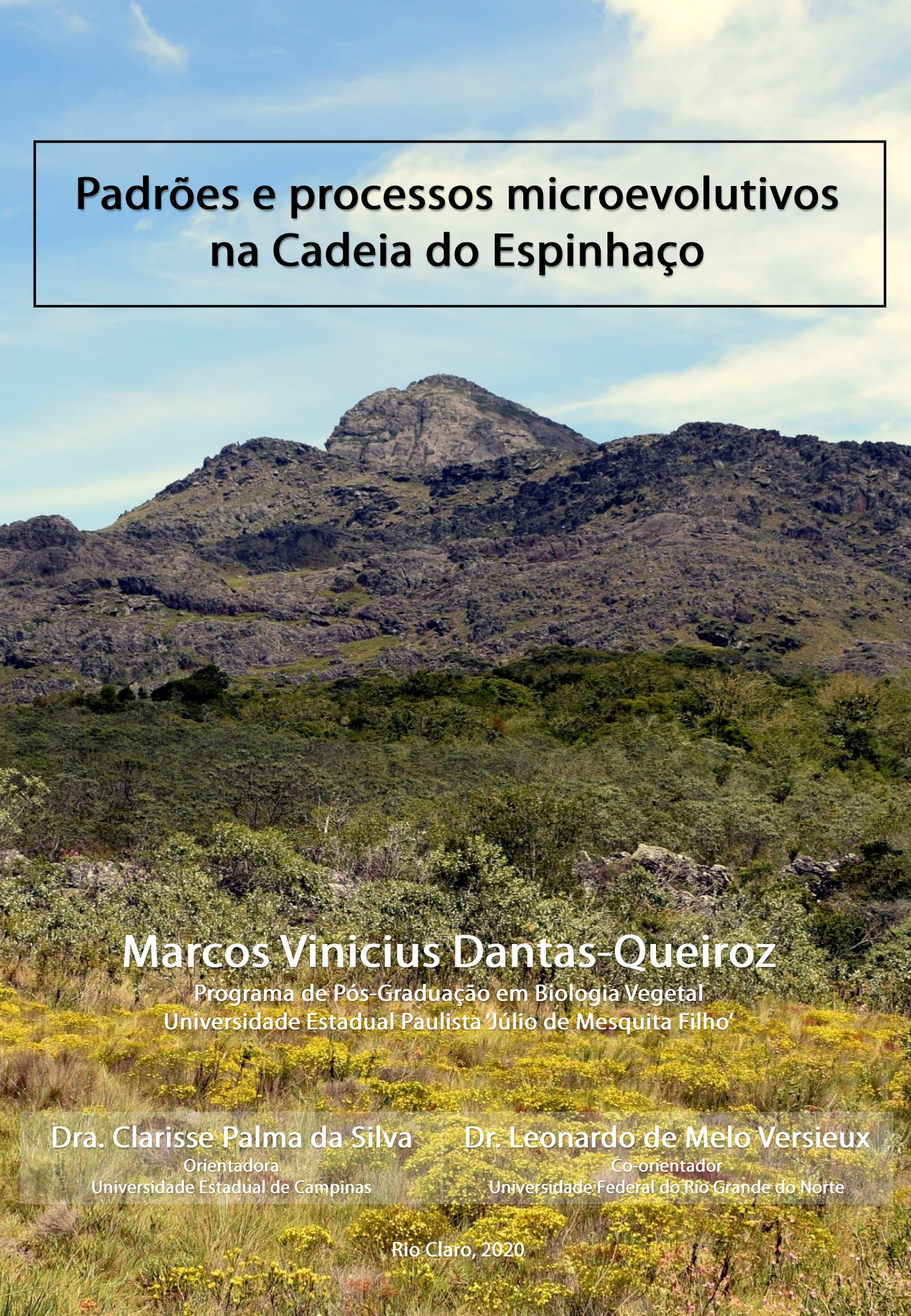
Padrões e processos microevolutivos na Cadeia do Espinhaço



Marcos Vinicius Dantas-Queiroz

Programa de Pós-Graduação em Biologia Vegetal
Universidade Estadual Paulista 'Júlio de Mesquita Filho'

Dra. Clarisse Palma da Silva
Orientadora
Universidade Estadual de Campinas

Dr. Leonardo de Melo Versieux
Co-orientador
Universidade Federal do Rio Grande do Norte

Rio Claro, 2020

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

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (BIOLOGIA VEGETAL)

PADRÕES E PROCESSOS MICROEVOLUTIVOS NA CADEIA DO ESPINHAÇO

MARCOS VINICIUS DANTAS-QUEIROZ

Tese apresentada ao Instituto de Biociências do *campus* de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de doutor em Ciências Biológicas (Biologia Vegetal).

Orientadora: Dra. Clarisse Palma-Silva
Co-orientador: Dr. Leonardo de Melo Versieux

Rio Claro – SP
2020

D192p

Dantas-Queiroz, Marcos Vinicius
Padrões e processos microevolutivos na Cadeia do Espinhaço / Marcos Vinicius Dantas-Queiroz. -- Rio Claro, 2020
209 p. : il., tabs., fotos, mapas

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, Rio Claro
Orientadora: Clarrise Palma-Silva
Coorientador: Leonardo de Melo Versieux

1. Botânica. 2. Evolução (Biologia). 3. Bromeliaceae. 4. Genética vegetal. 5. Ecologia das montanhas. I. Título.

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

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Rio Claro

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: PADRÕES E PROCESSOS MICROEVOLUTIVOS NA CADEIA DO ESPINHAÇO

AUTOR: MARCOS VINICIUS DANTAS DE QUEIROZ
ORIENTADORA: CLARISSE PALMA DA SILVA
COORIENTADOR: LEONARDO DE MELO VERSIEUX

Aprovado como parte das exigências para obtenção do Título de Doutor em CIÊNCIAS BIOLÓGICAS (BIOLOGIA VEGETAL), área: Biologia Vegetal pela Comissão Examinadora:

Profa. Dra. CLARISSE PALMA DA SILVA (Participaçao Virtual)

Departamento de Biologia Vegetal - Instituto de Biociências / UNICAMP - Universidade Estadual de Campinas / SP

Profa. Dra. CAROLINE TURCHETTO (Participaçao Virtual)
Departamento de Botânica - Instituto de Biociências / Universidade Federal do Rio Grande do Sul

Profa. Dra. ISABEL APARECIDA DA SILVA BONATELLI (Participaçao Virtual)
Campus Sorocaba / UFSCar - Universidade Federal de São Carlos - SP

Prof. Dr. MANOLO FERNANDO PEREZ (Participaçao Virtual)
Laboratório de Peixes / UFSCAR - São Carlos

Prof. Dr. MARCELO GEHARA (Participaçao Virtual)
Department of Earth and Environmental Sciences / Rutgers - University Newark

Rio Claro, 14 de dezembro de 2020

AGRADECIMENTOS

À minha orientadora Dra. Clarisse Palma da Silva pela paciência e dedicação nesta etapa tão importante da minha formação profissional.

Ao meu co-orientador Dr. Leonardo de Melo Versieux, pelas ideias iniciais de meu projeto de doutorado e por toda a ajuda fornecida durante minha breve passagem por Natal.

À Universidade Estadual Paulista, campus de Rio Claro, e ao Programa de Pós-Graduação em Biologia Vegetal pela infraestrutura concedida, as disciplinas ministradas e auxílios concedidos.

À minha família, meu pai Antonio Ribeiro de Queiroz, minha mãe Silvania Luiza Dantas de Queiroz, meu irmão Samuel Dantas de Queiroz e minha avó Otacília Luiza Dantas que foram fundamentais não apenas nestes quatros anos de doutorado, mas por toda minha vida; sem o apoio deles eu não seria o que sou hoje.

Aos meus companheiros unespianos, Dra. Bárbara Simões Santos Leal, Dr. Cléber Juliano Neves Chaves, Dra. Fernanda Hurbath Pita Brandão, Msc. Felipe Aoki-Gonçalves, Dr. Kléber Resende Silva e Msc. Mateus Ribeiro Mota, pela convivência, ajuda, confissões e apoios, sempre nos momentos oportunos.

Aos meus companheiros unicampianos, Dra. Bárbara Simões Santos Leal, Dr. Cléber Juliano Neves Chaves, Dr. Fábio Pinheiro, Msc. María Alejandra Buitrago Aristizábal, Marília Manuppella Tavares, Karolyne Wanessa de Jesus, Msc. Nathália Susin Streher, Dr. Pedro Joaquim Bergamo, Msc. Raquel Moura Machado, Tami da Costa Cacossi e Msc. Wagner Luiz dos Santos, pela atmosfera produtiva e dinâmica, principalmente nos bolos e pães vespertinos.

Ao meu supervisor de doutorado sanduíche, Dr. Bryan Carstens, e meus colegas de doutorado-sanduíche, Benjamin Stone, Msc. Cole Thompson, Drew Duckett, Danielle Parsons, Msc. Emanuel Masiero da Fonseca, Msc. Flávia Mol Lanna, Jackie Duckett, Dr. Jon Einar Flatnes, Dra. Lisa Barrow, Dra. Megan Smith, Msc. Rosa Rodriguez Pena e Sungsik “Kevin” Kong, pelos seis intensos e incríveis meses de aprendizado, convivência e diversão em Columbus, OH, EUA.

Aos meus *hosts* em Columbus, Peter Megert, Ursula Megert e Laptop “Lepo” Megert, pelo acolhimento, conversas e apoio durante minha estadia em sua residência nos EUA.

Aos meus amigos de graduação e pós-graduação Dr. Climbiê Ferreira Hall, Nuiawa Coelho e Msc. Rodolph Delfino Sartin pelas discussões embasadas em evidência, bom senso, clareza e humor, tão ausentes hoje em dia.

Aos meus amigos, Carlos Henrique Moreira Machado (“Carlão”), Dônovan Ferreira Rodrigues (“Don”) e Gabriel Lassi Bastos (“Biel”), pelas horas de gargalhadas remotas, conselhos necessários, pensamentos disruptivos e discussões-sem-pé-nem-cabeça-que-não-levam-ninguém-a-lugar-nenhum-mas-ainda-assim-são-legais-só-pelo-esporte.

À minha gata Canjica “Jijica” Pituca, por ser tão carinhosa conosco, ainda mais num período de quarentena, amaciando nossas preocupações com seu ronronar relaxante.

À minha amada noiva Msc. Lia Costa Pinto Wentzel, pelo amor, paciência, carinho e respeito compartilhados nestes últimos seis anos. Sem sua presença seria inviável permanecer nesta longa empreitada.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001

“We are going to die, and that makes us the lucky ones. Most people are never going to die because they are never going to be born. The potential people who could have been here in my place but who will in fact never see the light of day outnumber the sand grains of Arabia. Certainly, those unborn ghosts include greater poets than Keats, scientists greater than Newton. We know this because the set of possible people allowed by our DNA so massively exceeds the set of actual people. In the teeth of these stupefying odds it is you and I, in our ordinariness, that are here.

Richard Dawkins, *Unweaving the Rainbow: Science, Delusion and the Appetite for Wonder*

Man, he took his time in the sun
Had a dream to understand
A single grain of sand
He gave birth to poetry
But one day'll cease to be
Greet the last light of the library

We were here!

Nightwish, *The Greatest Show on Earth* from *Endless Forms Most Beautiful*

RESUMO GERAL

No Capítulo I, começamos a investigar o padrão genético de *V. oligantha*, selecionando algumas de suas populações para testar a compatibilidade de marcadores microssatélites já desenvolvidos para Bromeliaceae. Satisfatoriamente, conseguimos amplificar 24 loci, sendo 20 deles polimórficos. Assim sendo, selecionamos os dez loci mais polimórficos e genotipamos 229 indivíduos pertencentes a 14 populações para utilizarmos em nossas análises subsequentes. O Capítulo II trata da filogeografia de *V. oligantha*. Aos microssatélites nucleares obtidos, incluímos 95 indivíduos sequenciados usando dois marcadores plastidiais. Com esses dados em mãos, datamos a origem e a diversificação intraespecífica de *V. oligantha*, além de estimarmos a diversidade genética, a estrutura populacional e taxas de migração entre populações. Também modelamos a distribuição ancestral de *V. oligantha* utilizando Modelagem de Nicho Ecológico, inferindo possíveis corredores ancestrais entre as populações atualmente fragmentadas. Conseguimos notar uma alta estruturação genética, característica usual de organismos de ambientes naturalmente fragmentados; entretanto, a estruturação parece estar de acordo com o padrão biogeográfico, sugerindo assim como os processos microevolutivos envolvidos na diversificação de linhagens puderam resultar nos padrões de endemismo observados hoje no Espinhaço. Por fim, a modelagem de nicho ecológico confirma nossa suspeita de que, no Último Glacial Máximo (LGM), *V. oligantha* tinha uma adequabilidade muito maior, ocupando áreas mais baixas, conectando populações antes isoladas, conforme demonstramos nas análises de corredores. Para verificarmos mais a fundo o efeito das mudanças climáticas passadas na demografia e na diversificação das espécies das montanhas quartzíticas brasileiras (BQM), nós partimos para uma abordagem de filogeografia comparada, o pano de fundo do Capítulo III. Lá, buscamos na literatura organismos endêmicos destas montanhas e que tivessem dados genéticos depositados no Genbank. Também utilizamos os dados da própria *V. oligantha* e geramos mais sequências de duas espécies típicas das BQM mineiras e baianas: *Euphorbia attastoma* (Euphorbiaceae) e *Neoregelia bahiana* (Bromeliaceae). Ao final, além dessas três espécies, analisamos os dados das plantas *Lychnophora ericoides* e *Richterago discoidea* (Asteraceae), *Tibouchina papyrus* (Melastomataceae) e *Vellozia auriculata* (Velloziaceae) e também dois anuros, *Bokermmanohyla saxicola* (Hylidae) e *Pleurodema alium* (Leptodactylidae). Com estas nove espécies, verificamos a dinâmica populacional da comunidade, encontrando que grande parte delas passou por uma expansão síncrona durante o LGM, um sinal da força das mudanças climáticas na demografia nessas espécies endêmicas. Por outro lado, os dois anuros não seguiram este padrão, onde *P. alium* permaneceu constante enquanto *B. saxicola* teve um bottleneck durante o LGM. O padrão demográfico encontrado também se reflete na modelagem da adequabilidade de cada espécie para o presente e o LGM, com exceção de *B. saxicola*, que apresentou uma adequabilidade maior do que aquela estimada pelos nossos dados genéticos. Outra análise que fizemos foi comparar a diversificação interespecífica de espécies co-distribuídas do Espinhaço com os padrões biogeográficos para a região, onde encontramos um padrão congruente, mas ao adicionarmos espécies não exclusivas dali o cenário congruente se desfez. Dessa forma, os padrões de endemismo no Espinhaço parecem ser idiossincráticos, onde os processos microevolutivos ocorrentes ali modelaram os padrões biogeográficos existentes hoje.

Palavras-chave: Bromeliaceae, demografia, filogeografia, hABC, microssatélites, Último Glacial Máximo.

GENERAL ABSTRACT

In Chapter I, we investigated the genetic patterns of *V. oligantha*, selecting some of its populations to test the compatibility of previously developed microsatellites. Satisfactorily, we amplified 24 loci, although 20 of them were polymorphic. Thus, we selected the top ten most polymorphic loci and genotyped 229 individuals belonging to 14 populations. Chapter II is about the phylogeography of *V. oligantha.* With the nuclear microsatellites, we added 95 sequenced individuals, using two plastidial markers. With both datasets, we dated the origin and intraspecific diversification of *V. oligantha*, estimated its genetic diversity, population structure, and migration rates among populations. We also modeled the ancient distribution of *V. oligantha* using Ecological Niche Modelling, using it to inferring possible ancient corridors between the extant fragmented populations. We found a high genetic structure, a usual trait of species with a natural fragmented distribution; however, the structure seems to follow the biogeographic pattern, suggesting how the microevolutionary processes could shape the current patterns of endemism of the Espinhaço Range. Finally, the niche modeling confirmed our suspicion that, in the Last Glacial Maximum (LGM), the suitability of *V. oligantha* was wider than the current one, where it occupied lowlands, connecting isolated populations, as we showed with the corridor analysis. To deeply investigate the role of ancient climatic changes in the demography and diversification of species from the Brazilian Quartzitic Mountains (BQM), we used a comparative phylogeographic approach, the background of Chapter III. We conducted a literature survey seeking for endemic organisms of the BQM with available genetic data. Besides the data of *V. oligantha*, we also yielded sequences for more two species: *Euphorbia attastoma* (Euphorbiaceae) and *Neoregelia bahiana* (Bromeliaceae). Besides these three plant species, we incorporated genetic data of *Lychnophora ericoides* and *Richterago discoidea* (Asteraceae), *Tibouchina papyrus* (Melastomataceae), and *Vellozia auriculata* (Velloziaceae) and two anuran species, *Bokermmanohyla saxicola* (Hylidae) and *Pleurodema alium* (Leptodactylidae). Thus, with these nine species, we investigated their population dynamics, finding that most of them had a synchronous population expansion over the LGM, a result that supports the strength of climatic oscillations on the demography of these endemic species. Besides, we also compared the lineage diversification with the biogeographic patterns found in the Espinhaço Range, where a congruent diversification pattern was found. However, when we compared species non-endemic of the Espinhaço, this congruent pattern is blurred, suggesting that microevolutionary processes of the Espinhaço-endemic species are idiosyncratic.

Key-words: Bromeliaceae, demography, hABC, Last Glacial Maximum, microsatellites, phylogeography.

SUMÁRIO

PREFÁCIO 10

INTRODUÇÃO GERAL 15
MONTANHAS NEOTROPICAIS: ARENAS PARA A EVOLUÇÃO DE ESPÉCIES 15
MONTANHAS DO LESTE DA AMÉRICA DO SUL 17
MONTANHAS QUARTZÍTICAS BRASILEIRAS 20
BROMELIACEAE: UM MODELO PARA ESTUDOS FILOGEOGRÁFICOS NOS NEOTRÓPICOS 21
OBJETIVOS DA TESE 22
REFERÊNCIAS DA INTRODUÇÃO GERAL 25

CAPÍTULO I: TRANSFERABILITY OF NUCLEAR MICROSATELLITES MARKERS TO *VRIESEA OLIGANTHA* (BROMELIACEAE), AN ENDEMIC SPECIES FROM ESPINHAÇO RANGE, BRAZIL 31
1 INTRODUCTION 32
2 MATERIAL AND METHODS 34
3 RESULTS AND DISCUSSION 35
REFERENCES 37

CAPÍTULO II: UNDERLYING MICROEVOLUTIONARY PROCESSES PARALLEL MACROEVOLUTIONARY PATTERNS IN ANCIENT NEOTROPICAL MOUNTAINS 39
ABSTRACT 41
1 INTRODUCTION 42
2 MATERIAL AND METHODS 45
2.1 SAMPLING AND DNA EXTRACTION 45
2.2 PLASTIDIAL DNA SEQUENCING AND NUCLEAR MICROSATELLITE GENOTYPING 45
2.3 PHYLOGENETIC INFERENCES AND DIVERGENCE TIMES 49
2.4 GENETIC DIVERSITY 50
2.5 POPULATION STRUCTURE AND MIGRATION 50
2.6 PALEODISTRIBUTION AND ANCESTRAL POPULATION CONNECTIONS 51
2.7 DEMOGRAPHIC RECONSTRUCTION 51
2.8 ROLES OF CLIMATE AND GEOGRAPHY ON POPULATION STRUCTURE 52
3 RESULTS 53
3.1 PHYLOGENETIC INFERENCES AND DIVERGENCE TIMES 53
3.2 GENETIC DIVERSITY 53
3.3 POPULATION STRUCTURE 57
3.4 PALAEOCLIMATIC DISTRIBUTION AND ANCESTRAL POPULATION CONNECTIONS 60
3.5 DEMOGRAPHIC ANALYSES 60
3.6 RELATIVE ROLES OF CLIMATE AND GEOGRAPHY 61
4 DISCUSSION 65
4.1 DIVERSIFICATION OF *VRIESEA OLIGANTHA* 65
4.2 PHYLOGEOGRAPHY OF *VRIESEA OLIGANTHA*: INSIGHTS FROM MICRO TO MACROEVOLUTION IN THE ESPINHAÇO RANGE 66
4.3 MICROEVOLUTIONARY PROCESSES OF *VRIESEA OLIGANTHA* ARE DRIVEN BY GEOGRAPHIC DISTANCE AND CLIMATE GRADIENT 67
4.4 MICROEVOLUTIONARY PROCESSES AS A PROXY FOR UNDERSTANDING MACROEVOLUTIONARY PATTERNS 69
REFERENCES 70

CAPÍTULO III: CLIMATIC FLUCTUATIONS DROVE SYNCHRONIC DEMOGRAPHIC EXPANSION IN NEOTROPICAL ANCIENT MOUNTAINS 77
ABSTRACT 79
1 INTRODUCTION 80
2 MATERIALS AND METHODS 84

2.1 Data processing 84
2.2 Population structure 84
2.3 Demographic models 85
2.4 Co-expansion analysis 87
2.5 Current and ancient distribution modelling 88
2.6 Spatial phylogeographic patterns 89
3 RESULTS 91
3.1 Data retrieved 91
3.2 Population assignment and lineages-specific demography 91
3.3 Co-expansion analysis 92
3.4 Ecological niche modelling 96
3.5 Phylogeographic spatial patterns 96
4 DISCUSSION 99
4.1 Synchronic expansion and its consequences for speciation in the BQM 99
4.2 Diversity of demographic responses to climate changes 100
4.3 Phylogeographic spatial patterns 102
4.4 Beyond climatic oscillations 103
4.5 Conclusion and prospects 104
REFERENCES 106

CONSIDERAÇÕES FINAIS 116

ANEXOS 119

Materiais Suplementares do Capítulo II 119
S1 - Genbank accession number used for estimate of the Maximum clade credibility tree of Tillandsioideae. 119
S2 - Material and Methods of Paleodistribution and ancestral population Connections 124
S3 - Material and Methods of Roles of climate and geography on population structure 125
S4 – Supplementary Figures 126
S5 – Supplementary Tables 129
Supplementary References 136

Materiais Suplementares do Capítulo III 138
S1 - Supplementary Tables 138
S2 – Supplementary Figures 145
S3 – Supplementary Material and Methods 154
Supplementary References 156

Artigo: Plant species complexes as models to understand speciation and evolution: a review of South American studies 160

Artigo: Does habitat stability structure intraspecific genetic diversity? It's complicated… 188

PREFÁCIO

A trajetória desta tese começa nos idos de 2012, quando eu ainda era um mestrando do Instituto de Botânica de São Paulo. Naquele momento, minha vida profissional estava focada em entender a taxonomia de um grupo de Iridaceae, “queimando a retina” nas oculares de um microscópio ao medir e descrever grãos de pólen. Mas foi nos momentos de almoço no orquidário com o Dr. Climbiê Ferreira Hall que pela primeira vez ouvi falar sobre filogeografia. Pendurados por todo o prédio, havia pôsteres de congressos pregressos, repletos de mapas que ilustravam a diversidade e evolução dos *Epidendrum*, trabalhos do Dr. Fábio Pinheiro com sua companheira Dra. Clarisse Palma-Silva. E ali que fui fisgado: estudar a evolução na sua mais detalhada escala, na genética entre indivíduos e suas populações. Mas apesar de meu flerte com a filogeografia, ainda haveria algum tempo para amadurecer minhas ideias.

2014, fim do mestrado com muitas incertezas sobre o meu próprio futuro acadêmico. Mas felizmente tive uma “pausa”: em uma ensolarada Natal, repleta de gringos enrolados em bandeiras curtindo os jogos da Copa de Mundo de Futebol, passei em um concurso para professor substituto na Universidade Federal do Rio Grande do Norte. Durante um ano e meio como professor universitário tive também algumas oportunidades de auxiliar e orientar alguns alunos em pequenos projetos e, através de muita reflexão, concluí que era aquilo que eu gostava: ensinar e pesquisar sobre botânica, ecologia e evolução. Tive a sorte de contar com o apoio do Dr. Leonardo de Melo Versieux durante minha estada em Natal. Durante algumas conversas, ele comentou sobre os incríveis níveis de endemismo das bromélias dos campos rupestres brasileiros e como era necessário um melhor entendimento do porquê desse padrão.

Nesse momento, minha namorada Msc. Lia Costa Pinto Wentzel já estava em seu primeiro ano de mestrado na UNESP de Rio Claro, trabalhando com fungos da Antártida. E qual foi minha surpresa ao saber que a Dra. Clarisse Palma-Silva, aquela dos trabalhos de filogeografia, também estava trabalhando na mesma instituição, no Departamento de Ecologia. Assim, em uma das minhas primeiras visitas a Rio Claro, já entrei em contato com Clarisse, perguntando sobre um possível doutorado utilizando algumas bromélias que Leonardo já havia comentado.

Ela não apenas me deu forças para começar a escrever um projeto de doutorado, mas também foi super atenciosa ao me apresentar a todos os membros do que seriam então meus futuros colegas de doutorado.

Enfim decidi não continuar nos seis meses restantes de meu trabalho na UFRN. O namoro à distância e a saudade de Lia com certeza foi um fator decisivo para ir logo para Rio Claro, associado à vontade de começar o quanto antes minha pós-graduação. Nesse ínterim, conseguir uma bolsa não foi fácil. Entretanto, naquele primeiro semestre, Clarisse conseguiu uma bolsa de apoio técnico para mim; uma ajuda e tanto para alguém vivendo apenas do que conseguiu juntar em Natal. Durante este período, fui aprendendo pouco a pouco todas as técnicas, jargões e macetes presentes em um laboratório de biologia molecular: extração de DNA, gel de agarose, eletroforese, primers, PCR, sequenciamento, genotipagem, microssatélites... Graças à paciência de Msc. Felipe Aoki-Gonçalvez, Dra. Bárbara Simões Santos Leal, Dra. Fernanda Hurbath Pita Brandão e Dr. Cléber Juliano Neves Chaves, fui desbravando esse admirável mundo novo. Também foi nesse primeiro semestre de 2016 que fiz minha primeira viagem de campo aos campos rupestres baianos, para enfim conhecer as plantas que iriam me acompanhar durante os próximos quatros anos, as bromélias *Neoregelia bahiana* e *Vriesea oligantha*. Leonardo e o meu colega de pós-graduação, Dr. Kléber Resende Silva, que já estudava a anatomia das mesmas espécies, me acompanharam nessa primeira expedição.

Mas finalmente, com uma bolsa disponível pelo Programa de Pós-Graduação em Biologia Vegetal, me tornei oficialmente um aluno de doutorado em julho de 2016. E assim prossegui com os afazeres de um aluno de pós-graduação: escrever projetos para pleitear bolsas e *grants*, redigir manuscritos, cursar disciplinas, encontrar um estágio docência obrigatório, tudo isso enquanto conciliava com as horas de bancada. Devo ser sincero: as inúmeras tentativas de extração do DNA de minhas bromélias não me propiciaram uma experiência agradável. Seja por falta de sorte ou de experiência (ou um misto das duas coisas!), demorei bastante para conseguir uma quantidade de DNA adequada, fosse usando kits prontos ou protocolos *in house*. Mas finalmente, com muita insistência, conseguimos prosseguir para os sequenciamentos.

Pedidos de bolsas negadas, *grants* não concedidos. Foi difícil permanecer resoluto e impassível com o cenário econômico e científico brasileiro degringolando. Em janeiro de 2017 fiz mais uma expedição de coleta com Kléber para os campos rupestres mineiros e são em viagens de campo memoráveis como essa é que me dou conta do privilégio (um dos poucos, na verdade...) de morar num país tropical (abençoado por Deus?) com essa biota riquíssima. As paisagens deslumbrantes dos campos rupestres me deram um novo fôlego para continuar pesquisando os fatores que moldaram aquele ecossistema que encantam qualquer biólogo que ousa pisar em seus domínios.

O meio do doutorado se aproximava e os primeiros frutos de se trabalhar em um grupo dinâmico como o de Clarisse já surgiam. Aproveitando minhas leituras sobre complexos de espécies e novas técnicas de delimitação taxonômica, co-autorei conjuntamente com Clarisse e Fábio o primeiro artigo de meu doutorado (disponível nos Anexos desta tese). Durante esse período, perspectivas de um doutorado-sanduíche surgiam em minha mente e felizmente um edital PDSE (Programa de Doutorado-Sanduíche no Exterior) foi aberto em meu programa de pós. Com essa possibilidade em vista, entrei em contato com o Dr. Bryan Carstens, da Universidade Estadual de Ohio, nos EUA. Ele foi muito prestativo em querer conhecer melhor meu trabalho e topando me receber em seu laboratório, caso a bolsa fosse aprovada. E assim foi: no começo de 2018 uma bolsa de seis meses foi concedida para que eu pudesse passar uma temporada no laboratório do Dr. Carstens, onde eu iria desenvolver bibliotecas genômicas das duas espécies de bromélia, propiciando o sequenciamento de milhares de loci, o que deixaria os resultados de meu trabalho bem mais robustos. Dessa forma, o primeiro semestre daquele ano foi dedicado a tentar extrair o melhor DNA, da maior quantidade possível dos indivíduos já coletados. Em agosto, parti para meu doutorado-sanduíche.

A experiência de viver em um outro país foi maravilhosa. Conheci pessoas incríveis, de várias nacionalidades e com certeza fiz amigos que, apesar da distância, guardarei pra sempre. Mas as dificuldades de se extrair uma grande quantidade de DNA de boa qualidade no Brasil voltou para me assombrar. Realizei todos os procedimentos para preparar a biblioteca que, infelizmente, não foram suficientes para produzir os resultados tão aguardados. A empresa sul-coreana me

alertou para a baixa concentração do material e os riscos de prosseguir com o sequenciamento. Não quis correr o risco de perder o rico dinheirinho do projeto de Clarisse e abortei o plano. Mas minha experiência profissional não foi em vão: junto com Bryan e meus colegas de laboratório, realizamos muitas discussões sobre filogeografia, culminando enfim em um artigo publicado (também nos Anexos desta tese). Também foi lá que aprendi as primeiras lições sobre *Approximate Bayesian Computation* e melhorei minhas habilidades bioinformáticas, realizando várias análises em Python, R e até em Perl...

Quase ao final de minha estadia em Columbus, Lia me visitou nos EUA e fugimos das gélidas planícies de Ohio para as florestas de sequoias da Califórnia, um dos lugares mais incríveis que já visitei. Como botânico, estar imerso em uma "catedral de árvores" foi uma experiência indescritível. Foi também nessa floresta ancestral, onde, completamente sozinhos, declarei meu desejo de me casar com Lia: ela aceitou e fomos abençoados pela neblina úmida do Pacífico condensada em nossos olhos marejados.

Mas nem tudo foram flores: o frio e a falta de luz, somados aos sequenciamentos mal sucedidos no laboratório gringo estimularam um quadro depressivo em mim. O congelante janeiro de 2019 em Columbus se desfez em dias e noites improdutivas, enquanto eu tentava sair de minha cama. Mas enfim chegou o dia de minha volta para as terras tupiniquins. A luz do sol do verão brasileiro com certeza me ajudou um pouco, mas não foi o suficiente. Naquele momento, Clarisse já havia se mudado para Campinas, tomando posse de sua vaga na UNICAMP, enquanto meus amigos de laboratório também já haviam defendido suas teses. Dias e dias sozinhos no laboratório deserto, em um Departamento de Ecologia também praticamente às moscas não é uma experiência muito agradável. Observando minha situação, Lia me aconselhou a consultar um psiquiatra. De fato, era depressão. Felizmente, o contínuo tratamento vem dado resultados positivos até os dias de hoje.

Em junho de 2019, resolvemos ficar mais próximos de Clarisse e assim nos mudamos para Campinas. A dinâmica funcionaria bem melhor agora com a presença da orientadora por perto, além dos velhos amigos Cléber e Bárbara, agora como pós-docs, no laboratório de Clarisse e Fábio. E foi a melhor decisão que

poderíamos ter tomado até então, pois já nos primeiros dias como campineiros fomos agraciados pelo “achado” de Cléber e Bárbara: perambulando pelo Parque do Taquaral, uma gatinha perdida foi acolhida por nós. Canjica, foi assim então batizada.

A vivaz atmosfera do novo laboratório, com a presença de novos colegas, foi um estímulo e tanto. Discussões acadêmicas mescladas com reuniões regadas a bolos vespertinos devem fazer parte de qualquer laboratório que preze pela saúde mental de seus membros. A não ser é claro que tenhamos uma pandemia global: aí fica difícil manter essas atividades. E aqui começa o ano de 2020, com o distanciamento obrigatório pela pandemia causada pelo vírus SARS-CoV-2. Apesar da falta que as pessoas fazem, os trabalhos foram concluídos na medida do possível, através de dezenas de reuniões remotas, e-mails trocados e Whatsapps respondidos.

Qualquer um que já se aventurou no mundo acadêmico sabe o quão difícil é perseguir esta carreira e meu percurso durante estes quatro anos de doutorado não foi diferente. Felizmente tive pessoas queridas que puderam me apoiar, aconselhar, criticar (sim, é preciso!) e motivar durante todo o período. Sem elas, não seria possível concluir esta tese que agora está em suas mãos. Boa leitura!

INTRODUÇÃO GERAL

Montanhas Neotropicais: arenas para a evolução de espécies

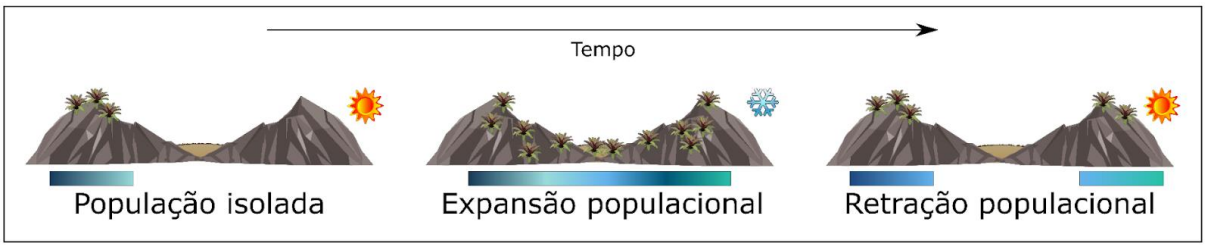
A região Neotropical é notavelmente reconhecida por sua alta diversidade biológica (ANTONELLI; SANMARTÍN, 2011; HUGHES; PENNINGTON; ANTONELLI, 2013; GUEDES et al., 2020). E dentro desta exuberante área, formações montanhosas usualmente se destacam ainda mais por abrigarem uma alta biodiversidade, com muitas espécies endêmicas (ANTONELLI et al., 2009; LAGOMARSINO et al., 2016; ALCANTARA; REE; MELLO-SILVA, 2018), um padrão já notado pelos primeiros naturalistas europeus do século XVIII (HUMBOLDT, 1807). A formação da Cordilheira dos Andes, por exemplo, alterou drasticamente a dinâmica populacional neotropical, causando múltiplos eventos de vicariância, que por sua vez levaram a abundantes episódios de especiação alopátrica e adaptação radiativa (GIVNISH et al., 2004; ANTONELLI et al., 2009; SEDANO; BURNS, 2010). Dessa forma, o soerguimento dos Andes tornou possível a emergência de novos nichos ecológicos ao gerar um gradiente altitudinal expressivo, uma característica importante em ambientes montanhosos que permite suportar a presença de tantas espécies em uma área relativamente restrita (MADRIÑÁN; CORTÉS; RICHARDSON, 2013; FLANTUA et al., 2020). Apesar dos Andes serem a maior cadeia de montanhas da América do sul, outras formações geológicas também se destacam pela sua impressionante riqueza de espécies, como por exemplo os Tepuis, no norte da América do Sul e montanhas do leste brasileiro (SILVEIRA et al., 2016; RULL et al., 2019).

Dada a origem pré-Cambriana das montanhas do leste do Brasil e sua relativa estabilidade orogênica desde o Mioceno (PEDREIRA; DE WAELE, 2008), fenômenos orogênicos possuem relativamente baixa importância em detrimento a outros fatores ambientais na formação dos padrões de biodiversidade e endemismo destas montanhas. Nos últimos dois milhões de anos, várias oscilações climáticas ocorreram por todo o globo, influenciando diretamente a dinâmica populacional especialmente das espécies de montanhas (DOWSETT et al., 2012; MARTÍNEZ-BOTÍ et al., 2015). As espécies adaptadas a ambientes montanhosos normalmente possuem uma tolerância ambiental bem restrita, onde temperaturas baixas estão diretamente associadas ao ganho de altitude (PERRIGO; HOORN; ANTONELLI,

2020). Assim, em um período de temperaturas mais baixas (i.e., períodos glaciais), essas espécies tenderiam a modificar sua distribuição para altitudes mais baixas, em direção ao seu ótimo ecológico, enquanto que em períodos de temperaturas mais altas (i.e., períodos interglaciais), as espécies ficariam restritas aos topos das montanhas, onde encontrariam temperaturas mais propícias a sua sobrevivência. Estes inúmeros ciclos de expansões e contrações populacionais são chamados de "conectividade oscilante" (*flickering connectivity*; Flantua; Henry, 2018; Flantua et al., 2019) e favoreceriam, portanto, diversos pulsos de conexão e fragmentação entre populações, propiciando a atuação de processos microevolutivos nestas espécies (Figura 1).

Em um cenário de prolongada fragmentação populacional com baixo (ou nenhum) fluxo gênico, as populações potencialmente se diferenciariam por meio da deriva genética e também através da seleção natural de indivíduos adaptados às pressões ecológicas locais. Ao retomar uma conexão durante períodos mais favoráveis, este novo contato entre populações poderia tanto eliminar as diferenças acumuladas ao longo do tempo, diminuindo a diversidade genética, quanto disseminar novas variantes genéticas entre populações distintas, aumentando a diversidade genética intrapopulacional (PALMA-SILVA et al., 2011; MOTA et al., 2019, 2020; DUCHEN et al., 2020; MAGALHÃES et al., 2020). Caso o isolamento reprodutivo entre populações divergentes tenha sido completo, o processo de especiação entre duas linhagens terá sido alcançado (LEAL et al., 2016; FERRIS; WILLIS, 2018; MOTA et al., 2020). Esta intricada dinâmica evolutiva é conhecida como "pulsação evolutiva" (*species-pump*; Haffer, 1969) ou "pulsação da biodiversidade" (*biodiversity pump*; Rull, 2005).

Figura 1 - Modelo esquemático do *flickering connectivity*, fenômeno ocasionado pelas oscilações climáticas ao longo do tempo. Uma população isolada em uma montanha, durante um período interglacial (representado pelo sol), possui uma dada constituição genética (representado pela barra em gradiente). Conforme o clima se torna mais frio (representado pelo floco de neve), as populações buscam seu ótimo ecológico, expandindo-se para altitudes menores. Dessa forma a espécie hipotética consegue alcançar outros ambientes. Durante o período de isolamento geográfico, caso haja pouco (ou nenhum) fluxo gênico entre as populações, a deriva genética e também a seleção natural contribuem para a diferenciação genética entre as populações. Repetidos ciclos de expansão e retração podem, eventualmente, dar origem a linhagens divergentes que já não conseguem se reproduzir, originando novas espécies. Este fenômeno é conhecido como *species pump*.



Fonte: o autor.

Montanhas do leste da América do Sul

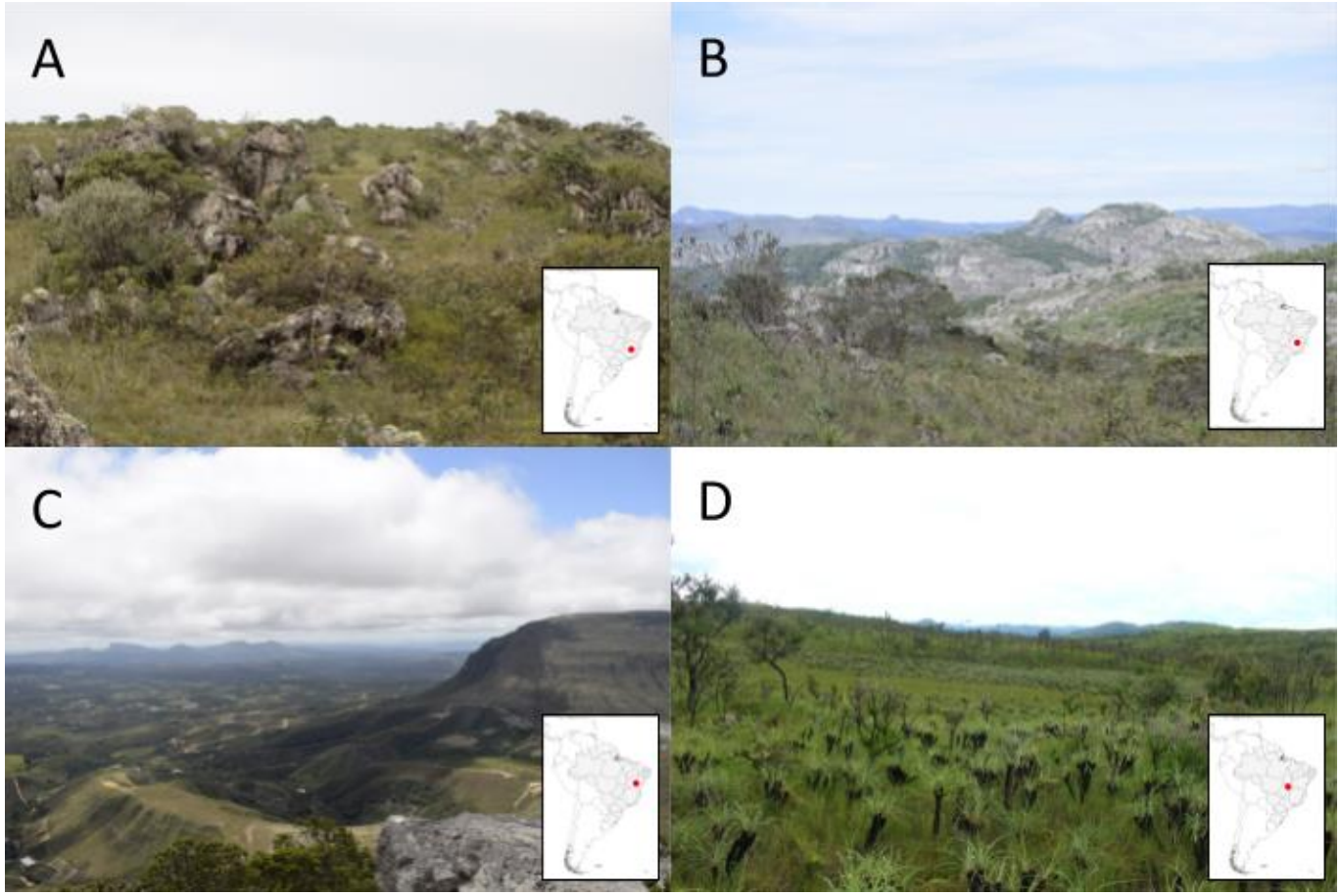
Apesar da relativa estabilidade geomorfológica das montanhas pré-cambrianas do leste da América do Sul e o papel das mudanças climáticas no desenvolvimento de sua biota (SCHAEFER et al., 2016), os estudos biogeográficos realizados em diferentes montanhas desta região apontam padrões distintos. Por exemplo, a história da biota das montanhas graníticas do sudeste brasileiro, como as Serra da Mantiqueira e do Mar, exibe uma similaridade florística e faunística com outras vegetações típicas de montanha e de ambientes temperados, como os *páramos* andinos (SAFFORD, 2007; CHAVES et al., 2015). Desta forma, a história evolutiva da biota dessas montanhas está mais relacionada àquela região Patagônica-Chacoana (FIASCHI; PIRANI, 2009; CHAVES et al., 2015).

Por outro lado, a história evolutiva da biota das montanhas quartzíticas brasileiras, como a Cadeia do Espinhaço e demais serras isoladas no Platô do Brasil Central (e.g., Serra dos Pireneus e Chapada dos Veadeiros) (Figura 2), está intimamente relacionada com os biomas que as circundam (i.e., o Cerrado, a Caatinga e a Mata Atlântica) (NEVES et al., 2018; COLLI-SILVA; VASCONCELOS; PIRANI, 2019). Neste contexto, estas montanhas apresentam-se como um excelente modelo para testar o papel do *species-pump* na dinâmica demográfica das espécies

ao longo do tempo, como também para verificar a influência das oscilações climáticas na formação de sua biota.

.

Figura 2 - Áreas de campos rupestres. A) Típica formação de campo rupestre, com afloramentos rochosos e vegetação majoritariamente herbáceo-arbustiva, no Parque Estadual do Biribiri (Diamantina – MG). B) Campo rupestre em área quartzítica (montanhas ao fundo), em Serro – MG. C) Contraste altitudinal entre uma montanha quartzítica e o entorno, em Barra da Estiva – BA. D) Campo rupestre no Platô Central Brasileiro, na Chapada dos Veadeiros (Alto Paraíso de Goiás – GO).



Fonte: Foto D por Rodolph Delfino Sartin. As demais pertencem ao autor.

Montanhas Quartzíticas Brasileiras

As Montanhas Quartzíticas Brasileiras (*Brazilian Quartzitic Mountains*, BQM) abrigam um dos mais biodiversos ecossistemas no planeta, os campos rupestres (CONCEIÇÃO; PIRANI, 2016; VASCONCELOS et al., 2020). Este ecossistema é encontrado primariamente acima dos 900 m de altitude nas BQM, sendo constituído por uma vegetação xerófita, dominada por espécies herbáceas e arbustivas. As plantas dos campos rupestres geralmente apresentam diversas adaptações para sobreviver aos solos rasos e pobres em nutrientes, mas também para suportar a alta incidência solar somada a uma intensa variação de temperatura ao longo do dia (GIULIETTI; PIRANI, 1988; ZAPPI et al., 2017). Os campos rupestres tem atraído a atenção de gerações de cientistas interessados em entender e desvendar os processos evolutivos que puderam gerar esta incrível biota, sendo considerados a estabilidade geológica juntamente com as oscilações climáticas do passado os fatores preponderantes que levaram à alta biodiversidade endêmica destas montanhas (ANTONELLI et al., 2010; RIBEIRO et al., 2014; BARRES et al., 2019).

A Cadeia do Espinhaço, localizada no leste do Brasil, é uma das principais formações quartzíticas e abriga a maior parte da vegetação de campos rupestres do Brasil (SILVEIRA et al., 21016). Nestas montanhas, padrões de endemismo associados a diferentes áreas já foram revelados por estudos anteriores (ECHTERNACHT et al., 2011; CHAVES et al., 2015; CAMPOS et al., 2019), levando ao reconhecimento de regiões biogeográficas distintas, como na sua porção setentrional, a província da Chapada Diamantina, e na porção centro-sul, a província do Sul do Espinhaço, essa última ainda subdividida em três distritos: Grão-Mogol, Platô de Diamantina e Quadrilátero Ferrífero (COLLI-SILVA; VASCONCELOS; PIRANI, 2019).

Mas será que as flutuações climáticas do passado foram capazes de moldar esses padrões biogeográficos atuais? Seguindo a hipótese da *flickering connectivity*, se os ciclos de contração e retração estão envolvidos nos padrões biogeográficos do Espinhaço, então seria esperado observar processos microevolutivos (e.g., deriva genética, fluxo gênico restrito e seleção natural) agindo a nível populacional, acarretando nos primeiros passos para a divergência entre linhagens (LI et al., 2018) (Figura 1).

Bromeliaceae: um modelo para estudos filogeográficos nos Neotrópicos

A família Bromeliaceae é reconhecida como um excelente exemplo de radiação adaptativa na região Neotropical (BENZING, 2000; GIVNISH et al., 2014). O metabolismo fotossintético CAM, os tricomas peltados, o epifitismo, a polinização por aves e as folhas em forma de tanque capazes de armazenar água são exemplos de inovações-chave que possibilitaram as Bromeliaceae colonizar diversos ambientes em curto período de tempo (GIVNISH et al., 2014; SILVESTRO et al., 2014).

As Bromeliaceae possivelmente alcançaram o leste do Brasil através de eventos de dispersão oriundos dos Andes há ca. 8.5 milhões de anos atrás (GIVNISH et al. 2011). De acordo com esta hipótese, a circulação das massas de ar vindas do Oceano Pacífico naquele período foi bloqueada pelo soerguimento dos Andes e o clima no leste do Brasil se tornou mais ameno e chuvoso com a elevação da Serra do Mar e a consequente estagnação da umidade vinda do Oceano Atlântico (DE ALMEIDA; CARNEIRO, 1998; INSEL et al., 2009). Este clima proporcionou um cenário ideal para a proliferação e radiação de bromélias na Floresta Atlântica, colonizando posteriormente áreas adjacentes, como os campos rupestres do Espinhaço (GIVNISH et al. 2011; VERSIEUX et al. 2012).

Na cadeia do Espinhaço, diversos levantamentos florísticos (RAPINI et al. 2008) demonstraram que a família Bromeliaceae possui um elevado número de espécies endêmicas, sendo os campos rupestres do Espinhaço considerados como um dos centros de diversificação para a família (VERSIEUX et al. 2008). Contudo, ainda não há estudos que indiquem quais fatores desencadearam a abundância e o endemismo deste grupo no Espinhaço. Assim, entender quais processos estão envolvidos na distribuição atual e prévia de Bromeliaceae para o Espinhaço possibilitaria testar hipóteses biogeográficas ocorrentes não apenas para a família, como também para a biota do Espinhaço como um todo.

Dessa forma, nós utilizamos abordagens filogeográficas para compreender o papel das oscilações climáticas do passado na formação dos padrões de estruturação genética e na diversificação da biota das BQM. Para tanto, nós selecionamos uma espécie endêmica e distribuída por todo o Espinhaço, a bromélia *Vriesea oligantha* (Figura 3) como modelo para investigar os fatores que moldaram a

diversidade e estruturação genética da biota da Cadeia do Espinhaço. Além disso, também buscamos outros organismos endêmicos do Espinhaço (e de outras áreas de campos rupestres do Brasil) para avaliar o papel que as oscilações climáticas do passado tiveram na demografia desta comunidade como um todo. Com essas duas abordagens combinadas, tentamos compreender os padrões de diversificação da Cadeia do Espinhaço.

Objetivos da Tese

Capítulo I:

(i) testar e otimizar marcadores moleculares microssatélites nucleares informativos em *V. oligantha*;

(ii) verificar os níveis de polimorfismos em diferentes populações;

Capítulo II:

(iii) investigar a importância das mudanças climáticas do fim do Plioceno e início do Pleistoceno na divergência intraespecífica de *V. oligantha*;

(iv) revelar os padrões da diversidade e estruturação genética de *V. oligantha*, bem como inferir sua dinâmica demográfica utilizando marcadores nucleares e plastidiais e modelagem de nicho ecológico;

(v) testar se a estruturação populacional de *V. oligantha* é fruto do isolamento por distância ou isolamento ambiental;

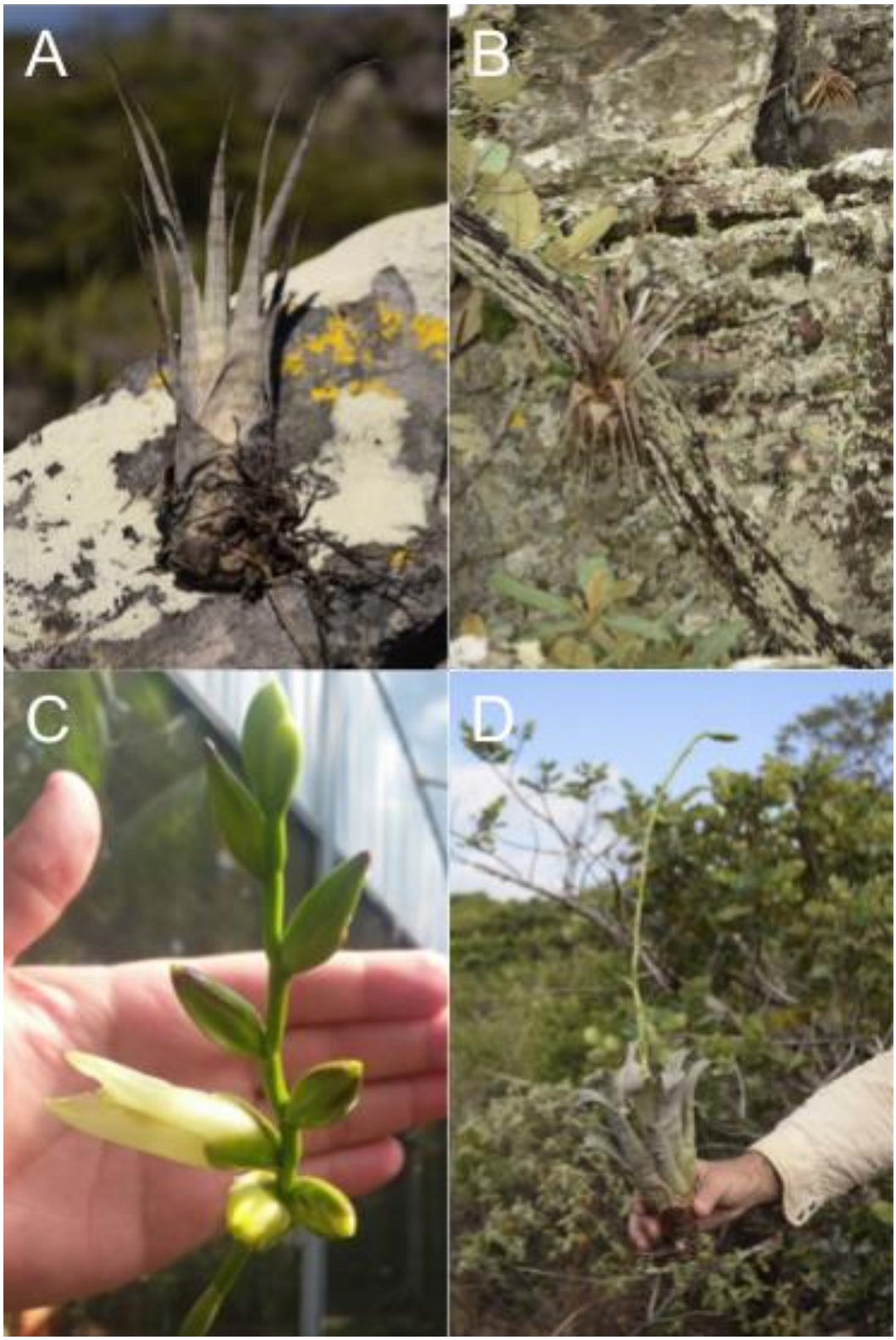
(vi) investigar se os padrões de filogeográficos de *V. oligantha* refletem os padrões macroevolutivos observados nas diferentes biorregiões da Cadeia do Espinhaço;

Capítulo III:

(vii) Avaliar se as oscilações climáticas ocorridas durante o último glacial máximo afetaram padrões demográficos da biota das BQM, através de uma abordagem de filogeografia comparada, utilizando dados inéditos conjuntamente com outros disponíveis na literatura, bem como modelagem de nicho ecológico;

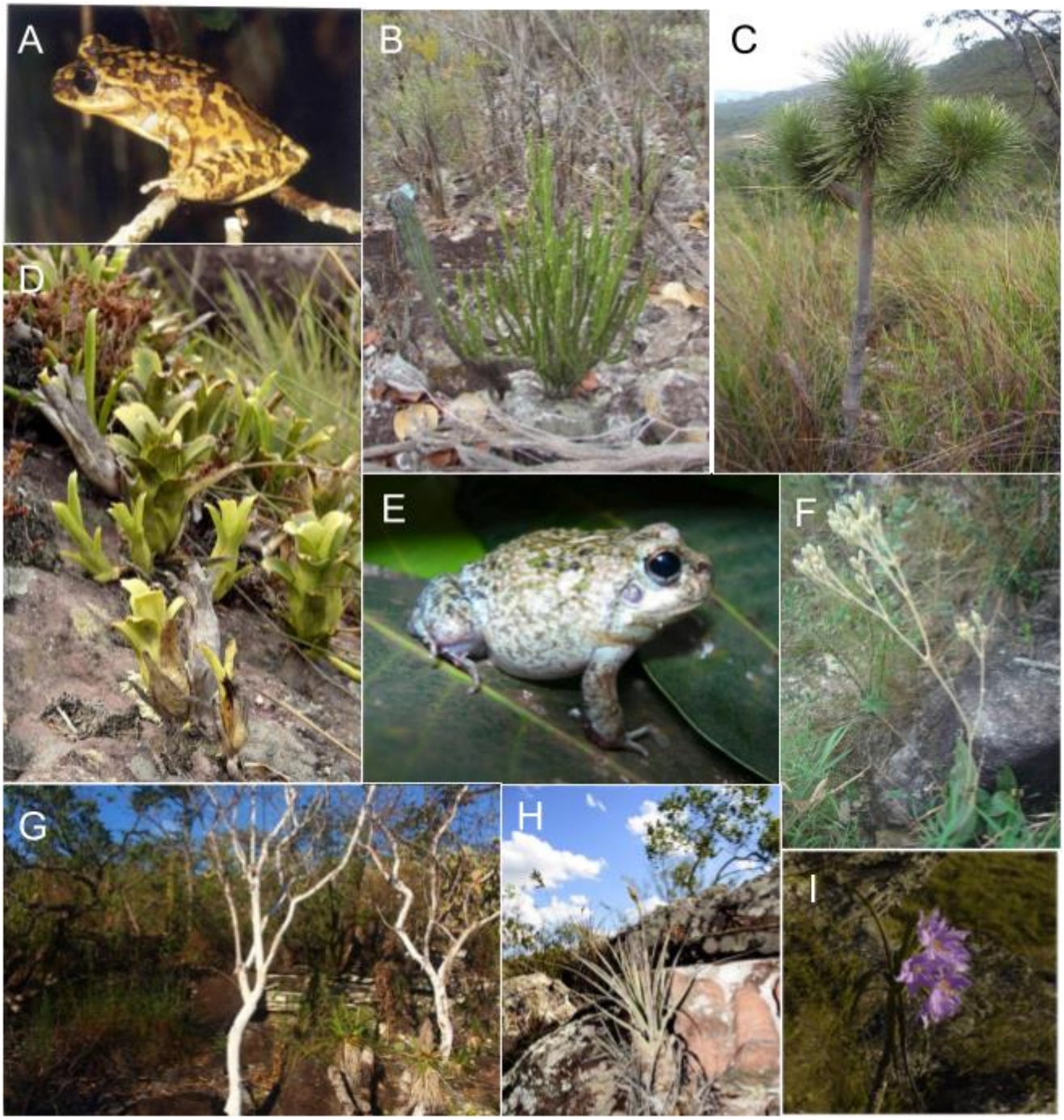
(viii) investigar se as oscilações climáticas foram capazes de causar uma congruência entre espécies endêmicas das biorregiões observadas da Cadeia do Espinhaço;

Figura 3 - A bromélia *Vriesea oligantha*, organismo modelo dos capítulos I e II. A) V. oligantha em seu hábito rupícola e B) epifítico. C) Flor e D) Inflorescência de *V. oligantha*.



Fonte: Foto C por Kléber Rezende Silva. As demais pertencem ao autor.

Figura 4 - Espécies endêmicas das BQM analisadas no Capítulo III. A) *Bokermannohyla saxicola*; B) *Euphorbia attastoma*; C) *Lychnophora ericoides*; D) *Neoregelia bahiana*; E) *Pleurodema alium*; F) *Richterago discoidea*; G) *Tibouchina papyrus*; H) *Vriesea oligantha*; I) *Vellozia auriculata.*



Fontes: A) Lucas Grandinetti; B) Fernanda Hurbath; C) Mauro Cruz; D) o autor; E) L. B. Nascimento; F) Nádia Roque; G) Rodolph Delfino Sartin; H) o autor; I) Cecília Fiorini.

Referências da Introdução Geral

ALCANTARA, S.; REE, R. H.; MELLO-SILVA, R. Accelerated diversification and functional trait evolution in Velloziaceae reveal new insights into the origins of the campos rupestres' exceptional floristic richness. *Annals of Botany*, Londres, v. 122, n. 1, p. 165–180, 2018.

ANTONELLI, A. E.; VEROLA, C. F.; PARISOD, C. N.; GUSTAFSSON, A. L. S. Climate cooling promoted the expansion and radiation of a threatened group of South American orchids (Epidendroideae: Laeliinae). *Biological Journal of the Linnean Society*, Londres, v. 100, n. 3, p. 597–607, 2010.

ANTONELLI, A.; NYLANDER, J. A. A.; PERSSON, C.; SANMARTÍN, I. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences of the United States of America*, Washington, v. 106, n. 24, p. 9749–54, 2009.

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

BARRES, L.; BATALHA-FILHO, H.; SCHNADELBACH, A. S.; ROQUE, N. 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*, Londres, v. 189, n. 2, p. 132–152, 2019.

BENZING, D. H. *Bromeliaceae - profile of an adaptive radiation*. Cambridge: Cambridge University Press, 2000.

CAMPOS, L.; FREIRE MORO, M.; FUNK, V. A.; ROQUE, N. Biogeographical review of Asteraceae in the Espinhaço Mountain Range, Brazil. *The Botanical Review*, Nova Iorque, v. 85, n. 4, p. 293–336, 2019.

CARNAVAL, A. C.; MORITZ, C. Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic Forest. *Journal of Biogeography*, Oxford, v. 35, n. 7, p. 1187–1201, 2008.

CHAVES, A. V.; FREITAS, G. H. S.; VASCONCELOS, M. F.; SANTOS, F. R. Biogeographic patterns, origin and speciation of the endemic birds from eastern Brazilian mountaintops: a review. *Systematics and Biodiversity*, Cambridge, v. 13, n. 1, p. 1–16, 2015.

CHAVES, A. V.; VASCONCELOS, M. F.; FREITAS, G. H. S.; SANTOS, F. R. Vicariant events in the montane hummingbird genera *Augastes* and *Schistes* in South America. *Ibis*, Londres, p. ibi.12777, 2019.

COLLI-SILVA, M.; VASCONCELOS, T. N. C.; PIRANI, J. R. Outstanding plant endemism levels strongly support the recognition of campo rupestre provinces in mountaintops of eastern South America. *Journal of Biogeography*, Oxford, v. 46, n. 8, p. 1723–1733, 2019.

CONCEIÇÃO, A. A.; PIRANI, J. R. Succession on the rocky outcrop Vegetation: a rupestrian grassland scheme. In: FERNANDES, G. W. (Ed.). *Ecology and Conservation of Mountaintop Grasslands in Brazil*. Cham: Springer, 2016. p. 181–206.

DE ALMEIDA, F. F.; CARNEIRO, C. R. Origem e evolução da Serra do Mar. *Revista Brasileira de Geociências,* São Paulo, v. 28, n. 2, p. 135 - 150, 1998.

DOWSETT, H. J.; ROBINSON, M. M.; HAYWOOD, A. M.; HILL, D. J.; DOLAN, A. M.; STOLL, D. K.; CHAN, W.; ABE-OUCHI, A.; CHANDLER, M. A.; ROSENBLOOM, N. A.; OTTO-BLIESNER, B. L.; BRAGG, F. J.; LUNT, D. J.; FOLEY, K. M.; RIESSELMAN, C. R. Assessing confidence in Pliocene sea surface temperatures to evaluate predictive models. *Nature Climate Change*, Londres, v. 2, n. 5, p. 365-371, 2012.

DUCHEN, P.; HAUTPHENNE, S.; LEHMANN, L.; SALAMIN, N. Linking micro and macroevolution in the presence of migration. *Journal of Theoretical Biology*, Londres, v. 486, p. 110087, 2020.

ECHTERNACHT, L.; TROVÓ, M.; OLIVEIRA, C. T.; PIRANI, J. R. Areas of endemism in the Espinhaço Range in Minas Gerais, Brazil. *Flora: Morphology, Distribution, Functional Ecology of Plants*, Jena, v. 206, n. 9, p. 782–791, 2011.

FERRIS, K. G.; WILLIS, J. H. Differential adaptation to a harsh granite outcrop habitat between sympatric *Mimulus* species. *Evolution*, Lancaster, v. 72, n. 6, p. 1225–1241, 2018.

FIASCHI, P.; PIRANI, J. R. Review of plant biogeographic studies in Brazil. *Journal of Systematics and Evolution*, Beijing, v. 47, n. 5, p. 477–496, 2009.

FLANTUA, S. G. A.; O'DEA, A.; ONSTEIN, R. E.; GIRALDO, C.; HOOGHIEMSTRA, H. The flickering connectivity system of the north Andean páramos. *Journal of Biogeography*, Oxford, v. 46, n. 8, p. 1808–1825, 2019.

FLANTUA, S.; HENRY, H. Historical connectivity and mountain biodiversity. In: HOORN, C.; PERRIGO, A.; (Eds.). *Mountains, climate and biodiversity*. Hoboken: John Wiley & Sons, 2018. p. 171-185.

FLANTUA, S.; PAYNE, D.; BORREGAARD, MICHAEL BEIERKUHNLEIN, C.; STEINBAUER, M.; DULLINGER, S.; ESSL, F.; IRL, S.; KIENLE, D.; KREFT, H.; LENZNER, B.; NORDER, S.; RIJSDIJK, K.; RUMPF, S.; WEIGELT, P.; FIELD, R. Snapshot isolation and isolation history challenge the analogy between mountains and islands used to understand endemism*. Global Ecology and Biogeography*, Oxford, v. 29, n. 10, p. 1–23, 2020.

GIULIETTI, A. M. A. M.; PIRANI, J. R. J. R. Patterns of geographic distribution of some plant species from the Espinhaco Range, Minas Gerais and Bahia, Brazil. In: VANZOLINI, P. E.; HEYER, W. R. (Eds.). *Proceedings of a Workshop*

on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências, 1988. p. 39-69.

GIVNISH, T. J.; MILLAM, K. C.; EVANS, T. M.; HALL, J. C.; PIRES, J. C.; BERRY, P. E.; SYTSMA, K. J. Ancient vicariance or recent long-distance dispersal? Inferences about phylogeny and South American–African disjunctions in Rapateaceae and Bromeliaceae based on *ndhF* sequence data. *International Journal of Plant Sciences*, Chicago, v. 165, n. 4, p. S35–S54, 2004.

GIVNISH, T. J.; BARFUSS, M. H. J.; EE, B. V.; RIINA, R.; SCHULTE, K.; HORRES, R.; GONSISKA, P. a.; JABAILY, R. S.; CRAYN, D. M.; SMITH, J. A. C.; WINTER, K.; BROWN, G. K.; EVANS, T. M.; HOLST, B. K.; LUTHER, H.; TILL, W.; ZIZKA, G.; BERRY, P. E.; SYTSMA, K. J.; VAN EE, B.; RIINA, R.; SCHULTE, K.; HORRES, R.; GONSISKA, P. a.; JABAILY, R. S.; CRAYN, D. M.; SMITH, J. A. C.; WINTER, K.; BROWN, G. K.; EVANS, T. M.; HOLST, B. K.; LUTHER, H.; TILL, W.; ZIZKA, G.; BERRY, P. E.; SYTSMA, K. J. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. *American Journal of Botany*, Baltimore, v. 98, n. 5, p. 872–95, 2011.

GIVNISH, T. J.; BARFUSS, M. H. J.; EE, B. Van; RIINA, R.; SCHULTE, K.; HORRES, R.; GONSISKA, P. A.; JABAILY, R. S.; CRAYN, D. M.; SMITH, J. A. C.; WINTER, K.; BROWN, G. K.; EVANS, T. M.; HOLST, B. K.; LUTHER, H.; TILL, W.; ZIZKA, G.; BERRY, P. E.; SYTSMA, K. J. Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. *Molecular Phylogenetics and Evolution*, Orlando, v. 71, n. 1, p. 55–78, 2014.

GUEDES, T. B.; AZEVEDO, J. A. R.; BACON, C. D.; PROVETE, D. B.; ANTONELI, A. Diversity, Endemism, and Evolutionary History of Montane Biotas Outside the Andean Region. In: RULL, V.; CARNAVAL, A. *Neotropical Diversification: Patterns and Processes.* Cham: Springer, 2020. p. 299-328.

HAFFER, J. Speciation in Amazonian Forest Birds. *Science*, Washington, v. 165, n. 3889, p. 131–137, 1969.

HUGHES, C. E.; PENNINGTON, R. T.; ANTONELLI, A. Neotropical plant evolution: assembling the big picture. *Botanical Journal of the Linnean Society*, Londres, v. 171, n. 1, p. 1-18, 2013.

HUMBOLDT, A. von. *Ideen zu einer Geographie der Pflanzen: nebst einem Naturgemälde der Tropenländer: auf Beobachtungen und Messungen gegründet, welche vom 10ten Grade nördlicher bis zum 10ten Grade südlicher Breite, in den Jahren 1799, 1800, 1801, 1802 und 1803 anges*. Tübingen: Cotta.1807.

INSEL, N.; POULSEN, C. J.; EHLERS, T. A. Influence of the Andes Mountains on South American moisture, transport, convection, and precipitation. C*limate Dynamics*, Amsterdam, Cambridge, v. 35, p. 1477 - 1492, 2010.

LAGOMARSINO, L. P.; CONDAMINE, F. L.; ANTONELLI, A.; MULCH, A.; DAVIS, C. C. The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). *New Phytologist*, Cambridge, v. 210, n. 4, p. 1430–1442, 2016.

LEAL, B. S. S.; CHAVES, C. J. N.; KOEHLER, S.; BORBA, E. L. When hybrids are not hybrids: a case study of a putative hybrid zone between *Cattleya coccinea* and *C. brevipedunculata* (Orchidaceae*). Botanical Journal of the Linnean Society*, Londres, v. 181, n. 4, p. 621–639, 2016.

LEAL, B. S. S.; PALMA-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*, Filadélfia, v. 35, n. 4, p. 215–230, 2016.

LI, J.; HUANG, J. P.; SUKUMARAN, J.; KNOWLES, L. L. Microevolutionary processes impact macroevolutionary patterns. *BMC Evolutionary Biology*, Londres, v. 18, n. 1, p. 1–8, 2018.

MADRIÑÁN, S.; CORTÉS, A. J.; RICHARDSON, J. E. Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics*, Lausanne, v. 4, n. OCT, p. 1–7, 2013.

MAGALHÃES, R. F.; LEMES, P.; SANTOS, M. T. T.; MOL, R. M.; RAMOS, E. K. S.; OSWALD, C. B.; PEZZUTI, T. L.; SANTOS, F. R.; BRANDÃO, R. A.; GARCIA, P. C. A. Evidence of introgression in endemic frogs from the campo rupestre contradicts the reduced hybridization hypothesis. *Biological Journal of the Linnean Society*, Londres, in advance, p. 1–16, 2020.

MARTÍNEZ-BOTÍ, M. A.; FOSTER, G. L.; CHALK, T. B.; ROHLING, E. J.; SEXTON, P. F.; LUNT, D. J.; PANCOST, R. D.; BADGER, M. P. S.; SCHMIDT, D. N. Plio-Pleistocene climate sensitivity evaluated using high-resolution CO2 records. *Nature*, Londres, v. 518, n. 7537, p. 49-54, 2015.

MOTA, M. R.; PINHEIRO, F.; LEAL, B. S. dos S.; SARDELLI, C. H.; WENDT, T.; PALMA-SILVA, C. From micro- to macroevolution: insights from a Neotropical bromeliad with high population genetic structure adapted to rock outcrops. *Heredity*, Londres, v. 125, n. 5, p. 353-370, 2020.

MOTA, M. R.; PINHEIRO, F.; LEAL, B. S. S.; WENDT, T.; PALMA-SILVA, C. The role of hybridization and introgression in maintaining species integrity and cohesion in naturally isolated inselberg bromeliad populations. *Plant Biology*, Stuttgart, v. 21, n. 1, p. 122–132, 2019.

NEVES, D. M.; DEXTER, K. G.; PENNINGTON, R. T.; BUENO, M. L.; DE MIRANDA, P. L. S.; OLIVEIRA-FILHO, A. T. Lack of floristic identity in campos rupestres — A hyperdiverse mosaic of rocky montane savannas in South America. *Flora: Morphology, Distribution, Functional Ecology of Plants*, Jena, v. 238, p. 24–31, 2018.

PALMA-SILVA, C.; WENDT, T.; PINHEIRO, F.; BARBARÁ, T.; FAY, M. F.; COZZOLINO, S.; LEXER, C. Sympatric bromeliad species (*Pitcairnia* spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. *Molecular Ecology*, Oxford, v. 20, n. 15, p. 3185–3201, 2011.

PEDREIRA, A. J.; DE WAELE, B. Contemporaneous evolution of the palaeoproterozoic mesoproterozoic sedimentary basins of the São Francisco-Congo craton. *Geological Society, London, Special Publication*, Londres, v. 294, n. 1, p. 33–48, 2008.

PERRIGO, A.; HOORN, C.; ANTONELLI, A. Why mountains matter for biodiversity. *Journal of Biogeography*, Oxford, v. 47, n. 2, p. 315 – 325, 2020.

PINHEIRO, F.; DANTAS-QUEIROZ, M. V.; PALMA-SILVA, C. Plant species complexes as models to understand speciation and evolution: a review of South American studies. *Critical Reviews in Plant Sciences*, Filadélfia, v. 37, n. 1, p. 54–80, 2018.

RAPINI, A.; RIBEIRO, P.; LAMBERT, S.; PIRANI, J. A flora dos campos rupestres da Cadeia do Espinhaço. *Megadiversidade*, Belo Horizonte, v. 4, n. 1–2, p. 16–24, 2008.

RIBEIRO, P. L.; RAPINI, A.; DAMASCENA, L. S.; VAN DEN BERG, C. Plant diversification in the Espinhaço Range: Insights from the biogeography of *Minaria* (Apocynaceae). *Taxon*, Utrechtt, v. 63, n. 6, p. 1253–1264, 2014.

RULL, V. Biotic diversification in the Guayana Highlands: a proposal. *Journal of Biogeography*, Oxford, v. 32, n. 6, p. 921–927, 2005.

RULL, V.; MONTOYA, E.; NOGUÉ, S.; SAFONT, E.; VEGAS-VILARRÚBIA, T. Climatic and ecological history of Pantepui and surrounding areas. In: RULL, V.; VEGAS-VILARRÚBIA, T.; HUBER, O.; SEÑARIS, C. (Eds.). *Biodiversity of Pantepui: the pristine "Lost World" of the Neotropical Guiana Highlands.* Amsterdã: Elsevier, 2019. p. 33–54.

SAFFORD, H. Brazilian Páramos IV. Phytogeography of the campos de altitude. *Journal of Biogeography*, Oxford, v. 34, n. 10, p. 1701–1722, 2007.

SEDANO, R. E.; BURNS, K. J. Are the Northern Andes a species pump for Neotropical birds? Phylogenetics and biogeography of a clade of Neotropical tanagers (Aves: Thraupini). *Journal of Biogeography*, Oxford, v. 37, n. 2, p. 325–343, 2010.

SÉRSIC, A. N.; COSACOV, A.; COCUCCI, A. A.; JOHNSON, L. A.; POZNER, R.; AVILA, L. J.; SITES, J. W.; MORANDO, M. Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. *Biological Journal of the Linnean Society*, Londres, v. 103, n. 2, p. 475–494, 2011.

SCHAEFER, C. E.; CANDIDO, H. G.; CORRÊA, G. R.; NUNES, J. A.; ARRUDA, D. M. Soils associated with rupestrian grasslands. In: FERNANDES, G. W. (Ed.). *Ecology and Conservation of Mountaintop Grasslands in Brazil*. Cham: Springer, 2016. p. 55–69.

