UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E BIODIVERSIDADE

FILOGEOGRAFIA DO COMPLEXO *Pitcairnia flammea* (BROMELIACEAE)

MATEUS RIBEIRO MOTA

Rio Claro - SP
Outubro de 2019

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E BIODIVERSIDADE

Filogeografia do complexo *Pitcairnia flammea* (Bromeliaceae)

MATEUS RIBEIRO MOTA

Orientadora:

Dra. Clarisse Palma da Silva

Dissertação apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Ecologia e Biodiversidade.

Rio Claro – SP
Outubro de 2019

M917f

Mota, Mateus Ribeiro
Filogeografia do complexo Pitcairnia flammea (Bromeliaceae) / Mateus Ribeiro Mota. -- Rio Claro, 2019
48 p. : il., tabs., mapas

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, Rio Claro
Orientadora: Clarisse Palma da Silva

1. Filogeografia. 2. Bromeliaceae. 3. Ecologia molecular. 4. Afloramentos rochosos. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Biociências, Rio Claro. Dados fornecidos pelo autor(a).

unesp

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Rio Claro

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: Filogeografia do complexo Pitcairnia flammea (Bromeliaceae)

AUTOR: MATEUS RIBEIRO MOTA
ORIENTADORA: CLARISSE PALMA DA SILVA

Aprovado como parte das exigências para obtenção do Título de Mestre em ECOLOGIA E BIODIVERSIDADE, área: Biodiversidade pela Comissão Examinadora:

Clarisse Palma
Profa. Dra. CLARISSE PALMA DA SILVA
Instituto de Biologia - Departamento de Ecologia / UNICAMP - Universidade Estadual de Campinas / SP

Prof. Dr. FÁBIO SARUBBI RAPOSO DO AMARAL
Departamento de Ciências Exatas e da Terra / Universidade Federal de São Paulo - Campus Diadema / SP

Profa. Dra. KARINA LUCAS DA SILVA BRANDÃO
Centro de Ciências Naturais e Humanas / Universidade Federal do ABC - Santo André/SP

Rio Claro, 21 de agosto de 2019

AGRADECIMENTOS

Agradeço primeiramente as agências financiadoras, que foram de extrema importância e que contribuíram diretamente para o desenvolvimento e concretização desse trabalho. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001. Agradeço também à Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), processo nº 2018/07596-0. Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), processo 475937/2013-9. Por fim, ao Internacional Association for Plant Taxonomy (IAPT).

Agradeço principalmente aos meus pais Vladimir José Mota e Silvana Ribeiro Mota, por possibilitarem todas as condições emocionais e financeiras para o desenvolvimento da minha vida pessoal e acadêmica; sempre incentivando o cumprimento dos meus objetivos. Sem a confiança que eles depositaram em mim eu não teria conseguido concluir essa importante fase da vida

Agradeço à minha Orientadora Drª Clarisse Palma da Silva por todo o apoio técnico concedido, pela excelente orientação, e pela participação ativa no desenvolvimento do trabalho. Obrigado também pelo apoio emocional e principalmente, pela confiança e paciência depositados em minha pessoa, mesmo nos momentos de dúvidas e dificuldades.

Agradeço também ao pessoal do Laboratório de Ecologia Molecular – Unesp, RC, os quais foram de grande ajuda para o desenvolvimento desse trabalho. Especialmente à Barbara Simões dos Santos Leal, que me ajudou ativamente em todos os momentos.

Agradeço por fim ao programa de Pós-Graduação em Ecologia e Biodiversidade, ao Departamento de Ecologia – Unesp, RC, incluindo seus funcionários, e a Seção Técnica de Pós-graduação da Universidade Estadual Paulista, através da Supervisora Ivana Terezinha Brandt, pelo auxílio técnico e burocrático necessários ao desenvolvimento deste trabalho.

DECLARAÇÃO

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 (processo nº 2018/07596-0). Declaro que as opiniões, hipóteses e conclusões ou recomendações expressas neste material são de responsabilidade do(s) autor(es) e não necessariamente refletem a visão da CAPES e FAPESP.

Resumo

A filogeografia surgiu como uma ponte entre várias disciplinas evolutivas, como a genética populacional, filogenia e biogeografia, estabelecendo ligações entre os estudos micro e macro evolutivos. A filogeografia tem sido cada vez mais utilizada para estudar a evolução de regiões com altos índices de diversidade, como a região Neotrópical. Os Neotrópicos apresentam uma grande variedade de biomas, incluindo a Floresta Atlântica, a segunda maior floresta tropical da América do Sul, contendo mais de 60% de todas as espécies terrestres do planeta. Um número crescente de estudos filogeográficos com espécies da Floresta Atlântica nos ajudado a entender os processos evolutivos que gradualmente formaram essa grande biodiversidade. Realizamos análises filogeográficas, avaliando padrões de estrutura e diversidade genética, tempo de divergência das linhagens e a demografia histórica do complexo de espécies *Pitcairnia flammea* (Bromeliaceae), grupo adaptado a inselbergs neotropicais naturalmente fragmentados, baseados em conjuntos de dados de microssatélites nucleares e sequências plastidiais. Nossos resultados mostraram baixa a moderada diversidade genética nuclear dentro de populações de *P. flammea*, e alta estrutura genética populacional, com poucos haplótipos de DNA plastidial compartilhados entre populações, indicando fluxo gênico limitado e baixa conectividade entre afloramentos rochosos. Não encontramos nenhuma estrutura filogeográfica clara, além de duas linhagens evolutivas que divergiram aproximadamente 2 Mya., sugerindo um papel importante das mudanças climáticas no início do Pleistoceno na diversificação do complexo de espécies de *P. flammea*. Além disso, não encontramos congruência direta entre divergência genética populacional e delimitação taxonômica. Este estudo vem apoiar o fato de que afloramentos rochosos são centros de diversidade de espécies e endemismo, por serem naturalmente isolados, contribuindo para a grande diversidade genética e morfológica observada atualmente nestas regiões. Em conjunto com estudos de genética de populações e filogeografia de outros organismos, as informações aqui geradas ajudam a esclarecer os processos complexos responsáveis pela origem e manutenção da biodiversidade dos Neotrópicos, especificamente da Floresta Atlântica.

Palavras-chaves: Filogeografia. Bromélias. Ecologia molecular. Especiação. Floresta Atlântica.

Abstract

Phylogeography has emerged as a bridge between several evolutionary disciplines, such as population genetics, phylogeny and biogeography, establishing the link between micro and macroevolutionary studies. Phylogeography has been increasingly used to study the evolution of regions with high diversity indexes, such as the Neotropic region. The Neotropics presents a wide variety of biomes, including the Atlantic Rainforest, the second largest tropical rainforest in South America containing more than 60% of all terrestrial species on the planet. An increasing number of phylogeographic studies in Atlantic Rainforest species have helped us to understand the evolutionary process that gradually formed such great biodiversity. We performed phylogeographic analyses, assessing genetic structure patterns, timing of lineage divergence and historical demography of *Pitcairnia flammea* species complex (Bromeliaceae), which are adapted to naturally fragmented Neotropical inselbergs, based on nuclear microsatellites and plastid sequence data sets. Our results showed low to moderate nuclear genetic diversity within *P. flammea* populations, and high population genetic structure, with few plastid DNA haplotype shared among populations, indicating limited gene flow and low connectivity among rock outcrops. We found no clear phylogeographic structure, besides two evolutionary lineage which diverged approximately 2 Mya., suggesting an important role of early Pleistocene climatic changes in the diversification of *P. flammea* species complex. Additionally, we find no direct congruence between population genetic divergence and taxonomic delimitation. This study supports the fact that rocky outcrops are centers of species diversity and endemism, as they are naturally isolated, contributing to the great genetic and morphological diversity currently observed in these regions. In conjunction with studies of population genetics and phylogeography of other organisms, the information generated here helps to clarify the complex processes responsible for the origin and maintenance of Neotropic biodiversity, specifically the Atlantic Forest.

Keywords: Phylogeography. Bromeliads. Molecular ecology. Speciation. Atlantic Rainforest.

SUMÁRIO

INTRODUÇÃO GERAL

O continente Sul Americano é composto por diversas regiões biogeográficas que contém os maiores índices de biodiversidade do planeta, e nele estão contidos cinco dos *hotspots* de biodiversidade mundial (MYERS et al., 2000). Dentro do continente, a região Neotropical é definida como a área que se estende entre o México Central e a Argentina, apresentando diversos biomas com histórias evolutivas distintas e conexões complexas (HUGHES; PENNINGTON; ANTONELLI, 2013). Essa região abriga uma biodiversidade extraordinária, tanto em termos taxonômicos, como em termos filogenéticos e funcionais (ANTONELLI et al., 2018a). Uma vasta gama de processos evolutivos foram responsáveis pela biodiversidade atual do continente, onde diferentes mecanismos, bióticos e abióticos, têm sido propostos para explicar a evolução da biodiversidade nos Neotrópicos (ANTONELLI et al., 2018b; ANTONELLI; SANMARTÍN, 2011; HUGHES; PENNINGTON; ANTONELLI, 2013; RULL, 2011, 2018; TURCHETTO-ZOLET et al., 2013). Eventos climáticos e orogênicos, como soerguimento de montanhas no Neógeno, e as mudanças climáticas ocorridas durante o Quaternário têm sido frequentemente associadas aos processos de formação, diversificação e manutenção da biota neotropical (ANTONELLI; SANMARTÍN, 2011; LEAL; PALMA DA SILVA; PINHEIRO, 2016; TURCHETTO-ZOLET et al., 2013). Na América do Sul, a distribuição geográfica de espécies associadas a ambientes florestais foi reduzida principalmente durante os ciclos glaciais; por outro lado, espécies associadas a domínios de vegetação aberta tiveram respostas variáveis as flutuações climáticas (TURCHETTO-ZOLET et al., 2013). Floresta Atlântica foi um bioma que sofreu forte influência de oscilações climáticas originadas pelos períodos glaciais e interglaciais, que promoveram eventos de expansão e retração com subsequente fragmentação florestal. Diversos estudos filogeográficos têm procurado revelar a complexa história evolutiva e biogeográfica da Floresta Atlântica brasileira, responsáveis pelos altos níveis de biodiversidade encontrados neste bioma (CARNAVAL et al., 2014; LEAL; PALMA DA SILVA; PINHEIRO, 2016; PAZ et al., 2019; RAPOSO DO AMARAL et al., 2018; TURCHETTO-ZOLET et al., 2013, 2016).

A Floresta Atlântica é a segunda maior floresta úmida tropical da América do Sul, e consiste em um complexo de vegetações que abrigam vários tipos de comunidades vegetais e está entre os cinco mais importantes *hotspots* da biodiversidade do mundo (MYERS et al., 2000; OLIVEIRA-FILHO; FONTES, 2006). Além dos ambientes florestais, este bioma também apresenta mosaicos de ambientes abertos (ex. restingas, afloramentos rochosos, campos de altitude) (SCARANO, 2009). Afloramentos rochosos, também chamados de inselbergs, são formações geológicas isolados ou em grupos, separados por poucos ou muitos quilômetros por uma matriz florestal, e são considerados "ilhas terrestres", por conta do isolamento espacial e ecológico que representa uma barreira contra a dispersão e migração, determinando assim que as populações sejam naturalmente fragmentadas (POREMBSKI, 2007). A natureza isolada destas ilhas terrestres é uma das características que as tornam modelos interessantes para estudos da conectividade genética de populações. Estas características edafoclimáticas promovem a ocorrência de vegetação especializada, compreendendo um grande número de espécies endêmicas (POREMBSKI, 2007).

Quase exclusivamente neotropicais, a família Bromeliaceae é um grupo amplamente diversificado, que representa um excelente exemplo de radiação adaptativa em plantas vasculares, apresentando uma enorme variabilidade anatômica, morfológica e ecológica (mais de 3300 espécies distribuídas em 58 gêneros), sendo distribuídas em uma ampla faixa latitudinal, elevacional e climática (GIVNISH et al., 2011, 2014; LUTHER, 2012; PALMA-SILVA et al., 2016). O Brasil é o maior centro de diversidade de bromélias, onde podemos encontrar cerca de 50% das espécies conhecidas. Regiões com alta riqueza de espécies e altos níveis de endemismo oferecem excelentes oportunidades para a investigação de hipóteses sobre evolução do isolamento reprodutivo, e as bromélias vêm sendo estudadas como modelos para a compreensão dos processos de microevolução e especiação na região Neotropical (AGUIAR-MELO et al., 2019; BARBARÁ et al., 2009; GOETZE et al., 2016; LEAL et al., 2018; LEXER et al., 2016; MOTA et al., 2019; PALMA-SILVA et al., 2009, 2011, 2015; ZANELLA et al., 2016).

Nesta dissertação, utilizamos uma espécie rupícola do gênero *Pitcairnia* (Bromeliaceae) como modelo para estudar os padrões e processos de diversificação na Floresta Atlântica. *Pitcairnia* é o gênero com a maior amplitude de distribuição e é também o maior e mais diversificado gênero da subfamília Pitcairnioideae, com cerca de 450 espécies atualmente aceitas (SARAIVA; MANTOVANI; CAMPOSTRINI FORZZA, 2015). *Pitcairnia flammea* é uma espécie exclusivamente saxícola e endêmica de afloramentos rochosos graníticos e gnáissicos no sul e sudeste do Brasil, assim ocorrendo de forma naturalmente fragmentada ao longo da Floresta Atlântica (MARTINELLI et al., 2018). Esta espécie possui uma extensa variabilidade morfológica entre populações, principalmente nas estruturas vegetativas da planta, característica utilizada por diferentes autores para a descrição de várias sub-espécies: *P. flammea* var. *flammea* Lindl.; *P. flammea* var. *glabrior* L.B. Smith; *P. flammea* var. *roezlii* L.B. Smith; *P. flammea* var. *floccosa* Lindl; *P. flammea* var. *macropoda* L.B. Smith & Reitz; *P.*

flammea var. *pallida* L.B. Smith (MARTINELLI et al., 2018; SMITH; DOWNS, 1979). Saraiva et al. (2015) publicaram uma hipótese filogenética, baseada em caracteres macromorfológicos e micromorfológicos, onde os autores reconheceram o grupo *Pitcairnia flammea,* dentro do gênero *Pitcairnia*, composto pelas espécies *P. beycalema*, *P. corcovadensis*, *P. flammea*, *P. norteflumenesis*, *P abyssicola*, *P. carinata*, *P. decidua* e *P. encholirioides*. Mais recentemente, Schubert (2017), baseado em uma filogenia molecular, reconheceu o complexo *P. flammea* como um grupo monofilético porém com ramos não resolvidos, destacando a dificuldade em resolver a delimitação de espécies dentro do complexo.

Objetivos

Este trabalho tem como objetivo estudar a diversidade de linhagens e os padrões filogeográficos do grupo *Pitcairnia flammea*, um complexo de espécies adaptadas a afloramentos rochosos da Floresta Atlântica Brasileira, e para isso, pretendemos responder às seguintes questões:

(1) O que os padrões filogeográficos de *P. flammea* nos dizem sobre os mecanismos evolutivos envolvidos na especiação e diversificação de táxons adaptados a afloramentos rochosos? Esses padrões são consistentes com os relatados anteriormente para outras plantas e animais da Floresta Atlântica?

(2) Qual é a influência das oscilações climáticas na diversificação de linhagens de *P. flammea*?

(3) Quantas populações geneticamente diferenciadas existem ao longo da distribuição das espécies, e estas podem ser consideradas diferentes linhagens evolutivas? As populações geneticamente diferenciadas correspondem às variedades taxonômicas e espécies propostas para o complexo *P. flammea*? O que o fluxo genético interespecífico nos diz sobre a diversificação em populações simpátricas?

Para responder essas questões, reconstruímos a história evolutiva e demográfica do complexo *P. flammea* usando abordagens filogeográficas, através de métodos de sequenciamento de Sanger com marcadores plastidiais, e genotipagem de marcadores microssatélites nucleares.

REFERÊNCIAS

AGUIAR-MELO, C. et al. 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**, v. 106, n. 7, p. 971-983, 2019.

ANTONELLI, A. et al. Amazonia is the primary source of Neotropical biodiversity. **Proceedings of the National Academy of Sciences**, v. 115, n. 23, p. 6034–6039, 2018a.

ANTONELLI, A. et al. Conceptual and empirical advances in Neotropical biodiversity research. **PeerJ**, 2018b.

ANTONELLI, A.; SANMARTÍN, I. Why are there so many plant species in the Neotropics? **Taxon**, , v. 60, n. 2, p. 403-414, 2011.

AVISE, J. C. et al. Intraspecific Phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. **Annual Review of Ecology and Systematics**, v. 18, p. 489-522, 1987.

AVISE, J. C. Phylogeography: Retrospect and prospect. **Journal of Biogeography**, v. 36, n. 1, p. 3-15, 2009.

BARBARÁ, T. et al. Population differentiation and species cohesion in two closely related plants adapted to neotropical high-altitude “inselbergs”, *Alcantarea imperialis* and *Alcantarea geniculata* (Bromeliaceae). **Molecular Ecology**, v. 16, n. 10, p. 1981–1992, 2007.

