 |
| -60.15 | -31.19 | -55.90 | -34.82 | -50.07 | -29.59 |
| -59.26 | -29.15 | -56.03 | -34.82 | -48.78 | -28.48 |
| -57.17 | -28.54 | -56.03 | -34.88 | -52.34 | -31.77 |

| | | | | | |
|---|---|---|---|---|---|
| -57.72 | -35.73 | -55.72 | -34.73 | -49.73 | -29.34 |
| -58.44 | -34.54 | -54.20 | -31.87 | -51.34 | -27.63 |
| -59.19 | -25.61 | -53.67 | -32.72 | -51.13 | -29.68 |
| -43.75 | -19.51 | -55.28 | -32.63 | -50.70 | -26.93 |
| -43.71 | -19.17 | -56.65 | -34.50 | -50.76 | -27.90 |
| -43.71 | -19.17 | -54.98 | -34.33 | -50.22 | -29.45 |
| -43.74 | -19.51 | -54.57 | -34.78 | -52.10 | -32.04 |
| -46.57 | -22.07 | -55.27 | -34.80 | -50.38 | -29.42 |
| -43.88 | -21.68 | -54.95 | -34.97 | -51.18 | -28.33 |
| -43.41 | -20.07 | -56.35 | -34.80 | -51.98 | -31.36 |
| -44.18 | -17.87 | -56.83 | -31.83 | -50.01 | -28.84 |
| -44.20 | -20.14 | -57.62 | -31.82 | -50.22 | -30.20 |
| -43.43 | -19.04 | -55.55 | -31.03 | -55.36 | -30.77 |
| -43.60 | -18.25 | -55.60 | -31.32 | -52.99 | -32.05 |
| -43.31 | -18.47 | -53.45 | -33.68 | -57.18 | -29.95 |
| -43.61 | -20.45 | -53.58 | -33.68 | -55.34 | -29.60 |
| -43.47 | -20.08 | -53.53 | -33.98 | -54.54 | -29.92 |
| -46.53 | -23.67 | -53.77 | -34.28 | -56.47 | -30.18 |
| -46.53 | -23.67 | -53.78 | -34.33 | -56.01 | -28.62 |
| -47.82 | -22.25 | -53.78 | -34.35 | -52.52 | -31.74 |
| -48.44 | -22.88 | -54.33 | -34.48 | -50.92 | -31.11 |
| -47.35 | -21.92 | -54.15 | -34.60 | -48.43 | -22.87 |
| -44.75 | -22.57 | -57.87 | -30.93 | -57.54 | -30.90 |
| -46.32 | -23.78 | -57.53 | -30.95 | -53.77 | -34.35 |
| -48.13 | -22.28 | -56.67 | -34.73 | -49.55 | 28.40 |
| -48.77 | -24.22 | -56.97 | -34.38 | -47.80 | -22.27 |
| -53.81 | -29.68 | -56.37 | -34.82 | -46.48 | -20.35 |
| -51.02 | -30.08 | -55.47 | -32.43 | -46.66 | -20.15 |
| -51.46 | -29.69 | -54.40 | -32.97 | -46.56 | -21.79 |
| -51.18 | -29.92 | -54.62 | -33.00 | -44.27 | -17.70 |
| -52.34 | -31.77 | -54.48 | -33.22 | -43.80 | -17.79 |
| -52.15 | -32.02 | -54.38 | -33.23 | -43.51 | -19.25 |
| -50.16 | -25.10 | -54.83 | -34.00 | -51.56 | -27.00 |

| | | | | | |
|---|---|---|---|---|---|
| -51.99 | -26.48 | -53.87 | -33.03 | -51.02 | -26.78 |
| -50.62 | -24.32 | -48.00 | -22.25 | -52.04 | -32.01 |
| -49.18 | -25.92 | -44.61 | -22.72 | -53.73 | -27.18 |
| -52.62 | -27.19 | -43.51 | -20.29 | -47.47 | -14.08 |
| -47.82 | -22.25 | -49.62 | -28.34 | -47.93 | -15.78 |
| -44.57 | -22.64 | -56.48 | -21.12 | -58.45 | -27.20 |
| -50.16 | -25.10 | -51.99 | -26.48 | | |

**Table S1.8** Details on Atmosphere-Ocean Global Circulation Models (AOGCMs) used for ecological niche modelling of *Scinax squalirostris*

| Model ID | Modelling Centre | Resolution* | Source | Year |
|---|---|---|---|---|
| CCSM4 | University of Miami – RSMAS, USA | 0.9° × 1.25° | CMIP5/PMIP3 | 2012 |
| CNRM-CM5 | Centre National de Recherches Meteorologiques / Centre Europeen de Recherche et Formation Avancees en Calcul Scientifique, France | 1.4° x 1.4° | CMIP5/PMIP3 | 2012 |
| MIROC-ESM | Atmosphere and Ocean Research Institute (University of Tokyo), National Institute for Environmental Studies, and Japan Agency for Marine-Earth Science and Technology, Japan | 2.8° × 2.8° | CMIP5/PMIP3 | 2012 |
| MPI-ESM-P | Max Planck Institute for Meteorology, Germany | 1.9° × 1.9° | CMIP5/PMIP3 | 2011 |
| MRI-CGCM3 | Meteorological Research Institute, Japan | 1.1° x 1.1° | CMIP5/PMIP3 | 2012 |

* longitude × latitude

CMIP5 –Coupled Model Intercomparison Project, Phase 5 (http://cmip-pcmdi.llnl.gov/)

PMIP3 –Paleoclimate Modelling Intercomparison Project, Phase 3 (http://pmip3.lsce.ipsl.fr/)

**Table S1.9** Ecological niche modeling methods used to estimate *Scinax squalirostris* potential distribution.

| Method | Species data type |
|---|---|
| Bioclimatic Envelope (BioClim) | Presence only |
| Ecological Niche Factor Analysis (ENFA) | Presence only |
| Euclidian Distance (EuclidDist) | Presence only |
| Flexible discriminant analysis (FDA) | Presence and absence |
| Generalized additive models (GAM) | Presence and absence |
| Generalized Linear Models (GLM) | Presence and absence |
| Gower Distance (GowerDist) | Presence only |
| Mahalanobis Distance (MahalDist) | Presence only |
| Multivariate adaptive regression splines (MARS) | Presence and absence |
| Maximum Entropy (MaxEnt) | Presence/background |
| Neural Networks (NNet) | Presence and absence |
| Random Forest (RNDFOR) | Presence and absence |

**Table S1.10** Characteristics of the 26 populations sampled for the analysis Phylogeography of *Scinax squalirostris*. Individuals (N), haplotypes by population (K) and relation of the haplotypes by population. Haplotypes in bold show the haplotype shared among some populations.

| **Population** | **mtDNA** | | | **nrDNA** | | |
|---|---|---|---|---|---|---|
| **Code** | **N** | ***k*** | **Haplotypes** | **N** | ***k*** | **Haplotypes** |
| ISP | 5 | 2 | **h01**, **h02** | 5 | 2 | **h01**, **h02** |
| BSP | 12 | 8 | **h01**, **h02**, h03, h04, **h05**, h06, h07, h08 | 11 | 4 | **h02**, h03, **h04**, h05 |
| SRMG | 11 | 4 | h09, h10, h11, h12 | 12 | 1 | **h04** |
| OMG | 16 | 5 | h13, **h14**, h15, h16, h17 | 16 | 2 | **h06**, **h07** |
| CMG | 3 | 1 | **h14** | 3 | 3 | **h06**, h08, h09 |
| PMG | 1 | 1 | h18 | - | - | - |
| SMG | 16 | 7 | h19, h20, h21, h22, h23, h24, h25 | 16 | 5 | **h06**, **h10**, h11, h12, h13 |
| SVMG | 22 | 5 | **h26**, h27, h28, h29, h30 | 21 | 8 | **h06**, **h10**, h14, h15, h16, h17, h18, h19 |
| SCMG | 13 | 2 | **h26**, h31 | 12 | 3 | **h06**, h20, h21 |
| CGO | 24 | 5 | h32, h33, h34, h35, h36 | 23 | 3 | h22, h23, h24 |
| BDF | 19 | 4 | h37, h38, h39, h40 | 18 | 1 | h25 |
| BMS | 7 | 3 | **h41**, h42, h43 | 7 | 5 | **h06**, **h07**, **h26**, h27, h28 |
| ISC | 26 | 12 | h44, **h45**, h46, h47, h48, h49, h50, h51, h52, h53, h54, h55 | 24 | 18 | **h01**, **h29**, **h30**, **h31**, **h32**, h33, h34, h35, **h36**, **h37**, h38, h39, h40, h41, h42, h43, h44, h45 |
| SJRS | 10 | 2 | h44, h46 | 10 | 10 | **h26**, **h30**, **h32**, **h36**, **h37**, h46, h47, h48, h49, h50 |

| | | | | | | |
|---|---|---|---|---|---|---|
| SFRS | 2 | 2 | h56, h57 | 2 | 2 | **h51**, h52 |
| BRS | 1 | 1 | h58 | 1 | 1 | h53 |
| PPR | 2 | 2 | **h59**, h60 | 2 | 2 | **h06**, h54 |
| PGPR | 8 | 3 | **h01**, **h05**, h61 | 8 | 6 | h55, h56, h57, h58, h59, h60 |
| CBSC | 1 | 1 | **h62** | 1 | 1 | **h51** |
| CNSC | 2 | 1 | **h01** | 2 | 2 | h61, h62 |
| BSC | 5 | 2 | **h01**, **h62** | 5 | 5 | **h31**, h63, h64, h65, h66 |
| CSC | 3 | 2 | **h59** | 2 | 2 | h67, h68 |
| ASC | 1 | 2 | **h59** | 2 | 2 | **h29**, h69 |
| EPY | 1 | 1 | **h41** | 1 | 1 | h70 |
| APY | 7 | 2 | **h41**, h63 | 4 | 3 | h71, h72, h73 |
| URU | 1 | 1 | **h45** | 1 | 1 | **h30** |

**Table S1.11** Analysis of Molecular Variance (AMOVA) for populations of *Scinax squalirostris*.

| DNA Region | Source of Variation | d.f | SSD | Variance components | % of variation | Fixation Index |
|---|---|---|---|---|---|---|
| Mitochondrial | Among populations | 25 | 1075.276 | 5.24081 Va | 93.78 | FST= 0.93 |
| | Within populations | 193 | 67.075 | 0.34754 Vb | 6.22 | |
| Nuclear | Among populations | 24 | 380.776 | 1.83940 Va | 65.11 | FST= 0.65 |
| | Within populations | 184 | 181.329 | 0.98548 Vb | 34.89 | |

Significant test with 10,100 permutations; p <0.000.

**Table S1.12** Coefficients of MRM analyses for calculated isolation by environmental. Environmental variables Bio4 - 'Temperature Seasonality', Bio6 - 'Minimum Temperature of Coldest Month', Bio11 - 'Mean Temperature of Coldest Quarter', Bio14 - 'Precipitation of Driest Month', Bio19 - 'Precipitation of Coldest Quarter'. Values in bold are significant *p < 0.05.

| MRM | Mitochondrial | | | Nuclear | | |
|---|---|---|---|---|---|---|
| | Coefficients | p-value | $R^2$ | Coefficients | p-value | $R^2$ |
| Intercept | -12.1328 | 0.874 | | -8.2777 | 0.505 | |
| Geographic Distance | 18.9238 | 0.312 | 0.0086 | 4.9931 | 0.202 | 0.043 |
| Bio4 | -1.8132 | 0.876 | 0.0002 | 2.2835 | 0.363 | 0.006 |
| Bio6 | -3.7484 | 0.743 | 0.0005 | 0.1250 | 0.0962 | 0.00001 |
| Bio11 | **-20.2499** | **0.048** | **0.0190** | -0.6186 | 0.787 | 0.0003 |
| Bio14 | -9.9699 | 0.491 | 0.0030 | 3.1278 | 0.290 | 0.006 |
| Bio19 | 5.1204 | 0.373 | 0.0040 | 0.9448 | 0.407 | 0.003 |
| $R^2$ (p-value) | 0.0583 (0.171) | | | 0.0762 (0.061) | | |
| *F*-test (p-value) | 3.2856 (0.171) | | | 4.0298 (0.061) | | |

*p-value calculated with 10,000 random permutations.

**Table S1.13** Values of true skill statistics (TSS) and values of area under the curve (AUC), with mean and standard deviation, for all ecological niche models (ENM) and Atmosphere-Ocean Global Circulation Model (AOGCM) combinations (ENM x AOGCM) in *Scinax squalirostris* paleodistribution modelling.

| ENM/AOGCM | CCSM | | CNRM | | MIROC | | MPI | | MRI | | **Mean** | | **SD** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TSS | AUC | TSS | AUC | TSS | AUC | TSS | AUC | TSS | AUC | TSS | AUC | TSS | AUC |
| BioClim | 0.698 | 0.937 | 0.690 | 0.936 | 0.678 | 0.920 | 0.722 | 0.949 | 0.647 | 0.927 | 0.690 | 0.936 | 0.028 | 0.011 |
| ENFA | 0.515 | 0.849 | 0.480 | 0.798 | 0.503 | 0.840 | 0.521 | 0.841 | 0.541 | 0.841 | 0.515 | 0.841 | 0.022 | 0.020 |
| EuclidDist | 0.645 | 0.895 | 0.465 | 0.825 | 0.625 | 0.888 | 0.673 | 0.903 | 0.551 | 0.883 | 0.625 | 0.888 | 0.084 | 0.031 |
| FDA | 0.759 | 0.961 | 0.730 | 0.945 | 0.724 | 0.940 | 0.793 | 0.963 | 0.751 | 0.950 | 0.751 | 0.950 | 0.027 | 0.010 |
| GAM | 0.768 | 0.960 | 0.780 | 0.967 | 0.767 | 0.942 | 0.821 | 0.966 | 0.758 | 0.956 | 0.768 | 0.960 | 0.025 | 0.010 |
| GowerDist | 0.662 | 0.921 | 0.647 | 0.889 | 0.683 | 0.900 | 0.746 | 0.927 | 0.616 | 0.915 | 0.662 | 0.915 | 0.049 | 0.016 |
| MahalonobisDit | 0.725 | 0.935 | 0.561 | 0.845 | 0.545 | 0.867 | 0.724 | 0.927 | 0.707 | 0.923 | 0.707 | 0.923 | 0.091 | 0.041 |
| GLM | 0.714 | 0.942 | 0.761 | 0.952 | 0.704 | 0.915 | 0.820 | 0.967 | 0.754 | 0.954 | 0.754 | 0.952 | 0.046 | 0.020 |
| MARS | 0.747 | 0.951 | 0.761 | 0.952 | 0.780 | 0.955 | 0.837 | 0.969 | 0.761 | 0.954 | 0.761 | 0.954 | 0.035 | 0.007 |
| MaxEnt | 0.762 | 0.958 | 0.727 | 0.957 | 0.768 | 0.960 | 0.836 | 0.978 | 0.777 | 0.972 | 0.768 | 0.960 | 0.039 | 0.009 |
| NNet | 0.564 | 0.882 | 0.686 | 0.962 | 0.688 | 0.951 | 0.694 | 0.920 | 0.670 | 0.966 | 0.686 | 0.951 | 0.054 | 0.035 |
| RndFor | 0.792 | 1.000 | 0.767 | 1.000 | 0.789 | 1.000 | 0.827 | 1.000 | 0.798 | 1.000 | 0.792 | 1.000 | 0.022 | 0.000 |
| **Mean** | 0.720 | 0.939 | 0.709 | 0.948 | 0.696 | 0.930 | 0.769 | 0.956 | 0.729 | 0.952 | - | - | - | - |
| **SD** | 0.085 | 0.041 | 0.111 | 0.064 | 0.091 | 0.044 | 0.093 | 0.043 | 0.089 | 0.043 | - | - | - | - |

**Table S1.14** Relative contributions of each modeling component (time, methods, AOGCMs and their interaction) to the variability of the predictions of the ENMs for *Scinax squalirostris*. The suitability column shows the median proportion and amplitude of the sum of squares from the hierarchical ANOVA calculated for each cell of the grid covering the Neotropical region.

| Sources of uncertainty | Median (SS)% | Min-Máx |
|---|---|---|
| ENMs*Time | 0.26 | 0.00 - 0.78 |
| AOGCMs*Time | 0.14 | 0.00 - 0.76 |
| Time | 0.04 | 0.02 - 0.92 |
| Residual | 0.46 | 0.06 - 0.78 |

**Table S1.15** Probability values and 95% confidence intervals for the 10 subsets of closest simulated data. We use the logistic approach in Approximate Bayesian Computation (ABC) to evaluate all demographic scenarios (8 scenarios). In bold the best-supported scenarios in this comparison.

| | N | Scenario 1 | | Scenario 2 | | Scenario 3 | | Scenario 4 | | Scenario 5 | | Scenario 6 | | Scenario 7 | | Scenario 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mitochondrial** | 1000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 2000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 3000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 4000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 5000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 6000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 7000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 8000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 9000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 10000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| **Nuclear** | 1000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 2000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 3000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 4000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 5000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 6000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 7000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 8000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 9000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |
| | 10000 | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] | **1.00** | **[1.0000,1.0000]** | 0.00 | [0.0000,1.0000] | 0.00 | [0.0000,1.0000] |

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**SUPPORTING INFORMATION**

# Inferring responses to Neogene-Quaternary climate dynamics from historical demography in a South American grassland treefrog

Tatianne P. F. Abreu-Jardim | Rafael F. Magalhães | Natan M. Maciel | Matheus Souza Lima-Ribeiro | Guarino R. Colli | Célio F. B. Haddad and Rosane G. Collevatti.

**Appendix S2** Supplementary additional information of Details of Material and Methods.

## Genetic data

We extracted the DNA of muscle and liver tissue samples preserved in ethanol, using the Dneasy Blood & Tissue Kit (Quiagen®, InR., Chatsworth, CA). DNA were amplified by polymerase chains reaction (PCR), and sequenced in an ABI 3500 automated DNA sequencer (Applied Biosystems®, CA) using the BigDye® Terminator Cycle Sequencing kit, according to the manufacturer's instructions. We sequenced all fragments in the forward and reverse directions.

The consensus sequences were assembled for each individual using the software SEQSCAPE 2.7 (Applied Biosystems) and to obtain the multiple sequence alignments, we used CLUSTALX (Thompson et al., 1997) implemented in BIOEDIT 7.2.5 (Hall, 1999). *Coding sequences were tested for saturation by plotting transitions and transversions with* TN93 distance (Tamura & Nei, 1993) using DAMBE software (Xia, 2013). As third codon positions of CytB showed high levels of saturation (see Appendix S3: Figure S3.1; in supporting information), they were excluded from the final alignment. The sequences of RAG-1 showed heterozygous individuals (138 individuals, 47 characters), therefore,

we used the most frequent haplotype, estimated by PHASED implemented in DNAsp 5.0 (Librado & Rozas, 2009). No signal of recombination was founded.

**Structure of populations: IBD and IBE test**

Environmental distance matrices were calculated as pairwise Euclidian distances for each environmental variable. We selected the variables that explained the most of the variance among populations: (Bio4- 'Temperature Seasonality' (16%), Bio6- 'Minimum Temperature of Coldest Month' (27%), Bio11- 'Mean Temperature of Coldest Quarter' (17%), Bio14- 'Precipitation of Driest Month' (66%), Bio19- 'Precipitation of Coldest Quarter' (67%), obtained in the EcoClimate database (www.ecoclimate.org; Lima-Ribeiro *et al.*, 2015). Before obtaining the environmental distance matrix, we standardized each variable to zero mean and unity variance (Z standardization) (Diniz-Filho et al., 2013). All distance matrices were log-scaled.

**Demographic population history: Lamarc**

The analyses were run with 20 initial chains of 15,000 steps and three final chains of 80,000 steps, sampling by each 100 steps. The demographic parameters were estimated with populations that had at least five individuals, due to the limitation in the number of populations for reliable estimation in LAMARC. The convergence, stability and the effective sample size (ESS $\geq$ 200) were checked using TRACER 1.6 (Rambaut *et al*., 2013).

**Species distribution modeling**

To overcome the multicollinearity problem among all bioclimatic variables in the ENMs predictions, we performed a factorial analysis using Varimax rotation from 19

bioclimatic variables obtained in the EcoClimate database (www.ecoclimate.org; Lima-Ribeiro *et al.*, 2015) which are available at 0.5° x 0.5° of resolution.

The distribution of *S. squalirostris* was inferred used presence-only and presence–absence algorithms (Table S10), represented by 12 algorithms: bioclimatic envelopes (Bioclim), ecological niche factor analysis (ENFA), Euclidean distance, flexible discriminant analysis (FDA), generalized additive models (GAM), generalized linear models (GLM), Gower distance, Mahalanobis distance, multivariate adaptive regression splines (MARS), maximum entropy (Maxent), neural networks (NNET), and random forest (RNDFOR). There are not reliable real absence data available, so we used pseudo-absences were randomly generated for all Neotropical grid cells excluding cells with presence, maintaining prevalence equal to 0.5 to maximize model performance. To assess the model stability, we used a random data partitioning 75% training (calibration) and 25% testing (evaluation), replicated 50 repetitions for each model, due to the absence of independent testing data. This procedure and ENMs were run in the integrated computational platform BIOENSEMBLES (Diniz-Filho *et al.*, 2009). Model accuracy was assessed though two accuracy metrics, the calculation of the area under the receiver operator characteristics (ROC) curve – AUC, which is a threshold-independent statistic that is considered a highly effective measure for the performance of ordinal score models whose AUC ranges from 0 to 1(Allouche *et al.***,** 2006). The other metric is the true skill statistic (TSS), that take into account both omission and commission errors, with range from -1 to 1 and values close to 1 indicate a perfect fit (Allouche *et al.*, 2006). We used an ensemble approach (Araújo & New, 2006) to calculate a consensus map that included only models with AUC > 0.75 and TSS>0.5, and the models with poor performance were eliminated from the ensemble process (values of AUC and TSS in Table S11). We used the remaining models to compute the

frequency of occurrence in the cells covering the Neotropical, after truncation using specific thresholds based on the ROC curve (maximum specificity + sensibility). The frequency of occurrence in each cell provide a surrogate for the environmental suitability and was used for *Scinax squalirostris* across the Neotropical grid cells.

The combination of all ENMs and AOGCMs resulted in 60 independent predictive maps (12 ENMs x 5 AOGCMs) for each time period (pre-industrial, 6 Ka, 21 Ka). We applied a hierarchical ANOVA using the predicted suitability from all models (12 ENMs x 5 AOGCMs x 3 times periods) as response variables to identify and map the uncertainties due to modeling components. For this, the ENMs and AOGCMs components were nested into the time component, but crossed by a two-way factorial design within each time period (see Terrible *et al*., 2012). The 60 predictive maps were combined to obtain the consensus map for each time period, and then they were combined to generate a map of the historical refuge (stable environments through time). We considered all grid cells with suitability values $\geq 0.5$ in the three time periods as refuge. To quantify the range sizes between the three time periods, we first tested 0.5 and 0.75 binary thresholds and selected 0.5 because it better recovered the current distribution area of *S. squalirostris*. Then, we used this threshold to transform occurrence frequency maps into presence and absence maps, and thus we calculated range size by summing the numbers of cells predicted as presence for each of the 60 predictive maps. After that, we subtracted the range sizes between current and Holocene, current and LGM, Holocene and LGM to calculate range shift.

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**SUPPORTING INFORMATION**

# Inferring responses to Neogene-Quaternary climate dynamics from historical demography in a South American grassland treefrog

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**Appendix S3** Supplementary figures (Figs S3.1-S3.13) with saturation analysis, genetic diversities maps, Bayesian Analysis of Population Structure, EBSP, location diffusion tree, details of ecological niche modelling, demography simulations and quantile regressions.

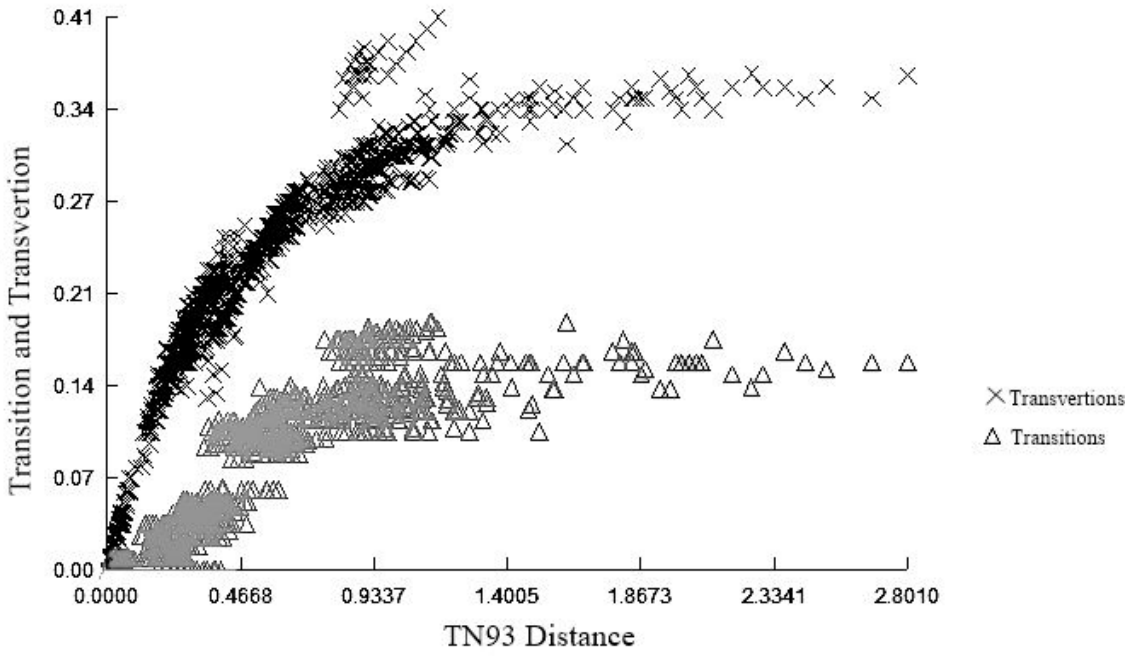


**Figure S3.1** Transition and transversion saturation plot for third codon positions of Cytochrome b sequences.

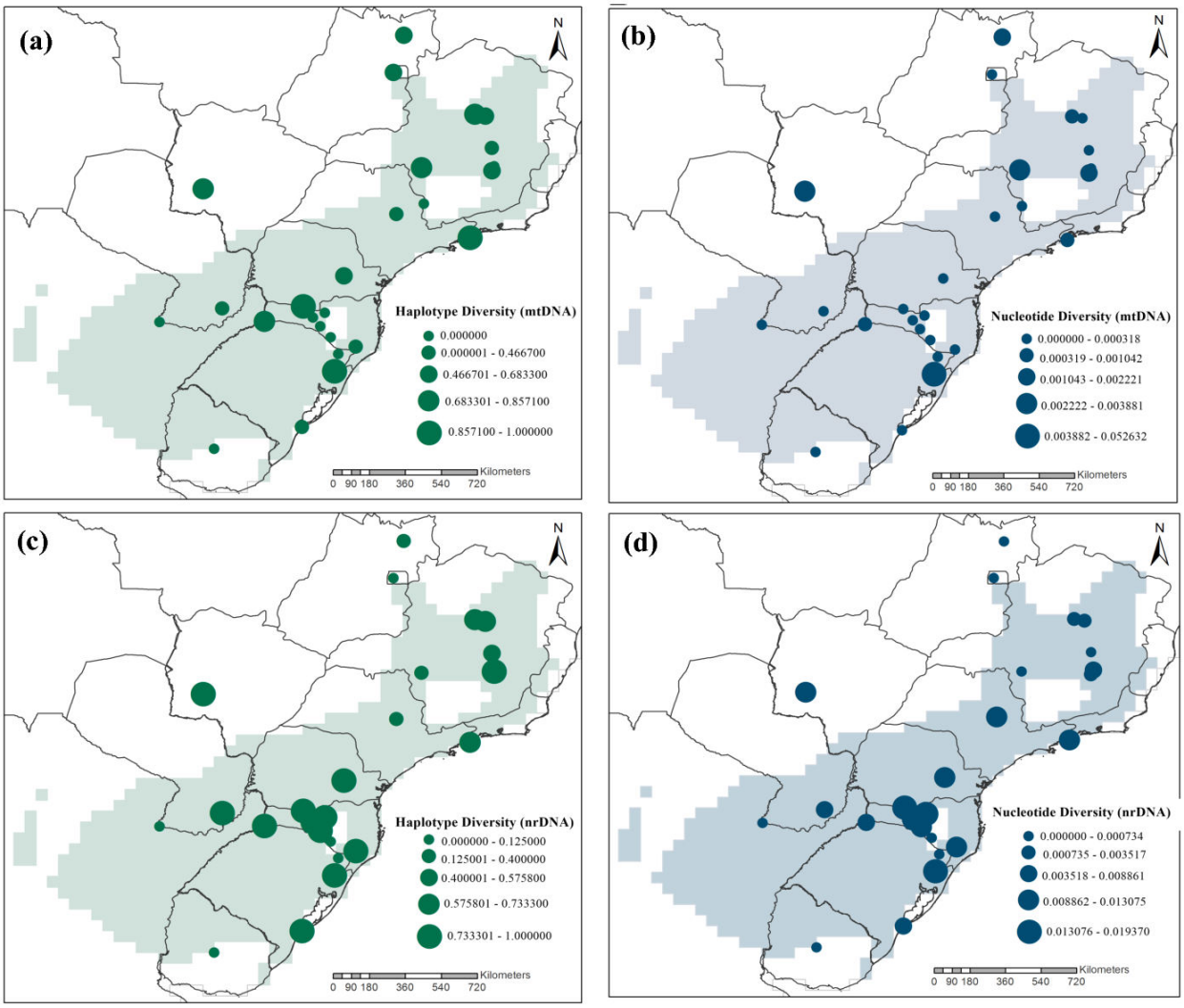


**Figure S3.2** Maps of geographic distribution of mitochondrial (mtDNA) and nuclear (nDNA) genetic diversity (nucleotide and haplotype) in the historical refuge predicted by paleodistribution modelling for *Scinax squalirostris*: (a) haplotype diversity for mtDNA, (b) nucleotide diversity for mtDNA, (c) haplotype diversity for nDNA, and (d) nucleotide diversity for nDNA.

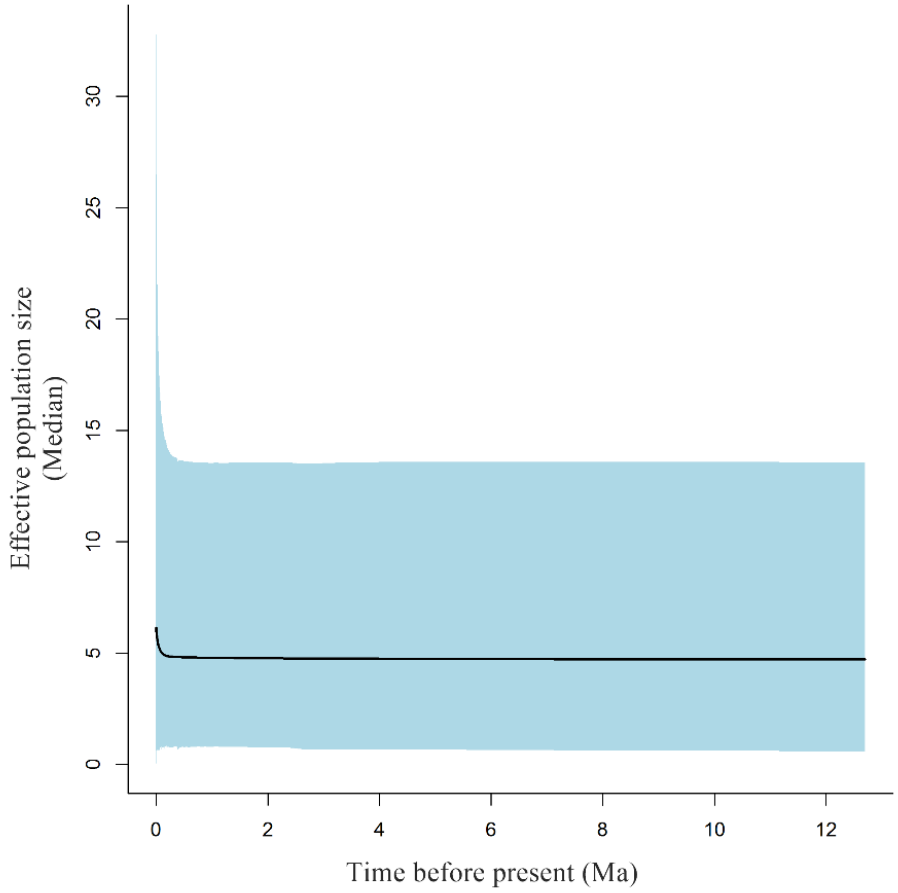


**Figure S3.3** Extended Bayesian Skyline Plot of *Scinax squalirostris* populations of concatenated fragments (mtDNA and nDNA). The black line represents the median of the effective population size estimates and the blue bands indicate the upper and lower credibility interval (95%). The scale is in millions of years (Ma) before present.

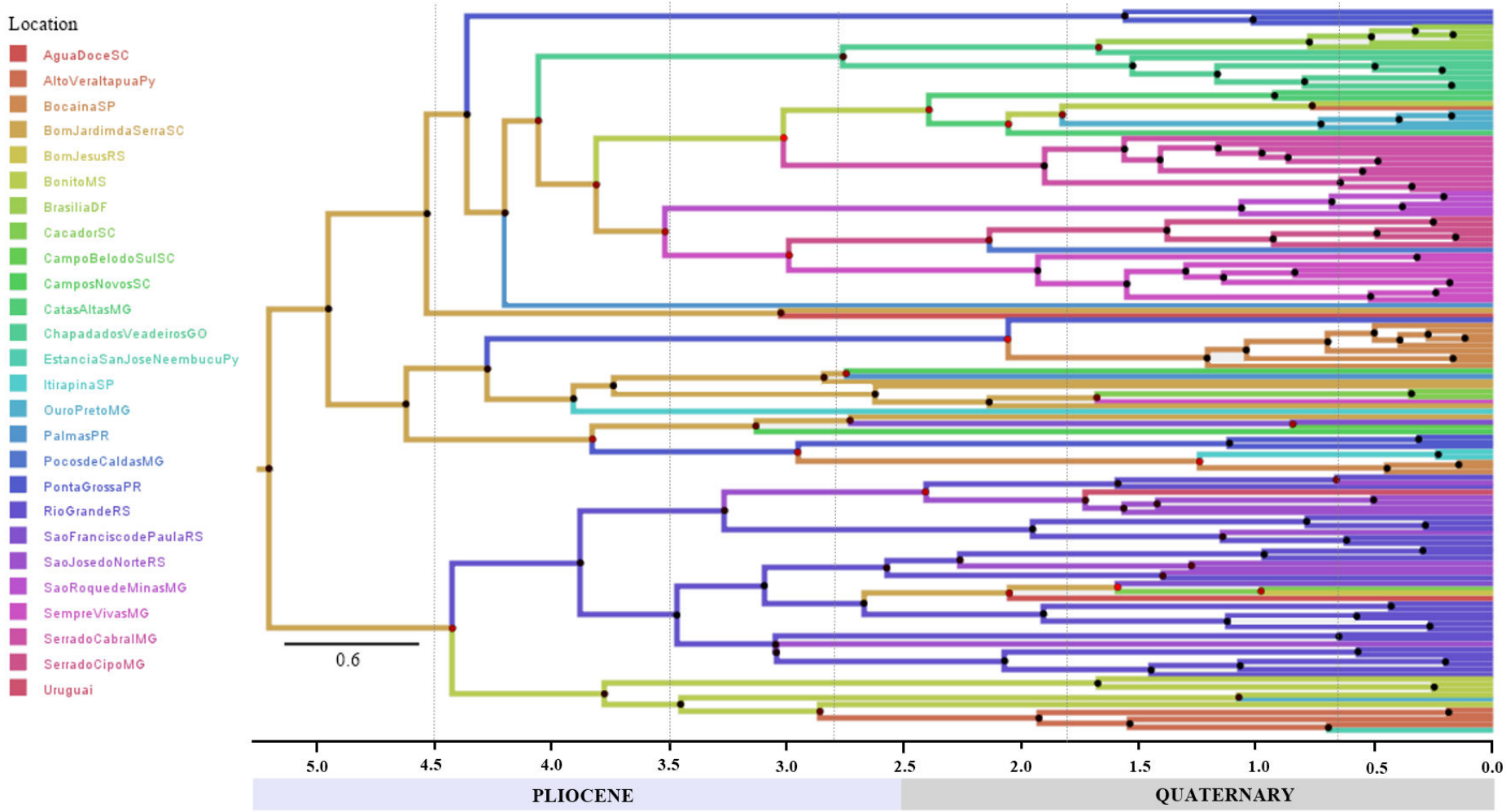


**Figure S3.4** Annotated tree showing the spatio-temporal dynamics of *Scinax squalirostris* lineages diffusion among the 26 sampled populations. The black dots represent the location probability upper to 0.8 and the red dots represent the location probability below to 0.79.

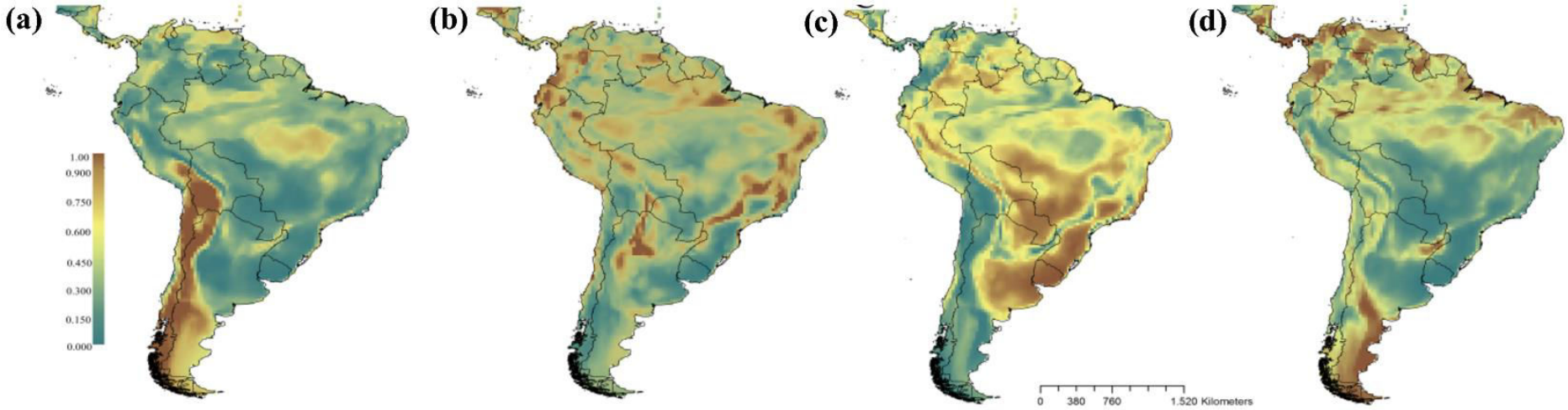


**Figure S3.5** Maps of uncertainty (relative sum of squares) for the modelling components for *Scinax squalirostris*: (a) ecological niche models (ENMs), (b) atmosphere–ocean global circulation models (AOGCMs), (c) time, and (d) residual (ENM * AOGCM interaction).

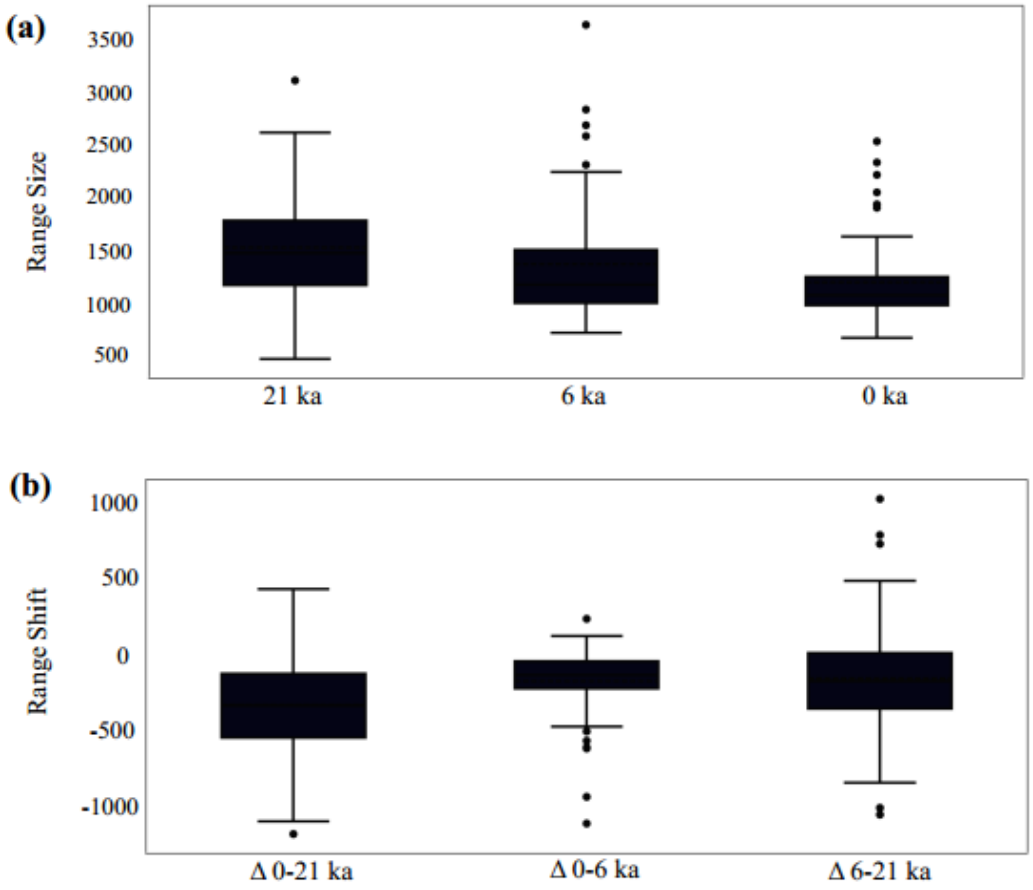


**Figure S3.6** Median and 0.05 – 0.95 quantiles among the 60 maps of (a) range size and (b) range shift (difference of range size among time periods in number of cells) predicted for *Scinax squalirostris* at present-day (0 Ka), mid-Holocene (6 Ka) and LGM (21 Ka).

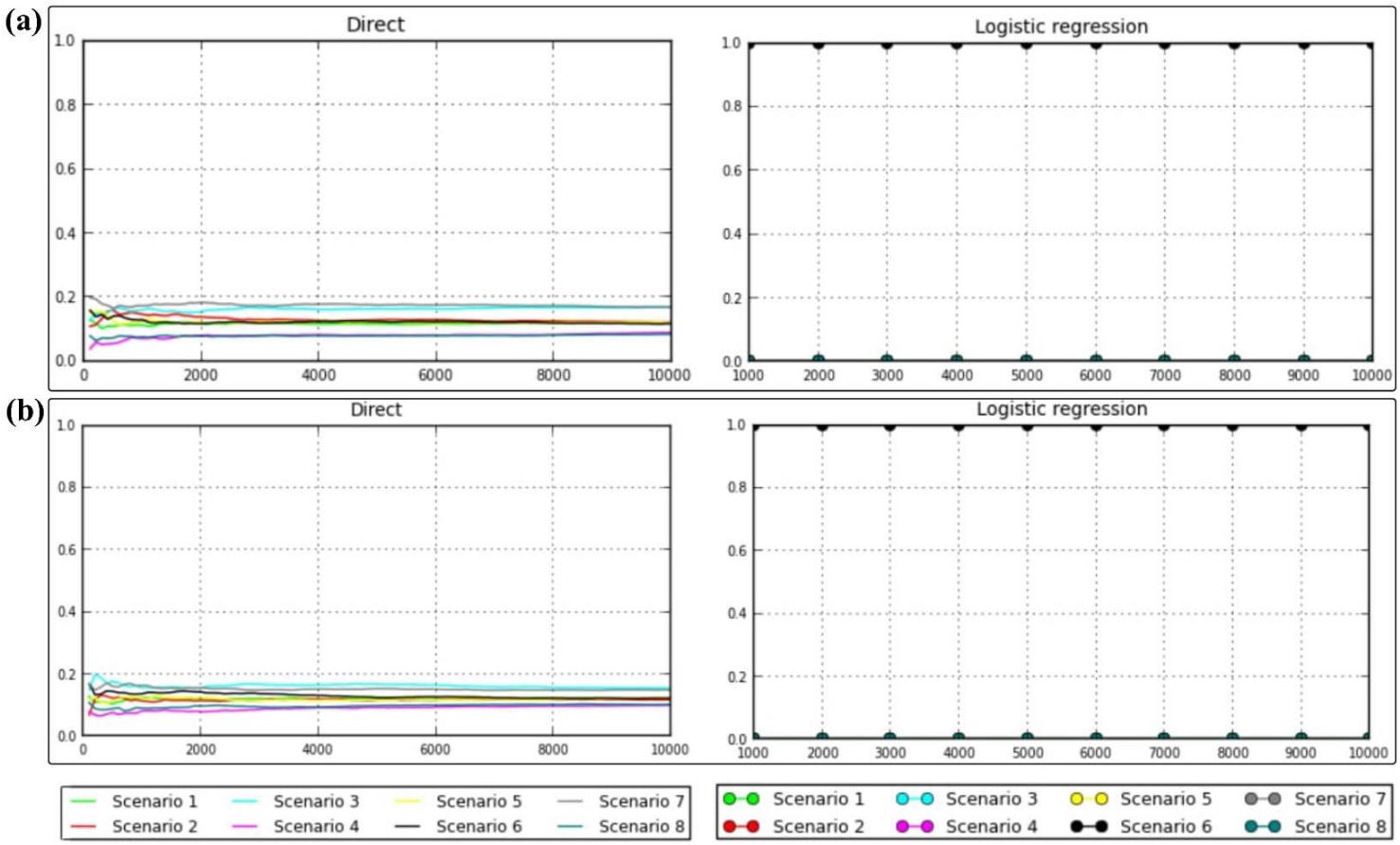


**Figure S3.7** Posterior probabilities of demographic scenarios for populations of *Scinax squalirostris* using approximate Bayesian computation (ABC), (a) scenarios tested with mtDNA and (b) scenarios tested with nrDNA. Scenarios without admixture: range expansion (scenario 1), range retraction (scenario 2), range stability (scenario 3) and multiples refuges (scenario 4). Scenarios with admixture: range expansion (scenario 3), range retraction (scenario 6), range stability (scenario 7) and multiples refuges (scenario 8).

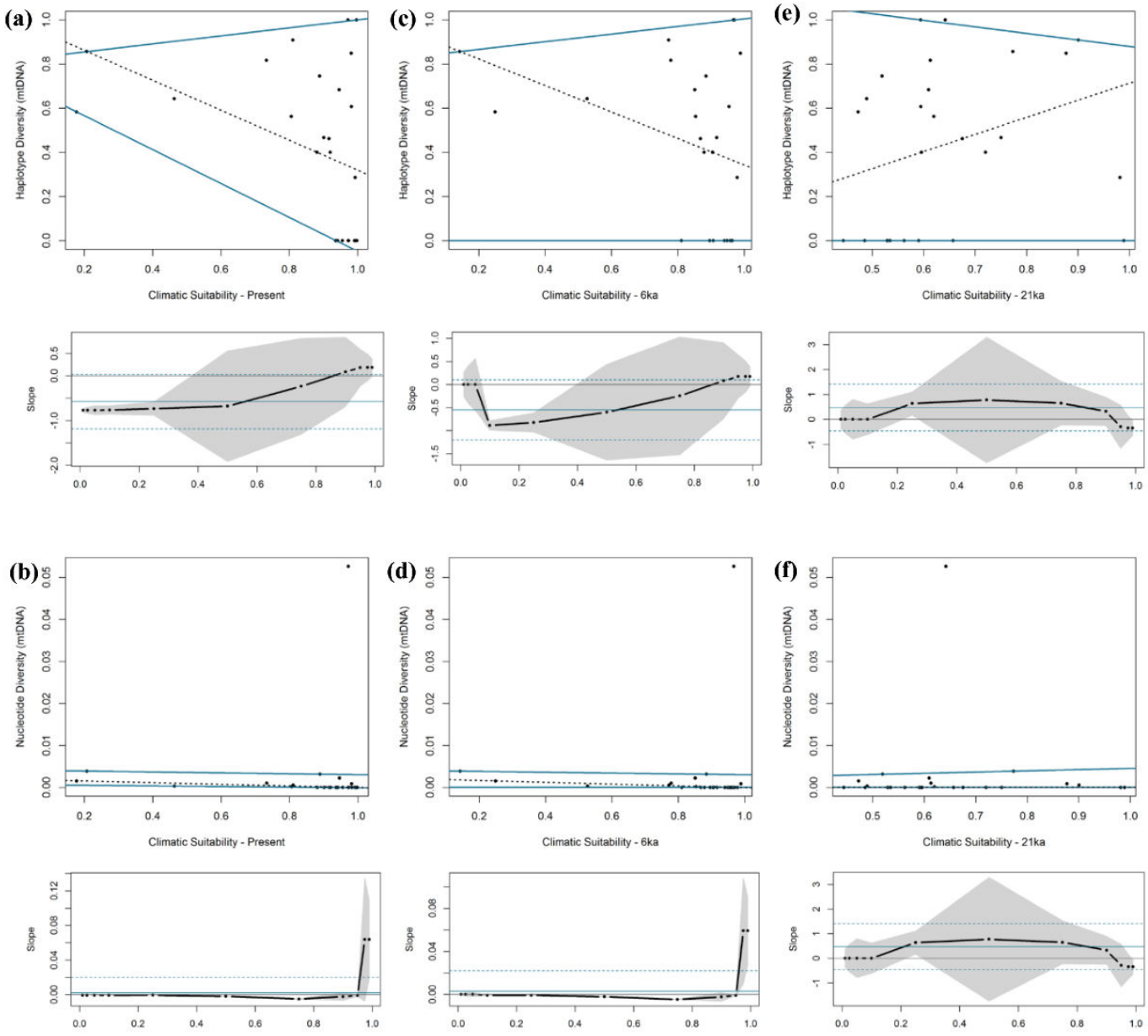


**Figure S3.8** Quantile regression for the relationships of haplotype and nucleotide diversities for mtDNA among the 26 populations of *Scinax squalirostris* with suitability in climatic conditions: (a) and (b) climatic suitability in present; (c) and (d) climatic suitability in 6 Ka; (e) and (f) climatic suitability in 21 Ka. The upper figures show the triangle-shaped envelopes from 0.05 (lower line) and 0.95 (upper line) quantiles. The lower figures show the slope estimate for 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 0.9, 0.95, 0.975 and 0.99 quantiles (area in grey indicates 95% confidence interval around estimates).

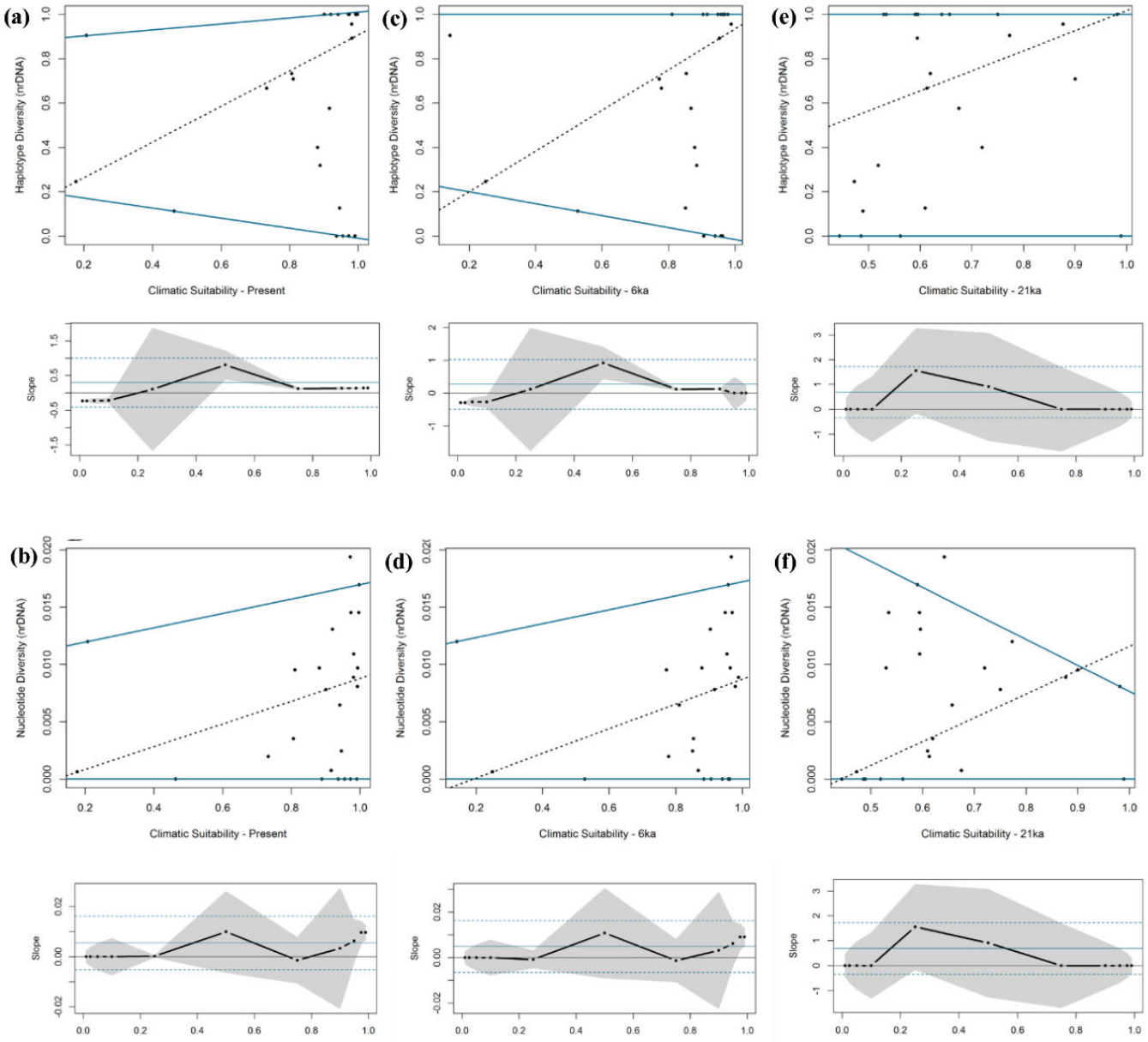


**Figure S3.9** Quantile regression for the relationships of haplotype and nucleotide diversities for nDNA among the 26 populations of *Scinax squalirostris* with suitability in climatic conditions: (a) and (b) climatic suitability in present; (c) and (d) climatic suitability in 6 Ka; (e) and (f) climatic suitability in 21 Ka. The upper figures show the triangle-shaped envelopes from 0.05 (lower line) and 0.95 (upper line) quantiles. The lower figures show the slope estimate for 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 0.9, 0.95, 0.975 and 0.99 quantiles (area in grey indicates 95% confidence interval around estimates).