SILVEIRA, F. A. O.; NEGREIROS, D.; BARBOSA, N. P. U.; BUISSON, E.; CARMO, F. F.; CARSTENSEN, D. W.; CONCEIÇÃO, A. A.; CORNELISSEN, T. G.; ECHTERNACHT, L.; FERNANDES, G. W.; GARCIA, Q. S.; GUERRA, T. J.; JACOBI, C. M.; LEMOS-FILHO, J. P.; LE STRADIC, S.; MORELLATO, L. P. C.; NEVES, F. S.; OLIVEIRA, R. S.; SCHAEFER, C. E.; VIANA, P. L.; LAMBERS, H. Ecology and evolution of plant diversity in the endangered campo rupestre: a neglected conservation priority. *Plant and Soil*, The Hague, v. 403, n. 1–2, p. 129–152, 2016.

SILVESTRO, D.; ZIZKA, G.; SCHULTE, K. Disentangling the effects of key innovations on the diversification of Bromelioideae (Bromeliaceae). *Evolution*, Lancaster, v. 68, n. 1, p. 163–175, 2014.

TURCHETTO-ZOLET, A. C.; PINHEIRO, F.; SALGUEIRO, F.; PALMA-SILVA, C. Phylogeographical patterns shed light on evolutionary process in South America. *Molecular Ecology*, Oxford, v. 22, n. 5, p. 1193–213, 2013.

VASCONCELOS, T. N. C.; ALCANTARA, S.; ANDRINO, C. O.; FOREST, F.; REGINATO, M.; SIMON, M. F.; PIRANI, J. R. Fast diversification through a mosaic of evolutionary histories characterizes the endemic flora of ancient Neotropical mountains. *Proceedings of the Royal Society B: Biological Sciences*, Londres, v. 287, n. 1923, p. 20192933, 2020.

VERSIEUX, L. M.; WENDT, T. T. Checklist of Bromeliaceae of Minas Gerais, Brazil, with notes on taxonomy and endemism. *Selbyana*, Sarasota, v. 27, n. 2, p. 107–146, 2006.

VERSIEUX, L. M.; WENDT, T.; LOUZADA, R. B.; WANDERLEY, M. G. L. Bromeliaceae da Cadeia do Espinhaço. *Megadiversidade*, Belo Horizonte, v. 4, p. 99–110, 2008.

ZAPPI, D. C.; MORO, M. F.; MEAGHER, T. R.; NIC LUGHADHA, E. Plant biodiversity drivers in Brazilian campos rupestres: insights from phylogenetic structure. *Frontiers in Plant Science*, Lausanne, v. 8, n. December, p. 1–15, 2017.

Capítulo I: Transferability of nuclear microsatellites markers to *Vriesea oligantha* (Bromeliaceae), an endemic species from Espinhaço Range, Brazil

Tami Cacossi, Marcos Vinicius Dantas-Queiroz, Clarisse Palma-Silva

Brazilian Journal of Botany 42 (4): 727 – 733, 2019
DOI: 10.1007/s40415-019-00560-z

Brazilian Journal of Botany
https://doi.org/10.1007/s40415-019-00560-z

SHORT COMMUNICATION

Transferability of nuclear microsatellites markers to *Vriesea oligantha* (Bromeliaceae), an endemic species from Espinhaço Range, Brazil

Tami Cacossi[1] · Marcos Vinicius Dantas-Queiroz[2] · Clarisse Palma-Silva[1,2]

Received: 17 May 2019 / Revised: 19 September 2019 / Accepted: 23 September 2019

Abstract
The Espinhaço Range is a center of biodiversity and endemism located in Eastern Brazil, and our knowledge is still scarce for the genetic diversity, structure and phylogeography of species from these mountains. *Vriesea oligantha* (Baker) Mez is an endemic bromeliad distributed along the Espinhaço Range with naturally fragmented populations. Here, the transferability of 30 microsatellites loci previously developed for seven Bromeliaceae species was tested from three different subfamilies in eight populations of *V. oligantha*. The amplification of 24 loci was successfully accomplished and 20 of them were polymorphic. Ten highly polymorphic microsatellite loci were selected to be amplified and genotyped in two population of *V. oligantha.* The number of alleles per locus ranged from 1 to 11, the expected and observed heterozygosities ranged from 0 to 0.905 and from 0 to 0.750, respectively. Our results endorse the cross-amplification between deeply divergent lineages of Bromeliaceae and provide useful markers for further phylogeographic, population genetics and mating systems studies to better understand the evolutionary history of an endemic species of a naturally fragmented area.

Keywords Cross-amplification · Monocots · Phylogeography · Population genetics · Rocky fields · SSR markers

1 Introduction

The Espinhaço Range is the second largest mountain range of South America located in Eastern Brazil and its high biodiversity is historically known by the scientific community (Giulietti and Pirani 1988; Silveira et al. 2016; Fernandes et al. 2018). However, compared with other Neotropical areas, there are proportionally few populational studies focused on endemic species from these mountains, which would explain genetic diversity and demography patterns for such organisms.

Bromeliaceae is one of the most species-rich flowering families native to Neotropical region, bearing several key innovation traits, making this an ecologically diverse and morphologically distinctive family with various examples of adaptive radiations (Givnish et al. 2011; Silvestro et al. 2014). In Espinhaço, several floristic surveys were carried out (Rapini et al. 2008) and the Bromeliaceae stands out by its high number of endemic species (49.5%) and because it has one of its diversification centers in Espinhaço Range (Versieux et al. 2008, 2010).

The genus *Vriesea* Lindl. belongs to Tillandsioideae and is recognized as one of the largest genus in number of species of the family, with 226 species (Gouda et al. 2018). Recently, *Vriesea* passed through a deep rearrangement (Barfuss et al. 2016) and most species are now exclusive of Eastern Brazil, with a high percentage of endemic species registered for the Espinhaço Range (Versieux et al. 2008, 2010).

Vriesea oligantha (Baker) Mez (Fig. 1) is an epiphytic and rupicolous species usually found associated with *Vellozia* spp., with naturally fragmented distribution throughout the entire Espinhaço Range. The study of the structure and genetic diversity in populations of *V. oligantha* could benefit the understanding of local adaptation processes due

Tami Cacossi and Marcos Vinicius Dantas-Queiroz have contributed equally to this work and are co-first authors on this paper.

✉ Marcos Vinicius Dantas-Queiroz
marvin.danque@gmail.com

[1] Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas (Unicamp), Campinas, SP 13083-862, Brazil

[2] Departamento de Ecologia, Programa de Pós-graduação em Biologia Vegetal, Universidade Estadual Paulista (Unesp), Rio Claro, SP 13506-900, Brazil

Published online: 05 October 2019

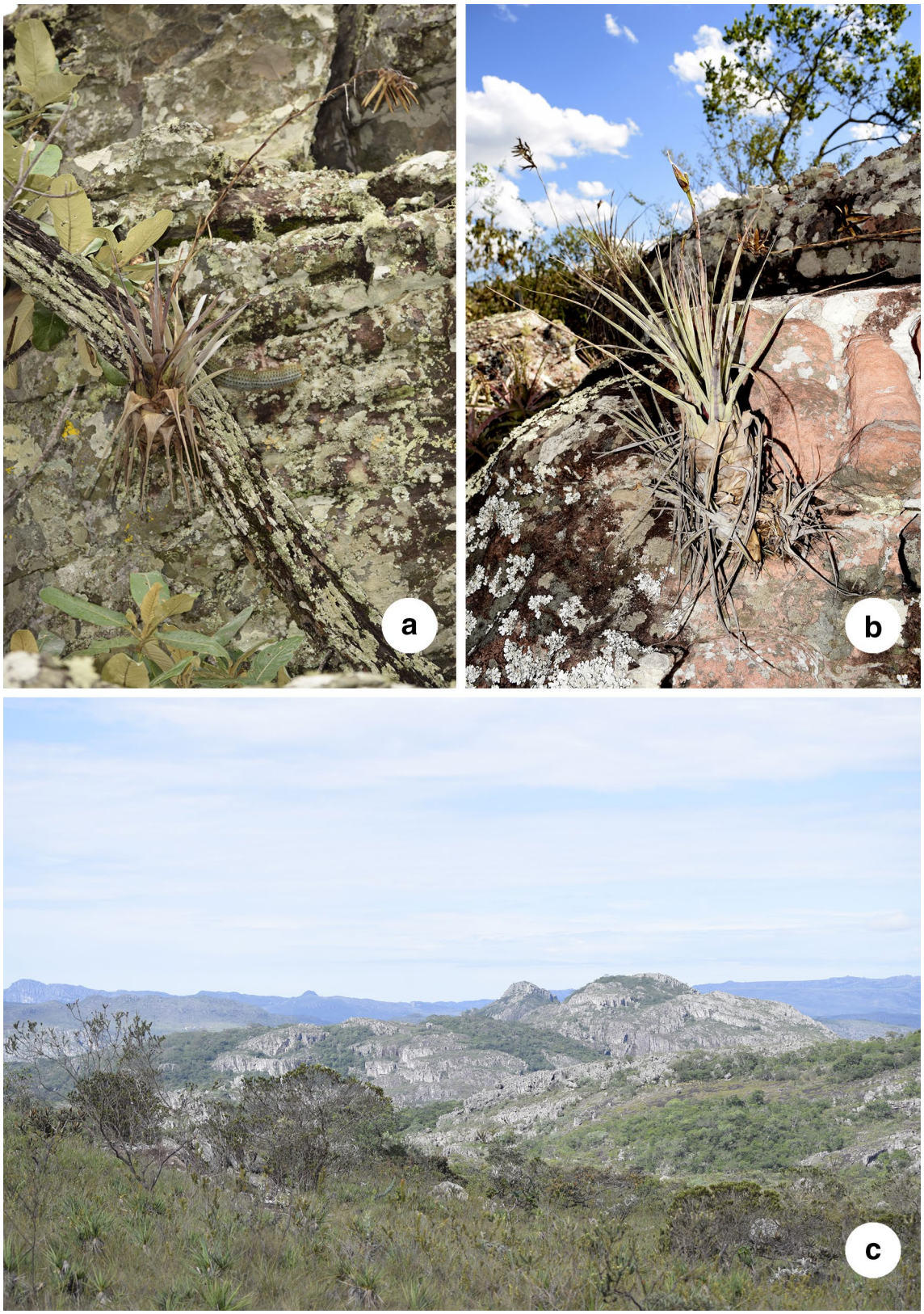


Fig. 1 Epiphytic (**a**) and rupicolous (**b**) individuals of *Vriesea oligantha*, an endemic species of rock outcrops from the Espinhaço Range (**c**)

to environmental and biotic pressures, verifying prior climatic and geological scenarios involved in the communities' dynamics in this region, as well as the biotic factors associated with the current distribution of the organisms from naturally fragmented areas of the Neotropics.

One of the most commonly used markers in analysis of genetic diversity in plants are the simple sequence repeats (SSR) or microsatellites, due to its highly informative multiallelic and codominant nature, reproducibility, heritability, relative abundance and extensive genome coverage (Parida et al. 2009). However, marker developing for specific organisms is the main limitation on the use of these markers. Bromeliaceae is a family with known successful cross-amplification studies among different subfamilies (Barbará et al. 2007a; Zanella et al. 2012), including *Vriesea*, with notable cross-amplification studies as well (e.g., Palma-Silva et al. 2007; Lavor et al. 2013; Neri et al. 2015; Todeschini et al. 2018).

We believe that the markers tested here will benefit forthcoming studies concerning the genetic structure and diversity, demography, phylogeography and conservation status of this rocky field endemic bromeliad. Therefore, in this study, the transferability of microsatellite loci previously developed for other bromeliads was tested in two populations of *V. oligantha*.

2 Materials and methods

Plant sampling and DNA extraction – A total of 40 individual from eight populations of *Vriesea oligantha* was sampled (Table 1). Fresh leaves were collected and stored in silica gel. Samples were kept under − 20 °C refrigeration until DNA extraction. Genomic DNA was extracted following the Tel-Zur et al. (1999) protocol.

Cross-amplification and genotyping – Thirty potential nuclear microsatellites markers developed for several species of Bromeliaceae in different subfamilies were selected (Table 2). Initially, cross-amplification testing was performed using eight individuals, one per population (Table 1). The polymerase chain reactions were conducted in a Veriti 96-Well Thermal Cycler (Applied Biosystems) using a touchdown program as described by Palma-Silva et al. (2007) in a reaction volume of 12 µL containing 2 ng of genomic DNA, 5x GoTaq Master Mix (Promega Corporation), 5 pmol forward primer, 10 pmol reverse primer and 1 pmol universal M13 universal primers tagged with different fluorochromes (FAM, VIC, PET or NED). Amplification products were verified by electrophoresis on 2.0% agarose gel stained with GelRed (Biotium, Hayward, California, USA). Fragments were visualized under UV light using MiniBis Pro (DNR Bio-Imaging System) and were considered successfully amplified when one band of expected size was clearly visualized. Successfully amplified fragments were genotyped using an ABI 3500 DNA Analyzer Sequencer (Applied Biosystem) precisely sized against GeneScan 500 LIZ (Applied Biosystem), molecular size standard using GeneMarker software version 1.97 Demo (Softgenetics LLC State College, PA, USA). Raw alleles sizes of the polymorphic markers were then both automated binned into discrete classes using FlexBin (Amos et al. 2007) and manually inspected.

From the initial test of the eight individuals from all sampled populations, we selected polymorphic markers with best pattern of genotyping to be amplified and genotyped in 34 individuals, from two populations (19 individuals in Rio de Contas (RCO) and 15 individuals in Diamantina (DIC) (Table 1), following the protocols previously described.

Data analysis – For each population, the number of alleles per locus (*A*), allelic richness per locus (AR), the observed (H_O) and the expected (H_E) heterozygosity and the

Table 1 Sampled localities and number of individuals collected of *Vriesea oligantha*

Population	State	Municipality	Location	Latitude	Longitude	*n*
ABA	BA	Abaíra	Distrito de Catolés, trilha para Serra do Barbado	− 13.2838	− 41.9004	1
DIC	MG	Diamantina	Estrada para Conselheiro Mata	− 18.3012	− 43.8224	15
GMO	MG	Grão Mogol	Parque Estadual Grão Mogol	− 16.5447	− 42.8911	1
JAC	BA	Jacobina	Morro do Tabor	− 11.1714	− 40.5103	1
LIC	BA	Licínio de Almeida	Trilha para o Morro do Cascarrento	− 14.5873	− 42.5402	1
MKA	BA	Miguel Calmon	Parque Estadual das Sete Passagens	− 11.3924	− 40.5403	1
MUC	BA	Mucugê	Parque Municipal de Mucugê	− 12.994387	− 41.352608	1
RCO	BA	Rio de Contas	Trilha para o Pico das Almas	− 13.5206	− 41.9361	19

n = number of sampled tested. Latitude and longitude in decimal degrees

Brazilian states *BA* Bahia, *MG* Minas Gerais

Table 2 Cross-amplification of 30 nuclear microsatellite markers previously developed for different Bromeliaceae species in *Vriesea oligantha*

Loci	Target species	Subfamily	References	Amplification	Polymorphism
Acom_12.12	*Ananas comosus* (L.) Merr.	Bromelioideae	Wöhrmann and Weising (2011)	+	–
Acom_82.8	*Ananas comosus* (L.) Merr.	Bromelioideae	Wöhrmann and Weising (2011)	+	–
Op77B	*Orthophytum ophiuroides* Louzada & Wand.	Bromelioideae	Aoki-Gonçalves et al. (2014)	–	
PaA10	*Pitcairnia albiflos* Herb.	Pitcairnioideae	Paggi et al. (2008)	+	–
PaC05	*Pitcairnia albiflos* Herb.	Pitcairnioideae	Paggi et al. (2008)	+	+
PaD07	*Pitcairnia albiflos* Herb.	Pitcairnioideae	Paggi et al. (2008)	+	–
PaZ01	*Pitcairnia albiflos* Herb.	Pitcairnioideae	Paggi et al. (2008)	+	+
e6b	*Tillandsia fasciculata* Sw.	Tillandsioideae	Boneh et al. (2003)	–	
CT5	*Guzmania monostachya* Sw.	Tillandsioideae	Boneh et al. (2003)	+	+
VgA04	*Vriesea gigantea* Mart. ex Schult. f.	Tillandsioideae	Palma-Silva et al. (2007)	+	+
VgA06	*Vriesea gigantea* Mart. ex Schult. f.	Tillandsioideae	Palma-Silva et al. (2007)	–	
VgB01	*Vriesea gigantea*	Tillandsioideae	Palma-Silva et al. (2007)	+	+
VgB06	*Vriesea gigantea* Mart. ex Schult. f.	Tillandsioideae	Palma-Silva et al. (2007)	+	+
VgB10	*Vriesea gigantea* Mart. ex Schult. f.	Tillandsioideae	Palma-Silva et al. (2007)	+	+
VgC01	*Vriesea gigantea* Mart. ex Schult. f.	Tillandsioideae	Palma-Silva et al. (2007)	+	+
VgF01	*Vriesea gigantea* Mart. ex Schult. f.	Tillandsioideae	Palma-Silva et al. (2007)	–	
VgF02	*Vriesea gigantea* Mart. ex Schult. f.	Tillandsioideae	Palma-Silva et al. (2007)	+	+
VgG02	*Vriesea gigantea* Mart. ex Schult. f.	Tillandsioideae	Palma-Silva et al. (2007)	+	+
VgG03	*Vriesea gigantea* Mart. ex Schult. f.	Tillandsioideae	Palma-Silva et al. (2007)	+	+
VgG05	*Vriesea gigantea* Mart. ex Schult. f.	Tillandsioideae	Palma-Silva et al. (2007)	+	+
Vs01	*Vriesea simplex* (Vell.) Beer	Tillandsioideae	Neri et al. (2015)	–	
Vs02	*Vriesea simplex* (Vell.) Beer	Tillandsioideae	Neri et al. (2015)	+	+
Vs06	*Vriesea simplex* (Vell.) Beer	Tillandsioideae	Neri et al. (2015)	+	+
Vs08	*Vriesea simplex* (Vell.) Beer	Tillandsioideae	Neri et al. (2015)	+	+
Vs09	*Vriesea simplex* (Vell.) Beer	Tillandsioideae	Neri et al. (2015)	+	+
Vs10	*Vriesea simplex* (Vell.) Beer	Tillandsioideae	Neri et al. (2015)	+	+
Vs17	*Vriesea simplex* (Vell.) Beer	Tillandsioideae	Neri et al. (2015)	+	+
Vs18	*Vriesea simplex* (Vell.) Beer	Tillandsioideae	Neri et al. (2015)	–	
Vs19	*Vriesea simplex* (Vell.) Beer	Tillandsioideae	Neri et al. (2015)	+	+
Vs20	*Vriesea simplex* (Vell.) Beer	Tillandsioideae	Neri et al. (2015)	+	+

inbreeding coefficient (F_{IS}) was estimated using the MSA software version 4.05 (Dieringer and Schlötterer 2003). Deviations from the Hardy–Weinberg equilibrium (HWE) per locus were also evaluated using Genepop software v3.5 (Raymond and Rousset 1995). Linkage disequilibrium between all pairs of loci was tested in FSTAT version 2.9.3.2 (Goudet 1995).

3 Results and discussion

From the 30 loci tested, 24 exhibited successful cross-amplification products and were subsequently genotyped (Table 2). Of these, 20 were polymorphic and the 10 loci with the best genotyping pattern were chosen for population analysis in two populations of *V. oligantha* (Table 3). The number of alleles ranged from one to nine per locus in the

Table 3 Characterization of the ten polymorphic microsatellite markers transferred to two populations, named Rio de Contas (RCO) and Diamantina (DIC), of *Vriesea oligantha* (Bromeliaceae)

Loci	RCO ($N=19$)					DIC ($N=15$)				
	A	AR	H_O	H_E	F_{IS}	A	AR	H_O	H_E	F_{IS}
Vs19	1	1.00	0.000	0.000	NA	2	1.99	0.200	0.287	0.311
VgG02	4	3.99	0.210	0.699	0.705***	5	4.67	0.750	0.659	−0.145
VgC01	2	1.99	0.166	0.246	0.329	4	3.64	0.266	0.632	0.587**
PaC05	2	1.99	0.210	0.273	0.234	5	4.72	0.538	0.747	0.288
Vs06	4	3.82	0.062	0.538	0.887***	11	9.60	0.571	0.886	0.364**
VgG03	2	2.00	0.052	0.422	0.878***	3	2.56	0.200	0.190	−0.050
Vs09	2	2.00	0.000	0.477	1.000***	9	8.55	0.545	0.870	0.385**
Vs10	3	2.99	0.058	0.639	0.911***	5	4.81	0.181	0.645	0.728***
Vs17	9	7.42	0.105	0.733	0.860***	3	3.00	0.272	0.662	0.600*
PaZ01	5	4.78	0.266	0.645	0.596**	9	9.00	0.200	0.905	0.788***
Overall	34				0.763***	56				0.437***

Number of alleles per locus (A), allelic richness per locus (AR), observed heterozygosity (H_O), expected heterozygosity (H_E) and within inbreeding coefficient (F_{IS}) are shown. *NA* not applied

Departures of within-population inbreeding coefficients (F_{IS}) from Hardy–Weinberg equilibrium (HWE) are indicated by asterisks, $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

RCO population and two to 11 per locus in DIC population (Table 3), summing up 90 alleles.

About 80% of the tested markers were amplified, while 66% of the loci were polymorphic. As reported in previous studies, the transferability between subfamilies confirms that the markers can be transferred between largely divergent Bromeliaceae species (e.g., Barbará et al. 2007b; Wöhrmann and Weising 2011; Chaves et al. 2018). Comparing the number of alleles from the target species with the observed in *Vriesea oligantha* (Table 3), most of the loci showed fewer alleles than those originally reported, a pattern already found in other cross-amplification studies in Bromeliaceae (e.g., Ferreira et al. 2017). Two *Pitcairnia albiflos* Herb. loci presented a higher number of alleles in *V. oligantha* than the number reported by Paggi et al. (2008) (PaC05 = 5 alleles in *V. oligantha* vs. 3 alleles in *P. albiflos* Herb. and PaZ01 = 9 alleles in *V. oligantha* vs. 7 alleles in *P. albiflos* Herb.).

The allelic richness varied from 1.00 to 4.78 per locus in RCO and 1.99 to 9.60 per locus in DIC (Table 3). The values of observed (H_O) and expected heterozygosity (H_E) in RCO ranged from 0.000 to 0.266 (average = 0.113) and 0.000 to 0.699 (average = 0.467), respectively. While in DIC, the H_O and H_E ranged from 0.181 to 0.750 (average = 0.372) and 0.190 to 0.905 (average = 0. 648), respectively (Table 3). The values of expected heterozygosity (H_E) in both populations of *V. oligantha* are within the values found in other Bromeliaceae. However, the observed heterozygosity (H_O) was considered low due to a deficit of heterozygotes. Bromeliads with similar naturally fragmented habitats, especially those restricted to rock outcrops and inselbergs, also show a similar pattern of low H_O (e.g., *Alcantarea imperialis* (Carrière) Harms, *A. geniculata* (Wawra) J.R. Grant, *A. glaziouana* (Lem.) Leme (Barbará et al. 2007b, 2009) and *A. patriae* Versieux & Wand. (Pereira et al. 2017), *Sincoraea ophiuroides* (Louzada & Wand.) Louzada & Wand. (Aoki-Gonçalves et al. 2014), *Pitcairnia albiflos* Herb. (Paggi et al. 2008) and *Vriesea minarum* L.B. Sm. (Lavor et al. 2013).

The inbreeding coefficient was high and significant for most of the loci in *Vriesea oligantha*, ranging from 0.234 to 1.00 in RCO and −0.145 to 0.788. Most of the loci showed a significant departure from HWE, due to a deficit of heterozygotes (Table 3). This scenario may be a consequence of a possible self-compatible mating system, commonly observed in populations of other *Vriesea* species (Matallana et al. 2010; Wolowski et al. 2013; Paggi et al. 2015; Neri et al. 2017) or due to historical genetic structure of this species in high altitude rock outcrops along the Espinhaço Range (Versieux et al. 2008). High levels of inbreeding coefficients were also observed in species of deferent plant families in the Espinhaço Range (e.g., *Comanthera elegans* (Bong.) L.R. Parra & Giul., $F_{IS}=0.308$ [Eriocaulaceae] (Leal et al. 2014), *Vellozia plicata* Mart., $F_{IS}=0.234$ [Velloziaceae] (de Paula et al. 2017).

The use of SSR markers in Bromeliaceae has proved to be an excellent way to investigate genetic structure since successful cross-amplification between species of same and other subfamilies is an indication of rapid speciation with low levels of divergence in DNA sequences (Barbará et al. 2007a). The results obtained here demonstrate that these tested loci are useful in future studies with population genetics, species delimitation and phylogeographic approaches. Forthcoming studies on population genetic diversity and structure are crucial not only to better understand how naturally fragmented areas foster high levels of diversity, but also are fundamental to support conservation policies since the Espinhaço Range has been threatening by human

activities for centuries (Silveira et al. 2016; Fernandes et al. 2018). Thus, description of the levels of genetic diversity and structure of endemic species are the first steps to better understand the evolutionary history of such organisms and consequently develop useful and efficiently conservational efforts for the Espinhaço biota.

Acknowledgements We thank Dr. Kléber Resende Silva and Dr. Leonardo Versieux (CNPq 455510/2014-8) for helping in field works and scientific discussion, including the project design and choice of the focal species. This study is part of MVDQ' Ph.D. thesis conducted in the Programa de Pós-Graduação em Biologia Vegetal (PPGBV) from the Universidade Estadual Paulista 'Júlio de Mesquita Filho' (UNESP), granted by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). The authors thank Fundação de Apoio à Pesquisa do Estado de São Paulo (FAPESP 2018/07596-0), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Fellowship Research to CPS (300819/2016-1), and Fundação de Apoio à Extensão e Pesquisa da UNICAMP (FAEPEX) to scientific initiation fellowship (2002/19).

Author contributions CPS and MVDQ designed the study. TC and CPS performed the experiments and analyzed the data. MVDQ and CPS wrote the manuscript. All authors reviewed the last version of this manuscript.

References

Amos W, Hoffman JI, Frodsham A, Zhang L, Best S, Hill AVS (2007) Automated binning of microsatellite alleles: problems and solutions. Mol Ecol Notes 7:10–14. https://doi.org/10.1111/j.1471-8286.2006.01560.x

Aoki-Gonçalves F, Louzada RB, Souza LM, Palma-Silva C (2014) Microsatellite loci for *Orthophytum ophiuroides* (Bromelioideae, Bromeliaceae) species adapted to Neotropical Rock Outcrops. Appl Plant Sci 2:1300073. https://doi.org/10.3732/apps.1300073

Barbará T, Martinelli G, Fay MF, Mayo SJ, Lexer C (2007a) Population differentiation and species cohesion in two closely related plants adapted to neotropical high-altitude "inselbergs", *Alcantarea imperialis* and *Alcantarea geniculata* (Bromeliaceae). Mol Ecol 16:1981–1992. https://doi.org/10.1111/j.1365-294X.2007.03272.x

Barbará T, Palma-Silva C, Paggi GM, Bered F, Fay MF, Lexer C (2007b) Cross-species transfer of nuclear microsatellite markers: potential and limitations. Mol Ecol 16:3759–3767. https://doi.org/10.1111/j.1365-294X.2007.03439.x

Barbará T, Martinelli G, Palma-Silva C, Fay MF, Mayo S, Lexer C (2009) Genetic relationships and variation in reproductive strategies in four closely related bromeliads adapted to neotropical 'inselbergs': *Alcantarea glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* (Bromeliaceae). Ann Bot 103:65–77. https://doi.org/10.1093/aob/mcn226

Barfuss MHJ, Till W, Leme EMC, Pinzón JP, Manzanares JM, Halbritter H, Samuel R, Brown GK (2016) Taxonomic revision of Bromeliaceae subfam. Tillandsioideae based on a multi-locus DNA sequence phylogeny and morphology. Phytotaxa 279:1–97

Boneh L, Kuperus P, van Tienderen H (2003) Microsatellites in the bromeliads *Tillandsia fasciculata* and *Guzmania monostachya*. Mol Ecol Notes 3:302–303. https://doi.org/10.1046/j.1471-8286.2003.00432.x

Chaves CJN, Aoki-Gonçalves F, Leal BSS, Rossatto DR, Palma-Silva C (2018) Transferability of nuclear microsatellite markers to the atmospheric bromeliads *Tillandsia recurvata* and *T. aeranthos* (Bromeliaceae). Rev Bras Bot 41:931–935. https://doi.org/10.1007/s40415-018-0494-4

de-Paula LFA, Leal BSS, Rexroth J, Porembski S, Palma-Silva C (2017) Transferability of microsatellite loci to *Vellozia plicata* (Velloziaceae), a widespread species on Brazilian inselbergs. Rev Bras Bot 40:1071–1075. https://doi.org/10.1007/s40415-017-0396-x

Dieringer D, Schlötterer C (2003) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Mol Ecol Notes 3:167–169. https://doi.org/10.1046/j.1471-8286.2003.00351.x

Fernandes GW, Alberton NPUBB, Dirzo ABR (2018) The deadly route to collapse and the uncertain fate of Brazilian rupestrian grasslands. Biodivers Conserv. https://doi.org/10.1007/s10531-018-1556-4

Ferreira DMC, Neri J, Palma-Silva C, Pinangé DS, Benko-Iseppon AM, Louzada RB (2017) Cross-amplification of nuclear microsatellite markers in two species of *Cryptanthus* Otto & A. Dietr. (Bromeliaceae). Rev Bras Bot 40:475–480. https://doi.org/10.1007/s40415-017-0362-7

Giulietti AM, Pirani JR (1988) Patterns of geographic distribution of some plant species from the Espinhaco Range, Minas Gerais and Bahia, Brazil. In: Vanzolini PE, Heyer WR (eds) Proceedings of a workshop on neotropical distribution patterns. Academia Brasileira de Ciências, Rio de Janeiro, pp 39–69

Givnish TJ, Barfuss MHJ, Ee BV, Riina R, Schulte K, Horres R, Gonsiska PA, Jabaily RS, Crayn DM, Smith JAC, Winter K, Brown GK, Evans TM, Holst BK, Luther H, Till W, Zizka G, Berry PE, Sytsma KJ, Van Ee B, Riina R, Schulte K, Horres R, Gonsiska PA, Jabaily RS, Crayn DM, Smith JAC, Winter K, Brown GK, Evans TM, Holst BK, Luther H, Till W, Zizka G, Berry PE, Sytsma KJ (2011) Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. Am J Bot 98:872–895. https://doi.org/10.3732/ajb.1000059

Gouda EJ, Butcher D, Gouda CS (2018) Encyclopaedia of bromeliads, version 4. http://bromeliad.nl/encyclopedia/. Accessed 13 May 2019

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486

Lavor P, van den Berg C, Versieux LM (2013) Transferability of 10 nuclear microsatellite primers to *Vriesea minarum* (Bromeliaceae), a narrowly endemic and threatened species from Brazil. Rev Bras Bot 36:165–168. https://doi.org/10.1007/s40415-013-0012-7

Leal BSS, Tannure MP, dos Santos FR, Lovato MB, Ribeiro RA (2014) Isolation and characterization of microsatellite markers for the *endangered Comanthera elegans* (Eriocaulaceae) and cross-species amplification within the family. Biochem Syst Ecol 55:305–309. https://doi.org/10.1016/j.bse.2014.03.036

Matallana G, Godinho MAS, Guilherme FAG, Belisario M, Coser TS, Wendt T (2010) Breeding systems of Bromeliaceae species: evolution of selfing in the context of sympatric occurrence. Plant Syst Evol 289:57–65. https://doi.org/10.1007/s00606-010-0332-z

Neri J, Nazareno AG, Wendt T, Palma-Silva C (2015) Development and characterization of microsatellite markers for *Vriesea simplex* (Bromeliaceae) and cross-amplification in other species of Bromeliaceae. Biochem Syst Ecol 58:34–37. https://doi.org/10.1016/j.bse.2014.10.004

Neri J, Wendt T, Palma-Silva C (2017) Natural hybridization and genetic and morphological variation between two epiphytic bromeliads. AoB Plants 10:1–16. https://doi.org/10.1093/aobpla/plx061

Paggi GM, Palma-Silva C, Bered F, Cidade FW, Sousa ACB, Souza AP, Wendt T, Lexer C (2008) Isolation and characterization of microsatellite loci in *Pitcairnia albiflos* (Bromeliaceae), an endemic bromeliad from the Atlantic Rainforest, and cross-amplification

in other species. Mol Ecol Resour 8:980–982. https://doi.org/10.1111/j.1755-0998.2008.02126.x

Paggi GM, Palma-Silva C, Bodanese-Zanettini MH, Lexer C, Bered F (2015) Limited pollen flow and high selfing rates toward geographic range limit in an Atlantic forest bromeliad. Flora Morphol Distrib Funct Ecol Plants 211:1–10. https://doi.org/10.1016/j.flora.2015.01.001

Palma-Silva C, Cavallari MM, Barbará T, Lexer C, Gimenes MA, Bered F, Bodanese-Zanettini MH (2007) A set of polymorphic microsatellite loci for *Vriesea gigantea* and *Alcantarea imperialis* (Bromeliaceae) and cross-amplification in other bromeliad species. Mol Ecol Notes 7:654–657. https://doi.org/10.1111/j.1471-8286.2006.01665.x

Parida SK, Kalia SK, Kaul S, Dalal V, Hemaprabha G, Selvi A, Pandit A, Singh A, Gaikwad K, Sharma TR, Srivastava PS, Singh NK, Mohapatra T (2009) Informative genomic microsatellite markers for efficient genotyping applications in sugarcane. Theor Appl Genet 118:327–338. https://doi.org/10.1007/s00122-008-0902-4

Pereira AG, Bernardi UCS, Manhães VC, Ferreira RS, Miranda FD (2017) Cross-amplification and characterization of microsatellite markers in *Alcantarea patriae* Versieux & Wand. Genet Mol Res 16:1–6. https://doi.org/10.4238/gmr16029692

Rapini A, Ribeiro P, Lambert S, Pirani J (2008) A flora dos campos rupestres da Cadeia do Espinhaço. Megadiversidade 4:16–24

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Silveira FAO, Negreiros D, Barbosa NPU, Buisson E, Carmo FF, Carstensen DW, Conceição AA, Cornelissen TG, Echternacht L, Fernandes GW, Garcia QS, Guerra TJ, Jacobi CM, Lemos-Filho JP, Le Stradic S, Morellato LPC, Neves FS, Oliveira RS, Schaefer CE, Viana PL, Lambers H (2016) Ecology and evolution of plant diversity in the endangered campo rupestre: a neglected conservation priority. Plant Soil 403:129–152. https://doi.org/10.1007/s11104-015-2637-8

Silvestro D, Zizka G, Schulte K (2014) Disentangling the effects of key innovations on the diversification of Bromelioideae (Bromeliaceae). Evolution (NY) 68:163–175. https://doi.org/10.1111/evo.12236

Tel-Zur N, Abbo S, Myslabodski D, Mizrahi Y (1999) Modified CTAB procedure for DNA isolation from epiphytic cacti of genera *Hylocereus* and *Selenicereus* (Cactaceae). Plant Mol Biol 17:249–254

Todeschini CC, Parizotto JLB, Guzman F, Zanella CM, Margis R, Goetze M, Paggi GM, Santana Costa LM, de Aguiar Melo C, Hirsch LD, Bered F (2018) Development, characterization, and transferability of SSR markers for *Vriesea carinata* (Bromeliaceae) based on RNA sequencing. Appl Plant Sci 6:1–6. https://doi.org/10.1002/aps3.1184

Versieux LM, Wendt T, Louzada RB, Wanderley MGL (2008) Bromeliaceae da Cadeia do Espinhaço. Megadiversidade 4:99–110

Versieux LM, Elbl PM, Wanderley MGL, Menezes N (2010) *Alcantarea* (Bromeliaceae) leaf anatomical characterization and its systematic implications. Nord J Bot 28:385–397. https://doi.org/10.1111/j.1756-1051.2010.00727.x

Wöhrmann T, Weising K (2011) In silico mining for simple sequence repeat loci in a pineapple expressed sequence tag database and cross-species amplification of EST-SSR markers across Bromeliaceae. Theor Appl Genet 123:635–647. https://doi.org/10.1007/s00122-011-1613-9

Wolowski M, Saad CF, Ashman TL, Freitas L (2013) Predominance of self-compatibility in hummingbird-pollinated plants in the Neotropics. Naturwissenschaften 100:69–79. https://doi.org/10.1007/s00114-012-0995-0

Zanella CM, Janke A, Palma-Silva C, Kaltchuk-Santos E, Pinheiro FG, Paggi GM, Soares LES, Goetze M, Büttow MV, Bered F (2012) Genetics, evolution and conservation of Bromeliaceae. Genet Mol Biol 35:1020–1026

Capítulo II: Underlying microevolutionary processes parallel macroevolutionary patterns in ancient Neotropical Mountains

Marcos Vinicius Dantas-Queiroz, Tami da Costa Cacossi, Bárbara Simões Santos Leal, Cléber Juliano Neves Chaves, Thais N. C. Vasconcelos, Leonardo de Melo Versieux, Clarisse Palma-Silva

Submetido no *Journal of Biogeography*
Disponível como pre-print no bioRxiv
DOI: 10.1101/2020.08.26.268870

Title: Underlying microevolutionary processes parallel macroevolutionary patterns in ancient Neotropical Mountains

Running title: Microevolutionary processes parallel macroevolutionary patterns

Authors: Marcos Vinicius Dantas-Queiroz[1]*, Tami da Costa Cacossi[2], Bárbara Simões Santos Leal[2], Cleber Juliano Neves Chaves[2], Thais N. C. Vasconcelos[3], Leonardo de Melo Versieux[4], Clarisse Palma-Silva[1,2]

*corresponding author

[1]Programa de Pós-graduação em Biologia Vegetal, Universidade Estadual Paulista (UNESP), Rio Claro, SP, 13506-900, Brazil

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

[3]Biological Sciences Department, University of Arkansas, AR 72701, Fayetteville, USA.

[4]Departamento de Botânica e Zoologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte (UFRN), Natal, RN, 59078-900, Brazil

Acknowledgments

We thank Dr. Kléber Resende (CENA, USP) for fieldwork assistance.

Funding by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Grant numbers: 88881.128215/2016-01 and PDSE 88881.190071/2018-01, PROAP-2015/CAPES/PPGCB-BV), FAPESP (2018/07596-0), CNPq (produtividade 300819/2016-1 and 305398/2019-9), CNPq (produtividade 304778/2013-3, 303794/2019-4 and Universal Project 455510/2014-8).

ABSTRACT

Aim

The exceptional species-richness associated with mountains worldwide is linked to the fragmented topography of these areas, responsible for constantly isolating populations during periods of climatic fluctuations. Consequently, endemism and spatial turnover in mountains are very high and few species are widespread among entire mountain ranges, precluding population-level studies that help understanding how macroevolutionary patterns were shaped. Here, we used a species endemic to, but widespread in, one of the most species-rich ancient montane areas in the globe to test how environmental changes over time may have acted on the evolutionary history of this taxon, contributing to understanding how montane macroevolutionary patterns were shaped.

Location

Espinhaço Range, Eastern South America.

Taxon

Vriesea oligantha species complex.

Methods

Through analyses of plastidial and nuclear DNA of *Vriesea oligantha*, we dated its origin and intraspecific diversification, and estimated the genetic diversity, structure and migration rates among populations. Using climatic and geographic variables, we modeled suitable areas for the present and the past, estimating corridors between isolated populations. We also used demographic analyses to estimate ancient population dynamics of *V. oligantha*. Finally, we tested whether climatic variables or geographical distance explain the observed population structure.

Results

The origin and intraspecific diversification of *Vriesea oligantha* are related to early climatic oscillations during the Plio-Pleistocene. This species has a high population structure due to its low pollen and seed dispersibility. The analysis of species distribution modeling estimated corridors between populations in the past, whereas the structure of *V. oligantha* results from both models of isolation by distance and isolation by environment.

Main conclusions

The phylogeographic patterns of *Vriesea oligantha* reflect previously recognized spatial and temporal macroevolutionary patterns in the *Espinhaço* Range, providing insights into how microevolutionary processes may have given rise to this astonishing mountain biodiversity.

Keywords

Bromeliaceae, *campos rupestres*, epiphytes, *Espinhaço*, interglacial refugia, phylogeography, species-pump, *Vriesea*

1 INTRODUCTION

Mountains are remarkable models for evolutionary studies since they host a substantial proportion of the world's biodiversity and harbors high levels of endemism (Antonelli et al., 2018; Perrigo, Hoorn & Antonelli, 2020). The drivers of this diversity began to be explored by Alexander von Humboldt, who studied the relationship between mountain vegetation and its abiotic traits (Humboldt, 1807). But how this astonishing biodiversity arises is still a debatable subject.

Based on the hypothesis of Quaternary refugies (Haffer, 1969; Vanzolini & Williams, 1970), climatic fluctuations are one of the main factors that would explain the high biodiversity in mountains (Antonelli et al., 2018; Rull, 2011). Accordingly, extant species restricted to mountain tops are in interglacial refuges, while in glacial periods, their area of distribution would be larger, seeking their optimal niches towards lowlands (Perrigo et al., 2020). The Last Glacial Maximum (LGM, ~21 kyr) predominates in the literature as a common driver of diversification, mainly on Northern but also in Southern latitudes (Beheregaray, 2008; Feliner, 2011). However,repeated cycles of climatic oscillations from the last million years may also have contributed to isolate mountain populations, leading to genetic drift and local adaptation to new environments and potentially generating new species (Flantua, O'Dea, Onstein, Giraldo & Hooghiemstra, 2019). This phenomenon has been described as a species pump (Haffer, 1997), biodiversity pump (Rull, 2005), or isolation-cooling hypothesis (Rull & Vegas-Vilarrúbia, 2020), and is thought to be associated with the elevated speciation rates linked to mountain ranges.

An example of the link between increased diversification during periods of climatic fluctuations can be observed in the *Espinhaço* Range in Eastern South America. The *Espinhaço* Range harbors an astonishing plant diversity, accounting for nearly 15% of the entire Brazilian Flora, with ca. 2,000 endemic species, making these mountains home of one of the highest species richness and endemism rates of the world (Silveira, Dayrell, Fiorini, Negreiros & Borba, 2020). Many *Espinhaço* endemic angiosperms lineages exhibit high rates of diversification in the last 5 Myr (Vasconcelos et al., 2020), despite the ancient age of these mountains (640 Myr - Precambrian) (Pedreira & de Waele, 2008), indicating the species-pump phenomenon caused by the climatic oscillations of the Plio-Pleistocene period as one

of the most likely drivers of the *Espinhaço* biota formation (Alcantara, Ree & Mello-Silva, 2018; Ribeiro, Rapini, Damascena & van Den Berg, 2014).

The species-pump assumes that spatial isolation is one of the main drivers of speciation, and a presumable consequence is that distributions of endemic species are clustered in small areas (Vasconcelos et al., 2020), leading to high spatial turnover. This high degree of endemism associated to different portions of the *Espinhaço* Range is also remarkably congruent between different organisms (Chaves, Freitas, Vasconcelos & Santos, 2015; Echternacht, Trovó, Oliveira & Pirani, 2011) and has led to the recognition of distinct biogeographical regions in the northern (e.g., the *Chapada Diamantina* province) and in the mid-southern portions of the range (e.g., the *Diamantina* Plateau and the Iron Quadrangle districts) (Colli-Silva et al., 2019).

Are past climatic fluctuations common factors that could explain the origin of these congruent biogeographic and diversification patterns? If on a macroevolutionary scale the species-pump boosts diversification rates through population isolation events, one can expect to observe microevolutionary processes (i.e., genetic drift and restricted gene flow) acting at the population level in the early stages of divergence (Li, Huang, Sukumaran & Knowles, 2018). Confirm the effects of such processes in already diverged lineages may be unfeasible, but perhaps it might be possible to observe these first steps of speciation in extant populations of the same species distributed among isolated mountains (Pinheiro et al., 2013). Even though there have been important contributions testing the species-pump hypothesis in other montane areas (e.g., Andes: Sedano & Burns, 2010; Himalayas: Liu et al., 2016; Sierra Nevada: Schoville, Roderick & Kavanaugh, 2012) studies in the *Espinhaço* Range are often prevented by the narrow distributions of endemic species, precluding the understanding of how underlying microevolutionary processes have promoted the emergence of macroevolutionary patterns of these mountains.

In this context, we used the wide-distributed *Espinhaço*-endemic bromeliad *Vriesea oligantha* species complex as a model system to investigate how climatic oscillations have shaped evolution of endemic lineages in this mountain range. Following the wide distribution and high-altitude suitability of *V. oligantha*, we

hypothesized that its evolutionary history likely follows the history of its habitat, varying in area and connectivity due to ancient climate oscillations. To test this premise, we predict that (i) isolation by distance together with isolation by environment are the main factors driving diversification, in accordance with the species-pump model, and (ii) past corridors connecting populations may have existed when climate was cooler. If both hypotheses are corroborated, we can expect that (iii) the genetic structure of populations reflect the macroevolutionary patterns found in lineages endemic to the *Espinhaço* Range. To answer these questions, we adopted different approaches, including population genetics, dated phylogenies and ecological niche modeling.

2 MATERIAL AND METHODS

2.1 Sampling and DNA extraction

The species complex *Vriesea oligantha* comprises morphologically similar species (the "*limae*" clade (Machado et al., 2019), all rupicolous and epiphytic bromeliads endemic to the *Espinhaço* Range. For the purpose of this work, we considered all sampled individuals as *Vriesea oligantha,* sampling 229 individuals from 14 populations (Table 1), covering its entire distribution range (Figure 1).

We extracted the total genomic DNA from silica-gel-dried leaves following a modified CTAB protocol described by Tel-Zur, Abbo, Myslabodski & Mizrahi (1999). Initially we tested ten plastidial markers (*petA-psbJ, petG-trnC, psbA-trnH, rpoB-trnC, psbM-trnD, rpoB-trnC-petN, trnK-matK-trnK, trnL-trnF, ycf1, ycf6-trnC*) and one nuclear (*phyC*), selecting two plastidial markers based on the higher polymorphism levels, *ycf1* (Barfuss et al., 2016) and *trnL-trnF* (Barfuss, Samuel, Till & Stuessy, 2005) to be amplified in 95 samples from 14 populations.

2.2 Plastidial DNA sequencing and nuclear microsatellite genotyping

Amplifications for the *ycf1* and *trnL-trnF* markers were performed in a 30 µL reaction using 3 ng of genomic DNA, 5x GoTaq Green Master Mix (Promega, Madison, USA), 0.5 µM of each primer and 1% of DMSO. Polymerase chain reaction (PCR) was conducted using a Veriti 96-well Thermal Cycler (Applied Biosystems, Foster City, USA) following the protocols described in Barfuss et al. (2005, 2016). PCR products were then purified and sequenced both forward and reverse directions on Macrogen (Seoul, South Korea). Consensus sequences and the alignment matrix were assembled on Geneious R10, using the default aligner algorithm. Indels longer than 1 base pair were removed due to uncertain homology. Both markers were concatenated for subsequent analyses. Sequences are deposited in GenBank (XXXXXX-YYYYYY[1]).

For nuclear microsatellites (nrSSR) loci, we genotyped nine polymorphic markers previously transferred from other bromeliads (Cacossi, Dantas-Queiroz &

1 DATA AVAILABILITY STATEMENT - We will submit the DNA sequences used in this work on GenBank (NCBI) after the publication acceptance.

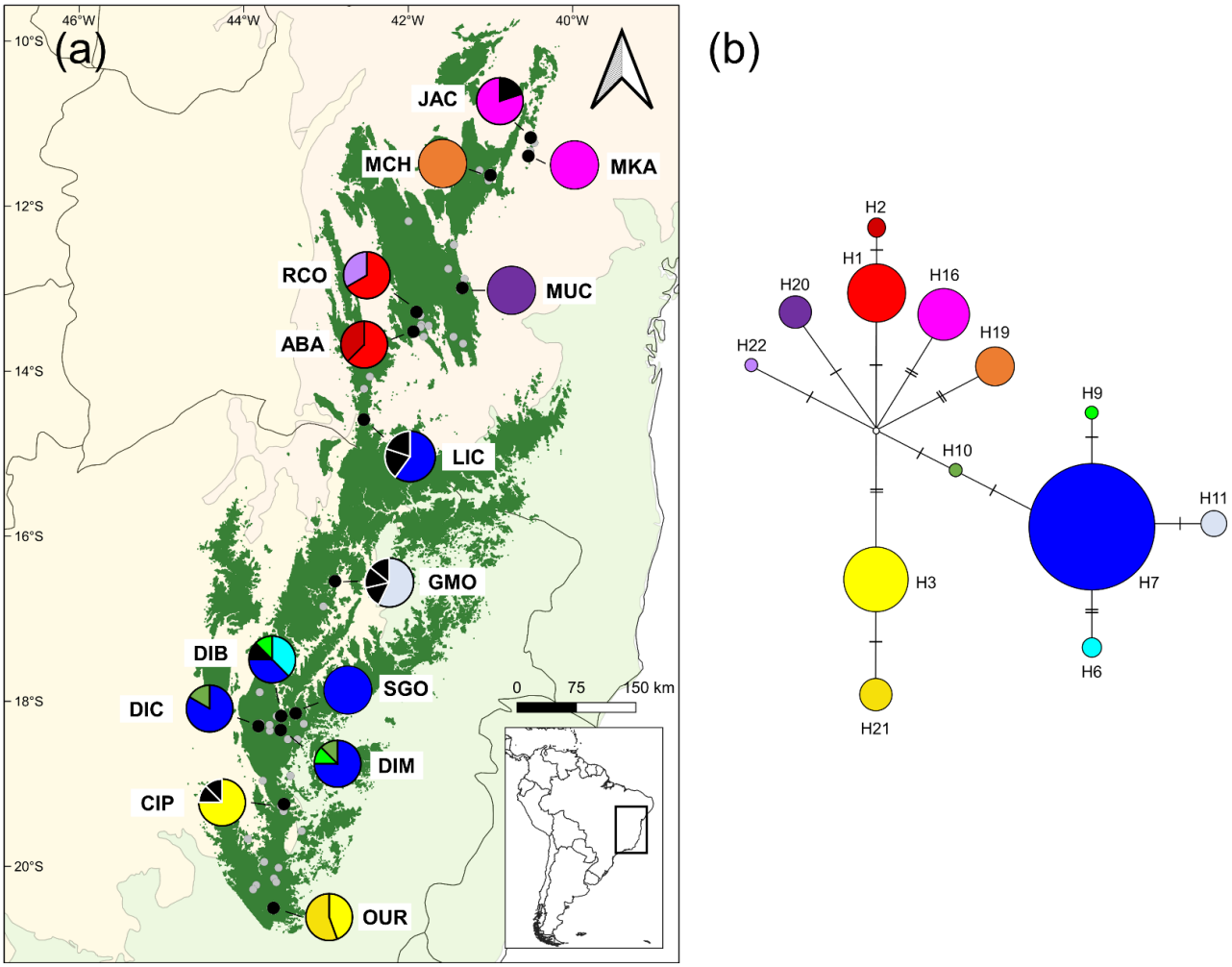
Palma-Silva, 2019) in 229 individuals from 12 populations. Protocols for genotyping followed those described in Cacossi et al. (2019).

Table 1 -Population names, geographic locality and tested individual samples for plastidial and nuclear data.

Population	Location	Latitude	Longitude	Altitude (m)	Plastidial *n*	Nuclear *n*
JAC	Jacobina - BA	-11.1704	-40.5112	713	5	16
MKA	Miguel Calmon - BA	-11.3933	-40.5393	1003	4	-
MCH	Morro do Chapéu - BA	-11.6263	-41.0000	904	6	19
MUC	Mucugê - BA	-12.9915	-41.3440	980	5	19
RCO	Rio de Contas - BA	-13.5244	-41.9567	1577	6	30
ABA	Abaíra - BA	-13.2838	-41.8998	1654	8	19
LIC	Licínio de Almeida - BA	-14.5874	-42.5400	915	5	5
GMO	Grão Mogol - MG	-16.5447	-42.8910	1088	7	20
DIB	Diamantina (Biribiri) - MG	-18.1787	-43.5447	1339	8	20
DIC	Diamantina (Cons. Mata) - MG	-18.3012	-43.8224	1313	6	18
DIM	Diamantina (Milho Verde) - MG	-18.3490	-43.5512	1206	8	-
SGO	São Gonçalo do Rio Preto - MG	-18.1460	-43.3687	906	10	15
CIP	Conceição do Mato Dentro - MG	-19.2467	-43.5116	1270	8	23
OUR	Ouro Branco - MG	-20.5051	-43.6390	1375	9	25

Fonte: o autor.

Figure 1 – (a) Map of the *Espinhaço* Range (dark green) and its surrounding phytogeographic domains: Caatinga (North, pink), Cerrado (West, beige) and Atlantic Forest (East and South, light green). Dark lines are the Brazilian States borders. This map shows the entire geographic distribution of *Vriesea oligantha*. Grey points represent occurrence data from herbarium specimens and black points are the sampled populations (See Table 1, for abbreviation codes). The pie charts represent the frequency of occurrence of each haplotype in each population; haplotype colors correspond to those shown in the network; black haplotypes are singletons. (b) Median-joining network depicting the relationships among the haplotypes of *V. oligantha*.



Fonte: o autor.

2.3 Phylogenetic inferences and divergence times

To determine the origin and intraspecific divergence time among *Vriesea oligantha* populations, we conducted time calibration analyses in two steps. First, we performed a dating analysis of the Tillandsioideae subfamily, including six individuals of *V. oligantha* from different populations in a matrix with 171 species of Tillandsioideae and seven species from other subfamilies of Bromeliaceae using three plastidial (*matK, rpoB-trnC* and *ycf1*) and one nuclear (*phyC*) markers downloaded from Genbank (see Appendix S1 in Supporting Information). The phylogenetic tree for Tillandsioideae was inferred using the GTR+I+G site model, estimated in jModelTest2 (Darriba, Taboada, Doallo & Posada, 2012), assuming a Yule speciation prior, a lognormal prior distribution for calibration points and a normally distributed prior root set to mean=19 Myr, SD=1.25 under an uncorrelated lognormal relaxed clock model. The calibration age was extracted from Givnish et al. (2011).

Secondly, using the crown age obtained for the *Vriesea oligantha* clade, we ran a calibration approach to infer the dates of the intraspecific diversification of *V. oligantha*. We used samples from the 95 individuals described above, using seven other Tillandsioideae as outgroups. We used a normal distribution prior for the calibration point at the root, the GTR+I+G substitution rate model selected using jModelTest2 and a coalescent constant size random tree under an uncorrelated exponential relaxed-clock model.

Both steps were run in BEAST 1.10.4 and executed in CIPRES Portal (Drummond & Rambaut, 2007; Miller, Pfeiffer & Schwartz, 2010). Each analysis had four independent MCMC runs of 200 million generations sampled every 1,000. We used Tracer 1.10.4 to assess the convergence and effective sample size (ESS>200) for each run. As a burn-in, we excluded 10% of trees for each step, combining the remaining trees in LogCombiner. Maximum clade credibility trees for Tillandsioideae and *Vriesea oligantha* were inferred using TreeAnnotator and visualized in FigTree 1.4.4 and in the Interactive Tree of Life platform (https://itol.embl.de/).

2.4 Genetic diversity

For cpDNA, we calculated the nucleotide diversity (π), haplotype diversity (Hd) and polymorphic sites (ss) per population in ARLEQUIN 3.5 (Excoffier & Lischer, 2010). A haplotype matrix was constructed based on ss detected, which were coded as single characters. To explore historical relationships among haplotypes, we built a median-joining-network (Bandelt, Forster & Rohl, 1999) on Network 5.0.1.1 (http://www.fluxus-engineering.com).

For nrSSR, putative clones were examined and removed using GenClone 2.0 (Arnaud-Haond & Belkhir, 2006). The number of alleles per locus (A), allelic richness per locus (AR), the observed (H_O) and the expected (H_E) heterozygosity, and the inbreeding coefficient (F_{IS}) were estimated per population using GenAlex 6.5 (Peakall & Smouse, 2012). We evaluated the deviations from the Hardy-Weinberg equilibrium (HWE) per population and per loci using Genepop software v3.5 (Raymond & Rousset, 1995). Linkage disequilibrium between all pairs of loci was tested in FSTAT 2.9.3.2 (Goudet, 1995).

2.5 Population structure and migration

For the population genetic structure of cpDNA, we employed a clustering analysis on GENELAND 4.0.7 (Guillot, Mortier & Estoup, 2005), testing 15 putative clusters. For nrSSR, we used STRUCTURE v.2.3.3 (Pritchard, Stephens & Donnelly, 2000) to assign individuals to genetic clusters (K) under the admixture model assuming independent allele frequencies. The number of K was set from 1 to 13, with 1,000,000 simulations for each K-value and a burn-in rate of 20%. The most probable number of K was examined using Structure Harvester v.6.0 (Earl & von Holdt, 2012), following the instructions of Evanno, Regnaut, & Goudet (2005).

Genetic differentiation was estimated using F-statistics (Weir & Cookham, 1984), based on cpDNA and nrSSR. Pairwise F_{ST} between localities were calculated using ARLEQUIN 3.5. We also conducted a molecular variance analysis (AMOVA) to evaluate the partition genetic variance in hierarchical models grouping by lineages obtained from the phylogenetic cpDNA tree by running 10,000 permutations between groups. AMOVA analyses were implemented on ARLEQUIN 3.5.

Recent migration events were estimated in BAYESASS 3.04 (Wilson & Rannala, 2003). Samples were run for $1.0x10^8$ interactions with a 10% burn-in, sampling every 1,000 interactions. Ancient migration events were estimated using MCMC and coalescence theory approach, implemented in MIGRATE 4.4.3 (Beerli & Felsenstein, 2001), testing two models, using the four phylogenetic lineages as groups: a panmictic model and a neighbor-only model, where migration was only allowed between adjacent groups. For both models, we used a Brownian motion model of mutation under the maximum-likelihood framework and 10 replicates of 1,000,000 runs with a 10% burn-in.

2.6 Paleodistribution and ancestral population connections

To predict the current and paleodistributions (i.e., Mid-Holocene, MH, 6 kyr; Last Glacial Maximum, LGM, 21 kyr; and Last Interglacial Maximum, LIG, 120-140 kyr) of *Vriesea oligantha*, we conduct species distribution modeling (SDMs) based on 55 unique occurrence records using an ensemble approach that combined the results from six distinct modeling algorithms (see Appendix S2 for detailed analyses). From the estimated SDMs, we generated population connectivity maps by summing the least-cost path (LCP) among all populations (Chan, Brown & Yoder, 2011) to investigate ancestral suitable connections. We created a Friction Layer by inverting the SDM to a dispersal cost layer on ArcMap 10.5 (ESRI, 2016). Next, we calculated corridors that minimize the cost of dispersal between populations by following paths of lowest frictions. To do so, we used the “Least-Cost Corridors and Paths>Pairwise: All Sites” tool from SDMtoolbox 1.1a (Brown, 2014), implemented on ArcMap 10.5.

2.7 Demographic reconstruction

To evaluate the demographic history of *Vriesea oligantha*, we used the cpDNA data to calculate the Fu’s Fs, Taijma’s D and Rozas’ R2 statistics and tested their departures from neutrality based on 100,000 coalescent simulations with DnaSP (Librado & Rozas, 2009). We also inferred changes in the species’ effective population size through time using a Coalescent Bayesian Skyline Plot (BSP) approach implemented in BEAST 1.10.4 and performed two independent MCMC runs of 200 million generations for each analysis, sampling every 2000 generations in the CIPRES. The BSP analysis was explored using the total cpDNA data set for each

clade of the species tree under a strict molecular clock using the substitution rate interval inferred by the divergence time estimated for the Tillandsioideae subfamily (see Results below). Best substitution models for both markers were defined according to the Akaike Information Criterion (AIC) on jModelTest 2.0.

For nrSSR data, we estimated contemporary effective population sizes using NEestimator 2.1, with the Molecular Coancestry Method (Do et al., 2014). Putative recent bottleneck events were estimated using the “Wilcoxon sign-rank test’ with ‘Two phased mutation model’, with a total of 5,000 simulation iterations using BOTTLENECK 1.2.02 (Piry, Luikart & Cornuet, 1999).

2.8 Roles of climate and geography on population structure

We first tested whether populations are differentiated following a model of isolation by distance by conducting a Mantel test (Wright, 1965) in R-package ‘adegenet’ with 10,000 permutations between geographic and genetic pairwise distances matrices, measured as $F_{ST}/(1-F_{ST})$. Then, we implemented a Bayesian generalized linear mixed modeling (GLMM) approach to test whether population genetic structure of *V. oligantha* is driven by isolation by environment (i.e., temperature and precipitation) and/or isolation by distance models (see Appendix S3 for detailed method).

3 RESULTS

3.1 Phylogenetic inferences and divergence times

The topology of the time-calibrated tree of Tillandsioideae indicates that the *Vriesea oligantha* complex is monophyletic (posterior probability, pp = 1.00) and belongs to Vrieseinae subtribe (Figure 2, S4.1, Table S5.1). This Bayesian inference indicated a nucleotide substitution rate mean of 0.0011135 Ma^{-1} (95% HPD=0.0009402-0.0012894). The estimated time to the most recent common ancestor (TMRCA) of *V. oligantha* was dated at 3.26 Myr (95% HPD=4.48-1.83 Myr) (Figure 2, Table S5.1). This tree shows four well-supported lineages in distinct geographic regions of the *Espinhaço* Range (Figure 2, S4.2). The Northern clade includes the JAC and MKA populations (pp=0.86, 0.702 Myr, 95% HPD=1.47-0.076 Myr) while *Chapada Diamantina* clade includes the MCH, MUC, RCO and ABA populations (pp=1.00) and it is estimated to be the oldest lineage (1.10 Myr, 95% HPD=1.17-1.04 Myr). *Diamantina* Plateau clade, includes the GMO, DIB, DIC, DIM, SGO and LIC populations (pp=0.98, 0.861 Myr, 95% HPD=1.06-0.494 Myr) and the Southern region of *Espinhaço* comprehends a clade with CIP and OUR populations (pp=0.99, 0.708 Myr, 95% HPD=0.999-0.274 Myr).

3.2 Genetic diversity

The final alignment matrix with consensus cpDNA sequences of *Vriesea oligantha* had 2,065 bp (*trnLF-trnF,* 753 and *ycf1,* 1312), showing 17 polymorphic sites and 22 haplotypes (Figure 1). The haplotype diversity per population ranged from zero to 0.785, and the nucleotide diversity from zero to 0.000761, with an overall haplotype and nucleotide diversity of 0.392 and 0.000280, respectively (Table 2). Few haplotypes are shared among populations*,* but the most frequent haplotype (H7) was found in five populations of the Diamantina Plateau clade, which also showed the highest values of nucleotide and haplotype diversity (Figure 1, Table 2).

We found a total of 162 alleles across loci and 67 were exclusive to single populations. Populations JAC and MUC presented the highest number of private alleles with 16 and 13 alleles, respectively (Table 2). Populations of *Vriesea oligantha* showed an averaged allelic richness ranging from 1.713 to 3.124, whereas variance in allele size ranged from 2.975 to 22.585 (Table 2). Observed and expected

heterozygosity per population varied from 0.034 to 0.550 and 0.266 to 0.627, respectively. The mean inbreeding coefficient (F_{IS}) was high and significant for most populations, with the exception of LIC population, ranging from 0.125 in DIB and 0.888 in MCH (Table 2).

Table 2 – Genetic diversity and tests of neutrality of 14 populations of *Vriesea oligantha.*

		plastidial						nuclear							
Pop	**Haplotypes**	**p**	**Hd**	**ss**	**Fu**	**D**	**R2**	**P**	**A**	**A_P**	**R**	**Var**	**H_O**	**H_E**	**F_{IS}**	**Ne**
JAC	H15-H16	0.000194	0.400	1	0.090	-0.8165	0.4	100	50	16	3.014	21.839	0.486	0.556	0.182**	13.0
MKA	H16	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-
MCH	H19	0	0	0	0	0	0	66.7	19	5	1.725	2.975	0.034	0.288	0.888**	2.4***
MUC	H20	0	0	0	0	0	0	100	53	13	3.124	17.813	0.471	0.627	0.281**	3.9
RCO	H1, H22	0.000517	0.533	2	1.723	1.03194	0.2667	66.7	21	0	1.713	3.694	0.181	0.266	0.357**	1.3
ABA	H1-H2	0.000259	0.535	1	0.866	1.1665	0.2679	88.9	36	7	2.248	13.705	0.147	0.444	0.679**	6.0
LIC	H7, H17-H18	0.000581	0.7	3	-0.185	-1.04849	0.2667	77.8	20	3	2.084	22.585	0.519	0.377	-0.258	∞
GMO	H11-H14	0.000484	0.714	2	-1.483*	0.92787	0.25	77.8	32	2	1.980	6.389	0.219	0.322	0.343**	∞
DIB	H6-H9	0.000761	0.785	4	-0.328	0.08124	0.1722	100	59	3	3.111	13.687	0.550	0.609	0.125**	3.8
DIC	H7, H10	0.000161	0.333	1	-0.002	-0.93302	0.3727	100	51	8	3.043	14.433	0.350	0.592	0.441**	20.4
DIM	H7, H9-H10	0.000242	0.464	2	-0.998	-1.31009	0.2165	-	-	-	-	-	-	-	-	-
SGO	H7	0	0	0	0	0	0	88.9	41	3	2.718	10.507	0.361	0.510	0.325**	8.4
CIP	H3-H5	0.00045	0.464	3	0.071	-0.81246	0.161*	88.9	32	3	2.251	12.496	0.145	0.430	0.676**	8.9
OUR	H3, H21	0.000269	0.555	1	1.015	1.40117	0.2778	55.6	23	4	1.773	9.245	0.071	0.291	0.766**	10.3***

p = nucleotide diversity; *H*d = haplotype diversity; *ss* = polymorphic sites; Fu = Fu's Fs statistics; D = Tajimas's D statistics; R2 = Ramos-Onsins-Rozas' statistics. **p-value* < 0.01

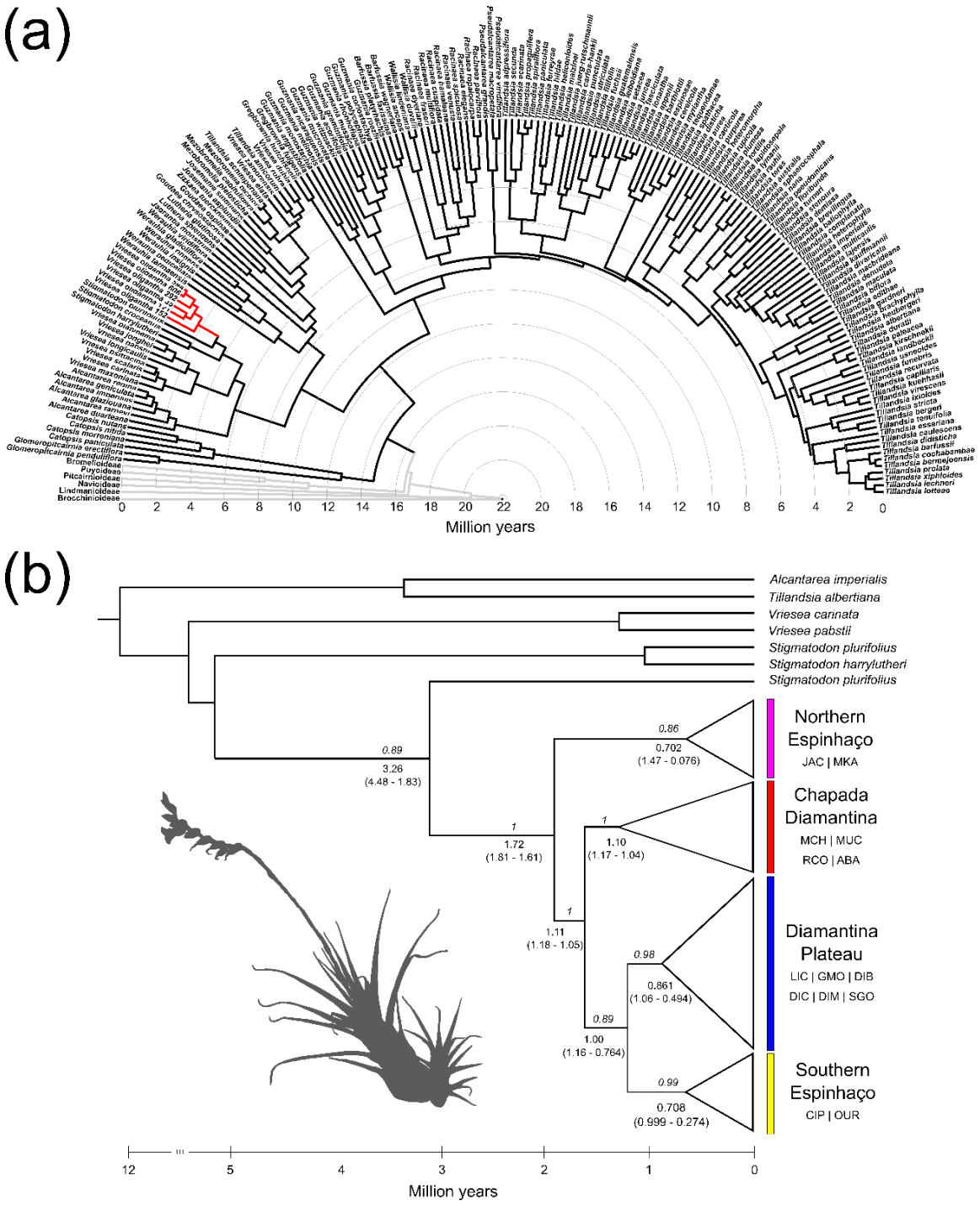
P = percentage of polymorphic loci; A = number of alleles; A_P = number of private alleles; R = allelic richness; Var = variance in allele size; H_O = Observed heterozygosity; H_E = Expected heterozygosity; F_{IS} = Inbreeding coefficient, Ne = Effective population size.

**departures of within-population inbreeding coefficients from Hardy-Weinberg equilibrium (*p-value* < 0.01).

***Wilcoxon test < 0.05

Fonte: o autor.

Figure 2 – (a) Maximum clade credibility tree of Tillandsioideae. Branches in light grey are outgroups, branches in black are Tillandsioideae and branches in red are the *Vriesea oligantha* lineage. (b) Maximum clade credibility tree of *Vriesea oligantha*, depicting four lineages: Northern *Espinhaço* (pink), *Chapada Diamantina* (red), *Diamantina* Plateau (blue) and Southern *Espinhaço*. Bayesian posterior probabilities are in italics above each node; clade ages (in million years) are written under each node; values in parentheses are the 95% highest posterior density (HPD). A silhouette of *Vriesea oligantha* is in the inset.



Fonte: o autor.

3.3 Population structure

GENELAND results, based on cpDNA markers, indicated five clustering among populations of *Vriesea oligantha* (Figure 3a-e). Northern populations (JAC, MKA and MCH) form a single cluster, whilst populations from *Chapada Diamantina* (ABA, MUC and RCO) are clustered together. Diamantina Plateau populations (DIB, DIC, DIM, LIC and SGO) belong to a separate cluster while GMO constitute a single cluster. The Southern *Espinhaço* populations (CIP and OUR) form the fifth cluster. STRUCTURE clustering analysis (Figure 3f), based on nrSSR revealed a large number of genetic clusters among all populations (k = 11).

The pairwise F_{ST} values among populations were significant for almost all combinations, ranging 0.117-1.000 for cpDNA data and 0.007-0.794 for nrSSR data, indicating a widely heterogeneous structure between populations (Figure 4a-b, Tables S5.2-5.3). AMOVA showed high values of structure for both markers (cpDNA, 0.822; nrSSR, 0.432) and, hierarchically, cpDNA showed that the largest proportion of variance was confined within population (0.853, $p<0.001$) (Table 3).

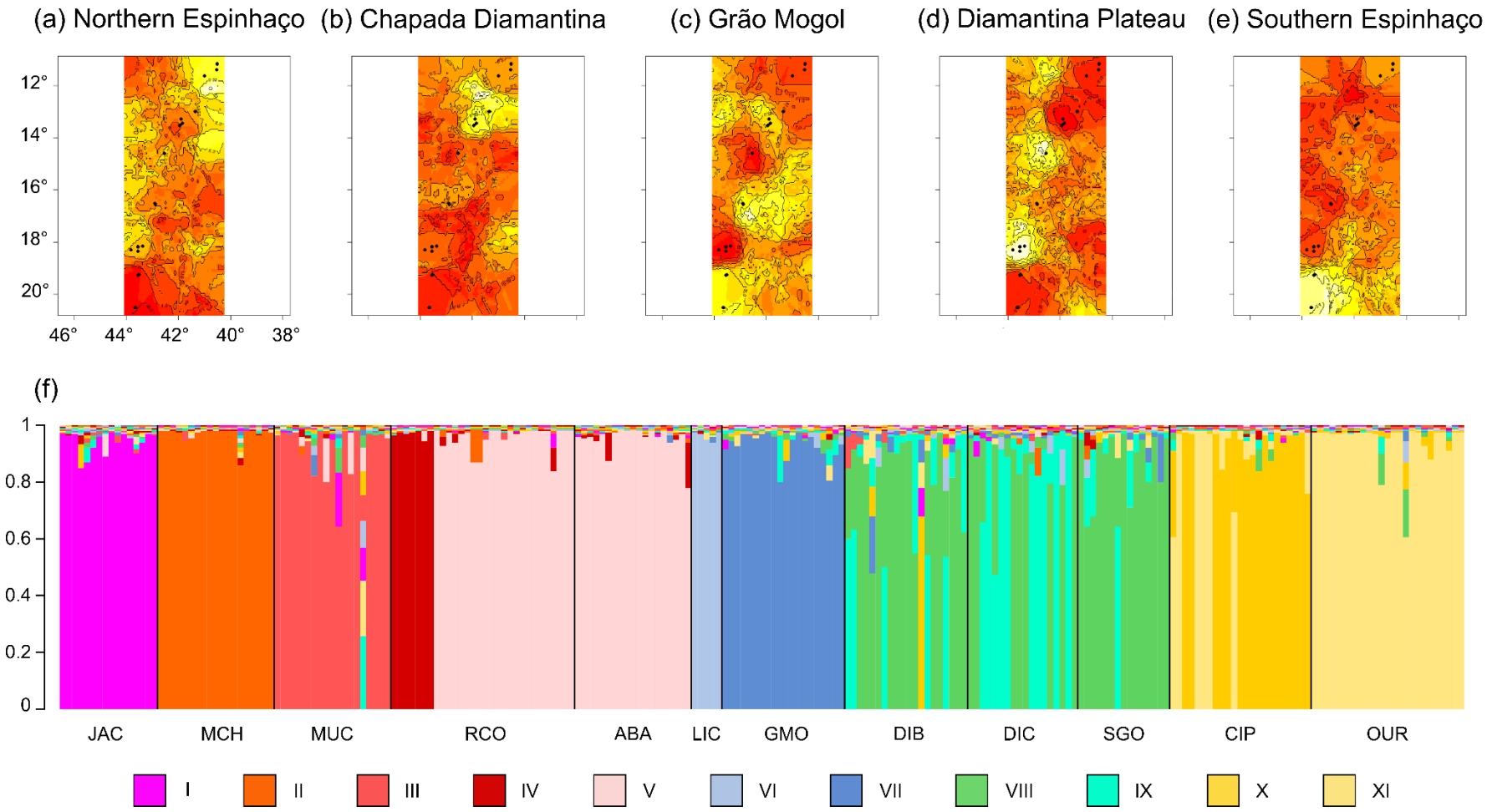
Gene flow estimation from BAYESASS shows very few contemporary migration events, only among nearby populations (Table S5.4). Similarly, MIGRATE estimated few ancient events between the tested models (Table S5.5), where the migration only between adjacent groups model was better-supported than the panmictic model (Table S5.6).

