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

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E BIODIVERSIDADE

FILOGEOGRAFIA E GENÔMICA DE POPULAÇÕES DE *Pitcairnia lanuginosa* (BROMELIACEAE)

BÁRBARA SIMÕES SANTOS LEAL

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

Orientadora: Clarisse Palma da Silva

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TÍTULO DA TESE: Filogeografia e genômica de populações de *Pitcairnia lanuginosa* (Bromeliaceae)

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"Toda a nossa ciência, comparada com a realidade, é primitiva e infantil – e, no entanto, é a coisa mais preciosa que temos."
(Albert Einstein)

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DECLARAÇÃO

Declaro que as opiniões, hipóteses e conclusões ou recomendações expressas neste material são de responsabilidade do(s) autor(es) e não necessariamente refletem a visão da CAPES e FAPESP.

SUMÁRIO

RESUMO GERAL

Os Neotrópicos possuem níveis de diversidade taxonômica, filogenética e funcional extremamente altos, mesmo quando comparados aos de outras regiões tropicais do mundo. O estudo dos processos envolvidos na diversificação de linhagens intra-específicas e no surgimento de novas espécies é essencial para a interpretação dos padrões de alta biodiversidade observados nessa região. Nesta tese, buscamos descrever os padrões de diversificação intra-específica e inferir os processos e mecanismos que governam a diversidade nos Neotrópicos, primeiramente, por meio de uma revisão de estudos filogeográficos de plantas no Brasil (CAPÍTULO 1), e, em seguida, por meio de estudos empíricos utilizando como modelo uma espécie de ampla porém fragmentada distribuição nos Neotrópicos, *Pitcairnia lanuginosa* (Bromeliaceae) (CAPÍTULOS 2 e 3). No CAPÍTULO 1, revisamos os resultados e sintetizamos o conhecimento proveniente de 41 estudos de filogeografia de plantas já publicados. Em suma, nossa revisão mostra que as oscilações climáticas do Pleistoceno afetaram diferentemente plantas associadas a tipos de vegetações contrastantes dentro de cada bioma. Além do clima, os estudos reunidos apontam que variação das condições de solos e o surgimento de barreiras geográficas (como montanhas e rios) também estão envolvidos na evolução da biota vegetal desta região. No CAPÍTULO 2, empregamos marcadores moleculares tradicionais (sequenciamento de sanger e microssatélites) e modelos de distribuição de espécies (SDMs) para verificar o papel dos processos demográficos históricos sobre a variação genética neutra e para verificar a existência de linhagens em *P. lanuginosa*, utilizando uma abordagem filogeográfica. Nossos dados apontam uma baixa diversidade dentro das populações e elevada estrutura genética entre populações da espécie, o que implica num papel determinante da deriva genética para a sua evolução. Além disso, os resultados indicam a existência de duas linhagens em *P. lanuginosa*, ocupando o Cerrado brasileiro e a porção Central dos Yungas andinos, respectivamente, que é potencialmente explicada pela colonização dos Yungas a partir da dispersão do Cerrado. Apesar dos SDMs mostrarem uma pequena expansão geográfica da espécie no último máximo glacial, as oscilações climáticas do Pleistoceno parecem ter tido apenas um papel pequeno da divergência de linhagens, uma vez os dados moleculares mostram uma divergência antiga seguido por persistência sem mudança de tamanho populacional das linhagens. Finalmente, no CAPÍTULO 3, integramos dados provenientes de centenas de marcadores SNPs isolados por ddRAD, e dados fenotípicos fisiológicos a nível populacional, para verificar o papel relativo de deriva genética e seleção natural na alta estruturação genética de *P. lanuginosa*. Os resultados confirmam o papel relevante da deriva apontada no capítulo anterior, mas sugerem que a seleção também pode ter contribuído para a diferenciação genética entre as populações apesar do forte efeito da deriva. Dentre os caracteres fenotípicos testados, apenas dois caracteres associados à tolerancia à seca estão potencialmente sob seleção. Padrões de diversificação de espécies herbáceas associadas a ambientes mésicos fragmentados, como *P. lanuginosa*, agregam informações importantes sobre conexões entre biomas e podem ajudar a traçar um panorama geral da história da biogeografia do continente sul-americano. Embora o estudo de evolução da biodiversidade nos Neotrópicos seja desafiador e o nosso conhecimento limitado por alguns obstáculos a pesquisa sobre biodiversidade, o incremento de informações independentes coletadas em uma ampla variedade de organismos tem o potencial de esclarecer os processos complexos que afetaram a biota neotropical.

Palavras-chave: adaptação local; Bromeliaceae; diversificação e especiação; filogeografia; Neotrópicos; sequenciamento RAD.

GENERAL ABSTRACT

The Neotropics harbors high levels of taxonomic, phylogenetic and functional diversity, even comparing to other tropical regions. The studies on processes driving diversification of intra-specific lineages and the origin of new species are essential to interpret the high levels of biodiversity found in this region. Here, we aimed to describe patterns of intra-specific diversification, and infer processes and mechanisms driving the Neotropical diversification by reviewing plant phylogeographic studies in Brazil (CHAPTER 1), and by performing empirical studies using a wide-spread, patchy distributed species, *Pitcairnia lanuginosa*, as a study model (CHAPTERS 2 and 3). In the first chapter, we reviewed and synthesized the knowledge from 41 previously published studies. In summary, we showed that Pleistocene climatic oscillations distinctly affected plants living in contrasting vegetation types within each biome. Despite past climate, these studies pointed that edaphic conditions and geographical barriers (such as mountains and rivers) have also influenced plant evolution patterns in this region. In the second chapter, we employed traditional molecular markers (sanger sequencing and microsatellites genotyping) and species distribution models (SDMs) to test the role of historical demographic processes on neutral genetic variation and verify the existence of lineages within *P. lanuginosa*, using a phylogeographic approach. Our data pointed to low genetic diversity within populations, and high genetic structure among populations within the species, which implies drift as a major force shaping the species evolution. Moreover, results evidenced two distinct *P. lanuginosa* lineages occupying the Brazilian Cerrado and the Central Andean Yungas that have likely diverged through dispersal from the Cerrado to the Central Andean Yungas. Although our SDMs showed a slight expansion of suitable range for *P. lanuginosa* during the Last Maximum Glacial, Pleistocene climatic oscillations seem to have played only a minor role on the diversification of the species, as molecular data show a signature of older divergence, followed by persistence in riparian forests. Finally, at the third chapter, we integrated hundreds of SNP markers defined through double-digest restriction-site associated DNA sequencing (ddRAD-Seq) and population-level phenotypic data, to infer the relative role of natural selection and genetic drift on the high genetic structure of *P. lanuginosa*. Results confirmed the relevant role of drift and suggested that selection plays an additional role on genetic differentiation despite strong drift effect. Among the tested phenotypic traits, only two traits associated to drought tolerance are potentially under selection. Diversification patterns of forbs associated to patchy mesic habitats, such as *P. lanuginosa*, add important information on past connections between biomes and may help to outline the biogeographic history of South America. Although studying biodiversity in the Neotropics remains challenging, and our knowledge is still limited by some obstacle to biodiversity research, increasing independent information from a wide variety of organisms have the potential to elucidate the complex processes driving the Neotropical biota.

Keywords: local adaptation; Bromeliaceae; diversification and speciation; phylogeography; Neotropics; RAD sequencing.

INTRODUÇÃO GERAL

Diversificação e especiação nos Neotrópicos

A região Neotropical é definida como a área que se estende entre o México Central e a Argentina, apresentando diversos biomas com histórias evolutivas distintas e conexões complexas (Morrone, 2013; Hughes et al., 2013). Essa região abriga uma biodiversidade extraordinária, tanto em termos taxonômicos, como em termos filogenéticos e funcionais. (Antonelli et al. 2018), que tem há muito tempo atraído a atenção de pesquisadores (e.g., Humboldt 1820; Gentry 1982). Para alguns grupos, como peixes e plantas, a diversidade de espécies na região é excepcionalmente alta mesmo quando comparada a outras regiões tropicais (Lundberg et al., 2000; Antonelli & Sanmartín, 2011). Dentre as angiospermas, por exemplo, 90-100 mil espécies ocorrem nessa região, o que representa cerca de um terço de toda biodiversidade terrestre, e mais do que o número de espécies somados das demais regiões tropicais (Antonelli & Sanmartín, 2011). Apesar da alta biodiversidade e das inúmeras regiões relevantes para a conservação (*hotspots* de biodiversidade, Myers et al. 2000) ocorrendo nas zonas tropicais das Américas, a maior parte das informações acumuladas sobre os mecanismos de diversificação ainda são provenientes de estudos em regiões temperadas (Beheregaray et al., 2015). De fato, o conhecimento sobre mecanismos e processos responsáveis por moldar a enorme biodiversidade dos Neotrópicos é ainda emergente, mas tem sido beneficiado pelo crescente número de estudos filogenéticos (Antonelli & Sanmartín, 2011; Antonelli et al. 2018) e filogeográficos nas últimas décadas (revisado por Turchetto-Zolet et al. 2013).

Diversos mecanismos bióticos e abióticos têm sido propostos para explicar a evolução da biodiversidade nos Neotrópicos (ver Antonelli & Sanmartin 2011; Hughes et al. 2013). Dentre os mecanismos abióticos, o tempo disponível para eventos de especiação, clima atual e passado, e eventos geológicos como soerguimento de montanhas e mudanças hidrológicas, têm sido frequentemente associados à formação e manutenção da biota neotropical (Antonelli & Sanmartin 2011). Apesar dos processos de diversificação não estarem restritos a um intervalo de tempo particular (Rull 2008, 2011; Antonelli et al. 2018), os eventos geológicos do Terciário e as oscilações climáticas do Quaternário são comumente apontados como períodos importantes para a diversificação entre linhagens intra-específicas e entre espécies proximamente relacionadas nesta região (Turchetto-Zolet et al. 2013). Dentre os eventos geológicos,

sabe-se que o soerguimento dos Andes (cujo pico de elevação ocorreu durante Mioceno tardio, cerca 12 Ma; e início do Pleistoceno, cerca de 4.5 Ma) teve um grande impacto na aceleração das taxas de diversificação do continente sul-americano (Gentry 1982; Hoorn et al., 2010), como documentado numa série de estudos evolutivos com distintos taxa (e.g., Hughes & Eastwood 2006; Chazot et al., 2016; Lagomarsino et al., 2016; Bacon et al., 2018). Outros eventos geológicos, como a formação das cadeiras montanhosas no leste do Brasil (i.e., Serra do Mar e Serra da Mantiqueira) e do planalto central brasileiro no Neogeno também têm sido apontados como eventos importantes para explicar a enorme biodiversidade na região (Lara & Patton 2000; Torres & Ribeiro 2009; Werneck et al. 2012). Além disso, estudos tem demonstrado que oscilações climáticas e transgressões marinhas, particularmente durante a segunda metade do Quaternário, também influenciaram os processos de diversificação nos Neotrópicos ao promover diversas mudanças ambientais (Rull 2011; Turchetto-Zolet et al. 2013).

Estrutura populacional, deriva e adaptação

O balanço entre os distintos fatores evolutivos - deriva, fluxo gênico, mutação e recombinação e seleção natural - levam a variação das frequências alélicas dentro das populações e, consequentemente, a estruturação de populações e divergência intra-específica e, eventualmente, especiação. Sob baixo fluxo de genes, populações podem estar geneticamente estruturadas devido a pressões seletivas impostas por condições ambientais contrastantes e/ou simplesmente por deriva genética (Rousset 2013). A distinção de ambos processos, seleção natural e deriva genética, é portanto, um dos grandes desafios da biologia evolutiva, e a contribuição relativa de ambas forças na evolução da vida na Terra tem sido há muito tempo debatida. Embora taxas de diferenciação genética e especiação não estejam sempre relacionadas (Kisel et al 2012; Singhal et al. 2018), populações altamente estruturadas, cuja divergência entre linhagens intra-específicas é considerável, podem eventualmente ser consideradas "espécies incipientes" (i.e., que não completaram o processo de especiação) ou espécies crípticas e representar modelos interessantes para os estudos de filogeografia e especiação.

A filogeografia é uma disciplina situada entre o estudo dos processos microevolutivos e macroevolutivos. Nas três décadas de existência (termo cunhado por Avise et al. 1987), a filogeografia se firmou como um importante elo entre filogenética e genética de populações, fornecendo um avanço conceitual que se situa na fronteira entre essas disciplinas (Avise 2009; Carstens et al. 2012). Métodos de filogeografia permitem

testar hipóteses biogeográficas, descrever a evolução do isolamento reprodutivo entre populações, inferir processos envolvidos na origem, distribuição e manutenção da biodiversidade e também fazer inferências sobre as mudanças temporais no ambiente físico e biótico de uma população, utilizando dados genéticos contemporâneos (Beheregaray 2008). O poder dessa disciplina está justamente em considerar o contexto geográfico da variação dos dados genéticos ao longo de um contínuo entre populações e espécies (Knowles 2004, Hickerson et al. 2010). Essa abordagem permite, portanto, compreender como os processos demográficos determinam a diversidade e estrutura genética dentro das espécies ou em um grupo de espécies relacionadas (Diniz-Filho et al. 2008).

Historicamente, os estudos de genética de populações e filogeografia estiveram restritos a utilização de um pequeno número de marcadores. O surgimento de novas tecnologias de sequenciamento de DNA (*Next Generation Sequencing*; NGS, ou ainda *hightroughtput sequencing*), entretanto, elevaram para outro patamar tais estudos, ajudando no entendimento sobre os mecanismos de diversificação, e permitindo que os pesquisadores possam responder uma série de perguntas ecológicas e evolutivas que eram antes intratáveis devido ao número limitado de marcadores moleculares (Seehausen et al. 2014; Andrews et al. 2016). Métodos empregando essas novas tecnologias permitem a descoberta, e genotipagem de centenas a milhares de marcadores SNPs em um único passo (Davey et al. 2011; Andrews et al. 2016). Dentre os vários métodos propostos, o de sequenciamento massivo de fragmentos associadas a sítios de restrição (*restriction site associated DNA sequencing*; RAD-seq) (Baird et al. 2008) e derivados (e.g., Petterson et al. 2012), têm se mostrado bastante promissores, especialmente para organismos não-modelo, que não possuem genomas de referência disponíveis. O aumento da cobertura do genoma desses organismos permitiu não só uma reconstrução mais precisa da história evolutiva, inclusive em grupos cuja radiação é recente (e.g Emerson et al. 2010), mas também a identificação de regiões especificas do DNA que contribuem para o isolamento reprodutivo ou estão envolvidas em processos de adaptação local (Payseur et al. 2016).

Para responder algumas das questões que fundamentam o conhecimento biológico sobre os processos de diversificação, é necessário distinguir a heterogeneidade causada por processos estocásticos (inferida a partir de marcadores neutros) da heterogeneidade causada por processos adaptativos (inferida a partir de marcadores sob

seleção). Enquanto marcadores neutros podem identificar diferenciação significativa entre as populações com base no fluxo limitado de genes ou deriva, marcadores de regiões genômicas sob seleção podem identificar populações localmente adaptadas a um habitat particular (Narum et al. 2013). Por gerar uma grande quantidade de sequências amplamente distribuídas no genoma, as técnicas de sequenciamento RAD permitem a identificação de regiões específicas do DNA que estão sob seleção natural. À medida que estudos de genética de populações e filogeografia se tornam mais genômicos, a tendência é que o estudo de adaptação local envolvendo marcadores sob seleção seja integrado a investigação dos processos demográficos utilizando marcadores neutros (Orsini et al. 2013, Andrews et al. 2016). Um grande número de estudos recentes tem demonstrado o potencial dessas técnicas em mapear características biologicamente importantes que podem fornecer novos *insights* sobre processos ecológicos, em adição as explicações envolvendo processos demográficos (e.g., Zellmer et al. 2012; Roda et al. 2013; Gaither et al. 2015).

