Universidade Estadual de Campinas
Instituto de Biologia

Tami da Costa Cacossi

GENOMIC PATTERNS OF *Pitcairnia flammea* (BROMELIACEAE) ACROSS AN ALTITUDINAL GRADIENT IN THE ATLANTIC FOREST

PADRÕES GENÔMICOS DE *Pitcairnia flammea* (BROMELIACEAE) AO LONGO DE UM GRADIENTE ALTITUDINAL DA FLORESTA ATLÂNTICA

CAMPINAS
2024

Tami da Costa Cacossi

GENOMIC PATTERNS OF *Pitcairnia flammea* (BROMELIACEAE) ACROSS AN ALTITUDINAL GRADIENT IN THE ATLANTIC FOREST

PADRÕES GENÔMICOS DE *Pitcairnia flammea* (BROMELIACEAE) AO LONGO DE UM GRADIENTE ALTITUDINAL DA FLORESTA ATLÂNTICA

Dissertation presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Plant Biology

Dissertação apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do Título de Mestra em Biologia Vegetal.

Orientador: Clarisse Palma da Silva

ESTE ARQUIVO DIGITAL CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO DEFENDIDA PELA ALUNA TAMI DA COSTA CACOSSI E ORIENTADA PELA PROFA. DRA. CLARISSE PALMA DA SILVA.

CAMPINAS

2024

Ficha catalográfica
Universidade Estadual de Campinas (UNICAMP)
Biblioteca do Instituto de Biologia
Mara Janaina de Oliveira - CRB 8/6972

C113g

Cacossi, Tami da Costa, 1999-
Genomic patterns of *Pitcairnia flammea* (Bromeliaceae) across an altitudinal gradient in the Atlantic Forest / Tami da Costa Cacossi. – Campinas, SP : [s.n.], 2024.

Orientador: Clarisse Palma da Silva.
Dissertação (mestrado) – Universidade Estadual de Campinas (UNICAMP), Instituto de Biologia.

1. Adaptação (Biologia). 2. Genética de populações. 3. Gradiente altitudinal. 4. Microevolução. 5. Polimorfismo de nucleotídeo único. I. Silva, Clarisse Palma da, 1978-. II. Universidade Estadual de Campinas (UNICAMP). Instituto de Biologia. III. Título.

Informações Complementares

Título em outro idioma: Padrões genômicos de *Pitcairnia flammea* (Bromeliaceae) ao longo de um gradiente altitudinal da Floresta Atlântica
Palavras-chave em inglês:
Adaptation (Biology)
Population genetics
Altitudinal gradient
Microevolution
Single nucleotide polymorphism
Área de concentração: Biologia Vegetal
Titulação: Mestra em Biologia Vegetal
Banca examinadora:
Clarisse Palma da Silva [Orientador]
Isabel Aparecida da Silva Bonatelli
Luiz Augusto Cauz dos Santos
Data de defesa: 26-08-2024
Programa de Pós-Graduação: Biologia Vegetal

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0001-6245-1382
- Currículo Lattes do autor: http://lattes.cnpq.br/9325834251402486

COMISSÃO EXAMINADORA

Profa. Dra. Clarisse Palma da Silva

Profa. Dra. Isabel Aparecida da Silva Bonatelli

Prof. Dr. Luiz Augusto Cauz dos Santos

Os membros da Comissão Examinadora acima assinaram a Ata de defesa, que se encontra no processo de vida acadêmica do aluno.

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa PPG-BV do Instituto de Biologia.

AGRADECIMENTOS

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001, e da Fundação de Amparo à pesquisa do Estado de São Paulo (FAPESP) - via processo 2022/04148-1, através do fornecimento de bolsas de estudo. O Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) também forneceu auxílios financeiros associados a este trabalho.

Agradeço à Universidade Estadual de Campinas (UNICAMP), ao Instituto de Biologia (IB), ao departamento de Biologia Vegetal (DBV), e especialmente ao Laboratório de Ecologia Evolutiva e Genômica de Plantas (LEEG), coordenado por Clarisse Palma da Silva e Fabio Pinheiro, nos quais vivi momentos tão importantes de aprendizado e convivência.

Agradeço à minha orientadora, Clarisse Palma da Silva e ao meu co-orientador, Cleber Chaves, que têm me ensinado e apoiado grandemente na trajetória acadêmica desde a graduação. Agradeço à Bárbara Leal, Marília Tavares e Paulo Aecyo, que contribuíram para o desenvolvimento deste trabalho e para meu desenvolvimento acadêmico.

Agradeço à Maria José Sanín, que me recebeu de braços abertos na Arizona State University, e além de me ensinar e supervisionar durante a o estágio no exterior, me mostrou as belezas infinitas do deserto. Agradeço ao Marcelo Gehara pela oportunidade de visitá-lo na Rutgers University e por todo aprendizado que me proporcionou.

Agradeço aos membros do comitê de acompanhamento, banca de qualificação e de análise prévia de defesa da dissertação por avaliarem o meu trabalho. Agradeço também às professoras e professores que influenciaram minha formação e aos funcionários que possibilitam o funcionamento da universidade.

Agradeço à minha família: minha mãe Yara, meu pai José Eduardo, meus irmãos Tiago e Tárcio, meu sobrinho Martino, e também aos meus avós, tias, tios, primas e primos, cunhadas, cunhado e sogra por todo amor e apoio de sempre. Sem eles, nada seria possível. Um agradecimento especial ao meu noivo, Ygor, que acompanhou bem de perto toda a trajetória deste mestrado e acolheu todas as minhas angústias e conquistas. Agradeço também às minhas amigas e amigos, que trouxeram leveza quando tudo parecia pesado demais.

À minha avó Izolina, que há pouco deixou este mundo: vó, se hoje eu posso correr atrás dos meus sonhos, é por causa de você, que aos quinze anos calçou seu primeiro par de sapatos e caminhou uma vida de muita luta e construção. **Obrigada**.

RESUMO

A Floresta Atlântica é a segunda maior floresta tropical da região Neotropical e é considerada um hotspot para conservação devido aos seus altos índices de endemismo. Devido à sua complexa história geológica, heterogeneidade ambiental, gradientes ecológicos, diversidade e *turnover* de espécies, os afloramentos rochosos da Floresta Atlântica são excelentes sistemas para estudar sua dinâmica evolutiva e ecológica. *Pitcairnia flammea* Lindl. (Bromeliaceae) é uma bromélia endêmica da Floresta Atlântica e ocorre em um gradiente de altitude desde o nível do mar até mais de 2.000 metros, apresentando grandes variações morfológicas e baixo fluxo gênico entre as populações. Neste estudo avaliamos os padrões de diversidade genética e a história evolutiva e demográfica das populações de *P. flammea* ao longo do gradiente altitudinal da Floresta Atlântica, analisando a divergência, o fluxo gênico e possíveis mudanças no tamanho populacional ao longo do tempo, e correlacionando com variações climáticas históricas. Utilizamos marcadores SNP derivados de RAD-seq de 159 indivíduos de oito populações de *P. flammea* distribuídas ao longo do gradiente altitudinal da Floresta Atlântica. O SNP *calling* foi realizado utilizando o genoma de referência da espécie. Nossos resultados revelam que as populações podem ser agrupadas em 3 grupos principais, apesar de serem grupos genéticos únicos. As duas populações de altitudes mais elevadas apresentam maiores níveis de diversidade quando comparadas às altitudes baixas e médias. São também as mais isoladas e têm tempos de divergência mais antigos, tendo divergido das demais há cerca de 300 mil anos, e entre si a cerca de 150 mil anos. As populações em altitudes baixas/médias estão mais conectadas, tendo divergido umas das outras nos últimos 60 mil anos. Nossos resultados sugerem diferentes histórias demográficas em altitudes altas e baixas/médias, tendo como cenário os ciclos climáticos do Pleistoceno. Aqui demonstramos que, entre outros componentes ambientais, a altitude foi e continua a ser importante ao moldar os padrões de diversidade nesta floresta tropical extraordinariamente complexa.

ABSTRACT

The Atlantic Forest is the second-largest tropical forest in the Neotropical region and is considered a hotspot for conservation due to its high levels of endemism. Because of its complex geological history, environmental heterogeneity, ecological gradients, and species diversity and turnover, the Atlantic Forest's rock outcrops are excellent for studying its evolutionary and ecological dynamics. *Pitcairnia flammea* Lindl. (Bromeliaceae) is an endemic bromeliad from the Atlantic Forest, and occurs across an elevational gradient from sea level to over 2,000 meters, exhibiting significant morphological variations and low gene flow among populations. In this study, we assessed the patterns of genetic diversity and the evolutionary and demographic history of *P. flammea* populations along the elevational gradients of the Atlantic Forest, analyzing divergence, gene flow, and potential changes in population size over time, while correlating with historical climatic variations. We used SNP markers derived from RAD-seq of 159 individuals from eight populations of *P. flammea* distributed along the altitudinal gradient of the Atlantic Forest. SNP calling was performed using the species' reference genome. Our results reveal that populations can be grouped into three main clusters, although they are unique genetic units. The two populations at higher altitudes show higher diversity levels than those at low and mid altitudes. They are also the most isolated and have older divergence times, having diverged from the others approximately 300 thousand years ago, and from each other about 150 thousand years ago. Populations at low/mid altitudes are more connected, having diverged from each other in the last 60 thousand years. Our results suggest different demographic histories at high and low/mid altitudes, influenced by the climatic cycles of the Pleistocene. Here we demonstrate that, among other environmental factors, altitude was and continues to be important in shaping diversity patterns in this extraordinarily complex tropical forest.

LISTA DE FIGURAS

Figure 1 - (A), (B) Distribution of the sampled *Pitcairnia flammea* populations in the Atlantic Forest; (C) elevation (in meters) of each population collected. Examples of sampled individuals from (D) MAR (Pico dos Marins, 2140 m a.s.l), (E) ITA (Itatiaia, 1139 m a.s.l), and (F) UBA (Ubatuba, 20 m a.s.l). Colors indicate: dark red, UBA (Ubatuba); red, RAN (Morro do Rangel); orange, COR (Corcovado - P. N. da Serra da Tijuca); green, MAC (Pico da Maria Comprida); light blue, ITA (P. N. de Itatiaia); blue, PES (Pedra do Sino - P. N. da Serra dos órgãos); dark blue, MAR (Pico dos Marins); navy blue, PAP (Serra do Papagaio).

Figure 2 - Heatmap of pairwise genetic divergence (F_{ST}) between eight *P. flammea* populations, based on 1561 SNPs (dataset II). Hotter colors mean higher F_{ST} values. For population details see Table 1.

Figure 3 - Results of STRUCTURE clustering for K = 2 (A) and K = 3 (B); Discriminant Analysis of Principal Components (DAPC) with 4 *K-mean* clusters (C); and ADMIXTURE results, assuming 8 clusters. All clustering analyses were done for 159 individuals of *Pitcairnia flammea* based on 1561 SNPs (dataset II). For population details see Table 1.

Figure 4 - Unrooted phylogenetic tree from SplitstTree5 neighbor-joining analysis for 159 individuals of *Pitcairnia flammea* based on 1561 SNPs (dataset II). Colored forms represent minor clusters (same as ADMIXTURE). For population details see Table 1.

Figure 5 - SNAPP results for SNPs tree of the eight *P. flammea* populations. Numbers indicate divergence estimations, in million years. For population details see Table 1.

Figure 6 - Fastsimcoal v2.7 simulated models for gene flow dynamics among *P. flammea* populations along the Atlantic Forest elevational gradient. **No** (no gene flow among populations); **Constant** (constant gene flow among populations over time); **Early** (only ancient gene flow among populations); **Recent** (only recent gene flow among populations); **Diff** (higher ancient gene flow reducing with isolation accumulation over time). **L/M** = UBA, RAN, COR, ITA, and PES; **H** = MAR and PAP. For population details see Table 1.

Figure 7 - PipeMaster simulated model (IMDL_M_H) for parameter estimation on demographic histories of *P. flammea* populations along the Atlantic Forest elevational gradient. The model reflects that MAC divergence was not considered for its evolution, and its relation to others was through migration). **L** = UBA, RAN, COR, ITA, and PES; **M** = MAC; and **H** = MAR and PAP. For population details see Table 1.

Figure 8 - Stairway Plot 2 results for changes in effective population size (*Ne*) for 3 clusters: populations from high elevations (MAR and PAP) MAC (*dark gray*), and populations from low/mid elevations (UBA, RAN, COR, ITA, and PES, *light gray*) (A). Stairway Plot 2 results within groups for high elevation populations PAP (*navy blue*) and MAR (*dark blue*) (B); and for low/mid elevations populations UBA (*dark red*), RAN (red), COR (*orange*), ITA (*light blue*), and PES (*blue*). For population details see Table 1.

Figure 1S - Flowchart representing dataset construction after filtering as required for each analysis. For Fastsimcoal and StairwayPlot 2, additional filtering was required as described in the Material and Methods session.

Figure 2S - Magnitude of ΔK (Delta K) as a function of K in STRUCTURE analysis. The modal value of these distributions indicates the true value of K (or the value corresponding to the highest level of population structure).

Figure 3S - Stairway Plot 2 results for populations from high elevations (MAR and PAP) (A); population MAC (B); and populations from low/mid elevations (UBA, RAN, COR, ITA, and PES. Red line: median of 200 inferences based on subsampling. Dark gray lines: 75% confidence interval of the inference. Light gray lines: 95% confidence interval. For population details see Table 1.

Figure 4S - Stairway Plot 2 results for populations ITA (P. N. de Itatiaia 1139 m a.s.l), PES (Pedra do Sino - P. N. da Serra dos órgãos 1496 m a.s.l), MAR (Pico dos Marins, 2037 m a.s.l), and PAP (Serra do Papagaio, 2140 m a.s.l). Red line: median of 200 inferences based on subsampling. Dark gray lines: 75% confidence interval of the inference. Light gray lines: 95% confidence interval. For population details see Table 1.

Figure 5S - Stairway Plot 2 results for populations UBA (Ubatuba, 20 m a.s.l), RAN (Morro do Rangel, 25 m a.s.l), and COR (Corcovado - P. N. da Serra da Tijuca, 425 m a.s.l). Red line: median of 200 inferences based on subsampling. Dark gray lines: 75% confidence interval of the inference. Light gray lines: 95% confidence interval. For population details see Table 1.

Figure 6S - Likelihood comparison for gene flow models tested for the 3 major clusters, in pairs: low/mid populations (UBA, RAN, COR, ITA, and PES), high elevation populations (MAR and PAP), and MAC, using FASTSIMCOAL v2.7. For population details see Table 1.

LISTA DE TABELAS

Table 1 – Information on *Pitcairnia flammea* collected and sequenced for each population.

Table 2 – Genetic diversity indices from STACKS for *Pitcairnia flammea* populations. *HO*, observed heterozygosity; *HE,* expected heterozygosity; F_{IS}, coefficient of inbreeding; π, nucleotide diversity. For population details see Table 1.

Table 3 – AIC (Akaike Information Criterion) values for the 5 gene flow models tested pairwise on Fastsimcoal v2.7 between the three major-scale clusters of *Pitcairnia flammea* populations.

Table 1S – Number of raw Illumina reads sequenced per individual of *Pitcairnia flammea* (Bromeliaceae).

Table 2S – Number of individuals and SNPs used for each major cluster (High-elevation, MAC or Low/mid-elevation) or population (PAP, MAR, PES, ITA, COR, RAN and UBA) used in the StairwPlot and Fastsimcoal analyses. Clusters were analyzed pairwise in Fastsimcoal. SNPs were used to generate MAF matrices (Minor Allele Frequency) used in both StairwayPlot and Fastsimcoal. For population details see Table 1.

Table 3S – Pairwise F_{ST} between the eight *Pitcairnia flammea* populations obtained using Stacks. For population details see Table 1.

SUMÁRIO

CAPÍTULO 1

1. INTRODUÇÃO GERAL

A região Neotropical é notavelmente reconhecida por sua alta diversidade biológica e endemismos (Antonelli et al. 2018), e diversos estudos recentes têm sido dedicados a desvendar os processos responsáveis pela criação e manutenção da biodiversidade neotropical em diferentes organismos e condições ambientais (Cacossi et al. 2019; Dantas-Queiroz et al. 2021; Antonelli, 2022; Palma-Silva et al. 2022; Pessoa et al. 2022; Romero-Soler et al. 2022; Souza et al. 2022; Vicente-Silva et al. 2022). Sendo a segunda maior floresta tropical úmida da América do Sul, a Floresta Atlântica é localizada no Neotrópico e é formada por um complexo de vegetações que abrigam vários tipos de comunidades vegetais, estando entre os cinco principais hotspots de biodiversidade do mundo (Myers et al., 2000; Oliveira-Filho & Fontes, 2000). Estimativas apontam que este bioma comporta cerca de 1 a 8% da biodiversidade global, com altos níveis de endemismo (Myers et al. 2000). Com cerca de 12% do território original, a Floresta Atlântica é, portanto, considerada uma prioridade para a conservação, uma vez que a alta fragmentação de origem antrópica aplicada na área coloca em risco sua megadiversidade (Morellato & Haddad 2000, Ribeiro et al. 2009).

Os habitats naturalmente fragmentados da Floresta Atlântica são consequência da heterogeneidade topográfica e ambiental imposta pelos amplos gradientes latitudinal (3-30°S) e altitudinal (0-2.900 m asl.) da sua distribuição (Ribeiro et al. 2011; Thom et al. 2020). A complexidade do relevo, a heterogeneidade ambiental e a fragmentação de habitat da Floresta Atlântica promovem a biodiversidade por aumentarem a divergência entre as populações como consequência do isolamento físico e reprodutivo, uma vez que a falta de fluxo gênico entre populações pode resultar no acúmulo de diferenças e culminar em especiação (Martin & McKay 2004; Polato et al. 2018). Neste sentido, espécies distribuídas neste bioma podem apresentar alterações fenotípicas relacionadas tanto à plasticidade quanto a adaptações locais ao longo desses gradientes latitudinais e altitudinais (Angilletta & Angilletta 2009; Moritz et al. 2012). Por exemplo, o estresse térmico e o estresse hídrico podem levar a pressões seletivas e consequentes modificações fenotípicas relacionadas a taxas de transpiração, fotossíntese e crescimento de plantas (Ataídes, 2018). De acordo com a hipótese de Janzen (1967), o gradiente de temperatura em montanhas tropicais está associado a microhabitats climaticamente estáveis, o que facilita a adaptação e aclimatação local, limitando a dispersão e aumentando isolamento reprodutivo nessas regiões. Estudar a distribuição dos padrões de diversidade genética, fluxo gênico e a história demográfica das populações pode contribuir para investigar hipóteses tais quais a de Janzen (1967), e melhorar o conhecimento sobre os processos micro e macroevolutivos na Floresta Atlântica (Hess et al. 2013; Nadeau et al. 2013; Harvey & Brumfield 2015; Hmeljevski et al. 2017; Palma-Silva et al., 2009, 2016; Pinto et al. 2019; Acha et al. 2021; Leal et al. 2021; Vieira et al. 2022).

A heterogeneidade consequente do relevo, juntamente com variações climáticas históricas, são os principais fatores responsáveis pelos padrões de diversidade observados na Floresta Atlântica e em outras regiões montanhosas tropicais e não tropicais (Flantua et al. 2019; Rahbek et al. 2019). A Floresta Atlântica foi altamente influenciada por eventos de expansão e retração originados por variações climáticas cíclicas do Quaternário, em especial do Pleistoceno (Prance 1982; Carnaval et al. 2009; Silva et al. 2014, Leite et al. 2016). Durante os períodos de retração, muitas espécies adaptadas a ambientes florestais permaneceram restritas a refúgios, ocasionando no isolamento reprodutivo entre populações; já os períodos de expansão possibilitaram o fluxo gênico e a conectividade entre populações (Prance 1982; Silva et al. 2014). No entanto, outros estudos sugerem que as oscilações climáticas contribuíram principalmente alterando a distribuição das espécies (Antonelli et al. 2018). O Quaternário teve um impacto ainda mais expressivo nas montanhas: montanhas tropicais foram refúgio ao aumento de temperatura dos períodos interglaciais, resultando no isolamento entre populações, enquanto nas glaciações as altitudes mais baixas e de temperaturas mais amenas eram habitats predominantes, o que aumentava a conectividade (Rahbek et el. 2019; Thom et al. 2020). Apesar destes padrões serem muitas vezes generalizados para toda a Floresta Atlântica, alguns estudos apontam que as porções Sul e Norte da Floresta foram afetadas de forma diferente pelas oscilações climáticas do Quaternário (Carnaval et al. 2014; Cazé et al. 2016; Damasceno et al. 2021; Abreu et al. 2022). Além disso, algumas espécies podem ter permanecido estáveis neste período, como é o caso do complexo de espécies *Vriesea incurvata* (Bromeliaceae), distribuída ao no sul da Floresta Atlântica, o que pode apontar para uma possível estabilidade climática na região de ocorrência da espécie (Aguiar-Melo et al. 2019). De forma geral, muitos fatores devem ser levados em consideração para explicar os altos níveis de diversidade e endemismo atualmente observados na Floresta Atlântica: eventos de retração e expansão, mudanças de habitat relacionadas oscilações climáticas, vicariância, heterogeneidade ambiental e adaptação; e diferentes grupos responderam diferentemente às pressões evolutivas (Antonelli et al. 2018; Peres et al. 2020).

Estudos de genética populacional têm sido empregados com finalidade de desvendar os padrões de diversidade e histórias evolutivas de populações da Floresta Atlântica. Atualmente, estes estudos utilizam especialmente dados de sequenciamento de alto rendimento, que fornecem de forma eficiênte, barata, e rápida, milhares de marcadores moleculares tais quais os nucleotídeos de polimorfismo único ou SNPs (*Single Nucleotide Polymorphism*) (Davey & Blaxter 2010). O sequenciamento de fragmentos de DNA associado a sítios de restrição ou RAD-seq (*Restriction site associated DNA sequencing*) é um método de sequenciamento de alto rendimento baseado no uso de enzimas para a fragmentação do DNA em pequenas sequências amostradas ao longo de todo genoma, fornecendo um conjunto de milhares de marcadores SNP (Baird et al. 2008, Peterson et al. 2012). Esta técnica permite a identificação de marcadores neutros – que evidenciam padrões de diferenciação populacional associados à deriva genética – e também de marcadores de regiões genômicas sob seleção natural – que indicam padrões de adaptação local de populações (Narum et al. 2013). Estudos prévios demonstraram a eficiência do RAD-seq para investigação de processos evolutivos em espécies neotropicais não modelo (por exemplo, Nadeau et al. 2013; Roda et al. 2013; Ebel et al. 2015; Pinto et al. 2019; Acha et al. 2021; Vieira et al. 2022) incluindo bromélias (Leal et al. 2021, Tavares et al. 2022).