Table 3 - Analysis of molecular variance on cpDNA sequences and nrSSR, based on GENELAND and STRUCTURE, respectively. All values of *F*-statistics are significant (*p-value* < 0.001).

Clustering method	Source of variation	df	Sum of Squares	Variance components	Variation (%)	*F*-statistics
cpDNA						
All populations	Among populations	13	125.787	1.38841	82.2	F_{ST} = 0.822
	Within populations	81	24.297	0.29997	17.7	
Phylogenetic lineages	Among lineages	3	95.702	1.35333	65.9	F_{CT} = 0.659
	Among populations within lineages	10	30.085	0.39953	19.4	F_{SC} = 0.571
	Within populations	81	24.297	0.29997	14.6	F_{ST} = 0.853
	Total	94	150.084	2.05283	100	
nrSSR						
All populations	Among populations	11	302.447	0.70207	43.2	F_{ST} = 0.432
	Within populations	446	410.732	0.92092	56.7	
	Total	457	88079.555	238.4214	100	

Fonte: o autor.

Figure 3 –Clustering methods used for *Vriesea oligantha* populations based on cpDNA (a-e) and nrDNA (f). In GENELAND analysis (a-e), yellow areas are associated with a high probability of distinct populations belonging to each cluster (See text for population assignment). In STRUCTURE analysis (f), each color represents a given cluster (I-XI) and each bar corresponds to a single individual.



Fonte: o autor.

3.4 Palaeoclimatic distribution and ancestral population connections

Our species distribution models (SDMs) indicated that 120-140 kyr ago (LIG), high suitable areas for *Vriesea oligantha* were concentrated in sparse fragments, southwards from the current distribution, although mid-suitability would be observed all over the *Espinhaço* Range. This suggests that the species might have survived in small areas in northwards and westwards of its distribution during this period (Figure 5d). Later, as the climate got colder, during the LGM (21 kyr), suitable areas of *V. oligantha* might have increased, becoming progressively connected (Figure 5c). According to our SDMs, the *V. oligantha*'s distribution became more fragmented once again during the Mid-Holocene (6 kyr), with suitable areas in the Northern *Espinhaço* (Figure 5b). The current distribution mainly reflects areas of high altitude in the *Espinhaço* Range (Figure 5a).

Analysis of the least-cost path (LCP) across isolated populations revealed suitable areas of ancestral connection over all investigated periods, especially between the *Chapada Diamantina* and the *Diamantina* Plateau populations (Figure 6). However, those connections seem broader in the LIG (Figure 6d), while they become progressively narrow until the current climate conditions, with high suitability among populations in the *Diamantina* Plateau (Figure 6a-c). Populations from the northern and southern peripheries of the distribution presented lower connections with the central groups in all periods.

3.5 Demographic analyses

All populations exhibited no departure from neutrality on demographic analyses, except for GMO (in Fu's FS) and CIP (in Roza's R2) (Table 2). Similarly, the Bayesian phylogenetic inference indicated a stable effective population size for the total dataset and to the four lineages during the last 100 kyr (Figure S4.3).

Contemporary effective population size ranged from 1.3 to 20.4, in populations RCO and DIC, respectively, while populations LIC and GMO showed infinite values (Table 2). Based on BOTTLENECK analysis, only MCH and OUR exhibited excess heterozygosity concerning the expectation under the TPM model (Wilcoxon test <

0.01, Table S5.7), evidencing that these two populations may have experienced population reduction.

3.6 Relative roles of climate and geography

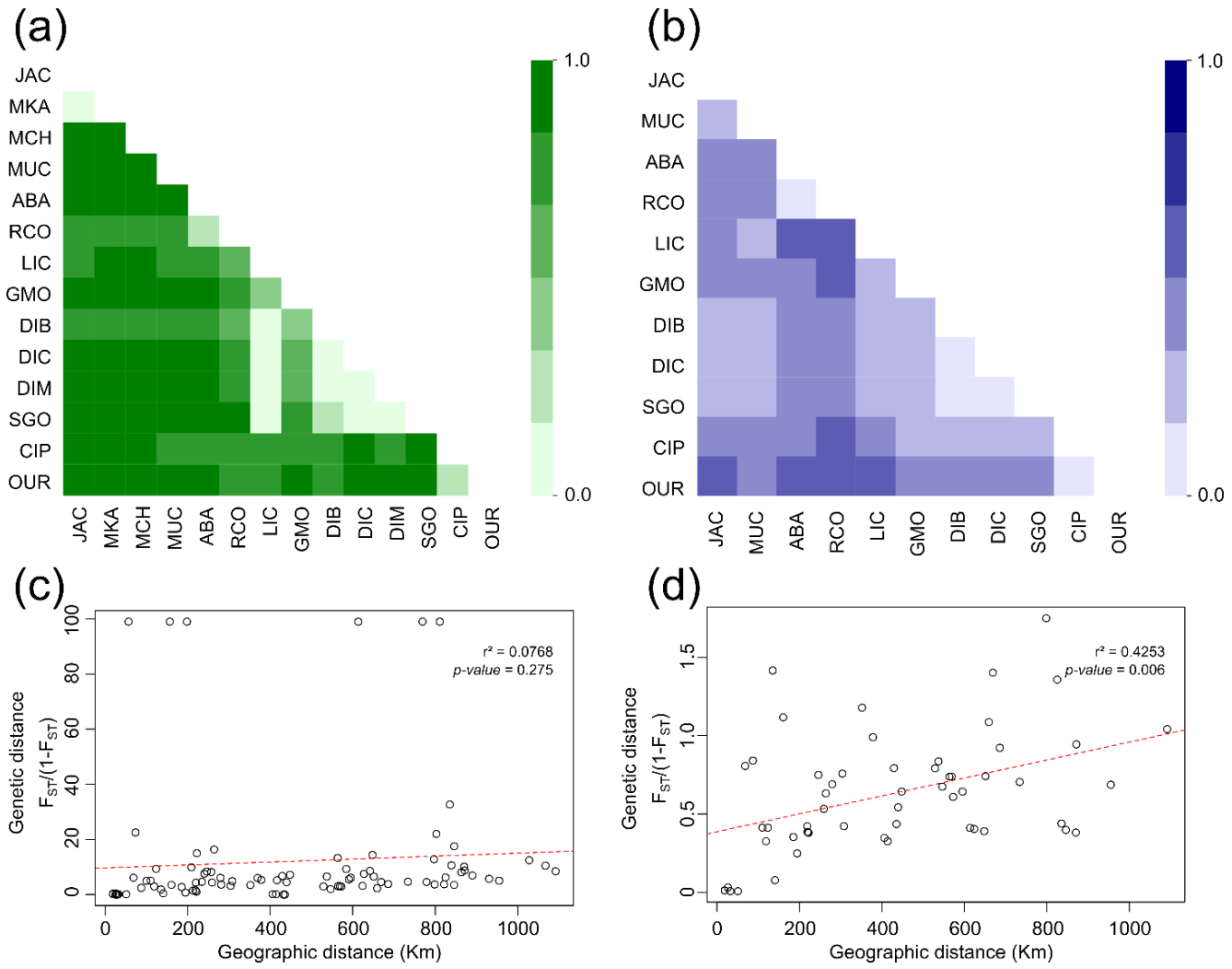
Mantel test showed a non-significant correlation between cpDNA differentiation and geographic distance (r^2=0.07, *p*=0.275) (Figure 4c) but a significant correlation between nrSSR differentiation and geographic distance (r^2=0.42, *p*=0.006) (Figure 4d), indicating isolation-by-distance in the nuclear genome (Diniz-Filho et al., 2013). Our GLMM analysis indicated the influence of both environmental and geographical distances on the genetic structure of *Vriesea oligantha*. Contrasted with other predictors, the model including both isolation by geographic distance (IBD) and by environment (IBE) received the highest DIC support for both plastidial and nuclear genomes (Table 4).

Table 4 - Results of generalized linear mixed models (GLMM) testing the influence of geographic distance and climatic differences, based on 19 bioclimatic variables, on the genetic divergence among populations of *Vriesea oligantha*. The best model for each data set is highlighted in bold.

	cpDNA			nrSSR		
Model	DIC	**Δ**DIC	DIC weight	DIC	**Δ**DIC	DIC weight
Null	810.1204	9.6164	0.0077	113.2596	9.5066	0.0045
Geography	811.7689	11.2648	0.0034	106.2901	2.5372	0.1461
Climate	806.3522	5.8482	0.0504	**103.7529**	**0.0000**	**0.5196**
Geography + Climate	**800.5041**	**0.0000**	**0.9386**	**104.6625**	**0.9096**	**0.3297**

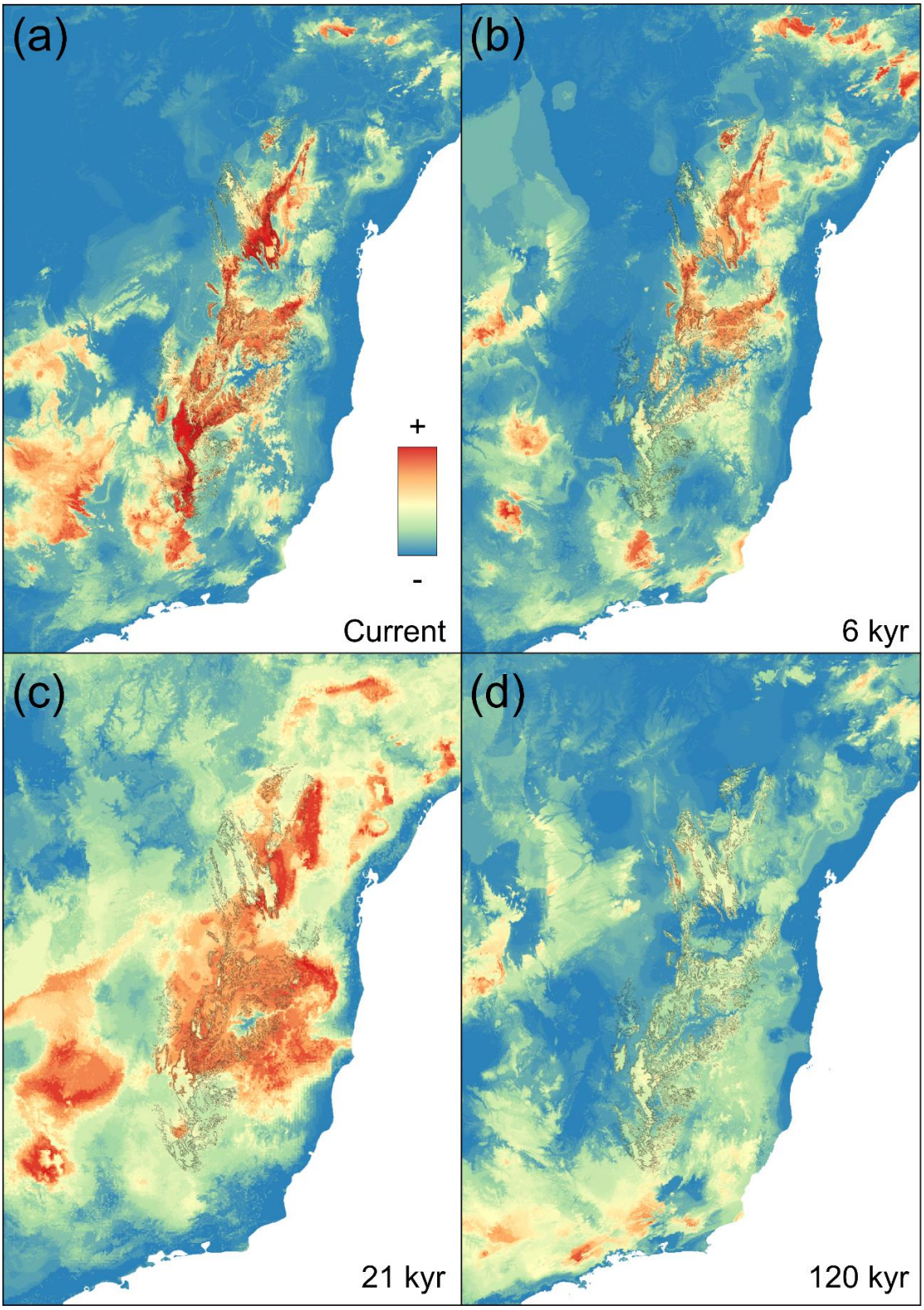
Fonte: o autor.

Figure 4 – Pairwise F_{ST} heatmap plots and relationship between genetic and geographic distances, from plastidial (a, c) and nuclear (b, d) data.



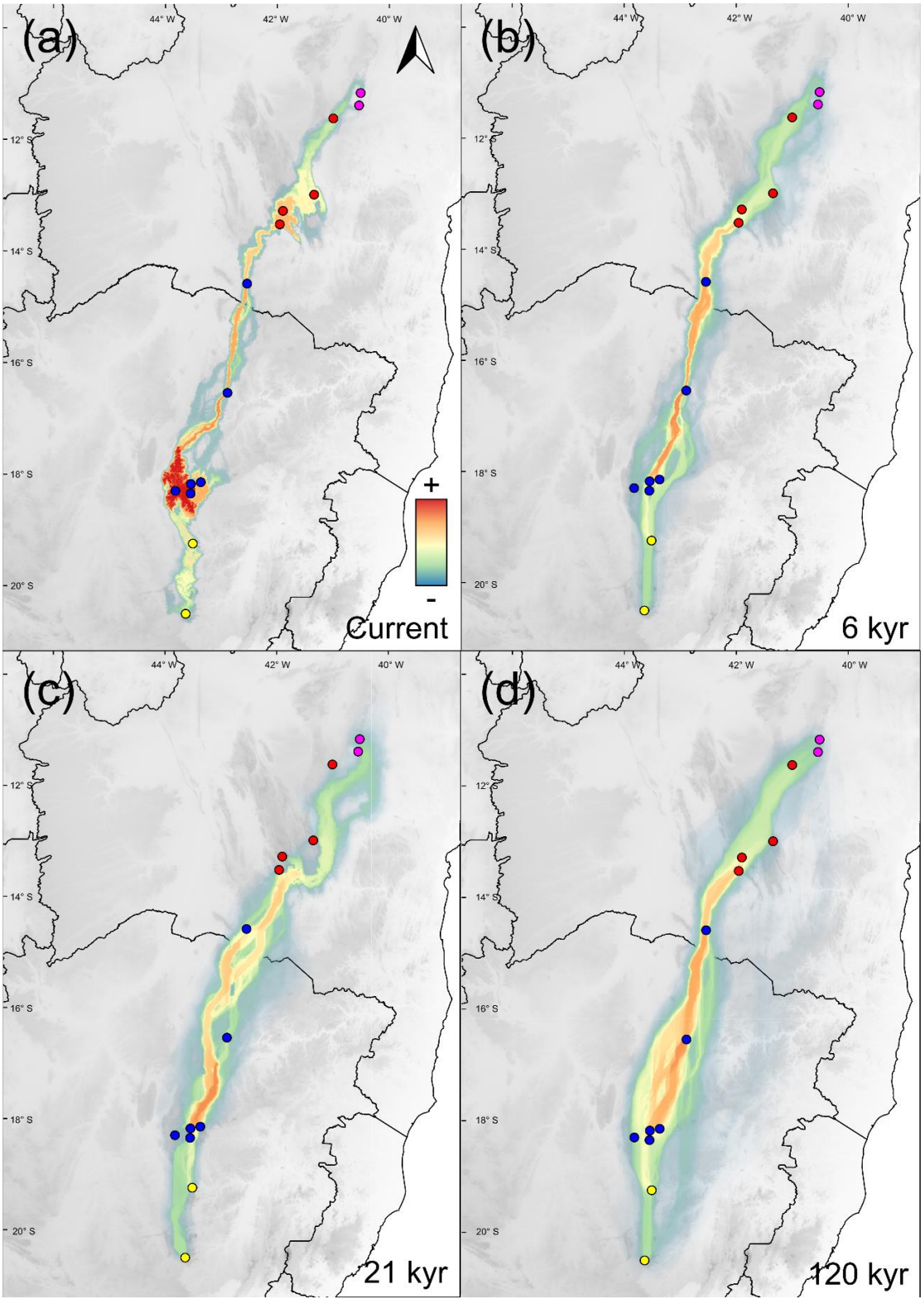
Fonte: o autor.

Figure 5 – Ensemble distribution models for *Vriesea oligantha* under (a) Current, (b) Mid-Holocene (6 kyr), (d) Last Glacial Maximum (21 kyr) and (D) Last Interglacial Maximum (120 kyr) climatic conditions. The thin line represents the current delimitation of the *Espinhaço* Range.



Fonte:o autor.

Figure 6 -Potential dispersal corridors between *Vriesea oligantha* populations (circles, each color represents a lineage from phylogenetic tree) based on friction layers, across four period times. (a). Current; (b). Mid-Holocene (6 kyr); (d). Last Glacial Maximum (21 kyr); and (d). Last Interglacial Maximum (120 kyr). Altitude is represented by the grey background.



Fonte: o autor.

4 DISCUSSION

4.1 Diversification of Vriesea oligantha

The divergence age between *Vriesea oligantha* and its outgroup dated back to the Pliocene-Pleistocene transition (3.26 Myr), as previously inferred in phylogenies of *Vriesea* (Kessous et al., 2019; Machado et al., 2019). This period is marked by a decrease in global temperature and the beginning of glaciations in the Northern Hemisphere (Prell, 1984). In this context, *V. oligantha* is a remarkable model to test effects of climatic oscillations, since this species remained consistent over the whole Pleistocene.

Our results show that the intraspecific divergence events of *Vriesea oligantha* are older than those associated with the latest Pleistocene climatic oscillations and cannot be explained solely by the late-Quaternary refugia hypothesis (Haffler, 1969; Rull, 2011). Altogether, extreme amplitude in climatic fluctuations were remarkable events in the mid-Pleistocene (Lisiecki & Raymo, 2007), when *V. oligantha* lineages diversified, supporting the view that climate fluctuations from the early-Quaternary are key components for understanding population differentiation processes and, eventually, the role of the environment in speciation events of Neotropical lineages (Silva, Antonelli, Lendel, Moraes & Manfrin, 2018). In fact, diversification events throughout the Quaternary are also congruent with other extant lineages from distinct Neotropical montane formations, as in the *Espinhaço* itself (e.g., Barres et al., 2019; Bonatelli et al., 2014; Chaves et al, 2019; Nascimento et al., 2018; the páramos (e.g., Hughes & Atchison, 2015; Madriñán, Cortés & Richardson, 2013) and the pantepuis (e.g., Rull et al, 2020; Salerno et al., 2012), reinforcing the importance of climate changes throughout the whole Pleistocene in the diversification of the montane biota in the Neotropics.

However, our demographic analyses usually assume panmictic populations, precluding a fine-scale visualization of demographic changes in highly structured populations, as in *V. oligantha* (Heller, Chikhi & Siegismund, 2013). Another caveat in the analyses is the scarcity of polymorphisms in our genetic markers, due to the low rates of molecular evolution found in Bromeliaceae (Smith and Donoghue, 2008). Furthermore, the high suitability of larger areas over the LGM inferred by our ENM differs from those of our demographic analyses, which were unable to demonstrate

significant changes in the effective population size over the past thousands of years. Therefore, distinct model-based analyses (e.g., approximate Bayesian computation; Gehara et al., 2017) could help us better understand the population dynamics of *V. oligantha* over the late-Quaternary.

4.2 Phylogeography of Vriesea oligantha*: insights from micro to macroevolution in the Espinhaço Range*

The phylogeographic results of *Vriesea oligantha* showed a remarkable congruence between the structure of different populations and the bioregionalization proposed by Colli-Silva et al. (2019), based on patterns of plant endemism in the *Espinhaço* Range. Similar biogeographic patterns were also pointed out by several other studies using floristic and faunistic composition and endemicity indexes (e.g., Bitencourt & Rapini, 2013; Bünger, Stehmann & Oliveira-Filho, 2014; Campos, Freire Moro, Funk & Roque, 2019; Chaves et al., 2015) and phylogenetic histories (e.g., Chaves et al., 2019; Ribeiro et al., 2014). Taken together, these studies suggest that particular drivers are leading the evolutionary history, community assembling, and physiognomy patterns of distinct areas in the *Espinhaço* Range (Zappi, Moro, Meagher, & Nic Lughadha, 2017).

Our results support the congruency between the Northern *Espinhaço* and the *Chapada* Diamantina lineages and clustering analysis within the *Chapada Diamantina* province (Colli-Silva et al., 2019). Populations from the Diamantina Plateau clade mainly mirrors the Southern *Espinhaço* province, particularly the Diamantina Plateau district. However, the GMO population is a noteworthy exception. In fact, as evidenced by GENELAND and STRUCTURE, GMO remained distinct on a single cluster, unarguably fitting into the *Grão-Mogol* district. The particularity of this district has already been reported elsewhere (e.g., Echternacht et al. 2011; Pirani, Mello-Silva & Giulietti, 2003), where its discontinuity seems to be the factor that leads to the singularity of these mountains. Another relevant factor is linked to its location as an ecotone between the Cerrado and Caatinga phytophysiognomies, affecting the dynamics of the biological community of this region and resulting in a particular evolutionary history.

The fourth estimated clade, the Southern *Espinhaço,* includes CIP and OUR populations, the latter coinciding with the Iron Quadrangle district, the southernmost bioregion of the *Espinhaço*. However, we could not directly link this clade with the Iron Quadrangle district, since its diagnostic characteristic is the presence of ironstone outcrops, while CIP and OUR are associated with quartzitic soils (Saadi, 1995). Despite the lack of putative soil differences, the relative proximity of CIP and OUR with the Iron Quadrangle district may promote the divergence of the Southern *Espinhaço* clade due to biotic interactions and constraints fostered by the singular environment and community of the Iron Quadrangle (Jacobi, do Carmo, Vincent & Stehmann, 2007; Zappi et al., 2017). Further analyses exploring the role of ecological interactions and how they affect speciation among communities could improve this hypothesis (Johnson & Stinchcombe, 2007)

4.3 Microevolutionary processes of Vriesea oligantha *are driven by geographic distance and climate gradient*

Our GLMM analysis demonstrates that populations of *Vriesea oligantha* are structured both by IBD and IBE. Indeed, both pollen and seed dispersal might be of great importance to explain the geographic component of the genetic structure pattern observed in nuclear and plastidial markers. However, assuming maternal inheritance of plastidial DNA and biparental inheritance of nuclear DNA as a rule for Angiosperm (Ennos, 1994), our genetic differentiation results indicate that gene flow via seeds is comparatively less efficient than gene exchange via pollen, in agreement with non-significant IBD obtained for the plastidial marker in the Mantel test. The discrepancy between the two markers may indicate the role of pollinators in maintaining the cohesion between close populations, since seeds are poorly dispersed, a common trend in other plant lineages of the *Espinhaço* Range (Silveira et al. 2020). Small and isolated populations may experience genetic depletion and local extinction, but if these populations persist, they might differentiate until the reproduction incompatibility and/or ecological divergence evolve, critical steps towards speciation (Harvey, Singhal & Rabosky, 2019). Thus, extant organisms with low dispersion that inhabit small and fragmented environments, such as the mountains of the *Espinhaço* Range, could be more prone to experience speciation events (Kisel & Timothy, 2010) and the described microevolutionary processes of *V. oligantha* might be the first steps of its diversification (Pinheiro et al., 2013).

Climatic variables along the *Espinhaço* Range was also a determinant component in the current genetic structure of the populations of *Vriesea oligantha.* The Northern *Espinhaço* climate is markedly drier and hotter, with long periods of low (or even absence of) precipitation while the climate of the mid-south *Espinhaço* has milder temperatures and higher humidity levels (Giulietti, Menezes, Pirani, Meguro & Wanderley, 1987; Zappi et al., 2003). Such variation has the potential to rapidly influence evolution by triggering ecological divergence (Campbell & Powers, 2015), especially in a scenario with reduced gene flow as observed in *V. oligantha*. Ancient climatic changes have a potential to strongly impact montane biological communities, as also reported in the Andes (Flantua et al., 2019). Paleopalinological studies of the *Espinhaço* have evidenced herbaceous vegetation expansion in the past towards lowlands (Barros, Lavarini, Lima & Magalhães-Júnior, 2011; Behling, 2002; Horák-Terra et al., 2015), supporting the putative role of ancestral climatic oscillations in the macroevolutionary patterns of endemic lineages in the *Espinhaço* Range (Vasconcelos et al., 2020). Nevertheless, considering the lower elevation of the *Espinhaço* Mountains (700-2,072 m) compared with the Andes (4,000-6,961 m), we expect the impacts of Pleistocene climate changes on *V. oligantha* diversification to be less associated with local extinctions due to glaciation events since there are few or no evidence that these mountaintops were once completely frozen (Luiz, Carneiro & Benitez, 2001). Thus, as estimated by the SDMs and the corridors between populations, events of secondary contact, hybridization and ecological interactions with other organisms are more likely to have promoted the current phylogeographic pattern in *V. oligantha*.

The evolution of species-rich biotas is remarkably known for its complex drivers (Antonelli et al., 2018; Rull, 2011) and other triggers might also help understanding the intra-specific lineage diversification of organisms from montane systems worldwide, such as edaphic adaptations (Alcantara et al., 2018), pollination strategies (Franceschinelli, Jacobi, Drummond & Resende, 2006) and niche conservatism (de Mattos, Morellato, Camargo & Batalha, 2019). In the future, the relative role of such processes could be tested by incorporating association analyses of the entire or partial genomes from multiple species with environmental datasets and functional ecological traits under a comparative framework.

4.4 Microevolutionary processes as a proxy for understanding macroevolutionary patterns

We showed that microevolutionary processes underlying the phylogeographic patterns of *Vriesea* oligantha are a consequence of IBD and IBE throughout its distribution. Considering the assumption that population differentiation is the basic mechanism of speciation, the concordant patterns between the diversification of *V. oligantha* and the *Espinhaço* biogeography generate powerful insights into how climatic variables and limited gene flow shaped early stages of macroevolutionary patterns. Studies rarely attempt to fill the gap between micro and macroevolutionary approaches and studies like ours are necessary to pave the way on the initial effects of population differentiation that ultimately lead to the origin of new species and spatial patterns of biodiversity distribution. Additional evidence using distinct organisms could also provide contrasting examples of how microevolutionary processes act and translate into the current biogeographic patterns of tropical montane biotas.

REFERENCES

Alcantara, S., Ree, R. H., & Mello-Silva, R. (2018). Accelerated diversification and functional trait evolution in Velloziaceae reveal new insights into the origins of the campos rupestres' exceptional floristic richness. *Annals of Botany, 122(*1), 1–16.

Antonelli, A., Kissling, W. D., Flantua, S. G. A., Bermúdez, M. A., Mulch, A., Muellner-Riehl, A. N., … Hoorn, C. (2018). Geological and climatic influences on mountain biodiversity. *Nature Geoscience,* 11(10), 718–725.

Arnaud-Haond, S., & Belkhir, K. (2006). GENCLONE: A computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes,* 7(1)*,* 15-17.

Bandelt, H. J., Forster, P., & Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16(1), 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Barfuss, M. H. J., Samuel, R., Till, W., & Stuessy, T. F. (2005). Phylogenetic relationships in subfamily Tillandsioideae (Bromeliaceae) based on DNA sequence data from seven plastid regions. *American Journal of Botany*, Baltimore, 92(2), 337–351.

Barfuss, M. H. J., Till, W., Leme, E. M. C., Pinzón, J. P., Manzanares, J. M., Halbritter, H., … Brown, G. K. (2016). Taxonomic revision of Bromeliaceae subfam. Tillandsioideae based on a multi-locus DNA sequence phylogeny and morphology. *Phytotaxa*, 279(1), 1–97.

Barres, L., Batalha-Filho, H., Schnadelbach, A. S., & Roque, N. (2019). 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*, 189(2), 132–152.

Barros, L. F. de P., Lavarini, C., Lima, L. S., & Magalhães-Júnior, A. P. (2011). Synthesis of the Late-Quaternary paleobioclimatic scenarios in Minas Gerais State/Southeastern Brazil. *Sociedade & Natureza*, 23(3), 371-385.

Beerli, P., & Felsenstein, J. (2001). Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the United States of America*, 98(8), 4563–4568.

Beheregaray, L. B. (2008). Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology*, 17(17), 3754–3774.

Behling, H. (2002). South and southeast Brazilian grassland during Late Quaternary times: a synthesis. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 177(1-2), 19–27.

Bitencourt, C., & Rapini, A. (2013). Centres of endemism in the Espinhaço Range: identifying cradles and museums of Asclepiadoideae (Apocynaceae). *Systematics and Biodiversity*, 11(4), 525–536.

Bonatelli, I. S., Perez, M. F., Peterson, A. T., Taylor, N. P., Zappi, D. C., Machado, M. C., … Moraes, E. M. (2014). Interglacial microrefugia and diversification of a cactus

species complex: Phylogeography and palaeodistributional reconstructions for *Pilosocereus aurisetus* and allies. *Molecular Ecology*, 23(12), 3044–3063.

Brown, J. L. (2014). SDMtoolbox: A python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. *Methods in Ecology and Evolution*, 5(7), 694-700.

Bünger, M. D. O., Stehmann, J. R., & Oliveira-Filho, A. T. (2014). Myrtaceae throughout the Espinhaço Mountain Range of central- eastern Brazil: floristic relationships and geoclimatic controls*. Acta Botanica Brasilica*, 28(1), 109–119.

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

Campbell, D. R., & Powers, J. M. (2015). Natural selection on floral morphology can be influenced by climate. *Proceedings of the Royal Society B: Biological Sciences*, 282(1808), 1-7.

Campos, L., Moro, M. F., Funk, V. A., & Roque, N. (2019). Biogeographical review of Asteraceae in the Espinhaço Mountain Range, Brazil. *The Botanical Review*, 85(4), 293-336.

Chan, L. M., Brown, J. L., & Yoder, A. D. (2011). Integrating statistical genetic and geospatial methods brings new power to phylogeography. *Molecular Phylogenetics and Evolution*, 59(2), 523–537.

Chaves, A. V., Freitas, G. H. S., Vasconcelos, M. F., & Santos, F. R. (2015). Biogeographic patterns, origin and speciation of the endemic birds from eastern Brazilian mountaintops: a review. *Systematics and Biodiversity*, 13(1), 1–16.

Chaves, A. V., Vasconcelos, M. F., Freitas, G. H. S., & Santos, F. R. (2019). Vicariant events in the montane hummingbird genera *Augastes* and *Schistes* in South America. *Ibis*, ibi.12777.

Colli-Silva, M., Vasconcelos, T. N. C., & Pirani, J. R. (2019). Outstanding plant endemism levels strongly support the recognition of campo rupestre provinces in mountaintops of eastern South America. *Journal of Biogeography*, 46(8), 1723–1733.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9(8), 772.

de Mattos, J. S., Morellato, L. P. C., Camargo, M. G. G., & Batalha, M. A. (2019). Plant phylogenetic diversity of tropical mountaintop rocky grasslands: local and regional constraints. *Plant Ecology*, 220(12), 1119–1129.

Diniz-Filho, J. A. F., Soares, T. N., Lima, J. S., Dobrovolski, R., Landeiro, V. L., Telles, M. P. de C., … Bini, L. M. (2013). Mantel test in population genetics. *Genetics and Molecular Biology*, 36(4), 475–485.

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources*, 14(1), 209-214.

Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7(1), 214.

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

Echternacht, L., Trovó, M., Oliveira, C. T., & Pirani, J. R. (2011). Areas of endemism in the Espinhaço Range in Minas Gerais, Brazil. *Flora*, 206(9), 782–791.

Ennos, R. A. (1994). Estimating the relative rates of pollen and seed migration among plant populations. *Heredity*, 72(3), 250-259.

ESRI. (2016). ArcGis desktop. Redlands, CA.

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

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver. 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3), 564–567.

Feliner, G. N. (2011). Southern European glacial refugia: A tale of tales. *Taxon*, 60(2), 365–372.

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

Franceschinelli, E. V., Jacobi, C. M., Drummond, M. G., & Resende, M. F. S. (2006). The genetic diversity of two Brazilian *Vellozia* (Velloziaceae) with different patterns of spatial distribution and pollination biology. *Annals of Botany*, 97(4), 585–592.

Gehara, M.; Garda, A. A.; Werneck, F. P.; Oliveira, E. F.; Da Fonseca, E. M.; Camurugi, F.; Magalhães, F. M.; Lanna, F. M.; Sites, J. W.; Marques, R.; Silveira-Filho, R.; São Pedro, V. A.; Colli, G. R.; Costa, G. C.; Burbrink, F. T. Estimating synchronous demographic changes across populations using hABC and its application for a herpetological community from northeastern Brazil. *Molecular Ecology*, v. 26, n. 18, p. 4756–4771, 2017.

Giulietti, A. M., Menezes, N. L., Pirani, J. R., Meguro, M., & Wanderley, M. G. L. (1987). Flora da Serra do Cipó, Minas Gerais: caracterização e lista das espécies. *Boletim de Botânica Da Universidade de São Paulo*, 9, 1–151.

Givnish, T. J., Barfuss, M. H. J., Ee, B. V., Riina, R., Schulte, K., Horres, R., … Sytsma, K. J. (2011). Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. *American Journal of Botany*, Baltimore, 98(5), 872–895.

Goudet, J. (1995). FSTAT (Version 1.2): a computer program to calculate F-statistics. *Journal of Heredity*, 86(6), 485–486.

Guillot, G., Mortier, F., & Estoup, A. (2005). GENELAND: a program for landscape genetics. *Molecular Ecology Notes*, 5(3), 712-715.

Haffer, J. (1969). Speciation in Amazonian Forest Birds. *Science*, 165(3889), 131–137.

Haffer, J. (1997). Alternative models of vertebrate speciation in Amazonia: An overview. *Biodiversity & Conservation*, 6, 451-476.

Harvey, M. G., Singhal, S., & Rabosky, D. L. (2019). Beyond reproductive isolation: demographic controls on the speciation process. *Annual Review of Ecology, Evolution, and Systematics*, 50(1), 75–95.

Heller, R., Chikhi, L., & Siegismund, H. R. (2013). The confounding effect of population structure on Bayesian Skyline Plot inferences of demographic history. *PLoS ONE*, 8(5), e62992.

Horák-Terra, I., Martínez Cortizas, A., da Luz, C. F. P., Rivas López, P., Silva, A. C., & Vidal-Torrado, P. (2015). Holocene climate change in central-eastern Brazil reconstructed using pollen and geochemical records of Pau de Fruta mire (Serra do Espinhaço Meridional, Minas Gerais). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 437, 117–131.

Hughes, C. E., & Atchison, G. W. (2015). The ubiquity of alpine plant radiations: from the Andes to the Hengduan Mountains. *New Phytologist*, 207(2), 275–282.

Humboldt, A. von. (1807). *Ideen zu einer Geographie der Pflanzen nebst einem Naturgemälde der Tropenländer : auf Beobachtungen und Messungen gegründet, welche vom 10ten Grade nördlicher bis zum 10ten Grade südlicher Breite, in den Jahren 1799, 1800, 1801, 1802 und 1803 angestellt worden sind, von Al. von Humboldt und A. Bonpland. Bearbeitet und herausgegeben von dem erstern*. Tübingen : Cotta

Jacobi, C. M., do Carmo, F. F., Vincent, R. C., & Stehmann, J. R. (2007). Plant communities on ironstone outcrops: a diverse and endangered Brazilian ecosystem. *Biodiversity and Conservation*, 16(7), 2185–2200.

Johnson, M. T. J., & Stinchcombe, J. R. (2007). An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology and Evolution*, 22(5), 250–257.

Kessous, I. M., Neves, B., Couto, D. R., Paixão-Souza, B., Pederneiras, L. C., Moura, R. L., … Costa, A. F. (2019). Historical biogeography of a Brazilian lineage of Tillandsioideae (subtribe Vrieseinae, Bromeliaceae): the Paranaean Sea hypothesized as the main vicariant event. *Botanical Journal of the Linnean Society*, 192(4), 625-641.

Kisel, Y., & Timothy, T. G. (2010). Speciation has a spatial scale that depends on levels of gene flow. American Naturalist, 175(3), 316-334.

Li, J., Huang, J. P., Sukumaran, J., & Knowles, L. L. (2018). Microevolutionary processes impact macroevolutionary patterns. *BMC Evolutionary Biology*, 18(1), 1–8.

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25(11), 1451-1452.

Lisiecki, L. E., & Raymo, M. E. (2007). Plio-Pleistocene climate evolution: trends and transitions in glacial cycle dynamics. *Quaternary Science Reviews*, 26(1-2), 56-69.

Liu, Y., Hu, J., Li, S. H., Duchen, P., Wegmann, D., & Schweizer, M. (2016). Sino-Himalayan mountains act as cradles of diversity and immigration centres in the diversification of parrotbills (Paradoxornithidae). Journal of Biogeography 43(8), 1488-1501.

Luiz, M., Carneiro, D. S., & Benitez, L. (2001). Depósitos superficiais diamantíferos da região De Diamantina, Serra Do Espinhaço (Minas Gerais). *Revista Geociências Unesp*, (1), 31–42.

Machado, T. M., Loiseau, O., Paris, M., Weigand, A., Versieux, L. M., Stehmann, J. R., … Salamin, N. (2019). Systematics of *Vriesea* (Bromeliaceae): phylogenetic relationships based on nuclear gene and partial plastome sequences. *Botanical Journal of the Linnean Society*, 192(4), 656–674.

Madriñán, S., Cortés, A. J., & Richardson, J. E. (2013). Páramo is the world's fastest evolving and coolest biodiversity hotspot*. Frontiers in Genetics*, 4, 1–7.

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In 2010 *Gateway Computing Environments Workshop, GCE 2010*. (pp. 1-8).

Nascimento, A. C., Chaves, A. V., Fortes Leite, F. S., Eterovick, P. C., & Santos, F. R. dos. (2018). Past vicariance promoting deep genetic divergence in an endemic frog species of the espinhaço range in Brazil: The historical biogeography of *Bokermannohyla saxicola* (Hylidae). *PLoS ONE*, 13(11), 1–19.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics*, 28(19), 2537–2539.

Pedreira, A. J., & De Waele, B. (2008). Contemporaneous evolution of the palaeoproterozoic mesoproterozoic sedimentary basins of the São Francisco-Congo craton. *Geological Society Special Publicatio*n, 294, 33–48.

Perrigo, A., Hoorn, C., & Antonelli, A. (2020). Why mountains matter for biodiversity. *Journal of Biogeography*, 47(2), 315–325.

Pinheiro, F., Cozzolino, S., de Barros, F., Gouveia, T. M. Z. M., Suzuki, R. M., Fay, M. F., & Palma-Silva, C. (2013). Phylogeographic structure and outbreeding depression reveal early stages of reproductive isolation in the neotropical orchid *Epidendrum denticulatum*. *Evolution*, 67(7), 2024–2039.

Pirani, J. R., Mello-Silva, R., & Giulietti, A. M. (2003). Flora de Grão-Mogol, Minas Gerais, Brasil. *Boletim de Botânica Da Universidade de São Paulo*, 21(1), 1–24.

Piry, S., Luikart, G., & Cornuet, J. M. (1999). BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, 90(4) 502-503.

Prell, W. L. (1984). Covariance patterns of foraminiferal dgr18O: an evaluation of Pliocene ice volume changes near 3.2 million years ago. *Science*, 226(4675), 692–694.

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

Raymond, M., & Rousset, F. (1995). GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86(3), 248–249.

Ribeiro, P. L., Rapini, A., Damascena, L. S., & van Den Berg, C. (2014). Plant diversification in the Espinhaço Range: Insights from the biogeography of *Minaria* (Apocynaceae). *Taxon*, 63(6), 1253–1264.

Rull, V. (2005). Biotic diversification in the Guayana Highlands: A proposal. *Journal of Biogeography*, 32(6), 921–927

Rull, V. (2011). Neotropical biodiversity: Timing and potential drivers. *Trends in Ecology and Evolution*, 26(10), 508–513.

Rull, V., & Vegas-Vilarrúbia, T. (2020). The Pantepui "Lost World": towards a biogeographical, ecological and evolutionary synthesis of a pristine Neotropical sky-island archipelago. In V. Rull & A. C. Carnaval (Eds.), *Neotropical Diversification: Patterns and Processes* (pp. 369–413). Basel: Springer, Charm.

Saadi, A. (1995). A geomorfologia da Serra do Espinhaço em Minas Gerais e de suas margens. *Geonomos*, 43(1), 41-63.

Salerno, P. E., Ron, S. R., Señaris, J. C., Rojas-Runjaic, F. J. M., Noonan, B. P., & Cannatella, D. C. (2012). Ancient tepui summits harbor young rather than old lineages of endemic frogs. *Evolution*, 66(10), 3000–3013.

Schoville, S. D., Roderick, G. K., & Kavanaugh, D. H. (2012). Testing the "Pleistocene species pump" in alpine habitats: lineage diversification of flightless ground beetles (Coleoptera: Carabidae: *Nebria*) in relation to altitudinal zonation. *Biological Journal of the Linnean Society*, 107(1), 95–111.

Sedano, R. E., & Burns, K. J. (2010). Are the Northern Andes a species pump for Neotropical birds? Phylogenetics and biogeography of a clade of Neotropical tanagers (Aves: Thraupini). *Journal of Biogeography*, 37(2), 325–343.

Silva, G. A. R., Antonelli, A., Lendel, A., Moraes, E. de M., & Manfrin, M. H. (2018). The impact of early Quaternary climate change on the diversification and population dynamics of a South American cactus species. *Journal of Biogeography*, 45(1), 76–88.

Silveira, F. A. O., Dayrell, R. L. C., Fiorini, C. F., Negreiros, D., & Borba, E. L. (2020). Diversification in ancient and nutrient-poor Neotropical ecosystems: how geological and climatic buffering shaped plant diversity in some of the world's neglected hotspots. In V. Rull & A. C. Carnaval (Eds.), *Neotropical Diversification: Patterns and Processes* (pp. 329–368). Basel: Springer, Charm.

Tel-Zur, N., Abbo, S., Myslabodski, D., & Mizrahi, Y. (1999). Modified CTAB procedure for DNA isolation from epiphytic cacti of genera *Hylocereus* and *Selenicereus* (Cactaceae). *Plant Molecular Biology*, 17, 249–254.

Vanzolini, P. E., & Williams, E. E. (1970). South American anoles: the geographic differentiation and evolution of the anolis *Chrysolepis* species group (Sauria, Iguanidae). *Arquivos de Zoologia*, 19(3–4), 125.

Vasconcelos, T. N. C., Alcantara, S., Andrino, C. O., Forest, F., Reginato, M., Simon, M. F. & Pirani, J. R. (2020). Fast diversification through a mosaic of evolutionary

histories characterizes the endemic flora of ancient Neotropical mountains. *Proceedings of the Royal Society B: Biological Sciences*, 287(1923), 20192933.

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

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, 38(6), 1358.

Wilson, G. A., & Rannala, B. (2003). Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, 163(3), 1177-1191.

Wright, S. (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution*, 19(3), 395.

Zappi, D. C., Lucas, E., Stannards, B. L., Lughadha, E. N., Pirani, J. R., Queiroz, L. P. de, … Carvalho, A. M. de. (2003). Lista das plantas vasculares de Catolés, Chapada Diamantina, Bahia, Brasil. *Boletim de Botânica Da Universidade de São Paulo*, 21(2), 345–398.

Zappi, D. C., Moro, M. F., Meagher, T. R., & Nic Lughadha, E. (2017). Plant biodiversity drivers in Brazilian campos rupestres: insights from phylogenetic structure. *Frontiers in Plant Science*, 8, 1–15.

Capítulo III: Climatic fluctuations drove synchronic demographic expansion in Neotropical ancient mountains

Marcos Vinicius Dantas-Queiroz, Fernanda Hurbath, Flávia Mol Lanna,
Leonardo de Melo Versieux, Clarisse Palma-Silva

Title: Climatic fluctuations drove synchronic demographic expansion in Neotropical ancient mountains

Authors: Marcos Vinicius Dantas-Queiroz[1]*; Fernanda Hurbath[2]; Flávia Mol Lanna[3]; Leonardo de Melo Versieux[4]; Clarisse Palma-Silva[1,5]

*corresponding author

1. Departamento de Ecologia, Programa de Pós-Graduação em Biologia Vegetal, Universidade Estadual Paulista (UNESP), Av. 24-A, 1515, Bela Vista, Rio Claro, SP, Brazil. 13506-900.

2. Universidade do Estado de Minas Gerais - Unidade Passos, Av. Juca Stockler, 1130, bairro Belo Horizonte, Passos, Minas Gerais - MG, Brazil. 37900-106.

3. Department of Evolution, Ecology and Organismal Biology. Museum of Biological Diversity, The Ohio State University, 1315 Kinnear Rd., Columbus, OH, USA. 43212.

4. Departamento de Botânica e Zoologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil. 59072-970.

5. Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil. 13083-862.

Agradecimentos

We thank Dr. Kléber Resende (CENA, USP, Brazil) for fieldwork assistance and Dr. Marcelo Gehara (Rutgers University-Newark, USA) for his availability and help with the analyses. Funding by Coordination for the improvement of Higher Education Personnel (CAPES) (88881.128215/2016-01; PDSE 88881.190071/2018-01, PROAP-2015/CAPES/PPGCB-BV), The São Paulo Research Foundation (FAPESP) (2019/24823-2; 2018/07596-0; 2015/11812-1; 2014/15588-6). National Council for Scientific and Technological Development (CNPq) (300819/2016-1; 305398/2019-9; 304778/2013-3; 303794/2019-4; 455510/2014-8).

ABSTRACT

Mountains are remarkable models for evolutionary studies since they host a substantial proportion of the world's biodiversity and harbors high levels of endemism. In the Neotropics, mountains play an important role in the development of the American biota. For instance, the Brazilian Quartzitic Mountains (BQM) in Eastern South America, harbor high levels of faunal and floristic endemism and biodiversity. Although orogenic events are important drivers that help explain the origin of high endemic montane biodiversity, the relative geological stability of the BQM usually implies further interpretations for the origin of their biota. Among the main explanations for the high species richness and endemism in the BQM are the cycles of climatic changes over the Quaternary, as explained in the "flickering connectivity" concept, which would repeatedly isolate and reconnect adjacent populations, potentially accelerating speciation events due to range fragmentation, dispersion (colonization and re-colonization), secondary contact and hybridization. To evaluate the role of the climatic fluctuations over the late-Quaternary in the BQM biota, we estimated the ancient demography of different endemic species of animals and plants using hierarchical approximate Bayesian computation analysis and Ecological Niche Modeling, but also evaluated if climatic oscillations could drive a genetic spatial congruence of co-distributed species from the *Espinhaço* Range, one of the main BQM massifs. Based on our demographic and modeling analysis, the majority of plant lineages exhibited a high probability of synchronous expansion over the Last Glacial Maximum (LGM, 20 thousand years ago), although the animal community did not follow this pattern. We also obtained an evidential signal of a congruent evolutionary history between lineages endemic to the bioregions of the Espinhaço Range, suggesting that similar environmental constraints, like the ancient climatic oscillations, are driving the evolutionary history of the *Espinhaço*'s biota. Moreover, the synchronous expansion over the LGM illustrates a crucial implication for conservation biology, since elucidating which past factors drove the current and ancient patterns of endangered populations are one of the most compelling ways to circumvent the loss of suitable areas of the BQM community.

Key-words: *campos rupestres*, comparative phylogeography, demographic inference, *Espinhaço*, hABC, Pleistocene, population expansion.

1 INTRODUCTION

It is ubiquitous the high biodiversity that mountainous regions harbor all over the globe (HUGHES; ATCHISON, 2015; ANTONELLI et al., 2018; PERRIGO; HOORN; ANTONELLI, 2020). This ecological pattern was brilliantly noticed by Humboldt on his travels from South America to Russia (NORDER, 2019) and continues to fascinate generations of scientists who keep looking for explanations to understand how this biodiversity arose and persists. The plethora of explanations consists mainly of two (non-antagonistic) fronts: intrinsic characteristics of species, such as the emergence of adaptive key-traits (e.g., HUGHES; ATCHISON, 2015; SCHWERY et al., 2015) or due to extrinsic factors, such as mountain orogeny and ancient climatic fluctuations (e.g., FLANTUA et al., 2019; MUELLNER-RIEHL, 2019a).

In the Neotropics, mountains play an important role in the development of the American biota (ANTONELLI et al., 2018). For instance, the relatively recent uplift of the Andes (Miocene-Oligocene) is an important driver in the development of the Neotropical biodiversity, which made possible the emergence of new areas for colonization, consequently leading to new ecological niches and to allopatric speciation (ANTONELLI et al., 2009; LAGOMARSINO et al., 2016). Although the Andes are the largest mountain range in South America, other geological formations also stand out for their high levels of faunal and floristic endemism and biodiversity, such as the Tepuis, in northern South America and several mountain complexes in Mid-Eastern South America (CHAVES et al., 2015; SILVEIRA et al., 2016; KOK et al., 2017; RULL et al., 2019).

Although orogenic events are strong drivers that help explain the origin of high endemic montane biodiversity (ANTONELLI et al., 2009; MUELLNER-RIEHL et al., 2019a), the relative geological stability of the Precambrian mountains of the Mid-Eastern South America usually implies further interpretations for the origin of their associated biota (SCHAEFER et al., 2016). Such explanations commonly rely on ancestral connections between surrounding ecosystems, directly influenced by ancient climatic fluctuations combined with the naturally fragmented nature of these mountains (BARRES et al., 2019; RULL et al., 2019; VASCONCELOS et al., 2020).

In the last 2.8 million years (Pliocene-Pleistocene), several climatic oscillations events took place in the globe, resulting in a high vegetation turnover, favoring pulses of expansion and retraction of the montane biological community over time, a phenomenon known as flickering connectivity (FLANTUA; HENRY, 2018; FLANTUA et al., 2019). The concept of flickering connectivity implies continually interconnection and disconnection of adjacent populations associated with shifts of altitudinal limits, providing suitable conditions to the emergence of genetic drift, local adaptations, secondary contact and hybridization, accelerating diversification rates (i.e., the 'species-pump' effect) (RULL, 2005; MCCORMACK; HUANG; KNOWLES, 2009; SILVEIRA et al., 2016).

Despite the relative stability of the Precambrian Mid-Eastern South American mountains, the biogeographic histories from these formations are contrasting. On the one hand, biogeographic studies carried out in granitic mountains of Southeastern Brazil (e.g., *Serra da Mantiqueira, Serra do Mar*) depict floristic and faunistic similarities between them, the *páramos* in the Andes, and to southern temperate grasslands (SAFFORD, 2007; CHAVES et al., 2015). Therefore, the evolutionary history from these mountains are linked to dispersion events through ancestral corridors, like the Patagonian-Chacoan region that may have bridged the two extremes of the Southern American continent (FIASCHI; PIRANI, 2009; CHAVES et al., 2015). On the other hand, the biogeographic history of the Brazilian Quartzitic Mountains (BQM) (e.g., *Espinhaço* Range, *Serra dos Pireneus, Chapada dos Veadeiros*) is intertwined with their surrounding biomes (i.e., Cerrado, Caatinga and Atlantic Forest - Neves et al., 2018), providing a good model for testing the role of flickering connectivity into the demography and species formation of biota endemic to these ancient mountains.

The BQM are home to one of the most biodiverse ecosystems on the globe, the *campos rupestres* (CONCEIÇÃO et al., 2016; VASCONCELOS et al., 2020). Such phytophisionomy is found scattered along the BQM, primarily at > 900 m elevation and are composed by a xerophytic vegetation, dominated by herbaceous and shrubby species well-adapted to harsh conditions, since most of the soils on the *campos rupestres* are shallow and well-drained, with an intense solar radiation and a striking daily temperature variation (GIULIETTI; PIRANI, 1988; ZAPPI et al., 2017). This mountain ecosystem has attracted the attention of generations of scientists

interested in unveiling the evolutionary dynamics that generated such an amazing endemic biota. The geological stability together with ancient climatic oscillations on the BQM are considered the main drivers leading to its high endemism, although there are still few papers dealing with endemic species under a phylogeographic and evolutionary approach (ANTONELLI et al., 2010; RIBEIRO et al., 2014; BARRES et al., 2019). Paleopalinological studies support the view of a vegetation turnover over the late-Quaternary (BARBERI; SALGADO-LABOURIAU; SUGUIO, 2000; HORÁK-TERRA et al., 2015, 2020; SILVA et al., 2020) suggesting the flickering connectivity between adjacent BQM as an elegant concept to explain how a rich endemic biota could emerge in these ancient neotropical mountains. In this context, studies focusing on the early stages of speciation could shed light into how microevolutionary processes (e.g., genetic drift, gene flow, natural selection) can generate such rich biodiversity.

A prevailing idea in comparative phylogeography is that co-distributed organisms under the effect of similar historical events should exhibit a concordance in several aspects (AVISE et al., 1987; SOLTIS et al., 2006). Accordingly, extrinsic factors, such as the last climatic fluctuations, could trigger a concordance history from genes among individuals of the same species to congruent patterns of entire biogeographic regions (AVISE, 2000). Therefore, analyzing the natural history of discrepant species in a comparative approach could show us if climatic oscillations resulted in synchronous reorganization in entire communities, suggesting a stronger effect in their demography, genetic structure and, finally, unveiling which processes are driving the diversification on these high biodiverse mountains (ORNELAS et al., 2013). Besides, understanding how putative climatic changes shaped the extant biodiversity patterns in the past, is a main guide to better elucidate how the forthcoming anthropogenic climatic changes will alter the endemic biota from montane environments.

In this study, we estimated the ancient demography of different species of animals and plants endemic to the BQM using hierarchical approximate bayesian computation analysis (hABC) and Ecological Niche Modeling (ENM). Based on the flickering connectivity concept, we hypothesized that (i) the BQM community showed a synchronous expansion over cold periods of the late-Quaternary age since the climate at such times would be more suitable to organisms adapted to montane

conditions. If the climate is a strong constraint to the BQM community, we inferred, using the Phylogeographic Concordant Factor analysis (PCF), that the (ii) climatic oscillation would shape a genetic spatial congruence of co-distributed species among reported biogeographic areas of the BQM.

2 MATERIALS AND METHODS

2.1 Data processing

We searched for published studies using online databases (Google Scholar, Periódico CAPES and ISI Web of Science) on March 2020, looking for the terms *phylogeograph**, *population genetics*, *biogeograph** and *genetics* followed by the expressions *campos rupestres,* rupestrian fields and *Espinhaço*. From the retrieved results, we selected papers that used sanger sequences and excluded papers using other techniques (e.g., RAPD, allozymes, SSR). We excluded papers that did not explicitly include the accession numbers of the deposited sequences, as well as those papers with less than 10 sequences analyzed, since so few sequences could compromise our subsequent analyses. Besides, we also generated new data for two endemic plants of the BQM: *Euphorbia attastoma* (Euphorbiaceae), *Neoregelia bahiana* - See Supplementary Material (S3) for description of DNA extraction, amplification and sequencing.

Plastidial, mitochondrial and/or nuclear data were downloaded from Genbank for each species (Table S1.1). Sequences were then aligned and visually inspected using Geneious 10.2.3 (Biomatters, New Zealand), where we subsequently concatenated sequences when multiple plastidial and mitochondrial markers were available for a single species.

Geographic locality information for all species was collected from original papers when it was possible. When the specific localities were unavailable, we looked for them in the metadata associated with the GenBank accession numbers. If specific coordinates could not be determined from either source, we generate coordinates using the ‘geoLoc’ tool (specieslink, CRIA), using any gazetteers provided by the authors and/or available in the metadata. Finally, once the data was compiled, we visually inspected all location points, including those from original sources, using Google Earth.

2.2 Population structure

Since we retrieved species from multiple sources with distinct methods for population structure and phylogeographic inferences, we standardized the population

structure using a Bayesian Generalized Mixed Yule Coalescent approach (bGMYC; REID; CARSTENS, 2012; FUJISAWA; BARRACLOUGH, 2013). To do so, we generate ultrametric trees for each species in BEAST 1.10.4, implemented on CIPRES Science Gateway (DRUMMOND; RAMBAUT, 2007; MILLER; PFEIFFER; SCHWARTZ, 2010) using the sequences alignments with substitution models estimated on jModeltest2 (DARRIBA et al., 2012), and an uncorrelated relaxed clock with a Yule speciation process. Because we are using distinct organisms, with distinct genes, we searched in the literature for general substitution rates, assuming a normal distribution prior to accommodate such variations (mitochondrial for amphibians, mean: 1.0×10^{-8}; SD: 1.5×10^{-9}; Gehara et al. (2017); plastidial for monocots, mean: 3.9×10^{-8}; SD: 1×10^{-8} (DROUIN; DAOUD; XIA, 2008); plastidial for eudicots, mean: 5.2×10^{-8}; SD: 2×10^{-9} (DROUIN; DAOUD; XIA, 2008). We ran the MCMC for 100 million steps, sampling every 10,000 steps, ensuring that results were reliable using Tracer v1.10.4, when the effective sample size (ESS) were ≥ 200.

The estimated trees were then used as inputs for the bGMYC analysis, implemented in the script written by Gehara et al. (2017) available on GITHUB (`github.com/gehara`). We ran a MCMC chain of 50,000 steps, with 40,000 steps of burn-in, using 100 trees randomly sampled from an amount of 8,000 trees. From the results, we kept all defined populations that contained at least 10 individuals with one or more polymorphisms. We excluded all other defined populations to avoid bias on our subsequent demographic analyses.

2.3 Demographic models

In order to estimate demographic changes in our species from BQM over the late-Quaternary, we tested three demographic models: population expansion, contraction and no demographic changes. Demographic inferences were conducted using an approximate bayesian computation (ABC) in the `'abc'` package (CSILLÉRY; FRANÇOIS; BLUM, 2012) in R 3.6.1 (R Core Team, 2019). With this approach, we first simulate genetic data for all species using wide prior uniform distributions for the effective population size (Ne; 1,000 – 100,000), the effective ancestral size of the population, estimated as the ratio of current to ancestral size (Ne/NeA; 1.0, for constant size; 0.001 – 0.1, for expansion; and 2.0 - 20, for

bottleneck) and the period when the populations had (or not) some demographic change (Te; 10 – 200 Ka).

We simulated sequences of the same length and the same number of individuals observed in each empirical lineage, calculating the specific mutation rate per generation, using a normal distribution (Table 1). We conducted 200,000 simulations of each model for each lineage using the function ‘single.pop.demog’ of the ‘PIPEMASTER’ package (GEHARA et al., 2017) in R 3.6.1 (R Core Team, 2019). Generation lengths for the amphibians were based on Gehara et al. (2017), but we could not find a reliable estimative for the generation lengths for the plants. Thus, we based these parameters on 15 years, as also estimated by the *Pilosocereus* genus, cacti species that usually inhabit the *campos rupestres* (BONATELLI et al., 2014; PEREZ et al., 2016).

With the simulated data in hands, we calculated seven summary statistics for each lineage and for each model, doing the same with the observed data set. The summary statistics were nucleotide diversity (pi), number of polymorphisms (S), haplotype diversity (h), Tajima’s D (D) and the frequency of the three most common alleles (ss1, ss2 and ss3). We confirmed if our observed data fell within the range of the parameter space of the simulated data using a principal component analysis (PCA), estimated with the summary statistics for both data sets. To verify the fit of the models, we used the ‘gfit’ function and estimated the posterior probability of the three demographic scenarios for each lineage with the ‘postpr’ function in package ‘abc’. Based on these results, we selected the lineages with the highest posterior probability under the expansion model (posterior probability ≥ 45%) to conduct a co-expansion analysis. Posterior distributions for Ne and Te for each expanding lineage were estimated using the minimum and maximum values retrieved from the ‘abc’ function in ‘abc’ package, using a simple rejection method with a tolerance level of 0.01.

Table 1- Species and their lineages estimated by bGMYC. Lineages shown in bold were included in the co-expansion analysis.

			Subst. Rate		
Species/Lineage	**Type**	**n**	**Mean**	**SD**	**p (Exp)**
Bokermannohyla saxicola 1	Amphibian	171	1.00E-08	1.50E-09	0.02
Bokermannohyla saxicola 2	Amphibian	20	1.00E-08	1.50E-09	0.08
Bokermannohyla saxicola 3	Amphibian	16	1.00E-08	1.50E-09	0.21
Euphorbia attastoma 1	Eudicot	45	5.20E-08	2.00E-09	**0.64**
Euphorbia attastoma 2	Eudicot	15	5.20E-08	2.00E-09	**0.56**
Lychnophora ericoides 1	Eudicot	159	5.20E-08	2.00E-09	**0.84**
Lychnophora ericoides 2	Eudicot	16	5.20E-08	2.00E-09	0.30
Lychnophora ericoides 3	Eudicot	16	5.20E-08	2.00E-09	0.29
Pleurodema allium	Amphibian	10	1.00E-08	1.50E-09	0.07
Richterago discoidea 1	Eudicot	15	5.20E-08	2.00E-09	**0.62**
Richterago discoidea 2	Eudicot	39	5.20E-08	2.00E-09	**0.83**
Tibouchina papyrus 1	Eudicot	19	5.20E-08	2.00E-09	**0.72**
Tibouchina papyrus 2	Eudicot	16	5.20E-08	2.00E-09	**0.61**
Tibouchina papyrus 3	Eudicot	17	5.20E-08	2.00E-09	0.36
Tibouchina papyrus 4	Eudicot	19	5.20E-08	2.00E-09	0.42
Vellozia auriculata	Monocot	27	3.90E-08	1.00E-08	**0.45**
Vriesea oligantha 1	Monocot	12	3.90E-08	1.00E-08	**0.61**
Vriesea oligantha 2	Monocot	41	3.90E-08	1.00E-08	**0.70**
Vriesea oligantha 3	Monocot	27	3.90E-08	1.00E-08	**0.67**

n, number of samples; SD, standard deviation; p (Exp), posterior probability of expansion.

Fonte: o autor.

2.4 Co-expansion analysis

We used the hierarchical approximate bayesian computation method (hABC) developed by Chan, Schanzenbach and Hickerson (2014) to determine the proportion of lineages expanding synchronously and when such expansion may have occurred. Under this framework, we estimated the proportion of lineages (zeta, ζ) expanding synchronously at the same period (*Ts*), whereas the remaining lineages expand asynchronously (*ts*). Accordingly, we used a uniform distribution for the hyperparameter ζ varying from complete asynchrony (ζ = 0) to complete synchrony (ζ = 1), while the intermediate values are distinct scenarios where random lineages expand synchronically and others don't. The simulations used a prior uniform distribution for the current population size and for the time of expansion based on the lineage-specific parameters described above for the ABC analysis. Ancestral population size was once again drawn from a uniform prior as the ratio of current to ancestral size and we use the previous genetic priors under a normal distribution.

We ran six million simulations and calculated 16 hypersummary statistics (mean, variance, skew and kurtosis for pi, S, H and D) for simulated and observed data sets, using the ‘sim.coexp’ and ‘observed.coexp.sumstat’ functions, respectively, in ‘PIPEMASTER’ (GEHARA et al., 2017). Since values of zeta close to 1 (i.e., high synchronic expansion) tend to bias the co-expansion estimation (GEHARA et al., 2017; REID et al., 2019), we also assigned asynchronous expansion times based on three different models, besides the Chan, Schanzenbach and Hickerson (2014) model (CEA): the narrow co-expansion model (NCT) - where *a priori* co-expansion time period (Ts) is used (i.e., during the LGM); the portioned-time model (PT) - where the co-expansion time prior (Ts) is equally partitioned between blocks that match the number of lineages in the data set; and the threshold model (TH) - where a threshold buffer (10 Ka) is set between the co-expansion time (Ts) and the asynchronous time periods (ts). Finally, we accessed posterior distributions of ζ, Ts, the mean time of demographic changes (E[t]) and the coefficient of variation of expansion times (DI) using once more the function ‘abc’ with a rejection method, under a tolerance value of 0.01. We evaluated which of the four models best fit our observed data using the ‘postpr’ function from the ‘abc’ package with a tolerance value of 0.0005, under a rejection method. All steps of co-expansion analyses were conducted in the Center for Scientific Computing (NCC/GridUNESP) of the São Paulo State University (UNESP).

2.5 Current and ancient distribution modelling

We conduct species distribution models (SDMs) to check if suitable areas from the past are congruent with our results from the demographic models. We used the occurrence records available from the original papers as well as those retrieved from our georeferencing method (see above). Also, to increase the number of coordinates points, we downloaded occurrence records from GBIF.org (GBIF, 2020), selecting only data with a preserved specimen source, with available coordinates and gazetteers. We visually inspected all coordinate records using QGIS 3.8.2, removing any overlapping points.

Coordinate points were mapped in a geographical grid of ca. 3,600,000 km² and 30’’ resolution (ca. 1 km²). The environmental conditions were selected from the 19 bioclimatic variables in Worldclim database, using the five less correlated

variables (≥ 70%) (Bio3, Isothermality; Bio7, Temperature Annual Range; Bio10, Mean Temperature of Warmest Quarter; Bio16, Precipitation of Wettest Quarter; Bio17, Precipitation of Driest Quarter), estimated by a factorial analysis, implemented in 'psych' R package (Revelle, 2015). Six distinct algorithms were used to predict the current potential distribution of all species: (i) Bioclim (NIX, 1986), (ii) Mahalanobis distance (FARBER; KADMON, 2003), (iii) Domain (Gower distance) (CARPENTER; GILLISON; WINTER, 1993); (iv) Maximum Entropy (MAXENT) (PHILLIPS; DUDÍK, 2008), (v) Support Vector Machines (SVM) (TAX; DUIN, 2004) and (vi) Random Forest (BREIMAN, 2001). Predictions of potential distributions were estimated using randomized occurrence records, divided in two subsets (75% and 25% of train and test records, respectively), maintaining the true skill statistics (TSS) (ALLOUCHE; TSOAR; KADMON, 2006) with values of TSS > 0.5. Past predictions used the paleoclimatic scenarios simulated by the Community Climate System Model (CCSM4) (HIJMANS et al., 2005) for the LGM (ca. 20 ka). To increase the reliability of potential predictions, we employed an ensemble approach of all models using average suitability weighted by the TSS values of each model.

2.6 Spatial phylogeographic patterns

Due to the intrinsic nature of endemic species, few taxa are actually widely distributed along the BQM, hampering a broad genetic spatial comparison among species from different communities (Chapter II). Nonetheless, the *Espinhaço* Range, mountains from Eastern Brazil, is one of the main BQM massifs and some biogeographic patterns were previously recognized based mainly on plant endemism (ECHTERNACHT et al., 2011; CAMPOS et al., 2019; COLLI-SILVA; VASCONCELOS; PIRANI, 2019). The proposal of Colli-Silva, Vasconcelos and Pirani (2019) consists in the following divisions (hereafter defined as bioregions): the northern province, known as the *Chapada Diamantina* (CHD) and the southern province, known as the Southern *Espinhaço*, this latter was even subdivided in three districts: the *Grão-Mogol* (GMO), the *Diamantina* Plateau (PLD) and the Iron Quadrangle (IQD).

We select four plant species that are distributed along the bioregions, two eudicots, *Richterago discoidea* (Asteraceae) (BARRES et al., 2019) and *Euphorbia attastoma* (Euphorbiaceae) and two monocots, *Neoregelia bahiana* and *Vriesea*

oligantha (Bromeliaceae) to estimate putative concordant patterns between them. Besides their wide distributions, these four species are good representatives of the major clades of angiosperms (APG IV, 2016), presenting contrasting evolutionary histories and distinct patterns of dispersion.

We use the bioregions to gather samples from each particular locality into operational taxonomic units (OTUs), in order to test the presence of phylogeographic concordant patterns following the Phylogeographic Concordance Factors (PCFs), an index developed by Satler and Carstens (2016). This analysis compares the similarity (or concordance) between trees topologies from species inhabiting the same bioregion. If two or more species live in the same area and are influenced by similar constraints (e.g., ancient climatic fluctuations), one can expect a similar divergence across the space, resulting in congruent intraspecific trees.

To calculate the PCFs, we estimated the posterior distributions for each species tree independently with BEAST v2.5.1 via the CIPRES Science Gateway (DRUMMOND; RAMBAUT, 2007; MILLER; PFEIFFER; SCHWARTZ, 2010). However, a limitation of the PCF analysis is that all OTUs require the representation of all species, but the southernmost bioregion (IQD) has only two of the four species and consequently was not analyzed here. We ran 100,000,000 generations, sampling every 1,000 runs, discarding 10% of trees as burn-in, checking the convergence by importing the log files into Tracer v1.7.1. We used the PCF python script written by Satler and Carstens (2016), available on GITHUB (`github.com/jordansatler/`), which uses `'MBSUM'` (LARGET et al., 2010) to summarize species tree distributions, and `'BUCKY'` (ANE et al., 2006) to generate the concordant trees.

Since the bioregions delimitation is based on levels of endemism of the *Espinhaço* Range species, we performed a two-fold analysis of PCF. Firstly, we used only species restricted to the *Espinhaço* Range (i.e., *E. attastoma*, *N. bahiana* and *V. oligantha*) and secondly, we incorporated *R. discoidea* to the comparison, which has a wider distribution outside of the *Espinhaço* Range (e.g., *Chapada dos Veadeiros, Serra dos Pireneus*). Therefore, we were able to compare the relative contribution of each species to a putative pattern of congruence between the lineages and the bioregions.

3 RESULTS

3.1 Data retrieved

In the literature survey we identified 30 papers focused on phylogeography and/or population genetics of endemic species from the BQM (Table S1.2), plus our Chapter II (*Vriesea oligantha*), from those 11 fitted in our criteria of having at least 10 individual sequences deposited in public databases (Table S1.3). Summing up with our two newly generated data (*Neoregelia bahiana* and *Euphorbia attastoma*), and *V. oligantha* (Chapter II), we end up with 13 species (three amphibians, one bird, six eudicots and three monocots) (Table S1.3).

The number of sequences per species ranged from 11 to 213 and the length ranged from 438 to 3,222 base pairs. Geographic data for individuals showed a good sampling of the BQM, occurring from west, in the Brazilian Plateau (e.g., *Serra Dourada, Serra dos Pireneus, Chapada dos Veadeiros*), to east, in the *Espinhaço* Range, with highest density in the latter region (Figure 1).

3.2 Population assignment and lineages-specific demography

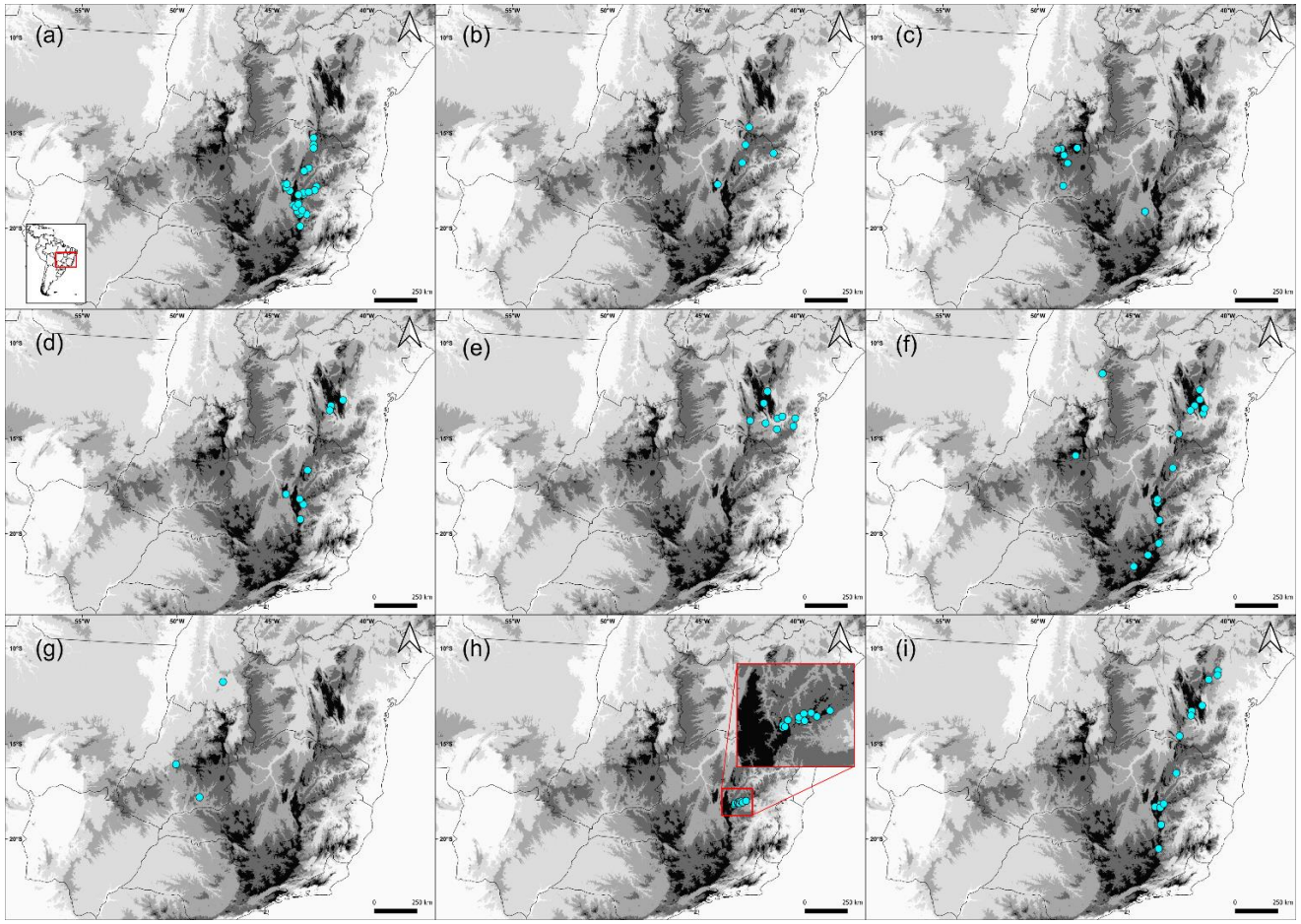
All defined lineages in our bGMYC analysis of the bird *Cinclodes espinhaensis*, the plants *Neoregelia bahiana*, *Pilosocereus aurisetus, P. vilaboensis* and the anuran *Pithecopus megacephalus* showed lineages with less than ten sequences and were not included on the subsequent demographic analyses. The remaining eight species formed 19 lineages (Table 1), all fitting within the simulated data, as observed by the PCA analysis (Figure S2.1).

Of the eight species included, most showed signals of expansion under the ABC analysis (Table 1). However, all lineages of the anuran *Bokermannohyla saxicola* exhibited signals of bottleneck, while all lineages of *Pleurodema allium* and two lineages of each plant *Lychnophora ericoides* and *Tibouchina papyrus* exhibited constant population sizes over the LGM (Table S1.4). Thus, these non-expanding lineages were not included in the co-expansion simulations.