Espécie modelo

A família Bromeliaceae (58 gêneros, ca. 3140 espécies) constitui um dos clados mais morfologicamente distinto, ecologicamente diverso e rico em espécies entre as angiospermas de distribuição neotropical (Givnish et al. 2011). A família representa um excelente exemplo de radiação adaptativa em plantas vasculares, ocupando uma grande variedade de nichos, nos extremos de umidade, altitude e exposição (Benzing 2000; Crayn et al. 2004; Givnish, 2015). Esse cenário de radiação recente das bromélias fornece um excelente modelo para estudar a divergência entre populações (e.g., Sarthou et al. 2001; Palma-Silva et al. 2009; Hmeljevski et al. 2017; Leal et al. 2018), bem como para examinar a possível existência de fluxo gênico entre espécies simpátricas de plantas (e.g., Palma-Silva et al. 2011; Zanella et al 2016; Neri et al 2017; Mota et al. 2018).

Nesta tese, utilizamos uma espécie herbácea e perene do gênero *Pitcairnia* (Bromeliaceae) como modelo para estudar os padrões e processos que governam a diversidade nos Neotrópicos. *Pitcairnia lanuginosa* Ruiz & Pav. (*sensu* Smith & Downs, 1974) está distribuída principalmente no Cerrado brasileiro e na porção central dos Yungas andinos (Bolívia e Peru), mas também são encontradas sobre afloramentos rochosos azonais localizados entre esses dois biomas/ecorregiões (Figura 1). Forzza et

al. (2018) reconheceram espécimes ocorrendo em território brasileiro como *P. burchellii* Mez (amplamente distribuída no Cerrado) e *P. caldasiana* Baker (rara e restrita ao estado de Minas Gerais) na Flora do Brasil, restringido a ocorrência de *P. lanuginosa* aos países vizinhos Bolívia e Peru. Neste trabalho, porém, incluímos uma amostragem de populações ocorrendo no Brasil e Peru e adotamos o tratamento de Smith & Downs 1974, que sinonimiza todos os nomes a *P. lanuginosa*, devido a ausência de variações morfológicas óbvias para separar os espécimes.

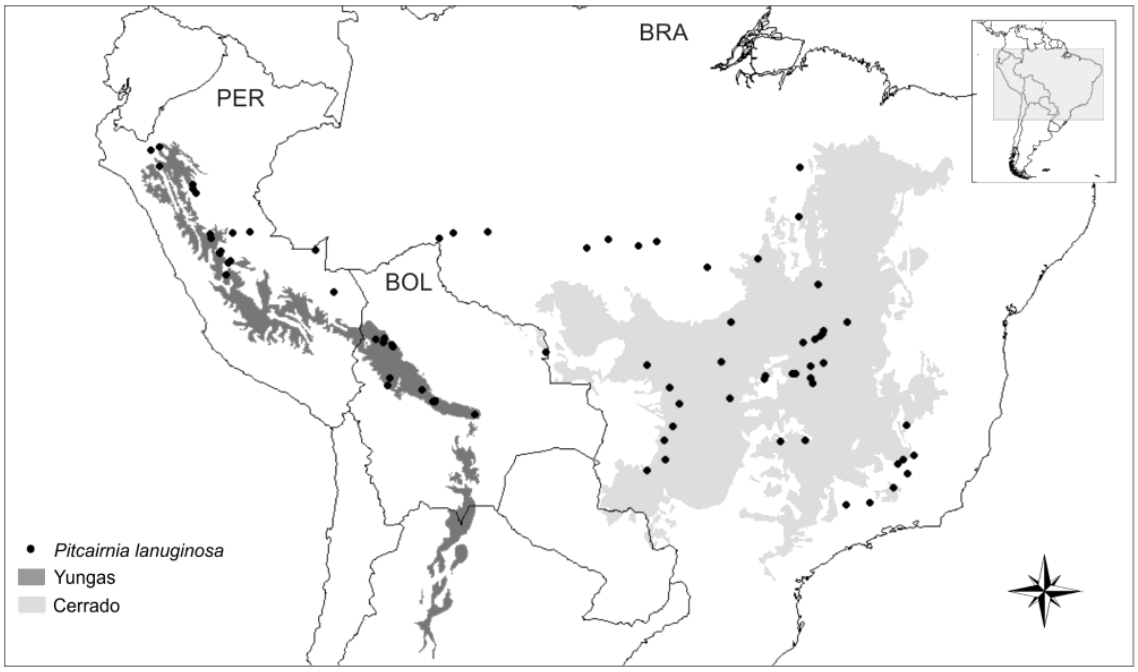

Figura 1. Distribuição de *Pitcairnia lanuginosa* (Bromeliaceae) conforme registros obtidos em herbários. Acrônimos para os países: BOL=Bolívia, BRA=Bolívia; PER=Peru. Delimitação dos biomas/ecorregiões segue a proposta de Olson et al. (2001).

Pitcairnia lanuginosa é geralmente encontrada em matas de galeria (ou matas ripárias), sobre rochas ou barrancos localizados próximo a cachoeiras ou margens de cursos d'água (Figura 2). A espécie possui flores branco-amareladas com manchas vináceas, corola tubular, abundante produção de néctar e flores com antese noturna (Figura 2). Ao longo da realização desse trabalho, observamos que a fenologia floral das plantas mantidas sob cultivo em casa de vegetação não é sincronizada entre populações, com picos de floração concentrados na primavera ou outono (Figura 3). Além disso, verificamos por meio de experimentos de cruzamentos manuais em casa de vegetação que a espécie apresenta altissimas taxas de auto-polinização espontânea (Tabela 1). Estudos recentes demonstraram que a espécie é notadamente tolerante a dessecação, e

reconheceram que se tratam de plantas revivescentes (Vieira et al, 2017a,b). Esta característica é relativamente rara em angiospermas (Oliver & Mishler 2000; Porembski & Barthlott 2000; Gaff & Oliver 2013) e parece determinada pela presença de mecanismos de proteção únicos que estão constantemente ativos (Alamillo et al. 1995; Gechev et al. 2012). Tais características biológicas, acoplada com a distribuição geográfica ampla e naturalmente fragmentada, tornam *P. lanuginosa* um interessante modelo para explorar os padrões de diversidade e estrutura genética, e para avaliar a importância da adaptação local como fator de divergência entre linhagens.

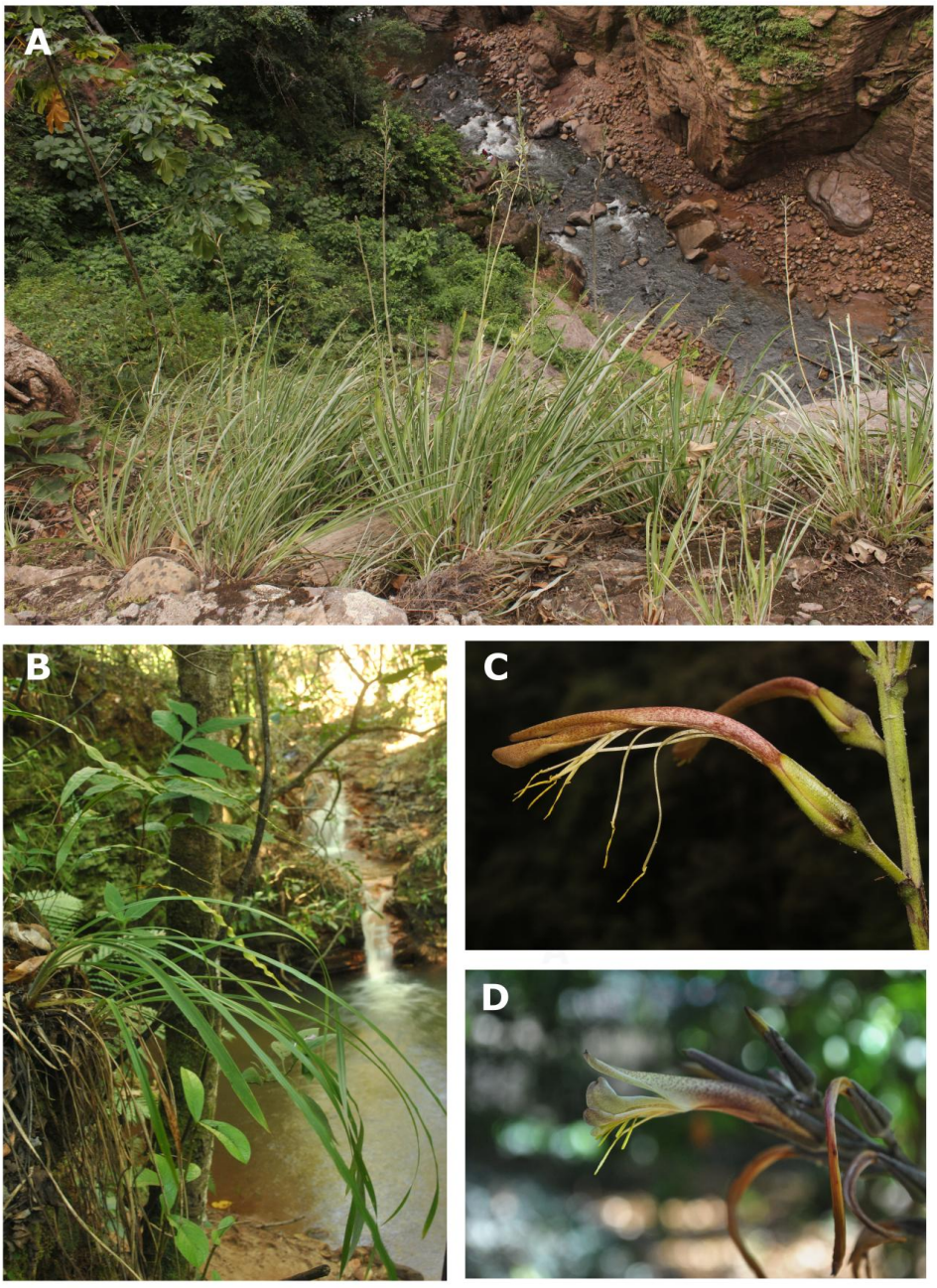

Figura 2. Hábito (A-B) e morfologia floral (C-D) de *Pitcairnia lanuginosa*.

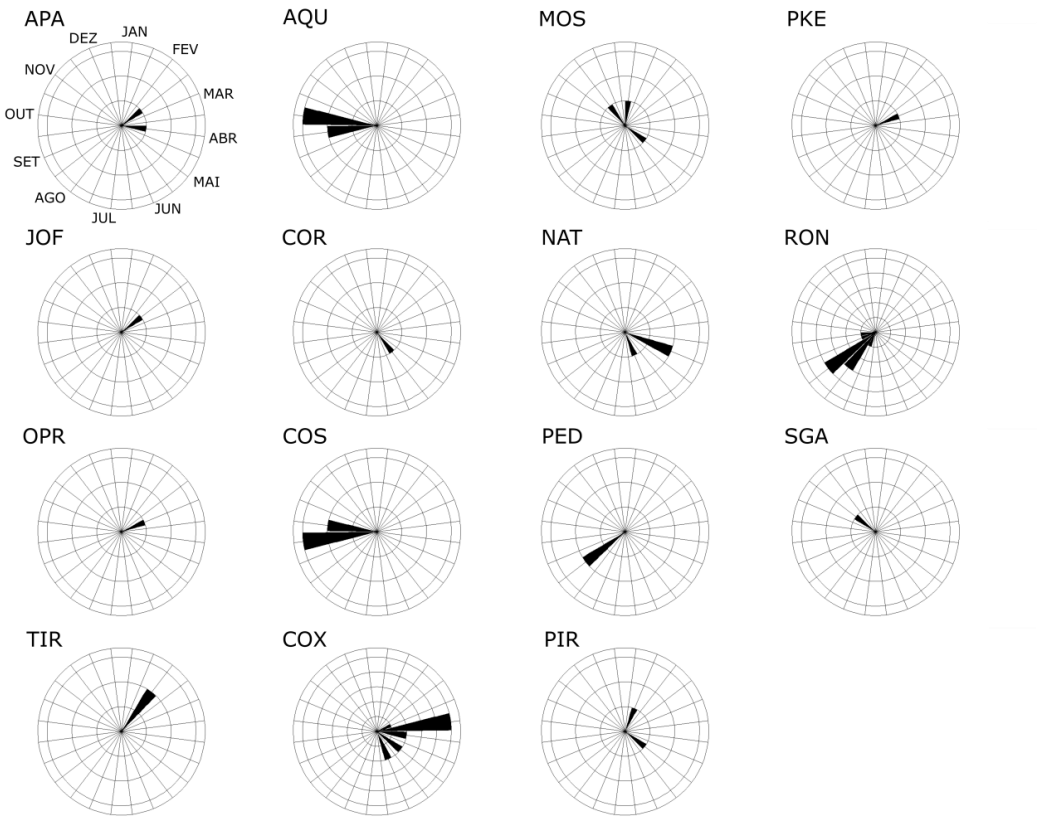

Figura 3. Variação do pico de floração de indivíduos coletados em 15 populações de *Pitcairnia lanuginosa* mantidos em cultivo em casa de vegetação na UNESP, Rio Claro, SP, Brasil. Dados não publicados.

Tabela 1. Resultados dos experimentos de cruzamento realizados em 2015 em indivíduos de *Pitcairnia lanuginosa* provenientes de uma mesma população e cultivados em casa de vegetação na UNESP, Rio Claro, Brasil. N = número de flores por tratamento. Dados não publicados.

Tratamento	**N**	**Produção de frutos (%)**	**Taxa de germinação média (%)**
Auto-polinização espontânea	21	86 (18/21)	18%
Auto-polinização manual	17	100 (17/17)	11.30%
Polinização cruzada	19	100 (3/3)*	4%
Emasculação	12	0	-

*Maioria das flores submetidas ao tratamento foram abortadas precocemente.

Além de revisar estudos de filogeografia realizados com espécies de plantas no território brasileiro e sumarizar o conhecimento sobre padrões e processos de

diversificação em cada bioma brasileiro (CAPÍTULO 1), nesta tese respondemos as seguintes questões associadas ao nosso modelo de estudo integrando diferentes abordagens metodológicas:

(1) Populações de *P. lanuginosa* distribuídas nos biomas/ecorregiões sul-americanos, Yungas e Cerrado, representam duas linhagens geneticamente divergentes? A atual distribuição pode ser explicada por vicariância ou dispersão? (CAPÍTULO 2);

(2) Qual o papel das oscilações climáticas do Pleistoceno sobre os padrões de variação genética do complexo? (CAPÍTULO 2);

(3) Qual o papel relativo dos processos estocásticos e adaptativos na divergência de linhagens e na colonização de ambientes mésicos do Cerrado? (CAPÍTULO 3).

Para responder essas questões, reconstruímos a história evolutiva da espécie *P. lanuginosa* utilizando duas diferentes abordagens. Inicialmente, integramos métodos tradicionais a partir de sequenciamento de Sanger com marcadores universais e genotipagem de marcadores microssalélites, e modelos de distribuição de espécies (SDMs) para verificar o papel dos processos demográficos históricos sobre a variação genética neutra de *P. lanuginosa*. Em seguida, genotipamos centenas de SNPs sequenciados por ddRAD (Petterson et al. 2012), para investigar os papeis relativos de deriva e da seleção na estruturação genética da espécie. Na tentativa de agregar conhecimento biológico importante para interpretar os padrões encontrados também levantamos informações sobre caracteres fenotípicos potencialmente associados a adaptação a seca e a altas temperaturas. Padrões de diversificação de espécies herbáceas associadas a matas de galeria, como *P. lanuginosa*, ainda são pouco conhecidos na região neotropical, e podem ajudar a traçar um panorama geral da história da biogeografia do continente sul-americano.

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CAPÍTULO I

Phylogeographic studies depict the role of space and time scales of plant speciation in a highly diverse Neotropical region

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Figura: http://www.leep.ufv.br/

Phylogeographic studies depict the role of space and time scales of plant speciation in a highly diverse Neotropical region

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Abstract

Phylogeographic studies have merged different disciplines to explain speciation processes at both spatial and time scales. Although the number of phylogeographic extant studies has increased almost exponentially, few have been conducted in tropical countries, especially using plants. Plants are interesting models for such studies because their responses to different habitats conditions are stronger than those observed in animals, enabling direct tests of alternative demographic scenarios. Here, we review phylogeographic studies using plant species occurring in different vegetation domains within Brazil, which has the greatest number of plant species in the world. Based on a detailed examination of 41 published articles, we synthesized the current knowledge and discussed the main processes driving the high levels of plant diversity within Brazilian domains. General patterns of diversification could be inferred due to the number of species studied, especially in the Cerrado and Atlantic Forest, the most intensively studied domains (34.1 and 17.1% of the studies, respectively). Distinct vegetation types within both biomes were affected differently by the Pleistocene climatic oscillations. Edaphic conditions and geographical barriers (rivers and mountains) have also influenced the phylogeographical patterns of plants species from Amazonia and the Atlantic Forest. Other Brazilian domains, such as the Caatinga, Pantanal, and Pampas, have been studied to a lesser extent and no common phylogeographic pattern across species could be inferred. Issues regarding past connections between distinct domains also remain unclear, including those affecting the two main forest domains in South America. Future research on plant species will fill these information gaps, improving our understanding of the complex diversification processes affecting the South American biota.