Uma forma de testar como a biodiversidade respondeu às mudanças históricas ambientais e de paisagem é testar modelos de divergência, mudança no tamanho populacional, e fluxo gênico ao longo do tempo, espaço e gradientes ambientais (Gavin et al. 2014). Reconstruções da história evolutiva e demográfica de populações já foram realizadas utilizando marcadores SNP derivados de RAD-seq e diferentes abordagens para construção e seleção de modelos, tais quais Computação Bayesiana Aproximada ou ABC (*Aproximate Bayesian Computation*) (Cornille et al. 2016; Sanín et al. 2017; Rougemont & Bernatchez, 2018; Gehara et al. 2020); máxima verossimilhança, como a teoria coalescente implementada no FASTSIMCOAL (Sovic et al. 2019; Rödin-Mörch et al. 2019; Teixeira &Nazareno, 2021); e o Aprendizado Supervisionado de Máquinas ou SML (sigla para *Supervised Machine-Learning*) (Gehara et al. 2020; Coelho et al. 2022). Através do uso de dados genômicos, diferentes cenários de divergência, migração e mudanças no tamanho da população podem ser testados (Cornille et al. 2016; Rougemont & Bernatchez, 2018; Rödin-Mörch et al. 2019; Sovic et al. 2019). Por exemplo, Coelho et al. (2022) utilizaram tanto ABC quanto SML para testar a hipótese do Rio São Francisco, revelando que este atua como barreira para populações de diversas espécies de vertebrados e promove a divergência por vicariância. Tavares et al. (2022), com base no uso de marcadores SNP e na análise ABC, demonstraram que o isolamento com redução gradual da migração é o melhor modelo para explicar o processo de divergência entre as bromélias hibridizantes *Pitcairnia staminia* e *Pitcairnia albiflus*.

A família Bromeliaceae é altamente diversa, com grande variabilidade anatômica, morfológica, ecológica e genética. É reconhecida como um excelente exemplo de radiação adaptativa no Neotrópico, com distribuição ampla em escala climática, latitudinal, e altitudinal (Benzing 2000; Givnish et al. 2014; Palma-Silva et al. 2016). Alguns exemplos de inovações-chave que possibilitaram às bromélias alcançar essa extraordinária radiação adaptativa são: metabolismo fotossintético CAM, associado à colonização de ambientes xéricos; tricomas peltados que permitem a absorção foliar de água e nutrientes; colonização de nichos ecológicos epifíticos; polinização por aves; e folhas em forma de tanque armazenadores de água (Quezada & Gianoli 2011; Givnish et al. 2014; Silvestro et al. 2014). O Neotrópico é o maior centro de diversidade de bromélias (Givnish et al. 2014), no qual alcançaram Escudo das Guianas ca. 100 milhões de anos atrás, e se espalharam rapidamente pelos Andes, Amazônia, América Central, Caribe e Escudo Brasileiro, se divergindo em um período relativamente curto (ca. 15-10 milhões de anos). Com o soerguimento dos Andes, a circulação das massas de ar oriundas do Oceano Pacífico foi bloqueada, fazendo com que o clima no leste do continente se tornasse mais ameno e chuvoso (Givnish et al. 2014; Versieux et al. 2012). Além disso, a presença da Serra do Mar e da Mantiqueira na costa leste do continente promoveu a estagnação da umidade vinda do Oceano Atlântico. Este clima altamente estável e úmido propiciou a radiação de bromélias na Floresta Atlântica (Givnish et al. 2014).

A subfamília Pitcairnoideae s s. é uma das oito subfamílias de Bromeliaceae e compreende cinco gêneros – *Dickya*, *Encholirium*, *Deuterocohnia*, *Fosterella* e *Pitcairnia* (Schutz et al 2016), sendo *Pitcairnia* o mais diverso, com cerca de 450 espécies (Saraiva et al 2015) que se diversificaram há aproximadamente 12 milhões de anos (Givnish et al 2014). *Pitcairnia flammea* Lindl. é uma espécie endêmica com alta variação fenotípica e com ampla distribuição em um gradiente altitudinal da Floresta Atlântica (Smith e Downs 1974). *P. flammea* (Figura 1) é encontrada em afloramentos rochosos isolados (ou inselbergs; Porembski, 2007) em populações naturalmente fragmentadas. Como consequência, o baixo fluxo gênico entre suas populações (Mota et al. 2020) torna *P. flammea* um bom modelo para estudar o padrão de distribuição de diversidade e a história evolutiva de populações associada à influência das oscilações climáticas do Quaternário (Hughes e Hollingsworth 2008; Damasceno et al. 2014).

Um estudo anterior apontou a existência de estratégias ecológicas divergentes em populações de *P. flammea* associadas ao gradiente de altitude da Floresta Atlântica. Chaves et al. (2024) demonstraram que em altitudes baixas, populações tendem a investir em armazenamento de água, como por exemplo alta suculência e densidade de tricomas abaxiais em função de evitar a perda de água para o ambiente por transpiração. Já populações de maiores altitudes têm maior tolerância térmica, apresentando em suas membranas lipídios associados ao aumento da fluidez, característica importante à redução do efeito do enrijecimento das folhas em baixas temperaturas. Estes resultados revelam a diversidade de respostas das populações às diferentes condições climáticas ao longo do gradiente, mas também instigam a investigação de como as oscilações climáticas históricas podem ter influenciado na história evolutiva destas populações.

2. OBJETIVOS

O presente estudo buscou entender os padrões de diversidade genética e a história evolutiva e demográfica de populações de *P. flammea* ao longo do gradiente de altitude da Floresta Atlântica, analisando a divergência, o fluxo gênico e possíveis mudanças no tamanho populacional ao longo do tempo, correlacionando com as variações climáticas históricas. Neste sentido, os objetivos específicos deste estudo foram:

1) Identificar os padrões de diversidade e estrutura genética das populações de *P. flammea* ao longo do gradiente altitudinal;

2) Avaliar quando se deu a divergência entre as populações;

3) Desvendar como se deu a história demográfica em relação a mudanças nos tamanhos populacionais e ao fluxo gênico entre populações;

4) Relacionar a história evolutiva e demográfica das populações com as mudanças climáticas do Quaternário;

5) Relacionar padrões genéticos com diferentes estratégias ecológicas encontradas em baixas e altas altitudes.

Para tanto nós utilizamos dados de RAD-seq obtidos para 159 indivíduos amostrados em oito populações da espécie, distribuídas entre 20-2140 m de altitude. As populações utilizadas neste trabalho foram as mesmas avaliadas por Chaves et. al., 2024, o que permitiu a comparação de resultados de genética populacional com as estratégias ecológicas das populações. Nosso estudo ajudou na compreensão dos padrões e processos responsáveis pela alta diversidade atual observada em uma espécie endêmica do gradiente altitudinal da Floresta Atlântica. Nossos resultados referentes à história evolutiva das populações podem ser motivadores à adoção de políticas públicas de conservação de populações frente às mudanças climáticas atuais e futuras.

REFERÊNCIAS

Abreu, E. F., Dalapicolla, J., Furini, P. R., De La Sancha, N. U., Jansa, S. A., & Percequillo, A. R. (2022). Habitat stability shaped the demographic history of the Atlantic Forest endemic rodent *Euryoryzomys russatus* (Cricetidae: Sigmodontinae) during the Pleistocene. *Biological Journal of the Linnean Society*, 136(4), 520-535.

Acha, S., Linan, A., MacDougal, J., & Edwards, C. (2021). The evolutionary history of vines in a neotropical biodiversity hotspot: Phylogenomics and biogeography of a large passion flower clade (*Passiflora* section Decaloba). *Molecular phylogenetics and evolution,* 164, 107260.

Aguiar‐Melo, C., Zanella, C. M., Goetze, M., Palma‐Silva, C., Hirsch, L. D., Neves, B., ... & Bered, F. (2019). Ecological niche modeling and a lack of phylogeographic structure in *Vriesea incurvata* suggest historically stable areas in the southern Atlantic Forest. *American Journal of Botany,* 106(7), 971-983.

Angilletta Jr, M. J., & Angilletta, M. J. (2009). Thermal adaptation: a theoretical and empirical synthesis.

Antonelli, A., Ariza, M., Albert, J., Andermann, T., Azevedo, J., Bacon, C., ... & Matos-Maraví, P. (2018). Conceptual and empirical advances in Neotropical biodiversity research. *PeerJ*, 6, e5644.

Antonelli, A. (2022). The rise and fall of Neotropical biodiversity. *Botanical Journal of the Linnean Society,* 199(1), 8-24.

Ataídes, D. S., Vasconcelos-Filho, S. C., Costa, A. C. Alterações na fisiologia de plantas de feijoeiro croulos como indicadores de vulnerabilidade ao déficit hídrico e ao aumento de temperatura do ambiente de crescimento, 2018. 51 f. Dissertação (Mestrado em Biodiversidade e Convervação) – Instituto Federal Goiano, Campus Rio Verde.

Benzing, D. H., & Bennett, B. (2000). Bromeliaceae: profile of an adaptive radiation. *Cambridge University Press.*

Cacossi, T., Dantas-Queiroz, M. V., & Palma-Silva, C. (2019). Transferability of nuclear microsatellites markers to *Vriesea oligantha* (Bromeliaceae), an endemic species from Espinhaço Range, Brazil. *Brazilian Journal of Botany,* 42(4), 727-733.

Carnaval, A. C., Hickerson, M. J., Haddad, C. F., Rodrigues, M. T., & Moritz, C. (2009). Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science,* 323(5915), 785-789.

Carnaval, A. C., Waltari, E., Rodrigues, M. T., Rosauer, D., VanDerWal, J., Damasceno, R., ... & Moritz, C. (2014). Prediction of phylogeographic endemism in an environmentally complex biome. *Proceedings of the Royal Society B: Biological Sciences*, 281(1792), 20141461.

Caze, A. L. R., Mäder, G., Nunes, T. S., Queiroz, L. P., de Oliveira, G., Diniz-Filho, J. A. F., ... & Freitas, L. B. (2016). Could refuge theory and rivers acting as barriers explain the genetic variability distribution in the Atlantic Forest? *Molecular Phylogenetics and Evolution,* 101, 242-251.

Chaves, C. J., Cacossi, T. C., Matos, T. S., Bento, J. P., Gonçalves, L. N., Silva, S. F., ... & Palma‐Silva, C. (2024). Bromeliad populations perform distinct ecological strategies across a tropical elevation gradient. *Functional Ecology,* 38(4), 910-925.

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

Cornille, A., Salcedo, A., Kryvokhyzha, D., Glémin, S., Holm, K., Wright, S. I., & Lascoux, M. (2016). Genomic signature of successful colonization of Eurasia by the allopolyploid shepherd's purse (*Capsella bursa‐pastoris*). *Molecular ecology,* 25(2), 616-629

Damasceno, R., Strangas, M. L., Carnaval, A. C., Rodrigues, M. T., & Moritz, C. (2014). Revisiting the vanishing refuge model of diversification. *Frontiers in genetics*, 5, 353.

Damasceno, R. P., Carnaval, A. C., Sass, C., Recoder, R. S., Moritz, C., & Rodrigues, M. T. (2021). Geographic restriction, genetic divergence, and morphological disparity in the Brazilian Atlantic Forests: Insights from *Leposoma* lizards (Gymnophthalmidae, Squamata). *Molecular Phylogenetics and Evolution*, 154, 106993.

Dantas‐Queiroz, M. V., Cacossi, T. D. C., Leal, B. S. S., Chaves, C. J. N., Vasconcelos, T. N., Versieux, L. D. M., & Palma‐Silva, C. (2021). Underlying microevolutionary processes parallel macroevolutionary patterns in ancient neotropical mountains. *Journal of Biogeography,* 48(9), 2312-2327.

Davey, J. W., & Blaxter, M. L. (2010). RADSeq: next-generation population genetics. *Briefings in functional genomics*, 9(5-6), 416-423.

Flantua, S. G., O'Dea, A., Onstein, R. E., Giraldo, C., & Hooghiemstra, H. (2019). The flickering connectivity system of the north Andean páramos. *Journal of Biogeography,* 46(8), 1808-1825.

Gehara, M., Mazzochinni, G. G., & Burbrink, F. (2020). PipeMaster: inferring population divergence and demographic history with approximate Bayesian computation and supervised machine-learning in R. *BioRxiv,* 2020-12.

Givnish, T. J., Barfuss, M. H., Van Ee, B., Riina, R., Schulte, K., Horres, R., ... & Sytsma, K. J. (2014). Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. *Molecular phylogenetics and evolution,* 71, 55-78.

Ebel, E. R., DaCosta, J. M., Sorenson, M. D., Hill, R. I., Briscoe, A. D., Willmott, K. R., & Mullen, S. P. (2015). Rapid diversification associated with ecological specialization in Neotropical *Adelpha* butterflies. *Molecular ecology,* 24(10), 2392-2405.

Harvey, M. G., & Brumfield, R. T. (2015). Genomic variation in a widespread Neotropical bird (*Xenops minutus*) reveals divergence, population expansion, and gene flow. *Molecular Phylogenetics and Evolution,* 83, 305-316.

Hess, J. E., Campbell, N. R., Close, D. A., Docker, M. F., & Narum, S. R. (2013). Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. *Molecular Ecology,* 22(11), 2898-2916.

Hmeljevski KV, Nazareno AG, Leandro Bueno M, dos Reis MS, Forzza RC (2017). Do 761 plant populations on distinct inselbergs talk to each other? A case study of genetic 762 connectivity of a bromeliad species in an Ocbil landscape. *Ecol Evol* 7: 4704-4716 .

Hughes, M., & Hollingsworth, P. M. (2008). Population genetic divergence corresponds with species‐level biodiversity patterns in the large genus Begonia. *Molecular ecology,* 17(11), 2643-2651.

Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *The American Naturalist,* 101(919), 233-249.

Leal, B. S. S., Chaves, C. J. N., Graciano, … & Palma-Silva, C. (2021). Evidence of local adaptation despite strong drift in a Neotropical patchily distributed bromeliad. *Heredity,* 127(2), 203-218.

Leite, Y. L., Costa, L. P., Loss, A. C., Rocha, R. G., Batalha-Filho, H., Bastos, A. C., ... & Pardini, R. (2016). Neotropical forest expansion during the last glacial period challenges refuge hypothesis. P*roceedings of the National Academy of Sciences,* 113(4), 1008-1013.

Martin, P. R., & McKay, J. K. (2004). Latitudinal variation in genetic divergence of populations and the potential for future speciation. *Evolution,* 58(5), 938-945.

Morellato, L. P. C., & Haddad, C. F. (2000). Introduction: The Brazilian Atlantic Forest 1. *Biotropica,* 32(4b), 786-792.

Mota, M. R., Pinheiro, F., Leal, B. S. D. S., Sardelli, C. H., Wendt, T., & Palma-Silva, C. (2020). From micro-to macroevolution: insights from a Neotropical bromeliad with high population genetic structure adapted to rock outcrops. *Heredity,* 125(5), 353-370

Moritz, C., Langham, G., Kearney, M., Krockenberger, A., VanDerWal, J., & Williams, S. (2012). Integrating phylogeography and physiology reveals divergence of thermal traits between central and peripheral lineages of tropical rainforest lizards. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 367(1596), 1680- 1687.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature, 403(6772), 853.

Nadeau, N. J., Martin, S. H., Kozak, K. M., Salazar, C., Dasmahapatra, K. K., Davey, J. W., ... & Jiggins, C. D. (2013). Genome‐wide patterns of divergence and gene flow across a butterfly radiation. *Molecular ecology*, 22(3), 814-826.

Narum, S. R., Buerkle, C. A., Davey, J. W., Miller, M. R., & Hohenlohe, P. A. (2013). Genotyping-by-sequencing in ecological and conservation genomics. *Molecular ecology,* 22(11), 2841.

Oliveira‐Filho, A. T., & Fontes, M. A. L. (2000). Patterns of floristic differentiation among Atlantic Forests in Southeastern Brazil and the influence of climate 1. *Biotropica*, 32(4b), 793-810.

Palma-Silva, C., Lexer, C., Paggi, G. M., Barbará, T., Bered, F., & Bodanese-Zanettini, M. H. (2009). Range-wide patterns of nuclear and chloroplast DNA diversity in *Vriesea gigantea* (Bromeliaceae), a neotropical forest species. *Heredity,* 103(6), 503-512. 11

Palma-Silva, C., Leal, B. S., Chaves, C. J., & Fay, M. F. (2016). Advances in and perspectives on evolution in Bromeliaceae. *Botanical Journal of the Linnean Society,* 181(3), 305-322.

Palma-Silva, C., Turchetto-Zolet, A. C., Fay, M. F., & Vasconcelos, T. (2022). Drivers of exceptional Neotropical biodiversity: an updated view. B*otanical Journal of the Linnean Society,* 199(1), 1-7.

Pessoa, E. M., Cordeiro, J. M., Felix, L. P., Lemes, P., Viruel, J., Alves, M., ... & Van Den Berg, C. (2022). The role of Quaternary glaciations in shaping biogeographic patterns in a recently evolved clade of South American epiphytic orchids. *Botanical Journal of the Linnean Society,* 199(1), 252-266.

Peres, E. A., Pinto-da-Rocha, R., Lohmann, L. G., Michelangeli, F. A., Miyaki, C. Y., & Carnaval, A. C. (2020). Patterns of species and lineage diversity in the Atlantic rainforest of Brazil. *Neotropical diversification: patterns and processes,* 415-447.

Peterson, B. K., Weber, J. N., Kay, … & Hoekstra, H. E. (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PloS one,* 7(5), e37135.

Pinto, B. J., Colli, G. R., Higham, T. E., … & Gamble, T. (2019). Population genetic structure and species delimitation of a widespread, Neotropical dwarf gecko. *Molecular phylogenetics and evolution,* 133, 54-66

Polato, N. R., Gill, B. A., Shah, A. A., Gray, M. M., Casner, K. L., Barthelet, A., ... & Kondratieff, B. C. (2018). Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains. *Proceedings of the National Academy of Sciences, 1*15(49), 12471-12476.

Porembski, S. (2007). Tropical inselbergs: habitat types, adaptive strategies and diversity patterns. *Brazilian Journal of Botany,* 30(4), 579-586.

Prance, G. T. (1982). A review of the phytogeographic evidences for Pleistocene climate changes in the Neotropics. *Annals of the Missouri Botanical Garden,* 594-624.

Quezada, I. M., & Gianoli, E. (2011). Crassulacean acid metabolism photosynthesis in Bromeliaceae: an evolutionary key innovation. *Biological Journal of the Linnean Society,* 104(2), 480-486.

Rahbek, C., Borregaard, M. K., Antonelli, A., Colwell, R. K., ... & Fjeldså, J. (2019). Building mountain biodiversity: Geological and evolutionary processes. *Science,* 365(6458), 1114-1119

Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J., & Hirota, M. M. (2009). The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological conservation,* 142(6), 1141-1153.

Ribeiro, M. C., Martensen, A. C., Metzger, J. P., Tabarelli, M., Scarano, F., & Fortin, M. J. (2011). The Brazilian Atlantic Forest: a shrinking biodiversity hotspot. In *Biodiversity hotspots* (pp. 405-434). Springer, Berlin, Heidelberg.

Roda, F., Ambrose, L., Walter, G. M., Liu, H. L., Schaul, A., Lowe, A., ... & Ortiz‐Barrientos, D. (2013). Genomic evidence for the parallel evolution of coastal forms in the *Senecio lautus* complex. *Molecular ecology,* 22(11), 2941-2952.

Rödin‐Mörch, P., Luquet, E., Meyer‐Lucht, Y., Richter‐Boix, A., Höglund, J., & Laurila, A. (2019). Latitudinal divergence in a widespread amphibian: Contrasting patterns of neutral and adaptive genomic variation. *Molecular Ecology,* 28(12), 2996-3011.

Romero-Soler, K. J., Ramírez-Morillo, I. M., Ruiz-Sanchez, E., Hornung-Leoni, C. T., & Carnevali, G. (2022). Historical biogeography and comparative phylogeography of the Mexican genus *Bakerantha* (Bromeliaceae): insights into evolution and diversification. *Botanical Journal of the Linnean Society,* 199(1), 109-127.

Rougemont, Q., & Bernatchez, L. (2018). The demographic history of Atlantic salmon (*Salmo salar*) across its distribution range reconstructed from approximate Bayesian computations. *Evolution,* 72(6), 1261-1277.

Sanín, M. J., Zapata, P., Pintaud, J. C., Galeano, G., Bohórquez, A., Tohme, J., & Hansen, M. M. (2017). Up and down the blind alley: Population divergence with scant gene flow in an endangered tropical lineage of Andean palms (*Ceroxylon quindiuense* Clade: Ceroxyloideae). *Journal of Heredity,* 108(3), 288-298.

Saraiva, D. P., Mantovani, A., & Forzza, R. C. (2015). Insights into the evolution of *Pitcairnia* (Pitcairnioideae Bromeliaceae), based on morphological evidence. Systematic Botany, 40(3), 726-736.

Schütz, N., Krapp, F., Wagner, N., & Weising, K. (2016). Phylogenetics of Pitcairnioideae ss.(Bromeliaceae): evidence from nuclear and plastid DNA sequence data. *Botanical Journal of the Linnean Society,* 181(3), 323-342.

Silva, F. R., Almeida-Neto, M., & Arena, M. V. N. (2014). Amphibian beta diversity in the Brazilian Atlantic Forest: contrasting the roles of historical events and contemporary conditions at different spatial scales. *PLoS One,* 9(10).

Silvestro, D., Zizka, G., & Schulte, K. (2014). Disentangling the effects of key innovations on the 12 diversification of Bromelioideae (Bromeliaceae). *Evolution,* 68(1), 163-175.

Souza, A., Giudicelli, G. C., Teixeira, M. C., Turchetto, C., Bonatto, S. L., & Freitas, L. B. (2022). Genetic diversity in micro-endemic plants from highland grasslands in southern Brazil. *Botanical Journal of the Linnean Society,* 199(1), 235-251.

Sovic, M., Fries, A., Martin, S. A., & Lisle Gibbs, H. (2019). Genetic signatures of small effective population sizes and demographic declines in an endangered rattlesnake, *Sistrurus catenatus*. *Evolutionary Applications,* 12(4), 664-678.

Smith, L. B., & Downs, R. J. (1974). Bromeliaceae (Pitcairnioideae). *Flora Neotropica Monograph*

Tavares, M. M., Ferro, M., Leal, B. S. S., & Palma‐Silva, C. (2022). Speciation with gene flow between two Neotropical sympatric species (*Pitcairnia spp.*: Bromeliaceae). *Ecology and evolution,* 12(5), e8834.

Thom, G., Smith, B. T., Gehara, M., Montesanti, J., Lima-Ribeiro, M. S., Piacentini, V. Q., ... & do Amaral, F. R. (2020). Climatic dynamics and topography control genetic variation in Atlantic Forest montane birds. *Molecular phylogenetics and evolution,* 148, 106812.

Vicente-Silva, L., De Russo Godoy, F. M., Faggioni, G. P., Lorenz, A. P., & Paggi, G. M. (2022). Patchily distributed but not necessarily isolated populations of *Deuterocohnia meziana:* a threatened bromeliad from rock outcrops. *Botanical Journal of the Linnean Society,* 199(1), 312-330.

Vieira, L. D., Silva-Junior, O. B., Novaes, E., & Collevatti, R. G. (2022). Comparative population genomics in *Tabebuia* alliance shows evidence of adaptation in Neotropical tree species. *Heredity*, 1-13.

Versieux, L. M., Barbará, T., Wanderley, M. D. G. L., Calvente, A., Fay, M. F., & Lexer, C. (2012). Molecular phylogenetics of the Brazilian giant bromeliads (*Alcantarea,* Bromeliaceae): implications for morphological evolution and biogeography. *Molecular Phylogenetics and Evolution,* 64(1), 177-189.

CAPÍTULO 2

Note: This paper is evolving based on the findings from this Master's research.