BARBARÁ, T. et al. 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**, v. 103, n. 1, p. 65–77, 2009.

BENZING, D. H. **Bromeliaceae: Profile of an Adaptive Radiation.** Cambridge University Press, 2000.

CARNAVAL, A. C. et al. Prediction of phylogeographic endemism in an environmentally complex biome. **Proceedings of the Royal Society B: Biological Sciences**, v. 281, n. 1792, 2014.

CARNAVAL, A. C.; MORITZ, C. Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. **Journal of Biogeography**, v. 35, p. 1187-1201, 2008.

GIVNISH, T. et al. Phylogeny, Adaptive Radiation, and Historical Biogeography of Bromeliaceae Inferred from ndhF Sequence Data. **Aliso**, v. 23, n. 1, p. 3-26, 2007.

GIVNISH, T. J. et al. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. **American Journal of Botany**, v. 98, n. 5, p. 872–895, 2011.

GIVNISH, T. J. et al. Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. **Molecular Phylogenetics and Evolution**, v. 71, n. 1, p. 55–78, 2014.

GOETZE, M. et al. Diversification of Bromelioideae (Bromeliaceae) in the Brazilian Atlantic rainforest: A case study in *Aechmea* subgenus *Ortgiesia*. **Molecular Phylogenetics and Evolution**, v. 98, p. 346–357, 2016.

HMELJEVSKI, K. V. et al. Do plant populations on distinct inselbergs talk to each other? A case study of genetic connectivity of a bromeliad species in an Ocbil landscape. **Ecology and Evolution**, v. 7, n. 13, p. 4704-4716, 2017.

HUGHES, C. E.; PENNINGTON, R. T.; ANTONELLI, A. Neotropical Plant Evolution: Assembling the Big Picture. **Botanical Journal of the Linnean Society**, v. 171, n. 1, p. 1-18, 2013.

LEAL, B. S. S. et al. Insights into the evolutionary dynamics of Neotropical biomes from the phylogeography and paleodistribution modeling of Bromelia balansae. **American Journal of Botany**, v. 105, n. 10, p. 1725–1734, 2018.

LEAL, B. S. S.; PALMA DA SILVA, C.; PINHEIRO, F. Phylogeographic Studies Depict the Role of Space and Time Scales of Plant Speciation in a Highly Diverse Neotropical Region. **Critical Reviews in Plant Sciences**, v. 35, n. 4, p. 215-230, 2016.

LEXER, C. et al. Gene flow and diversification in a species complex of Alcantarea inselberg bromeliads. **Botanical Journal of the Linnean Society**, v. 181, n. 3, p. 505–520, 2016.

LUTHER, H. E. An alphabetical list of bromeliad binomials. **The Marie Selby Botanical Gardens**, 2012.

MARTINELLI, G. et al. Bromeliaceae da mata atlântica brasileira: lista de espécies, distribuição e conservação. **Rodriguésia**, v. 59, n. 1, p. 209-258, 2018.

MOTA, M. R. et al. The role of hybridization and introgression in maintaining species integrity and cohesion in naturally isolated inselberg bromeliad populations. **Plant Biology**, v. 21, p. 122–132, 2019.

MYERS, N. et al. Biodiversity hotspots for conservation priorities. **Nature**, v. 403, p. 853-858, 2000.

OLIVEIRA-FILHO, A. T.; FONTES, M. A. L. Patterns of Floristic Differentiation among Atlantic Forests in Southeastern Brazil and the Influence of Climate. **Biotropica**, v. 32, n. 4b, p. 793-810, 2006.

PALMA-SILVA, C. et al. Range-wide patterns of nuclear and chloroplast DNA diversity in Vriesea gigantea (Bromeliaceae), a neotropical forest species. **Heredity**, v. 103, n. 6, p. 503–512, 2009.

PALMA-SILVA, C. et al. Sympatric bromeliad species (*Pitcairnia* spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. **Molecular Ecology**, v. 20, n. 15, p. 3185–3201, 2011.

PALMA-SILVA, C. et al. Mating system variation and assortative mating of sympatric bromeliads (*Pitcairnia* spp.) endemic to neotropical inselbergs. **American Journal of Botany**, v. 102, n. 5, p. 758–764, 2015.

PALMA-SILVA, C. et al. Advances in and perspectives on evolution in Bromeliaceae. **Botanical Journal of the Linnean Society**, v. 181, n. 3, p. 305-322, 2016.

PAZ, A. et al. Phylogeography of Atlantic Forest glassfrogs (*Vitreorana*): when geography, climate dynamics and rivers matter. **Heredity**, v. 122, n. 5, p. 545–557, 2019.

POREMBSKI, S. Tropical inselbergs: habitat types, adaptive strategies and diversity patterns. **Revista Brasileira de Botânica**, v. 30, n. 4, p. 579-586, 2007.

RAPOSO DO AMARAL, F. et al. Recent chapters of Neotropical history overlooked in phylogeography: Shallow divergence explains phenotype and genotype uncoupling in *Antilophia manakins*. **Molecular Ecology**, v. 27, n. 20, p. 4108–4120, 2018.

RULL, V. Neotropical biodiversity: Timing and potential drivers. **Trends in Ecology and Evolution**, v. 26, n. 10, p. 508-513, 2011.

RULL, V. Neotropical diversification: historical overview and conceptual insights. **PeerJ Preprints**, 2018.

SARAIVA, D. P.; MANTOVANI, A.; CAMPOSTRINI FORZZA, R. Insights into the Evolution of *Pitcairnia* (Pitcairnioideae-Bromeliaceae), Based on Morphological Evidence. **Systematic Botany**, v. 40, n. 3, p. 726–736, 2015.

SCARANO, F. R. Plant communities at the periphery of the Atlantic rain forest: Rare-species bias and its risks for conservation. **Biological Conservation**, v. 142, n. 6, p. 1201-1208, 2009.

SCHUBERT, K. **Systematik und Evolution der Gattung *Pitcairnia* L'Heritier (Bromeliaceae)**. [s.l.] Universität Kassel, 2017.

SCHÜTZ, N. et al. Phylogenetics of Pitcairnioideae s.s. (Bromeliaceae): evidence from nuclear and plastid DNA sequence data. **Botanical Journal of the Linnean Society**, v. 181, n. 3, p. 323-342, 2016.

SMITH, L. B.; DOWNS, R. J. **Flora Neotropica, Vol. 14, No. 3, Bromelioideae (Bromeliaceae).** 1974.

TURCHETTO-ZOLET, A. C. et al. Phylogeographical patterns shed light on evolutionary process in South America. **Molecular Ecology**, v. 22, n. 5, p. 1193-1213, 2013.

TURCHETTO-ZOLET, A. C. et al. Phylogeography and ecological niche modelling in Eugenia uniflora (Myrtaceae) suggest distinct vegetational responses to climate change between the southern and the northern Atlantic Forest. **Botanical Journal of the Linnean Society**, v. 182, n. 3, p. 670–688, 2016.

ZANELLA, C. M. et al. Hybridization between two sister species of Bromeliaceae: *Vriesea carinata* and *V. incurvata*. **Botanical Journal of the Linnean Society**, v. 181, n. 3, p. 491–504, 2016.

CAPÍTULO 1

Original Article

Lack of phylogeographic structure despite extremely high genetic population differentiation in *Pitcanirnia flammea* species complex (Bromeliaceae) adapted to rock outcrops in the Brazilian Atlantic Rainforets

Mateus Ribeiro Mota[1], Fabio Pinheiro[2], Barbara Simões dos Santos Leal[2], Juliana Santin[1], Carla Sardelli, Tania Wendt, Clarisse Palma-Silva [1; 2*]

[1]Departamento de Ecologia, Instituto de Biociências, Universidade Estadual Paulista, 13506-900, Rio Claro, SP, Brazil

[2]Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, 13083-862, Campinas, SP, Brazil

*Correspondence: Clarisse Palma-Silva, Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, 13083-862, Campinas, SP, Brazil

E-mail: clarissepalma@yahoo.com.br

Introduction

Phylogeography has emerged as a bridge between several evolutionary disciplines, such as population genetics, phylogeny and biogeography, being in essence the study of the relationship between genealogy and geography (AVISE, 2009; AVISE et al., 1987). Phylogeography facilitated a revolution in biodiversity sciences through its role in establishing the link between micro and microevolutionary studies (PAPADOPOULOU; KNOWLES, 2016; RIDDLE, 2009). Based on the coalescence theory, phylogeography methods allow to test biogeographic hypotheses and to infer past demographic events that may have directly influenced the current distribution of a group of species, in addition to identifying lineages that may have evolved independently, each representing possible species (AVISE, 2009; FUJITA et al., 2012)

Phylogeography has been increasingly used to study the evolution of regions with high diversity indexes, such as the Neotropic region. Many studies indicated that orogenic events during the Neogene, such as the emergence of ridges, mountains and rivers, together with the climatic characteristics of the Quaternary, may have played an important role in influencing population structure and species diversification in the region (TURCHETTO-ZOLET et al., 2013). South America is a topographically complex continent, and the upheavals of mountains, have been reported as events that contributed to the organization of the biota and can act as phylogeographical barriers. Thus, the Neotropics presents a wide variety of biomes, including the humid forests of the Amazon and Atlantic Rainforest (LEAL; PALMA DA SILVA; PINHEIRO, 2016; TURCHETTO-ZOLET et al., 2013). The Atlantic Rainforest is the second largest tropical rainforest in South America, recognized as one of the five most important biodiversity hotspots in the world, containing more than 60% of all terrestrial species on the planet (MITTERMEIER et al., 2005; MYERS et al., 2000; OLIVEIRA-FILHO; FONTES, 2006). According to the survey of phylogeographic studies by Turchetto-Zolet et al. (2013), the Atlantic Forest is the third most studied biome in South America, and the second biome most studied in relation to plant organisms according to Leal et al. (2016). These studies attempt to reveal the complex evolutionary history of the Brazilian Atlantic Rainforest biota, taking into account different groups of organisms as models to understand historical patterns of diversification, seeking to clarify the geomorphological and climatic processes responsible for the high levels of diversity found in the biome (AGUIAR-MELO et al., 2019; CABANNE; SANTOS; MIYAKI, 2007; CARNAVAL; MORITZ, 2008; COLOMBI; LOPES;

FAGUNDES, 2010; HMELJEVSKI et al., 2017; MARTINS, 2011; MENEZES et al., 2016; PAZ et al., 2019; PINHEIRO et al., 2011, 2014).

Geographic barriers, such as the elevation of Serra do Mar and Serra da Mantiqueira, and formation of rivers like Doce River, have been evoked to explain the biogeographic history and evolutionary processes for the Atlantic Rainforest biota (LARA; PATTON, 2000; MENEZES et al., 2016; THOMÉ et al., 2010; TORRES; RIBEIRO, 2009). In addition to the geomorphological factors, the current set of studies suggest that Pleistocene climatic fluctuations have led to the fragmentation of forest formations, and that forest refuges may have played an important role in diversification within the Biome (CARNAVAL; MORITZ, 2008; MARTINS, 2011). Paleopalynological records (BEHLING, 1998; GARCIA et al., 2004; LEDRU et al., 2005) showed that during the last glaciation a large area in the Atlantic Forest was covered by pastures, predicting the contraction of the forest in the south and southeast and the existence of a large forest refuge along the north-eastern coast of Brazil (CARNAVAL; MORITZ, 2008; LEAL; PALMA DA SILVA; PINHEIRO, 2016; MARTINS, 2011; PALMA-SILVA et al., 2009; TURCHETTO-ZOLET et al., 2013).

The Atlantic Forest consists of a complex of vegetation that shelters various types of plant communities (SCARANO, 2002, 2009). In addition to the predominant semi-deciduous and humid forest types, the complex has open tree vegetation of sandy coastal plains, marsh forests and vegetation growing in high-altitude rocky outcrops (SCARANO, 2002). In this complex of vegetations, rocky outcrops are considered islands of terrestrial habitats because of the spatial and ecological isolation. This provides a barrier against dispersal and migration, providing the occurrence of species with naturally fragmented populations (POREMBSKI, 2007). Within the flora specialized to rock outcrops, bromeliads species have attracted the interest of molecular ecologists and evolutionary biologists, since they represent extreme cases to understand species cohesion and speciation process in fragmented environments (BARBARÁ et al., 2009; HMELJEVSKI et al., 2017; PALMA-SILVA et al., 2011).

Bromeliaceae is a monocot family (58 genera, about 3350 species) that underwent an extensive adaptive radiation, constituting one of the clades of flowering plants with greater morphological and ecological diversity (GIVNISH et al., 2014; PALMA-SILVA et al., 2016; LUTHER, 2012). It is distributed throughout the Americas, mainly in the Neotropical region, inhabiting from the canopy of the rainforests to cracks of rock in the desert regions (BENZING, 2000; LUTHER, 2012). Based on phylogenetic studies, currently eight subfamilies are recognized: Brocchinoioideae, Lindmanioideae, Tillandsioideae, Hechtioideae, Navioideae, Pitcairnioideae, Puyoideae and Bromelioideae (GIVNISH et al., 2007, 2011). In addition to phylogenetic (macroevolutionary) studies, a growing number of studies addressing a population scale (microevolutionary) of bromeliads have been published (GOETZE et al., 2016; LEAL et al., 2018; MOTA et al., 2019; PALMA-SILVA et al., 2009; SANTOS LEAL et al., 2019), thus being a great group for the establishment of connections between scales of micro and macroevolutionary processes.

Pitcairnioideae includes the terrestrial genera *Pitcairnia* L'Hér., *Fosterella*, *Deuterocohnia* Mez, *Dyckia* and *Encholirium* Mart. ex Schult.f. (SCHÜTZ et al., 2016). As pointed out by phylogenetic studies (GIVNISH et al., 2011; SCHUBERT, 2017; SCHÜTZ et al., 2016), the subfamily stem group arose c. 13.4 Mya in the Andes; and the genus *Pitcairnia* diverged about 11.8 Mya, with diversification apparently beginning in the Upper Miocene, between 5 and 4 Mya (SCHUBERT, 2017; SCHÜTZ et al., 2016). The genus *Pitcairnia* has large radiation in Neotropics, with a single species (*P. feliciana*) found in tropical West Africa (BENZING, 2000; GIVNISH et al. al., 2007). With 399 species currently accepted, *Pitcairnia* is the largest and most diverse genus of the subfamily Pitcairnioideae (LUTHER, 2012). The genus is characterized by herbaceous plants, of terrestrial habit, rockery or more rarely epiphyte. The inflorescences are spikes, racemes or panicles. The flowers are monoclinic, long-pedicellate to sessile, secondarily zygomorphic by the twisting of the petals. The fruit is a capsule with dehiscent septicidal or loculicidal. The seeds are small, numerous, linear and bi-billed or oval and winged or rarely without appendages (BENZING, 2000).

The *Pitcairnia flammea* occupy naturally fragmented rocky outcrops along the Atlantic Forest, from Bahia to Santa Catarina brazillian federal states (latitudes 16°S to 26°S). This taxon has one of the largest geographical range and morphological variation among the species in the genus *Pitcairnia* (SMITH; DOWNS, 1979). According to Flora Neotropica (SMITH; DOWNS, 1979), seven varieties of *P. flammea* (var *flammea*, var *roezlii*, var *corcovadensis*, var *glabrior*, var *pallida*, var *floccosa*, and var *macropoda*) are recognized. *Pitcairnia corcovadensis* was raised to the species category by Wendt et al., (2000). Saraiva et al. (2015) published a phylogenetic hypothesis, based on macromorphological and micromorphological characters, for the genus *Pitcairnia,* and in this study, the authors recognized the *Pitcairnia flammea* group (*P. beycalema*, *P. corcovadensis*, *P. flammea*, *P. norteflumenesis*, *P. abyssicola*, *P. carinata*, *P. decidua*, and *P. encholirioides*). More recently, Schubert (2017), based on a molecular phylogeny, recognized *P. flammea* as a monophyletic clade, with unresolved branches. Furthermore, the species and varieties are found both in allopatry and sympatry throughout its distribution, and records of interspecific hybridization between sympatric populations highlight the role of introgression and/or incomplete lineage sorting for the diversification of the group (MOTA et al., 2019). In fact, *P. flammea* complex present one of the most difficult problems in species delimitation in the genus. Here, we threated the *Pitcairnia flammea* group as proposed by Saraiva et al (2015), and the varieties proposed by Smith & Downs (1979) as *Pitcairnia flammea* species complex.

In this work, we intend to answer the following questions:

(1) What phylogeographic and genetic structure patterns of *P. flammea* complex tell us about the evolutionary mechanisms involved in speciation and diversification of rock outcrops taxa? And are these patterns consistent with those reported previously for other dwelling-forest plants and animals in the Atlantic Forest?

(2) What is the influence of the climatic oscillations on diversification of *P. flammea* lineages, and when it has occurred?

(3) How many genetically differentiated populations exist along the distribution of the species, and can these be considered different evolutionary lineages? Are the genetically differentiated populations corresponding to taxonomic varieties and species proposed for the *P. flammea* complex? What interspecific gene flow tells us about diversification in sympatric populations of the group?