Keywords: biodiversity, microevolution, neotropics, phylogeography, population structure, species diversification

Introduction

Species distribution patterns in space and time are critical for understanding speciation processes (Sanmartín, 2012). Major evolutionary forces such as gene flow, genetic drift, and natural selection will be directly influenced by landscape elements, leading to different degrees of divergence among populations and incipient species. For example, geographic barriers would prevent gene exchange among conspecific populations, which in turn may increase their differentiation due to drift and natural selection associated with different environmental conditions. The action of gene flow, drift, and natural selection could be related to historical climatic oscillation events and past geologic processes that changed landscape features and, consequently, the demography of populations and species. For example, glacial cycles dramatically changed the distribution of vegetation domains in South America (Clapperton, 1993), reducing gene exchange and increasing the effects of drift in fragmented populations. Thus, to find support for speciation models, such as the refugee theory (Haffer, 1969), the associations between past climatic oscillations and both the time and intensity of evolutionary forces involved in lineage diversification must be explored. According to Bennett and Provan (2008), researchers aiming to provide support for the refugee theory need to provide evidence of changes in population sizes and, consequently, the time periods in which drift and gene exchange were affected by such demographic changes.

The role of geographic space and time in speciation has been intensively studied within the field of biogeography (Lomolino *et al*., 2010; Sanmartín, 2012). Scientific expeditions to tropical regions in the eighteenth century have yielded explanations of the evolution of floras in different continents, and interest in biogeography again increased in the 1950s, when the theory of continental drift was fully accepted (De Queiroz, 2005). The use of molecular markers to investigate geographical patterns of variation, from populations to higher taxa, enabled a reconstruction of the evolutionary history of various groups, especially those with a poor fossil record (Soltis *et al*., 2009). At the end of the 1980s, a new discipline emerged aiming to investigate how the effects of time and geographic space on speciation events: phylogeography. In his seminal study, Avise *et al*. (1987) coined the term phylogeography, in an attempt to provide direction to those interested in the geographic context of speciation events. Later, Avise (2000) provide the basic agenda of phylogeography, guiding population-level studies interested in speciation.

Phylogeographic studies have increasingly merged different disciplines to explain the history and formation of species at geographic and time scales. Initial studies mostly used molecular datasets, using DNA polymorphisms as the primary source of information to infer past demographic events and the existence of multiple lineages in different environments (Olsen and Schaal, 1999; Collevatti *et al*., 2003). The characterization of highly informative nuclear and plastid regions enabled the testing of alternative demographic scenarios in plant populations (i.e., Palma-Silva *et al*., 2009; Bonatelli *et al*., 2014, and others). The role of historical refuges in the diversification of lineages and species has been confirmed for several plant species (Ramos *et al*., 2007; Ribeiro *et al*., 2011). The inclusion of species distribution models greatly enhanced the potential to infer past demographic events (Collevatti *et al*.; 2015). By retrieving the potential past distribution area, the results obtained independently from the models could be crosschecked with the molecular evidence of population expansion and/or bottlenecks (Collevatti *et al*., 2012a; Lima *et al*., 2014). Natural hybrid zones have also been the subject of phylogeographic studies, which have involved spatial distribution patterns, genetic composition of parental and hybrid plants, and reproductive isolation across species boundaries (Hewitt, 2001; Lorenz-Lemke *et al*., 2006; Palma-Silva *et al*., 2011). Because species formation is a population-level process, the study of reproductive isolation within species may shed light on the processes operating during the earliest stages of speciation (Scopece *et al*., 2010). To achieve this, a detailed phylogeographic picture is crucial to understand how reproductive isolation evolves among lineages, in a geographic context (Pinheiro *et al*., 2013).

Phylogeography has been growing vigorously as an integrative discipline, bridging different sources of data (Diniz-Filho *et al*., 2008). The number of extant studies has increased almost exponentially (Beheregaray, 2008). However, until 2006, most studies were performed in the northern hemisphere, despite the fact that most biodiversity is concentrated in the tropics (Beheregaray, 2008). Indeed, Brazil is the 15th most productive country in terms of phylogeography studies (Beheregaray, 2008), but has the greatest number of angiosperm species (BFG - The Brazil Flora Group, 2015). Therefore, few phylogeographic studies have been conducted in tropical countries with a rich biota, such as Brazil. There is also an imbalance between the number of studies of plants and animals. Turchetto-Zolet *et al*. (2013) reported that

most phylogeographic studies in South America, across all vegetation domains of the continent, involved animals.

Various hypotheses regarding the origin of the considerable levels of plant diversity in tropical countries have been formulated (Antonelli and Sanmartin, 2011). Much effort has been devoted to investigating broad patterns of diversification, using plant phylogenies at higher taxonomic levels to determine the mechanisms of plant speciation (Hoorn *et al*., 2010; Hughes *et al*., 2013). In contrast, population-level studies have received less attention, and little is known of the microevolutionary mechanisms during the first stages of speciation. Here, we review phylogeographic studies of plant species in Brazil as biological models. Compared to other megadiverse countries, Brazil has the greatest number of angiosperms, with 32,086 species (BFG, 2015). Thus, using plant phylogeographic studies performed in Brazil, we discuss the main processes driving the diversification of lineages and species in this highly biodiverse region. Specifically, we had the following goals: (i) to review the current knowledge of plant phylogeography in Brazil; (ii) to verify the occurrence of demographic processes (expansion and contraction) related to glacial/interglacial climatic oscillations; (iii) to assess hypothesized refugia types (multiple or single); and (iv) to discuss the role of geological and climatic changes during the Tertiary and Quaternary in shaping the phylogeographic patterns of plants in hyperdiverse vegetation domains in South America.

Phylogeographic studies in Brazil

This review focuses on the historical distribution and dynamics of Brazilian plant species based on phylogeography and the genetic signatures of vegetation history. For the purpose of this review we consider all vegetation domains found in Brazilian territory. We review and compare studies on the open and seasonal vegetation domains known as Caatinga, Cerrado, and Pampas, and mesic domains such as the Brazilian Atlantic Forest, Amazon, and Pantanal. The literature survey was conducted in the Web of Science using the keywords "phylogeograph*" + "plant" and one of the following domains: "Amazon", "Atlantic Forest", "Caatinga", "Cerrado", "Pampas" and “Pantanal”. Following this non-exhaustive survey, we selected 41 papers (Table S1) based on an inspection of their title, abstract, and keywords.

The first study using one of the reference phrases was published in 1999, and since then the number of studies has increased (Figure 1), albeit at a different rate than

that for South America as a whole (Turcheto-Zolet *et al*., 2013). The studies involved 20 plant families and 30 genera. The four most studied families were Fabaceae (seven studies), Solanaceae (four), Orchidaceae (three), and Bignoniaceae (three) (Figure 2A). Approximately 78% of the studies focused on only 11 families. Similarly, most genera were studied only once, while 30% were investigated twice or more (Table S1, Figure 2B). Most phylogeographic studies used trees (53.7%) and herbs (36.6%) as models (Table S1). Most studies (75.6%) sampled species in only one phytogeographic domain (Figure 3). Cerrado was the most studied domain (34.1% of the studies), followed by the Brazilian Atlantic Forest (17.1%), Amazonia (14.6%), Pampas (7.3%), and Caatinga (2.4%, one study). To the best of our knowledge, no phylogeographic study of plant species from the Pantanal domain has been published. Species occurring in more than one phytogeographic domain were the focus of 11 studies, most of which included populations distributed within the Cerrado domain. Species distributed across Caatinga and Cerrado were the most intensively studied (three studies), followed by the Brazilian Atlantic Forest and Cerrado (two), Amazonia and Cerrado (two), and the Brazilian Atlantic Forest and Amazonia (two). A comparative approach, using two or more co-distributed species, was used in only 17.1% of the studies. Niche models were included in ten studies, and hybridization events were detected in approximately one third of the studies. Genic/intergenic DNA region sequencing was the most common method used in the phylogeographic studies in this review (Figure 4A). Microsatellite markers were used in nine studies, and in most cases were combined with sequence data. Eighteen studies combined nuclear and plastid regions, and the remaining used only nuclear (6 studies) or plastid markers (13 studies) (Figure 4B).

Phylogeographic patterns in tropical forest domains

The Brazilian Atlantic Forest and Amazonia are the two main forest domains in South America (Ab'Saber, 1977). The Brazilian Atlantic Forest is restricted to the Atlantic coast of Brazil. Phylogeographic studies conducted in the Brazilian Atlantic Forest support the idea that this domain was deeply affected by historical climatic oscillation events, such as glacial/interglacial cycles, as well by geographic factors, such as mountains and rivers. The existence of multiple refuges, for example, is reported in almost all plant phylogeographic studies conducted in the Brazilian Atlantic Forest (Table S1). Palma-Silva *et al*. (2009) found multiple lineages along the distribution of *Vriesea gigantea*, an epiphyte bromeliad species distributed across the Brazilian

Atlantic Forest. The different demographic signatures of northern and southern populations suggest regions in which the forest was fragmented (in the southern populations) and continuous (in the northern populations) during the Last Glacial Maximum (LGM), following the pattern expected for glacial refuges (Bennett and Provan, 2008). The same pattern has been reported for other plant species, including a tree species, *Dalbergia nigra* (Ribeiro *et al*., 2011) and a liana, *Passiflora actinia* (Teixeira *et al*., 2016). Contrasting results were provided by Pinheiro *et al*. (2011) using an herbaceous orchid species that occurs in sand dune vegetation close to the seashore. In this study, increasing levels of genetic diversity and signs of demographic stability were observed in populations distributed in the southern portion of the Brazilian Atlantic Forest. These results are in agreement with the notion that the Brazilian Atlantic Forest is a mosaic of different physiognomies, and each vegetation type was probably differently influenced by past climatic oscillations and geographic barriers (Leite *et al*., 2016). Considering this heterogeneous scenario, phylogeographic studies using species occurring in different environments may reveal novel and informative patterns.

The heterogeneous nature of the southern and northern regions of the Brazilian Atlantic Rain Forest, as determined by floristic inventories (Oliveira-Filho and Fontes, 2000) and niche models (Carnaval and Moritz, 2008), has been confirmed by phylogeographic studies. Northern Rio de Janeiro, Espirito Santo, and southern Bahia are a transition zone between lineages, which show marked genetic divergences in this region (Ribeiro *et al*., 2011, Turchetto-Zolet *et al*., 2012, Pinheiro *et al*., 2013). The fragmentation of the Brazilian Atlantic Forest during glacial cycles, splitting these large portions of forest is a possible explanation for this pattern (Carnaval and Moritz, 2008). The existence of large river basins that act as geographic barriers for intraspecific gene exchange, such as the Doce River Basin, is an alternative explanation of the marked floristic differences between the northern and southern portions of the Brazilian Atlantic Forest (Bigarella *et al*., 1975, Prance, 1982). Recently, Cazé *et al*. (2016) provided evidence supporting the divergence of lineages of *Passiflora contracta* associated with the major river basins within the Brazilian Atlantic Forest, including the Doce River.

The Brazilian Atlantic Forest is influenced by several mountain chains in its range (Morellato and Haddad, 2000). Severe restrictions in gene exchange may lead to genetic divergence between populations on different mountains, which would explain

the high levels of species diversity in this forest domain (Scarano, 2002). This hypothesis has been confirmed by the few studies of species occurring at high elevations within the Brazilian Atlantic Forest. For example, Lorenz-Lemke *et al*. (2010) found multiple lineages of *Petunia* within a species clade restricted to high altitudes. High levels of genetic divergence were found among *Pitcairnia* lineages endemic to rock outcrops separated by a few kilometers (Palma-Silva *et al*., 2011). These studies support the view that restrictions of gene exchange in these naturally fragmented environments likely contributes to lineage diversification on mountains. The extent to which the restriction of gene exchange increases the reproductive isolation among these incipient lineages and species, and the role of drift, instead of natural selection, in the fragmented environments, should be addressed by future studies using mountain plants as models. Palma-Silva *et al*. (2011) reported the permeability of the reproductive barriers between co-occurring *Pitcairnia* species growing in inselbergs. Extensive levels of haplotype sharing were detected on individual mountains, indicating old hybridization events. Moreover, despite the limited gene flow between conspecific populations, introgression was relatively low and did not affect the cohesion of parental species. In the absence of high levels of gene exchange, selection may play an important role in maintaining species cohesion, particularly in naturally fragmented populations (Palma-Silva *et al*., 2011; Southcott and Ostevik, 2011).

The Amazonian domain encompasses several countries, but the majority lies in Brazil. Several hypotheses to explain the biogeographical history have been proposed in the Amazon domain (reviewed by Antonelli and Sanmartin, 2011). The effect of soil diversity on levels of plant diversity, tectonic activity, and refuges are considered important drivers of speciation in the Amazonia domain, and were evaluated by phylogeographic studies. However, despite the broad extension and high levels of plant diversity in Amazonia (Forzza et al., 2012), few phylogeographic studies using a lower species level approach have been published (Figure 3A). Fine *et al*. (2013) investigated two *Protium* species with different degrees of edaphic specialization: *P. alvarezianum*, an edaphic specialist of white-sand soils distributed throughout the Amazonia domain; and *P. subserratum*, an edaphic generalist found in different soil types, including white-sand soils. The results confirmed the genetic differentiation of both species, which was partially correlated with the soil types inhabited. The Andean uplift was the most important tectonic event in the recent history of South America. However, a number of

other tectonic and geomorphological processes were influenced by the Andean uplift, such as the formation of arches or ridges and changes in the direction and volume of rivers within the Amazon basin (Marroig and Cerqueira, 1997). Such processes have caused genetic breaks in some species (da Silva and Patton, 1998), and plant phylogeographic studies have tested some of these hypotheses. For example, Roncal *et al*. (2015) studied *Astrocaryum* species and found evidence of allopatric speciation driven by contrasting geological activity in the Fitzcarrald Arch uplift and subsidence of the northern Amazonian foreland basin.

The Refugia Theory is an influential explanation for the origin of species diversity (Bennett and Provan, 2008), including within the Amazonia domain (Hooghiemstra and van der Hammen 1998). This theory is controversial due to several inconsistencies (reviewed by Rull, 2011). However, the theory has been abandoned in the absence of population-level studies using plants as models. Phylogeographic studies would enable an evaluation of the role of refuge theory in lineage and species diversification in the Amazonia domain, as in other regions (e.g., Petit *et al*., 2003, Liu *et al*., 2012, Poncet *et al*., 2013). According to Bennett and Provan (2008), studies of refuges should consider both the size and abundance of populations to detect demographic changes due to glacial/interglacial cycles. Unfortunately, only Lemes *et al*. (2010) discussed the role of forest refuges in lineage diversification of the tree *Swietenia macrophylla*. This study detected populations with high levels of differentiation and diversity, which indicates the presence of multiple refuges in the Amazonian basin (Lemes *et al*., 2010). However, different species may show contrasting patterns of genetic variation due to different pollination and dispersion mechanisms. For example, Dick *et al*. (2007) detected very low levels of genetic differentiation among populations of *Ceiba pentandra*, which did not support any past fragmentation of the Amazonia domain. The high level of diversity detected in populations of *Jacaranda copaia* within the Amazon basin was interpreted as indicative of a zone of secondary contact between divergent lineages (Scotti-Saintagne *et al*., 2013). Studies of multiple co-occurring species have been used as natural replicates to support, or not, the persistence of forest refuges during glacial cycles. Consequently, comparative phylogeographic studies would provide important information about the potential effect of past refuges in lineage diversification, as for previous works on tropical forest trees (Poelchau and Hamrick, 2013).