3.3 Co-expansion analysis

From the remaining 11 lineages of the six species (*E. attastoma, L. ericoides, R. discoidea, T. papyrus, V. auriculata* and *V.* oligantha) (Table S1.5), estimated values for the ζ parameter were consistent with a high synchronous expansion. The mode for the posterior distribution of ζ was 0.90 in models CEA, PT and NCT and 0.91 in TH (Table 2, Figure 2). Credible interval of ζ for all models was wide (0.09 – 1), but mode, median and mean values all fitted in high values of ζ (Table 2, Figure 2). Posterior distribution of expansion times (Ts) match within values close to LGM (i.e., 20 Ka), with mode for CEA and PT 23 Ka (12 - 180 and 12 - 181 Ka HDP 2.5 – 97.5%, respectively); 24 Ka for TH and NCT (12 – 182 and 20 – 25 Ka HDP 2.5 – 97.5%, respectively). Estimates of dispersion index for expansion times was low, with a high density near zero (CEA: 6 Ka (0 – 72); TH 3 Ka (0 – 71); PT 2 Ka (0 – 71); NCT 2 Ka (0 – 73). Expected expansion times (E[t]) overlap Ts, although the mode refers to periods when the global temperatures had already started to decline. Model evaluation indicated NCT as the most probable, with 0.42 probability using the rejection method (Table 2).

Figure 1 – Coordinate points retrieved from the original sources. (a) *Bokermannohyla saxicola*, (b) *Euphorbia attastoma*, (c) *Lychnophora ericoides*, (d) *Neoregelia bahiana*, (e) *Pleurodema allium*, (f) *Richterago discoidea*, (g) *Tibouchina papyrus*, (h) *Vellozia auriculata* and (i) *Vriesea oligantha*. Altitude is represented by the grey altitude, where white is lower and black is higher.



Fonte: o autor.

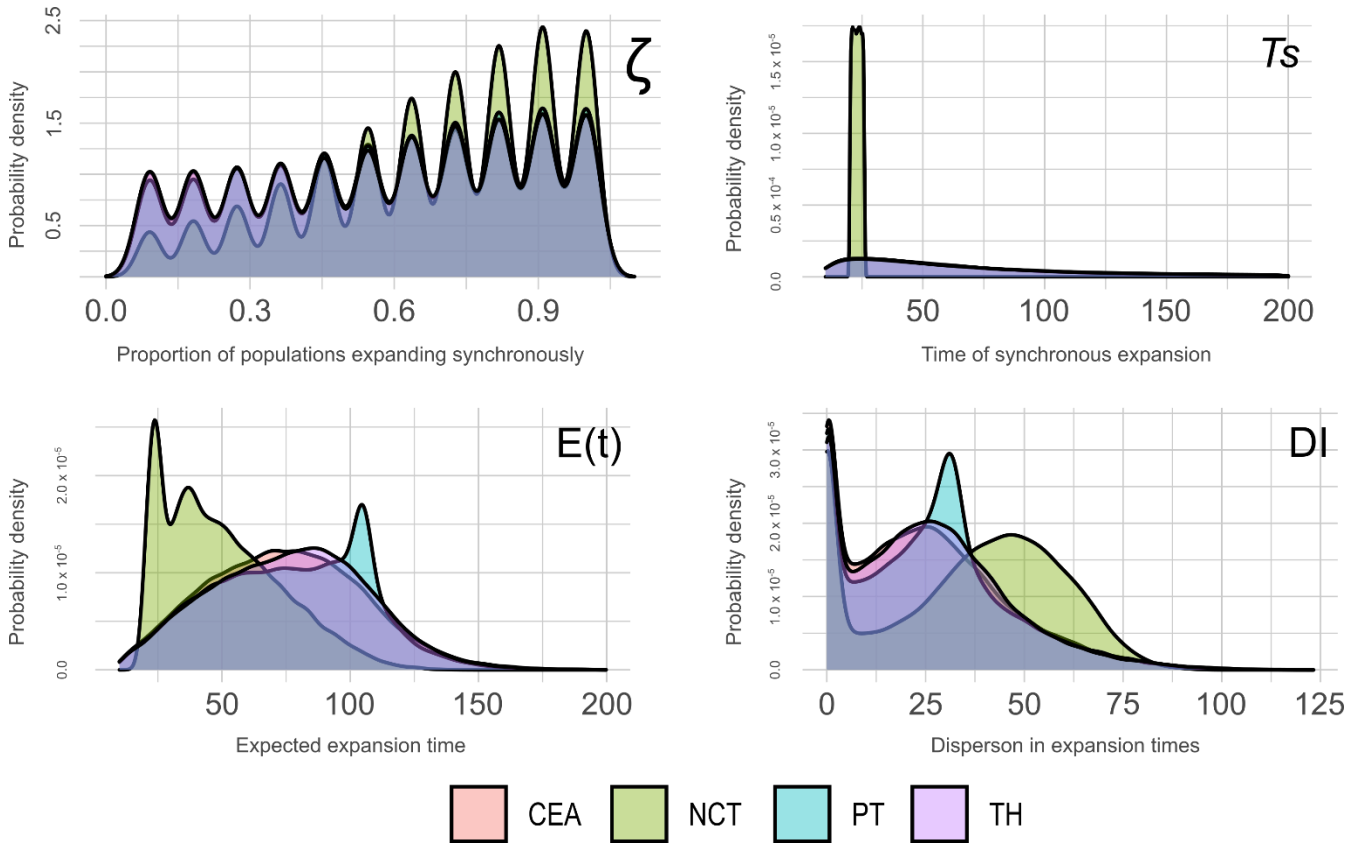
Table 2 – Mode, mean, median and credible intervals (CI) of posterior distributions of parameters estimated by each of the four models. Values of Ts (time of simultaneous change), E(t) (mean time of all demographic changes) and DI (dispersion across time) are in thousand years. Prob. is the probability value of each model.

		zeta				**Ts**				**E(t)**				**DI**			
	Prob.	mode	mean	median	CI	mode	mean	median	CI	mode	mean	median	CI	mode	mean	median	CI
CEA	0.19	0.90	0.59	0.63	0.09 – 1	23	65	52	12 – 180	70	74	73	20 – 135	6.0	24	22	0 – 72
TH	0.20	0.91	0.59	0.63	0.09 – 1	24	67	54	12 – 182	85	76	76	21 – 134	3.8	25	23	0 – 71
PT	0.18	0.90	0.60	0.63	0.09 – 1	23	66	52	12 – 181	104	77	78	21 – 132	2.9	25	26	0 – 71
NCT	0.42	0.90	0.68	0.72	0.09 – 1	24	22	23	20 – 25	23	50	46	21 – 101	2.8	35	39	**0 – 73**

CEA – Chan et al. (2014) model; TH – Threshold model; PT – Partitioned Time model; NCT – Narrow Coexpansion Time model.

Fonte: o autor.

Figure 2 – Posterior densities for zeta, Ts, E(t) and DI for the four models tested (CEA, NCT, PT and TH). Times of Ts, E(t) and DI are in thousand years.



Fonte: o autor.

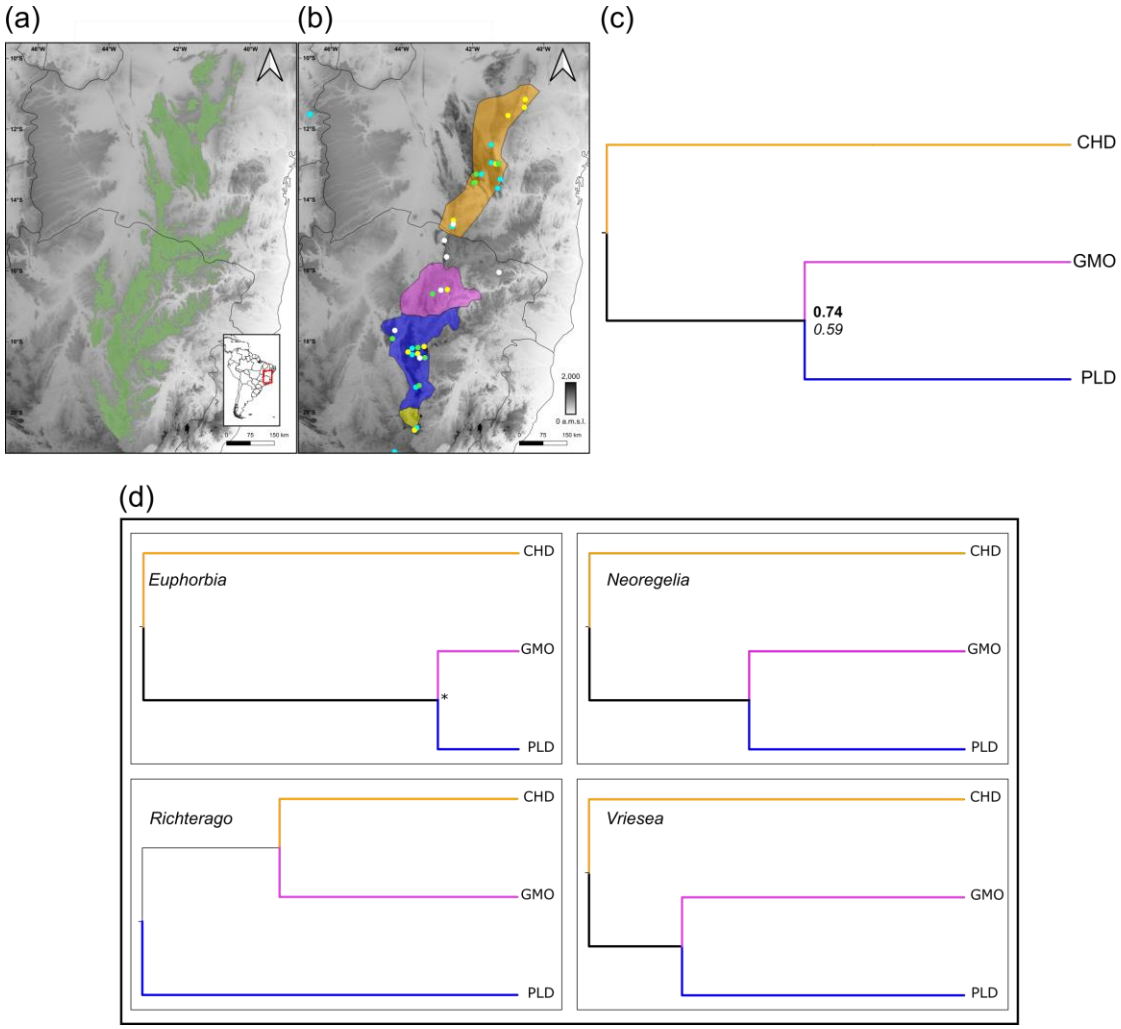
3.4 Ecological niche modelling

Most species showed high suitable areas over the LGM when compared with the current predicted distribution areas (Figure 4). Suitable areas for *Pleurodema alium* mainly overlap its current distribution. On the other hand, *Bokermannohyla saxicola*, showed a high suitable area during the LGM, reaching distant areas in relation to its current endemic distribution along the *Espinhaço* Range. *Euphorbia attastoma* presented high suitability during the LGM, mainly towards the Brazilian Northeastern. *Lychnophora ericoides*, *Richterago discoidea* and *Tabebuia papyrus* showed the broadest suitable areas over the LGM, while the plant species endemic to the *Espinhaço* Range (*Neoregelia bahiana*, *Vellozia auriculata* and *Vriesea oligantha*) exhibited a more limited suitability, although still more widespread than their current predictions.

3.5 Phylogeographic spatial patterns

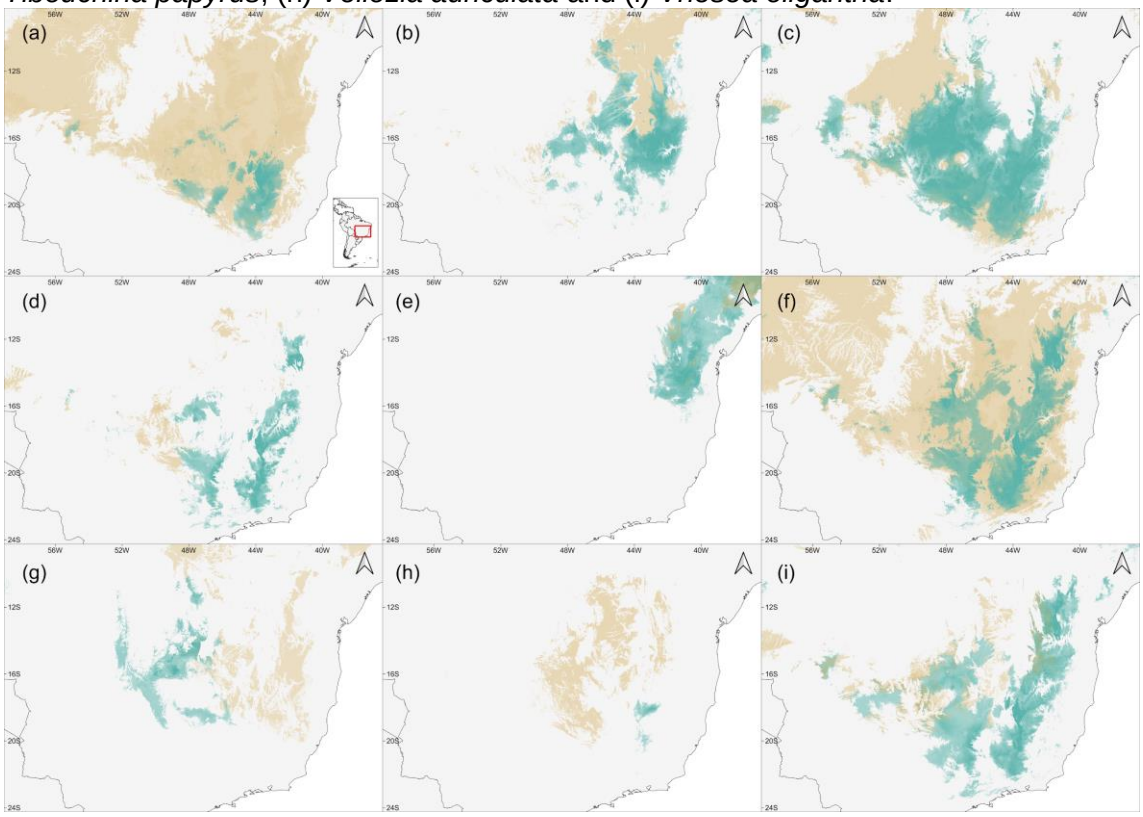
Both PCFs analyses showed contrasting values of concordance (Figure 3). When comparing only *Espinhaço*-endemic species (*E. attastoma, N. bahiana* and *V. oligantha),* nodal value of the concordant tree supports a high congruent scenario between species and bioregions (0.74 - Figure 3C, following the threshold of ≥ 0.71, Satler and Carstens, 2016). However, when incorporating *R. discoidea*, a non-endemic plant of the *Espinhaço* Range, the nodal value drops significantly, discarding the congruent scenario (0.59 - Figure 3C). Pairwise comparison between each species also demonstrates a high concordant association in the *Espinhaço*-endemic species, except for the two bromeliads that displayed a relatively low value of congruence (0.62). When incorporating all species, only the pairwise value between *E. attastoma* and *N. bahiana* exhibited a congruent pattern (0.83) (Figure S2.2).

Figure 3 – a) The *Espinhaço* Range (green); b) The *Espinhaço* Range bioregionalization by Colli-Silva et al. (2019) is constituted of two provinces, *Chapada Diamantina* (orange, CHD) and the Southern *Espinhaço*, which is subdivided in three districts: *Grão-Mogol* (purple, GMO), *Diamantina* Plateau (blue, PLD) and the Iron Quadrangle (yellow, IQD). Circles represent four species, the *Espinhaço*-endemics *Euphorbia attastoma* (white); *Neoregelia bahiana* (green) and *Vriesea oligantha* (yellow) and the wide-distributed *Richterago discoidea* (light blue); c) Comparison community tree, depicting the Phylogeographic Concordance Factor (PCF), an index that indicates the proportion of posterior trees that support each node across species of the *Espinhaço* Range. Bold number is the concordant value when only comparing endemic species, while in italic is the value when comparing all species. d) Maximum Clade Credibility (MCC) tree of the BEAST results from each species. Posterior probability values greater than 0.95 represented by an asterisk



Fonte: o autor.

Figure 4 - Ensemble distribution models for current (green) and the LGM (beige) periods. (a) *Bokermannohyla saxicola*, (b) *Euphorbia attastoma*, (c) *Lychnophora ericoides*, (d) *Neoregelia bahiana*, (e) *Pleurodema allium*, (f) *Richterago discoidea*, (g) *Tibouchina papyrus*, (h) *Vellozia auriculata* and (i) *Vriesea oligantha*.



Fonte: o autor.

4 DISCUSSION

4.1 Synchronic expansion and its consequences for speciation in the BQM

Our study uniquely models the historical demography at the community level, and, to our knowledge, this is the first study supporting a major spatial reorganization in the community of the BQM under a comparative phylogeographic approach. Here we assessed the role of climatic fluctuations during the late-Quaternary using an hABC approach to infer the demographic history of two anurans and six plant species endemic to the BQM. Based on our demographic analysis, all plant lineages of *Euphorbia attastoma*, *Richterago discoidea*, *Vellozia auriculata* and *Vriesea oligantha*, and some lineages of *Lychnophora ericoides* and *Tibouchina papyrus* exhibited a high probability of synchronous expansion over the LGM (Table 1). On the other hand, constant population size was found in the anuran *Pleurodema allium* and in two plant lineages *of L. ericoides* and *T. papyrus*, while the frog *Bokermannohyla saxicola* was the only taxon that showed a bottleneck over the LGM (Table 1). The ENM confirms our demographic inferences, except for *B. saxicola*, that exhibited a high suitability over an area larger than its current distribution (Figure 4). In agreement with our findings, the expansion pattern of the plant community during ancient cold periods are corroborated by paleopalinological studies over the region, that have evidenced expansion in the herbaceous vegetation in the past towards lowlands, supporting the role of ancient climatic oscillations on the biological community structure (BARBERI; SALGADO-LABOURIAU; SUGUIO, 2000; HORÁK-TERRA et al., 2015, 2020; SILVA et al., 2020).

Although we used species with distinct dispersal abilities and independent evolutionary histories, the synchronic demographic expansion suggests that climatic fluctuations are important drivers that shaped the species demography in the BQM community. Contrary to other mountain areas of the globe, where orogenic events might have played a significant role to fragmenting populations over time (MUELLNER-RIEHL et al., 2019b), the outcomes of past climatic changes by continuously connecting and disrupting populations provides an exemplary factor that explains how the extraordinary endemic biodiversity of the BQM could have arisen. According to the flickering connectivity concept, species well-adapted to mountain environments usually remain fragmented and isolated on mountaintops during

warmer periods (FLANTUA et al., 2019), where the interplay among microevolutionary processes such as genetic drift, limited gene flow and local adaptation generate divergence within species lineages, promoting species formation (LI et al., 2018). As the climate cools, suitable areas for the BQM species expand towards lowlands, enabling the contact of previously isolated populations. If populations are still capable of maintaining gene flow, this new contact could (i) vanish putative differences accumulated over the isolated time or could also (ii) disseminate new genetic variants among populations (DUCHEN et al., 2020). Otherwise, the accumulated differences could, (iii) generate hybrid lineages (PALMA-SILVA et al., 2011; MOTA et al., 2019, 2020; MAGALHÃES et al., 2020) or inhibiting viable mating, where (iv) the speciation process between two lineages has already been achieved (LEAL et al., 2016; FERRIS; WILLIS, 2018; MOTA et al., 2020). This intricate evolutionary dynamic provides a scenario of distinct processes of lineage divergence, genetic diversity and speciation events. Therefore, the proper evaluation of population variation over time is a crucial step to estimate the role of microevolutionary processes that yields the rich endemic biodiversity that inhabits the BQM (LANFEAR; KOKKO; EYRE-WALKER, 2014).

4.2 Diversity of demographic responses to climate changes

A study carried out with *Lychnophora ericoides* on the BQM, pointed out contrasting results among distinct lineages (COLLEVATTI; RABELO; VIEIRA, 2009). Accordingly, some lineages expanded while others remained constant or decreased over the LGM, as shown by our ABC results (Table S1.4). A similar pattern was also observed with *Tibouchina papyrus* as a model species, where some population sizes were constant and some others were under expansion (COLLEVATTI et al., 2012), which is also congruent with our ABC results. Such discrepant changes among lineages were indicated as possible effects of the variation in the current effective population size, since some populations showed a large number of adult and juveniles individuals, while others only exhibited a small number of adults (COLLEVATTI; RABELO; VIEIRA, 2009). Indeed, the effective population size is a limiting factor when accessing the coalescence time, narrowing the possibility of estimating realistic demographic histories of that lineage (PANNELL, 2003). In this case, secondary lines of evidence like the ENM provide a complementary and finer evaluation of past demographic history, as illustrated by the expansion scenario

inferred by our analysis for *L. ericoides* and *T. papyrus* (Figure 4), reinforcing the utility of integrating multi-model inferences to investigate complex demographic dynamics in response to climatic changes over the LGM (COLLEVATTI et al., 2015).

The animal community, represented here by two anurans, did not follow the synchronic expansion of the vegetation. Lineages of one anuran species (*Bokermannohyla saxicola)* showed a bottleneck while we detected constant population size in the other (*Pleurodema allium*). Anurans are well-known for their high dependence on microhabitats and low mobility behavior (GOMES et al., 2009; ETEROVICK et al., 2010). Even with high suitable areas outside its current distribution (Figure 4), *B. saxicola* exhibited a reduced population size over the LGM (Table S1.4). If its natural habitats, small ponds and streams, are strong constraints to this species, a bottleneck model would be expected for this species, since such water bodies might have been vanishing from the highlands due to increased low temperatures and aridity. Besides, the limited mobility of *B. saxicola* could also preclude its dispersion to distant and more suitable areas (NASCIMENTO et al., 2018).

On the other hand, *Pleurodema alium* has maintained its population size constant during the last 20 thousand years, according to our demographic and environmental modelling analyzes. Indeed, Thomé and Carstens (2016) also showed that this species did not display substantial demographic changes over the last thousand years. This anuran inhabits one of the most septentrional areas of the BQM, the *Chapada Diamantina*, a mountain massif immersed in a sea of seasonally dry forests, the Caatinga biome. Gehara et al. (2017) estimated an increased expansion in the herpetological community from the Caatinga over the last 200 thousand years, although our results did not show any expansion scenario over this period for *P. alium*. The lack of demographic changes during the late-Quaternary could be a consequence of the *P. alium* distribution in microhabitats of the *Chapada Diamantina*, which might have acted as a buffer for the effects of climatic changes over the last 200 thousand years, enabling suitable areas within these mountains for the endurance of this anuran.

4.3 Phylogeographic spatial patterns

Our hypothesis that climatic oscillations would generate a congruent phylogeographic pattern between local bioregions and co-distributed lineages was partially confirmed, according to the PCF analysis (Figure 3). By comparing only *Espinhaço*-endemic lineages (*E. attastoma, N. bahiana* and *V. oligantha*) in the PCF analysis, we obtained an evidential signal of a congruent evolutionary history between lineages. Such congruence might suggest a more cohesive evolutionary history within the *Espinhaço*-endemic species likely due to more similar environmental constraints that they are subject to, as the climatic oscillations inferred here. When inserting a species that is not endemic to the *Espinhaço* Range habitats, other environmental and ecological factors intermix, blurring how the endemic patterns of the *Espinhaço* Range could be originated. The congruence between intraspecific lineages and biogeographic patterns for the *Espinhaço* Range was firstly noted for the bromeliad *Vriesea oligantha* (in the Chapter II). In that work, the authors explicit this fact by suggesting the microevolutionary processes involved in that species, such as the low gene flow between isolated populations and the consequent genetic drift, as the initial steps that could shape the high levels endemism in the *Espinhaço* Range, ultimately leading to macroevolutionary patterns observed today. Besides, natural selection is probably a strong component in the evolution of species from the BQM as exemplified by *V. oligantha*, since its populations are strongly structured by the *Espinhaço* Range environment variation, a compelling driver potentially triggering local adaptations (CAMPBELL; POWERS, 2015).

Although our demographic results corroborate a highly synchronic expansion in *Espinhaço*-endemic species, the phylogeographic congruent pattern between lineages could not necessarily imply congruent temporal concordance. As suggested by the flickering connectivity concept, several other climatic oscillations over the Pleistocene could prompt cycles of connection and isolation between adjacent populations of the BQM (BONATELLI et al., 2014; THOMÉ; CARSTENS, 2016). Differently from the effects of glacial periods on high latitude areas of the globe, climatic oscillations didn’t provide extreme elements such as glacial sheets or frozen mountaintops on Eastern South America, phasing out a ‘*tabula rasa*’ scenario on these mountains (LUIZ; CARNEIRO; BENITEZ, 2001). On the contrary, pulses of connectivity and contraction over the Pleistocene allowed a complex evolutionary

arena of isolation, secondary contact, and hybridization (ANTONELLI et al., 2010; RIBEIRO et al., 2014). In consequence, climatic oscillations prior to the LGM must be accounted to unveil how they promoted the divergence and spatial structure on BQM species and if biogeographic patterns could be explained by older climatic fluctuations (RIBEIRO et al., 2014; VASCONCELOS et al., 2020). Therefore, our work provides a glimpse of how ancient climatic oscillations could act at the population level, eventually leading to divergent lineages fragmented among mountain tops.

Moreover, we acknowledge some caveats of using single-locus data to estimate fine temporal differences in expansion times, such as in the LGM. Still, despite the relative weakness to access single-species inferences, single-locus data may provide important insights under a biogeographic approach when combined into a single comparative framework, as conducted in our study and elsewhere (LEITE et al., 2016; GEHARA et al., 2017; REID et al., 2019; WIERINGA et al., 2020). Nonetheless, the stochasticity of the coalescent process yields wide credible intervals of posteriors probabilities, a common output in this type of demographic analysis (CHAN; SCHANZENBACH; HICKERSON, 2014; GEHARA et al., 2017; REID et al., 2019). With the advent of next-generation sequencing technology, collection of multiple loci data is becoming more feasible and more detailed and comprehensive demographic analyses under a coalescent framework will enhance our ability to infer demographic changes of the BQM community (BEICHMAN; HUERTA-SANCHEZ; LOHMUELLER, 2018).

4.4 Beyond climatic oscillations

Our work has primarily focused on estimating the influence of the LGM over the demography and spatial patterns of the BQM community. For certain, climatic changes are one of the main causes for population size changes in temperate regions and tropical regions (CARNAVAL; MORITZ, 2008; PALMA-SILVA et al., 2009; SÉRSIC et al., 2011; TURCHETTO-ZOLET et al., 2013; LEAL; PALMA-SILVA; PINHEIRO, 2016; PINHEIRO; DANTAS-QUEIROZ; PALMA-SILVA, 2018; YOU et al., 2018; CHAVES et al., 2020; WIERINGA et al., 2020). However, taxon-specific traits and ecological interactions can also contribute to unveil how they influence ancient demographic dynamics and how they affected the current biogeographic

patterns (PAPADOPOULOU; KNOWLES, 2016; NÜRK et al., 2020; ORTEGO; KNOWLES, 2020). In this sense, there is a need to untangle the role of demographic changes from species' intrinsic traits (SMITH et al., 2011). For instance, montane lineages of Ericaceae exhibited higher diversification rates than other non-montane lineages due to the presence of reduced leaves (SCHWERY et al., 2015); the evolution of perennial life in *Lupinus* increased speciation rates among montane lineages (DRUMMOND et al., 2012); the capability of use crassulacean acid metabolism (CAM) and C_4 photosynthesis is recognized as an adaptive response to aridity, increasing speciation rates within *Euphorbia* and Bromeliaceae (HORN et al., 2014; SILVESTRO et al., 2014); and the pollination syndrome and fruit types played a significant role in diversification of Andean Campanulaceae (LAGOMARSINO et al., 2016).

Besides, putative microhabitat affinities, such as soil types and the presence of water bodies, very common ecological constraints on montane endemic species, must also be taken into account to guiding us to a better understanding of how co-distributed species experience demographic shifts over time (MASSATTI; KNOWLES, 2014; MORELLATO; SILVEIRA, 2018; FIORINI et al., 2019). Indeed, Papadoupoulous and Knowles (2016) advocate to incorporate trait data into comparative phylogeographic studies and we strongly support this view, encouraging future studies interested in understanding the species diversification from the BQM mountains to incorporate biotic traits and ecological data to unveil their natural histories.

4.5 Conclusion and prospects

Comprehending the organisms' past demographic history is the first step in understanding the formation of the current biodiversity patterns. Our work presents evidence supporting the role that late-Quaternary climatic fluctuations played in the BQM community, providing a compelling framework of how microevolutionary processes involved in lineage diversification prompt extant macroevolutionary patterns. However, further studies are needed to refine the role of ancient climatic oscillations in diversification, since these climatic changes also shaped the BQM species' evolutionary history (Chapter II). With the increasing accession to genomic data and the development of sophisticated demographic inferences (BEICHMAN;

HUERTA-SANCHEZ; LOHMUELLER, 2018; FLAGEL; BRANDVAIN; SCHRIDER, 2019), combined with intrinsic and extrinsic traits, future studies that integrate such frameworks can robustly characterize past population history, shedding light onto details in how demographic dynamics of each species can influence the rise of macroevolutionary patterns observed today (LI et al., 2018; ORTEGO; KNOWLES, 2020).

Nonetheless, the synchronous expansion over the LGM illustrates a crucial implication for conservation biology. Suitable areas for the BQM community were predicted to be smaller in the future (BARBOSA; FERNANDES, 2016; BITENCOURT et al., 2016). However, areas with reduced historic climatic variation may hold stable lineages, a signal of a resilient environment facing climatic oscillations and could be, for instance, candidate areas to implement conservation efforts, although local resilience will depend on how the forthcoming Anthropocene climatic changes will affect the BQM community (PRATES et al., 2016). Elucidating which past factors drove the current and ancient patterns of endangered populations are one of the most compelling ways to circumvent the loss of suitable areas of the BQM community.

REFERENCES

ALLOUCHE, O.; TSOAR, A.; KADMON, R. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology*, Oxford, v. 43, n. 6, p. 1223–1232, 2006.

ANE, C.; LARGET, B.; BAUM, D. A.; SMITH, S. D.; ROKAS, A. Bayesian estimation of concordance among gene trees*. Molecular Biology and Evolution*, Chicago, v. 24, n. 2, p. 412–426, 2006.

ANTONELLI, A. E.; VEROLA, C. F.; PARISOD, C. N.; GUSTAFSSON, A. L. S. Climate cooling promoted the expansion and radiation of a threatened group of South American orchids (Epidendroideae: Laeliinae). *Biological Journal of the Linnean Society*, London, v. 100, n. 3, p. 597–607, 2010.

ANTONELLI, A.; KISSLING, W. D.; FLANTUA, S. G. A.; BERMÚDEZ, M. A.; MULCH, A.; MUELLNER-RIEHL, A. N.; KREFT, H.; LINDER, H. P.; BADGLEY, C.; FJELDSÅ, J.; FRITZ, S. A.; RAHBEK, C.; HERMAN, F.; HOOGHIEMSTRA, H.; HOORN, C. Geological and climatic influences on mountain biodiversity. *Nature Geoscience*, London, v. 11, n. 10, p. 718–725, 2018.

ANTONELLI, A.; NYLANDER, J. A. A.; PERSSON, C.; SANMARTÍN, I. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences of the United States of America*, Washington, v. 106, n. 24, p. 9749–54, 2009.

APG IV. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, London, v. 181, n. 1, p. 1-20, 2016.

AVISE, J. C. *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press, 2000.

AVISE, J. C.; ARNOLD, J.; BALL, R. M.; BERMINGHAM, E.; LAMB, T.; NEIGEL, J. E.; REEB, C. a; SAUNDERS, N. C. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, Palo Alto, v. 18, n. 1, p. 489–522, 1987.

BARBERI, M.; SALGADO-LABOURIAU, M. L.; SUGUIO, K. Paleovegetation and paleoclimate of “Vereda de Águas Emendadas”, Central Brazil. *Journal of South American Earth Sciences*, Oxford, v. 13, n. 3, p. 241–254, 2000.

BARBOSA, N. P. U.; FERNANDES, G. W. Rupestrian grassland: past, present and future distribution. In: FERNANDES, G. W. (Ed.). *Ecology and Conservation of Mountaintop grasslands in Brazil*. Cham: Springer International Publishing, 2016. p. 531–544.

BARRES, L.; BATALHA-FILHO, H.; SCHNADELBACH, A. S.; ROQUE, N. 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*, London, v. 189, n. 2, p. 132–152, 25.

BEICHMAN, A. C.; HUERTA-SANCHEZ, E.; LOHMUELLER, K. E. Using genomic data to infer historic population dynamics of nonmodel organisms. *Annual Review of Ecology, Evolution, and Systematics*, Palo Alto, v. 49, n. 1, p. 433–456, 2018.

BITENCOURT, C.; RAPINI, A.; SANTOS DAMASCENA, L.; DE MARCO JUNIOR, P. The worrying future of the endemic flora of a tropical mountain range under climate change. *Flora - Morphology, Distribution, Functional Ecology of Plants*, Jena, v. 218, p. 1–10, 2016.

BONATELLI, I. S.; PEREZ, M. F.; PETERSON, A. T.; TAYLOR, N. P.; ZAPPI, D. C.; MACHADO, M. C.; KOCH, I.; PIRES, A. H. C.; MORAES, E. M. Interglacial microrefugia and diversification of a cactus species complex: Phylogeography and palaeodistributional reconstructions for *Pilosocereus aurisetus* and allies. *Molecular Ecology*, Oxford, v. 23, n. 12, p. 3044–3063, 2014.

BREIMAN, L. Random forests. *Machine Learning*, Boston, v. 45, p. 5–32, 2001.

CAMPOS, L.; FREIRE MORO, M.; FUNK, V. A.; ROQUE, N. Biogeographical review of Asteraceae in the Espinhaço Mountain Range, Brazil. *The Botanical Review*, New York, v. 85, n. 4, p. 293–336, 2019.

CARNAVAL, A. C.; MORITZ, C. Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic Forest. *Journal of Biogeography*, Oxford, v. 35, n. 7, p. 1187–1201, 2008.

CARPENTER, G.; GILLISON, A. N.; WINTER, J. DOMAIN: a flexible modelling procedure for mapping potential distributions of plants and animals. *Biodiversity and Conservation*, London, v. 2, n. 6, p. 667–680, 1993.

CHAN, Y. L.; SCHANZENBACH, D.; HICKERSON, M. J. Detecting concerted demographic response across community assemblages using hierarchical approximate Bayesian computation. *Molecular Biology and Evolution*, Chicago, v. 31, n. 9, p. 2501–2515, 2014.

CHAVES, A. V.; FREITAS, G. H. S.; VASCONCELOS, M. F.; SANTOS, F. R. Biogeographic patterns, origin and speciation of the endemic birds from eastern Brazilian mountaintops: a review. *Systematics and Biodiversity*, Cambridge, v. 13, n. 1, p. 1–16, 2015.

CHAVES, A. V.; VASCONCELOS, M. F.; FREITAS, G. H. S.; SANTOS, F. R. Vicariant events in the montane hummingbird genera *Augastes* and *Schistes* in South America. *Ibis*, London, v. 162, n. 3, p. 1060–1067, 2019.

COLLEVATTI, R. G.; DE CASTRO, T. G.; LIMA, J. D. S.; TELLES, M. P. D. C. Phylogeography of *Tibouchina papyrus* (Pohl) toledo (Melastomataceae), an endangered tree species from rocky savannas, suggests bidirectional expansion due

to climate cooling in the Pleistocene. *Ecology and Evolution*, Oxford, v. 2, n. 5, p. 1024–1035, 2012.

COLLEVATTI, R. G.; RABELO, S. G.; VIEIRA, R. F. Phylogeography and disjunct distribution in *Lychnophora ericoides* (Asteraceae), an endangered cerrado shrub species. *Annals of Botany*, London, v. 104, n. 4, p. 655–664, 2009.

COLLEVATTI, R. G.; TERRIBILE, L. C.; DINIZ-FILHO, J. A. F.; LIMA-RIBEIRO, M. S. Multi-model inference in comparative phylogeography: an integrative approach based on multiple lines of evidence. *Frontiers in Genetics*, Lausanne, v. 6, n. February, p. 1–8, 2015.

COLLI-SILVA, M.; VASCONCELOS, T. N. C.; PIRANI, J. R. Outstanding plant endemism levels strongly support the recognition of campo rupestre provinces in mountaintops of eastern South America. *Journal of Biogeography*, Oxford, v. 46, n. 8, p. 1723–1733, 2019.

CONCEIÇÃO, A. A.; RAPINI, A.; DO CARMO, F. F.; BRITO, J. C.; SILVA, G. A.; NEVES, S. P. S.; JACOBI, C. M. Rupestrian Grassland Vegetation, Diversity, and Origin. In: FERNANDES, G. W. (Ed.). *Ecology and Conservation of Mountaintop grasslands in Brazil*. Cham: Springer International Publishing, 2016. p. 105–127.

CSILLÉRY, K.; FRANÇOIS, O.; BLUM, M. G. B. Abc: An R package for approximate Bayesian computation (ABC). *Methods in Ecology and Evolution*, Oxford, v. 3, n. 3, p. 475–479, 2012.

DARRIBA, D.; TABOADA, G. L.; DOALLO, R.; POSADA, D. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods,* London, v. 9, n. 8, p. 772, 2012.

DROUIN, G.; DAOUD, H.; XIA, J. Relative rates of synonymous substitutions in the mitochondrial, chloroplast and nuclear genomes of seed plants*. Molecular Phylogenetics and Evolution*, Orlando, v. 49, n. 3, p. 827–831, 2008.

DRUMMOND, A. J.; RAMBAUT, A. BEAST: Bayesian Evolutionary Analysis by Sampling Trees. *BMC Evolutionary Biology*, London, v. 7, n. 1, p. 214, 2007.

DRUMMOND, C. S.; EASTWOOD, R. J.; MIOTTO, S T. S.; HUGHES, C. E. Multiple continental radiations and correlates of diversification in Lupinus (Leguminosae): testing for key innovation with incomplete taxon sampling. *Systematic Biology*, Oxford, v. 61, n. 3, p. 443-460, 2012.

DUCHEN, P.; HAUTPHENNE, S.; LEHMANN, L.; SALAMIN, N. Linking micro and macroevolution in the presence of migration. *Journal of Theoretical Biology*, London, v. 486, p. 110087, 2020.

ECHTERNACHT, L.; TROVÓ, M.; OLIVEIRA, C. T.; PIRANI, J. R. Areas of endemism in the Espinhaço Range in Minas Gerais, Brazil. *Flora: Morphology, Distribution, Functional Ecology of Plants*, Jena, v. 206, n. 9, p. 782–791, 2011.

ETEROVICK, P. C.; RIEVERS, C. R.; KOPP, K.; WACHLEVSKI, M.; FRANCO, B. P.; DIAS, C. J.; BARATA, I. M.; FERREIRA, A. D. M.; AFONSO, L. G. Lack of phylogenetic signal in the variation in anuran microhabitat use in southeastern Brazil. *Evolutionary Ecology*, London, v. 24, n. 1, p. 1–24, 2010.

FARBER, O.; KADMON, R. Assessment of alternative approaches for bioclimatic modeling with special emphasis on the Mahalanobis distance. *Ecological Modelling*, Amsterdam, v. 160, n. 1–2, p. 115–130, 2003.

FERRIS, K. G.; WILLIS, J. H. Differential adaptation to a harsh granite outcrop habitat between sympatric *Mimulus* species. *Evolution*, Lancaster, v. 72, n. 6, p. 1225–1241, 2018.

FIASCHI, P.; PIRANI, J. R. Review of plant biogeographic studies in Brazil. *Journal of Systematics and Evolution*, Beijing, v. 47, n. 5, p. 477–496, 2009.

FIORINI, C. F.; MIRANDA, M. D.; SILVA-PEREIRA, V.; BARBOSA, A. R.; OLIVEIRA, U. De; KAMINO, L. H. Y.; MOTA, N. F. D. O.; VIANA, P. L.; BORBA, E. L. The phylogeography of *Vellozia auriculata* (Velloziaceae) supports low zygotic gene flow and local population persistence in the campo rupestre, a Neotropical OCBIL. *Botanical Journal of the Linnean Society*, London, v. 191, n. 3, p. 381–398, 2019.

FLAGEL, L.; BRANDVAIN, Y.; SCHRIDER, D. R. The unreasonable effectiveness of convolutional neural networks in population genetic inference. *Molecular Biology and Evolution*, Chicago, v. 36, n. 2, p. 220–238, 2019.

FLANTUA, S. G. A.; O'DEA, A.; ONSTEIN, R. E.; GIRALDO, C.; HOOGHIEMSTRA, H. The flickering connectivity system of the north Andean páramos. *Journal of Biogeography*, Oxford, v. 46, n. 8, p. 1808–1825, 2019.

FLANTUA, S.; HENRY, H. Historical connectivity and mountain biodiversity. In: HOORN, C.; PERRIGO, A.; ANTONELLI, A. (Eds.). *Mountains, Climate and Biodiversity*. Hoboken: Wiley Blackwell, 2019. p. 171–185.

FUJISAWA, T.; BARRACLOUGH, T. G. Delimiting species using single-locus data and the generalized mixed yule coalescent approach: A revised method and evaluation on simulated data sets. *Systematic Biology*, Washington, v. 62, n. 5, p. 707 – 724, 2013.

GBIF. *The Global Biodiversity Information Facility*. 2020. Available in: <www.gbif.org>. Date accession: November 12th, 2020.

GEHARA, M.; GARDA, A. A.; WERNECK, F. P.; OLIVEIRA, E. F.; DA FONSECA, E. M.; CAMURUGI, F.; MAGALHÃES, F. de M.; LANNA, F. M.; SITES, J. W.; MARQUES, R.; SILVEIRA-FILHO, R.; SÃO PEDRO, V. A.; COLLI, G. R.; COSTA, G. C.; BURBRINK, F. T. Estimating synchronous demographic changes across populations using hABC and its application for a herpetological community from northeastern Brazil. *Molecular Ecology*, Oxford, v. 26, n. 18, p. 4756–4771, 2017.

GIULIETTI, A. M.; PIRANI, J. R. Patterns of geographic distribution of some plant species from the Espinhaço. *Proceedings of a Workshop on Neotropical Distribution Patterns*, Rio de Janeiro, n. May, p. 39–69, 1988.

GOMES, F. R.; REZENDE, E. L.; GRIZANTE, M. B.; NAVAS, C. A. The evolution of jumping performance in anurans: morphological correlates and ecological implications. *Journal of Evolutionary Biology*, Basel, v. 22, n. 5, p. 1088–1097, 2009.

HIJMANS, R. J.; CAMERON, S. E.; PARRA, J. L.; JONES, P. G.; JARVIS, A. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, Chichester, v. 25, n. 15, p. 1965–1978, 2005.

HORÁK-TERRA, I.; CORTIZAS, A. M.; DA LUZ, C. F. P.; SILVA, A. C.; MIGHALL, T.; DE CAMARGO, P. B.; MENDONÇA-FILHO, C. V.; DE OLIVEIRA, P. E.; CRUZ, F. W.; VIDAL-TORRADO, P. Late Quaternary vegetation and climate dynamics in central-eastern Brazil: insights from a ~35k cal a bp peat record in the Cerrado biome. *Journal of Quaternary Science*, Harlow, v. 35, n. 5, p. 664–676, 2020.

HORÁK-TERRA, I.; MARTÍNEZ CORTIZAS, A.; DA LUZ, C. F. P.; RIVAS LÓPEZ, P.; SILVA, A. C.; VIDAL-TORRADO, P. Holocene climate change in central-eastern Brazil reconstructed using pollen and geochemical records of Pau de Fruta mire (Serra do Espinhaço Meridional, Minas Gerais). *Palaeogeography, Palaeoclimatology, Palaeoecology*, Amsterdam, v. 437, p. 117–131, 2015.

HORN, J. W.; ZHENXIANG, X.; RIINA, R.; PEIRSON, J. A.; YANG, Y.; DORSEY, B. L.; BERRY, P. E.; DAVIS, P. E.; WURDACK, K. J. Evolutionary bursts in *Euphorbia* (Euphorbiaceae) are linked with photosynthetic pathway. *Evolution*, Lancaster, v. 68, n. 12, p. 3485-3504, 2014.

HUGHES, C. E.; ATCHISON, G. W. The ubiquity of alpine plant radiations: From the Andes to the Hengduan Mountains. *New Phytologist*, Cambridge, v. 207, n. 2, p. 275–282, 2015.

KOK, P. J. R.; RUSSO, V. G.; RATZ, S.; MEANS, D. B.; MACCULLOCH, R. D.; LATHROP, A.; AUBRET, F.; BOSSUYT, F. Evolution in the South American 'Lost World': insights from multilocus phylogeography of stefanias (Anura, Hemiphractidae, *Stefania*). *Journal of Biogeography*, Oxford, v. 44, n. 1, p. 170–181, 2017.

KÖRNER, C.; JETZ, W.; PAULSEN, J.; PAYNE, D.; RUDMANN-MAURER, K.; M. SPEHN, E. A global inventory of mountains for bio-geographical applications. *Alpine Botany*, Basel, v. 127, n. 1, p. 1 – 15, 2017.

LAGOMARSINO, L. P.; CONDAMINE, F. L.; ANTONELLI, A.; MULCH, A.; DAVIS, C. C. The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). *New Phytologist*, Cambridge, v. 210, n. 4, p. 1430–1442, 2016.

LANFEAR, R.; KOKKO, H.; EYRE-WALKER, A. Population size and the rate of evolution. *Trends in Ecology and Evolution*, Amsterdam, v. 29, n. 1, p. 33–41, 2014.

LARGET, B. R.; KOTHA, S. K.; DEWEY, C. N.; ANÉ, C. BUCKy: Gene tree/species tree reconciliation with Bayesian concordance analysis. *Bioinformatics*, Oxford, v. 26, n. 22, p. 2910–2911, 2010.

LEAL, B. S. S.; CHAVES, C. J. N.; KOEHLER, S.; BORBA, E. L. When hybrids are not hybrids: a case study of a putative hybrid zone between *Cattleya coccinea* and *C. brevipedunculata* (Orchidaceae). *Botanical Journal of the Linnean Society*, London, v. 181, n. 4, p. 621–639, 2016.

LEAL, B. S. S.; PALMA-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*, Philadelphia, v. 35, n. 4, p. 215–230, 2016.

LEITE, Y. L. R.; COSTA, L. P.; LOSS, A. C.; ROCHA, R. G.; BATALHA-FILHO, H.; BASTOS, A. C.; QUARESMA, V. S.; FAGUNDES, V.; PARESQUE, R.; PASSAMANI, M.; PARDINI, R. Neotropical forest expansion during the last glacial period challenges refuge hypothesis. *Proceedings of the National Academy of Sciences*, Washington, v. 113, n. 4, p. 1008–1013, 2016.

LI, J.; HUANG, J. P.; SUKUMARAN, J.; KNOWLES, L. L. Microevolutionary processes impact macroevolutionary patterns. *BMC Evolutionary Biology*, London, v. 18, n. 1, p. 1–8, 2018.

LUIZ, M.; CARNEIRO, D. S.; BENITEZ, L. Depósitos superficiais diamantíferos da região de Diamantina, Serra do Espinhaço (Minas Gerais). *Revista Geociências Unesp*, Rio Claro, n. 1, p. 31–42, 2001.

MAGALHÃES, R. F.; LEMES, P.; SANTOS, M. T. T.; MOL, R. M.; RAMOS, E. K. S.; OSWALD, C. B.; PEZZUTI, T. L.; SANTOS, F. R.; BRANDÃO, R. A.; GARCIA, P. C. A. Evidence of introgression in endemic frogs from the campo rupestre contradicts the reduced hybridization hypothesis. *Biological Journal of the Linnean Society*, London, in advance, p. 1–16, 2020.

MASSATTI, R.; KNOWLES, L. L. Microhabitat differences impact phylogeographic concordance of codistributed species: Genomic evidence in montane sedges (*Carex* L.) from the Rocky Mountains. *Evolution*, Lancaster, v. 68, n. 10, p. 2833–2846, 2014.

MCCORMACK, J. E.; HUANG, H.; KNOWLES, L. L. Sky Islands. In: GILLESPIE, R. G.; CLAGUE, D. A. (Ed.). *Encyclopedia of Islands*. Berkeley: University of California Press, 2009. p. 839–843.

MILLER, M. A.; PFEIFFER, W.; SCHWARTZ, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop, GCE 2010, Anais...2010.

MORELLATO, L. P. C.; SILVEIRA, F. A. O. Plant life in campo rupestre: new lessons from an ancient biodiversity hotspot. *Flora: Morphology, Distribution, Functional Ecology of Plants*, Jena, v. 238, p. 1–10, 2018.

MOTA, M. R.; PINHEIRO, F.; LEAL, B. S. S.; SARDELLI, C. H.; WENDT, T.; PALMA-SILVA, C. From micro- to macroevolution: insights from a Neotropical bromeliad with high population genetic structure adapted to rock outcrops. *Heredity*, London, v. 125, n. 5, p. 353–370, 2020.

MOTA, M. R.; PINHEIRO, F.; LEAL, B. S. S.; WENDT, T.; PALMA-SILVA, C. The role of hybridization and introgression in maintaining species integrity and cohesion in naturally isolated inselberg bromeliad populations. *Plant Biology*, Stuttgart, v. 21, n. 1, p. 122–132, 2019.

MUELLNER-RIEHL, A. N.; SCHNITZLER, J.; KISSLING, W. D.; MOSBRUGGER, V.; RIJSDIJK, K. F.; SEIJMONSBERGEN, A. C.; VERSTEEGH, H.; FAVRE, A. Origins of global mountain plant biodiversity: Testing the 'mountain-geobiodiversity hypothesis'. *Journal of Biogeography*, Oxford, v. 46, n. 12, p. 2826–2838, 2019a.

MUELLNER-RIEHL, A. N. Mountains as evolutionary arenas: patterns, emerging approaches, paradigm shifts, and their implications for plant phylogeographic research in the Tibeto-himalayan region. *Frontiers in Plant Science*, Lausanne, v. 10, n. March, p. 1–18, 2019b.

NASCIMENTO, A. C.; CHAVES, A. V.; FORTES LEITE, F. S.; ETEROVICK, P. C.; SANTOS, F. R. Past vicariance promoting deep genetic divergence in an endemic frog species of the Espinhaço range in Brazil: The historical biogeography of *Bokermannohyla saxicola* (Hylidae). *PLoS ONE*, San Francisco, v. 13, n. 11, p. 1–19, 2018.

NEVES, D. M.; DEXTER, K. G.; PENNINGTON, R. T.; BUENO, M. L.; DE MIRANDA, P. L. S.; OLIVEIRA-FILHO, A. T. Lack of floristic identity in campos rupestres — A hyperdiverse mosaic of rocky montane savannas in South America. *Flora: Morphology, Distribution, Functional Ecology of Plants*, Jena, v. 238, p. 24–31, 2018.

NIX, H. A. A biogeographic analysis of Australian elapid snakes. In: LONGMORE, R. (Ed.). *Atlas of Elapid Snakes of Australia*. Camberra: Australian Government Publishing Service, 1986. p. 4–15.

NORDER, S. J. Alexander von Humboldt (1769–1859): Connecting geodiversity, biodiversity and society. *Journal of Biogeography*, Oxford, v. 46, n. 8, p. 1627 – 1630, 2019.

NÜRK, N. M.; LINDER, H. P.; ONSTEIN, R. E.; LARCOMBE, M. J.; HUGHES, C. E.; FERNÁNDEZ, L. P.; SCHLÜTER, P. M.; VALENTE, L.; BEIERKUHNLEIN, C.; CUTTS, V.; DONOGHUE, M. J.; EDWARDS, E. J.; FIELD, R.; FLANTUA, S. G. A.; HIGGINS, S. I.; JENTSCH, A.; LIEDE-SCHUMANN, S.; PIRIE, M.D. Diversification in evolutionary arenas—Assessment and synthesis. *Ecology and Evolution*, Amsterdam, v. 10, n. 12, p. 6163-6182, 2020.

ORNELAS, J. F. J.; SOSA, V.; SOLTIS, D. E. DE; DAZA, J. M. J.; GONZÁLEZ, C.; SOLTIS, P. S.; GUTIÉRREZ-RODRÍGUEZ, C.; DE LOS MONTEROS, A. E.; CASTOE, T. A; BELL, C.; RUIZ-SANCHEZ, E. Comparative phylogeographic

analyses illustrate the complex evolutionary history of threatened cloud forests of northern Mesoamerica. *PloS One*, San Francisco, v. 8, n. 2, p. e56283, 2013.

ORTEGO, J.; KNOWLES, L. L. Incorporating interspecific interactions into phylogeographic models: A case study with Californian oaks. *Molecular Ecology*, Oxford, n. February, p. 1–15, 2020.

PALMA-SILVA, C.; LEXER, C.; PAGGI, G. M.; BARBARÁ, T.; BERED, F.; BODANESE-ZANETTINI, M. H. Range-wide patterns of nuclear and chloroplast DNA diversity in *Vriesea gigantea* (Bromeliaceae), a Neotropical forest species. *Heredity,* London, v. 103, n. 6, p. 503–512, 2009.

PALMA-SILVA, C.; WENDT, T.; PINHEIRO, F.; BARBARÁ, T.; FAY, M. F.; COZZOLINO, S.; LEXER, C. Sympatric bromeliad species (*Pitcairnia* spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs*. Molecular Ecology*, Oxford, v. 20, n. 15, p. 3185–3201, 2011.

PANNELL, J. R. Coalescence in a metapopulation with recurrent local extinction and recolonization. *Evolution*, Lancaster, v. 57, n. 5, p. 949 – 961, 2003.

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

PEREZ, M. F.; BONATELLI, I. A. S.; MORAES, E. M.; CARSTENS, B. C. Model-based analysis supports interglacial refugia over long-dispersal events in the diversification of two South American cactus species. *Heredity*, London, v. 116, n. 6, p. 550–557, 2016.

PERRIGO, A.; HOORN, C.; ANTONELLI, A. Why mountains matter for biodiversity. *Journal of Biogeography*, Oxford, v. 47, n. 2, p. 315–325, 2020.

PHILLIPS, S. J.; DUDÍK, M. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, Copenhagen, v. 31, n. 2, p. 161–175, 2008.

PINHEIRO, F.; DANTAS-QUEIROZ, M. V.; PALMA-SILVA, C. Plant species complexes as models to understand speciation and evolution: a review of South American studies. *Critical Reviews in Plant Sciences*, Philadelphia, v. 37, n. 1, p. 54–80, 2018.

PRATES, I.; XUE, A. T.; BROWN, J. L.; ALVARADO-SERRANO, D. F.; RODRIGUES, M. T.; HICKERSON, M. J.; CARNAVAL, A. C. Inferring responses to climate dynamics from historical demography in Neotropical forest lizards. *Proceedings of the National Academy of Sciences*, Washington, v. 113, n. 29, p. 7978–7985, 2016.

REID, B. N.; NARO-MACIEL, E.; HAHN, A. T.; FITZSIMMONS, N. N.; GEHARA, M. Geography best explains global patterns of genetic diversity and postglacial co-

expansion in marine turtles. *Molecular Ecology*, Oxford, v. 28, n. 14, p. 3358–3370, 2019.

REID, N. M.; CARSTENS, B. C. Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. *BMC Evolutionary Biology*, London, v. 12, n. 1, p. 196, 2012.

RIBEIRO, P. L.; RAPINI, A.; DAMASCENA, L. S.; VAN DEN BERG, C. Plant diversification in the Espinhaço Range: insights from the biogeography of *Minaria* (Apocynaceae). *Taxon*, Utrecht, v. 63, n. 6, p. 1253–1264, 2014.

RULL, V. Biotic diversification in the Guayana Highlands: a proposal. *Journal of Biogeography*, Oxford, v. 32, n. 6, p. 921–927, 2005.

RULL, V.; MONTOYA, E.; NOGUÉ, S.; SAFONT, E.; VEGAS-VILARRÚBIA, T. Climatic and ecological history of Pantepui and surrounding areas. In: RULL, V.; VEGAS-VILARRÚBIA, T.; HUBER, O.; SEÑARIS, C. (Eds.). *Biodiversity of Pantepui: the pristine "Lost World" of the Neotropical Guiana Highlands*. Basel: Elsevier, 2019. p. 33–54.

SAFFORD, H. Brazilian Páramos IV. Phytogeography of the campos de altitude. *Journal of Biogeography*, Oxford, v. 34, n. 10, p. 1701–1722, 2007.

SATLER, J. D.; CARSTENS, B. C. Phylogeographic concordance factors quantify phylogeographic congruence among co-distributed species in the *Sarracenia alata* pitcher plant system. *Evolution*, Lancaster, v. 70, n. 5, p. 1105–1119, 2016.

SCHAEFER, C. E.; CANDIDO, H. G.; CORRÊA, G. R.; NUNES, J. A.; ARRUDA, D. M. Soils associated with rupestrian grasslands. In: FERNANDES, G. W. (Ed.). *Ecology and Conservation of Mountaintop Grasslands in Brazil*. Cham: Springer, 2016. p. 55–69.

SCHWERY, O.; ONSTEIN, R. E.; BOUCHENAK-KHELLADI, Y.; XING, Y.; CARTER, R. J.; LINDER, H. P. As old as the mountains: the radiations of the Ericaceae. *New Phytologist*, Cambridge, v. 207, n. 2, p. 355–367, 2015.

SÉRSIC, A. N.; COSACOV, A.; COCUCCI, A. A.; JOHNSON, L. A.; POZNER, R.; AVILA, L. J.; SITES, J. W.; MORANDO, M. Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. *Biological Journal of the Linnean Society*, London, v. 103, n. 2, p. 475–494, 2011.

SILVA, A. C.; HORÀK-TERRA, I.; BARRAL, U. M.; COSTA, C. R.; GONÇALVES, S. T.; PINTO, T.; CHRISTÓFARO SILVA, B. P.; CUNHA FERNANDES, J. S.; MENDONÇA FILHO, C. V.; VIDAL-TORRADO, P. Altitude, vegetation, paleoclimate, and radiocarbon age of the basal layer of peatlands of the Serra do Espinhaço Meridional, Brazil. *Journal of South American Earth Sciences*, Oxford, v. 103, n. March, p. 102728, 2020.

SILVEIRA, F. A. O.; NEGREIROS, D.; BARBOSA, N. P. U.; BUISSON, E.; CARMO, F. F.; CARSTENSEN, D. W.; CONCEIÇÃO, A. A.; CORNELISSEN, T. G.;

ECHTERNACHT, L.; FERNANDES, G. W.; GARCIA, Q. S.; GUERRA, T. J.; JACOBI, C. M.; LEMOS-FILHO, J. P.; LE STRADIC, S.; MORELLATO, L. P. C.; NEVES, F. S.; OLIVEIRA, R. S.; SCHAEFER, C. E.; VIANA, P. L.; LAMBERS, H. Ecology and evolution of plant diversity in the endangered campo rupestre: a neglected conservation priority. *Plant and Soil*, The Hague, v. 403, n. 1–2, p. 129–152, 2016.

SMITH, C. I.; TANK, S.; GODSOE, W.; LEVENICK, J.; STRAND, E.; ESQUE, T.; PELLMYR, O. Comparative phylogeography of a coevolved community: concerted population expansions in Joshua trees and four Yucca moths. *PLoS ONE*, San Francisco, v. 6, n. 10, p. e25628, 2011.

SOLTIS, D. E.; MORRIS, A. B.; MCLACHLAN, J. S.; MANOS, P. S.; SOLTIS, P. S. Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*, Oxford, v. 15, n. 14, p. 4261–4293, 2006.

TAX, D. M. J.; DUIN, R. P. W. Support Vector Data Description. *Machine Learning*, Boston, v. 54, n. 1, p. 45–66, 2004.

THOMÉ, M. T. C.; CARSTENS, B. C. Phylogeographic model selection leads to insight into the evolutionary history of four-eyed frogs. *Proceedings of the National Academy of Sciences*, Washington, v. 113, n. 29, p. 8010–8017, 2016.

TURCHETTO-ZOLET, A. C.; PINHEIRO, F.; SALGUEIRO, F.; PALMA-SILVA, C. Phylogeographical patterns shed light on evolutionary process in South America. *Molecular Ecology*, Oxford, v. 22, n. 5, p. 1193–213, 2013.

VASCONCELOS, T. N. C.; ALCANTARA, S.; ANDRINO, C. O.; FOREST, F.; REGINATO, M.; SIMON, M. F.; PIRANI, J. R. Fast diversification through a mosaic of evolutionary histories characterizes the endemic flora of ancient Neotropical mountains. *Proceedings of the Royal Society B: Biological Sciences*, London, v. 287, n. 1923, p. 20192933, 2020.

WIERINGA, J. G.; BOOT, M. R.; DANTAS-QUEIROZ, M. V.; DUCKETT, D.; FONSECA, E. M.; GLON, H.; HAMILTON, N.; KONG, S.; LANNA, F. M.; MATTINGLY, K. Z.; PARSONS, D. J.; SMITH, M. L.; STONE, B. W.; THOMPSON, C.; ZUO, L.; CARSTENS, B. C. Does habitat stability structure intraspecific genetic diversity? It’s complicated... *Frontiers of Biogeography*, Westlake Village, v. 12, n. 2, 2020.

ZAPPI, D. C.; MORO, M. F.; MEAGHER, T. R.; NIC LUGHADHA, E. Plant Biodiversity drivers in Brazilian campos rupestres: insights from phylogenetic structure. *Frontiers in Plant Science*, Lausanne, v. 8, n. December, p. 1–15, 2017.

CONSIDERAÇÕES FINAIS

Nesta tese, utilizamos distintas abordagens filogeográficas para responder como processos microevolutivos puderam gerar os padrões macroevolutivos existentes nas BQM. A espécie modelo utilizada neste trabalho, a bromélia *Vriesea oligantha*, com populações distribuídas ao longo de todo a cadeia do Espinhaço, oferece um grande potencial para compreender os processos microevolutivos responsáveis pela diversificação da biota endêmica destas montanhas.

No Capítulo I foram otimizados marcadores nucleares, que posteriormente, no Capítulo II, foram aplicados juntamente com marcadores plastidiais bem como as análises de modelagem de nicho ecológico, para investigar a história evolutiva de *V. oligantha.* Através da datação realizada, verificamos que a espécie se originou há ca. de três milhões de anos, demonstrando que *V. oligantha* é um bom modelo para testar os efeitos das flutuações climáticas do passado, perdurando desde o final do Plioceno até os dias de hoje. De fato, constatamos que a diversificação intraespecífica de *V. oligantha* é bem antiga, remontando ao meio do Pleistoceno. Esta antiga divergência é possivelmente fruto da deriva genética ocorrente nas populações, já que o fluxo gênico interpopulacional é bem baixo (ou até ausente), mas também devido à seleção natural, pois a diferença ambiental entre as populações é um fator importante na estruturação das populações. Nossas predições feitas através das análises demográficas não foram capazes de captar mudanças significativas no tamanho populacional durante os últimos 120 mil anos em *V. oligantha*, período bem conhecido pela influência das oscilações climáticas na demografia de espécies de montanhas. Contudo, nossas análises de modelagem de nicho ecológico demonstraram uma dinâmica populacional de expansão e contração durante os últimos interglacial e glacial máximos, suscitando dúvidas se realmente as populações *V. oligantha* passaram incólumes às mudanças climáticas durante este período.

Assim, no Capítulo III, investigamos o efeito das oscilações climáticas mais recentes (fim do Pleistoceno e começo do Holoceno) na dinâmica populacional ao nível da comunidade, utilizando técnicas distintas daquelas do Capítulo II. Notavelmente, inferimos uma expansão demográfica síncrona entre diversas espécies das BQM durante o último glacial máximo, incluindo *V. oligantha*.

Majoritariamente, as espécies vegetais apresentaram expansão populacional, enquanto a comunidade animal não seguiu este padrão. As modelagens de nicho ecológico realizadas também corroboram nossas inferências sobre a dinâmica populacional, com exceção do anuro *Bokermannohyla saxicola*, que teve uma enorme área adequada inferida para o último glacial máximo, indo em desacordo à redução populacional estimada pelos dados genéticos.

Além do mais, utilizando regiões biogeográficas do Espinhaço baseadas em níveis de endemismo, encontramos um padrão concordante entre as linhagens de três espécies vegetais. Este padrão concordante é um indício de que os fatores ambientais ocorrentes na Cadeia do Espinhaço estão influenciando fortemente a dinâmica demográfica das espécies. Pressões ambientais que possam limitar assim os tamanhos populacionais, como as flutuações climáticas históricas, são fortes indícios da origem e diversificação das espécies do Espinhaço, explicando a formação dos padrões biogeográficos ocorrentes nestas montanhas.

Através dos resultados presentes nesta tese demonstramos a importância que as oscilações climáticas, tanto aquelas do Plio-Pleistoceno (Capítulo II), quanto aquelas do Pleistoceno-Holoceno (Capítulo III), possuem na origem e diversificação da biota das BQM. Com estes resultados, acreditamos contribuir para elucidarmos os processos evolutivos responsáveis pelos altos níveis de endemismo e diversidade biológica das BQM. Contudo, ainda há muito a ser feito. A próxima frente a ser tomada nos trabalhos de filogeografia e da evolução das espécies nas BQM é a incorporação de dados ecológicos às análises. Ao utilizarmos características como tipos de síndromes de polinização e outras interações ecológicas poderíamos compreender melhor como as relações das espécies com seu ambiente pode influenciar na demografia. Como modelo, *Vriesea oligantha* possui o potencial de elucidar como as interações ecológicas afetaram sua história. Por exemplo, *V. oligantha* possui hábito predominantemente epifítico, vivendo principalmente sobre plantas do gênero *Vellozia*. Neste sentido, será que a dinâmica populacional de espécies de *Vellozia* poderia também influenciar a demografia de *V. oligantha* ao longo do tempo? Será que poderíamos encontrar uma resposta demográfica síncrona entre *V. oligantha* e seus forófitos? Essas são apenas algumas questões que poderão ser elucidadas ao conectarmos as análises

filogeográficas com dados ecológicos, suscitando hipóteses sobre pressões locais das quais os organismos das BQM estariam suscetíveis.

Além disso, as adaptações locais como fatores de divergência intrapopulacional também serão pormenorizadamente investigadas com o uso de dados genômicos e transcriptômicos. Estudos utilizando diversos locus resultam em análises demográficas mais detalhadas e compreensivas, principalmente quando sob uma abordagem de coalescência. Ao conectar os fatores abióticos e bióticos que desencadearam as mudanças no genoma das espécies, poderemos entender com mais precisão como a evolução ocorre não apenas nas BQM, mas em qualquer sistema de montanhas pelo globo.

ANEXOS

Materiais Suplementares do Capítulo II

S1 - Genbank accession number used for estimate of the Maximum clade credibility tree of Tillandsioideae.