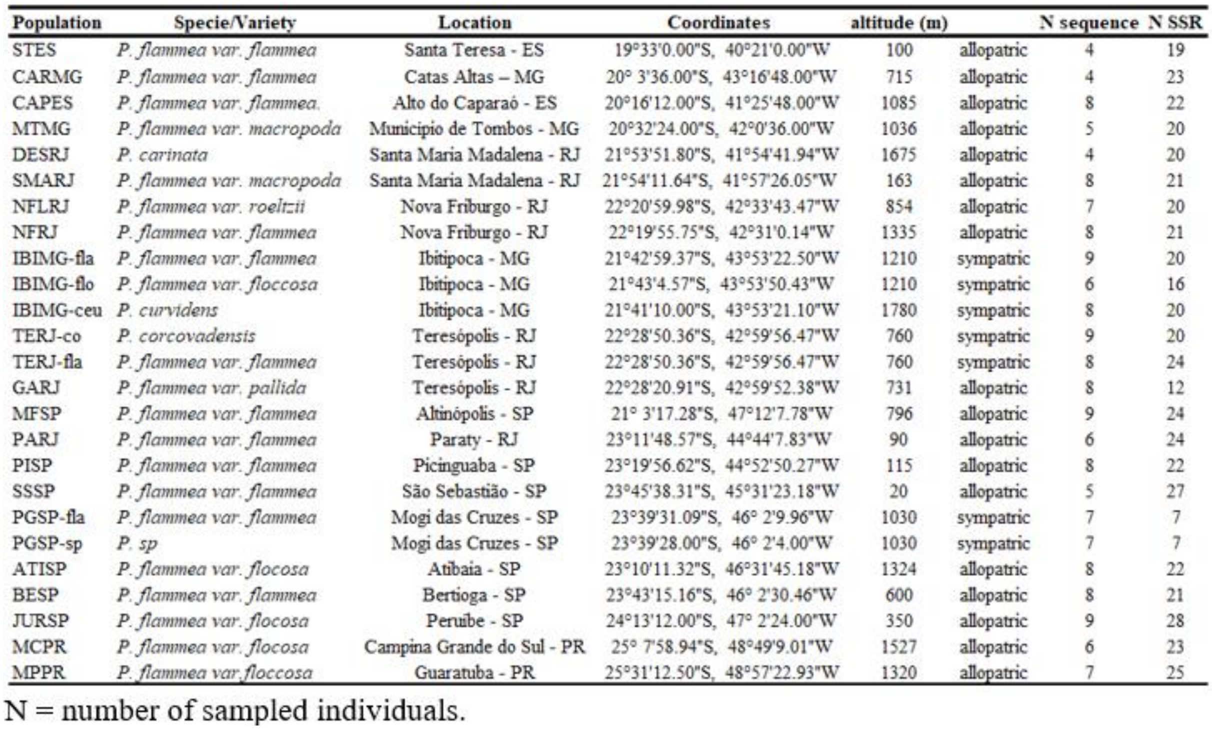
To answer these questions, we will reconstruct the evolutionary and demographic history of the *P. flammea* complex using phylogeographic approaches based on nuclear and plastidial DNA analysis.

Materials and methods

Species studied and sampling

Here, we studied a total of 501 samples, from 21 localities, including 18 allopatric and three sympatric populations covering the most geographic range of *Pitcairnia flammea* (Bromeliaceae) species complex. Different varieties collected in sympatry were considered as different populations for the following analyses, totalling 25 populations. Five varieties of the *P. flammea* complex were sampled, and three other morphologically similar species: *P. carinata*, *P. corcovadensis* and *P. curvidens*, which were included to test the degree of genetic differentiation among the populations of *P. flammea* (Table 1). Outgroups species were included from the early diverging species in the genus *Pitcairnia: P. heterophyla* and *P. lanuginose.* Sequences of *P. heterophyla* were obtained from the GenBank database (KJ188920/AF537904) and sequences of *P. lanuginose* were obtained from Leal et al. (2019). Information on each sampling locality and the number of individuals from each population used in DNA sequencing is in Table 1. Voucher specimens were deposited in the Herbarium Rioclarense (HRCB), Rio Claro, Brazil.

Table 1. Sample information of 25 populations of *Pitcairnia flammea* complex (Bromeliaceae).



Population	Specie/Variety	Location	Coordinates	altitude (m)		N sequence	N SSR
STES	*P. flammea var. flammea*	Santa Teresa - ES	19°33'0.00"S, 40°21'0.00"W	100	allopatric	4	19
CARMG	*P. flammea var. flammea*	Catas Altas – MG	20° 3'36.00"S, 43°16'48.00"W	715	allopatric	4	23
CAPES	*P. flammea var. flammea.*	Alto do Caparaó - ES	20°16'12.00"S, 41°25'48.00"W	1085	allopatric	8	22
MTMG	*P. flammea var. macropoda*	Municipio de Tombos - MG	20°32'24.00"S, 42°0'36.00"W	1036	allopatric	5	20
DESRJ	*P. carinata*	Santa Maria Madalena - RJ	21°53'51.80"S, 41°54'41.94"W	1675	allopatric	4	20
SMARJ	*P. flammea var. macropoda*	Santa Maria Madalena - RJ	21°54'11.64"S, 41°57'26.05"W	163	allopatric	8	21
NFLRJ	*P. flammea var. roeltzii*	Nova Friburgo - RJ	22°20'59.98"S, 42°33'43.47"W	854	allopatric	7	20
NFRJ	*P. flammea var. flammea*	Nova Friburgo - RJ	22°19'55.75"S, 42°31'0.14"W	1335	allopatric	8	21
IBIMG-fla	*P. flammea var. flammea*	Ibitipoca - MG	21°42'59.37"S, 43°53'22.50"W	1210	sympatric	9	20
IBIMG-flo	*P. flammea var. floccosa*	Ibitipoca - MG	21°43'4.57"S, 43°53'50.43"W	1210	sympatric	6	16
IBIMG-ceu	*P. curvidens*	Ibitipoca - MG	21°41'10.00"S, 43°53'21.10"W	1780	sympatric	8	20
TERJ-co	*P. corcovadensis*	Teresópolis - RJ	22°28'50.36"S, 42°59'56.47"W	760	sympatric	9	20
TERJ-fla	*P. flammea var. flammea*	Teresópolis - RJ	22°28'50.36"S, 42°59'56.47"W	760	sympatric	8	24
GARJ	*P. flammea var. pallida*	Teresópolis - RJ	22°28'20.91"S, 42°59'52.38"W	731	allopatric	8	12
MFSP	*P. flammea var. flammea*	Altinópolis - SP	21° 3'17.28"S, 47°12'7.78"W	796	allopatric	9	24
PARJ	*P. flammea var. flammea*	Paraty - RJ	23°11'48.57"S, 44°44'7.83"W	90	allopatric	6	24
PISP	*P. flammea var. flammea*	Picinguaba - SP	23°19'56.62"S, 44°52'50.27"W	115	allopatric	8	22
SSSP	*P. flammea var. flammea*	São Sebastião - SP	23°45'38.31"S, 45°31'23.18"W	20	allopatric	5	27
PGSP-fla	*P. flammea var. flammea*	Mogi das Cruzes - SP	23°39'31.09"S, 46° 2'9.96"W	1030	sympatric	7	7
PGSP-sp	*P. sp*	Mogi das Cruzes - SP	23°39'28.00"S, 46° 2'4.00"W	1030	sympatric	7	7
ATISP	*P. flammea var. flocosa*	Atibaia - SP	23°10'11.32"S, 46°31'45.18"W	1324	allopatric	8	22
BESP	*P. flammea var. flammea*	Bertioga - SP	23°43'15.16"S, 46° 2'30.46"W	600	allopatric	8	21
JURSP	*P. flammea var. flocosa*	Peruibe - SP	24°13'12.00"S, 47° 2'24.00"W	350	allopatric	9	28
MCPR	*P. flammea var. flocosa*	Campina Grande do Sul - PR	25° 7'58.94"S, 48°49'9.01"W	1527	allopatric	6	23
MPPR	*P. flammea var.floccosa*	Guaratuba - PR	25°31'12.50"S, 48°57'22.93"W	1320	allopatric	7	25

N = number of sampled individuals.

DNA extraction, Sanger sequencing and genotyping of microsatellites

From each individual plant, fresh leaves were sampled, dried on silica gel and stored at –20°C in a freezer until DNA extraction. Total genomic DNA was extracted using the DNeasy 96 Plant Kit (Qiagen), according to the manufacturer's instructions.

Two plastid DNA regions (*rpl32-trnL* and *rps16-trnK* – Table S1) were amplified and sequenced for a subset of 176 individual from the populations sampled, using the primers described by Shaw et al. (2007). All polymerase chain reaction (PCR) amplifications were carried out in a total volume of 20 µL containing 10 ng DNA template, 1× GoTaq buffer, 2.5 mM MgCl2, 0.25 mM dNTP mix, 5 pmol forward and reverse primers and 0.5 U GoTaq DNA polymerase (Promega) and run using the following parameters: an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 54 °C (rpl32-trnL) or 58 °C (rps16-trnK)for 1 min, and 72 °C for 1 min. There was a final extension for 10 min at 72 °C. PCR products were sent for sequencing for both forward and reverse to Macrogen (South Korea). The *rpl32-trnL* and *rps16-trnK* sequences were visualized, edited and manually verified using Geneious 10.2.3 software (http://www.geneious.com, KEARSE et al., 2012). The alignments were made using the MUSCLE algorithm with default parameters and checked by eyes for ambiguous alignments. Mononucleotide repeats were removed because of uncertain homology, and indels longer than 1 base pair were recorded as single characters. Sequence data of both plastid regions were then concatenated for subsequent analyses.

We amplified ten nuclear microsatellite loci that were previously developed for other bromeliad species (E6B: BONEH et al., 2003; PaA05, PaA09, PaA10, PaC05, PaD07, PaB12: PAGGI et al., 2008; VgA04: PALMA-SILVA et al., 2007; Acom12.12: WOHRMANN; WEISING, 2011; ngFos22: WOHRMANN et al., 2012) in a total of 501 individuals. For each microsatellite locus, the forward primer was synthesised with a 19-bp M13 tail (50-CACGACGTTGTAAAACGAC- 30), following a method that uses three primers: a forward SSR-specific primer with the M13 tail added at its 5´-end, a reverse locus-specific primer and a universal M13 primer labelled with one of four fluorescent dyes: FAM, VIC, PET or NED (Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction (PCR) amplifications of nuclear microsatellites were carried out in 10 µL reaction volumes comprising 1x GoTaq Colorless Master Mix (Promega, Madison, WI, USA), 10 pmol of each forward and reverse microsatellite primer, 1 pmol of M13 universal primer and 10 ng of template DNA. We used a ‘touchdown’ cycling programme, following the protocol described by Palma-Silva et al. (2007).

All reactions were performed using the Veriti 96-well thermal cycler (Applied Biosystems). Amplifications of nuclear microsatellites were conducted on an ABI 3500 sequencer (Applied Biosystems, Warrington, UK) and compared with the GeneScan LIZ 500 molecular size standard (Applied Biosystems). The loci were visually analysed using GeneMarker version 1.95 Demo software (SoftGenetics, State College, Pennsylvania, USA).

Molecular analyses on cpDNA sequence data

Genetic diversity and structure analyses

The number of haplotypes were identified using DnaSP (Librado and Rozas 2017). The haplotype (Hd) and nucleotide diversity(π), and number of polymorphic sites (S)were estimatedusing ARLEQUIN 3.5 software (EXCOFFIER; LISCHER, 2010). This analysis was conducted for each population, as well as for the whole data set. The evolutionary relationships between the haplotypes were estimated with Network 4.6.1.1 (available at http://www.fluxus-engineering.com) using the median-joining (MJ) method (BANDELT; FORSTER; RÖHL, 1999). The "Frequency>1" criterion was used for MJ calculations, to ignore haplotypes which are unique in the dataset.

We examined the population genetic structure using "Clustering with linked loci" implemented in BAPS 6.0 (CORANDER et al., 2013). To determine the most probable number of genetic groups (K), we performed ten algorithm repetitions for each K between 1 and 24 using the default software parameters. We assessed the population structure with pairwise F_{ST} values calculated on Arlequin 3.5 software. We also performed an analysis of molecular variance (AMOVA) to test the structure between populations, using the whole data set, and different hierarchical groups based on groups detected by the phylogenetic tree inferred in BEAST, and sorting by varieties.

Phylogenetic inference and divergence times

Phylogenetic trees were inferred based on Bayesian analyses, as implemented in BEAST v1.8.4 software (DRUMMOND et al., 2012). Two phylogenetic trees were inferred: individuals tree for the entire dataset, and a haplotype tree. The divergence time for individuals and haplotypes were estimated using *Pitcairnia lanuginosa* and *P. heterophyla* as outgroups. The best-fit model of nucleotide substitution for each locus partition was estimated using jModelTest (DARRIBA et al., 2012) under default settings (NumModels = 88, +F, +I, +G, ML optimized, BEST) and following the corrected Akaike information criterion.

Analyses in BEAST consisted of three independent runs, each with a Markov chain Monte Carlo (MCMC) analysis performed for 10 x 10^7 steps, sampling every 10000 steps. We estimated speciation times using a relaxed Bayesian molecular clock with uncorrelated lognormal rates (DRUMMOND et al., 2012; DRUMMOND; RAMBAUT, 2007), a coalescent constant population size prior, and using the divergence-time as inferred by Schubert (2017), set as a normal distribution. We verified the stationary posterior distributions of parameters (ESS >200) and compared the likelihood of runs using the MCMC Akaike information criterion (AICM) in TRACER version1.6.

Demographic analyses

To assess population demographic history, Tajima's D and Fu's Fs neutrality tests were carried out in Arlequin, based on 10 000 simulations. In addition, changes in population size over time were estimated with an Extended Bayesian Skyline Plot (EBSP) analysis. The analysis was run in the software BEAST version 2.4.0, with the following priors: the HKY DNA substitution models (selected prior using jModelTest 2.1.10); and a strict clock prior using substitution rate intervals inferred by the BEAST individuals tree (1.706E-3±0.826). Two independent runs of 200,000,000 generations, with a thinning interval of 20,000, were combined using LogCombiner to generate the final output after checking for convergence and stationarity in Tracer 1.7. To avoid bias in the demographic analysis interpretation due to the genetic structure found in *P. flammea* (see Results), we also performed the neutrality tests and EBSP considering the two genetic groups recovered by BEAST, using the same parameters described above.

Species delimitation

To determine genetic clusters representing possible independent evolving entities in the *P. flammea* complex, we applied the generalised mixed Yule coalescent method (GMYC). GMYC uses an ultrametric single-locus MCC tree to find a threshold in time between inter- and intraspecific branching rates, by maximizing the model likelihood. The analysis used the phylogenetic tree generated on BEAST, inferred for the entire data set, and was implemented in software R using the package "splits" under the single threshold version (https://r-forge.r-project.org/projects/splits/).

Molecular analyses on microsatellite data

Genetic diversity and structure analyses

The nuclear genetic diversity of microsatellite loci was characterised based on the number of alleles (A), allelic richness (A_R), observed heterozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (F_{IS}) calculated for each locus using MSAnalyzer version 4.05 software (DIERINGER; SCHLÖTTERER, 2003). Deviations from Hardy-Weinberg equilibrium (HWE) was calculated in GENEPOP on the Web 4.6 (RAYMOND; ROUSSET, 2006).

To estimate the population genetic structure and admixture proportion of nuclear DNA, we performed a model-based Bayesian clustering analyse using STRUCTURE 2.3.2 software (Pritchard et al. 2000). It was carried out under the admixture model assuming independent allele frequencies and using a burn-in period of 250,000 and run length of 1,000,000 with the Markov Chain Monte Carlo (MCMC) and 10 replicates per K, ranging from 1 to 30. No prior population information was used for analysis. To determine the most probable number of genetic groups (K) we used the method of Evanno et al. (2005) through the program STRUCTURE-HARVESTER Web v0.6.94 (EARL; VONHOLDT, 2012). We also assessed the population genetic differentiation with pairwise F_{ST} values and performed an AMOVA to test the hierarquical structure within populations and among populations, using the whole data set and sorting by varieties, all calculated on Arlequin 3.5. To determine whether divergence among populations is an effect of isolation by distance (IBD) we tested the correlation between geographical and genetic distance matrices (F_{ST}) with a standardized Mantel test using GENEPOP on the Web 4.6 (RAYMOND; ROUSSET, 2006). The significance was assessed through a randomization test using 10,000 Monte Carlo simulations.