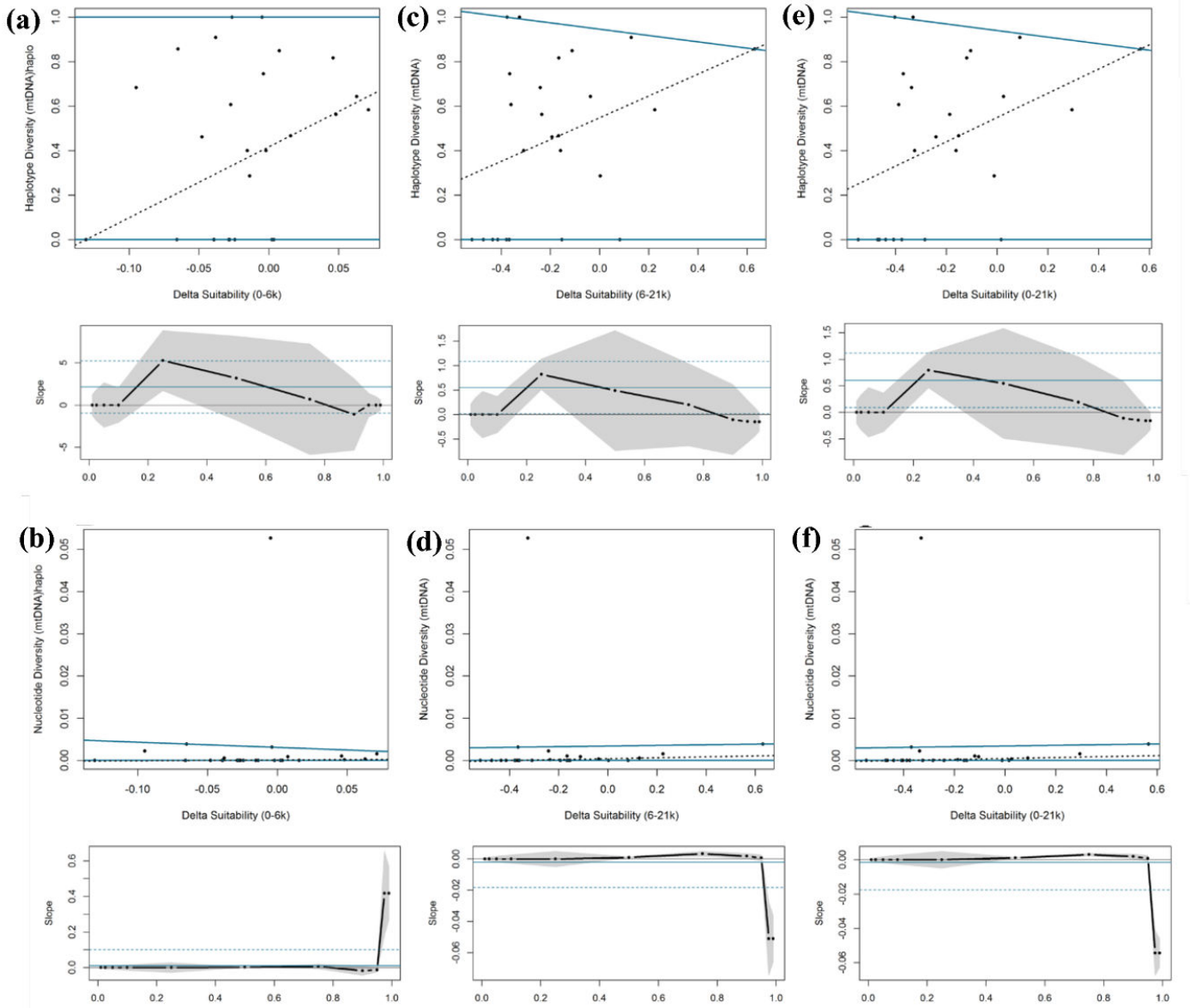


**Figure S3.10** Quantile regression for the relationships of haplotype and nucleotide diversities for mtDNA among the 26 populations of *Scinax squalirostris* with stability and suitability in climatic conditions: (a) and (b) climatic stability through 6 Ka to present; (c) and (d) climatic stability through 21 Ka to 6 Ka; (e) and (f) climatic stability through 21 Ka to present. The upper figures show the triangle-shaped envelopes from 0.05 (lower line) and 0.95 (upper line) quantiles. The lower figures show the slope estimate for 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 0.9, 0.95, 0.975 and 0.99 quantiles (area in grey indicates 95% confidence interval around estimates).

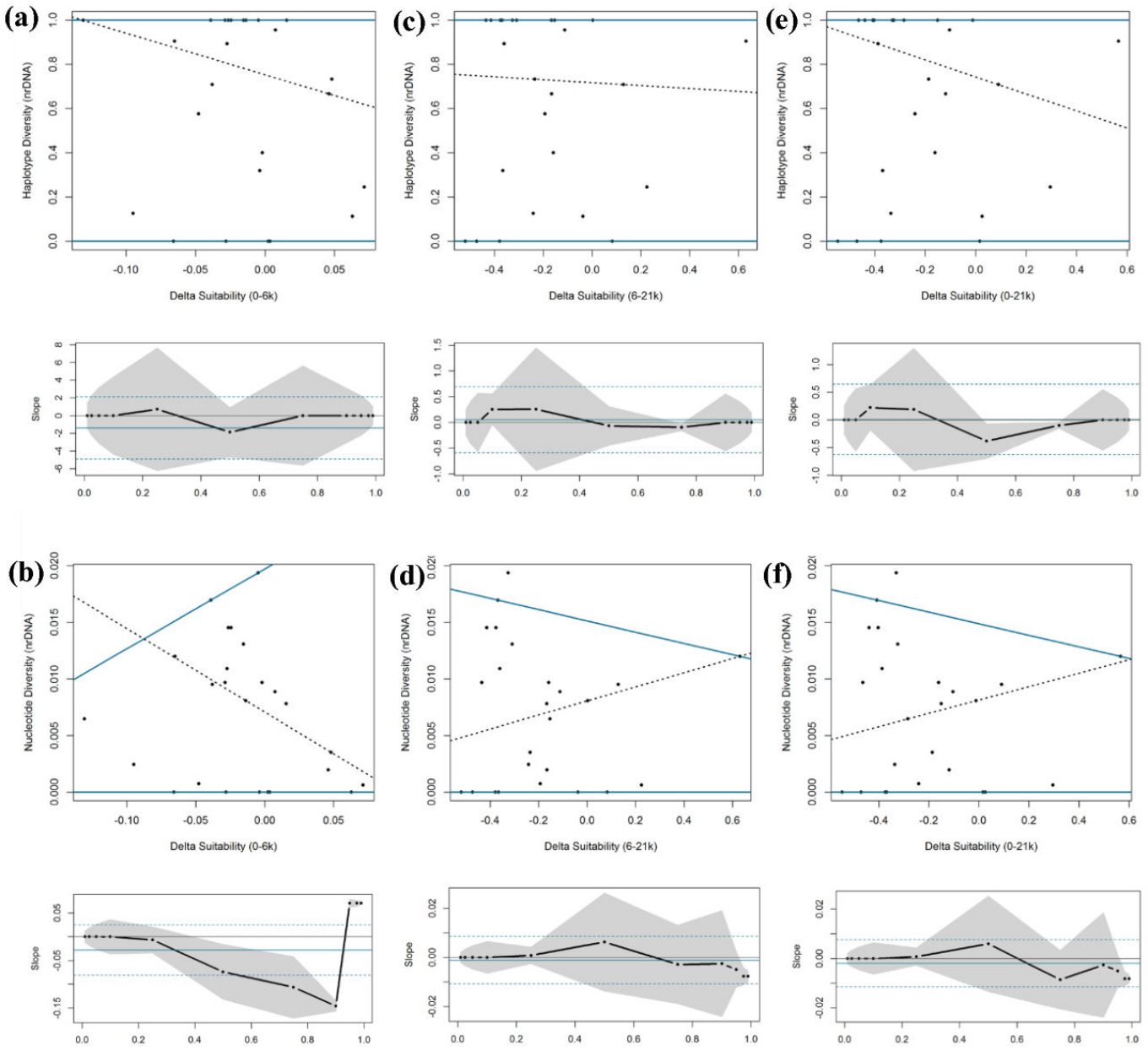


**Figure S3.11** Quantile regression for the relationships of haplotype and nucleotide diversities for nDNA among the 26 populations of *Scinax squalirostris* with stability and suitability in climatic conditions: (a) and (b) climatic stability through 6 Ka to present; (c) and (d) climatic stability through 21 Ka to 6 Ka; (e) and (f) climatic stability through 21 Ka to present. The upper figures show the triangle-shaped envelopes from 0.05 (lower line) and 0.95 (upper line) quantiles. The lower figures show the slope estimate for 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 0.9, 0.95, 0.975 and 0.99 quantiles (area in grey indicates 95% confidence interval around estimates).

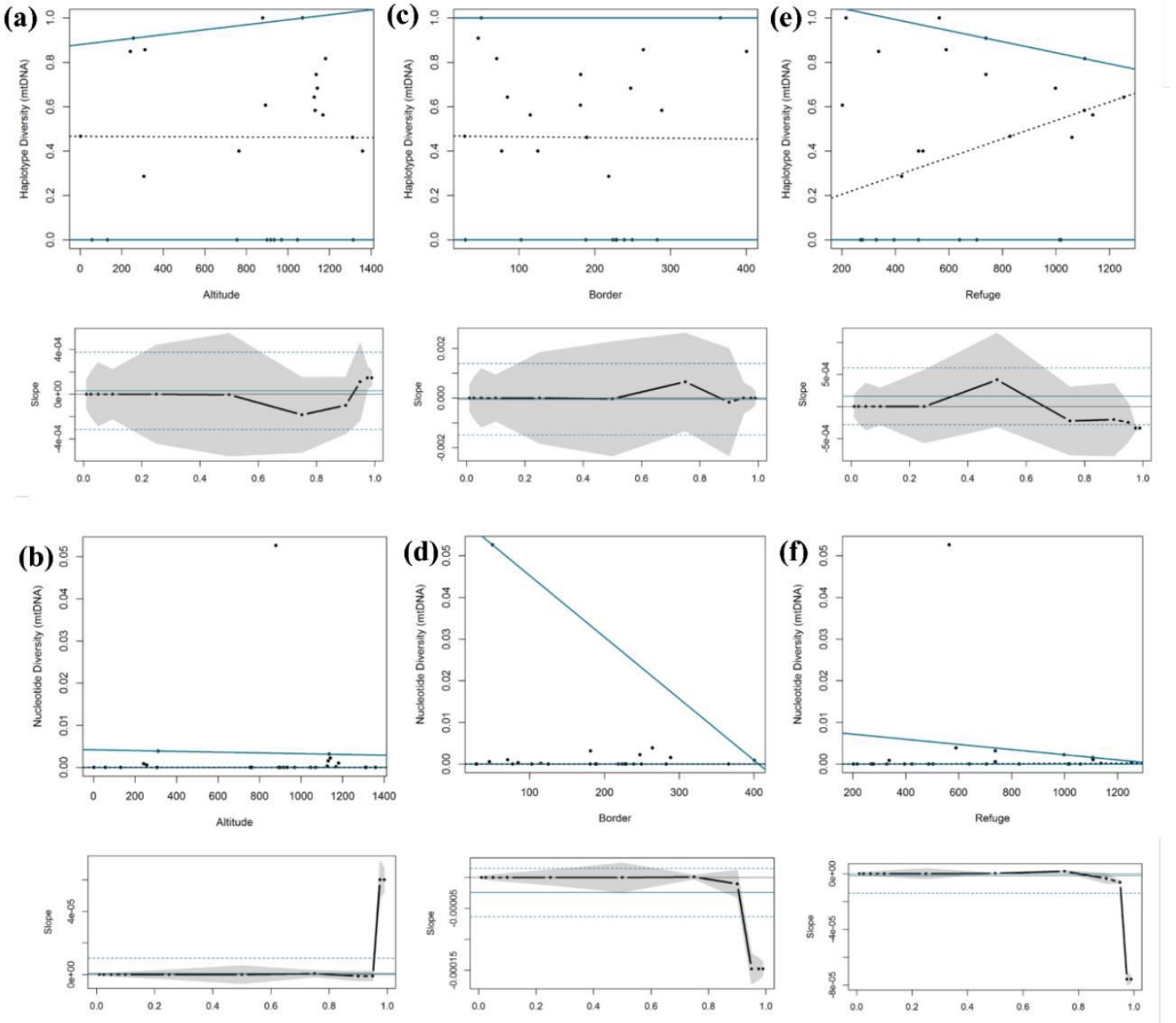


**Figure S3.12** Quantile regression for the relationships of haplotype and nucleotide diversities (mtDNA) among the 26 populations with altitude, edges and centroid distances of the historical refuge occupied by *S. squalirostris* through the last glacial period: (a) and (b) altitude (in meters), (c) and (d) distance from the edge of the historical refuge (in kilometers), and (e) and (f) distance from the centroid of the historical refuge (in kilometers). The upper figures show the triangle-shaped envelopes from 0.05 (lower line) and 0.95 (upper line) quantiles. The lower figures show the slope estimates for 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 0.9, 0.95, 0.975 and 0.99 quantiles (area in grey indicates 95% confidence interval around estimates).

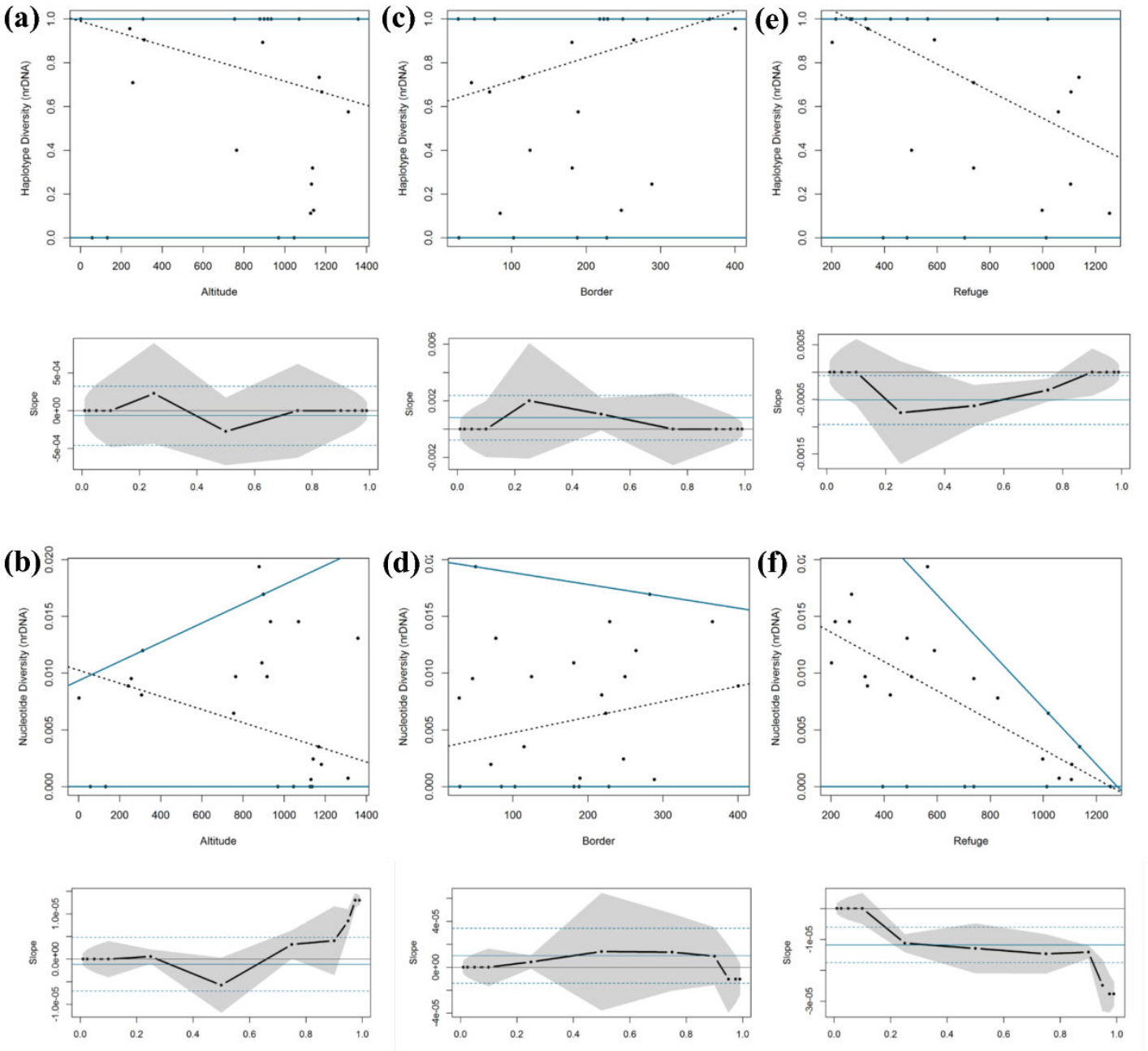


**Figure S3.13** Quantile regression for the relationships of haplotype and nucleotide diversities (nDNA) among the 26 populations with altitude, edges and centroid distances of the historical refuge occupied by *S. squalirostris* through the last glacial period: (a) and (b) altitude (in meters), (c) and (d) distance from the edge of the historical refuge (in kilometers), and (e) and (f) distance from the centroid of the historical refuge (in kilometers). The upper figures show the triangle-shaped envelopes from 0.05 (lower line) and 0.95 (upper line) quantiles. The lower figures show the slope estimates for 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 0.9, 0.95, 0.975 and 0.99 quantiles (area in grey indicates 95% confidence interval around estimates).

# Capítulo 2

RESEARCH PAPER

# Revelando a diversidade do complexo de espécies de *Scinax squalirostris* (Lutz, 1925) (Anura, Hylidae), uma espécie com ampla distribuição disjunta

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**Título de cabeçalho:** Revelando o complexo *Scinax squalirostris*.

## Resumo

A falta de conhecimento sobre as espécies pode afetar a compreensão de aspectos relacionados à biodiversidade em diversos níveis, como o entendimento dos padrões e processos que a regem. A ausência do conhecimento sobre espécies é denominada de lacuna Linneana. Um dos problemas associados a essa lacuna é o número elevado de

espécies não descritas. A presença de espécies crípticas (i.e. espécies diferentes com morfologias similares) podem elevar a complexidade do conhecimento real dos organismos. A taxonomia integrativa busca, através da utilização de várias fontes independentes, compreender a riqueza de espécies existentes. Assim, estratégias como a utilização de dados ditos não tradicionais como os moleculares, que levam em conta a história demográfica passada das espécies, e o uso de caracteres morfométricos e análises estatísticas, provém uma maior robustez na delimitação de uma espécie e na busca para revelar a cripticidade existente em nossa biodiversidade. Neste trabalho utilizamos medidas morfométricas e sequências de duas regiões mitocondriais e uma nuclear para verificar a possível existência de mais de uma espécie sob o mesmo nome de uma perereca Sul-Americana amplamente distribuída e com ocorrência disjunta, *Scinax squalirostris*. Os dados moleculares indicaram a existência de três linhagens evolutivamente distintas como possíveis candidatas a espécie. Entretanto, ao combinar as evidências destes dados como análises morfométricas revelaram a existência de duas linhagens, sendo uma candidata a espécie, em uma abordagem mais conservativa. A espécie candidata ocorre na região Centro-Oeste do Brasil, e não teria ainda nome constituindo uma nova espécie a ser descrita. Além disso, nossos resultados revelam a necessidade da realização de testes adicionais de hipóteses de relação entre as linhagens, com utilização de modelos de delimitação independentes e ainda a inclusão de dados morfológicos para melhor compreender esse complexo de espécie.

**Palavras-chave:** anfíbios, coalescente, delimitação, taxonomia integrativa, multiespécies.

## 1. INTRODUÇÃO

A ausência de informação e conhecimento sobre as espécies é um problema que pode afetar o acesso da biodiversidade, estudos ecológicos e evolutivos (Beheregaray & Caccone, 2007), pois ainda existe uma quantidade (desconhecida) de espécies a serem identificadas e descritas (Wilson, 2003). Essa falta de informação em relação ao conhecimento taxômico de espécies é denominada como lacuna Linneana (Lomolino, 2004; Hortal et al., 2015). Essa lacuna abrange duas categorias de ausência de informação sobre espécies, uma delas é que ainda existem espécies que não foram amostradas e a outra é que existem espécies que foram amostradas, mas não foram descritas (Hortal et al., 2015). Essa segunda categoria está em constante mudança, pois novas espécies são descritas, há um grande número de revisões taxonômicas e sinonimização de espécies. Um outro desafio ainda é a dificuldade em estabelecer um conceito unificado de espécie e como delimitá-las (De Queiroz, 2007; Freudenstein et al., 2017).

A utilização da taxonomia tradicional para delimitar espécies pode ser problemática, por basear-se apenas em caracteres fenotípicos, pois grupos de organismos podem apresentar pouca ou nenhuma variação morfológica (morfologia conservada), o que impede a sua descrição como espécie distinta, podendo gerar problemas de ações de conservação e o conhecimento real da riqueza global (Bickford et al., 2007). Esses grupos morfologicamente indistinguíveis são denominados de espécies crípticas, e geralmente são erroneamente associados a um único táxon (Beheregaray & Caccone, 2007). Métodos de taxonomia integrativa podem ajudar a revelar a diversidade críptica dos organismos, utilizando múltiplas fontes de dados (molecular, morfologia, comportamental, ecológico, etc) (Dayrat, 2005, Fujita et al., 2012). Com isso, houve o aumento do número de estudos utilizando dados morfológicos, morfométricos e genéticos na última década (Pante et al., 2015). Essa integração metodológica é funcional, pois cada método apresenta limites

intrínsecos para o delineamento das espécies, e mesmo quando dados morfológicos ou moleculares, por si só sejam capazes de delimitar uma unidade evolutiva como espécie, outras abordagens podem auxiliar e promover robustez na denominação de espécie (Bickford et al., 2007).

Recentemente, um dos métodos de delimitação mais utilizados tem sido inferir árvores de espécies através de múltiplas árvores gênicas (Knowles & Carstens, 2007). A abordagem de múltiplos genes pode fornecer hipóteses mais robustas, uma vez que combinam regiões do DNA com diferentes taxas evolutivas (Yang & Rannala, 2010). Métodos moleculares sob abordagens baseadas em modelos estatísticos vêm sendo utilizados para delimitar os limites de espécies, usando por exemplo, o modelo coalescente de multiespécies (Yang & Rannala, 2010).

A inferência sob o modelo coalescente de multiespécies fornece poder considerável na identificação de limites dos táxons recentemente divergidos e leva em conta a alta variância estocástica de processos genéticos (Sukumaran & Knowles, 2017). Assim, os dados genéticos podem ser utilizados para delimitar a espécie através de um critério de monofiletismo recíproco (Knowles & Carstens, 2007), em que se espera que haplótipos amostrados em uma espécie sejam mais intimamente relacionados entre si do qualquer haplótipo da outra espécie, devido ao longo tempo de divergência após a interrupção do fluxo gênico (Avise, 2000).

A ausência reciprocidade monofilética não indica a ausência de divergência de espécies, sendo que as espécies recentemente derivadas tenderão a não ser reveladas sob um critério monofilético (Knowles & Carstens, 2007). Eventos como esse, são caracterizados pela existência de um arranjo incompleto de linhagens, devido à ausência de tempo substancial para a separação completa das linhagens (Knowles & Carstens,

2007; Sukumaran & Knowles, 2017). Assim, o método de coalescência multiespécies, estima a árvore de espécie com múltiplas árvores gênicas, considerando a incongruência das árvores gênicas devido a retenção de polimorfismo ancestral, variação nas sequências moleculares e variação nos parâmetros demográficos (Yang & Rannala, 2010; Leaché & Fujita, 2010). Além disso, este método fornece um modelo apropriado para investigar e determinar linhagens separadas dentro de complexos de espécies (Yang & Rannala, 2010; Heled e Drummond, 2010), juntamente com a utilização de fontes de dados alternativos (Fujita et al., 2012; Olivier et al., 2014).

Anfíbios são um grupo de vertebrados com cerca de 7932 espécies reconhecidas (Frost, 2018), e com uma alta taxa de descoberta e descrição de espécies (Köhler et al., 2005). Porém, muitos de seus grupos refletem uma morfologia conservada, tornando-os um grupo desafiadores em sua taxonomia, o que poderia indicar que as espécies ainda se encontram no processo contínuo de especiação (De Queiroz, 2007). Assim, anfíbios que possuem ampla distribuição, que apresentam baixa capacidade de dispersão e alta filopatria (Duellman & Trueb, 1994; Blaustein *et al*., 1994), podem apresentar uma alta estruturação geográfica, o que pode levar a representar um complexo de espécies. A utilização da taxonomia integrativa com dados moleculares pode trazer a luz essa diversidade críptica e categorizar as espécies candidatas, além de entender os processos que envolveram a diversificação dessas espécies (e.g. Gehara et al., 2014; Correia et al., 2017).

O gênero *Scinax* Wagler, 1830 possui ampla distribuição geográfica desde o México até a Argentina (Frost, 2018). Além disso apresenta uma taxonomia complexa devido ao grande número de espécies que compõem o grupo, inclusive morfologicamente semelhantes (Faivovich, 2002). Segundo Faivovich (2002), o gênero tem dois clados, *Scinax ruber* e *Scinax catharinae*, e alguns estudos mostram que o clado *S. ruber* pode

possuir uma alta diversidade críptica (Duellman et al., 2016, Ferrão et al., 2016). Entre essa diversidade críptica do clado *S. ruber*, encontra-se o caso de *Scinax squalirostris* (Lutz, 1925), uma pequena perereca (23 to 28mm) (Cei, 1980), que possui uma ampla distribuição geográfica, porém disjunta em formações de Campos nos Pampas, Chaco, Mata Atlântica e Cerrado. Essa espécie possui alta variação acústica (Faria et al., 2013) e morfológica, e vem sendo considerada como um complexo de espécies crípticas (Eterovick & Sazima, 2004). Assim neste trabalho, utilizamos a abordagem de taxonomia integrativa com *Scinax squalirostris* como modelo, combinando dados moleculares e morfométricos, com o objetivo de identificar e delimitar linhagens evolutivamente distintas que possam ser candidatas a espécie.

## 2. MATERIAL E MÉTODOS

### 2.1 Amostragem e dados moleculares

Foram amostrados 219 indivíduos de *Scinax squalirostris* em 26 localidades (Tabela 1), ao longo de sua distribuição geográfica no Brasil, Uruguai e Paraguai (Figura 1; Tabela 1). A extração do DNA foi realizada a partir de amostras de fígado ou tecido muscular preservados em etanol absoluto, utilizando o Kit Dneasy Blood & Tissue (Qiagen®, InR Chatsworth, CA). O DNA foi amplificado por reação em cadeia de polimerase (PCR) e sequenciado em um sequenciador automático de DNA ABI 3500 (Applied Biosystems® CA) usando o kit BigDye® Terminator Cycle Sequencing. Foi utilizado dois fragmentos mitocondriais (mtDNA), 12S e citocromo B (Cytb), e um nuclear (nDNA) gene de ativação de recombinação (RAG1) (para detalhes sobre primers, amplificações e condições de sequenciamento, consulte o Apêndice S1: Tabelas S1.1-S1.3 no material suplementar). As sequências consenso foram montadas para cada indivíduo usando o software Seqscape 2.7 (Applied Biosystems) e o alinhamento múltiplo foi feito pelo

software ClustalW (Thompson et al., 1997) implementado no software Bioedit 7.2.5 (Hall, 1999). Foi verificado a existência de saturação na segunda e terceira posição do códon, pelas transições e transversões com a distância TN93 (Tamura & Nei, 1993) usando o software Dambe (Xia, 2013). Como as posições do terceiro códon de CytB mostraram altos níveis de saturação (ver Apêndice S2: Figura S2.1 em material suplementar), elas foram excluídas do alinhamento final. As sequências de RAG1 mostraram indivíduos heterozigotos (138 indivíduos, 47 caracteres), portanto, utilizamos o haplótipo mais frequente, estimado pelo Phased implementado no DNAsp 5.0 (Librado & Rozas, 2009).

**2.2 Dados morfométricos**

Medidas morfométricas de 365 indivíduos, sendo 269 machos, de oito museus/coleções (Apêndice S1: Tabela S1.4; Apêndice S3 para detalhes de números dos tombos e localidades), foram obtidas para integrar em uma análise discriminante baseado nos clados gerados a partir da análise de delimitação (veja resultados). A identificação do sexo foi baseada na presença do saco vocal em machos. Os indivíduos representaram populações de toda a distribuição geográfica de *Scinax squalirostris* (Figura 1). As medidas foram baseadas em Heyer et al. (1990) e Watters et al. (2016), aferindo 15 caracteres morfométricos: comprimento rostro-cloacal (SVL), desde a ponta do focinho até a abertura cloacal; comprimento da cabeça (HL), distância perpendicular desde o tímpano até a ponta da boca; largura da cabeça (HW), entre na porção média dos tímpanos; diâmetro do olho (ED), entre a borda anterior e posterior do olho; diâmetro do tímpano (TD), entre a borda anterior e posterior do tímpano; distância interorbital (IOD), entre a base das pálpebras; distância internarinas (IND), entre as bordas das narinas; distância da narina à focinho (NSD), desde a borda da narina até a ponta do focinho;

distância olho-narina (E-N), desde a borda anterior à olho até a narina; comprimento do antebraço (FaL), desde a articulação braço-antebraço até a borda posterior do tubérculo palmar; comprimento da mão (HaL), desde a borda posterior do tubérculo palmar até a ponta do dedo III; comprimento da coxa (THL), desde a abertura cloacal até a articulação fêmur-tíbia; comprimento da tíbia (TiL), desde a articulação fêmur-tíbia até a articulação tíbia-tarso; comprimento do tarso (TaL), desde a articulação fêmur-tíbia até a articulação tarso-metatarso; comprimento do pé (FoL), desde a borda posterior do tubérculo metatarsal até a ponta do artelho IV. Todas as medidas foram realizadas utilizando um paquímetro digital (Mitutoyo) de precisão 0,01 mm.

**2.3 Análises moleculares**

Para a análise de delimitação molecular, foi utilizado os dados genéticos combinados (mtDNA + nDNA), totalizando 1002pb. Foi utilizado duas espécies como grupo externo *Sphaenorhynchus lacteus* (12S: EF217472.1; RAG1: AY844527.1 e Cytb: AY549420.1) e *Pseudis paradoxa* (RAG1: AY844506.1), baseado em Duellman et al. (2016) e na disponibilidade dos dados no GenBank. O melhor modelo de substituição para cada partição (12S, Cytb e RAG) foi selecionado com base no Critério de Informação de Akaike corrigido (AICc) implementado no software jModelTest 2 (Darriba *et al*., 2012) (Apêndice S1: Tabela S1.5). Para a datação molecular, foi usado taxas de mutação previamente estimadas para fragmentos mitocondriais e nuclear para a datação molecular de anfíbios anuros (Apêndice S1: Tabela S1.6) devido à falta de fósseis de Scinaxinae.

Para levar em conta o arranjo incompleto de linhagens, a análise de árvores de espécies foi realizada usando o modelo coalescente de multiespécies implementado no Beast 2.4.8 configurado no template *Beast (*StarBeast*) (Bouckaert et al., 2014). Para

selecionar as possíveis linhagens, alocamos grupos de espécies de acordo com a localidade, sem a utilização de uma árvore guia previamente gerada, e utilizamos o template *StarBeastwithStacey*, que considera que cada haplótipo ou população pode ser uma linhagem independente. Foi selecionado o relógio molecular lognormal não correlacionado e o modelo de processo de especiação de Yule como *prior* para a árvore. Duas corridas independentes foram realizadas, cada uma com 150 milhões de gerações, amostrando a cada 1.500 gerações. A convergência e estacionariedade foram avaliadas (Tamanho efetivo amostral - ESS ≥ 200) usando o software Tracer 1.6 (Rambaut et al., 2014). As corridas e árvores foram combinadas após a remoção de 20% do *burn-in* com software LogCombiner 2.4.8 (Bouckaert *et al*., 2014) e a árvore de *Maximum Clade Credibility* (MCC) foi obtida com o software TreeAnnotator 2.4.8 (Bouckaert *et al*., 2014). A árvore foi visualizada e editada no software Figtree 1.4.3 (Rambaut, 2012). A análise foi realizada na plataforma Cipres 3.3 - *Cyberinfrastructure for Phylogenetic Research* (Miller et al., 2010).

**2.4 Análises morfométricas**

Considerando que existe variação no tamanho corporal por dimorfismo sexual, nas análises só foram incluídos os machos (Hayek et al., 2001), pois apresentavam uma amostra mais representativa das populações em relação às fêmeas. Assim, variáveis morfométricas, foram integradas em uma análise discriminante (AD) com a função *lda* do pacote MASS (Venables & Ripley 2002). O AD é um método estatístico multivariado usado para discriminar e classificar grupos (variável categórica), o qual se baseia na combinação das variáveis que mais contribuem na separação desses grupos. O AD foi combinado com uma seleção de variáveis passo-a-passo segundo o critério de Lambda de Wilk's (função *greedy.wilks*, no pacote klaR: Weihs et al., 2005), com nível de 0.05 para

a decisão do teste de F. Esta seleção passo-a-passo baseia-se no desvio das variáveis dentro de cada grupo em relação ao desvio total sem distinção dos grupos. A decisão de entrada da variável é feita de acordo com o Lambda de Wilk's, assim, a primeira variável a entrar no modelo será aquela que proporciona a melhor diferenciação dos grupos. Além disso, foram feitas análises específicas para as três primeiras variáveis que mais contribuíram na discriminação dos grupos. Para essas variáveis foram feitas análises de variância (ANOVA) ou teste de Kruskal-Wallis, e posteriormente análises *post-hoc* para determinar de onde estava vindo essa variação entre os grupos. Todos os testes estatísticos foram feitos no programa R (R Core Team, 2016).

## 3. RESULTADOS

### 3.1 Análises moleculares

As análises do *Beast alcançaram convergência aparente, com ESS de pelo menos 300 para todos os parâmetros, mostrando convergência entre as corridas. Mas ainda assim, baixos valores de probabilidade a posteriori foram estimados para os clados formados (Figura 2). Contudo, não podemos desconsiderar a relação e a possível conformação dessas linhagens. Assim, para a realização das análises morfométricas, consideramos clados suportados acima de 60% de probabilidade a posteriori, adotando uma medida mais conservadora. A árvore de espécie estimada sugere a existência de três possíveis linhagens candidatas a espécie (Figura 2). Os iniciais eventos de divisão foram antigos, ~6 milhões de anos atrás (Ma) para o clado 1 em relação aos clados 2 e 3. Os outros eventos de cladogenese foram mais recentes, datando de ~ 4.0 Ma, entre os clados 2 e 3. O clado 1 inclui as populações do Paraguai e de Poços de Caldas em Minas Gerais (PMG). O clado 2 inclui as populações do Centro-Oeste brasileiro (BDF e CGO). Enquanto, o clado 3 agrupa o restante de populações presentes no Sudeste e Sul do Brasil (Figura 2).

### 3.2 Análises morfométricas

Indivíduos das populações medidas foram distribuídas nos três agrupamentos gerados na análise de delimitação (ver subtópico 3.1). Como o número de populações medidas foi maior que nas amostras genéticas, populações não amostradas pelos dados genéticos foram alocadas nesses agrupamentos por similaridade morfológica e distância geográfica. Um resumo das medidas morfométricas para cada agrupamento está disponível na Tabela 2. As populações do clado 2 em média foram menores do que as outras populações (Tabela 2).

O AD incluiu em seus dois primeiros eixos o 99.97% da variação, sendo que o primeiro compilou o 96.8% (Tabela 3). Segundo o AD, só foi possível observar dois agrupamentos dos clados obtidos pelo método de delimitação de espécies (Figura 3). O clado 3, inclui dentro de sua variação o clado 1, incorporando indivíduos de populações do sul e sudeste do Brasil, junto com as populações de Paraguai, Uruguai e Argentina. Enquanto o clado 2 foi mais consistente, incluindo indivíduos somente do Centro-Oeste do Brasil (Figura 3). As variáveis mais importantes nessa separação foram a distância interorbital (IOD), comprimento da mão (HaL) e largura da cabeça (HW) (Tabela 4), as quais variaram entre os três clados (IOD: chi-quadrado=137.38, $p<0.001$; HaL: $F=106.6$, $p<0.001$; HW: $F=104.2$, $p<0.001$). Essa variação foi dada principalmente pelo clado 2, em relação aos outros dois clados (Tabela 5), sendo as populações desse clado em média, menores que os outros dois (Figura 4).

## 4. DISCUSSÃO

Nossos resultados indicaram a formação três possíveis linhagens candidatas à espécie, porém com baixo suporte. De acordo os dados morfométricos, apenas dois clados podem

ser suportados a partir dessa evidência externa. Uma formação seria composta por populações da região Centro-Oeste e outra composta por populações das regiões Sul/ Sudeste do Brasil, Paraguai e Uruguai.

### 4.1 Delimitação Molecular

A utilização do critério coalescente de múltiplas espécies pode gerar uma delimitação precisa de espécies (Maddison & Knowles, 2007), juntamente com dados independentes, como os morfométricos, morfológicos, acústicos, etc (Sólis-Lemus & Knowles, 2015). No caso de *Scinax squalirostris*, com a utilização dos dados moleculares e morfométricos foi possível categorizar duas linhagens como candidatas a espécie. Como o modelo coalescente de multiespécies considera um arranjo incompleto de linhagens, se espera incongruências genéticas na árvore de espécies (Sukumaran & Knowles, 2017), pois o evento de divisão de linhagens não corresponde um evento de especiação instantânea, mas sim uma especiação primária, que representa o isolamento inicial das populações (Sukumaran & Knowles, 2017). Isso ocorre, porque é necessária uma quantidade substancial de tempo após a divergência inicial para que haja uma monofiletismo recíproco observado na árvore de espécies (Knowles & Carstens, 2007; Mehta et al., 2016). Na árvore de espécies, o clado 2 foi monofilético, bem suportado e corroborado pelas diagnoses morfométrica. A cladogenese desse clado foi ~4.0Ma, que mostrou-se suficiente, indicando a ausência de fluxo gênico atual e de retenção de polimorfismo ancestral. Essas linhagens podem estar isoladas a mais tempo que os outros clados (clado 1 e clado 3), e de fato, a ocorrência dessas populações estão restritas a região do Centro-Oeste do Brasil, sendo geograficamente distantes das outras populações, o que facilitaria a divergência dessa linhagem em relação às outras. Em um estudo feito por Faria e colaboradores (2013) com avaliação canto de anúncio de *S. squalirostris* entraram

divergência de canto em populações da região Centro-Oeste do Brasil em relação as outras regiões, indicando a existência de uma linhagem críptica e reforçando nossos resultados de uma linhagem candidata a espécie. Essa linhagem se torna uma candidata a espécie, e ainda, sem um nome disponível para a sua associação, podendo ser descrito (ver abaixo em 4.3 Nomenclatura).

A árvore estimada neste trabalho foi baseada em uma abordagem não restritiva, ou seja, não foram estabelecidos e nem testados possíveis *constrains* de linhagens como espécies para testes de hipóteses de relações e nem utilizamos uma árvore guia a *priori*. Com essa abordagem obtivemos a formação dos clados 1 e 3, porém com baixo suporte e que não foram separados pela análise morfométrica. Como a delimitação de espécie consiste nessa etapa inicial de verificação de possíveis linhagens evolutivamente distintas sem a atribuição à *priori* e depois o teste de hipóteses baseado nas linhagens já atribuídas (Ence & Carstens, 2011), se faz necessário testar diferentes hipóteses de relação e conformação dos clados 1 e 3. Assim, ainda devemos testar modelos de espécies hipotéticas e analisar sob diferentes modelos de especiação (*Yule process* ou *Birth-Death model*) com os modelos coalescentes, levando em conta a estocasticidade genética. Com isso, a abordagem de limite e validação de espécies será mais robusta (Carstens et al., 2013).

## 4.2 Análise Morfométrica

As análises morfométricas permitiram confirmar duas das linhagens obtidas pelas análises moleculares dentro do nome *S. squalirostris*. O clado 1 indicou variação morfométrica similar ao clado 3, sendo assim, difícil assimilar esses dois agrupamentos como linhagens evolutivas diferentes. Porém, isso não descarta a possibilidade dos clados

1 e 3 serem espécies candidatas diferentes. Dentro de anfíbios anuros, existem diferentes casos de espécies com diferenciação genética, mas sem clara evidência morfológica/morfométrica, e conformam unidades taxonômicas consistentes a partir de outras evidências (ver o caso de *Dendropsophus luddekei* e *D. molitor* [Guarnizo et al., 2012] ou das espécies de *Melanophryniscus* [Rojas-Zamora et al., 2018]). No entanto, no caso *S. squalirostris* para os clados 1 e 3, considerando o baixo suporte das análises de delimitação, seguimos uma abordagem conservadora na ausência de outras evidências disponíveis. O clado 2, que incluiu nas análises morfométricas populações do centro-oeste brasileiro de Goiás e de São Paulo, pode ser diagnosticado morfometricamente do clado 1+ clado 3 em tamanho, sendo a distância interorbital (IOD), a largura da cabeça (HW) e a longitude da mão (HaL) menores. Essa diferenciação pode vir acompanhada por mudança no padrão morfológico dos indivíduos (Bookstein, 1991; Kaliontzopoulou, 2011). Como por exemplo, o formato da cabeça (Figura 5), é subovóide (*subovoid*) para os clados 1 e 3, e pontuda (*pointed*) para o clado 2 segundo os formatos de Heyer et al. (1990) (Figura 5). No entanto, a morfometria necessita de análises morfológicas mais específicas, como variação do padrão de coloração e membranas interdigitais dos pés, acompanhados de outras linhas de evidências comportamentais e ecológicas, que permitam suportar mais robustamente as espécies candidatas.

### 4.3 Nomenclatura

Considerando a provável nomenclatura dos dois clados suportados pelas análises morfométricas, temos quatro nomes associados para *S. squalirostris*. (1) O sinônimo senior da espécie, *Hyla leucotaenia* descrita pelo Burmeister (1861) de lagos ao longo do rio Paraná, na Argentina, sendo um nome recuperado e designado como um nome esquecido (*nomem oblitum*) associado com *S. squalirostris* por Pinheiro et al. (2014). (2)

O nome *Hyla squalirostris* descrito pelo Lutz (1925) de Serra da Bocaina, São Paulo, Brazil. (3) O nome *Hyla lindneri* descrito pelo Müller & Hellmich (1936) em Formosa, Argentina. E (4) o nome *Hyla evelynae* descrito pelo Schmidt (1944) em San Carlos, Uruguai. Todos os nomes encontram-se limitados para o sudeste do Brasil, Uruguai e Argentina, o que incluiria as populações geograficamente limitadas nos clados 1 e 3, assumindo assim, o nome válido para a espécie, que seria *Scinax squalirostris*. Enquanto que, as populações do clado 2, não possuem nenhum nome descrito na sua área geográfica, portanto, ficariam sem nome disponível. Assim, o clado 2 poderia ser nomeado e descrito formalmente para as populações do Centro-Oeste brasileiro.

Em conclusão nossos resultados indicam a existência de duas possíveis linhagens candidata a espécie: (i) as populações de Centro-Oeste brasileiro e (ii) as populações presentes no Sudeste e Sul do Brasil, Uruguai, Paraguai e Argentina. Mesmo que com baixo suporte e levando em conta a estocasticidade genética, não podemos descartar a formação desses grupos acima mencionados, que foram corroborados pelos dados morfométricos. Além disso, nossos resultados mostram que ainda é necessário a utilização de métodos e teste de hipóteses de relação de espécies, bem como a utilização de mais dados como fonte externa para a corroboração da delimitação molecular, tais como a inclusão de dados morfológicos e acústicos.

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Agradecemos a todas as pessoas e suas instituições que gentilmente coletaram, doaram espécimes, enviaram amostras de tecidos e amostras de empréstimos.

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## MATERIAL SUPLEMENTAR

**Apêndice S1** Tabelas Suplementares (Tabelas S1.1 - S1.6) contendo informações sobre primers, amplificação, sequenciamento, locais das amostras cedidas para as medidas morfométricas, modelos evolutivos e taxas de mutação.