Taxon	Dna isolate	PHYC	rpoB-trnC-petN	trnK-matK-trnK	ycf1	Subfamily
Aechmea nudicaulis	B118	KX753971	KX754314	AY614024	KX753761	Bromelioideae
Alcantarea duarteana	B59	KX753933	KX754276	AY614031	KX753723	Tillandsioideae
Alcantarea farneyi	B170	KX753988	KX754331	KX754121	KX753778	Tillandsioideae
Alcantarea geniculata	B153	KX753986	KX754329	KX754119	KX753776	Tillandsioideae
Alcantarea glaziouana	B167	KX753987	KX754330	KX754120	KX753777	Tillandsioideae
Alcantarea imperialis	B1	KX753891	KX754234	AY614032	KX753681	Tillandsioideae
Alcantarea regina	B136	KX753978	KX754321	KX754111	KX753768	Tillandsioideae
Barfussia laxissima	B294	KX754009	KX754352	KX754142	KX753799	Tillandsioideae
Barfussia platyrhachis	B753	KX754062	KX754405	KX754195	KX753852	Tillandsioideae
Barfussia wagneriana	B58	KX753932	KX754275	AY614067	KX753722	Tillandsioideae
Brocchinia micrantha	B150	KX753984	KX754327	KX754117	KX753774	Brocchinioideae
Bromelia karatas	B119	KX753972	KX754315	AY614023	KX753762	Bromelioideae
Catopsis morreniana	B106	KX753967	KX754310	AY614025	KX753757	Tillandsioideae
Catopsis nitida	B463	KX754033	KX754376	KX754166	KX753823	Tillandsioideae
Catopsis nutans	B2	KX753892	KX754235	KX754101	KX753682	Tillandsioideae
Catopsis paniculata	B507	KX754038	KX754381	KX754171	KX753828	Tillandsioideae
Glomeropitcairnia erectiflora	B30	KX753910	KX754253	AY614029	KX753700	Tillandsioideae
Glomeropitcairnia penduliflora	B13	KX753899	KX754242	KX754103	KX753689	Tillandsioideae
Goudaea chrysostachys	B1300	KX754094	KX754437	KX754227	KX753884	Tillandsioideae
Goudaea ospinae	B54	KX753929	KX754272	AY614040	KX753719	Tillandsioideae
Gregbrownia fulgens	B195	KX753990	KX754333	KX754123	KX753780	Tillandsioideae
Gregbrownia hutchisonii	B3	KX753893	KX754236	KX754102	KX753683	Tillandsioideae
Guzmania acorifolia	B52	KX753928	KX754271	AY614060	KX753718	Tillandsioideae
Guzmania angustifolia	B93	KX753959	KX754302	AY614052	KX753749	Tillandsioideae
Guzmania coriostachya	B481	KX754035	KX754378	KX754168	KX753825	Tillandsioideae
Guzmania graminifolia	B120	KX753973	KX754316	AY614057	KX753763	Tillandsioideae
Guzmania melinonis	B32	KX753911	KX754254	AY614051	KX753701	Tillandsioideae
Guzmania monostachia	B22	KX753907	KX754250	AY614054	KX753697	Tillandsioideae
Guzmania mucronata	B858	KX754072	KX754415	KX754205	KX753862	Tillandsioideae
Guzmania musaica	B14	KX753900	KX754243	AY614058	KX753690	Tillandsioideae
Guzmania nicaraguensis	B479	KX754034	KX754377	KX754167	KX753824	Tillandsioideae
Guzmania polycephala	B651	KX754054	KX754397	KX754187	KX753844	Tillandsioideae
Guzmania rhonhofiana	B96	KX753960	KX754303	AY614064	KX753750	Tillandsioideae
Guzmania roezlii	B286	KX754006	KX754349	KX754139	KX753796	Tillandsioideae
Guzmania wittmackii	B12	KX753898	KX754241	AY614056	KX753688	Tillandsioideae
Jagrantia monstrum	B108	KX753969	KX754312	KX754106	KX753759	Tillandsioideae
Josemania asplundii	B588	KX754049	KX754392	KX754182	KX753839	Tillandsioideae
Josemania singularis	B64	KX753937	KX754280	AY614039	KX753727	Tillandsioideae
Lindmania guianensis	B112	KX753970	KX754313	AY614019	KX753760	Lindmanioideae

Taxon	Dna isolate	PHYC	rpoB-trnC-petN	trnK-matK-trnK	ycf1	Subfamily
Lutheria glutinosa	B130	KX753976	KX754319	KX754109	KX753766	Tillandsioideae
Lutheria splendens	B37	KX753915	KX754258	AY614045	KX753705	Tillandsioideae
Mezobromelia bicolor	B578	KX754048	KX754391	KX754181	KX753838	Tillandsioideae
Mezobromelia capituligera	B978	KX754074	KX754417	KX754207	KX753864	Tillandsioideae
Mezobromelia pleiosticha	B274	KX754005	KX754348	KX754138	KX753795	Tillandsioideae
Navia saxicola	B182	KX753989	KX754332	KX754122	KX753779	Navioideae
Pitcairnia punicea	B77	KX753948	KX754291	AY614021	KX753738	Pitcairnioideae
Pseudalcantarea grandis	B125	KX753974	KX754317	KX754107	KX753764	Tillandsioideae
Pseudalcantarea macropetala	B742	KX754059	KX754402	KX754192	KX753849	Tillandsioideae
Pseudalcantarea viridiflora	B6	KX753896	KX754239	AY614066	KX753686	Tillandsioideae
Puya laxa	B78	KX753949	KX754292	AY614022	KX753739	Puyoideae
Racinaea cuspidata	B1034	KX754077	KX754420	KX754210	KX753867	Tillandsioideae
Racinaea dyeriana	B151	KX753985	KX754328	KX754118	KX753775	Tillandsioideae
Racinaea elegans	B51	KX753927	KX754270	AY614084	KX753717	Tillandsioideae
Racinaea fraseri	B547	KX754043	KX754386	KX754176	KX753833	Tillandsioideae
Racinaea hamaleana	B251	KX754004	KX754347	KX754137	KX753794	Tillandsioideae
Racinaea multiflora	B426	KX754031	KX754374	KX754164	KX753821	Tillandsioideae
Racinaea parviflora	B335	KX754015	KX754358	KX754148	KX753805	Tillandsioideae
Racinaea ropalocarpa	B57	KX753931	KX754274	AY614083	KX753721	Tillandsioideae
Racinaea spiculosa	B99	KX753962	KX754305	AY614082	KX753752	Tillandsioideae
Racinaea venusta	B7	KX753897	KX754240	AY614081	KX753687	Tillandsioideae
Stigmatodon croceanus	B802	KX754067	KX754410	KX754200	KX753857	Tillandsioideae
Stigmatodon harrylutheri	B1282	KX754086	KX754429	KX754219	KX753876	Tillandsioideae
Stigmatodon plurifolius	B376	KX754021	KX754364	KX754154	KX753811	Tillandsioideae
Tillandsia adpressiflora	B597	KX754050	KX754393	KX754183	KX753840	Tillandsioideae
Tillandsia albertiana	B33	KX753912	KX754255	AY614117	KX753702	Tillandsioideae
Tillandsia amicorum	B318	KX754011	KX754354	KX754144	KX753801	Tillandsioideae
Tillandsia appenii	B66	KX753939	KX754282	AY614077	KX753729	Tillandsioideae
Tillandsia aurea	B250	KX754003	KX754346	KX754136	KX753793	Tillandsioideae
Tillandsia australis	B203	KX753993	KX754336	KX754126	KX753783	Tillandsioideae
Tillandsia baliophylla	B101	KX753963	KX754306	AY614114	KX753753	Tillandsioideae
Tillandsia barfussii	B387	KX754023	KX754366	KX754156	KX753813	Tillandsioideae
Tillandsia barthlottii	B35	KX753914	KX754257	AY614076	KX753704	Tillandsioideae
Tillandsia bergeri	B97	KX753961	KX754304	AY614134	KX753751	Tillandsioideae
Tillandsia bermejoensis	B34	KX753913	KX754256	AY614123	KX753703	Tillandsioideae
Tillandsia biflora	B90	KX753957	KX754300	AY614107	KX753747	Tillandsioideae
Tillandsia brachyphylla	B82	KX753951	KX754294	AY614105	KX753741	Tillandsioideae
Tillandsia brevilingua	B56	KX753930	KX754273	AY614113	KX753720	Tillandsioideae
Tillandsia cacticola	B44	KX753922	KX754265	AY614070	KX753712	Tillandsioideae
Tillandsia capillaris	B916	KX754073	KX754416	KX754206	KX753863	Tillandsioideae
Tillandsia carlos-hankii	B62	KX753936	KX754279	AY614089	KX753726	Tillandsioideae
Tillandsia caulescens	B71	KX753943	KX754286	AY614126	KX753733	Tillandsioideae
Tillandsia cereicola	B134	KX753977	KX754320	KX754110	KX753767	Tillandsioideae
Tillandsia cochabambae	B392	KX754025	KX754368	KX754158	KX753815	Tillandsioideae
Tillandsia complanata	B562	KX754044	KX754387	KX754177	KX753834	Tillandsioideae

Taxon	Dna isolate	PHYC	rpoB-trnC-petN	trnK-matK-trnK	ycf1	Subfamily
Tillandsia demissa	B75	KX753947	KX754290	AY614115	KX753737	Tillandsioideae
Tillandsia denudata	B750	KX754060	KX754403	KX754193	KX753850	Tillandsioideae
Tillandsia didisticha	B38	KX753916	KX754259	AY614127	KX753706	Tillandsioideae
Tillandsia disticha	B48	KX753925	KX754268	AY614068	KX753715	Tillandsioideae
Tillandsia divaricata	B68	KX753940	KX754283	AY614108	KX753730	Tillandsioideae
Tillandsia duratii	B88	KX753955	KX754298	AY614119	KX753745	Tillandsioideae
Tillandsia ecarinata	B237	KX754000	KX754343	KX754133	KX753790	Tillandsioideae
Tillandsia edithae	B425	KX754030	KX754373	KX754163	KX753820	Tillandsioideae
Tillandsia espinosae	B462	KX754032	KX754375	KX754165	KX753822	Tillandsioideae
Tillandsia esseriana	B69	KX753941	KX754284	AY614120	KX753731	Tillandsioideae
Tillandsia fasciculata	B717	KX754056	KX754399	KX754189	KX753846	Tillandsioideae
Tillandsia ferreyrae	B241	KX754001	KX754344	KX754134	KX753791	Tillandsioideae
Tillandsia filifolia	B790	KX754066	KX754409	KX754199	KX753856	Tillandsioideae
Tillandsia floribunda	B351	KX754018	KX754361	KX754151	KX753808	Tillandsioideae
Tillandsia fuchsii	B391	KX754024	KX754367	KX754157	KX753814	Tillandsioideae
Tillandsia funebris	B89	KX753956	KX754299	AY614118	KX753746	Tillandsioideae
Tillandsia gardneri	B41	KX753919	KX754262	AY614104	KX753709	Tillandsioideae
Tillandsia guatemalensis	B103	KX753965	KX754308	AY614094	KX753755	Tillandsioideae
Tillandsia heliconioides	B1289	KX754087	KX754430	KX754220	KX753877	Tillandsioideae
Tillandsia heteromorpha	B224	KX753998	KX754341	KX754131	KX753788	Tillandsioideae
Tillandsia heterophylla	B47	KX753924	KX754267	KX754104	KX753714	Tillandsioideae
Tillandsia heubergeri	B42	KX753920	KX754263	AY614106	KX753710	Tillandsioideae
Tillandsia hildae	B763	KX754065	KX754408	KX754198	KX753855	Tillandsioideae
Tillandsia imperialis	B292	KX754008	KX754351	KX754141	KX753798	Tillandsioideae
Tillandsia ionantha	B84	KX753953	KX754296	AY614099	KX753743	Tillandsioideae
Tillandsia ixioides	B43	KX753921	KX754264	AY614129	KX753711	Tillandsioideae
Tillandsia juerg-rutschmannii	B715	KX754055	KX754398	KX754188	KX753845	Tillandsioideae
Tillandsia juncea	B73	KX753945	KX754288	AY614097	KX753735	Tillandsioideae
Tillandsia kauffmannii	B74	KX753946	KX754289	AY614103	KX753736	Tillandsioideae
Tillandsia kirschnekii	B384	KX754022	KX754365	KX754155	KX753812	Tillandsioideae
Tillandsia kuehhasii	B396	KX754026	KX754369	KX754159	KX753816	Tillandsioideae
Tillandsia lajensis	B546	KX754042	KX754385	KX754175	KX753832	Tillandsioideae
Tillandsia landbeckii	B423	KX754029	KX754372	KX754162	KX753819	Tillandsioideae
Tillandsia lechneri	B808	KX754069	KX754412	KX754202	KX753859	Tillandsioideae
Tillandsia leiboldiana	B323	KX754012	KX754355	KX754145	KX753802	Tillandsioideae
Tillandsia lepidosepala	B219	KX753997	KX754340	KX754130	KX753787	Tillandsioideae
Tillandsia lotteae	B820	KX754070	KX754413	KX754203	KX753860	Tillandsioideae
Tillandsia lymanii	B1098	KX754079	KX754422	KX754212	KX753869	Tillandsioideae
Tillandsia macbrideana	B70	KX753942	KX754285	AY614109	KX753732	Tillandsioideae
Tillandsia maculata	B574	KX754047	KX754390	KX754180	KX753837	Tillandsioideae
Tillandsia malzinei	B145	KX753979	KX754322	KX754112	KX753769	Tillandsioideae
Tillandsia multicaulis	B107	KX753968	KX754311	AY614112	KX753758	Tillandsioideae
Tillandsia myriantha	B760	KX754063	KX754406	KX754196	KX753853	Tillandsioideae
Tillandsia nana	B343	KX754016	KX754359	KX754149	KX753806	Tillandsioideae
Tillandsia paleacea	B404	KX754027	KX754370	KX754160	KX753817	Tillandsioideae

Taxon	Dna isolate	PHYC	rpoB-trnC-petN	trnK-matK-trnK	ycf1	Subfamily
Tillandsia paniculata	B102	KX753964	KX754307	AY614086	KX753754	Tillandsioideae
Tillandsia plumosa	B86	KX753954	KX754297	AY614075	KX753744	Tillandsioideae
Tillandsia prolata	B418	KX754028	KX754371	KX754161	KX753818	Tillandsioideae
Tillandsia propagulifera	B310	KX754010	KX754353	KX754143	KX753800	Tillandsioideae
Tillandsia pseudomicans	B347	KX754017	KX754360	KX754150	KX753807	Tillandsioideae
Tillandsia punctulata	B61	KX753935	KX754278	AY614087	KX753725	Tillandsioideae
Tillandsia purpurea	B246	KX754002	KX754345	KX754135	KX753792	Tillandsioideae
Tillandsia rauhii	B92	KX753958	KX754301	AY614101	KX753748	Tillandsioideae
Tillandsia recurvata	B529	KX754041	KX754384	KX754174	KX753831	Tillandsioideae
Tillandsia rupicola	B39	KX753917	KX754260	AY614073	KX753707	Tillandsioideae
Tillandsia schimperiana	B334	KX754014	KX754357	KX754147	KX753804	Tillandsioideae
Tillandsia secunda	B527	KX754039	KX754382	KX754172	KX753829	Tillandsioideae
Tillandsia setacea	B1246	KX754081	KX754424	KX754214	KX753871	Tillandsioideae
Tillandsia spathacea	B565	KX754045	KX754388	KX754178	KX753835	Tillandsioideae
Tillandsia sphaerocephala	B366	KX754020	KX754363	KX754153	KX753810	Tillandsioideae
Tillandsia spiraliflora	B762	KX754064	KX754407	KX754197	KX753854	Tillandsioideae
Tillandsia stenoura	B635	KX754052	KX754395	KX754185	KX753842	Tillandsioideae
Tillandsia stricta	B81	KX753950	KX754293	KX754105	KX753740	Tillandsioideae
Tillandsia tenuifolia	B26	KX753909	KX754252	AY614132	KX753699	Tillandsioideae
Tillandsia tequendamae	B569	KX754046	KX754389	KX754179	KX753836	Tillandsioideae
Tillandsia teres	B201	KX753991	KX754334	KX754124	KX753781	Tillandsioideae
Tillandsia tortilis	B49	KX753926	KX754269	AY614074	KX753716	Tillandsioideae
Tillandsia turneri	B650	KX754053	KX754396	KX754186	KX753843	Tillandsioideae
Tillandsia usneoides	B83	KX753952	KX754295	AY614122	KX753742	Tillandsioideae
Tillandsia utriculata	B807	KX754068	KX754411	KX754201	KX753858	Tillandsioideae
Tillandsia virescens	B1591	KX754098	KX754441	KX754231	KX753888	Tillandsioideae
Tillandsia xiphioides	B40	KX753918	KX754261	AY614125	KX753708	Tillandsioideae
Vriesea carinata	B21	KX753906	KX754249	AY614033	KX753696	Tillandsioideae
Vriesea dubia	B982	KX754075	KX754418	KX754208	KX753865	Tillandsioideae
Vriesea elata	B1297	KX754091	KX754434	KX754224	KX753881	Tillandsioideae
Vriesea jonghei	B65	KX753938	KX754281	AY614037	KX753728	Tillandsioideae
Vriesea longicaulis	B290	KX754007	KX754350	KX754140	KX753797	Tillandsioideae
Vriesea maxoniana	B490	KX754036	KX754379	KX754169	KX753826	Tillandsioideae
Vriesea pabstii	B357	KX754019	KX754362	KX754152	KX753809	Tillandsioideae
Vriesea platynema	B146	KX753980	KX754323	KX754113	KX753770	Tillandsioideae
Vriesea psittacina	B20	KX753905	KX754248	AY614034	KX753695	Tillandsioideae
Vriesea rubra	B983	KX754076	KX754419	KX754209	KX753866	Tillandsioideae
Vriesea scalaris	B147	KX753981	KX754237	KX754114	KX753771	Tillandsioideae
Vriesea zamorensis	B45	KX753923	KX754266	AY614043	KX753713	Tillandsioideae
Wallisia anceps	B741	KX754058	KX754401	KX754191	KX753848	Tillandsioideae
Wallisia duvalii	B23	KX753908	KX754251	AY614080	KX753698	Tillandsioideae
Wallisia lindeniana	B216	KX753995	KX754338	KX754128	KX753785	Tillandsioideae
Werauhia gladioliflora	B149	KX753983	KX754326	KX754116	KX753773	Tillandsioideae
Werauhia insignis	B17	KX753902	KX754245	AY614049	KX753692	Tillandsioideae
Werauhia pedicellata	B752	KX754061	KX754404	KX754194	KX753851	Tillandsioideae

Taxon	Dna isolate	PHYC	rpoB-trnC-petN	trnK-matK-trnK	ycf1	Subfamily
Werauhia ringens	B19	KX753904	KX754247	AY614047	KX753694	Tillandsioideae
Werauhia tarmaensis	B18	KX753903	KX754246	AY614046	KX753693	Tillandsioideae
Werauhia viridiflora	B325	KX754013	KX754356	KX754146	KX753803	Tillandsioideae
Zizkaea tuerckheimii	B148	KX753982	KX754325	KX754115	KX753772	Tillandsioideae

Fonte: o autor.

This dataset was generated by BARFUSS, M. H. J.; TILL, W.; LEME, E. M. C.; PINZÓN, J. P.; MANZANARES, J. M.; HALBRITTER, H.; SAMUEL, R.; BROWN, G. K. Taxonomic revision of Bromeliaceae subfam. Tillandsioideae based on a multi-locus DNA sequence phylogeny and morphology. *Phytotaxa*, Auckland, v. 279, n. 1, p. 1–97, 2016.

S2 - Material and Methods of Paleodistribution and ancestral population Connections

To predict the current and paleodistributions of *V. oligantha*, we conduct species distribution models (SDMs). We first listed 55 unique occurrence records of *V. oligantha* from field trips and SpeciesLink database (http://splink.cria.org.br), which we mapped in a squared geographical grid of c.a. 3,500,00 km² and 30'' of resolution (ca. 1 km²). To characterize the background environmental conditions, we selected the following five less correlated bioclimatic variables of the 19 available in Worldclim database (http://worldclim.org; HIJMANS et al. (2005), through a factorial analysis, using 'psych' R package (REVELLE, 2015): annual mean temperature (°C), temperature seasonality (°C), temperature annual range (°C), annual precipitation (mm), and precipitation of driest month (mm). To predict the current potential distribution of *V. oligantha*, we adopted six distinct algorithms: (i) Bioclim (NIX, 1986), (ii) Mahalanobis distance (FARBER; KADMON, 2003), (iii) Domain (Gower distance; CARPENTER; GILLISON; WINTER (1993), (iv) Maximum Entropy (MAXENT; PHILLIPS; DUDÍK (2008), (v) Support Vector Machines (SVM; TAX; DUIN (2004), and (vi) Random Forest (BREIMAN, 2001).

To evaluate the predictions of potential *V. oligantha* distribution, we randomized the occurrence records in two subsets (75 and 25 % of train and test records, respectively) and estimated the true skill statistics (TSS; ALLOUCHE; TSOAR; KADMON (2006). From a range of -1 (worse) to 1 (ideal), the acceptable models showed a TSS > 0.5 (ALLOUCHE; TSOAR; KADMON, 2006). To predict the effects of past climatic oscillations on *V. oligantha* distribution, we projected the models onto the paleoclimatic scenarios simulated by the Community Climate System Model (CCSM4; HIJMANS et al. (2005) for the Mid-Holocene (MH, 6 Ka), Last Glacial Maximum (LGM, 21 Ka), and Last Interglacial (LIG,120-140 Ka; (OTTO-BLIESNER et al., 2006). To increase the reliability of potential distribution predictions, we employed an ensemble approach of all models using the average suitability weighted by the TSS value of each model (see BARRY; ELITH (2006); DINIZ-FILHO et al. (2009).

S3 - Material and Methods of Roles of climate and geography on population structure

We implemented a Bayesian generalized linear mixed modeling (GLMM) approach to test whether genetic structure among *V. oligantha* populations is driven by environmental variables, i.e. temperature and precipitation, and/or geographic distance among populations. We used the pairwise linear F_{ST} matrices [measured as $F_{ST}/1(1-F_{ST})$] from both cpDNA and nrSSR as the response variable. As predictor variables, we tested a matrix of the natural logarithm of geographic distances among populations to assess the effect of isolation by distance (IBD) and a matrix of Euclidean distances along the first and second axis of a principal component analysis (PCA) of 19 WorldClim variables (HIJMANS et al., 2005) at a resolution of 30 arc seconds, to access the effect of isolation by environment (IBE). The first and second axes of the PCA accounted for 62.77 and 21.64% of the data variance. This first axis was mostly correlated with Precipitation of Wettest Quarter, Precipitation of Wettest Month and Minimal Temperature of Coldest Month, while the second axis were more associated with the Precipitation of Driest Month and Precipitation of Driest Quarter.

We confronted models including only IBD, only IBE or both IBD and IBE as predictors against a null model. We performed the analysis with the R packages MCMCGLMM (HADFIELD, 2010), following scripts available in Lexer et al. (2014). We ran 2,000,000 MCMC iterations with a 500,000 burn-in with a thinning interval of 750, under standard priors. The lack of independence between pairs of populations was accounted by fitting a multiple membership model. Chain convergences were checked using ‘CODA’ R packages. We used the Deviance Information Criterion (DIC) to compare models and determine the role of IBD versus IBE in the population structure of populations of *V oligantha*.

S4 – Supplementary Figures

Figure S4.1 - Phylogenetic tree of Tillandsioideae. Branch colors are the posterior probability of each node, varying from 0 (red) to 1 (blue). The bottom scale is in million years.

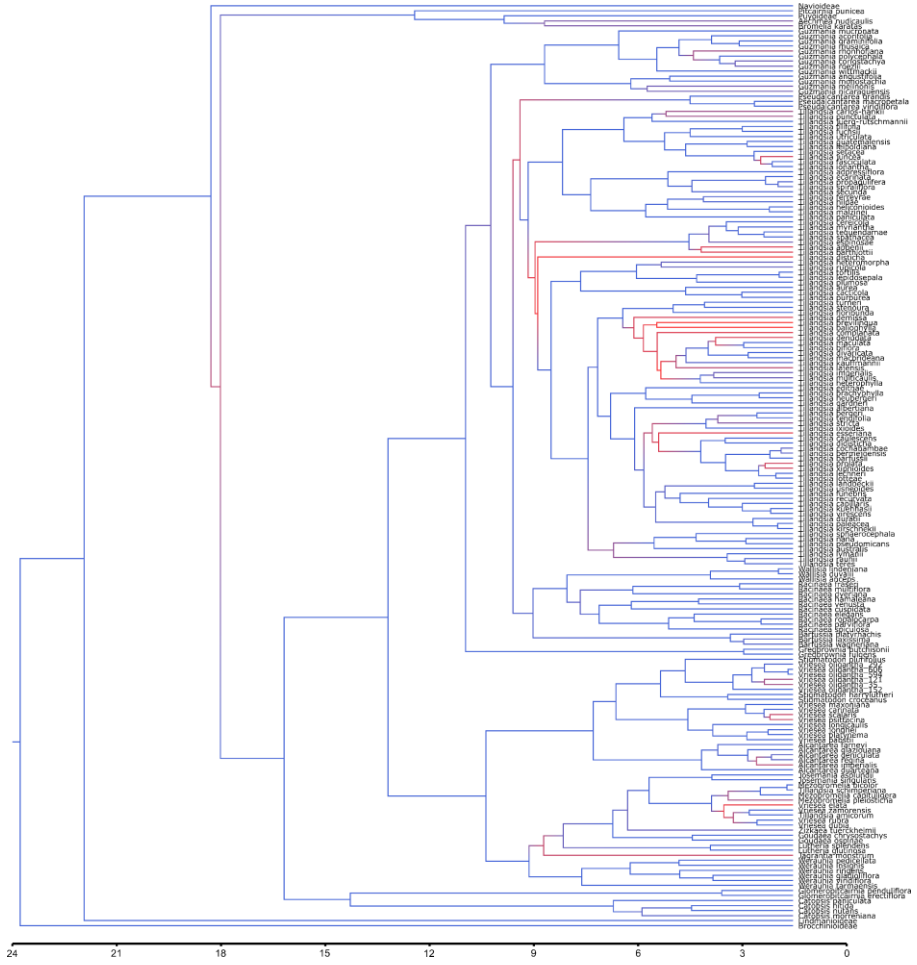


Figure S4.2 – Phylogenetic tree of *Vriesea oligantha*. Branch colors are the posterior probability of each node, varying from 0 (red) to 1 (blue). The bottom scale is in million years

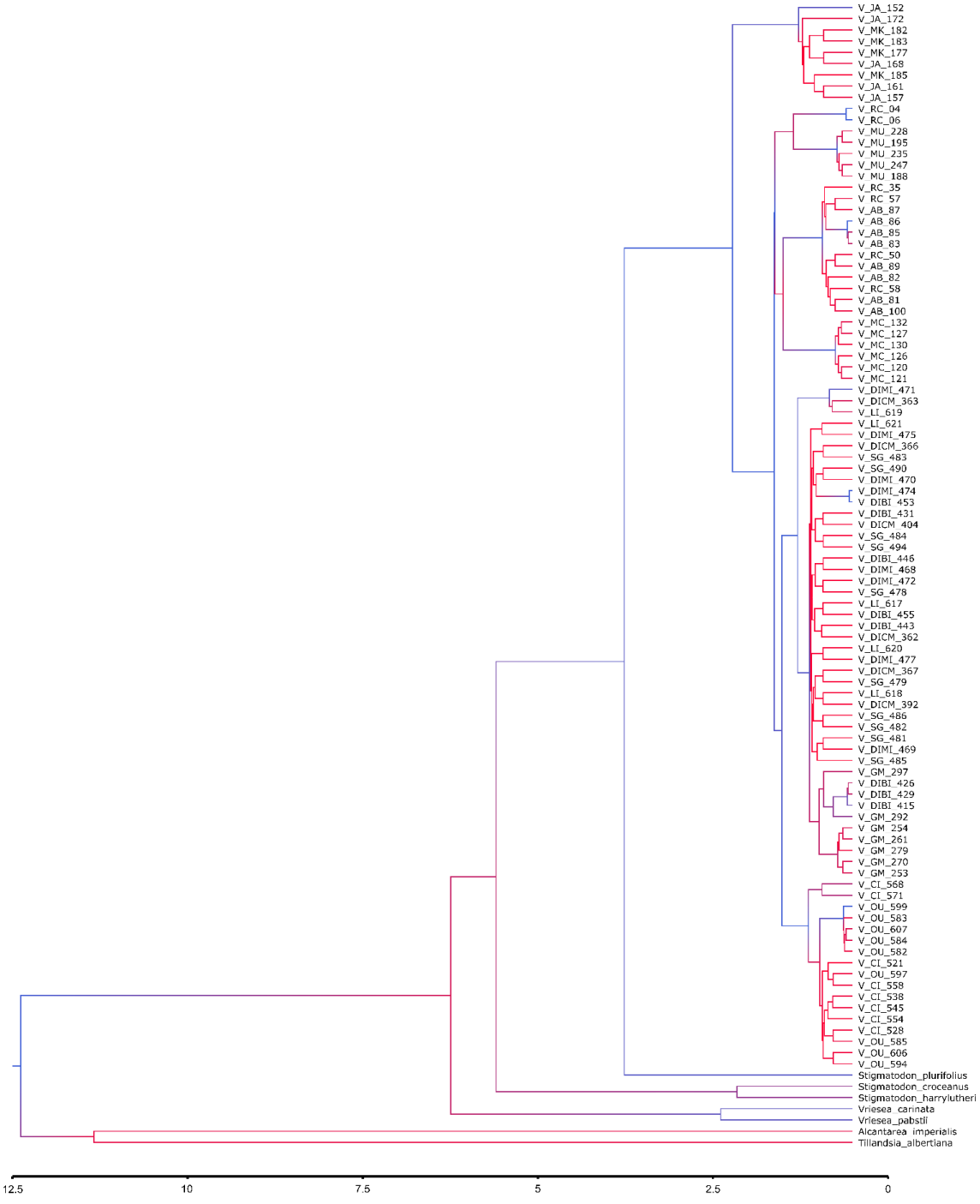
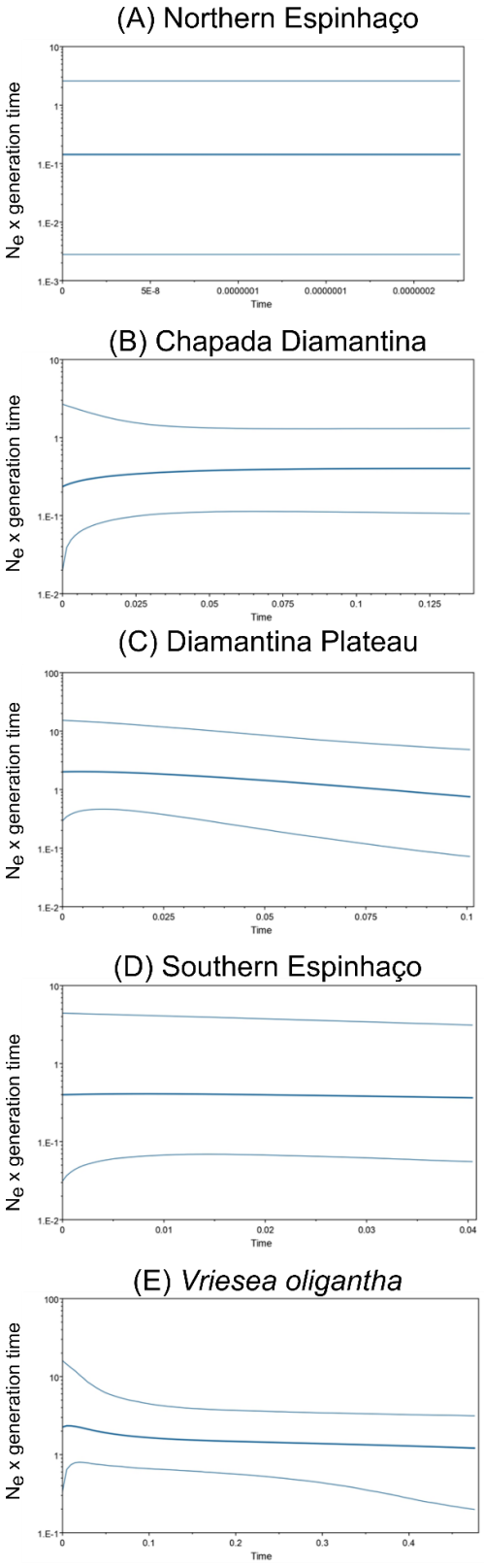


Figure S4.3 – Bayesian Skyline Plots (ESP) from each of the phylogenetic lineages (A-D) and for the total dataset of *Vriesea oligantha*, with 95% HPD intervals (thinner lines). They-axis indicates population size; the x-axis indicates the time in millions of years



S5 – Supplementary Tables

Table S5.1 - Estimated ages (Mya) in Tillandsioideae using a relaxed-clock model approach. The values in brackets are the 95% highest posterior density (HPD). Tribes and subtribes following Barfuss et al. 2016.

Taxon	**Crown age (Mya)**	**bpp**
Tillandsioideae	14.6 (16.0 – 13.2)	1
Glomeropitcairnieae + Catopsideae	12.7 (14.8 – 10.4)	0.99
Vrieseeae	8.8 (10.3 – 7.3)	1
Cipuropsidinae	7.5 (9.1 – 6.1)	1
Vrieseinae	5.7 (10.3 – 7.3)	1
Alcantarea	2.6 (3.8 – 1.4)	1
Vriesea	3.0 (4.2 – 1.9)	1
Stigmatodon + *Vriesea oligantha*	3.8 (5.2 – 2.5)	0.99
Vriesea oligantha	1.7 (2.6 – 0.9)	1
Tillandsieae	9.4 (10.7 – 8.0)	1
Guzmania	7.1 (8.6 – 5.6)	1
Gregbrownia	1.4 (2.6 – 0.4)	1
Barfussia	1.7 (2.9 – 0.7)	1
Wallisia	2.3 (4.0 – 0.9)	1
Racinaea	6.1 (7.3 – 4.8)	0.87
Pseudoalcantarea	2.9 (5.0 – 1.2)	1
Tillandsia	7.6 (8.8 – 6.3)	0.96

Clade		
Northern Espinhaço (JAC | MKA)	0.702 (1.473 – 0.076)	0.86
Chapada Diamantina (MCH | MUC | RCO | ABA)	1.108 (1.177 – 1.040)	1
Diamantina Plateau (LIC | GMO | DIB | DIC | DIM | SGO)	0.861 (1.065 – 0.494)	0.98
Southern Espinhaço (CIP | OUR)	0.708 (0.999 – 0.274)	0.99

JAC: Jacobina - BA; MKA: Miguel Calmon - BA; MCH: Morro do Chapéu - BA; MUC: Mucugê - BA; RCO: Rio de Contas - BA; ABA: Abaíra - BA; LIC: Licínio de Almeida - BA; GMO: Grão Mogol - MG; DIB: Diamantina (Biribiri) - MG; DIC: Diamantina (Cons. Mata) - MG; DIM: Diamantina (Milho Verde) - MG; SGO: São Gonçalo do Rio Preto - MG; CIP: Conceição do Mato Dentro - MG; OUR: Ouro Branco – MG.

Table S5.2 - Pairwise F_{ST} among 14 populations of *Vriesea oligantha* based on cpDNA sequences. All significant values are in bold.

	JAC	MKA	MCH	MUC	ABA	RCO	LIC	GMO	DIB	DIC	DIM	SAI	CIP	OUR
JAC	**0**													
MKA	-0.05263	**0**												
MCH	**0.95752**	**1**	**0**											
MUC	**0.9375**	**1**	**1**	**0**										
ABA	**0.85853**	**0.89116**	**0.90829**	**0.85948**	**0**									
RCO	**0.7611**	**0.78378**	**0.82222**	**0.70944**	0.25011	**0**								
LIC	**0.81818**	**0.83871**	**0.85799**	**0.8125**	**0.77866**	**0.6476**	**0**							
GMO	**0.86663**	**0.8826**	**0.90267**	**0.87069**	**0.84106**	**0.77451**	**0.49947**	**0**						
DIB	**0.77809**	**0.78872**	**0.8201**	**0.75904**	**0.74264**	**0.66231**	0.12173	**0.40906**	**0**					
DIC	**0.90995**	**0.94602**	**0.95652**	**0.93492**	**0.85984**	**0.75294**	-0.07579	**0.60394**	0.15005	**0**				
DIM	**0.88986**	**0.91391**	**0.92763**	**0.8956**	**0.84656**	**0.75178**	-0.02914	**0.59559**	0.15018	-0.11796	**0**			
SGO	**0.9703**	**1**	**1**	**1**	**0.93001**	**0.86799**	0.14894	**0.73231**	**0.25487**	0.09091	0.02946	**0**		
CIP	**0.83454**	**0.85132**	**0.87422**	**0.82301**	**0.79267**	**0.6958**	**0.74785**	**0.82954**	**0.74843**	**0.8348**	**0.83193**	**0.90337**	**0**	
OUR	**0.89449**	**0.91292**	**0.92573**	**0.89773**	**0.86103**	**0.78583**	**0.81997**	**0.87776**	**0.81378**	**0.89335**	**0.88378**	**0.94245**	**0.30238**	**0**

JAC: Jacobina - BA; MKA: Miguel Calmon - BA; MCH: Morro do Chapéu - BA; MUC: Mucugê - BA; RCO: Rio de Contas - BA; ABA: Abaíra - BA; LIC: Licínio de Almeida - BA; GMO: Grão Mogol - MG; DIB: Diamantina (Biribiri) - MG; DIC: Diamantina (Cons. Mata) - MG; DIM: Diamantina (Milho Verde) - MG; SGO: São Gonçalo do Rio Preto - MG; CIP: Conceição do Mato Dentro - MG; OUR: Ouro Branco – MG.

Table S5.3 - Pairwise F_{ST} among 12 populations of *Vriesea oligantha* based on nrDNA microsatellites. All significant values are in bold.

	JAC	MCH	MUC	ABA	RCO	LIC	GMO	DIB	DIC	SGO	CIP	OUR
JAC	**0**											
MCH	**0.60448**	**0**										
MUC	**0.27558**	**0.55063**	**0**									
ABA	**0.40826**	**0.58123**	**0.44628**	**0**								
RCO	**0.43111**	**0.68538**	**0.45668**	0.03048	**0**							
LIC	**0.35194**	**0.73545**	**0.27868**	**0.5275**	**0.58596**	**0**						
GMO	**0.4258**	**0.71828**	**0.44233**	**0.4974**	**0.54094**	**0.27769**	**0**					
DIB	**0.28499**	**0.60217**	**0.28804**	**0.37852**	**0.40288**	**0.24587**	**0.19927**	**0**				
DIC	**0.2765**	**0.63076**	**0.28076**	**0.39132**	**0.42489**	**0.30363**	**0.297**	0.00813	**0**			
SGO	**0.30511**	**0.62592**	**0.29139**	**0.42439**	**0.45532**	**0.258**	**0.26111**	0.01276	0.00786	**0**		
CIP	**0.40719**	**0.70189**	**0.41326**	**0.47976**	**0.52067**	**0.44173**	**0.29706**	**0.24608**	**0.2926**	**0.29227**	**0**	
OUR	**0.51005**	**0.79466**	**0.48566**	**0.57567**	**0.63612**	**0.58351**	**0.39143**	**0.34761**	**0.42841**	**0.38705**	**0.07238**	**0**

JAC: Jacobina - BA; MCH: Morro do Chapéu - BA; MUC: Mucugê - BA; RCO: Rio de Contas - BA; ABA: Abaíra - BA; LIC: Licínio de Almeida - BA; GMO: Grão Mogol - MG; DIB: Diamantina (Biribiri) - MG; DIC: Diamantina (Cons. Mata) - MG; SGO: São Gonçalo do Rio Preto - MG; CIP: Conceição do Mato Dentro - MG; OUR: Ouro Branco – MG.

Table S5.4 - Contemporary migration rates estimated in BAYESASS among 12 populations of *Vriesea oligantha.* Estimated migration rates > 0.10 are displayed in italics.

From/To	JAC	MCH	MUC	RCO	ABA	LIC	GMO	DIB	DIC	SGO	CIP	OUR
JAC	0.8666	0.0107	0.0112	0.0079	0.0107	0.0196	0.0104	0.0104	0.0112	0.0124	0.0095	0.009
MCH	0.0119	0.8819	0.0106	0.0081	0.0107	0.0196	0.0104	0.0104	0.0112	0.0124	0.0096	0.009
MUC	0.0124	0.0107	0.8777	0.008	0.0107	0.0196	0.0105	0.0104	0.0111	0.0124	0.0096	0.009
RCO	0.0119	0.0107	0.0107	0.9125	*0.2153*	0.0196	0.0104	0.0105	0.0111	0.0125	0.0095	0.009
ABA	0.0122	0.0108	0.011	0.0079	0.6774	0.0196	0.0104	0.0104	0.0111	0.0124	0.0095	0.009
LIC	0.0122	0.0107	0.0108	0.0079	0.0107	0.6863	0.0104	0.0104	0.0112	0.0124	0.0096	0.009
GMO	0.0119	0.0108	0.0123	0.008	0.0107	*0.1176*	0.8854	0.0127	0.0112	0.0136	0.0095	0.0089
DIB	0.0123	0.0108	0.011	0.008	0.0107	0.0196	0.0105	0.8709	*0.2107*	*0.1954*	0.0095	0.009
DIC	0.0122	0.0107	0.0109	0.0079	0.0107	0.0196	0.0105	0.0104	0.6778	0.0124	0.0095	0.009
SGO	0.0122	0.0107	0.011	0.0079	0.0107	0.0196	0.0104	0.0105	0.0111	0.6791	0.0096	0.009
CIP	0.0122	0.0108	0.011	0.008	0.0107	0.0196	0.0104	0.0105	0.0111	0.0124	0.6762	0.0091
OUR	0.0119	0.0107	0.0117	0.0079	0.0108	0.0196	0.0104	0.0227	0.0111	0.0126	*0.2283*	0.901

JAC: Jacobina - BA; MCH: Morro do Chapéu - BA; MUC: Mucugê - BA; RCO: Rio de Contas - BA; ABA: Abaíra - BA; LIC: Licínio de Almeida - BA; GMO: Grão Mogol - MG; DIB: Diamantina (Biribiri) - MG; DIC: Diamantina (Cons. Mata) - MG; SGO: São Gonçalo do Rio Preto - MG; CIP: Conceição do Mato Dentro - MG; OUR: Ouro Branco – MG

Table S5.5 -Posterior distribution values for the Neighbor groups only, estimated on Migrate.

Parameter	2.5%	25%	Mode	75%	97.50%	Median	Mean
Θ_1	0.800	1.400	1.760	2.066	2.466	1.766	1.681
Θ_2	2.000	2.333	2.633	2.933	3.666	2.766	2.511
Θ_3	4.200	4.200	4.300	4.733	5.533	4.566	4.370
Θ_4	0.800	1.400	1.766	2.066	2.533	1.766	1.707
$M_{2->1}$	1.200	1.640	1.823	1.960	2.160	1.763	1.733
$M_{1->2}$	0.020	0.120	0.190	0.247	0.373	0.203	0.202
$M_{3->2}$	0.287	0.427	0.503	0.580	0.713	0.510	0.506
$M_{2->3}$	0	0.113	0.170	0.173	0.173	0.137	0.135
$M_{4->3}$	0.073	0.187	0.257	0.327	0.473	0.277	0.273
$M_{3->4}$	0.380	0.540	0.623	0.760	0.967	0.677	0.676

Θ – Effective population size; $M_{A \rightarrow B}$ – Migration from group A to B. Group numbers are 1. Northern Espinhaço (JAC and MKA); 2. Chapada Diamantina (MCH, MUC, RCO and ABA); 3. Diamantina Plateau (LIC, GMO, DIB, DIC, DIM and SGO); 4. Southern Espinhaço (CIP and OUR). JAC: Jacobina - BA; MCH: Morro do Chapéu - BA; MUC: Mucugê - BA; RCO: Rio de Contas - BA; ABA: Abaíra - BA; LIC: Licínio de Almeida - BA; GMO: Grão Mogol - MG; DIB: Diamantina (Biribiri) - MG; DIC: Diamantina (Cons. Mata) - MG; SGO: São Gonçalo do Rio Preto - MG; CIP: Conceição do Mato Dentro - MG; OUR: Ouro Branco – MG.

Table S5.6 – Probability of the data of the two tested models (marginal likelihood) estimated on MIGRATE.

Model	**RTS**	**BAS**	**HM**	**LBF**	**MPP**
Panmictic	-472245	-78485.9	-1738.12	0	0
Neighboors only	-428574	-71604.3	-1717.89	-6881.56	1.00

RTS - Raw thermodynamic score; BAS - Bezier approximation score; HM – Harmonic mean; LBF – Log Bayes Factor; MPP – Model posterior probability

Table S5.7 – Probability of population reduction, estimated with BOTTLENECK 1.2.02, using all loci fitting the two phases model (TPM).

Population	Wilcoxon test (TPM)
JAC	0.15039
MCH	0.00098
MUC	0.02441
RCO	0.21289
ABA	0.27344
LIC	0.15625
GMO	0.41016
DIB	0.28516
DIC	0.15039
SGO	0.08203
CIP	0.32617
OUR	0.00195

JAC: Jacobina - BA; MCH: Morro do Chapéu - BA; MUC: Mucugê - BA; RCO: Rio de Contas - BA; ABA: Abaíra - BA; LIC: Licínio de Almeida - BA; GMO: Grão Mogol - MG; DIB: Diamantina (Biribiri) - MG; DIC: Diamantina (Cons. Mata) - MG; SGO: São Gonçalo do Rio Preto - MG; CIP: Conceição do Mato Dentro - MG; OUR: Ouro Branco – MG

Supplementary References

ALLOUCHE, O.; TSOAR, A.; KADMON, R. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology*, Oxford, v. 43, n. 6, p. 1223–1232, 2006.

BARRY, S.; ELITH, J. Error and uncertainty in habitat models. *Journal of Applied Ecology*, Oxford, v. 43, n. 3, p. 413–423, 2006.

BREIMAN, L. Random forests. *Machine Learning*, Boston, v. 45, p. 5–32, 2001.

CARPENTER, G.; GILLISON, A. N.; WINTER, J. DOMAIN: a flexible modelling procedure for mapping potential distributions of plants and animals. *Biodiversity and Conservation*, London, v. 2, n. 6, p. 667–680, dez. 1993.

DINIZ-FILHO, J. A. F.; MAURICIO BINI, L.; FERNANDO RANGEL, T.; LOYOLA, R. D.; HOF, C.; NOGUÉS-BRAVO, D.; ARAÚJO, M. B. Partitioning and mapping uncertainties in ensembles of forecasts of species turnover under climate change. *Ecography*, Copenhagen, v. 32, n. 6, p. 897–906, 2009.

FARBER, O.; KADMON, R. Assessment of alternative approaches for bioclimatic modeling with special emphasis on the Mahalanobis distance. *Ecological Modelling*, Amsterdam, v. 160, n. 1–2, p. 115–130, 2003.

HADFIELD, J. D. MCMC Methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software*, v. 33, n. 2, p. 1–22, 2010.

HIJMANS, R. J.; CAMERON, S. E.; PARRA, J. L.; JONES, P. G.; JARVIS, A. Very high resolution interpolated climate surfaces for global land areas*. International Journal of Climatology*, Innsbruck, v. 25, n. 15, p. 1965–1978, 2005.

LEXER, C.; WÜEST, R. O.; MANGILI, S.; HEUERTZ, M.; STÖLTING, K. N.; PEARMAN, P. B.; FOREST, F.; SALAMIN, N.; ZIMMERMANN, N. E.; BOSSOLINI, E. Genomics of the divergence continuum in an African plant biodiversity hotspot, I: drivers of population divergence in *Restio capensis* (Restionaceae). *Molecular Ecology*, Oxford, v. 23, n. 17, p. 4373–4386, 2014.

NIX, H. A. A biogeographic analysis of Australian elapid snakes. In: LONGMORE, R. (Ed.). *Atlas of Elapid Snakes of Australia*. Camberra: Australian Government Publishing Service, 1986. p. 4–15.

OTTO-BLIESNER, B. L.; MARSHALL, S. J.; OVERPECK, J. T.; MILLER, G. H.; HU, A. Simulating arctic climate warmth and icefield retreat in the last interglaciation. *Science*, Washington, v. 311, n. 5768, p. 1751-1753, 2006.

PHILLIPS, S. J.; DUDÍK, M. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, Copenhagen, v. 31, n. 2, p. 161–175, 2008.

REVELLE, W. “psych”: Procedures for psychological, psychometric, and personality research. *Northwestern University, Evanston, Illinois*, v. 165, p. 1-10, 2014.

TAX, D. M. J.; DUIN, R. P. W. Support Vector Data Description. *Machine Learning*, Boston, v. 54, n. 1, p. 45–66, 2004.

Materiais Suplementares do Capítulo III

S1 - Supplementary Tables

Table S1.1 - Genbank accessions, coordinates, gazeteers and vouchers[2]

[2] Para acessar esta tabela, entre no link ESTE LINK

Table S1.2 – Retrieved species from our search using online databases. Species with an asterisk were selected to subsequent analyses.

	Species	Type	Reference	Data type	Sequences
1	*Baccharis concinna*	eudicot	(GOMES et al., 2004)	RAPD	-
2	*Bokermmanohyla saxicola**	frog	(NASCIMENTO et al., 2018)	mtDNA (COI;cytb)	213
3	*Bulbophyllum exaltatum*	monocot	(RIBEIRO et al., 2008)	alozyme	0
4	*Cattleya elongata*	monocot	(DA CRUZ et al., 2011)	allozyme/ISSR	-
5	*Cattleya liliputana*	monocot	(LELES et al., 2015)	SSR	-
6	*Chamaecrista* spp.	eudicot	(SILVA; FERNANDES; LOVATO, 2007)	RAPD	-
7	*Cinclodes espinhacensis**	bird	(FREITAS et al., 2012)	mtDNA(COI;COII;cytb;ND3)	11
8	*Coccoloba cereifera*	eudicot	(MOREIRA et al., 2010)	SSR	-
9	*Encholirium* spp.	monocot	(CAVALLARI et al., 2006)	RAPD	-
	*Euphorbia attastoma**	eudicot	this work	cpDNA;nrDNA (psbA;ycf6)(ITS)	80;80
10	*Eurolophosaurus nanuzae*	lizard	(PASSONI; BENOZZATI; RODRIGUES, 2008)	mtDNA (cytb, COI, 12S, 16S)	9
11	*Lippia rotundifolia*	eudicot	(MEIRA; MARTINS; RESENDE, 2019)	ISSR	-
12	*Lychnophora ericoides**	eudicot	(COLLEVATTI; RABELO; VIEIRA, 2009)	cpDNA;nrDNA (psbA-trnH;trnL)(ITS)	191;25
13	*Minasia* spp.	eudicot	(JESUS et al., 2009)	allozyme	-

	Species	Type	Reference	Data type	Sequences
	*Neoregelia bahiana**	monocot	this work	nDNA (phyC; g3pdH)	46;31
14	*Pilosocereus aurisetus**	eudicot	(BONATELLI et al., 2014)	cpDNA;nDNA (trnS-trnG;trnT-trnL)(phyC)	52;11
14	*Pilosocereus villaboensis**	eudicot	(BONATELLI et al., 2014)	cpDNA;nDNA (trnS-trnG;trnT-trnL)(phyC)	9;3
15	*Pithecopus megacephalus**	frog	(RAMOS et al., 2018)	mtDNA (cytb)	25
16	*Pleurodema alium**	frog	(THOMÉ; CARSTENS, 2016)	mtDNA (coi)	26
17	*Richterago discoidea**	eudicot	(BARRES et al., 2019)	cpDNA;nrDNA (psba-trnh;rpl32-trnL;trnk-rps16;ycf3-trnS)(ITS)	92;51
18	*Rupirana cardosoi*	frog	(FOUQUET et al., 2013)	mtDNA (12s;16s; COI, cytb)	8
19	*Sophronitis sincorana*	monocot	(BORBA et al., 2007)	allozyme	
20	*Strabomantis aramunha*	frog	(AMARO et al., 2013)	mtDNA (12S;16S)	6
21	*Thoropa megatympanum*	frog	(SABBAG et al., 2018)	mtDNA (12S;16S; COI; ND2)	4
22	*Tibouchina papyrus**	eudicot	(COLLEVATTI et al., 2012)	cpDNA (psba-trnh; trnc-ycf6; trns-trng)	93
23	*Uebelmannia* spp.	eudicot	(SILVA et al., 2020)	SSR	-
24	*Vellozia auriculata**	monocot	(FIORINI et al., 2019)	cpDNA (psbd-trnt; rpl32-trnl)	129
25	*Vellozia compacta*	monocot	(LOUSADA; LOVATO; BORBA, 2013)	ISSR	-
26	*Vellozia gigantea*	monocot	(LOUSADA et al., 2011)	ISSR	-

	Species	Type	Reference	Data type	Sequences
27	*Vellozia hirsuta*	monocot	(BARBOSA et al., 2012)	no genbank accessions	-
28	*Vellozia* spp.	monocot	(FRANCESCHINELLI et al., 2006)	allozyme	-
29	*Vriesea oligantha**	monocot	(DANTAS-QUEIROZ et al., 2020)	cpDNA (trnL-trnF; ycf6)	95
30	*Wunderlichia mirabilis*	eudicot	(FERES et al., 2009)	no genbank accessions	-

Table S1.3 - Species used in our demographic analysis. Substitution models were estimated using jModelTest2.

Species	**Type**	**Subst. Model**
Bokermannohyla saxicola	frog	HKY + G
Cinclodes espinhacensis	bird	F81
Euphorbia attastoma	plant	F81
Lychnophora ericoides	plant	F81
Neoregelia bahiana	plant	K80 + I
Pilosocereus aurisetus	plant	F81
Pilosocereus villaboensis	plant	F81
Pithecopus megacephalus	frog	HKY + I
Pleurodema allium	frog	HKY
Richterago discoidea	plant	GTR + I
Tibouchina papyrus	plant	GTR + I +G
Vellozia auriculata	plant	F81 + I
Vriesea oligantha	plant	HKY + I

Table S1.4 – Posterior probability of each of the tested demographic models. Expansion lineages ≥ 0.45 (in bold) were used in the co-expansion analyses.

Species/Lineage	Bottleneck	Constant	Expansion
Bokermannohyla saxicola 1	0.9408333	0.0355	0.0236667
Bokermannohyla saxicola 2	0.757	0.1548333	0.0881667
Bokermannohyla saxicola 3	0.4476667	0.3356667	0.2166667
Euphorbia attastoma 1	0.0938667	0.2607333	**0.6454**
Euphorbia attastoma 2	0.1363	0.3033333	**0.5603667**
Lychnophora ericoides 1	0.0286667	0.1311667	**0.8401667**
Lychnophora ericoides 2	0.115	0.5795	0.3055
Lychnophora ericoides 3	0.1361667	0.5718333	0.292
Pleurodema allium	0	0.9235	0.0765
Richterago discoidea 1	0.1006667	0.2781667	**0.6211667**
Richterago discoidea 2	0.0606667	0.1063333	**0.833**
Tibouchina papyrus 1	0.0602667	0.2166667	**0.7230667**
Tibouchina papyrus 2	0.0578667	0.3248333	**0.6173**
Tibouchina papyrus 3	0.0821333	0.5578667	0.36
Tibouchina papyrus 4	0.1311	0.4446667	0.4242333
Vellozia auriculata	0.1683769	0.3785862	**0.4530368**
Vriesea oligantha 1	0.0471667	0.341	**0.6118333**
Vriesea oligantha 2	0.0098333	0.2855	**0.7046667**
Vriesea oligantha 3	0.0636667	0.265	**0.6713333**

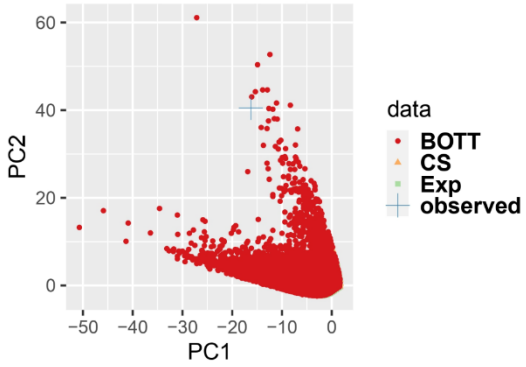
Table S1.5 – Inferred prior parameters of effective population size (Ne) and expansion times in years (Te) for the expansion lineages, used in the co-expansion analysis.

	Ne		Te	
Species/Lineages	**Min**	**Max**	**Min**	**Max**
Euphorbia attastoma 1	1391.92	99679.58	10392.97	199983.66
Euphorbia attastoma 2	1002.30	99985.46	10044.33	199986.23
Lychnophora ericoides 1	2942.16	99993.61	10076.51	199943.25
Richterago discoidea 1	1611.23	99761.14	10095.56	199984.19
Richterago discoidea 2	3252.72	99961.38	10053.35	199618.05
Tibouchina papyrus 1	3371.92	99863.00	10284.63	199969.95
Tibouchina papyrus 2	1003.69	99850.96	10007.26	199950.35
Vellozia auriculata	1029.58	99313.33	10015.35	199965.89
Vriesea oligantha 1	1006.56	99869.98	10013.23	199952.63
Vriesea oligantha 2	1177.08	99920.89	10083.24	199866.00
Vriesea oligantha 3	4915.00	99885.14	10426.18	199980.95

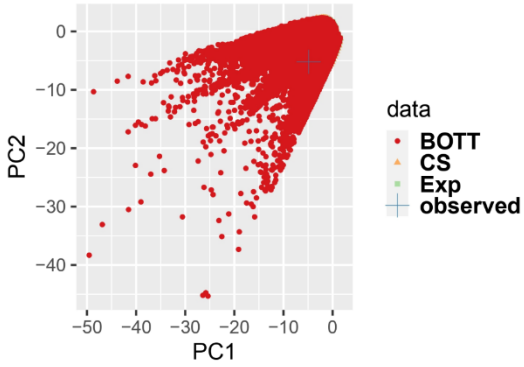
S2 – Supplementary Figures

S2.1 – PCA

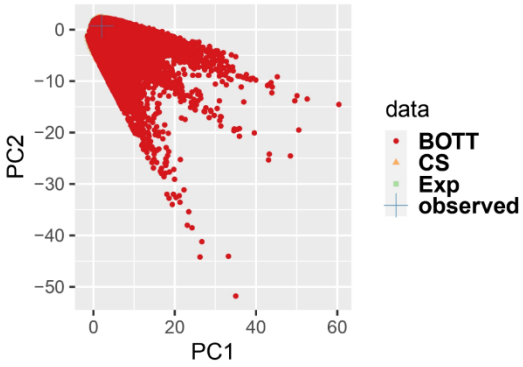
(a) *Bokermannohyla saxicola*



Bokermannohyla_1



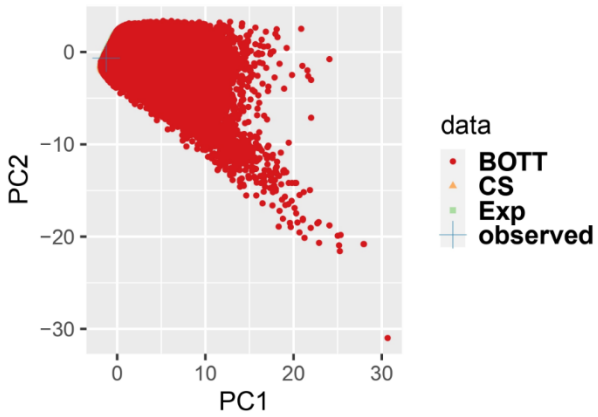
Bokermannohyla_2



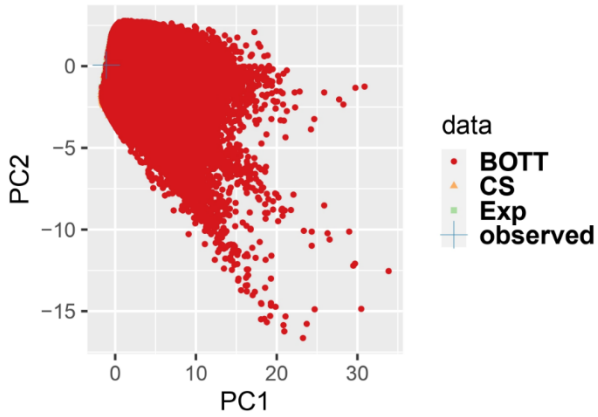
Bokermannohyla_3

Fonte: o autor

(b) *Euphorbia attastoma*



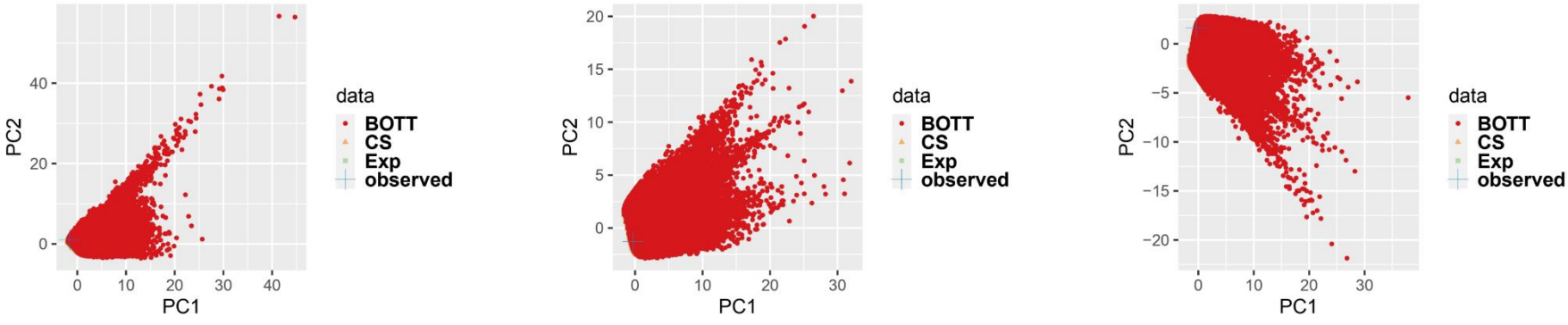
Euphorbia_1



Euphorbia_2

Fonte: o autor.

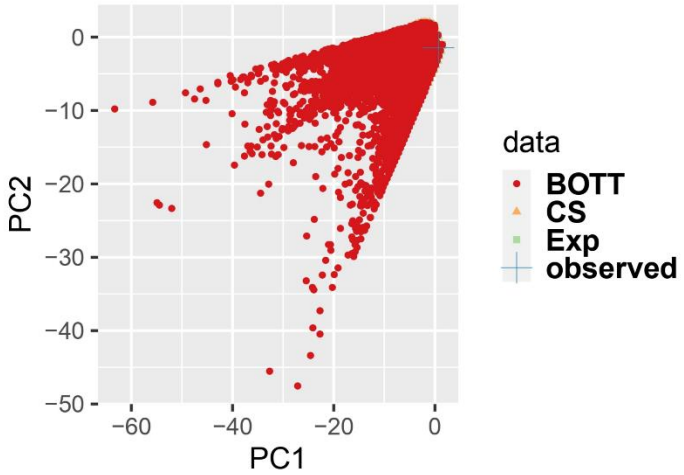
(c) *Lychnophora ericoides*



Lychnophora_1 Lychnophora_2 Lychnophora_3

Fonte: o autor.

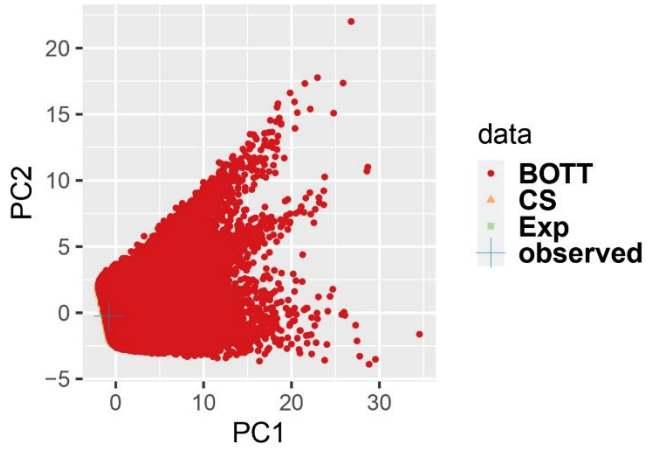
(d) *Pleurodema alium*



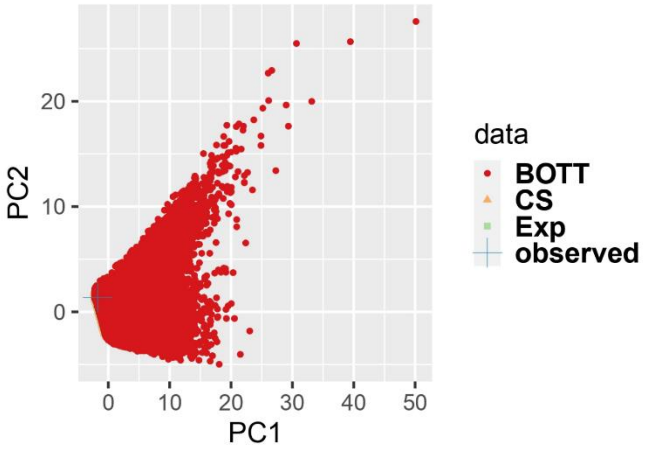
Pleurodema_1

Fonte: o autor.

(e) *Richterago discoidea*



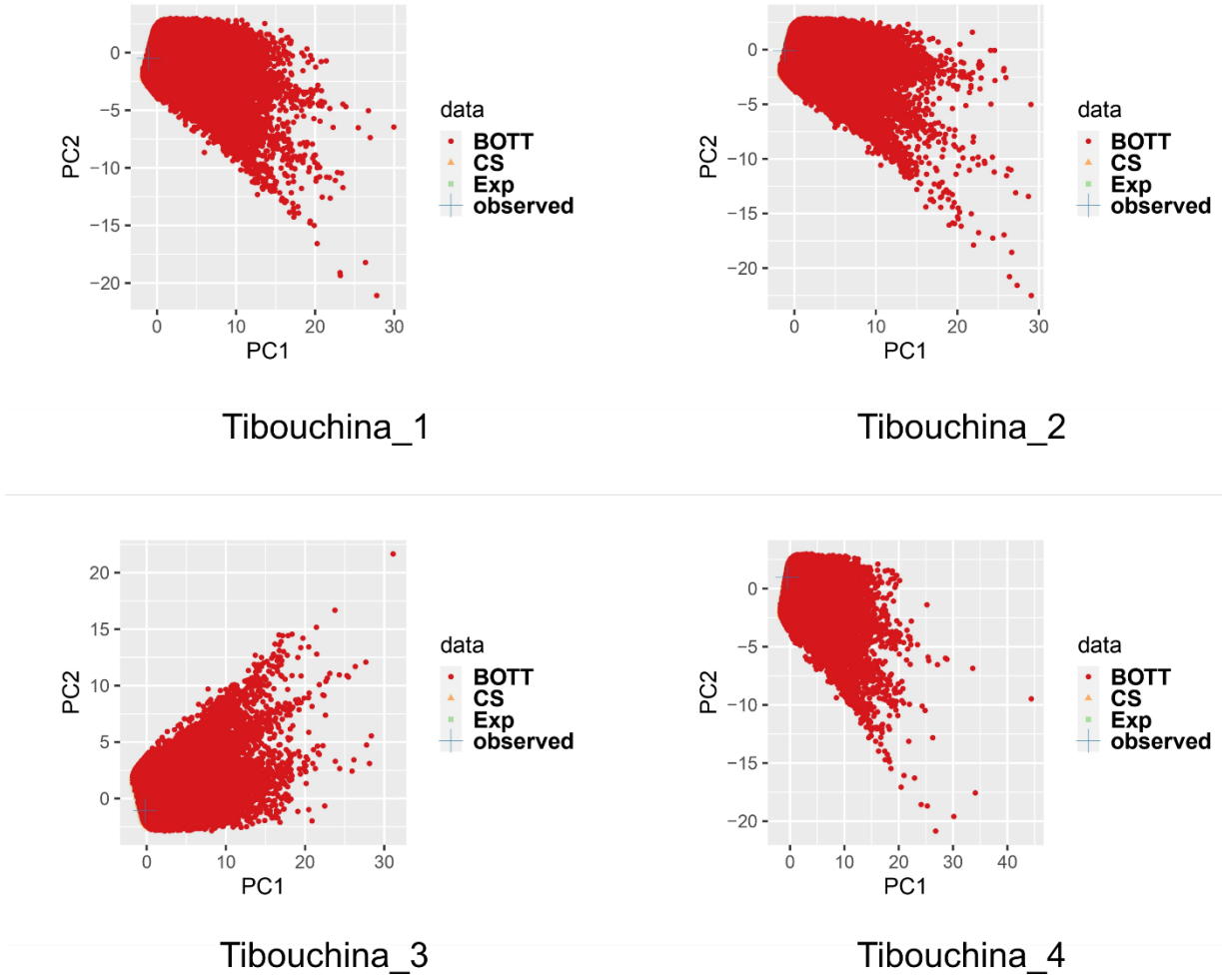
Richterago_1



Richterago_2

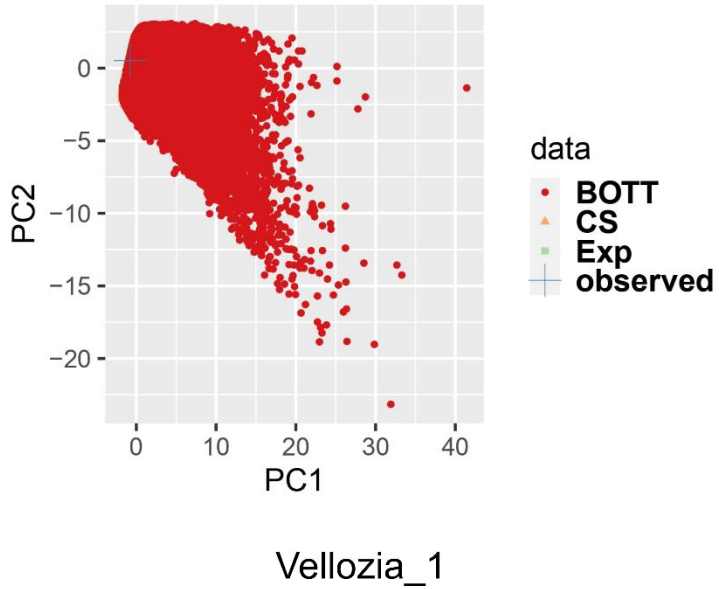
Fonte: o autor.

(f) *Tibouchina papyrus*



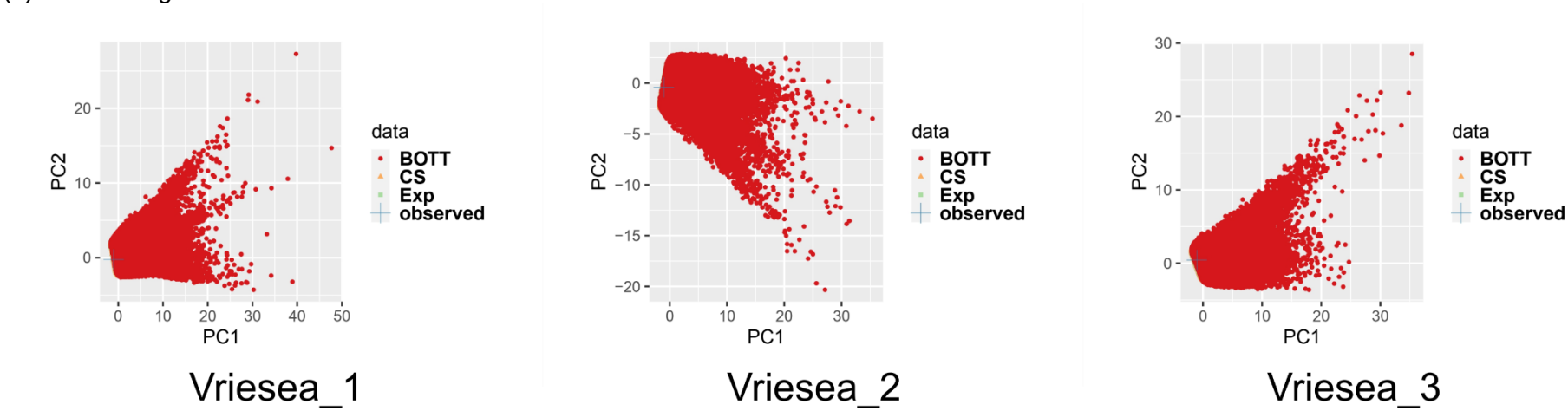
Fonte: o autor.

(g) *Vellozia auriculata*



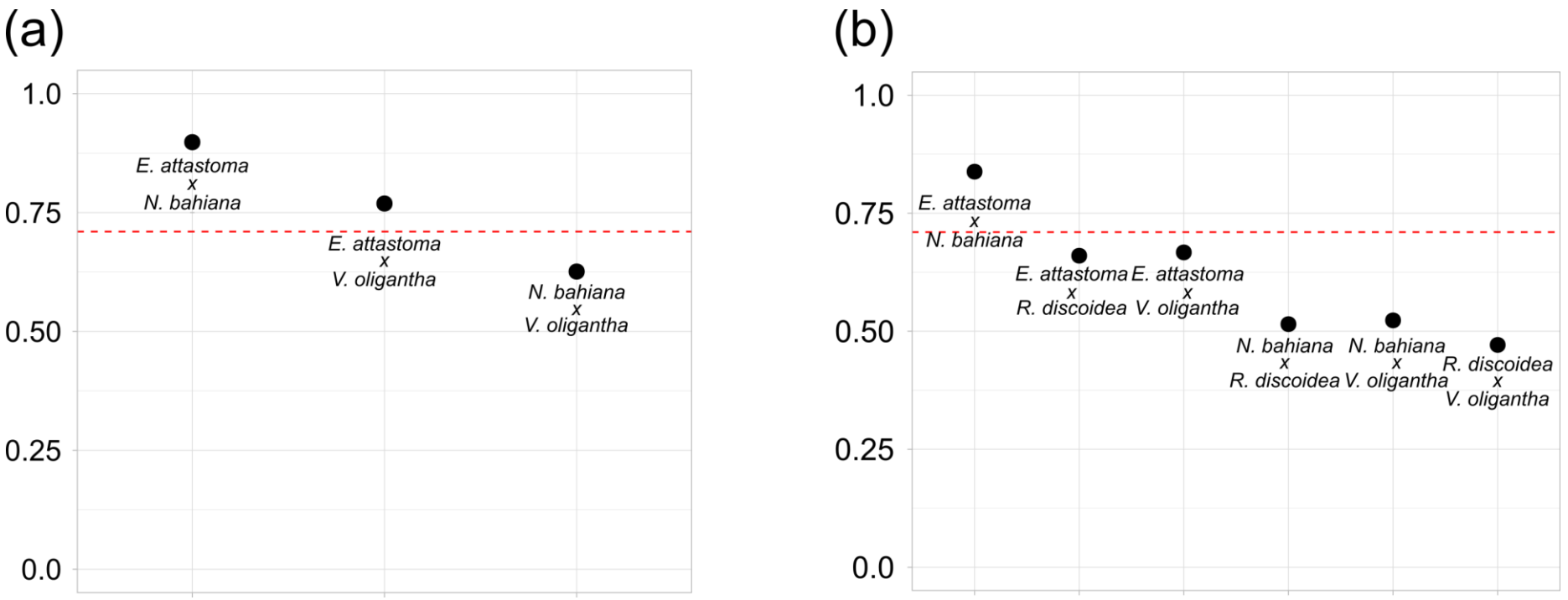
Fonte: o autor.

(h) *Vriesea oligantha*



Fonte: o autor.

S2.2 – PCFs pairwises, comparing (a) only Espinhaço-endemic species and (b) with all species. The red dotted line marks the lower limit (0.71) of the 90% HPD produced from PCF simulations in Satler and Carstens (2016). Points above this line are a signal of shared history of diversification.



Fonte: o autor.

S3 – Supplementary Material and Methods

Sampling and DNA extraction

Euphorbia attastoma

We sampled 80 individuals of *E. attastoma* from five populations, covering its entire distribution. Total genomic DNA was extracted using epidermal tissue obtained from branches, since this species have caducous leaves. Epidermal tissues underwent a combination of five pre-washing steps, with sorbitol extraction buffer, following TEL-ZUR et al. (1999) in order to remove significant amounts of secondary compounds from samples, using the Dneasy Plant Mini Kit (Qiagen, California) following the manufacturer's protocol. Polymorphic sites were investigated using six plastidial (*psbA – trnH, trnC – ycf6, trnQ – rps16x1, atpI – atpH, pbsJ – petA*) and one ribosomal (*ITS1*) markers, although we only found significant polymorphisms with *psbA – trnH* and *trnC – ycf6*.

Amplifications were performed in a final volume of 30 µL, following the final concentrations: 1X buffer solution, 1.5 - 3 mM $MgCl_2$, 0.2 - 0.4 mM of dNTP (Promega), 0.33 - 0.5 µM of each primer,1U Taq polymerase (Biolase and Promega), DMSO 0.02%, 0.1 - 0.2 mM of BSA and ca. 5 ng of DNA. PCRs were performed using a Veriti 96-Well Thermal Cycler (Applied Biosystems) following the cycling conditions described and referenced in Hurbath et al. (2018).

PCR products were examined in agarose gels (1%) by electrophoresis. All PCR products were sent to Macrogen (Seoul, South Korea) for purification and sequencing, using the same primers as for PCR, on an ABI3730XL DNA sequencer. Consensus sequences were assembled using De Novo Assembly in the software Geneious 6.1.8. (KEARSE et al., 2012), and checked base by base with the chromatogram. The alignment was done using MUSCLE (EDGAR, 2004) also in Geneious, with posterior manual adjustment based on similarity criteria (SIMMONS, 2004).

Neoregelia bahiana

We sampled 46 individuals of *N. bahiana* from eight populations, covering its entire distribution range. Total genomic DNA was extracted from silica-gel-dried leaves, following the protocol described by TEL-ZUR ET AL. (1999). We looked for polymorphic sites using six plastidial markers (*petG-trnP, rpl16, rpoB-trnC, trnC-ycf6, trnV intron, trnV – ndhC, ycf*) and two nuclear markers (*G3pdH and phyC*). Only the nuclear markers exhibited higher polymorphism levels and were selected to subsequent amplifications.

Amplifications were performed in a 30 µL reacting using ca. 3 ng of genomic DNA and final concentrations of: 1x Buffer solution, 2mM $MgCl_2$, 0.2 mM of dNTP 96 (Promega, Madison, WI, USA), 1.0 µM of each primer and 1U GoTaq DNA Polymerase (Promega). PCRs were conducted using a Veriti 96-Well Thermal Cycler (Applied Biosystems) following the protocols described in BARFUSS et al. (2005, 2016). We visualized successful amplifications by electrophoresis on 2.0% agarose gel stained with GelRed (Biotium, Hayward, California, USA). Successful amplifications were purified and sequenced both forward and reverse direction on Macrogen (Seoul, South Korea). Consensus sequences and alignment matrix were assembled on Geneious R10 (Biomatters, Auckland, New Zealand), using the default parameters of the aligner toolbox. Sequences of both species will be deposited in GenBank after the publication acceptance.

Supplementary References

AMARO, R. C.; NUNES, I.; CANEDO, C.; NAPOLI, M. F.; JUNCÁ, F. A.; VERDADE, V. K.; HADDAD, C. F. B.; RODRIGUES, M. T. A molecular phylogeny recovers *Strabomantis aramunha* Cassimiro, Verdade and Rodrigues, 2008 and *Haddadus binotatus* (Spix, 1824) (Anura: Terrarana) as sister taxa. *Zootaxa*, Auckland, v. 3741, n. 4, p. 569, 2013.

BARBOSA, A. R.; FIORINI, C. F.; SILVA-PEREIRA, V.; MELLO-SILVA, R.; BORBA, E. L. Geographical genetic structuring and phenotypic variation in the *Vellozia hirsuta* (Velloziaceae) ochlospecies complex. *American Journal of Botany*, Baltimore, v. 99, n. 9, p. 1477–1488, 2012.

BARFUSS, M. H. J.; SAMUEL, R.; TILL, W.; STUESSY, T. F. Phylogenetic relationships in subfamily Tillandsioideae (Bromeliaceae) based on DNA sequence data from seven plastid regions. *American Journal of Botany*, Baltimore, v. 92, n. 2, p. 337–351, 2005.

BARFUSS, M. H. J.; TILL, W.; LEME, E. M. C.; PINZÓN, J. P.; MANZANARES, J. M.; HALBRITTER, H.; SAMUEL, R.; BROWN, G. K. Taxonomic revision of Bromeliaceae subfam. Tillandsioideae based on a multi-locus DNA sequence phylogeny and morphology. *Phytotaxa*, Auckland, v. 279, n. 1, p. 1–97, 2016.

BARRES, L.; BATALHA-FILHO, H.; SCHNADELBACH, A. S.; ROQUE, N. 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*, London, v. 189, n. 2, p. 132–152, 2019.

BONATELLI, I. S.; PEREZ, M. F.; PETERSON, A. T.; TAYLOR, N. P.; ZAPPI, D. C.; MACHADO, M. C.; KOCH, I.; PIRES, A. H. C.; MORAES, E. M. Interglacial microrefugia and diversification of a cactus species complex: Phylogeography and palaeodistributional reconstructions for *Pilosocereus aurisetus* and allies*. Molecular Ecology*, Oxford, v. 23, n. 12, p. 3044–3063, 2014.

BORBA, E. L.; FUNCH, R. R.; RIBEIRO, P. L.; SMIDT, E. C.; SILVA-PEREIRA, V. Demography, and genetic and morphological variability of the endangered *Sophronitis sincorana* (Orchidaceae) in the Chapada Diamantina, Brazil. *Plant Systematics and Evolution*, Berlin, v. 267, n. 1–4, p. 129–146, 2007.

CAVALLARI, M. M.; FORZZA, R. C.; VEASEY, E. A.; ZUCCHI, M. I.; OLIVEIRA, G. C. X. Genetic variation in three endangered species of *Encholirium* (Bromeliaceae) from Cadeia do Espinhaço, Brazil, detected using RAPD markers. *Biodiversity and Conservation*, London, v. 15, n. 14, p. 4357–4373, 2006.

COLLEVATTI, R. G.; DE CASTRO, T. G.; LIMA, J. D. S.; TELLES, M. P. D. C. Phylogeography of *Tibouchina papyrus* (Pohl) toledo (Melastomataceae), an endangered tree species from rocky savannas, suggests bidirectional expansion due to climate cooling in the pleistocene. *Ecology and Evolution*, Oxford, v. 2, n. 5, p. 1024–1035, 2012.

COLLEVATTI, R. G.; RABELO, S. G.; VIEIRA, R. F. Phylogeography and disjunct distribution in *Lychnophora ericoides* (Asteraceae), an endangered cerrado shrub species. *Annals of Botany*, London, v. 104, n. 4, p. 655–664, 2009.

DA CRUZ, D. T.; SELBACH-SCHNADELBACH, A.; LAMBERT, S. M.; RIBEIRO, P. L.; BORBA, E. L. Genetic and morphological variability in *Cattleya elongata* Barb. Rodr. (Orchidaceae), endemic to the campo rupestre vegetation in northeastern Brazil. *Plant Systematics and Evolution*, Berlin, v. 294, n. 1–2, p. 87–98, 2011.

DANTAS-QUEIROZ, M. V.; CACOSSI, T. C.; LEAL, B. S. S.; CHAVES, C. J. N.; VASCONCELOS, T. N. C.; VERSIEUX, L. M.; PALMA-SILVA, C. Underlying microevolutionary processes parallel macroevolutionary patterns in ancient Neotropical Mountains. bioRxiv, 2020. DOI: 10.1101/2020.08.26.268870.