Demographic analyses

To detect a recent and pronounced reduction in effective population size (Bottleneck effect) the two-phase mutation model (TPM) was implemented in BOTTLENECK 1.2 software using the recommendation for microsatellite data by the user manual (12% variance and 95% stepwise mutations) – (PIRY; LUIKART; CORNUET, 1999). Statistical significance was assessed in 10,000 replicates using a one tailed 'Wilcoxon signed-rank' test of heterozygosity excess for each population. We also calculated the M-ratio of Garza and Williamson (2001) for each population to detect for recent reductions in effective population size using the software M_P_Val.exe for each population. The M-value represents the ratio between the total number of alleles and the range in allele size for each locus (GARZA; WILLIAMSON, 2001). When random alleles are lost due to a reduction in effective population size, the number of alleles tends to reduce faster than the overall allelic range (GARZA; WILLIAMSON, 2001). Significance for each population was assessed by comparisons of M-ratios and critical values (MC values) obtained by simulating the M-ratio under specific demographic and mutational conditions using the software Critical_M.exe (available at http://swfsc.noaa.gov/textblock.aspx?Division=FED&id =3298). M-ratios below the critical M represent a strong sign of recent past bottleneck events.

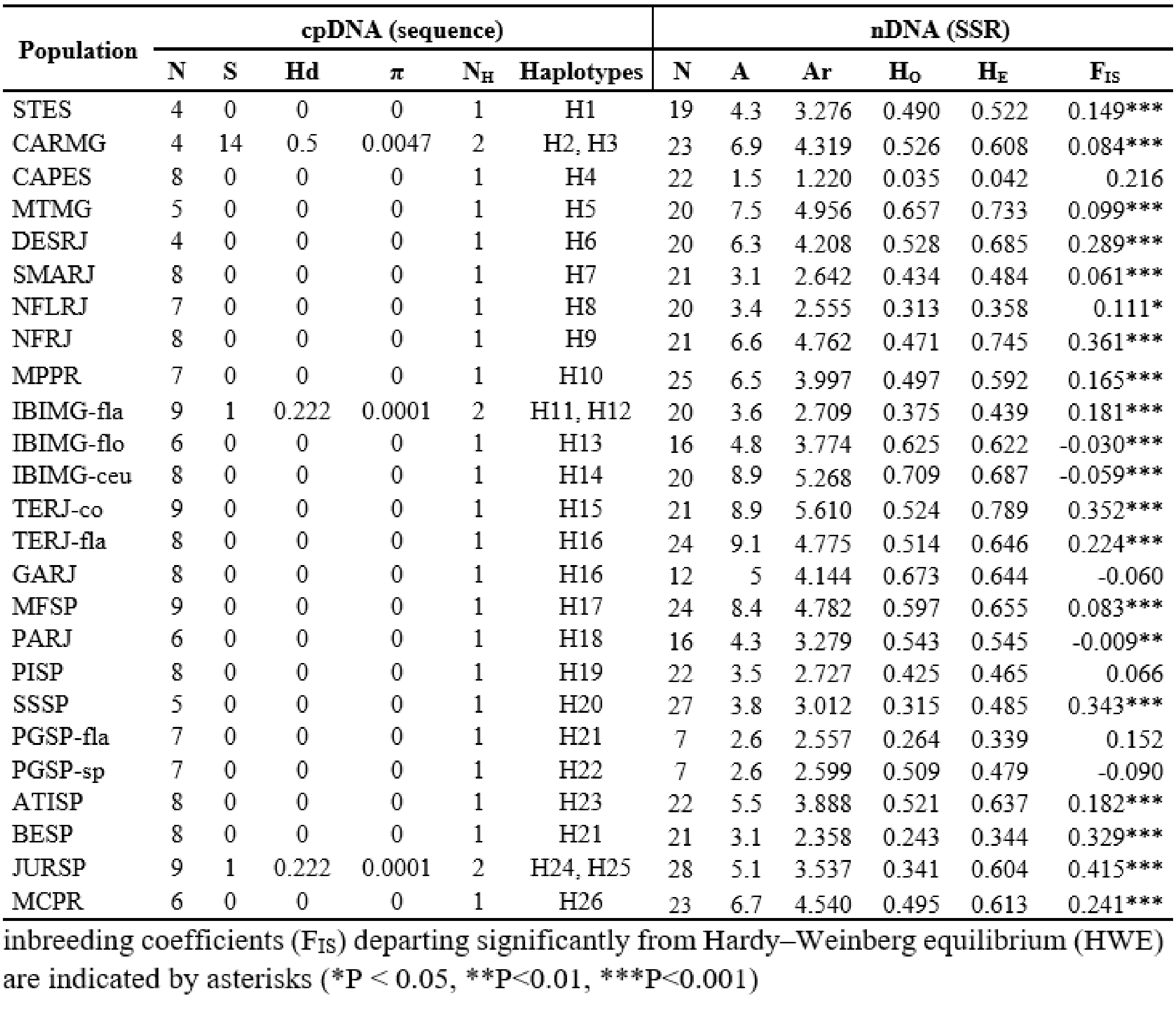
Results

Sanger sequencing of cpDNA

Genetic diversity

The final alignment with 176 *P. flammea* consensus sequences, from the two concatenated plastid regions, measured 1480 base pairs (< 5.00 % missing data), with 74 polymorphic sites resulting in 26 haplotypes (Figures 1 and 2). Extremely low intrapopulation diversity was observed in *P. flammea*, with few to no variations within populations, so the genetic diversity indexes were zero for most populations (Figure 2; Table 2). Hence, most populations were monomorphic with only three exceptions (CARMG, IBIMG-fla, and JURSP, with two haplotypes each). Only two haplotypes were shared between different populations: TERJ-fla and GARJ shared haplotype H16, and PGSP-fla and BESP shared haplotype H21.The overall haplotype diversity for the whole data set was h = 0.957 (SD = 0.0035) and nucleotide diversity was π = 0.0072 (SD = 0.0036) – (Table 2).

Table 2. Genetic diversity indices for *Pitcairnia flammea* based on cpDNA (sequence) and nDNA (SSR) data. N = Number of samples, S = number of polymorphic sites, NH = number of haplotypes, Hd = haplotype diversity, π = nucleotide diversity, A = number of alleles, Ar = allele richness, H_O = mean observed heterozygosity, H_E = mean expected heterozygosity, F_{IS} = inbreeding coefficient.



Population	cpDNA (sequence)						nDNA (SSR)					
	N	S	Hd	π	N_H	Haplotypes	N	A	Ar	H_O	H_E	F_{IS}
STES	4	0	0	0	1	H1	19	4.3	3.276	0.490	0.522	0.149***
CARMG	4	14	0.5	0.0047	2	H2, H3	23	6.9	4.319	0.526	0.608	0.084***
CAPES	8	0	0	0	1	H4	22	1.5	1.220	0.035	0.042	0.216
MTMG	5	0	0	0	1	H5	20	7.5	4.956	0.657	0.733	0.099***
DESRJ	4	0	0	0	1	H6	20	6.3	4.208	0.528	0.685	0.289***
SMARJ	8	0	0	0	1	H7	21	3.1	2.642	0.434	0.484	0.061***
NFLRJ	7	0	0	0	1	H8	20	3.4	2.555	0.313	0.358	0.111*
NFRJ	8	0	0	0	1	H9	21	6.6	4.762	0.471	0.745	0.361***
MPPR	7	0	0	0	1	H10	25	6.5	3.997	0.497	0.592	0.165***
IBIMG-fla	9	1	0.222	0.0001	2	H11, H12	20	3.6	2.709	0.375	0.439	0.181***
IBIMG-flo	6	0	0	0	1	H13	16	4.8	3.774	0.625	0.622	-0.030***
IBIMG-ceu	8	0	0	0	1	H14	20	8.9	5.268	0.709	0.687	-0.059***
TERJ-co	9	0	0	0	1	H15	21	8.9	5.610	0.524	0.789	0.352***
TERJ-fla	8	0	0	0	1	H16	24	9.1	4.775	0.514	0.646	0.224***
GARJ	8	0	0	0	1	H16	12	5	4.144	0.673	0.644	-0.060
MFSP	9	0	0	0	1	H17	24	8.4	4.782	0.597	0.655	0.083***
PARJ	6	0	0	0	1	H18	16	4.3	3.279	0.543	0.545	-0.009**
PISP	8	0	0	0	1	H19	22	3.5	2.727	0.425	0.465	0.066
SSSP	5	0	0	0	1	H20	27	3.8	3.012	0.315	0.485	0.343***
PGSP-fla	7	0	0	0	1	H21	7	2.6	2.557	0.264	0.339	0.152
PGSP-sp	7	0	0	0	1	H22	7	2.6	2.599	0.509	0.479	-0.090
ATISP	8	0	0	0	1	H23	22	5.5	3.888	0.521	0.637	0.182***
BESP	8	0	0	0	1	H21	21	3.1	2.358	0.243	0.344	0.329***
JURSP	9	1	0.222	0.0001	2	H24, H25	28	5.1	3.537	0.341	0.604	0.415***
MCPR	6	0	0	0	1	H26	23	6.7	4.540	0.495	0.613	0.241***

inbreeding coefficients (F_{IS}) departing significantly from Hardy–Weinberg equilibrium (HWE) are indicated by asterisks (*P < 0.05, **P<0.01, ***P<0.001)

cpDNA population structure

The haplotype network (Figure 1) showed a star-like pattern, with H22 as the central haplotype. Most of the haplotypes are connected by one or two mutational steps. However, some haplotypes have more mutational steps from central H22.Bayesian clustering analysis (BAPS) of the cpDNA sequences revealed an optimal structure of nine genetic clusters (K = 9) for *P. flammea* individuals (Figure S1). The defined groups recovered the patterns observed in the haplotype network, where the most distant haplotypes were clustered together (Figure 1).

Pairwise F_{ST} analyse with cpDNA ranged from 0.745 to 1,000, the latter being most of the values obtained between the populations. For nDNA, the variation was greater, ranging from 0.116 to 0.750 (Figure 5). All AMOVA tests (Table 3) indicated extremely high and significant genetic differentiation among populations and groups of *P. flammea*. For the whole data set, the genetic variation was the highest among population (F_{ST} = 0.985, P< 0.001). AMOVA also showed a significant genetic structure when contrasting genetic clusters indicated by BEAST (F_{CT} = 0.434, P< 0.001). Such a high level of genetic differentiation among groups clearly indicate limited gene flow among populations. On the other hand, the sorting by varieties were not significant among the groups (F_{CT} = 0.065, P> 0.05), suggesting that taxonomy was not a good predictor of population genetic differentiation. Regardless the clustering method utilized in the hierarquical AMOVA, differentiation among populations were always higher than between groups and within groups.

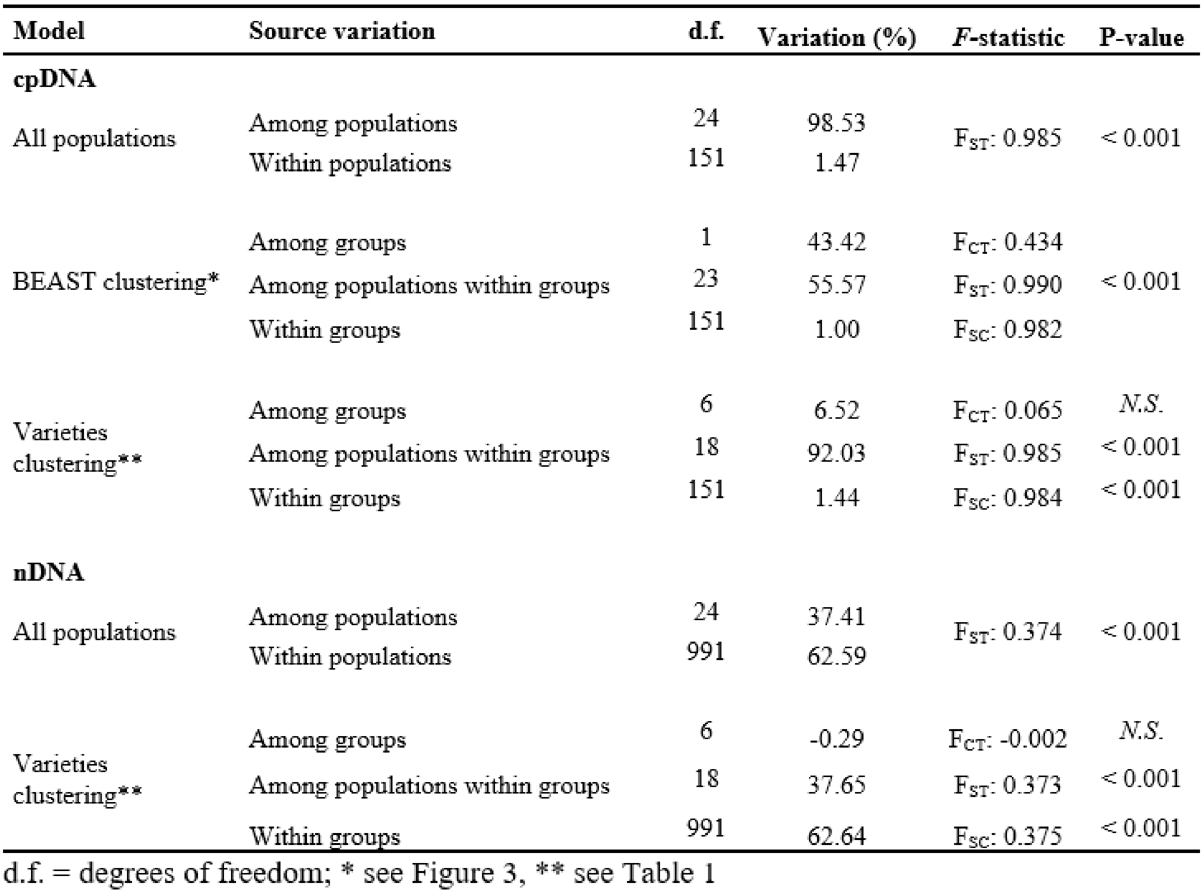
Divergence times and demographic analysis

The Bayesian phylogenetic analysis of the cpDNA region, for both specie and haplotype trees, clustered most of the populations in two strongly supported groups (posterior > 0.95), one comprising populations DESRJ, NFLRJ, NFRJ and SMARJ, distributed in north region of Serra do Mar, in the state of Rio de Janeiro, and the second with the remaining populations, including populations in the Northern most of the geographic distribution in Minas Gerais and Espirio Santos states (Figure 3). The haplotypes of *P. flammea* diverged from a common ancestor with *P. heterophylla* at 3.866 Mya, during the Pliocene. The time of the most recent common ancestor (TMRCA) of all *P. flammea* haplotypes was estimated at 2.008 Mya [95% highest posterior density (HPD)] in the early Pleistocene (Figure 4), which corresponds to the divergence time between the two possible main haplotypes lineages (Northern Rio de Janeiro with haplotypes H6, H7, H8 and H9, and all others). All the other divergence events are suggested to have occurred in less than 1 Mya. The species tree constructed with all individuals recovered the same pattern of groups and times of divergence: 4.454 Mya for divergence with *P. heterophylla*, 2.089 for the TMRCA among *P. flammea* species complex (Figure 3).

The results of the Tajima's D and Fu's FS neutrality tests for cpDNA were not significant (P> 0.005). Hence, no demographic changes were detected for the *P. flammea* populations included in this study. The results were also not significant when tested for the two

BEAST genetic groups. EBSP analyses, with the present dataset, did not generate satisfactory results for any level of population clustering. The BEAST runs did not converge, presenting ESS<200 in any cases, likely due to the high population structure observed in among populations. Thus, we were unable to assess changes in populations size over time.

Table 3. Analysis of molecular variance (AMOVA) based on cpDNA sequences and nDNA microsatellites. For cpDNA, groups of populations were defined according to BEAST phylogenetic tree, and by the varieties of *Pitcairnia flammea.* For the nDNA, only varieties groups were defined.



Model	Source variation	d.f.	Variation (%)	*F*-statistic	P-value
cpDNA					
All populations	Among populations	24	98.53	F_{ST}: 0.985	< 0.001
	Within populations	151	1.47		
BEAST clustering*	Among groups	1	43.42	F_{CT}: 0.434	< 0.001
	Among populations within groups	23	55.57	F_{ST}: 0.990	
	Within groups	151	1.00	F_{SC}: 0.982	
Varieties clustering**	Among groups	6	6.52	F_{CT}: 0.065	*N.S.*
	Among populations within groups	18	92.03	F_{ST}: 0.985	< 0.001
	Within groups	151	1.44	F_{SC}: 0.984	< 0.001
nDNA					
All populations	Among populations	24	37.41	F_{ST}: 0.374	< 0.001
	Within populations	991	62.59		
Varieties clustering**	Among groups	6	-0.29	F_{CT}: -0.002	*N.S.*
	Among populations within groups	18	37.65	F_{ST}: 0.373	< 0.001
	Within groups	991	62.64	F_{SC}: 0.375	< 0.001

d.f. = degrees of freedom; * see Figure 3, ** see Table 1

The GMYC single-threshold results recovered 24 molecular operational taxonomic units (MOTUs) and identified a coalescence time threshold at 0.157 Mya. Thus, most populations were identified as a single independent MOTU, with few exceptions. CAPES was divided in two MOTUs; the geographic close populations of TERJ-fla and GARJ were grouped together in two MOTUs, as the same for IBIMG-fla/ceu/flo. The sympatric populations of PGSP-sp and PGSP-fla were each grouped in different MOTUs: PGSP-sp was grouped together with JURSP; and PGSP-fla together with BESP.