**Apêndice S2** Figura suplementar (Figuras S3.1) gráfico de saturação com as taxas de transição e transversão.

**Apêndice S3** Detalhes de número tombo e localidades das espécimes utilizadas na análise morfométrica.

**Declaração de conflito de interesse:** Os autores declaram não ter conflito de interesses.

**Tabela 1.** Locais de amostragem das 26 populações de *Scinax squalirostris*. Nome das localidades, abreviação de estados brasileiros e departamento paraguaio. Coluna de coleção nós apresentamos a abreviação de instituições doadoras. (Coleção de Anfíbios Célio F. B. Haddad (CFBH), Coleção da Universidade Federal de Minas Gerais (UFMG), Coleção Zoológica da Universidade Federal de Goiás (ZUFG), Coleção Elaine Maria Lucas Gonsales (EMLG), Museu de Ciências e Tecnologia da PUCRS (MCT), Coleção da Universidade Federal do Rio Grande do Sul (UFRGS), Coleção de Herpetologia do Instituto de Investigação Biológica do Paraguai (IIBPH).

| Localidade | Código da População | Número de indivíduos | Coleção | Latitude | Longitude |
|---|---|---|---|---|---|
| Itirapina -São Paulo | ISP | 5 | CFBH | -22.2744 | -47.796 |
| Bocaina - São Paulo | BSP | 12 | CFBH | -23.3458 | -44.483 |
| São Roque -Minas Gerais | SRMG | 11 | CFBH | -20.1461 | -46.663 |
| Ouro Preto - Minas Gerais | OMG | 16 | CFBH | -20.2875 | -43.508 |
| Catas Altas - Minas Gerais | CMG | 3 | UFMG | -20.0747 | -43.408 |
| Poços de Caldas - Minas Gerais | PMG | 1 | UFMG | -21.7878 | -46.561 |
| Serra do Cabral - Minas Gerais | SMG | 16 | LOD | -17.6992 | -44.271 |
| Sempre Vivas - Minas Gerais | SVMG | 22 | ZUFG | -17.7883 | -43.798 |
| Serra do Cipó - Minas Gerais | SCMG | 13 | ZUFG | -19.2469 | -43.510 |
| Chapada dos Veadeiros - Goiás | CGO | 24 | ZUFG | -14.0833 | -47.467 |
| Brasília- Goiás | BDF | 19 | ZUFG | -15.7797 | -47.930 |
| Bonito- Mato Grosso do Sul | BMS | 7 | ZUFG | -21.1211 | -56.482 |
| Itapiranga - Santa Catarina | ISC | 26 | CFBH | -27.1833 | -53.733 |
| São José do Norte - Rio Grande do Sul | SJRS | 10 | UFRGS | -32.0147 | -52.042 |
| São Francisco de Paula - Rio Grande do Sul | SFRS | 2 | MCP | -29.4481 | -50.584 |
| Bom Jesus - Rio Grande do Sul | BRS | 1 | CFBH | -28.6678 | -50.417 |
| Palmas- Paraná | PPR | 2 | ZUFG | -26.4842 | -51.991 |
| Ponta Grossa - Paraná | PGPR | 8 | ZUFG | -25.095 | -50.162 |
| Campo Belo -Santa Catarina | CBSC | 1 | MCP | -27.8992 | -50.761 |
| Campos Novos - Santa Catarina | CNSC | 2 | CFBH | -27.4017 | -51.225 |
| Bom Jardim -Santa Catarina | BSC | 5 | CFBH | -28.3369 | -49.625 |
| Caçador- Santa Catarina | CSC | 3 | EMLG | -26.7753 | -51.015 |
| Água Doce - Santa Catarina | ASC | 1 | EMLG | -26.9978 | -51.556 |
| Estancia San José -Ñeembucú - Paraguai | EPY | 1 | IIBPH | -27.2016 | -58.449 |
| Alto Vera - Paraguai | APY | 7 | IIBPH | -26.5833 | -55.633 |
| Uruguai | URU | 1 | - | -33.0000 | -56.000 |

**Tabela 2**. Resumo das medidas morfométricas de machos de *Scinax squalirostris*. Para cada variável é mostrado a média com o desvio padrão, e entre parênteses o range com o valor máximo e mínimo. Siglas das medidas: comprimento focinho-cloaca (**SVL**), comprimento da cabeça (**HL**), largura da cabeça (**HW**), diâmetro do olho (**ED**), diâmetro do tímpano (**TD**), distância interorbital (**IOD**), distância internarinas (**IND**), distância da narina à focinho (**NSD**), distância olho-narina (**E-N**), comprimento do antebraço (**FaL**), comprimento da mão (**HaL**), comprimento da coxa (**THL**), comprimento da tíbia (**TiL**), comprimento do tarso (**TaL**), comprimento do pé (**FoL**).

| **Variável** | **Clado 1 (n=12)** | **Clado 2 (n=76)** | **Clado 3 (n=181)** |
|---|---|---|---|
| SVL | 22.9 ± 1.01 (21.62–24.86) | 20.74 ± 1.13 (17.84–23.48) | 23.24 ± 1.51 (18.99–26.54) |
| HL | 8.47 ± 0.39 (7.92–9.3) | 7.47 ± 0.77 (5.51–9.11) | 8.3 ± 0.64 (6.36–10.18) |
| HW | 5.65 ± 0.4 (5.1–6.18) | 5.11 ± 0.39 (4.21–5.97) | 5.99 ± 0.49 (4.9–7.42) |
| ED | 2.19 ± 0.21 (1.87–2.52) | 2.08 ± 0.28 (1.58–2.7) | 2.34 ± 0.27 (1.54–3.1) |
| TD | 1.24 ± 0.08 (1.12–1.34) | 1.13 ± 0.25 (0.75–2.39) | 1.25 ± 0.18 (0.72–1.67) |
| IOD | 2.47 ± 0.26 (2.08–2.98) | 1.95 ± 0.17 (1.55–2.42) | 2.47 ± 0.28 (1.95–3.59) |
| IND | 1.81 ± 0.18 (1.47–2.05) | 1.54 ± 0.2 (1.11–2.08) | 1.76 ± 0.22 (1–2.69) |
| E-N | 2.81 ± 0.2 (2.43–3.07) | 2.66 ± 0.35 (1.97–3.5) | 2.95 ± 0.36 (2.04–3.83) |
| NSD | 1.4 ± 0.21 (1.05–1.72) | 1.44 ± 0.23 (0.93–1.89) | 1.5 ± 0.2 (0.96–1.98) |
| THL | 10.12 ± 0.66 (9.28–11.07) | 9.44 ± 0.7 (6.92–10.81) | 10.41 ± 0.82 (8.28–12.79) |
| TiL | 12.19 ± 0.68 (11.06–13.21) | 11.23 ± 0.67 (9.76–12.56) | 12.33 ± 0.93 (9.99–15.06) |
| TaL | 6.2 ± 0.47 (5.49–7.18) | 6.12 ± 0.58 (4.75–7.33) | 6.61 ± 0.7 (4.76–8.48) |
| FoL | 9.73 ± 0.91 (8.08–11.07) | 9.27 ± 0.8 (7.46–11.63) | 10.21 ± 1.03 (7.39–13.33) |
| FaL | 3.42 ± 0.3 (2.96–3.87) | 3.29 ± 0.3 (2.64–4.12) | 3.68 ± 0.39 (2.64–5.03) |
| HaL | 5.45 ± 0.36 (5.02–6.23) | 4.51 ± 0.42 (3.09–5.2) | 5.45 ± 0.5 (3.66–6.62) |

**Tabela 3**. Coeficientes de variação da análise discriminante (AD) para os indivíduos machos das populações de *Scinax squalirostris*. Siglas: distância interorbital (**IOD**), comprimento da mão (**HaL**), largura da cabeça (**HW**), distância da narina à focinho (**NSD**), distância internarinas (**IND**), diâmetro do olho (**ED**), comprimento do antebraço (**FaL**), comprimento do pé (**FoL**).

| **Variáveis** | **LD1** | **LD2** |
|---|---|---|
| IOD | -1.9677 | -0.298 |
| HaL | -1.156 | -1.4178 |
| HW | -0.9416 | 0.92573 |
| NSD | 1.65699 | 0.74628 |
| IND | -0.6072 | -2.5382 |
| ED | -0.6141 | 1.68076 |
| FaL | -0.5215 | 1.23947 |
| FoL | 0.22177 | 0.34809 |
| Proporção dos eixos | 0.9683 | 0.0317 |

**Tabela 4**. Variáveis que mais contribuem na discriminação dos agrupamentos segundo o critério do Lambda de Wilk´s com 0.05 para decisão para a estatística F. A estatística F da diferença compara o modelo incluindo a nova variável com o modelo que não a incluem. Siglas: distância interorbital (**IOD**), comprimento da mão (**HaL**), largura da cabeça (**HW**), distância da narina à focinho (**NSD**), distância internarinas (**IND**), diâmetro do olho (**ED**), comprimento do antebraço (**FaL**), comprimento do pé (**FoL**).

| | **Variáveis** | **Lambda de Wilk's** | **Estatística F** | **p** | **F da diferença** | **p da diferença** |
|---|---|---|---|---|---|---|
| 1 | IOD | 0.5441142 | 111.43398 | <0.0001 | 111.433981 | <0.0001 |
| 2 | HaL | 0.4138502 | 73.46541 | <0.0001 | 41.705864 | <0.0001 |
| 3 | HW | 0.3688121 | 56.90391 | <0.0001 | 16.119403 | <0.0001 |
| 4 | NSD | 0.349366 | 45.48858 | <0.0001 | 7.319427 | 0.0008 |
| 5 | IND | 0.3397214 | 37.5021 | <0.0001 | 3.71907 | 0.0255 |
| 6 | ED | 0.331049 | 32.10371 | <0.0001 | 3.418644 | 0.0342 |
| 7 | FAL | 0.3228558 | 28.22602 | <0.0001 | 3.29906 | 0.0385 |
| 8 | FoL | 0.3153119 | 25.28035 | <0.0001 | 3.098312 | 0.0468 |

**Tabela 5**. Valores de significância das comparações múltiplas das médias das três variáveis que mais contribuíram na diferenciação dos clados ($p > 0.05$). Siglas: distância interorbital (**IOD**), comprimento da mão (**HaL**), largura da cabeça (**HW**).

| **Comparação** | **IOD** | **HaL** | **HW** |
|---|---|---|---|
| Clado 1 vs Clado 2 | <0.0001 | <0.0001 | <0.0001 |
| Clado 1 vs Clado 3 | 0.9928 | 0.9999 | 0.0501 |
| Clado 2 vs Clado 3 | <0.0001 | <0.0001 | <0.0001 |

**Lista de Figuras**

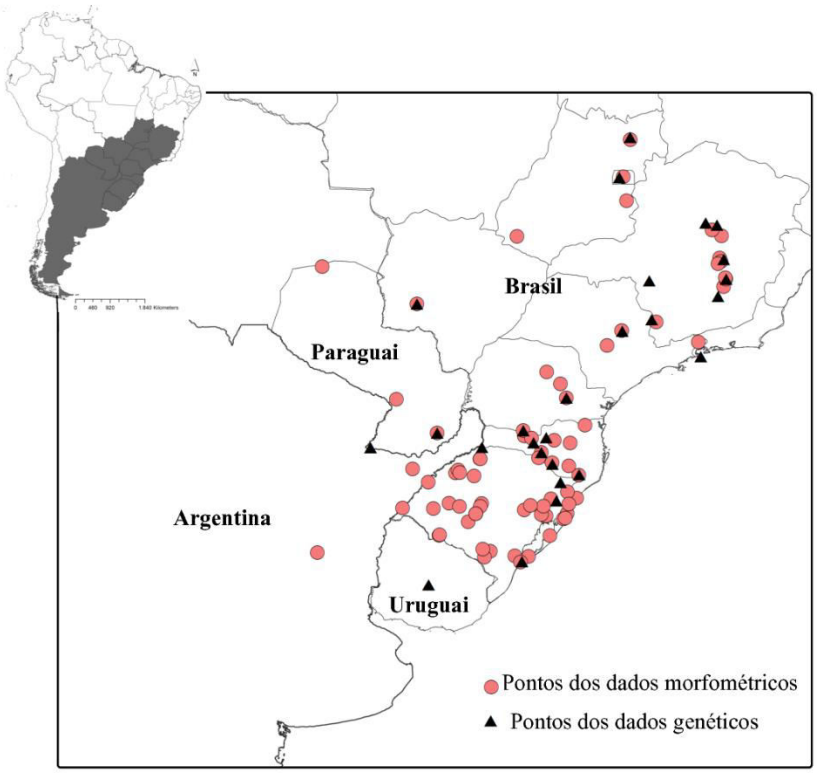
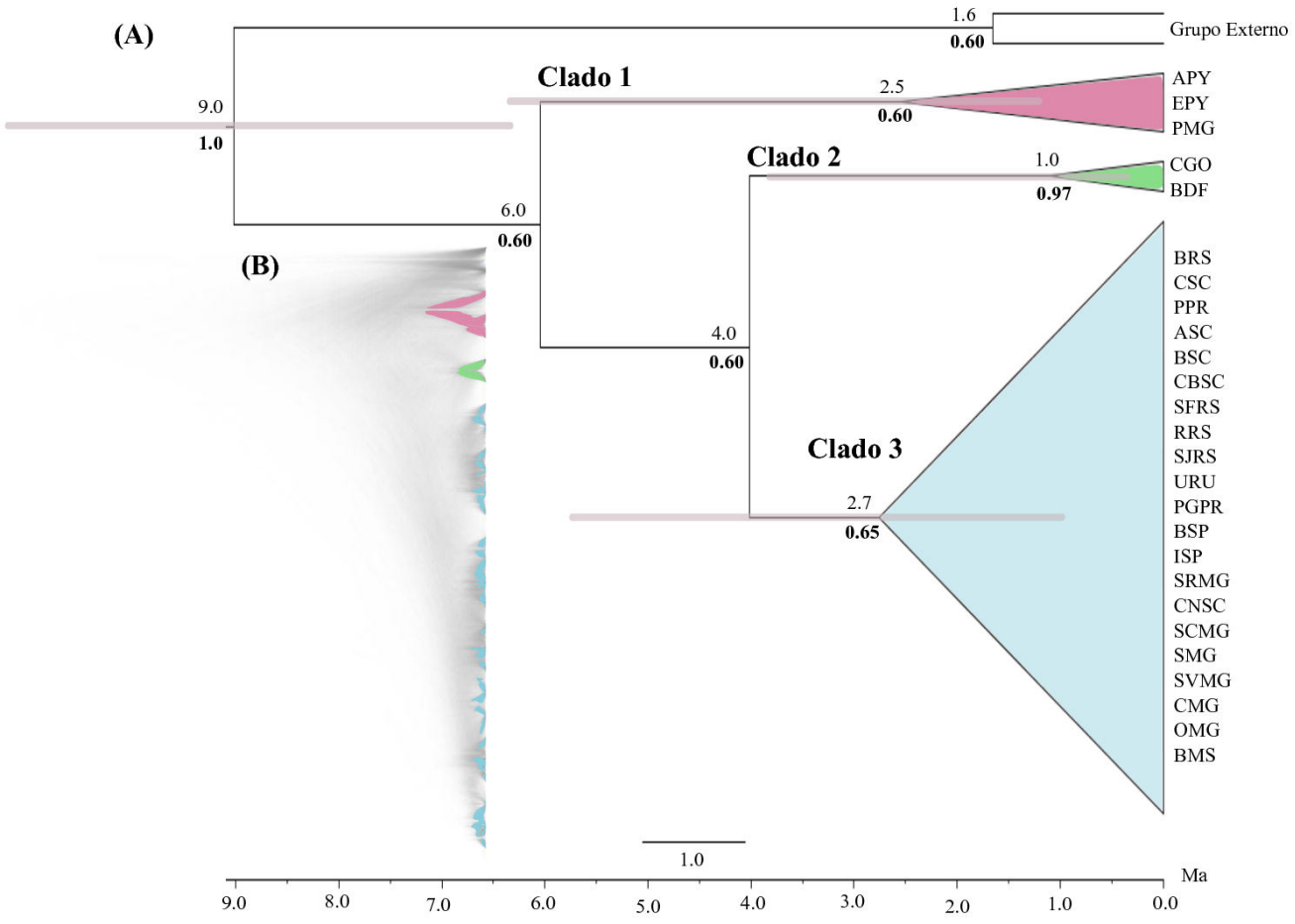

Brasil
Paraguai
Argentina
Uruguai
Pontos dos dados morfométricos
Pontos dos dados genéticos

**Figura 1**


(A)
(B)
1.6
0.60
Grupo Externo
Clado 1
2.5
0.60
APY
EPY
PMG
9.0
1.0
Clado 2
1.0
0.97
CGO
BDF
6.0
0.60
4.0
0.60
Clado 3
2.7
0.65
BRS
CSC
PPR
ASC
BSC
CBSC
SFRS
RRS
SJRS
URU
PGPR
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ISP
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SCMG
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**Figura 2**

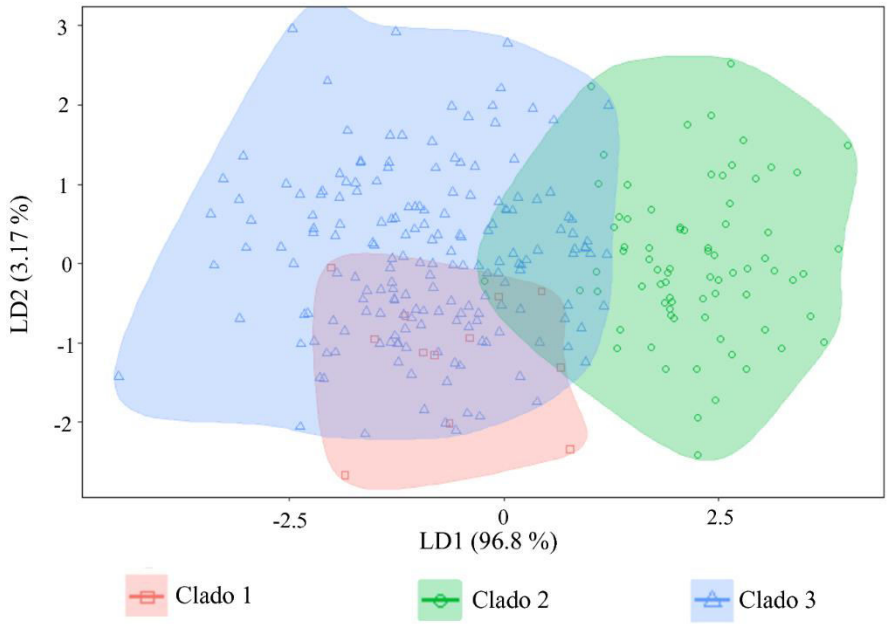


**Figura 3**

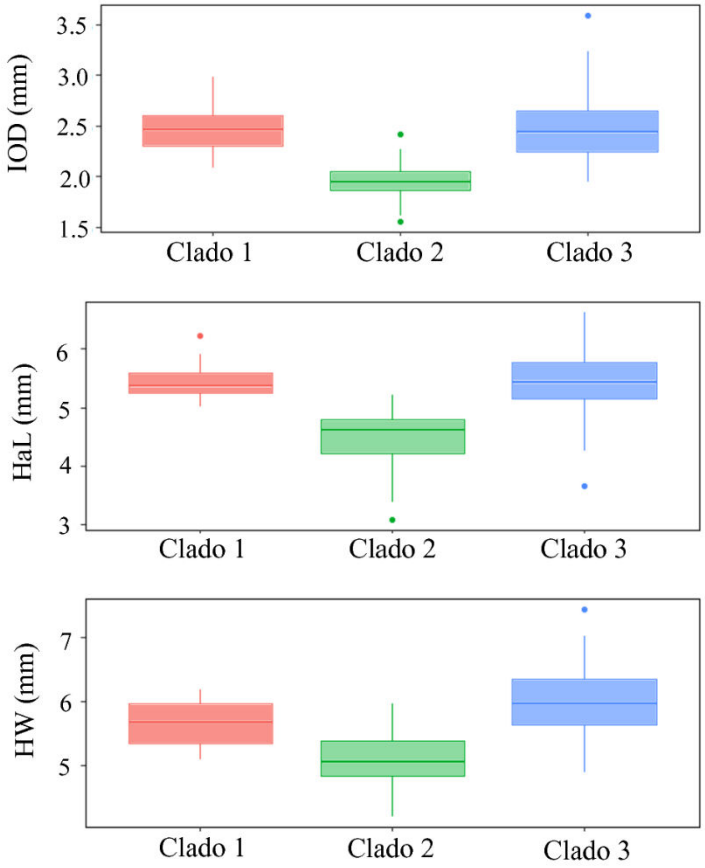


**Figura 4**

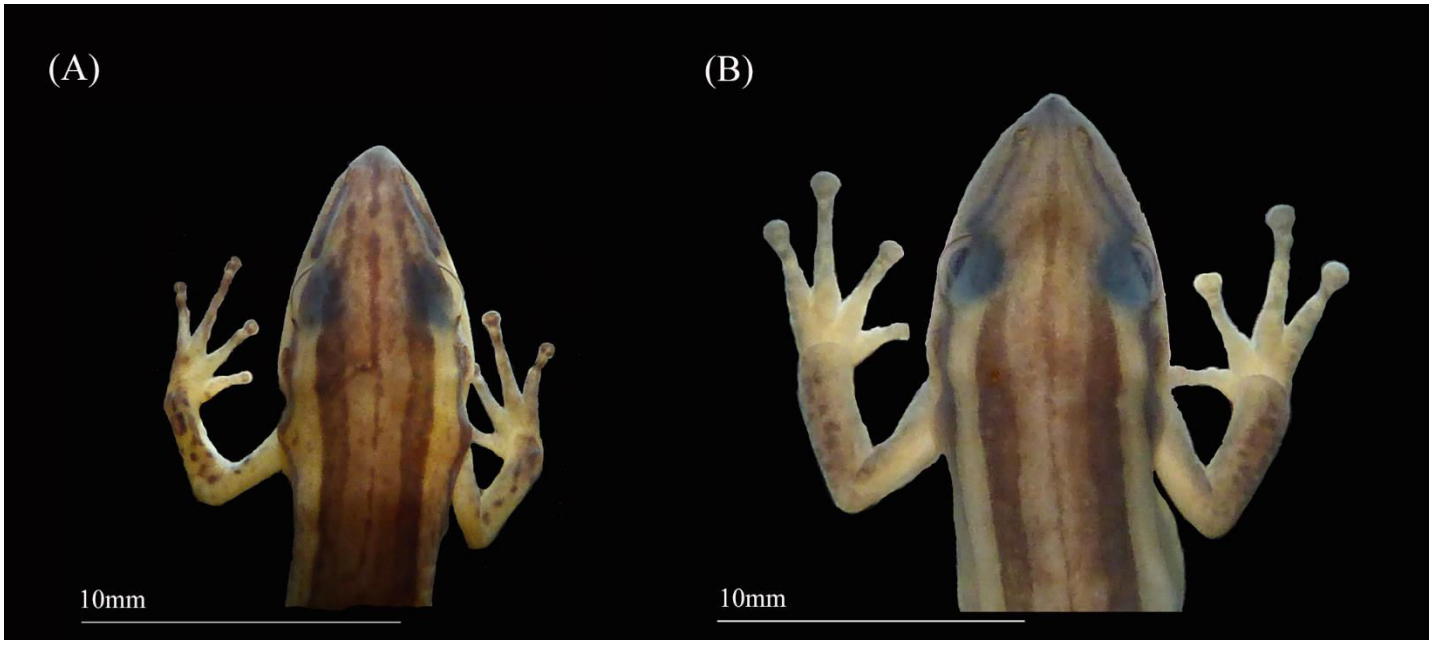


**Figura 5**

**Material Suplementar**

**Revelando a diversidade do complexo de espécies de *Scinax squalirostris* (Lutz, 1925) (Anura, Hylidae).**

Tatianne P. F. Abreu-Jardim, Alejandro Valencia-Zuleta, Natan M. Maciel e Rosane G. Collevatti.

**Apêndice S1** Suplementar de Tabelas S1: Tabelas S1.1-S1.6 contendo informações sobre *primers*, amplificação, sequenciamento, locais das amostras cedidas para as medidas morfométricas, modelos evolutivos e taxas de mutação.

**Tabela S1.1** Pares de pimers usados PCR e sequenciamento: CytB, 12S e RAG-1

| Região | Primer | Direção | Sequência Nucleotídica (5'-3') |
|---|---|---|---|
| CytB | MVZ15 | *forward* | GAACTAATGGCCCACACWWTACGNAA |
| | H1415 (cyt-b2) (H15149) | *reverse* | AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA |
| 12S | 12S | *forward* | AAACTGGGATTAGATACCCCACTAT |
| | 12S | *reverse* | GAGGGTGACGGGCGGTGTGT |
| RAG | RAG1 | *forward* | ATGCATCRAAAATTCARCAAT |
| | RAG1 | *reverse* | CCYCCTTTRTTGATAKGGWCATA |

**Tabela S1.2** Componentes de quantidades dos reagentes para o mix de PCR para cada um dos fragmentos utilizados nesse estudo.

| **Reagentes** | **Cytb, 12S e RAG-1** |
|---|---|
| Deionized water | 8.5 µL |
| DNA | 2.0 µL |
| Forward primer (2 mM) | 3.0 µL |
| Reverse primer (2mM) | 3.0 µL |
| Buffer 1X* | 2.0 µL |
| DNTPs (2,5 mM) | 1,2 µL |
| Taq polymerase (5u/µL) | 0.3 µL |
| Total volume | 20 µL |

*Buffer 1X (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2)

**Tabela S1.3** Programa do termo de PCR para cada um dos fragmentos utilizados nesse estudo.

| **Região** | **Aquecimento Inicial** | **Desnaturação** | **Anelamento** | **Extensão** | **Extensão final** |
|---|---|---|---|---|---|
| | | **35 cycles** | | | |
| CytB | 2 min at 94°C | 60 sec at 94°C | 60 sec at 56°C | 90 sec at 72°C | 6 min at 72°C |
| 12S | 2 min at 94°C | 60 sec at 94°C | 60 sec at 54°C | 90 sec at 72°C | 6 min at 72°C |
| RAG | 2 min at 94°C | 60 sec at 94°C | 60 sec at 58°C | 90 sec at 72°C | 6 min at 72°C |

**Tabela S1.4** Museus/Coleções onde foi revisado o material morfométricos de *Scinax squalirostris*.

| Museu/Coleção | Sigla |
|---|---|
| Coleção Celio F. B. Haddad, Departamento de Zoologia, Universidade Estadual Paulista, Campus de Rio Claro, São Paulo, Brasil | CFBH |
| Coleção herpetológica, Universidade de Brasília, Brasília, D.F., Brasil. | CHUNB |
| Colección Biológica Arnoldo da Winkelried Bertoni, Instituto de Investigación Biológica del Paraguay, Asunción, Paraguai. | IIBP-H |
| Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil | MCP |
| Museu Nacional do Rio de Janeiro, Universidade Federal de Rio de Janeiro, Rio de Janeiro, Brasil. | MNRJ |
| Coleção do setor de herpetologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil | UFRGS |
| Coleção de herpetologia, Universidade Federal de Goiás, Goiânia, Goiás, Brasil. | ZUFG |
| Coleção herpetológica, setor de Zoologia, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brasil. | ZUFSM |

**Tabela S1.5** Modelos evolutivos e parâmetros utilizado para *S. squalirostris*.

| **Dados** | **Cytb** | **12S** | **RAG-1** |
|---|---|---|---|
| **Model0** | K80+I | K80+G | JC |
| **p-inv** | 0.8790 | - | - |
| **Kappa** | 17.7938 | 5.4465 | |
| **gamma shape** | - | 0.1200 | - |
| **Nst** | 2 | 2 | 1 |
| **Ncat** | - | 4 | - |

**Tabela S1.6** Taxa de mutação para cada região utilizado no estudo para gerar a árvore de espécie.

| | Taxa de mutação (substituição/milhão de anos) Média - Desvio | Referência |
|---|---|---|
| 12S | 0.0026 - 0.0001 | Evans *et al*., 2004 |
| CytB | 0.0069 - 0.0010 | Macey *et al*., 1998 |
| RAG | 0.0015 - 0.0001 | Heinicke *et al*., 2007 |


Evans, B. J., Kelley, D. B., Tinsley, R. C., Melnick, D. J. & Cannatella, D. C. (2004) A mitochondrial DNA phylogeny of African clawed frogs: phylogeography and implications for polyploid evolution. *Molecular Phylogenetics and Evolution,* 33, 197-213.

Macey, J. R., Schulte, J. A., Larson, A., Fang, Z. L., Wang, Y. Z., Tuniyev, B.S. & Papenfuss, T. J. (1998) Phylogenetic relationships of toads in the *Bufo bufo* species group from the eastern escarpment of the Tibetan Plateau: a case of vicariance and dispersal. *Molecular Phylogenetics and Evolution,* 9, 80-87.

Heinicke, M. P., Duellman, W. E. & Hedges, S. B. (2007) Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. *Proceedings of the National Academy of Sciences,* 104, 10092–10097.

**Material Suplementar**

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**Apêndice S2** Suplementar de Figura S2 gráfico de saturação com as taxas de transição e transversão.

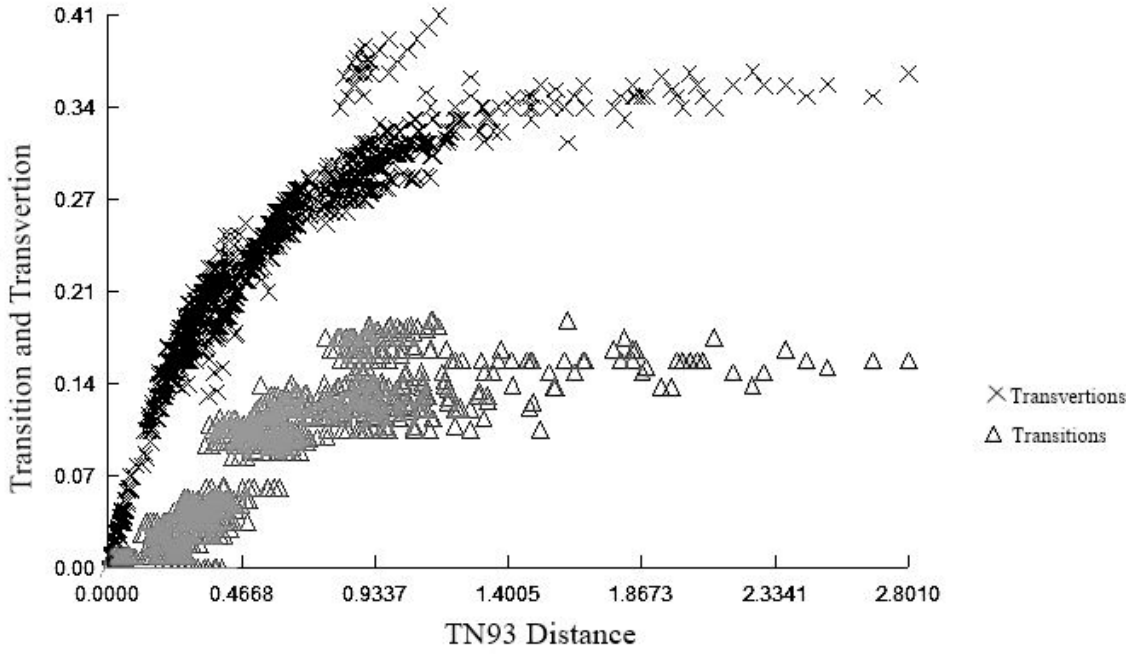


**Figure S3.1** Gráfico de saturação de transição e transversão para as posições dos terceiros códons das sequências do Citocromo b (Cytb).

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**Apêndice S3** Detalhes de número tombo e localidades das espécimes utilizadas na análise morfométrica.

Clado 1: **Brasil**: MINAS Gerais: Poços de Caldas: MNRJ 35579-81, 87812-16. **Paraguai**: Distrito Alto Verá: IIBP-H 1215-17, 2773-75; Emboscada: IIBP-H 1953-1955; Estancia San José: IIBP-H 267.

Clado 2: **Brasil**: DISTRITO FEDERAL: Brasília: CHUNB 16894-95, 16897, 16899-02, 16904-06, 25058, 42501-03, 50308-10; ZUFG 4754-59, 5476-88, 5651. GOIÁS: Alto Paraíso de Goiás: CHUNB 58392, 58395, 58397, 58768-73, 72679-80, ZUFG 5414-29, 6266, 6268; Cristalina: ZUFG 2717-24, 4949-53. SÃO PAULO: Botucatu: MNRJ 67337-52, 69864-65; Itirapina: CFBH 5709, 5666-69, 5708, 6551, 7067, 7069-70, 7072-74, 7076-77, 12847.

Clado 3: **Argentina**: CORRIENTES: Galarza: MNRJ 39989. SANTA FE: Villa Constitución: MCP 6505. **Brasil**: MINAS GERAIS: Alto Palácio: ZUFG 4740-41, 4767-69, 4771-79; Catas Altas: MNRJ 60634-42, 60655-59, 77745-65; Diamantina ZUFG 6309, 6350, 6353, 6357, 6364-81, 7625; Ibitipoca: MNRJ 59363-69; Jaboticatubas: MNRJ,45341,45344; Ouro Preto: CFBH 24377, 34626-29; Santa Barbara: MNRJ 72979-80; Santana do Riacho: CFBH 35053; Serra do Cipó: ZUFG 4785-87. MATO GROSSO DO SUL: Bonito: CHUNB 44058-59, 49342-44, 49347-50, 49352. PARANÁ: Guaíra: MNRJ 85613; Palmas: ZUFG 5660-61; Ponta Grossa: ZUFG 5652-59; Tibagi: ZUFG 7316, 7513. RIO GRANDE DO SUL: Alegrete: UFRGS 4244, ZUFSM 8509; Bagé: UFRGS 5715; Balneario Pinhal: MCP 3267, 3919, 4226-27; Braga: ZUFSM 4436;

Caibaté: UFRGS 4908; Cambara do Sul: CHUNB 69058-61, 69201, MCP 11408; Campos Novos: MCP 9463; Candiota: MCP 4059; Canela: UFRGS 2379; Canguçu: UFRGS 5393-94; Cerro Largo: ZUFSM 3333; Cidreira: MCP 11744; Coronel Barros: UFRGS 4915; General Câmara: MCP 3852-54; Itaára: ZUFSM 4149; Manuel Viana: UFRGS 4453, ZUFSM 2430-31; Mato Queimado: UFRGS 4878-79; Mostardas: MCP 12639-40; Muitos Capois: MCP 11265; Nonoia: UFRGS 4074-75; Novo Hamburgo: MCP 9125; Pedras Altas: UFRGS 4834; Pinheiro Machado: UFRGS 5210, 5795, 6794; Porto Alegre: MCP 3290; Rio Grande: MCP 3214, 10803; Rolador: ZUFSM 3965, 4039; Santa Maria: ZUFSM 3119, 3737, 5157; Santana do Livramento: MCP 12138; São Borja: MCP 5771-73, ZUFSM 5009; São Francisco de Assis: UFRGS 5772, 5776, ZUFSM 3048; São Francisco de Paula: MCP 2473, 3430, 3482, 3655, 11037; São Gabriel : ZUFSM 9769; São João: ZUFSM 4097; São Joaquim: MCP 10364; São José do Norte: UFRGS 5271; São Lourenço do Sul: MCP 11290-92; São Luiza Conzaga: ZUFSM 4052; São Vicente: ZUFSM 2414; Santa Margarida do Sul: UFRGS 2534; Terra de Areia: MCP 5224-25; Torres: MCP 8688, ZUFSM 9992; Tramandaí: UFRGS 2129, 2131; Uruguaiana: UFRGS 1803-05; Viamão: UFRGS 1371. SÃO PAULO: São José do Barreiro: CFBH 21981, 28778-79, 28781, 30884, 30888-90, 35247. SANTA CATARINA: Água Doce: ZUFSM 8920; Bom Jardim da Serra: CFBH 9858, 11009, 11024, 11026; Campo Belo do Sul: MCP 10600-01, UFRGS 4044; Campos Novos: CFBH 14082; Celso Ramos: MCP 9006-07; Içara: UFRGS 6338, 6340; Lebon Regis: MCP 9663-64; Painel: ZUFSM 2346; Passos Maia: CFBH 25775, UFRGS 5931; São Bento do Sul: CFBH 39252-53. **Uruguai**: Rivera: CHUNB 48026, ZUFG 13340-42.

# Capítulo 3

Original Article

# Predicting impacts of global climatic change on distribution and genetic diversity of the treefrog *Scinax squalirostris* (Lutz, 1925) (Anura, Hylidae)

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## Abstract

Future climate changes caused by the increasing emission of greenhouse gases can affect negatively species distribution and their genetic diversity, hampering species adaption to the new climate or range shifting tracking the environment changes. Amphibians have

high sensitivity to environmental degradation and changes in temperature and humidity. Thus, the expected climatic changes to the end-of-century (EOC - 2100) may affect their ability to adapt to local conditions and may cause the local extinction of some species. In this work, we used Ecological Niche Modelling (ENM) and genetic simulations to predict the effects of climate change on the geographical distribution, genetic diversity and structure of *Scinax squalirostris*, a South American treefrog, using two scenarios of different $CO_2$ emission from the RCP database v 2.0.5 (Representative Concentration Pathways). We also performed a spatial analysis to verify how Protection Areas (PAs) are protecting the genetic diversity of the species. We found a reduction in the potential distribution of *S. squalirostris* under a future climate 8.5 RCP scenario. Genetic diversity and the number of haplotypes will decrease in both future scenarios, leading to a homogenization of genetic diversity across the geographical range of *S. squalirostris*. Populations may have difficulty adapting to new climatic conditions due to the displacement of genetic ancestry clusters from their ideal conditions. Although PAs conserve the current genetic diversity of *S. squalirostris*, only PAs in Southern Brazil and West Argentina will maintain high genetic diversity in the future, since most PAs will be in areas that will no longer be suitable for *S. squalirostris*.

**Keywords:** Bayesian clustering, conservation, ENMs, genetic clusters, global warming, South America.

**Short running head:** Climatic changing and genetic diversity in *Scinax squalirostris*.

## 1. INTRODUCTION

Genetic diversity represents the evolutionary potential of the species, in which natural selection acts to promote the evolution of organisms and their adaptation to environmental changes (Urban *et al*., 2012, 2013). High levels of genetic diversity may favour a rapid evolutionary response, but populations with low genetic diversity have their evolutionary potential reduced and making them prone to local extinction (Spielman, *et al*., 2004; Frankham, 2005). Anthropogenic impacts such as habitat fragmentation, invasive species, hunting pressures and climatic changes, have been associated with current extinctions (Purvis *et al*., 2000; Fahrig, 2003; Loyola *et al*., 2014; Bellard *et al*., 2016). For instance, climatic dynamics (e.g. increase in temperature and variation in precipitation) may increase the effect of genetic stochasticity (e.g. loss of genetic diversity and inbreeding) in small populations and, therefore, fixation of poorly adapted haplotypes, causing extinction (Urban *et al*., 2013; Pauls *et al*., 2013). Furthermore, climatic changes force species to track suitable areas (Chen *et al*., 2011), but some species cannot follow their optimal climatic niche because they have little dispersal capacity or cannot disperse through natural or anthropic barriers (Urban *et al*., 2012). Species with low dispersal capacity have high extinction rates and population declines (Duan *et al*., 2016).

Climatic changes can modify aspects of the biological system from the level of genes to ecosystems, thus, identifying populations that will be more vulnerable to these changes is a concern of biology and genetic conservation. Perhaps, one of the most worrisome groups are amphibians, with about 50% of all populations declining or threatened of extinction (Stuart *et al*., 2004; Gibbons *et al*., 2000; Houlahan *et al*., 2000; Alroy, 2015; Duan *et al*., 2016), amongst the most vulnerable vertebrates, declining even faster than birds and mammals (Stuart *et al*., 2004). Because amphibians are highly reliable on environmental conditions (Duellman & Trueb, 1994), climatic change will

cause the extinction of approximately 40% of the species, with greater impact in the endemic groups (Gibbons *et al*., 2000; Thomas *et al*., 2004), making them the most threatened group of animals (Stuart *et al*., 2004, Mendelson *et al*., 2006).

Amphibians have low vagility, sensitivity to environmental degradation and changes in environmental temperature and humidity, mainly due to their permeable skin and life cycle, which includes stages in water and land (Duellman & Trueb, 1994, Stuart *et al*., 2004; Storfer *et al*., 2009). Changes in temperature and precipitation can lead to changes in geographic distribution and abundance (Chen *et al*., 2011) of amphibian populations. Furthermore, climate changes could affect the hydro period available for amphibian populations, i.e. the time period that a temporary pond retains water for mating and metamorphosis (Rowe & Dunson, 1995; Carey & Alexander, 2003).

The global climate is changing drastically due to the emission of greenhouse gases, cause by anthropogenic action (Solomon, 2007; IPCC, 2014; 2018), since the last century there has been an increase of 0.6ºC (Jones *et* al., 2001) and climate models predict an increase of 2ºC - 4ºC in world temperature by the middle of the century (New *et al*., 2011; Fischer *et al*., 2018). Ecological niche modelling (ENM) is widely used to predict changes in the geographical distribution of species under climate change (e.g. Thomas *et al*., 2004; Lima *et al*., 2014; Collevatti *et al*., 2015; Lima *et al*., 2017). This framework uses current distribution data of species to model their fundamental environmental niches and, then, forecast potential distributions under various climatic scenarios (Peterson *et al*., 2011), enabling the understanding of spatial dynamics and the impact of climatic changes on species in the future (Zhang *et al*., 2012; Simon *et al*., 2013; Mendoza-González *et al*., 2013; Vasconcelos *et al*., 2018). Future distributions are forecasted projecting the current suitable climatic conditions of species onto a map of future climatic scenarios (Midgley *et al*., 2002; Lima *et al*., 2017), assuming that species conserve their

current environmental niche and that they will be able to track future suitable climatic conditions (Elith & Leathwick, 2009). Coupling ENM to simulations of genetic parameters within and among populations can improve our understanding of climate change effects on genetic diversity and genetic clusters (Lima *et al*., 2017). So, our knowledge about how climate change will affect genetic diversity will help predictions concerning how amphibian populations will cope with rapid future changes (Corn, 2005), especially guiding appropriate conservation policy scenarios (Duan *et al*., 2016), protecting the future viability and genetic diversity of species and their ecosystems (Mendelson *et al*., 2006; Schwartz *et al*., 2007).

As the decline of amphibians is already a conservation concern, because of their great risk of extinction, it becomes urgent to understand how climate change will affect amphibian genetic diversities. In this work, we aimed to understand if and how the effects of future climate change on the distribution of the South American treefrog *Scinax squalirostris* Lutz, 1925 as a model group. This treefrog is widely distributed throughout the southeast and south of South America, occurring from the Central-West to the northern region of Argentina (Frost, 2018). We performed ecological niche modelling (ENM) to forecast the future geographical distribution based on two different scenarios of greenhouse gas emissions, 4.5 and 8.5 RCP. In order to understand the effects of climate change on genetic diversity, we performed genetic simulations conditioned to climatic and topographic variables, restricted to dispersal and establishment of anuran amphibians. In addition, since conservation of genetic diversity is a worldwide concern (Convention on Biological Diversity-Target 13 – CBD, Strategic Goal, https://www.cbd.int/sp/targets/), we performed a spatial analysis of the current genetic diversity and analyzed how much of this diversity is covered by Protection Areas (PAs) and predict PAs that will maintain genetic diversity even with future climate changes.

## 2. MATERIAL AND METHODS

### 2.1 Population sampling and genetic diversity

We sampled 26 localities across the distribution of *S. squalirostris* (Brazil, Uruguay and Paraguay) and collected 228 individuals with a sampling effort ranging between 1 and 10 individuals per locality (Table 1; Figure 1). Genetic data was obtained of tissue (muscle and liver) take from specimens and preserved in ethanol 95%. DNA samples was obtained using Dneasy Blood & Tissue Kit (Quiagen®, InR. Chatsworth, CA). We sequenced two fragments of the mitochondrial DNA (mtDNA henceforward), 12S (353 bp, primers 12Sa-12Sb; Reeder, 1995), Cytochrome B (236 bp, primers MVZ15L, Moritz *et al*., 1992, and H15149, Kocher *et al*., 1989) and the nuclear (nDNA henceforward) RAG-1 gene (413 bp, Heinicke *et al*., 2007). Fragments were amplified by polymerase chains reaction (details for the primers, PCR conditions and amplifications are summarized in Supporting Information, see Appendix S1: Tables S1.2-S1.4) and sequenced on an ABI GS3500 Genetic Analyser (Applied Biosystems®, CA) using the BigDye® Terminator Cycle Sequencing kit, according to the manufacturer's instructions. We sequenced all fragments in forward and reverse directions and consensus were obtained using the software SEQSCAPE 2.7 (Applied Biosystems, CA). Further, we used CLUSTALW (Thompson *et al*., 1997) to obtain the multiple sequence alignments. Coding sequences were tested for saturation by plotting transitions and transversions with TN93 distance (Tamura & Nei, 1993) using DAMBE software (Xia, 2013). We excluded from the final alignment the third codon position of Cytb, which showed high levels of saturation (Appendix S2: Figure S2.1 in Supporting Information).

We estimated the overall and population nucleotide ($\pi$) and haplotype ($h$) diversities, using the concatenated fragments (mtDNA and nDNA = 1002 bp) in

ARLEQUIN 3.11 (Excoffier *et al*., 2005). We also estimated the number of population and overall haplotypes (k) in DNAsp 5.0 (Librado & Rozas, 2009).

### 2.2 Ecological niche modelling: Future predictions

To model the present and future potential geographical distributions of *S. squalirostris* in the Neotropics, we obtained occurrence records in literature and in open-access digital databases, such as VerNet (http://portal.vertnet.org), GBIF (the Global Biodiversity Information Facility-http://www.gbif.org/) and SpeciesLink project (http://splink.cria.org.br/). All records were examined for errors and duplicate occurrences were removed, thus, we obtained 257 unique occurrence records for *S. squalirostris* (Figure 1A; Table S1.5 in Appendix S1). We mapped the occurrence records in a grid cells of 0.5 x 0.5° (longitude x latitude) of spatial resolution, encompassing the Neotropics to generate the matrix of presences used to calibrate the ENMs. The environmental climatic conditions were characterized by climatic models for pre-industrial (representing current climate conditions) and two future scenarios of different $CO_2$ emission from the RCP database v 2.0.5 (Representative Concentration Pathways, http://tntcat.iiasa.ac.at/RcpDb). The scenario 4.5 RCP (rising radioactive forcing pathway leading to 4.5 W/m$^2$ in 2100), predicts a temperature increase of 1.8ºC and stabilization before the EOC (end-of-century, 2100) due to the decrease in emissions of greenhouse gas. The scenario 8.5 RCP (rising radioactive forcing pathway leading to 8.5 W/m$^2$ in 2100), predicts a temperature increase of 3.7°C until the year 2100 and a continuing rising, due to a constant increase in representative concentration pathways (IPCC, 2013). These climatic conditions are derived from four Atmosphere-Ocean General Circulation Models (AOGCMs) (see Appendix S1: Table S1.6), with spatial resolution of 0.5º (ca. 55 km cell size). To overcome the multicollinearity problem among all bioclimatic variables

in the ENMs predictions, we performed a factorial analysis using *Varimax rotation* from 19 bioclimatic variables obtained in the EcoClimate database (www.ecoclimate.org) which are available at 0.5° x 0.5° of resolution (Lima-Ribeiro *et al*., 2015). We obtained five bioclimatic variables: annual mean temperature, mean diurnal range, isothermality-mean diurnal range/temperature annual range, precipitation of wettest three months and precipitation of driest three months. The current and future potential distributions of the treefrog were inferred using presence-only and presence–absence algorithms (Appendix S1: Table S1.7), represented by 12 algorithms. Some ENM algorithms based on presence-absence need absence data for model fitting and testing, however, there are not reliable real absence data available for this species. Therefore, we generated pseudo-absences randomly across the Neotropical area excluding cells with presence, maintaining prevalence equal to 0.5 to maximize model performance (Barbet-Massin *et al*, 2012). To assess the model stability, we used a random data partitioning 75% training (calibration) and 25% testing (evaluation), replicated 50 repetitions for each model, due to the absence of independent testing data. This procedure and ENMs were run in the integrated computational platform BIOENSEMBLES (Diniz-Filho *et al*., 2009). Model accuracy was assessed using the calculation of the area under the receiver operator characteristics (ROC) curve AUC (Allouche *et al*., 2006) and with the metric true skill statistic (TSS), which take into account both omission and commission errors (Allouche *et* al., 2006). We used an ensemble approach (Araújo & New, 2007) to calculate a consensus map that included only models with AUC > 0.75 and TSS>0.5 (Appendix S1: Table S1.8), and the models with poor performance were eliminated from the ensemble process. The frequency of occurrence in each cell provided a surrogate of the environmental suitability and was used for *Scinax squalirostris* across the Neotropical grid cells.

The combination of all ENMs and AOGCMs resulted in 48 independent predictive maps (12 ENMs x 4 AOGCMs) for each period of time (pre-industrial, 4.5 and 8.5 RCPs). We applied a hierarchical ANOVA using the predicted suitability from all models (12 ENMs x 4 AOGCMs x 3 times periods) as response variables to identify and map the uncertainties due to modelling components. For this, the ENMs and AOGCMs components were nested into the time component, but crossed by a two-way factorial design within each period of time (see Terrible *et al*., 2012). The 48 predictive maps were combined to obtain the consensus map for each period of time. To quantify the range sizes and range shift between the three time periods, we first tested 0.5 and 0.75 binary thresholds and selected 0.5 because it better recovered the current distribution area of *S. squalirostris*. Then, we used this threshold to transform occurrence frequency maps into presence and absence maps, and then we calculated range size by summing the number of cells predicted as presence for each of the 48 predictive maps.

**2.3 Simulations of genetic diversity under climatic changes**

To understand how climate change can affect genetic diversity, we simulated the extinction of some populations according to the occurrence thresholds estimated for two future scenarios (4.5 and 8.5 RCP). Ecological niche modelling predicts climatically suitable areas for species occurrence given predicted climate changes (see results below). Based on this, we assume that only populations occurring in climatically suitable areas in 2100 will persist and contribute to the gene pool for the next generations (see Collevatti *et al*., 2011). Then, we simulated the extinction of populations below suitability thresholds ranging from 0.1 to 0.9 (Table 1) and re-calculated genetic diversity ($\pi$, $h$ and k) for the remaining populations under two future scenarios (4.5 and 8.5 RCP), using the software ARLEQUIN 3.11 (Excoffier *et al*., 2005).

### 2.4 Forecasting and dynamic genetic clusters in future scenarios

We performed spatially explicit simulations to predict the dynamics of climatic clusters conditioned to climatic changes using the software POPs (Jay *et al*., 2015). In this analysis, Bayesian grouping is based on genetic, geographic and environmental variables, assigning individuals to genetic clusters, based on genetic coancestry. POPs models the effects of climatic and landscape variables in such cluster assignments and predicts changes in the genetic structure in response to climatic changes (Jay *et al*., 2012). For the simulations, we selected two environmental variables for current and future scenarios: minimum temperature of the coldest month and precipitation of the coldest quarter, which were the two climatic predictors with the highest variance among populations. In addition, we selected topographic variables that may affect the dispersal and occurrence of anurans, such as slope and altitude (Table 1). We performed simulations for mitochondrial and nuclear haplotypes separated and excluded rare haplotypes (Appendix S1: Table S1.9). In order to define the rare haplotypes, we made an abundance rank curve and set a sample selection of 85% of the relative frequency, that is, haplotypes present in less than 15% of the individuals were excluded.

We simulated genetic clusters under current climatic conditions combining genetic, climatic and topographic variables. The analysis was performed using Markov Chain Monte Carlo (MCMC) implemented in POPS (Jay *et al*., 2015). The MCMC runtime was set for 50,000 sweeps and the burn-in period for 5,000 sweeps using models with admixture. To define the number of clusters (K), we run the simulations four times for each K varying between 2 and 23 for both mitochondrial and nuclear data. The subset of runs minimizing the deviation information criterion (DIC, Spiegelhalter *et al*., 2002) and the lowest DIC values were selected. Finally, we predicted the genetic clusters for both future scenarios (4.5 and 8.5 RCP) conditioned on future climatic and topographic

conditions. We measured the changes in ancestry among contemporary and predicted ancestry coefficients (intraspecific turnover, Jay *et al*. 2012) for both 4.5 and 8.5 RCP future scenarios to understand the impact of climate change on spatial genetic structure. Topographic variables were extracted from the occurrence points of the *S. squalirostris* populations in ArcGIS® 10.2 (ESRI, 2014) using a Digital Elevation Model (DEM) raster from the ALOS – Advanced Land Observing Satellite database (https://www.eorc.jaxa.jp/ALOS/en/index.htm). Since we expect no changes to altitude and slope in a few years (up to 2100), we use the same values of the present to make the future simulations.

### 2.5 Accessing the genetic richness and conservation of genetic diversity

To obtain the genetic richness of *S. squalirostris*, we defined the genetic richness as the number of haplotypes in a population divided by the number of individuals sampled within population. Then, we performed a spatial interpolation of genetic richness over a raster of the current distribution area predicted by ENM with a resolution of 0.5º. We fitted a spherical variogram model and performed interpolation using R package *gstat* (Gräler *et al*., 2016).

We also verified the effectiveness of the current PAs across the geographical range of *S. squalirostris* to conserve genetic richness. We obtained CU data from Protect Planet website (www.protectedplanet.net) and maintained only PAs with area higher than 1% of the cell of the interpolated genetic richness raster. Then, we calculated the mean genetic richness covered by each PAs and performed PAs clustering using k-means method (Hartigan & Wong 1979), implemented in *kmeans* function of R package *stats* (R Core Team, 2016). The clusters were optimized by minimizing the sum of squares within

groups. The number of groups were optimized by a grid search ranging from 2 to 15 clusters.

## 3. RESULTS

### 3.1 Ecological niche modelling: Future predictions

*Scinax squalirostris* shows a preference to cooler and drier habitats (Figure 2). The ENMs predicted range shift and loss of suitability in the future for both climatic scenarios (Figure 1; Appendix S2: Figure S2.3A). The average number of cells for current geographical range is 1,286 and decreases to 1,050 cells under 4.5 RCP scenario and to 901 cells under 8.5 RCP (Appendix S2: Figure S2.3A). The ENMs also predicted a higher range shift between the current conditions and the 8.5 RCP scenario than for the 4.5 RCP (Appendix S2: Figure S2.3B) with a potential loss of climatic suitability mainly at the Eastern Brazil (Figure 1C, D). At 8.5 RCP scenario (Figure 1D) the potential geographical range is restricted to the Southern Brazil to Central Argentina and eastern Andean slopes (Figure 1D).

The analysis of uncertainty using hierarchical ANOVA showed high proportional variance from both ENMs algorithms and AOGCM (Appendix S1: Table S1.10). However, variation was higher on cells outside *S. squalirostris*' occurrence range (Figure S2.2 in Appendix S2). Variation through time was higher within *S. squalirostris* range, therefore, the ENMs were able to detect the effects of climatic changes on the distribution dynamics of *S. squalirostris* through time, despite the AOGCM variation.

### 3.2 Simulations of genetic diversity under climatic changes

Although number of haplotypes will be rapidly lost at both scenarios (Figure 3), haplotype diversity will decrease only under 8.5 RCP scenario (Figure 3). The number of haplotypes will decrease, with a steeper decrease for the 8.5 RCP scenario and there is not a drastic decrease for the 4.5 RCP scenario, since the suitability thresholds cover the areas with the most haplotypes (Figure 3 and 4). Nucleotide diversity is shown to be constant over the course of climate change, being little affected by range retraction, for both 4.5 and 8.5 RCP scenarios (Figure 3).

### 3.3 Forecasting and dynamics in genetic clusters in future scenarios v

The most likely number of mitochondrial genetic clusters conditioned to environmental and topographic variables (precipitation, temperature, altitude and slope) was K = 8 (DIC = 980.274) (Appendix S1: Tables S1.11-S1.13) and for nuclear genetic clusters conditioned to environmental variables was K = 9 (DIC = 700.11) (Appendix S1: Tables S1.14-S1.16). The correlation between estimated and predicted admixture coefficients of geographic and environmental covariates was 0.99 for both mitochondrial and nuclear data, these values indicate that predictions of environmental variables were accurate.

Overall, spatial explicit simulations showed loss of variation over time due to environmental climate changes (Figure 5). Some climatic clusters were lost for mitochondrial (clusters 1, 2, 4, 5, 6 and 8) predictions for both climate change scenarios (4.5 and 8.5 RCP) (Figure 5A, B and C) leading to a genetic homogenization in the South Brazil, Paraguay and Uruguay and in the Central, West Brazil and Northwest South America (Figures 5B and 5C). Nuclear data showed a displacement in clusters (5D, E and F) and loss of clusters for all scenarios (clusters 7, 8 and 9) (Figure 5D, E and F). In

addition, the simulations evidenced a homogenization in the spatial conformation of the genetic variability in Central, Northeast and East Brazil (cluster 1) (Figure 5F).

Turnover analysis showed the impact of climatic changes in spatial genetic structure, but the clusters, in general, showed congruent responses (Figure 5G, H). The mitochondrial genetic clusters 3 and 7, remained constant from the current to 4.5 RCP and 8.5 RCP scenarios (Appendix S2: Figure S2.4A). The other clusters showed a change from the current to the 4.5 RCP scenario, but did not show great changes and remained practically constant, except clusters 1 and 5 (Appendix S2: Figure S2.4A). For nuclear sequences, clusters 3 and 5 had little or no shifts in ancestry. Most clusters shifted the ancestry from current scenario to 4.5 RCP and 8.5 RCP, with increase (clusters 1 and 6) or decrease (clusters 2, 4, 7, 8 and 9) (Appendix S2: Figure S2.4B).

### 3.4 Conservation of genetic diversity

Populations at the South and Southeast Brazil showed higher genetic richness (Figure 6A, B). Spatial interpolation (Figure 6C) showed that the genetic richness is lower in the northeaster geographical distribution of *S. squalirostris*. Higher genetic richness can be found mainly in the Southern region of the *S. squalirostris* distribution area.

The cluster analysis found 5 groups (Appendix S2: Figure S2.4) classified according to the amount of genetic richness and size of the PAs area (Figure 7A; Table S1.17 in Appendix S1). Four groups were composed by PAs smaller than 4000 km², but with different genetic richness (clusters 1, 2, 3 and 4, Figure 7A). Cluster 5 comprises PAs larger than 4000 km² with high genetic richness. However, PAs clusters had no spatial pattern (Figure 7B), with Protection Areas with high or low genetic richness spread throughout *S. squalirostris* geographical distribution.

## 4. DISCUSSION

Our findings showed that climatic changes can affect *S. squalirostris* populations, leading to a reduction in its geographical distribution and loss of genetic diversity. However, loss of genetic diversity will be greater and faster at 8.5 RCP scenario, although haplotypes will be rapidly lost at both scenarios 4.5 and 8.5 RCP.

ENMs showed a decrease in suitability in the Central-West and Southeastern region of *S. squalirostris,* leading to a shift in suitable areas towards the South Brazil and Central and West Argentina. The scenarios 4.5 RCP will cause 18% reduction of suitable areas for *S. squalirostris* and the scenario 8.5 RCP will reach 30% of loss in suitable areas. South American amphibians and reptiles also showed retraction and shift in geographical distribution due to future climatic changes, affecting both widely-distributed (Mesquita *et al*., 2010; Vasconcelos *et al*., 2014; 2016; 2018) and locally restricted species (Vilela *et al*., 2018). The co-generic widely-distributed species *Scinax fuscomarginatus* and *S. fuscovarius* showed reduction of 31 to 43% of suitable areas by 2050 (Vasconcelos *et al*., 2014). Thus, the scenario of reduction in suitable areas in the future due to climatic changes seem common among amphibians and reptiles, two taxa highly affected by environment conditions (e.g. Mesquita *et al*., 2010; Vasconcelos *et al*., 2014; López-Alcaide & Macip-Ríos, 2014).