Phylogeography in seasonal vegetation domains

The central Brazilian Cerrado and the Caatinga in north-eastern Brazil form, together with the Argentinean, Bolivian, and Paraguayan Chaco, the so-called 'dry diagonal of open vegetation' of eastern South America (Sarmiento, 1975; Pennington *et al*., 2006; Werneck, 2011). The Cerrado is a savanna that covers a huge area of central Brazil and has dystrophic soils, a marked seasonality of precipitation, and experiences frequent fires (Pennington *et al*. 2006). The Caatinga is the largest nucleus of seasonally dry tropical forest in the country (SDTF; Prado and Gibbs, 1993) and typically harbors fertile soils under the drought-prone climate of northeast Brazil (Prado, 2003; Queiroz, 2006). These open vegetation domains are poorly characterized in terms of biogeographical relationships (see review of Werneck, 2011), and the response patterns of organisms from these domains are unclear (Werneck, 2011; Turchetto-Zolet *et al*. 2013). Phylogeographic studies of plants from the Brazilian open and seasonal domains are biased toward the Cerrado vegetation. Twelve studies focused on this domain, while three focused on species distributed in SDTFs, including the Caatinga (Figure 3A).

The Cerrado is not a uniform savanna but a mosaic of different physiognomies, including savannas, *campos rupestres* (rocky fields) and humid formations, such as *veredas* and gallery forests (Eiten, 1972; Furley and Ratter, 1988). Both geological and paleoclimatic events have contributed to the diversification of plant and animal lineages within this domain (e.g., Moraes *et al*. 2009; Prado *et al*., 2012; Bonatelli *et al*., 2014). However, studies of plant species from the Cerrado (Table S1) have indicated a pre-eminent role of the climatic oscillations of the Quaternary on the phylogeographic structure of many species. Although plant community responses to climate are heterogeneous (see above), some diversification patterns have emerged due to the increasing number of species studied, including lineages with distinct ecological requirements.

Tree species in Cerrado show evidence of a recent colonization of the southern Cerrado after a range retraction during the LGM (Collevatti *et al*., 2003; Ramos *et al*., 2007; Novaes *et al*., 2010, 2013; Collevatti *et al*., 2015; Ribeiro *et al*., 2016). This pattern is supported by paleopalynological studies (Behling and Lichte, 1997; Behling, 1998; Behling, 2002) and a recent paleodistribution modelling study (Werneck *et al*., 2012). Nevertheless, retraction with population subdivision in multiple refugia during the LGM has been recorded for a savanna tree species from central Brazil (Collevatti *et*

al., 2012a) and a palm species restricted to wetlands (Lima *et al*., 2014). Additionally, a single species presented only a slight range retraction, with a large stable area maintained since the LGM (Souza *et al*., 2016). Higher genetic diversity in the core distribution of diverse plants has also been reported by these phylogeographic studies (Ramos *et al*., 2007; Novaes *et al*., 2010, 2013; Ribeiro *et al*., 2016; Souza *et al*., 2016).

Variable genetic effects can be determined by differences in natural-history traits among species, especially those in the contrasting formations of the Cerrado domain. Climate fluctuations have affected plants restricted to the *campos rupestres* formations within the Cerrado in a different manner. Instead of retraction, the evidence suggests range expansion of these plants during glaciations (Collevatti *et al*., 2009; Barbosa *et al*., 2012; Collevatti *et al*., 2012b; Bonatelli *et al*., 2014). Fossil evidence indicates that the decreasing temperatures and humidity during the glacial period drove a reduction in forest cover and favored the expansion of savanna and grassland vegetation (e.g., Salgado-Labouriau *et al*., 1998; Behling and Hooghiemstra, 2001; Mayle *et al*., 2000). Such climatic conditions favored range expansion of dry-adapted species to lower altitudes during the LGM. A warmer and wetter climate then resulted in the fragmentation of a broader distribution, which is currently restricted to multiple interglacial microrefugia (see Bonatellii *et al*., 2014).

Despite these contrasting patterns, studies of the flora of Cerrado have commonly reported a high level of population genetic differentiation (e.g., Collevatti *et al*., 2003; Ramos *et al*., 2007; Collevatti *et al*., 2012a, Novaes *et al*., 2013; Bonatelli *et al*., 2014). A notable exception is the widely distributed tree species, *Dimorphandra mollis*, which has lower levels of genetic diversity and genetic differentiation (Souza *et al*., 2016). Additionally, an east–west split in genetic structure was observed in both widely distributed tree species (e.g., Collevatti *et al*., 2003; Ramos *et al*., 2007, 2009; Novaes *et al*., 2010; 2013; Ribeiro *et al*. 2016) and rocky field specialists (e.g., Collevati *et al*., 2009; Bonatelli *et al*., 2014).

SDTFs are scattered throughout other vegetation types, occurring as enclaves within the Cerrado and Chaco domains, and as isolated nuclei, the largest being located in the Caatinga domain (Prado, 2000; Werneck, 2011). In fact, the Caatinga domain is one of the least studied Brazilian vegetation region (Turquetto-Zolet *et al*., 2013), and vegetation shifts in the SDTF during the Pleistocene are unclear (Thomé *et al*., 2016). The few extant phylogeographic studies that focused on species from SDTFs (Table S1)

reported distinct responses to Quaternary climate changes. Two studies of SDTF plant species (i.e., Caetano *et al*., 2008; Collevatti *et al*., 2012c) revealed patterns similar to those predicted by the Pleistocene Arc hypothesis (Prado and Gibbs, 1993). According to this hypothesis, SDTFs were widely and continuously distributed during the dry and cold periods of the Pleistocene, and the present-day range of SDTF nuclei is a relic of a wider distribution (Prado and Gibbs, 1993; Pennington *et al*., 2000). Phylogeographic analyses of *Ficus bonijesulapensis* (Vieira *et al*., 2015) and *Cedrela fissilis* (Garcia *et al*., 2011), however, showed genetic signatures of a recent expansion (during warmer and wetter periods), which is in accordance with the results of palaeodistribution modelling (Werneck *et al*., 2011) and the dispersal scenarios proposed by Mayle *et al*. (2004). According to Werneck *et al*. (2011) the distribution of SDTFs was more fragmented during the LGM than the Holocene, when a southern expansion of this vegetation type occurred. Future research on plant species of these dry and seasonal forests will increase our understanding of the diversification processes of the South American biota.

Seasonal formations also occur in the subtropical region of Brazil. The Pampas is one of the largest warm grassland areas globally and occurs in east-central Argentina, Uruguay and the extreme south of Brazil (Fregonezi *et al*., 2013). This subtropical domain is characterized by a seasonality in precipitation patterns and soil heterogeneity (Overbeck *et al*., 2007; Roeschet *et al*., 2009). Few studies have attempted to investigate the phylogeographic structure of the grassland plant species of Southern Brazil (Figure 3A). Diversification of some typical Pampas vegetation species was influenced by ecological factors to a greater degree than historical factors (forest range shifts or ice-sheet advances) (Fregonezi *et al*., 2013). For example, Longo *et al*. (2014) found that morphological variants within the *Petunia integrifolia* complex may be associated with the variation in soil salinity between mainland and coastal regions. Moreover, an expansion after a size reduction resulted in the establishment of two allopatric groups within this complex, one of them associated with a geologically ancient area and the other in areas under the influence of marine transgressions/regressions. Climatic and sea level changes have also been reported to influence the evolutionary history of *Calibrachoa heterophylla* and *Petunia integrifolia*, herbaceous species from the South Atlantic Coastal Region (Mader *et al*., 2013; Ramos-Fregonezi *et al*., 2015). Both species exhibited a pattern of recent population expansion

associated with colonization of coastal regions. There is a clear need to increase the number of studies of specific grassland formations to assess the impact of climate and ecological barriers on speciation in subtropical South America.

Phylogeography of species occurring in multiple vegetation domains

The Amazonia and Brazilian Atlantic Forest domains are separated by a broad corridor of open vegetation physiognomies, comprising the Chaco, the Caatinga, and the Cerrado (see above). This corridor of dry vegetation is an important barrier to species interchange and migration between the two tropical forest domains (Rizzini, 1979; Mori *et al*., 1981). There is much debate in the literature regarding the biogeographic history of both forest domains. Some authors agree with the view that both Amazonia and the Brazilian Atlantic Forest were more connected in the past, before the LGM (Bigarella *et al*., 1975; Prance, 1982). During drier periods, gallery forests and seasonal forests may have acted as floristic connections between Amazonia and the Brazilian Atlantic Forest (Oliveira-Filho and Ratter, 1995). Alternatively, despite the overall similarities in structure and physiognomy, Amazonia and the Atlantic Forest have markedly different species compositions, which does not support strong biogeographic connections in the past (Oliveira-Filho and Fontes, 2000).

Few plant phylogeographic studies have explored the potential past connection between Amazonia and the Brazilian Atlantic Forest. De Oliveira *et al*. (2010) reported marked differences between lineages of *Carapichea ipecacuanha* in Amazonia and the Brazilian Atlantic Forest, suggesting long periods of isolation rather than past connections. Turcheto-Zolet *et al*. (2012) reported similar results for the widespread tropical tree species *Schizolobium parahyba*. Intriguingly, the phylogeographic evidence of past connections between these two forest domains came from studies using animals as models (Costa, 2003, Batalha-Filho *et al*., 2013, Thomé *et al*., 2016). Future plant phylogeographic studies should explore the potential existence of past connections between Amazonia and the Brazilian Atlantic Forest, because few empirical data are available. Of special interest are species that occur in both forests (Oliveira-Filho and Fontes, 2000), which should be used as models to evaluate the evolution of these tropical forest domains.

Some phylogeographic studies in Brazil have focused on plant species associated with both mesic and seasonal domains (Figure 3B). Advances in understanding the complex shifts among contrasting biomes have resulted from

palinologic (e.g., Behling and Negrelle, 2001; Pessenda *et al*., 2009) and phylogeographic (e.g., Franco *et al*., 2012; Martins *et al*., 2009) evidence, but further work is needed. Studies to date have suggested that forest and grassland expansion/fragmentation and dispersal events explain the differentiation among plant populations. Species from the Cerrado-Atlantic Forest were the focus of the phylogeographic studies by Novaes *et al*. (2010) and Pinheiro *et al*. (2013). The tree *Plathymenia reticulata* shows a pattern of recent expansion from the central Cerrado to northeastern Brazil via eastern (Atlantic coast) and western (inland) colonization routes (Novaes *et al*., 2010). In contrast, recent population contraction events were detected in populations of *Epidendrum denticulatum*, particularly at the margins of its distribution range (in the Atlantic rainforest), suggesting that it was restricted to multiple refuges during forest expansion events (Pinheiro *et al*., 2013). Past floristic connections between the Atlantic Forest and Cerrado domains are suggested by this study, because there is no differentiation between *E. denticulatum* populations in such biomes. Such connections are also indicated by the extensive haplotype sharing among populations of *Epidendrum cinnabarinum*, a species associated with the Atlantic Forest and Caatinga inselbergs (Pinheiro *et al*. 2014). This orchid species shows signs of expansion from inland towards dry coastal vegetation zones as a result of long-distance dispersal events. Only two studies sampled populations in Amazonia and Cerrado vegetation (Figure 3B). These studies reported the geographical origins of the cassava (*Manihot esculenta*) (Olsen *et al*., 1999), and inferred fragmentation events consistent with post-Pleistocene habitat shifts in the rainforest–Cerrado ecotone (Olsen *et al*., 2002). We claim here that the historical connection dynamics among Brazilian biomes should take advantage of the emergence of model-based methods of phylogeographic inference. The evidence mentioned above will be confirmed or refuted using other evolutionary scenarios (e.g., Collevatti *et al*., 2012c; Lima *et al*., 2014).

Final remarks and Conclusions

Plants are long held recognized as models to track climate change over different time-scales (Holdridge, 1947). Due to their lack of movement, plant responses to different habitats are stronger than those of animals (Dansereau, 1957; Whitaker, 1975). In this context, plants could be used as models to test the effects of climate change on species distribution under different time scales, from thousands of years to the last

century. A detailed record based on fossilized pollen grains has been used for paleovegetation reconstruction (reviewed by Behling, 2002, Ledru *et al*., 2015). By studying the genetic architecture and lineage history of plant groups present in the fossilized pollen record, phylogeographic studies using plant species may enable testing of the demographic scenarios inferred by paleovegetation reconstructions and by current patterns of genetic diversity. Plants have also provided important insights into the recent and rapid climate changes (Root *et al*., 2003), and phylogeographic studies are crucial for understanding the effect of such drastic changes on plant populations.

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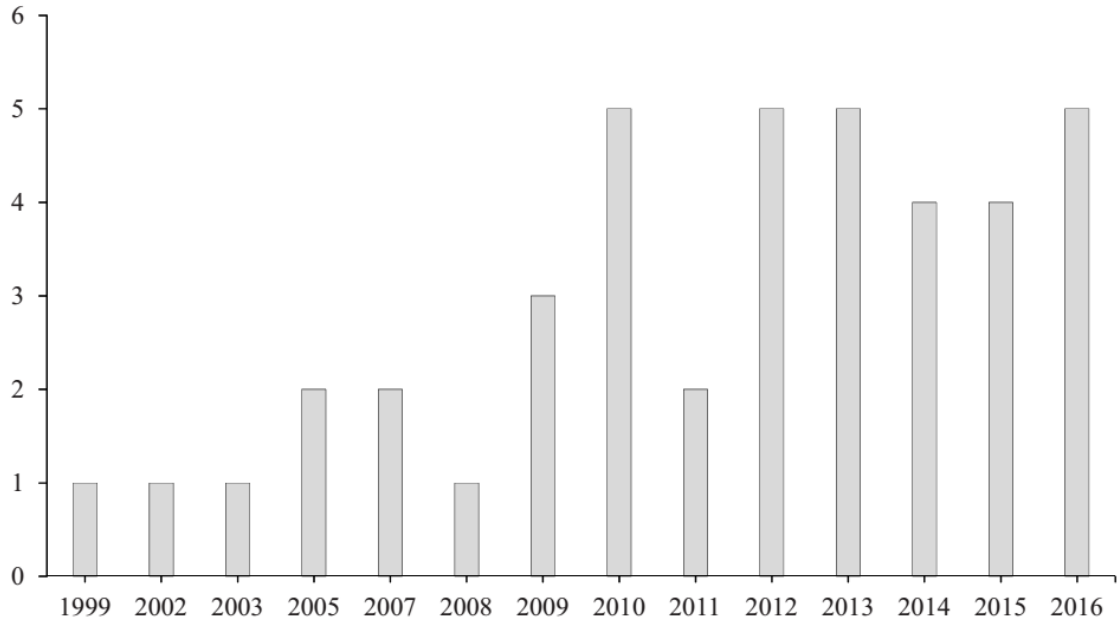

Figure 1. Number of phylogeographic articles published between 1999 and June 2016, referring primarily to plant species occurring in vegetation domains in Brazil.