Microsatellite data of nDNA

Genetic diversity

Twenty-five populations of *Pitcairnia flammea* were analysed, totalling 501 individuals, with a mean of 21 individuals per population (Table 1). Allelic richness (A_R) varied from 1.220 to 5.610; the mean number of alleles (A) ranged from 1.5 to 9.1; the observed heterozygosity (H_O) ranged from 0.035 to 0.709; and the expected heterozygosity (H_E) ranged from 0.042 to 0.789 (Table 2).The population-level inbreeding coefficient (F_{IS}) was high and significant for most of populations, ranging from -0.090 to 0.414 (Table 2).

nDNA population structure

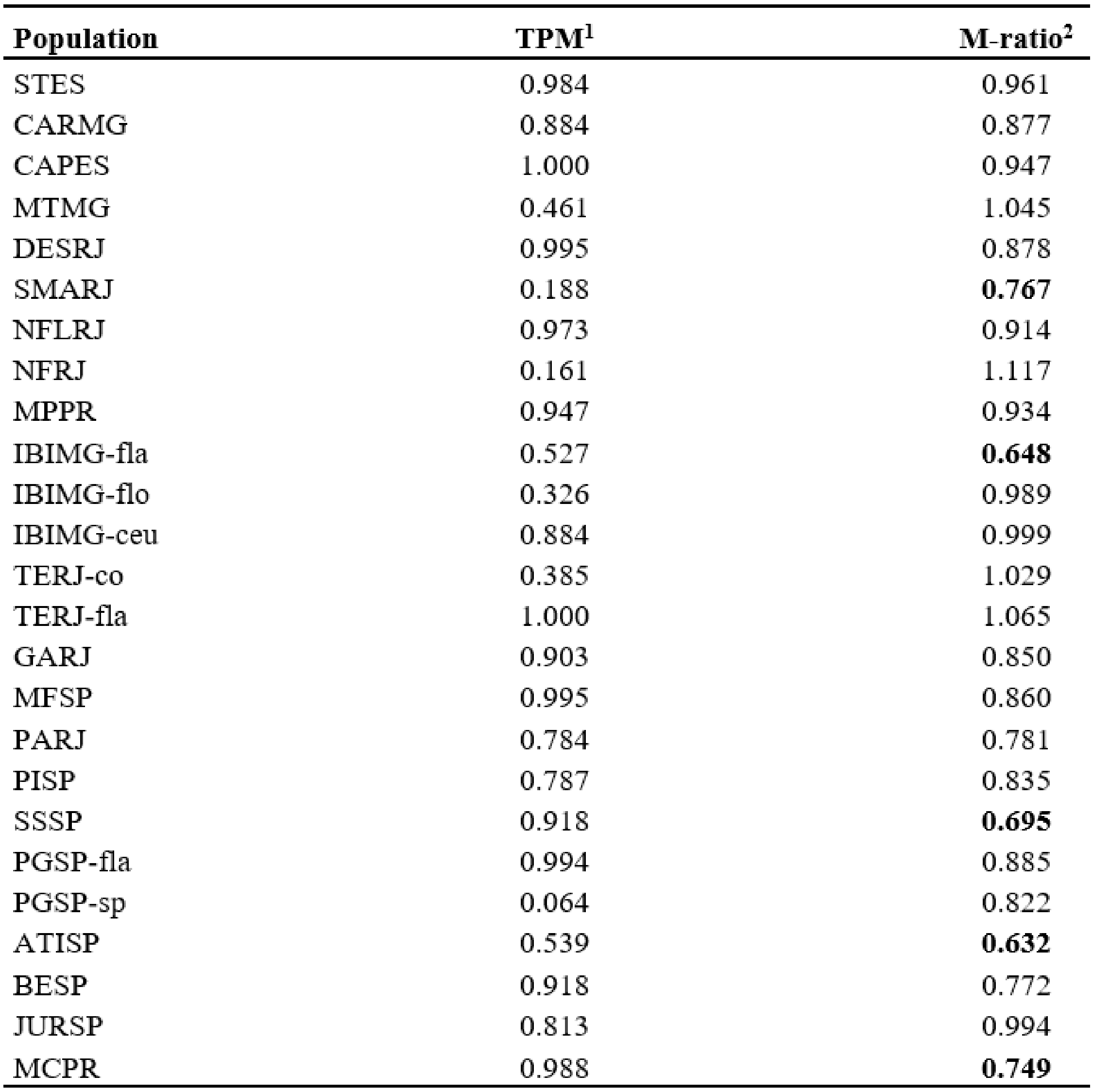
Bayesian admixture analyse revealed K = 22 genetic clusters. Most of the populations were grouped in independent genetic clusters, indicating extremely low gene flow among populations. Few geographically close populations of BESP, PISP and PGSP-fla; IBIMG-fla and TERJ-fla; IBIMG-flo and IBIMG-ceu; MPPR and MCPR were grouped together in same genetic cluster. But Mantel test was not significant, showing that genetic differentiation were not correlated with geographic distance ($P = 0.369$, > 0.05), thus suggesting that populations were not differentiated under isolated-by-distance model. Among the three sympatric populations, only one (TERJ-fla and TERJ-co) showed genetic admixture (Figure S1), suggesting hybridization and introgression between these two species, as reported previously by Mota et al. (2019).

High and significant genetic differentiation was estimated among all populations for nuclear microsatellite in AMOVA ($F_{ST} = 0.374$, $P < 0.001$). For the hierarchical AMOVA, considering the different varieties/species of the *P. flammea* complex, the genetic differentiation among the varieties was low and non-significant ($F_{CT} = -0.002$, $P > 0.05$) - (Table 2), in agreement with results found in the cpDNA. In all cases, differentiation within population were higher than among population, contrasting with the results pointed out by cpDNA.

Demographic analysis

No excess of heterozygosity was detected in the bottleneck analysis for any of the 25 populations investigated with microsatellite loci, suggesting no recent changes in population sizes (Table 4). M-ratio values ranged from 0.632 and 1.117 across all populations. The simulation conducted generated a threshold critical M-ratio (Mc) of 0.771. At this Mc threshold value, five populations (SMARJ, IBIMG-fla, SSSP, ATISP and MCPR) showed signs of recent past genetic bottleneck events (Table 4).

Table 4. Bottleneck Probability Test results (TPM: Two Phase Mutation Model) and M-ratio values based on 10 nuclear microsatellite loci.



Population	TPM[1]	M-ratio[2]
STES	0.984	0.961
CARMG	0.884	0.877
CAPES	1.000	0.947
MTMG	0.461	1.045
DESRJ	0.995	0.878
SMARJ	0.188	**0.767**
NFLRJ	0.973	0.914
NFRJ	0.161	1.117
MPPR	0.947	0.934
IBIMG-fla	0.527	**0.648**
IBIMG-flo	0.326	0.989
IBIMG-ceu	0.884	0.999
TERJ-co	0.385	1.029
TERJ-fla	1.000	1.065
GARJ	0.903	0.850
MFSP	0.995	0.860
PARJ	0.784	0.781
PISP	0.787	0.835
SSSP	0.918	**0.695**
PGSP-fla	0.994	0.885
PGSP-sp	0.064	0.822
ATISP	0.539	**0.632**
BESP	0.918	0.772
JURSP	0.813	0.994
MCPR	0.988	**0.749**

[1] Significant value for $P < 0.05$

[2] A population is considered to have undergone a bottleneck if its M-ratio value falls below the threshold of critical M-ratio (Mc = 0.771)

Discussion

In this study, we investigated phylogeographic patterns and genetic structure and diversity of *P. flammea* species complex, an endemic Bromeliaceae adapted to rock outcrops in the Atlantic Rainforest. Our main results showed low to moderate nuclear genetic diversity within *P. flammea* populations, and high population genetic structure, with few plastid DNA haplotype shared among populations and K=22 nuclear genetic clusters, indicating limited gene flow, low connectivity and evolutionary history of long-term isolation and persistence among rock outcrops populations. Despite the high genetic structure, we found no clear northern and southern phylogeographic structure commonly reported for the Atlantic rain forest organisms. However, we found two divergent lineages which diverged approximately 2 Mya, one comprising the majority of populations all over the Southern and Northern most of the geographic distribution, and the other including four populations located in the northern part Serra do Mar. Thus, suggesting an important role of early Pleistocene climatic changes in the diversification of *P. flammea* species complex. Moreover, we could not find direct congruence between population genetic divergence and taxonomic delimitation. The strong population genetic structure in nuclear and plastidial markers acknowledged most of the populations as independent evolving entities in the species delimitation analysis. And finally, in the three sympatric populations we detected limited interspecific gene flow although some hybridization and introgression were observed.

Genetic structure and phylogeography patterns

We found a high and strong genetic structure at population level for both cpDNA and nDNA (Figure 5), with the almost complete fixation of haplotypes within each population, and a high number of clusters obtained in Structure (K = 22). Our results show low gene flow and genetic connectivity between *P. flammea* populations and the absence of isolation by distance, reinforcing the geographic isolation of populations restricted to the naturally fragmented inselbergs and rock outcrops. The strong genetic structure and high haplotypic diversity suggest low gene flow for both nuclear and plastidial markers, and consequently limited pollen and seed dispersal. *Pitcairnia flammea* has small and inconspicuous seeds, and without any flight appendages and known disperser, with the wind as the primary vector of dispersion. This indicates that the plants have a limited dispersal range. Pollination is done by hummingbirds, and mating system is self-compatible (Wendt et al 2001). The fixation of cpDNA haplotypes is an observed pattern in angiosperms, since cpDNA is inherited maternally in most flowering plants, including bromeliads (WAGNER et al., 2015). This, coupled with the naturally fragmented nature of inselbergs, hinders gene flow among populations, and consequently contributes to high genetic differentiation. These results are in consonance with the common pattern related for populations located in rock outcrops landscapes: low gene flow, extremely high population differentiation, prolonged isolation, absence of isolation by distance and long term population persistence (BARBARÁ et al., 2007; HMELJEVSKI et al., 2017; PALMA-

SILVA et al., 2011; PINHEIRO et al., 2011, 2014; BARRES et al., 2019; FRANCO et al., 2017; GONÇALVES-OLIVEIRA et al., 2017; LAVOR et al., 2014; LELES et al., 2015; NISTELBERGER et al., 2014; RAMOS et al., 2018)

Despite the strong genetic structure observed in *P. flammea* populations, no phylogeographic structure was evident in our results for both nDNA and cpDNA data sets. The Brazilian Atlantic Forest is a mosaic of different dynamics and patterns of diversification, and each organism was probably differently influenced by past climatic oscillations and geographic barriers (AGUIAR-MELO et al., 2019; CABANNE; SANTOS; MIYAKI, 2007; CARNAVAL; MORITZ, 2008; COLOMBI; LOPES; FAGUNDES, 2010; MARTINS, 2011; MENEZES et al., 2016; PAZ et al., 2019; RIBEIRO et al., 2011; THOMÉ et al., 2010). The heterogeneous nature of the southern and northern regions of the Brazilian Atlantic Rainforest is a well-observed fact, for both animals and plant groups (CABANNE; SANTOS; MIYAKI, 2007; CARNAVAL; MORITZ, 2008; COSTA, 2003; GRAZZIOTIN et al., 2006; PALMA-SILVA et al., 2009; PAZ et al., 2019; PINHEIRO; COZZOLINO, 2013; TCHAICKA et al., 2007). Unlike most of the studies, we could not detect clear sign of phylogeographic breaks between northern and southern populations. The lack of phylogeographic structure was reported for few other taxas such as *Vriesea incurvate* (plant) - (AGUIAR-MELO et al., 2019), *Schiffornis virescens* (bird) - (CABANNE et al., 2013), and *Mycetophylax simplex* (ants) - (CARDOSO et al., 2015).

We could not detect significant demographic changes in population sizes through time and signs of recent demographic variation for *P. flammea* except for five populations that indicated signs of contraction, suggesting a long-term persistence in *P. flammea* populations across time (Table 4). Moreover, the variable responses of populations demographic changes observed all over the species range suggest the effects might be due to variable ecological and local adaptations responses in each population and not much influenced by altitudinal and latitudinal gradients. Some authors have (SCARANO, 2002; SPEZIALE; EZCURRA, 2012; TAPPER et al., 2014) also suggested that inselbergs may have acted as refugia for xerophilic or cold-adapted species during interglacial periods, where Pleistocene forest expansion events would have caused the fragmentation of populations and a consequent diversification of lineages. However, quaternary climatically changes had no clear effect in the demographic patterns of the taxa, suggesting that climatically stable micro-habitats could have contributed to the maintenance of these inselberg populations. Even though, it is important to acknowledge the high genetic structure and low gene flow might promote a bias in the interferes of demographic analysis

Lineages diversification age during early Pleistocene

The results indicate the existence of two evolutionary lineages for *P. flammea*, which apparently diverged c. 2 Mya, during the early Pleistocene (posterior < 0.90), revealing a distinct genetic grouping in the northern region of Serra do Mar. Thus, our results indicate a plausible role of the first Quaternary glaciations in shaping population divergence of this species complex. Other studies, as Silva et al (2017), have identified major transitions in climate system dynamics during the Plio-Pleistocene transition about 2.5 million years. Despite many studies pointing to climatic oscillations connected with the late Pleistocene and Last Glacial Maximum (LGM- about 21,000 ka) as major forces on promoting population divergence of South American species (BATALHA-FILHO; MIYAKI, 2016; CARNAVAL et al., 2014; VERGARA et al., 2017), other works have indicated the importance of older geological and climatic events as main drivers of the lineages diversification and speciation events on the Atlantic Forest (MENEZES et al., 2017; PENNINGTON et al., 2004; PERES et al., 2019; SILVA et al., 2018).

Species delimitation

The GMYC species delimitation analyses with cpDNA acknowledged most populations as independent evolving entities (24 MOTUs). Accordingly, nDNA Bayesian admixture analyses in Structure revealed K = 22 independent clusters out of 25 populations. AMOVA sorting by varieties were not significant among the groups but was high and significant among populations within groups. This indicates that genetic clusters do not agree with the typological/morphological descriptions. The genetic differentiation can be possibly related to historical population isolation, and the morphological variances found between the different varieties are probably the result of local adaptations or phenotypic plasticity. Thus, the pattern observed was the absence of congruence in genetic patterns and the species or taxonomic varieties described in the literature, therefore, our data do not support the classification in seven varieties. The species category is a fundamental unit in biology, and the development of robust and highly replicable measures for the identification of distinct evolutionary lineages is a central objective of species delimitation (DE QUEIROZ, 2007).

Our Bayesian phylogenetic results supported the existence of two evolutionary lineages for the *P. flammea* species complex (Figure 3). One comprising four populations distributed in north of Serra do Mar, in state of Rio de Janeiro, and the second with the remaining populations. This lineage is composed of four distinct morphological taxa: *P. flammea* var. *flammea, P. flammea* var. *roeltzii*, *P. flammea* var. *macropoda* and *P. carinata* (Table 1). AMOVA showed high genetic among populations of each lineages (F_{ST} = 0.990 – Table 3), indicating that the population continue to diversify as an independent unit.

Populations IBIMG-flo and IBIMG-ceu, although described as being of different taxa (*P. flammea* var. *floccosa* and *P. curvidens*, respectively) – (MONTEIRO; FORZZA, 2008), were not delimited as distinct lineages in the haplotype and species trees, and they were assigned into the same cluster in the Structure analysis using nDNA. In addition, BAPS analyses with cpDNA classified both in a single cluster. All together, these evidences indicated those two taxa are in fact belonging to the same population and species. Moreover, *Pitcairnia carinata* (DESRJ population) was the only population with a significant statistical support in the phylogenetic trees. In agreement, Structure classified it as a single cluster, indicating this species as an independent lineage.

Besides the genetic differentiation and structure among populations, results of nDNA showed genetic admixtures between some sympatric populations (Figure S1). Species in the sympatric population of Teresopolis (TERJ-fla and TERJ-co) had signs of gene flow and allele sharing between then, as was previously reported by Mota et al. (2019). Populations of Ibitipoca (IBIMG) and Mogi das Cruzes (PGSP) showed no gene flow for nuclear DNA among the species present in each locality. In addition, none of the taxa in sympatric populations shared haplotypes, as pointed out in cpDNA analyses, suggesting the strength of reproductive isolation barriers among *P. flammea* taxa. Studies with Bromeliaceae adapted to inselberg has reported low levels of intraspecific gene flow among rock outcrops, but significant levels of interspecific geneflow among closed related species in sympatry (BARBARÁ et al., 2007; LEXER et al., 2016; MOTA et al., 2019; PALMA-SILVA et al., 2011). Specific for *Pitcairnia* genus, it has been already reported strong but permeable reproductive isolation barriers for species occurring in sympatry (MOTA et al., 2019; PALMA-SILVA et al., 2011; WENDT et al., 2001), supporting the studies in which inselberg bromeliad species are maintained as discrete evolutionary units despite the presence of low levels of genetic connection. Understand the interspecific gene flow and the role of hybridization is crucial in assessing the process of speciation and species delimitation among recently radiated taxa. (LEXER et al., 2016; MOTA et al., 2019; VERSIEUX et al., 2012; ZANELLA et al., 2016).