The decrease in suitable areas will lead to a negative effect on the genetic diversity of *S. squalirostris*. Although nucleotide diversity will not reduce, haplotype diversity and the number of different haplotypes will decrease sharply for 8.5 RCP. Populations of *S. squalirostris* from the South Brazil have higher genetic diversity, thus, haplotype diversity at 4.5 RCP scenario will not decrease, because this area will be the most stable with the least reduction of climatic suitability. Thus, with the displacement of suitable areas towards the South and Southeast Brazil for the 4.5 RCP scenario, populations with

high genetic diversity will remain. On the other hand, many haplotypes will be potentially lost, and even if the remaining populations can maintain high diversity, this loss may hinder response to climate change and lead to local extinction risk (Wright *et al*., 2008; Foden *et al*., 2013). The low genetic diversity and richness in population from the Central-West and Southeast region of the distribution of *S. squalirostris* may be due to demographic history and habitat fragmentation. The loss of habitat in the past may have led to the loss of haplotypes in the Central-West populations, reducing the genetic diversity (Abreu-Jardim *et al*., 2018).

Due to the low genetic diversity of *S. squalirostris* populations in less suitable areas, the adaptation to new environmental conditions may be difficult (Hoffmann & Willi, 2008). In addition, there is no guarantee that those vulnerable *S. squalirostris* populations will track its adequate climatic conditions, due to the speed of climate changes (Chen *et al*., 2011; Arenas *et al*., 2012), the low dispersion capacity (Blaustein *et al*., 1994; Hillman *et al*., 2014) or by failing to disperse through natural or anthropogenic barriers (Urban *et al*., 2012). The ENMs predicted a change and spatial restriction on habitat suitability for *S. squalirostri*s from Southern Brazil to the West of Argentina. In these areas the anthropogenic disturbances as urbanization (Lopéz *et al*., 2015), agriculture (Moreira & Maltchik, 2015; Lopéz *et al*., 2015; Laufer & Gobel, 2017) and cattle grazing cropping (Medan *et al*., 2011; Lopéz *et al*., 2015) are strong and the tendency is to increase in the future. These factors can affect population persistence through time under the scenarios of climatic changes, despite their high genetic diversity.

Our simulations showed a loss of genetic variation among populations. *Scinax squalirostris* preferes cold and dry climates, so temperature increasing and changes in precipitation regimes predicted for the EOC will affect the geographical distribution and the spatial patterns of genetic diversity distribution. Temperature regimes are expected to

change dramatically according to 8.5 RCP scenario (Figure 2), particularly colder month temperatures will increase in some regions resulting in the loss of some genetic clusters and homogenization of climatic genetic clusters through the distribution range of *S. squalirostris*. Species require a diverse gene pool to rapid adaptation to environmental changes (Barrett & Schluter, 2008). The predicted loss of variation among populations and within populations will potentially threat *S. squalirostris* long-term conservation under climatic change.

During the evolution of the species the climatic niches may evolve (Peterson *et al*., 1999; Wiens & Graham, 2005; Wiens *et al*., 2010), but as climatic changes will happen rapidly and abruptly due to high intensity of anthropic disturbs, niche conservatism can disable vulnerable populations to respond to climatic changes, leading to local extinction (Wiens, 2004). Thus, climatic change will challenge the adaptation of vulnerable populations to new environmental conditions due to the displacement of genetic ancestry clusters from their ideal conditions.

Our results indicate that even small PAs can preserve high levels of genetic richness of *S. squalirostris*. However, climatic change will decrease suitability in areas with higher density of PAs through *S. squalirostris* geographical range, such as the Central-West and Southeast Brazil (Figure 7). In addition, the areas with higher density of PAs have lower levels of genetic richness. Thus, PAs at the Southern, the more climatic stable area through time and with higher genetic richness are more important to maintain the genetic diversity of *S. squalirostris* in the future. However, it is important note that the Southern has low density of PAs, which may potentially threat the species long-term conservation. The reduction of suitable areas and the decline of amphibian species within PAs due to climatic changes are of major concern in conservation planning (e.g. Loyola *et al*., 2008; Loyola *et al*., 2014; Vasconcelos *et al*., 2018). Amphibians from the Atlantic

Forest will be misrepresented by current PAs, because those PAs will be in climatically inadequate places to sustain species richness and diversity (Loyola *et al*., 2014). Further, global warming and the reduction of suitable areas for species coupled with the loss of genetic diversity may lead to the extinction of undescribed and cryptic species (Bálint *et al*., 2011). *Scinax squalirostris* has high genetic differentiation among populations and populations in Central-West Brazil are specially highly differentiated (Abreu-Jardim *et al*., 2018). This region is characterized by low levels of genetic diversity, with low suitability through the Quaternary. Our findings show that this region will also have low suitability in the EOC. Therefore, we have a pessimistic scenario, in which lineages in Central-West will probably extinct or be highly threaten. The problem of losing cryptic lineages is that it can affect ongoing diversification processes and thus affect future biodiversity (Bálint *et al*., 2011). Thus, under a more unpredictable climate and with very rapid changes, leading to a decrease and shift in the geographical range the genetic diversity becomes increasingly important for the survival of populations and species.

In conclusion, our results show that populations of *S. squalirostris* can be negatively affected by climatic changes, mainly by reducing and shifting suitable areas in EOC. The contraction of suitable areas can lead to local extinction and range retraction and, in turn, decrease the genetic diversity, becoming *S. squalirostris* less able to respond to the fast climatic changes. Thus, *S. squalirostris* will become more threatened in future, as distribution size is the main criteria to assign extinction risk category of species. Moreover, populations of the Central-West and Southeast Brazil have low genetic diversity, and may lose their suitable area due to climatic changes leading to a potential extinction of lineages in the EOC. These regions will lose climatic suitability in the areas with high density of PAs, making it difficult to preserve this species and its genetic diversity. Furthermore, climatic changes will result in loss and changes in genetic

clusters, leading to a homogenization of diversity, which will decrease evolutionary potential of *S. squalirostris* to deal with future climate changes.

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**BIOSKETCH**

The authors work with bridging macroecology and population genetics, with particular emphasis on phylogeography and geographical patterns of genetic diversity in Neotropical species.

**Author contributions**: RGC, NMM, and TPFAJ conceived the study; TPFAJ generated the genetic data, and performed the genetic analysis and niche modelling analyses. LBM and T PFAJ performed the forecasting and dynamics in genetic clusters in future scenarios. LBM, LJ and TPFAJ performed kriging and spatial genetic analysis. All authors contributed and approved the final version of the manuscript.

## SUPPORTING INFORMATION

Additional Supporting information may be found in the online version of this article:

**Appendix S1** Supplementary tables (Tables S1.1- S1.17) with details of sampling, PCR amplification, sampling and models used in ecological niche modelling, number of haplotypes and results of genetic ancestry clusters.

**Appendix S2** Supplementary figures (Figures S2.1-S2.4) with saturation result, maps of uncertainty, graph of range size and range shift and Sum of squares within clusters by number of clusters.

**Conflict of Interest Statement:** The authors declare no conflict of interest.

**Table 1** Genetic diversity and environmental variables of *Scinax squalirostris* populations. Population – Population code, Coordinates (longitude e latitude), N – sample size, *h* – haplotype diversity, $\pi$ - nucleotide diversity and k - number of haplotypes, Altitude, Slope, aspect, and suitability values for each time (Current, 4.5 RCP and 8.5 RCP future scenarios).

| Population | Coordinates | | N | Genetic Diversities | | | Altitude | Slope | Suitability | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Longitude | Latitude | | *h* | $\pi$ | k | | | Current | Future 4.5 RCP | Future 8.5 RCP |
| ISP | -47.7958 | -22.2744 | 5 | 0.70000 | 0.006163 ± 0.004322 | 3 | 764 | 1.159 | 0.861 | 0.333 | 0.091 |
| BSP | -44.4825 | -23.3458 | 13 | 0.98718 | 0.006163 ± 0.004322 | 12 | 256 | 0.000 | 0.772 | 0.334 | 0.061 |
| SRMG | -46.6628 | -20.1461 | 12 | 0.83333 | 0.001527 ± 0.001107 | 5 | 1136 | 2.565 | 0.914 | 0.398 | 0.083 |
| OMG | -43.5081 | -20.2875 | 16 | 0.68333 | 0.002304 ± 0.001490 | 5 | 1141 | 3.646 | 0.939 | 0.659 | 0.190 |
| CMG | -43.4075 | -20.0747 | 3 | 1.00000 | 0.001527 ± 0.001107 | 2 | 755 | 2.100 | 0.935 | 0.638 | 0.165 |
| PMG | -46.5614 | -21.7878 | 1 | 0.00000 | 0.000000 ± 0.000000 | 1 | 1314 | 4.174 | 0.950 | 0.632 | 0.180 |
| SMG | -44.2714 | -17.6992 | 16 | 0.93333 | 0.002016 ± 0.001486 | 11 | 1181 | 0.292 | 0.728 | 0.140 | 0.070 |
| SVMG | -43.7977 | -17.7883 | 22 | 0.86147 | 0.001408 ± 0.000999 | 11 | 1169 | 3.146 | 0.828 | 0.189 | 0.071 |
| SCMG | -43.5100 | -19.2469 | 13 | 0.79487 | 0.000256 ± 0.000341 | 5 | 1311 | 1.428 | 0.901 | 0.386 | 0.115 |
| CGO | -47.4667 | -14.0833 | 24 | 0.64855 | 0.001146 ± 0.000856 | 8 | 1131 | 5.362 | 0.223 | 0.064 | 0.050 |
| BDF | -47.9297 | -15.7797 | 19 | 0.70175 | 0.000187 ± 0.000279 | 5 | 1127 | 0.529 | 0.535 | 0.118 | 0.059 |
| BMS | -56.4819 | -21.1211 | 7 | 1.00000 | 0.007311 ± 0.004469 | 6 | 312 | 0.896 | 0.195 | 0.081 | 0.062 |
| RRS | -53.7333 | -27.1833 | 27 | 0.99715 | 0.003332 ± 0.001965 | 25 | 242 | 0.157 | 0.997 | 0.858 | 0.360 |
| SJRS | -52.0417 | -32.0147 | 10 | 1.00000 | 0.003216 ± 0.002048 | 10 | 2 | 0.048 | 0.890 | 0.753 | 0.609 |
| SFRS | -50.5836 | -29.4481 | 2 | 1.00000 | 0.038922 ± 0.039418 | 2 | 879 | 2.609 | 0.965 | 0.860 | 0.732 |
| BRS | -50.4167 | -28.6678 | 1 | 0.00000 | 0.000000 ± 0.000000 | 1 | 1046 | 1.197 | 0.922 | 0.826 | 0.745 |
| PPR | -51.9906 | -26.4842 | 2 | 1.00000 | 0.005988 ± 0.006468 | 2 | 1070 | 0.454 | 0.996 | 0.965 | 0.692 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PGPR | -50.1619 | -25.095 | 8 | 0.94290 | 0.004491 ± 0.002815 | 7 | 892 | 0.723 | 0.977 | 0.875 | 0.619 |
| CBSC | -50.7608 | -27.8992 | 1 | 0.00000 | 0.000000 ± 0.000000 | 1 | 970 | 0.424 | 0.949 | 0.895 | 0.759 |
| CNSC | -51.2250 | -27.4017 | 2 | 1.00000 | 0.006163 ± 0.006891 | 2 | 917 | 0.435 | 0.998 | 0.946 | 0.757 |
| BSC | -49.6247 | -28.3369 | 5 | 1.00000 | 0.008320 ± 0.005639 | 5 | 1359 | 0.464 | 0.901 | 0.810 | 0.644 |
| CSC | -51.0150 | -26.7753 | 3 | 1.00000 | 0.003082 ± 0.002905 | 3 | 934 | 0.896 | 0.997 | 0.949 | 0.748 |
| ASC | -51.5561 | -26.9978 | 2 | 1.00000 | 0.010786 ± 0.011531 | 2 | 899 | 1.763 | 0.999 | 0.953 | 0.720 |
| EPY | -58.4486 | -27.2016 | 1 | 0.00000 | 0.000000 ± 0.000000 | 1 | 57 | 0.045 | 0.964 | 0.336 | 0.236 |
| APY | -55.6333 | -26.5833 | 7 | 0.95238 | 0.002054 ± 0.001651 | 5 | 306 | 0.864 | 0.992 | 0.334 | 0.186 |
| URU | -56.0000 | -33.0000 | 1 | 0.00000 | 0.000000 ± 0.000000 | 1 | 132 | 0.249 | 0.986 | 0.991 | 0.946 |

**Figures Labels**

**Figure 1.** Current geographical distribution of *Scinax squalirostris* and environmental suitability modelled using ecological niche modelling. A) The 257 occurrence records (black dots) used in ecological niche modelling. B) Distribution of suitability for the current climate conditions. C) Distribution of suitability for the future climatic scenario of $CO_2$ emission for 4.5 RCP and D) Distribution of suitability for the future climate scenarios of $CO_2$ emission for 8.5 RCP. Black dots in B, C and D represent the 26 sampled populations from where genetic data were collected.

**Figure 2.** Ecological space of climatic conditions across the Neotropics during current time (light blue), 4.5 RCP future scenario (blue) and 8.5 RCP future scenario (purple). The climatic preferences from current occurrence records of *Scinax squalirostris* are represented by black dots. The bioclimatic variable values used here were obtained from AOGCM CCSM4.

**Figure 3.** Simulations of shifts in genetic diversity for sequence data under different levels of suitability threshold for A) 4.5 RCP and B) 8.5 scenarios, based on 26 populations of *Scinax squalirostris*. *h* – haplotype diversity, $\pi$ - nucleotide diversity and k - number of haplotypes.

**Figure 4.** Geographical distribution of nucleotide and haplotype genetic diversity and number of haplotypes for *Scinax squalirostris* populations: A) nucleotide diversity, B) haplotype diversity, C) number of haplotypes. For details on population codes and localities see Table S1.1 in Appendix S1.

**Figure 5.** Spatial distribution of genetic clusters conditioned to environmental variables simulated for *Scinax squalirostris*, for mitochondrial (A, B and C) and nuclear data (D, E and F), for present-day (A and D), 4.5 RCP scenario (B and E), 8.5 RCP scenario (C and F). Each colour set represents a cluster in the figure legend. Shifts in ancestry between contemporary and predicted ancestry coefficients for both future scenarios based on the simulation of genetic clusters of all individuals of *Scinax squalirostris*. G) Shifts in ancestry for the eight genetic clusters based on mitochondrial data. H) Shifts in ancestry for the three genetic clusters based on nuclear data.

**Figure 6.** Spatial distributions of genetic information from nuclear and mitochondrial sequences. A) Raw data of spatial distribution of haplotypes; B) Observed genetic richness measured by the number haplotypes divided by number of individuals per cell. C) Estimated genetic richness in 0.5º cell size measured as the estimated genetic richness using kriging.

**Figure 7.** Spatial distributions of clusters of Protection Areas, based on *k-mean* clusters analysis. A) Relationship of estimated genetic richness and Protection Areas size; B) Spatial distribution of genetic richness and the Protection Areas clusters. The colour of polygons representing Protection Areas corresponds to the clusters in the *k-mean* cluster analysis following the figure legends.

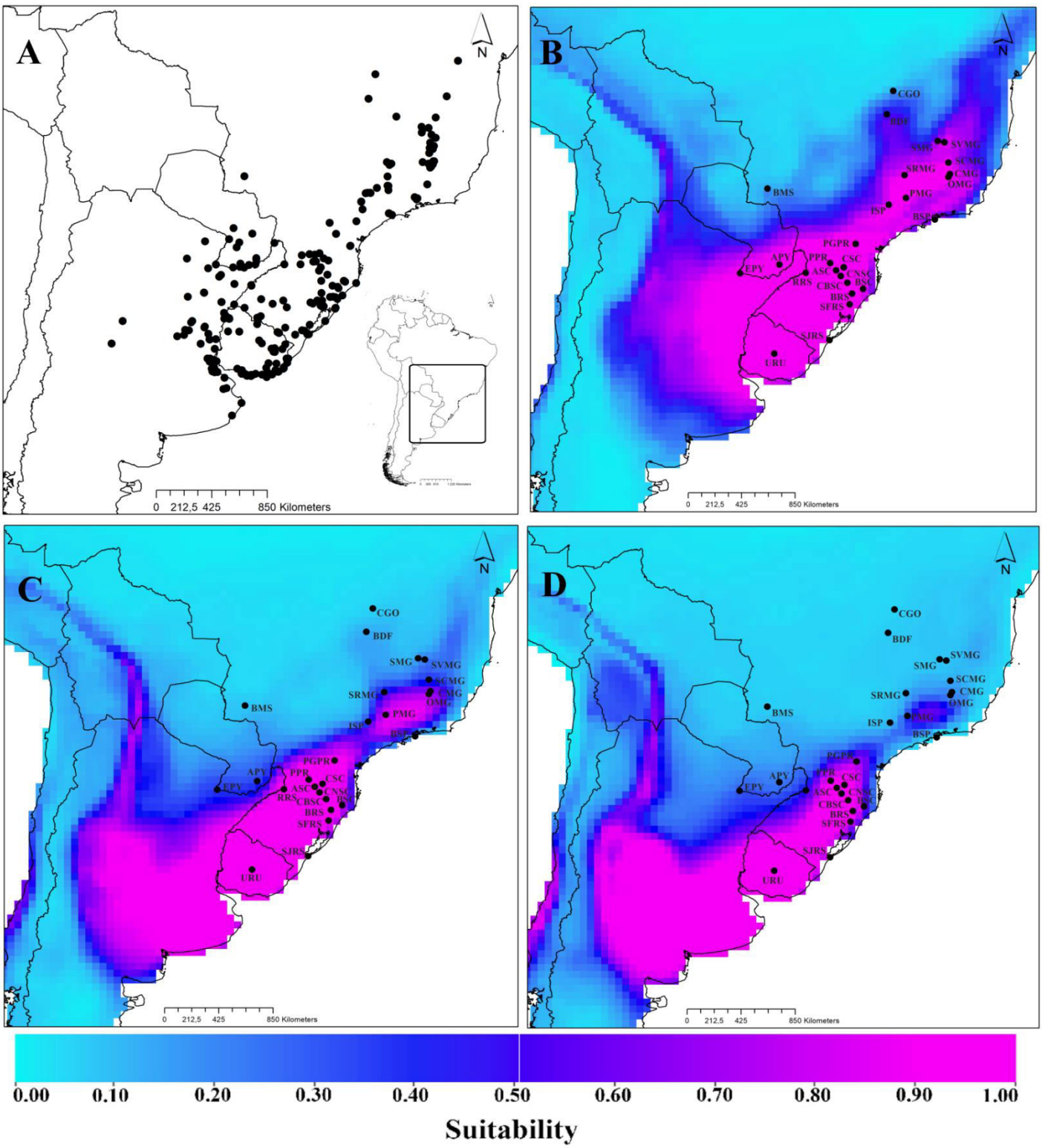
A
B
C
D
N
0 212,5 425 850 Kilometers
CGO
BDF
SMG
SVMG
SCMG
SRMG
CMG
OMG
BMS
PMG
ISP
BSP
PGPR
APY
PPR
CSC
EPY
ASC
CNSC
RRS
CBSC
BSC
BRS
SFRS
SJRS
URU
0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00
Suitability

**Figura 1**

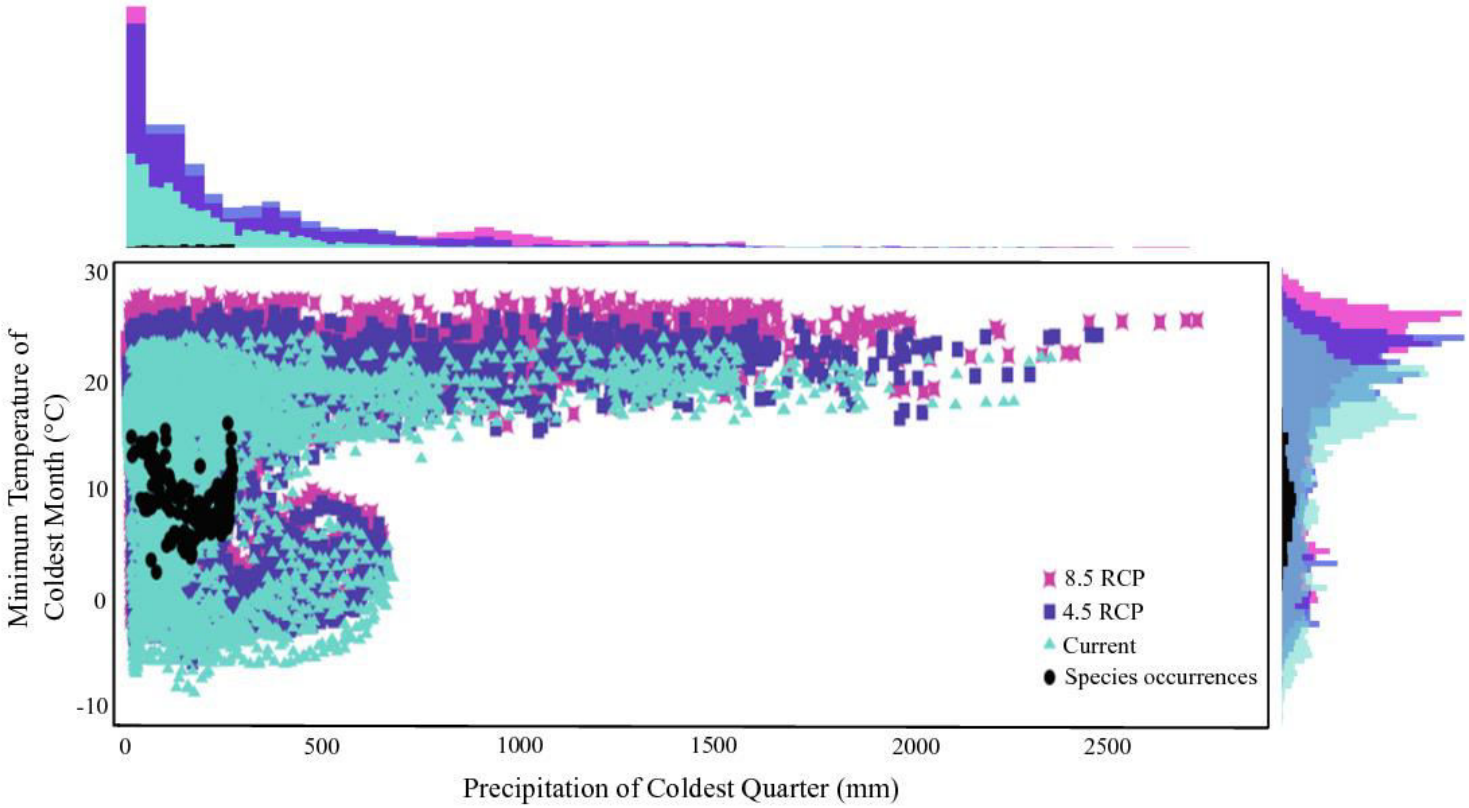
Minimum Temperature of Coldest Month (°C)
30
20
10
0
-10
0
500
1000
1500
2000
2500
Precipitation of Coldest Quarter (mm)
8.5 RCP
4.5 RCP
Current
Species occurrences

**Figura 2**

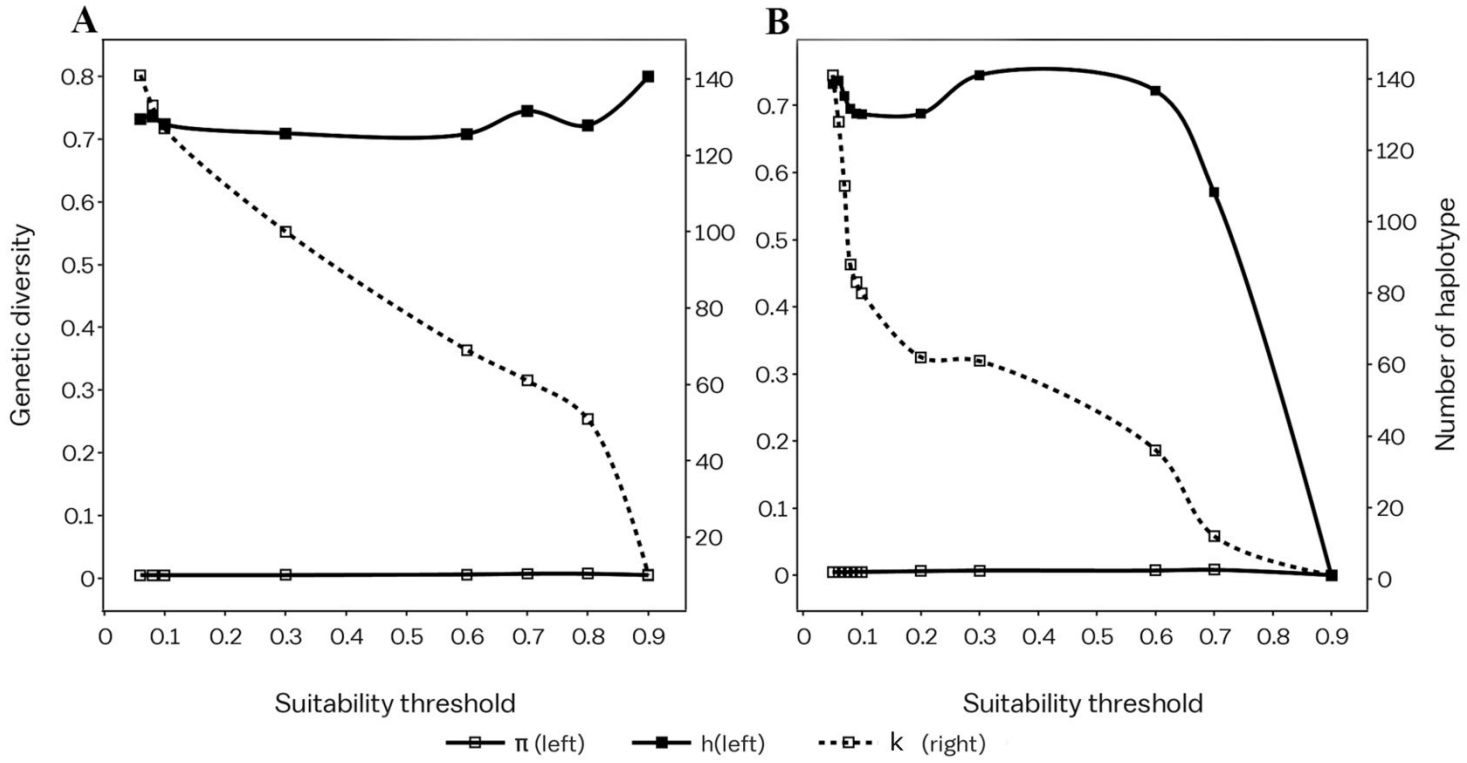
A
B
Genetic diversity
Number of haplotype
Suitability threshold
Suitability threshold
π (left)
h(left)
k (right)

**Figura 3**

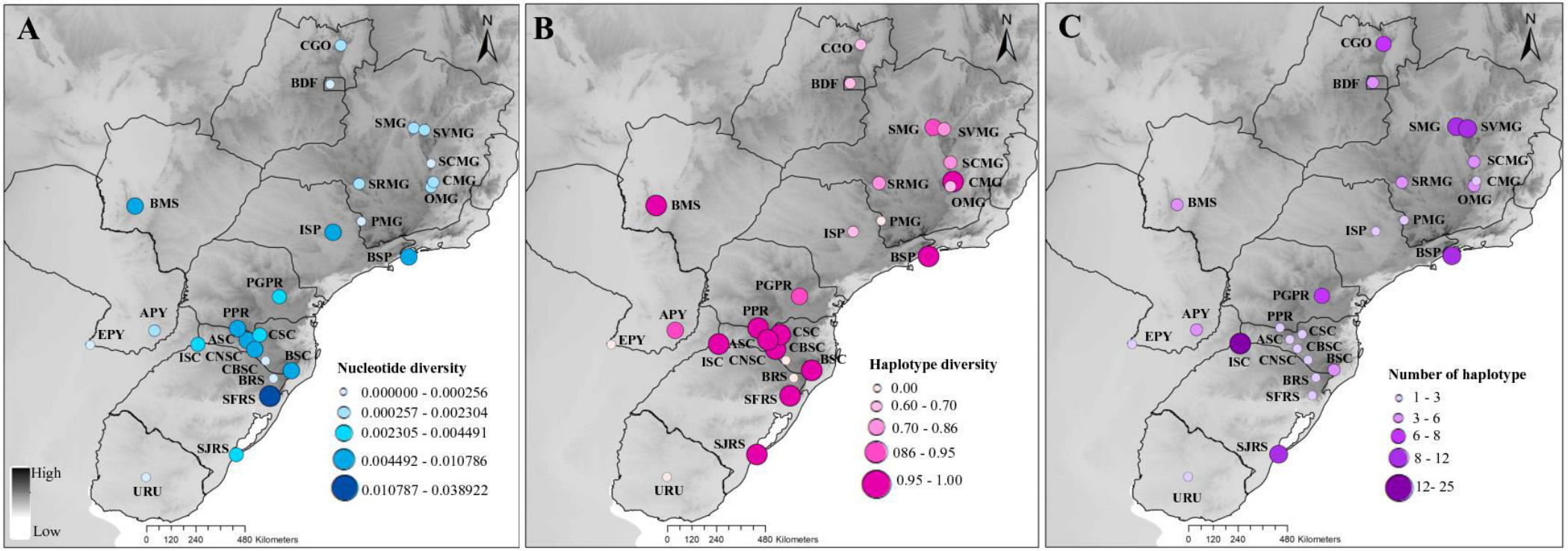
A
B
C
N
CGO
BDF
SMG
SVMG
SCMG
SRMG
CMG
OMG
BMS
ISP
PMG
BSP
PGPR
APY
PPR
EPY
ASC
CSC
ISC
CNSC
CBSC
BSC
BRS
SFRS
SJRS
URU
High
Low
Nucleotide diversity
0.000000 - 0.000256
0.000257 - 0.002304
0.002305 - 0.004491
0.004492 - 0.010786
0.010787 - 0.038922
Haplotype diversity
0.00
0.60 - 0.70
0.70 - 0.86
086 - 0.95
0.95 - 1.00
Number of haplotype
1 - 3
3 - 6
6 - 8
8 - 12
12- 25
0 120 240 480 Kilometers

**Figura 4**

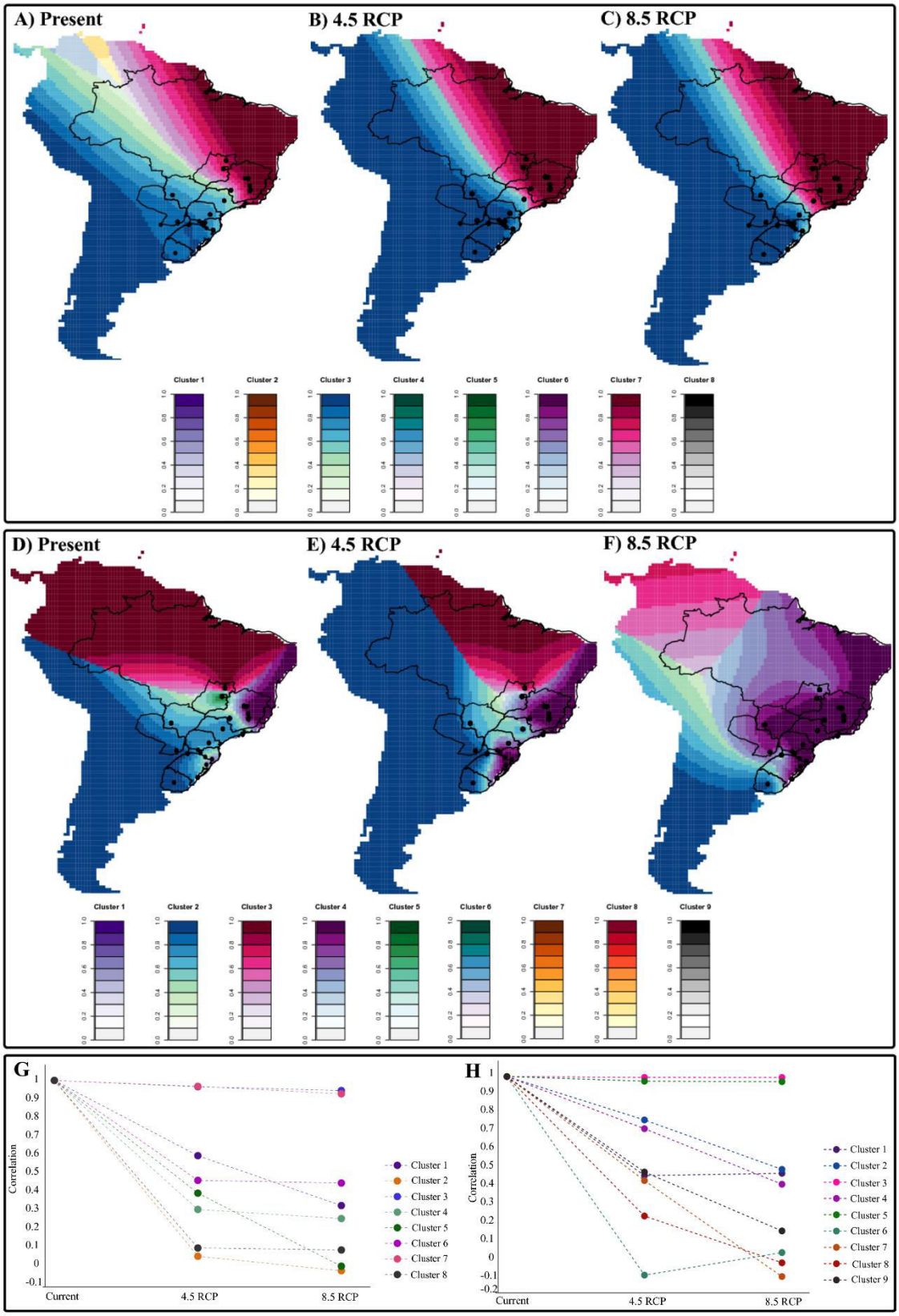
A) Present
B) 4.5 RCP
C) 8.5 RCP
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
D) Present
E) 4.5 RCP
F) 8.5 RCP
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
Cluster 9
G
Correlation
Current
4.5 RCP
8.5 RCP
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
H
Correlation
Current
4.5 RCP
8.5 RCP
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
Cluster 9

**Figura 5**

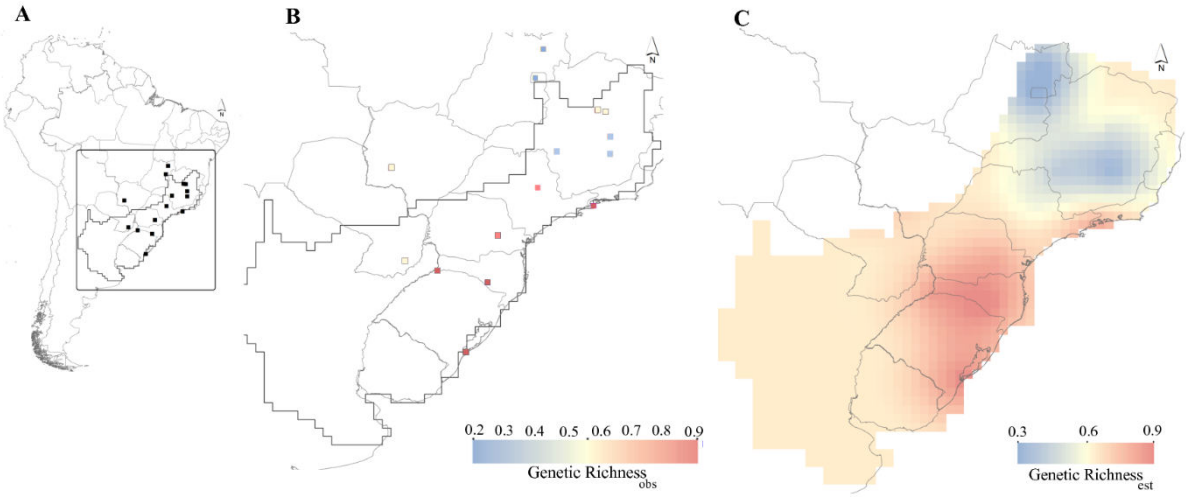


**Figura 6**

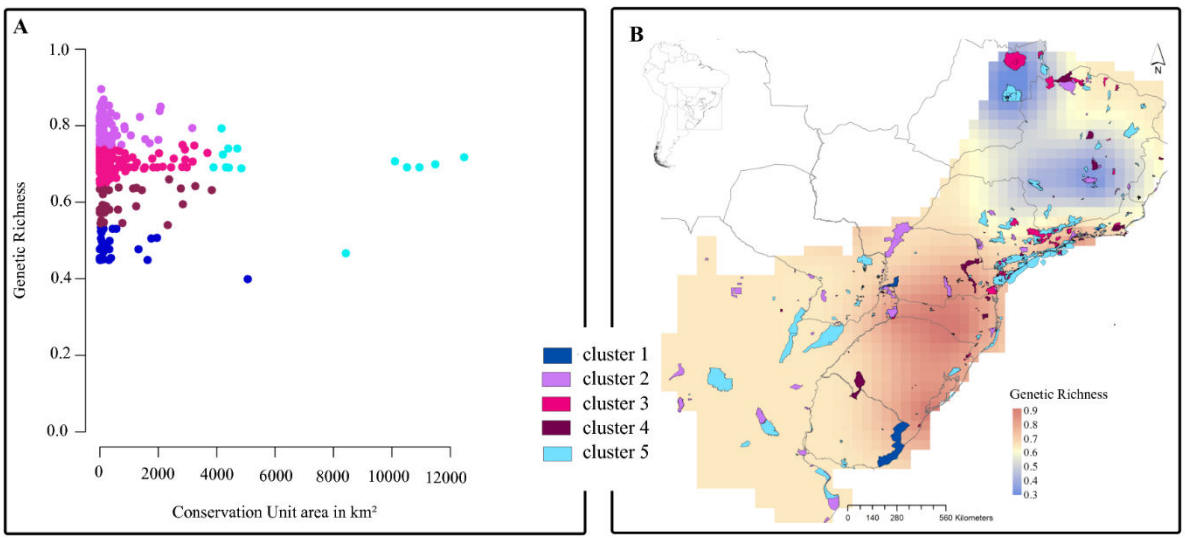


**Figura 7**

**SUPPORTING INFORMATION**

# Predicting impacts of global climatic change on distribution and genetic diversity of the treefrog *Scinax squalirostris* (Lutz, 1925) (Anura, Hylidae)

Tatianne P. F. Abreu-Jardim, Lucas Jardim, Liliana Ballesteros-Meija, Natan M. Maciel, Rosane G. Collevatti

**Appendix S1** Supplementary tables (Tables S1- S11) with details of sampling, PCR amplification, sampling and models used in ecological niche modelling, number of haplotypes and results of genetic ancestry clusters.

**Table S1.1** Sampling locations of the 26 populations of *Scinax squalirostris*. Name of localities in the Population column we presented the abbreviation of Brazilian states and a Paraguayan

department. Collection column we presented the abbreviation of donor institutions. (Amphibian Collection Célio F. B. Haddad (CFBH), Collection of the Universidade Federal de Minas Gerais (UFMG), Zoological Collection of the Universidade Federal de Goiás (ZUFG), Collection Elaine Maria Lucas Gonsales (EMLG), Museum of Ciências e Tecnologia da PUCRS (MCT), Collection of the Universidade Federal do Rio Grande do Sul (UFRGS), Herpetology Collection of the Instituto de Investigación Biológica del Paraguay (IIBPH).

| Population | Population Code | Collection | Latitude | Longitude |
|---|---|---|---|---|
| Itirapina-SP | ISP | CFBH | -22.2744 | -47.7958 |
| PARNA Bocaina-SP | BSP | CFBH | -23.3458 | -44.4825 |
| São Roque de Minas-MG | SRMG | CFBH | -20.1461 | -46.6628 |
| Ouro Preto-MG | OMG | CFBH | -20.2875 | -43.5081 |
| Catas Altas-MG | CMG | UFMG | -20.0747 | -43.4075 |
| Poços de Caldas-MG | PMG | UFMG | -21.7878 | -46.5614 |
| Serra do Cabral-MG | SMG | LOD | -17.6992 | -44.2714 |
| PARNA Sempre Vivas-MG | SVMG | ZUFG | -17.7883 | -43.7977 |
| PARNA Serra do Cipó-MG | SCMG | ZUFG | -19.2469 | -43.5100 |
| PARNA Chapada dos Veadeiros-GO | CGO | ZUFG | -14.0833 | -47.4667 |
| Brasília-DF | BDF | ZUFG | -15.7797 | -47.9297 |
| Bonito-MS | BMS | ZUFG | -21.1211 | -56.4819 |
| Itaparinga-SC | ISC | CFBH | -27.1833 | -53.7333 |
| São José do Norte-RS | SJRS | UFRGS | -32.0147 | -52.0417 |
| São Francisco de Paula-RS | SFRS | MCP | -29.4481 | -50.5836 |
| Bom Jesus-RS | BRS | CFBH | -28.6678 | -50.4167 |
| Palmas-PR | PPR | ZUFG | -26.4842 | -51.9906 |
| Ponta Grossa-PR | PGPR | ZUFG | -25.095 | -50.1619 |
| Campo Belo do Sul-SC | CBSC | MCP | -27.8992 | -50.7608 |
| Campos Novos-SC | CNSC | CFBH | -27.4017 | -51.2250 |
| Bom Jardim da Serra-SC | BSC | CFBH | -28.3369 | -49.6247 |
| Caçador-SC | CSC | EMLG | -26.7753 | -51.0150 |
| Água Doce-SC | ASC | EMLG | -26.9978 | -51.5561 |
| Estancia San José-Ñeembucú-Py | EPY | IIBPH | -27.2016 | -58.4486 |
| Alto Vera, Yataí-Itapuá-Py | APY | IIBPH | -26.5833 | -55.6333 |
| Uruguai | URU | - | -33.0000 | -56.0000 |

**Table S1.2** PCR Primers used to sequence CytB, 12S rRNA, and RAG-1 genes used in this study.

| Gene region | Primer | Direction | Nucleotide Sequence (5'-3') |
|---|---|---|---|
| CytB | MVZ15 | forward | GAACTAATGGCCCACACWWTACGNAA |
| | H1415 (cyt-b2) (H15149) | reverse | AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA |
| 12S | 12S | forward | AAACTGGGATTAGATACCCCACTAT |
| | 12S | reverse | GAGGGTGACGGGCGGTGTGT |
| RAG | RAG1 | forward | ATGCATCRAAAATTCARCAAT |
| | RAG1 | reverse | CCYCCTTTRTTGATAKGGWCATA |

**Table S1.3** PCR mix components and quantities for each of the amplified fragments used in this study.

| **Reagent** | **Cytb, 12S and RAG-1** |
|---|---|
| Deionized water | 8.5 µL |
| DNA | 2.0 µL |
| Forward primer (2 mM) | 3.0 µL |
| Reverse primer (2mM) | 3.0 µL |
| Buffer 1X* | 2.0 µL |
| DNTPs (2,5 mM) | 1,2 µL |
| Taq polymerase (5u/µL) | 0.3 µL |
| Total volume | 20 µL |

*Buffer 1X (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2)

**Table S1.4** PCR thermal program for each of the amplified fragments used in this study.

| **Gene region** | **Initial heating** | **Desnaturation** | **Annealing** | **Extension** | **Final extension** |
|---|---|---|---|---|---|
| | | **35 cycles** | | | |
| CytB | 2 min at 94°C | 60 sec at 94°C | 60 sec at 56°C | 90 sec at 72°C | 6 min at 72°C |
| 12S | 2 min at 94°C | 60 sec at 94°C | 60 sec at 54°C | 90 sec at 72°C | 6 min at 72°C |
| RAG | 2 min at 94°C | 60 sec at 94°C | 60 sec at 58°C | 90 sec at 72°C | 6 min at 72°C |

**Table S1.5** Geographic coordinates of 257 occurrence records of *Scinax squalirostris* used in ecological niche modelling.

| longitude | latitude | longitude | latitude | longitude | latitude |
|---|---|---|---|---|---|
| -54.80 | -34.70 | -55.67 | -26.83 | -50.42 | -28.67 |
| -56.46 | -34.75 | -56.17 | -27.23 | -51.23 | -27.40 |
| -54.92 | -34.80 | -49.63 | -28.33 | -53.57 | -30.16 |
| -54.10 | -31.92 | -56.77 | -27.14 | -43.31 | -17.06 |
| -54.28 | -34.03 | -57.02 | -26.01 | -44.27 | -18.11 |
| -56.69 | -34.71 | -58.40 | -33.87 | -46.01 | -17.01 |
| -53.77 | -34.35 | -58.58 | -34.33 | -50.58 | -29.45 |
| -57.54 | -30.90 | -58.23 | -28.05 | -51.18 | -29.17 |
| -55.67 | -26.76 | -64.87 | -31.10 | -52.53 | -26.56 |
| -56.74 | -27.36 | -54.42 | -25.72 | -43.68 | -18.81 |
| -56.50 | -27.42 | -65.63 | -32.65 | -43.69 | -20.52 |
| -56.17 | -27.23 | -56.68 | -36.75 | -42.54 | -15.61 |
| -55.94 | -25.07 | -58.54 | -34.63 | -44.18 | -17.87 |
| -56.76 | -27.14 | -58.05 | -29.79 | -41.77 | -13.15 |
| -57.23 | -24.69 | -59.26 | -29.14 | -43.80 | -20.25 |
| -55.73 | -27.18 | -61.11 | -32.16 | -55.06 | -34.83 |
| -56.83 | -25.68 | -58.60 | -33.78 | -58.52 | -33.01 |
| -57.27 | -27.40 | -58.88 | -32.37 | -60.08 | -31.10 |
| -57.54 | -25.46 | -58.54 | -34.58 | -46.37 | -20.25 |
| -55.67 | -26.83 | -58.52 | -33.02 | -46.62 | -21.13 |
| -46.52 | -23.67 | -60.44 | -26.79 | -48.35 | -23.40 |
| -47.35 | -21.92 | -60.64 | -32.06 | -56.33 | -34.86 |
| -44.75 | -22.57 | -60.57 | -31.71 | -44.57 | -22.64 |
| -49.55 | -28.40 | -59.02 | -33.69 | -58.99 | -27.47 |
| -46.32 | -23.78 | -60.30 | -31.73 | -46.56 | -21.79 |
| -35.73 | -84.83 | -58.44 | -32.35 | -51.32 | -264.28 |
| -48.43 | -22.87 | -59.22 | -31.48 | -49.20 | -25.93 |
| -59.50 | -32.67 | -60.52 | -31.73 | -51.32 | -26.43 |
| -58.73 | -34.38 | -57.85 | -35.02 | -51.63 | -26.52 |
| -58.84 | -33.69 | -57.33 | -37.62 | -46.62 | -21.78 |

| | | | | | |
|---|---|---|---|---|---|
| -58.87 | -32.37 | -54.91 | -34.80 | -44.58 | -22.65 |
| -58.96 | -31.99 | -54.28 | -34.04 | -51.74 | -27.01 |
| -59.24 | -31.54 | -54.13 | -34.58 | -46.37 | -20.25 |
| -58.88 | -33.91 | -57.53 | -30.95 | -51.02 | -30.08 |
| -57.17 | -28.54 | -56.45 | -34.74 | -50.23 | -30.25 |
| -58.70 | -34.58 | -54.17 | -31.87 | -50.21 | -30.18 |
| -58.10 | -27.92 | -55.06 | -34.83 | -52.10 | -32.04 |
| -58.64 | -34.36 | -56.30 | -34.87 | -51.23 | -30.03 |
| -60.38 | -29.42 | -55.60 | -30.87 | -50.07 | -28.75 |
| -58.06 | -29.79 | -53.80 | -34.39 | -51.93 | -29.83 |
| -58.87 | -33.89 | -57.88 | -30.53 | -51.76 | -29.91 |
| -57.93 | -35.78 | -57.87 | -30.58 | -53.67 | -31.56 |
| -58.35 | -34.61 | -57.73 | -30.78 | -56.00 | -28.66 |
| -60.15 | -31.19 | -55.90 | -34.82 | -50.07 | -29.59 |
| -59.26 | -29.15 | -56.03 | -34.82 | -48.78 | -28.48 |
| -57.17 | -28.54 | -56.03 | -34.88 | -52.34 | -31.77 |
| -57.72 | -35.73 | -55.72 | -34.73 | -49.73 | -29.34 |
| -58.44 | -34.54 | -54.20 | -31.87 | -51.34 | -27.63 |
| -59.19 | -25.61 | -53.67 | -32.72 | -51.13 | -29.68 |
| -43.75 | -19.51 | -55.28 | -32.63 | -50.70 | -26.93 |
| -43.71 | -19.17 | -56.65 | -34.50 | -50.76 | -27.90 |
| -43.71 | -19.17 | -54.98 | -34.33 | -50.22 | -29.45 |
| -43.74 | -19.51 | -54.57 | -34.78 | -52.10 | -32.04 |
| -46.57 | -22.07 | -55.27 | -34.80 | -50.38 | -29.42 |
| -43.88 | -21.68 | -54.95 | -34.97 | -51.18 | -28.33 |
| -43.41 | -20.07 | -56.35 | -34.80 | -51.98 | -31.36 |
| -44.18 | -17.87 | -56.83 | -31.83 | -50.01 | -28.84 |
| -44.20 | -20.14 | -57.62 | -31.82 | -50.22 | -30.20 |
| -43.43 | -19.04 | -55.55 | -31.03 | -55.36 | -30.77 |
| -43.60 | -18.25 | -55.60 | -31.32 | -52.99 | -32.05 |
| -43.31 | -18.47 | -53.45 | -33.68 | -57.18 | -29.95 |
| -43.61 | -20.45 | -53.58 | -33.68 | -55.34 | -29.60 |
| -43.47 | -20.08 | -53.53 | -33.98 | -54.54 | -29.92 |

| | | | | | |
|---|---|---|---|---|---|
| -46.53 | -23.67 | -53.77 | -34.28 | -56.47 | -30.18 |
| -46.53 | -23.67 | -53.78 | -34.33 | -56.01 | -28.62 |
| -47.82 | -22.25 | -53.78 | -34.35 | -52.52 | -31.74 |
| -48.44 | -22.88 | -54.33 | -34.48 | -50.92 | -31.11 |
| -47.35 | -21.92 | -54.15 | -34.60 | -48.43 | -22.87 |
| -44.75 | -22.57 | -57.87 | -30.93 | -57.54 | -30.90 |
| -46.32 | -23.78 | -57.53 | -30.95 | -53.77 | -34.35 |
| -48.13 | -22.28 | -56.67 | -34.73 | -49.55 | 28.40 |
| -48.77 | -24.22 | -56.97 | -34.38 | -47.80 | -22.27 |
| -53.81 | -29.68 | -56.37 | -34.82 | -46.48 | -20.35 |
| -51.02 | -30.08 | -55.47 | -32.43 | -46.66 | -20.15 |
| -51.46 | -29.69 | -54.40 | -32.97 | -46.56 | -21.79 |
| -51.18 | -29.92 | -54.62 | -33.00 | -44.27 | -17.70 |
| -52.34 | -31.77 | -54.48 | -33.22 | -43.80 | -17.79 |
| -52.15 | -32.02 | -54.38 | -33.23 | -43.51 | -19.25 |
| -50.16 | -25.10 | -54.83 | -34.00 | -51.56 | -27.00 |
| -51.99 | -26.48 | -53.87 | -33.03 | -51.02 | -26.78 |
| -50.62 | -24.32 | -48.00 | -22.25 | -52.04 | -32.01 |
| -49.18 | -25.92 | -44.61 | -22.72 | -53.73 | -27.18 |
| -52.62 | -27.19 | -43.51 | -20.29 | -47.47 | -14.08 |
| -47.82 | -22.25 | -49.62 | -28.34 | -47.93 | -15.78 |
| -44.57 | -22.64 | -56.48 | -21.12 | -58.45 | -27.20 |
| -50.16 | -25.10 | -51.99 | -26.48 | | |

**Table S1.6** Details on atmosphere-ocean global circulation models (AOGCMs) used for ecological niche modelling of *Scinax squalirostris*

| Model ID | Modelling Centre | Resolution* | Source | Year |
|---|---|---|---|---|
| CCSM4 | University of Miami – RSMAS, USA | 0.9° × 1.25° | CMIP5/PMIP3 | 2012 |
| CNRM-CM5 | Centre National de Recherches Meteorologiques / Centre Europeen de Recherche et Formation Avancees en Calcul Scientifique, France | 1.4° x 1.4° | CMIP5/PMIP3 | 2012 |
| MIROC-ESM | Atmosphere and Ocean Research Institute (University of Tokyo), National Institute for Environmental Studies, and Japan Agency for Marine-Earth Science and Technology, Japan | 2.8° × 2.8° | CMIP5/PMIP3 | 2012 |
| MPI-ESM-P | Max Planck Institute for Meteorology, Germany | 1.9° × 1.9° | CMIP5/PMIP3 | 2011 |
| MRI-CGCM3 | Meteorological Research Institute, Japan | 1.1° x 1.1° | CMIP5/PMIP3 | 2012 |