EDGAR, R. C. MUSCLE: a multiple sequence alignment method with reduced time and space complexity*. BMC Bioinformatics*, London, v. 5, p. 113, 2004.

FERES, F.; ZUCCHI, M. I.; SOUZA, A. P. de; AMARAL, M. D. C. E.; BITTRICH, V. Phylogeographic studies of Brazilian “campo-rupestre” species: *Wunderlichia mirabilis* Riedel ex Baker (Asteraceae). *Biotemas*, Florianópolis, v. 22, n. 1, p. 17–26, 2009.

FIORINI, C. F.; MIRANDA, M. D.; SILVA-PEREIRA, V.; BARBOSA, A. R.; OLIVEIRA, U. De; KAMINO, L. H. Y.; MOTA, N. F. D. O.; VIANA, P. L.; BORBA, E. L. The phylogeography of *Vellozia auriculata* (Velloziaceae) supports low zygotic gene flow and local population persistence in the campo rupestre, a Neotropical OCBIL. *Botanical Journal of the Linnean Society*, London, v. 191, n. 3, p. 381–398, 2019.

FOUQUET, A.; BLOTTO, B. L.; MARONNA, M. M.; VERDADE, V. K.; JUNCÁ, F. A.; DE SÁ, R.; RODRIGUES, M. T. Unexpected phylogenetic positions of the genera *Rupirana* and *Crossodactylodes* reveal insights into the biogeography and reproductive evolution of leptodactylid frogs. *Molecular Phylogenetics and Evolution*, Orlando, v. 67, n. 2, p. 445–457, 2013.

FRANCESCHINELLI, E. V.; JACOBI, C. M.; DRUMMOND, M. G.; RESENDE, M. F. S. The genetic diversity of two Brazilian *Vellozia* (Velloziaceae) with different patterns of spatial distribution and pollination biology. *Annals of Botany*, London, v. 97, n. 4, p. 585–592, 2006.

FREITAS, G. H. S. S.; CHAVES, A. V.; COSTA, L. M.; SANTOS, F. R.; RODRIGUES, M. A new species of *Cinclodes* from the Espinhaço Range, southeastern Brazil: Insights into the biogeographical history of the South American highlands. *Ibis*, London, v. 154, n. 4, p. 738–755, 2012.

GOMES, V.; COLLEVATTI, R. G.; SILVEIRA, F. A. O.; FERNANDES, G. W. The distribution of genetic variability in *Baccharis concinna* (Asteraceae), an endemic, dioecious and threatened shrub of rupestrian fields of Brazil. *Conservation Genetics*, Berlin, v. 5, n. 2, p. 157–165, 2004.

HURBATH, F.; LEAL, B. S. S.; SILVA, O. L. M.; PALMA-SILVA, C.; CORDEIRO, I. A new species and molecular phylogeny of Brazilian succulent *Euphorbia* sect. *Brasilienses. Systematics and Biodiversity*, Cambridge, v. 16, n. 7, p. 658 – 667, 2018.

JESUS, F. F.; ABREU, A. G.; SEMIR, J.; SOLFERINI, V. N. Low genetic diversity but local genetic differentiation in endemic *Minasia* (Asteraceae) species from Brazil. *Plant Systematics and Evolution,* Berlin, v. 277, n. 3–4, p. 187–196, 2009.

KEARSE, M.; MOIR, R.; WILSON, A.; STONES-HAVAS, S.; CHEUNG, M.; STURROCK, S.; BUXTON, S.; COOPER, A.; MARKOWITZ, S.; DURAN, C.; THIERER, T.; ASHTON, B.; MEINTJES, P.; DRUMMOND, A. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, Oxford, v. 28, n. 12, p. 1647–1649, 2012.

LELES, B.; CHAVES, A. V.; RUSSO, P.; BATISTA, J. A. N.; LOVATO, M. B. 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*, San Francisco, v. 10, n. 3, p. e0120645, 2015.

LOUSADA, J. M.; BORBA, E. L.; RIBEIRO, K. T.; RIBEIRO, L. C.; LOVATO, M. B. Genetic structure and variability of the endemic and vulnerable *Vellozia gigantea* (Velloziaceae) associated with the landscape in the Espinhaço Range, in southeastern Brazil: Implications for conservation. *Genetica*, Berlin, v. 139, n. 4, p. 431–440, 2011.

LOUSADA, J. M.; LOVATO, M. B.; BORBA, E. L. High genetic divergence and low genetic variability in disjunct populations of the endemic *Vellozia compacta* (Velloziaceae) occurring in two edaphic environments of Brazilian campos rupestres. *Revista Brasileira de Botanica*, São Paulo, v. 36, n. 1, p. 45–53, 2013.

MEIRA, M. R.; MARTINS, E. R.; RESENDE, L. V. Genetic diversity of *Lippia rotundifolia* Cham. in Minas Gerais, Brazil. *Acta Scientiarum. Biological Sciences*, Maringá, v. 41, n. 1, p. 42754, 2019.

MOREIRA, R. G.; MCCAULEY, R. A.; CORTÉS-PALOMEC, A. C.; FERNANDES, G. W.; OYAMA, K. Spatial genetic structure of *Coccoloba cereifera* (Polygonaceae), a critically endangered microendemic species of Brazilian rupestrian fields. *Conservation Genetics*, Berlin, v. 11, n. 4, p. 1247–1255, 2010.

NASCIMENTO, A. C.; CHAVES, A. V.; FORTES LEITE, F. S.; ETEROVICK, P. C.; SANTOS, F. R. Past vicariance promoting deep genetic divergence in an endemic frog species of the espinhaço range in Brazil: The historical biogeography of *Bokermannohyla saxicola* (Hylidae). *PLoS ONE*, San Francisco, v. 13, n. 11, p. 1–19, 2018.

PASSONI, J. C.; BENOZZATI, M. L.; RODRIGUES, M. T. Phylogeny, species limits, and biogeography of the Brazilian lizards of the genus *Eurolophosaurus* (Squamata: Tropiduridae) as inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, Orlando, v. 46, n. 2, p. 403–414, 2008.

RAMOS, E. K. S.; DE MAGALHÃES, R. F.; SARI, E. H. R.; ROSA, A. H. B.; GARCIA, P. C. A.; SANTOS, F. R. Population genetics and distribution data reveal conservation concerns to the sky island endemic *Pithecopus megacephalus* (Anura, Phyllomedusidae). *Conservation Genetics*, Berlin, v. 19, n. 1, p. 99–110, 2018.

RIBEIRO, P. L.; BORBA, E. L.; DE CAMARGO SMIDT, E.; LAMBERT, S. M.; SCHNADELBACH, A. S.; VAN DEN BERG, C. Genetic and morphological variation in the *Bulbophyllum exaltatum* (Orchidaceae) complex occurring in the Brazilian "campos rupestres": Implications for taxonomy and biogeography. *Plant Systematics and Evolution*, Berlin, v. 270, n. 1–2, p. 109–137, 2008.

SABBAG, A. F.; LYRA, M. L.; ZAMUDIO, K. R.; HADDAD, C. F. B.; FEIO, R. N.; LEITE, F. S. F.; GASPARINI, J. L.; BRASILEIRO, C. A. Molecular phylogeny of Neotropical rock frogs reveals a long history of vicariant diversification in the Atlantic forest. *Molecular Phylogenetics and Evolution*, Orlando, v. 122, p. 142–156, 2018.

SILVA, R. M. da; FERNANDES, G. W.; LOVATO, M. B. Genetic variation in two *Chamaecrista species* (Leguminosae), one endangered and narrowly distributed and another widespread in the Serra do Espinhaço, Brazil. *Canadian Journal of Botany*, Ontario, v. 85, n. 7, p. 629–636, 2007.

SILVA, G. A. R.; KHAN, G.; RIBEIRO-SILVA, S.; AONA, L. Y. S.; MACHADO, M. C.; BONATELLI, I. A. S.; MORAES, E. M. Extreme genetic structure in a relict cactus genus from campo rupestre landscapes: implications for conservation. *Biodiversity and Conservation*, London, v. 29, n. , p. 1263-1281, 2020.

SIMMONS, M. P. Independence of alignment and tree search. *Molecular Phylogenetics and Evolution,* Orlando, v. 31, n. 3, p. 874–879, 2004.

TEL-ZUR, N.; ABBO, S.; MYSLABODSKI, D.; MIZRAHI, Y. Modified CTAB procedure for DNA isolation from epiphytic cacti of genera *Hylocereus* and *Selenicereus* (Cactaceae). *Plant Molecular Biology*, Berlin, v. 17, p. 249–254, 1999.

THOMÉ, M. T. C.; CARSTENS, B. C. Phylogeographic model selection leads to insight into the evolutionary history of four-eyed frogs. *Proceedings of the National Academy of Sciences*, Washington, v. 113, n. 29, p. 8010–8017, 2016.

Artigo: Plant species complexes as models to understand speciation and evolution: a review of South American studies

Fábio Pinheiro, Marcos Vinicius Dantas-Queiroz, Clarisse Palma-Silva

Critical Reviews in Plant Sciences, v. 37, n. 1, p. 54 – 80, 2018.
DOI: 10.1080/07352689.2018.1471565

CRITICAL REVIEWS IN PLANT SCIENCES
https://doi.org/10.1080/07352689.2018.1471565

Taylor & Francis
Taylor & Francis Group

Plant Species Complexes as Models to Understand Speciation and Evolution: A Review of South American Studies

Fábio Pinheiro [a], Marcos Vinicius Dantas-Queiroz[b], and Clarisse Palma-Silva[a]

[a]Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil; [b]Departamento de Ecologia, Programa de Pós-graduação em Biologia Vegetal, Universidade Estadual Paulista, UNESP, Rio Claro, Brazil

ABSTRACT

Identifying discontinuous entities within species complexes is a major topic in systematic and evolutionary biology. Comprehensive inventories describing and identifying species rapidly and correctly before they or their habitats disappear is especially important in megadiverse regions, such as South America continent, where a large part of the biodiversity is still unknown and remains to be discovered. Species complexes may account for a substantial number of plant groups in the South American flora, and studies investigating species boundaries in such challenging groups are needed. In this context, multidisciplinary approaches are crucial to understanding the species integrity and boundaries within species complexes. Morphometrics, cytogenetics, anatomy, crossing experiments, and molecular markers have been combined in different ways to investigate species complexes and have helped depict the mechanisms underlying the origin of South American species. Here, we review the current knowledge about plant species complexes on the hyperdiverse South American continent based on a detailed examination of the relevant literature. We discuss the main findings in light of the potential evolutionary mechanisms involved in speciation and suggest future directions in terms of integrating multispecies coalescence methods with several complementary types of morphological, ecological, and geographical data in this research field.

KEYWORDS

Biosystematics; cryptic; species; experimental taxonomy; integrative taxonomy; sibling species; species limits

I. Introduction

Species identification, delimitation, and description constitute a long and controversial debate in the fields of systematic and evolutionary biology. Species are the most relevant unit of biodiversity; thus, cautiously deciding how species entities are defined will influence crucial conclusions related to ecological and evolutionary analyses (de Queiroz, 2007; Wiens, 2007; Freudenstein et al., 2017). Furthermore, today's global biodiversity crisis has caused an urgent need for comprehensive inventories describing and identifying species rapidly and correctly before they or their habitats disappear. This effort is especially important in megadiverse regions, such as the South America, where a large part of the biodiversity remains to be discovered, and where the patterns and processes responsible for generating and maintaining the rich biota are poorly understood.

Species delimitation is not always difficult. In cases where speciation occurred a long time ago, newly discovered species will fit into most species concepts. The major difficulty in delimiting species occurs at the beginning of species formation. It is particularly complicated in the following situations: (1) when diversification (or lineage formation) arises with little morphological change, known as cryptic or sibling species (Bickford et al., 2007); (2) when species exhibit extensive morphological diversity (Figure 1) and little genetic divergence, commonly occurring in evolutionary radiations (Shaffer and Thomson, 2007; Barley et al., 2013); or (3) when a subset of populations becomes morphologically and genetically distinct, forming a new lineage from ancestral population types, known as progenitor-derivative speciation (Crawford, 2010; Freudenstein et al., 2017). In these "species complexes," studying species delimitation is most important because we can better understand the first steps of species formation. Furthermore, in species complexes, incomplete knowledge of the biodiversity status of the whole group will lead to erroneous taxon sampling and flawed assessments of biodiversity, biogeography, and speciation processes (Heath et al., 2008).

CONTACT Fábio Pinheiro biopinheiro@yahoo.com.br Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas 13083-862, Brazil.

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bpts

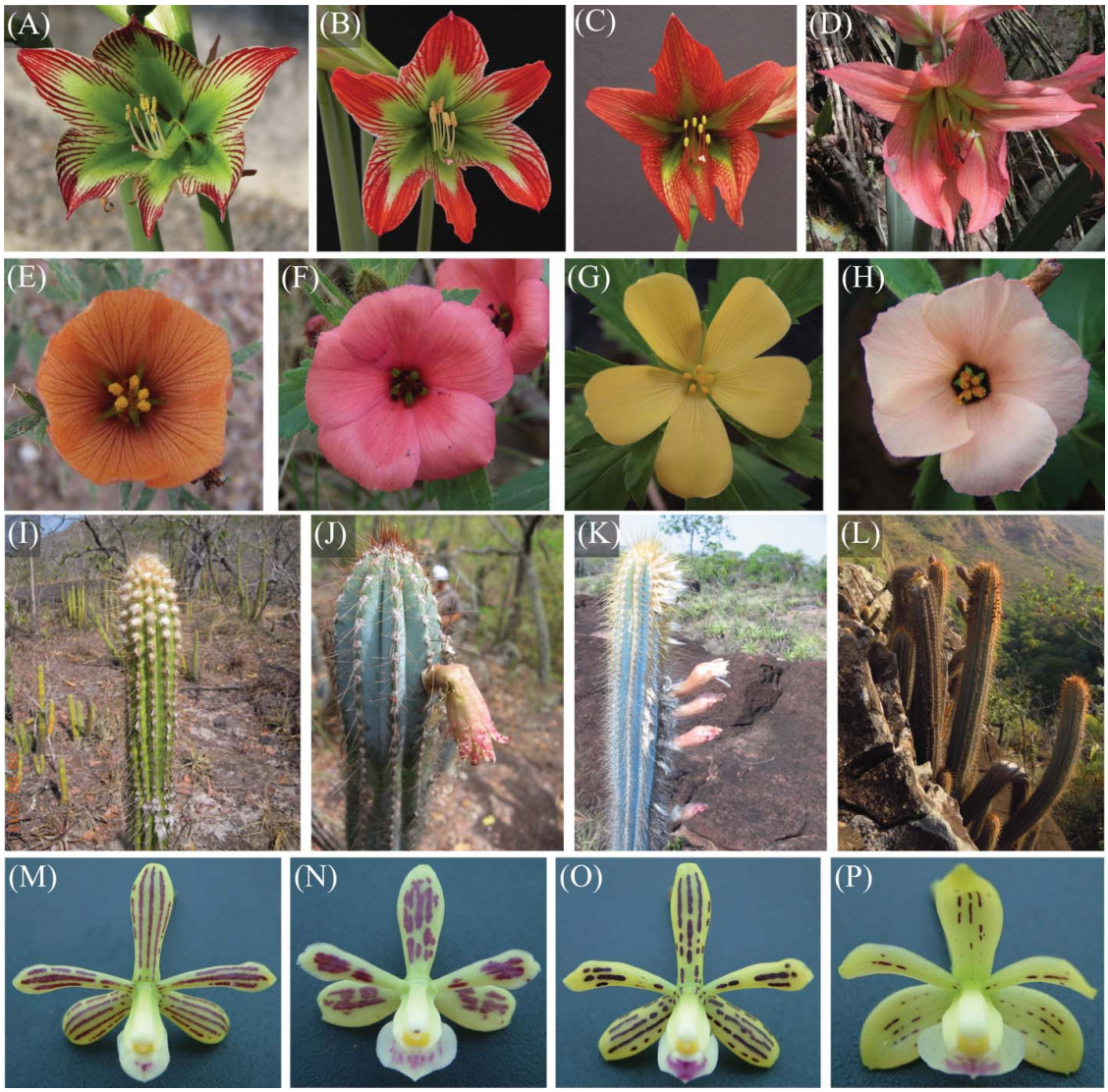


Figure 1. Examples of extensive morphological variability found in plant species complexes occurring within South America. (A–D) *Hippeastrum glaucescens* Herb. complex (Amaryllidaceae), (E–H) *Turnera sidoides* Vell. complex (Passifloraceae), (I–L) *Pilosocereus jauruensis* (Buining & Brederoo) P. J. Braun complex (Cactaceae), (M–P) *Prosthechea vespa* (Vell.) W. E. Higgins complex (Orchidaceae). Photo credits: (A–D) Mauro Peixoto, (E–H) Viviana G. SolísNeffa, (I–L) Evandro Marsola de Moraes, and (M–P) Fábio Pinheiro.

The biological species concept, created at the middle of the 20th century (Mayr, 1942), was one of the most influential ideas regarding species delimitation. The definition of species coined by Ernst Mayr (i.e., groups of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups) was influenced by the work of Dobzhansky (1937), who explored the association between barriers to reproductive isolation and species formation. According to this concept, groups of closely related species do not interbreed when growing sympatrically. If hybridization occurs, low hybrid sterility and/or viability would maintain the integrity of the parental species. The biological species concept strongly influenced scientists for generations. Many evolutionary biologists advocated that total reproductive isolation is crucial to maintaining species integrity and thus to recognize different taxonomic entities.

Since the biological species concept was proposed in 1942, many other authors have attempted to formulate alternative definitions to answer the same question: What are species? These alternative concepts have emphasized different sources of data (i.e., morphology assessments, DNA analyses, ecological measures, etc.) and different methods (i.e., morphometry, population genetics, phylogenetics, etc.) to delineate different species concepts. Here, we will not describe these alternative concepts, as this subject has been reviewed intensely in the literature (Luckow and Hortorium, 1995; Mayden, 1997; de Queiroz, 1998, 2007; Wilkins, 2009; Freudenstein et al., 2017). Because different species concepts

often refer to different stages of speciation, the concepts *per se* are not mutually exclusive (de Queiroz, 1998). In fact, most of the concepts are complementary, as each has been applied to particular stages of the continuous process of species formation. This idea led the same author to formulate the General Lineage Concept of Species (de Queiroz, 2007), in which species are treated as distinct evolving metapopulation lineages. According to the General Lineage Concept of Species, the former species concepts are treated as operational criteria used to explore the magnitude of evolutionary lineage separation. In this context, there are several advantages to using multiple lines of evidence to explore species delimitation, and some recent reviews on this subject encourage this approach (de Queiroz, 2007; Padial and de la Riva, 2010; Hausdorf, 2011; Carstens et al., 2013; Freudenstein et al., 2017). In addition, few species criteria will support and/or show full congruence during the early stages of speciation (de Queiroz, 1998), as is often observed in species complexes.

Research in the field of species delimitation and its conceptual changes have been flourishing in the last decades, producing several methods and techniques to achieve this goal (Sites and Marshall, 2003; Knowles and Carstens, 2007; Wiens, 2007; Flot, 2015). Discussion on species delimitation still persists, but today, with the development of informative molecular tools and computational resources capable of processing large amounts of data, the methods have become more meticulous and capable of reconciling the micro- and macroevolutionary dimensions. However, distinguishing among emergent species from populations is especially difficult in species complexes. The line between these two categories is tenuous: divergent species generally do not reach total monophyly, which may be considered gene flow between populations (Naciri and Linder, 2015; Willis, 2017). To overcome such difficulties, multispecies coalescent-based methods have been used, because these approaches do not require reciprocal monophyly of alleles to delimit species, mainly when taxa originate from recent speciation events (Kingman, 1982; Yang and Rannala, 2010). Delimitation methods incorporating multispecies coalescence theory (e.g., BEAST, Heled and Drummond, 2010; BPP, Yang and Rannala, 2010; Yang, 2015; spedeSTEM, Ence and Carstens, 2011; DISSECT, Jones et al., 2015; among others) are already recognized for their empiricism in species complexes (e.g., Carstens and Satler, 2013; Tomasello et al., 2015; Folk and Freudenstein, 2015). Each approach attempts to identify and delimit species via different statistical strategies generally utilizing multiple unlinked loci to reach this goal (for reviews, see Fujita et al., 2012; Camargo et al., 2012).

Using the approach of multiple unlinked loci was important for the development of species tree-based methods and for documenting the important evolutionary role of natural hybridization and speciation with gene flow in species complexes (Nosil, 2008; Jackson et al., 2017). Levin (1978) suggested that biological species are distinct entities generally separated by multiple barriers, which may reduce gene exchange between them. Coyne and Orr (2004) introduced the notion that different species can maintain lower levels of gene exchange without losing their integrity, which was further developed by the idea of "porous genomes" (Wu, 2001). The idea that species can maintain their integrity even in the presence of interspecific gene exchange is crucial in the study of species complexes. In general, incomplete reproductive barriers and gene flow blur species boundaries between sympatric populations, as hybrids may exhibit intermediate morphological characters (Soltis and Soltis, 2009). However, in several cases, parental species maintain their integrity as separate entities, even in cases in which introgression (gene flow) is found. Thus, partial reproductive barriers could be considered the rule, rather than the exception, for most species complexes. Nonetheless, only recently, the coalescent methods for assessing lineage independence have started to explicitly account for gene flow when inferring species boundaries (Chan et al., 2017; Jackson et al., 2017; Morales and Carstens, 2018).

II. Plant diversity in South America

Compared with other megadiverse regions, South America is the most biodiverse region on Earth in terms of the number of angiosperm species (Govaerts, 2001). Moreover, South America harbors different plant diversity centers, which surpass 5000 species/10,000 km^2 (Barthlott et al., 2005). Considering the total number of endemic species observed in 16 megadiverse countries (145,610), 28% occur exclusively in South America (Forzza et al., 2012). This high diversity has attracted the attention of the scientific community, which has launched different hypotheses to explain the origin of this diversity (reviewed by Antonelli and Sanmartín, 2011). Much effort has been expended to investigate broad patterns of diversification, using plant phylogenies at higher taxonomic levels to depict the mechanisms involved in plant diversification (Hughes et al., 2013). On the other hand, population-level aspects have received less attention, and little is known regarding microevolutionary mechanisms occurring during the first stages of speciation. Common methods used to investigate species complexes, such as transplant

experiments (Hagen, 1984) and measures of reproductive isolation in hybrid zones (Baack et al., 2015), have rarely been employed in studies of South American plants. Population-level studies are crucial steps toward understanding speciation mechanisms, as lineages diverge, and incipient reproductive isolation barriers arise, which play an important role in maintaining species diversity (Scopece et al., 2010).

In this context, the study of species complexes plays a crucial role in understanding speciation because our knowledge of this process is incomplete (Via, 2009). Thus, species complexes offer a great opportunity for connecting the micro- and macroevolution scales using coalescent models within a phylogenetic framework (Pons et al., 2006; Knowles and Carstens, 2007; O'Meara, 2010; Yang and Rannala, 2010; Ence and Carstens, 2011). Furthermore, the study of species complexes under an integrative analytical approach will be useful for testing the hypothesis of species delimitation and for better understanding the processes that have promoted neotropical diversification (Padial et al., 2010; Fujita et al., 2012; Guillot et al., 2012). Most studies that have used species complexes as models for understanding speciation originated from temperate countries (Briggs and Walters, 1997), and little is known about this issue in tropical regions, particularly South America.

Species complexes may account for a substantial number of plant groups in the South American flora (Figure 1), and studies investigating species boundaries in such challenging groups are needed. Different South American research teams have been working on species complexes, focusing on different plant groups and using diverse sources of data and analytical tools. The main objective of this study was to investigate the way species complexes have been studied on the megadiverse South American continent. Here, we review the current knowledge about plant species complexes in South America based on a detailed examination of the literature, discussing the main findings in light of the potential evolutionary mechanisms involved in speciation. Specifically, we aimed to (1) review the current knowledge of South American species complexes, (2) examine study distribution among plant groups, (3) investigate the main approaches, sources of data, and analytical tools used to study such groups, and (4) discuss future directions to stimulate this approach as a primary choice for those interested in understanding speciation mechanisms in highly diverse plant groups to guide future studies in the field.

The database used for this review was compiled by conducting searches in the Web of Science® (Institute of Scientific Information, Thomson Scientific). We searched articles published from 1900 (initial coverage of the Web of Science database) to June 2016, combining the key phrases: 'South America' and "species complex" and/or "cryptic species" and/or "sibling species" and/or "species flock." These phrases needed to be cited in the title, abstract, keywords, or the main body of the article. We filtered only studies performed only with angiosperms and excluded studies of invasive species in their nonnative ranges. In addition, we performed a nonexhaustive Google Scholar database search with the same terms (Table 1). We recorded the following information for all articles retrieved: genus and family names; habit; number of populations sampled; whether a multidisciplinary approach was adopted (i.e., molecular, morphometry, cytogenetics, anatomy, etc.); the inclusion of taxonomic decisions; main research fields involved in the study; and main analytical tools adopted when using molecular markers. Traditional taxonomic methods based mainly on analyses of qualitative diagnostic characters, morphometry using quantitative characters, anatomy, cytogenetics, and transplant and crossing experiments, as well as other ecological analyses, such as pollination, phenology, niche models, and molecular markers, were the main research fields considered in this study. Studies using molecular markers were further classified according to three main analytical strategies of phylogenetic, population genetic, and phylogeographic methods. We used the chi-square test with Yates correction to assess the strength of the associations between adopting a multidisciplinary approach and including taxonomic decisions regarding the species complexes. The same test was also used to explore associations between individual data types (e.g., molecular data alone) and taxonomic decisions. Descriptions of new species and the splitting and/or lumping of current species groups were all interpreted as taxonomic decisions to clarify the taxon delimitation within the species complexes. Associations among different research fields were summarized into a network calculated using Gephi software (Bastian et al., 2009).

The Web of Science® and Google Scholar survey of the literature from 1900 to June 2016 identified 129 articles matching our criteria (Table 1). The first study that used one of the reference phrases was published in 1968. Studies were performed on 44 different plant families and 84 genera. Overall, the studies included a range of plant species, from herbs (89 studies), trees (23 studies), lianas (12 studies), and shrubs (8 studies); yet, they still are not a taxonomically representative sample of plant species from South America. Approximately 70% of the studies were concentrated in 13 families. The seven most studied families were Fabaceae (13), Orchidaceae (13), Poaceae (13), Solanaceae (12), Asteraceae (9), Turneraceae (6), and Bromeliaceae (5) (Figure 2).

Table 1. Summary of studies on plant species complexes occurring within South America.

Reference	Family	Genus	Habit	Number of populations	Multiple methods	Taxonomic decisions	Traditional taxonomic methods	Morphometry/ Anatomy	Cytogenetics	Reproductive/ crossing experiments	Ecological data	Phylogenetic approach	Population genetic approach	Phylogeographic approach	Molecular markers	Nuclear	Plastid
Allred and Gould, 1983	Poaceae	*Bothriochloa*	herb	—	yes	yes		yes	yes	yes							
Alvarez et al., 2008	Solanaceae	*Solanum*	herb	59	yes	no		yes					yes		RAPD, RFLP, AFLP	yes	
Alves et al., 2014	Iridaceae	*Sisyrinchium*	herb	—	no	no						yes			sequencing / barcoding	yes	yes
Ames and Spooner, 2010	Solanaceae	*Solanum*	herb	—	no	no						yes			sequencing	yes	
Ames et al., 2008	Solanaceae	*Solanum*	herb	37	no	no		yes									
Amico and Nickrent, 2009	Loranthaceae	*Tristerix*	herb	30	no	no								yes	sequencing		yes
André *et al.*, 2015	Costaceae	*Chamaecostus*	herb	12	yes	yes		yes				yes				yes	yes
Ballard and Iltis, 2012	Violaceae	*Viola*	herb	—	no	yes	yes										
Barbosa et al., 2012	Velloziaceae	*Vellozia*	herb	25	yes	yes	yes							yes			yes
Berg et al., 1998	Solanaceae	*Solanum*	herb	—	no	no		yes									
Bonatelli et al., 2014	Cactaceae	*Pilosocereus*	herb	33	yes	no					niche models			yes		yes	yes
Borba et al., 2002	Orchidaceae	*Acianthera*	herb	21	yes	yes		yes		yes	flower visitors	yes	yes		allozime loci	yes	
Borges et al., 2012	Fabaceae	*Libidibia*	tree	2	yes	yes		yes	yes	yes							
Borsch et al., 2011	Amaranthaceae	*Pedersenia*	liana	—	yes	yes	yes					yes			sequencing		yes
Bünger et al., 2015	Myrtaceae	*Eugenia*	tree	—	yes	yes	yes	yes									
Caddah et al., 2012	Calophyllaceae	*Kielmeyera*	tree	4	yes	yes	yes	yes					yes			yes	
Caddah et al., 2013	Calophyllaceae	*Kielmeyera*	tree	3	yes	yes					phenology		yes			yes	
Caetano et al., 2008	Anacardiaceae	*Astronium*	tree	7	no	yes							yes		SSR and sequencing	yes	yes
Canela et al., 2003	Bromeliaceae	*Aechmea*	herb	—	no	yes	yes										
Cardim et al., 2001	Orchidaceae	*Oncidium*	herb	5	no	no		yes									
Cardozo et al., 2015	Araceae	*Anthurium*	herb	12	no	yes		yes									
Carlini-Garcia et al., 2002	Orchidaceae	*Miltonia*	herb	8	yes	yes		yes			phenology						

(*Continued on next page*)

Table 1. (*Continued*)

Reference	Family	Genus	Habit	Number of populations	Multiple methods	Taxonomic decisions	Traditional taxonomic methods	Morphometry/ Anatomy	Cytogenetics	Reproductive/ crossing experiments	Ecological data	Phylogenetic approach	Population genetic approach	Phylogeographic approach	Molecular markers	Nuclear	Plastid
Catling and Porebski, 1998	Rosaceae	*Fragaria*	herb	—	no	yes		yes									
Cavallari et al., 2010	Salicaceae	*Casearia*	tree	9	no	yes							yes		SSR	yes	
Ceolin and Miotto, 2012	Fabaceae	*Galactia*	herb	82	yes	yes	yes	yes			environment characterization						
Conceição et al., 2008	Fabaceae	*Chamaecrista*	shrub	33	yes	yes		yes					yes		15 allozime loci	yes	
Cooley et al., 2008	Phrymaceae	*Mimulus*	herb	6	yes	no		yes		yes							
Costa et al., 2007	Fabaceae	*Chamaecrista*	herb	2	yes	yes				yes	reproductive behaviour, flower phenology, flower visitors		yes		4 allozime loci	yes	
Costa et al., 2009	Bromeliaceae	*Vriesea*	herb	6	yes	yes	yes	yes									
Costea and Stefanovic, 2010	Convolvulaceae	*Cuscuta*	liana	—	yes	yes	yes	yes				yes			sequencing	yes	yes
Costea et al., 2011	Convolvulaceae	*Cuscuta*	liana	—	yes	yes	yes	yes				yes			sequencing	yes	yes
Costea et al., 2015	Convolvulaceae	*Cuscuta*	liana	—	yes	yes		yes				yes			sequencing	yes	yes
Dematteis, 2004	Asteraceae	*Vernonia*	herb	—	no	yes	yes										
Díaz et al., 1996	Convolvulaceae	*Ipomoea*	liana	—	yes	no		yes		yes							
Díaz, 2013	Calophyllaceae	*Calophyllum*	tree	—	yes	yes	yes	yes									
Elías et al., 2011	Turneraceae	*Turnera*	herb	26	yes	no			yes		climatic characteristics						
Esteves and Vicentini, 2013	Rubiaceae	*Pagamea*	tree	3	yes	yes		yes			flower phenology, environmental characterization						
Farinaccio and Mello-Silva et al., 2006	Apocynaceae	*Oxypetalum*	shrub	—	no	yes	yes										
Ferreyra et al., 2013	Fabaceae	*Prosopis*	tree	—	yes	no		yes					yes		RAPD	yes	
Filardi et al., 2013	Fabaceae	*Machaerium*	tree,	shrub, and lianas	—	no	yes	yes									
Firetti-Leggieri et al., 2011	Bignoniaceae	*Anemopaegma*	shrub		no	no			yes								

Freitas-Mansano and Tozzi, 2001	Fabaceae	*Swartzia*	tree	—	no	yes	yes											
Gagnon et al., 2015	Fabaceae	*Caesalpinia*	tree	—	yes	yes		yes				yes				sequencing	yes	yes
Gentry, 1981	Passifloraceae	*Passiflora*	liana	—	no	yes	yes											
Giussani et al., 1996	Poaceae	*Poa*	herb	10	yes	no		yes	yes		climatic characteristics							
Gomes-da-Silva *et al.*, 2011	Bromeliaceae	*Vriesea*	herb		no	yes	yes											
Gomes-da-Silva *et al.*, 2012	Bromeliaceae	*Vriesea*	herb	—	no	no		yes										
Gonella et al., 2012	Droseraceae	*Drosera*	herb	1	no	yes	yes											
Gonella et al., 2014	Droseraceae	*Drosera*	herb		no	yes	yes											
Graham and Cavalcanti, 2013	Lythraceae	*Cuphea*	herb	—	yes	yes	yes		yes									
Grombone-Guaratini et al., 2005	Asteraceae	*Bidens*	herb	12	no	no							yes			16 allozime loci	yes	
Grombone-Guaratini et al., 2006	Asteraceae	*Bidens*	herb	—	no	no			yes									
Henderson and Martins, 2002	Arecaceae	*Geonoma*	tree	—	no	no		yes										
Holanda et al., 2015	Humiriaceae	*Humiria*	tree	—	yes	no		yes		yes								
Ispizúa et al., 2015	Solanaceae	*Solanum*	herb	4	yes	no		yes	yes	yes								
Lammers and Hensold, 1992	Campanulaceae	*Lobelia*	herb	—	no	no			yes									
Lima et al., 2015	Myrtaceae	*Myrcia*	tree	17	yes	yes		yes					yes			6 primers and 59 ISSR Loci	yes	
Lombardi, 2006	Celastraceae	*Tontelea*	lianas,	shrubs	—	no	yes	yes										
Longo et al., 2014	Solanaceae	*Petunia*	herb	47	no	no								yes		two cpDNA: trnS-trnG and trnH-psbA		yes
López et al., 2002	Asteraceae	*Senecio*	herb	—	no	no			yes									
López et al., 2012	Apiaceae	*Pozoa*	herb	22	no	yes						yes	yes			sequencing / AFLP	yes	yes
López-Sepúlveda et al., 2013a	Asteraceae	*Hypochaeris*	herb	54	yes	no		yes					yes			AFLP	yes	

(*Continued on next page*)

Table 1. (*Continued*)

Reference	Family	Genus	Habit	Number of populations	Multiple methods	Taxonomic decisions	Traditional taxonomic methods	Morphometry/ Anatomy	Cytogenetics	Reproductive/ crossing experiments	Ecological data	Phylogenetic approach	Population genetic approach	Phylogeographic approach	Molecular markers	Nuclear	Plastid
López-Sepúlveda et al., 2013b	Myrtaceae	*Myrceugenia*	tree	33	no	no									AFLP / SSR	yes	
López-Sepúlveda et al., 2015a	Asteraceae	*Erigeron*	herb	50	no	no							yes		AFLP / SSR	yes	
López-Sepúlveda et al., 2015b	Winteraceae	*Drimys*	tree	44	no	no							yes		AFLP / SSR	yes	
Lousada et al., 2013	Velloziaceae	*Vellozia*	herb	10	no	no							yes		8 primers and 141 ISSR Loci	yes	
Marques et al., 2014	Orchidaceae	*Epidendrum*	herb	10	yes	no			yes	yes	niche models		yes		AFLP/sequencing	yes	yes
Marquete and Mansano, 2012	Salicaceae	*Casearia*	tree	—	no	yes	yes										
Melo and Borba, 2011	Orchidaceae	*Acianthera*	herb	7	no	no		yes									
Meneguzzo et al., 2015	Orchidaceae	*Aganisia*	herb	—	no	yes	yes										
Miller and Spooner, 1999	Solanaceae	*Solanum*	herb	59	no	yes						yes			RAPD / RFLP	yes	
Miz et al., 2008	Solanaceae	*Solanum*	herb	—	no	no						yes			sequencing	yes	yes
Moraes et al., 2012	Cactaceae	*Pilosocereus*	herb	10	no	no							yes		11 SSR	yes	
Moreno et al., 2015	Turneraceae	*Turnera*	herb	—	yes	no		yes	yes	yes			yes		RAPD/RFLP		yes
Norrmann, 2009	Poaceae	*Andropogon*	herb	—	yes	yes	yes		yes	yes							
O'Leary et al., 2012	Verbenaceae	*Lippia*	shrub	—	yes	yes	yes	yes									
Oliveira and Valls, 2002	Poaceae	*Paspalum*	herb	—	no	yes	yes										
Oliveira et al., 2007	Asteraceae	*Vernonia*	herb	8	no	no			yes								
Oliveira et al., 2008a	Poaceae	*Raddia*	herb	14	yes	yes		yes					yes		10 Allozime loci	yes	
Oliveira et al., 2008b	Poaceae	*Raddia*	herb	14	no	yes		yes									
Oliveira et al., 2013	Myrtaceae	*Campomanesia*	tree	—	no	yes	yes										
Oliveira et al., 2015	Poaceae	*Paspalum*	herb	—	yes	yes	yes	yes	yes								

Palacios et al., 2012	Fabaceae	*Prosopis*	shrub	—	yes	yes	yes	yes				yes		AFLP	yes	
Paula-Souza et al., 2011	Violaceae	*Hybanthus*	herb	—	no	yes	yes									
Pelegrin et al., 2009	Poaceae	*Briza*	herb	—	no	no		yes								
Peraza-Flores et al., 2011	Orchidaceae	*Polystachya*	herb	—	no	yes	yes									
Pereira et al., 2007	Eriocaulaceae	*Syngonanthus*	herb	10	yes	yes	yes					yes		14 allozime loci	yes	
Pessoa et al., 2012	Orchidaceae	*Epidendrum*	herb	20	yes	yes	yes	yes			yes	yes			yes	yes
Pinheiro and Barros, 2007	Orchidaceae	*Epidendrum*	herb	18	no	no		yes								
Pinheiro and Barros, 2009	Orchidaceae	*Brasiliorchis*	herb	30	no	no		yes								
Pinheiro and Miotto, 2005	Fabaceae	*Lupinus*	herb	—	no	yes	yes									
Planchuelo and Dunn, 1989	Fabaceae	*Lupinus*	herb	—	no	yes	yes									
Porter-Utley, 2014	Passifloraceae	*Passiflora*	liana	—	yes	yes	yes	yes			yes			sequencing	yes	
Prohens et al., 2006	Solanaceae	*Solanum*	herb	—	no	no						yes		AFLP	yes	
Ribeiro et al., 2008	Orchidaceae	*Bulbophyllum*	herb	33	yes	yes		yes				yes		9 allozime loci	yes	
Ritz et al., 2007	Cactaceae	*Rebutia*	herb	—	no	no					yes			sequencing		yes
Rivadavia et al., 2014	Droseraceae	*Drosera*	herb	—	no	yes	yes									
Rodrigues et al., 2015	Orchidaceae	*Cattleya*	herb	8	no	no					yes	yes		two cpDNA:rps16-trnKandrpL32-trnL and 295 ISSR loci	yes	yes
Roncal et al., 2007	Arecaceae	*Geonoma*	tree	4	no	yes						yes		ISSR	yes	
Särkinen et al., 2015	Solanaceae	*Solanum*	herb	—	yes	yes	yes				yes			sequencing	yes	yes
Sawyer and Rojas, 1998	Solanaceae	*Deprea*	herb	—	yes	yes	yes	yes								
Schmidt-Lebuhn, 2007	Lamiaceae	*Minthostachys*	shrub	—	no	no					yes			AFLP	yes	
Schulman et al., 2004	Melastomataceae	*Clidemia*	liana	50	yes	yes	yes	yes	soil analysis							
Scotti-Saintagne *et al.*, 2013	Meliaceae	*Carapa*	tree	40	no	no							yes	SSR / sequencing	yes	yes

(*Continued on next page*)

Table 1. (*Continued*)

Reference	Family	Genus	Habit	Number of populations	Multiple methods	Taxonomic decisions	Traditional taxonomic methods	Morphometry/ Anatomy	Cytogenetics	Reproductive/ crossing experiments	Ecological data	Phylogenetic approach	Population genetic approach	Phylogeographic approach	Molecular markers	Nuclear	Plastid
Scrivanti et al., 2010	Poaceae	*Bothriochloa*	herb	19	no	no			yes								
Seijo and Fernández, 2003	Fabaceae	*Lathyrus*	herb	37	no	no			yes								
Semir et al., 2014	Asteraceae	*Lychnophora*	herb	—	no	yes	yes										
Silberbauer-Gottsberger et al., 1992	Rubiaceae	*Tocoyena*	tree	—	yes	yes	yes	yes			distribution						
Solís-Neffa and Fernández, 2001	Turneraceae	*Turnera*	herb	48	no	no			yes								
Solís-Neffa and Fernández, 2002	Turneraceae	*Turnera*	herb	12	no	no			yes								
Solís-Neffa et al., 2004	Turneraceae	*Turnera*	herb	41	no	no			yes								
Souza et al., 2012	Alliaceae	*Nothoscordum*	herb	—	yes	no			yes			yes			one cpDNA: trnL-trnF and one nuDNA: ITS	yes	yes
Speranza et al., 2003	Poaceae	*Paspalum*	herb	—	no	no			yes								
Speranza et al., 2007	Turneraceae	*Turnera*	herb	79	no	no								yes	sequencing		yes
Strelin et al., 2013	Calceolariaceae	*Calceolaria*	herb	18	no	no		yes									
Tacuatiá et al., 2012	Iridaceae	*Sisyrinchium*	herb	27	yes	no		yes	yes				yes		ISSR	yes	
Takayama et al., 2015	Asteraceae	*Robinsonia*	herb	28	no	no							yes		AFLP/SSR	yes	
Turchetto et al., 2014	Solanaceae	*Petunia*	herb	64	yes	yes		yes			climatic characteristics				two cpDNA: rnS-trnG and trnH-psbA and one nuDNA: 6 CAPs loci	yes	yes
Urbani et al., 2002	Poaceae	*Paspalum*	herb	32	yes	no		yes	yes								
Vázquez-Garcidueñas et al., 2003	Fabaceae	*Prosopis*	tree	5	yes	yes		RAPD	yes								
Vega et al., 2013	Orchidaceae	*Epidendrum*	herb	50	yes	no		yes	yes	yes	phenology		yes	yes	AFLP/sequencing	yes	yes

Versieux and Machado, 2012	Bromeliaceae	*Vriesea*	herb	—	no	yes	yes						
Viruel et al., 2010	Dioscoreaceae	*Dioscorea*	liana	25	yes	yes	yes	yes					
Walter et al., 2015	Portulacaceae	*Portulaca*	herb	178	yes	no		yes	yes				
Welker et al., 2015	Poaceae	*Saccharum*	herb	—	no	no				yes		5 nuDNA: apo 1, d 8, ep 2 -ex 7, ep 2 -ex 8, and rep 1	yes
Wet, 1968	Poaceae	*Bothriochloa*	herb	—	yes	yes		yes	yes				
Zaitlin and Pierce, 2010	Gesneriaceae	*Sinningia*	herb	25	no	no			yes				
Zuntini et al., 2015	Bignoniaceae	*Bignonia*	liana	—	no	yes	yes						

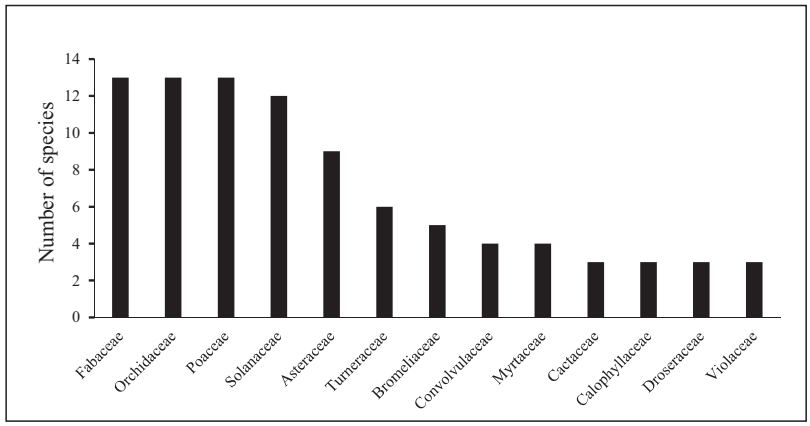


Figure 2. The best represented plant families considering studies of species complexes performed within South America.

Similarly, most of the genera were studied only once, and only 28% were studied more than once (Table 1, Figure 3). The use of molecular markers was the most common approach, adopted by 42.6% of the studies, followed by morphometry (37.9%) and traditional taxonomy (35.6%) (Figure 4). Cytogenetic analyses, mostly meiotic and/or mitotic chromosome counting, were performed in 22% of the studies. Genome sizes estimated using flow cytometry were assessed in four papers. Karyotypes were analyzed in three studies. Cytogenetic molecular techniques (such as *in situ* hybridization) were used in one of the studies retrieved in our review. Ecological data were used in 14 studies, encompassing different methods, such as phenological analyses (5), climatic variables (5), niche models (2), pollination ecology (2), and soil analysis (1). Anatomy was used in 9.3% of the studies.

A large number of the studies used molecular markers (42.6%). Of those, 56.4% employed population genetic methods to analyze the data, whereas 36% used phylogenetic approaches, and only 12.7% used phylogeographic analyses to delimit species (Figure 5A). Eighteen studies combined nuclear and plastid data. Nuclear markers were the primary choice in studies that used molecular markers (87.3%), and plastid markers were used by 45.4% of the studies (Figure 5B). Among the molecular markers used, sequences from different intergenic regions were used most commonly (23 studies); however, most of these studies used few plastidial regions, usually one or two, and only three studies used four or more plastidial regions (López et al., 2012; Vega et al., 2013; Marques et al., 2014). The majority of studies (11 cases) evaluated only a single-copy nuclear locus, in contrast to three studies that evaluated at least four unlinked nuclear loci (Ames and Spooner, 2010; Andre et al., 2015; Welker et al., 2015). Dominant markers, such as random amplification of polymorphic DNA, amplified fragment length polymorphism, and intersimple sequence repeat, were applied in 20 studies. Co-dominant and unlinked nuclear microsatellite loci and allozyme loci were assessed in 11 and 7 studies, respectively (Table 1).

Less than half of the studies (43.4%) used more than one source of data to analyze species complexes. However, a significant proportion of studies that used a multidisciplinary approach also included taxonomic decisions associated with the species complexes investigated (69.6%, d.f. = 1, χ^2 [Yates corrected] = 8.529, $P <$ 0.01). Considering only studies that adopted only one

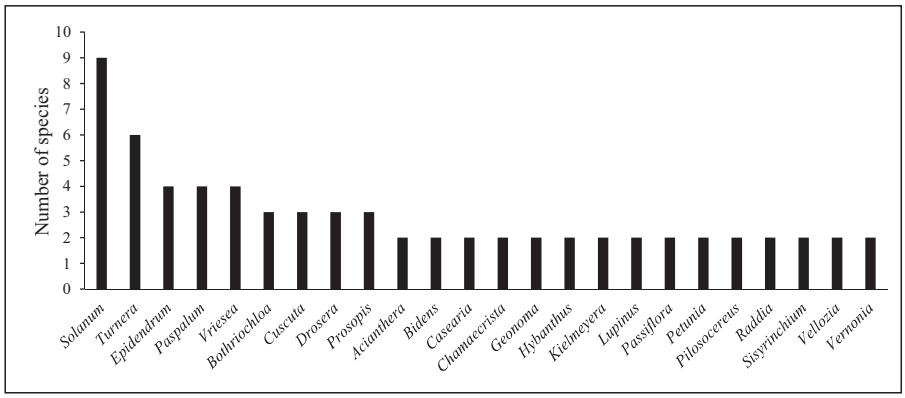


Figure 3. The most well-represented represented plant genera in studies on species complexes in South America.

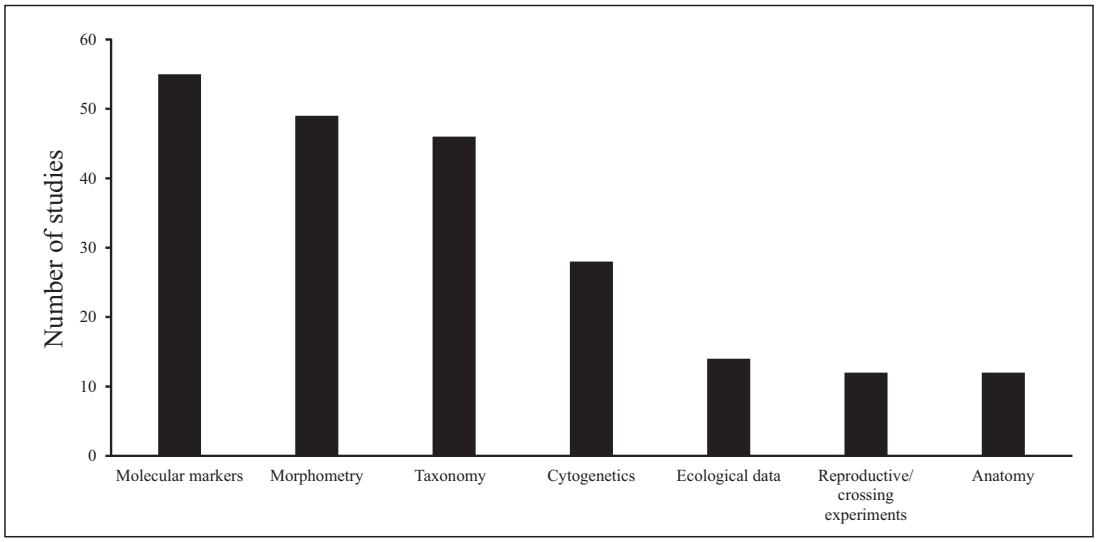


Figure 4. The most widely used methods in studies of species complexes performed within South America.

data type, the use of traditional taxonomic methods always resulted in taxonomic decisions (100%, d.f. = 1, χ^2 [Yates corrected] = 42.476, $P = 0.00$). The use of molecular markers alone was negatively associated with the adoption of taxonomic decisions [79.1%, d.f. = 1, χ^2 [Yates corrected] = 6.355, $P < 0.05$], and a similar pattern was detected in studies that used only cytogenetics [100.0%, d.f. = 1, χ^2 [Yates corrected] = 9.179, $P < 0.01$]. Taxonomic decisions were not significantly associated with studies that used morphometry [27.2%, d.f. = 1, χ^2 [Yates corrected] = 0.76, $P = 0.383$]. Associations between the presence of taxonomic decisions and studies using anatomy, reproductive experiments, and ecological data were not assessed due to the low number of studies in these categories.

According to the network (Figure 6), among the studies that adopted a multidisciplinary approach, molecular markers were used 31 times and were combined more often with morphometry (19), morphologic taxonomy (10), and ecological data (7) (Figure 6). Other common associations between research fields were taxonomy and morphometry (12 studies), taxonomy and molecular markers (10 studies), morphometry and cytogenetics (9 studies), and morphometry and ecology (9 studies, Table 2). In contrast, ecological, anatomical, and cytogenetic studies showed considerably less interaction with other disciplines. Reproductive/crossing experiments and ecological data were never used as a unique source of information to study species complexes and were always combined with other research fields.

The study of species complexes deserves special attention because of the potential to reveal novel and informative results. Most species complexes are potentially composed of recently diverged lineages, which may provide important information regarding the first steps in species radiation, such as colonization of new habitats (Lagomarsino et al., 2016) and selective pressures in different environments (Simon et al., 2011). Owing to their importance, studies of species complexes have guided most of the research on species variation from

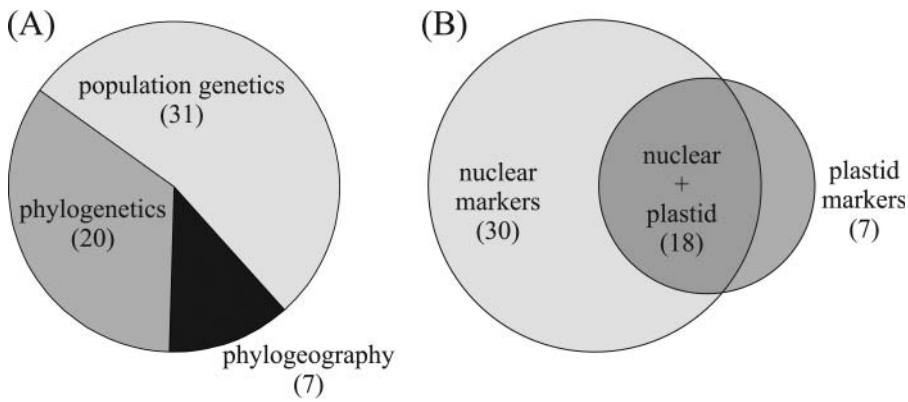


Figure 5. The main methods used to analyze molecular data in studies of species complexes (A) and the types of markers used in these studies (B). Numbers within parentheses indicate the numbers of studies.

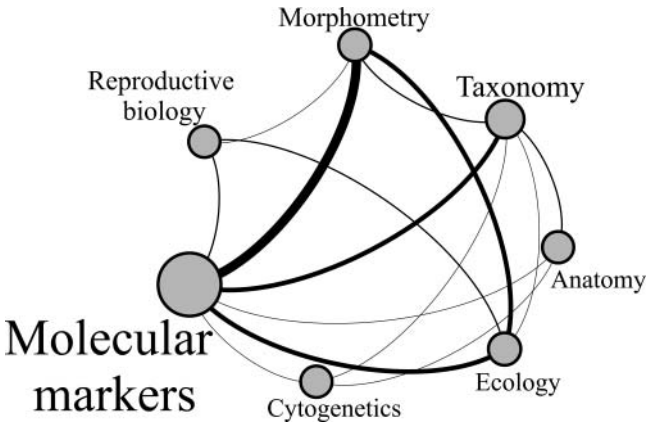


Figure 6. Network showing connections between research fields addressed in studies of species complexes, when adopting a multidisciplinary approach. The size of the circle is proportional to the number of studies in each field, and the line thickness is proportional to the number of connections between fields. See Table 2 for details.

morphological to molecular traits. Thus, these species groups may serve as models to understand speciation events, mainly in the Neotropical region, where mechanisms of species formation remain poorly understood (Antonelli and Sanmartín, 2011). The patterns observed here revealed strong discrepancies among studies of species complexes in South America, showing that such studies have preferentially focused on a few plant families and genera (Table 1). Many studies have been performed on orchids, legumes, and grasses, which are families with great economic importance. Surprisingly, no studies on Euphorbiaceae or Malvaceae species were detected by our survey. Melastomataceae, which is one of the richest plant families in South America, was addressed by only one study (Figure 2). A strong disparity was also observed among the methods chosen by different studies, which focused on molecular markers, morphometry, and traditional taxonomy (Figure 4). Although, most studies were performed using only one line of evidence (Table 1), taxonomic decisions were significantly associated with studies that used a multidisciplinary approach. We explored the patterns identified by our survey to provide a clear picture of studies of species complex and help guide future efforts in this field.

Table 2. Number of connections between different research fields in studies of species complexes performed in South America. Details are show in Figure 6.

Source	Target	Number of interactions
Anatomy	Cytogenetics	4
Anatomy	Molecular markers	4
Anatomy	Morphometry	4
Anatomy	Reproductive biology	3
Cytogenetics	Molecular markers	5
Cytogenetics	Morphometry	9
Ecology	Molecular markers	7
Morphometry	Ecology	9
Morphometry	Molecular markers	19
Morphometry	Reproductive biology	8
Reproductive biology	Ecology	4
Reproductive biology	Molecular markers	5
Reproductive biology	Cytogenetics	7
Taxonomy	Anatomy	6
Taxonomy	Cytogenetics	3
Taxonomy	Ecology	3
Taxonomy	Molecular markers	10
Taxonomy	Morphometry	12
Taxonomy	Reproductive biology	1

III. Taxonomic bias

Most of the studies on plant species complexes focused on the most diverse plant families found in South America (Figure 2). For example, six of the most studied plant families were also included among the top 10 most diverse families found in Brazil (Zappi et al., 2015). The Orchidaceae family has been a focus of morphological variation studies since Darwin (1882). Large living collections in scientific institutions and of amateur orchidologists have stimulated studies of species complexes worldwide, particularly in Brazil (Pinheiro and Barros, 2007). The same effect is evident in studies on Bromeliaceae (Costa et al., 2009). With some exceptions (Costa et al., 2007; Oliveira et al., 2015), most studies performed on Fabaceae, Poaceae, Solanaceae, and Asteraceae focused on economically important genera. For example, most Solanaceae studies focused on tuber-bearing *Solanum* L. (potato) species (Figure 3). Grasses commonly used for animal pastures, such as *Paspalum* L. and *Bothriochloa* Kuntze, have also been studied extensively. These families show extensive advantages in the study of species complexes, such as short life cycles, ease of cultivation and manipulation in crossing experiments, and high availability of molecular markers.

IV. Use of molecular markers to investigate species complexes

Studies using molecular markers also showed a preference for population genetic methods (Figure 5A). In many cases, these studies commonly reflected associations between the botany and genetics departments of different institutions (Moraes et al., 2012; Caddah et al., 2013). In these studies, genetic differentiation among populations and lineages was crucial to identify the existence of different species. Use of highly informative markers such as microsatellites, coupled with Bayesian assignment methods (i.e., Pritchard et al., 2000), holds great promise for studying species delimitation and speciation processes. For example, Moraes et al. (2012) and Caddah et al. (2013) depicted complex patterns of

variation, enabling recognition of divergent lineages that did not correspond exactly to the species limits traditionally recognized. For instance, hybridization between *Kielmeyera coriacea* Mart. and *K. grandiflora* A.St.-Hil. has blurred species boundaries and challenged the identification of species throughout history (Caddah et al., 2013). The long-term isolation of *Pilosocereus aurisetus* (Werderm.) Byles & G.D. Rowley populations may have promoted divergence of narrow endemic lineages, which occur in a naturally fragmented landscape that imposes strong restrictions on gene exchange and enhances genetic drift (Moraes et al., 2012). Similar patterns were observed in two studies on Asteraceae, which is endemic to oceanic islands, in which multiple events of lineage divergence were driven by strong restrictions on gene exchange (López-Sepúlveda et al., 2013a; Takayama et al., 2015).

Nuclear markers have been highlighted as ideal for interspecific comparisons and have been useful for testing hypotheses of species delimitations (Palma-Silva et al., 2011). Molecular markers with higher levels of gene flow are less prone to introgression and thus more effective for species delimitation (Petit and Excoffier, 2009). In angiosperms, nuclear genomes are usually inherited biparentally and dispersed (from seed and pollen grains), unlike plastidial genomes, which are usually only maternally inherited and dispersed from seeds. This led us to expect that nuclear markers, in angiosperms, would be more suitable for species delimitation than plastidial markers. In fact, most studies that we identified here used nuclear markers (Figure 5B) and conducted better species delimitation in *Sisyrinchium* (Alves et al., 2014) and *Astronium* (Caetano et al., 2008). On the other hand, plastid markers show lower levels of gene exchange, often reflecting patterns of geographic isolation (Bonatelli et al., 2014; Turchetto et al., 2014). Furthermore, using plastidial markers exclusively may be problematic because they have limited utility for many of the coalescent methods, as the chloroplast represents only one locus. In addition, divergence among nuclear and plastidial markers could occur as a consequence of hybridization among species, as reported for two species complexes in the *Cuscuta* genus (Costea et al., 2011, 2015). Thus, despite that only 32% (18) of studies in our review used a combination of both genomes, authors should attempt to combine both nuclear and plastidial markers when studying species complexes to provide a clearer picture of the species boundaries, and processes involved in their diversification.

One interesting approach employed to investigate species complexes using molecular markers is the "blind sampling" strategy, described by Duminil et al. (2006). In special cases, such as species with a short flowering period and/or a lack of morphological diagnostic characters, authors suggest skipping previous morphological identification methods performed by most studies and proceeding directly to molecular genetic analyses when studying species complexes. Some studies identified in our review adopted the same strategy and were able to identify the species effectively *a posteriori* (Caetano et al., 2008; Marques et al., 2014; Gagnon et al., 2015). However, it is worth mentioning that without appropriate recording of phenotypes, it is presumably more difficult to identify lineages based on previous hypotheses in the literature or translating results into taxonomic updates.

V. Multidisciplinary approaches

One advantage of adopting a multidisciplinary approach is the possibility of identifying potential mechanisms underlying the diversification and speciation processes within species complexes. In fact, data from multiple sources can form a reciprocal illumination (Hennig, 1966; Blaxter, 2004; Sukumaran and Knowles, 2017), in which different disciplines inform and refine others. In this context, all sources of data are combined to help describe the diversity of life. For example, despite the efficiency of molecular markers in describing levels of biodiversity and evolutionary relationships, studies using this approach exclusively have limited the explanatory power regarding the mechanisms leading to speciation (Lipscomb et al., 2003; Huang and Knowles, 2016). According to Lipscomb et al. (2003), a multidisciplinary approach has the power to expose inconsistencies, which is an important means of identifying important evolutionary processes, such as hybridization and introgression, and phenotypic convergent plasticity. In fact, recent studies have emphasized the importance of integrative approaches in species delimitation (de Queiroz, 1998; Padial and de la Riva, 2010; Puillandre et al., 2012; Zapata and Jiménez, 2012). This trend was detected based on taxonomic decisions, which were significantly associated with studies that employed different methods. Species complexes are often composed of recently diverged lineages, in which the speciation process is incomplete. In such situations, using a single method may not be sufficient to detect the mechanisms involved in lineage divergence (de Queiroz, 1998). On the other hand, different methods increase the power to detect early stages of divergence, improving further attempts to delimit species.

The joint use of molecular markers and morphometry was observed in 15% (19 studies) of all retrieved papers (Figure 6). This pattern may reflect the intention of these authors to investigate the extent to which the morphological similarities reflect the evolutionary relationships

in such groups. This is a fundamental question in species complex studies, as mentioned previously (Briggs and Walters, 1997). In general, the lack of a correlation between morphometric and genetic data is interpreted as evidence of convergent evolution. For example, the results obtained by Borba et al. (2002) support the hypothesis that floral similarities among *Acianthera* Scheidw. species represent convergence driven by flower fidelity to the same pollinators. Indeed, species with similar flowers are allopatric and show extensive genetic differences (Borba et al., 2002). On the other hand, some studies have found strong correlations between observed morphometric patterns and genetic data. The agreement between morphological and genetic differences has been used to recognize different species within the *Chamaecrista cytisoides* (Coll) H.S. Irwin & Barneby complex (Conceição et al., 2008) and within the *Chamaecostus subsessilis* (Nees & Mart.) C. D. Specht & D. W. Stev. complex (Andre et al., 2015).

Crossing experiments were always used in combination with other methods in the studies evaluated in this review (Table 1). Despite the importance of crossability for the biological species concept (Mayr, 1942), such experiments have rarely been used (only 9.3%, 12 papers) to study species complexes in South America. Experimental pollination provides important information regarding mating systems and inbreeding depression (Borba et al., 2011; Sampaio et al., 2012). This information is also crucial to explore the evolution of reproductive barriers among populations and species (Costa et al., 2007; Pinheiro et al., 2013). For example, species with different mating systems may show greater reproductive isolation (Palma-Silva et al., 2015; Pinheiro et al., 2015; Twyford et al., 2015; Neri et al., 2017, 2018). The evolution of reproductive isolation has been central to species recognition since the influential works of Dobzhansky (1937) and Mayr (1942), which articulated the biological species concept. In this context, hybrid zones have been used as natural laboratories to study the evolution of reproductive barriers (Soltis and Soltis, 2009; Baack et al., 2015). However, several studies conducted in hybrid zones have revealed incomplete barriers to gene exchange (Rieseberg et al., 2004), increasing uncertainty regarding species boundaries, mainly in species complexes (Cavallari et al., 2010; Costea et al., 2011, 2015; Vega et al., 2013; Marques et al., 2014). The idea that species integrity is maintained even in the presence of low levels of interspecific gene exchange has altered the concept of "complete reproductive isolation," initially postulated in the biological species concept (Mayr, 1942), to "partially permeable reproductive isolation," known as a porous genome (Wu, 2001). Recent approaches have shown that a combination of multiple reproductive barriers to gene exchange act in concert, preventing the collapse of species (Palma-Silva et al., 2011; Vega et al., 2013; Marques et al., 2014; Pinheiro et al., 2016; Neri et al., 2017, 2018). Therefore, a multidisciplinary approach is crucial when studying barriers to gene exchange, which in turn will clarify the ecological and genetic mechanisms acting during species and lineage diversification. For instance, interspecific gene exchange may result in transfer of adaptive traits between closely related species, reducing extinction risks during environmental changes (Cannon and Lerdau, 2015).

It is important to highlight that speciation is a population-level process, which involves the evolution of reproductive isolation among different populations (Scopece et al., 2010). The study of reproductive barriers within species, or within species complexes, would help determine the first stages of the speciation process in situations in which species formation is still incomplete. The population-level analysis of the ecological and genetic causes of reproductive isolation was named "the magnifying glass" approach (Via, 2009). This approach is suitable for determining whether ecological and genetic mechanisms are activated before speciation is complete, avoiding confusion with the changes that accumulate after species formation. Very few studies have used this approach in tropical plants as models (but see Pinheiro et al., 2013). One potential reason for this is that most authors still interpret the biological species concept in its original version, in which reproductive isolation would be completely absent among populations within species. A change in this perception and the inclusion of "speciation in a continuum" and "speciation with gene flow" ideas (Wu, 2001; Nosil, 2012; Seehausen et al., 2014) are urgently needed to encourage investigations of reproductive barriers during the early stages of divergence: within species and/or species complexes.

Ecological data were included in 14 of the 56 studies that involved multidisciplinary approaches (Figure 6, Table 1). Eight of these studies included a range of methods, such as the analysis of single lines of environmental data including temperature and/or rainfall, or included more climatic variables to characterize the environments in which the populations were sampled. Actually, the history of species complex studies is strongly associated with characterizing the heterogeneous environments in which the populations were found (Turesson, 1922). The study of ecotypes and races stimulated the use of novel and creative approaches to disentangle the effects of different habitats on variations in morphological characters. For example, transplant experiments are crucial to understand how plasticity and/or heritable characters are responsible for the variation found among populations

growing in divergent habitats (Clausen et al., 1939). In our survey, 38% of the studies applied morphometric analyses to investigate species complexes, indicating potential phenotypic variation within the species complexes. In the studies that employed morphometric analyses, 49 studies in total, it would help to know whether the morphological variation was caused by phenotypic plasticity, local adaptation, or both. Classical reciprocal transplant experiments are not always feasible, but alternatives, such as rearing/raising/growing divergent phenotypes in controlled climatic environments, (e.g., common garden studies) could be implemented more easily (Franks et al., 2014). Nevertheless, Esteves and Vicentini (2013) used morphometry and habitat characterization to explore the existence of plasticity and/or selection for different environments in the *Pagamea coriacea* Benth. complex. They found two discrete morphological groups in this complex, associated with different habitat preferences and reproductive behaviors. Turchetto et al. (2014) reported strong connections among morphological, genetic, and environmental variables, suggesting that selection for different habitats, rather than plasticity, is responsible for the diversification of lineages in the *Petunia axillaris* (Lam.) Britton, Sterns & Poggenb. complex. Salinity, elevation, and climate were important environmental drivers for lineage diversification in the *Hypochaeris apargioides* Hook. & Arn. complex (López-Sepúlveda et al., 2013a).

Phylogeographic studies have played a central role in interpreting how past orogenic changes and climatic fluctuations have affected the diversification of lineages and species in South America (Turchetto-Zolet et al., 2013; Smith et al., 2014; Leal et al., 2016b; among others). Unfortunately, in our review, only seven studies used a phylogeographic approach to evaluate species complexes (Figure 5A). For example, morphological and molecular data have been combined to study the *Vellozia hirsuta* Goeth.& Henrard complex, a highly polymorphic group found among disjointed mountains within the Espinhaço Mountain Range in Brazil (Barbosa et al., 2012). Despite strong morphological differentiation among populations, no associations with the genetic lineages were found, suggesting that *V. hirsuta* should be considered a single polymorphic species. Bonatelli et al. (2014) concluded that diversification of the *P. aurisetus* complex has been affected by long-term isolation in microrefugia composed of xerophytic vegetation, by combining detailed demographic analyses, divergence time estimates, and niche modeling. Integrating different types of data is commonly performed in phylogeographic studies to obtain a clear picture of the processes involved in lineage and species diversification. Considering the growing number of phylogeographic studies in South America (Turchetto-Zolet et al., 2013), several groups remain poorly studied, and many questions remain unanswered. Of special interest are studies exploring how reproductive isolation evolves in a geographic context, and how selective pressures for divergent environments contribute to the formation of barriers preventing gene exchange among populations (Vallejo-Marín and Hiscock, 2016).

Phylogenetic inferences are crucial to recognize broad evolutionary relationships among lineages and species, enabling the recognition of groups that may refer to species complexes. For instance, the phylogeny published by Pinheiro et al. (2009) provided the basic framework for a detailed study of a particular group of *Epidendrum* showing unclear species boundaries (Pessoa et al., 2012). The same strategy is observed in other groups, such as the phylogenetic study published for the tribe Bignonieae (Bignoniaceae, Lamiales, Lohmann, 2006), allowing further population-level studies in species complexes (Firetti-Leggieri et al., 2011; Zuntini et al., 2015). One of the advantages of this approach is to identify cases of trait convergence, to avoid the study of unrelated paraphyletic groups. Rodrigues et al. (2015) constructed a phylogenetic inference for the *Cattleya coccinea* complex, and the recognition of different clades was important for the detailed study of Leal et al. (2016a), using morphometric and microsatellite analyses. The study performed by Welker et al. (2015) also identified several clades within the genus *Saccharum*, allowing future studies to explore particular microevolutionary processes within each species group of this genus. Species complexes belonging to monophyletic groups would facilitate the identification of drivers that fuel diversification events such as lineage dating (Perret et al., 2007; Breitkopf et al., 2015) and trait evolution (Silvestro et al., 2014; Lagomarsino et al., 2016).

In our review, species tree-based methods for species delimitation were limited to the application of a single or few universal markers, thus not incorporating tree-based coalescent methods. However, the accuracy of species delimitation based on a few or single gene trees may be limited and prone to incongruences due to incomplete lineage sorting, hybridization, and introgression (Naciri and Linder, 2015). Thus, unlinked loci and multispecies coalescent methods, which combine concepts from phylogenetic and population genetics, may help to overcome these difficulties. In general, the application of such methods is advantageous because they (1) are highly replicable and capable of being used by researchers in different areas (e.g., ecology, physiology, and systematics) (Flot et al., 2010); (2) are based on falsifiable hypotheses,

removing possible biases inherent to the taxonomist (Fujita et al., 2012); (3) incorporate hypothetical species with incomplete lineage sorting and gene flow (see Camargo et al., 2012, Jackson et al., 2017 and Chan et al., 2017), and (4) have an objective statistical framework (Huang and Knowles, 2016). The study of cryptic diversity in *Cerrado* endemic lizards was a good example of using high-throughput phylogenomic data and coalescent techniques to delimit species and to investigate patterns of species diversity in a geographically widespread animal species (Domingos et al., 2017).

However, the application of multispecies coalescent methods solely to identify evolutionarily independent lineages to delimit species has been criticized (Freudenstein et al., 2017; Sukumaran and Knowles, 2017). Again, the thin line that distinguishes a species population at the beginning of divergence raises the question of the epistemological concept of multispecies coalescence techniques that use only molecular data, showing that this method elucidates the genetic structure, not the delimitation of the species itself (Sukumaran and Knowles, 2017), and the ecological role that a given species plays is crucial for better characterization of the taxa in question (Freudenstein et al., 2017).

Therefore, no method is a panacea, and any approach to species delimitation is unable to distinguish precisely certain taxa given the complexity of evolutionary history (Carstens et al., 2013). Species delimited in multispecies coalescent methods should be considered as hypotheses, and their delimitation should be based on the greatest possible numbers of lines of evidence and methods, as discussed above, with the aim of achieving congruence between them, resulting in a greater robustness of the *a priori* hypothesis (Carstens et al., 2013; Chan et al., 2017; Sukumaran and Knowles, 2017). Thus, methods that incorporate different and independent data, such as ecological, phenotypic, and geographic data, with multilocus analyses and coalescence theory (e.g., iBPP—integrated Bayesian Phylogenetics and Phylogeography, Solís-Lemus et al., 2015; the Guillot model, Guillot et al., 2012) are the new frontier for studies of species complexes. In such studies, these methods may enhance the prediction of species delimitation by contributing independent datasets, seeking the congruence (or the lack thereof) of distinct hypotheses (Sukumaran and Knowles, 2017).

VI. Concluding remarks and perspectives

Our review retrieved 129 studies that explicitly investigated species complexes in South America, which is probably very low considering the levels of plant diversity on this continent. The actual number of biological species is likely to be greater than the current list of nominal species. There is likely a long list of reasons for this, including the fact that species divergence is not always followed by morphological changes, or that morphological and/or ecological variation can occur with low genetic divergence.

Thus, multidisciplinary approaches are crucial to understanding the integrity and boundaries within species complexes (Stebbins, 1950). Integrative taxonomy has fueled discussions about strategies to combine different data sources to achieve comprehensive species delineation (Constance, 1964; Padial et al., 2010). This approach is central for planning future studies of species complexes and may help guide efforts to understand plant speciation in tropical regions. In addition, the creative use of available databases holds great promise for investigating species complexes. Inexpensive approaches, such as crossing experiments and ecological niche modeling, in addition to the data available in public databases, such as TreeBASE (for published phylogenies), GenBank (for molecular data), GBIF (for georeferenced collections), and Dryad (for alignments and morphological matrices), can be associated with multispecies coalescent data, generated from cost-effective sequencing (RAD-seq, GBS, exon capture, among others; Herrera and Shank, 2016), yielding unprecedented resolution of species boundaries among South American plant species complexes. Furthermore, using genome scanning methods, it is also possible to identify loci and genomic regions under adaptive divergence that may be responsible for reproductive isolation and ecological speciation among populations (Hoban et al. 2016)

We thus believe that the application of new methodologies, such as multispecies coalescence techniques, integrated with different source data (Guillot et al., 2012; Solís-Lemus et al., 2015) in species delimitation of South American species complexes will greatly improve our knowledge of the lineage diversification and speciation processes in this rich biota. In fact, the history of plant variation studies shows that investigating a species complex depends on a creative combination of diverse informative methods. In this context, species complexes should be viewed as important models to understand plant speciation and adaptation, inspiring new generations of botanists, ecologists, and evolutionary biologists.

Acknowledgments

The authors are grateful to Dr. Rafael Batista Louzada for helpful discussions regarding the evolution of species complexes. The authors also thank Mauro Peixoto for use of pictures A–D, Viviana G. Solís Neffa for use of pictures E—H, and

Evandro Marsola de Moraes for use of pictures I–L, all included in Figure 1. Grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CPS: CNPq-Universal 475937/2013–9, and Fellowship 300819/2016-1; FP: CNPq-Universal 440367/2014–0 and fellowship 300927/2016-9) and Fundação de Amparo à Pesquisa do Estado de São Paulo (CPS: FAPESP-2014/15588–6; FP: FAPESP 2016/22785-8) funded this study.