Conclusion

Our study adds important information on how rock outcrops species have been affected by past climate oscillations in Brazilian Atlantic Rainforest, and how naturally fragmented species are maintained as discrete evolutionary units despite the long isolation, low levels of genetic connection and low intraspecific gene flow. The present study revealed no phylogeographical pattern between populations of *P. flammea*. Our study has revealed that the high genetic structure found in this species complex are the result of random genetic drift and inbreeding, consequence of a long-time isolation between populations, and demographic stability and persistence. *P. flammea* maintained constant population sizes during the climatic oscillations of the Pleistocene. Our study also indicates an impact of the early Quaternary glaciations on the distribution and population divergence of South American plant species, contrary to the dominant research focus on the LGM. It can be concluded that there is a high genetic structuring between populations, independent of the varieties, reinforcing the idea that these populations have been evolving and diversifying for a long time independently of each other; and the morphological variances found between the different varieties are probably the result of local adaptations, consequential from a phenotypic plasticity. Thus, our data do not support the morphological classification in seven taxonomic varieties or species described in the literature.

References

AGUIAR-MELO, C. et al. 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,** v. 106, n. 7, p. 971-983, 2019.

ANTONELLI, A. et al. Amazonia is the primary source of Neotropical biodiversity. **Proceedings of the National Academy of Sciences**, v. 115, n. 23, p. 6034–6039, 2018a.

ANTONELLI, A. et al. Conceptual and empirical advances in Neotropical biodiversity research. **PeerJ**, 2018b.

ANTONELLI, A.; SANMARTÍN, I. **Why are there so many plant species in the Neotropics?** Taxon, v. 60, n. 2, p. 403-414, 2011.

AVISE, J. C. et al. Intraspecific Phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. **Annual Review of Ecology and Systematics**, v. 18, p. 489-522, 1987.

AVISE, J. C. **Phylogeography: Retrospect and prospectJournal of Biogeography**, v. 36, n. 1, p. 3-15, 2009.

BANDELT, H. J.; FORSTER, P.; RÖHL, A. Median-joining networks for inferring intraspecific phylogenies. **Molecular Biology and Evolution**, v. 16, n. 1, p. 37-48, 1999.

BARBARÁ, T. et al. Population differentiation and species cohesion in two closely related plants adapted to neotropical high-altitude “inselbergs”, *Alcantarea imperialis* and *Alcantarea geniculata* (Bromeliaceae). **Molecular Ecology**, v. 16, n. 10, p. 1981–1992, 2007.

BARBARÁ, T. et al. 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**, v. 103, n. 1, p. 65–77, 2009.

BARRES, L. et al. Pleistocene climatic changes drove dispersal and isolation of *Richterago discoidea* (Asteraceae), an endemic plant of campos rupestres in the central and eastern Brazilian sky islands. **Botanical Journal of the Linnean Society**, v. 189, n. 2, p. 132–152, 2019.

BATALHA-FILHO, H.; MIYAKI, C. Y. 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**, v. 54, n. 2, p. 137-147, 2016.

BEHLING, H. Late Quaternary vegetational and climatic changes in Brazil. **Review of Palaeobotany and Palynology**, v. 99, n. 2, p. 143-156, 1998.

BENZING, D. H. **Bromeliaceae: Profile of an Adaptive Radiation.** Cambridge University Press, 2000.

BONEH, L.; KUPERUS, P.; VAN TIENDEREN, P.H. Microsatellites in the bromeliads *Tillandsia fasciculate* and *Guzmania monostachya*. **Molecular Ecology Notes**, v. 3, p. 302–303, 2003.

CABANNE, G. S. et al. Matrilineal evidence for demographic expansion, low diversity and lack of phylogeographic structure in the Atlantic forest endemic Greenish Schiffornis

Schiffornis virescens (Aves: Tityridae). **Journal of Ornithology**, v. 154, n. 2, p. 371–384, 2013.

CABANNE, G. S.; SANTOS, F. R.; MIYAKI, C. Y. Phylogeography of *Xiphorhynchus fuscus* (Passeriformes, Dendrocolaptidae): Vicariance and recent demographic expansion in southern Atlantic forest. **Biological Journal of the Linnean Society**, v. 91, n. 1, p. 73–84, 2007.

CARDOSO, D. C. et al. Phylogeography of the sand dune ant *Mycetophylax simplex* along the Brazilian Atlantic Forest coast: Remarkably low mtDNA diversity and shallow population structure. **BMC Evolutionary Biology**, v. 15, n. 1, p. 1-13, 2015.

CARNAVAL, A. C. et al. Prediction of phylogeographic endemism in an environmentally complex biome. **Proceedings of the Royal Society B: Biological Sciences**, , v. 281, n. 1792, 2014.

CARNAVAL, A. C.; MORITZ, C. Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. **Journal of Biogeography**, v. 35, p. 1187-1201, 2008.

COLOMBI, V. H.; LOPES, S. R.; FAGUNDES, V. Testing the Rio Doce as a riverine barrier in shaping the atlantic rainforest population divergence in the rodent Akodon cursor. **Genetics and Molecular Biology**, v. 33, n. 4, p. 785-789, 2010.

CORANDER, J. et al. BAPS : Bayesian Analysis of Population Structure. Manual v 6.0 **Bioinformatics**, 2013.

COSTA, L. P. The historical bridge between the Amazon and the Atlantic Forest of Brazil: A study of molecular phylogeography with small mammals. **Journal of Biogeography**, v. 30, n. 1, p 71-86, 2003.

DARRIBA, D. et al. JModelTest 2: More models, new heuristics and parallel computing**.** **Nature Methods**, v. 9, n. 8, p. 772, 2012.

DE QUEIROZ, K. Species concepts and species delimitation. **Systematic Biology**, v. 56, n. 6, p. 879–886, 2007.

DIERINGER, D.; SCHLÖTTERER, C. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. **Molecular Ecology Notes**, v. 3, p. 167–169, 2003.

DRUMMOND, A. J. et al. Bayesian phylogenetics with BEAUti and the BEAST 1.7. **Molecular Biology and Evolution**, v. 29, n. 8, p. 1969–1973, 2012.

DRUMMOND, A. J.; RAMBAUT, A. BEAST: Bayesian evolutionary analysis by sampling trees. **BMC Evolutionary Biology**, v. 7, n. 1, 2007.

EARL, D. A.; VONHOLDT, B. M. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. **Conservation Genetics Resources**, v. 4, n. 2, p. 359–361, 2012.

EXCOFFIER, L.; LISCHER, H. E. L. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. **Molecular Ecology Resources**, v. 10, n. 3, p. 564-567, 2010.

FRANCO, F. F. et al. The xeric side of the Brazilian Atlantic Forest: The forces shaping phylogeographic structure of cacti. **Ecology and Evolution**, v. 7, n. 22, p. 9281-9293, 2017.

FUJITA, M. K. et al. Coalescent-based species delimitation in an integrative taxonomy. **Trends in Ecology and Evolution**, v. 27, n. 9, p. 480-488, 2012.

GARCIA, M. J. et al. A Holocene vegetational and climatic record from the Atlantic rainforest belt of coastal State of São Paulo, SE Brazil. **Review of Palaeobotany and Palynology**, v. 131, n. 3–4, p. 181-199, 2004.

GARZA, J. C.; WILLIAMSON, E. G. Detection of reduction in population size using data from microsatellite loci. **Molecular Ecology**, v. 10, n. 2, p. 305-318, 2001.

GIVNISH, T. et al. Phylogeny, Adaptive Radiation, and Historical Biogeography of Bromeliaceae Inferred from ndhF Sequence Data. **Aliso**, v. 23, n. 1, p. 3-26, 2007.

GIVNISH, T. J. et al. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: Insights from an eight-locus plastid phylogeny. **American Journal of Botany**, v. 98, n. 5, p. 872–895, 2011.

GIVNISH, T. J. et al. Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. **Molecular Phylogenetics and Evolution**, v. 71, n. 1, p. 55–78, 2014.

GOETZE, M. et al. Diversification of Bromelioideae (Bromeliaceae) in the Brazilian Atlantic rainforest: A case study in *Aechmea* subgenus Ortgiesia. **Molecular Phylogenetics and Evolution**, v. 98, p. 346–357, 2016.

GONÇALVES-OLIVEIRA, R. C. et al. Population genetic structure of the rock outcrop species *Encholirium spectabile* (Bromeliaceae): The role of pollination vs. seed dispersal and evolutionary implications. **American Journal of Botany**, v. 104, n. 6, p. 868–878, 2017.

GRAZZIOTIN, F. G. et al. Phylogeography of the *Bothrops jararaca* complex (Serpentes: Viperidae): Past fragmentation and island colonization in the Brazilian Atlantic Forest. **Molecular Ecology**, v. 15, n. 13, p. 3969-3982, 2006.

HMELJEVSKI, K. V. et al. Do plant populations on distinct inselbergs talk to each other? A case study of genetic connectivity of a bromeliad species in an Ocbil landscape. **Ecology and Evolution**, v. 7, n. 13, p. 4704-4716, 2017.

HUGHES, C. E.; PENNINGTON, R. T.; ANTONELLI, A. **Neotropical Plant Evolution: Assembling the Big PictureBotanical Journal of the Linnean Society**, v. 171, n. 1, p. 1-18, 2013.

LARA, M. C.; PATTON, J. L. Evolutionary diversification of spiny rats (genus *Trinomys*, Rodentia: Echimyidae) in the Atlantic Forest of Brazil. **Zoological Journal of the Linnean Society**, v. 130, n. 4, p. 661–686, 2000.

LAVOR, P. et al. Population genetics of the endemic and endangered *Vriesea minarum* (Bromeliaceae) in the Iron Quadrangle, Espinhaço Range, Brazil. **American Journal of Botany**, v. 101, n. 7, p. 1167-1175, 2014.

LEAL, B. S. S. et al. Insights into the evolutionary dynamics of Neotropical biomes from the phylogeography and paleodistribution modeling of Bromelia balansae. **American Journal of Botany**, v. 105, n. 10, p. 1725–1734, 2018.

LEAL, B. S. S.; PALMA DA SILVA, C.; PINHEIRO, F. Phylogeographic Studies Depict the Role of Space and Time Scales of Plant Speciation in a Highly Diverse Neotropical Region. **Critical Reviews in Plant Sciences**, v. 35, n. 4, p. 215-230, 2016.

LEDRU, M. P. et al. Paleoclimate changes during the last 100,000 yr from a record in the Brazilian Atlantic rainforest region and interhemispheric comparison. **Quaternary Research**, v. 64, n. 3, p. 444-450, 2005.

LEITE, Y. L. R. et al. Neotropical forest expansion during the last glacial period challenges refuge hypothesis. **Proceedings of the National Academy of Sciences**, v. 113, n. 4, p. 1008-1013, 2016.

LELES, B. et al. Genetic structure is associated with phenotypic divergence in floral traits and reproductive investment in a high-altitude orchid from the iron Quadrangle, Southeastern Brazil. **PLoS ONE**, v. 10, n. 3, p. 1-19, 2015.

LEXER, C. et al. Gene flow and diversification in a species complex of *Alcantarea inselberg* bromeliads. **Botanical Journal of the Linnean Society**, v. 181, n. 3, p. 505–520, 2016.

LUTHER, H. E. An alphabetical list of bromeliad binomials. **The Marie Selby Botanical Gardens**, 2012.

MARTINELLI, G. et al. Bromeliaceae da mata atlântica brasileira: lista de espécies, distribuição e conservação. **Rodriguésia**, v. 59, n. 1, p. 209-258, 2018.

MARTINS, F. DE M. Historical biogeography of the Brazilian Atlantic forest and the Carnaval-Moritz model of Pleistocene refugia: what do phylogeographical studies tell us? **Biological Journal of the Linnean Society**, v. 104, n. 3, p. 499–509, 2011.

MENEZES, L. et al. Multilocus phylogeography of the treefrog *Scinax eurydice* (Anura, Hylidae) reveals a plio-pleistocene diversification in the atlantic forest. **PLoS ONE**, v. 11, n. 6, 2016.

MENEZES, R. S. T. et al. The roles of barriers, refugia, and chromosomal clines underlying diversification in Atlantic Forest social wasps. **Scientific Reports**, v. 7, n. 1, 2017.

MITTERMEIER, R. et al. **Hotspots revisited: earth's biologically richest and most endangered terrestrial ecoregions.** Conservation International, Arlington, 2005.

MONTEIRO, R. F.; FORZZA, R. C. A Família Bromeliaceae no Parque Estadual do Ibitipoca, Minas Gerais, Brasil. **Boletim de Botânica da Universidade de São Paulo**, v. 26 n. 1, 2008.

MOTA, M. R. et al. The role of hybridization and introgression in maintaining species integrity and cohesion in naturally isolated inselberg bromeliad populations. **Plant Biology**, v. 21, p. 122–132, 2019.

MYERS, N. et al. Biodiversity hotspots for conservation priorities. **Nature**, v. 403, p. 853-858, 2000.

NISTELBERGER, H. et al. Strong phylogeographic structure in a millipede indicates pleistocene vicariance between populations on banded iron formations in semi-arid Australia. **PLoS ONE**, 2014.

OLIVEIRA-FILHO, A. T.; FONTES, M. A. L. Patterns of Floristic Differentiation among Atlantic Forests in Southeastern Brazil and the Influence of Climate1. **BIOTROPICA**, v. 32, n. 4b, p. 793-810, 2006.

PAGGI, G.M.; PALMA-SILVA, C.; BERED, F. Isolation and characterization of microsatellite loci in *Pitcairnia albiflos* (Bromeliaceae), an endemic bromeliad from the Atlantic Rainforest, and cross-amplification in other species. **Molecular Ecology Resources**,

v. 8, p. 980–982, 2008.

PALMA-SILVA, C. et al. A set of polymorphic microsatellite loci for *Vriesea gigantea* and *Alcantarea imperialis* (Bromeliaceae) and cross-amplification in other bromeliad species. **Molecular Ecology Notes**, v. 7, p. 654–657, 2007.

PALMA-SILVA, C. et al. Range-wide patterns of nuclear and chloroplast DNA diversity in Vriesea gigantea (Bromeliaceae), a neotropical forest species. **Heredity**, v. 103, n. 6, p. 503–512, 2009.

PALMA-SILVA, C. et al. Sympatric bromeliad species (*Pitcairnia* spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. **Molecular Ecology**, v. 20, n. 15, p. 3185–3201, 2011.

PALMA-SILVA, C. et al. Mating system variation and assortative mating of sympatric bromeliads (*Pitcairnia* spp.) endemic to neotropical inselbergs. **American Journal of Botany**, v. 102, n. 5, p. 758–764, 2015.

PALMA-SILVA, C. et al. Advances in and perspectives on evolution in Bromeliaceae. **Botanical Journal of the Linnean Society**, v. 181, n. 3, p. 305-322, 2016.

PAPADOPOULOU, A.; KNOWLES, L. L. Toward a paradigm shift in comparative phylogeography driven by trait-based hypotheses. **Proceedings of the National Academy of Sciences**, v. 113, n. 29, p. 8018-8024, 2016.

PASSAMANI, M. et al. Neotropical forest expansion during the last glacial period challenges refuge hypothesis. **Proceedings of the National Academy of Sciences**, v. 113, n. 4, p. 1008-1013, 2016.

PAZ, A. et al. Phylogeography of Atlantic Forest glassfrogs (*Vitreorana*): when geography, climate dynamics and rivers matter. **Heredity**, v. 122, n. 5, p. 545–557, 2019.

PENNINGTON, R. T. et al**.** Historical climate change and speciation: Neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. **Phil. Trans. R. Soc. Lond.B.** v. 359, n. 1443, p. 515–537, 2004

PERES, E. A. et al. Phylogeography of *Sodreaninae harvestmen* (Arachnida: Opiliones: Gonyleptidae): Insights into the biogeography of the southern Brazilian Atlantic Forest. **Molecular Phylogenetics and Evolution**, v. 138, n. May, p. 1–16, 2019.

PINHEIRO, F. et al. Phylogeography and genetic differentiation along the distributional range of the orchid *Epidendrum fulgens*: A neotropical coastal species not restricted to glacial refugia. **Journal of Biogeography**, v. 38, n. 10, p. 1923–1935, 2011.

PINHEIRO, F. et al. Rock outcrop orchids reveal the genetic connectivity and diversity of inselbergs of northeastern Brazil. **BMC Evolutionary Biology**, v. 14, n. 1, 2014.

PINHEIRO, F.; COZZOLINO, S. *Epidendrum* (Orchidaceae) as a model system for ecological and evolutionary studies in the Neotropics. **Taxon**, v. 62, n. 1, p. 77-88, 2013.

PIRY, S.; LUIKART, G.; CORNUET, J. M. BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. **Journal of Heredity**, v. 90, n. 4, p. 502-503, 1999.