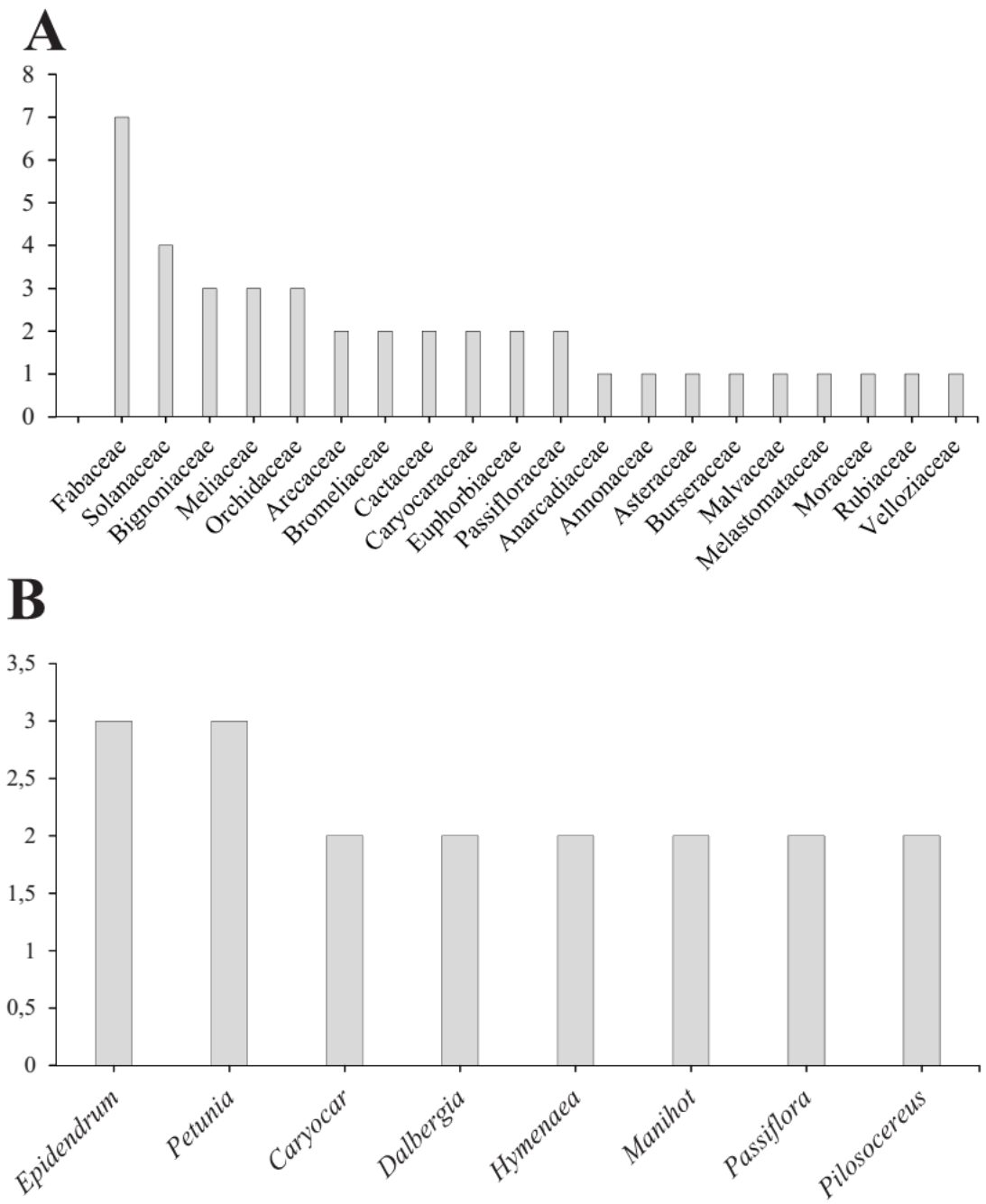

Figure 2. Most frequently investigated plant families (A) and genera that are included more than once (B) in phylogeographic studies of plant species occurring within Brazil.

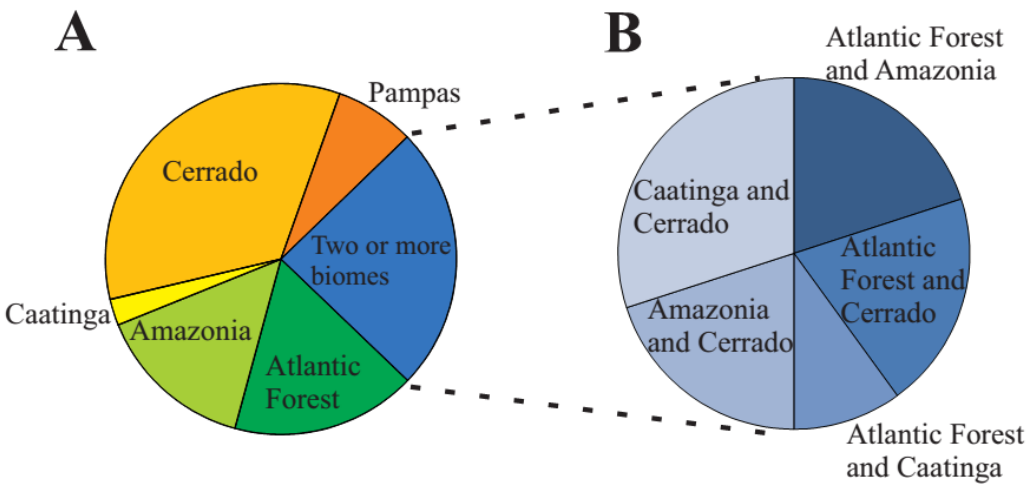

Figure 3. Number of phylogeographic studies using species distributed in single (A) and multiple phylogeographic domains (B).

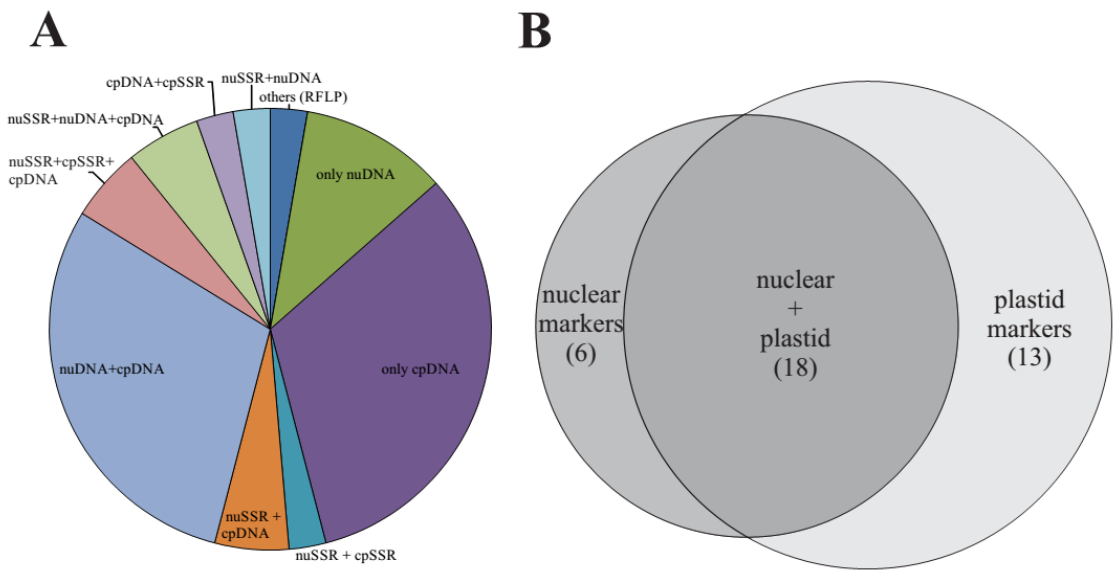

Figure 4. Major molecular markers used in phylogeographic studies of plant species occurring within Brazil (A), and the nuclear and/or organelle origin of markers used in such studies (B). The number of studies is shown in parentheses.

Table 1. Summary of plant phylogeographic studies for different Brazilian vegetation.

Reference	Journal	Family	Species	Life-form	Domain(s)[a]	Climate	Markers[b]	Cryptic speciation	Demographic processes[c]	Refugium type	Comparative approach	Niche modeling	Hybridi-zation or introgression	Reference
Oslen and Schaal 1999	PNAS	Euphorbiaceae	*Manihot esculenta; M. pruinosa*	Herb	AM; CE	tropical	one nuDNA: G3pdh	no	NA	NA	no	no	no	1
Olsen 2002	Molecular Ecology	Euphorbiaceae	*Manihot esculenta* ssp. *flabellifolia; M. pruinosa*	Herb	AM; CE	tropical	one nuDNA: G3pdh	no	NA	NA	no	no	yes	2
Collevatti et al. 2003	Molecular Ecology	Caryocaraceae	*Caryocar brasiliense*	Tree	CE	tropical	two cpDNA: trnT and trnF; 10 cpSSR	no	NA	multiple	no	no	yes	3
Lorenz-Hemke et al. 2005	Annals of Botany	Passifloraceae	*Passiflora actinia; P. elegans*	Liana	AF	subtropical	two cpDNA: trnL-trnF and psbA-trnH; nrDNA: ITS	no	Expansion	NA	yes	no	yes	4
Cloutier et al. 2005	Silvae Genetica	Meliaceae	*Carapa guianensis*	Tree	AM	tropical	RFLP	yes	NA	multiple	no	no	no	5
Ramos et al. 2007	Annals of Botany	Fabaceae	*Hymenaea stigonocarpa*	Tree	CE	tropical	one cpDNA: psbC-trnS3	no	Expansion of southern from north after LGM	multiple	no	no	no	6
Dick et al. 2007	Molecular Ecology	Malvaceae	*Ceiba pentandra*	Tree	AM	tropical	one cpDNA: psbB-psbF; one nrDNA: ITS	no	NA	NA	no	no	no	7
Caetano et al. 2008	Molecular Ecology	Anarcadiaceae	*Astronium urundeuva*	Tree	CA; CE; CH (SDTF)	tropical and subtropical	two cpDNA: trnH-psbA and trnS-trnG; 9 nuSSR	no	Expansion	NA	no	no	yes ("secondary contact")	8

Palma-Silva et al. 2009	Heredity	Bromeliaceae	*Vriesea gigantea*	Herb	AF	tropical and subtropical	one cpDNA: rpoB; four cpSSR; 11 nuSSR	no	Spatial expansion towards southern after LGM	multiple	no	no	no
Collevatti et al. 2009	Annals of Botany	Asteraceae	*Lychnophora ericoides*	Shrub	CE	tropical	two cpDNA: trnL intron and psbA-trnH; one nrDNA: ITS	no	Expansion during the Kansan Glacial period and retraction in interglacial	multiple	no	no	yes
Ramos et al. 2009	Journal of Heredity	Fabaceae	*Hymenaea courbaril* var. *stilbocarpa*; *Hymenaea stigonocarpa*	Tree	CE	tropical	one cpDNA: psbC-trnS3	no	Expansion of southern from north after LGM	multiple	yes	no	yes
Lorenz-Hemke et al. 2010	Molecular Ecology	Solanaceae	*Petunia altiplana*, *P. bonjardinensis*, *P. guarapuavensis*, *P. mantiqueirensis*, *P. reitzii*, *P. saxicola* and *P. scheideana*	Herb	AF	subtropical	two cpDNA: trnH-psbA and trnS-trnG	no	*Petunia andiplatina*: Expansion during LGM	multiple	yes	no	yes
Novaes et al. 2010	Molecular Ecology	Fabaceae	*Plathymenia reticulata*	Tree	AF; CE	tropical	two cpDNA: trnL-trnL-trnF and trnS-trnG	no	Expansion	NA	no	no	no
Lemes et al. 2010	Tropical Plant Biology	Meliaceae	*Swietenia macrophylla*	Tree	AM	tropical	six cpSSR	no	Refuges in Amazonian basin	multiple	no	no	no
de Oliveira et al. 2010	Molecular Ecology	Rubiaceae	*Carapichea ipecacuanha*	Shrub	AF; AM	tropical	one cpDNA: trnT-trnL; one nrDNA: ITS	no	Expansion	multiple	no	no	yes

Ribeiro et al. 2011	Heredity	Fabaceae	*Dalbergia nigra*	Tree	AF	tropical	two cpDNA: rnV-trnM and trnL	no	Weak population expansion	multiple	no	no	no
Pinheiro et al. 2011	Journal of Biogeography	Orchidaceae	*Epidendrum fulgens*	Herb	AF	tropical and subtropical	four cpSSR and nine nuSSR	no	Expansion after LGM	multiple	no	no	no
Garcia et al. 2011	Molecular Phylogenetics and Evolution	Meliaceae	*Cedrela fissilis*	Tree	CA; CE (SDTF)	tropical	three cpDNA: trnT–trnL, trnS–trnG, psbB-psbF; nrDNA: ITS	yes	expansion	multiple	no	no	yes
Turchetto-Zolet et al. 2013	Molecular Phylogenetics and Evolution	Fabaceae	*Schizolobium parahyba*	Tree	AF; AM	tropical	three cpDNA: psbA–trnH, trnL–trnF and matK; one nrDNA: ITS	no	Population expansion toward south after LGM	multiple	no	no	no
Barbosa et al. 2012	American Journal of Botany	Velloziaceae	*Vellozia hirsuta*	Herb	CE	tropical	one cpDNA: rpl32-trnL	no	Expansion during glaciation	multiple	no	no	yes
Collevatti et al. 2012a	Natureza & Conservacao	Caryocaraceae	*Caryocar brasiliense*	Tree	CE	tropical	three cpDNA: trnL intron, psbA-trnH and trnC-ycf6	no	Retraction during LGM	multiple	no	yes	no
Collevatti et al. 2012b	Ecology and Evolution	Melastomataceae	*Tibouchina papyrus*	Tree	CE	tropical	three cpDNA: psbA-trnH, trnS-trnG and trnC-ycf6; 10 nuSSR	no	Expansion during Pre-Illinoian glaciation	multiple	no	no	no

Collevatti et al. 2012c	Molecular Ecology	Bignoniaceae	*Tabebuia impetiginosa*	Tree	CE; CA (SDTF)	tropical	three cpDNA: trnS-trnG, psbA-trnH and ycf6-trnC ; one nrDNA: ITS	no	Expansion during LGM	NA	no	yes	no	23
Pinheiro et al. 2013	Evolution	Orchidaceae	*Epidendrum denticulatum*	Herb	AF; CE	tropical	cpDNA: five cpSSR; one SNP; nine nuSSR	no	Expansion after LGM	multiple	no	no	no	24
Fine et al. 2013	Journal of Biogeography	Burseraceae	*Protium alvarezianum; Protium subserratum*	Tree	AM	tropical	one nuDNA: PhyC; two nrDNA: ITS and ETS	no	Expansion during Pleistocene or Pliocene Glacial Maxima	NA	yes	no	yes	25
Scotti-Saintagne et al. 2013	Journal of Biogeography	Bignoniaceae	*Jacaranda copaia*	Tree	AM	tropical	twp cpDNA: trnH–psbA and trnC–ycf6; eight cpSSR; nine nuSSR	no	NA	NA	no	no	yes	26
Novaes et al. 2013	Plos One	Fabaceae	*Dalbergia miscolobium*	Tree	CE	tropical	one cpDNA: trnL intron; one nrDNA: ITS	no	Spatial and demographic expansion after LGM	NA	no	no	no	27
Mader et al. 2013	BMC Evolutionary Biology	Solanaceae	*Calibrachoa heterophylla*	Herb	PA	tropical	two cpDNA: trnH-psbA and trnS-trnG	no	Expansion after LGM	single (inland)	no	no	no	28

Pinheiro et al. 2014	BMC Evolutionary Biology	Orchidaceae	*Epidendrum cinnabarinum; E. secundum*	Herb	AF; CA	tropical	one cpDNA: rps16–trnK; six cpSSR; six nuSSR	no	E. secundum - demographic stability *E. denticulatum* - northern expansion after LGM	multiple	yes	yes	no	29
Bonatelli et al. 2014	Molecular Ecology	Cactaceae	*Pilosocereus aurisetus* complex: *P. aurisetus, P. machrisii, P. vilaboensis, P. jauruensis, P. aureispinus, P. parvus* and *P. bohlei*	Herb	CE	tropical	two cpDNA: trnT-trnL and trnS-trnG; one nuDNA: PhyC; 10 nuSSR	no	Expansion during LGM and retraction during interglacial periods	multiple	no	yes	yes ("secondary contact")	30
Lima et al. 2014	Journal of Biogeography	Arecaceae	*Mauritia flexuosa*	Palm	CE	tropical	three cpDNA: psbA–trnH, trnS–trnG and trnC–ycf6	no	Retraction during LGM	multiple	no	yes	no	31
Longo et al. 2014	Botanical Journal of Linnean Society	Solanaceae	*Petunia integrifolia* complex: *Petunia integrifolia; P. riograndensis; P. littoralis; P. inflata*	Herb	PA	subtropical	two cpDNA: trnH-psbA and trnS-trnG; one nrDNA: ITS	no	Expansion earlier than 0.5 Mya	NA	no	no	no	32