ORCID

Fábio Pinheiro http://orcid.org/0000-0003-3243-2652

References

Allred, K. W., and Gould, F. W. 1983. Systematics of the *Bothriochloa saccharoides* complex (Poaceae: Andropogoneae). *Syst. Bot.* **8**: 168. doi:10.2307/2418692.

Alvarez, N. M. B., Peralta, I. E., Salas, A., and Spooner, D. M. 2008. A morphological study of species boundaries of the wild potato *Solanum brevicaule* complex: replicated field trials in Peru. *Plant Syst. Evol.* **274**: 37–45. doi:10.1007/s00606-008-0023-1.

Alves, T. L. S., Chauveau, O., Eggers, L., and Souza-Chies, T. T. 2014. Species discrimination in *Sisyrinchium* (Iridaceae): assessment of DNA barcodes in a taxonomically challenging genus. *Mol. Ecol. Resour.* **14**: 324–335. doi:10.1111/1755-0998.12182.

Ames, M., Salas, A., and Spooner, D. M. 2008. A morphometric study of species boundaries of the wild potato *Solanum* series *piurana* (Solanaceae) and putatively related species from seven other series in *Solanum* sect. *Petota*. *Syst. Bot.* **33**: 566–578. doi:10.1600/036364408785679789.

Ames, M., and Spooner, D. M. 2010. Phylogeny of *Solanum* series *Piurana* and related species in *Solanum* section *Petota* based on five conserved ortholog sequences. *Taxon* **59**: 1091–1101.

Amico, G. C., and Nickrent, D. L. 2009. Population structure and phylogeography of the mistletoes *Tristerix corymbosus* and *T. aphyllus* (Loranthaceae) using chloroplast DNA sequence variation. *Am. J. Bot.* **96**: 1571–1580. doi:10.3732/ajb.0800302.

Andre, T. J. C., Specht, C., Salzman, S., Palma-Silva, C., and Wendt, T. 2015. Evolution of species diversity in the genus *Chamaecostus* (Costaceae): molecular phylogenetics and morphometric approaches. *Phytotaxa* **204**: 265–276. doi:10.11646/phytotaxa.204.4.3.

Antonelli, A., and Sanmartín, I. 2011. Why are there so many plant species in the Neotropics? *Taxon* **60**: 403–414.

Baack, E., Melo, M. C., Rieseberg, L. H., and Ortiz-Barrientos, D. 2015. The origins of reproductive isolation in plants. *New Phytol.* **207**: 968–984. doi:10.1111/nph.13424.

Ballard, H. E., and Iltis, H. H. 2012. *Viola lilliputana* sp. nov. (*Viola* sect. *Andinium*, Violaceae), one of the world's smallest violets, from the Andes of Peru. *Brittonia* **64**: 353–358. doi:10.1007/s12228-012-9238-0.

Barbosa, A. R., Fiorini, C. F., Silva-Pereira, V., Mello-Silva, R., and Borba, E. L. 2012. Geographical genetic structuring and phenotypic variation in the *Vellozia hirsuta* (Velloziaceae) ochlospecies complex. *Am. J. Bot.* **99**: 1477–1488. doi:10.3732/ajb.1200070.

Barley, A. J., White, J., Diesmos, A. C., and Brown, R. M. 2013. The challenge of species delimitation at the extremes: diversification without morphological change in Philippine sun skinks. *Evolution* **67**: 3556–3572. doi:10.1111/evo.12219.

Barthlott, W., Mutke, J., Rafiqpoor, D., Kier, G., and Kreft, H. 2005. Global centers of vascular plant diversity. *Nov. Acta Leopoldina* **92**: 61–83.

Bastian, M., Heymann, S., and Jacomy, M. 2009. Gephi: an open source software for exploring and manipulating networks. *Third Int. AAAI Conf. Weblogs Soc. Media*. 361–362.

Berg, R. G. van den, Miller, J. T., Ugarte, M. L., Kardolus, J. P., Villand, J., Nienhuis, J., and Spooner, D. M. 1998. Collapse of morphological species in the wild potato *Solanum brevicaule* complex (Solanaceae: Sect. *Petota*). *Am. J. Bot.* **85**: 92–109. doi:10.2307/2446559.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., and Das, I. 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* **22**: 148–155. doi:10.1016/j.tree.2006.11.004.

Blaxter, M. L. 2004. The promise of a DNA taxonomy. *Philos. Trans. R. Soc. B Biol. Sci.* **359**: 669–679. doi:10.1098/rstb.2003.1447.

Bonatelli, I. S., Perez, M. F., Peterson, A. T., Taylor, N. P., Zappi, D. C., Machado, M. C., Koch, I., Pires, A. H. C., and Moraes, E. M. 2014. Interglacial microrefugia and diversification of a cactus species complex: Phylogeography and palaeodistributional reconstructions for *Pilosocereus aurisetus* and allies. *Mol. Ecol.* **23**: 3044–3063. doi:10.1111/mec.12780.

Borba, E. L., Shepherd, G. J., Berg, C., and Semir, J. 2002. Floral and vegetative morphometrics of five *Pleurothallis* (Orchidaceae) species: correlation with taxonomy, phylogeny, genetic variability and pollination systems. *Ann. Bot.* **90**: 219–230. doi:10.1093/aob/mcf168.

Borba, E. L., Barbosa, A. R., Melo, M. C., Gontijo, S. L., and Oliveira, H. O. 2011. Mating systems in the Pleurothallidinae (Orchidaceae): evolutionary and systematic implications. *Lankesteriana* **11**: 207–221.

Borges, L. A., Souza, L. G. R., Guerra, M., Machado, I. C., Lewis, G. P., and Lopes, A. V. 2012. Reproductive isolation between diploid and tetraploid cytotypes of *Libidibia ferrea* (= *Caesalpinia ferrea*) (Leguminosae): Ecological and taxonomic implications. *Plant Syst. Evol.* **298**: 1371–1381. doi:10.1007/s00606-012-0643-3.

Borsch, T., Limarino, T. O., and Nee, M. H. 2011. Phylogenetics of the neotropical liana genus *Pedersenia* (Amaranthaceae: Gomphrenoideae) and discovery of a new species from Bolivia based on molecules and morphology. *Willdenowia* **41**: 5–14. doi:10.3372/wi.41.41101.

Breitkopf, H., Onstein, R. E., Cafasso, D., Schlüter, P. M., and Cozzolino, S. 2015. Multiple shifts to different pollinators fuelled rapid diversification in sexually deceptive *Ophrys* orchids. *New Phytol.* **207**: 377–389. doi:10.1111/nph.13219.

Briggs, D., and Walters, S. 1997. *Plant Variation and Evolution*. Cambridge University Press, Cambridge.

Bünger, M. O., Einsehlor, P., Figueiredo, M. L. N., and Stehmann, J. R. 2015. Resolving species delimitations in the *Eugenia involucrata* group (*Eugenia* sect. *Phyllocalyx* –

Myrtaceae) with morphometric analysis. *Syst. Bot.* **40**: 995–1002. doi:10.1600/036364415X690030.

Caddah, M. K., Mayer, J. L. S., Bittrich, V., and Amaral, M. C. E. 2012. Species limits in the *Kielmeyera coriacea* complex (Calophyllaceae) – a multidisciplinary approach. *Bot. J. Linn. Soc.* **168**: 101–115. doi:10.1111/j.1095-8339.2011.01192.x.

Caddah, M. K., Campos, T., Zucchi, M. I., Souza, A. P. de, Bittrich, V., and Amaral, M. C. E. do. 2013. Species boundaries inferred from microsatellite markers in the *Kielmeyera coriacea* complex (Calophyllaceae) and evidence of asymmetric hybridization. *Plant Syst. Evol.* **299**: 731–741. doi:10.1007/s00606-012-0755-9.

Caetano, S., Nusbaumer, L., and Naciri, Y. 2008. Chloroplast and microsatellite markers in *Astronium urundeuva* (Allemão) Engl. and close species of Anacardiaceae: toward the definition of a species complex? *Candollea* **63**: 115–130.

Camargo, A., Morando, M., Avila, L. J., and Sites, J. W. 2012. Species delimitation with ABC and other coalescent-based methods: a test of accuracy with simulations and an empirical example with lizards of the *Liolaemus darwinii* complex (Squamata: Liolaemidae). *Evolution (N. Y).* **66**: 2834–2849.

Canela, M. B. F., Prado-Lopez, N. P., and Wendt, T. 2003. Revision of the *Aechmea multiflora* complex (Bromeliaceae). *Bot. J. Linn. Soc.* **143**: 189–196. doi:10.1046/j.1095-8339.2003.00202.x.

Cannon, C. H., and Lerdau, M. 2015. Variable mating behaviors and the maintenance of tropical biodiversity. *Front. Genet.* **6**: 183. doi:10.3389/fgene.2015.00183.

Cardim, D. C., Carlini-Garcia, L. A., Mondin, M., Martins, M., Veasey, E. A., and Ando, A. 2001. Variabilidade intra-específica em cinco populações de *Oncidium varicosum* Lindl. (Orchidaceae – Oncidiinae) em Minas Gerais. *Rev. Bras. Botânica* **24**: 553–560. doi:10.1590/S0100-84042001000500010.

Cardozo, A. P., Godinho-Temponi, L., Moreira de Andrade, I., Mayo, S. J., and Smidt, E. C. 2015. A morphometric and taxonomic study of *Anthurium augustinum* complex (Araceae), endemic to the Brazilian Atlantic Forest. *Feddes Repert.* **125**: 43–58. doi:10.1002/fedr.201400025.

Carlini-Garcia, L. A., Berg, C. van den, and Martins, P. S. 2002. A morphometric analysis of floral characters in *Miltonia spectabilis* and *Miltonia spectabilis* var. *moreliana* (Maxillarieae: Oncidiinae). *Lindleyana* **17**: 122–129.

Carstens, B. C., Pelletier, T. A., Reid, N. M., and Satler, J. D. 2013. How to fail at species delimitation. *Mol. Ecol.* **22**: 4369–4383. doi:10.1111/mec.12413.

Carstens, B. C., and Satler, J. D. 2013. The carnivorous plant described as *Sarracenia alata* contains two cryptic species. *Biol. J. Linn. Soc.* **109**: 737–746. doi:10.1111/bij.12093.

Catling, P. M., and Porebski, S. 1998. A morphometric evaluation of the subspecies of *Fragaria chiloensis*. *Can. J. Bot.* **76**: 290–297.

Cavallari, M. M., Gimenes, M. A., Billot, C., Torres, R. B., Zucchi, M. I., Cavalheiro, A. J., and Bouvet, J. M. 2010. Population genetic relationships between *Casearia sylvestris* (Salicaceae) varieties occurring sympatrically and allopatrically in different ecosystems in southeast Brazil. *Ann. Bot.* **106**: 627–636. doi:10.1093/aob/mcq151.

Ceolin, G. B., and Miotto, S. T. S. 2012. Combining ecological and morphometrical approaches to increase the resolution within the *Galactia neesii* (Leguminosae) complex. *Plant Syst. Evol.* **298**: 645–652. doi:10.1007/s00606-011-0573-5.

Chan, K. O., Alexander, A. M., Grismer, L. L., Su, Y. C., Grismer, J. L., Quah, E. S. H., and Brown, R. M. 2017. Species delimitation with gene flow: a methodological comparison and population genomics approach to elucidate cryptic species boundaries in Malaysian Torrent Frogs. *Mol. Ecol.* **26**: 5435–5450. doi:10.1111/mec.14296.

Clausen, J., Keck, D. D., and Hiesey, W. M. 1939. The concept of species based on experiment. *Am. J. Bot.* **26**: 103. doi:10.1002/j.1537-2197.1939.tb12875.x.

Conceição, A. S., Queiroz, L. P., Lambert, S. M., Pereira, A. C. S., and Borba, E. L. 2008. Biosystematics of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* (Leguminosae-Caesalpinioideae) based on allozyme and morphometric analyses. *Plant Syst. Evol.* **270**: 183–207. doi:10.1007/s00606-007-0604-4.

Constance, L. 1964. Systematic Botany – an unending synthesis. *Taxon* **13**: 257–273. doi:10.2307/1216193.

Cooley, A. M., Carvallo, G., and Willis, J. H. 2008. Is floral diversification associated with pollinator divergence? Flower shape, flower colour and pollinator preference in Chilean *Mimulus*. *Ann. Bot.* **101**: 641–650. doi:10.1093/aob/mcn014.

Costa, C. B. N., Lambert, S. M., Borba, E. L., and Queiroz, L. P. de. 2007. Post-zygotic reproductive isolation between sympatric taxa in the *Chamaecrista desvauxii* complex (Leguminosae-Caesalpinioideae). *Ann. Bot.* **99**: 625–635. doi:10.1093/aob/mcm012.

Costa, A. F. da, Rodrigues, P. J. F. P., and Wanderley, M. G. L. 2009. Morphometric analysis and taxonomic revision of the *Vriesea paraibica* complex (Bromeliaceae). *Bot. J. Linn. Soc.* **159**: 163–181. doi:10.1111/j.1095-8339.2008.00919.x.

Costea, M., and Stefanović, S. 2010. Evolutionary history and taxonomy of the *Cuscuta umbellata* complex (Convolvulaceae): evidence of extensive hybridization from discordant nuclear and plastid phylogenies. *Taxon* **59**: 1783–1800.

Costea, M., Ruiz, I. G., and Stefanović, S. 2011. Systematics of "horned" dodders: phylogenetic relationships, taxonomy, and two new species within the *Cuscuta chapalana* complex (Convolvulaceae). *Botany* **89**: 715–730. doi:10.1139/b11-049.

Costea, M., García, M. A., Baute, K., and Stefanović, S. 2015. Entangled evolutionary history of *Cuscuta pentagona* clade: A story involving hybridization and Darwin in the Galapagos. *Taxon* **64**: 1225–1242. doi:10.12705/646.7.

Coyne, J., and Orr, H. 2004. *Speciation*. Sinauer Associates, Inc, Sunderland, MA.

Crawford, D. J. 2010. Progenitor-derivative species pairs and plant speciation. *Taxon* **59**: 1413–1423.

Darwin, C. 1882. *The Various Contrivances by Which Orchids are Fertilised by Insects*. J. Murray, London.

de Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. In: Howard, D. J., and S. H. Berlocher, Eds., *Endless Forms: Species and Speciation*. Oxford University Press, New York: pp. 57–75.

de Queiroz, K. 2007. Species concepts and species delimitation. *Syst. Biol.* **56**: 879–886. doi:10.1080/10635150701701083.

Dematteis, M. 2004. Taxonomía del complejo *Vernonia rubricaulis* (Vernonieae, Asteraceae). *Bonplandia* **13**: 5–13.

Díaz, D. M. V. 2013. Multivariate analysis of morphological and anatomical characters of *Calophyllum* (Calophyllaceae) in South America. *Bot. J. Linn. Soc.* **171**: 587–626. doi:10.1111/boj.12012.

Diaz, J., Schmiediche, P., and Austin, D. F. 1996. Polygon of crossability between eleven species of *Ipomoea*: section *Batatas* (Convolvulaceae). *Euphytica* **88**: 189–200. doi:10.1007/BF00023890.

Dobzhansky, T. 1937. *Genetics and the Origin of Species*. Columbia University Press, New York.

Domingos, F. M. C. B., Colli, G. R., Lemmon, A., Lemmon, E. M., and Beheregaray, L. B. 2017. In the shadows: phylogenomics and coalescent species delimitation unveil cryptic diversity in a Cerrado endemic lizard (Squamata: *Tropidurus*). *Mol. Phylogenet. Evol.* **107**: 455–465. doi:10.1016/j.ympev.2016.12.009.

Duminil, J., Caron, H., Scotti, I., Cazal, S. O., and Petit, R. J. 2006. Blind population genetics survey of tropical rainforest trees. *Mol. Ecol.* **15**: 3505–3513. doi:10.1111/j.1365-294X.2006.03040.x.

Elías, G., Sartor, M., and Solís-Neffa, V. G. 2011. Patterns of cytotype variation of *Turnera sidoides* subsp. *pinnatifida* (Turneraceae) in mountain ranges of central Argentina. *J. Plant Res.* **124**: 25–34. doi:10.1007/s10265-010-0347-0.

Ence, D. D., and Carstens, B. C. 2011. SpedeSTEM: A rapid and accurate method for species delimitation. *Mol. Ecol. Resour.* **11**: 473–480. doi:10.1111/j.1755-0998.2010.02947.x.

Esteves, S. M., and Vicentini, A. 2013. Cryptic species in *Pagamea coriacea* sensu lato (Rubiaceae): evidence from morphology, ecology and reproductive behavior in a sympatric context. *Acta Amaz.* **43**: 415–428. doi:10.1590/S0044-59672013000400003.

Farinaccio, M. A., and Mello-Silva, R. 2006. *Oxypetalum gyrophyllum* and *O. oblanceolatum*, new species of Asclepiadoideae (Apocynaceae) from Brazil, and a key for the *O. insigne* group. *Novon* **16**: 235–239. doi:10.3417/1055-3177(2006)16%5b235:OGAOON%5d2.0.CO;2.

Ferreyra, L. I., Vilardi, J. C., Verga, A., López, V., and Saidman, B. O. 2013. Genetic and morphometric markers are able to differentiate three morphotypes belonging to Section *Algarobia* of genus *Prosopis* (Leguminosae, Mimosoideae). *Plant Syst. Evol.* **299**: 1157–1173. doi:10.1007/s00606-013-0786-x.

Filardi, F. L. R., Lima, H. C. de, Klitgaard, B. B., and Sartori, Â. L. B. 2013. Taxonomy and nomenclature of the neotropical *Machaerium hirtum* complex (Leguminosae, Papilionoideae). *Brittonia* **65**: 154–170. doi:10.1007/s12228-012-9276-7.

Firetti-Leggieri, F., Ribeiro da Costa, I., Lohmann, L. G., Semir, J., and Forni-Martins, E. R. 2011. Chromosome studies in Bignonieae (Bignoniaceae): the first record of polyploidy in *Anemopaegma*. *Cytologia* **76**: 185–191. doi:10.1508/cytologia.76.185.

Flot, J. F., Couloux, A., and Tillier, S. 2010. Haplowebs as a graphical tool for delimiting species: a revival of Doyle's "field for recombination" approach and its application to the coral genus *Pocillopora* in Clipperton. *BMC Evol. Biol.* **10**: 372. doi:10.1186/1471-2148-10-372.

Flot, J. F. 2015. Species delimitation's coming of age. *Syst. Biol.* **64**: 897–899. doi:10.1093/sysbio/syv071.

Folk, R. A., and Freudenstein, J. V. 2015. "Sky islands" in the eastern U.S.A.? — Strong phylogenetic structure in the *Heuchera parviflo*ra group (Saxifragaceae). *Taxon* **64**: 254–271. doi:10.12705/642.3.

Forzza, R. C., Baumgratz, J. F. A., Bicudo, C. E. M., Canhos, D. A. L., Carvalho, A. A., Coelho, M. A. N., Costa, A. F., Costa, D. P., Hopkins, M. G., Leitman, P. M., Lohmann, L. G., Lughadha, E. N., Maia, L. C., Martinelli, G., Menezes, M., Morim, M. P., Peixoto, A. L., Pirani, J. R., Prado, J., Queiroz, L. P., Souza, S., Souza, V. C., Stehmann, J. R., Sylvestre, L. S., Walter, B. M. T., Zappi, D. C. 2012. New Brazilian floristic list highlights conservation challenges. *Bioscience* **62**: 39–45. doi:10.1525/bio.2012.62.1.8.

Franks, S. J., Weber, J. J., and Aitken, S. N. 2014. Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evol. Appl.* **7**: 123–139. doi:10.1111/eva.12112.

Freitas-Mansano, V., and Azevedo-Tozzi, A. M. G. 2001. *Swartzia* Schreb. (Leguminosae: Papilionoideae: Swartzieae): a taxonomic study of the *Swartzia acutifolia* complex including a new name and a new species from Southeastern Brazil. *Kew Bull.* **56**: 917. doi:10.2307/4119303.

Freudenstein, J. V., Broe, M. B., Folk, R. A., and Sinn, B. T. 2017. Biodiversity and the species concept – lineages are not enough. *Syst. Biol.* **66**: 644–656.

Fujita, M. K., Leaché, A. D., Burbrink, F. T., McGuire, J. A., and Moritz, C. 2012. Coalescent-based species delimitation in an integrative taxonomy. *Trends Ecol. Evol.* **27**: 480–488. doi:10.1016/j.tree.2012.04.012.

Gagnon, E., Hughes, C. E., Lewis, G. P., and Bruneau, A. 2015. A new cryptic species in a new cryptic genus in the *Caesalpinia* group (Leguminosae) from the seasonally dry inter-Andean valleys of South America. *Taxon* **64**: 468–490. doi:10.12705/643.6.

Gentry, A. H. 1981. Distributional patterns and an additional species of the *Passiflora vitifolia* complex: Amazonian species diversity due to edaphically differentiated communities. *Plant Syst. Evol.* **137**: 95–105. doi:10.1007/BF00983208.

Giussani, L. M., Martinez, A. J., and Collantes, M. B. 1996. Morphological variation associated with the environment in four dioecious Patagonian *Poa* species: the *Poa rigidifolia* complex. *Can. J. Bot.* **772**: 762–772. doi:10.1139/b96-095.

Gomes-da-Silva, J., and da Costa, A. F. 2011. A taxonomic revision of *Vriesea corcovadensis* group (Bromeliaceae: Tillandsioideae) with description of two new species. *Syst. Bot.* **36**: 291–309. doi:10.1600/036364411X569499.

Gomes-da-Silva, J., Vargens, F. A. C., Arruda, R. C. O., and da Costa, A. F. 2012. A morphological cladistic analysis of the *Vriesea corcovadensis* group (Bromeliaceae: Tillandsiodeae), with anatomical descriptions: new evidence of the non-monophyly of the genus. *Syst. Bot.* **37**: 641–654. doi:10.1600/036364412X648599.

Gonella, P. M., Rivadavia, F., and Sano, P. T. 2012. Re-establishment of *Drosera spiralis* (Droseraceae), and a new circumscription of *D. graminifolia*. *Phytotaxa* **75**: 43–57. doi:10.11646/phytotaxa.75.1.4.

Gonella, P. M., Rivadavia, F., Sano, P. T., and Fleischmann, A. 2014. Exhuming Saint-Hilaire: revision of the *Drosera villosa* complex (Droseraceae) supports 200 year-old neglected species concepts. *Phytotaxa* **156**: 1–40. doi:10.11646/phytotaxa.156.1.1.

Govaerts, R. 2001. How many species of seed plants are there? *Taxon* **50**: 1085–1090. doi:10.2307/1224723.

Graham, S. A., and Cavalcanti, T. B. 2013. Taxonomic revision of *Cuphea* sect. *Euandra* subsect. *Oidemation* (Lythraceae). *Phytotaxa* **113**: 1–86. doi:10.11646/phytotaxa.113.1.1.

Grombone-Guaratini, M. T., Semir, J., and Solferini, V. N. 2005. Low allozymic variation in the *Bidens pilosa* L. complex (Asteraceae). *Biochem. Genet.* **43**: 335–345. doi:10.1007/s10528-005-6774-2.

Grombone-Guaratini, M. T., Solferini, V. N., Mansanares, M. E., and Semir, J. 2006. Chromosomal studies of three species of *Bidens* L. (Asteraceae). *Caryologia* **59**: 14–18. doi:10.1080/00087114.2006.10797892.

Guillot, G., Renaud, S., Ledevin, R., Michaux, J., and Claude, J. 2012. A unifying model for the analysis of phenotypic, genetic, and geographic data. *Syst. Biol.* **61**: 897–911. doi:10.1093/sysbio/sys038.

Hagen, J. B. 1984. Experimentalists and naturalists in twentieth-century botany: experimental taxonomy, 1920–1950. *J. Hist. Biol.* **17**: 249–270. doi:10.1007/BF00143734.

Hausdorf, B. 2011. Progress toward a general species concept. *Evolution* **65**: 923–931. doi:10.1111/j.1558-5646.2011.01231.x.

Heath, T. A., Hedtke, S. M., and Hillis, D. M. 2008. Taxon sampling and the accuracy of phylogenetic analyses. *J. Syst. Evol.* **46**: 239–257.

Heled, J., and Drummond, A. J. 2010. Bayesian inference of species trees from multilocus data. *Mol. Biol. Evol.* **27**: 570–580. doi:10.1093/molbev/msp274.

Henderson, A., and Martins, R. 2002. Classification of specimens in the *Geonoma stricta* (Palmae) complex: the problem of leaf size and shape. *Brittonia* **54**: 202–212. doi:10.1663/0007-196X(2002)054%5b0202:COSITG%5d2.0.CO;2.

Hennig, W. 1966. *Phylogenetic Systematics*. University of Illinois Press, Urbana.

Herrera, S., and Shank, T. M. 2016. RAD sequencing enables unprecedented phylogenetic resolution and objective species delimitation in recalcitrant divergent taxa. *Mol. Phylogenet. Evol.* **100**: 70–79. doi:10.1016/j.ympev.2016.03.010.

Hoban, S., Kelley, J. L., Lotterhos, K. E., Antolin, M. F., Bradburd, G., Lowry, D. B., Poss, M. L., Reed, L. K., Storfer, A., and Whitlock, M. C. 2016. Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. *Am. Nat.* **188**: 379–397. doi:10.1086/688018.

Holanda, A. S. S. de, Vicentini, A., Hopkins, M. J. G., and Zartman, C. E. 2015. Phenotypic differences are not explained by pre-zygotic reproductive barriers in sympatric varieties of the *Humiria balsamifera* complex (Humiriaceae). *Plant Syst. Evol.* **301**: 1767–1779. doi:10.1007/s00606-014-1191-9.

Huang, J. P., and Knowles, L. L. 2016. The species versus subspecies conundrum: quantitative delimitation from integrating multiple data types within a single Bayesian approach in hercules beetles. *Syst. Biol.* **65**: 685–699. doi:10.1093/sysbio/syv119.

haHughes, C. E., Pennington, R. T., and Antonelli, A. 2013. Neotropical plant evolution: assembling the big picture. *Bot. J. Linn. Soc.* **171**: 1–18. doi:10.1111/boj.12006.

Ispizúa, V. N., Camadro, E. L., and Clausen, A. M. 2015. Variation patterns in natural populations of wild potatoes along Quebrada de Inca Cueva, Northwestern Argentina. *Genet. Resour. Crop Evol.* **62**: 235–253. doi:10.1007/s10722-014-0149-7.

Jackson, N. D., Morales, A. E., Carstens, B. C., and O'Meara, B. C. 2017. PHRAPL: phylogeographic inference using approximate likelihoods. *Syst. Biol.* **66**: 1045–1053. doi:10.1093/sysbio/syx001.

Jones, G., Aydin, Z., and Oxelman, B. 2015. DISSECT: An assignment-free Bayesian discovery method for species delimitation under the multispecies coalescent. *Bioinformatics* **31**: 991–998. doi:10.1093/bioinformatics/btu770.

Kingman, J. F. C. 1982. The coalescent. *Stoch. Process. Their Appl.* **13**: 235–248. doi:10.1016/0304-4149(82)90011-4.

Knowles, L. L., and Carstens, B. C. 2007. Delimiting species without monophyletic gene trees. *Syst. Biol.* **56**: 887–895. doi:10.1080/10635150701701091.

Lagomarsino, L. P., Condamine, F. L., Antonelli, A., Mulch, A., and Davis, C. C. 2016. The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). *New Phytol.* **210**: 1430–1442. doi:10.1111/nph.13920.

Lammers, T. G., and Hensold, N. 1992. Chromosome numbers of Campanulaceae II. The *Lobelia-Tupa* complex of Chile. *Am. J. Bot.* **79**: 585–588. doi:10.1002/j.1537-2197.1992.tb14596.x.

Leal, B. S. S., Chaves, C. J. N., Koehler, S., and Borba, E. L. 2016a. When hybrids are not hybrids: a case study of a putative hybrid zone between *Cattleya coccinea* and *C. brevipedunculata* (Orchidaceae). *Bot. J. Linn. Soc.* **181**: 621–639. doi:10.1111/boj.12437.

Leal, B. S. S., Palma-Silva, C., and Pinheiro, F. 2016b. Phylogeographic studies depict the role of space and time scales of plant speciation in a highly diverse neotropical region. *Crit. Rev. Plant Sci.* **35**: 215–230. doi:10.1080/07352689.2016.1254494.

Levin, D. A. 1978. The origin of isolating mechanisms in flowering plants. In: *Evolutionary Biology*. Springer US, Boston, MA. pp. 185–317.

Lima, D. F., Mauad, A. V. S., Silva-Pereira, V., Smidt, E. C., and Goldenberg, R. 2015. Species boundaries inferred from ISSR markers in the *Myrcia laruotteana* complex (Myrtaceae). *Plant Syst. Evol.* **301**: 353–363. doi:10.1007/s00606-014-1078-9.

Lipscomb, D., Platnick, N., and Wheeler, Q. 2003. The intellectual content of taxonomy: a comment on DNA taxonomy. *Trends Ecol. Evol.* **18**: 65–66. doi:10.1016/S0169-5347(02)00060-5.

Lohmann, L. G. 2006. Untangling the phylogeny of neotropical lianas (Bignonieae, Bignoniaceae). *Am. J. Bot.* **93**: 304–318. doi:10.3732/ajb.93.2.304.

Lombardi, J. A. 2006. A new species of *Tontelea* from Amazonian Peru and Ecuador, and notes on the *Tontelea attenuata* species group (Celastraceae, Hippocrateoideae). *Brittonia* **58**: 52–58. doi:10.1663/0007-196X(2006)58%5b52:ANSOTF%5d2.0.CO;2.

Longo, D., Lorenz-Lemke, A. P., Mäder, G., Bonatto, S. L., and Freitas, L. B. 2014. Phylogeography of the *Petunia integrifolia* complex in Southern Brazil. *Bot. J. Linn. Soc.* **174**: 199–213. doi:10.1111/boj.12115.

López-Sepúlveda, P., Tremetsberger, K., Ortiz, M. Á., Baeza, C. M., Peñailillo, P., and Stuessy, T. F. 2013a. Radiation of the *Hypochaeris apargioides* complex (Asteraceae: Cichorieae) of southern South America. *Taxon* **62**: 550–564. doi:10.12705/623.14.

López-Sepúlveda, P., Takayama, K., Greimler, J., Peñailillo, P., Crawford, D. J., Baeza, M., Ruiz, E., Kohl, G., Tremetsberger, K., Gatica, A., Letelier, L., Novoa, P., Novak, J., and Stuessy, T. F. 2013b. Genetic variation (Aflps and nuclear microsatellites) in two anagenetically derived endemic species of myrceugenia (Myrtaceae) on the juan fernández

Islands, Chile. *Am. J. Bot.* **100**: 722–734. doi:10.3732/ajb.1200541.

López-Sepúlveda, P., Takayama, K., Greimler, J., Crawford, D. J., Peñailillo, P., Baeza, M., Ruiz, E., Kohl, G., Tremetsberger, K., Gatica, A., Letelier, L., Novoa, P., Novak, J., and Stuessy, T. F. 2015a. Speciation and Biogeography of *Erigeron* (Asteraceae) in the Juan Fernández Archipelago, Chile, based on AFLPs and SSRs. *Syst. Bot.* **40**: 888–899. doi:10.1600/036364415X689311.

López-Sepúlveda, P., Takayama, K., Greimler, J., Crawford, D. J., Peñailillo, P., Baeza, M., Ruiz, E., Kohl, G., Tremetsberger, K., Gatica, A., Letelier, L., Novoa, P., Novak, J., and Stuessy, T. F. 2015b. Progressive migration and anagenesis in *Drimys confertifolia* of the Juan Fernández Archipelago, Chile. *J. Plant Res.* **128**: 73–90. doi:10.1007/s10265-014-0666-7.

López, M. G., Wulff, A. F., Wulff, A. F., Xifreda, C. C., and Xifreda, C. C. 2002. Chromosome contribution to Andean polyploid species of *Senecio* (Asteraceae), from Argentina. *Caryologia* **55**: 27–35. doi:10.1080/00087114.2002.10589255.

López, P., Tremetsberger, K., Kohl, G., and Stuessy, T. 2012. Progenitor–derivative speciation in *Pozoa* (Apiaceae, Azorelloideae) of the southern Andes. *Ann. Bot.* **109**: 351–363. doi:10.1093/aob/mcr291.

Lousada, J. M., Lovato, M. B., and Borba, E. L. 2013. High genetic divergence and low genetic variability in disjunct populations of the endemic *Vellozia compacta* (Velloziaceae) occurring in two edaphic environments of Brazilian campos rupestres. *Rev. Bras. Bot.* **36**: 45–53. doi:10.1007/s40415-013-0001-x.

Luckow, M., and Hortorium, L. H. B. 1995. Species concepts: assumptions, methods, and applications. *Syst. Bot.* **20**: 589–605. doi:10.2307/2419812.

Marques, I., Draper, D., Riofrío, L., and Naranjo, C. 2014. Multiple hybridization events, polyploidy and low postmating isolation entangle the evolution of neotropical species of *Epidendrum* (Orchidaceae). *BMC Evol. Biol.* **14**: 20. doi:10.1186/1471-2148-14-20.

Marquete, R., and Mansano, V. F. 2012. Taxonomic rrevision of the *Casearia ulmifolia* complex (Salicaceae). *Novon* **22**: 196–206. doi:10.3417/2011011.

Mayden, R. L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: Claridge, M. F., H. A. Dawah, and M. R. Wilson, Eds., *Species: the Units of Biodiversity*. Chapman and Hall, London. pp. 381–424.

Mayr, E. 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.

Melo, M. C., and Borba, E. L. 2011. Morphological variability in rupicolous species of the *Acianthera prolifera* complex (Orchidaceae) occurring in Southeastern Brazil. *Plant Syst. Evol.* **293**: 135–145. doi:10.1007/s00606-011-0435-1.

Meneguzzo, T. E. C., Baumgratz, J. F. A., and Berg, C. van den. 2015. Taxonomic studies in the *Aganisia* complex (Orchidaceae, Zygopetalinae). *Phytotaxa* **238**: 1. doi:10.11646/phytotaxa.238.1.1.

Miller, J. T., and Spooner, D. M. 1999. Collapse of species boundaries in the wild potato *Solanum brevicaule* complex (Solanaceae, *S.* sect. *Petota*): molecular data. *Plant Syst. Evol.* **214**: 103–130. doi:10.1007/BF00985734.

Miz, R. B., Mentz, L. A., and Souza-Chies, T. T. 2008. Overview of the phylogenetic relationships of some southern Brazilian species from section *Torva* and related sections of "spiny *Solanum*" (*Solanum* subgenus *Leptostemonum*, Solanaceae). *Genetica* **132**: 143–158. doi:10.1007/s10709-007-9156-3.

Moraes, E. M., Perez, M. F., Téo, M. F., Zappi, D. C., Taylor, N. P., and Machado, M. C. 2012. Cross-species amplification of microsatellites reveals incongruence in the molecular variation and taxonomic limits of the *Pilosocereus aurisetus* group (Cactaceae). *Genetica* **140**: 277–285. doi:10.1007/s10709-012-9678-1.

Morales, A. E., and Carstens, B. C. 2018. Evidence that *Myotis lucifugus* "subspecies" are five non-sister species, despite gene flow. *Syst. Biol.* **Ahead of print**: 1–14.

Moreno, E. M. S., Speranza, P. R., Roggero-Luque, J. M., and Solís-Neffa, V. G. 2015. Natural hybridization among subspecies of *Turnera sidoides* L. (Passifloraceae) revealed by morphological and genetic evidence. *Plant Syst. Evol.* **301**: 883–892. doi:10.1007/s00606-014-1122-9.

Naciri, Y., and Linder, H. P. 2015. Species delimitation and relationships: the dance of the seven veils. *Taxon* **64**: 3–16. doi:10.12705/641.24.

Neri, J., Wendt, T., Leles, B., Santos, M. F., and Palma-Silva, C. 2017. Variation in reproductive systems facilitates species boundaries of sympatric *Vriesea* (Bromeliaceae). *Bot. J. Linn. Soc.* **184**: 272–279. doi:10.1093/botlinnean/box026.

Neri, J., Wendt, T., and Palma-Silva, C. 2018. Natural hybridization and genetic and morphological variation between two epiphytic bromeliads. *AoB Plants* **10**: plx061. doi:10.1093/aobpla/plx061.

Norrmann, G. A. 2009. Natural hybridization in the *Andropogon lateralis* complex (Andropogoneae, Poaceae) and its impact on taxonomic literature. *Bot. J. Linn. Soc.* **159**: 136–154. doi:10.1111/j.1095-8339.2008.00904.x.

Nosil, P. 2008. Speciation with gene flow could be common. *Mol. Ecol.* **17**: 2103–2106. doi:10.1111/j.1365-294X.2008.03715.x.

Nosil, P. 2012. *Ecological Speciation*. Oxford University Press, Oxford.

O'Leary, N., Denham, S. S., Salimena, F., and Múlgura, M. E. 2012. Species delimitation in *Lippia* section *Goniostachyum* (Verbenaceae) using the phylogenetic species concept. *Bot. J. Linn. Soc.* **170**: 197–219. doi:10.1111/j.1095-8339.2012.01291.x.

O'Meara, B. C. 2010. New heuristic methods for joint species delimitation and species tree inference. *Syst. Biol.* **59**: 59–73. doi:10.1093/sysbio/syp077.

Oliveira, M. I. U., Landrum, L. R., Oliveira, R. P., and Funch, L. S. 2013. A new species of *Campomanesia* (Myrtaceae) from Bahia, Brazil, and its relationships with the *C. xanthocarpa* complex. *Phytotaxa* **149**: 19–26. doi:10.11646/phytotaxa.149.1.3.

Oliveira, R. C., and Valls, J. F. M. 2002. Taxonomia de *Paspalum* L., grupo *Linearia* (Gramineae – Paniceae) do Brasil. *Rev. Bras. Botânica* **25**: 371–389. doi:10.1590/S0100-84042002012000001.

Oliveira, R. C. de, Valls, J. F. M., Gomes, S. M., Fagg, C. W., and Toniolo Pozzobon, M. 2015. A new species of *Paspalum*, Notata group (Poaceae, Paspaleae), from the Cerrado biome, Brazil: description, chromosome number, and leaf

blade anatomy. *Phytotaxa* **203**: 159–168. doi:10.11646/phytotaxa.203.2.5.

Oliveira, R. P., Borba, E. L., Longhi-Wagner, H. M., Pereira, A. C. S., and Lambert, S. M. 2008a. Genetic and morphological variability in the *Raddia brasiliensis* complex (Poaceae: Bambusoideae). *Plant Syst. Evol.* **274**: 25–35. doi:10.1007/s00606-008-0034-y.

Oliveira, R. P., Borba, E. L., and Longhi-Wagner, H. M. 2008b. Morphometrics of herbaceous bamboos of the *Raddia brasiliensis* complex (Poaceae — Bambusoideae): implications for the taxonomy of the genus and new species from Brazil. *Plant Syst. Evol.* **270**: 159–182. doi:10.1007/s00606-007-0614-2.

Oliveira, V. M., Forni-Martins, E. R., and Semir, J. 2007. Cytotaxonomy of species of *Vernonia*, section *Lepidaploa*, group Axilliflorae (Asteraceae, Vernonieae). *Bot. J. Linn. Soc.* **154**: 99–108. doi:10.1111/j.1095-8339.2007.00633.x.

Padial, J. M., and de la Riva, I. 2010. A response to recent proposals for integrative taxonomy. *Biol. J. Linn. Soc.* **101**: 747–756. doi:10.1111/j.1095-8312.2010.01528.x.

Padial, J. M., Miralles, A., de la Riva, I., and Vences, M. 2010. The integrative future of taxonomy. *Front. Zool.* **7**: 1–14. doi:10.1186/1742-9994-7-16.

Palacios, R. A., Burghardt, A. D., Frías-Hernández, J. T., Olalde-Portugal, V., Grados, N., Alban, L., and Martínez-de la Vega, O. 2012. Comparative study (AFLP and morphology) of three species of *Prosopis* of the Section *Algarobia*: *P. juliflora, P. pallida*, and *P. limensis*. Evidence for resolution of the "*P. pallida–P. juliflora* complex." *Plant Syst. Evol.* **298**: 165–171. doi:10.1007/s00606-011-0535-y.

Palma-Silva, C., Wendt, T., Pinheiro, F., Barbará, T., Fay, M. F., Cozzolino, S., and Lexer, C. 2011. Sympatric bromeliad species (*Pitcairnia* spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. *Mol. Ecol.* **20**: 3185–3201. doi:10.1111/j.1365-294X.2011.05143.x.

Palma-Silva, C., Cozzolino, S., Paggi, G. M., Lexer, C., and Wendt, T. 2015. Mating system variation and assortative mating of sympatric bromeliads (*Pitcairnia* spp.) endemic to neotropical inselbergs. *Am. J. Bot.* **102**: 758–764. doi:10.3732/ajb.1400513.

Paula-Souza, J., Pirani, J. R., and Feliciano, C. D. 2011. Taxonomic and geographic notes on the *Hybanthus lanatus* (A. St.-Hil.) Baill. complex (Violaceae). *Candollea* **66**: 367–375. doi:10.15553/c2011v662a14.

Pelegrin, C. M. G. de, Longhi-Wagner, H. M., and Oliveira, P. L. 2009. Anatomia foliar como subsídio à taxonomia de espécies do complexo *Briza* L. (Poaceae: Pooideae: Poeae). *Acta Bot. Brasilica* **23**: 666–680. doi:10.1590/S0102-33062009000300006.

Peraza-Flores, L. N., Fernández-Concha, G. C., and Romero-González, G. A. 2011. Taxonomic notes in American *Polystachya* (Orchidaceae): the identity of *P. foliosa* (Hook.) Rchb.f. and the reestablishment of *P. caracasana* Rchb.f. *J. Torrey Bot. Soc.* **138**: 366–380. doi:10.3159/TORREY-D-11-00029.1.

Pereira, A. C. S., Borba, E. L., and Giulietti, A. M. 2007. Genetic and morphological variability of the endangered *Syngonanthus mucugensis* Giul. (Eriocaulaceae) from the Chapada Diamantina, Brazil: implications for conservation and taxonomy. *Bot. J. Linn. Soc.* **153**: 401–416. doi:10.1111/j.1095-8339.2007.00624.x.

Perret, M., Chautems, A., Spichiger, R., Barraclough, T. G., and Savolainen, V. 2007. The geographical pattern of speciation and floral diversification in the neotropics: The tribe Sinningieae (Gesneriaceae) as a case study. *Evolution* **61**: 1641–1660. doi:10.1111/j.1558-5646.2007.00136.x.

Pessoa, E. M., Alves, M., Alves-Araújo, A., Palma-Silva, C., and Pinheiro, F. 2012. Integrating different tools to disentangle species complexes: a case study in *Epidendrum* (Orchidaceae). *Taxon* **61**: 721–734.

Petit, R. J., and Excoffier, L. 2009. Gene flow and species delimitation. *Trends Ecol. Evol.* **24**: 386–393. doi:10.1016/j.tree.2009.02.011.

Pinheiro, F., and Barros, F. de. 2007. Morphometric analysis of *Epidendrum secundum* (Orchidaceae) in southeastern Brazil. *Nord. J. Bot.* **25**: 129–136. doi:10.1111/j.0107-055X.2007.00010.x.

Pinheiro, F., and Barros, F. de. 2009. Morphometric analysis of the *Brasiliorchis picta* complex (Orchidaceae). *Rev. Bras. Botânica* **32**: 11–21.

Pinheiro, F., Koehler, S., Corrêa, A. M., Salatino, M. L. F., Salatino, A., and Barros, F. de. 2009. Phylogenetic relationships and infrageneric classification of *Epidendrum* subgenus *Amphiglottium* (Laeliinae, Orchidaceae). *Plant Syst. Evol.* **283**: 165–177. doi:10.1007/s00606-009-0224-2.

Pinheiro, F., Cozzolino, S., Barros, F. de, Gouveia, T. M. Z. M., Suzuki, R. M., Fay, M. F., and Palma-Silva, C. 2013. Phylogeographic structure and outbreeding depression reveal early stages of reproductive isolation in the neotropical orchid *Epidendrum denticulatum*. *Evol. Int. J. Org. Evol.* **67**: 2024–39. doi:10.1111/evo.12085.

Pinheiro, F., Cafasso, D., Cozzolino, S., and Scopece, G. 2015. Transitions between self-compatibility and self-incompatibility and the evolution of reproductive isolation in the large and diverse tropical genus *Dendrobium* (Orchidaceae). *Ann. Bot.* **116**: 457–467. doi:10.1093/aob/mcv057.

Pinheiro, F., Gouveia, T. M. Z. de M., Cozzolino, S., Cafasso, D., Cardoso-Gustavson, P., Suzuki, R. M., and Palma-Silva, C. 2016. Strong but permeable barriers to gene exchange between sister species of *Epidendrum*. *Am. J. Bot.* **103**: 1472–1482. doi:10.3732/ajb.1600064.

Pinheiro, M., and Miotto, S. T. S. 2005. *Lupinus reitzii* (Fabaceae-Faboideae), a new species of the *Lupinus lanatus* complex from southern Brazil. *Novon* **15**: 346–349.

Planchuelo, A. M., and Dunn, D. B. 1989. Two new species of the *Lupinus lanatus* complex. *Ann. Missouri Bot. Gard.* **76**: 303. doi:10.2307/2399349.

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W. D., and Vogler, A. P. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Syst. Biol.* **55**: 595–609. doi:10.1080/10635150600852011.

Porter-Utley, K. 2014. A revision of *Passiflora* L. subgenus *Decaloba* (DC.) Rchb. supersection *Cieca* (Medik.) J. M. MacDougal & Feuillet (Passifloraceae). *PhytoKeys* **43**: 1–224. doi:10.3897/phytokeys.43.7804.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–59.

Prohens, J., Anderson, G. J., Blanca, J. M., Cañizares, J., Zuriaga, E., and Nuez, F. 2006. The implications of AFLP data for the systematics of the wild species of *Solanum* section

Basarthrum. *Syst. Bot.* **31**: 208–216. doi:10.1600/036364406775971822.

Puillandre, N., Modica, M. V., Zhang, Y., Sirovich, L., Boisselier, M. C., Cruaud, C., Holford, M., and Samadi, S. 2012. Large-scale species delimitation method for hyperdiverse groups. *Mol. Ecol.* **21**: 2671–2691. doi:10.1111/j.1365-294X.2012.05559.x.

Ribeiro, P. L., Borba, E. L., Smidt, E. C., Lambert, S. M., Schnadelbach, A. S., and Berg, C. van den. 2008. Genetic and morphological variation in the *Bulbophyllum exaltatum* (Orchidaceae) complex occurring in the Brazilian "campos rupestres": Implications for taxonomy and biogeography. *Plant Syst. Evol.* **270**: 109–137. doi:10.1007/s00606-007-0603-5.

Rieseberg, L. H., Church, S. A., and Morjan, C. L. 2004. Integration of populations and differentiation of species. *New Phytol.* **161**: 59–69. doi:10.1046/j.1469-8137.2003.00933.x.

Ritz, C. M., Martins, L., Mecklenburg, R., Goremykin, V., and Hellwig, F. H. 2007. The molecular phylogeny of *Rebutia* (Cactaceae) and its allies demonstrates the influence of paleogeography on the evolution of South American mountain cacti. *Am. J. Bot.* **94**: 1321–1332. doi:10.3732/ajb.94.8.1321.

Rivadavia, F., Gonella, P. M., Sano, P. T., and Fleischmann, A. 2014. Elucidating the controversial *Drosera montana* complex (Droseraceae): a taxonomic revision. *Phytotaxa* **172**: 141–175. doi:10.11646/phytotaxa.172.3.1.

Rodrigues, J. F., Berg, C., Abreu, A. G., Novello, M., Veasey, E. A., Oliveira, G. C. X., and Koehler, S. 2015. Species delimitation of *Cattleya coccinea* and *C. mantiqueirae* (Orchidaceae): insights from phylogenetic and population genetics analyses. *Plant Syst. Evol.* **301**: 1345–1359. doi:10.1007/s00606-014-1156-z.

Roncal, J., Francisco-Ortega, J., and Lewis, C. E. 2007. An evaluation of the taxonomic distinctness of two *Geonoma macrostachys* (Arecaceae) varieties based on intersimple sequence repeat (ISSR) variation. *Bot. J. Linn. Soc.* **153**: 381–392. doi:10.1111/j.1095-8339.2007.00619.x.

Sampaio, J. A. T., Paggi, G. M., Zanella, C. M., Bruxel, M., Palma-Silva, C., Goetze, M., Büttow, M. V., and Bered, F. 2012. Inbreeding depression in *Vriesea gigantea*, a perennial bromeliad from southern Brazil. *Bot. J. Linn. Soc.* **169**: 312–319. doi:10.1111/j.1095-8339.2012.01240.x.

Särkinen, T., Barboza, G. E., and Knapp, S. 2015. True black nightshades: phylogeny and delimitation of the morelloid clade of *Solanum*. *Taxon* **64**: 945–958. doi:10.12705/645.5.

Sawyer, N. W., and Rojas, C. E. B. 1998. Morphological analysis of three equivocal sibling species of *Deprea* (Solanaceae). *Brittonia* **50**: 524. doi:10.2307/2807764.

Schmidt-Lebuhn, A. N. 2007. Using amplified fragment length polymorphism (AFLP) to unravel species relationships and delimitations in *Minthostachys* (Labiatae). *Bot. J. Linn. Soc.* **153**: 9–19. doi:10.1111/j.1095-8339.2007.00588.x.

Schulman, L., Koivunen, H., and Ruokolainen, K. 2004. Spatio-ecological niche segregation of two sympatric species of *Clidemia* (Melastomataceae) in western Amazonian non-flooded rainforests. *Folia Geobot.* **39**: 143–160. doi:10.1007/BF02805243.

Scopece, G., Lexer, C., Widmer, A., and Cozzolino, S. 2010. Polymorphism of postmating reproductive isolation within plant species. *Taxon* **59**: 1367–1374.

Scotti-Saintagne, C., Dick, C. W., Caron, H., Vendramin, G. G., Guichoux, E., Buonamici, A., Duret, C., Sire, P., Valencia, R., Lemes, M. R., Gribel, R., and Scotti, I. 2013. Phylogeography of a species complex of lowland Neotropical rain forest trees (*Carapa*, Meliaceae). *J. Biogeogr.* **40**: 676–692. doi:10.1111/j.1365-2699.2011.02678.x.

Scrivanti, L. R., Caponio, I., Anton, A. M., and Norrmann, G. A. 2010. Chromosome number in South American species of *Bothriochloa* (Poaceae: Andropogoneae) and evolutionary history of the genus. *Plant Biol.* **12**: 910–916. doi:10.1111/j.1438-8677.2009.00314.x.

Seehausen, O., Butlin, R. K., Keller, I., Wagner, C. E., Boughman, J. W., Hohenlohe, P. A., Peichel, C. L., Saetre, G.-P., Bank, C., Brännström, Å., Brelsford, A., Clarkson, C. S., Eroukhmanoff, F., Feder, J. L., Fischer, M. C., Foote, A. D., Franchini, P., Jiggins, C. D., Jones, F. C., Lindholm, A. K., Lucek, K., Maan, M. E., Marques, D. A., Martin, S. H., Matthews, B., Meier, J. I., Möst, M., Nachman, M. W., Nonaka, E., Rennison, D. J., Schwarzer, J., Watson, E. T., Westram, A. M., and Widmer, A. 2014. Genomics and the origin of species. *Nat. Rev. Genet.* **15**: 176–192. doi:10.1038/nrg3644.

Seijo, J. G., and Fernández, A. 2003. Karyotype analysis and chromosome evolution in South American species of *Lathyrus* (Leguminosae). *Am. J. Bot.* **90**: 980–987. doi:10.3732/ajb.90.7.980.

Semir, J., Loeuille, B., and Monge, M. 2014. The *Lychnophora granmogolensis* (Asteraceae-Vernonieae) species complex: two new species and comments on the identity of *Lychnophora granmogolensis*. *Syst. Bot.* **39**: 988–996. doi:10.1600/036364414X682193.

Shaffer, H. B., and Thomson, R. C. 2007. Delimiting species in recent radiations. *Syst. Biol.* **56**: 896–906. doi:10.1080/10635150701772563.

Silberbauer-Gottsberger, I., Gottsberger, G., and Ehrendorfer, F. 1992. Hybrid speciation and radiation in the neotropical woody genus *Tocoyena* (Rubiaceae). *Plant Syst. Evol.* **181**: 143–169. doi:10.1007/BF00937441.

Silvestro, D., Zizka, G., and Schulte, K. 2014. Disentangling the effects of key innovations on the diversification of Bromelioideae (Bromeliaceae). *Evolution* **68**: 163–175. doi:10.1111/evo.12236.

Simon, M. F., Grether, R., Queiroz, L. P. de, Särkinen, T. E., Dutra, V. F., and Hughes, C. E. 2011. The evolutionary history of *Mimosa* (Leguminosae): toward a phylogeny of the sensitive plants. *Am. J. Bot.* **98**: 1201–1221. doi:10.3732/ajb.1000520.

Sites, J. W., and Marshall, J. C. 2003. Delimiting species: a renaissance issue in systematic biology. *Trends Ecol. Evol.* **18**: 462–470. doi:10.1016/S0169-5347(03)00184-8.

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

Solís-Lemus, C., Knowles, L. L., and Ané, C. 2015. Bayesian species delimitation combining multiple genes and traits in

a unified framework. *Evolution* **69**: 492–507. doi:10.1111/evo.12582.

Solís-Neffa, V. G., and Fernández, A. 2001. Cytogeography of the South American *Turnera sidoides* L. complex (Turneraceae, Leiocarpae). *Bot. J. Linn. Soc.* **137**: 189–196. doi:10.1006/bojl.2001.0479.

Solís-Neffa, V. G., and Fernández, A. 2002. Karyotypic studies in *Turnera sidoides* complex (Turneraceae, Leiocarpae). *Am. J. Bot.* **89**: 551–558. doi:10.3732/ajb.89.4.551.

Solís-Neffa, V. G., Panseri, A. F., Reynoso, W., and Seijo, J. G. 2004. Variación del color de flores y números cromosómicos en el noroeste del área de distribución de *Turnera sidoides*. *Bonplandia* **13**: 117–128.

Soltis, P. S., and Soltis, D. E. 2009. The role of hybridization in plant speciation. *Annu. Rev. Plant Biol.* **60**: 561–588. doi:10.1146/annurev.arplant.043008.092039.

Souza, L. G. R., Crosa, O., Speranza, P., and Guerra, M. 2012. Cytogenetic and molecular evidence suggest multiple origins and geographical parthenogenesis in *Nothoscordum gracile* (Alliaceae). *Ann. Bot.* **109**: 987–999. doi:10.1093/aob/mcs020.

Speranza, P. R., Vaio, M., and Mazzella, C. 2003. Karyotypes of two cytotypes of *Paspalum quadrifarium* Lam. (Poaceae): an alternative technique for small chromosomes in plants. *Genet. Mol. Biol.* **26**: 449–503. doi:10.1590/S1415-47572003000400013.

Speranza, P. R., Seijo, J. G., Grela, I. A., and Solís-Neffa, V. G. 2007. Chloroplast DNA variation in the *Turnera sidoides* L. complex (Turneraceae): biogeographical implications. *J. Biogeogr.* **34**: 427–436. doi:10.1111/j.1365-2699.2006.01622.x.

Stebbins, G. L. 1950. *Variation and Evolution in Plants*. Columbia University Press, New York.

Strelin, M. M., Cosacov, A., Diller, M., and Sérsic, A. N. 2013. Study of the polymorphism of the Patagonian *Calceolaria polyrhiza* (Calceolariaceae) using decision tree and sequential covering rule induction. *Bot. J. Linn. Soc.* **173**: 487–500. doi:10.1111/boj.12078.

Sukumaran, J., and Knowles, L. L. 2017. Multispecies coalescent delimits structure, not species. *Proc. Natl. Acad. Sci.* **114**: 1607–1612. doi:10.1073/pnas.1607921114.

Tacuatiá, L. O., Souza-Chies, T. T., Flores, A. M., Eggers, L., Siljak-Yakovlev, S., and Kaltchuk-Santos, E. 2012. Cytogenetic and molecular characterization of morphologically variable *Sisyrinchium micranthum* (Iridaceae) in southern Brazil. *Bot. J. Linn. Soc.* **169**: 350–364. doi:10.1111/j.1095-8339.2012.01229.x.

Takayama, K., López-Sepúlveda, P., Greimler, J., Crawford, D. J., Peñailillo, P., Baeza, M., Ruiz, E., Kohl, G., Tremetsberger, K., Gatica, A., Letelier, L., Novoa, P., Novak, J., Stuessy, T. F. 2015. Relationships and genetic consequences of contrasting modes of speciation among endemic species of *Robinsonia* (Asteraceae, Senecioneae) of the Juan Fernández Archipelago, Chile, based on AFLPs and SSRs. *New Phytol.* **205**: 415–428. doi:10.1111/nph.13000.

Tomasello, S., Álvarez, I., Vargas, P., and Oberprieler, C. 2015. Is the extremely rare Iberian endemic plant species *Castrilanthemum debeauxii* (Compositae, Anthemideae) a "living fossil"? Evidence from a multi-locus species tree reconstruction. *Mol. Phylogenet. Evol.* **82**: 118–130. doi:10.1016/j.ympev.2014.09.007.

Turchetto-Zolet, A. C., Pinheiro, F., Salgueiro, F., and Palma-Silva, C. 2013. Phylogeographical patterns shed light on evolutionary process in South America. *Mol. Ecol.* **22**: 1193–213. doi:10.1111/mec.12164.

Turchetto, C., Segatto, A. L. A., Telles, M. P. C., Diniz-Filho, J. A. F., and Freitas, L. B. 2014. Infraspecific classification reflects genetic differentiation in the widespread *Petunia axillaris* complex: a comparison among morphological, ecological, and genetic patterns of geographic variation. *Perspect. Plant Ecol. Evol. Syst.* **16**: 75–82. doi:10.1016/j.ppees.2014.01.002.

Turesson, G. 1922. The genotypical response of the plant species to the habitat. *Hereditas* **3**: 211–350. doi:10.1111/j.1601-5223.1922.tb02734.x.

Twyford, A. D., Kidner, C. A., and Ennos, R. 2015. Maintenance of species boundaries in a Neotropical radiation of *Begonia*. *Mol. Ecol.* **24**: 4982–4993. doi:10.1111/mec.13355.

Urbani, M., Quarin, C., Espinoza, F., Penteado, M., and Rodrigues, I. 2002. Cytogeography and reproduction of the *Paspalum simplex* polyploid complex. *Plant Syst. Evol.* **236**: 99–105. doi:10.1007/s00606-002-0237-6.

Vallejo-Marín, M., and Hiscock, S. J. 2016. Hybridization and hybrid speciation under global change. *New Phytol.* **211**: 1170–1187. doi:10.1111/nph.14004.

Vázquez-Garcidueñas, S., Palacios, R. A., Segovia-Quiroz, J., Frías-Hernández, J. T., Olalde-Portugal, V., Martínez-de la Vega, O., Mollard, F. P. O., and Vázquez-Marrufo, G. 2003. Morphological and molecular data to determine the origin and taxonomic status of *Prosopis chilensis* var. *riojana* (Fabaceae, Mimosoideae). *Can. J. Bot.* **81**: 905–917. doi:10.1139/b03-078.

Vega, Y., Marques, I., Castro, S., and Loureiro, J. 2013. Outcomes of extensive hybridization and introgression in *Epidendrum* (Orchidaceae): can we rely on species boundaries? *PLoS One* **8**: e80662. doi:10.1371/journal.pone.0080662.

Versieux, L. M., and Machado, T. M. 2012. A new ornithophilous yellow-flowered *Vriesea* (Bromeliaceae) from Serra do Caraça, Minas Gerais, Brazil. *Phytotaxa* **71**: 36–41. doi:10.11646/phytotaxa.71.1.7.

Via, S. 2009. Natural selection in action during speciation. *Proc. Natl. Acad. Sci.* **106**: 9939–9946. doi:10.1073/pnas.0901397106.

Viruel, J., Segarra-Moragues, J. G., Pérez-Collazos, E., Villar, L., and Catalán, P. 2010. Systematic revision of the *Epipetrum* group of *Dioscorea* (Dioscoreaceae) endemic to Chile. *Syst. Bot.* **35**: 40–63. doi:10.1600/036364410790862579.

Walter, J., Vekslyarska, T., and Dobeš, C. 2015. Flow cytometric, chromosomal and morphometric analyses challenge current taxonomic concepts in *the Portulaca oleracea* complex (Portulacaeae, Caryophyllales). *Bot. J. Linn. Soc.* **179**: 144–156. doi:10.1111/boj.12309.

Welker, C. A. D., Souza-Chies, T. T., Longhi-Wagner, H. M., Peichoto, M. C., McKain, M. R., and Kellogg, E. A. 2015. Phylogenetic analysis of *Saccharum* s.l. (Poaceae; Andropogoneae), with emphasis on the circumscription of the South American species. *Am. J. Bot.* **102**: 248–263. doi:10.3732/ajb.1400397.

Wet, J. M. J. 1968. Biosystematics of the *Bothriochloa barbinodis* complex (Gramineae). *Am. J. Bot.* **55**: 1246–1250. doi:10.1002/j.1537-2197.1968.tb07492.x.

Wiens, J. J. 2007. Species delimitation: new approaches for discovering diversity. *Syst. Biol.* **56**: 875–878., doi:10.1080/10635150701748506.

Wilkins, J. S. 2009. *Species: A History of the Idea*. University of California Press, Berkeley.

Willis, S. C. 2017. One species or four? Yes!… and, no. Or, arbitrary assignment of lineages to species obscures the diversification processes of Neotropical fishes. *PLoS One* **12**: 1–26. doi:10.1371/journal.pone.0172349.

Wu, C. I. 2001. The genic view of the process of speciation. *J. Evol. Biol.* **14**: 851–865. doi:10.1046/j.1420-9101.2001.00335.x.

Yang, Z. 2015. The BPP program for species tree estimation and species delimitation. *Curr. Zool.* **61**: 854–865. doi:10.1093/czoolo/61.5.854.

Yang, Z., and Rannala, B. 2010. Bayesian species delimitation using multilocus sequence data. *Proc. Natl. Acad. Sci.* **107**: 9264–9269. doi:10.1073/pnas.0913022107.

Zaitlin, D., and Pierce, A. J. 2010. Nuclear DNA content in *Sinningia* (Gesneriaceae); intraspecific genome size variation and genome characterization in *S. speciosa*. *Genome* **53**: 1066–1082. doi:10.1139/G10-077.

Zapata, F., and Jiménez, I. 2012. Species delimitation: inferring gaps in morphology across geography. *Syst. Biol.* **61**: 179–194. doi:10.1093/sysbio/syr084.

Zappi, D. C., Ranzato Filardi, F. L., Leitman, P., Souza, V. C., Walter, B. M. T., Pirani, J. R., Morim, M. P., Queiroz, L. P., Cavalcanti, T. B., Mansano, V. F., Forzza, R. C. 2015. Growing knowledge: an overview of seed plant diversity in Brazil. *Rodriguesia* **66**: 1085–1113. doi:10.1590/2175-7860201566411.

Zuntini, A. R., Taylor, C. M., and Lohmann, L. G. 2015. Deciphering the neotropical *Bignonia binata* species complex (Bignoniaceae). *Phytotaxa* **219**: 069–077. doi:10.11646/phytotaxa.219.1.5.

Artigo: Does habitat stability structure intraspecific genetic diversity? It's complicated…

Jamin G. Wieringa, Mathhew R. Boot, Marcos V. Dantas-Queiroz, Drew Duckett, Emanuel M. Fonseca, Heather Glon, Natalie Hamilton, Sungsik Kong, Flavia M. Lanna, Kali Z. Mattingly, Danielle J. Parsons, Megan L. Smith, Benjamin W. Stone, Coleen Thompson, Lei Zuo, Bryan Carstens

Frontiers of Biogeography, v. 12, n. 2, p. e45377, 2020
DOI: 10.21425/F5FBG45377

RESEARCH ARTICLE

Frontiers of Biogeography
the scientific journal of
the International Biogeography Society

Does habitat stability structure intraspecific genetic diversity? It's complicated…

Jamin G. Wieringa[1], Matthew R. Boot[1,2], Marcos V. Dantas-Queiroz[3], Drew Duckett[1,2], Emanuel M. Fonseca[1,2], Heather Glon[1,2], Natalie Hamilton[1,2], Sungsik Kong[1], Flavia M. Lanna[1,2], Kali Z. Mattingly[1], Danielle J. Parsons[1,2], Megan L. Smith[1,2], Benjamin W. Stone[1], Coleen Thompson[1,2], Lei Zuo[1,2] and Bryan C. Carstens[1,2]*

[1]*Department of Evolution, Ecology and Organismal Biology*, The Ohio State University, 318 W. 12th Ave., Columbus, OH, USA. 43210; [2]*Museum of Biological Diversity*, The Ohio State University, 1315 Kinnear Rd., Columbus, OH, USA, 43212; [3]*Departamento de Ecologia, Programa de Pós-graduação em Biologia Vegetal,* Universidade Estadual Paulista, Av. 24-A, 1515, Bela Vista, Rio Claro, SP, Brazil. 13506–900. *correspondence: carstens.12@osu.edu; https://carstenslab.osu.edu/

Abstract

Regional phylogeographic studies have long been conducted in the southeastern United States for a variety of species. With some exceptions, many of these studies focus on single species or single clades of organisms, and those considering multiple species tend to focus on deep historical breaks causing differentiation. However, in many species more recent factors may be influencing genetic diversity. To understand the roles of historic and contemporary processes in structuring genetic diversity, we reanalyzed existing genetic data from Southeast of North America using approaches gleaned from phylogeographic and landscape genetic literature that were implemented across species including AMOVAs, PCoAs, Species Distribution Modelling, and tests of isolation by distance, environment, and habitat instability. Genetic variance was significantly partitioned by ecoregions, watersheds, and across phylogeographic breaks in the majority of species. Similarly, genetic variation was significantly associated with some combination of geographic or environmental distance or habitat instability in most species. Patterns of genetic variation were largely idiosyncratic across species. While habitat instability over time is significantly correlated with genetic diversity in some species, it appears generally less important than isolation by geographic or environmental distance. Our results suggest that many factors, both historical and contemporary, impact genetic diversity within a species, and more so, that these patterns aren't always similar in closely related species. This supports the importance of species-specific factors and cautions against assumptions that closely related species will respond to historical and contemporary forces in similar ways.

Highlights

- We used a variety of approaches to quantify factors that potentially influence genetic structure across multiple species in the Southeastern United States
- Our analyses indicate that both contemporary and historical factors promote genetic divergence across multiple taxa
- Machine learning analysis indicated that taxa are largely idiosyncratic in terms of which factors are most important, suggesting that specific factors regulate how particular taxa respond to external factors
- Our results highlight the importance of considering both landscape and phylogeographic methodology when investigating genetic structure

Keywords: Phylogeography, data repurposing, Pleistocene glaciation, comparative phylogeography, mtDNA, cpDNA, barriers

e-ISSN: 1948-6596 https://escholarship.org/uc/fb doi:10.21425/F5FBG45377

Introduction

Phylogeography aims to understand how climatic and geologic events have structured genetic diversity; while comparative phylogeography seeks this understanding in multiple species. Traditionally, this discipline interprets similar patterns (e.g., genealogies, STRUCTURE plots, etc.) across species as evidence for a shared species response to climate and/or geologic events (Avise 1987, Sullivan et al. 2000). One region that has served as the setting for many early phylogeographic studies (e.g., Avise 2000; Soltis 2006) and has continued to capture the interest of researchers (e.g., Satler and Carstens 2016; Barrow et al. 2017) is the Southeastern United States. The geologic and climatic history of this region is complex, involving diverse processes acting over millions of years, including mountain orogeny, changing river basins, and climate cycles, and species may have been affected in diverse ways by these factors. Soltis et al. (2006) reviewed phylogeographic patterns from the Southeastern US and suggested that these patterns could be categorized into three categories: populations structured by 1) river basins, 2) mountain ranges, and 3) the locations of glacial refugia. However, these categories are not independent or exclusive; for example, species with population structure across a given river or the Appalachian Mountains may have been isolated across these barriers in separate refugia during Pleistocene glaciation.

Studies of congruence in phylogeography have often aimed to identify identical breakpoints and population structure (e.g. Avise 2000, Satler and Carstens 2016), a task that is complicated by differences in species-specific attributes, such as dispersal ability and ecological niche (Papadopoulou and Knowles 2016). Another strategy for identifying similarity in pattern across species is to assess the relative importance of different factors in structuring genetic diversity. This approach is popular in landscape genetics (Rissler 2016), where many studies aim to evaluate the relative effects of isolation-by-distance (IBD; Wright 1943) and isolation-by-environment (IBE). By considering habitat stability in such an approach we can potentially disentangle the relative contribution of both contemporary and historical factors structuring genetic diversity (e.g., Vansconcellos et al. 2019).

Comparative phylogeographic investigations have long used climate modeling to identify regions of stable habitat that putatively served as climate refugia (e.g., Hugall et al. 2002, Carstens & Richards 2007, Carnaval et al. 2009). The general interpretation of such results is that similarity in genetic structure across species is a product of species persistence during periods of climatic change in these regions of stable habitat. The relationship between habitat and genetic diversity is a feature of landscape genetic investigations (e.g., Orsini et al. 2013), particularly in combination with species distribution modeling of habitat suitability (e.g., Geber 2011). Therefore, analytical methods that explicitly consider the suitability of the environment in the context of the genetic structure of species enable integration between phylogeography and landscape genetics (Rissler 2016). One promising analysis was proposed by Vasconcellos et al. (2019), who investigated the effects of climatic shifts on the genetic structure of a savanna frog from Brazil. Their strategy used an evaluation of the relative contributions of isolation by distance and isolation by habitat suitability modeled over evolutionary time (habitat instability). Vasconcellos et al. (2019) argue that the second measure, their 'isolation by instability (IBI)' model, is a special case of the isolation by resistance model developed by McRae (2006). Like McRae's model, which posits that genetic diversity is structured by the separation of individuals using corridors of suitable habitat rather than a straight line over geographic space, Vasconcellos et al.'s model predicts that genetic diversity is structured by the connectedness of individuals over evolutionary time. Use of a multiple matrix regression with randomization (MMRR) approach (Wang 2013) allowed Vasconcellos et al. to evaluate the relative contribution of habitat instability in comparison to other factors.

While habitat instability is one important component of historical effects on genetic diversity, it is not the only factor to consider. In addition to the effects of Pleistocene glacial cycles, the effects of river formation and mountain orogeny are evident in phylogeographic datasets from eastern North America (Soltis 2000, Avise 2006, Satler and Carstens 2016). To evaluate the effects of these and other phylogeographic breaks on structuring genetic diversity, we conduct analyses of molecular variance (AMOVAs; Excoffier 1992) and habitat stability in order to evaluate whether genetic diversity is significantly structured across river basins, ecoregions, or proposed phylogeographic breaks. In combination, these approaches allow us to evaluate the relative contributions of contemporary and various historic factors across species from eastern North America. Because they provide easily comparable metrics across species, the degree to which species have similar phylogeographic patterns and responses to the factors that lead to incongruence across species can be assessed. Here, we use repurposed data collected from a variety of sources to test the factors influencing genetic variation in a comparative framework in the Southeastern United States.

Methods

Data Processing

Fifty-seven species were identified from published phylogeographic studies in the Southeastern United States, with most of the taxa included by Soltis et al. (2006) supplemented by data from several more recent investigations (Table 1). Overall guidelines for processing are presented in Fig. S1. To start, genetic data were downloaded from GenBank for each species. Genetic data were then aligned using CLUSTAL (Thompson et al. 1994) before selecting appropriate models of sequence evolution using the Akaike Information Criterion implemented in jModeltest ver 2.1.10 (Darriba et al. 2012, Guindon and Gascuel 2003) and/or MrModeltest ver 2.4 (Nylander 2004). Models implemented (Table S1) included K80 (Kimura

Table 1. Species used in analysis. For each species, the scientific name, type of organism, type of data, number of sequences, and reference of original publication is shown.

Species	Broad Taxon	Type of Data	# sequences	Original publication
Bryopsis sp.	Green Algae	cpDNA	66	Krellwitz et al. (2001)
Gracilaria tikvahiae	Red Algae	cpDNA	20	Gurgel et al.(2004)
Xerula furfuracea	Fungi	nuDNA	41	Yang et al.(2009) & Petersen and Hughes (2010) & Hao et al.(2016)
Sphagnum bartlettianum	Bryophyta	cpDNA + nuDNA	12	Shaw et al.(2005)
Acer rubrum	Angiosperm	cpDNA	38	McLachlan et al.(2005)
Apios americana	Angiosperm	nuDNA	18	Joly & Bruneau (2004)
Dicerandra spp	Angiosperm	cpDNA	30	Oliveira et al.(2007)
Fagus grandifolia	Angiosperm	cpDNA	23	McLachlan et al.(2005)
Liquidambar styraciflua	Angiosperm	cpDNA	109	Morris et al.(2008)
Prunus spp	Angiosperm	cpDNA	226	Shaw & Small (2005)
Tilia americana	Angiosperm	cpDNA	297	McCarthy and Mason-Gamer (2016)
Trillium cuneatum	Angiosperm	cpDNA	281	Gonzales et al.(2008)
Uniola paniculata	Angiosperm	cpDNA	131	Hodel & Gonzales (2013)
Bugula neritina	Bryozoa	mtDNA	30	McGovern & Hellberg (2003)
Daphnia obtusa	Crustacean	mtDNA	36	Penton et al.(2004)
Emerita talpoida	Crustacean	mtDNA	4	Tam et al.(1996)
Farfantepenaeus aztecus	Crustacean	mtDNA	76	McMillen-Jackson and Bert (2003)
Litopenaeus setiferus	Crustacean	mtDNA	92	McMillen-Jackson and Bert (2003) & Maggioni et al. (2001) &Vazquez-Bader et al.(2004) & Bremer et al.(2010)
Pagarus longicarpus	Crustacean	mtDNA	67	Young et al.(2002)
Pagarus pollicaris	Crustacean	mtDNA	13	Young et al.(2002)
Busycon sinistrum	Gastropod	mtDNA	31	Wise et al.(2004)
Lampsilis altilis	Mollusk	mtDNA	5	Roe et al.(2001)
Lampsilis australis	Mollusk	mtDNA	5	Roe et al.(2001)
Lampsilis ovata	Mollusk	mtDNA	2	Roe et al.(2001) & Campbell et al.(2005)
Lampsilis perovalis	Mollusk	mtDNA	5	Roe et al.(2001)
Lampsilis teres	Mollusk	mtDNA	2	Roe et al.(2001) & Lydeard et al.(2000)
Spisula solidissima	Mollusk	mtDNA	52	Hare and Weinberg (2005)
Ambystoma tigrinum	Amphibian	mtDNA	56	Church et al.(2003)
Desmognathus wrightii	Amphibian	mtDNA	29	Crespi et al.(2003)
Eumeces fasciatus	Amphibian	mtDNA	82	Howes et al.(2006)
Eurycea bislineata	Amphibian	mtDNA	56	Kozak et al.(2006)
Eurycea cirrigera	Amphibian	mtDNA	251	Kozak et al.(2006)
Eurycea junaluska	Amphibian	mtDNA	6	Kozak et al.(2006)
Eurycea multiplicata	Amphibian	mtDNA	46	Bonett & Chippindale (2004)
Eurycea tymerensis	Amphibian	mtDNA	16	Bonett & Chippindale (2004)
Eurycea wilderae	Amphibian	mtDNA	129	Kozak et al.(2006)

Table 1. Continued...