* longitude × latitude

CMIP5 –Coupled Model Intercomparison Project, Phase 5 (http://cmip-pcmdi.llnl.gov/)

PMIP3 –Paleoclimate Modelling Intercomparison Project, Phase 3 (http://pmip3.lsce.ipsl.fr/)

**Table S1.7** Ecological niche modeling methods used to estimate *Scinax squalirostris* potential distribution.

| Method | Species data type |
|---|---|
| Bioclimatic Envelope (BioClim) | Presence only |
| Ecological Niche Factor Analysis (ENFA) | Presence only |
| Euclidian Distance (EuclidDist) | Presence only |
| Flexible discriminant analysis (FDA) | Presence and absence |
| Generalized additive models (GAM) | Presence and absence |
| Generalized Linear Models (GLM) | Presence and absence |
| Gower Distance (GowerDist) | Presence only |
| Mahalanobis Distance (MahalDist) | Presence only |
| Multivariate adaptive regression splines (MARS) | Presence and absence |
| Maximum Entropy (MaxEnt) | Presence/background |
| Neural Networks (NNet) | Presence and absence |
| Random Forest (RNDFOR) | Presence and absence |

**Table S1.8** Values of true skill statistics (TSS) and values of area under the curve (AUC), with mean and standard deviation, for all ecological niche models (ENM) and atmosphere-ocean global circulation model (AOGCM) combinations (ENM x AOGCM) in *Scinax squalirostris* paleodistribution modelling.

| ENM/AOGCM | CCSM | | CNRM | | MIROC | | MPI | | MRI | | **Mean** | | **SD** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TSS | AUC | TSS | AUC | TSS | AUC | TSS | AUC | TSS | AUC | TSS | AUC | TSS | AUC |
| BioClim | 0.698 | 0.937 | 0.690 | 0.936 | 0.678 | 0.920 | 0.722 | 0.949 | 0.647 | 0.927 | 0.690 | 0.936 | 0.028 | 0.011 |
| ENFA | 0.515 | 0.849 | 0.480 | 0.798 | 0.503 | 0.840 | 0.521 | 0.841 | 0.541 | 0.841 | 0.515 | 0.841 | 0.022 | 0.020 |
| EuclidDist | 0.645 | 0.895 | 0.465 | 0.825 | 0.625 | 0.888 | 0.673 | 0.903 | 0.551 | 0.883 | 0.625 | 0.888 | 0.084 | 0.031 |
| FDA | 0.759 | 0.961 | 0.730 | 0.945 | 0.724 | 0.940 | 0.793 | 0.963 | 0.751 | 0.950 | 0.751 | 0.950 | 0.027 | 0.010 |
| GAM | 0.768 | 0.960 | 0.780 | 0.967 | 0.767 | 0.942 | 0.821 | 0.966 | 0.758 | 0.956 | 0.768 | 0.960 | 0.025 | 0.010 |
| GowerDist | 0.662 | 0.921 | 0.647 | 0.889 | 0.683 | 0.900 | 0.746 | 0.927 | 0.616 | 0.915 | 0.662 | 0.915 | 0.049 | 0.016 |
| MahalonobisDist | 0.725 | 0.935 | 0.561 | 0.845 | 0.545 | 0.867 | 0.724 | 0.927 | 0.707 | 0.923 | 0.707 | 0.923 | 0.091 | 0.041 |
| GLM | 0.714 | 0.942 | 0.761 | 0.952 | 0.704 | 0.915 | 0.820 | 0.967 | 0.754 | 0.954 | 0.754 | 0.952 | 0.046 | 0.020 |
| MARS | 0.747 | 0.951 | 0.761 | 0.952 | 0.780 | 0.955 | 0.837 | 0.969 | 0.761 | 0.954 | 0.761 | 0.954 | 0.035 | 0.007 |
| MaxEnt | 0.762 | 0.958 | 0.727 | 0.957 | 0.768 | 0.960 | 0.836 | 0.978 | 0.777 | 0.972 | 0.768 | 0.960 | 0.039 | 0.009 |
| NNet | 0.564 | 0.882 | 0.686 | 0.962 | 0.688 | 0.951 | 0.694 | 0.920 | 0.670 | 0.966 | 0.686 | 0.951 | 0.054 | 0.035 |
| RndFor | 0.792 | 1.000 | 0.767 | 1.000 | 0.789 | 1.000 | 0.827 | 1.000 | 0.798 | 1.000 | 0.792 | 1.000 | 0.022 | 0.000 |
| **Mean** | 0.720 | 0.939 | 0.709 | 0.948 | 0.696 | 0.930 | 0.769 | 0.956 | 0.729 | 0.952 | - | - | - | - |
| **SD** | 0.085 | 0.041 | 0.111 | 0.064 | 0.091 | 0.044 | 0.093 | 0.043 | 0.089 | 0.043 | - | - | - | - |

**Table S1.9** Characteristics of the 26 populations sampled for the analysis Phylogeography of *Scinax squalirostris*. Individuals (N), haplotypes by population (K) and relation of the haplotypes by population. Haplotypes in bold show the haplotype shared among some populations.

| Population Codes | mtDNA | | | nrDNA | | |
|---|---|---|---|---|---|---|
| | N | *k* | Haplotypes | N | *k* | Haplotypes |
| ISP | 5 | 2 | h01, h02 | 5 | 2 | h01, h02 |
| BSP | 12 | 8 | h01, h02, h03, h04, h05, h06, h07, h08 | 11 | 4 | h02, h03, h04, h05 |
| SRMG | 11 | 4 | h09, h10, h11, h12 | 12 | 1 | h04 |
| OMG | 16 | 5 | h13, h14, h15, h16, h17 | 16 | 2 | h06, h07 |
| CMG | 3 | 1 | h14 | 3 | 3 | h06, h08, h09 |
| PMG | 1 | 1 | h18 | - | - | - |
| SMG | 16 | 7 | h19, h20, h21, h22, h23, h24, h25 | 16 | 5 | h06, h10, h11, h12, h13 |
| SVMG | 22 | 5 | h26, h27, h28, h29, h30 | 21 | 8 | h06, h10, h14, h15, h16, h17, h18, h19 |
| SCMG | 13 | 2 | h26, h31 | 12 | 3 | h06, h20, h21 |
| CGO | 24 | 5 | h32, h33, h34, h35, h36 | 23 | 3 | h22, h23, h24 |
| BDF | 19 | 4 | h37, h38, h39, h40 | 18 | 1 | h25 |
| BMS | 7 | 3 | h41, h42, h43 | 7 | 5 | h06, h07, h26, h27, h28 |
| ISC | 26 | 12 | h44, h45, h46, h47, h48, h49, h50, h51, h52, h53, h54, h55 | 24 | 18 | h01, h29, h30, h31, h32, h33, h34, h35, h36, h37, h38, h39, h40, h41, h42, h43, h44, h45 |

| | | | | | | |
|---|---|---|---|---|---|---|
| SJRS | 10 | 2 | h44, h46 | 10 | 10 | h26, h30, h32, h36, h37, h46, h47, h48, h49, h50 |
| SFRS | 2 | 2 | h56, h57 | 2 | 2 | h51, h52 |
| BRS | 1 | 1 | h58 | 1 | 1 | h53 |
| PPR | 2 | 2 | h59, h60 | 2 | 2 | h06, h54 |
| PGPR | 8 | 3 | h01, h05, h61 | 8 | 6 | h55, h56, h57, h58, h59, h60 |
| CBSC | 1 | 1 | h62 | 1 | 1 | h51 |
| CNSC | 2 | 1 | h01 | 2 | 2 | h61, h62 |
| BSC | 5 | 2 | h01, h62 | 5 | 5 | h31, h63, h64, h65, h66 |
| CSC | 3 | 2 | h59 | 2 | 2 | h67, h68 |
| ASC | 1 | 2 | h59 | 2 | 2 | h29, h69 |
| EPY | 1 | 1 | h41 | 1 | 1 | h70 |
| APY | 7 | 2 | h41, h63 | 4 | 3 | h71, h72, h73 |
| URU | 1 | 1 | h45 | 1 | 1 | h30 |

**Table S1.10** Relative contributions of each modeling component (time, methods, AOGCMs and their interaction) to the variability of the predictions of the ENMs for *Scinax squalirostris*. The suitability column shows the median proportion and amplitude of the sum of squares from the hierarchical ANOVA calculated for each cell of the grid covering the Neotropical region.

| Sources of uncertainty | Median (SS)% | Min-Máx |
|---|---|---|
| ENMs*Time | 0.26 | 0.00 - 0.78 |
| AOGCMs*Time | 0.14 | 0.00 - 0.76 |
| Time | 0.04 | 0.02 - 0.92 |
| Residual | 0.46 | 0.06 - 0.78 |

**Table S1.11** Proportion on ancestry for individuals of *Scinax squalirostris*, conditioned to environmental variables, inferred by Bayesian analysis of mitochondrial for present climatic conditions.

| | **Inferred Cluster** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Individual** | **Cluster 1** | **Cluster 2** | **Cluster 3** | **Cluster 4** | **Cluster 5** | **Cluster 6** | **Cluster 7** | **Cluster 8** |
| 1 | 0.004344828 | 0.007167816 | 0.593778544 | 0.004300383 | 0.005538697 | 0.201199234 | 0.178868199 | 0.004802299 |
| 2 | 0.004407663 | 0.007630651 | 0.596 | 0.004133333 | 0.005888889 | 0.200182375 | 0.177249808 | 0.00450728 |
| 3 | 0.004271264 | 0.00762682 | 0.59643908 | 0.004165517 | 0.00560613 | 0.200652107 | 0.17651954 | 0.00471954 |
| 4 | 0.004250575 | 0.007413027 | 0.594255172 | 0.004298851 | 0.005630651 | 0.200624521 | 0.178816092 | 0.004711111 |
| 5 | 0.004195402 | 0.007236782 | 0.594556322 | 0.004347126 | 0.006088123 | 0.199836782 | 0.178898851 | 0.004840613 |
| 6 | 0.005784674 | 0.004825287 | 0.290354023 | 0.006108812 | 0.020458238 | 0.006009962 | 0.660121073 | 0.006337931 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7 | 0.005827586 | 0.004735632 | 0.288455939 | 0.00597931 | 0.02034023 | 0.006071264 | 0.662344061 | 0.006245977 |
| 8 | 0.00574023 | 0.004747893 | 0.288927203 | 0.006095785 | 0.020748659 | 0.005927969 | 0.661275096 | 0.006537165 |
| 9 | 0.005836782 | 0.004922605 | 0.287701916 | 0.005921839 | 0.020900383 | 0.005985441 | 0.662208429 | 0.006522605 |
| 10 | 0.005527203 | 0.004824521 | 0.287843678 | 0.005839847 | 0.020557854 | 0.006023755 | 0.663285057 | 0.006098084 |
| 11 | 0.005359387 | 0.005091188 | 0.288165517 | 0.005822989 | 0.020461303 | 0.005966284 | 0.663057471 | 0.006075862 |
| 12 | 0.005625287 | 0.005043678 | 0.289311111 | 0.006016858 | 0.020701916 | 0.006016092 | 0.661153257 | 0.006131801 |
| 13 | 0.005550192 | 0.005045977 | 0.288970881 | 0.00617931 | 0.020388506 | 0.005790805 | 0.661572414 | 0.006501916 |
| 14 | 0.005414559 | 0.004969349 | 0.289145594 | 0.006422989 | 0.020476628 | 0.006224521 | 0.660870498 | 0.006475862 |
| 15 | 0.0050659 | 0.007944828 | 0.357798467 | 0.003902682 | 0.004614559 | 0.211132567 | 0.404449808 | 0.005091188 |
| 16 | 0.005363218 | 0.007902682 | 0.356860536 | 0.004134866 | 0.004497318 | 0.211509579 | 0.404496552 | 0.005235249 |
| 17 | 0.005011494 | 0.008087356 | 0.357956322 | 0.003933333 | 0.004485824 | 0.211491954 | 0.403898851 | 0.005134866 |
| 18 | 0.005143295 | 0.007944061 | 0.356460536 | 0.004083525 | 0.004229885 | 0.211537931 | 0.405425287 | 0.005175479 |
| 19 | 0.005002299 | 0.007750958 | 0.356767816 | 0.004046743 | 0.004543295 | 0.21100613 | 0.40577318 | 0.005109579 |
| 20 | 0.00489272 | 0.008150192 | 0.356413793 | 0.004282759 | 0.004704981 | 0.211198467 | 0.405017625 | 0.005339464 |
| 21 | 0.004870498 | 0.008216858 | 0.357268966 | 0.003679693 | 0.004673563 | 0.210915709 | 0.405391571 | 0.004983142 |
| 22 | 0.005216858 | 0.007790038 | 0.356971648 | 0.003962452 | 0.004753257 | 0.211382375 | 0.404828352 | 0.005095019 |
| 23 | 0.00512567 | 0.007924904 | 0.359766284 | 0.0042 | 0.00472567 | 0.211154023 | 0.40194023 | 0.005163218 |
| 24 | 0.004987739 | 0.008050575 | 0.357062069 | 0.003976245 | 0.00431954 | 0.211240613 | 0.405375479 | 0.004987739 |
| 25 | 0.002547893 | 0.00647433 | 0.036866667 | 0.002298084 | 0.002284291 | 0.015028352 | 0.931223755 | 0.003276628 |
| 26 | 0.002477395 | 0.006499617 | 0.036362452 | 0.002249808 | 0.002232184 | 0.015263602 | 0.931894253 | 0.00302069 |
| 27 | 0.002652874 | 0.006723372 | 0.036400766 | 0.002227586 | 0.002198467 | 0.014619157 | 0.932207663 | 0.002970115 |
| 28 | 0.002580843 | 0.00663295 | 0.036370881 | 0.002294253 | 0.002269732 | 0.015127969 | 0.931712644 | 0.003010728 |
| 29 | 0.002577778 | 0.006222222 | 0.037219157 | 0.002299617 | 0.002213793 | 0.01511954 | 0.931424521 | 0.002923372 |
| 30 | 0.002535632 | 0.006551724 | 0.036463602 | 0.002285824 | 0.002316475 | 0.015599234 | 0.931177011 | 0.003070498 |
| 31 | 0.002602299 | 0.006591571 | 0.035731034 | 0.002285057 | 0.002285824 | 0.014816858 | 0.932737931 | 0.002949425 |
| 32 | 0.002431418 | 0.006695019 | 0.036096552 | 0.00242069 | 0.002243678 | 0.01550728 | 0.931532567 | 0.003072797 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 33 | 0.002600766 | 0.006354023 | 0.037070498 | 0.002390038 | 0.002224521 | 0.015182375 | 0.931150192 | 0.003027586 |
| 34 | 0.002454406 | 0.006518774 | 0.03614636 | 0.002229885 | 0.002354789 | 0.015590805 | 0.931691188 | 0.003013793 |
| 35 | 0.002547126 | 0.006331034 | 0.036642146 | 0.002303448 | 0.002324904 | 0.015425287 | 0.93145977 | 0.002966284 |
| 36 | 0.0024659 | 0.006385441 | 0.036079693 | 0.002236015 | 0.002161686 | 0.015222989 | 0.932549425 | 0.002898851 |
| 37 | 0.002341762 | 0.006539464 | 0.036412261 | 0.002301149 | 0.002243678 | 0.015310345 | 0.931704215 | 0.003147126 |
| 38 | 0.003997701 | 0.01102069 | 0.049704215 | 0.003458238 | 0.014724904 | 0.003428352 | 0.909532567 | 0.004133333 |
| 39 | 0.004273563 | 0.011411494 | 0.050548659 | 0.003652107 | 0.015298084 | 0.003342529 | 0.9071341 | 0.004339464 |
| 40 | 0.00414023 | 0.01108659 | 0.050728736 | 0.003716475 | 0.015645211 | 0.003137165 | 0.907510345 | 0.004035249 |
| 41 | 0.001463602 | 0.00097931 | 0.011255172 | 0.001454406 | 0.068785441 | 0.004529502 | 0.910097318 | 0.001435249 |
| 42 | 0.001427586 | 0.00105977 | 0.01145364 | 0.001258238 | 0.068837548 | 0.004655939 | 0.910029119 | 0.001278161 |
| 43 | 0.001345594 | 0.001087356 | 0.011242912 | 0.001315709 | 0.068523372 | 0.004717241 | 0.910343295 | 0.001424521 |
| 44 | 0.001436015 | 0.001096552 | 0.011043678 | 0.001426054 | 0.068283525 | 0.00471341 | 0.910759387 | 0.001241379 |
| 45 | 0.001354789 | 0.001008429 | 0.011540996 | 0.001343295 | 0.068930268 | 0.004783142 | 0.909636015 | 0.001403065 |
| 46 | 0.001386207 | 0.001061303 | 0.011214559 | 0.001403831 | 0.069731034 | 0.004568582 | 0.909260536 | 0.001373946 |
| 47 | 0.001362452 | 0.00111954 | 0.010893487 | 0.001324138 | 0.068560153 | 0.004619923 | 0.910663602 | 0.001456705 |
| 48 | 0.001318008 | 0.00107433 | 0.011006897 | 0.001337931 | 0.06947433 | 0.004535632 | 0.909854406 | 0.001398467 |
| 49 | 0.00132567 | 0.001104215 | 0.011213793 | 0.001349425 | 0.068176245 | 0.004882759 | 0.910610728 | 0.001337165 |
| 50 | 0.001377778 | 0.001029119 | 0.011485824 | 0.001305747 | 0.068694253 | 0.00459387 | 0.910132567 | 0.001380843 |
| 51 | 0.001422989 | 0.001030651 | 0.011216858 | 0.001251341 | 0.069818391 | 0.004628352 | 0.909310345 | 0.001321073 |
| 52 | 0.001472031 | 0.001049808 | 0.01112567 | 0.00136705 | 0.068603065 | 0.004564751 | 0.910321073 | 0.001496552 |
| 53 | 0.000948659 | 0.005016858 | 0.001969349 | 0.000834483 | 0.001970881 | 0.002636782 | 0.985649042 | 0.000973946 |
| 54 | 0.000931801 | 0.005033716 | 0.001954789 | 0.00083295 | 0.002042146 | 0.002467433 | 0.985757854 | 0.00097931 |
| 55 | 0.000975479 | 0.005038314 | 0.002000766 | 0.000852874 | 0.001927203 | 0.002433716 | 0.985839847 | 0.000931801 |
| 56 | 0.000854406 | 0.00477931 | 0.001883525 | 0.000912644 | 0.001870498 | 0.002580843 | 0.986251341 | 0.000867433 |
| 57 | 0.000901916 | 0.004961686 | 0.00185977 | 0.0008659 | 0.001940996 | 0.002781609 | 0.985748659 | 0.000939464 |
| 58 | 0.00092567 | 0.004849042 | 0.001950192 | 0.000805364 | 0.002004598 | 0.002580843 | 0.985942529 | 0.000941762 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 59 | 0.000893487 | 0.005081992 | 0.00203908 | 0.000836015 | 0.002064368 | 0.002601533 | 0.985427586 | 0.001055939 |
| 60 | 0.000887356 | 0.004898084 | 0.002028352 | 0.000785441 | 0.001770115 | 0.002414559 | 0.986212261 | 0.001003831 |
| 61 | 0.00096092 | 0.004849042 | 0.002016092 | 0.000855172 | 0.001943295 | 0.002488889 | 0.985964751 | 0.000921839 |
| 62 | 0.000953257 | 0.005072031 | 0.002061303 | 0.000743295 | 0.001896552 | 0.002530268 | 0.985783142 | 0.000960153 |
| 63 | 0.000954023 | 0.004989272 | 0.00192567 | 0.000838314 | 0.001832184 | 0.002641379 | 0.985896552 | 0.000922605 |
| 64 | 0.000910345 | 0.005037548 | 0.001898084 | 0.000967816 | 0.001911877 | 0.002629119 | 0.985649042 | 0.000996169 |
| 65 | 0.00096092 | 0.005042912 | 0.001990805 | 0.000839847 | 0.001954789 | 0.002695019 | 0.985434483 | 0.001081226 |
| 66 | 0.000844444 | 0.005008429 | 0.002024521 | 0.000802299 | 0.001966284 | 0.002809962 | 0.985509579 | 0.001034483 |
| 67 | 0.000868199 | 0.005228352 | 0.001917241 | 0.00087433 | 0.001931034 | 0.002470498 | 0.985731801 | 0.000978544 |
| 68 | 0.000878161 | 0.005003831 | 0.001977778 | 0.000908812 | 0.002006897 | 0.00231341 | 0.985941762 | 0.000969349 |
| 69 | 0.000918008 | 0.005186973 | 0.001901916 | 0.000785441 | 0.001924904 | 0.002458238 | 0.985835249 | 0.000989272 |
| 70 | 0.000995402 | 0.005091188 | 0.002003831 | 0.000798467 | 0.001878927 | 0.002460536 | 0.985861303 | 0.000910345 |
| 71 | 0.000910345 | 0.005069732 | 0.00187433 | 0.000795402 | 0.00194636 | 0.002624521 | 0.98587433 | 0.000904981 |
| 72 | 0.001684291 | 0.001450575 | 0.015961686 | 0.001478161 | 0.009900383 | 0.015608429 | 0.95217318 | 0.001743295 |
| 73 | 0.001665134 | 0.001511877 | 0.01642069 | 0.001459004 | 0.010038314 | 0.015574713 | 0.951672797 | 0.001657471 |
| 74 | 0.001744061 | 0.001225287 | 0.016487356 | 0.001425287 | 0.009871264 | 0.015586207 | 0.951970881 | 0.001689655 |
| 75 | 0.001634483 | 0.001436015 | 0.016295019 | 0.001314176 | 0.01054023 | 0.015630651 | 0.951530268 | 0.001619157 |
| 76 | 0.001696552 | 0.001422222 | 0.016354023 | 0.001427586 | 0.009891188 | 0.0161341 | 0.951359387 | 0.001714943 |
| 77 | 0.001651341 | 0.001340996 | 0.016301149 | 0.001366284 | 0.010186207 | 0.015999234 | 0.951357088 | 0.001797701 |
| 78 | 0.001652874 | 0.001398467 | 0.016659004 | 0.001388506 | 0.009882759 | 0.016394636 | 0.950964751 | 0.001659004 |
| 79 | 0.001744828 | 0.001318774 | 0.016344061 | 0.001402299 | 0.010206897 | 0.016005364 | 0.951233716 | 0.001744061 |
| 80 | 0.001823755 | 0.00142069 | 0.016408429 | 0.00134023 | 0.010121073 | 0.015977011 | 0.951175479 | 0.001733333 |
| 81 | 0.001607663 | 0.001363985 | 0.016524904 | 0.001347893 | 0.010177011 | 0.015813027 | 0.951380843 | 0.001784674 |
| 82 | 0.001785441 | 0.001378544 | 0.016254406 | 0.001481992 | 0.01010728 | 0.015683525 | 0.951545594 | 0.001763218 |
| 83 | 0.001632184 | 0.001377778 | 0.016641379 | 0.00151341 | 0.009943295 | 0.016004598 | 0.95119387 | 0.001693487 |
| 84 | 0.00162069 | 0.001396935 | 0.016266667 | 0.001466667 | 0.010151724 | 0.015550958 | 0.951836015 | 0.001710345 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 85 | 0.001329502 | 0.192845977 | 0.006281992 | 0.001485824 | 0.004344828 | 0.00257931 | 0.789097318 | 0.002035249 |
| 86 | 0.00136705 | 0.192629119 | 0.006145594 | 0.001517241 | 0.004318774 | 0.002491954 | 0.789443678 | 0.00208659 |
| 87 | 0.001455939 | 0.19367433 | 0.00605977 | 0.001448276 | 0.004181609 | 0.002609195 | 0.788526437 | 0.002044444 |
| 88 | 0.001562452 | 0.192793103 | 0.006009195 | 0.001542529 | 0.004311111 | 0.002527203 | 0.789162452 | 0.002091954 |
| 89 | 0.001596935 | 0.192937165 | 0.006277395 | 0.001523372 | 0.004248276 | 0.002570881 | 0.788681992 | 0.002163985 |
| 90 | 0.001375479 | 0.193332567 | 0.005956322 | 0.001520307 | 0.004278927 | 0.002505747 | 0.789 | 0.002030651 |
| 91 | 0.001517241 | 0.193277395 | 0.005977778 | 0.001669732 | 0.004043678 | 0.002245211 | 0.789273563 | 0.001995402 |
| 92 | 0.001564751 | 0.192625287 | 0.006345594 | 0.001491188 | 0.004291188 | 0.002504215 | 0.789087356 | 0.002090421 |
| 93 | 0.001386207 | 0.192962452 | 0.006003065 | 0.001539464 | 0.003996169 | 0.002735632 | 0.789309579 | 0.002067433 |
| 94 | 0.001499617 | 0.19334636 | 0.006062835 | 0.001471264 | 0.003935632 | 0.002586207 | 0.78895249 | 0.002145594 |
| 95 | 0.001429885 | 0.193544061 | 0.005936398 | 0.001504215 | 0.004126437 | 0.002568582 | 0.788784674 | 0.002105747 |
| 96 | 0.001508046 | 0.193445977 | 0.006065134 | 0.00148046 | 0.004236015 | 0.002463602 | 0.788741762 | 0.002059004 |
| 97 | 0.001482759 | 0.192856705 | 0.006057471 | 0.001409962 | 0.004308812 | 0.002308046 | 0.789468199 | 0.002108046 |
| 98 | 0.001603065 | 0.193012261 | 0.00615249 | 0.001514176 | 0.00415249 | 0.002708812 | 0.788780843 | 0.002075862 |
| 99 | 0.001433716 | 0.193061303 | 0.005936398 | 0.001583908 | 0.004180077 | 0.00239387 | 0.789306513 | 0.002104215 |
| 100 | 0.001596935 | 0.193002299 | 0.006327969 | 0.001462069 | 0.00396705 | 0.002468199 | 0.789117241 | 0.002058238 |
| 101 | 0.00151341 | 0.193373946 | 0.006067433 | 0.001462069 | 0.004170115 | 0.002285824 | 0.78917318 | 0.001954023 |
| 102 | 0.001399234 | 0.192163218 | 0.006171648 | 0.001488889 | 0.004376245 | 0.002493487 | 0.789733333 | 0.002173946 |
| 103 | 0.001496552 | 0.193340996 | 0.006171648 | 0.001553257 | 0.004103448 | 0.002503448 | 0.788678161 | 0.00215249 |
| 104 | 0.001468199 | 0.193099617 | 0.006109579 | 0.001426054 | 0.004291188 | 0.002577011 | 0.788895019 | 0.002133333 |
| 105 | 0.001462835 | 0.193381609 | 0.006162452 | 0.001423755 | 0.004321839 | 0.00251341 | 0.78862682 | 0.00210728 |
| 106 | 0.00144751 | 0.192764751 | 0.006145594 | 0.001527969 | 0.004235249 | 0.002541762 | 0.789248276 | 0.002088889 |
| 107 | 0.00303908 | 0.006829885 | 0.022931034 | 0.002891954 | 0.17891341 | 0.011808429 | 0.770701149 | 0.002885057 |
| 108 | 0.002953257 | 0.0066659 | 0.022414559 | 0.002826054 | 0.178403065 | 0.012190038 | 0.77185977 | 0.002687356 |
| 109 | 0.002856705 | 0.006977011 | 0.022409195 | 0.002652107 | 0.178345594 | 0.011905747 | 0.77188046 | 0.00297318 |
| 110 | 0.003001533 | 0.006914176 | 0.022729502 | 0.00290728 | 0.17771954 | 0.012121073 | 0.771479693 | 0.003127203 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 111 | 0.002898084 | 0.00656092 | 0.022544061 | 0.002967816 | 0.179234483 | 0.012354789 | 0.770676628 | 0.002763218 |
| 112 | 0.002773946 | 0.006740996 | 0.022483525 | 0.002970115 | 0.178488123 | 0.012132567 | 0.77134023 | 0.003070498 |
| 113 | 0.003017625 | 0.006889655 | 0.022749425 | 0.00296705 | 0.178285057 | 0.012084291 | 0.771130268 | 0.002876628 |
| 114 | 0.002934866 | 0.006687356 | 0.02270728 | 0.002953257 | 0.179482759 | 0.01154636 | 0.770730268 | 0.002957854 |
| 115 | 0.00305364 | 0.00691341 | 0.022843678 | 0.002881992 | 0.178506513 | 0.012407663 | 0.77054636 | 0.002846743 |
| 116 | 0.002727969 | 0.006682759 | 0.022549425 | 0.00282069 | 0.178150192 | 0.012421456 | 0.771675096 | 0.002972414 |
| 117 | 0.00282682 | 0.00635249 | 0.022836015 | 0.002850575 | 0.179436782 | 0.012308812 | 0.770386973 | 0.003001533 |
| 118 | 0.002682759 | 0.007358621 | 0.022640613 | 0.002734866 | 0.178613793 | 0.012184674 | 0.770910345 | 0.00287433 |
| 119 | 0.002839847 | 0.006796935 | 0.022628352 | 0.002915709 | 0.179396169 | 0.011704215 | 0.770809195 | 0.002909579 |
| 120 | 0.00294636 | 0.006790038 | 0.02228659 | 0.002747893 | 0.178798467 | 0.011831418 | 0.771660536 | 0.002938697 |
| 121 | 0.002889655 | 0.006753257 | 0.022786207 | 0.002831418 | 0.17822682 | 0.012373946 | 0.771364751 | 0.002773946 |
| 122 | 0.002957854 | 0.006318008 | 0.02283295 | 0.002815326 | 0.178967816 | 0.012052874 | 0.771139464 | 0.002915709 |
| 123 | 0.002908046 | 0.006586973 | 0.022467433 | 0.002757088 | 0.179314943 | 0.011418391 | 0.771593103 | 0.002954023 |
| 124 | 0.005399234 | 0.010770115 | 0.762583908 | 0.004333333 | 0.009008429 | 0.168145594 | 0.03496092 | 0.004798467 |
| 125 | 0.005479693 | 0.010310345 | 0.76316092 | 0.004308046 | 0.009574713 | 0.167731801 | 0.034580843 | 0.00485364 |
| 126 | 0.005521839 | 0.010655172 | 0.762199234 | 0.004481226 | 0.009311877 | 0.167708046 | 0.035068199 | 0.005054406 |
| 127 | 0.005573946 | 0.010370115 | 0.762417625 | 0.004336398 | 0.009308046 | 0.167927969 | 0.035097318 | 0.004968582 |
| 128 | 0.005388506 | 0.010403065 | 0.761311877 | 0.004493487 | 0.009485057 | 0.168362452 | 0.035597701 | 0.004957854 |
| 129 | 0.005072031 | 0.01040613 | 0.76191954 | 0.004332567 | 0.009666667 | 0.167914176 | 0.035592337 | 0.005096552 |
| 130 | 0.001208429 | 0.004745594 | 0.954174713 | 0.001477395 | 0.006563985 | 0.027392337 | 0.003211494 | 0.001226054 |
| 131 | 0.001245977 | 0.004417625 | 0.954858238 | 0.001568582 | 0.006392337 | 0.026983142 | 0.003367816 | 0.001166284 |
| 132 | 0.001154023 | 0.00452567 | 0.95480613 | 0.001649042 | 0.006566284 | 0.026922605 | 0.003137165 | 0.00123908 |
| 133 | 0.001259004 | 0.004659004 | 0.954695019 | 0.001624521 | 0.006506513 | 0.0268659 | 0.003157088 | 0.00123295 |
| 134 | 0.001222222 | 0.004445977 | 0.954485824 | 0.001497318 | 0.006803831 | 0.026932567 | 0.003357088 | 0.001255172 |
| 135 | 0.001240613 | 0.00437931 | 0.954560153 | 0.001454406 | 0.00644751 | 0.02768046 | 0.003190038 | 0.00104751 |
| 136 | 0.001206897 | 0.004518774 | 0.954899617 | 0.001541762 | 0.006575479 | 0.02692567 | 0.003178544 | 0.001153257 |

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|---|---|---|---|---|---|---|---|---|
| 137 | 0.001209195 | 0.004624521 | 0.954697318 | 0.001475862 | 0.006498084 | 0.027101149 | 0.003188506 | 0.001205364 |
| 138 | 0.00117318 | 0.00454023 | 0.954694253 | 0.001634483 | 0.006359387 | 0.027240613 | 0.0032659 | 0.001091954 |
| 139 | 0.001217625 | 0.00468659 | 0.954771648 | 0.001516475 | 0.006550958 | 0.026788506 | 0.003391571 | 0.001076628 |
| 140 | 0.001182375 | 0.004726437 | 0.954363218 | 0.001604598 | 0.006567816 | 0.026914943 | 0.003384674 | 0.001255939 |
| 141 | 0.001203065 | 0.004436015 | 0.954583908 | 0.001612261 | 0.006396935 | 0.027216092 | 0.003372414 | 0.00117931 |
| 142 | 0.001216092 | 0.004347126 | 0.95462069 | 0.001636015 | 0.006595402 | 0.026976245 | 0.003430651 | 0.001177778 |
| 143 | 0.001168582 | 0.004498851 | 0.955441379 | 0.001583908 | 0.006176245 | 0.026749425 | 0.003229119 | 0.00115249 |
| 144 | 0.001236015 | 0.004398467 | 0.954862835 | 0.001599234 | 0.006695785 | 0.026758621 | 0.0032 | 0.001249042 |
| 145 | 0.001147893 | 0.004565517 | 0.95496092 | 0.001558621 | 0.006521839 | 0.026862835 | 0.003162452 | 0.001219923 |
| 146 | 0.001273563 | 0.004597701 | 0.954283525 | 0.001529502 | 0.006339464 | 0.027385441 | 0.003386207 | 0.001204598 |
| 147 | 0.00168046 | 0.003603065 | 0.942478927 | 0.001922605 | 0.001267433 | 0.008656705 | 0.038934866 | 0.001455939 |
| 148 | 0.001533333 | 0.00363295 | 0.943449042 | 0.001756322 | 0.001160153 | 0.008563218 | 0.038572414 | 0.001332567 |
| 149 | 0.001730268 | 0.003586973 | 0.942553257 | 0.001731801 | 0.001290421 | 0.008436015 | 0.039317241 | 0.001354023 |
| 150 | 0.001706513 | 0.003575479 | 0.943085057 | 0.00183295 | 0.001190805 | 0.008381609 | 0.038875096 | 0.00135249 |
| 151 | 0.001715709 | 0.003657471 | 0.942498084 | 0.001852874 | 0.001302682 | 0.008574713 | 0.038956322 | 0.001442146 |
| 152 | 0.001729502 | 0.003862835 | 0.94229272 | 0.001956322 | 0.001396169 | 0.008268199 | 0.039093487 | 0.001400766 |
| 153 | 0.00163295 | 0.0036 | 0.942087356 | 0.001938697 | 0.001344828 | 0.008743295 | 0.039222989 | 0.001429885 |
| 154 | 0.001777011 | 0.003671264 | 0.942298851 | 0.001921073 | 0.001378544 | 0.008041379 | 0.039495019 | 0.001416858 |
| 155 | 0.0016659 | 0.003642146 | 0.942695019 | 0.001860536 | 0.00129272 | 0.008354789 | 0.039055172 | 0.001433716 |
| 156 | 0.001676628 | 0.003768582 | 0.941648276 | 0.001904981 | 0.00125977 | 0.008594636 | 0.039721073 | 0.001426054 |
| 157 | 0.000687356 | 0.000990038 | 0.739391571 | 0.001042146 | 0.001 | 0.247595402 | 0.008527203 | 0.000766284 |
| 158 | 0.002123372 | 0.003956322 | 0.732327969 | 0.00282682 | 0.007002299 | 0.216891954 | 0.03239387 | 0.002477395 |
| 159 | 0.002171648 | 0.003949425 | 0.731008429 | 0.002947893 | 0.00665364 | 0.218170881 | 0.032659004 | 0.00243908 |
| 160 | 0.002265134 | 0.003988506 | 0.731337931 | 0.003088123 | 0.006688889 | 0.217601533 | 0.032514943 | 0.002514943 |
| 161 | 0.002136398 | 0.004036782 | 0.731944061 | 0.002911111 | 0.006684291 | 0.217682759 | 0.031968582 | 0.002636015 |
| 162 | 0.002324138 | 0.004042146 | 0.732332567 | 0.002947126 | 0.006771648 | 0.217304215 | 0.031965517 | 0.002312644 |

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|---|---|---|---|---|---|---|---|---|
| 163 | 0.002345594 | 0.00411954 | 0.731478927 | 0.002929502 | 0.006938697 | 0.217215326 | 0.032390038 | 0.002582375 |
| 164 | 0.002327203 | 0.004073563 | 0.731493487 | 0.003045211 | 0.00708659 | 0.217408429 | 0.031992337 | 0.00257318 |
| 165 | 0.002329502 | 0.004045977 | 0.732350958 | 0.002865134 | 0.00702069 | 0.217439847 | 0.031594636 | 0.002353257 |
| 166 | 0.002295019 | 0.00595249 | 0.779038314 | 0.003608429 | 0.010196169 | 0.178060536 | 0.018256705 | 0.002592337 |
| 167 | 0.001547126 | 0.003881226 | 0.775214559 | 0.002367816 | 0.006998467 | 0.196785441 | 0.011582375 | 0.001622989 |
| 168 | 0.001504981 | 0.003636782 | 0.774332567 | 0.002468199 | 0.00671341 | 0.198109579 | 0.0114659 | 0.001768582 |
| 169 | 0.004516475 | 0.005412261 | 0.658881992 | 0.006629885 | 0.008150192 | 0.212267433 | 0.098751724 | 0.005390038 |
| 170 | 0.004629885 | 0.005455172 | 0.658843678 | 0.006585441 | 0.008271264 | 0.212025287 | 0.098705747 | 0.005483525 |
| 171 | 0.004481992 | 0.00569272 | 0.659852107 | 0.006673563 | 0.008151724 | 0.211287356 | 0.09823908 | 0.005621456 |
| 172 | 0.004557088 | 0.005629885 | 0.659145594 | 0.006731801 | 0.008744828 | 0.211362452 | 0.098046743 | 0.005781609 |
| 173 | 0.004675862 | 0.005843678 | 0.659592337 | 0.006360153 | 0.008305747 | 0.210988506 | 0.098605364 | 0.005628352 |
| 174 | 0.001648276 | 0.003845977 | 0.756729502 | 0.002221456 | 0.003966284 | 0.215950958 | 0.013867433 | 0.001770115 |
| 175 | 0.001488123 | 0.003915709 | 0.754914176 | 0.002284291 | 0.004073563 | 0.216951724 | 0.014591571 | 0.001780843 |
| 176 | 0.001557088 | 0.003918008 | 0.756717241 | 0.002088889 | 0.004016092 | 0.215154023 | 0.014672797 | 0.001875862 |
| 177 | 0.001272031 | 0.00334023 | 0.750560153 | 0.001799234 | 0.001139464 | 0.229381609 | 0.011193103 | 0.001314176 |
| 178 | 0.000211494 | 0.000436015 | 0.815682759 | 0.000268199 | 0.000305747 | 0.182842146 | 5.05747E-05 | 0.000203065 |
| 179 | 0.000587739 | 0.001673563 | 0.82076705 | 0.000672797 | 0.000531801 | 0.174353257 | 0.000774713 | 0.00063908 |
| 180 | 0.000586207 | 0.001754789 | 0.820947126 | 0.00063908 | 0.000548659 | 0.174270498 | 0.000668199 | 0.000585441 |
| 181 | 0.000581609 | 0.001643678 | 0.820898084 | 0.000670498 | 0.000570115 | 0.174239847 | 0.000829119 | 0.00056705 |
| 182 | 0.00055249 | 0.001721839 | 0.820737931 | 0.000682759 | 0.00055249 | 0.174422222 | 0.000758621 | 0.000571648 |
| 183 | 0.000524138 | 0.001649808 | 0.821162452 | 0.00070728 | 0.000574713 | 0.17399387 | 0.000842912 | 0.000544828 |
| 184 | 0.000572414 | 0.001666667 | 0.82122682 | 0.000632184 | 0.000568582 | 0.173992337 | 0.000772414 | 0.000568582 |
| 185 | 0.000121073 | 0.000267433 | 0.796537165 | 0.000123372 | 3.06513E-05 | 0.202780843 | 4.90421E-05 | 9.04215E-05 |

**Table S1.12** Proportion on ancestry for individuals of *Scinax squalirostris*, conditioned to environmental variables, inferred by Bayesian analysis of mitochondrial for 4.5RCP climatic conditions.