The Atlantic Forest's elevation gradient provides an arena for contrasting demographic histories of an endemic bromeliad

ABSTRACT

The Atlantic Forest is the second-largest rainforest in the Neotropical region and is considered a hotspot for conservation due to its high rates of endemism. Because of its complex geological history, environmental heterogeneity, ecological gradients, and species diversity and turnover, the rocky mountains of the Atlantic Forest are excellent systems to study the environmental and evolutionary dynamics of the Atlantic Forest. *Pitcairnia flammea* Lindl. (Bromeliaceae) is endemic to the Atlantic Forest, and occurs at an elevation gradient from sea level to more than 2000 meters a.s.l., presenting high morphological differences and low gene flow among populations. In this study, we evaluated the patterns of genetic diversity and the evolutionary and demographic history of *P. flammea* populations across the elevational gradient of the Atlantic Forest, analyzing patterns of divergence, gene flow, and possible changes in population size over time, and also correlating our findings with historical climate variations. We obtained SNP markers derived from RAD-seq of 159 individuals from eight populations of *P. flammea* distributed across the Atlantic Forest altitudinal gradient, by using the species' reference genome. Our results reveal that populations can be clustered into 3 major groups, despite each of them acting as a single genetic unit. The two populations from the highest elevations have the highest diversity levels compared to the others, are the most isolated (including between themselves), and show the earliest divergence from the others (around 300 kya). Populations at low/mid-elevations are more interconnected, having diverged from each other in the last 60 kya. Our results suggest different demographic histories between high and low/mid elevations, influenced by the climatic cycles of the Pleistocene. Our results indicate that elevation variation is an important driver of diversity in tropical rainforests, showing how local ecological strategies can reflect the demographic and evolutionary patterns of populations undergoing a potential speciation process.

1. INTRODUCTION

The Neotropical region is known for its high diversity and endemism patterns, embracing critical hotspots for conservation (Antonelli et al. 2018). The Atlantic Forest is the second-largest tropical rainforest and a hotspot of conservation in the Neotropics, composed of a mosaic of vegetation (Myers et al., 2000; Oliveira-Filho & Fontes, 2000), and contains from 1% to 8% of all world species inhabiting a largely fragmented territory (Ribeiro et al. 2009). The Atlantic Forest's naturally fragmented habitats are sustained by relief heterogeneity and encompass a consequent huge environmental heterogeneity, a key factor for in situ diversification and endemisms (Thom et al. 2020). With large latitudinal (3-30°S) and elevational (0-2.900 m asl.) gradients, the Atlantic Forest is exposed to temperature and precipitation gradients as well (Ribeiro et al. 2011), which affect species' responses to the different environmental pressures (Angilletta &Angilletta 2009; Moritz et al. 2012). In this sense, strategies such as phenotypic plasticity and local adaptation can evolve (Angilletta & Angilletta 2009; Moritz et al. 2012). For example, temperature and drought stress can act as selective pressures for plants, resulting in phenotypic modifications (Ataídes, 2018).

Relief complexity and habitat fragmentation promote biodiversity by enhancing divergence between populations. This occurs due to spatial isolation and lack of gene flow, which can potentially lead to speciation (Martin & McKay 2004; Polato et al. 2018). According to Janzen's (1967) hypothesis, the temperature gradient in tropical mountains, such as those found in the Atlantic Forest, is associated with climatically stable microhabitats. This facilitates local adaptation and acclimatization, limits dispersion, and increases reproductive isolation in these regions. Studying the distribution of genetic diversity, genomic signs of reproductive isolation, and historical demographic changes in populations distribution, can contribute to the investigation of hypotheses such as Janzen’s (1967) and improve the knowledge about micro and macroevolutionary processes from the Atlantic Forest (Hess et al. 2013; Nadeau et al. 2013; Harvey & Brumfield 2015; Hmeljevski et al 2017; Palma-Silva et al., 2009, 2016; Pinto et al. 2019; Acha et al. 2021; Leal et al. 2021; Vieira et al. 2022).

Topographic heterogeneity and historical climatic oscillations are the main drivers of the diversity patterns observed in tropical and non-tropical mountain regions (Flantua et al. 2019; Rahbek et al. 2019). For instance, the evolutionary history of the Atlantic Forest is marked by multiple expansion and retraction events during the climatic oscillations of the Quaternary (Prance

1982; Carnaval et al. 2009; Silva et al. 2014). In periods of retraction, many species remained isolated into scattered refugia, with low gene flow among populations, resulting in reproductive isolation and subsequent speciation events (Prance 1982; Silva et al. 2014; Aguiar-Melo et al. 2019). However, studies have also suggested that climatic oscillations mostly shifted species distribution (Antonelli et al. 2018). Climatic changes from the Quaternary had an even more expressive impact on mountains. During warmer periods many species were prompted to higher elevations, increasing isolation. The vegetation tended to shift downslope in colder periods, increasing spatial connectivity (Thom et al. 2020). Understanding the effect of these oscillatory climatic events on the demographic history of species currently distributed across a wide elevational range would help us to understand the speciation mechanisms in tropical mountains.

One way to test how biodiversity has responded to environmental and landscape change is by testing models of population change across time, space, and environmental gradients (Gavin et al. 2014). Demography, divergence, and gene flow histories have been investigated in model-based studies using SNP markers derived from RAD sequencing and relying on different model selection methods, such as Bayesian approaches - Approximate Bayesian Computation (ABC) (Cornille et al. 2016; Sanín et al. 2017; Rougemont & Bernatchez, 2018) and maximum-likelihood, like the continuous-time coalescent framework implemented in FASTSIMCOAL (Sovic et al. 2019; Rödin-Mörch et al. 2019; Teixeira & Nazareno, 2021). Supervised Machine-Learning is emerging as a model selection tool in such studies (Gehara et al. 2020; Coelho et al. 2022). Using genomic data, it is possible to test different scenarios of migration, divergence, and changes in population size (Cornille et al. 2016; Rougemont & Bernatchez, 2018; Rödin-Mörch et al. 2019; Sovic et al. 2019). For example, Coelho et al. 2022 used both ABC and SML to test the São Francisco River hypothesis, revealing that the São Francisco River is a barrier for populations of several vertebrate species, and promotes divergence through vicariance. Tavares et al. 2022 used SNP markers and ABC analysis to test hybridizing species. They found that isolation with decreased migration best explains the divergence between the hybridizing bromeliads *Pitcairnia staminia* and *Pitcairnia albiflos*.

The Bromeliaceae family is highly diverse, with great anatomical, morphological, and ecological variability, being an excellent example of adaptive radiation in the Neotropics, and distributing broadly across climatic, latitudinal, and elevational scales (Benzing 2000; Givnish et al. 2014; Palma-Silva et al. 2016). *Pitcairnia flammea* Lindl. is a bromeliad endemic to the Atlantic

Forest and has high morphological diversity, and a wide distribution along an elevational gradient (Smith and Downs 1974). The populations of this species are distributed on rocky outcrops naturally isolated (or inselbergs; Porembski, 2007), which results in low gene flow among them (Mota et al. 2020). Therefore, *P. flammea* is a good model for population genetics studies taking place at the Atlantic Forest's gradients, which are one of the main explanations (combined with the Quaternary climatic oscillations) for the diversification of lineages in tropical forests (Damasceno et al. 2014). Chaves et al. 2024 pointed out the existence of divergent ecological strategies in *P. flammea* populations associated with the elevational gradient of the Atlantic Forest. These results indicate local adaptions of *P. flammea* populations across elevations and also encourage the investigation of how historical climate oscillations may have influenced the evolutionary history of these populations. In this sense, we evaluated the current patterns of genetic diversity and the evolutionary and demographic histories of *P. flammea* populations along the elevational gradient of the Atlantic Forest, analyzing divergence, gene flow, and possible changes in population size over time, and correlating with historical climate variations. We aimed to answer the following questions: 1) How are the diversity patterns and genetic structure of *P. flammea* populations along the elevational gradient? 2) When did the divergence between populations occur? 3) Are there any changes in population size or gene flow between populations over time? If positive, how did it occur? 4) How can we relate evolutionary and demographic histories of populations to Quaternary climate changes? 5) How can we relate genetic patterns to different ecological strategies at high and low elevations? Our study helped to understand the processes responsible for the high diversity observed in an endemic species of the Atlantic Forest Mountain populations, uncovering how they interact in molding patterns in this remarkably complex tropical forest.

2. MATERIAL AND METHODS

2.1 Data collection, DNA extraction, and RAD-sequencing

We collected eight *Pitcairnia flammea* populations distributed from sea level to 2140 meters in the Atlantic Forest (Table 1, Figure 1). The genomic DNA was extracted from the fresh leaves of 159 individuals (approximately 20 per population) following the procedures by Qiagen DNA plant mini kit (Qiagen, Finland). Library preparation and sequencing of RAD markers from the genomic DNA of the samples were performed by Floragenex Inc. (Eugene, Oregon) using the restriction enzyme *SbfI* and sample-specific barcodes. The amplification and single-ended sequencing were performed on a single lane of the Illumina 150 SE HiSeq 2000 platform.

Table 1 – Information on *Pitcairnia flammea* collected and sequenced for each population.

Population	**Nº of samples**	**State**	**City**	**Locality**	**Elevation**	**Latitude**	**Longitude**
UBA	23	SP	Ubatuba	Praia do Lázaro	20	-23.50845	-45.13502
RAN	23	RJ	Rio de Janeiro	Morro do Rangel	25	-22.96413	-43.45646
COR	19	RJ	Rio de Janeiro	P.N. Serra da Tijuca	425	-22.94998	-43.22385
MAC	22	RJ	Petrópolis	Pico da Maria Comprida	959	-22.40893	-43.20572
ITA	23	SP	Itatiaia	P. N. de Itatiaia	1139	-22.42762	-44.61919
PES	10	RJ	Teresópolis	P. N. da Serra dos Órgãos	1496	-22.44797	-43.01323
MAR	22	SP	Piquete	Pico dos Marins	2037	-22.49830	-45.13075
PAP	22	MG	Aiuruoca	Serra do Papagaio	2140	-22.0605	-44.6938

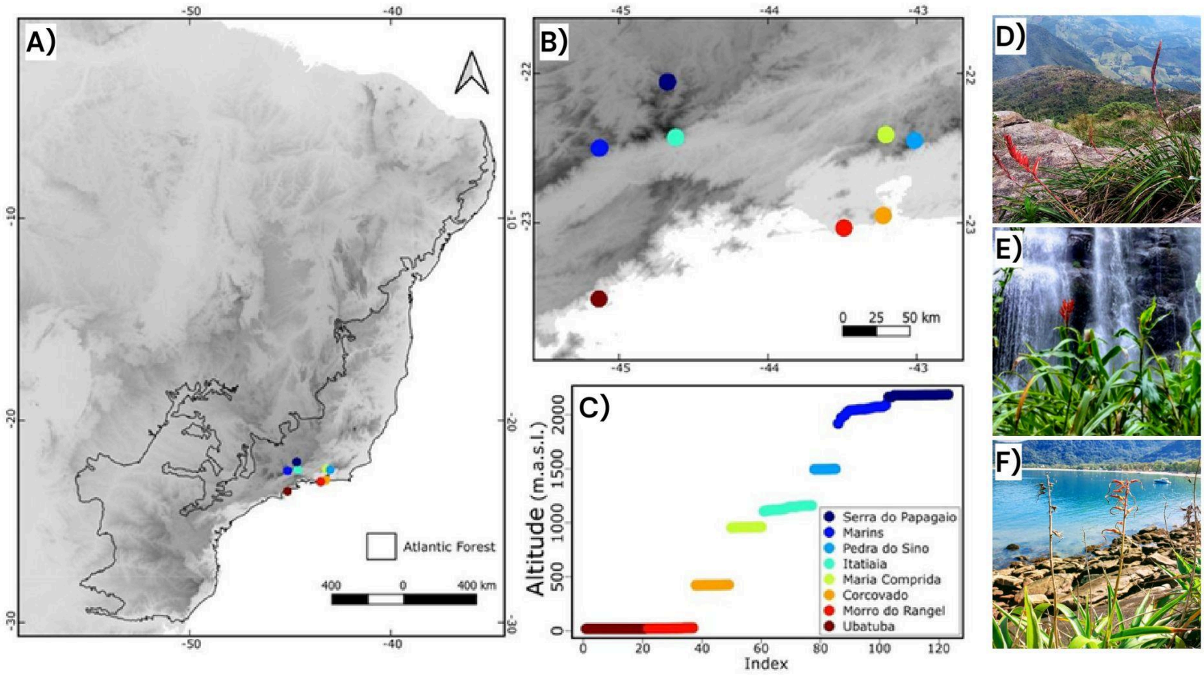

Figure 1 - (A), (B) Distribution of the sampled *Pitcairnia flammea* populations in the Atlantic Forest; (C) elevation (in meters) of each population collected. Examples of sampled individuals from (D) MAR (Pico dos Marins, 2140 m a.s.l), (E) ITA (Itatiaia, 1139 m a.s.l), and (F) UBA (Ubatuba, 20 m a.s.l). Colors indicate: dark red, UBA (Ubatuba); red, RAN (Morro do Rangel); orange, COR (Corcovado - P. N. da Serra da Tijuca); green, MAC (Pico da Maria Comprida); light blue, ITA (P. N. de Itatiaia); blue, PES (Pedra do Sino - P. N. da Serra dos órgãos); dark blue, MAR (Pico dos Marins); navy blue, PAP (Serra do Papagaio).

2.2 Data quality verification, filtering, and SNP calling

The quality of raw reads was checked using FastQC v.0.11.3 (Andrews, 2014) and alignments were sorted and indexed using iPyrad v.0.7.30 (Eaton, 2014). We also used iPyrad v.0.7.30 (Eaton, 2014) to remove low-quality reads and the adapters used in sequencing and perform the alignment reads from each individual to the *P. flammea* reference genome. We used a custom Python script to pick up one random SNP per locus for analyses that require non-linked SNPs. VCFTOOLS v.0.1.17 (Danecek et al. 2011) was used to remove indels, filter non-biallelic loci (dataset I), and filter loci for a maximum of 25% of missing data (dataset II), and no missing data (dataset III).

2.3 Genetic diversity and structure

We calculated the diversity indices using dataset II, obtaining the number of private alleles, percentage of polymorphic loci, observed heterozygosity (HO), expected heterozygosity (HE), nucleotide diversity (π), and Wright's *F* statistics, such as the coefficient of inbreeding (F_{IS}) and genetic divergence (pairwise F_{ST}) between populations using Stacks (Catchen et al., 2011). We also estimated the genomic divergence between populations using a model-based Bayesian clustering method implemented in STRUCTURE version 2.3.4 software (Pritchard et al., 2000). We assumed an admixture model, and independent allele frequencies, and used a burn-in period of 250.000 and 1.000.000 MCMC iterations. We obtained the graphic to visualize genotype clustering using CLUMPAK - Cluster Markov Packager Across K (Kopelman et al. 2015). The method of Evanno et al. (2005) implemented in STRUCTURE HARVESTER v0.6.97 (Earl and vonHoldt, 2012) was further employed to determine the most likely number of clusters (K). In addition, we investigated the genetic structure using the Discriminant Analysis of Principal Components (DAPC) in R with the Adegenet package (Jombart et al. 2010). This multivariate method uses sequential K-means and model selection to infer genetic clusters (Jombart et al. 2010). We also implemented a maximum likelihood estimation method, ADMIXTURE 1.3 (Alexander et al. 2015), with its default settings. In ADMIXTURE, the best K value is determined by the lowest cross-validation error (Alexander et al. 2015). Lastly, we performed a neighbor-joining analysis using SPLITSTREE5 (Huson & Bryant, 2006) to create a bootstrapped phylogenetic tree from the evolutionary distance of our data.

2.4 Geographic and elevational effect under genetic differentiation

We estimated Isolation by distance (IBD) and Isolation by Elevation (IBE) by doing mantel tests using the R package ‘vegan’ (Oksanen et al. 2022). We calculated the pairwise geographical distances of populations through their latitude and longitude coordinates, and pairwise Euclidean distances for their per-site elevation. Pairwise F_{ST} estimated by Stacks was linearized by calculating $F_{ST}/(1-F_{ST})$. As sampling sites follow a bimodal distribution, we run each mantel test using Spearman correlation and test for their significance using 999 permutations with ‘vegan’. We calculated a partial mantel to evaluate the effect of the elevation on the genetic differentiation controlling for the geographical distance, also considering the Spearman correlation.

2.5 Detection of outlier loci

We used dataset I and two different approaches based on population differentiation to investigate outlier loci and remove them for gene flow and demographic analyses. First, we used PCAdapt, based on principal components analysis, which identifies outlier SNPs based on the Mahalanobis distance. In this sense, SNPs significantly distinct from the population’s average are listed as outliers (Luu, Bazin & Blum 2017). We then used Bayescan, which decomposes the F_{ST}

calculated for the loci in each population into specific components: population-specific (β) and locus-specific (α) using logistic regression (Foll, 2012). In this case, outlier loci with positive α are candidates for positive selection, while those with negative α are potentially under either balancing or purifying selection (Foll & Gaggiotti, 2008). All outlier loci found in at least one of the analyses were excluded from demographic modeling. The outlier loci found both using PCAdapt and Bayescan were blasted against National Center for Biotechnology Information (NCBI) nucleotide collection. The sequences related to protein synthesis were annotated and their function was explored.

2.5 Gene flow dynamics and demographic modeling

STRUCTURE, Admixture, and Neighbor-Joining analyses (Evanno et al., 2015; Alexander et al. 2015; Huson & Bryant, 2006), revealed the genetic structure and distance of the eight *P. flammea* populations from this study. To further investigate in which way individual SNPs were responsible for the genetic structure found, we inferred the SNPs tree of the eight populations using SNAPP (SNP and AFLP Package for Phylogenetic analysis) from BEAST 2 (Bouckaert & Bryant 2012). We used dataset I and 8 randomly chosen individuals per population, totaling 579 SNPs. We implemented 1,000,000 Markov chains Monte Carlo (MCMC), storing every 1,000 MCMC, and three different runs. We checked for chain convergence for each run using Tracer (Rambaut et al. 2018). We then used Logcombiner to combine searches and trees of each replicate. We considered 10% of burn-in and loaded this combined tree at DENSITREE v. 2.2.6 (Bouckaert and Heled 2014), being able to visualize the topology, as well as infer the time of divergence events following the method implemented by Brandrud et al. 2020. For that, we calibrated the tree by multiplying the mutation rate (μ) of 3e-9 base substitutions per site per generation (as inferred for *Puya raimondii* (Bromeliaceae); Liu et al. 2021), the generation time (g) of 5 years, based on observations for *Pitcairnia* genus (Tavares et al. 2022), and the total length of investigated sites, and dividing its result to the total number of polymorphic sites across the loci. This way, we could rescale the tree at DENSITREE. Furthermore, we used TreeAnnotator to create a maximum credibility tree from the three replicates tree. By loading this tree at FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/), we were able to extract the node heights values and estimate the exact divergence of each node by diving node heights to the value obtained from the calculation of (g x μ x number of sites)/number of SNPs (Brandrud et al. 2020). Likewise, we obtained the confidence interval of divergence by dividing the values of heights 95% to the result of (g x µ x number of sites)/number of SNPs (Brandrud et al. 2020).

Based on SNAPP analyses (Figures 2 to 4), we used FASTSIMCOAL v2.7 (Excoffier et al., 2021) to build different models for gene flow dynamics among populations along the Atlantic Forest elevational gradient. Fastsimcoal v2.7 is a simulation-based approach that infers demographic parameters from site frequency spectra (SFS), which we obtained by transforming the VCF file from our data using the Python script easySFS (https://github.com/isaacovercast/easySFS). Gene flow models simulated under FASTSIMCOAL v.2.7 are shown in Figure 6. Models were built considering, in pairs, the three major-scale clusters found in structure analyses. We tested 5 gene flow models: **No** (no gene flow among populations); **Constant** (constant gene flow among populations over time); **Recent** (only recent flow among populations); **Early** (only ancient gene flow among populations); **Diff** (higher ancient gene flow reducing with isolation accumulation over time). For each one of the models we performed 100 replicates with 200,000 coalescent simulations, considering 60 optimization cycles (EMC), using a MAF (Minor Allele Frequency), with no monomorphic sites, and no entries with less than 10 SNPS. We used a bash script available in (https://github.com/speciationgenomics/scripts/blob/master/fsc-selectbestrun.sh) to select the replicate with the best likelihood. Finally, we compared and selected the model with the best fit to our data by calculating the AIC (Akaike Information Criterium) for the best run of each model simulated.

Considering all previous analyses, we used the R-package PipeMaster (https://github.com/gehara/PipeMaster) to build and simulate a more complex demographic model (Figure 7) for parameter estimation, integrating the investigation of both gene flow and demographic histories of *P. flammea* along the elevational gradient. First, we transformed raw alleles output from iPyrad v.0.7.30 into individual fasta files for each locus. It's important to highlight that both variable and monomorphic loci were considered for this analysis. Second, we implemented a custom R-function to eliminate short sequences, loci present in less than 50 samples, and loci with more than 50% of segregation sites; removed gaps, and trimmed alignments. We removed loci that were absent in one of the populations, ending up with 1550 loci. Finally, we built a model considering SNAPP results (model IMDL_M_H), where MAC divergence was not considered for its evolution, and its relation to others was through migration (Figure 7). We simulated 100,000 times using the msABC simulator (controlled by PipeMaster), considering a mutation rate of 3e-9 (as inferred for *Puya raimondii* (Bromeliaceae); Liu et al. 2021), with standard deviations of 1e-9 and 11e-9. We considered a uniform distribution to parametrize the mutation rate. We used SML (Supervised Machine-Learning) using keras R-package (https://cran.r-project.org/web/packages/keras/) for parameter estimation, considering 100 repetitions.

Stairway Plot 2 (Liu & Fu, 2020) was used to estimate the changes in population size over time through individual population site frequency spectra (SFS) (https://github.com/isaacovercast/easySFS). Stairway Plot 2 is recommended for non-model species, as it does not require previous knowledge of SNP markers' ancestral alleles (Liu & Fu, 2020). Most priors were kept as default settings, except ones that depend on the data information. This analysis was performed following the tutorial found at https://github.com/xiaoming-liu/stairway-plot-v2.

3. RESULTS

3.1 Genetic diversity and structure

We obtained raw Illumina reads from 159 individuals of *Pitcairnia flammea* (Supplementary Table 1S). After quality verification, assembly, and filtering, we created three custom datasets: dataset I, with 1643 SNPs (one SNP per locus), dataset II, with 1,561 SNPs (25% of missing data, one SNP per locus), and Dataset III (no missing data, one SNP per locus and randomly chosen individuals per population) (Supplementary Figure 1S). Dataset III had a variable number of loci and SNPs, depending on the individuals included in the analyses (Supplementary Table 2S). The genetic diversity was evaluated from 1,561 loci (dataset II) (Table 2). We found that the two populations from higher elevations (MAR and PAP; Table 1) have more private alleles (240 and 245, respectively). They also have higher percentages of polymorphic loci (19.30 and 18.10), nucleotide diversity (0.042 and 0.040), and higher numbers of expected and observed heterozygosities (MAR: HE = 0.041 and HO = 0.042; PAP: HE = 0.043 and HO = 0.039). Population-level inbreeding coefficients (F_{IS}) were low for all populations, but even lower in higher altitudes (ITA: F_{IS} = 0.011; PES: F_{IS} = 0.003; MAR: F_{IS} =0.010; PAP: F_{IS} = 0.015). Overall, the genetic diversity was higher for MAR (2037 m a.s.l) and PAP (2140 m a.s.l) (Table 2). Another population, MAC, from intermediate elevation, showed a genetic diversity similar to high altitude populations.