Collevatti et al 2015	Frontiers in Plant Science	Bignoniaceae	*Tabebuia aurea*	Tree	CE	tropical	three cpDNA: psbA-trnH, trnC-ycf6 and trnS-trnG; one nrDNA: ITS1+5.8S + ITS2	no	Range retraction during the LGM	single	no	yes	no
Ramos-Fregonezi et al. 2015	BMC Evolutionary Biology	Solanaceae	*Petunia integrifolia* ssp. *depauperata*	Herb	PA	subtropical	two cpDNA: trnH-psbA and trnS-trnG	no	Recent range expansion	single	no	no	no
Roncal et al. 2015	Journal of Biogeography	Arecaceae	*Astrocaryum* sect. Huicungo	Tree	AM	tropical	five cpDNA: psbM–trnD, rps16 intron, rps16–trnQ, trnD–trnT and trnG intron; two nuDNA: WRKY7; PRK	no	Expansion	multiple	no	no	no
Vieira et al. 2015	Botanical Journal of Linnean Society	Moraceae	*Ficus bonijesulapensis*	Tree	CA	tropical	one cpDNA: trnQ–5′rps16	no	Southward expansion after LGM	single	no	yes	no
Goetze et al. 2016	Botanical Journal of Linnean Society	Bromeliaceae	*Aechmea calyculata*	Herb	AF	subtropical	two cpDNA: rpl32-trnL and rps16-trnK; one nuDNA: PhyC; 12 nuSSR	no	Recent expansion (East distribution) / Stability (West distribution)	NA	no	no	no

Teixera et al. 2016	Botanical Journal of the Linnean Society	Passifloraceae	*Passiflora actinia*	Herb	AF	tropical	one cpDNA: trnS-trnG; one nrDNA: ITS	no	Expansion	multiple	no	yes	no	38
Perez et al. 2016	Molecular Phylogenetics and Evolution	Cactaceae	*Pilosocereus aurisetus* complex*: P. machrisii; P. aurisetus; P. vilaboensis; P. jauruensis*	Herb	CE	tropical	25 nuDNA loci; 367 SNPs	no	NA	NA	no	yes	no	39
Ribeiro et al. 2016	Botanical Journal of Linnean Society	Annonaceae	*Annona crassiflora; Annona coriaceae*	Tree	CE	tropical	two cpDNA: trnL-trnF and rpl32-trnL	no	Demographic and spatial expansion of southern from north after LGM	multiple	yes	no	no	40
Souza et al. 2016	Annals of Botany	Fabaceae	*Dimorphandra mollis*	Tree	CE	tropical	one nrDNA: ITS; 7 nuSSR (SSR)	no	Constant sizes in the last 10 Ma (ITS), with recent demographical retraction	single	no	yes	no	41

[a]Abbreviations for "Domains" column listed alphabetically: AF, Brazilian Atlantic Forest; AM, Amazon Forest; CA, Caatinga; CE, Cerrado; CH, Chaco; SDTF, Seasonal Dry Tropical Forest. [b]Abbreviations for "Markers" column listed alphabetically: cpDNA, plastid DNA sequence; cpSSR, plastid microsatellite; nuDNA, nuclear DNA sequence; nuSSR, nuclear microsatellite; RFLP, Restriction Fragment Length Polymorphism; SNP, single nucleotide polymorphism. [c]Abbreviations for "Demographic processes" column: LGM, Last Glacial Maximum (~ 20,000 YBP).

CAPÍTULO II

Dispersal and local persistence shape the genetic structure of a wide-spread, patchy distributed Neotropical plant species

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Title

Dispersal and local persistence shape the genetic structure of a wide-spread, patchy distributed Neotropical plant species

Short running title

Phylogeography of *Pitcairnia lanuginosa*

Authors and affiliations

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Abstract

Aim: Isolated populations constitute an ideal laboratory to study the consequences of intra-specific divergence, considering that intrinsic incompatibilities are more likely to accumulate under reduced gene flow. Here, we use a widespread but patchy distributed bromeliad, *Pitcairnia lanuginosa* Ruiz & Pav, as a model to infer processes driving Neotropical diversification and, thus, to improve our understanding on the biodiversity dynamics in this highly speciose region.

Location: Brazilian Cerrado and Central Andean Yungas, in South America.

Methods: We assessed genetic structure patterns, timing of lineage divergence and historical demography of *P. lanuginosa*, based on microsatellites, plastid and nuclear sequence data sets in combination with coalescent analyses and an Approximate Bayesian Computation (ABC) framework. Additionally, we used species distribution models (SDMs) under current and past climate to independently estimate potential changes in habitat suitability.

Results: Despite the morphological uniformity, our plastid and nuclear DNA data evidenced two distinct *P. lanuginosa* lineages that have likely diverged through dispersal from the Cerrado to the Central Andean Yungas, following the final uplift of the Andes, and passed through a long-term isolation with no evidence of migration. Microsatellite data points to restricted gene flow, low genetic diversity and high levels of inbreeding within populations, which are a likely consequence of life history features such as high selfing rates promoting population persistence in isolation, as well as population-level bottlenecks and founder events. Although our SDMs show slight expansion of suitable range for *P. lanuginosa* during the Last Maximum Glacial, Pleistocene climatic oscillations seem to have played only a minor role on the diversification of the species, as molecular data show a signature of older divergence, followed by persistence in riparian forests.

Main conclusions: Our results imply drift as a major force shaping the evolution of *P. lanuginosa*, and suggest that the strong genetic structure found in the species may not be linked to an incipient speciation.

Keywords: bromeliads, diversification, Central Andean Yungas, Cerrado, genetic drift.

INTRODUCTION

Evolutionary biologists have long recognized speciation as a continuous process of genetic differentiation, culminating in reproductively isolated organisms called 'species' (Dobzhansky, 1940; Mayr, 1942). The evolution of barriers to gene flow (reproductive barriers) is driven by divergent ecological or sexual selection and by the accumulation of genetic incompatibilities, which may be a by-product of natural selection or a result of genetic drift (Seehausen et al., 2014). Isolated populations may constitute an ideal laboratory to study the consequences of intra-species divergence, considering that intrinsic incompatibilities are more likely to accumulate under reduced gene flow among populations. In such systems, the high levels of within-species divergence could represent a step in the continuum of speciation (Coyne & Orr, 2004), or rather result in ephemeral entities, in which differentiation rates are unlinked to speciation rates over phylogenetic timescales (Kisel et al., 2012, Rosenblum et al., 2012; Singhal et al., 2018). Although persistence of intra-species lineages may explain the lack of links between both rates, studies have commonly underappreciated persistence as a control of speciation rates (Dyonesius & Jasson, 2014).

Identifying the drivers of speciation is crucial for understanding the biodiversity patterns and dynamics in highly speciose regions. Evolutionary processes responsible for shaping one of the most species-rich region of the world, the Neotropics, have been debated intensively in the last decade (Turchetto-Zolet, Pinheiro, Salgueiro & Palma-Silva, 2013; Smith et al., 2014; Antonelli et al., 2018). The huge comparative richness and intriguing distribution patterns of the Neotropical biodiversity are assumed to be a product of complex interactions between historical and ecological processes (Antonelli & Sanmartin, 2011; Hughes, Pennington & Antonelli, 2013; Smith et al., 2014). Vicariance resulting from uplift of the Andes and subsequent changing in the drainage and distribution of Amazonian rivers has been long considered an important driver of Neotropical diversification (Hoorn et al., 2010; Ribas, Aleixo, Nogueira, Miyaki & Cracraft, 2012). However, recent studies suggested that speciation can be initiated to a greater extent by dispersal than through vicariance events; thus the likelihood of diversification should be intimately linked to species' specific abilities to colonize new habitats and persist into them (Smith et al., 2014). Although challenging, distinguishing dispersal from vicariance-mediated speciation remains as one of the big questions to be addressed through phylogeographic studies (e.g., Paulo et al., 2008;

Winger et al., 2015), preferably in combination with model-based approaches (e.g., Hickerson & Meyer, 2008; Perez, Bonatelli, Moraes & Carstens, 2016).

An increasing number of phylogeographic studies has shown that both geological events and past climate changes strongly affected the distribution and genetic structure of Neotropical species (revised by Turchetto-Zolet et al., 2013; Leal, Pinheiro & Palma-Silva, 2016). In the highly heterogeneous environmental conditions of the Neotropical region, species persistence in multiple refugia rather than in a single climatic refugium has often been raised to explain intra-species diversification (e.g., Collevatti et al., 2014; Bonatelli et al., 2014; reviewed by Turchetto-Zolet et al., 2013). Because most Neotropics remained unaffected by ice sheets, these refugia may be mainly associated with areas buffered from environmental extremes. In the Cerrado, a savanna-like biome, riparian (or gallery) forests may represent a buffered environment for a number of species with mesic preferences. Phylogenetic and phylogeographic studies on vertebrates have provided useful information on the role of such forest patches within the large area covered by savannas as a historical bridge connecting forested biomes (e.g., Costa, 2003; Ledo & Coli, 2017; Trujillo-Arias et al., 2018), but we still lack information on processes explaining the evolution of riparian plants (but see Resende-Moreira et al., 2017). The evolutionary history of lineages associated with such habitats shall be thus important for understanding the dynamics among the Cerrado and South-American forest formations.

In this study, we use the forb *Pitcairnia lanuginosa* Ruiz & Pav. (Bromeliaceae) as a model to infer processes driving intra-specific diversification in the Neotropics. *Pitcairnia lanuginosa* features a wide-range, yet patchy distribution of small populations that are mostly associated to riparian forests in the Brazilian Cerrado, and in the Central Andean Yungas, a transitional zone between the Andean highlands and eastern Amazonia. Despite its apparent preference for mesic habitats, *P. lanuginosa* (here treated as synonymous of *P. burchellii* Mez; Smith and Downs, 1974) exhibits a number of physiological, biochemical and morphological responses to withstand water deficit, and has recently been described as a resurrection plant (Vieira, Centeno, Freschi, Silva & Braga, 2017; Vieira, Silva, Oriani, Moro, & Braga, 2017). Studies have shown that individuals subjected to water deficit present a dual strategy to cope with drought based on vegetative desiccation tolerance and rhizome starch storage (Vieira, Centeno et al., 2017). Indeed, many *Pitcairnia* species have broad hydrological habitat ranges

(Males, 2018) and those inhabiting seasonal environments, such as the Cerrado, frequently display morphological adaptations (Males, 2017). Such ecological features, coupled with a widespread patchy distribution, make *P. lanuginosa* a particularly interesting model to investigate processes resulting from the dynamic history of South America, including putative barriers/connections between the Central Andean Yungas and the Cerrado via vicariance and/or dispersal events.

Here, we infer the evolutionary history and phylogeographical structure of *P. lanuginosa* across its range by using a coupled phylogeographical and paleodistributional modeling approach. We assessed genetic structure patterns, timing of lineage divergence and historical demography using coalescent analyses and an Approximate Bayesian Computation (ABC) approach. Additionally, we used species distribution models (SDMs) under current and past climate to independently estimate potential changes in the suitability of habitat. We aimed to answer the following questions: 1) Does the species harbour major genealogical lineages and do they coincide with distinct geographical regions, or differential habitat history? 2) May vicariance and/or dispersal processes explain divergence between lineages occupying the Brazilian Cerrado and the Central Andean Yungas? 3) Is the current distribution of the species a consequence of climate- or orogenic-driven events? Based on the known shifts between South-American forested and open biomes (Souza-Neto, Cianciaruso & Collevatti, 2016; Antonelli et al. 2018), we hypothesize that riparian forests may have facilitated persistence of *P. lanuginosa* in the Cerrado and bridged naturally fragmented areas thought dispersal events. We also hypothesize that Pleistocene climate may have affected the distribution of *P. lanuginosa*, leading to the current low connectivity among patchy areas, and consequently to a high differentiation among populations.

MATERIAL AND METHODS

Target species and population sampling

Pitcairnia lanuginosa is widely distributed in tropical regions of South America, occurring mainly in a large area of the Cerrado biome and in a narrow area in the Central Andean Yungas, along the eastern slopes of these mountains. Plant material was collected in 22 populations of *P. lanuginosa* covering most of its distribution range (Table 1, Figure 1). We found small and isolated populations on rocky outcrops within riparian forests, mainly near streams and waterfalls. We avoided collecting clones by

sampling individuals >5m apart. Voucher specimens are deposited in the herbarium HBRC, Universidade Estadual Paulista–UNESP, Rio Claro, São Paulo, Brazil.

DNA extraction, sequencing and genotyping of microsatellites

Genomic DNA was extracted from silica gel-dried leaves using the DNeasy Plant Mini Kit (Qiagen). Among a total of eight plastid (cpDNA) and five nuclear DNA (nDNA) markers (see description and primer sequences in Appendix S1 in Supporting Information), we selected the intergenic spacer *rps16-trnK* (Shaw et al. 2007), and two putative single-copy nDNA markers, the phytochrome C (*PHYC)* and the *cpNGS* genes, which were successfully amplified and sequenced in a total of 169 (nDNA) and 174 (cpDNA) individuals (Table 1). Amplifications of *rps16-trnK* were performed in a total volume of 30 uL containing 10 ng of genomic DNA, 1X PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.3 µM of forward and reverse primers, 1U of BIOLASE™ DNA Polymerase (Bioline) and 2% DMSO. PCR amplifications of both nDNA regions were performed in a total volume of 30 uL containing 10 ng of genomic DNA, 1X PCR buffer, 2.0 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.6 µM of forward and reverse primers and 2U of BIOLASE™ DNA Polymerase (Bioline). PCRs were performed in a Veriti 96-Well Thermal Cycler (Applied Biosystems) following a program that consisted of an initial 5 min denaturation step at 95°C; followed by 35 cycles of denaturation at 95°C for 60 s, annealing at 58°C for *rps16-trnK* and 54°C for *cpNGS* for 60 s, and extension at 72°C for 90 s for *rps16-trnK* and 120 s for *cpNGS*; followed by a final extension at 72°C for 7 min. Cycling conditions for *PHYC* followed Louzada et al. (2014). PCR products were double-strand sequenced by Macrogen Inc. (Seoul, South Korea). We used single specimens of *P. flammea* Lindl. and *P. breedlovei* L.B.Sm (GenBank accession numbers: HQ913754 and HQ913666) as outgroups in phylogenetic reconstructions. All sequences were assembled and edited using the Staden Package (Staden 1996) and an alignment matrix was constructed using ClustalW (Thompson et al. 1997) in software MEGA 7 (Kumar, Stecher. & Tamura, 2016). Each indel were recorded as a single character and homonucleotide repeats were removed due to uncertain homology. Sequence ambiguities at heterozygous sites in both nuclear markers were resolved under a no recombination model using PHASE implemented in DnaSP 5.10.01 (Librado & Rozas, 2009).

We tested the amplification and polymorphism of 51 microsatellite (simple sequence repeats, SSR) loci previously described for other bromeliad species and amplified eight selected loci (see Appendix S1) in a total of 318 individuals (Table 1). PCR was carried out in a total of 10 μL containing ~10 ng of DNA, 2.5X GoTaq Master Mix (Promega), 0.5 μM forward primer, 1 μM reverse primer, and 1 μM universal M13 primer tagged with FAM, VIC, PET or NED fluorochromes. Reactions were performed in a Veriti 96-Well Thermal Cycler (Applied Biosystems) using a touchdown protocol as described by Palma-Silva et al. (2007). The PCR products were subjected to fragment analysis on an ABI PRISM 3500 sequencer (Applied Biosystems) and sized in comparison with the GeneScan 500 LIZ size standard (Applied Biosystems) using Genemarker 1.95 (SoftGenetics). Raw allele sizes were automatically binned into discrete classes using Flexibin (http://www.zoo.cam.ac.uk/zoostaff/meg/amos.htm) (Amos et al., 2007).

Molecular analyses on sequence data

We calculated molecular diversity indices, such as number of polymorphic sites (*S*), number of haplotypes (*h*), haplotype diversity (*H*d), and nucleotide diversity (*p*) using DnaSP for the species, as well as for clades recovered by the phylogenetic reconstruction. We combined sequence variations (substitutions and indels) into haplotypes and conducted a median joining network analysis (Bandelt, Forster & Röhl, 1999), as implemented in Network 5.01 (http://www.fluxus-engineering.com) to assess the historical relationships among the haplotypes.