Species	Broad Taxon	Type of Data	# sequences	Original publication
Pseudacris brachyphona	Amphibian	mtDNA	25	Lemmon et al.(2007)
Pseudacris brimleyi	Amphibian	mtDNA	8	Lemmon et al.(2007)
Pseudacris clarkii	Amphibian	mtDNA	9	Lemmon et al.(2007)
Pseudacris crucifer	Amphibian	mtDNA	62	Austin et al.(2004)
Pseudacris feriarium	Amphibian	mtDNA	61	Lemmon et al.(2007)
Pseudacris nigrita	Amphibian	mtDNA	19	Lemmon et al.(2007)
Pseudacris spnov	Amphibian	mtDNA	27	Lemmon et al.(2007)
Rana catesbeiana	Amphibian	mtDNA	213	Austin et al.(2004)
Tryphlotriton spelaeus	Amphibian	mtDNA	14	Bonett & Chippindale (2004)
Acipenser oxyrinchus	Fish	mtDNA	615	Grunwald et al.(2008)
Carcharhinus limbatus	Fish	mtDNA	10	Keeney et al.(2005)
Hypentelium nigricans	Fish	mtDNA	85	Berendzen et al.(2003)
Percina caprodes	Fish	mtDNA	15	Near (2008)
Percina nasuta	Fish	mtDNA	6	Robison et al.(2014)
Percina phoxocephala	Fish	mtDNA	34	Robison et al.(2014)
Seriola dumerli	Fish	mtDNA	16	Gold & Richardson (1998)
Blarina brevicauda	Mammal	mtDNA	74	Brant et al.(2003)
Apalone ferox	Reptile	mtDNA	9	Weisrock and Jenzen (2000)
Apalone mutica	Reptile	mtDNA	9	Weisrock and Jenzen (2000)
Apalone spinifera	Reptile	mtDNA	30	Weisrock and Jenzen (2000)
Pantherophis obsoletus	Reptile	mtDNA	39	Burbrink et al. 2000

1980), K81 (Kimura 1981), F81 (Felsenstein 1981), or TN93 (Tamura and Nei 1993), with gamma correction for rate variation in some species. For species with multiple genes, we chose the model that applied to the greatest number of base pairs and concatenated all cases of multiple genes. FASTA formatted data alignments were used to analyze sequence diversity and polymorphism using DnaSP ver 6 software (Rozas et al. 2017). Prior to calculating genetic distance matrices, we removed outgroups and dealt with missing data as follows. For 44 / 57 species, we deleted sites with at least one missing base across all sequences. Because this procedure deleted most sites for 13 species with many missing data, for these species we instead performed pairwise deletion of missing bases, and, for 10 of these, also replaced remaining undefined pairwise distances with the mean distance across all other sequence comparisons for that site. Adopting procedures for addressing missing data (Jombart 2008, Jombart and Ahmed 2011) allowed us to define comparable distance matrices for all species.

Geographic locality information was collected from source publications where possible. In cases where specific coordinates were not provided, we used sample accession numbers to check GenBank for sample-associated metadata. If specific coordinates could not be determined from either source, we used GEOLocate, an online platform for georeferencing data, to generate coordinates based on the most specific locality available (Rios and Bart 2010). Once the data was complied, all coordinates, including those from original sources, were double-checked using GEOLocate and then visually inspected using Google Earth. Generation length and substitution rate were obtained for each species from the literature (Supplementary Table S2).

Nucleotide Mapping

In order to develop a qualitative understanding of how nucleotide diversity is distributed across the landscape, we created a heat map of nucleotide diversity across all species. To calculate nucleotide diversity, we divided North America into a 1° grid and, using geographic coordinates for each species, assigned individuals to a cell in this grid. As nucleotide diversity requires multiple individuals for comparison, grid cells with fewer than two individuals of a species were removed. In order to avoid sampling bias, species with fewer than three occupied cells were also removed from subsequent analyses. Nucleotide diversity (π) was calculated within each grid cell using the R package "pegas" (Emmanuel et al. 2018). Given the challenges associated with using different molecular markers, we normalized each individual raster to ensure that species richness did not exert an undue influence on nucleotide diversity (π) as follows:

$$\frac{\pi - \min(\pi)}{\max(\pi) - \min(\pi)} \quad (1)$$

Subsequently, GIS layers for each species were summed to create a final heat map that included all species to indicate the overall level of nucleotide

diversity across the region. While this conservative approach provides a simplistic impression of nucleotide diversity, it prevents overinterpretation that may result from differences in species richness or the use of genetic markers across species that differ in their rates of evolution and as such may exhibit different levels of haplotype diversity. In addition, Moran's I was calculated using the R package "ape" (Paradis et al. 2004) to assess autocorrelation under the assumption that significant positive autocorrelation in nucleotide diversity may be indicative of shared refugia across species.

Analysis of molecular variation

We used analysis of molecular variance (AMOVA; Excoffier et al. 1992) to compare genetic variation across geographic features thought to structure populations. For each georeferenced species, we matched GPS data for individuals to geographic features delineated by shapefiles (sp::over, Pebesma and Bivand 2005). We used tools in ArcMap 10.3 (ESRI 2014) to create shapefiles from preexisting spatial data for two current landscape features, Ecoregion (CECWG 1997; Spalding et al. 2007; USEPA 2012; AAFC 2017) and Watershed (Spalding et al. 2007; NRC, USGS, and INEG 2010a), and four phylogeographic breaks described in Soltis et al. (2006): the Atlantic-Gulf Coast discontinuity (Spalding et al. 2007), the Apalachicola River-Tombigbee River discontinuity (which separates rivers flowing into the Gulf of Mexico from those flowing into the Atlantic Ocean; Spalding et al. 2007; NRC, USGS, and INEG 2010a), the Appalachian Mountains (UNEP-WCMC 2002), and the Mississippi River (NRC, USGS, and INEG 2010b). We implemented AMOVA in Arlequin using *arlecore* with 1000 permutations to test for significance (Excoffier et al. 2005). Outgroups and species lacking geographic data were omitted from these analyses.

Population assignment

Intraspecific genetic variation is often structured by populations, and the characterization of population genetic structure was prominently featured by many of the original publications that described the data we use here (Table 1). This type of information is also often necessary for other types of analysis, as was the case here. To facilitate comparison across species, we inferred the number of genetic populations using an assignment protocol implemented in the adegenet R package (Jombart 2008, Jombart and Ahmed 2011). This protocol utilizes principal components (PCs) to calculate successive K-means (adegenet::find.clusters, Jombart and Collins 2015) which are then used to infer the number of population groups. We modified this method such that it utilized principal coordinates analysis (PCoA, adegenet::dudi.pco) to analyze a distance matrix calculated according to a model of sequence evolution. Note that species with < 4 individuals (*Emerita talpoida, Lampsilis ovata, Lampsilis teres*) were not evaluated using this algorithm, but were assigned a value of K = 1 for other analyses.

We explored how well population assignments explained the genetic variation by performing a permutational multivariate analysis of variance (PERMANOVA, adonis::vegan, Oksanen et al. 2018). The dispersion of clusters relative to each other (vegan::betadisper) was also assessed. Here, a significant beta-dispersion value indicates violation of PERMANOVA's assumption of homogeneity of variance (Anderson 2006).

Species Distribution Modelling

Species distribution modelling (SDM) was used to characterize habitat suitability in the present by associating species locality data with maps of environmental variables (limited to climate and ocean depth), and to estimate habitat stability over time. We downloaded the GBIF Records in November 2018 using the R package "dismo" (Hijmans et al. 2017) and retained records that passed the following filters: preserved specimen, living specimen, material sample from 1900 to present, present in the United States and Canada. We combined the downloaded GBIF records with the specimen localities associated with the genetic data to create a complete presence record dataset. These were reduced to one occurrence per grid cell within the boundary of [-130, -60, 23, 50] at 5 arcmin resolution using the R package "raster" (Hijmans et al. 2019); species that had less than 25 occurrences were removed from the analysis following results from van Proosdij et al. (2016). Three sets of environmental variables were used: WorldClim 2.0 bioclimatic variables for the current (Fick and Hijmans 2017) and WorldClim 1.4 for past climate at the Mid-Holocene and LGM (Higmans et al. 2005) under the CCSM4 model for all terrestrial and freshwater species, and MARSPEC (Sbrocco and Barber 2013), including bathymetry, also at 5 arcmin resolution and under the current and LGM conditions (all models) for the marine species. All bioclimatic variables were downloaded at a resolution of 5 arcmin and trimmed to the same constraints as the occurrence records using the R packages "raster", "maptools" (Bivand et al. 2019a), "rgdal" (Bivand et al. 2019b), and "rgeos" (Bivand et al. 2019c). We performed a correlation analysis on the raster layers under current conditions using the "layerStats" function and subsequently removed highly correlated variables ($r > 0.8$; Mateo et al. 2013).

SDM analyses were conducted in R using the packages "randomForest" (Liaw and Wiener 2018), "raster", "rgeos", "maptools", "dismo", "sp" (Pevesma et al. 2018), "ecospat" (Cola et al. 2017), and "rJava" (Urbanek 2019). Pseudo absence points were drawn randomly from the background layers, and we partitioned the model into testing and training data using the "kfold" function. Distributional models were estimated for each species using the present environmental layers and cast onto the last glacial maximum layers using the MaxEnt, Random Forest, and GLM approaches (Phillips et al. 2017 and Hijmans 2017). In order to combine model results for an ensemble model, we evaluated the AUC of each present-day model to weight the contribution of each to the final output. We calculated a separate binary output using a

threshold determined using 'ecospat.mpa' (Cola et al. 2017), resulting in a total of six raster layers for each species: present-day continuous and binary rasters, mid-Holocene continuous and binary rasters, and last glacial maximum continuous and binary rasters.

As different species have different life history traits, species were combined into functional groups (Reptiles, Amphibians, Plants, Mammals, Freshwater, and Marine) in order to build cumulative maps of suitable habitat for the present day and the LGM using raster addition. Additionally, we determined potential refugia for each species through the identification of the areas of overlap between the present day and LGM binary models for each species. Using the functional groups defined as above, we took the overlapped species models and compared areas of interspecific overlap within each major grouping to determine areas of multi-species refugia, and we added these maps together for cumulative Terrestrial, Freshwater, and Marine groupings.

Multiple Matrix Regression with Randomization

Three factors are often highlighted in phylogenetic and landscape genomic studies: Isolation by Distance (IBD), Isolation by Environment (IBE), and Isolation by Instability (IBI). To examine the impact of each force as independent predictors on genetic distance (calculated above in the AMOVA), we used multiple matrix regression with randomization (MMRR; Wang 2013). We performed the MMRR as outlined in Legendre et al. (1994) and implemented by Wang (2013) using the MRM command in the 'ecodist' R package with 1000 permutations (Goslee and Urban 2007). One advantage of MMRR is the ability to account for small amounts of covariation (less than 0.5) in the data through inclusion of randomization (Wang 2013). For use with the MRM command, each independent factor (IBD, IBE, and IBI) was transformed to distance matrices using the 'dist' function present in the stats R package. Values for IBD were determined from geographic coordinates collected with the genetic data, IBE and IBI values were extracted at sampling geographic points from species distribution models in the present, and stability maps, respectively. To generate stability maps we averaged the species distribution models from the present, Mid-Holocene, and LGM for each species. Using the averaged model gives us the relative stability of the environment over this time period, and using the 'extract' function, we sampled this stability surface at each sampling point.

The rational for each metrics inclusion are as follows: IBD is the isolation by Euclidean distance between two geographic points (Wright 1943); two individuals that are closer together should be more similar genetically than those that are not as close, as there is a greater chance of gene flow between two close individuals. IBE implies that large differences in the environment prevent movement across the landscape and therefore gene flow between individuals (Nosil 2004). Under IBI, movement across the landscape is affected by the relative stability of different regions. Where there is high instability, movement is restricted, preventing gene flow between individuals separated by unsuitable areas (Vasconcellos et al. 2019). Due to the possibility of strong correlation (greater than 0.5) between some predictors, correlations were checked for all variables. Those that were found to be significant using MMRR and were strongly correlated were checked using partial mantel tests to more accurately determine which predictors were important. MMRR also gives a value for the correlation coefficient (R^2) for each species. This value gives the amount of genetic variation explained by IBD, IBE, and IBI.

Random Forest

To evaluate whether some factors were predictive of species-specific responses to geographic and environmental factors across species, we constructed a Random Forest (RF) classifier. RF is a machine learning approach that uses multiple decision trees to predict the response based on many predictor variables (Liaw and Wiener 2002, Biau 2012). Random forest samples the data for each decision tree with replacement and uses the unsampled data to test the model. This is used to build a confusion matrix for the prediction and calculate the out of bag error rates (OOB). Each variable's importance is determined by measuring the mean decrease in accuracy of the prediction after the removal of a given variable.

We designed different models that attempted to predict whether or not a species would have significant genetic structuring based on the categories used in the AMOVA and MMRR tests (Appalachian, Apalachicola, Coastal splits and Mississippi phylogeographic breaks; Ecoregion or Watershed; IBD, IBE, and IBI) as response variables. For predictor variables, we included organismal traits of body size, aquatic vs terrestrial, and generation length, and we included broad taxonomic groups (Table 1) in an attempt to identify results being driven by phylogeny. Additionally, we included the mean climatic variables for each species across their sampling range. Finally, we included the proportion of North America determined via SDM analysis to be suitable for both current and LGM timeframes, and the change in proportional area between LGM and the present.

Results

Data processing

The number of sequences per species ranged from three to 615 and the length ranged from 255 to 2395 base pairs (Table S2). The best fitted models of sequence evolution identified for each sequence alignment in either jModeltest or MrModeltest as well as the diversity and polymorphism calculations (i.e., Tajima D, Pi, θ) are recorded in Table S2 for lowest AIC by jmodeltest or mrmodeltest, and a narrowed list in Table S1 for analyses that couldn't use all possible models of substitution. Geographic data for individuals with associated genetic data showed widely distributed sampling across the eastern US, with the highest density in the south east (Fig. 1), suggesting our dataset has coverage adequate for testing our hypotheses. This

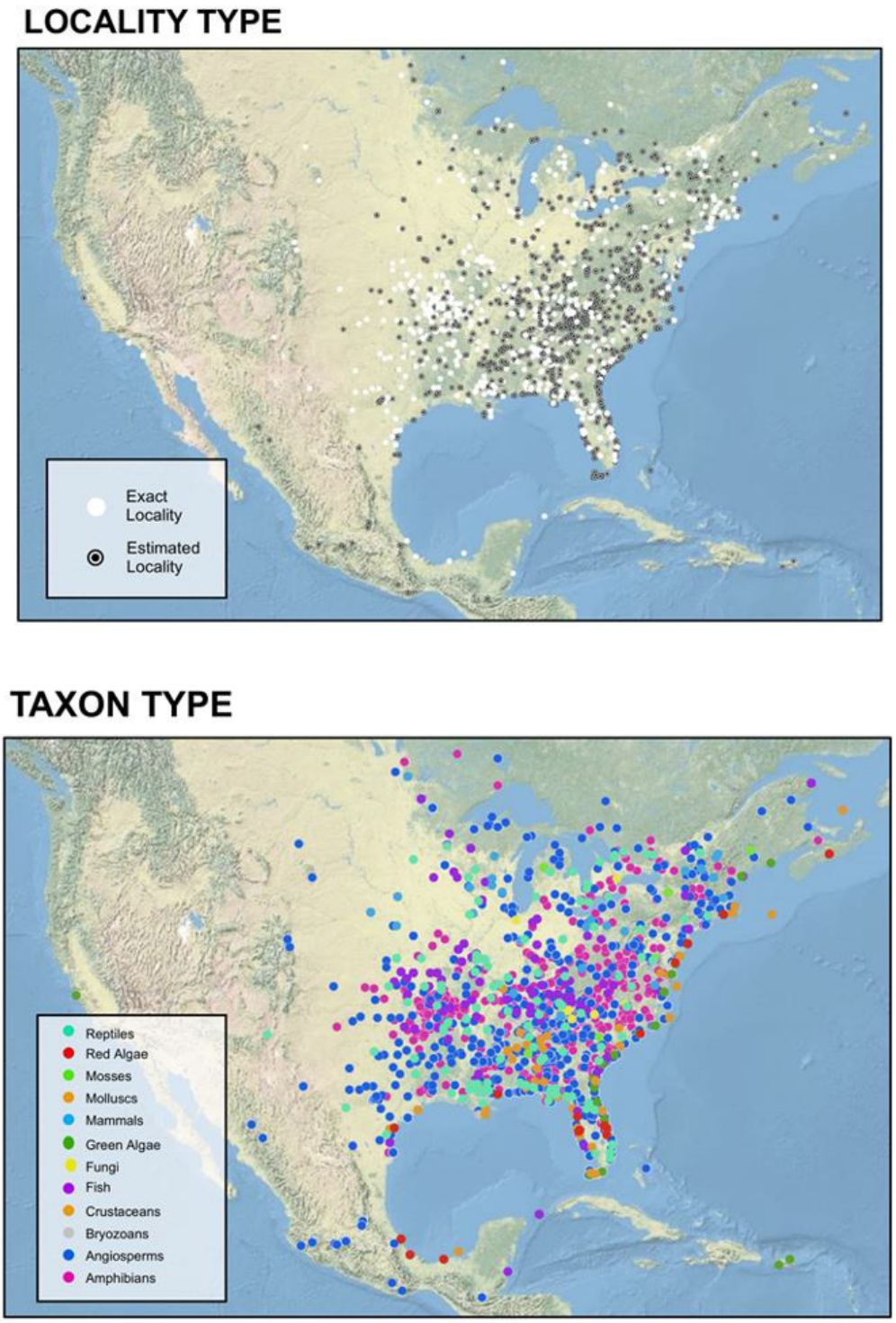


Figure 1. Summary of samples analyzed here. The upper figure shows the location of all samples, color coded by how the sample location was determined. The lower figure shows the location of all samples, color coded by the type of organism.

was regardless of sampling method (exact location vs estimated location), although there is a higher density of estimated localities in northern reaches. There is also no apparent effect of sampling based on taxon type (Fig. 1). While angiosperms clearly had the widest range, the data do not suggest that taxa are unevenly sampled across regions.

Nucleotide Mapping

Nucleotide diversity for the taxa analyzed in this study is heterogeneously distributed across southeastern North America. While some regions contain high levels of nucleotide diversity, there is no evidence for a geographical trend in our final diversity map (Fig. 2). Moran's I was

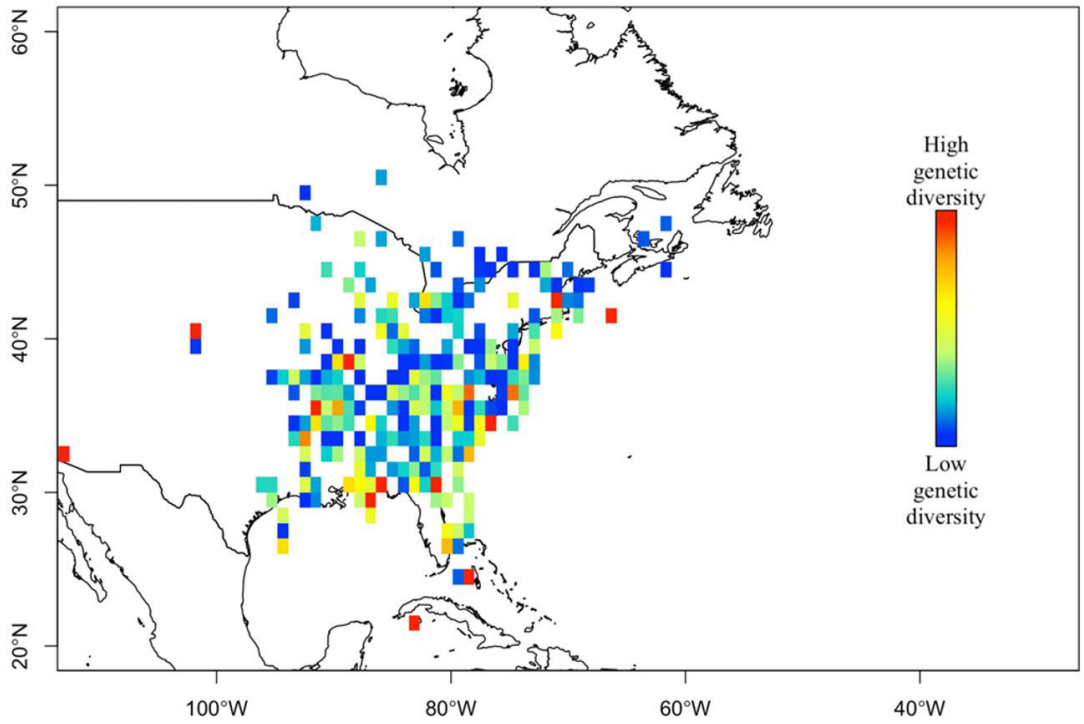


Figure 2. Nucleotide diversity mapping across the eastern United States. Estimates of nucleotide diversity across species were visualized by summarizing values within a 1° grid cell for cells with two or more individuals and then normalized.

found to be close to 0 (0.037, p=0.32), indicating little positive spatial autocorrelation. This indicates that grid cells that are near one another in geographic space are not any more similar in their nucleotide diversity than would be expected by chance. An out of refugia hypothesis would predict that areas of refugia would have higher nucleotide diversity. We also compared this to rasters based on predictions of trends (glacial refugia, null and random), and none were found to be significant.

AMOVA

We used analysis of molecular variance to explore the influence of ecological regions and putative phylogeographic breaks on intraspecific diversity. Samples were partitioned according to regions (i.e., Ecoregion, Watershed) or by putative phylogeographic breaks (i.e., Coast, Appalachian Mountains, Apalachicola, Mississippi River). For example, if population genetic structuring results from historical breaks, we expect significant differentiation across phylogeographic breaks, while if genetic structure results from contemporary ecological barriers, we expect that ecoregions would be an important promotor of genetic variance. Ecoregion AMOVA analyses indicate that 15 (37%) species had significant proportion of the observed variation occurring within groups and that 4 (10%) species had significant proportion of the observed variation occurring among groups (Fig. 3; Table S3). The results of our watershed AMOVA analyses for groups assigned by watersheds indicate that 14 (34%) species had significant proportion of the variation within group and three species (7%) had significant proportion of the observed variation occurring among groups (Table S4). An AMOVA that tested the impact of phylogeographic breaks on genetic structure was conducted in 4 analyses that were named after the putative physical barrier: Coast, Appalachian Mountains, Apalachicola, and Mississippi River. For our Coast AMOVA analyses for groups either along the coast of the Gulf of Mexico or the coast of the Atlantic Ocean indicate that 10 species (34%) had significant portion of the variation within groups and 7 species (17%) had significant proportion of the observed variation among groups (Table S5). Results of our Appalachian AMOVA analyses for groups either on the west or east side of the Appalachian Mountains indicate that 7 species (17%) had significant portion of the variation within groups and one species (2%) had significant proportion of the observed variation among groups (Table S6). The AMOVA analyses for groups either on the west or east side of the Apalachicola River indicate that 10 species (24%) had significant portion of the variation within groups and four species (10%) had significant proportion of the observed variation among groups (Table S7). Lastly, the Mississippi River AMOVA split show that six species (15%) had significant portion of the variation within groups and five species (12%) had significant proportion of the observed variation among groups (Table S8). Results of all AMOVA analyses are summarized in Table 2.

Population assignment

The K-means procedure typically produced population assignments that agreed with visual examinations of BIC vs. K plots (Fig. S2). For seven species, we chose a K that differed from that identified via the automated

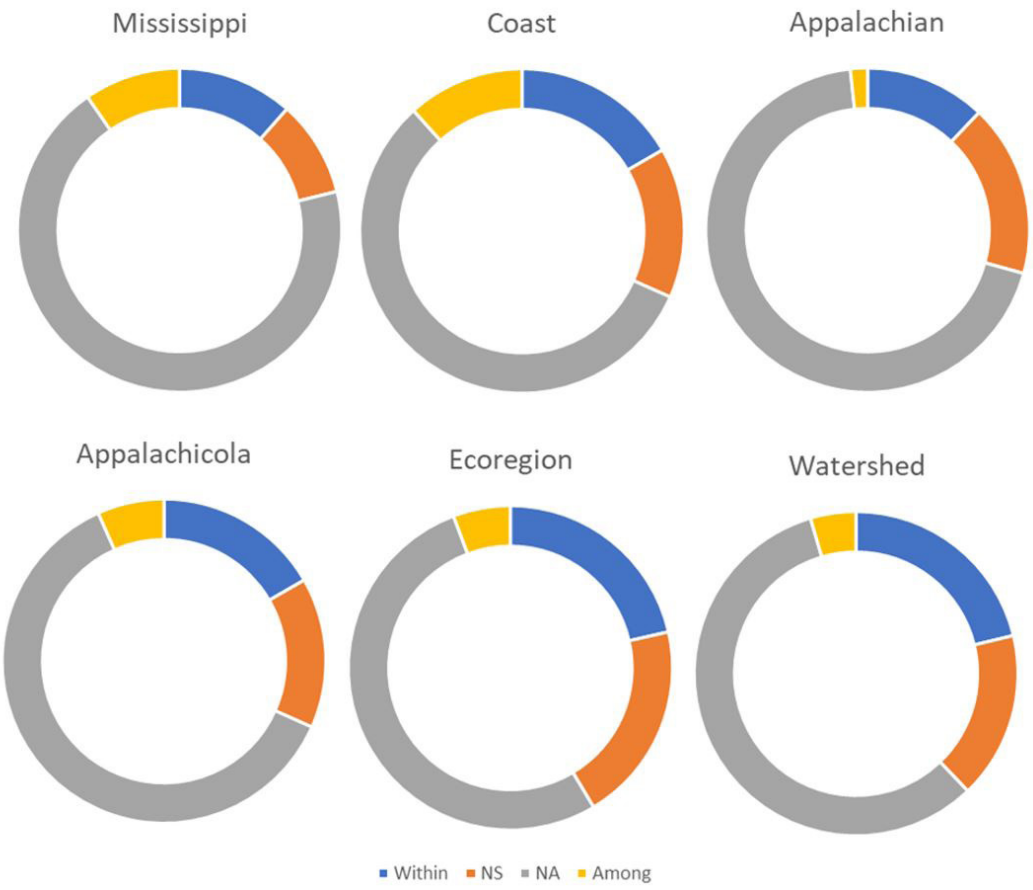


Figure 3. Pie charts summarizing AMOVA results with a variety of different potential breaks/factors. Grey indicates species that could not be tested due to not being in multiple regions or on both sides of a break (NA). Orange are those species that were tested but were found to not be significant (NS). Species that were significant among breaks are in yellow. Blue gives species that were found to be significant within a break or region.

Table 2. Summary of AMOVA for all species. In each analysis, samples were partitioned according to regions (i.e., Ecoregion, Watershed) or by putative phylogeographic breaks (i.e., Coast, Appalachian Mountains, Apalachicola, and Mississippi River).

Species	Mississippi	Coast	Appalachian	Apalachicola	Ecoregion	Watershed
Acipenser oxyrinchus	NA	**<0.001***	**<0.001***	NA	**<0.001***	**<0.001***
Apalone ferox	NA	0.829	NA	0.618	0.246	0.618
Apalone mutica	0.033*	0.027*	NA	0.648	0.179	0.139
Apalone spinifera	0.313	<0.001*	NA	NA	0.172	**0.003***
Blarina brevicauda	<0.001*	**<0.001***	**<0.001***	**<0.001***	<0.001*	**<0.001***
Bugula neritina	0.131	0.077	0.281	1	**0.031***	0.316
Busycon sinistrum	<0.001*	<0.001*	NA	**<0.001***	**<0.001***	**<0.001***
Carcharhinus limbatus	NA	0.094	NA	0.11	0.045*	**0.043***
Desmognathsu wrightii	NA	NA	NA	0.010*	NA	**0.004***
Eumeces fasciatus	<0.001*	**<0.001***	**0.001***	**<0.001***	<0.001*	**<0.001***
Eurycea bislineata	NA	NA	0.071	0.224	0.109	0.216
Eurycea cirrigera	NA	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
Eurycea junaluska	NA	NA	0.059	NA	NA	NA

For each test, the *p*-value is reported, with significant results denoted by an '*'. Those denoted by 'NA' are species that were not sampled on both sides of a split or in multiple ecoregions or watersheds. Blue denotes significant within, yellow among.

Table 2. Continued...

Species	Mississippi	Coast	Appalachian	Apalachicola	Ecoregion	Watershed
Eurcyea multiplicata	NA	NA	NA	NA	0.089	NA
Eurycea tymerensis	NA	NA	NA	NA	0.286	NA
Eurycea wilderae	NA	**0.001***	**<0.001***	**<0.001***	**0.001***	**0.001***
Farfantepenaeus aztecus	0.639	0.594	NA	0.611	0.648	0.671
Hypentelium nigricans	<0.001*	**0.025***	0.189	0.435	**<0.001***	0.221
Lampsilis altilis	NA	0.824	0.342	NA	0.51	NA
Lampsilis australis	NA	NA	NA	NA	NA	NA
Lampsilis ovata	NA	NA	NA	NA	NA	NA
Lampsilis perovalis	NA	NA	NA	NA	0.764	NA
Lampsilis teres	NA	NA	NA	NA	NA	NA
Litopenaeus setiferus	**0.024***	**0.003***	NA	**0.001***	**0.011***	**0.009***
Pagarus longicarpus	**<0.001***	<0.001*	0.919	<0.001*	**<0.001***	**<0.001***
Pagarus pollicaris	0.087	<0.001*	0.146	<0.001*	<0.001*	<0.001*
Pantherophis obsoletus	**0.002***	0.065	0.364	0.068	**0.024***	0.208
Percina caprodes	1	0.724	0.72	0.859	0.543	0.498
Percina nasuta	NA	NA	NA	NA	NA	NA
Percina phoxocephala	NA	NA	NA	NA	<0.001*	NA
Pseudacris brachyphone	NA	**0.017***	0.206	NA	<0.001*	**<0.001***
Pseudacris brimleyi	NA	NA	NA	NA	1	NA
Pseudacris clarkii	NA	0.242	NA	NA	0.347	0.244
Pseudacris crucifer	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**0.001***	**<0.001***
Pseudacris feriarium	0.813	<0.001*	<0.001*	<0.001*	0.089	**<0.001***
Pseudacris nigrita	NA	0.545	NA	0.552	0.664	0.664
Pseudacris spnov	**0.018***	0.448	NA	NA	0.087	0.383
Rana catesbeiana	<0.001*	**<0.001***	**<0.001***	**<0.001***	**<0.001***	**<0.001***
Seriola dumerli	NA	NA	NA	NA	<0.001*	<0.001*
Spisula solidissima	NA	<0.001*	NA	0.001*	<0.001*	<0.001*
Tryphlotriton spelaeus	NA	NA	NA	NA	0.081	NA

For each test, the *p*-value is reported, with significant results denoted by an '*'. Those denoted by 'NA' are species that were not sampled on both sides of a split or in multiple ecoregions or watersheds. Blue denotes significant within, yellow among.

procedure. The majority of species were assigned to two or three populations (Table 3). Results of PERMANOVA suggested population assignments were efficacious. For 42 of the 53 species that were split into populations ($K > 1$), we found that population assignments explained significant portions of the genetic variation ($p < 0.05$, Table 3). Those with non-significant population assignments had either low sample sizes or low overall genetic variation. Fifteen species had significant PERMANOVA results but had beta-dispersion values (Table 3) that suggested assigned groupings were not significantly differentiated. About half (27/53) of the species split into populations which had significant assignments that were not over-dispersed.

Species Distribution Modelling

We retained 31 species for species distribution modelling after removing those species with <25 unique occurrence records (Table S9). Output maps for each of these species display habitat suitability indices for

Table 3. Summary of population assignment results and assessment of population assignment utility. Shown for each species are the number of individuals, the model of sequence evolution, the final 'K' chosen, the *P*-value from the PERMANOVA, and the mean population membership probability. For each species, populations were assigned by calculating a distance matrix based on an appropriate model of sequence evolution, analyzing this matrix with PCoA, using the principal components to calculate successive K-means, and choosing an optimum K based on BIC vs. K plots (Fig. S1). Efficacy of population assignments were assessed via PERMANOVA and DAPC. Complete records found in Table S11.

Species	Number of individuals	Model of sequence evolution	K selected by comparing BIC vs. K plots	*P* from PERMANOVA	population membership probability
Acer rubrum	37	F81	2	<0.001	0.972
Acipenser oxyrinchus	614	K80	1	NA	1.000
Ambystoma tigrinum	54	K81	3	<0.001	0.840
Apalone ferox	8	K81	2	0.250	NA
Apalone mutica	7	K81	2	0.029	1.000
Apalone spinifera	21	K81	2	<0.001	0.971
Apios americana	63	K81	2	<0.001	1.000
Blarina brevicauda	74	K81	3	<0.001	1.000
Bryopsis sp.	65	F81	5	<0.001	1.000
Bugula neritina	20	K80	2	0.006	1.000
Busycon sinistrum	31	K81	2	<0.001	0.971
Carcharhinus limbatus	10	K81	2	0.008	1.000
Daphnia obtusa	36	K81	2	<0.001	1.000
Desmognathus wrightii	28	TN93	2	<0.001	1.000
Dicerandra spp.	28	F81	3	1.000	NA
Emerita talpoida	3	F81	1	NA	NA
Eumeces fasciatus	82	TN93	5	<0.001	1.000
Eurycea bislineata	52	TN93	2	<0.001	1.000
Eurycea cirrigera	247	TN93	12	<0.001	1.000
Eurycea junaluska	6	TN93	2	0.167	1.000
Eurycea multiplicata	44	TN93	2	<0.001	0.982
Eurycea tymerensis	14	TN93	2	0.070	1.000
Eurycea wilderae	125	TN93	3	<0.001	1.000
Fagus grandifolia	22	F81	2	<0.001	1.000
Farfantepenaeus aztecus	76	K80	2	<0.001	1.000
Gracilaria tikvahiae	21	TN93	2	0.047	1.000
Hypentelium nigricans	72	TN93	2	<0.001	1.000
Lampsilis altilis	5	F81	2	0.200	NA
Lampsilis australis	5	F81	2	0.200	1.000
Lampsilis ovata	2	F81	1	NA	NA
Lampsilis perovalis	4	F81	2	0.250	NA
Lampsilis teres	2	F81	1	NA	NA
Liquidambar styraciflua	111	F81	2	<0.001	0.984
Litopenaeus setiferus	91	K80	2	<0.001	1.000
Pagarus longicarpus	67	K81	3	<0.001	1.000
Pagarus pollicaris	21	K81	2	<0.001	1.000

Table 3. Continued...

Species	Number of individuals	Model of sequence evolution	K selected by comparing BIC vs. K plots	*P* from PERMANOVA	population membership probability
Pantherophis obsoletus	37	K81	2	<0.001	1.000
Percina caprodes	13	TN93	2	0.007	1.000
Percina nasuta	6	TN93	2	0.133	1.000
Percina phoxocephala	34	TN93	3	<0.001	0.830
Prunus spp.	223	K81	2	<0.001	1.000
Pseudacris brachyphona	25	K81	2	<0.001	1.000
Pseudacris brimleyi	8	K81	2	0.036	1.000
Pseudacris clarkii	9	TN93	2	0.012	1.000
Pseudacris crucifer	61	K80	5	<0.001	1.000
Pseudacris feriarium	60	TN93	2	<0.001	1.000
Pseudacris nigrita	19	TN93	2	0.006	1.000
Pseudacris spnov	27	TN93	2	<0.001	1.000
Rana catesbeiana	211	TN93	2	<0.001	1.000
Seriola dumerli	16	K81	2	<0.001	1.000
Sphagnum bartlettianum	11	F81	2	0.090	1.000
Spisula solidissima	52	K81	3	<0.001	1.000
Tilia americana	298	K81	3	<0.001	0.992
Trillium cuneatum	281	F81	7	1.000	1.000
Tryphlotriton spelaeus	12	K81	2	0.084	1.000
Uniola paniculata	130	TN93	4	<0.001	1.000
Xerula furfuracea	25	K80	2	0.041	1.000

the models based on these data (Individual maps in Fig. S4). The output maps displaying the cumulative stable habitat (including combinations of major functional groups of Amphibians, Reptiles, Terrestrial, Terrestrial & Freshwater), that existed in both the current day and LGM predictions are in (Fig. 4). Amphibians and Reptiles had the greatest amount of stable habitat extending in a band from Texas across the Florida panhandle to North Carolina, while the Freshwater and Plants categories were mainly localized within the Gulf region and in the Carolinas. The complete Terrestrial & Freshwater group had the greatest amount of stable habitat along the Gulf Coast and near North Carolina. In marine species there was stable habitat along the Gulf of California, along the tip of the Florida Peninsula, and localized areas throughout the Caribbean and up into the east coast; these models were based on three species, so there was a maximum overlap of three.

Multiple Matrix Regression with Randomization

The MMRR required both a genetic distance matrix (requiring more than 3 individuals that exhibit genetic differences) and a species distribution model, generated previously. Using these criteria, we were able to include 28 species in this analysis. The multiple matrix regression highlighted the differences between species, with varying combinations of our independent factors (IBD, IBE, and IBI) being found significant (Fig. 5). Additionally, those variables that were correlated (>0.5) and were both significant, were checked using partial mantel tests. This analysis was able to explain between 3% and 86% of the variation in genetic distances using varying combinations of IBD, IBE and IBI, averaging 27% and a median of 14% variation explained. In short, IBD was found to be a significant factor in 71% of species, IBE in 29%, and IBI in 32% of species. Interestingly, 14% of species' genetic diversity was not significantly influenced by any of these factors. There were no apparent trends based on taxonomy or other characteristics of species in terms of which factors explained genetic variation in a given species.

Random Forest

Across all tests the random forest analysis was generally not effective at building a predictive model that could correctly assign species to the predetermined categories (Table S11), a result that may result from the small number of species present in our data matrix (Table S12). The use of the machine learning framework provides a check against overinterpretation of similar results in particular species.

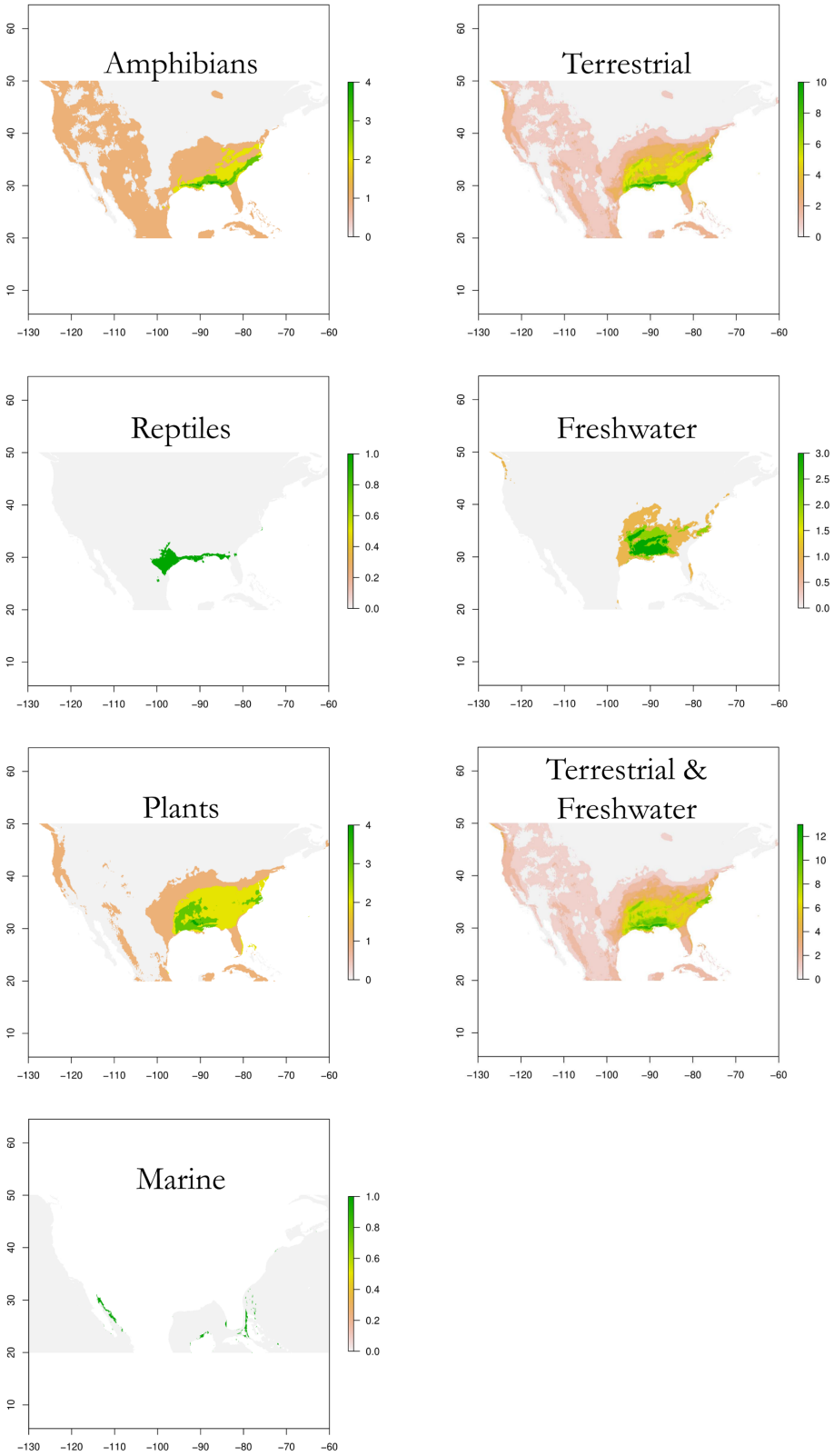


Figure 4. Regions of habitat stability mapped for terrestrial (Amphibians/Plants/Reptiles) and aquatic (Freshwater/Marine) organisms. Each figure depicts the stability of the habitat in each grid cell since the last glacial maximum. Scales bars (to right of each figure) show the color scale representing the number of species with overlapping stable habitat within each taxon type.

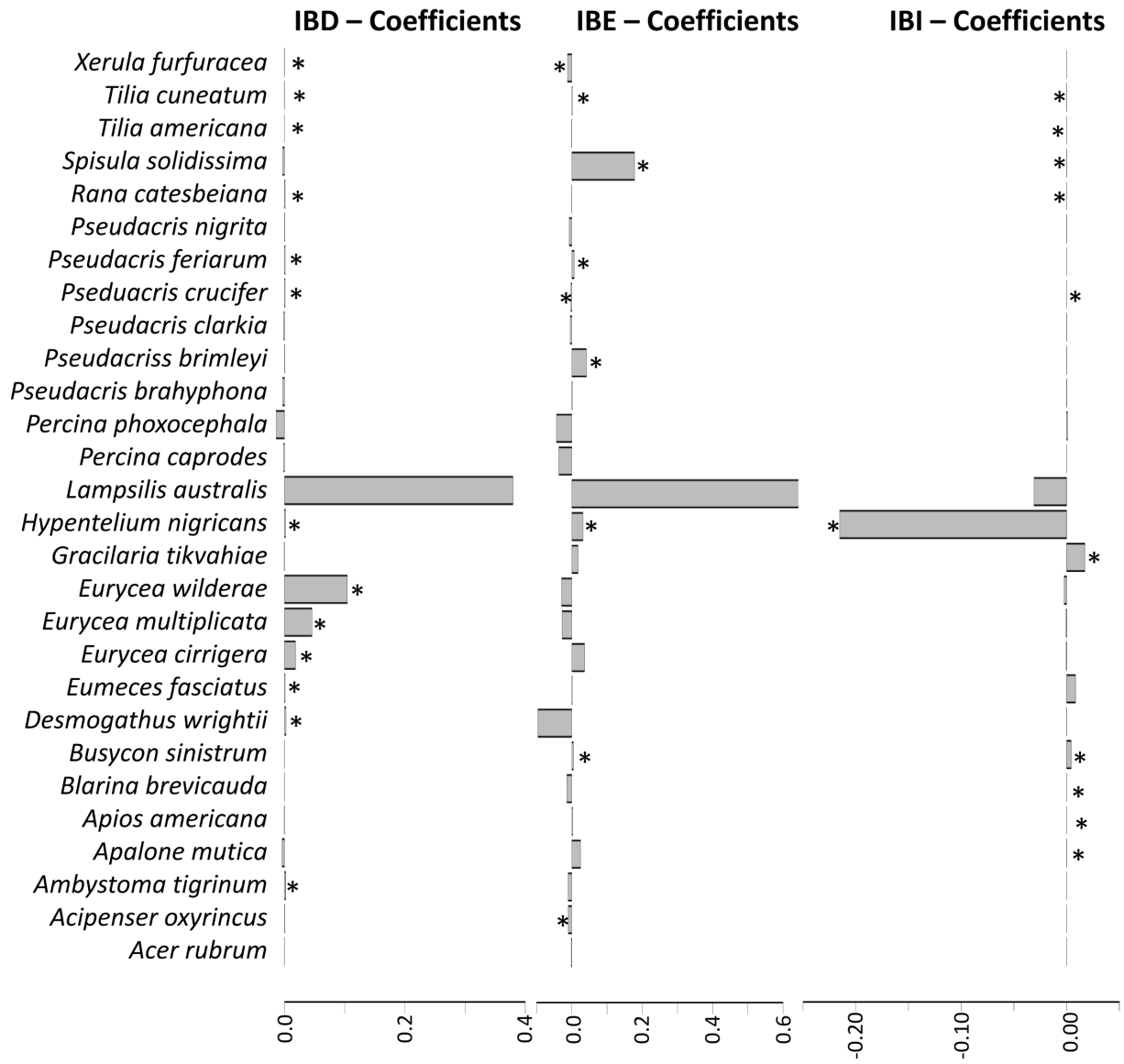


Figure 5. Results of multiple matrix regression across species. Shown for each species are the *p*-values and coefficients for three tests (IBD, IBE, IBI) with significant results with an *. Full detailed numbers can be found in Table S11.

Discussion

Data repurposing (Sidlauskas et al. 2010) provides a more comprehensive approach than meta-analysis for comparative phylogeography because data collected from species that share a geographic distribution can be analyzed and interpreted in a common framework. While the species examined here have all been investigated previously (e.g., Soltis 2000, Avise 2006), the specific contexts of these studies differ, which makes it difficult to evaluate common factors. Our results support the viewpoint that many forces combine to influence genetic diversity within species (e.g., Zhang et al. 2018). While this result is not unexpected, it is striking to observe in a comparative context across disparate taxa. Three factors (geographic separation, habitat stability, and barriers to dispersal) largely explain gross patterns of intraspecific genetic variation in the species analyzed here.

The first factor that contributes to intraspecific genetic structure is the physical separation of individuals. Genetic isolation by geographic distance (e.g., Wright, 1943) is statistically significant in all but five cases (82%; Fig. 5), and these species generally contain fewer samples, which may lead to false negatives and is consistent with previously reported biases (Meirmans 2012, Pelletier and Carstens 2018). We identified contemporary climatic conditions as a significant factor in 8/28 (29%) species exhibited significant IBE (Fig. 5), a result that is broadly consistent with the AMOVAs that were conducted with species partitioned by 'ecoregions' (19/35 species, a higher proportion

than were significantly partitioned by 'watershed'; Table 2). Within many species, genetic diversity is correlated with differences in the current environment across the range of the species, as assumed by many landscape genetic investigations (Sexton et al. 2013).

Following previous work, we also explored the impact of the phylogeographic breaks that are expected to structure genetic diversity over deeper time scales. In a slight majority of species (18/35; 51%), genetic variance was significantly partitioned across putative biogeographic barriers in our AMOVA results (Table 2). Since these barriers were derived from the literature on species included in this comparison set, this result is circular for some species (see Soltis et al. 2006 for these species). However, many of these same species also exhibited isolation by habitat instability, which also speaks to the relative importance of historical processes in structuring genetic diversity. Unlike Vasconcellos et al. (2019), we found that habitat instability over time does not appear to explain how intraspecific genetic diversity is distributed across the landscape. While 9/28 species exhibited significant isolation by instability, in nearly all of these cases, other factors (i.e., IBD or IBE) were also found to be significant. The nucleotide diversity map (Fig.2) supports this idea. If habitat stability were an important predictor, we would see an "out of refugia" pattern due to those areas being the most stable over long periods of time. Although the combined regression models explained a large (>50%) proportion of the variation in several species, only one of these (*Xerula furfuracea*) included IBI. Overall, this suggests that IBI is not an important contributor to population genetic structure, at least in this region. However, it may play a role in other regions that were more directly impacted by glaciation.

In most cases, either geographic distance or physical barriers were important in determining genetic differentiation; this is supported by both broad-scale phylogeographic and fine-scale landscape approaches. Despite the near-ubiquitous importance of physical barriers and geographic distance, the influence of environmental factors in the process of genetic differentiation should not be ignored. On the contrary, our results implicate a complex mixture of processes related to geographic distance, environment, and instability, which are responsible for affecting within-species genetic differentiation. A more concise view of these complexities may be attained by observing species that were in both the AMOVA and the MMRR analyses (see Table S13). Each species with a significant inter-population AMOVA result (7 species; Table S13) also had significant MMRR results. In only one of these cases was IBD the only significant MMRR result (*Apalone mutica*), supporting the role of processes other than geographic distance or geographic barriers in structuring genetic diversity. In several cases (11; Table S14), the MMRR analyses found support for an environmental (IBE), distance-related (IBD), and/or instability-related (IBI) factor without support for a phylogeographic barrier hypothesis. Clearly, geographic distance/phylogeographic breaks are important facets to intraspecific genetic differentiation, but an overreliance on these types of hypotheses would ignore other variables explaining this differentiation, and in turn could prevent the recognition of patterns that may exist between taxonomic groups of interest. These results highlight the need for the integration of both phylogeographic and landscape genetic approaches in investigations that seek to discover processes affecting genetic differentiation within species. They also suggest that sampling bias is a potential limitation. For example, data from some of the species used here were able to be included in only some analyses due to low sampling numbers in some regions.

The mixed results presented here offer the possibility for hypotheses that could be tested in future studies in this region. In closely related species, we could observe differences between both time scales influencing genetic structuring but also the factors present in the same time scale. To us, this indicates that we cannot simply apply understanding from one species and expect it to hold for another species, even a closely related one. Evolution is a complex process and it can be difficult to measure every feature that may be under selection, leading to genetic structuring, especially for non-model systems. For example, two species of frogs may be evolutionarily similar, exist in the same time and space, but have experienced vastly different evolutionary pressures leading to variations in the factors influencing their genetic structure. One of the two species may be slightly more impacted by environmental pressures that are hard to detect, so it may be affected by IBE where the other species may not be, but these types of relationships are hard to detect when only using one type of analysis.

Our results suggest that both broad historical and fine scale contemporary processes are likely to influence genetic diversity within a given species. Rissler (2016) suggested that landscape genetics and phylogeography were headed towards a conceptual unification due to advances in sequencing technology and analytical approaches. As observed here, a more complete understanding of the forces that influence genetic diversity might be gained through a conceptual unification of these two methods, particularly if data from a given species is analyzed using approaches drawn from each. Our study highlights the multiple processes that likely influence genetic structure in a given species and suggests that any investigation conducted from a single perspective (i.e., historical or contemporary; phylogeographic or landscape genetic) is likely to provide an incomplete understanding of the focal system.

Data Accessibility

All code used is freely available for download at https://github.com/jgwieringa/IBI_and_others and all sequencing data can be collected from the source manuscripts listed in Table 1.

Acknowledgements

The authors thank Soltis et al. (2006) for compiling the review that served as a starting point for our own analyses. This work is supported by the following means: EMF (process #88881.170016/2018-1) is supported through the Coordenação de Apoio ã Formacção de Pessoal de Nivel Superior (CAPES) for his doctoral fellowship, MVD (process #88881.190071/2018-01) is supported by CAPES Programa de Doutorado-sanduíche no Exterior (PDSE), MLS is support by the National Science Foundation Graduate Research Fellowship Program under Grant #: DG-1343012, HG is supported by the Ohio State University Helen M and Milton O Lee Fellowship, DJP is supported by the Ohio State University Graduate Enrichment Fellowship, and DD is supported by the OSU University Fellowship. MRB is supported by National Science Foundation Graduate Research Fellowships Program #DGE-1343012. Some analyses were conducted using computational resources from the Ohio Supercomputer Center (PAA0202).

Supplementary Materials

The following materials are available as part of the online article from https://escholarship.org/uc/fb

Table S1. Population assignment analyses using four methods.

Table S2. Data collection for all species and estimated parameter values.

Table S3. AMOVA results for ecoregion analyses.

Table S4. AMOVA results for watershed analyses.

Table S5. AMOVA results for coastal analyses.

Table S6. AMOVA results for Appalachian analyses.

Table S7. AMOVA results for Apalachicola analyses.

Table S8. AMOVA results for Mississippi analyses.

Table S9. Georeferenced localities for all individuals used in manuscript.

Table S10. AUC evaluation for each species from the SDM analyses.

Table S11. Detailed results from the MMR analysis.

Table S12. Detailed results from the Random Forest analysis.

Table S13. Data matrix used in the Random Forest analysis.

Table S14. Summary of results from MMR and AMOVA.

Figure S1. Graphical summary of data analysis strategy.

Figure S2. BIC vs K plots for all species giving the recommended number of K for each species.

Figure S3. PCOA visualization for population groupings.

Figure S4. Species distribution models for all species in present and last glacial maximum climates.

Appendix S1. Supplementary bibliography.

Appendix S2. Analysis pipeline scripts and associated readme files.

References

Agriculture and Agri-Food Canada [AAFC] (2017) Terrestrial ecoprovinces of Canada. National Ecological Framework for Canada. Available at http://sis.agr.gc.ca/cansis/nsdb/ecostrat/gis_data.html

Anderson, M. J. (2006) Distance-based tests for homogeneity of multivariate dispersions. Biometrics, 62, 245-253.

Austin, J. D., Lougheed, S. C. & Boag, P. T. (2004) Discordant temporal and geographic patterns in maternal lineages of eastern north American frogs, *Rana catesbeiana* (Ranidae) and *Pseudacris crucifer* (Hylidae). Molecular Phylogenetics and Evolution, 32, 799-816.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A. & Saunders, N. C. (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics, 18, 489-522.

Avise, J.C. (2000) Phylogeography: the history and formation of species. Harvard University Press.

Barrow, L. N., Soto-Centeno, J. A., Warwick, A. R., Lemmon, A. R., Lemmon, E. M. (2017) Evaluating hypotheses of expansion from refugia through comparative phylogeography of south-eastern Coastal Plain amphibians. Journal of Biogeography, 44, 2692-2705.

Berendzen, P. B., Simons, A. M. & Wood, R. M. (2003) Phylogeography of the northern hogsucker, *Hypentelium nigricans* (Teleostei: Cypriniformes): Genetic evidence for the existence of the ancient Teays River. Journal of Biogeography, 30, 1139-1152.

Biau, G. (2012) Analysis of a random forests model. Journal of Machine Learning Research, 13, 1063-1095.

Bivand, R., Archer, E., Baddeley, A., Bearman, N., Callahan, J., Forrest, D., Friendly, M., Giraudoux, P., Golicher, D., Gomez, V. R., Hausmann, P., Hufthammer, K. O., Jagger, T., Johnson, K., Luque, S., MacQueen, D., Niccolai, A., Pebesma, E., Lamigueiro, O. P., Plunkett, E., Short, T., Snow, G., Stabler, B., Stokely, M. & Turner, R. (2019) R Package 'maptools.' https://cran.r-project.org/web/packages/maptools/index.html

Bivand, R., Keitt, T., Rowlingson, B., Pebesma, E., Summer, M., Higmans, R., Rouault, E., Warmerdam, F., Ooms, J. & Rundel, C. (2019)

R Package 'rgdal.' https://CRAN.R-project.org/package=rgdal

Bivand, R., Stuetz, R., Ove, K., Giraudoux, P., Davis, M. & Santilli, S. (2019) R Package 'rgeos'. https://cran.r-project.org/web/packages/rgeos/index.html

Bonett, R. M. & Chippindale, P. T. (2004) Speciation, phylogeography and evolution of life history and morphology in plethodontid salamanders of the *Eurycea multiplicata* complex. Molecular Ecology, 13, 1189-1203.

Brant, S. V. & Ortí, G. (2003) Phylogeography of the Northern short-tailed shrew, *Blarina brevicauda* (Insectivora: Soricidae): past fragmentation and postglacial recolonization. Molecular Ecology, 12, 1435-1449.

Bremer, J. R. A., Ditty, J. G., Turner, J. S. & Saxton, B. L. (2010) Molecular species identification of commercially important penaeid shrimp from the Gulf of Mexico using a multiplex haplotype-specific PCR assay. Biochemical Systematics and Ecology, 38, 715-721.

Burbrink, F. T., Lawson, R. & Slowinski, J. B. (2000) Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution, 54, 2107-2118.

Campbell, D. C., Serb, J. M., Buhay, J. E., Roe, K. J., Minton, R. L. & Lydeard, C. (2005) Phylogeny of North American amblemines (Bivalvia, Unionoida): prodigious polyphyly proves pervasive across genera. Invertebrate Biology, 124, 131-164.

Carnaval, A. C., Hickerson, M. J. & Haddad, C. F. B. (2009) Stability predicts genetic diversity in the Brazilian Atlantic Forest hotspot. Science, 323, 785–790.

Carstens, B. C. & Richards, C. L. (2007) Integrating coalescent and ecological niche modeling in comparative phylogeography. Evolution, 61, 1439–1454.

Cola, V. Di, Broennimann, O., Petitpierre, B., Breiner, F. T., Amen, M. D., Randin, C., Engler, R., Pottier, J., Pio, D., Dubuis, A., Mateo, R. G., Hordijk, W., Salamin, N. & Guisan, A. (2017) ecospat: an R package to support spatial analyses and modeling of species niches and distributions. Ecography, 40, 774–787.

Commission for Environmental Cooperation Working Group [CECWG] (1997) Ecological Regions of North America: Toward a Common Perspective.

Crespi, E. J., Rissler, L. J. & Browne, R. A. (2003) Testing Pleistocene refugia theory: phylogeographical analysis of *Desmognathus wrighti*, a high-elevation salamander in the southern Appalachians. Molecular Ecology, 12, 969-984.

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2015) jModelTest 2: more models, new heuristics and high- performance computing. Nature Methods, 9, 6–9.

Emmanuel, P., Jombart, T., Brian, K., Schliep, K. & Potts, A. (2018) R Package 'pegas.' Environmental Systems Research Institute [ESRI] (2014) ArcGIS Desktop: Release 10. Redlands, CA.

Excoffier, L., Smouse, P. E. & Quattro, J. M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131, 479-491.

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary bioinformatics, 1, 117693430500100003.

Felsenstein, J. (1981) Evolutionary Trees from DNA Sequences: A Maximum Likelihood Approach, Journal of Molecular Evolution, 368–376.

Fick, S. E. & Hijmans, R. J. (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology, 37, 4302-4315.

Geber, M. A. (2011) Ecological and evolutionary limits to species geographic ranges. The American Naturalist, 178, S1-S5.

Gold, J. R. & Richardson, L. R. (1998) Population structure in greater amberjack, *Seriola dumerili*, from the Gulf of Mexico and the western Atlantic Ocean. Fishery Bulletin, 96, 767-778.

Gonzales, E., Hamrick, J. L. & Chang, S. M. (2008) Identification of glacial refugia in south-eastern North America by phylogeographical analyses of a forest understorey plant, *Trillium cuneatum*. Journal of Biogeography, 35, 844-852.

Goslee, S. C. & Urban, D. L. (2007) The ecodist Package for Dissimilarity-based Analysis of Ecological Data. Journal of Statistical Software, 22, 1-19.

Graham, C. H., VanDerWal, J., Phillips, S. J., Moritz, C. & Williams, S. E. (2010) Dynamic refugia and species persistence: tracking spatial shifts in habitat through time. Ecography, 33(6), 1062-1069.

Grunwald, C., Maceda, L., Waldman, J., Stabile, J. & Wirgin, I. (2008) Conservation of Atlantic

sturgeon *Acipenser oxyrinchus oxyrinchus*: delineation of stock structure and distinct population segments. Conservation Genetics, 9, 1111-1124.

Guindon, S. & Gascuel, O. (2003) A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies. Systematic Biology, 52, 696–704.

Gurgel, C. F. D., Fredericq, S. & Norris, J. N. (2004) Phylogeography of *Gracilaria tikvahiae* (Gracilariaceae, Rhodophyta): a study of genetic discontinuity in a continuously distributed species based on molecular evidence. Journal of Phycology, 40, 748-758.

Hao, Y. J., Zhao, Q., Wang, S. X. & Yang, Z. L. (2016) What is the radicate *Oudemansiella* cultivated in China? Phytotaxa, 286, 1-12.

Hare, M. P. & Weinberg, J. R. (2005) Phylogeography of surfclams, *Spisula solidissima*, in the western North Atlantic based on mitochondrial and nuclear DNA sequences. Marine Biology, 146, 707-716.

Hijmans, R. J., van Etten, J., Sumner, M., Cheng, J., Bevan, A., Bivand, R., … Hijmans, M. R. J. (2019) R Package 'raster'.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, G. & Jarvis, A. (2005) Very high-resolution interpolated climate surfaces for global land areas. International Journal of Climatology, 25, 1965–1978.

Hijmans, R. J. & Elith, J. (2017) Species distribution modeling with R. CRAN Project, pgs. 1-79. https://cran.r-project.org/web/packages/dismo/vignettes/sdm.pdf

Hijmans, R. J., Phillips, S., Leathwick, J. & Elith, J. (2017) R Package 'dismo'. https://cran.r-project.org/web/packages/dismo/index.html

Hijmans, R., Sumner, M., Macqueen, D., Lemon, J., Brien, J. O. & Rourke, J. O. (2018) R Package 'sp'.

Hodel, R. G. & Gonzales, E. (2013) Phylogeography of sea oats (*Uniola paniculata*), a dune-building coastal grass in southeastern North America. Journal of Heredity, 104, 656-665.

Howes, B. J., Lindsay, B. & Lougheed, S. C. (2006) Range-wide phylogeography of a temperate lizard, the five-lined skink (*Eumeces fasciatus*). Molecular Phylogenetics and Evolution, 40, 183-194.

Hugall, A., Moritz, C., Moussalli, A. & Stanisic, J. (2002) Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). Proceedings of the National Academy of Sciences USA, 99, 6112–6117.

Joly, S. & Bruneau, A. (2004) Evolution of triploidy in *Apios americana* (Leguminosae) revealed by genealogical analysis of the histone H3-D gene. Evolution, 58, 284-295.

Jombart, T. & Ahmed, I. (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics, 27, 3070–3071.

Jombart, T. & Collins, C. (2015) A tutorial for Discriminant Analysis of Principal Components (DAPC) using adegenet 2.0.0.

Jombart, T., Lyon, D. & Biome, L. De. (2008) adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics, 24, 1403–1405.

Keeney, D. B., Heupel, M. R., Hueter, R. E. & Heist, E. J. (2005) Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. Molecular Ecology, 14, 1911-1923.

Kimura, M. (1981) Estimation of evolutionary distances between homologous nucleotide sequences b. Proceedings of the National Academy of Sciences USA, 78, 454–458.

Kimura, M. (1980) A Simple Method for Estimating Evolutionary Rates of Base Substitutions Through Comparative Studies of Nucleotide Sequences. Journal of Molecular Evolution, 16, 111-120.

Kozak, K. H., Blaine, R. A. & Larson, A. (2006) Gene lineages and eastern North American palaeodrainage basins: phylogeography and speciation in salamanders of the *Eurycea bislineata* species complex. Molecular Ecology, 15, 191-207.

Krellwitz, E. C., Kowallik, K. V. & Manos, P. S. (2001) Molecular and morphological analyses of *Bryopsis* (Bryopsidales, Chlorophyta) from the western North Atlantic and Caribbean. Phycologia, 40, 330-339.

Legendre, P., Lapointe, F. I. & Casgrain, P. (1994) Modeling Brain Evolution from Behavior: A Permutational Regression Approach. Evolution, 48, 1487–1499.

Lemmon, E. M., Lemmon, A. R., Collins, J. T., Lee-Yaw, J. A. & Cannatella, D. C. (2007) Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs

(*Pseudacris*). Molecular Phylogenetics and Evolution, 44, 1068-1082.

Liaw, A. & Wiener, M. (2002) Classification and regression by random Forest. R news, 2, 18-22.

Lydeard, C., Minton, R. L. & Williams, J. D. (2000) Prodigious polyphyly in imperiled freshwater pearly-mussels (Bivalvia: Unionidae): a phylogenetic test of species and generic designations. Geological Society, London, Special Publications, 177, 145-158.

Maggioni, R., Rogers, A. D., Maclean, N. & D'Incao, F. (2001) Molecular phylogeny of western Atlantic Farfantepenaeus and Litopenaeus shrimp based on mitochondrial 16S partial sequences. Molecular Phylogenetics and Evolution, 18, 66-73.

Mateo, G., Vanderpoorten, A. & Laenen, B. (2013) Modeling species distributions from heterogeneous data for the biogeographic regionalization of the European bryophyte flora. PLoS ONE, 8, e55648.

McCarthy, D. M. & Mason-Gamer, R. J. (2016) Chloroplast DNA-based phylogeography of *Tilia americana* (Malvaceae). Systematic Botany, 41, 865-880.

Mcgovern, T. M. & Hellberg, M. E. (2003) Cryptic species, cryptic endosymbionts, and geographical variation in chemical defences in the bryozoan *Bugula neritina*. Molecular Ecology, 12, 1207-1215.

McLachlan, J. S., Clark, J. S. & Manos, P. S. (2005) Molecular indicators of tree migration capacity under rapid climate change. Ecology, 86, 2088-2098.

Mcmillen-Jackson, A. L. & Bert, T. M. (2003) Disparate patterns of population genetic structure and population history in two sympatric penaeid shrimp species (*Farfantepenaeus aztecus* and *Litopenaeus setiferus*) in the eastern United States. Molecular Ecology, 12, 2895-2905.

McRae, B. H. (2006) Isolation by resistance. Evolution, 60, 1551–1561.

Meirmans, P. G. (2012) The trouble with isolation by distance. Molecular Ecology, 21, 2839-2846.

Morris, A. B., Ickert-Bond, S. M., Brunson, D. B., Soltis, D. E. & Soltis, P. S. (2008) Phylogeographical structure and temporal complexity in American sweetgum (*Liquidambar styraciflua*; Altingiaceae). Molecular Ecology, 17, 3889-3900.

Natural Resources Canada, United States Geological Survey, and Instituto Nacional de Estadística y Geografía [NRC, USGS, and INEG] (2010a) North American Atlas – Basin Watersheds. Available at: https://www.sciencebase.gov/catalog/item/4fb697b2e4b03ad19d64b47f

Natural Resources Canada, United States Geological Survey, and Instituto Nacional de Estadística y Geografía [NRC, USGS, and INEG] (2010b) North American Atlas – Hydrography. Available at: https://www.sciencebase.gov/catalog/item/4fb55df0e4b04cb937751e02

Near, T. J. (2008) Rescued from synonymy: a redescription of *Percina bimaculata* Haldeman and a molecular phylogenetic analysis of logperch darters (Percidae: Etheostomatinae). Bulletin of the Peabody Museum of Natural History, 49, 3-19.

Nosil, P. (2004) Reproductive isolation caused by visual predation. Proceedings of the Royal Society B, 271, 1521–1528.

Nylander, J. A. A. (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Oliveira, L. O., Huck, R. B., Gitzendanner, M. A., Judd, W. S., Soltis, D. E. & Soltis, P. S. (2007) Molecular phylogeny, biogeography, and systematics of Dicerandra (Lamiaceae), a genus endemic to the southeastern United States. American Journal of Botany, 94, 1017-1027.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., … Szoecs, E. (2018) R Package 'vegan'.

Orsini, L., Vanoverbeke, J., Swillen, I., Mergeay, J. & De Meester, L. (2013) Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. Molecular Ecology, 22, 5983-5999.

Papadopoulou, A. &Knowles, L.L. (2016) Toward a paradigm shift in comparative phylogeography driven by trait-based hypotheses. Proceedings of the National Academy of Sciences USA 113, 29, 8018-8024.

Paradis, E., J. Claude & K. Strimmer. (2004) APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics, 20, 289-290.

Pebesma, E. & Bivand, R. S. (2005) Classes and Methods for Spatial Data: the sp Package. 21pp.

Penton, E. H., Hebert, P. D. N. & Crease, T. J. (2004) Mitochondrial DNA variation in North American populations of *Daphnia obtusa*: continentalism

or cryptic endemism? Molecular Ecology, 13, 97-107.

Pelletier, T. A. & Carstens, B. C. (2018) Geographical range size and latitude predict population genetic structure in a global survey. Biology letters, 14, 20170566.

Petersen, R. H. & Hughes, K. W. (2010) The *Xerula*/*Oudemansiella* complex (Agaricales). Nova Hedwigia, Beihefte Beih, 137.

Phillips, S. J., Dudík, M. & Schapire, R. E. (2017) Maxent software for modeling species niches and distributions (Version 3.4.1). Available at http://biodiversityinformatics.amnh.org/open_source/maxent/

Rios, N. E. & Bart, H. L. (2010) GEOLocate (Version 3.22) Belle Chasse, LA: Tulane University Museum of Natural History.

Rissler, L. J. (2016) Union of phylogeography and landscape genetics. Proceedings of the National Academy of Sciences USA, 113, 8079–8086.

Robison, H. W., Cashner, R. C., Raley, M. E. & Near, T. J. (2014) A new species of darter from the Ouachita Highlands in Arkansas related to *Percina nasuta* (Percidae: Etheostomatinae). Bulletin of the Peabody Museum of Natural History, 55, 237-253.

Roe, K. J., Hartfield, P. D. & Lydeard, C. (2001) Phylogeographic analysis of the threatened and endangered superconglutinate-producing mussels of the genus *Lampsilis* (Bivalvia: Unionidae). Molecular Ecology, 10, 2225-2234.

Rozas, J., Ferrer-mata, A., S, J. C., Guirao-rico, S., Librado, P., Ramos-onsins, E., … & Gene, D. De. (2017) DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Molecular Biology and Evolution, 34, 3299–3302.

Satler, J. D., Carstens, B. C. (2016) Phylogeographic concordance factors quantify phylogeographic congruence among co-distributed species in the *Sarracenia alata* pitcher plant system. Evolution, 70, 1105-1119.

Sbrocco, E. J. & Barber, P. H. (2013) MARSPEC: ocean climate layers for marine spatial ecology. Ecological Archives, 94, 27705.

Sexton, J. P., Hangartner, S. B. & Hoffmann, A. A. (2014) Genetic isolation by environment or distance: which pattern of gene flow is most common? Evolution, 68, 1-15.

Shaw, A. J., Cox, C. J. & Boles, S. B. (2005) Phylogeny, species delimitation, and recombination in *Sphagnum* section *Acutifolia*. Systematic Botany, 30, 16-33.

Shaw, J. & Small, R. L. (2005) Chloroplast DNA phylogeny and phylogeography of the North American plums (*Prunus* subgenus *Prunus* section *Prunocerasus*, Rosaceae). American Journal of Botany, *92*, 2011-2030.

Sidlauskas, B., Ganapathy, G., Hazkani-Covo, E., Jenkins, K. P., Lapp, H., McCall, L. W., ... & Kidd, D. M. (2010) Linking big: the continuing promise of evolutionary synthesis. Evolution, 64, 871-880.

Soltis, P. S. (2006) Comparative phylogeography of unglaciated eastern North America. Molecular Ecology, 15, 4261–4293.

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M. A. X., … Robertson, J. (2007) Marine Ecoregions of the World: A bioregionalization of coastal and shelf areas. BioScience, 57, 573–583.

Sullivan, J., Arellano, E. & Rogers, D. S. (2000) Comparative phylogeography of Mesoamerican highland rodents: concerted versus independent response to past climatic fluctuations. The American Naturalist, 155, 755-768.

Tam, Y. K., Kornfield, I. & Ojeda, F. P. (1996) Divergence and zoogeography of mole crabs, *Emerita* spp. (Decapoda: Hippidae), in the Americas. Marine Biology, 125, 489-497.

Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular biology and evolution, 10, 512-526.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22, 4673–4680.

United Nations Environment Programme-World Conservation Monitoring Centre [UNEP-WCMC] (2002) Mountains and tree cover in mountain regions. Available at https://www.unep-wcmc.org/resources-and-data/mountains-and-tree-cover-in-mountain-regions

United States Environmental Protection Agency [USEPA] (2012) U.S. Level III and IV Ecoregions. Research Triangle Park, NC. Available at https://catalog.data.gov/dataset/u-s-level-iii-and-iv-ecoregions-u-s-epa

Urbanek, S. (2019) R Package 'rJava'. van Proosdij, A. S. J., Sosef, M. S. M., Wieringa, J. J. & Raes, N. (2016) Minimum required number of specimen records to develop accurate species distribution models. Ecography, 39, 542–552.

Vasconcellos, M. M., Ortiz, E. M., Weber, J. N., Cannatella, D. C. & Rodrigues, M. T. (2019) Isolation by instability: Historical climate change shapes population structure and genomic divergence of treefrogs in the Neotropical Cerrado savanna. Molecular Ecology, 28, 1748–1764.

Vazquez-Bader, A. R., Carrero, J. C., Garcia-Varela, M., Gracia, A. & Laclette, J. P. (2004) Molecular phylogeny of superfamily Penaeoidea Rafinesque-Schmaltz, 1815, based on mitochondrial 16S partial sequence analysis. Journal of Shellfish Research, 23, 911-918.

Wang, I. J. (2013) Examining the Full Effects of Landscape heterogeneity on spatial genetic variation: A multiple matrix regression approach for quantifying geographic and ecological isolation. Evolution, 67, 3403–3411.

Weisrock, D. W. & Janzen, F. J. (2000) Comparative molecular phylogeography of North American softshell turtles (Apalone): implications for regional and wide-scale historical evolutionary forces. Molecular Phylogenetics and Evolution, 14, 152-164.

Wise, J., Harasewych, M. G. & Dillon, R. T. (2004) Population divergence in the sinistral whelks of North America, with special reference to the east Florida ecotone. Marine Biology, 145, 1167-1179.

Wright, S. (1943) Isolation by distance. Genetics, 28, 114-138.

Yang, Z. L., Zhang, L. F., Mueller, G. M., Kost, G. W. & Rexer, K. H. (2009) A new systematic arrangement of the genus *Oudemansiella* s. str. (Physalacriaceae, Agaricales). Mycosystema, 28, 1-13.

Young, A., Torres, C., Mack, J. & Cunningham, C. (2002) Morphological and genetic evidence for vicariance and refugium in Atlantic and Gulf of Mexico populations of the hermit crab *Paguruslongicarpus*. Marine Biology, 140, 1059-1066.

Zhang, Y. H., Wang, I. J., Comes, H. P., Peng, H. & Qiu, Y. X. (2016) Contributions of historical and contemporary geographic and environmental factors to phylogeographic structure in a Tertiary relict species, *Emmenopterys henryi* (Rubiaceae). Scientific reports, 6, 24041.

Submitted: 01 October 2019
First decision: 20 November 2019
Accepted: 07 January 2020

Edited by Janet Franklin