Table 2 – Genetic diversity indices from STACKS for *Pitcairnia flammea* populations. *HO*, observed heterozygosity; *HE,* expected heterozygosity; *F*IS, coefficient of inbreeding; *π,* nucleotide diversity. For population details see Table 1.

Population	Nº of samples	Altitude	N of Private alleles	HO	HE	*F*IS	π	% of Polymorphic loci
UBA	23	20	138	0,028	0,038	0,038	0,039	17,23
RAN	23	25	72	0,026	0,031	0,026	0,032	13,58
COR	17	425	46	0,018	0,032	0,039	0,033	11,53
MAC	20	959	208	0,023	0,030	0,033	0,031	15,97
ITA	22	1139	102	0,035	0,037	0,011	0,038	15,95
PES	10	1496	63	0,031	0,030	0,003	0,032	11,53
MAR	22	2037	240	0,040	0,041	0,010	0,042	19,38
PAP	22	2140	245	0,039	0,042	0,015	0,043	18,1

The pairwise genetic divergence (*F*ST) ranged from 0.08 to 0.35 (Supplementary Table 3S), revealing high divergence of the high-altitude populations (Figure 2). High *F*ST values were also found for MAC, from the middle of the elevational gradient (Figure 2). The lowest differentiation was found among RAN and COR (*F*ST = 0.08), two populations very close geographically (around 35 km) (Figure 1; Figure 2). STRUCTURE clustering analysis (Pritchard et al., 2000) revealed K = 2 as the most likely genetic clustering (Figure 3A). High-altitude populations (MAR and PAP) clustered with MAC, and the other five populations of intermediate and low altitudes formed a second cluster (Figure 3A). Because STRUCTURE has the “The K= 2 conundrum”, which means a tendency to identify two clusters as the most probable model (Janes et al. 2017), we also considered K = 3 clustering as a possible genetic structure because of its high ΔK value (Supplementary Figure

2S). In this case, clustering remains the same, except for MAC, which clusters alone (Figure 3B). DAPC revealed a similar pattern, with 4 *K-mean* clusters, as MAR and PAP are shown as different clusters (Figure 3C). Except for MAR, PAP, and MAC, populations are considerably geographically close, suggesting potential admixture. ADMIXTURE revealed a higher population structure, with eight genetic clusters, each corresponding to a single population, except for RAN and COR that grouped, and for two genetic groups of PAP (Figure 3D). SplitsTree neighbor-joining analysis evidenced three major splits, same as STRUCTURE and DAPC, despite all populations differentiated from each other with highly supported bootstraps, as evidenced by ADMIXTURE (Figure 3D). SplitsTree evidenced that the genetic distance of each population is consonant with structure analyses, given the existence of three major splits, in which each split population can be considered as single units at a minor clustering scale (Figure 4).

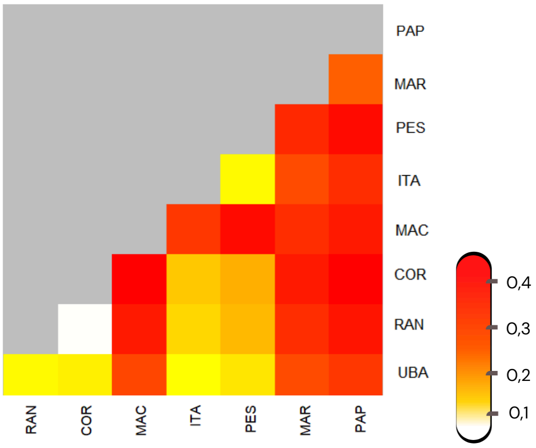

Figure 2 - Heatmap of pairwise genetic divergence (F_{ST}) between eight *P. flammea* populations, based on 1561 SNPs (dataset II). Hotter colors mean higher F_{ST} values. For population details see Table 1.

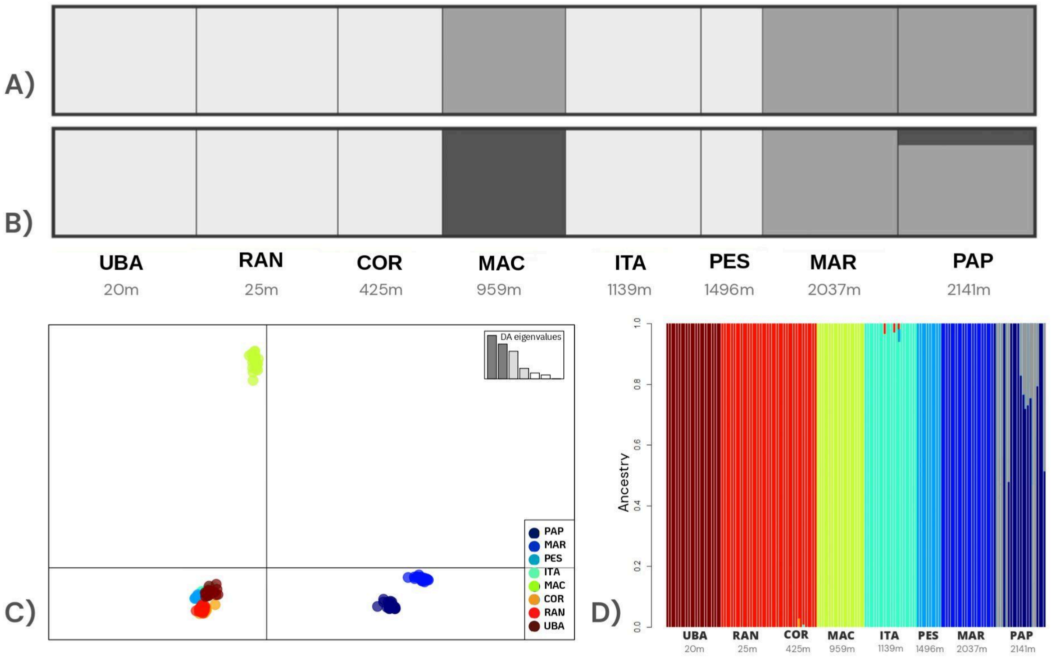

Figure 3 - Results of STRUCTURE clustering for K = 2 (A) and K = 3 (B); Discriminant Analysis of Principal Components (DAPC) with 4 *K-mean* clusters (C); and ADMIXTURE results, assuming 8 clusters. All clustering analyses were done for 159 individuals of *Pitcairnia flammea* based on 1561 SNPs (dataset II). For population details see Table 1.

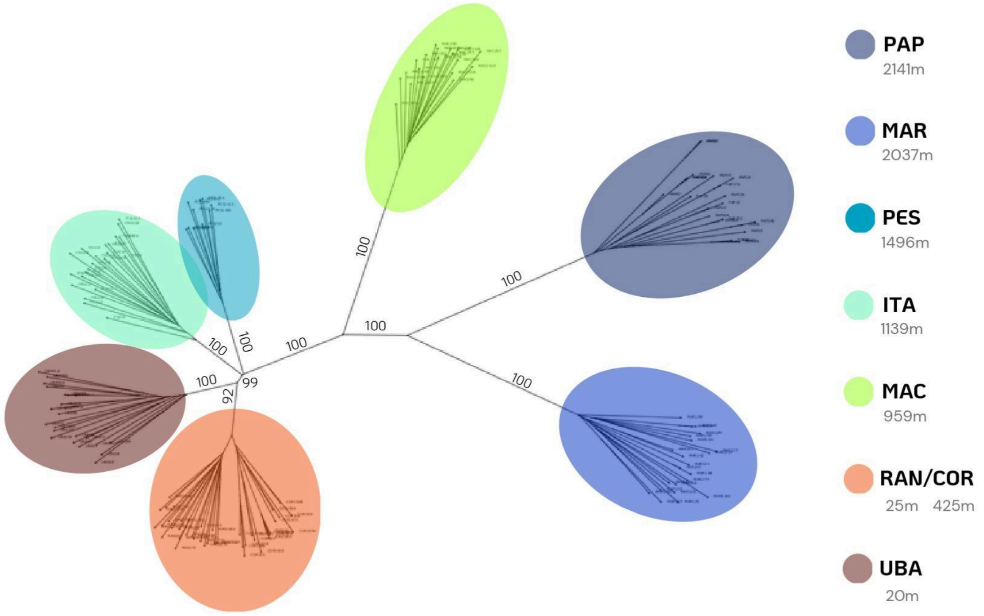

Figure 4 - Unrooted phylogenetic tree from SplitstTree5 neighbor-joining analysis for 159 individuals of *Pitcairnia flammea* based on 1561 SNPs (dataset II). Colored forms represent minor clusters (same as ADMIXTURE). For population details see Table 1.

3.2 Geographic and elevational effect under genetic differentiation

Mantel analysis revealed no significant correlation between geographical and genetic distances (r: -0.06542; p = 0.604). Equivalently, there is no significant correlation between elevation and genetic distances (r: -0.003832; p = 0.475), as well as when controlled for the geographical distance (r: 0.007911; p = 0.452).

3.3 Outlier loci candidates to positive selection

The outlier loci were investigated from 1,632 loci (dataset I). PCAdapt pointed out 174 outlier loci, while Bayescan, a more stringent analysis, indicated 13 loci candidates to positive selection and 19 loci potentially under either balancing or purifying selection. All except one genomic region candidates to positive selection from Bayescan were also found for PCAdapt, totalizing 12 genomic regions from both analyses. Outliers from both analyses were removed from Dataset III, used for gene flow and demographic analysis. Further, we blasted the 12 outliers found for both analyses against the National Center for Biotechnology Information (NCBI) nucleotide collection. Three outlier loci could be annotated: 1) L-type lectin-domain containing receptor kinase IV.1-like; 2) Callose synthase 12-like, mRNA; and 3) Protein DETOXIFICATION 40-like, mRNA.

3.4 Gene flow dynamics and demographic modeling

SNAPP phylogeny relationships were compatible with our structure analyses (Figure 5). Divergence times calculated following Brandrud et al. 2020 revealed a first split between low and high elevations occurring at 275 kya, with a confidence interval of 226 to 325 kya. The divergence of MAC (959 m a.s.l) from the ancestral population of those of low elevations was suggested to have occurred around 250 kya, ranging from 180 to 297 kya. MAC shares ancestry with low and high-elevation population groups, which can be explained by old, constant, or recent gene flow or due to incomplete lineage sorting (Qu et al. 2012). Populations from high elevations, MAR (2037 m a.s.l) and PAP (2140 m a.s.l) diverged 155 kya. More recently, populations from low and mid elevations diverged around 60 kya, with UBA (20 m a.s.l) diverging from RAN (25 m a.s.l) and COR (425 m a.s.l) 60 kya, and ITA (1139 m a.s.l) diverged from PES (1496 m a.s.l) 57 kya.

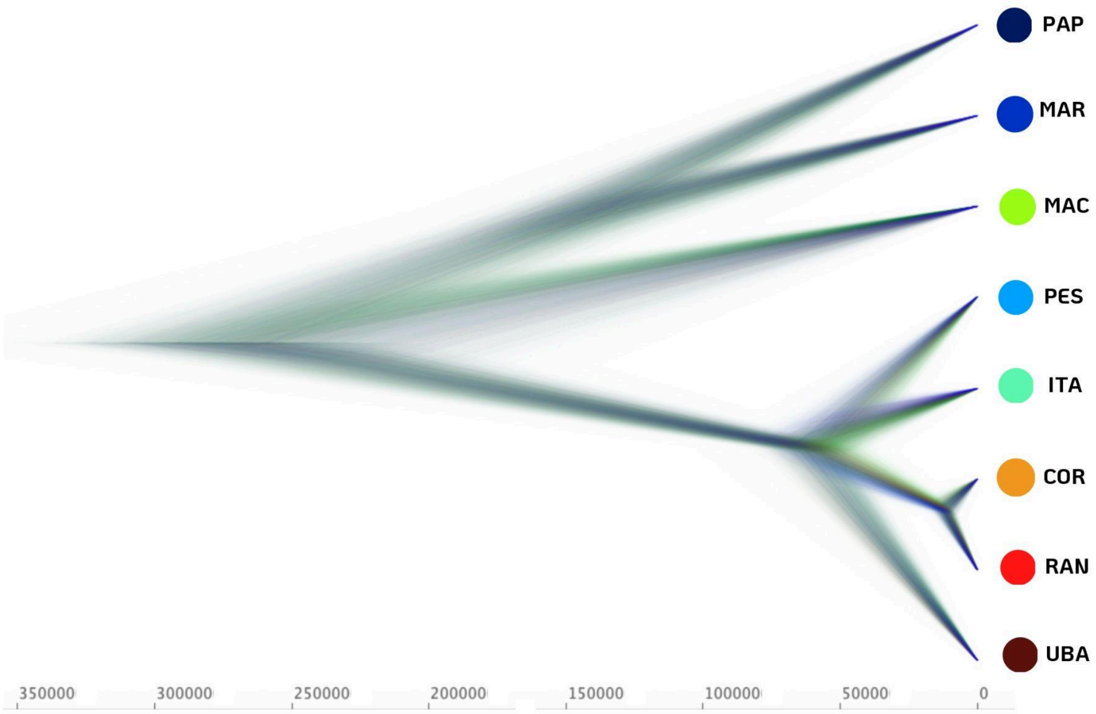

Figure 5 - SNAPP results for SNPs tree of the eight *P. flammea* populations. Numbers indicate divergence estimates, in million years. For population details see Table 1.

Structure and SNAPP results set the base for the gene flow and demographic models built and simulated using FASTSIMCOAL v.2.7 (Figure 6). **Early** gene flow was the best-fit model for gene flow between populations from high and low/mid elevations, with the lowest AIC value (16.203). Similarly, this was also the best-fit model for gene flow between populations from high elevations (MAR and PAP) and MAC (IAC = 17.961), besides differential gene flow had a similar AIC (18.015) and Likelihood (Supplementary Figure 6S). Differential gene flow (**Diff**) was the best-fit model for gene flow between MAC and populations from low and mid-elevation (AIC = 0.233). All AIC values are shown in Table 2.

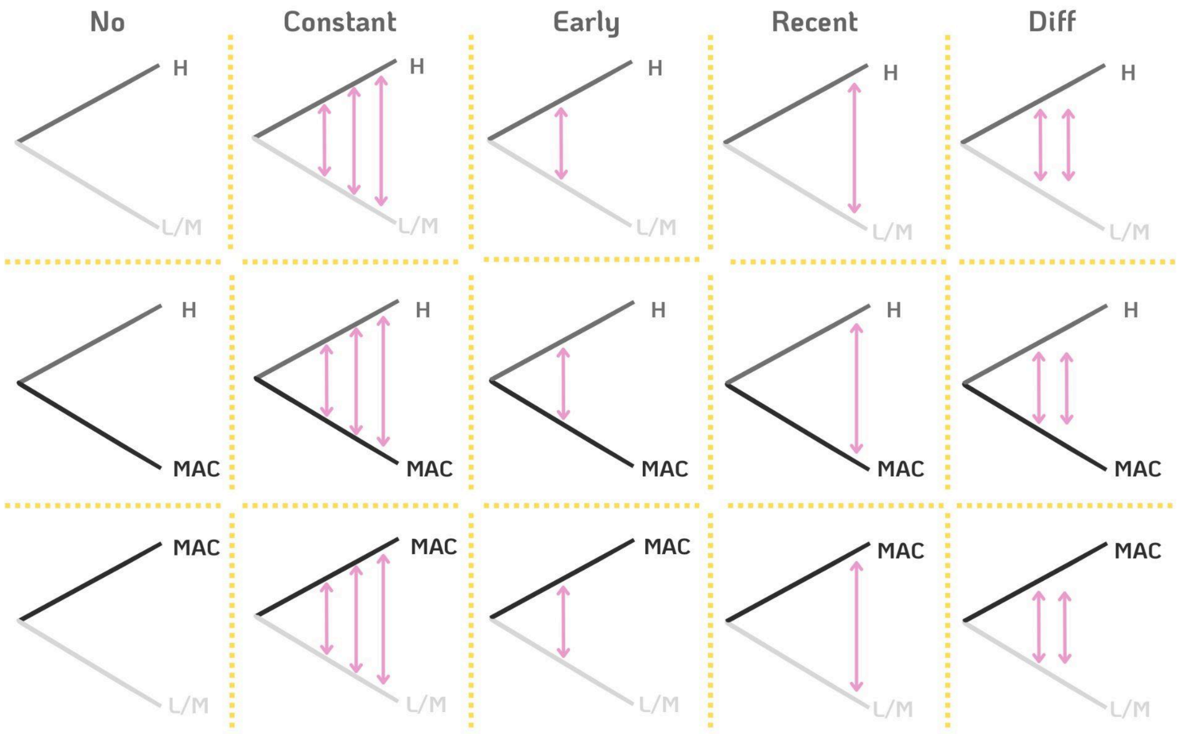

Figure 6 - Fastsimcoal v2.7 simulated models for gene flow dynamics among *P. flammea* populations along the Atlantic Forest elevational gradient. **No** (no gene flow among populations); **Constant** (constant gene flow among populations over time); **Early** (only ancient gene flow among populations); **Recent** (only recent gene flow among populations); **Diff** (higher ancient gene flow reducing with isolation accumulation over time). **L/M** = UBA, RAN, COR, ITA, and PES; **H** = MAR and PAP. For population details see Table 1.

Table 3 – AIC (Akaike Information Criterion) values for the 5 gene flow models tested pairwise on Fastsimcoal v2.7 between the three major-scale clusters of *Pitcairnia flammea* populations.

Models of gene flow	**AIC values**		
	Low/mid-elevation vs. high-elevation	MAC vs. low/mid-elevation	MAC vs. high-elevation
Constant	17.142	0.245	21.453
Early	**16.203**	286.4	**17.961**
No	17.868	0.506	20.758
Recent	17.214	0.301	19.888
Diff	16.256	**0.233**	18.015

Parameter estimation of the IMDL_M_H model simulated using PipeMaster revealed a light bottleneck for populations for low/mid elevations, occurring around 120,000 years, followed by recent expansion, while only one expansion was found for MAC, also starting around 120,000 years ago. Likewise, populations from high elevations (MAR and PAP) started decreasing in population sizes around 120,000 years ago. Consistent with Fastsimcoal results, the migration rate was higher between populations from low/mid-elevations to MAC (mean = 0,255) and vice-versa (mean = 0,259), when compared to populations from high elevations and MAC (0,167, 0,179). As the model does not include MAC divergence, we could track divergence times only between populations from low/mid and high elevations. Our results show that low and mid-elevation populations diverged from high elevations around 200,000 years ago.

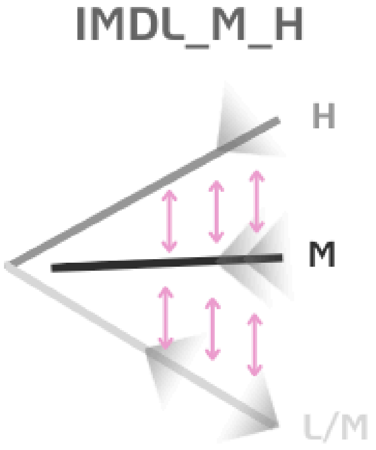

Figure 7 - PipeMaster simulated model (IMDL_M_H) for parameter estimation on demographic histories of *P. flammea* populations across the Atlantic Forest elevational gradient. The model reflects that MAC divergence was not considered for its evolution, and its relation to others was through migration). **L** = UBA, RAN, COR, ITA, and PES; **M** = MAC; and **H** = MAR and PAP. For population details see Table 1.

Stairway Plot 2 results (Figure 8) for the 3 major clusters indicated a bottleneck for populations from high elevations around 150k years ago, possibly related to a divergence event, followed by expansion and a recent decrease in the population size starting around 30k years ago. Currently, *Ne* in high-elevation is around 40k individuals. For MAC, Stairway Plot 2 revealed expansion, starting around 80k years ago, with a current *Ne* of approximately 50k individuals. Finally, populations from low/mid elevations showed population expansion from 300k to 100k years ago, followed by stability, with a *Ne* of approximately 60k (Figure 8A). However, it is important to highlight that this result also pointed to a high deviation of the confidence interval (Supplementary Figure 3S), possibly because this cluster gathers together five populations. Because this cluster combines so many populations, we also analyzed the population growth within each cluster separately, highlighting population patterns (Figure 8, Supplementary Figure 4S, Figure 5S). In high elevations, one population is decreasing and the other is increasing in population size (Figure 8B). In low/mid elevation, *Ne* patterns also vary among populations with time. UBA (20m a.s.l), with a current Ne of approximately 18k individuals, remained stable until 5k years ago when it started decreasing. RAN (20m a.s.l) and COR (425m a.s.l), which always cluster together, have the same pattern, with a bottleneck starting around 40k years ago, and a recent growth since 6k years ago. Despite having similar patterns, COR always had a larger *Ne* than RAN (around 30k and 20k in the present, respectively). ITA (1139m a.s.l) *Ne* decreased around 80k years ago, and remained stable since then, with a current Ne of 25k. Lastly, PES *Ne* (1496m a.s.l) remained stable until 15k years ago, then increased rapidly, being stable again since 6k years ago. Currently, PES has a *Ne* of 50k individuals (Figure 8C). Although they vary among populations, all *Ne* changes happened in the last 100,000 years.

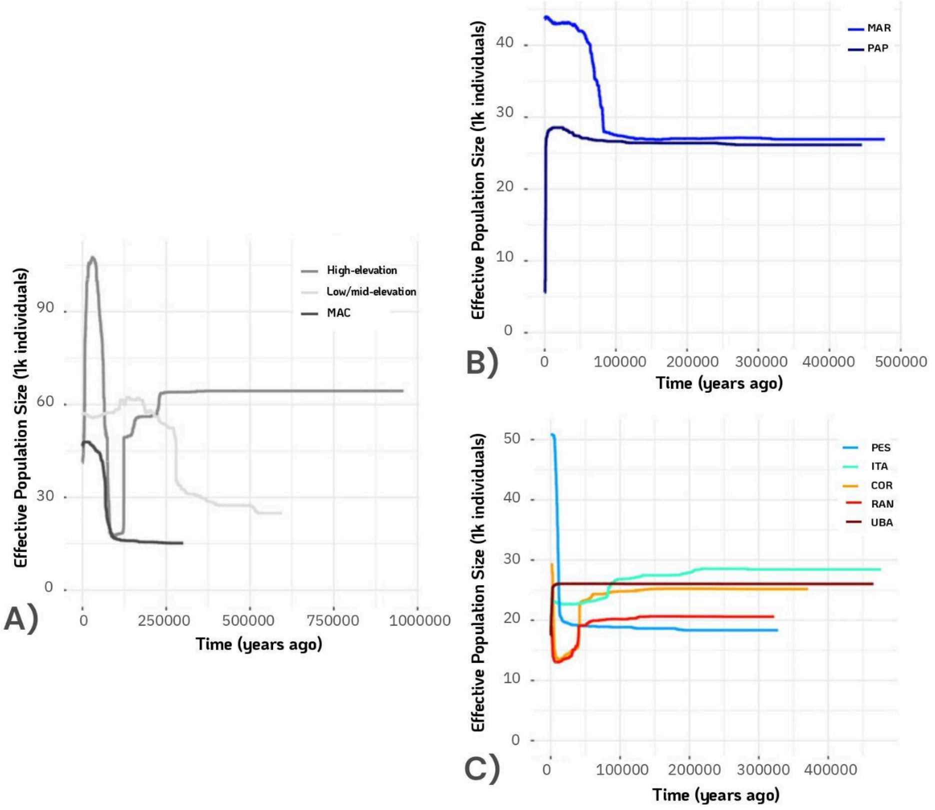

Figure 8 - Stairway Plot 2 results for changes in effective population size (*Ne*) for 3 clusters: populations from high elevations (MAR and PAP) MAC (*dark gray*), and populations from low/mid elevations (UBA, RAN, COR, ITA, and PES, *light gray*) (A). Stairway Plot 2 results within groups for high elevation populations PAP (*navy blue*) and MAR (*dark blue*) (B); and for low/mid

elevations populations UBA (*dark red*), RAN (red), COR (*orange*), ITA (*light blue*), and PES (*blue*). For population details see Table 1.