| | Inferred Cluster | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Individual** | **Cluster 1** | **Cluster 2** | **Cluster 3** | **Cluster 4** | **Cluster 5** | **Cluster 6** | **Cluster 7** | **Cluster 8** |
| 1 | 1.91E-06 | 0.00E+00 | 0.64226 | 2.10E-14 | 0.00E+00 | 0.03833 | 0.319371 | 3.96E-05 |
| 2 | 0.00E+00 | 0.00E+00 | 0.69923 | 2.00E-15 | 0.00E+00 | 0.00000 | 0.273014 | 2.78E-02 |
| 3 | 1.68E-07 | 7.60E-14 | 0.51629 | 4.38E-13 | 3.61E-07 | 0.00114 | 0.482572 | 0.00E+00 |
| 4 | 2.00E-15 | 1.01E-06 | 0.46421 | 6.03E-03 | 3.33E-12 | 0.01787 | 0.511246 | 6.39E-04 |
| 5 | 3.42E-08 | 0.00E+00 | 0.48759 | 2.63E-02 | 2.74E-09 | 0.02938 | 0.456125 | 5.74E-04 |
| 6 | 2.50E-02 | 4.01E-09 | 0.02723 | 2.70E-10 | 0.00E+00 | 0.00000 | 0.947788 | 1.52E-11 |
| 7 | 2.72E-04 | 3.68E-05 | 0.02333 | 9.00E-07 | 6.70E-03 | 0.00000 | 0.968184 | 1.47E-03 |
| 8 | 1.61E-10 | 5.57E-08 | 0.03495 | 7.43E-11 | 2.03E-02 | 0.00000 | 0.944371 | 3.52E-04 |
| 9 | 1.31E-12 | 0.00E+00 | 0.03458 | 4.73E-13 | 3.06E-03 | 0.00279 | 0.959567 | 9.50E-08 |
| 10 | 3.20E-04 | 0.00E+00 | 0.05797 | 0.00E+00 | 0.00E+00 | 0.00000 | 0.941701 | 7.87E-06 |
| 11 | 3.16E-12 | 0.00E+00 | 0.06618 | 2.44E-04 | 0.00E+00 | 0.00005 | 0.933531 | 1.17E-07 |
| 12 | 1.34E-07 | 0.00E+00 | 0.04238 | 1.57E-07 | 1.00E-15 | 0.00000 | 0.953744 | 3.87E-03 |
| 13 | 3.58E-06 | 0.00E+00 | 0.02941 | 0.00E+00 | 5.54E-11 | 0.00000 | 0.970587 | 1.31E-08 |
| 14 | 8.90E-10 | 0.00E+00 | 0.01236 | 2.97E-07 | 1.47E-05 | 0.00000 | 0.987625 | 1.00E-15 |
| 15 | 7.57E-09 | 5.66E-08 | 0.12568 | 2.23E-10 | 0.00E+00 | 0.11636 | 0.757961 | 4.62E-10 |
| 16 | 2.01E-09 | 2.67E-11 | 0.08749 | 5.80E-14 | 3.00E-15 | 0.00069 | 0.911817 | 0.00E+00 |
| 17 | 1.17E-07 | 0.00E+00 | 0.06781 | 3.18E-10 | 0.00E+00 | 0.00011 | 0.928914 | 3.17E-03 |
| 18 | 1.30E-03 | 0.00E+00 | 0.01116 | 1.14E-08 | 0.00E+00 | 0.00000 | 0.987538 | 6.20E-08 |
| 19 | 1.05E-03 | 0.00E+00 | 0.11430 | 0.00E+00 | 2.16E-03 | 0.00000 | 0.882490 | 2.69E-06 |
| 20 | 5.10E-14 | 0.00E+00 | 0.09867 | 1.72E-09 | 0.00E+00 | 0.00005 | 0.901284 | 8.00E-12 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 21 | 0.00E+00 | 3.58E-07 | 0.00361 | 1.00E-10 | 0.00E+00 | 0.00013 | 0.996258 | 0.00E+00 |
| 22 | 2.97E-04 | 1.60E-03 | 0.10186 | 0.00E+00 | 0.00E+00 | 0.01600 | 0.880233 | 0.00E+00 |
| 23 | 1.39E-12 | 0.00E+00 | 0.01663 | 4.20E-14 | 0.00E+00 | 0.00000 | 0.983366 | 9.90E-14 |
| 24 | 2.04E-05 | 5.97E-04 | 0.15773 | 4.90E-04 | 2.05E-06 | 0.00385 | 0.837305 | 0.00E+00 |
| 25 | 6.61E-09 | 1.43E-08 | 0.00119 | 2.45E-03 | 2.61E-08 | 0.00000 | 0.996348 | 1.07E-05 |
| 26 | 0.00E+00 | 1.54E-08 | 0.01390 | 4.66E-12 | 1.10E-03 | 0.00000 | 0.984999 | 1.09E-10 |
| 27 | 1.06E-08 | 1.45E-08 | 0.00570 | 1.22E-11 | 2.65E-10 | 0.00000 | 0.994303 | 4.06E-11 |
| 28 | 0.00E+00 | 1.43E-10 | 0.00237 | 1.09E-07 | 0.00E+00 | 0.00000 | 0.997602 | 2.39E-05 |
| 29 | 1.33E-04 | 1.23E-06 | 0.00000 | 8.72E-13 | 0.00E+00 | 0.00000 | 0.997751 | 2.11E-03 |
| 30 | 2.36E-05 | 6.26E-10 | 0.00009 | 2.72E-06 | 5.77E-11 | 0.00018 | 0.999701 | 1.25E-13 |
| 31 | 0.00E+00 | 1.85E-03 | 0.00433 | 6.40E-05 | 0.00E+00 | 0.00000 | 0.993758 | 1.30E-13 |
| 32 | 4.21E-11 | 3.65E-10 | 0.00005 | 5.78E-10 | 0.00E+00 | 0.00000 | 0.999949 | 0.00E+00 |
| 33 | 4.13E-11 | 0.00E+00 | 0.00000 | 0.00E+00 | 4.05E-11 | 0.00000 | 0.999999 | 6.83E-10 |
| 34 | 1.11E-09 | 2.11E-05 | 0.00000 | 2.73E-05 | 2.53E-11 | 0.00050 | 0.999448 | 1.00E-15 |
| 35 | 0.00E+00 | 0.00E+00 | 0.00000 | 1.62E-03 | 6.09E-10 | 0.00002 | 0.997196 | 1.16E-03 |
| 36 | 3.60E-14 | 1.04E-07 | 0.00319 | 5.35E-06 | 0.00E+00 | 0.00010 | 0.996699 | 0.00E+00 |
| 37 | 0.00E+00 | 5.82E-10 | 0.00105 | 4.47E-03 | 2.61E-04 | 0.00000 | 0.993775 | 4.42E-04 |
| 38 | 0.00E+00 | 0.00E+00 | 0.00000 | 1.50E-14 | 2.66E-04 | 0.00000 | 0.999730 | 3.92E-12 |
| 39 | 2.33E-05 | 0.00E+00 | 0.02095 | 0.00E+00 | 5.40E-03 | 0.00000 | 0.973627 | 0.00E+00 |
| 40 | 0.00E+00 | 7.90E-12 | 0.00000 | 0.00E+00 | 4.22E-06 | 0.00000 | 0.999996 | 0.00E+00 |
| 41 | 1.10E-09 | 1.90E-12 | 0.00007 | 2.60E-10 | 8.12E-09 | 0.00000 | 0.999927 | 1.83E-06 |
| 42 | 1.35E-09 | 1.68E-05 | 0.00000 | 0.00E+00 | 2.28E-04 | 0.00000 | 0.999755 | 7.12E-11 |
| 43 | 5.59E-06 | 0.00E+00 | 0.00000 | 0.00E+00 | 1.32E-05 | 0.00001 | 0.999973 | 7.00E-15 |
| 44 | 2.49E-09 | 0.00E+00 | 0.00000 | 3.70E-08 | 1.45E-06 | 0.00225 | 0.997746 | 2.49E-11 |
| 45 | 3.07E-09 | 0.00E+00 | 0.00000 | 1.96E-11 | 4.03E-05 | 0.00001 | 0.999930 | 1.63E-05 |
| 46 | 3.08E-10 | 5.14E-07 | 0.00053 | 0.00E+00 | 1.23E-12 | 0.00000 | 0.999266 | 2.01E-04 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 47 | 0.00E+00 | 5.47E-08 | 0.00001 | 5.98E-05 | 5.54E-08 | 0.00000 | 0.999925 | 7.86E-07 |
| 48 | 1.60E-06 | 0.00E+00 | 0.00000 | 0.00E+00 | 3.89E-04 | 0.00000 | 0.999605 | 4.98E-07 |
| 49 | 4.35E-05 | 0.00E+00 | 0.00000 | 3.15E-13 | 7.32E-04 | 0.00001 | 0.999219 | 1.65E-12 |
| 50 | 2.49E-06 | 1.84E-09 | 0.00000 | 1.72E-06 | 1.05E-08 | 0.00000 | 0.999996 | 1.88E-10 |
| 51 | 2.78E-09 | 6.96E-08 | 0.00000 | 2.57E-07 | 8.87E-04 | 0.00000 | 0.999110 | 1.38E-09 |
| 52 | 8.00E-15 | 0.00E+00 | 0.00000 | 3.32E-06 | 2.74E-07 | 0.00000 | 0.999996 | 2.95E-11 |
| 53 | 6.94E-13 | 0.00E+00 | 0.00011 | 0.00E+00 | 5.77E-05 | 0.00011 | 0.999709 | 8.52E-06 |
| 54 | 2.68E-05 | 2.31E-06 | 0.00018 | 1.04E-10 | 1.35E-08 | 0.00022 | 0.999446 | 1.25E-04 |
| 55 | 8.88E-06 | 3.15E-05 | 0.00000 | 3.28E-05 | 0.00E+00 | 0.00000 | 0.999927 | 1.06E-07 |
| 56 | 6.55E-05 | 3.09E-09 | 0.00001 | 1.99E-06 | 5.69E-10 | 0.00000 | 0.999918 | 2.16E-10 |
| 57 | 1.49E-07 | 5.90E-04 | 0.00005 | 1.47E-08 | 5.86E-11 | 0.00000 | 0.999357 | 1.65E-12 |
| 58 | 0.00E+00 | 0.00E+00 | 0.00010 | 0.00E+00 | 1.52E-04 | 0.00000 | 0.999749 | 0.00E+00 |
| 59 | 0.00E+00 | 5.16E-13 | 0.00014 | 0.00E+00 | 3.63E-05 | 0.00000 | 0.999828 | 2.00E-14 |
| 60 | 5.37E-13 | 0.00E+00 | 0.00000 | 2.42E-07 | 9.37E-08 | 0.00000 | 1.000000 | 5.56E-09 |
| 61 | 3.66E-13 | 0.00E+00 | 0.00000 | 8.23E-06 | 0.00E+00 | 0.00000 | 0.999992 | 0.00E+00 |
| 62 | 4.85E-09 | 6.60E-04 | 0.00000 | 1.93E-07 | 0.00E+00 | 0.00000 | 0.999336 | 4.11E-07 |
| 63 | 1.28E-08 | 8.18E-04 | 0.00000 | 3.52E-05 | 7.60E-07 | 0.00018 | 0.998856 | 1.06E-04 |
| 64 | 1.10E-13 | 1.17E-03 | 0.00000 | 0.00E+00 | 0.00E+00 | 0.00000 | 0.998827 | 0.00E+00 |
| 65 | 1.05E-08 | 2.04E-07 | 0.00000 | 0.00E+00 | 5.96E-09 | 0.00014 | 0.999809 | 5.00E-05 |
| 66 | 5.99E-05 | 4.01E-06 | 0.00000 | 0.00E+00 | 5.10E-14 | 0.00012 | 0.999821 | 2.44E-12 |
| 67 | 4.65E-08 | 1.73E-08 | 0.00122 | 3.60E-10 | 3.19E-10 | 0.00000 | 0.998780 | 6.50E-11 |
| 68 | 0.00E+00 | 0.00E+00 | 0.00003 | 3.89E-05 | 5.43E-04 | 0.00000 | 0.999360 | 2.84E-05 |
| 69 | 0.00E+00 | 2.25E-04 | 0.00001 | 0.00E+00 | 2.68E-08 | 0.00000 | 0.999769 | 0.00E+00 |
| 70 | 1.31E-04 | 1.55E-04 | 0.00041 | 7.25E-12 | 1.69E-03 | 0.00000 | 0.997614 | 4.29E-07 |
| 71 | 3.00E-10 | 0.00E+00 | 0.00000 | 1.74E-05 | 0.00E+00 | 0.00000 | 0.999978 | 0.00E+00 |
| 72 | 8.56E-11 | 0.00E+00 | 0.00000 | 1.57E-10 | 2.76E-05 | 0.00070 | 0.996372 | 2.90E-03 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 73 | 5.13E-09 | 2.21E-13 | 0.00000 | 0.00E+00 | 3.28E-05 | 0.00001 | 0.999951 | 1.06E-06 |
| 74 | 2.01E-12 | 0.00E+00 | 0.00002 | 2.17E-03 | 0.00E+00 | 0.00002 | 0.997790 | 3.64E-11 |
| 75 | 1.86E-08 | 0.00E+00 | 0.00000 | 2.46E-05 | 1.61E-03 | 0.00000 | 0.996762 | 1.60E-03 |
| 76 | 0.00E+00 | 1.06E-10 | 0.00000 | 0.00E+00 | 1.53E-12 | 0.00000 | 0.999997 | 1.11E-07 |
| 77 | 2.34E-05 | 0.00E+00 | 0.00004 | 9.15E-05 | 4.32E-08 | 0.00227 | 0.997582 | 1.61E-10 |
| 78 | 1.06E-03 | 2.04E-08 | 0.00040 | 1.50E-14 | 8.13E-06 | 0.00000 | 0.998531 | 1.50E-11 |
| 79 | 5.28E-06 | 0.00E+00 | 0.00405 | 0.00E+00 | 2.05E-09 | 0.00000 | 0.995948 | 0.00E+00 |
| 80 | 0.00E+00 | 0.00E+00 | 0.00000 | 0.00E+00 | 1.10E-09 | 0.00054 | 0.999464 | 0.00E+00 |
| 81 | 0.00E+00 | 0.00E+00 | 0.00036 | 1.46E-07 | 1.13E-04 | 0.00000 | 0.999526 | 6.88E-11 |
| 82 | 0.00E+00 | 0.00E+00 | 0.00057 | 4.05E-03 | 2.61E-10 | 0.00000 | 0.995373 | 1.69E-07 |
| 83 | 1.86E-09 | 3.12E-05 | 0.00000 | 0.00E+00 | 2.35E-03 | 0.00001 | 0.997607 | 0.00E+00 |
| 84 | 4.11E-13 | 2.75E-06 | 0.00000 | 3.38E-05 | 4.01E-09 | 0.00242 | 0.997542 | 0.00E+00 |
| 85 | 3.82E-04 | 2.42E-07 | 0.00000 | 3.00E-15 | 2.14E-05 | 0.00281 | 0.996789 | 0.00E+00 |
| 86 | 5.15E-05 | 2.18E-03 | 0.00002 | 3.59E-09 | 4.30E-14 | 0.00000 | 0.997748 | 7.27E-08 |
| 87 | 5.20E-11 | 1.21E-05 | 0.00061 | 1.21E-07 | 0.00E+00 | 0.00000 | 0.999375 | 4.07E-07 |
| 88 | 0.00E+00 | 3.36E-04 | 0.00000 | 3.12E-07 | 0.00E+00 | 0.00000 | 0.999663 | 5.35E-08 |
| 89 | 0.00E+00 | 2.29E-05 | 0.00000 | 9.92E-11 | 3.14E-12 | 0.00000 | 0.999977 | 1.28E-12 |
| 90 | 0.00E+00 | 8.68E-04 | 0.00001 | 0.00E+00 | 3.64E-03 | 0.00000 | 0.995120 | 3.66E-04 |
| 91 | 9.44E-04 | 1.68E-04 | 0.00000 | 2.00E-15 | 2.47E-03 | 0.00000 | 0.996421 | 1.40E-09 |
| 92 | 1.20E-03 | 4.65E-04 | 0.00000 | 5.31E-05 | 2.05E-07 | 0.00000 | 0.998275 | 2.71E-06 |
| 93 | 1.21E-06 | 1.17E-04 | 0.00000 | 7.10E-14 | 8.43E-10 | 0.00000 | 0.999881 | 8.50E-14 |
| 94 | 0.00E+00 | 1.07E-04 | 0.00124 | 0.00E+00 | 0.00E+00 | 0.00000 | 0.998655 | 1.20E-11 |
| 95 | 0.00E+00 | 1.39E-05 | 0.00000 | 1.33E-09 | 5.58E-04 | 0.00000 | 0.999409 | 1.82E-05 |
| 96 | 0.00E+00 | 2.87E-07 | 0.00000 | 9.00E-15 | 0.00E+00 | 0.00000 | 0.999998 | 4.43E-07 |
| 97 | 3.26E-08 | 8.75E-04 | 0.00000 | 1.81E-03 | 4.54E-05 | 0.00000 | 0.997267 | 5.38E-10 |
| 98 | 1.30E-05 | 3.42E-06 | 0.00000 | 4.15E-07 | 3.05E-05 | 0.00000 | 0.999950 | 3.34E-09 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 99 | 1.08E-04 | 3.54E-03 | 0.00000 | 2.02E-07 | 1.76E-12 | 0.00033 | 0.995976 | 3.99E-05 |
| 100 | 2.04E-11 | 0.00E+00 | 0.00000 | 2.76E-05 | 1.82E-06 | 0.00000 | 0.999970 | 0.00E+00 |
| 101 | 0.00E+00 | 5.46E-04 | 0.00000 | 0.00E+00 | 2.42E-06 | 0.00000 | 0.999452 | 4.16E-13 |
| 102 | 0.00E+00 | 1.17E-05 | 0.00000 | 0.00E+00 | 1.13E-04 | 0.00000 | 0.999876 | 3.56E-10 |
| 103 | 5.24E-03 | 8.92E-04 | 0.00001 | 1.00E-15 | 0.00E+00 | 0.00000 | 0.993866 | 1.68E-08 |
| 104 | 0.00E+00 | 2.37E-06 | 0.00000 | 3.53E-06 | 2.38E-05 | 0.00000 | 0.997673 | 2.30E-03 |
| 105 | 2.14E-09 | 1.02E-03 | 0.00000 | 3.00E-14 | 0.00E+00 | 0.00000 | 0.998974 | 4.08E-08 |
| 106 | 0.00E+00 | 6.70E-06 | 0.00000 | 2.52E-07 | 5.21E-08 | 0.00000 | 0.999499 | 4.93E-04 |
| 107 | 7.00E-15 | 4.80E-03 | 0.00163 | 2.34E-02 | 3.14E-05 | 0.00000 | 0.970175 | 0.00E+00 |
| 108 | 1.46E-07 | 3.60E-04 | 0.01305 | 0.00E+00 | 1.02E-02 | 0.00002 | 0.976198 | 1.58E-04 |
| 109 | 0.00E+00 | 4.60E-11 | 0.00000 | 0.00E+00 | 2.77E-06 | 0.00627 | 0.993731 | 0.00E+00 |
| 110 | 1.61E-11 | 0.00E+00 | 0.00000 | 0.00E+00 | 4.81E-03 | 0.00000 | 0.995184 | 2.34E-06 |
| 111 | 3.48E-09 | 9.78E-09 | 0.00000 | 1.97E-12 | 4.33E-03 | 0.00000 | 0.995664 | 0.00E+00 |
| 112 | 0.00E+00 | 1.04E-04 | 0.00000 | 1.49E-04 | 2.63E-02 | 0.00000 | 0.973394 | 2.33E-06 |
| 113 | 8.70E-08 | 3.88E-07 | 0.00292 | 3.80E-14 | 9.35E-05 | 0.00000 | 0.996987 | 0.00E+00 |
| 114 | 3.79E-04 | 3.08E-07 | 0.00010 | 1.69E-03 | 2.81E-03 | 0.00553 | 0.986583 | 2.91E-03 |
| 115 | 2.03E-13 | 3.61E-12 | 0.00061 | 1.26E-09 | 1.86E-10 | 0.00000 | 0.999394 | 0.00E+00 |
| 116 | 0.00E+00 | 4.93E-05 | 0.00579 | 3.78E-05 | 3.51E-08 | 0.00000 | 0.992790 | 1.33E-03 |
| 117 | 9.83E-05 | 1.58E-09 | 0.00029 | 1.02E-06 | 1.01E-04 | 0.00009 | 0.997880 | 1.54E-03 |
| 118 | 1.45E-13 | 1.81E-07 | 0.00022 | 6.45E-05 | 1.80E-03 | 0.00145 | 0.996459 | 1.15E-06 |
| 119 | 1.43E-02 | 0.00E+00 | 0.04467 | 0.00E+00 | 2.06E-08 | 0.00000 | 0.941030 | 2.53E-05 |
| 120 | 0.00E+00 | 6.96E-04 | 0.00293 | 1.20E-03 | 1.59E-04 | 0.00000 | 0.995011 | 0.00E+00 |
| 121 | 1.77E-05 | 8.15E-07 | 0.00467 | 0.00E+00 | 8.16E-03 | 0.00001 | 0.987145 | 4.61E-12 |
| 122 | 4.67E-05 | 0.00E+00 | 0.00001 | 2.11E-09 | 2.01E-03 | 0.00000 | 0.997934 | 0.00E+00 |
| 123 | 0.00E+00 | 1.53E-03 | 0.00000 | 0.00E+00 | 6.76E-11 | 0.00000 | 0.998466 | 2.37E-10 |
| 124 | 4.46E-05 | 1.83E-03 | 0.97345 | 1.12E-03 | 1.48E-06 | 0.01308 | 0.010431 | 4.71E-05 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 125 | 1.05E-09 | 1.76E-04 | 0.96958 | 1.81E-06 | 7.67E-03 | 0.00973 | 0.012836 | 1.13E-05 |
| 126 | 2.17E-04 | 1.17E-05 | 0.98084 | 2.04E-05 | 8.57E-04 | 0.00007 | 0.017374 | 6.06E-04 |
| 127 | 2.69E-02 | 4.97E-05 | 0.93871 | 1.17E-05 | 1.29E-05 | 0.00279 | 0.031504 | 1.73E-05 |
| 128 | 0.00E+00 | 1.88E-06 | 0.93831 | 3.39E-04 | 5.00E-15 | 0.01277 | 0.048564 | 1.47E-05 |
| 129 | 1.97E-07 | 4.47E-03 | 0.94214 | 2.21E-05 | 1.00E-14 | 0.00188 | 0.051485 | 1.00E-15 |
| 130 | 2.61E-08 | 2.50E-08 | 0.99510 | 1.31E-04 | 4.22E-09 | 0.00002 | 0.004584 | 1.67E-04 |
| 131 | 0.00E+00 | 1.78E-08 | 0.99936 | 1.21E-08 | 1.01E-04 | 0.00038 | 0.000165 | 0.00E+00 |
| 132 | 1.67E-06 | 2.06E-06 | 0.99447 | 1.00E-05 | 4.79E-07 | 0.00000 | 0.005511 | 8.14E-09 |
| 133 | 0.00E+00 | 4.83E-04 | 0.99644 | 2.92E-06 | 4.10E-07 | 0.00073 | 0.002299 | 4.06E-05 |
| 134 | 1.08E-06 | 1.64E-06 | 0.99974 | 0.00E+00 | 2.87E-07 | 0.00026 | 0.000001 | 4.97E-07 |
| 135 | 0.00E+00 | 1.06E-04 | 0.99848 | 1.95E-12 | 3.23E-11 | 0.00002 | 0.000573 | 8.27E-04 |
| 136 | 3.12E-05 | 4.45E-03 | 0.99494 | 0.00E+00 | 0.00E+00 | 0.00001 | 0.000569 | 2.90E-07 |
| 137 | 5.17E-07 | 1.05E-06 | 0.99407 | 1.00E-15 | 8.00E-04 | 0.00026 | 0.004864 | 1.39E-09 |
| 138 | 1.06E-11 | 4.08E-09 | 0.99945 | 1.14E-10 | 3.33E-04 | 0.00008 | 0.000137 | 1.10E-14 |
| 139 | 2.34E-08 | 4.89E-03 | 0.99444 | 5.92E-04 | 4.76E-07 | 0.00000 | 0.000075 | 6.41E-10 |
| 140 | 0.00E+00 | 1.42E-06 | 0.99548 | 3.41E-05 | 5.05E-12 | 0.00096 | 0.003524 | 7.77E-11 |
| 141 | 9.85E-05 | 7.14E-08 | 0.99808 | 4.45E-09 | 1.82E-03 | 0.00000 | 0.000000 | 0.00E+00 |
| 142 | 3.47E-10 | 3.34E-04 | 0.99965 | 1.13E-05 | 3.14E-10 | 0.00000 | 0.000000 | 7.37E-12 |
| 143 | 1.07E-13 | 1.48E-05 | 0.99982 | 1.18E-09 | 1.48E-12 | 0.00015 | 0.000011 | 9.41E-06 |
| 144 | 5.55E-05 | 1.64E-04 | 0.99665 | 4.22E-06 | 4.40E-12 | 0.00098 | 0.002145 | 1.38E-10 |
| 145 | 0.00E+00 | 2.15E-03 | 0.99705 | 4.58E-04 | 1.36E-07 | 0.00000 | 0.000337 | 0.00E+00 |
| 146 | 0.00E+00 | 8.96E-05 | 0.99870 | 0.00E+00 | 9.12E-09 | 0.00000 | 0.001208 | 3.49E-07 |
| 147 | 5.59E-05 | 1.61E-05 | 0.99418 | 1.09E-04 | 2.36E-03 | 0.00029 | 0.002971 | 2.34E-05 |
| 148 | 1.46E-09 | 1.84E-06 | 0.99585 | 1.39E-04 | 0.00E+00 | 0.00000 | 0.004008 | 7.56E-12 |
| 149 | 2.00E-15 | 1.08E-13 | 0.99941 | 1.56E-04 | 1.64E-04 | 0.00023 | 0.000045 | 2.24E-09 |
| 150 | 4.03E-04 | 5.09E-04 | 0.98825 | 3.29E-06 | 0.00E+00 | 0.00996 | 0.000663 | 2.09E-04 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 151 | 2.30E-10 | 1.69E-05 | 0.99536 | 2.07E-05 | 6.60E-14 | 0.00004 | 0.004556 | 0.00E+00 |
| 152 | 2.52E-08 | 3.41E-10 | 0.99822 | 4.19E-07 | 2.40E-14 | 0.00000 | 0.001769 | 7.96E-06 |
| 153 | 2.23E-12 | 6.76E-04 | 0.99360 | 5.92E-04 | 8.73E-11 | 0.00000 | 0.004410 | 7.21E-04 |
| 154 | 7.43E-08 | 4.90E-05 | 0.99296 | 3.21E-07 | 6.21E-04 | 0.00000 | 0.006373 | 0.00E+00 |
| 155 | 8.85E-05 | 6.91E-12 | 0.99966 | 2.13E-06 | 4.21E-11 | 0.00005 | 0.000199 | 8.93E-07 |
| 156 | 4.34E-08 | 5.54E-08 | 0.99598 | 7.86E-08 | 9.96E-06 | 0.00000 | 0.004014 | 2.86E-07 |
| 157 | 0.00E+00 | 2.00E-15 | 0.98793 | 3.94E-13 | 6.75E-08 | 0.00625 | 0.005822 | 3.47E-06 |
| 158 | 7.56E-08 | 2.10E-11 | 0.99289 | 0.00E+00 | 4.86E-04 | 0.00193 | 0.004696 | 2.14E-13 |
| 159 | 4.24E-05 | 1.00E-15 | 0.99710 | 1.98E-03 | 2.95E-09 | 0.00000 | 0.000864 | 0.00E+00 |
| 160 | 0.00E+00 | 0.00E+00 | 0.95787 | 6.10E-10 | 1.79E-12 | 0.00000 | 0.042127 | 0.00E+00 |
| 161 | 9.29E-03 | 5.65E-04 | 0.87225 | 0.00E+00 | 8.64E-06 | 0.00417 | 0.113718 | 1.55E-06 |
| 162 | 4.07E-11 | 7.75E-08 | 0.93806 | 1.97E-02 | 2.39E-06 | 0.00050 | 0.041711 | 9.26E-08 |
| 163 | 2.32E-05 | 1.07E-08 | 0.98707 | 5.08E-07 | 1.67E-04 | 0.00034 | 0.012394 | 0.00E+00 |
| 164 | 4.67E-04 | 2.85E-04 | 0.97074 | 3.42E-12 | 3.42E-12 | 0.02429 | 0.004215 | 0.00E+00 |
| 165 | 0.00E+00 | 1.60E-05 | 0.84311 | 4.44E-03 | 4.12E-05 | 0.00000 | 0.152392 | 1.86E-09 |
| 166 | 0.00E+00 | 1.02E-08 | 0.90739 | 1.46E-04 | 7.80E-04 | 0.05084 | 0.040847 | 1.17E-11 |
| 167 | 7.50E-10 | 4.40E-11 | 0.96926 | 2.00E-15 | 2.01E-05 | 0.00009 | 0.012411 | 1.82E-02 |
| 168 | 1.00E-15 | 1.45E-06 | 0.98819 | 0.00E+00 | 1.00E-15 | 0.00014 | 0.000597 | 1.11E-02 |
| 169 | 1.13E-05 | 0.00E+00 | 0.87566 | 3.88E-04 | 5.96E-04 | 0.00378 | 0.119570 | 1.09E-06 |
| 170 | 1.97E-09 | 6.08E-09 | 0.60549 | 0.00E+00 | 5.16E-10 | 0.00129 | 0.361592 | 3.16E-02 |
| 171 | 1.86E-02 | 1.01E-06 | 0.88443 | 3.73E-04 | 8.82E-11 | 0.00002 | 0.096559 | 1.63E-05 |
| 172 | 1.73E-06 | 2.93E-03 | 0.72747 | 1.87E-02 | 1.63E-05 | 0.00000 | 0.222391 | 2.85E-02 |
| 173 | 7.73E-03 | 9.59E-05 | 0.62929 | 2.04E-02 | 6.73E-13 | 0.00000 | 0.342456 | 2.71E-08 |
| 174 | 2.15E-12 | 1.47E-05 | 0.98481 | 3.27E-03 | 1.79E-12 | 0.00002 | 0.011884 | 8.22E-11 |
| 175 | 3.04E-06 | 0.00E+00 | 0.94448 | 9.18E-12 | 1.49E-03 | 0.00000 | 0.052625 | 1.40E-03 |
| 176 | 3.85E-10 | 0.00E+00 | 0.96866 | 9.86E-03 | 7.91E-07 | 0.00023 | 0.006825 | 1.44E-02 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 177 | 1.30E-05 | 2.11E-02 | 0.95519 | 1.00E-15 | 2.25E-07 | 0.00091 | 0.022782 | 3.43E-12 |
| 178 | 2.53E-06 | 5.73E-08 | 0.99983 | 4.68E-06 | 4.27E-13 | 0.00015 | 0.000001 | 1.83E-05 |
| 179 | 4.70E-05 | 1.25E-09 | 0.99739 | 3.12E-12 | 2.30E-11 | 0.00237 | 0.000191 | 1.25E-06 |
| 180 | 1.07E-05 | 3.20E-04 | 0.99819 | 1.35E-08 | 2.76E-04 | 0.00086 | 0.000087 | 2.59E-04 |
| 181 | 5.33E-07 | 6.22E-08 | 0.99845 | 9.16E-04 | 5.00E-15 | 0.00005 | 0.000584 | 2.00E-14 |
| 182 | 0.00E+00 | 1.71E-06 | 0.99870 | 0.00E+00 | 9.04E-04 | 0.00039 | 0.000000 | 6.58E-10 |
| 183 | 4.00E-15 | 2.49E-03 | 0.99299 | 3.18E-03 | 2.57E-08 | 0.00001 | 0.001220 | 1.00E-04 |
| 184 | 1.95E-06 | 1.51E-06 | 0.99955 | 9.09E-11 | 0.00E+00 | 0.00000 | 0.000443 | 1.80E-14 |
| 185 | 0.00E+00 | 6.72E-05 | 0.99987 | 4.93E-09 | 0.00E+00 | 0.00007 | 0.000000 | 4.85E-10 |

**Table S1.13** Proportion on ancestry for individuals of *Scinax squalirostris*, conditioned to environmental variables, inferred by Bayesian analysis of mitochondrial for 8.5RCP climatic conditions.

| | Inferred Cluster | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Individual** | **Cluster 1** | **Cluster 2** | **Cluster 3** | **Cluster 4** | **Cluster 5** | **Cluster 6** | **Cluster 7** | **Cluster 8** |
| 1 | 5.88E-07 | 2.44E-07 | 1.72E-01 | 3.67E-03 | 2.30E-12 | 1.25E-05 | 8.24E-01 | 1.30E-08 |
| 2 | 4.06E-13 | 1.00E-15 | 2.71E-01 | 7.83E-07 | 0.00E+00 | 6.26E-07 | 7.29E-01 | 0.00E+00 |
| 3 | 7.13E-05 | 8.92E-13 | 1.67E-01 | 0.00E+00 | 2.29E-06 | 6.39E-05 | 8.32E-01 | 3.45E-13 |
| 4 | 2.60E-11 | 6.28E-05 | 3.94E-01 | 1.28E-03 | 2.26E-12 | 2.85E-03 | 6.02E-01 | 0.00E+00 |
| 5 | 4.78E-12 | 2.03E-05 | 3.32E-01 | 2.05E-02 | 0.00E+00 | 1.46E-08 | 6.47E-01 | 1.41E-07 |
| 6 | 3.84E-09 | 0.00E+00 | 2.88E-02 | 4.73E-09 | 3.71E-08 | 2.18E-06 | 9.71E-01 | 2.20E-14 |
| 7 | 0.00E+00 | 6.34E-12 | 6.74E-03 | 3.85E-08 | 0.00E+00 | 6.11E-07 | 9.93E-01 | 4.19E-10 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8 | 1.90E-14 | 1.05E-13 | 1.45E-02 | 0.00E+00 | 4.34E-12 | 0.00E+00 | 9.85E-01 | 2.66E-13 |
| 9 | 0.00E+00 | 3.35E-04 | 1.31E-02 | 1.46E-04 | 5.97E-05 | 8.44E-11 | 9.86E-01 | 3.74E-10 |
| 10 | 9.62E-04 | 8.39E-09 | 1.69E-02 | 7.75E-05 | 3.43E-04 | 2.83E-09 | 9.80E-01 | 1.49E-03 |
| 11 | 9.40E-03 | 1.59E-03 | 6.12E-03 | 4.02E-03 | 2.06E-03 | 9.37E-09 | 9.77E-01 | 4.28E-06 |
| 12 | 9.70E-07 | 1.93E-07 | 1.03E-02 | 2.00E-15 | 8.25E-05 | 0.00E+00 | 9.89E-01 | 2.19E-04 |
| 13 | 4.73E-08 | 3.72E-11 | 7.69E-03 | 2.01E-10 | 2.99E-11 | 0.00E+00 | 9.92E-01 | 0.00E+00 |
| 14 | 5.30E-05 | 1.80E-03 | 2.19E-03 | 3.04E-04 | 3.56E-04 | 2.40E-14 | 9.92E-01 | 2.96E-03 |
| 15 | 6.50E-14 | 0.00E+00 | 4.77E-03 | 9.66E-07 | 8.65E-06 | 1.64E-03 | 9.94E-01 | 5.18E-10 |
| 16 | 1.02E-03 | 0.00E+00 | 5.25E-03 | 0.00E+00 | 0.00E+00 | 1.73E-03 | 9.92E-01 | 1.99E-07 |
| 17 | 5.66E-03 | 4.36E-13 | 3.70E-03 | 5.37E-10 | 9.10E-14 | 2.08E-03 | 9.89E-01 | 2.47E-05 |
| 18 | 0.00E+00 | 0.00E+00 | 1.61E-02 | 0.00E+00 | 2.00E-15 | 2.19E-03 | 9.82E-01 | 0.00E+00 |
| 19 | 3.98E-08 | 2.88E-04 | 1.61E-03 | 9.07E-13 | 0.00E+00 | 1.02E-04 | 9.98E-01 | 4.00E-15 |
| 20 | 6.49E-04 | 1.07E-12 | 1.94E-02 | 7.42E-05 | 2.02E-09 | 6.61E-04 | 9.79E-01 | 0.00E+00 |
| 21 | 8.70E-14 | 1.92E-07 | 8.47E-03 | 0.00E+00 | 0.00E+00 | 2.38E-04 | 9.91E-01 | 7.02E-13 |
| 22 | 0.00E+00 | 7.51E-11 | 4.18E-03 | 0.00E+00 | 2.34E-02 | 2.39E-04 | 9.72E-01 | 9.43E-07 |
| 23 | 2.55E-06 | 4.11E-06 | 5.33E-03 | 0.00E+00 | 0.00E+00 | 2.08E-05 | 9.90E-01 | 4.66E-03 |
| 24 | 0.00E+00 | 1.20E-14 | 3.83E-02 | 0.00E+00 | 0.00E+00 | 7.25E-05 | 9.62E-01 | 1.84E-12 |
| 25 | 0.00E+00 | 1.61E-06 | 2.05E-06 | 2.32E-05 | 0.00E+00 | 3.12E-11 | 1.00E+00 | 1.48E-11 |
| 26 | 2.68E-06 | 2.22E-04 | 2.35E-04 | 1.68E-12 | 1.35E-11 | 1.12E-07 | 9.99E-01 | 6.07E-05 |
| 27 | 0.00E+00 | 0.00E+00 | 4.88E-04 | 2.83E-07 | 0.00E+00 | 3.20E-08 | 9.99E-01 | 4.97E-04 |
| 28 | 1.16E-11 | 2.02E-08 | 1.37E-05 | 5.42E-06 | 0.00E+00 | 0.00E+00 | 1.00E+00 | 5.47E-05 |
| 29 | 7.29E-05 | 2.90E-14 | 1.14E-04 | 1.00E-15 | 0.00E+00 | 0.00E+00 | 1.00E+00 | 3.48E-08 |
| 30 | 8.65E-06 | 6.16E-09 | 1.70E-03 | 0.00E+00 | 0.00E+00 | 1.60E-09 | 9.98E-01 | 5.93E-09 |
| 31 | 5.21E-07 | 0.00E+00 | 3.16E-09 | 0.00E+00 | 2.15E-10 | 1.71E-10 | 9.99E-01 | 8.70E-04 |
| 32 | 6.90E-07 | 2.56E-06 | 3.25E-08 | 2.39E-06 | 7.68E-04 | 1.64E-03 | 9.98E-01 | 1.65E-11 |
| 33 | 3.97E-11 | 8.08E-11 | 7.26E-04 | 4.00E-14 | 1.16E-04 | 1.02E-05 | 9.99E-01 | 6.17E-10 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 34 | 2.02E-05 | 5.98E-13 | 1.61E-03 | 1.99E-07 | 0.00E+00 | 6.37E-11 | 9.98E-01 | 5.49E-05 |
| 35 | 6.52E-08 | 1.34E-04 | 1.57E-07 | 1.44E-06 | 3.06E-11 | 1.71E-09 | 1.00E+00 | 5.37E-06 |
| 36 | 4.55E-09 | 3.03E-13 | 1.25E-04 | 0.00E+00 | 0.00E+00 | 5.49E-11 | 1.00E+00 | 1.68E-04 |
| 37 | 0.00E+00 | 1.12E-05 | 1.50E-03 | 0.00E+00 | 0.00E+00 | 3.74E-09 | 9.98E-01 | 8.00E-15 |
| 38 | 0.00E+00 | 9.74E-11 | 3.95E-04 | 1.20E-09 | 2.67E-08 | 3.94E-03 | 9.96E-01 | 0.00E+00 |
| 39 | 0.00E+00 | 0.00E+00 | 1.25E-05 | 1.51E-03 | 3.88E-10 | 3.59E-06 | 9.98E-01 | 7.00E-15 |
| 40 | 0.00E+00 | 0.00E+00 | 7.37E-07 | 2.17E-03 | 2.34E-09 | 4.97E-04 | 9.97E-01 | 9.70E-06 |
| 41 | 1.15E-12 | 7.07E-05 | 2.80E-08 | 0.00E+00 | 1.34E-04 | 0.00E+00 | 1.00E+00 | 0.00E+00 |
| 42 | 1.34E-04 | 2.90E-07 | 3.64E-07 | 2.83E-08 | 1.31E-08 | 3.70E-08 | 1.00E+00 | 1.16E-09 |
| 43 | 1.98E-07 | 0.00E+00 | 0.00E+00 | 1.06E-06 | 1.08E-04 | 2.07E-06 | 1.00E+00 | 1.30E-14 |
| 44 | 4.13E-05 | 0.00E+00 | 1.59E-06 | 3.19E-11 | 7.01E-04 | 2.59E-06 | 9.99E-01 | 2.30E-12 |
| 45 | 0.00E+00 | 0.00E+00 | 7.00E-15 | 3.28E-13 | 3.72E-05 | 3.27E-12 | 1.00E+00 | 3.95E-11 |
| 46 | 3.93E-12 | 3.33E-07 | 1.60E-14 | 2.53E-09 | 2.78E-07 | 5.77E-07 | 1.00E+00 | 3.23E-08 |
| 47 | 3.91E-10 | 0.00E+00 | 7.59E-06 | 1.29E-04 | 6.71E-07 | 3.34E-12 | 1.00E+00 | 0.00E+00 |
| 48 | 0.00E+00 | 5.00E-15 | 3.94E-04 | 4.06E-10 | 2.08E-07 | 7.93E-06 | 1.00E+00 | 7.94E-09 |
| 49 | 1.04E-04 | 0.00E+00 | 1.84E-03 | 3.74E-13 | 2.51E-04 | 0.00E+00 | 9.98E-01 | 3.16E-08 |
| 50 | 1.14E-06 | 4.83E-07 | 1.21E-05 | 4.85E-05 | 1.28E-04 | 1.07E-10 | 1.00E+00 | 1.12E-05 |
| 51 | 9.75E-10 | 1.13E-10 | 6.88E-07 | 1.05E-09 | 1.69E-05 | 4.68E-07 | 1.00E+00 | 3.74E-04 |
| 52 | 2.24E-07 | 3.99E-09 | 3.70E-11 | 0.00E+00 | 3.63E-11 | 1.90E-11 | 1.00E+00 | 2.33E-06 |
| 53 | 0.00E+00 | 0.00E+00 | 9.83E-07 | 7.70E-09 | 5.80E-08 | 2.37E-10 | 1.00E+00 | 6.95E-05 |
| 54 | 0.00E+00 | 1.37E-11 | 1.27E-08 | 5.17E-04 | 4.85E-11 | 3.52E-06 | 9.99E-01 | 0.00E+00 |
| 55 | 1.00E-15 | 5.31E-05 | 2.64E-12 | 5.72E-05 | 5.27E-13 | 9.46E-06 | 1.00E+00 | 4.01E-06 |
| 56 | 3.86E-13 | 2.74E-10 | 0.00E+00 | 2.81E-05 | 2.33E-05 | 0.00E+00 | 1.00E+00 | 6.63E-05 |
| 57 | 1.21E-08 | 0.00E+00 | 2.67E-08 | 1.37E-05 | 3.61E-12 | 5.21E-06 | 1.00E+00 | 3.94E-07 |
| 58 | 2.83E-07 | 2.61E-06 | 3.90E-14 | 1.58E-12 | 0.00E+00 | 1.63E-09 | 1.00E+00 | 1.53E-08 |
| 59 | 0.00E+00 | 1.18E-04 | 0.00E+00 | 3.45E-06 | 1.74E-06 | 4.89E-08 | 1.00E+00 | 3.93E-06 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 60 | 3.54E-06 | 0.00E+00 | 0.00E+00 | 4.11E-12 | 1.62E-04 | 1.17E-04 | 1.00E+00 | 6.88E-07 |
| 61 | 9.13E-07 | 9.54E-07 | 0.00E+00 | 0.00E+00 | 4.66E-09 | 0.00E+00 | 1.00E+00 | 0.00E+00 |
| 62 | 7.10E-10 | 1.06E-11 | 4.31E-11 | 1.79E-04 | 1.86E-10 | 2.48E-12 | 1.00E+00 | 1.35E-07 |
| 63 | 2.79E-05 | 3.02E-07 | 0.00E+00 | 2.58E-11 | 3.62E-12 | 2.59E-07 | 1.00E+00 | 3.16E-05 |
| 64 | 2.00E-15 | 5.57E-09 | 0.00E+00 | 1.32E-05 | 8.40E-08 | 1.50E-06 | 1.00E+00 | 4.67E-04 |
| 65 | 2.48E-09 | 4.64E-04 | 2.67E-08 | 8.34E-05 | 1.07E-04 | 5.70E-09 | 9.99E-01 | 1.13E-09 |
| 66 | 0.00E+00 | 1.15E-03 | 7.52E-07 | 0.00E+00 | 0.00E+00 | 2.90E-14 | 9.99E-01 | 3.06E-05 |
| 67 | 0.00E+00 | 0.00E+00 | 2.88E-05 | 9.88E-07 | 0.00E+00 | 3.71E-06 | 1.00E+00 | 5.71E-10 |
| 68 | 2.69E-04 | 0.00E+00 | 1.00E-15 | 0.00E+00 | 1.10E-14 | 5.32E-08 | 1.00E+00 | 1.70E-06 |
| 69 | 2.54E-07 | 0.00E+00 | 0.00E+00 | 2.59E-07 | 4.60E-13 | 0.00E+00 | 1.00E+00 | 5.35E-06 |
| 70 | 0.00E+00 | 3.12E-05 | 5.38E-07 | 8.30E-06 | 0.00E+00 | 3.10E-14 | 1.00E+00 | 1.83E-08 |
| 71 | 5.86E-08 | 1.51E-04 | 8.38E-12 | 3.74E-10 | 4.00E-11 | 0.00E+00 | 1.00E+00 | 5.00E-15 |
| 72 | 0.00E+00 | 0.00E+00 | 8.29E-05 | 0.00E+00 | 1.67E-08 | 3.98E-07 | 1.00E+00 | 5.04E-08 |
| 73 | 9.00E-15 | 7.89E-05 | 2.54E-07 | 0.00E+00 | 1.15E-08 | 3.55E-05 | 1.00E+00 | 0.00E+00 |
| 74 | 2.36E-05 | 1.57E-06 | 2.87E-07 | 3.80E-14 | 2.22E-09 | 1.25E-07 | 1.00E+00 | 1.00E-15 |
| 75 | 9.87E-07 | 6.44E-10 | 1.10E-05 | 0.00E+00 | 0.00E+00 | 1.72E-07 | 1.00E+00 | 1.22E-07 |
| 76 | 1.60E-11 | 0.00E+00 | 2.62E-05 | 1.09E-05 | 0.00E+00 | 1.19E-09 | 1.00E+00 | 1.98E-11 |
| 77 | 4.77E-05 | 1.70E-11 | 0.00E+00 | 4.64E-05 | 0.00E+00 | 1.91E-12 | 1.00E+00 | 4.77E-05 |
| 78 | 2.95E-05 | 9.01E-10 | 5.44E-13 | 4.29E-08 | 1.92E-08 | 8.18E-10 | 9.99E-01 | 5.12E-04 |
| 79 | 2.45E-12 | 0.00E+00 | 1.27E-06 | 1.03E-09 | 3.57E-08 | 2.16E-05 | 1.00E+00 | 0.00E+00 |
| 80 | 1.34E-11 | 3.50E-14 | 1.85E-06 | 1.48E-05 | 0.00E+00 | 4.40E-14 | 1.00E+00 | 0.00E+00 |
| 81 | 3.18E-10 | 0.00E+00 | 7.18E-04 | 9.90E-14 | 1.52E-08 | 1.80E-14 | 9.99E-01 | 3.41E-13 |
| 82 | 6.63E-07 | 1.06E-03 | 1.14E-04 | 0.00E+00 | 7.21E-04 | 2.63E-07 | 9.98E-01 | 1.43E-09 |
| 83 | 0.00E+00 | 0.00E+00 | 1.60E-06 | 0.00E+00 | 1.70E-06 | 5.95E-04 | 9.99E-01 | 5.71E-04 |
| 84 | 9.16E-09 | 0.00E+00 | 4.80E-06 | 9.10E-09 | 2.35E-13 | 5.46E-08 | 1.00E+00 | 4.02E-12 |
| 85 | 0.00E+00 | 3.30E-05 | 5.60E-14 | 6.86E-08 | 2.34E-04 | 0.00E+00 | 1.00E+00 | 6.86E-13 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 86 | 0.00E+00 | 2.60E-05 | 1.90E-06 | 3.86E-06 | 0.00E+00 | 4.84E-06 | 1.00E+00 | 1.62E-04 |
| 87 | 6.28E-08 | 6.55E-04 | 0.00E+00 | 8.30E-05 | 7.13E-06 | 6.22E-09 | 9.99E-01 | 1.84E-04 |
| 88 | 2.35E-13 | 4.87E-10 | 8.29E-06 | 1.37E-09 | 3.78E-13 | 4.33E-12 | 1.00E+00 | 3.85E-12 |
| 89 | 2.87E-09 | 1.74E-06 | 4.22E-09 | 1.85E-06 | 3.42E-09 | 3.05E-08 | 1.00E+00 | 1.51E-10 |
| 90 | 7.55E-04 | 1.91E-05 | 0.00E+00 | 1.38E-09 | 2.25E-07 | 0.00E+00 | 9.99E-01 | 8.82E-05 |
| 91 | 2.01E-06 | 7.15E-04 | 2.36E-04 | 4.50E-04 | 0.00E+00 | 7.74E-08 | 9.99E-01 | 3.26E-10 |
| 92 | 4.33E-12 | 2.75E-04 | 0.00E+00 | 2.00E-06 | 1.38E-04 | 0.00E+00 | 9.99E-01 | 7.52E-04 |
| 93 | 4.20E-14 | 3.86E-06 | 0.00E+00 | 0.00E+00 | 2.64E-12 | 1.94E-05 | 1.00E+00 | 2.31E-10 |
| 94 | 6.43E-09 | 9.23E-06 | 2.28E-06 | 5.49E-05 | 9.70E-14 | 4.53E-06 | 1.00E+00 | 2.00E-10 |
| 95 | 1.35E-07 | 7.68E-05 | 0.00E+00 | 2.13E-11 | 5.38E-09 | 5.00E-15 | 1.00E+00 | 1.35E-09 |
| 96 | 3.60E-07 | 8.97E-05 | 1.26E-08 | 1.29E-04 | 7.70E-05 | 2.54E-08 | 1.00E+00 | 8.04E-08 |
| 97 | 0.00E+00 | 9.84E-07 | 0.00E+00 | 3.30E-07 | 1.00E-14 | 6.92E-06 | 1.00E+00 | 6.46E-06 |
| 98 | 0.00E+00 | 3.50E-05 | 1.08E-06 | 5.08E-08 | 1.20E-06 | 0.00E+00 | 1.00E+00 | 5.72E-05 |
| 99 | 2.13E-06 | 1.25E-04 | 0.00E+00 | 1.21E-09 | 2.27E-08 | 0.00E+00 | 1.00E+00 | 5.80E-14 |
| 100 | 7.89E-13 | 2.11E-07 | 0.00E+00 | 3.70E-10 | 8.00E-04 | 4.00E-15 | 9.99E-01 | 6.04E-04 |
| 101 | 5.90E-14 | 8.05E-06 | 3.65E-08 | 7.00E-15 | 2.38E-06 | 5.01E-09 | 1.00E+00 | 5.46E-11 |
| 102 | 1.00E-14 | 3.70E-05 | 2.25E-10 | 0.00E+00 | 1.55E-10 | 1.00E-15 | 1.00E+00 | 4.71E-05 |
| 103 | 3.81E-07 | 1.14E-06 | 7.08E-09 | 4.16E-07 | 0.00E+00 | 0.00E+00 | 1.00E+00 | 1.49E-04 |
| 104 | 6.64E-11 | 1.47E-09 | 3.05E-13 | 4.67E-08 | 0.00E+00 | 1.64E-04 | 1.00E+00 | 9.33E-06 |
| 105 | 1.04E-10 | 2.07E-04 | 0.00E+00 | 0.00E+00 | 5.16E-11 | 1.57E-05 | 1.00E+00 | 5.15E-13 |
| 106 | 1.27E-11 | 1.25E-05 | 2.17E-08 | 1.28E-07 | 4.31E-10 | 1.00E-15 | 1.00E+00 | 2.87E-13 |
| 107 | 3.46E-05 | 0.00E+00 | 7.04E-05 | 0.00E+00 | 2.51E-06 | 8.86E-05 | 9.99E-01 | 9.12E-04 |
| 108 | 0.00E+00 | 0.00E+00 | 3.90E-07 | 1.00E-15 | 4.14E-04 | 2.17E-04 | 9.99E-01 | 1.55E-06 |
| 109 | 2.29E-12 | 2.82E-12 | 2.09E-08 | 5.87E-08 | 1.01E-04 | 2.31E-08 | 1.00E+00 | 1.88E-06 |
| 110 | 1.27E-04 | 0.00E+00 | 3.09E-04 | 1.61E-09 | 5.36E-06 | 0.00E+00 | 1.00E+00 | 0.00E+00 |
| 111 | 3.76E-04 | 2.91E-04 | 7.62E-04 | 3.32E-06 | 1.15E-05 | 7.81E-06 | 9.99E-01 | 0.00E+00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 112 | 2.10E-05 | 1.92E-10 | 3.79E-07 | 1.15E-06 | 5.29E-05 | 1.70E-12 | 1.00E+00 | 1.00E-15 |
| 113 | 0.00E+00 | 4.32E-08 | 2.45E-05 | 2.86E-09 | 1.68E-10 | 8.00E-15 | 1.00E+00 | 1.68E-08 |
| 114 | 1.60E-05 | 5.73E-07 | 2.81E-05 | 3.84E-04 | 4.93E-04 | 6.92E-03 | 9.92E-01 | 1.97E-12 |
| 115 | 2.27E-08 | 6.00E-15 | 6.41E-04 | 5.70E-12 | 5.27E-04 | 0.00E+00 | 9.99E-01 | 2.84E-13 |
| 116 | 4.98E-08 | 0.00E+00 | 6.84E-09 | 6.87E-07 | 2.13E-06 | 0.00E+00 | 1.00E+00 | 4.46E-07 |
| 117 | 6.50E-07 | 1.12E-06 | 3.89E-04 | 1.41E-07 | 4.71E-06 | 1.13E-04 | 9.99E-01 | 3.86E-05 |
| 118 | 1.20E-06 | 1.43E-04 | 1.01E-03 | 4.83E-04 | 4.47E-07 | 4.26E-04 | 9.89E-01 | 8.52E-03 |
| 119 | 0.00E+00 | 1.11E-03 | 3.25E-08 | 1.64E-05 | 3.00E-04 | 6.86E-07 | 9.99E-01 | 2.35E-08 |
| 120 | 3.86E-08 | 1.59E-03 | 8.56E-07 | 0.00E+00 | 3.16E-03 | 2.52E-04 | 9.95E-01 | 0.00E+00 |
| 121 | 9.02E-12 | 2.59E-11 | 1.60E-14 | 0.00E+00 | 4.75E-05 | 5.37E-06 | 1.00E+00 | 8.18E-10 |
| 122 | 1.89E-03 | 2.79E-04 | 5.91E-06 | 0.00E+00 | 1.45E-04 | 3.59E-10 | 9.98E-01 | 3.14E-09 |
| 123 | 1.16E-08 | 1.80E-04 | 3.68E-05 | 3.56E-05 | 2.58E-06 | 4.83E-04 | 9.99E-01 | 4.89E-06 |
| 124 | 3.10E-05 | 1.87E-07 | 9.11E-01 | 3.07E-03 | 8.04E-08 | 9.63E-03 | 7.66E-02 | 5.69E-07 |
| 125 | 4.30E-03 | 1.31E-04 | 9.10E-01 | 5.24E-05 | 4.61E-06 | 1.56E-04 | 8.49E-02 | 7.96E-07 |
| 126 | 2.57E-03 | 1.31E-03 | 8.86E-01 | 5.78E-04 | 1.53E-03 | 1.12E-08 | 1.08E-01 | 3.49E-06 |
| 127 | 1.52E-03 | 1.32E-05 | 8.26E-01 | 5.74E-05 | 2.09E-04 | 8.10E-04 | 1.70E-01 | 1.38E-03 |
| 128 | 8.48E-04 | 2.93E-05 | 9.00E-01 | 7.35E-08 | 0.00E+00 | 9.44E-04 | 9.81E-02 | 1.03E-05 |
| 129 | 1.15E-04 | 5.35E-03 | 8.22E-01 | 1.65E-02 | 0.00E+00 | 7.01E-03 | 1.49E-01 | 1.34E-06 |
| 130 | 1.69E-03 | 1.04E-06 | 9.97E-01 | 6.59E-09 | 1.17E-08 | 2.87E-04 | 1.46E-04 | 1.36E-03 |
| 131 | 9.20E-05 | 1.85E-04 | 9.95E-01 | 1.40E-04 | 1.26E-07 | 2.47E-05 | 5.00E-03 | 1.40E-14 |
| 132 | 1.06E-04 | 4.14E-06 | 9.84E-01 | 3.71E-10 | 3.33E-05 | 6.12E-06 | 1.37E-02 | 2.04E-03 |
| 133 | 1.56E-03 | 3.19E-07 | 9.98E-01 | 0.00E+00 | 0.00E+00 | 2.46E-05 | 6.39E-06 | 6.07E-13 |
| 134 | 1.74E-03 | 4.97E-09 | 9.82E-01 | 9.93E-08 | 3.78E-03 | 5.46E-03 | 6.26E-03 | 8.29E-04 |
| 135 | 1.80E-09 | 0.00E+00 | 9.91E-01 | 4.06E-05 | 1.56E-07 | 2.30E-03 | 7.12E-03 | 8.25E-07 |
| 136 | 4.83E-09 | 1.57E-10 | 9.94E-01 | 4.89E-05 | 3.67E-04 | 1.29E-06 | 5.87E-03 | 4.06E-05 |
| 137 | 0.00E+00 | 7.37E-12 | 9.98E-01 | 1.60E-07 | 1.81E-10 | 7.59E-05 | 2.17E-03 | 1.04E-11 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 138 | 1.21E-08 | 1.06E-08 | 9.96E-01 | 5.23E-06 | 3.38E-03 | 5.26E-08 | 2.86E-04 | 5.88E-09 |
| 139 | 1.00E-15 | 2.77E-05 | 9.92E-01 | 7.29E-09 | 4.90E-05 | 4.67E-03 | 2.65E-03 | 3.80E-04 |
| 140 | 3.12E-05 | 3.02E-08 | 9.99E-01 | 1.51E-07 | 1.41E-10 | 2.12E-11 | 5.64E-04 | 1.02E-13 |
| 141 | 2.00E-15 | 1.80E-14 | 9.96E-01 | 1.16E-08 | 5.20E-11 | 2.67E-04 | 3.63E-03 | 6.37E-10 |
| 142 | 8.99E-10 | 0.00E+00 | 9.96E-01 | 2.27E-09 | 2.26E-03 | 7.97E-07 | 2.00E-03 | 2.84E-09 |
| 143 | 8.15E-08 | 0.00E+00 | 9.96E-01 | 1.00E-15 | 7.46E-04 | 9.27E-06 | 2.98E-03 | 0.00E+00 |
| 144 | 3.43E-03 | 2.42E-04 | 9.93E-01 | 2.11E-07 | 2.69E-08 | 3.30E-04 | 2.51E-03 | 0.00E+00 |
| 145 | 3.75E-06 | 1.79E-03 | 9.92E-01 | 4.29E-03 | 6.72E-07 | 4.85E-05 | 1.11E-03 | 7.40E-04 |
| 146 | 8.07E-05 | 1.92E-10 | 9.99E-01 | 7.03E-08 | 5.27E-12 | 3.28E-05 | 4.11E-04 | 2.41E-07 |
| 147 | 5.45E-04 | 4.12E-04 | 9.86E-01 | 2.15E-03 | 2.02E-05 | 1.55E-03 | 9.02E-03 | 4.25E-04 |
| 148 | 1.53E-04 | 1.66E-05 | 9.92E-01 | 1.13E-07 | 2.08E-12 | 2.73E-06 | 7.35E-03 | 1.95E-04 |
| 149 | 4.04E-12 | 1.00E-15 | 9.88E-01 | 3.21E-03 | 7.25E-06 | 0.00E+00 | 8.09E-03 | 1.12E-03 |
| 150 | 8.50E-09 | 1.43E-05 | 9.81E-01 | 4.59E-04 | 0.00E+00 | 2.54E-12 | 1.83E-02 | 3.95E-06 |
| 151 | 5.89E-05 | 2.76E-06 | 9.85E-01 | 3.19E-03 | 1.60E-08 | 2.83E-04 | 1.11E-02 | 1.70E-08 |
| 152 | 0.00E+00 | 2.77E-05 | 9.89E-01 | 8.56E-07 | 9.93E-05 | 1.44E-09 | 1.09E-02 | 2.77E-04 |
| 153 | 8.25E-06 | 6.91E-13 | 9.76E-01 | 4.21E-06 | 5.74E-03 | 1.24E-11 | 1.35E-02 | 4.72E-03 |
| 154 | 8.77E-04 | 2.42E-05 | 9.86E-01 | 1.27E-07 | 3.00E-09 | 7.66E-12 | 1.36E-02 | 4.57E-09 |
| 155 | 7.52E-05 | 4.38E-04 | 9.90E-01 | 1.39E-05 | 0.00E+00 | 5.57E-08 | 9.56E-03 | 1.21E-04 |
| 156 | 5.54E-08 | 7.29E-04 | 9.94E-01 | 8.89E-06 | 9.28E-05 | 1.27E-05 | 5.19E-03 | 2.00E-15 |
| 157 | 8.85E-04 | 1.19E-08 | 9.45E-01 | 4.12E-02 | 7.00E-05 | 3.43E-05 | 1.26E-02 | 2.20E-09 |
| 158 | 0.00E+00 | 4.73E-06 | 8.46E-01 | 3.56E-03 | 1.99E-06 | 1.10E-03 | 1.49E-01 | 2.95E-06 |
| 159 | 2.35E-07 | 0.00E+00 | 7.42E-01 | 2.04E-08 | 1.10E-03 | 2.42E-03 | 2.13E-01 | 4.11E-02 |
| 160 | 2.74E-07 | 5.07E-07 | 8.06E-01 | 5.00E-15 | 2.44E-05 | 3.02E-04 | 1.94E-01 | 8.33E-05 |
| 161 | 6.91E-03 | 6.19E-06 | 8.29E-01 | 6.10E-03 | 0.00E+00 | 1.21E-06 | 1.58E-01 | 3.72E-12 |
| 162 | 0.00E+00 | 7.30E-02 | 7.83E-01 | 2.98E-05 | 2.74E-05 | 2.36E-04 | 1.42E-01 | 1.81E-03 |
| 163 | 1.16E-07 | 6.94E-04 | 8.57E-01 | 1.52E-12 | 1.84E-07 | 5.44E-08 | 1.42E-01 | 3.72E-09 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 164 | 1.90E-09 | 1.13E-10 | 6.87E-01 | 5.69E-03 | 9.97E-05 | 6.28E-02 | 1.55E-01 | 8.92E-02 |
| 165 | 1.38E-09 | 8.75E-06 | 8.81E-01 | 8.15E-03 | 1.55E-10 | 1.38E-02 | 9.66E-02 | 0.00E+00 |
| 166 | 2.02E-04 | 0.00E+00 | 9.79E-01 | 1.00E-15 | 0.00E+00 | 1.01E-08 | 2.10E-02 | 0.00E+00 |
| 167 | 5.75E-04 | 1.83E-08 | 9.64E-01 | 9.71E-08 | 1.10E-14 | 5.77E-03 | 2.92E-02 | 6.77E-08 |
| 168 | 0.00E+00 | 1.67E-03 | 9.55E-01 | 7.66E-05 | 1.00E-14 | 4.15E-08 | 4.37E-02 | 0.00E+00 |
| 169 | 3.57E-05 | 3.15E-04 | 4.09E-01 | 1.29E-03 | 3.42E-04 | 3.46E-06 | 5.89E-01 | 6.51E-11 |
| 170 | 5.69E-04 | 5.87E-04 | 3.99E-01 | 2.43E-13 | 1.40E-05 | 1.49E-04 | 6.00E-01 | 2.90E-05 |
| 171 | 9.34E-06 | 0.00E+00 | 6.62E-01 | 1.68E-08 | 1.96E-10 | 1.49E-06 | 3.14E-01 | 2.41E-02 |
| 172 | 5.86E-11 | 8.04E-06 | 7.28E-01 | 8.91E-09 | 5.47E-08 | 1.35E-05 | 2.72E-01 | 0.00E+00 |
| 173 | 3.82E-02 | 4.81E-04 | 5.30E-01 | 6.18E-10 | 7.01E-04 | 6.18E-03 | 4.24E-01 | 1.05E-04 |
| 174 | 7.21E-07 | 6.62E-11 | 9.72E-01 | 0.00E+00 | 7.13E-11 | 4.74E-04 | 2.80E-02 | 0.00E+00 |
| 175 | 6.10E-04 | 5.48E-13 | 9.79E-01 | 1.03E-09 | 8.08E-10 | 9.44E-04 | 1.77E-02 | 1.56E-03 |
| 176 | 0.00E+00 | 2.62E-03 | 9.18E-01 | 0.00E+00 | 5.72E-04 | 2.99E-05 | 7.89E-02 | 4.93E-13 |
| 177 | 1.09E-02 | 2.82E-06 | 9.81E-01 | 4.20E-04 | 4.30E-10 | 5.08E-03 | 2.81E-03 | 0.00E+00 |
| 178 | 2.10E-14 | 3.00E-15 | 9.99E-01 | 4.73E-08 | 1.70E-05 | 5.17E-04 | 3.34E-06 | 1.91E-06 |
| 179 | 2.45E-03 | 1.38E-06 | 9.94E-01 | 1.07E-06 | 8.05E-10 | 5.97E-04 | 2.49E-03 | 0.00E+00 |
| 180 | 5.05E-06 | 3.78E-08 | 9.97E-01 | 3.70E-04 | 1.51E-03 | 4.99E-05 | 6.89E-04 | 1.35E-06 |
| 181 | 1.24E-04 | 0.00E+00 | 9.97E-01 | 3.32E-07 | 0.00E+00 | 1.05E-03 | 2.29E-03 | 9.37E-06 |
| 182 | 4.40E-04 | 4.43E-05 | 9.94E-01 | 7.42E-04 | 0.00E+00 | 1.63E-03 | 2.64E-03 | 2.79E-04 |
| 183 | 9.95E-05 | 6.76E-05 | 9.99E-01 | 7.34E-13 | 0.00E+00 | 1.50E-04 | 1.79E-04 | 2.73E-04 |
| 184 | 6.00E-04 | 4.68E-06 | 9.96E-01 | 2.60E-04 | 1.16E-03 | 1.12E-03 | 4.55E-04 | 5.34E-06 |
| 185 | 3.41E-04 | 2.06E-04 | 9.99E-01 | 2.96E-05 | 2.99E-04 | 3.68E-05 | 4.05E-12 | 4.15E-06 |