POREMBSKI, S. Tropical inselbergs: habitat types, adaptive strategies and diversity patterns. **Revista Brasileira de Botânica**, v. 30, n.4, p. 579-586, 2007.

RAMOS, E. K. S. et al. Population genetics and distribution data reveal conservation concerns to the sky island endemic *Pithecopus megacephalus* (Anura, Phyllomedusidae). **Conservation Genetics**, v. 19, n. 1, p. 99–110, 2018.

RAYMOND, M.; ROUSSET, F. An Exact Test for Population Differentiation. **Evolution**, v. 49, n. 6, p. 1280-1283, 2006.

RIBEIRO, R. A. et al. Phylogeography of the endangered rosewood *Dalbergia nigra* (Fabaceae): Insights into the evolutionary history and conservation of the Brazilian Atlantic Forest. **Heredity**, v. 106, n, 1, p. 46-57, 2011.

RIDDLE, B. R. What is modern biogeography without phylogeography? **Journal of Biogeography**, v. 36, n. 1, p. 1-2, 2009.

RULL, V. Neotropical biodiversity: Timing and potential drivers. **Trends in Ecology and Evolution**, v. 26, n. 10, p. 508-513, 2011.

RULL, V. Neotropical diversification: historical overview and conceptual insights. **PeerJ Preprints**, 2018.

SANTOS LEAL, B. S. et al. Dispersal and local persistence shape the genetic structure of a widespread Neotropical plant species with a patchy distribution. **Annals of Botany**, v. 20, n. 1, p. 1–14, 2019.

SARAIVA, D. P.; MANTOVANI, A.; CAMPOSTRINI FORZZA, R. Insights into the Evolution of *Pitcairnia* (Pitcairnioideae-Bromeliaceae), Based on Morphological Evidence. **Systematic Botany**, v. 40, n. 3, p. 726–736, 2015.

SCARANO, F. R. Structure, function and floristic relationships of plant communities in stressful habitats marginal to the Brazilian Atlantic rainforest**. Annals of Botany**, v. 90, n. 4, p. 517–524, 2002.

SCARANO, F. R. Plant communities at the periphery of the Atlantic rain forest: Rare-species bias and its risks for conservation. **Biological Conservation**, v. 142, n. 6, p. 1201-1208, 2009.

SCHUBERT, K. **Systematik und Evolution der Gattung *Pitcairnia* L'Heritier (Bromeliaceae)**. Universität Kassel, 2017.

SCHÜTZ, N. et al. Phylogenetics of Pitcairnioideae s.s. (Bromeliaceae): evidence from nuclear and plastid DNA sequence data. **Botanical Journal of the Linnean Society**, 2016.

SILVA, G. A. R. et al. The impact of early Quaternary climate change on the diversification and population dynamics of a South American cactus species. **Journal of Biogeography**, v. 45, n. 1, p. 76–88, 2018.

SMITH, L. B.; DOWNS, R. J. **Flora Neotropica, Vol. 14, No. 3, Bromelioideae (Bromeliaceae)** 1979.

SPEZIALE, K. L.; EZCURRA, C. The role of outcrops in the diversity of Patagonian vegetation: Relicts of glacial palaeofloras? **Flora: Morphology, Distribution, Functional Ecology of Plants**, v. 207, n. 2, p. 141-149, 2012.

TAPPER, S. L. et al. Prolonged isolation and persistence of a common endemic on granite outcrops in both mesic and semi-arid environments in south-western Australia. **Journal of Biogeography**, v. 41, n. 11, p. 2032-2044, 2014.

TCHAICKA, L. et al. Phylogeography and population history of the crab-eating fox

(Cerdocyon thous). **Molecular Ecology**, v. 16, n. 4, p. 819-838, 2007.

THOMÉ, M. T. C. et al. Phylogeography of endemic toads and post-Pliocene persistence of the Brazilian Atlantic Forest. **Molecular Phylogenetics and Evolution**, v. 55, n. 3, p. 1018-1031, 2010.

TORRES, R. A.; RIBEIRO, J. The remarkable species complex Mimagoniates microlepis (Characiformes: Glandulocaudinae) from the Southern Atlantic Rain forest (Brazil) as revealed by molecular systematic and population genetic analyses. **Hydrobiologia.** v. 617, n. 1, p. 157–170, 2009.

TURCHETTO-ZOLET, A. C. et al. Phylogeographical patterns shed light on evolutionary process in South America. **Molecular Ecology**, v. 22, n. 5, p. 1193-1213, 2013.

TURCHETTO-ZOLET, A. C. et al. Phylogeography and ecological niche modelling in *Eugenia uniflora* (Myrtaceae) suggest distinct vegetational responses to climate change between the southern and the northern Atlantic Forest. **Botanical Journal of the Linnean Society**, v. 182, n. 3, p. 670–688, 2016.

VERGARA, J. et al. The disjunct pattern of the Neotropical harvestman Discocyrtus dilatatus (Gonyleptidae) explained by climate-driven range shifts in the Quaternary: Paleodistributional and molecular evidence. **PLoS ONE**, v. 12, n. 11, p. 1-32, 2017.

VERSIEUX, L. M. et al. Molecular phylogenetics of the Brazilian giant bromeliads (Alcantarea, Bromeliaceae): Implications for morphological evolution and biogeography. **Molecular Phylogenetics and Evolution**, v. 64, n. 1, p. 177-189, 2012.

WAGNER, N. D. et al. Reproduction biology and chloroplast inheritance in Bromeliaceae: a case study in *Fosterella* (Pitcairnioideae). **Plant Systematics and Evolution**, v. 301, n. 9, p. 2231–2246, 2015.

WENDT, T. et al. Recognition of *Pitcairnia corcovadensis* (Bromeliaceae) at the Species Level. **Systematic Botany**, v. 25, n. 3, p. 389-398, 2000.

WENDT, T. et al. Reproductive biology and natural hybridization between two endemic species of *Pitcairnia* (Bromeliaceae). **Am J Bot**, v. 88, n. 10, p. 1760–1767, 2001.

WÖHRMANN, T. et al.Development of microsatellite markers in *Fosterella rusbyi* (Bromeliaceae) using 454 pyrosequencing. **Am J Bot**, v. 99, n. 4, e160–e163, Mar., 2012.

WÖHRMANN, T.; WEISING, K. In silico mining for simple sequence repeat loci in a pineapple expressed sequence tag database and cross-species amplification of ESTSSR markers across Bromeliaceae. **Theoretical and Applied Genetics**, v. 123, n. 4, p. 635-647, Maio 2011

ZANELLA, C. M. et al. Hybridization between two sister species of Bromeliaceae: *Vriesea carinata* and *V. incurvata*. **Botanical Journal of the Linnean Society**, v. 181, n. 3, p. 491–504, 2016.

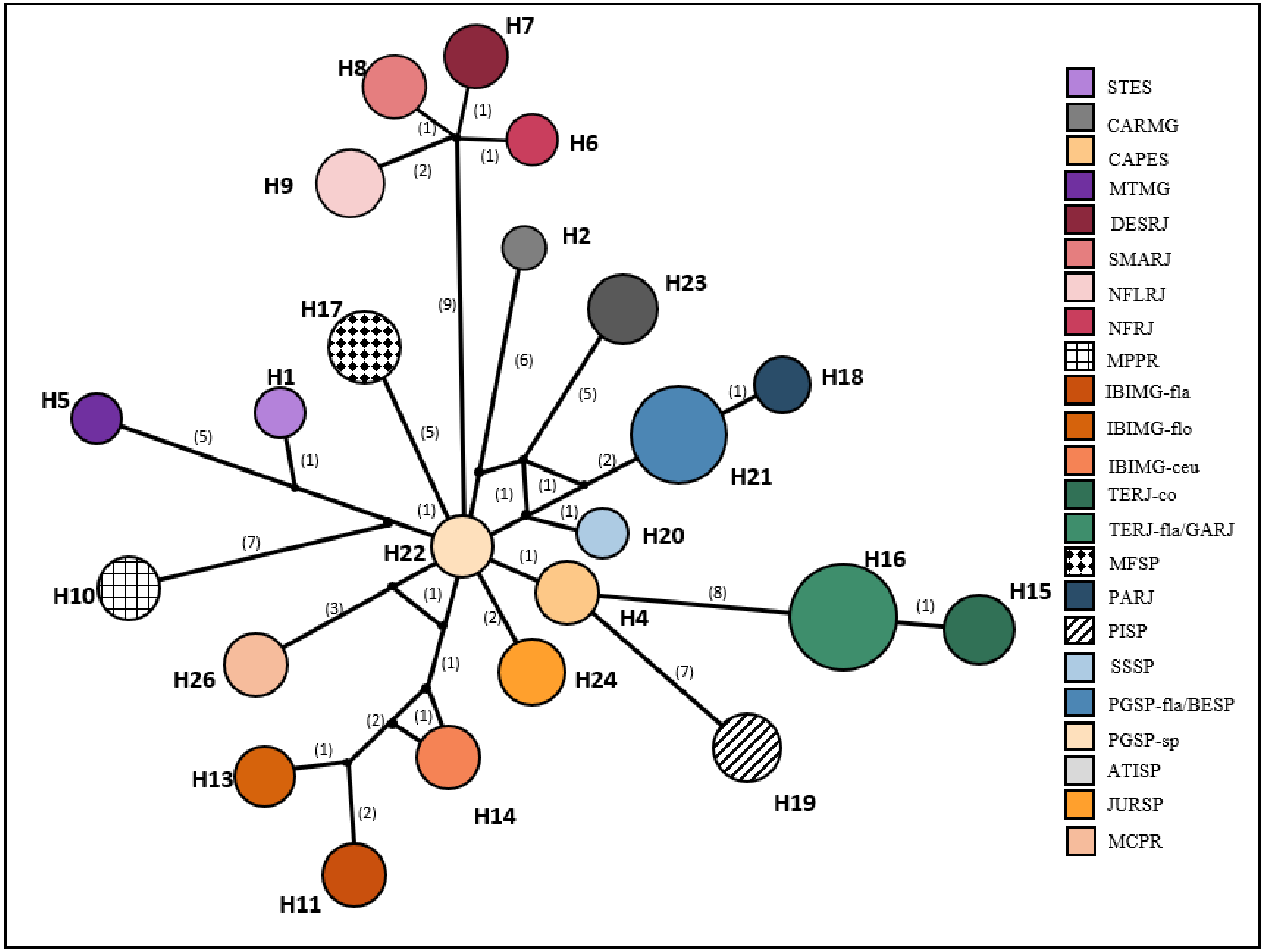


Figure 1. Median-joining network of cpDNA haplotypes of *P. flammea* complex. Filled circles indicate the haplotype. The size of each circle being proportional to the observed frequency of each haplotype and numbers represent the mutations steps between them.

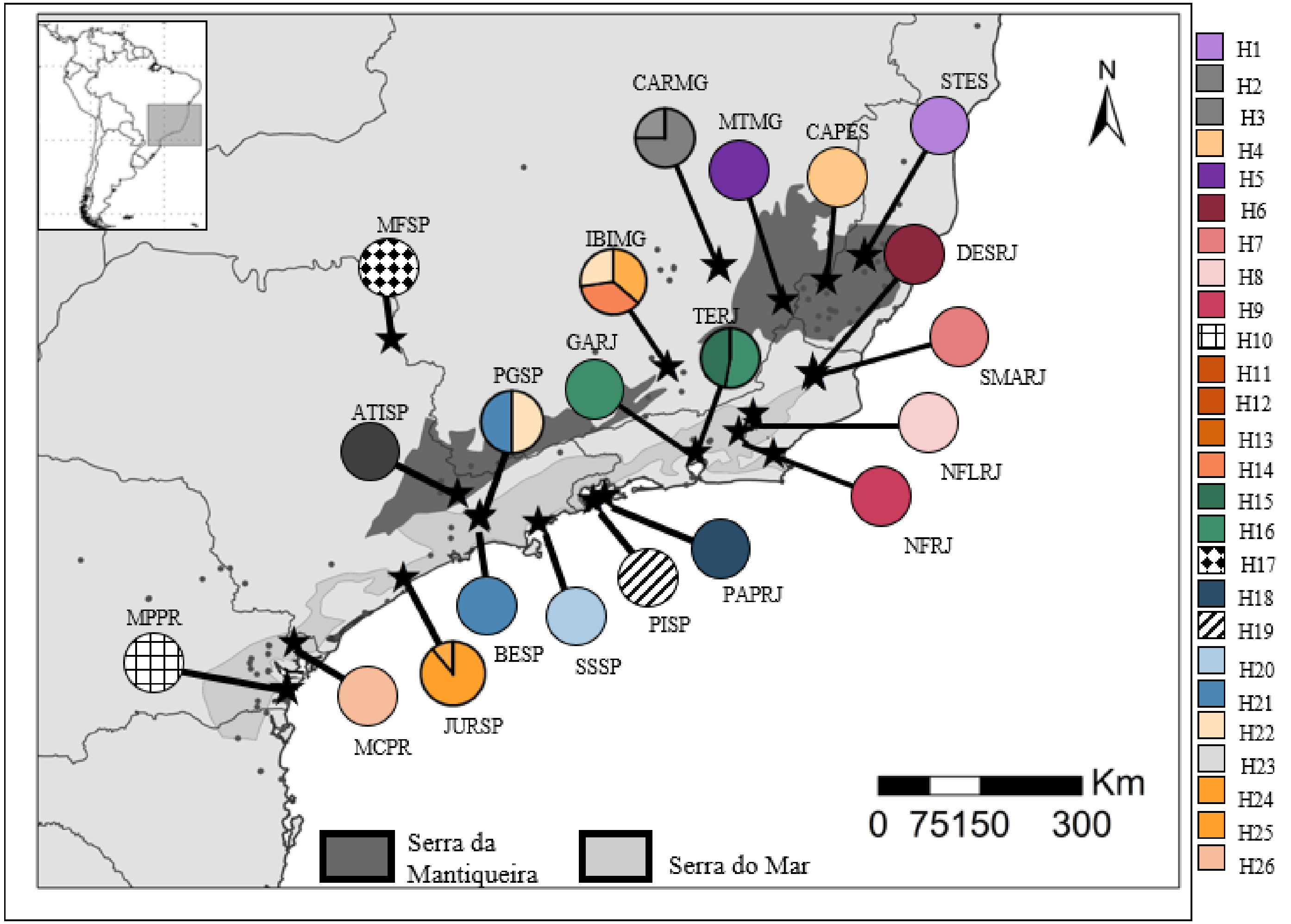


Figure 2. Geographic distribution of the cpDNA haplotypes. Records were obtained from GBIF (https://www.gbif.org/) and Species Link (http://splink.cria.org.br/) online databases and filtered according to the species checked distribution. Each haplotype assigned different colors and circle sections represents the haplotype frequency in each sampled population. The codes indicate the sampling localities (for full names of populations, see Table 2).

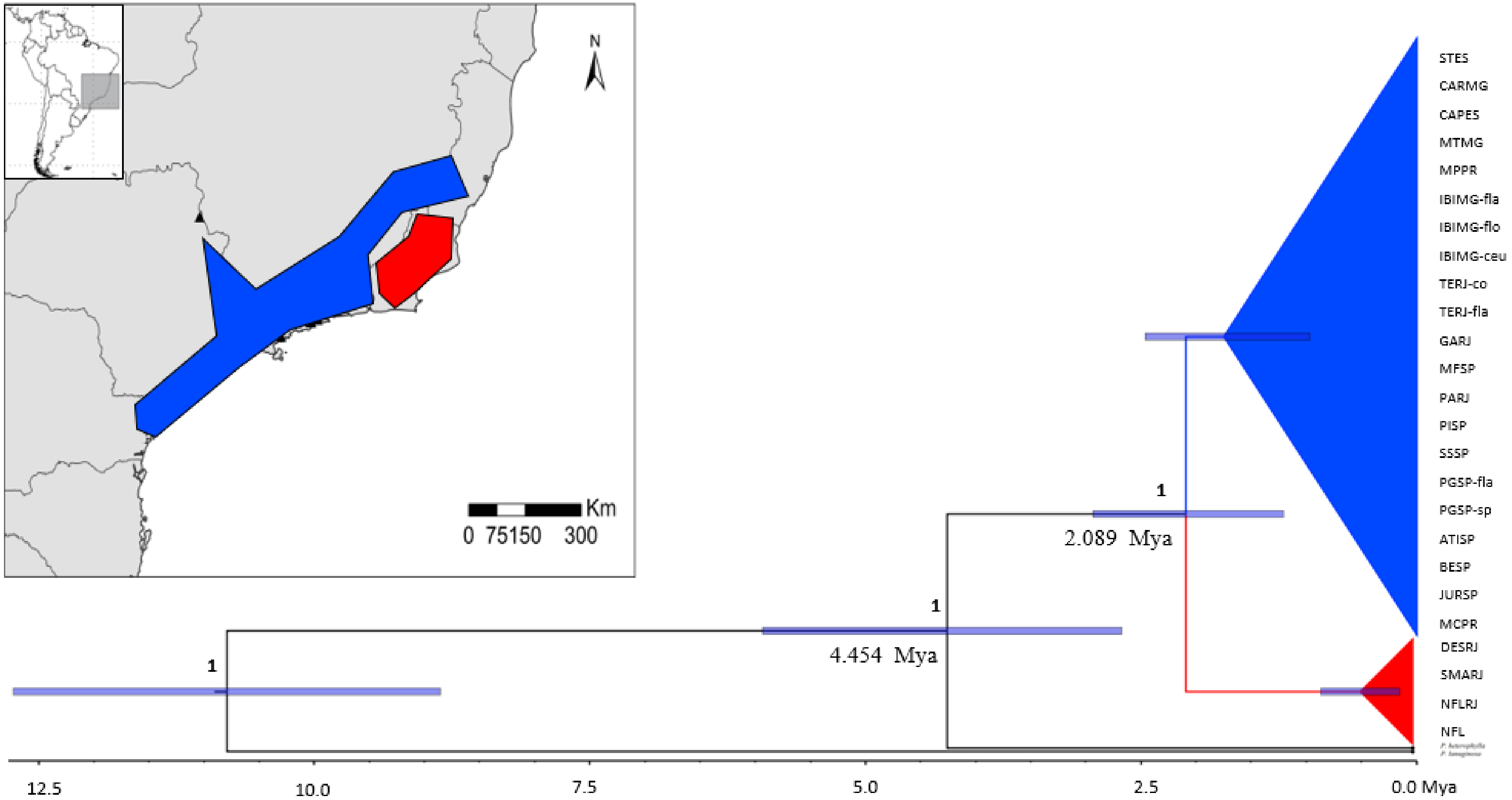


Figure 3. Species-tree resulting from the BEAST analysis of the cpDNA regions. Posterior probabilities are shown above the branches and estimated divergence times are shown with 95% HPD. Collapsed branches indicate the possible genetic lineages.