4. DISCUSSION

Our results revealed that *P. flammea* exhibits significant genetic structure along the Atlantic Forest elevational gradient. Individuals are grouped into three major clusters; however, they are also grouped into minor clusters that indicate most populations function as independent genetic units. This pattern of genetic structure aligns with divergence times, as the divergence between high-elevation and low/mid-elevation populations is much older than the divergence within the low/mid-elevation group. The genetic structure also aligns with the best model for gene flow between high and low/mid elevations, as indicated by coalescent demographic inferences, which suggest gene flow occurred only in the early stages after divergence. Populations at high elevations are more isolated from one another, while those at low and intermediate elevations are more interconnected. The divergence process appears to be associated with climatic cycles during the Late Pleistocene. Additionally, we detected variations in effective population sizes that may also have been influenced by Pleistocene climatic cycles. Finally, we identified three outlier loci associated with tolerance to abiotic stresses, and potentially related to natural selection across the elevational gradient.

We found that *P. flammea* exhibits a high genetic structure and can be clustered into three major groups, with most populations functioning as independent entities (Figure 4). The Mantel test revealed no evidence of isolation by distance or elevation, which reinforces the genetic structure found between populations. A previous study using few genomic markers and investigating phylogeographical patterns in *P. flammea* also identified most populations as independent evolving entities, highlighting the role of isolated rock outcrops in lineage diversification (Mota et al. 2020). Due to their isolation, rock outcrops are referred to as 'sky islands' and are considered drivers of population divergence, acting as barriers to gene flow (Porembski 2007). In this sense, high to moderate genetic structure patterns are not unusual for species inhabiting the Atlantic Forest rock outcrops, as observed for other bromeliads (*Alcantarea imperialis* (Carrière) Harms and *A. geniculata* (Wawra) J.R.Grant, Barbará et al. 2007; *Encholirium horridum* L.B.Sm., Hmeljevski et al. 2015; *Pitcairnia azouryi* Martinelli and Forzza, Manhaes et al. 2019; *Pitcairnia albiflos* Herb., *P. staminea* G. Lodd.*)*, and *P. corcovadensis* Wawra, Palma-Silva et al. 2011), and other plant species (e.g. *Portulaca hatschbachii* D.Legrand, Feliciano et al. 2022; *Bathysa australis* (A. St.-Hil.) Hook. f. ex K. Schum. (Rubiaceae), Reis et al. 2015). Similarly, high to moderate genetic structure can be found in rock outcrops associated with elevational gradients in other regions, demonstrating that this is a broader pattern. It was observed, for example, for species in Pantanal (e.g. *Bromelia hieronymi* Mez (Bromeliaceae), Godoy et al. 2018; *Dyckia excelsa* Leme (Bromeliaceae), Ruas et al. 2020); in the Espinhaço Range, that encompass the Cerrado, Caatinga and the Atlantic Forest (e.g. *Vellozia compacta* Mart. ex Schult. (Veloziaceae), Lousada et al. 2013; *Uebelmannia* Buining (Cactaceae), Silva et al. 2020; *Vriesea oligantha* complex, Dantas-Queiroz et al. 2021); and beyond Brazilian biomes, such as Rocky Mountains of Wyoming and Montana, in the U.S (e.g *Penstemon caryi* Pennell (Plantaginaceae), Stone et al. 2019) and the Yilgarn Banded Iron Formations in Australia (e.g. *Grevillea georgeana* McGill. (Proteaceae) Nistelberger et al. 2015).

Our results indicate that low and intermediate-elevation populations, with the exception of MAC, are more closely related to each other than to high-elevation populations, as demonstrated by structure, neighbor-joining, and phylogenetic analyses (Figures 4, 5, and 6). Similarly, populations from low elevations are more closely related to one another, and the same pattern is observed among populations from mid-elevations. Our SNAPP phylogeny results suggest that populations at higher elevations diverged from lower elevations around 300,000 years ago, and the two higher-elevated populations diverged from each other around 150,000 years ago. Divergence events occurred later (around 60,000 years ago) at low/mid-elevations, first between low and mid-elevations, then within low and mid-elevations. The deep divergence between populations at high and low/mid-elevations aligns with our demographic inferences based on coalescence, which revealed that gene flow happened only early times after the divergence. The Late Pleistocene, when divergence events between the *P. flammea* populations happened, was associated with intensified climatic cycles that started 900 kya due to orbital variations (Maslin & Brierley, 2015). Contrary to our results, Mota et al. (2020), based on a limited number of plastid markers, found no evidence of the Late Pleistocene glacial cycle's impact on *P. flammea* diversification. Their findings supported the existence of two main clades within *P. flammea* that diverged around 2 million years ago, reflecting the effects of the early Quaternary. Here, using more than a thousand SNP markers, we demonstrate the role of Late Pleistocene glaciations in the divergence of *P. flammea* populations across different elevations.

We detected variations in effective population sizes (*Ne*) using two different approaches: site frequency spectrum (SFS), with StairwayPlot 2; and Approximate Bayesian Computation (ABC) combined with Supervised Machine Learning (SML) through PipeMaster. Both methods revealed similar patterns of *Ne* changes, indicating a current decrease in population size at high elevations, MAC expansion over time, and a slight bottleneck at low/mid-elevations. Based on StairwayPlot results, we found evidence of population stability and growth at high elevations, except for the past 2,000 years (Figure 8), during which one population (PAP) has experienced a decrease in effective population size (*Ne*) from approximately 25,000 to 5,000. Population stability until very recent times, combined with old divergence events, suggests that *P. flammea* persisted in high elevations through both interglacial and glacial phases, either remaining in refugia or shifting their distribution upslope and downslope (Rahbek et al. 2019). At low/mid-elevations, light bottleneck events were observed in some populations (ITA, RAN, and COR), while others remained stable during the Pleistocene (UBA and PES). Current *Ne* values for low/mid-elevation populations fluctuate between 20,000 and 50,000. The *N*e variations in *P. flammea* and the later divergence times might reflect some effect of Late Pleistocene glaciations on low/mid-elevations, possibly associated with habitat availability and range shifts directed to lower elevations during glacial periods (Cabanne et al. 2016). However, most changes in *Ne* for populations from both high (PAP) and low/mid elevations occurred recently (less than 10,000 years), suggesting a strong contemporary climate influence on *P. flammea*, as already proposed for the evolutionary history of the Southern Atlantic Forest (Carnaval et al. 2014).

Our results show that populations exhibit moderate genetic diversity at low/mid-elevations and high genetic diversity at high elevations. Mota et al. (2020) found low to moderate diversity levels across the *P. flammea* distribution; however, populations above 2000 m a.s.l. (such as MAR and PAP, which here form the high-elevation cluster) were not sampled in their study. The diversity patterns in high and low/mid-elevation populations likely result from their distinct demographic histories. Higher diversity levels are associated with higher effective population sizes (Lande and Barrowclough, 1987), higher population structure, and long-term persistence (Carnaval et al. 2009), which aligns with the stability we found for *P. flammea* from high elevations. Besides, highlands were proposed to have acted as microrefugia in the Southern Atlantic Forest (Carnaval et al. 2014). The long-term persistence of *P. flammea* over time was also supported by demographic inferences based on microsatellite data from Mota et al. (2020), where only five out of 25 populations exhibited bottlenecks. They proposed that rock outcrops could have served as refugia for xerophilic and cold-adapted species during glacial and interglacial cycles, emphasizing the importance of including environmental gradients and high-throughput sequencing data to enhance the understanding of demographic dynamics in such fragmented environments. In this regard, our findings further validate their observations. Differences in diversity at high and low/mid elevations may also be related to pollinator behavior and the evolution of different flowering times and mating systems between populations, aspects that have already been investigated between hybridizing *Pitcairnia* spp. (Wendt et al. 2002; Rôças et al. 2004; Palma-Silva et al. 2011; Tavares et al. 2022), but not between populations of *P. flammea*. Further studies exploring the reproductive aspects of these populations would enhance our understanding of their evolution.

The different demographic histories of *P. flammea* populations along the Atlantic Forest elevational gradient also align with the tolerance-avoidance trade-off described by Chaves et al. (2024). Their study found that lower elevation populations tend to avoid thermal stress, while higher elevation populations exhibit thermal tolerance to both heat and cold, highlighting local adaptation through ecophysiological traits such as foliar traits, thermal tolerance, and membrane fluidity. High-elevation populations diverged earlier and persisted, allowing more time for accumulating genetic differences. These differences potentially led to adaptive traits such as cold and heat tolerance (Futuyma, 2010). Indeed, Carnaval et al. (2014) and Cabanne et al. (2016) suggested that cold tolerance — as found for *P. flammea* at high elevations — was related to stability during Pleistocene climatic cycles. Meanwhile, populations at low/mid elevations diverged more recently, possibly having not yet evolved tolerant traits and adopting avoidance as their strategy.

Finally, our results indicate three outlier loci that would be related to a natural selection in the *P. flammea* populations across altitudes: (i) the Lectin Receptor-like Kinase, such as other Receptor-like kinases, is associated with tolerance to abiotic stresses, particularly to cold tolerance (Vaid et al., 2013; Yang et al., 2014; Jung et al., 2015; Sun et al. 2020; Geng et al., 2021); (ii) the Callose synthase is involved in many plant processes (e.g., leaf development, pollen formation, plant growth, callose formation induced by pathogens; UniProt Consortium, 2015), including cold stress responses (Wang et al 2020); and (iii) the Protein DETOXIFICATION 40-like (MATE protein 40), is associated with responses to drought and metal stresses (Zhang et al. 2014; UniProt Consortium, 2015; Chen et al. 2022). These outlier loci align with the aforementioned tolerance-avoidance trade-off described by Chaves et al. (2024). Our results indicate potential loci related to these adaptations, including thermal tolerance and drought avoidance. They also suggest adaptations to other environmental factors acting simultaneously with the spatial thermal changes associated with altitudinal variations (Alonso-Amelot, 2008). Yet, the role of these outlier loci in *P. flammea* local adaptation requires to be thoroughly investigated.

5. REFERENCES

Acha, S., Linan, A., MacDougal, J., & Edwards, C. (2021). The evolutionary history of vines in a neotropical biodiversity hotspot: Phylogenomics and biogeography of a large passion flower clade (*Passiflora* section Decaloba). *Molecular phylogenetics and evolution*, 164, 107260.

Aguiar‑Melo, C., Zanella, C. M., Goetze, M., Palma‑Silva, C., Hirsch, L. D., Neves, B., ... & Bered, F. (2019). Ecological niche modeling and a lack of phylogeographic structure in *Vriesea incurvata* suggest historically stable areas in the southern Atlantic Forest. *American Journal of Botany*, 106(7), 971-983.

Alexander, D. H., Shringarpure, S. S., Novembre, J., & Lange, K. (2015). Admixture 1.3 software manual. Los Angeles: UCLA Human Genetics Software Distribution.

Alonso-Amelot, M. E. (2008). High altitude plants, chemistry of acclimation and adaptation. *Studies in natural products chemistry,* 34, 883-982.

Andrews, S. (2014). FastQC: A quality control tool for high throughput sequence data. Retrieved from http://www.bioinformatics.babraha m.ac.uk/projects/fastqc/

Angilletta Jr, M. J., & Angilletta, M. J. (2009). Thermal adaptation: a theoretical and empirical synthesis.

Antonelli, A., Ariza, M., Albert, J., Andermann, T., Azevedo, J., Bacon, C., ... & Matos-Maraví, P. (2018). Conceptual and empirical advances in Neotropical biodiversity research. PeerJ, 6, e5644. Antonelli, A. (2022). The rise and fall of Neotropical biodiversity. *Botanical Journal of the Linnean Society*, 199(1), 8-24.

Antonelli, A. (2022). The rise and fall of Neotropical biodiversity. *Botanical Journal of the Linnean Society*, 199(1), 8-24.

Ataídes, D. S., Vasconcelos-Filho, S. C., Costa, A. C. Alterações na fisiologia de plantas de feijoeiro croulos como indicadores de vulnerabilidade ao déficit hídrico e ao aumento de temperatura do ambiente de crescimento, 2018. 51 f. Dissertação (Mestrado em Biodiversidade e Convervação) – Instituto Federal Goiano, Campus Rio Verde.

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., ... & Johnson, E. A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PloS one*, 3(10), e3376.

Barbará, T., Martinelli, G., Palma-Silva, C., Fay, M. F., Mayo, S., & Lexer, C. (2009). Genetic relationships and variation in reproductive strategies in four closely related bromeliads adapted to neotropical 'inselbergs': *Alcantarea glaziouana, A. regina, A. geniculata and A. imperialis* (Bromeliaceae). *Annals of Botany*, 103(1), 65-77.

Batalha‑Filho, H., & Miyaki, C. Y. (2016). Late Pleistocene divergence and postglacial expansion in the Brazilian Atlantic Forest: multilocus phylogeography of *Rhopias gularis* (Aves: Passeriformes). *Journal of Zoological Systematics and Evolutionary Research*, 54(2), 137-147.

Benzing, D. H., & Bennett, B. (2000). Bromeliaceae: profile of an adaptive radiation. *Cambridge University Press.*

Brandrud, M. K., Baar, J., Lorenzo, M. T., Athanasiadis, A., Bateman, R. M., Chase, M. W., ... & Paun, O. (2020). Phylogenomic relationships of diploids and the origins of allotetraploids in Dactylorhiza (Orchidaceae). *Systematic Biology,* 69(1), 91-109.

Bouckaert, R., & Bryant, D. (2012). A rough guide to SNAPP. Website: https://github. com/BEAST2-Dev/SNAPP/releases/download/v1, 2.

Bouckaert, R. R., & Heled, J. (2014). DensiTree 2: Seeing trees through the forest. *BioRxiv,* 012401.

Carnaval, A. C., Hickerson, M. J., Haddad, C. F., Rodrigues, M. T., & Moritz, C. (2009). Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science*, 323(5915), 785-789.

Carnaval, A. C., Waltari, E., Rodrigues, M. T., Rosauer, D., VanDerWal, J., Damasceno, R., ... & Moritz, C. (2014). Prediction of phylogeographic endemism in an environmentally complex biome. *Proceedings of the Royal Society B: Biological Sciences*, 281(1792), 20141461.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis toolset for population genomics. *Mol Ecol* 22:3124–3140.

Chen, H., Wang, D., Zhu, Y., Li, W., Chen, J., & Li, Y. (2022). Integrative transcriptomics and proteomics elucidate the regulatory mechanism of Hydrangea macrophylla flower-color changes induced by exogenous aluminum. *Agronomy*, 12(4), 969.

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

Cornille, A., Salcedo, A., Kryvokhyzha, D., Glémin, S., Holm, K., Wright, S. I., & Lascoux, M. (2016). Genomic signature of successful colonization of Eurasia by the allopolyploid shepherd's purse (*Capsella bursa‐pastoris*). *Molecular ecology*, 25(2), 616-629.

Damasceno, R. P., Carnaval, A. C., Sass, C., Recoder, R. S., Moritz, C., & Rodrigues, M. T. (2021). Geographic restriction, genetic divergence, and morphological disparity in the Brazilian Atlantic Forests: Insights from *Leposoma* lizards (Gymnophthalmidae, Squamata). *Molecular Phylogenetics and Evolution*, 154, 106993.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., ... & 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, 27(15), 2156-2158.

Dantas‐Queiroz, M. V., Cacossi, T. D. C., Leal, B. S. S., Chaves, C. J. N., Vasconcelos, T. N., Versieux, L. D. M., & Palma‐Silva, C. (2021). Underlying microevolutionary processes parallel macroevolutionary patterns in ancient neotropical mountains. Journal of Biogeography, 48(9), 2312-2327.

Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation genetics resources*, 4(2), 359-361.

Eaton, D. A. (2014). PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics,* 30(13), 1844-1849.

Excoffier, L., Marchi, N., Marques, D. A., Matthey-Doret, R., Gouy, A., & Sousa, V. C. (2021). fastsimcoal2: demographic inference under complex evolutionary scenarios. *Bioinformatics,* 37(24), 4882-4885.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,* 14(8), 2611-2620.

Ferreira, D. M. C., Palma-Silva, C., Néri, J., Medeiros, M. C. M. P. D., Pinangé, D. S., Benko-Iseppon, A. M., & Louzada, R. B. (2021). Population genetic structure and species delimitation in the *Cryptanthus zonatus* complex (Bromeliaceae). *Botanical Journal of the Linnean Society*, 196(1), 123-140.

Flantua, S. G., O'Dea, A., Onstein, R. E., Giraldo, C., & Hooghiemstra, H. (2019). The flickering connectivity system of the north Andean páramos. *Journal of Biogeography*, 46(8), 1808-1825.

Feliciano, D. C., De Godoy, S. M., Da Silva, J. F. M., Góes, B. D., Ferraz, J. R., De Oliveira Santos, P., ... & De Fátima Ruas, C. (2022). Landscape genetics reveal low diversity and adaptive divergence in Portulaca hatschbachii (Portulacaceae): an endangered species endemic to rocky outcrops of the Atlantic Forest. Botanical Journal of the Linnean Society, 200(1), 116-141.

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics*, 180(2), 977-993.

Foll, M. (2012). BayeScan v2. 1 user manual. *Ecology,* 20(10), 1450-1462.

Futuyma, D. J. (2010). Evolutionary constraint and ecological consequences. *Evolution*, 64(7), 1865-1884.

Gavin, D. G., Fitzpatrick, M. C., Gugger, P. F., Heath, K. D., Rodríguez‐Sánchez, F., Dobrowski, S. Z., ... & Williams, J. W. (2014). Climate refugia: joint inference from fossil records, species distribution models and phylogeography. *New Phytologist*, 204(1), 37-54.

Gehara, M., Mazzochinni, G. G., & Burbrink, F. (2020). PipeMaster: inferring population divergence and demographic history with approximate Bayesian computation and supervised machine-learning in R. *BioRxiv*, 2020-12.

Geng, B., Wang, Q., Huang, R., Liu, Y., Guo, Z., & Lu, S. (2021). A novel LRR‐RLK (CTLK) confers cold tolerance through regulation on the C‐repeat‐binding factor pathway, antioxidants, and proline accumulation. *The Plant Journal*, 108(6), 1679-1689.

Givnish, T. J., Barfuss, M. H., Van Ee, B., Riina, R., Schulte, K., Horres, R., ... & Sytsma, K. J. (2014). Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. *Molecular phylogenetics and evolution*, 71, 55-78.

Godoy, F. M. D. R., Lenzi, M., Ferreira, B. H. D. S., Silva, L. V. D., Zanella, C. M., & Paggi, G. M. (2018). High genetic diversity and moderate genetic structure in the self-incompatible, clonal Bromelia hieronymi (Bromeliaceae). Botanical Journal of the Linnean Society, 187(4), 672-688.

Harvey, M. G., & Brumfield, R. T. (2015). Genomic variation in a widespread Neotropical bird (*Xenops minutus*) reveals divergence, population expansion, and gene flow. *Molecular Phylogenetics and Evolution*, 83, 305-316.

Hess, J. E., Campbell, N. R., Close, D. A., Docker, M. F., & Narum, S. R. (2013). Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. *Molecular Ecology*, 22(11), 2898-2916.

Hmeljevski, K. V., dos Reis, M. S., & Forzza, R. C. (2015). Patterns of gene flow in *Encholirium horridum* LB Sm., a monocarpic species of Bromeliaceae from Brazil. *Journal of Heredity*, 106(1), 93-101.

Hmeljevski KV, Nazareno AG, Leandro Bueno M, dos Reis MS, Forzza RC (2017). Do 761 plant populations on distinct inselbergs talk to each other? A case study of genetic 762 connectivity of a bromeliad species in an Ocbil landscape. *Ecol Evol* 7: 4704-4716.

Huson, D. H., & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular biology and evolution*, 23(2), 254-267.

Janes, J. K., Miller, J. M., Dupuis, J. R., Malenfant, R. M., Gorrell, J. C., Cullingham, C. I., & Andrew, R. L. (2017). The K= 2 conundrum. *Molecular ecology*, 26(14), 3594-3602.

Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *The American Naturalist*, 101(919), 233-249.

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC genetics*, 11, 1-15.

Jung, C. G., Hwang, S. G., Park, Y. C., Park, H. M., Kim, D. S., Park, D. H., & Jang, C. S. (2015). Molecular characterization of the cold-and heat-induced *Arabidopsis* PXL1 gene and its potential role in transduction pathways under temperature fluctuations. *Journal of plant physiology*, 176, 138-146.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular ecology resources*, 15(5), 1179-1191

Lande, R., & Barrowclough, G. F. (1987). Effective population size, genetic variation, and their use in population management. Viable populations for conservation, 87, 87-124.

Leal, B. S. S., Chaves, C. J. N., Graciano, … & Palma-Silva, C. (2021). Evidence of local adaptation despite strong drift in a Neotropical patchily distributed bromeliad. *Heredity*, 127(2), 203-218.

Liu, X., & Fu, Y. X. (2020). Stairway Plot 2: demographic history inference with folded SNP frequency spectra. *Genome biology*, 21(1), 280.

Liu, L., Tumi, L., Suni, M. L., Arakaki, M., Wang, Z. F., & Ge, X. J. (2021). Draft genome of *Puya raimondii* (Bromeliaceae), the Queen of the Andes. *Genomics*, 113(4), 2537-2546.

Lousada, J. M., Lovato, M. B., & Borba, E. L. (2013). High genetic divergence and low genetic variability in disjunct populations of the endemic Vellozia compacta (Velloziaceae) occurring in two edaphic environments of Brazilian campos rupestres. Brazilian Journal of Botany, 36, 45-53.

Luu, K., Bazin, E., & Blum, M. G. (2017). pcadapt: an R package to perform genome scans for selection based on principal component analysis. *Molecular ecology resources*, 17(1), 67-77.

Manhaes, V. D. C., de Miranda, F. D., Clarindo, W. R., & Carrijo, T. T. (2020). Genetic diversity and karyotype of *Pitcairnia azouryi*: an endangered species of Bromeliaceae endemic to Atlantic Forest inselbergs. *Molecular Biology Reports*, 47, 179-189.

Martin, P. R., & McKay, J. K. (2004). Latitudinal variation in genetic divergence of populations and the potential for future speciation. *Evolution*, 58(5), 938-945.

Maslin, M. A., & Brierley, C. M. (2015). The role of orbital forcing in the Early Middle Pleistocene Transition. Quaternary International, 389, 47-55.

Morellato, L. P. C., & Haddad, C. F. (2000). Introduction: The Brazilian Atlantic Forest 1. *Biotropica*, 32(4b), 786-792.

Moritz, C., Langham, G., Kearney, M., Krockenberger, A., VanDerWal, J., & Williams, S. (2012). Integrating phylogeography and physiology reveals divergence of thermal traits between central and peripheral lineages of tropical rainforest lizards. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1596), 1680- 1687.