**Table S1.14** Proportion on ancestry for individuals of *Scinax squalirostris*, conditioned to environmental variables, inferred by Bayesian analysis of nuclear for present climatic conditions.

| | Inferred Cluster | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Individual** | **Cluster 1** | **Cluster 2** | **Cluster 3** | **Cluster 4** | **Cluster 5** | **Cluster 6** | **Cluster 7** | **Cluster 8** | **Cluster 9** |
| 1 | 0.000560 | 0.772864 | 0.000259 | 0.004862 | 0.006075 | 0.000599 | 0.000536 | 0.000366 | 0.213879 |
| 2 | 0.000587 | 0.772552 | 0.000205 | 0.004832 | 0.006089 | 0.000619 | 0.000542 | 0.000407 | 0.214168 |
| 3 | 0.000566 | 0.772619 | 0.000232 | 0.004766 | 0.006171 | 0.000685 | 0.000481 | 0.000398 | 0.214081 |
| 4 | 0.000597 | 0.772906 | 0.000250 | 0.004941 | 0.005920 | 0.000629 | 0.000539 | 0.000336 | 0.213882 |
| 5 | 0.000577 | 0.772784 | 0.000254 | 0.004811 | 0.005954 | 0.000570 | 0.000537 | 0.000413 | 0.214100 |
| 6 | 0.003443 | 0.720549 | 0.004989 | 0.087267 | 0.008823 | 0.003751 | 0.004271 | 0.003619 | 0.163288 |
| 7 | 0.003394 | 0.721781 | 0.004405 | 0.086321 | 0.008574 | 0.003723 | 0.004460 | 0.003700 | 0.163643 |
| 8 | 0.003231 | 0.720129 | 0.004709 | 0.087689 | 0.009246 | 0.003708 | 0.004443 | 0.003656 | 0.163189 |
| 9 | 0.003383 | 0.720784 | 0.005014 | 0.086426 | 0.009449 | 0.003482 | 0.004479 | 0.003496 | 0.163486 |
| 10 | 0.003260 | 0.720860 | 0.004641 | 0.086392 | 0.008802 | 0.003694 | 0.004347 | 0.003577 | 0.164427 |
| 11 | 0.003384 | 0.719718 | 0.004789 | 0.088244 | 0.008509 | 0.003651 | 0.004544 | 0.003419 | 0.163743 |
| 12 | 0.003219 | 0.720187 | 0.004699 | 0.086702 | 0.008886 | 0.003834 | 0.004333 | 0.003683 | 0.164456 |
| 13 | 0.003315 | 0.719731 | 0.004681 | 0.087356 | 0.008881 | 0.003846 | 0.004514 | 0.003478 | 0.164198 |
| 14 | 0.003447 | 0.722399 | 0.004640 | 0.085743 | 0.008797 | 0.003507 | 0.004481 | 0.003546 | 0.163439 |
| 15 | 0.003196 | 0.719990 | 0.004755 | 0.088016 | 0.008747 | 0.003773 | 0.004512 | 0.003623 | 0.163388 |
| 16 | 0.003343 | 0.676647 | 0.004680 | 0.096811 | 0.021194 | 0.003594 | 0.003205 | 0.002601 | 0.187927 |
| 17 | 0.003623 | 0.674833 | 0.004786 | 0.097601 | 0.021544 | 0.003779 | 0.003089 | 0.002679 | 0.188067 |
| 18 | 0.003542 | 0.676503 | 0.004697 | 0.097837 | 0.020599 | 0.003782 | 0.002839 | 0.002610 | 0.187591 |
| 19 | 0.003490 | 0.676302 | 0.004535 | 0.097709 | 0.021093 | 0.003575 | 0.003035 | 0.002662 | 0.187599 |
| 20 | 0.003631 | 0.675996 | 0.004741 | 0.097032 | 0.021705 | 0.003459 | 0.003023 | 0.002638 | 0.187774 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 21 | 0.003512 | 0.675993 | 0.004883 | 0.097565 | 0.021141 | 0.003444 | 0.003101 | 0.002716 | 0.187646 |
| 22 | 0.003418 | 0.676255 | 0.004829 | 0.097467 | 0.021119 | 0.003535 | 0.002995 | 0.002497 | 0.187885 |
| 23 | 0.003501 | 0.676235 | 0.004773 | 0.096758 | 0.021040 | 0.003655 | 0.002924 | 0.002708 | 0.188406 |
| 24 | 0.003401 | 0.676063 | 0.004853 | 0.097179 | 0.020970 | 0.003550 | 0.002935 | 0.002675 | 0.188374 |
| 25 | 0.003364 | 0.676643 | 0.004800 | 0.097075 | 0.020631 | 0.003563 | 0.003005 | 0.002576 | 0.188344 |
| 26 | 0.003480 | 0.676868 | 0.004877 | 0.096272 | 0.021194 | 0.003626 | 0.002890 | 0.002576 | 0.188218 |
| 27 | 0.003331 | 0.675819 | 0.004989 | 0.097087 | 0.021207 | 0.003647 | 0.003018 | 0.002700 | 0.188202 |
| 28 | 0.002339 | 0.057844 | 0.019120 | 0.896778 | 0.001021 | 0.002262 | 0.002142 | 0.002168 | 0.016326 |
| 29 | 0.002240 | 0.057904 | 0.019545 | 0.896629 | 0.001026 | 0.002183 | 0.002162 | 0.002236 | 0.016075 |
| 30 | 0.002083 | 0.057806 | 0.019131 | 0.897392 | 0.000981 | 0.002003 | 0.002189 | 0.002260 | 0.016155 |
| 31 | 0.002200 | 0.057931 | 0.019473 | 0.896322 | 0.000941 | 0.002141 | 0.002070 | 0.002313 | 0.016609 |
| 32 | 0.002210 | 0.057956 | 0.018554 | 0.897747 | 0.001008 | 0.002098 | 0.002146 | 0.002252 | 0.016029 |
| 33 | 0.002116 | 0.057544 | 0.019254 | 0.897711 | 0.000969 | 0.002108 | 0.002117 | 0.002259 | 0.015922 |
| 34 | 0.002124 | 0.057576 | 0.018432 | 0.897869 | 0.001033 | 0.002093 | 0.002369 | 0.002362 | 0.016142 |
| 35 | 0.002108 | 0.057969 | 0.019141 | 0.896397 | 0.001122 | 0.002209 | 0.002192 | 0.002331 | 0.016531 |
| 36 | 0.002009 | 0.058809 | 0.019302 | 0.895307 | 0.001047 | 0.002100 | 0.002311 | 0.002298 | 0.016816 |
| 37 | 0.002137 | 0.058258 | 0.019108 | 0.896431 | 0.001093 | 0.002128 | 0.002233 | 0.002395 | 0.016217 |
| 38 | 0.002269 | 0.058445 | 0.019719 | 0.895174 | 0.000978 | 0.002231 | 0.002119 | 0.002476 | 0.016589 |
| 39 | 0.002158 | 0.057824 | 0.019565 | 0.896410 | 0.001079 | 0.002167 | 0.002197 | 0.002309 | 0.016291 |
| 40 | 0.002160 | 0.057012 | 0.018602 | 0.898952 | 0.000896 | 0.002108 | 0.002092 | 0.002306 | 0.015872 |
| 41 | 0.002202 | 0.057731 | 0.018981 | 0.897081 | 0.001020 | 0.002066 | 0.002082 | 0.002476 | 0.016360 |
| 42 | 0.002282 | 0.057314 | 0.019259 | 0.897781 | 0.000975 | 0.002108 | 0.002169 | 0.002249 | 0.015861 |
| 43 | 0.002043 | 0.057221 | 0.019230 | 0.897972 | 0.000956 | 0.002118 | 0.002081 | 0.002187 | 0.016192 |
| 44 | 0.011200 | 0.378496 | 0.135743 | 0.282617 | 0.084289 | 0.016584 | 0.014591 | 0.011541 | 0.064940 |
| 45 | 0.002091 | 0.009486 | 0.001753 | 0.890574 | 0.084220 | 0.002180 | 0.001920 | 0.002140 | 0.005636 |
| 46 | 0.002355 | 0.009518 | 0.001891 | 0.889564 | 0.084455 | 0.002107 | 0.001861 | 0.002202 | 0.006048 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 47 | 0.002046 | 0.009601 | 0.001857 | 0.889505 | 0.085205 | 0.002194 | 0.001774 | 0.002156 | 0.005663 |
| 48 | 0.002293 | 0.009556 | 0.001999 | 0.889547 | 0.084256 | 0.002195 | 0.001856 | 0.002296 | 0.006002 |
| 49 | 0.002219 | 0.009399 | 0.001785 | 0.888744 | 0.085495 | 0.002107 | 0.001844 | 0.002081 | 0.006324 |
| 50 | 0.002227 | 0.009344 | 0.001992 | 0.889812 | 0.084467 | 0.002225 | 0.001832 | 0.002281 | 0.005820 |
| 51 | 0.002286 | 0.009182 | 0.001877 | 0.889515 | 0.085068 | 0.002122 | 0.002010 | 0.002247 | 0.005693 |
| 52 | 0.002126 | 0.009341 | 0.001954 | 0.889368 | 0.084854 | 0.002256 | 0.001852 | 0.002173 | 0.006078 |
| 53 | 0.002156 | 0.009586 | 0.001897 | 0.890059 | 0.084382 | 0.002248 | 0.001837 | 0.002142 | 0.005693 |
| 54 | 0.002153 | 0.009351 | 0.001811 | 0.890676 | 0.083965 | 0.002087 | 0.001781 | 0.002170 | 0.006006 |
| 55 | 0.002312 | 0.009685 | 0.001877 | 0.889663 | 0.084746 | 0.002118 | 0.001638 | 0.002180 | 0.005781 |
| 56 | 0.002227 | 0.009679 | 0.002017 | 0.890413 | 0.083505 | 0.002220 | 0.001919 | 0.002197 | 0.005824 |
| 57 | 0.002183 | 0.009681 | 0.001978 | 0.889489 | 0.084627 | 0.002067 | 0.001781 | 0.002231 | 0.005964 |
| 58 | 0.002244 | 0.009304 | 0.001784 | 0.889831 | 0.084745 | 0.002181 | 0.001712 | 0.002134 | 0.006063 |
| 59 | 0.001447 | 0.004210 | 0.022730 | 0.962131 | 0.002322 | 0.001229 | 0.001403 | 0.001475 | 0.003053 |
| 60 | 0.001525 | 0.004325 | 0.022891 | 0.961918 | 0.002130 | 0.001105 | 0.001451 | 0.001542 | 0.003115 |
| 61 | 0.001516 | 0.004101 | 0.023158 | 0.961716 | 0.002186 | 0.001250 | 0.001418 | 0.001550 | 0.003106 |
| 62 | 0.001488 | 0.004157 | 0.023070 | 0.961635 | 0.002231 | 0.001203 | 0.001531 | 0.001531 | 0.003153 |
| 63 | 0.001379 | 0.004192 | 0.023103 | 0.961766 | 0.002281 | 0.001169 | 0.001441 | 0.001552 | 0.003119 |
| 64 | 0.001452 | 0.004129 | 0.023278 | 0.961546 | 0.002321 | 0.001223 | 0.001350 | 0.001554 | 0.003147 |
| 65 | 0.001446 | 0.004294 | 0.023057 | 0.961752 | 0.002268 | 0.001195 | 0.001311 | 0.001566 | 0.003111 |
| 66 | 0.001543 | 0.004063 | 0.023155 | 0.961721 | 0.002132 | 0.001271 | 0.001416 | 0.001553 | 0.003146 |
| 67 | 0.001533 | 0.004090 | 0.023238 | 0.961517 | 0.002271 | 0.001260 | 0.001406 | 0.001576 | 0.003108 |
| 68 | 0.001452 | 0.004114 | 0.022886 | 0.961968 | 0.002131 | 0.001252 | 0.001473 | 0.001567 | 0.003157 |
| 69 | 0.001499 | 0.004100 | 0.023304 | 0.961467 | 0.002240 | 0.001302 | 0.001481 | 0.001456 | 0.003153 |
| 70 | 0.001513 | 0.004072 | 0.023352 | 0.961740 | 0.002300 | 0.001185 | 0.001411 | 0.001573 | 0.002854 |
| 71 | 0.001408 | 0.004042 | 0.023339 | 0.961557 | 0.002369 | 0.001078 | 0.001437 | 0.001584 | 0.003186 |
| 72 | 0.001520 | 0.004060 | 0.023084 | 0.962217 | 0.002229 | 0.001222 | 0.001305 | 0.001422 | 0.002941 |

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|---|---|---|---|---|---|---|---|---|---|
| 73 | 0.001551 | 0.004226 | 0.023274 | 0.961466 | 0.002245 | 0.001168 | 0.001346 | 0.001513 | 0.003211 |
| 74 | 0.001398 | 0.003966 | 0.023122 | 0.962304 | 0.002227 | 0.001199 | 0.001326 | 0.001512 | 0.002946 |
| 75 | 0.000721 | 0.004485 | 0.000523 | 0.985403 | 0.004442 | 0.000591 | 0.000628 | 0.000610 | 0.002597 |
| 76 | 0.000681 | 0.004585 | 0.000583 | 0.985406 | 0.004302 | 0.000599 | 0.000589 | 0.000648 | 0.002606 |
| 77 | 0.000687 | 0.004745 | 0.000544 | 0.985039 | 0.004469 | 0.000535 | 0.000586 | 0.000722 | 0.002671 |
| 78 | 0.000693 | 0.004957 | 0.000611 | 0.984706 | 0.004570 | 0.000556 | 0.000583 | 0.000684 | 0.002639 |
| 79 | 0.000656 | 0.004534 | 0.000508 | 0.985170 | 0.004444 | 0.000677 | 0.000584 | 0.000725 | 0.002702 |
| 80 | 0.000634 | 0.004568 | 0.000541 | 0.985321 | 0.004370 | 0.000644 | 0.000609 | 0.000654 | 0.002658 |
| 81 | 0.000687 | 0.004723 | 0.000555 | 0.984784 | 0.004535 | 0.000685 | 0.000581 | 0.000734 | 0.002715 |
| 82 | 0.000607 | 0.004741 | 0.000588 | 0.985201 | 0.004534 | 0.000616 | 0.000569 | 0.000647 | 0.002496 |
| 83 | 0.000688 | 0.004548 | 0.000578 | 0.985240 | 0.004531 | 0.000575 | 0.000533 | 0.000639 | 0.002668 |
| 84 | 0.000619 | 0.004571 | 0.000589 | 0.984975 | 0.004461 | 0.000579 | 0.000549 | 0.000797 | 0.002859 |
| 85 | 0.001480 | 0.003372 | 0.944903 | 0.027527 | 0.016391 | 0.001569 | 0.001777 | 0.001493 | 0.001489 |
| 86 | 0.001512 | 0.003354 | 0.945492 | 0.027385 | 0.016359 | 0.001639 | 0.001543 | 0.001348 | 0.001369 |
| 87 | 0.001436 | 0.003393 | 0.945007 | 0.028079 | 0.015923 | 0.001639 | 0.001681 | 0.001508 | 0.001334 |
| 88 | 0.001382 | 0.003355 | 0.944976 | 0.027932 | 0.016124 | 0.001618 | 0.001610 | 0.001563 | 0.001440 |
| 89 | 0.001441 | 0.003583 | 0.944460 | 0.027775 | 0.016310 | 0.001623 | 0.001723 | 0.001579 | 0.001506 |
| 90 | 0.001440 | 0.003450 | 0.944798 | 0.028142 | 0.015804 | 0.001686 | 0.001705 | 0.001459 | 0.001517 |
| 91 | 0.001475 | 0.003482 | 0.945275 | 0.027383 | 0.016340 | 0.001551 | 0.001658 | 0.001445 | 0.001390 |
| 92 | 0.001379 | 0.003413 | 0.945244 | 0.027435 | 0.016588 | 0.001558 | 0.001560 | 0.001474 | 0.001348 |
| 93 | 0.001394 | 0.003465 | 0.944377 | 0.028418 | 0.016077 | 0.001591 | 0.001710 | 0.001555 | 0.001415 |
| 94 | 0.001444 | 0.003417 | 0.944694 | 0.028003 | 0.016192 | 0.001559 | 0.001673 | 0.001508 | 0.001510 |
| 95 | 0.001377 | 0.003582 | 0.944889 | 0.027808 | 0.016083 | 0.001687 | 0.001794 | 0.001391 | 0.001389 |
| 96 | 0.001572 | 0.003471 | 0.944001 | 0.028719 | 0.016010 | 0.001584 | 0.001772 | 0.001487 | 0.001383 |
| 97 | 0.001438 | 0.003377 | 0.944952 | 0.027943 | 0.016153 | 0.001422 | 0.001739 | 0.001491 | 0.001486 |
| 98 | 0.001422 | 0.003204 | 0.945966 | 0.027083 | 0.016119 | 0.001554 | 0.001791 | 0.001460 | 0.001402 |

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|---|---|---|---|---|---|---|---|---|---|
| 99 | 0.001403 | 0.003473 | 0.945928 | 0.027213 | 0.015776 | 0.001594 | 0.001778 | 0.001439 | 0.001397 |
| 100 | 0.001329 | 0.003280 | 0.944979 | 0.027809 | 0.016353 | 0.001558 | 0.001722 | 0.001669 | 0.001301 |
| 101 | 0.001469 | 0.003535 | 0.944130 | 0.028294 | 0.016295 | 0.001582 | 0.001665 | 0.001569 | 0.001462 |
| 102 | 0.001528 | 0.003396 | 0.945084 | 0.027681 | 0.015982 | 0.001676 | 0.001726 | 0.001420 | 0.001506 |
| 103 | 0.001483 | 0.003544 | 0.944679 | 0.027596 | 0.016487 | 0.001509 | 0.001722 | 0.001516 | 0.001464 |
| 104 | 0.001481 | 0.003200 | 0.945791 | 0.027248 | 0.016115 | 0.001481 | 0.001764 | 0.001495 | 0.001424 |
| 105 | 0.001501 | 0.003541 | 0.944095 | 0.028679 | 0.016134 | 0.001568 | 0.001708 | 0.001356 | 0.001419 |
| 106 | 0.001473 | 0.003451 | 0.944361 | 0.028253 | 0.016155 | 0.001639 | 0.001730 | 0.001394 | 0.001545 |
| 107 | 0.002834 | 0.016005 | 0.015480 | 0.034507 | 0.916956 | 0.003098 | 0.002792 | 0.002368 | 0.005961 |
| 108 | 0.002919 | 0.015692 | 0.015695 | 0.034574 | 0.916769 | 0.003131 | 0.002676 | 0.002302 | 0.006243 |
| 109 | 0.002896 | 0.015294 | 0.015379 | 0.033921 | 0.918635 | 0.002950 | 0.002576 | 0.002462 | 0.005887 |
| 110 | 0.002781 | 0.015565 | 0.015549 | 0.034006 | 0.918380 | 0.002704 | 0.002647 | 0.002484 | 0.005883 |
| 111 | 0.002782 | 0.015810 | 0.015648 | 0.033996 | 0.917740 | 0.002974 | 0.002717 | 0.002544 | 0.005788 |
| 112 | 0.002704 | 0.015673 | 0.015609 | 0.033727 | 0.917804 | 0.002914 | 0.002713 | 0.002505 | 0.006352 |
| 113 | 0.002872 | 0.015230 | 0.015787 | 0.033481 | 0.918287 | 0.003036 | 0.002860 | 0.002494 | 0.005954 |
| 114 | 0.002746 | 0.015508 | 0.015922 | 0.034034 | 0.917856 | 0.003070 | 0.002733 | 0.002338 | 0.005791 |
| 115 | 0.002846 | 0.015381 | 0.015577 | 0.033368 | 0.919095 | 0.002810 | 0.002494 | 0.002397 | 0.006032 |
| 116 | 0.002728 | 0.015834 | 0.015872 | 0.034090 | 0.917246 | 0.002986 | 0.002828 | 0.002332 | 0.006083 |
| 117 | 0.002736 | 0.015264 | 0.015606 | 0.034713 | 0.917279 | 0.003188 | 0.002730 | 0.002477 | 0.006008 |
| 118 | 0.002881 | 0.015772 | 0.015269 | 0.033593 | 0.918413 | 0.003081 | 0.002645 | 0.002426 | 0.005919 |
| 119 | 0.002709 | 0.016085 | 0.015529 | 0.034675 | 0.917064 | 0.002983 | 0.002535 | 0.002369 | 0.006051 |
| 120 | 0.002665 | 0.015716 | 0.015306 | 0.034095 | 0.918043 | 0.002994 | 0.002745 | 0.002362 | 0.006075 |
| 121 | 0.002748 | 0.015683 | 0.015643 | 0.035052 | 0.916584 | 0.002984 | 0.002739 | 0.002341 | 0.006226 |
| 122 | 0.002675 | 0.015344 | 0.015700 | 0.033518 | 0.918308 | 0.002943 | 0.002684 | 0.002405 | 0.006424 |
| 123 | 0.002968 | 0.015674 | 0.015737 | 0.033259 | 0.918676 | 0.002784 | 0.002739 | 0.002413 | 0.005750 |
| 124 | 0.002826 | 0.015615 | 0.015877 | 0.033811 | 0.917989 | 0.002981 | 0.002613 | 0.002356 | 0.005932 |

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|---|---|---|---|---|---|---|---|---|---|
| 125 | 0.009640 | 0.673896 | 0.054271 | 0.046647 | 0.069997 | 0.007722 | 0.009324 | 0.006563 | 0.121939 |
| 126 | 0.009482 | 0.673457 | 0.054484 | 0.045963 | 0.070356 | 0.007613 | 0.009481 | 0.006673 | 0.122490 |
| 127 | 0.009547 | 0.672744 | 0.054595 | 0.047057 | 0.070513 | 0.007292 | 0.009378 | 0.006495 | 0.122379 |
| 128 | 0.009687 | 0.673715 | 0.054275 | 0.046407 | 0.070318 | 0.007419 | 0.009382 | 0.006678 | 0.122119 |
| 129 | 0.009378 | 0.673768 | 0.054285 | 0.046646 | 0.070927 | 0.007671 | 0.009319 | 0.006314 | 0.121692 |
| 130 | 0.009496 | 0.673165 | 0.054470 | 0.046527 | 0.069670 | 0.007545 | 0.009327 | 0.006432 | 0.123367 |
| 131 | 0.009417 | 0.673208 | 0.054854 | 0.047132 | 0.070085 | 0.007494 | 0.009430 | 0.006481 | 0.121900 |
| 132 | 0.001331 | 0.937373 | 0.010036 | 0.000454 | 0.035746 | 0.002473 | 0.002760 | 0.001656 | 0.008170 |
| 133 | 0.001359 | 0.936846 | 0.009664 | 0.000405 | 0.036742 | 0.002309 | 0.002779 | 0.001775 | 0.008121 |
| 134 | 0.001400 | 0.936422 | 0.009965 | 0.000459 | 0.036550 | 0.002407 | 0.002833 | 0.001731 | 0.008232 |
| 135 | 0.001321 | 0.937577 | 0.009622 | 0.000479 | 0.036134 | 0.002527 | 0.002638 | 0.001718 | 0.007984 |
| 136 | 0.001268 | 0.937614 | 0.009569 | 0.000423 | 0.036198 | 0.002503 | 0.002731 | 0.001682 | 0.008011 |
| 137 | 0.001353 | 0.937192 | 0.009910 | 0.000377 | 0.035870 | 0.002442 | 0.002695 | 0.001768 | 0.008394 |
| 138 | 0.001355 | 0.936763 | 0.009817 | 0.000358 | 0.036305 | 0.002521 | 0.002490 | 0.001787 | 0.008605 |
| 139 | 0.001331 | 0.937883 | 0.009746 | 0.000469 | 0.035527 | 0.002371 | 0.002687 | 0.001767 | 0.008218 |
| 140 | 0.001348 | 0.937833 | 0.009660 | 0.000469 | 0.035904 | 0.002376 | 0.002706 | 0.001757 | 0.007946 |
| 141 | 0.001306 | 0.936978 | 0.009989 | 0.000462 | 0.036106 | 0.002405 | 0.002769 | 0.001781 | 0.008206 |
| 142 | 0.001309 | 0.937321 | 0.009852 | 0.000412 | 0.036154 | 0.002505 | 0.002682 | 0.001762 | 0.008003 |
| 143 | 0.001251 | 0.937862 | 0.009582 | 0.000418 | 0.035967 | 0.002480 | 0.002657 | 0.001748 | 0.008035 |
| 144 | 0.001349 | 0.937350 | 0.009768 | 0.000374 | 0.035931 | 0.002444 | 0.002705 | 0.001788 | 0.008292 |
| 145 | 0.007128 | 0.695919 | 0.019820 | 0.243230 | 0.000844 | 0.004313 | 0.010794 | 0.008225 | 0.009728 |
| 146 | 0.006864 | 0.697135 | 0.020156 | 0.242387 | 0.000786 | 0.004674 | 0.010635 | 0.008056 | 0.009307 |
| 147 | 0.007103 | 0.698399 | 0.019879 | 0.240835 | 0.000736 | 0.004599 | 0.010619 | 0.008213 | 0.009618 |
| 148 | 0.007225 | 0.696835 | 0.020099 | 0.242054 | 0.000713 | 0.004327 | 0.010991 | 0.008196 | 0.009560 |
| 149 | 0.007070 | 0.698719 | 0.019731 | 0.240339 | 0.000807 | 0.004597 | 0.010588 | 0.008358 | 0.009790 |
| 150 | 0.007256 | 0.205991 | 0.038508 | 0.720604 | 0.002920 | 0.004518 | 0.006618 | 0.009032 | 0.004554 |

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|---|---|---|---|---|---|---|---|---|---|
| 151 | 0.007377 | 0.546651 | 0.007587 | 0.273287 | 0.123168 | 0.007130 | 0.008367 | 0.008642 | 0.017793 |
| 152 | 0.004766 | 0.757357 | 0.009966 | 0.055624 | 0.128426 | 0.005679 | 0.005739 | 0.005271 | 0.027172 |
| 153 | 0.004736 | 0.755645 | 0.010244 | 0.055806 | 0.128936 | 0.005668 | 0.005851 | 0.005306 | 0.027809 |
| 154 | 0.004438 | 0.756970 | 0.009834 | 0.055601 | 0.129245 | 0.005562 | 0.005841 | 0.005352 | 0.027156 |
| 155 | 0.007385 | 0.391352 | 0.047973 | 0.279569 | 0.216203 | 0.014391 | 0.015171 | 0.013714 | 0.014242 |
| 156 | 0.003015 | 0.076719 | 0.002454 | 0.887592 | 0.021836 | 0.001354 | 0.002824 | 0.003094 | 0.001114 |
| 157 | 0.005921 | 0.762548 | 0.036770 | 0.122094 | 0.033539 | 0.007380 | 0.008490 | 0.007271 | 0.015987 |
| 158 | 0.002681 | 0.891463 | 0.011282 | 0.005825 | 0.010298 | 0.001837 | 0.002666 | 0.002013 | 0.071934 |
| 159 | 0.002627 | 0.892194 | 0.011317 | 0.005803 | 0.010447 | 0.001923 | 0.002609 | 0.001893 | 0.071187 |
| 160 | 0.000327 | 0.945769 | 0.000094 | 0.000095 | 0.000195 | 0.000181 | 0.000257 | 0.000349 | 0.052733 |

**Table S1.15** Proportion on ancestry for individuals of *Scinax squalirostris*, conditioned to environmental variables, inferred by Bayesian analysis of nuclear for 4.5RCP climatic conditions.

| | Inferred Cluster | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Individual** | **Cluster 1** | **Cluster 2** | **Cluster 3** | **Cluster 4** | **Cluster 5** | **Cluster 6** | **Cluster 7** | **Cluster 8** | **Cluster 9** |
| 1 | 0.000000 | 0.748363 | 0.000000 | 0.250356 | 0.000000 | 0.000000 | 0.000000 | 0.000001 | 0.001281 |
| 2 | 0.000002 | 0.811036 | 0.000000 | 0.187840 | 0.000191 | 0.000001 | 0.000000 | 0.000003 | 0.000927 |
| 3 | 0.000000 | 0.789702 | 0.000000 | 0.171008 | 0.000000 | 0.000000 | 0.000000 | 0.000001 | 0.039289 |
| 4 | 0.011692 | 0.802633 | 0.000000 | 0.185577 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000098 |
| 5 | 0.000000 | 0.719585 | 0.000000 | 0.273016 | 0.000005 | 0.000000 | 0.000000 | 0.000000 | 0.007394 |
| 6 | 0.000004 | 0.336543 | 0.000000 | 0.651662 | 0.000000 | 0.000000 | 0.000002 | 0.000000 | 0.011789 |
| 7 | 0.000000 | 0.197504 | 0.000000 | 0.802463 | 0.000001 | 0.000000 | 0.000000 | 0.000000 | 0.000033 |
| 8 | 0.000000 | 0.266425 | 0.000000 | 0.732886 | 0.000000 | 0.000000 | 0.000000 | 0.000001 | 0.000688 |

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|---|---|---|---|---|---|---|---|---|---|
| 9 | 0.000000 | 0.278994 | 0.000000 | 0.719808 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.001198 |
| 10 | 0.000000 | 0.217988 | 0.000000 | 0.668509 | 0.017277 | 0.000000 | 0.000001 | 0.000000 | 0.096225 |
| 11 | 0.000014 | 0.303278 | 0.000000 | 0.696707 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 12 | 0.000000 | 0.200940 | 0.000000 | 0.798783 | 0.000000 | 0.000075 | 0.000005 | 0.000006 | 0.000191 |
| 13 | 0.000000 | 0.239793 | 0.000000 | 0.747632 | 0.000000 | 0.000000 | 0.004736 | 0.007797 | 0.000042 |
| 14 | 0.000000 | 0.138237 | 0.000000 | 0.861702 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000061 |
| 15 | 0.001531 | 0.297267 | 0.000000 | 0.700792 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000410 |
| 16 | 0.000001 | 0.012969 | 0.000000 | 0.962732 | 0.000000 | 0.019457 | 0.000010 | 0.000026 | 0.004806 |
| 17 | 0.000735 | 0.040187 | 0.000000 | 0.954510 | 0.004562 | 0.000000 | 0.000005 | 0.000000 | 0.000002 |
| 18 | 0.000000 | 0.032467 | 0.000000 | 0.967533 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 19 | 0.000852 | 0.011815 | 0.000043 | 0.982875 | 0.000000 | 0.000000 | 0.000011 | 0.003942 | 0.000463 |
| 20 | 0.000000 | 0.047596 | 0.000000 | 0.949329 | 0.000000 | 0.000000 | 0.000002 | 0.000202 | 0.002871 |
| 21 | 0.000004 | 0.067479 | 0.000033 | 0.932351 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000133 |
| 22 | 0.000000 | 0.021336 | 0.000000 | 0.966873 | 0.000000 | 0.000000 | 0.002913 | 0.008143 | 0.000736 |
| 23 | 0.000000 | 0.024855 | 0.000000 | 0.961951 | 0.000000 | 0.000008 | 0.002171 | 0.000000 | 0.011015 |
| 24 | 0.000000 | 0.011261 | 0.000007 | 0.985954 | 0.002740 | 0.000000 | 0.000000 | 0.000000 | 0.000037 |
| 25 | 0.000000 | 0.036019 | 0.000000 | 0.963621 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000360 |
| 26 | 0.001142 | 0.021929 | 0.000000 | 0.976897 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000032 |
| 27 | 0.000477 | 0.006711 | 0.000000 | 0.992812 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 28 | 0.000001 | 0.000125 | 0.000013 | 0.999767 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000094 |
| 29 | 0.000000 | 0.000000 | 0.000715 | 0.999196 | 0.000000 | 0.000088 | 0.000000 | 0.000000 | 0.000001 |
| 30 | 0.000016 | 0.002639 | 0.000000 | 0.997341 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000004 |
| 31 | 0.000000 | 0.000001 | 0.000000 | 0.999997 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000003 |
| 32 | 0.000000 | 0.000156 | 0.000002 | 0.999652 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000190 |
| 33 | 0.000000 | 0.000014 | 0.000132 | 0.999749 | 0.000000 | 0.000003 | 0.000000 | 0.000001 | 0.000102 |
| 34 | 0.000146 | 0.000330 | 0.000000 | 0.999521 | 0.000000 | 0.000000 | 0.000000 | 0.000001 | 0.000002 |

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|---|---|---|---|---|---|---|---|---|---|
| 35 | 0.000000 | 0.000001 | 0.000000 | 0.999517 | 0.000000 | 0.000002 | 0.000000 | 0.000480 | 0.000000 |
| 36 | 0.000000 | 0.000000 | 0.000105 | 0.999895 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 37 | 0.000000 | 0.000033 | 0.000001 | 0.999966 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 38 | 0.000000 | 0.001500 | 0.000000 | 0.998312 | 0.000000 | 0.000148 | 0.000000 | 0.000039 | 0.000000 |
| 39 | 0.000026 | 0.000020 | 0.000012 | 0.999730 | 0.000000 | 0.000000 | 0.000179 | 0.000000 | 0.000034 |
| 40 | 0.000000 | 0.001162 | 0.000000 | 0.995582 | 0.000000 | 0.003110 | 0.000000 | 0.000146 | 0.000000 |
| 41 | 0.000000 | 0.000004 | 0.001230 | 0.998752 | 0.000000 | 0.000000 | 0.000014 | 0.000000 | 0.000000 |
| 42 | 0.000000 | 0.000140 | 0.000000 | 0.999579 | 0.000000 | 0.000000 | 0.000006 | 0.000273 | 0.000002 |
| 43 | 0.000000 | 0.000000 | 0.000785 | 0.998585 | 0.000000 | 0.000092 | 0.000290 | 0.000247 | 0.000001 |
| 44 | 0.000000 | 0.033420 | 0.000025 | 0.956459 | 0.004966 | 0.000985 | 0.000000 | 0.004144 | 0.000000 |
| 45 | 0.000302 | 0.000000 | 0.000000 | 0.999419 | 0.000262 | 0.000000 | 0.000000 | 0.000011 | 0.000006 |
| 46 | 0.000000 | 0.000000 | 0.000019 | 0.999740 | 0.000241 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 47 | 0.000000 | 0.000033 | 0.000000 | 0.999124 | 0.000842 | 0.000001 | 0.000000 | 0.000000 | 0.000000 |
| 48 | 0.000020 | 0.000000 | 0.000000 | 0.998206 | 0.001774 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 49 | 0.000009 | 0.000331 | 0.000000 | 0.998231 | 0.000938 | 0.000000 | 0.000000 | 0.000490 | 0.000000 |
| 50 | 0.000000 | 0.000000 | 0.000094 | 0.999137 | 0.000548 | 0.000000 | 0.000221 | 0.000000 | 0.000000 |
| 51 | 0.000000 | 0.000000 | 0.000008 | 0.999947 | 0.000029 | 0.000017 | 0.000000 | 0.000000 | 0.000000 |
| 52 | 0.000000 | 0.000000 | 0.000000 | 1.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 53 | 0.000000 | 0.000000 | 0.000000 | 0.999078 | 0.000641 | 0.000000 | 0.000000 | 0.000078 | 0.000203 |
| 54 | 0.000000 | 0.000000 | 0.000000 | 0.999901 | 0.000000 | 0.000000 | 0.000001 | 0.000098 | 0.000000 |
| 55 | 0.000000 | 0.000000 | 0.000000 | 0.999616 | 0.000166 | 0.000000 | 0.000000 | 0.000000 | 0.000218 |
| 56 | 0.000000 | 0.000004 | 0.000000 | 0.999873 | 0.000123 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 57 | 0.000004 | 0.000002 | 0.000000 | 0.999945 | 0.000038 | 0.000000 | 0.000000 | 0.000011 | 0.000000 |
| 58 | 0.000001 | 0.000000 | 0.000000 | 0.998440 | 0.001559 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 59 | 0.000001 | 0.000000 | 0.000005 | 0.999994 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 60 | 0.000442 | 0.000000 | 0.000228 | 0.999329 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 61 | 0.000000 | 0.000000 | 0.000991 | 0.998899 | 0.000000 | 0.000108 | 0.000001 | 0.000000 | 0.000000 |
| 62 | 0.000000 | 0.000000 | 0.000051 | 0.999946 | 0.000000 | 0.000000 | 0.000000 | 0.000002 | 0.000001 |
| 63 | 0.000000 | 0.000000 | 0.000253 | 0.999636 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000111 |
| 64 | 0.000000 | 0.000000 | 0.002659 | 0.997341 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 65 | 0.000000 | 0.000000 | 0.000073 | 0.999854 | 0.000003 | 0.000000 | 0.000000 | 0.000071 | 0.000000 |
| 66 | 0.000005 | 0.000000 | 0.000024 | 0.999960 | 0.000000 | 0.000000 | 0.000000 | 0.000009 | 0.000001 |
| 67 | 0.000000 | 0.000000 | 0.000115 | 0.999447 | 0.000438 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 68 | 0.000000 | 0.000000 | 0.000082 | 0.999496 | 0.000000 | 0.000325 | 0.000027 | 0.000000 | 0.000069 |
| 69 | 0.000001 | 0.000000 | 0.000565 | 0.999427 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000007 |
| 70 | 0.000000 | 0.000000 | 0.000000 | 1.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 71 | 0.000000 | 0.000000 | 0.000352 | 0.998879 | 0.000000 | 0.000001 | 0.000000 | 0.000769 | 0.000000 |
| 72 | 0.000000 | 0.000009 | 0.000089 | 0.999863 | 0.000000 | 0.000004 | 0.000035 | 0.000000 | 0.000000 |
| 73 | 0.000000 | 0.000000 | 0.000668 | 0.999192 | 0.000000 | 0.000124 | 0.000000 | 0.000001 | 0.000015 |
| 74 | 0.000000 | 0.000080 | 0.000481 | 0.999425 | 0.000000 | 0.000000 | 0.000000 | 0.000015 | 0.000000 |
| 75 | 0.000022 | 0.000000 | 0.000000 | 0.999717 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000261 |
| 76 | 0.000000 | 0.000000 | 0.000000 | 0.999976 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000024 |
| 77 | 0.000042 | 0.000000 | 0.000000 | 0.999958 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 78 | 0.000020 | 0.000000 | 0.000009 | 0.999898 | 0.000001 | 0.000066 | 0.000000 | 0.000006 | 0.000000 |
| 79 | 0.000000 | 0.000000 | 0.000000 | 1.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 80 | 0.000000 | 0.000000 | 0.000000 | 0.999989 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000010 |
| 81 | 0.000000 | 0.000003 | 0.000000 | 0.999996 | 0.000001 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 82 | 0.000042 | 0.000001 | 0.000000 | 0.999797 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000161 |
| 83 | 0.000001 | 0.000000 | 0.000000 | 0.999999 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 84 | 0.000000 | 0.000000 | 0.000000 | 0.999971 | 0.000000 | 0.000001 | 0.000000 | 0.000027 | 0.000000 |
| 85 | 0.000000 | 0.000000 | 0.833094 | 0.166785 | 0.000008 | 0.000010 | 0.000000 | 0.000067 | 0.000036 |
| 86 | 0.000000 | 0.000000 | 0.874003 | 0.110925 | 0.000000 | 0.015072 | 0.000000 | 0.000000 | 0.000000 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 87 | 0.006611 | 0.000000 | 0.857535 | 0.133859 | 0.001643 | 0.000000 | 0.000000 | 0.000348 | 0.000005 |
| 88 | 0.000000 | 0.000000 | 0.872510 | 0.127114 | 0.000005 | 0.000000 | 0.000337 | 0.000034 | 0.000000 |
| 89 | 0.000000 | 0.000000 | 0.883276 | 0.108676 | 0.000003 | 0.000000 | 0.003520 | 0.004525 | 0.000000 |
| 90 | 0.000000 | 0.000000 | 0.869316 | 0.130309 | 0.000374 | 0.000000 | 0.000001 | 0.000000 | 0.000000 |
| 91 | 0.000000 | 0.000000 | 0.869789 | 0.128859 | 0.000002 | 0.000000 | 0.000000 | 0.001346 | 0.000004 |
| 92 | 0.000000 | 0.000000 | 0.839961 | 0.156577 | 0.003198 | 0.000001 | 0.000036 | 0.000001 | 0.000228 |
| 93 | 0.000004 | 0.000000 | 0.835231 | 0.155287 | 0.000000 | 0.000000 | 0.009472 | 0.000004 | 0.000002 |
| 94 | 0.000000 | 0.000000 | 0.886197 | 0.113802 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 95 | 0.007080 | 0.000000 | 0.815686 | 0.177234 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 96 | 0.000000 | 0.000000 | 0.882174 | 0.113190 | 0.000000 | 0.000000 | 0.000001 | 0.004635 | 0.000000 |
| 97 | 0.000000 | 0.000000 | 0.803059 | 0.196204 | 0.000000 | 0.000000 | 0.000095 | 0.000641 | 0.000000 |
| 98 | 0.000000 | 0.000000 | 0.851876 | 0.148123 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 99 | 0.000000 | 0.000000 | 0.877299 | 0.122695 | 0.000001 | 0.000000 | 0.000005 | 0.000000 | 0.000000 |
| 100 | 0.000000 | 0.000000 | 0.911999 | 0.088000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000001 |
| 101 | 0.000000 | 0.000000 | 0.834255 | 0.160319 | 0.000000 | 0.004381 | 0.000000 | 0.000000 | 0.001044 |
| 102 | 0.000000 | 0.000000 | 0.851569 | 0.147991 | 0.000000 | 0.000043 | 0.000395 | 0.000000 | 0.000002 |
| 103 | 0.000000 | 0.000000 | 0.856286 | 0.143714 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 104 | 0.000000 | 0.000000 | 0.801907 | 0.198093 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 105 | 0.000000 | 0.000000 | 0.867090 | 0.132908 | 0.000001 | 0.000000 | 0.000000 | 0.000001 | 0.000000 |
| 106 | 0.000000 | 0.000000 | 0.894934 | 0.101781 | 0.003285 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 107 | 0.000000 | 0.000000 | 0.020709 | 0.502846 | 0.476445 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 108 | 0.000000 | 0.000000 | 0.001321 | 0.554068 | 0.444611 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 109 | 0.000000 | 0.000000 | 0.000216 | 0.626440 | 0.366358 | 0.000002 | 0.000000 | 0.006984 | 0.000000 |
| 110 | 0.000000 | 0.000000 | 0.001472 | 0.677522 | 0.313166 | 0.000000 | 0.000000 | 0.000000 | 0.007839 |
| 111 | 0.000000 | 0.000000 | 0.000062 | 0.744356 | 0.252779 | 0.000000 | 0.000000 | 0.000000 | 0.002803 |
| 112 | 0.000000 | 0.000003 | 0.000195 | 0.536893 | 0.456878 | 0.005040 | 0.000991 | 0.000000 | 0.000000 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 113 | 0.000000 | 0.000000 | 0.000416 | 0.613552 | 0.356298 | 0.000000 | 0.000000 | 0.029662 | 0.000072 |
| 114 | 0.000000 | 0.000049 | 0.000006 | 0.655536 | 0.344407 | 0.000000 | 0.000001 | 0.000000 | 0.000002 |
| 115 | 0.000000 | 0.000000 | 0.015807 | 0.519676 | 0.464515 | 0.000000 | 0.000000 | 0.000000 | 0.000001 |
| 116 | 0.000000 | 0.000000 | 0.014246 | 0.555688 | 0.426818 | 0.000000 | 0.000000 | 0.003248 | 0.000000 |
| 117 | 0.000000 | 0.000005 | 0.000238 | 0.545016 | 0.454740 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 118 | 0.000000 | 0.004557 | 0.000127 | 0.712675 | 0.280718 | 0.000000 | 0.000000 | 0.000000 | 0.001924 |
| 119 | 0.000000 | 0.000000 | 0.049675 | 0.408232 | 0.542093 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 120 | 0.002377 | 0.000000 | 0.000000 | 0.911637 | 0.084650 | 0.000000 | 0.000000 | 0.001327 | 0.000009 |
| 121 | 0.000000 | 0.000000 | 0.000017 | 0.668279 | 0.290590 | 0.023281 | 0.000000 | 0.000000 | 0.017833 |
| 122 | 0.000000 | 0.000000 | 0.001624 | 0.457210 | 0.522825 | 0.000000 | 0.000004 | 0.000000 | 0.018338 |
| 123 | 0.000000 | 0.024186 | 0.000000 | 0.541315 | 0.433794 | 0.000000 | 0.000677 | 0.000028 | 0.000000 |
| 124 | 0.000049 | 0.000000 | 0.000000 | 0.612493 | 0.387458 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 125 | 0.000693 | 0.527822 | 0.000263 | 0.300891 | 0.069692 | 0.000095 | 0.000086 | 0.057821 | 0.042636 |
| 126 | 0.022203 | 0.543592 | 0.004061 | 0.275140 | 0.000000 | 0.000000 | 0.000011 | 0.000219 | 0.154773 |
| 127 | 0.000870 | 0.612981 | 0.058702 | 0.258636 | 0.000122 | 0.009686 | 0.000023 | 0.047735 | 0.011244 |
| 128 | 0.000652 | 0.609855 | 0.003880 | 0.260087 | 0.060103 | 0.002022 | 0.000000 | 0.000115 | 0.063286 |
| 129 | 0.018761 | 0.777086 | 0.013269 | 0.083179 | 0.001290 | 0.001095 | 0.007164 | 0.000015 | 0.098142 |
| 130 | 0.017377 | 0.777739 | 0.000711 | 0.196619 | 0.000000 | 0.000035 | 0.000029 | 0.000892 | 0.006596 |
| 131 | 0.067363 | 0.782926 | 0.027344 | 0.060995 | 0.000001 | 0.000000 | 0.003353 | 0.000000 | 0.058018 |
| 132 | 0.000014 | 0.968260 | 0.014212 | 0.017372 | 0.000045 | 0.000000 | 0.000038 | 0.000059 | 0.000001 |
| 133 | 0.007702 | 0.974032 | 0.015314 | 0.000638 | 0.001643 | 0.000018 | 0.000000 | 0.000083 | 0.000569 |
| 134 | 0.000000 | 0.985745 | 0.005230 | 0.002856 | 0.005384 | 0.000348 | 0.000000 | 0.000000 | 0.000437 |
| 135 | 0.004068 | 0.925051 | 0.001286 | 0.006593 | 0.045644 | 0.000177 | 0.013913 | 0.000182 | 0.003086 |
| 136 | 0.000000 | 0.989384 | 0.001646 | 0.003419 | 0.003857 | 0.000033 | 0.001352 | 0.000001 | 0.000308 |
| 137 | 0.000000 | 0.967161 | 0.012830 | 0.000008 | 0.003215 | 0.003278 | 0.000169 | 0.013337 | 0.000001 |
| 138 | 0.001333 | 0.945377 | 0.047529 | 0.000880 | 0.001835 | 0.002674 | 0.000044 | 0.000328 | 0.000000 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 139 | 0.004380 | 0.992605 | 0.000017 | 0.002589 | 0.000088 | 0.000205 | 0.000046 | 0.000006 | 0.000064 |
| 140 | 0.000002 | 0.978439 | 0.001040 | 0.015327 | 0.000539 | 0.000000 | 0.001657 | 0.000000 | 0.002997 |
| 141 | 0.000001 | 0.945579 | 0.000344 | 0.005364 | 0.006459 | 0.001192 | 0.000002 | 0.040376 | 0.000682 |
| 142 | 0.024489 | 0.963016 | 0.003000 | 0.000000 | 0.003673 | 0.003710 | 0.000002 | 0.002109 | 0.000000 |
| 143 | 0.000571 | 0.985669 | 0.007969 | 0.001417 | 0.000596 | 0.003554 | 0.000000 | 0.000001 | 0.000222 |
| 144 | 0.000000 | 0.918633 | 0.008459 | 0.000939 | 0.028671 | 0.043263 | 0.000002 | 0.000000 | 0.000032 |
| 145 | 0.000036 | 0.044879 | 0.008330 | 0.940754 | 0.000000 | 0.000324 | 0.001650 | 0.004027 | 0.000000 |
| 146 | 0.000000 | 0.095820 | 0.003768 | 0.894770 | 0.000000 | 0.000009 | 0.000579 | 0.000170 | 0.004884 |
| 147 | 0.000010 | 0.060986 | 0.005313 | 0.925713 | 0.000000 | 0.007705 | 0.000015 | 0.000259 | 0.000000 |
| 148 | 0.000000 | 0.083372 | 0.001058 | 0.911821 | 0.000468 | 0.000015 | 0.000149 | 0.000340 | 0.002777 |
| 149 | 0.000000 | 0.033269 | 0.002210 | 0.954727 | 0.000000 | 0.000114 | 0.008362 | 0.000402 | 0.000915 |
| 150 | 0.000001 | 0.000008 | 0.002162 | 0.997803 | 0.000000 | 0.000000 | 0.000002 | 0.000024 | 0.000000 |
| 151 | 0.000000 | 0.043703 | 0.000000 | 0.953585 | 0.000068 | 0.002586 | 0.000000 | 0.000002 | 0.000055 |
| 152 | 0.000073 | 0.323245 | 0.000000 | 0.655610 | 0.019530 | 0.000192 | 0.000000 | 0.000018 | 0.001333 |
| 153 | 0.001474 | 0.316506 | 0.000000 | 0.678941 | 0.000013 | 0.003055 | 0.000001 | 0.000008 | 0.000004 |
| 154 | 0.000000 | 0.446718 | 0.000000 | 0.534017 | 0.000000 | 0.000000 | 0.015680 | 0.000000 | 0.003584 |
| 155 | 0.000021 | 0.010397 | 0.000392 | 0.985656 | 0.003511 | 0.000010 | 0.000012 | 0.000000 | 0.000000 |
| 156 | 0.000000 | 0.000000 | 0.000000 | 0.999999 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 157 | 0.000000 | 0.181842 | 0.024944 | 0.768523 | 0.000000 | 0.000000 | 0.024692 | 0.000000 | 0.000000 |
| 158 | 0.001047 | 0.961999 | 0.000000 | 0.029909 | 0.000000 | 0.000000 | 0.001688 | 0.002914 | 0.002442 |
| 159 | 0.000000 | 0.944947 | 0.020278 | 0.034603 | 0.000000 | 0.000000 | 0.000001 | 0.000002 | 0.000168 |
| 160 | 0.000002 | 0.999501 | 0.000000 | 0.000243 | 0.000000 | 0.000000 | 0.000074 | 0.000000 | 0.000180 |

**Table S1.16** Proportion on ancestry for individuals of *Scinax squalirostris*, conditioned to environmental variables, inferred by Bayesian analysis of nuclear for 8.5RCP climatic conditions.