We estimated the time of the most recent common ancestor (T_{MRCA}) for nodes of interest in a combined nDNA and cpDNA species tree inferred by BEAST 1.8.4 (Drummond, Suchard & Rambaut, 2012). DNA substitution models for each gene were selected prior to phylogenetic reconstructions using jModelTest 2.1.7 (Darriba, Taboada, Doallo & Posada, 2012), under the Bayesian information criterion (BIC). Due to the lack of fossils for primary calibrations in Bromeliaceae (see Baresch, Smith, Winter, Valerio & Jaramillo, 2011), we applied a normal distribution to secondary calibration points corresponding to the split between *P. flammea* and *P. breedlovei* + *P. lanuginosa* and to the split between *P. breedlovei* and *P. lanuginosa* (as inferred by Schubert, 2017). Substitution models and clock rates were unlinked, while trees were linked. We performed two independent runs with 100,000,000 iterations and trees sampled every 10,000 generations, under an uncorrelated lognormal relaxed molecular

clock, and a coalescent constant population size prior. We then checked for convergence with Tracer 1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018) and combined runs with LogCombiner discarding a burn-in of 10%.

In order to test for signatures of population expansion or decline for each inferred clade, we calculated Fu's FS, Tajima's D and Ramos-Onsins and Rozas' R2 statistics based on segregating sites and tested their departures from neutrality based on 10,000 coalescent simulations with DnaSP. In addition, we used an Extended Bayesian Skyline Plot (EBSP) analysis (Drummond, Rambaut, Shapiro & Pybus, 2005) to estimate changes in population size over time for each clade, combining both nuclear markers as independent partitions in BEAST 2 (Bouckaert et al., 2014). We set a strict clock prior using substitution rate intervals for each marker inferred by our BEAST species tree as priors (7.44E-4±0.834 for *rps16-trnK*; 2.475E-3±1.328 for *cpNGS* and 5.937±0.533 for *PHYC*). We then adjusted the weights of the analysis' operators according to the recommendations of the EBSP tutorial (available at http://beast.bio.ed.ac.uk/). Two independent runs of 200,000,000 generations, with a thinning interval of 20,000, were combined using LogCombiner to generate the final output after checking for convergence and stationarity in Tracer 1.7.

Molecular analyses on microsatellite data

For each population and SSR marker, we estimated the number of alleles (A), number of private alleles (PA), and observed (H_O) and expected (H_E) heterozygosities, and inbreeding coefficient (F_{IS}) using GenAlEx 6.5 (Peakall & Smouse, 2012). We also estimated the Garza-Williamson index (*M*) by population using Arlequin 3.5 (Excoffier & Lischer, 2010). Linkage disequilibrium between all pairs of SSR loci per population, and deviations from Hardy-Weinberg equilibrium (HWE) was calculated in GENEPOP on the Web 4.6 (Raymond & Rousset, 1995). In addition, we tested for genotyiping errors due to null alleles, stuttering or large allele dropout using MICRO-CHECKER (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004).

We used a principal coordinate analysis (PCoA) of population pairwise Nei's unbiased genetic distance, as implemented in GenAlEx. 6.5 to describe genetic structure within the species. Unlike model-based approaches, the distance-based method employed here works without any explicit biological assumption. Because HWE is likely violated in our target species (see Results), this method is more suitable to detect genetic clusters in our SSR data set than model-based approached that make

assumptions on HWE. The partition of genetic diversity within and between clusters detected by PCoA was then investigated by an analysis of molecular variance (AMOVA) using Arlequin 3.5. To determine whether divergence among populations is an effect of isolation by distance (IBD), we correlated pairwise genetic distance (measured as $F_{ST}/(1-F_{ST})$) and the logarithm of geographic distance matrices by a Mantel test using R-package 'adegenet' (Jombart, 2008).

ABC framework

We used an ABC framework to test whether vicariance or dispersal might better explain the existence of two infered lineages occupying the Cerrado and Central Andean Yungas (see Results). We generated 1,200,000 simulated SSR data sets under six distinct scenarios: (I) Vicariance; (II) Vicariance with past gene flow; (III) Dispersal from Cerrado to Yungas; (IV) Dispersal from Cerrado to Yungas with past gene flow; (V) Dispersal from Yungas to Cerrado; and (VI) Dispersal from Yungas to Cerrado with past gene flow (Figure 2). For this, we implemented a custom python script modified from Perez et al. (2016) to run the models based on a set of eight SSR markers genotyped in 318 individuals (274 from Cerrado and 44 from Yungas). To avoid sampling bias, we also performed four distinct simulations based on equal size samples (44 individuals from each Cerrado and Yungas lineages). The script uses ms software (Hudson, 2002) to simulate samples under the coalescent model, followed by microsat and microsat2arp scripts (Perez et al., 2016) to convert generated samples to SSR data sets in Arlequin format. Parameter values of theta (θ), time of divergence (T_2), time of past migration (T_1), bidirectional migration rates (m_{c-y} and m_{y-c}), growth rate (g) and size of founded population (N founded) were drawn from broad prior distributions (see Appendix S2 for details on prior settings).

The following summary statistics were then calculated for the simulated and observed data sets using arlsumstat software (Excoffier & Lischer, 2010): mean number of alleles over loci (A); mean expected heterozygosity over loci (H_E), modified Garza-Williamson index over loci (mM); and F_{ST} between lineages. We calculated summary statistics for five distinct observed data sets: one for the total number of samples from Cerrado (N=274), and four data sets based on distinct combinations of three out of 19 sampled localities from Cerrado (N=44 for each data set). We chose summary statistics based on informativeness and also on their fit to the observed data.

We used the 'abc' R package (Csilléry, François & Blum, 2012) to implement distinct ABC algorithms for model selection and estimation of parameters. To identify the most probable scenario, we estimated the posterior probabilities using multinomial logistic regression and neural network methods on retained simulations under a threshold of 0.01. We assessed confidence in scenario choice by using a cross-validation step implemented in the R-package 'abc' based on one hundred test data sets. Finally, after performing cross-validation, we estimated the parameters posterior (θ, T1 and T2) for the best-supported model retrieving 0.5% of simulations, and applying a log transformation and neural network method, as implemented in R-package 'abc'.

Species distribution modeling

We modeled the range of the climatic niches of the two main clades recovered by BEAST phylogenetic reconstruction using 67 and 16 spatially unique occurrence points for the Cerrado and Yungas range parts, respectively, obtained from GBIF (http://doi.org/10.15468/dl.ywhpmz) and Species Link (http://splink.cria.org.br/) online databases and from GPS measurements during field trips (see Appendix S3). Based on these records, current distribution models for each clade were developed using eight previously selected bioclimatic variables (see Appendix S3) at 30 arc s (1 km^2) spatial resolution, available from the WorldClim database (Hijmans, Cameron, Parra, Jones & Jarvis, 2005). We performed selection by excluding the highly correlated available variables through a stepwise procedure implemented in R-package 'usdm' (Naimi et al., 2014).

We used the machine-learning maximum entropy algorithm implemented in MAXENT 3.3.3e (Phillips, Anderson & Schapire, 2006; Phillips & Dudík, 2008) to perform species distribution models (SDMs) for each lineage under the current climatic scenario. We then projected the models onto the paleoclimatic dataset simulated by the Community Climate System Model (CCSM4) for the Holocene (6 kya) and Last Glacial Maximum (LGM, 21 kya) scenarios to test the effects of past climatic oscillations on the niche of each lineage. We set 50000 as the maximum number of iterations and specified a random seed for generating 20 replicates for each model, with 25% of training and 75% of test data. Average values of the replicates were used as final logistic outputs. A jackknife test was conducted to assess the relative importance of each climatic variable to the models. The algorithm performance was assessed by the threshold independent statistics areas under the receiver operating characteristic curves

(AUC; Phillips et al., 2006). As AUC values tend to be higher for species with narrow ranges relative to the study area, we also evaluate model accuracy by using the true skill statistics (TSS; Allouche, Tsoar & Kadmon, 2006).

We applied the minimum presence threshold, which prioritizes omission error over commission considerations (Pearson, Raxworthy, Nakamura & Peterson, 2007), to generate restrictive presence maps including all known occurrence points of each clade. We then mapped stable areas and the spatial overlap between predicted distributions of each clade to detect zones where conditions are suitable for both of them. We used R custom scripts and R-packages 'raster' (Hijmans & van Etten, 2013) and 'rgdal' (Bivand, Keitt & Rowlingson, 2018) to prepare raster layers for modeling and mapping results.

RESULTS

Phylogeographical and phylogenetic patterns

Consensus sequences were obtained for 169-174 *P. lanuginosa* individuals per marker (Table 1). For the cpDNA intergenic spacer *rps16-trnK*, we obtained an alignment of 673 base pairs (bp) and found seven polymorphic sites, which resulted in six haplotypes (Figure 1, Table 2). The alignments of the nDNA genes resulted in a 221 bp alignment with 14 polymorphic sites and eight haplotypes for *cpNGS*, and in a 981 pb alignment with 12 polymorphic sites and 11 haplotypes for *PHYC* (Figure 1, Table 2). *P. lanuginosa* showed low genetic diversity, as most populations presented no polymorphism and only a few of them presented more than one haplotype per marker (Figure 1). For all sequenced individuals, cpDNA and nDNA exhibited overall haplotype diversity of 0.677 and 0.796, respectively. Overall nucleotide diversity was 0.003 for both nuclear and plastid markers (Table 2).

No cpDNA or nDNA haplotypes were shared between Cerrado and Central Andean Yungas populations (Figure 1a-c). Yungas populations showed a remarkable cpDNA differentiation (Figure 1a). For both nDNA regions, however, we observed a ‘star-like’ pattern, with slightly longer terminal branches corresponding to haplotypes occurring in the Yungas populations (Hc6-Hc8) or the western Cerrado populations (Hc2-3; Figure 1b). Only central nDNA haplotypes (i.e., Hc1 and Hp2) were shared among populations occurring in the eastern and western Cerrado (Figure 1b-c). All eastern Cerrado populations retained the central and putatively ancestral haplotypes (Hr3, Hc1 and Hp2), showing no genetic variation (Figure 1a-c).

BEAST phylogenetic reconstruction suggested that Central Andean Yungas and Cerrado populations form two distinct monophyletic groups (Figure 3a), with high support (posterior probability ≥ 0.98). According to this analysis, *P. lanuginosa* diverged from a common ancestor with *P. breedlovei* in the late Miocene to early Pliocene (~4.03 Mya; Figure 3). The estimated divergence time between Yungas and Cerrado lineages dates back to the Pliocene, ~2.91 Mya ago (Figure 3a). The Bayesian inference failed to consistently resolve any divergence within the Cerrado clade. The Cerrado clade showed higher haplotype and nucleotide diversities than the Yungas clade for all sequenced markers (Table 2).

No departure from neutrality in the Cerrado or the Yungas clades was identified using Tajima's D, Fu's FS or Ramos-Onsins and Roza's R2 neutrality tests (Table 2). In addition, the Bayesian skyline plot approach identified no changes in the effective population size over time for the Cerrado clade (Figure 3b). Due to scarcity of polymorphisms, we couldn't test for population size changes in the Yungas clade using this approach.

Population diversity and structure

We detected a total of 97 SSR alleles across 19 *P. lanuginosa* populations. Our data points to extremely low genetic diversity within populations (with two notable exceptions: APA and SGA populations), which is consistently distributed across SSR loci (Appendix S4). The number of alleles per population ranged from ten to 43, and private alleles from zero to 7 (Table 3). Observed and expected heterozygosities per population ranged from zero to 0.605 and from 0.020 to 0.630, respectively (Table 3). Almost all populations showed significant deviation from Hardy–Weinberg equilibrium ($p<0.05$) due to heterozygosity deficiency, and high inbreeding coefficients (F_{IS}) (Table 3). Moreover, we found low values of Garza Williamson indexes per population, ranging from 0.289 to 0.600. These values lie below the critical value proposed by Garza and Williamson 2001, $M<0.68$, suggesting past bottlenecks or founder events within populations. We did not detect linkage disequilibrium between any pair of loci within populations. Almost all populations showed evidence of null alleles in one to four loci, but these loci vary accross populations.

The first two axes of the PCoA explained 26.56% and 22.26% of the variation, and identified two major genetic clusters along axis 2 (Figure 4a). The first cluster corresponded to Yungas populations and the other were formed by populations from

eastern, central and central-western Cerrado, showing a continuum of variation along axis 1 (Figure 4a). Sub-structuring within the Cerrado is more evident when excluding Yungas populations from the analysis (Figure 4b). AMOVA pointed to a significant genetic structure when contrasting Cerrado *versus* Yungas genetic clusters (F_{CT} = 0.17, $p<0.01$) and even higher levels of structure among the three Cerrado genetic clusters (F_{CT}=0.28, $p<0.001$; Table 4). Nevertheless, the largest proportion of the variance is partitioned among populations in both analysis (F_{ST} = 0.68 and F_{ST} = 0.71, $p<0.001$; Table 4), suggesting restricted gene flow among populations (for pairwise F_{ST} values see Appendix S4). The Mantel test showed that nuclear genetic differentiation within the species was not correlated with geographic distance (R^2 = -0.105, p = 0.875).

ABC results

The posterior probabilities clearly rejected all models assuming past migration between the Cerrado and Yungas clades, and pointed to dispersal from Cerrado to Yungas (Scenario III; Figure 2) as the best model. For 12,000 retained simulations (1% of the total simulations), the posterior probabilities obtained for Scenario III were 1 and 0.997, using multinomial logistic regression and neural network algorithms, respectively (Figure 2, see also Appendix S5). Nevertheless, cross-validation tests suggest that dispersal from Cerrado to Yungas (Scenario III) is not easily distinguishable from the vicariance model (Scenario I); specifically, Scenario III is misclassified as Scenario I in 25 out of 100 simulated samples (Appendix S5). Four distinct unbiased data sets that use the same sample sizes for Cerrado and Yungas lineages confirmed Scenario III as the most probable, despite the lower posterior probabilities (Appendix S5).

The posterior estimates of parameters θ, T1 and T2 parameters, based on ca. 1000 posterior samples simulated under Scenario III, are shown in Table S5.9 (Appendix S5). Assuming this model, the Yungas clade has diverged from the Cerrado clade (215.69 – 274.69) coalescent time units ago, which corresponds to 252.87 x $4N_0$ generations, from a dispersal event corresponding to 1.54% of the ancestral population size (N_0) (Appendix S5).

Paleodistributional reconstructions

The MAXENT algorithm performed satisfactorily for all models and replicates (see Appendix S2). For the Cerrado clade models, the bioclimatic variables that most contributed were isothermality, mean temperature of the wettest quarter, precipitation of

the wettest quarter and, especially, precipitation of the driest quarter (Appendix S2). For the Yungas clade models, the precipitation of coldest quarter showed the most important contribution (Appendix S2). Our paleodistributional modeling showed a larger suitable range for both clades during the last glacial maximum (LGM) than under current climatic conditions, with a greater predicted range overlap between clades during the LGM (Figure 5). Nevertheless, most of the distribution range of the Cerrado clade was little affected by past climate change (Figure 5), with the exception of peripheral areas and non-reliable areas predicted in northern South-America. In the Cerrado, multiple large areas of the clade's distribution range in the southeast, central and north-west Cerrado remained stable, although little connected, through the past 21 Kya. On the other hand, our models suggested that climatic oscillations caused bigger changes in the distribution of the Yungas clade, allowing migration towards lowland areas in south-western Amazonia in the LGM (Figure 5).

DISCUSSION

In this study, we integrated phylogeography and species distribution modeling to provide insights into the geographic context of diversification of *P. lanuginosa*, a Neotropical plant species with a wide but naturally fragmented distribution range. These independent approaches supported the divergence between Cerrado and Yungas populations in the Late Pliocene, with no evidence of genetic exchange after divergence, despite predicted overlap of suitable distribution ranges during the LGM. Our data indicates extremely strong population genetic structure, low within-population genetic diversity and high inbreeding coefficients. Our results are discussed below in the light of historical and ecological determinants that might have affected the diversification of the species, explaining the patterns of genetic diversity and structure found herein.