Mota, M. R., Pinheiro, F., Leal, B. S. D. S., Sardelli, C. H., Wendt, T., & Palma-Silva, C. (2020). From micro-to macroevolution: insights from a Neotropical bromeliad with high population genetic structure adapted to rock outcrops. *Heredity*, 125(5), 353-370.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403(6772), 853.

Nadeau, N. J., Martin, S. H., Kozak, K. M., Salazar, C., Dasmahapatra, K. K., Davey, J. W., ... & Jiggins, C. D. (2013). Genome‐wide patterns of divergence and gene flow across a butterfly radiation. *Molecular ecology*, 22(3), 814-826.

Nistelberger, H. M., Byrne, M., Coates, D., & Roberts, J. D. (2015). Genetic drift drives evolution in the bird-pollinated, terrestrial island endemic Grevillea georgeana (Proteaceae). Botanical Journal of the Linnean Society, 178(1), 155-168.

Ohsawa, T., & Ide, Y. (2008). Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Global Ecology and Biogeography,* 17(2), 152-163.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., & Suggests, M. A. S. S. (2007). The vegan package. *Community ecology package*, 10(631-637), 719.

Oliveira‐Filho, A. T., & Fontes, M. A. L. (2000). Patterns of floristic differentiation among Atlantic Forests in Southeastern Brazil and the influence of climate 1. *Biotropica,* 32(4b), 793-810.

Palma‐silva, C., Wendt, T., Pinheiro, F., Barbará, T., Fay, M. F., Cozzolino, S., & Lexer, C. (2011). Sympatric bromeliad species (Pitcairnia spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. Molecular Ecology, 20(15), 3185-3201.

Palma-Silva, C., Leal, B. S., Chaves, C. J., & Fay, M. F. (2016). Advances in and perspectives on evolution in Bromeliaceae. *Botanical Journal of the Linnean Society*, 181(3), 305-322.

Palma-Silva, C., Turchetto-Zolet, A. C., Fay, M. F., & Vasconcelos, T. (2022). Drivers of exceptional Neotropical biodiversity: an updated view. *Botanical Journal of the Linnean Society,* 199(1), 1-7.

Peres, E. A., Pinto-da-Rocha, R., Lohmann, L. G., Michelangeli, F. A., Miyaki, C. Y., & Carnaval, A. C. (2020). Patterns of species and lineage diversity in the Atlantic rainforest of Brazil. *Neotropical diversification: patterns and processes,* 415-447.

Pinto, B. J., Colli, G. R., Higham, T. E., … & Gamble, T. (2019). Population genetic structure and species delimitation of a widespread, Neotropical dwarf gecko. *Molecular phylogenetics and evolution,* 133, 54-66.

Pisa, S., Werner, O., Vanderpoorten, A., Magdy, M., & Ros, R. M. (2013). Elevational patterns of genetic variation in the cosmopolitan moss Bryum argenteum (Bryaceae). American journal of botany, 100(10), 2000-2008.

Polato, N. R., Gill, B. A., Shah, A. A., Gray, M. M., Casner, K. L., Barthelet, A., ... & Kondratieff, B. C. (2018). Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains. *Proceedings of the National Academy of Sciences,* 115(49), 12471-12476.

Porembski, S. (2007). Tropical inselbergs: habitat types, adaptive strategies and diversity patterns. *Brazilian Journal of Botany,* 30(4), 579-586.

Prance, G. T. (1982). A review of the phytogeographic evidences for Pleistocene climate changes in the Neotropics. *Annals of the Missouri Botanical Garden,* 594-624.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics, 155(2), 945-959.

Rahbek, C., Borregaard, M. K., Antonelli, A., Colwell, R. K., ... & Fjeldså, J. (2019). Building mountain biodiversity: *Geological and evolutionary processes. Science,* 365(6458), 1114-1119.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic biology,* 67(5), 901-904.

Reis, T. S., Ciampi‐Guillardi, M., Bajay, M. M., de Souza, A. P., & Dos Santos, F. A. M. (2015). Elevation as a barrier: genetic structure for an A tlantic rain forest tree (*Bathysa australis*) in the Serra do Mar mountain range, SE Brazil. *Ecology and Evolution,* 5(9), 1919-1931.

Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J., & Hirota, M. M. (2009). The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological conservation,* 142(6), 1141-1153.

Ribeiro, M. C., Martensen, A. C., Metzger, J. P., Tabarelli, M., Scarano, F., & Fortin, M. J. (2011). The Brazilian Atlantic Forest: a shrinking biodiversity hotspot. In *Biodiversity hotspots* (pp. 405-434). Springer, Berlin, Heidelberg.

Roças, G., Klein, D. E., & De Mattos, E. A. (2004). Artificial hybridization between Pitcairnia flammea and Pitcairnia corcovadensis (Bromeliaceae): analysis of the performance of parents and hybrids. Plant species biology, 19(1), 47-53.

Rödin‐Mörch, P., Luquet, E., Meyer‐Lucht, Y., Richter‐Boix, A., Höglund, J., & Laurila, A. (2019). Latitudinal divergence in a widespread amphibian: Contrasting patterns of neutral and adaptive genomic variation. *Molecular Ecology*, 28(12), 2996-3011.

Rougemont, Q., & Bernatchez, L. (2018). The demographic history of Atlantic salmon (*Salmo salar*) across its distribution range reconstructed from approximate Bayesian computations. *Evolution,* 72(6), 1261-1277.

Ruas, R. D. B., Paggi, G. M., Aguiar-Melo, C., Hirsch, L. D., & Bered, F. (2020). Strong genetic structure in Dyckia excelsa (Bromeliaceae), an endangered species found on ironstone outcrops in Pantanal, Brazil. Botanical Journal of the Linnean Society, 192(4), 691-705.

Sanín, M. J., Zapata, P., Pintaud, J. C., Galeano, G., Bohórquez, A., Tohme, J., & Hansen, M. M. (2017). Up and down the blind alley: Population divergence with scant gene flow in an endangered tropical lineage of Andean palms (*Ceroxylon quindiuense* Clade: Ceroxyloideae). *Journal of Heredity,* 108(3), 288-298.

Silva, G. A., Khan, G., Ribeiro-Silva, S., Aona, L. Y., Machado, M. C., Bonatelli, I. A., & Moraes, E. M. (2020). Extreme genetic structure in a relict cactus genus from campo rupestre landscapes: implications for conservation. Biodiversity and Conservation, 29, 1263-1281.

Smith, L. B., & Downs, R. J. (1974). Bromeliaceae (Pitcairnioideae). *Flora Neotropica Monograph.*

Sovic, M., Fries, A., Martin, S. A., & Lisle Gibbs, H. (2019). Genetic signatures of small effective population sizes and demographic declines in an endangered rattlesnake, *Sistrurus catenatus*. *Evolutionary Applications,* 12(4), 664-678.

Stone, B. W., Ward, A., Farenwald, M., Lutz, A. W., & Wolfe, A. D. (2019). Genetic diversity and population structure in Cary's Beardtongue Penstemon caryi (Plantaginaceae), a rare plant endemic to the eastern Rocky Mountains of Wyoming and Montana. Conservation Genetics, 20, 1149-1161.

Sun, Y., Qiao, Z., Muchero, W., & Chen, J. G. (2020). Lectin receptor-like kinases: the sensor and mediator at the plant cell surface. *Frontiers in plant science*, 11, 596301.

Tavares, M. M., Ferro, M., Leal, B. S. S., & Palma‐Silva, C. (2022). Speciation with gene flow between two Neotropical sympatric species (*Pitcairnia spp.*: Bromeliaceae). *Ecology and evolution,* 12(5), e8834.

Teixeira, T. M., & Nazareno, A. G. (2021). One step away from extinction: a population genomic analysis of a narrow endemic, tropical plant species. *Frontiers in Plant Science*, 12, 730258.

Thome, M. T. C., Lyra, M. L., Lemes, P., Teixeira, L. S., Carnaval, A. C., Haddad, C. F., & Canedo, C. (2020). Outstanding diversity and microendemism in a clade of rare Atlantic Forest montane frogs. *Molecular Phylogenetics and Evolution,* 149, 106813.

Thom, G., Smith, B. T., Gehara, M., Montesanti, J., Lima-Ribeiro, M. S., Piacentini, V. Q., ... & do Amaral, F. R. (2020). Climatic dynamics and topography control genetic variation in Atlantic Forest montane birds. *Molecular phylogenetics and evolution*, 148, 106812.

Vaid, N., Macovei, A., & Tuteja, N. (2013). Knights in action: lectin receptor-like kinases in plant development and stress responses. *Molecular plant,* 6(5), 1405-1418.

Versieux, L. M., Barbará, T., Wanderley, M. D. G. L., Calvente, A., Fay, M. F., & Lexer, C. (2012). Molecular phylogenetics of the Brazilian giant bromeliads (*Alcantarea,* Bromeliaceae): implications for morphological evolution and biogeography. *Molecular Phylogenetics and Evolution,* 64(1), 177-189.

Vicente-Silva, L., De Russo Godoy, F. M., Faggioni, G. P., Lorenz, A. P., & Paggi, G. M. (2022). Patchily distributed but not necessarily isolated populations of *Deuterocohnia meziana*: a threatened bromeliad from rock outcrops. *Botanical Journal of the Linnean Society,* 199(1), 312-330.

Vieira, L. D., Silva-Junior, O. B., Novaes, E., & Collevatti, R. G. (2022). Comparative population genomics in *Tabebuia alliance* shows evidence of adaptation in Neotropical tree species. *Heredity,* 1-13.

Wang, X., Chang, X., Jing, Y., Zhao, J., Fang, Q., Sun, M., ... & Li, Y. (2020). Identification and functional prediction of soybean CircRNAs involved in low-temperature responses. *Journal of plant physiology*, 250, 153188.

Wendt, T., Canela, M. B. F., Klein, D. E., & Rios, R. I. (2002). Selfing facilitates reproductive isolation among three sympatric species of Pitcairnia (Bromeliaceae). Plant Systematics and Evolution, 232, 201-212.

Yang, L., Wu, K., Gao, P., Liu, X., Li, G., & Wu, Z. (2014). GsLRPK, a novel cold-activated leucine-rich repeat receptor-like protein kinase from Glycine soja, is a positive regulator to cold stress tolerance. P*lant Science,* 215, 19-28.

Zhang, H., Zhu, H., Pan, Y., Yu, Y., Luan, S., & Li, L. (2014). A DTX/MATE-type transporter facilitates abscisic acid efflux and modulates ABA sensitivity and drought tolerance in Arabidopsis. *Molecular plant,* 7(10), 1522-1.

SUPPLEMENTARY MATERIAL

Table 1S – Number of raw Illumina reads sequenced per individual of *Pitcairnia flammea* (Bromeliaceae).

Population	Individual	Total of raw reads	Population	Individual	Total of raw reads	Population	Individual	Total of raw reads	Population	Individual	Total of raw reads
UBA	UBA01A	2875691	RAN	RAN238	3261996	MAC	MAC297	1169270	MAR	MAR126I	6220354
UBA	UBA02A	1960315	RAN	RAN243	2579176	MAC	MAC298	1940432	MAR	MAR127I	4898310
UBA	UBA03A	8049569	RAN	RAN244	2430543	MAC	MAC301	2542423	MAR	MAR129I	8361367
UBA	UBA04A	3017318	RAN	RAN247	3185247	ITA	ITA101A	7634736	MAR	MAR130I	4166373
UBA	UBA05B	2618581	RAN	RAN249	5692348	ITA	ITA102A	2816483	MAR	MAR131I	7118821
UBA	UBA06	2548008	RAN	RAN250	2858865	ITA	ITA103A	2496020	MAR	MAR133I	6065642
UBA	UBA07	7144394	COR	COR185B	2033292	ITA	ITA104	4491021	MAR	MAR134I	5336658
UBA	UBA08B	3259024	COR	COR186B	1740071	ITA	ITA108	6332619	MAR	MAR135	2895384
UBA	UBA09	2270948	COR	COR187B	3409845	ITA	ITA109	3856735	MAR	MAR137I	1678010
UBA	UBA10	3027604	COR	COR189B	2709766	ITA	ITA110	6711503	MAR	MAR138I	6395343
UBA	UBA11	3168056	COR	COR190B	2432542	ITA	ITA111	4387463	MAR	MAR139I	2970446
UBA	UBA12	1007989	COR	COR191A	4539405	ITA	ITA112	381907	MAR	MAR277I	7045564
UBA	UBA13	891812	COR	COR192B	6154814	ITA	ITA113	9165937	MAR	MAR338	1756538
UBA	UBA15	3872461	COR	COR194	1302274	ITA	ITA114	1750705	MAR	MAR340	3063310
UBA	UBA16	1840955	COR	COR195B	3817407	ITA	ITA116	3637302	MAR	MAR342	5216310
UBA	UBA18	4083277	COR	COR197A	3453100	ITA	ITA117	5947166	MAR	MAR343	9399142
UBA	UBA21	2697165	COR	COR198A	1481548	ITA	ITA118	6939046	MAR	MAR345	2099861
UBA	UBA22	1108532	COR	COR199A	6284432	ITA	ITA119	5136611	PAP	PAP01A	7844878

UBA	UBA329	1285858	COR	COR305	1575524	ITA	ITA311	2338515	PAP	PAP03	5135196
UBA	UBA330	1311455	COR	COR307	2398759	ITA	ITA312	3279116	PAP	PAP04A	3079530
UBA	UBA332	2018560	COR	COR308	1645504	ITA	ITA314	4291046	PAP	PAP05A	1947277
UBA	UBA333	2267199	COR	COR309	4008158	ITA	ITA315	3181992	PAP	PAP06	3251457
UBA	UBA334	2268974	COR	COR310	4264010	ITA	ITA316	3661519	PAP	PAP07	878983
RAN	RAN166A	3023386	MAC	MAC152A	2261226	ITA	ITA318	2307441	PAP	PAP08B	6350204
RAN	RAN167A	5557714	MAC	MAC153A	2639441	ITA	ITA99	2079353	PAP	PAP09	1031508
RAN	RAN168A	1309705	MAC	MAC154A	2791218	PES	PES142A	6711117	PAP	PAP12	3045476
RAN	RAN169A	2207259	MAC	MAC155A	6381561	PES	PES143A	4526509	PAP	PAP14	2380481
RAN	RAN170A	1522823	MAC	MAC157A	4884058	PES	PES144A	6221453	PAP	PAP15A	2345994
RAN	RAN171A	3581873	MAC	MAC158A	4119060	PES	PES145A	4706936	PAP	PAP16A	4208373
RAN	RAN172A	3132405	MAC	MAC159A	970315	PES	PES146A	4127825	PAP	PAP17A	6441365
RAN	RAN173A	1868200	MAC	MAC160	2200844	PES	PES148A	4025357	PAP	PAP18	8168404
RAN	RAN174A	2173775	MAC	MAC161A	4158547	PES	PES149A	3207788	PAP	PAP19	1540596
RAN	RAN175A	1477114	MAC	MAC162A	4517514	PES	PES150	6621285	PAP	PAP20	1299857
RAN	RAN176A	2968756	MAC	MAC163A	3955513	PES	PES253	1372680	PAP	PAP22	2601977
RAN	RAN177A	1345246	MAC	MAC289	1611575	PES	PES254	2013074	PAP	PAP24	3526171
RAN	RAN178A	2275773	MAC	MAC290	1379458	MAR	MAR120I	4593463	PAP	PAP25	3159415
RAN	RAN179A	3196296	MAC	MAC291	2598798	MAR	MAR121I	6440729	PAP	PAP349	5837685
RAN	RAN180A	1686563	MAC	MAC292	3057527	MAR	MAR123	2081601	PAP	PAP351	2428067
RAN	RAN189A	2217588	MAC	MAC293	1496528	MAR	MAR124	8810029	PAP	PAP352	3030554
RAN	RAN237	1154141	MAC	MAC295	1944768	MAR	MAR125I	7639074			

Table 2S – Number of individuals and SNPs used for each major cluster (High-elevation, MAC or Low/mid-elevation) or population (PAP, MAR, PES, ITA, COR, RAN and UBA) used in the StairwPlot and Fastsimcoal analyses. Clusters were analyzed pairwise in Fastsimcoal. SNPs were used to generate MAF matrices (Minor Allele Frequency) used in both StairwayPlot and Fastsimcoal. For population details see Table 1.

Cluster/ Population	**Number of individuals**	**Number of SNPs**	**Analysis**
High-elevation	20	16779	StairwayPlot
MAC	20	16131	StairwayPlot
Low/mid-elevation	20	16458	StairwayPlot
High-elevation vs. Low/mid-elevation	40	12356	Fastsimcoal
High-elevation vs. MAC	40	12018	Fastsimcoal
PAP	10	19038	StairwayPlot
MAR	10	18203	StairwayPlot
PES	10	21620	StairwayPlot
ITA	10	21567	StairwayPlot
COR	10	19098	StairwayPlot
RAN	10	21511	StairwayPlot
UBA	10	18328	StairwayPlot

Table 3S – Pairwise *F*ST between the eight *Pitcairnia flammea* populations obtained using Stacks. For population details see Table 1.

Population	**UBA**	**RAN**	**COR**	**MAC**	**ITA**	**PES**	**MAR**	**PAP**
UBA	0,000	0,152	0,158	0,293	0,149	0,166	0,289	0,304
RAN	0,152	0,000	0,082	0,328	0,179	0,201	0,315	0,333
COR	0,158	0,082	0,000	0,349	0,189	0,211	0,329	0,350
MAC	0,293	0,328	0,349	0,000	0,305	0,339	0,313	0,328
ITA	0,149	0,179	0,189	0,305	0,000	0,151	0,291	0,313
PES	0,166	0,201	0,211	0,339	0,339	0,000	0,316	0,341
MAR	0,289	0,315	0,329	0,313	0,291	0,316	0,000	0,273
PAP	0,304	0,333	0,350	0,328	0,313	0,341	0,273	0,000

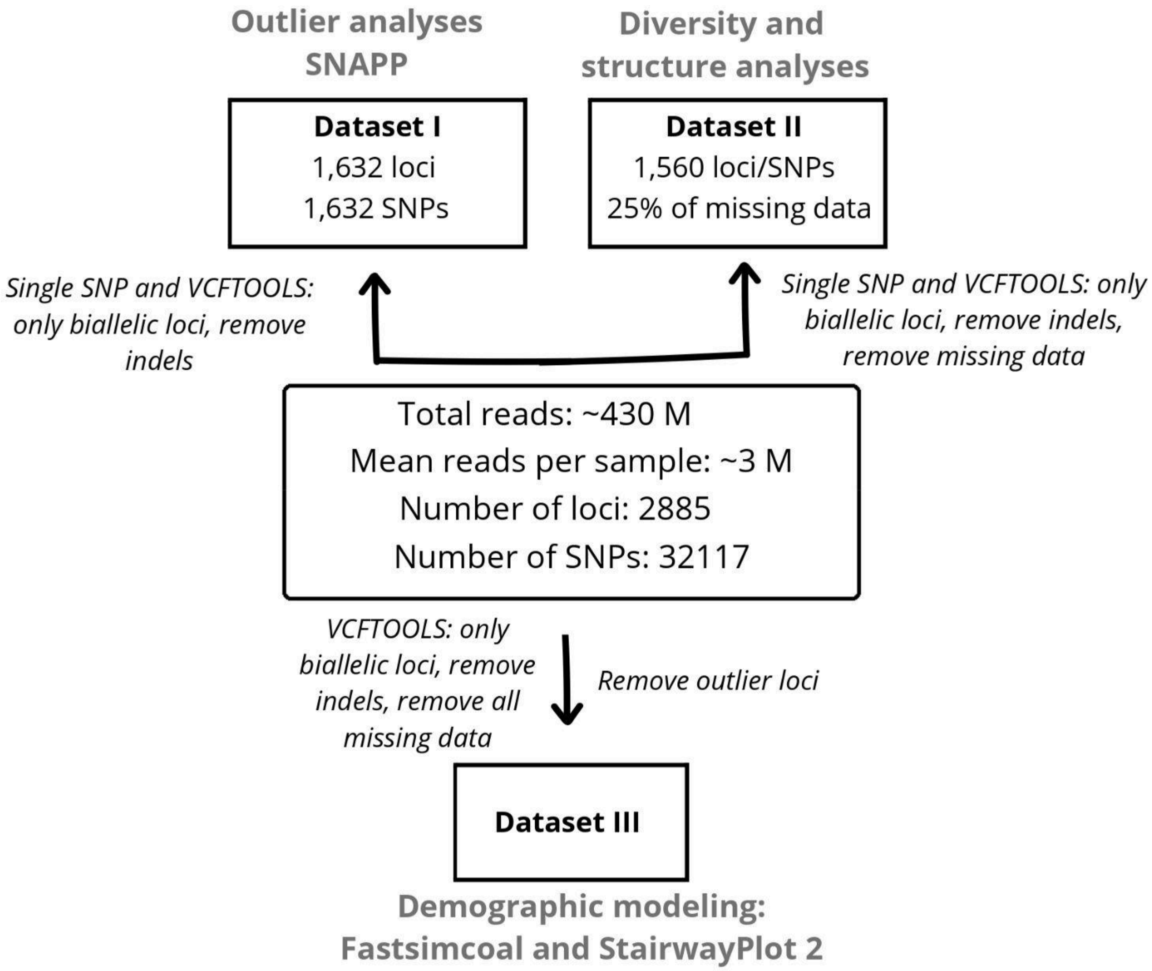

Figure 1S - Flowchart representing dataset construction after filtering as required for each analysis. For Fastsimcoal and StairwayPlot 2, additional filtering was required as described in the Material and Methods session.

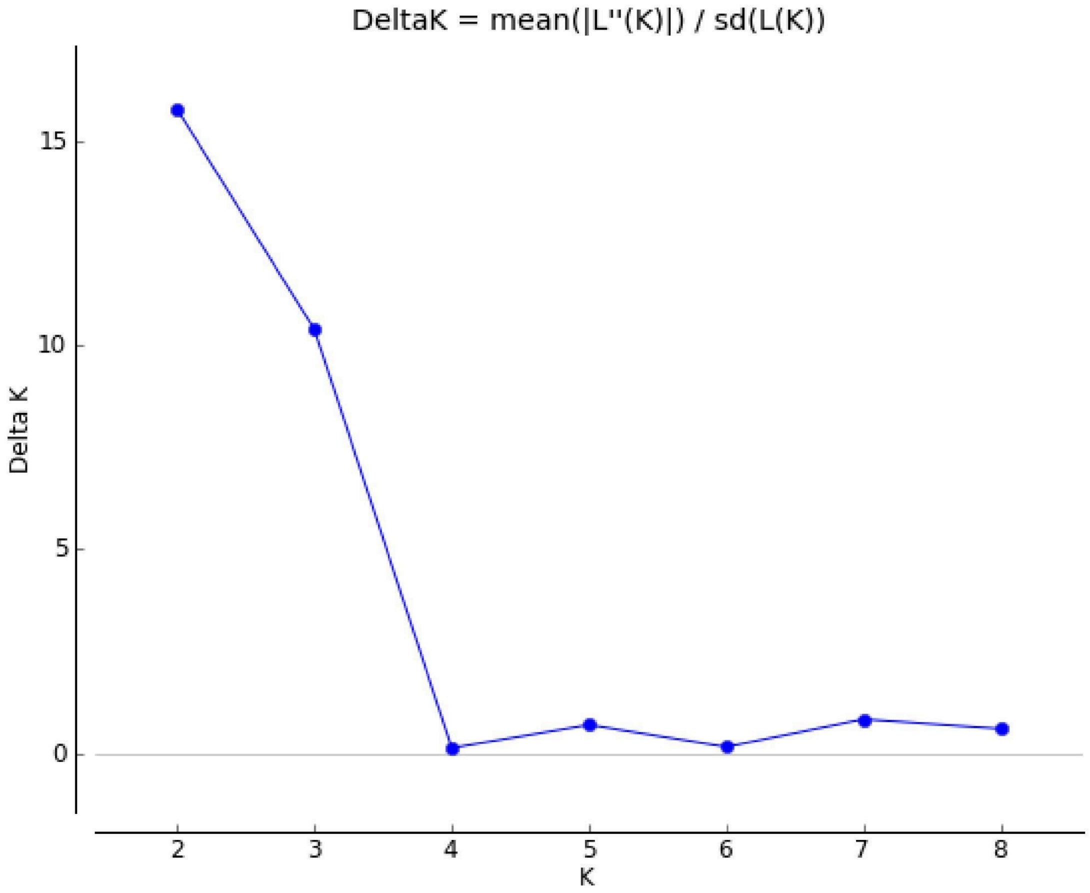

Figure 2S - Magnitude of ΔK (Delta K) as a function of K in STRUCTURE analysis. The modal value of these distributions indicates the true value of K (or the value corresponding to the highest level of population structure).