| | Inferred Cluster | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Individual** | **Cluster 1** | **Cluster 2** | **Cluster 3** | **Cluster 4** | **Cluster 5** | **Cluster 6** | **Cluster 7** | **Cluster 8** | **Cluster 9** |
| 1 | 1.9923E-05 | 2.2609E-02 | 0.0000E+00 | 9.7714E-01 | 0.0000E+00 | 7.2946E-09 | 0.0000E+00 | 1.8500E-13 | 2.2710E-04 |
| 2 | 1.8812E-06 | 6.4153E-03 | 3.8083E-09 | 9.9161E-01 | 1.5154E-03 | 0.0000E+00 | 1.2439E-10 | 1.7297E-04 | 2.8680E-04 |
| 3 | 9.2239E-04 | 1.3147E-02 | 1.0361E-06 | 9.8588E-01 | 0.0000E+00 | 6.0376E-09 | 0.0000E+00 | 0.0000E+00 | 4.8797E-05 |
| 4 | 9.1963E-10 | 2.4587E-02 | 0.0000E+00 | 9.7516E-01 | 0.0000E+00 | 1.4006E-08 | 0.0000E+00 | 5.3286E-05 | 1.9577E-04 |
| 5 | 2.3637E-07 | 6.3682E-03 | 0.0000E+00 | 9.9230E-01 | 0.0000E+00 | 8.4637E-10 | 6.6903E-06 | 6.1175E-08 | 1.3291E-03 |
| 6 | 0.0000E+00 | 1.3529E-02 | 0.0000E+00 | 9.8230E-01 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 2.5551E-07 | 4.1719E-03 |
| 7 | 5.4259E-11 | 5.5265E-03 | 0.0000E+00 | 9.9430E-01 | 0.0000E+00 | 2.8993E-05 | 3.3371E-08 | 8.9600E-13 | 1.4622E-04 |
| 8 | 4.8050E-05 | 1.0271E-02 | 0.0000E+00 | 9.8947E-01 | 0.0000E+00 | 1.3682E-05 | 3.4499E-05 | 3.6000E-14 | 1.6681E-04 |
| 9 | 1.7302E-10 | 6.8327E-03 | 1.3229E-10 | 9.9108E-01 | 0.0000E+00 | 0.0000E+00 | 1.5760E-05 | 1.8827E-11 | 2.0672E-03 |
| 10 | 4.0000E-15 | 8.7383E-03 | 0.0000E+00 | 9.9123E-01 | 0.0000E+00 | 5.3148E-10 | 1.0000E-15 | 2.8094E-05 | 7.3459E-08 |
| 11 | 6.7242E-05 | 1.2618E-02 | 0.0000E+00 | 9.8728E-01 | 0.0000E+00 | 1.0299E-06 | 4.5100E-13 | 1.4318E-11 | 2.9652E-05 |
| 12 | 1.2327E-09 | 1.5565E-02 | 0.0000E+00 | 9.8443E-01 | 0.0000E+00 | 3.0046E-07 | 1.4687E-07 | 0.0000E+00 | 2.7712E-07 |
| 13 | 5.4830E-03 | 4.8950E-03 | 0.0000E+00 | 9.8856E-01 | 0.0000E+00 | 9.4402E-10 | 5.7485E-07 | 4.0971E-10 | 1.0653E-03 |
| 14 | 7.5159E-09 | 3.1585E-03 | 0.0000E+00 | 9.9683E-01 | 0.0000E+00 | 4.2005E-08 | 1.8583E-07 | 1.0776E-05 | 2.5160E-06 |
| 15 | 8.6470E-07 | 3.9654E-03 | 3.9304E-07 | 9.9603E-01 | 0.0000E+00 | 2.9320E-12 | 0.0000E+00 | 1.4933E-07 | 4.0835E-11 |
| 16 | 2.7499E-09 | 6.7471E-04 | 0.0000E+00 | 9.9925E-01 | 0.0000E+00 | 0.0000E+00 | 1.8350E-12 | 6.2000E-14 | 7.8148E-05 |
| 17 | 2.0628E-08 | 6.0880E-06 | 0.0000E+00 | 9.9770E-01 | 0.0000E+00 | 3.1000E-14 | 4.8047E-04 | 2.2358E-04 | 1.5856E-03 |
| 18 | 3.6894E-09 | 9.8002E-04 | 0.0000E+00 | 9.9901E-01 | 7.2429E-09 | 0.0000E+00 | 0.0000E+00 | 8.4225E-06 | 7.8635E-10 |
| 19 | 1.4417E-11 | 6.1401E-04 | 0.0000E+00 | 9.9906E-01 | 0.0000E+00 | 2.0000E-15 | 1.6997E-08 | 2.4819E-08 | 3.2613E-04 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 20 | 2.6092E-06 | 9.9411E-04 | 0.0000E+00 | 9.9801E-01 | 1.7394E-09 | 4.0934E-07 | 5.8000E-14 | 2.6388E-04 | 7.2570E-04 |
| 21 | 7.3327E-08 | 2.4886E-03 | 2.9467E-06 | 9.9648E-01 | 0.0000E+00 | 2.2400E-08 | 0.0000E+00 | 1.0503E-05 | 1.0214E-03 |
| 22 | 5.1054E-09 | 2.2711E-03 | 1.5304E-03 | 9.9590E-01 | 0.0000E+00 | 3.0005E-08 | 5.5546E-10 | 1.5010E-12 | 2.9906E-04 |
| 23 | 0.0000E+00 | 2.4826E-04 | 1.0101E-08 | 9.9964E-01 | 0.0000E+00 | 4.0700E-13 | 0.0000E+00 | 1.6096E-10 | 1.0847E-04 |
| 24 | 0.0000E+00 | 8.4705E-05 | 0.0000E+00 | 9.9879E-01 | 0.0000E+00 | 0.0000E+00 | 7.9727E-06 | 0.0000E+00 | 1.1138E-03 |
| 25 | 3.4937E-04 | 1.9834E-04 | 0.0000E+00 | 9.9945E-01 | 0.0000E+00 | 0.0000E+00 | 2.1203E-10 | 0.0000E+00 | 9.4555E-07 |
| 26 | 5.6000E-13 | 3.8451E-04 | 0.0000E+00 | 9.9949E-01 | 0.0000E+00 | 0.0000E+00 | 4.8486E-07 | 2.2150E-12 | 1.2738E-04 |
| 27 | 1.1388E-06 | 1.4576E-03 | 2.2695E-06 | 9.9853E-01 | 0.0000E+00 | 3.1927E-08 | 1.3552E-06 | 3.1893E-08 | 3.9564E-06 |
| 28 | 4.2990E-12 | 7.8467E-06 | 1.9712E-06 | 9.9998E-01 | 0.0000E+00 | 0.0000E+00 | 3.2150E-12 | 0.0000E+00 | 1.4334E-05 |
| 29 | 0.0000E+00 | 2.4690E-12 | 2.1614E-06 | 1.0000E+00 | 0.0000E+00 | 0.0000E+00 | 3.9626E-09 | 2.7697E-09 | 9.5875E-10 |
| 30 | 3.0831E-08 | 2.1496E-05 | 5.7507E-06 | 9.9997E-01 | 0.0000E+00 | 7.6383E-08 | 3.1001E-06 | 1.1438E-09 | 2.1776E-11 |
| 31 | 2.5223E-08 | 0.0000E+00 | 3.4360E-07 | 1.0000E+00 | 0.0000E+00 | 0.0000E+00 | 1.2726E-08 | 0.0000E+00 | 0.0000E+00 |
| 32 | 1.3939E-11 | 9.6200E-13 | 4.4899E-07 | 9.9988E-01 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 1.2065E-04 |
| 33 | 1.5763E-07 | 2.2819E-07 | 2.1823E-08 | 9.9985E-01 | 0.0000E+00 | 1.7000E-14 | 0.0000E+00 | 5.7381E-11 | 1.5177E-04 |
| 34 | 5.4324E-10 | 1.0389E-05 | 2.1784E-05 | 9.9994E-01 | 0.0000E+00 | 0.0000E+00 | 1.3880E-12 | 9.4546E-07 | 2.6776E-05 |
| 35 | 3.8512E-10 | 3.5043E-07 | 1.3279E-11 | 1.0000E+00 | 0.0000E+00 | 5.1016E-08 | 1.0000E-15 | 5.5225E-09 | 1.0000E-15 |
| 36 | 1.8644E-05 | 9.1958E-10 | 3.8664E-08 | 9.9997E-01 | 0.0000E+00 | 2.2459E-08 | 1.6966E-08 | 2.5328E-10 | 1.2401E-05 |
| 37 | 4.9206E-10 | 1.1373E-08 | 7.8515E-08 | 1.0000E+00 | 0.0000E+00 | 2.1208E-07 | 2.8300E-13 | 1.1348E-09 | 1.4381E-08 |
| 38 | 1.3443E-06 | 6.6942E-08 | 1.8371E-06 | 1.0000E+00 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 1.7742E-08 | 0.0000E+00 |
| 39 | 3.8508E-05 | 6.6201E-07 | 1.8144E-07 | 9.9989E-01 | 0.0000E+00 | 1.7000E-14 | 7.3045E-07 | 2.1469E-08 | 7.2042E-05 |
| 40 | 7.9700E-13 | 5.7994E-10 | 7.5078E-07 | 9.9998E-01 | 0.0000E+00 | 1.5772E-05 | 1.7876E-07 | 3.2193E-07 | 6.8210E-10 |
| 41 | 3.4413E-08 | 0.0000E+00 | 1.1191E-04 | 9.9989E-01 | 0.0000E+00 | 1.2677E-09 | 4.7670E-12 | 5.7482E-09 | 5.7602E-07 |
| 42 | 7.8050E-10 | 4.0435E-11 | 1.1142E-05 | 9.9967E-01 | 0.0000E+00 | 3.2067E-04 | 6.6638E-08 | 9.0100E-09 | 6.4181E-08 |
| 43 | 5.7361E-10 | 3.7950E-12 | 2.3222E-07 | 9.9998E-01 | 0.0000E+00 | 8.1000E-14 | 1.8271E-05 | 7.3000E-14 | 1.3350E-08 |
| 44 | 3.4712E-04 | 2.5000E-13 | 7.6317E-04 | 9.9883E-01 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 5.6831E-05 | 0.0000E+00 |
| 45 | 0.0000E+00 | 1.0000E-15 | 0.0000E+00 | 9.9982E-01 | 1.2880E-12 | 0.0000E+00 | 2.0737E-05 | 1.5728E-04 | 6.3852E-09 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 46 | 8.2664E-10 | 0.0000E+00 | 0.0000E+00 | 9.9998E-01 | 1.8409E-08 | 0.0000E+00 | 6.1103E-10 | 2.0000E-15 | 1.5929E-05 |
| 47 | 2.4935E-06 | 2.0000E-14 | 0.0000E+00 | 9.9998E-01 | 1.1758E-05 | 0.0000E+00 | 3.5570E-07 | 4.9073E-07 | 1.0000E-15 |
| 48 | 3.8482E-11 | 1.4293E-09 | 0.0000E+00 | 9.9986E-01 | 1.3547E-04 | 2.0000E-15 | 0.0000E+00 | 2.0000E-15 | 2.6336E-11 |
| 49 | 0.0000E+00 | 0.0000E+00 | 1.7002E-07 | 9.9996E-01 | 9.7128E-08 | 4.4275E-05 | 2.8365E-07 | 9.9951E-11 | 0.0000E+00 |
| 50 | 3.8603E-11 | 0.0000E+00 | 0.0000E+00 | 9.9982E-01 | 2.5659E-05 | 1.5132E-04 | 0.0000E+00 | 1.8238E-08 | 0.0000E+00 |
| 51 | 7.7369E-10 | 0.0000E+00 | 2.8334E-11 | 9.9995E-01 | 7.6706E-08 | 1.0000E-15 | 9.0300E-12 | 5.0296E-05 | 1.4696E-09 |
| 52 | 1.9993E-06 | 1.7603E-08 | 4.5600E-13 | 1.0000E+00 | 4.5817E-07 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 |
| 53 | 2.6035E-05 | 0.0000E+00 | 0.0000E+00 | 9.9988E-01 | 8.3525E-05 | 9.2146E-10 | 1.1333E-05 | 6.4046E-07 | 0.0000E+00 |
| 54 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 9.9988E-01 | 7.7173E-05 | 5.3514E-11 | 5.4328E-10 | 4.7159E-05 | 2.5550E-12 |
| 55 | 9.5500E-13 | 0.0000E+00 | 0.0000E+00 | 1.0000E+00 | 1.8148E-08 | 0.0000E+00 | 1.3000E-14 | 1.4162E-06 | 1.8000E-14 |
| 56 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 1.0000E+00 | 4.2059E-06 | 9.1000E-14 | 5.9642E-08 | 6.8209E-10 | 3.4821E-07 |
| 57 | 4.9543E-09 | 0.0000E+00 | 0.0000E+00 | 1.0000E+00 | 3.0028E-07 | 0.0000E+00 | 0.0000E+00 | 1.1436E-10 | 7.0941E-10 |
| 58 | 1.0000E-15 | 0.0000E+00 | 4.9848E-09 | 9.9990E-01 | 3.3849E-06 | 0.0000E+00 | 5.0200E-13 | 1.0000E-15 | 1.0108E-04 |
| 59 | 3.1475E-06 | 4.0300E-13 | 3.3438E-06 | 9.9999E-01 | 7.5000E-14 | 0.0000E+00 | 1.0000E-15 | 9.4593E-09 | 1.8007E-10 |
| 60 | 0.0000E+00 | 0.0000E+00 | 1.3604E-04 | 9.9984E-01 | 0.0000E+00 | 0.0000E+00 | 1.9000E-14 | 2.4817E-05 | 3.7632E-06 |
| 61 | 0.0000E+00 | 2.7000E-14 | 1.3067E-04 | 9.9987E-01 | 0.0000E+00 | 1.5500E-13 | 6.1762E-10 | 2.5217E-07 | 0.0000E+00 |
| 62 | 6.7649E-07 | 2.0709E-06 | 8.3477E-05 | 9.9988E-01 | 0.0000E+00 | 1.5529E-09 | 1.1833E-05 | 2.4574E-05 | 1.3064E-07 |
| 63 | 5.9309E-08 | 0.0000E+00 | 1.3302E-06 | 1.0000E+00 | 0.0000E+00 | 1.0472E-10 | 1.2335E-08 | 7.8501E-10 | 7.1997E-09 |
| 64 | 4.0347E-10 | 1.7163E-09 | 1.1595E-05 | 9.9999E-01 | 0.0000E+00 | 1.9900E-13 | 1.7327E-08 | 3.3647E-08 | 1.2845E-09 |
| 65 | 4.5806E-08 | 0.0000E+00 | 3.8641E-05 | 9.9996E-01 | 3.5000E-14 | 1.9825E-09 | 2.6410E-12 | 0.0000E+00 | 3.3254E-06 |
| 66 | 1.0079E-04 | 0.0000E+00 | 8.9910E-06 | 9.9988E-01 | 0.0000E+00 | 1.7410E-12 | 1.1509E-09 | 8.1387E-06 | 1.2244E-07 |
| 67 | 5.9670E-12 | 3.4635E-09 | 4.1324E-05 | 9.9996E-01 | 0.0000E+00 | 0.0000E+00 | 1.0800E-13 | 3.8196E-09 | 3.6600E-13 |
| 68 | 1.3130E-05 | 0.0000E+00 | 4.1978E-05 | 9.9994E-01 | 0.0000E+00 | 0.0000E+00 | 1.6660E-12 | 4.3200E-13 | 0.0000E+00 |
| 69 | 5.9000E-14 | 7.8436E-05 | 1.8382E-04 | 9.9971E-01 | 0.0000E+00 | 2.5014E-05 | 0.0000E+00 | 0.0000E+00 | 2.6668E-07 |
| 70 | 3.9027E-07 | 0.0000E+00 | 3.2748E-05 | 9.9997E-01 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 8.9000E-13 | 2.0000E-15 |
| 71 | 3.4000E-14 | 4.8818E-09 | 1.8587E-06 | 1.0000E+00 | 0.0000E+00 | 0.0000E+00 | 3.1500E-13 | 1.3462E-08 | 0.0000E+00 |

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|---|---|---|---|---|---|---|---|---|---|
| 72 | 4.7727E-07 | 0.0000E+00 | 3.7972E-05 | 9.9996E-01 | 0.0000E+00 | 0.0000E+00 | 3.6620E-12 | 0.0000E+00 | 1.3724E-06 |
| 73 | 2.0000E-14 | 0.0000E+00 | 1.8608E-04 | 9.9981E-01 | 0.0000E+00 | 1.2934E-10 | 2.9776E-09 | 0.0000E+00 | 4.7020E-11 |
| 74 | 1.2809E-10 | 0.0000E+00 | 1.0482E-06 | 1.0000E+00 | 0.0000E+00 | 1.6779E-07 | 5.1021E-07 | 4.4072E-09 | 9.6452E-11 |
| 75 | 3.6457E-09 | 0.0000E+00 | 1.3000E-14 | 1.0000E+00 | 5.0000E-15 | 1.0670E-12 | 0.0000E+00 | 0.0000E+00 | 4.1793E-10 |
| 76 | 6.6168E-06 | 0.0000E+00 | 0.0000E+00 | 9.9999E-01 | 0.0000E+00 | 0.0000E+00 | 2.2930E-07 | 1.9591E-07 | 0.0000E+00 |
| 77 | 1.5586E-09 | 0.0000E+00 | 1.1681E-06 | 1.0000E+00 | 0.0000E+00 | 0.0000E+00 | 1.0000E-15 | 2.5949E-10 | 2.3622E-10 |
| 78 | 8.0323E-11 | 0.0000E+00 | 1.6600E-13 | 9.9998E-01 | 1.0637E-06 | 0.0000E+00 | 0.0000E+00 | 1.2883E-11 | 1.9630E-05 |
| 79 | 0.0000E+00 | 0.0000E+00 | 4.3000E-13 | 1.0000E+00 | 0.0000E+00 | 3.6335E-07 | 1.9115E-07 | 4.8398E-07 | 1.9000E-14 |
| 80 | 9.3267E-07 | 0.0000E+00 | 0.0000E+00 | 1.0000E+00 | 0.0000E+00 | 0.0000E+00 | 1.8723E-07 | 1.1500E-13 | 0.0000E+00 |
| 81 | 1.4163E-10 | 0.0000E+00 | 0.0000E+00 | 1.0000E+00 | 7.3100E-13 | 6.4310E-12 | 6.9000E-14 | 0.0000E+00 | 2.5048E-07 |
| 82 | 9.4052E-09 | 9.2340E-12 | 5.0020E-12 | 9.9999E-01 | 1.2239E-09 | 5.3561E-07 | 0.0000E+00 | 5.0730E-06 | 0.0000E+00 |
| 83 | 4.5156E-06 | 1.3138E-11 | 0.0000E+00 | 9.9998E-01 | 1.1070E-12 | 7.8138E-11 | 1.0599E-10 | 1.3766E-07 | 1.3072E-05 |
| 84 | 1.9097E-10 | 0.0000E+00 | 1.6062E-05 | 9.9998E-01 | 0.0000E+00 | 4.9736E-08 | 2.3152E-06 | 2.0090E-09 | 1.0393E-11 |
| 85 | 2.9727E-04 | 0.0000E+00 | 4.5527E-01 | 5.4442E-01 | 1.7888E-06 | 3.7980E-11 | 3.3451E-09 | 5.3652E-06 | 8.3271E-07 |
| 86 | 0.0000E+00 | 0.0000E+00 | 4.1262E-01 | 5.8737E-01 | 5.1349E-07 | 1.1018E-10 | 0.0000E+00 | 0.0000E+00 | 5.5489E-07 |
| 87 | 2.1830E-08 | 0.0000E+00 | 3.7501E-01 | 6.2349E-01 | 0.0000E+00 | 1.3827E-03 | 0.0000E+00 | 1.1798E-04 | 0.0000E+00 |
| 88 | 1.0000E-15 | 0.0000E+00 | 4.7862E-01 | 5.2138E-01 | 0.0000E+00 | 3.7000E-14 | 1.3824E-08 | 0.0000E+00 | 4.7100E-13 |
| 89 | 5.6564E-10 | 0.0000E+00 | 4.0613E-01 | 5.9370E-01 | 0.0000E+00 | 1.7054E-04 | 4.7580E-12 | 0.0000E+00 | 0.0000E+00 |
| 90 | 2.6406E-06 | 0.0000E+00 | 4.1727E-01 | 5.8256E-01 | 0.0000E+00 | 1.5884E-04 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 |
| 91 | 0.0000E+00 | 0.0000E+00 | 4.6480E-01 | 5.3504E-01 | 0.0000E+00 | 0.0000E+00 | 1.2315E-08 | 1.6115E-04 | 0.0000E+00 |
| 92 | 1.1153E-05 | 0.0000E+00 | 3.9739E-01 | 6.0165E-01 | 2.0001E-06 | 0.0000E+00 | 9.4024E-04 | 1.6750E-06 | 1.3423E-10 |
| 93 | 0.0000E+00 | 0.0000E+00 | 3.9888E-01 | 6.0112E-01 | 6.0300E-11 | 0.0000E+00 | 0.0000E+00 | 4.3095E-08 | 7.5008E-11 |
| 94 | 0.0000E+00 | 0.0000E+00 | 4.5316E-01 | 5.4683E-01 | 0.0000E+00 | 0.0000E+00 | 3.0906E-06 | 9.3247E-07 | 1.8533E-08 |
| 95 | 2.1221E-07 | 0.0000E+00 | 4.5039E-01 | 5.4388E-01 | 0.0000E+00 | 3.4647E-04 | 9.0000E-15 | 5.3780E-03 | 1.0389E-08 |
| 96 | 1.7782E-05 | 0.0000E+00 | 4.0579E-01 | 5.9419E-01 | 0.0000E+00 | 6.7243E-08 | 1.9721E-06 | 4.6300E-07 | 1.3229E-11 |
| 97 | 8.7675E-06 | 0.0000E+00 | 3.6608E-01 | 6.3382E-01 | 0.0000E+00 | 2.1279E-06 | 3.9500E-13 | 8.4623E-05 | 6.0000E-14 |

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|---|---|---|---|---|---|---|---|---|---|
| 98 | 1.2797E-10 | 0.0000E+00 | 4.1477E-01 | 5.8522E-01 | 0.0000E+00 | 5.3542E-06 | 0.0000E+00 | 6.4067E-09 | 1.8416E-07 |
| 99 | 7.2694E-05 | 0.0000E+00 | 4.2617E-01 | 5.7318E-01 | 2.2579E-04 | 0.0000E+00 | 3.4666E-04 | 2.7000E-14 | 6.0000E-15 |
| 100 | 3.2543E-04 | 0.0000E+00 | 4.5785E-01 | 5.4183E-01 | 2.2986E-07 | 0.0000E+00 | 1.0982E-09 | 7.5225E-08 | 5.0309E-10 |
| 101 | 1.8213E-06 | 0.0000E+00 | 4.0184E-01 | 5.9814E-01 | 0.0000E+00 | 1.4222E-09 | 5.0846E-06 | 2.9440E-08 | 8.3003E-06 |
| 102 | 2.3174E-05 | 0.0000E+00 | 4.1540E-01 | 5.8458E-01 | 0.0000E+00 | 0.0000E+00 | 7.5870E-08 | 1.0562E-07 | 0.0000E+00 |
| 103 | 3.3564E-10 | 0.0000E+00 | 4.0689E-01 | 5.9256E-01 | 0.0000E+00 | 0.0000E+00 | 1.9271E-10 | 5.5278E-04 | 6.6314E-10 |
| 104 | 7.0482E-07 | 0.0000E+00 | 4.2671E-01 | 5.7328E-01 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 2.0699E-06 | 0.0000E+00 |
| 105 | 0.0000E+00 | 0.0000E+00 | 4.2218E-01 | 5.7782E-01 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 0.0000E+00 | 1.0000E-15 |
| 106 | 4.7621E-05 | 0.0000E+00 | 4.3235E-01 | 5.6760E-01 | 0.0000E+00 | 3.3760E-09 | 1.7482E-07 | 0.0000E+00 | 0.0000E+00 |
| 107 | 2.2887E-10 | 0.0000E+00 | 1.4682E-04 | 9.9842E-01 | 1.3494E-03 | 7.9126E-05 | 1.0000E-15 | 0.0000E+00 | 1.0000E-15 |
| 108 | 4.4000E-13 | 0.0000E+00 | 3.6237E-03 | 9.7130E-01 | 2.3580E-02 | 1.4888E-03 | 6.3852E-06 | 2.9865E-11 | 7.6800E-13 |
| 109 | 0.0000E+00 | 2.7000E-14 | 3.4395E-06 | 9.7030E-01 | 1.5469E-02 | 4.9100E-13 | 1.4088E-02 | 9.9685E-10 | 1.4322E-04 |
| 110 | 9.5042E-05 | 0.0000E+00 | 2.6003E-10 | 9.7083E-01 | 2.9075E-02 | 1.1537E-08 | 8.7444E-07 | 4.1093E-11 | 6.9284E-10 |
| 111 | 2.5640E-04 | 1.8645E-10 | 8.7087E-03 | 9.8122E-01 | 9.8173E-03 | 5.1087E-10 | 1.5100E-13 | 0.0000E+00 | 0.0000E+00 |
| 112 | 3.5004E-06 | 4.6856E-06 | 4.9680E-08 | 9.8981E-01 | 3.8990E-03 | 8.5000E-14 | 2.4742E-03 | 3.7943E-03 | 1.1045E-05 |
| 113 | 1.6543E-05 | 0.0000E+00 | 2.8382E-05 | 9.6415E-01 | 2.7938E-02 | 7.5542E-06 | 7.4869E-08 | 7.8631E-03 | 2.4234E-11 |
| 114 | 5.5500E-13 | 0.0000E+00 | 6.9946E-03 | 9.7560E-01 | 1.7319E-02 | 1.2400E-13 | 8.7685E-05 | 7.1006E-07 | 6.8774E-07 |
| 115 | 6.3066E-09 | 0.0000E+00 | 7.2993E-04 | 9.3868E-01 | 6.0589E-02 | 7.6715E-11 | 5.7285E-07 | 2.6700E-13 | 0.0000E+00 |
| 116 | 3.6006E-05 | 0.0000E+00 | 4.3511E-04 | 9.9166E-01 | 7.3349E-03 | 1.1233E-05 | 1.3751E-08 | 5.1799E-04 | 0.0000E+00 |
| 117 | 2.2805E-07 | 0.0000E+00 | 5.5415E-04 | 9.9249E-01 | 6.9566E-03 | 5.3954E-07 | 0.0000E+00 | 3.7790E-11 | 1.0800E-13 |
| 118 | 0.0000E+00 | 0.0000E+00 | 7.6246E-04 | 9.9136E-01 | 7.7941E-03 | 0.0000E+00 | 8.4793E-05 | 8.4055E-07 | 0.0000E+00 |
| 119 | 2.0848E-11 | 0.0000E+00 | 8.7645E-05 | 9.6698E-01 | 2.7158E-02 | 6.2902E-04 | 1.8106E-07 | 5.1495E-03 | 4.4382E-11 |
| 120 | 3.0326E-08 | 0.0000E+00 | 5.2506E-03 | 9.8734E-01 | 7.4007E-03 | 3.6900E-06 | 0.0000E+00 | 3.3629E-06 | 0.0000E+00 |
| 121 | 3.4000E-14 | 0.0000E+00 | 2.2931E-03 | 9.7855E-01 | 1.9093E-02 | 1.0000E-15 | 4.9125E-05 | 6.9948E-09 | 1.4889E-05 |
| 122 | 1.4460E-04 | 0.0000E+00 | 2.2836E-04 | 9.6115E-01 | 3.7578E-02 | 2.5665E-06 | 9.5876E-10 | 8.9586E-04 | 0.0000E+00 |
| 123 | 1.7001E-09 | 3.5965E-06 | 5.9555E-10 | 9.8259E-01 | 1.7404E-02 | 4.4014E-07 | 6.7013E-07 | 6.0954E-09 | 0.0000E+00 |

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|---|---|---|---|---|---|---|---|---|---|
| 124 | 1.0000E-15 | 3.4004E-11 | 2.2784E-05 | 9.8621E-01 | 1.3767E-02 | 2.5781E-06 | 2.7900E-12 | 0.0000E+00 | 0.0000E+00 |
| 125 | 9.9641E-03 | 8.0334E-03 | 9.1630E-04 | 9.5067E-01 | 0.0000E+00 | 1.6076E-02 | 6.0036E-03 | 1.4906E-09 | 8.3332E-03 |
| 126 | 3.3922E-03 | 3.5791E-02 | 5.7002E-05 | 9.2571E-01 | 1.1729E-07 | 2.5435E-02 | 2.6608E-03 | 1.5918E-03 | 5.3625E-03 |
| 127 | 3.2240E-03 | 9.4926E-03 | 2.0516E-04 | 9.3267E-01 | 4.2015E-06 | 1.2658E-05 | 2.2420E-03 | 2.5511E-03 | 4.9597E-02 |
| 128 | 1.8437E-02 | 2.9242E-02 | 3.3585E-06 | 9.4385E-01 | 0.0000E+00 | 3.7883E-03 | 8.5650E-04 | 8.4303E-04 | 2.9763E-03 |
| 129 | 6.0617E-03 | 3.7337E-02 | 8.1497E-07 | 9.4852E-01 | 0.0000E+00 | 1.3908E-08 | 1.0172E-03 | 1.4849E-04 | 6.9121E-03 |
| 130 | 4.0809E-03 | 2.8053E-02 | 1.5701E-03 | 9.5419E-01 | 0.0000E+00 | 3.8562E-05 | 8.5130E-03 | 2.5518E-03 | 1.0018E-03 |
| 131 | 1.1838E-06 | 6.3672E-03 | 2.9898E-02 | 9.4954E-01 | 1.2700E-13 | 1.0000E-15 | 1.0768E-05 | 7.2215E-03 | 6.9615E-03 |
| 132 | 2.3649E-03 | 5.8062E-01 | 1.1492E-03 | 4.0192E-01 | 0.0000E+00 | 1.6179E-06 | 2.5425E-03 | 4.5493E-03 | 6.8465E-03 |
| 133 | 2.5617E-04 | 5.5801E-01 | 5.5682E-05 | 4.1216E-01 | 0.0000E+00 | 4.0816E-07 | 1.8251E-02 | 6.9401E-06 | 1.1261E-02 |
| 134 | 3.5493E-07 | 7.3641E-01 | 1.8623E-02 | 2.4452E-01 | 3.6981E-05 | 6.4644E-06 | 3.9610E-06 | 3.4119E-04 | 5.4983E-05 |
| 135 | 7.3640E-07 | 5.3677E-01 | 5.0311E-06 | 4.4705E-01 | 1.5737E-02 | 8.7796E-05 | 4.5896E-07 | 3.5318E-05 | 3.0872E-04 |
| 136 | 1.9620E-10 | 6.7954E-01 | 3.3801E-08 | 3.0948E-01 | 8.0997E-03 | 1.6870E-03 | 2.9538E-07 | 5.8736E-05 | 1.1305E-03 |
| 137 | 4.5678E-02 | 5.3948E-01 | 7.3321E-02 | 3.3075E-01 | 0.0000E+00 | 1.0142E-02 | 2.0896E-04 | 6.8916E-06 | 4.0563E-04 |
| 138 | 7.9463E-03 | 5.5917E-01 | 5.6107E-04 | 4.2821E-01 | 2.0014E-03 | 4.1894E-11 | 5.1279E-05 | 7.2982E-04 | 1.3221E-03 |
| 139 | 2.4098E-06 | 5.8404E-01 | 1.6395E-03 | 4.0799E-01 | 3.0000E-15 | 6.7290E-08 | 5.3734E-07 | 5.7601E-03 | 5.6822E-04 |
| 140 | 1.1974E-03 | 6.4028E-01 | 2.0807E-02 | 3.1817E-01 | 7.2142E-03 | 1.2335E-02 | 1.9871E-07 | 1.1922E-11 | 4.7910E-08 |
| 141 | 4.7485E-05 | 5.3440E-01 | 8.7023E-05 | 4.4804E-01 | 2.5600E-13 | 5.6993E-11 | 2.1509E-08 | 1.6791E-02 | 6.3509E-04 |
| 142 | 1.0172E-07 | 5.7092E-01 | 5.6035E-03 | 3.8186E-01 | 1.8294E-11 | 1.0224E-04 | 6.4199E-05 | 5.3025E-03 | 3.6145E-02 |
| 143 | 6.2320E-03 | 6.6849E-01 | 2.6500E-04 | 3.1581E-01 | 7.4395E-05 | 2.5860E-07 | 7.0745E-05 | 9.0339E-03 | 2.3165E-05 |
| 144 | 9.6248E-06 | 6.1519E-01 | 5.9378E-04 | 3.7771E-01 | 0.0000E+00 | 1.6376E-09 | 1.8998E-05 | 3.8688E-03 | 2.6091E-03 |
| 145 | 4.2736E-05 | 1.0104E-03 | 2.7844E-03 | 9.9392E-01 | 0.0000E+00 | 4.2703E-09 | 4.8686E-04 | 4.5511E-04 | 1.2956E-03 |
| 146 | 2.6365E-04 | 1.1162E-03 | 2.2547E-03 | 9.9617E-01 | 0.0000E+00 | 5.6941E-05 | 1.4823E-05 | 1.2623E-04 | 0.0000E+00 |
| 147 | 1.4159E-04 | 4.8394E-04 | 3.1798E-03 | 9.9614E-01 | 0.0000E+00 | 3.9574E-09 | 2.1293E-05 | 2.8933E-05 | 2.6029E-07 |
| 148 | 1.2748E-04 | 5.7296E-04 | 1.6209E-03 | 9.9553E-01 | 0.0000E+00 | 5.1592E-07 | 1.3821E-03 | 7.6774E-04 | 6.0000E-14 |
| 149 | 4.1535E-05 | 5.7592E-05 | 2.5596E-03 | 9.9713E-01 | 0.0000E+00 | 7.6023E-06 | 1.0671E-04 | 4.7163E-05 | 4.6427E-05 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 150 | 3.0553E-10 | 1.1300E-13 | 3.0306E-05 | 9.9995E-01 | 0.0000E+00 | 1.2769E-07 | 1.9381E-06 | 1.9036E-06 | 1.5857E-05 |
| 151 | 1.3355E-04 | 2.7916E-04 | 0.0000E+00 | 9.9943E-01 | 0.0000E+00 | 8.6568E-07 | 2.9483E-07 | 1.6002E-04 | 1.1046E-11 |
| 152 | 1.9491E-11 | 7.1169E-04 | 0.0000E+00 | 9.9924E-01 | 3.7600E-13 | 3.8032E-07 | 1.9257E-05 | 4.7305E-06 | 2.5649E-05 |
| 153 | 1.5561E-03 | 4.3867E-03 | 8.2758E-08 | 9.9314E-01 | 8.0400E-13 | 0.0000E+00 | 4.2462E-08 | 9.1625E-04 | 1.7469E-08 |
| 154 | 1.2279E-03 | 3.9359E-03 | 1.5202E-05 | 9.8712E-01 | 7.4520E-03 | 6.9922E-05 | 1.4303E-04 | 1.1320E-12 | 3.1169E-05 |
| 155 | 8.2612E-05 | 4.3558E-04 | 4.9482E-05 | 9.9918E-01 | 2.8312E-08 | 0.0000E+00 | 3.9277E-09 | 2.5294E-04 | 0.0000E+00 |
| 156 | 7.7575E-09 | 0.0000E+00 | 5.0803E-09 | 1.0000E+00 | 0.0000E+00 | 0.0000E+00 | 6.2279E-09 | 7.3217E-07 | 9.6408E-09 |
| 157 | 9.6920E-09 | 2.7757E-03 | 3.1877E-06 | 9.9695E-01 | 0.0000E+00 | 7.2978E-07 | 2.6387E-04 | 2.4201E-07 | 3.0427E-06 |
| 158 | 4.2612E-06 | 1.1427E-01 | 2.3244E-09 | 8.8086E-01 | 0.0000E+00 | 1.2909E-08 | 3.6347E-04 | 2.3241E-07 | 4.5002E-03 |
| 159 | 6.0177E-04 | 1.2522E-01 | 1.1296E-03 | 8.6825E-01 | 0.0000E+00 | 3.8940E-11 | 2.0673E-04 | 4.6462E-04 | 4.1322E-03 |
| 160 | 1.1336E-06 | 9.9265E-01 | 1.9166E-04 | 6.5176E-03 | 0.0000E+00 | 5.8084E-08 | 2.6586E-04 | 3.6779E-04 | 1.4263E-06 |

**Table S1.17** Protection Areas clusters based on *k-mean* cluster analysis.

| Cluster number | Cluster size | Protection Areas ($km^2$) | Genetic richness | Sum of Square within clusters |
|---|---|---|---|---|
| 1 | 41 | -0.12295820 | -2.60794521 | 18.10607 |
| 2 | 133 | -0.21967676 | 1.069904709 | 30.1519 |
| 3 | 311 | -0.16779237 | 0.03600683 | 68.01019 |
| 4 | 39 | 0.06994206 | -1.16858556 | 23.13335 |
| 5 | 20 | 4.18569915 | -0.04499571 | 95.65173 |

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**SUPPORTING INFORMATION**

**Predicting impacts of global climatic change on distribution and genetic diversity of the treefrog *Scinax squalirostris* (Lutz, 1925) (Anura, Hylidae)**

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**Appendix S2** Supplementary figures (Figs S2.1-S2.5) with saturation result, maps of uncertainty, graph of range size and range shift, shifts in ancestry between contemporary and predicted ancestry coefficients and Sum of squares within clusters by number of clusters.

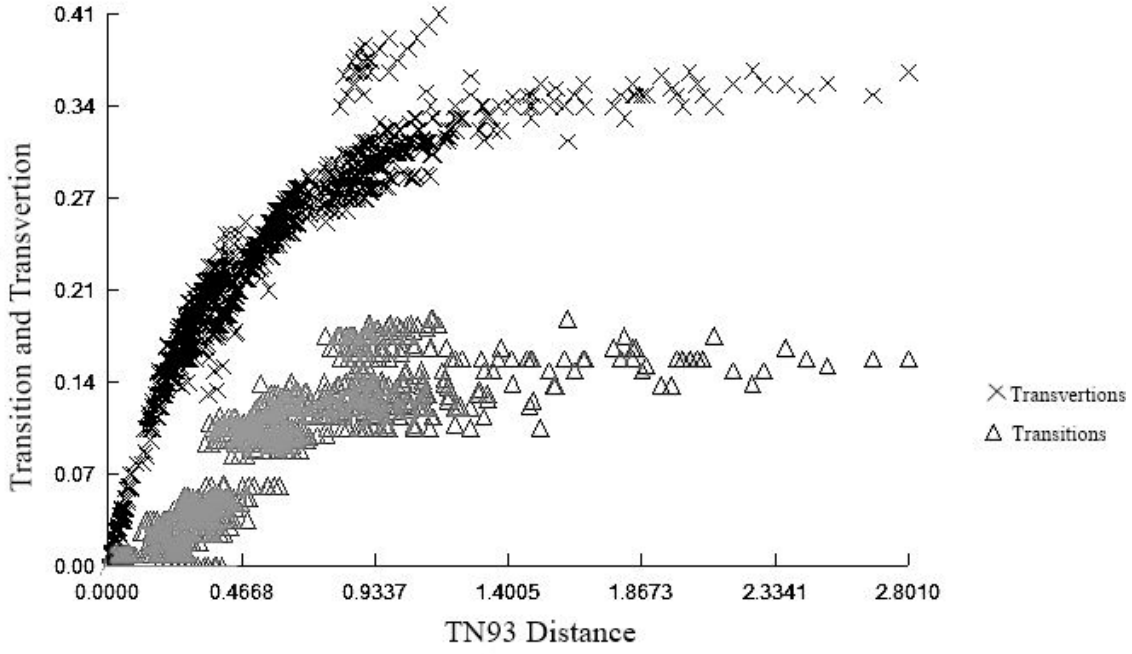


**Figure S2.1** Transition and transversion saturation plot for third codon positions of Cytochrome b sequences.

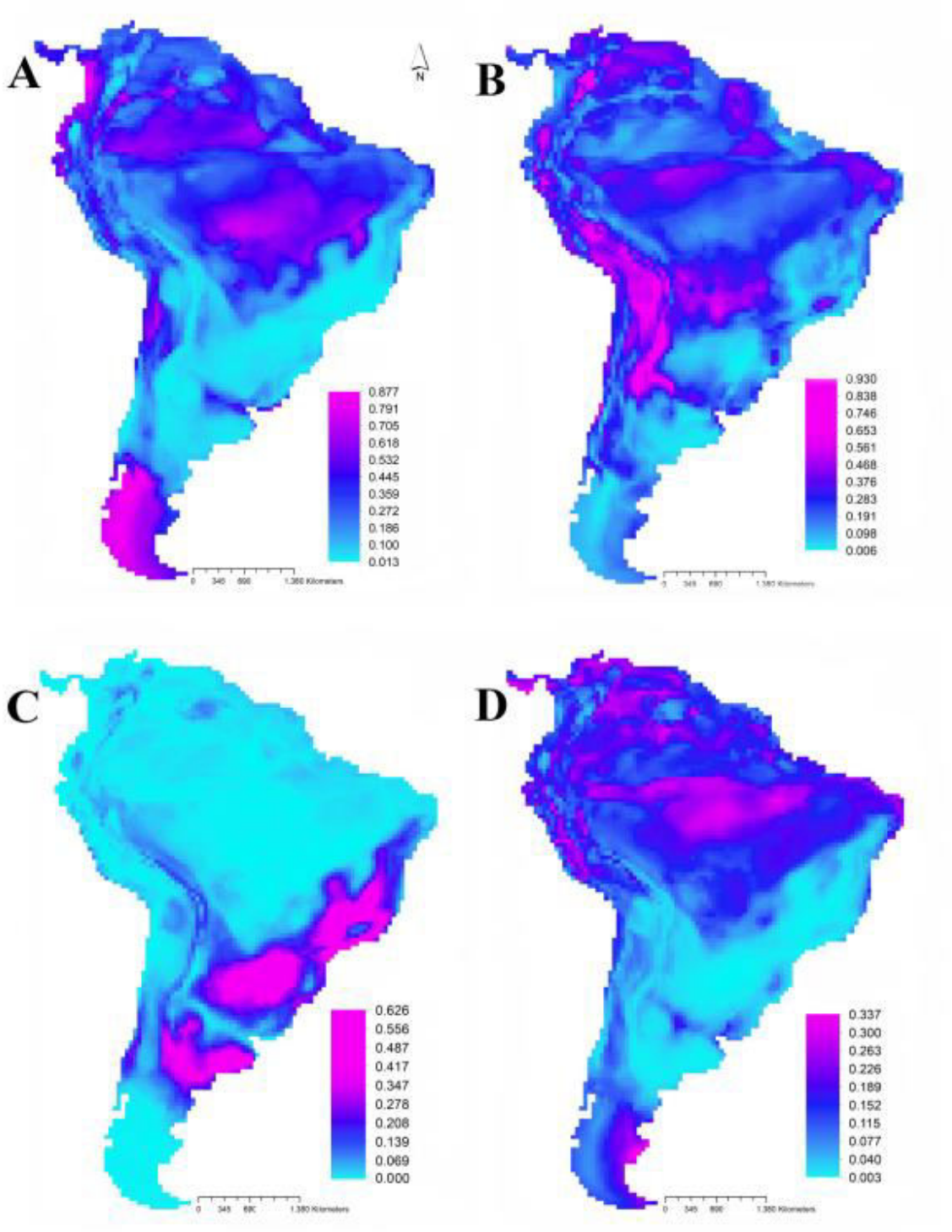


**Figure S2.2** Maps of uncertainty (relative sum of squares) for the modelling components for *Scinax squalirostris*: A) ecological niche models (ENMs), B) atmosphere–ocean global circulation models (AOGCMs), C) time, and D) residual (ENM * AOGCM interaction).

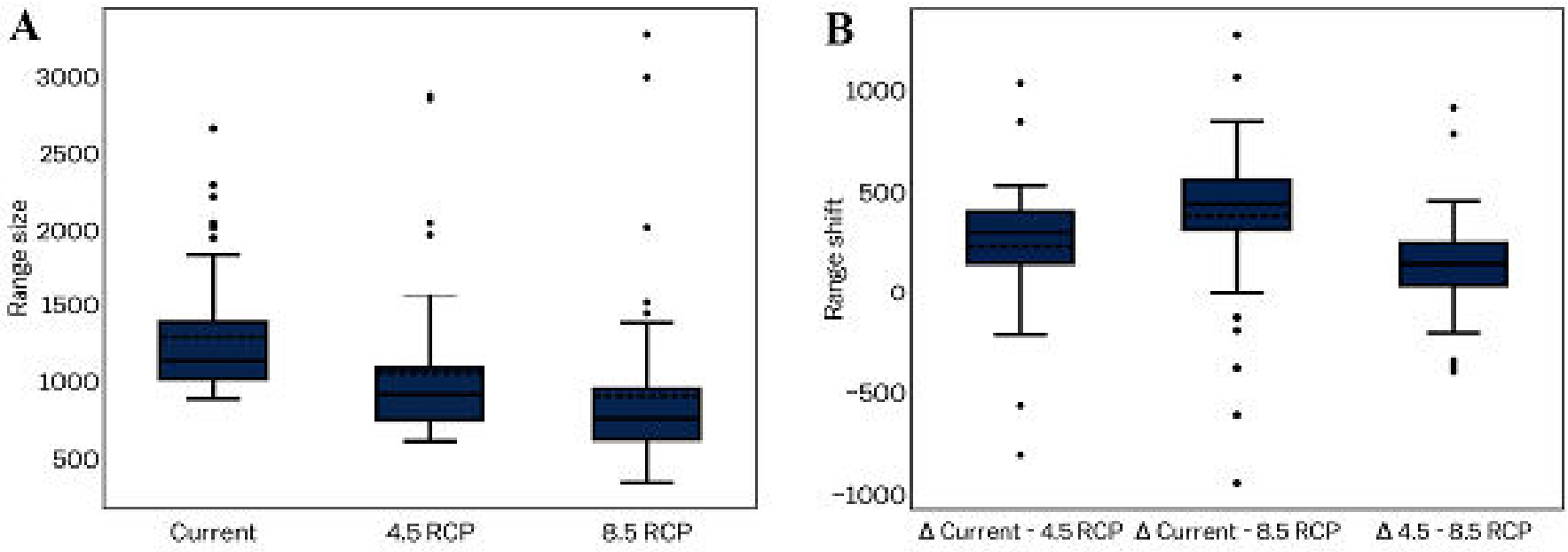


**Figure S2.3** Median and 0.05 – 0.95 quantile among the 48 maps of A) range size and B) shift (difference of range size among time periods in number of cells) predicted for *Scinax squalirostris* at present-day (0 Kya), 4.5 RCP scenario and 8.5 RCP scenario.

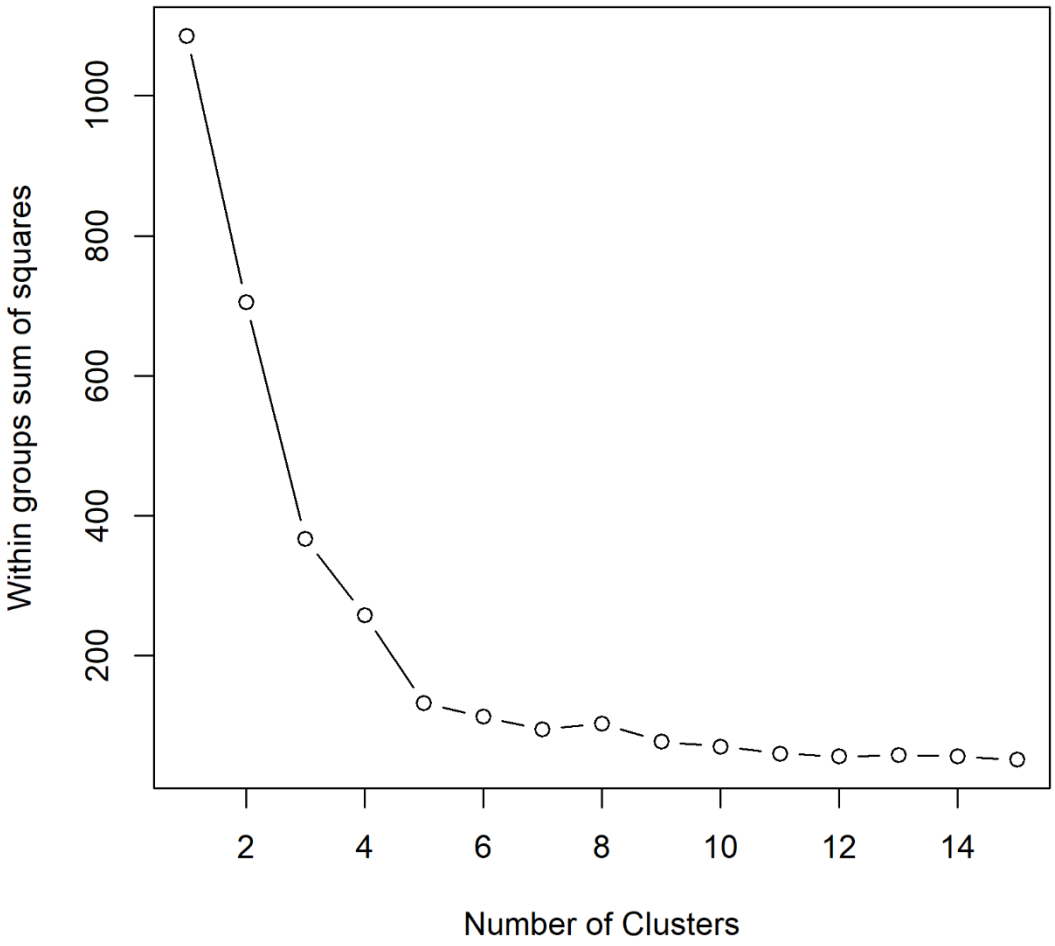


**Figure S2.4** Sum of squares within clusters by number of clusters.

# CONCLUSÃO GERAL

Nesta tese foi avaliado como os eventos históricos podem ter moldado e influenciado a atual distribuição disjunta e diversidade genética de *Scinax squalirostris*. Além disso, avaliamos se essa espécie pode apresentar mais de uma espécie sob o mesmo nome, devido ao isolamento geográfico e restrição ao fluxo gênico. E por fim, avaliamos como as mudanças climáticas esperadas para o fim do século (2100), irão afetar a distribuição geográfica das populações de *S. squalirostris* e se com a perda de áreas adequadas, poderia levar a perda da diversidade genética e assim, uma diminuição do seu potencial de se adaptar as mudanças climáticas, podendo levar a extinção local. Avaliamos essas questões utilizando abordagens de filogeográfica estatística, múltiplos genes de herança e taxas de evolução diferentes, simulações coalescentes e modelagem de nicho ecológico.

No primeiro capítulo verificamos que as populações de *S. squalirostris* apresentam alta diferenciação genética e história demográfica constante ao longo do tempo, com eventos de coalescência e origem de dispersão que datam no Plioceno-Pleistoceno, com compartilhamento de haplótipos entre populações geograficamente distantes, o que indicaria um arranjo incompleto de linhagens. Esses resultados nos levaram a concluir que a atual distribuição disjunta e diversidade genética de *S. squalirostris* é devido a contração de uma área amplamente distribuída no passado, gerada pela dinâmica de retração de *grasslands* nos períodos mais quentes devido à perda de áreas adequadas para sua ocorrência.

No segundo capítulo, através de uma abordagem de taxonomia integrativa, com a utilização de dados moleculares e dados morfométricos resgatou-se a formação de dois grupos evolutivamente distintos. A linhagem restrita a região Centro-Oeste do Brasil é uma candidata a espécie e sem nome para a sua associação, podendo ser descrita. O outro grupo abrange populações do Sul e Sudeste do Brasil, Paraguai, Uruguai e Argentina, e devido a essa relação com populações geograficamente distantes, ainda se faz necessário mais testes de hipóteses de delimitação, afim de verificar a existência de mais linhagens candidatas à espécie.

No terceiro capítulo, utilizamos simulações de dados genéticos e modelagem de nicho ecológico e assim fizemos predições para os efeitos das mudanças climáticas esperadas para o fim do século, que indicaram que *S. squalirostris* terá sua área de distribuição retraída e haverá perda da diversidade genética, que poderá levar a

diminuição do seu potencial em se adaptar as condições locais, principalmente devido as mudanças climáticas serem preditas a acontecer de forma rápida e brusca. Neste capítulo também verificamos que as populações da região Centro-Oeste do Brasil poderão ser extintas devido à ausência de área adequada para a sua ocorrência, essas são as populações que representam ser linhagens evolutivamente distintas candidatas a espécie. Refletindo a problemática em que uma nova espécie pode ser descrita, porém já como ameaçada de extinção. Como a área adequada para a ocorrência irá diminuir, as Unidades de Conservação presentes nessa região, não irão conseguir preservar a sua diversidade genética. Apenas as Unidades de Conservação na região Sul do Brasil, Paraguai, Uruguai e Argentina conseguiram manter a atual riqueza genética encontrada para essas espécies. Porém, nossas simulações genéticas indicaram que haverá perda dos clusters genéticos estimados e poderá haver uma homogeneização da diversidade, em toda a área de ocorrência de *S. squalirostris* e isso poderia levar a uma baixa capacidade de lidar com as mudanças climáticas.

Assim, nosso trabalho indica que *Scinax squalirostris* possui uma história evolutiva complexa, em que os eventos climáticos do passado levaram a uma diminuição de sua distribuição ampla no passado, moldando a sua distribuição atual e sua diversidade genética, e devido à restrição ao fluxo gênico essas populações apresentaram alta estruturação genética. E assim, com a essa estruturação e isolamento, essas populações começaram a se diversificar e hoje podem apresentar pelo menos uma espécie a mais sob o mesmo nome. Com isso, nos deparamos com um cenário em que uma nova espécie pode ser descrita e possivelmente em breve, se enquadrar em uma categoria de ameaça de extinção, devido à perda de áreas adequadas para a sua ocorrência e permanência, mediada pelas mudanças climáticas futuras.

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