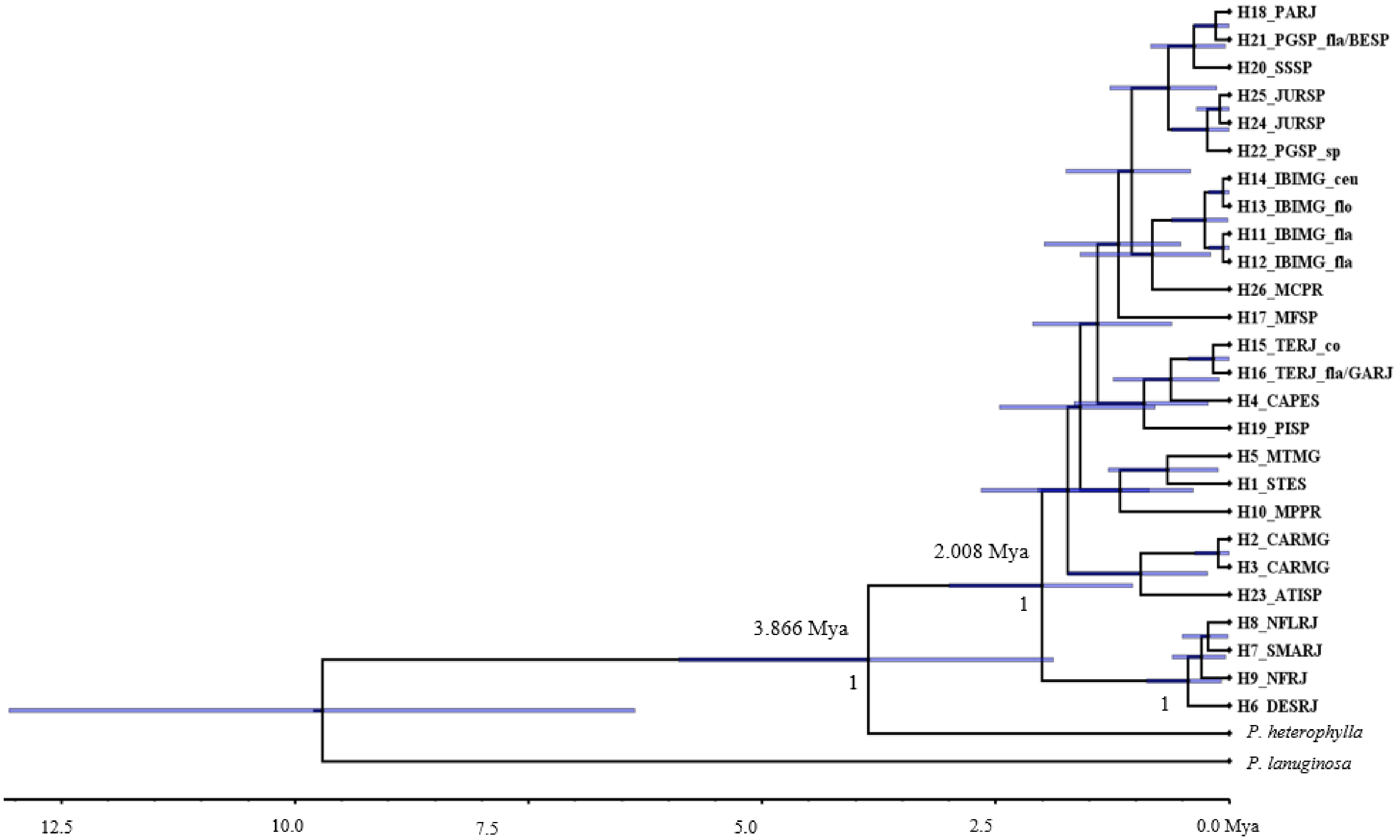


Figure 4. Bayesian phylogenetic tree of plastid DNA haplotypes with posterior probabilities (> 0.9) shown below the branches, and ages indicated for selected nodes (bars indicate 95% HPD). The time scale is in millions of years ago (Mya).

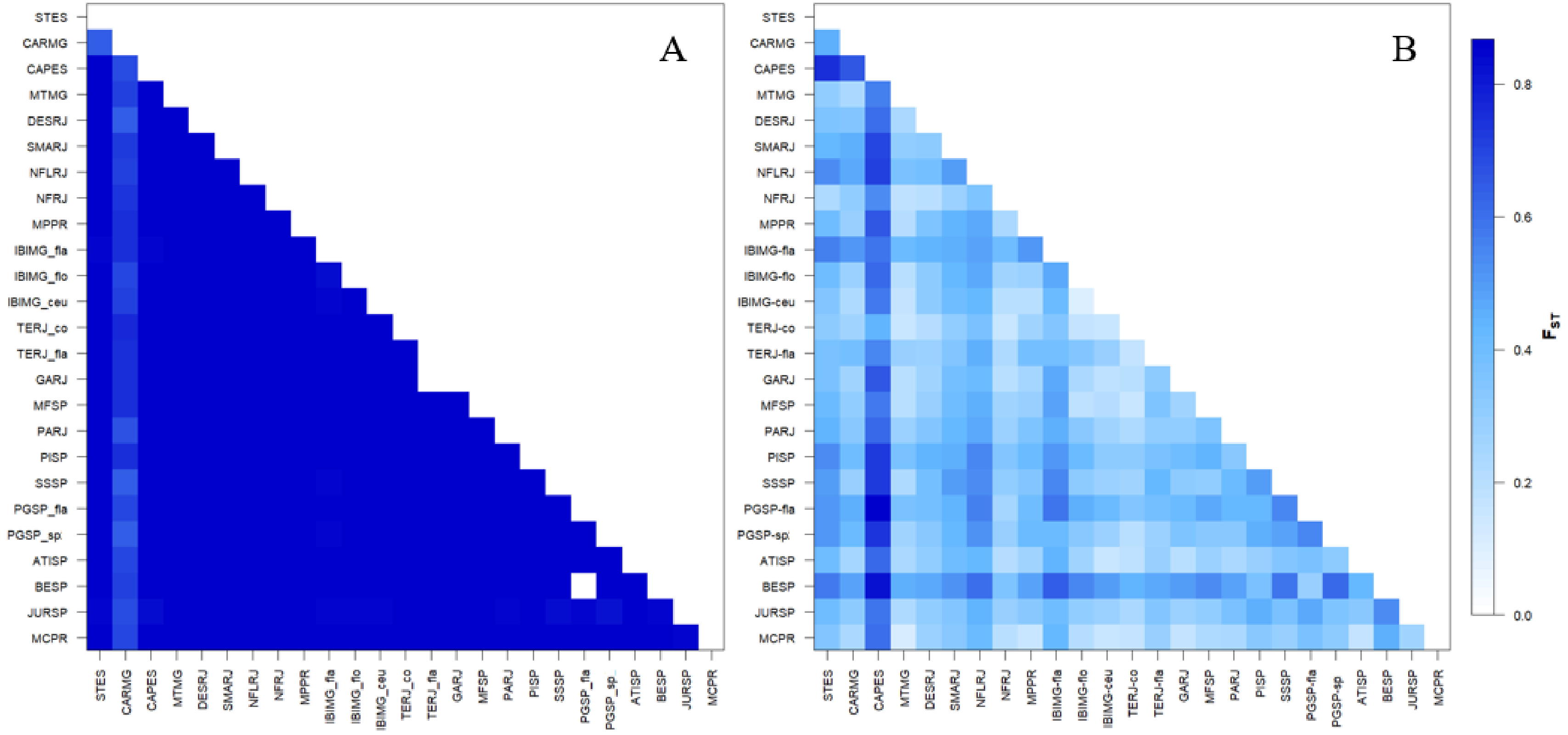
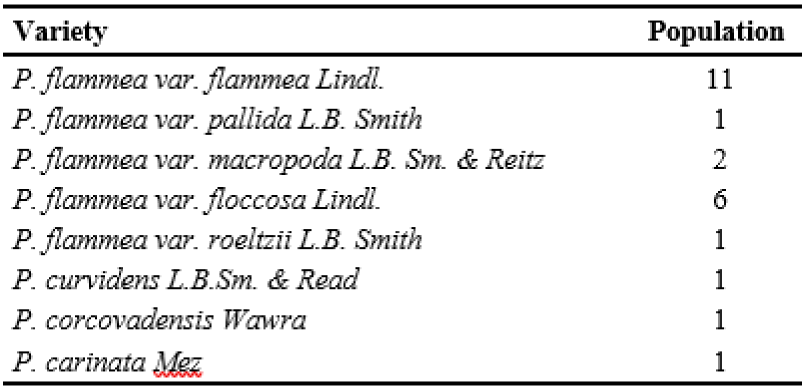


Figure 5. Pairwise F_{ST} between *P. flammea* populations for both (A) cpDNA and (B) nDNA.

Supplementary Table and Figure

Table S1. Varieties of the *Pitcairnia flammea* complex collected.



Variety	**Population**
P. flammea var. flammea Lindl.	11
P. flammea var. pallida L.B. Smith	1
P. flammea var. macropoda L.B. Sm. & Reitz	2
P. flammea var. floccosa Lindl.	6
P. flammea var. roeltzii L.B. Smith	1
P. curvidens L.B.Sm. & Read	1
P. corcovadensis Wawra	1
P. carinata Mez	1

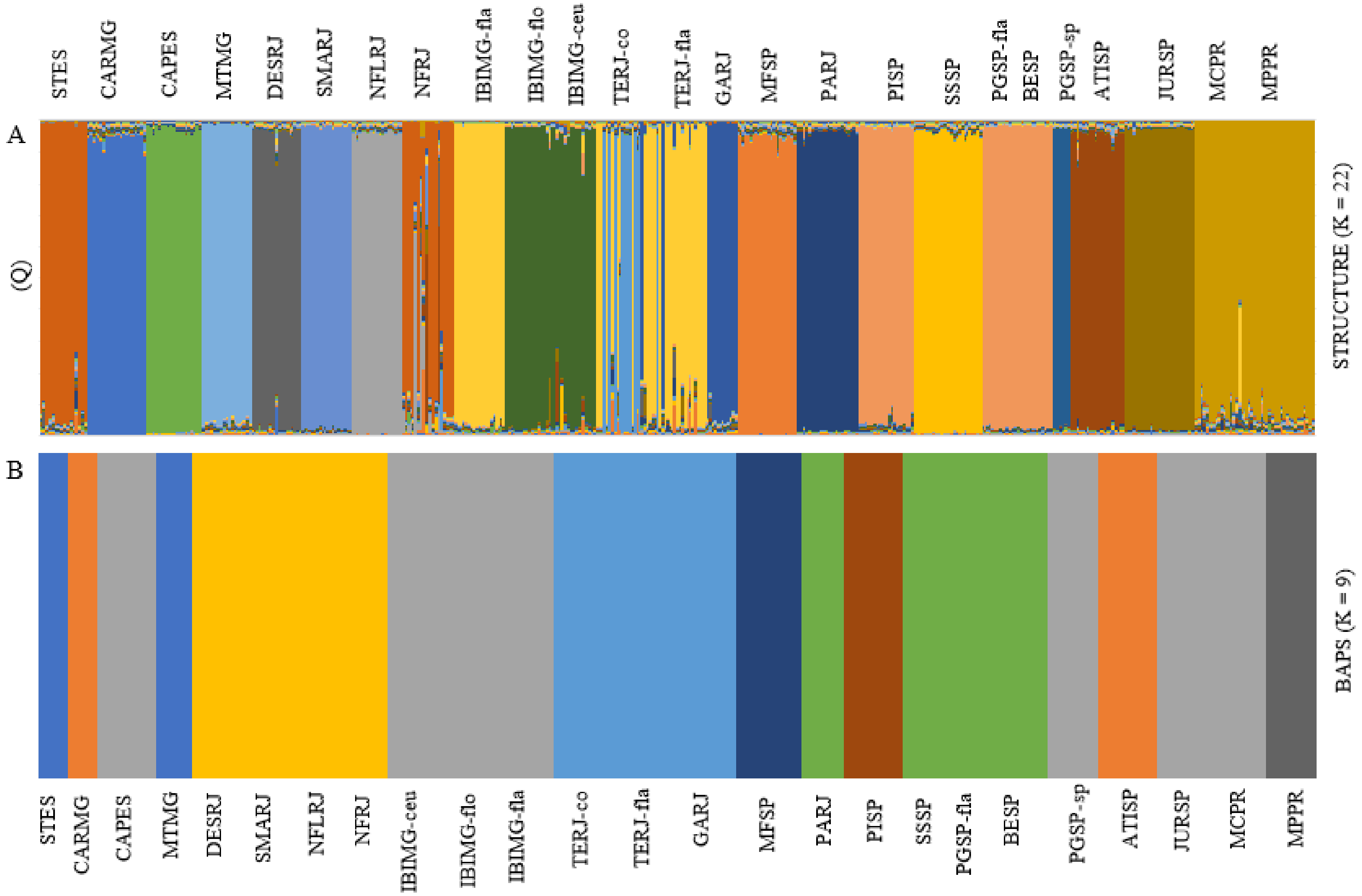


Figure S1. Clustering analyses of genomic variation across the range of *Pitcairnia flammea* populations. (A) Indicates results for STRUCTURE, with aa optimal K = 22, and (B) indicates results for BAPS, with an optimal partition of 9 groups. Individuals are arranged by population. Distinct colors represent distinct genetic groups. See Table 1 for abbreviations of populations.

CONSIDERAÇÕES FINAIS

Esta dissertação compõe um projeto amplo que engloba estudos que visam elucidar os mecanismos de diversificação e especiação na família Bromeliaceae; que têm como objetivo fornecer modelos importantes para o entendimento da dinâmica evolutiva de plantas do Neotrópico. O trabalho reúne dados genéticos que agregam informações sobre como os fatores históricos e evolutivos moldaram a distribuição geográfica e estrutura genética de populações em ambientes naturalmente fragmentados. Este estudo vem apoiar o fato de que afloramentos rochosos são centros de diversidade de espécies e endemismo, por serem naturalmente isolados, contribuindo para a grande diversidade genética e morfológica observada atualmente nestas regiões. A alta estruturação e diferenciação encontrada entre as populações simpátricas e alopátricas de *Pitcairnia. flammea* indicam pouco fluxo gênico e isolamento geográfico entre diferentes afloramentos rochosos, com grande efeito de deriva genética nos processos evolutivos deste grupo de plantas. Além disso, nosso estudo indica que a diversificação do grupo foi moldada pelas oscilações climáticas ocorridas no início do Pleistoceno, revelando o impacto das primeiras glaciações do Quaternário na distribuição e divergência populacional de espécies de plantas da América do Sul. Em conjunto com estudos de genética de populações e filogeografia de outros organismos, as informações aqui geradas ajudam a esclarecer os processos complexos responsáveis pela origem e manutenção da biodiversidade dos Neotrópicos, especificamente da Floresta Atlântica. Por fim, esforços futuros deverão ser direcionados de forma expandir o uso de dados genômicos, com técnicas de sequenciamento massivo que permitem uma abrangência maior do genoma, agregando dados ecológicos, como dispersão de sementes e sistema reprodutivo, dados fisiológicos, como amplitudes de tolerância térmica e hídrica, assim como dados morfológicos que podem disponibilizar informações sobre caracteres funcionais relacionados aos processos ecológicos e evolutivos, buscando melhorar a compreensão das dinâmicas demográficas e evolutivas dessas populações.