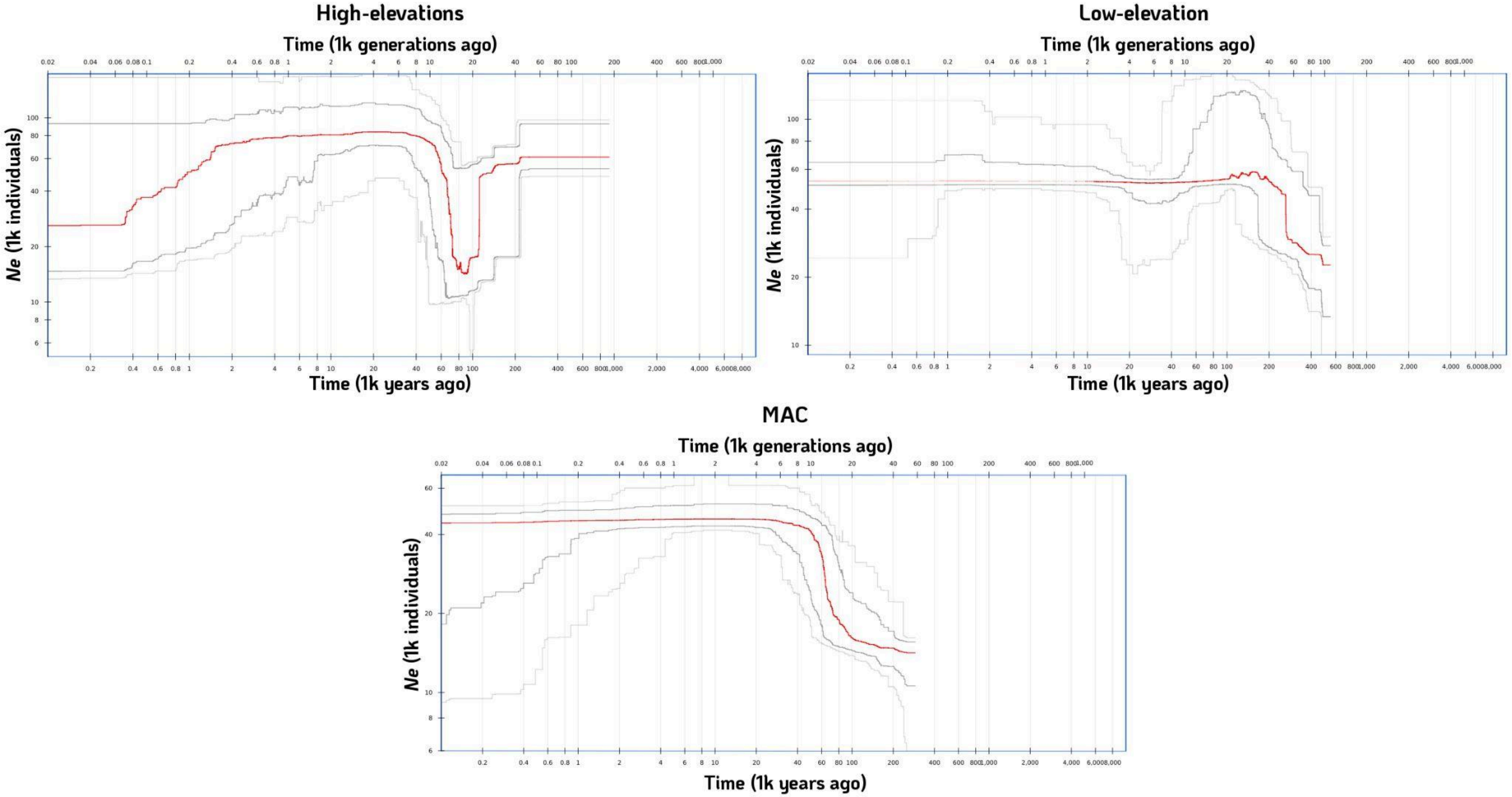

Figure 3S - Stairway Plot 2 results for populations from high elevations (MAR and PAP) (A); population MAC (B); and populations from low/mid elevations (UBA, RAN, COR, ITA, and PES. Red line: median of 200 inferences based on subsampling. Dark gray lines: 75% confidence interval of the inference. Light gray lines: 95% confidence interval. For population details see Table 1.

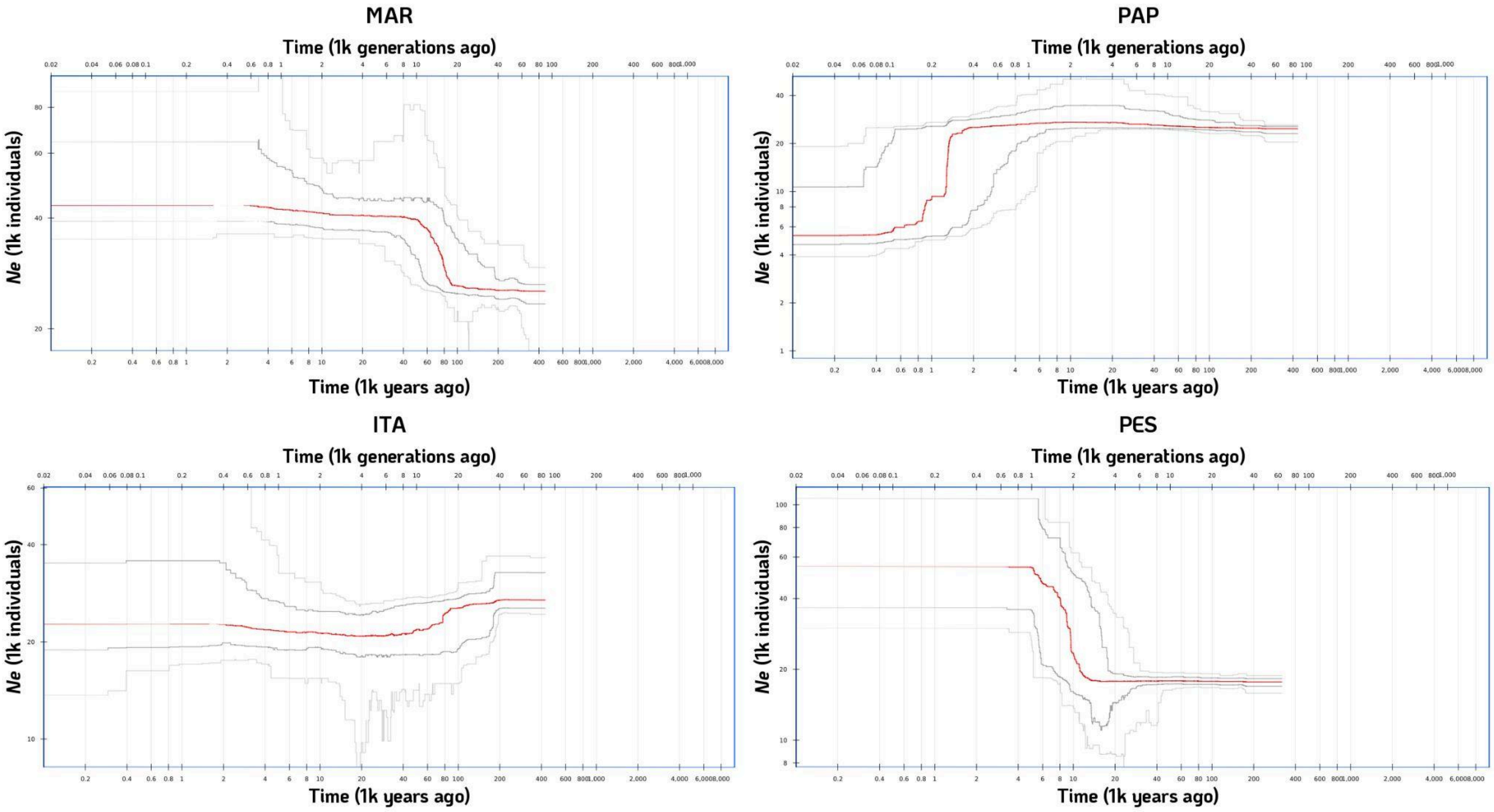

Figure 4S - Stairway Plot 2 results for populations ITA (P. N. de Itatiaia 1139 m a.s.l), PES (Pedra do Sino - P. N. da Serra dos órgãos 1496 m a.s.l), MAR (Pico dos Marins, 2037 m a.s.l), and PAP (Serra do Papagaio, 2140 m a.s.l). Red line: median of 200 inferences based on subsampling. Dark gray lines: 75% confidence interval of the inference. Light gray lines: 95% confidence interval. For population details see Table 1.

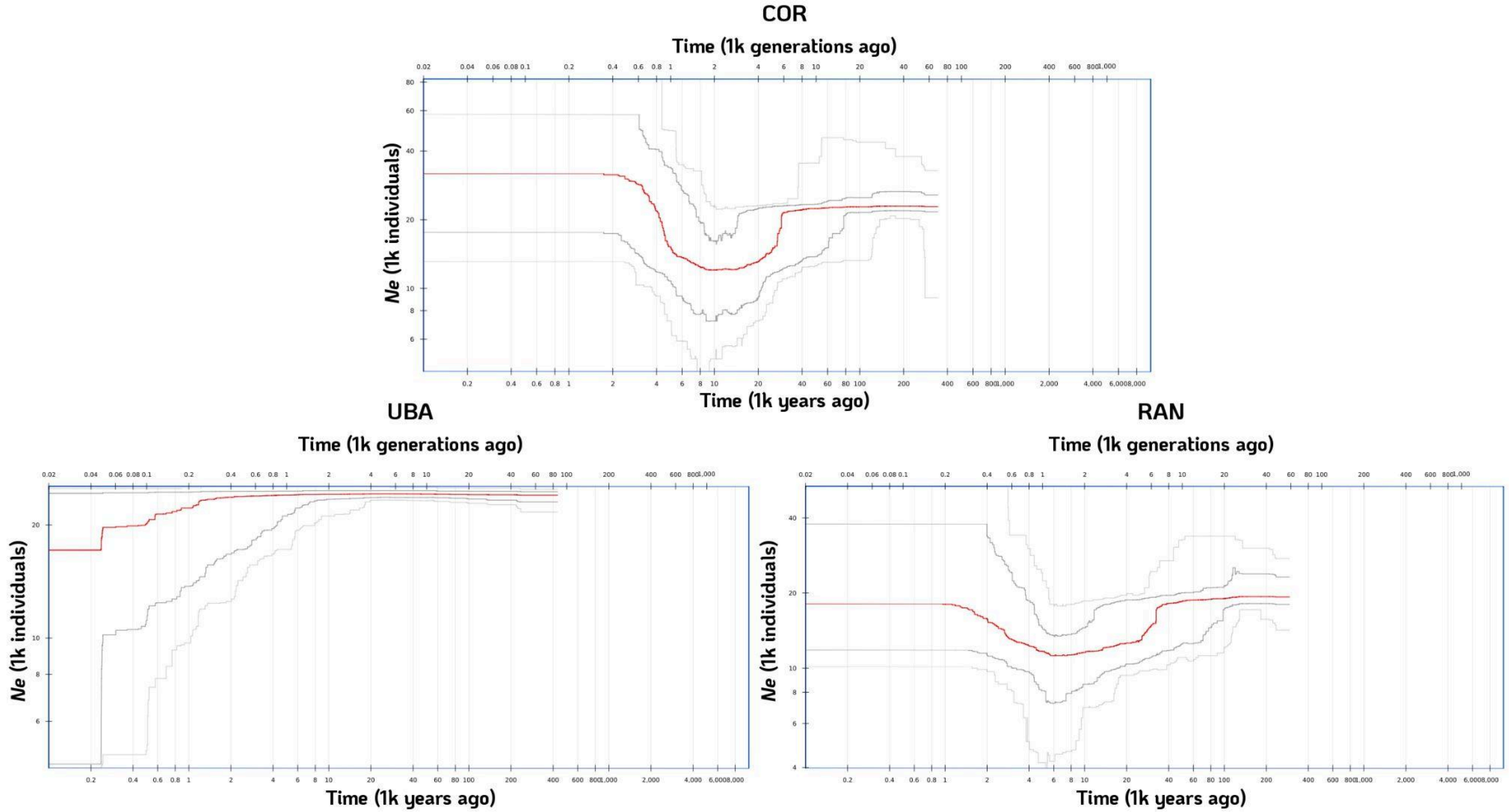

Figure 5S - Stairway Plot 2 results for populations UBA (Ubatuba, 20 m a.s.l), RAN (Morro do Rangel, 25 m a.s.l), and COR (Corcovado - P. N. da Serra da Tijuca, 425 m a.s.l). Red line: median of 200 inferences based on subsampling. Dark gray lines: 75% confidence interval of the inference. Light gray lines: 95% confidence interval. For population details see Table 1.

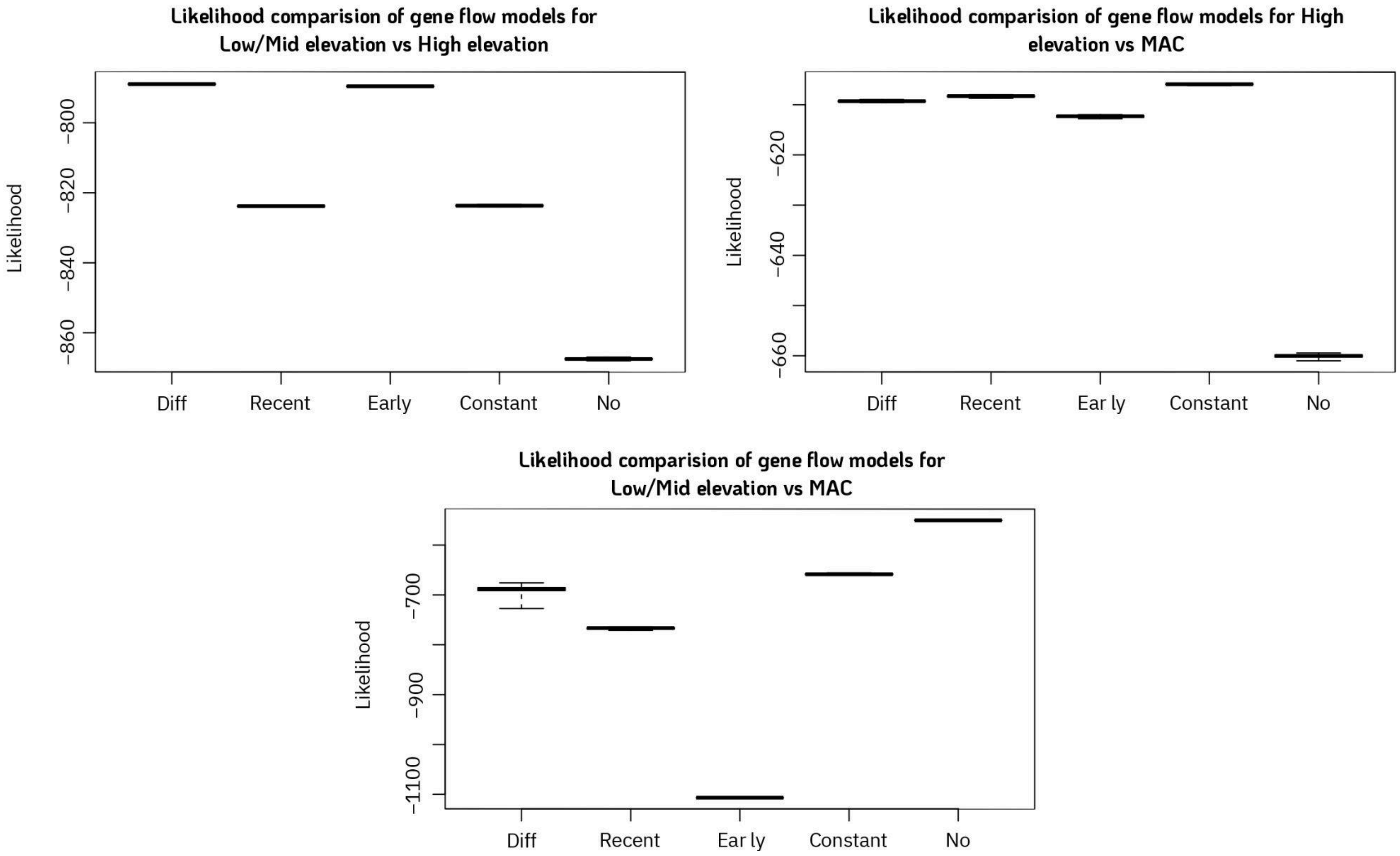

Figure 6S - Likelihood comparison for gene flow models tested for the 3 major clusters, in pairs: low/mid populations (UBA, RAN, COR, ITA, and PES), high elevation populations (MAR and PAP), and MAC, using FASTSIMCOAL v2.7. For population details see Table 1.

REFERÊNCIAS GERAIS

Abreu, E. F., Dalapicolla, J., Furini, P. R., De La Sancha, N. U., Jansa, S. A., & Percequillo, A. R. (2022). Habitat stability shaped the demographic history of the Atlantic Forest endemic rodent Euryoryzomys russatus (Cricetidae: Sigmodontinae) during the Pleistocene. *Biological Journal of the Linnean Society*, 136(4), 520-535.

Acha, S., Linan, A., MacDougal, J., & Edwards, C. (2021). The evolutionary history of vines in a neotropical biodiversity hotspot: Phylogenomics and biogeography of a large passion flower clade (Passiflora section Decaloba). *Molecular phylogenetics and evolution*, 164, 107260.

Aguiar‐Melo, C., Zanella, C. M., Goetze, M., Palma‐Silva, C., Hirsch, L. D., Neves, B., ... & Bered, F. (2019). Ecological niche modeling and a lack of phylogeographic structure in Vriesea incurvata suggest historically stable areas in the southern Atlantic Forest. *American Journal of Botany*, 106(7), 971-983.

Angilletta Jr, M. J., & Angilletta, M. J. (2009). *Thermal adaptation: a theoretical and empirical synthesis*.

Antonelli, A., Ariza, M., Albert, J., Andermann, T., Azevedo, J., Bacon, C., ... & Matos-Maraví, P. (2018). Conceptual and empirical advances in Neotropical biodiversity research. *PeerJ*, 6, e5644.

Antonelli, A. (2022). The rise and fall of Neotropical biodiversity. *Botanical Journal of the Linnean Society*, 199(1), 8-24.

Ataídes, D. S., Vasconcelos-Filho, S. C., Costa, A. C. (2018). Alterações na fisiologia de plantas de feijoeiro croulos como indicadores de vulnerabilidade ao déficit hídrico e ao aumento de temperatura do ambiente de crescimento. Dissertação (Mestrado em Biodiversidade e Conservação) – Instituto Federal Goiano, Campus Rio Verde.

Benzing, D. H., & Bennett, B. (2000). *Bromeliaceae: profile of an adaptive radiation*. Cambridge University Press.

Cacossi, T., Dantas-Queiroz, M. V., & Palma-Silva, C. (2019). Transferability of nuclear microsatellites markers to Vriesea oligantha (Bromeliaceae), an endemic species from Espinhaço Range, Brazil. *Brazilian Journal of Botany*, 42(4), 727-733.

Carnaval, A. C., Hickerson, M. J., Haddad, C. F., Rodrigues, M. T., & Moritz, C. (2009). Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science*, 323(5915), 785-789.

Carnaval, A. C., Waltari, E., Rodrigues, M. T., Rosauer, D., VanDerWal, J., Damasceno, R., ... & Moritz, C. (2014). Prediction of phylogeographic endemism in an environmentally complex biome. *Proceedings of the Royal Society B: Biological Sciences*, 281(1792), 20141461.

Caze, A. L. R., Mäder, G., Nunes, T. S., Queiroz, L. P., de Oliveira, G., Diniz-Filho, J. A. F., ... & Freitas, L. B. (2016). Could refuge theory and rivers acting as barriers explain the genetic variability distribution in the Atlantic Forest? *Molecular Phylogenetics and Evolution*, 101, 242-251.

Chaves, C. J., Cacossi, T. C., Matos, T. S., Bento, J. P., Gonçalves, L. N., Silva, S. F., ... & Palma‐Silva, C. (2024). Bromeliad populations perform distinct ecological strategies across a tropical elevation gradient. *Functional Ecology*, 38(4), 910-925.

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

Cornille, A., Salcedo, A., Kryvokhyzha, D., Glémin, S., Holm, K., Wright, S. I., & Lascoux, M. (2016). Genomic signature of successful colonization of Eurasia by the allopolyploid shepherd's purse (Capsella bursa‐pastoris). *Molecular ecology*, 25(2), 616-629.

Alexander, D. H., Shringarpure, S. S., Novembre, J., & Lange, K. (2015). *Admixture 1.3 software manual*. Los Angeles: UCLA Human Genetics Software Distribution.

Alonso-Amelot, M. E. (2008). High altitude plants, chemistry of acclimation and adaptation. *Studies in natural products chemistry*, 34, 883-982.

Andrews, S. (2014). FastQC: A quality control tool for high throughput sequence data. Retrieved from http://www.bioinformatics.babraha m.ac.uk/projects/fastqc/

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., ... & Johnson, E. A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PloS one*, 3(10), e3376.

Barbará, T., Martinelli, G., Palma-Silva, C., Fay, M. F., Mayo, S., & Lexer, C. (2009). Genetic relationships and variation in reproductive strategies in four closely related bromeliads adapted to neotropical 'inselbergs': Alcantarea glaziouana, A. regina, A. geniculata and A. imperialis (Bromeliaceae). *Annals of Botany*, 103(1), 65-77.

Batalha‐Filho, H., & Miyaki, C. Y. (2016). Late Pleistocene divergence and postglacial expansion in the Brazilian Atlantic Forest: multilocus phylogeography of Rhopias gularis (Aves: Passeriformes). *Journal of Zoological Systematics and Evolutionary Research*, 54(2), 137-147.

Brandrud, M. K., Baar, J., Lorenzo, M. T., Athanasiadis, A., Bateman, R. M., Chase, M. W., ... & Paun, O. (2020). Phylogenomic relationships of diploids and the origins of allotetraploids in Dactylorhiza (Orchidaceae). *Systematic Biology*, 69(1), 91-109.

Bouckaert, R., & Bryant, D. (2012). A rough guide to SNAPP. Website: https://github. com/BEAST2-Dev/SNAPP/releases/download/v1, 2.

Bouckaert, R. R., & Heled, J. (2014). DensiTree 2: Seeing trees through the forest. *BioRxiv*, 012401.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis toolset for population genomics. *Molecular Ecology*, 22:3124–3140.

Chen, H., Wang, D., Zhu, Y., Li, W., Chen, J., & Li, Y. (2022). Integrative transcriptomics and proteomics elucidate the regulatory mechanism of Hydrangea macrophylla flower-color changes induced by exogenous aluminum. *Agronomy*, 12(4), 969.

Damasceno, R. P., Carnaval, A. C., Sass, C., Recoder, R. S., Moritz, C., & Rodrigues, M. T. (2021). Geographic restriction, genetic divergence, and morphological disparity in the Brazilian Atlantic Forests: Insights from Leposoma lizards (Gymnophthalmidae, Squamata). *Molecular Phylogenetics and Evolution*, 154, 106993.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., ... & 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, 27(15), 2156-2158.

Dantas‐Queiroz, M. V., Cacossi, T. D. C., Leal, B. S. S., Chaves, C. J. N., Vasconcelos, T. N., Versieux, L. D. M., & Palma‐Silva, C. (2021). Underlying microevolutionary processes parallel macroevolutionary patterns in ancient neotropical mountains. *Journal of Biogeography*, 48(9), 2312-2327.

Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359-361.

Eaton, D. A. (2014). PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics*, 30(13), 1844-1849.

Excoffier, L., Marchi, N., Marques, D. A., Matthey-Doret, R., Gouy, A., & Sousa, V. C. (2021). fastsimcoal2: demographic inference under complex evolutionary scenarios. *Bioinformatics*, 37(24), 4882-4885.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14(8), 2611-2620.

Ferreira, D. M. C., Palma-Silva, C., Néri, J., Medeiros, M. C. M. P. D., Pinangé, D. S., Benko-Iseppon, A. M., & Louzada, R. B. (2021). Population genetic structure and species delimitation in the Cryptanthus zonatus complex (Bromeliaceae). *Botanical Journal of the Linnean Society*, 196(1), 123-140.

Flantua, S. G., O'Dea, A., Onstein, R. E., Giraldo, C., & Hooghiemstra, H. (2019). The flickering connectivity system of the north Andean páramos. *Journal of Biogeography*, 46(8), 1808-1825.

Feliciano, D. C., De Godoy, S. M., Da Silva, J. F. M., Góes, B. D., Ferraz, J. R., De Oliveira Santos, P., ... & De Fátima Ruas, C. (2022). Landscape genetics reveal low diversity and adaptive divergence in Portulaca hatschbachii (Portulacaceae): an endangered species endemic to rocky outcrops of the Atlantic Forest. *Botanical Journal of the Linnean Society*, 200(1), 116-141.

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics*, 180(2), 977-993.

Foll, M. (2012). BayeScan v2. 1 user manual. *Ecology*, 20(10), 1450-1462.

Futuyma, D. J. (2010). Evolutionary constraint and ecological consequences. *Evolution*, 64(7), 1865-1884.

Gavin, D. G., Fitzpatrick, M. C., Gugger, P. F., Heath, K. D., Rodríguez‐Sánchez, F., Dobrowski, S. Z., ... & Williams, J. W. (2014). Climate refugia: joint inference from fossil records, species distribution models and phylogeography. *New Phytologist*, 204(1), 37-54.

Gehara, M., Mazzochinni, G. G., & Burbrink, F. (2020). PipeMaster: inferring population divergence and demographic history with approximate Bayesian computation and supervised machine-learning in R. *BioRxiv*, 2020-12.

Geng, B., Wang, Q., Huang, R., Liu, Y., Guo, Z., & Lu, S. (2021). A novel LRR‐RLK (CTLK) confers cold tolerance through regulation on the C‐repeat‐binding factor pathway, antioxidants, and proline accumulation. *The Plant Journal*, 108(6), 1679-1689.

Givnish, T. J., Barfuss, M. H., Van Ee, B., Riina, R., Schulte, K., Horres, R., ... & Sytsma, K. J. (2014). Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. *Molecular Phylogenetics and Evolution*, 71, 55-78.

Godoy, F. M. D. R., Lenzi, M., Ferreira, B. H. D. S., Silva, L. V. D., Zanella, C. M., & Paggi, G. M. (2018). High genetic diversity and moderate genetic structure in the self-incompatible, clonal Bromelia hieronymi (Bromeliaceae). *Botanical Journal of the Linnean Society*, 187(4), 672-688.

Damasceno, R., Strangas, M. L., Carnaval, A. C., Rodrigues, M. T., & Moritz, C. (2014). Revisiting the vanishing refuge model of diversification. *Frontiers in Genetics*, 5, 353.

Davey, J. W., & Blaxter, M. L. (2010). RADSeq: next-generation population genetics. *Briefings in Functional Genomics*, 9(5-6), 416-423.

Ebel, E. R., DaCosta, J. M., Sorenson, M. D., Hill, R. I., Briscoe, A. D., Willmott, K. R., & Mullen, S. P. (2015). Rapid diversification associated with ecological specialization in Neotropical Adelpha butterflies. *Molecular Ecology*, 24(10), 2392-2405.

Harvey MG, Brumfield RT. Genomic variation in a widespread Neotropical bird (Xenops minutus) reveals divergence, population expansion, and gene flow. Mol Phylogenet Evol. 2015;83:305-16.

Hess JE, Campbell NR, Close DA, Docker MF, Narum SR. Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. Mol Ecol. 2013;22(11):2898-916.

Hmeljevski KV, Nazareno AG, Leandro Bueno M, dos Reis MS, Forzza RC. Do plant populations on distinct inselbergs talk to each other? A case study of genetic connectivity of a bromeliad species in an Ocbil landscape. Ecol Evol. 2017;7:4704-16.

Hughes M, Hollingsworth PM. Population genetic divergence corresponds with species-level biodiversity patterns in the large genus Begonia. Mol Ecol. 2008;17(11):2643-51.

Janzen DH. Why mountain passes are higher in the tropics. Am Nat. 1967;101(919):233-49.

Leal BSS, Chaves CJN, Graciano FM, Palma-Silva C. Evidence of local adaptation despite strong drift in a Neotropical patchily distributed bromeliad. Heredity. 2021;127(2):203-18.

Leite YL, Costa LP, Loss AC, Rocha RG, Batalha-Filho H, Bastos AC, et al. Neotropical forest expansion during the last glacial period challenges refuge hypothesis. Proc Natl Acad Sci U S A. 2016;113(4):1008-13.

Martin PR, McKay JK. Latitudinal variation in genetic divergence of populations and the potential for future speciation. Evolution. 2004;58(5):938-45.

Morellato LPC, Haddad CFB. Introduction: The Brazilian Atlantic Forest. Biotropica. 2000;32(4b):786-92.

Mota MR, Pinheiro F, Leal BSS, Sardelli CH, Wendt T, Palma-Silva C. From micro-to macroevolution: insights from a Neotropical bromeliad with high population genetic structure adapted to rock outcrops. Heredity. 2020;125(5):353-70.

Moritz C, Langham G, Kearney M, Krockenberger A, VanDerWal J, Williams SE. Integrating phylogeography and physiology reveals divergence of thermal traits between central and peripheral lineages of tropical rainforest lizards. Philos Trans R Soc Lond B Biol Sci. 2012;367(1596):1680-7.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature. 2000;403(6772):853-8.

Nadeau NJ, Martin SH, Kozak KM, Salazar C, Dasmahapatra KK, Davey JW, et al. Genome-wide patterns of divergence and gene flow across a butterfly radiation. Mol Ecol. 2013;22(3):814-26.

Narum SR, Buerkle CA, Davey JW, Miller MR, Hohenlohe PA. Genotyping-by-sequencing in ecological and conservation genomics. Mol Ecol. 2013;22(11):2841-7.

Oliveira-Filho AT, Fontes MAL. Patterns of floristic differentiation among Atlantic Forests in Southeastern Brazil and the influence of climate. Biotropica. 2000;32(4b):793-810.

Hmeljevski KV, dos Reis MS, Forzza RC. Patterns of gene flow in Encholirium horridum LB Sm., a monocarpic species of Bromeliaceae from Brazil. J Hered. 2015;106(1):93-101.

Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. Mol Biol Evol. 2006;23(2):254-67.

Janes JK, Miller JM, Dupuis JR, Malenfant RM, Gorrell JC, Cullingham CI, et al. The K=2 conundrum. Mol Ecol. 2017;26(14):3594-602.

Jombart T, Devillard S, Balloux F. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 2010;11(1):94.

Jung CG, Hwang SG, Park YC, Park HM, Kim DS, Park DH, et al. Molecular characterization of the cold-and heat-induced Arabidopsis PXL1 gene and its potential role in transduction pathways under temperature fluctuations. J Plant Physiol. 2015;176:138-46.

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour. 2015;15(5):1179-91.

Lande R, Barrowclough GF. Effective population size, genetic variation, and their use in population management. In: Soule ME, editor. Viable populations for conservation. Cambridge: Cambridge University Press; 1987. p. 87-124.

Liu X, Fu YX. Stairway Plot 2: demographic history inference with folded SNP frequency spectra. Genome Biol. 2020;21(1):280.

Liu L, Tumi L, Suni ML, Arakaki M, Wang ZF, Ge XJ. Draft genome of Puya raimondii (Bromeliaceae), the Queen of the Andes. Genomics. 2021;113(4):2537-46.

Lousada JM, Lovato MB, Borba EL. High genetic divergence and low genetic variability in disjunct populations of the endemic Vellozia compacta (Velloziaceae) occurring in two edaphic environments of Brazilian campos rupestres. Braz J Bot. 2013;36(1):45-53.

Luu K, Bazin E, Blum MG. pcadapt: an R package to perform genome scans for selection based on principal component analysis. Mol Ecol Resour. 2017;17(1):67-77.

Manhaes VDC, de Miranda FD, Clarindo WR, Carrijo TT. Genetic diversity and karyotype of Pitcairnia azouryi: an endangered species of Bromeliaceae endemic to Atlantic Forest inselbergs. Mol Biol Rep. 2020;47:179-89.

Maslin MA, Brierley CM. The role of orbital forcing in the Early Middle Pleistocene Transition. Quat Int. 2015;389:47-55.

Morellato, L. P. C., & Haddad, C. F. (2000). Introduction: The Brazilian Atlantic Forest. *Biotropica, 32*(4b), 786-792.

Moritz, C., Langham, G., Kearney, M., Krockenberger, A., VanDerWal, J., & Williams, S. (2012). Integrating phylogeography and physiology reveals divergence of thermal traits between central and peripheral lineages of tropical rainforest lizards. *Philosophical Transactions of the Royal Society B: Biological Sciences, 367*(1596), 1680-1687.

Mota, M. R., Pinheiro, F., Leal, B. S. D. S., Sardelli, C. H., Wendt, T., & Palma-Silva, C. (2020). From micro-to macroevolution: insights from a Neotropical bromeliad with high population genetic structure adapted to rock outcrops. *Heredity, 125*(5), 353-370.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature, 403*(6772), 853.

Nadeau, N. J., Martin, S. H., Kozak, K. M., Salazar, C., Dasmahapatra, K. K., Davey, J. W., ... & Jiggins, C. D. (2013). Genome-wide patterns of divergence and gene flow across a butterfly radiation. *Molecular Ecology, 22*(3), 814-826.

Nistelberger, H. M., Byrne, M., Coates, D., & Roberts, J. D. (2015). Genetic drift drives evolution in the bird-pollinated, terrestrial island endemic *Grevillea georgeana* (Proteaceae). *Botanical Journal of the Linnean Society, 178*(1), 155-168.

Ohsawa, T., & Ide, Y. (2008). Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Global Ecology and Biogeography, 17*(2), 152-163.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., & Suggests, M. A. S. S. (2007). The vegan package. *Community Ecology Package, 10*(631-637), 719.

Oliveira-Filho, A. T., & Fontes, M. A. L. (2000). Patterns of floristic differentiation among Atlantic Forests in Southeastern Brazil and the influence of climate. *Biotropica, 32*(4b), 793-810.

Palma-Silva, C., Lexer, C., Paggi, G. M., Barbará, T., Bered, F., & Bodanese-Zanettini, M. H. (2009). Range-wide patterns of nuclear and chloroplast DNA diversity in *Vriesea gigantea* (Bromeliaceae), a neotropical forest species. *Heredity*, 103(6), 503-512. https://doi.org/10.1038/hdy.2009.114.

Palma-Silva, C., Leal, B. S., Chaves, C. J., & Fay, M. F. (2016). Advances in and perspectives on evolution in Bromeliaceae. *Botanical Journal of the Linnean Society*, 181(3), 305-322. https://doi.org/10.1111/boj.12381.

Palma-Silva, C., Turchetto-Zolet, A. C., Fay, M. F., & Vasconcelos, T. (2022). Drivers of exceptional Neotropical biodiversity: an updated view. *Botanical Journal of the Linnean Society*, 199(1), 1-7. https://doi.org/10.1093/botlinnean/boab080.

Pessoa, E. M., Cordeiro, J. M., Felix, L. P., Lemes, P., Viruel, J., Alves, M., ... & Van Den Berg, C. (2022). The role of Quaternary glaciations in shaping biogeographic patterns in a recently evolved clade of South American epiphytic orchids. *Botanical Journal of the Linnean Society*, 199(1), 252-266. https://doi.org/10.1093/botlinnean/boac010.

Peres, E. A., Pinto-da-Rocha, R., Lohmann, L. G., Michelangeli, F. A., Miyaki, C. Y., & Carnaval, A. C. (2020). Patterns of species and lineage diversity in the Atlantic rainforest of Brazil. *Neotropical Diversification: Patterns and Processes*, 415-447. https://doi.org/10.1007/978-3-030-29256-5_14.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLOS ONE*, 7(5), e37135. https://doi.org/10.1371/journal.pone.0037135.

Pinto, B. J., Colli, G. R., Higham, T. E., Scantlebury, D. P., Rocha, R. C., & Gamble, T. (2019). Population genetic structure and species delimitation of a widespread, Neotropical dwarf gecko. *Molecular Phylogenetics and Evolution*, 133, 54-66. https://doi.org/10.1016/j.ympev.2019.01.020.

Polato, N. R., Gill, B. A., Shah, A. A., Gray, M. M., Casner, K. L., Barthelet, A., ... & Kondratieff, B. C. (2018). Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains. *Proceedings of the National Academy of Sciences*, 115(49), 12471-12476. https://doi.org/10.1073/pnas.1809326115.

Porembski, S. (2007). Tropical inselbergs: habitat types, adaptive strategies and diversity patterns. *Brazilian Journal of Botany*, 30(4), 579-586. https://doi.org/10.1590/S0100-84042007000400002.

Prance, G. T. (1982). A review of the phytogeographic evidences for Pleistocene climate changes in the Neotropics. *Annals of the Missouri Botanical Garden*, 69(3), 594-624. https://doi.org/10.2307/2399085.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945-959.

Rahbek, C., Borregaard, M. K., Antonelli, A., Colwell, R. K., ... & Fjeldså, J. (2019). Building mountain biodiversity: Geological and evolutionary processes. *Science, 365*(6458), 1114-1119.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology, 67*(5), 901-904.

Reis, T. S., Ciampi-Guillardi, M., Bajay, M. M., de Souza, A. P., & Dos Santos, F. A. M. (2015). Elevation as a barrier: Genetic structure for an Atlantic rain forest tree (*Bathysa australis*) in the Serra do Mar mountain range, SE Brazil. *Ecology and Evolution, 5*(9), 1919-1931.

Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J., & Hirota, M. M. (2009). The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation, 142*(6), 1141-1153.

Ribeiro, M. C., Martensen, A. C., Metzger, J. P., Tabarelli, M., Scarano, F., & Fortin, M. J. (2011). The Brazilian Atlantic Forest: A shrinking biodiversity hotspot. In *Biodiversity Hotspots* (pp. 405-434). Springer, Berlin, Heidelberg.

Roças, G., Klein, D. E., & De Mattos, E. A. (2004). Artificial hybridization between *Pitcairnia flammea* and *Pitcairnia corcovadensis* (Bromeliaceae): Analysis of the performance of parents and hybrids. *Plant Species Biology, 19*(1), 47-53.

Rödin-Mörch, P., Luquet, E., Meyer-Lucht, Y., Richter-Boix, A., Höglund, J., & Laurila, A. (2019). Latitudinal divergence in a widespread amphibian: Contrasting patterns of neutral and adaptive genomic variation. *Molecular Ecology, 28*(12), 2996-3011.

Rougemont, Q., & Bernatchez, L. (2018). The demographic history of Atlantic salmon (*Salmo salar*) across its distribution range reconstructed from approximate Bayesian computations. *Evolution, 72*(6), 1261-1277.

Ruas, R. D. B., Paggi, G. M., Aguiar-Melo, C., Hirsch, L. D., & Bered, F. (2020). Strong genetic structure in *Dyckia excelsa* (Bromeliaceae), an endangered species found on ironstone outcrops in Pantanal, Brazil. *Botanical Journal of the Linnean Society, 192*(4), 691-705.

Sanín, M. J., Zapata, P., Pintaud, J. C., Galeano, G., Bohórquez, A., Tohme, J., & Hansen, M. M. (2017). Up and down the blind alley: Population divergence with scant gene flow in an endangered tropical lineage of Andean palms (*Ceroxylon quindiuense* Clade: Ceroxyloideae). *Journal of Heredity, 108*(3), 288-298.

Silva, G. A., Khan, G., Ribeiro-Silva, S., Aona, L. Y., Machado, M. C., Bonatelli, I. A., & Moraes, E. M. (2020). Extreme genetic structure in a relict cactus genus from campo rupestre landscapes: Implications for conservation. *Biodiversity and Conservation, 29*, 1263-1281.

Smith, L. B., & Downs, R. J. (1974). *Bromeliaceae (Pitcairnioideae)*. Flora Neotropica Monograph.

Sovic, M., Fries, A., Martin, S. A., & Lisle Gibbs, H. (2019). Genetic signatures of small effective population sizes and demographic declines in an endangered rattlesnake, *Sistrurus catenatus*. *Evolutionary Applications, 12*(4), 664-678.

Stone, B. W., Ward, A., Farenwald, M., Lutz, A. W., & Wolfe, A. D. (2019). Genetic diversity and population structure in Cary's Beardtongue *Penstemon caryi* (Plantaginaceae), a rare plant endemic to the eastern Rocky Mountains of Wyoming and Montana. *Conservation Genetics, 20*, 1149-1161.

Sun, Y., Qiao, Z., Muchero, W., & Chen, J. G. (2020). Lectin receptor-like kinases: The sensor and mediator at the plant cell surface. *Frontiers in Plant Science, 11*, 596301.

Tavares, M. M., Ferro, M., Leal, B. S. S., & Palma-Silva, C. (2022). Speciation with gene flow between two Neotropical sympatric species (*Pitcairnia spp.*: Bromeliaceae). *Ecology and Evolution, 12*(5), e8834.

Teixeira, T. M., & Nazareno, A. G. (2021). One step away from extinction: A population genomic analysis of a narrow endemic, tropical plant species. *Frontiers in Plant Science, 12*, 730258.

Thome, M. T. C., Lyra, M. L., Lemes, P., Teixeira, L. S., Carnaval, A. C., Haddad, C. F., & Canedo, C. (2020). Outstanding diversity and microendemism in a clade of rare Atlantic Forest montane frogs. *Molecular Phylogenetics and Evolution, 149*, 106813.

Thom, G., Smith, B. T., Gehara, M., Montesanti, J., Lima-Ribeiro, M. S., Piacentini, V. Q., ... & do Amaral, F. R. (2020). Climatic dynamics and topography control genetic variation in Atlantic Forest montane birds. *Molecular Phylogenetics and Evolution, 148*, 106812.

Vaid, N., Macovei, A., & Tuteja, N. (2013). Knights in action: Lectin receptor-like kinases in plant development and stress responses. *Molecular Plant, 6*(5), 1405-1418.

Versieux, L. M., Barbará, T., Wanderley, M. D. G. L., Calvente, A., Fay, M. F., & Lexer, C. (2012). Molecular phylogenetics of the Brazilian giant bromeliads (*Alcantarea*, Bromeliaceae): Implications for morphological evolution and biogeography. *Molecular Phylogenetics and Evolution, 64*(1), 177-189.

Vicente-Silva, L., De Russo Godoy, F. M., Faggioni, G. P., Lorenz, A. P., & Paggi, G. M. (2022). Patchily distributed but not necessarily isolated populations of *Deuterocohnia meziana*: A threatened bromeliad from rock outcrops. *Botanical Journal of the Linnean Society, 199*(1), 312-330.

Vieira, L. D., Silva-Junior, O. B., Novaes, E., & Collevatti, R. G. (2022). Comparative population genomics in *Tabebuia* alliance shows evidence of adaptation in Neotropical tree species. *Heredity*, 1-13.

Wang, X., Chang, X., Jing, Y., Zhao, J., Fang, Q., Sun, M., ... & Li, Y. (2020). Identification and functional prediction of soybean CircRNAs involved in low-temperature responses. *Journal of Plant Physiology, 250*, 153188.

Wendt, T., Canela, M. B. F., Klein, D. E., & Rios, R. I. (2002). Selfing facilitates reproductive isolation among three sympatric species of *Pitcairnia* (Bromeliaceae). *Plant Systematics and Evolution, 232*, 201-212.

Yang, L., Wu, K., Gao, P., Liu, X., Li, G., & Wu, Z. (2014). GsLRPK, a novel cold-activated leucine-rich repeat receptor-like protein kinase from *Glycine soja*, is a positive regulator to cold stress tolerance. *Plant Science, 215*, 19-28.

Zhang, H., Zhu, H., Pan, Y., Yu, Y., Luan, S., & Li, L. (2014). A DTX/MATE-type transporter facilitates abscisic acid efflux and modulates

ANEXO I

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº A0A3189

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A0A3189**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Pitcairnia flammea

Título da Atividade:	**RAD-seq Pitcairnia**

Equipe

Clarisse Palma da Silva	**UNICAMP**
Tami da Costa Cacossi	**Unicamp**

Envios de Amostra

Espécie:	**Pitcairnia flammea**
Tipo do Patrimônio Genético:	**-**
Forma do Patrimônio Genético:	**Amostra líquida em tubo de microcentrífuga (eppendorf)**
Instituição Destinatária:	**Floragenex INC**
Sede da Instituição Destinatária:	**9590 SW Gemini Drive Beaverton, OR 97008-7166 USA, Beaverton, Oregon, 97008-7166, Estad**

Data do Cadastro:	**12/07/2021 17:31:07**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **13:27** de **03/05/2023**.

SISTEMA NACIONAL DE GESTÃO
DO PATRIMÔNIO GENÉTICO
E DO CONHECIMENTO TRADICIONAL
ASSOCIADO - SISGEN

ANEXO II

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada "PADRÕES GENÔMICOS DE *Pitcairnia flammea* (BROMELIACEAE) AO LONGO DE UM GRADIENTE ALTITUDINAL DA FLORESTA ATLÂNTICA", não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas,

Assinatura : Tami Cacossi
Nome do(a) autor(a): Tami da Costa Cacossi
RG n.° 50407453-2

Clarisse Palma

Assinatura : __________________________________
Nome do(a) orientador(a):
RG n.° 7075205901