Diversification patterns

Distinguishing between within-species lineages and true species is often challenging, considering that biological diversity exists at many non-discrete levels (Huang & Knowles, 2016). Here, we uncovered two divergent evolutionary lineages within *P. lanuginosa*, occurring in the Yungas and Cerrado biomes, respectively. The Bayesian phylogenetic analysis, as well as the patterns of genetic structure from SSR variation and genealogical relationships of nDNA and cpDNA haplotypes, revealed a long-term isolation without migration between these lineages. Although their common ancestor dates back to the late Pliocene (~2.9 Mya), there are no obvious morphological

characteristics distinguishing the lineages. A number of studies have found similar deep phylogeographic divisions within traditionally described Neotropical taxa, and commonly suggested the existence of cryptic species (e.g. Garcia et al., 2011; Prado, Haddad & Zamudio, 2012; Guarnizo et al., 2016). However, addressing whether lineages found herein are cryptic species is not a trivial task, and delimitation could be further determined under an integrative analytical approach (see Pinheiro, Dantas-Queiroz & Palma-Silva, 2018).

Despite the high levels of genetic differentiation among Cerrado populations detected for nDNA, cpDNA and particularly SSR data sets, we did not find any evidence of incipient lineages supported by our coalescent species tree analysis. Even though genetic differentiation is required for speciation, there are many situations in which these processes are not linked (Kisel et al., 2012). The low effective population size in *P. lanuginosa* likely determined the accumulation of genetic differences via drift, but such effect seems to be decoupled from speciation and may be instead associated to ephemeral entities/populations with elevated risk of extinction due to the accumulation of deleterious alleles (Lynch et al., 1995). Although drift could be associated to speciation events by providing initial population divergence before the action of selection (Uyeda et al., 2009), selection is less effective in small populations (Lynch et al., 1995; Collard and Mckill, 2008) and the idea of genetic drift solely driving the evolution of reproductive barriers is still controversial and mostly untested (Sobel, Chen, Watt & Schemske, 2010; The Marie Curie Speciation Network, 2012). The relative role of drift and selection on the diversification of *P. lanuginosa* will be further addressed by using a genome-wide marker sampling approach. Moreover, testing for reproductive isolation among these naturally isolated populations would be useful to provide a deeper insight into diversification patterns.

Demographic features

The evolutionary history of taxa in the Neotropics has been marked by geological and climatic events that altered the landscape of the region leaving an imprint on the biota's genetic diversity (e.g., Moraes, Yotoko, Manfrin, Solferini & Sene, 2009; Prado et al., 2012; Winger et al., 2015, revised by Turchetto-Zolet et al., 2013). Andean uplift, for instance, has long been recognized as a major force shaping Neotropical biodiversity by offering opportunities for vicariant speciation and by

providing several new environmental niches (Hoorn et al., 2010). Our results support dispersal rather than vicariance as a more plausible explanation for the current distribution of *P. lanuginosa*. The ABC framework based on SSR markers, and also the distribution patterns of the nDNA haplotypes, provide evidence of dispersal from Cerrado riparian forests to the Yungas forests. Accordingly, the split between lineages occupying these regions are dated to the late Pliocene, following the final uplift of the Andes during the Late Miocene and Pliocene (Gregory-Wodzicki, 2000; Garzione et al., 2008). The increasing habitat heterogeneity and rainfall along the eastern flanks promoted by the Andean uplift (Garreaud, Vuille, Compagnucci & Marengo, 2009; Hoorn et al., 2010) have likely caused the opening of niches for colonization of many preadapted plant lineages at the eastern slope. Furthermore, recent evidence points to a prominent role of dispersal events on shifts between Neotropical open and forest biomes (Antonelli et al., 2018), and also to connections between Andean and southern Atlantic Forests through Cerrado and Chaco corridors (Ledo et al., 2017; Trujillo-Arias et al., 2017, 2018). Nevertheless, our results should be interpreted with caution, considering that the ABC approach had a moderate power to discriminate between vicariance and dispersal from Cerrado to Yungas scenarios, putatively due to limitation of SSR-derived summary statistics. Furthermore, we cannot distinguish long-distance from stepwise dispersal events; it remains possible that the Yungas could have been colonized from shorter distance, un-sampled source populations in south-eastern Amazonia.

Our paleodistribution models revealed that LGM conditions enabled range expansion in both *P. lanuginosa* clades, with greater changes on the Yungas clade distribution toward western Amazon. However, phylogeographic patterns found in the Cerrado and Yungas clades are not indicative of population size changes, as demonstrated by neutrality tests and ESBP analysis. Moreover, ABC rejected any past gene flow between Cerrado and Yungas clades, even during the LGM, when SDMs inferred a slight overlap between both clades' distributions. In summary, climatic oscillations seem to have played only a minor role on the diversification of *P. lanuginosa*. Rather, the species shows a genetic signature of an older divergence (late Pliocene), followed by persistence into small and patchy populations throughout the Pleistocene. Although Pleistocene climatic dynamics is indicated as an important factor underlying the diversification in the Neotropics, studies have shown variable organism

responses to past climate changes (reviewed by Turchetto-Zolet et al., 2013; Leal et al., 2016). The resilience of the studied species to past climate oscillations may be enhanced by a combination of particular traits, such as spontaneous selfing reproduction and broad physiological tolerance (Vieira, Centeno et al., 2017; Vieira, Silva et al., 2017). Moreover, the species' preferred habitats (i.e. riparian forests) are ideal to buffer climatic variations due to the warmer and moister conditions.

Ecological determinants

Life history traits, especially mating system, are strong determinants of both within-population genetic diversity and population structure (Glemin, Bazin & Charlesworth, 2006; Duminil et al., 2007) and shall certainly explain genetic patterns uncovered here. We found extremely low population genetic diversity and significant differentiation among naturally fragmented populations of *P. lanuginosa*. The low levels of genetic variability, as revealed by SSR data for almost all populations (except for APA and SGA), suggested that *P. lanuginosa* could be highly selfing and/or apomictic. Hand-pollination experiments in *P. lanuginosa* have shown no evidences of apomixes in this species, and indicated that fruit set is produced mainly via spontaneous selfing (B.S.S. Leal; personal observation). In addition, only few individuals were observed flowering during field work, which results in limited pollen-mediated gene flow in this species. The reproductive assurance provided by spontaneous selfing (Baker, 1955; Herlihy & Eckert, 2002) may be important to explain *P. lanuginosa* population persistence within small patches of riparian forests despite restricted gene exchange, and also its broad distribution in South America, putatively caused by successful but rare seed dispersal events.

Our results also indicated a strong population structure resulting from low gene flow among Cerrado populations, as demonstrated by the extremely high SSR differentiation, and the existence of exclusive nDNA and cpDNA haplotypes and private SSR alleles in most populations. Indeed, AMOVA showed that differentiation among populations at SSR loci (F_{ST}=0.73) was even higher than between the long-term isolated intra-specific lineages occurring in the Yungas and Cerrado (F_{CT}=0.17). Similar genetic patterns have been detected in a number of plants occurring in naturally fragmented terrestrial habitats (e.g, Nistelberger, Byrne, Coates & Roberts, 2015; Maia, Sujii, Silva-Pereira & Goldenberg, 2017), including other bromeliads (e.g., Boisselier-Dubayle, Leblois, Samadi, Lobourdière & Sarthou, 2010; Palma-Silva et al., 2011;

Hmeljevski, Nazareno, Bueno, Reis & Forzza, 2017). Such levels of population structure are also expected due to high levels of self-fertilization, and restricted dispersal, as suggested from the small seeds of *P. lanuginosa* that lack any apparent adaptation to dispersal. Despite the high population differentiation, AMOVA, PCoA and haplotypes network suggested an incipient east-western divergence within the Cerrado distribution, following a longitudinal pattern also observed in other animal and plant species (e.g. Prado et al., 2012; Collevatti, Rabelo & Vieira, 2009; Ribeiro, Lemos-Filho, Buzatti, Lovato, & Heuertz, 2016). The low levels of gene flow among *P. lanuginosa* populations, coupled with the high inbreeding rates and putative bottlenecks or founder events, suggest that populations are exposed to the effects of strong genetic drift.

In summary, we showed that Cerrado and Yungas populations have likely diverged in the Late Pliocene following dispersal events, and that the extremely strong genetic structure may not be related to incipient speciation in the naturally fragmented *P. lanuginosa*. Besides the occurrence of historical population bottlenecks (or founder events) following dispersion events, the species genetic diversity and structure may have been strongly influenced by the combined effect of high rates of selfing and low seed-mediated gene flow among populations. The scenario highlighted here implies strong genetic drift as the major force underlying the species diversification. Whether selection may contribute or not to the divergence within the naturally fragmented distribution of *P. lanuginosa* will be further addressed by an ongoing study using genome-wide data.

Biosketch

B.S.S.L. and C.P.S. conceived the ideas; B.S.S.L, V.G.A., C.J.N.C, L.A.P.H and C.P.S. sampled the populations; B.S.S.L and VG.A. collected molecular data; B.S.S.L, and C.J.N.C analyzed the data; and B.S.S.L., M. H. and C.P.S. led the writing. All authors read and accepted the final version. Our research group is focused on describing patterns and inferring evolutionary processes underlying neotropical biodiversity, particularly in plant systems, integrating ecological and genetic tools.

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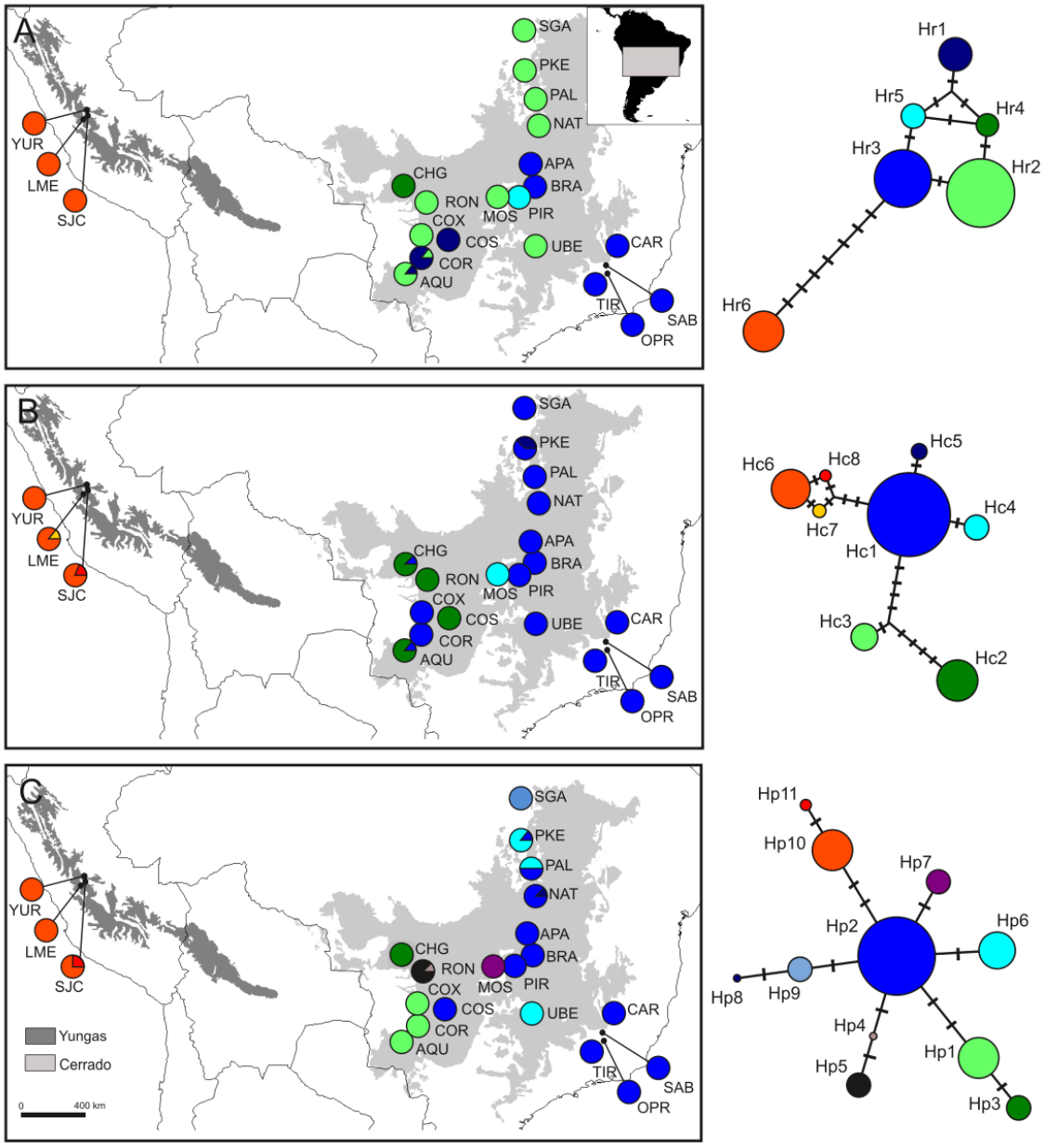

Figure 1. Geographic distribution and median-joining networks depicting relationships among (a) *rps16-trnK*, (b) *cpNGS* and (c) *PHYC* haplotypes of *Pitcairnia lanuginosa*. Each haplotype is depicted with a different color and circle sections represent the haplotype frequency in each sampled population in the map. The codes indicate the sampling localities according to Table 1. The sizes of the circles in the networks are proportional to the numbers of individuals bearing the haplotype and dashes represent the number of mutations.

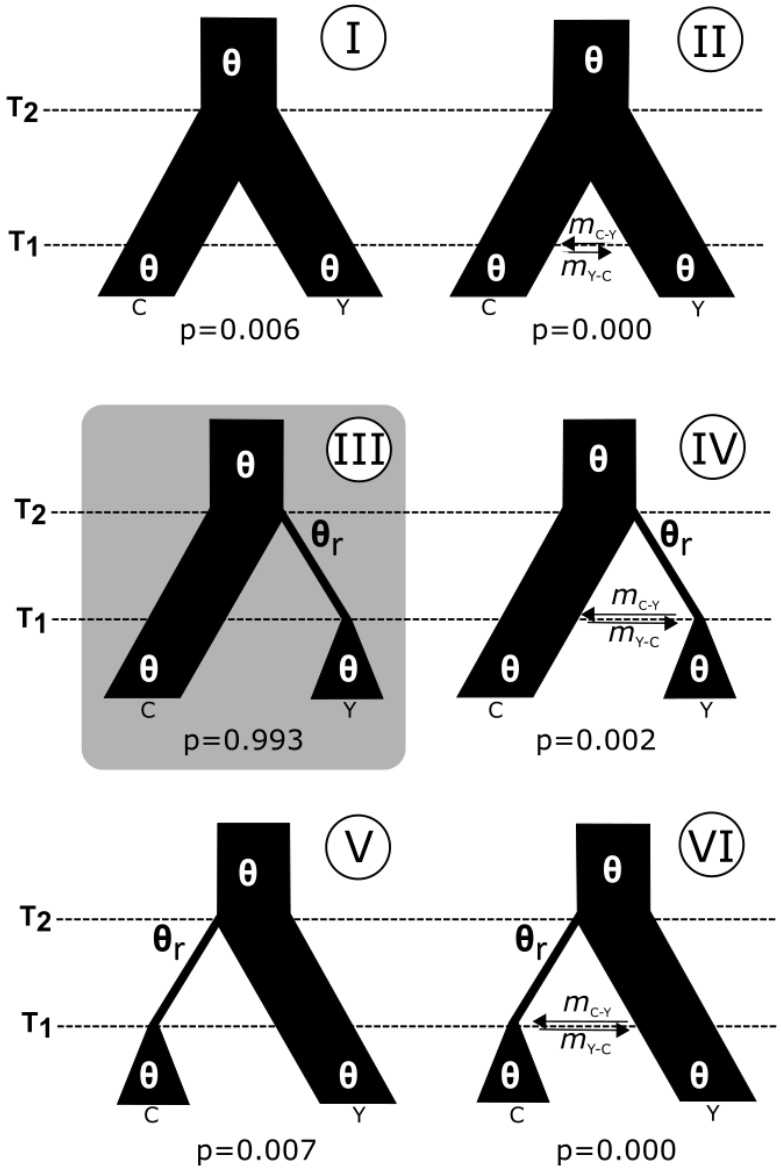

Figure 2. Phylogeographic hypotheses explicitly tested using the Approximate Bayesian computation (ABC) framework, with estimated posterior probabilities based on 12,000 retained simulations using the neural network algorithm. θ =theta; T_2 = divergence time between Cerrado and Yungas lineages; T_1 = time bounded by Pleistocene events, θ_r = ratio of theta moved from ancestral population N_0 to the lineage.

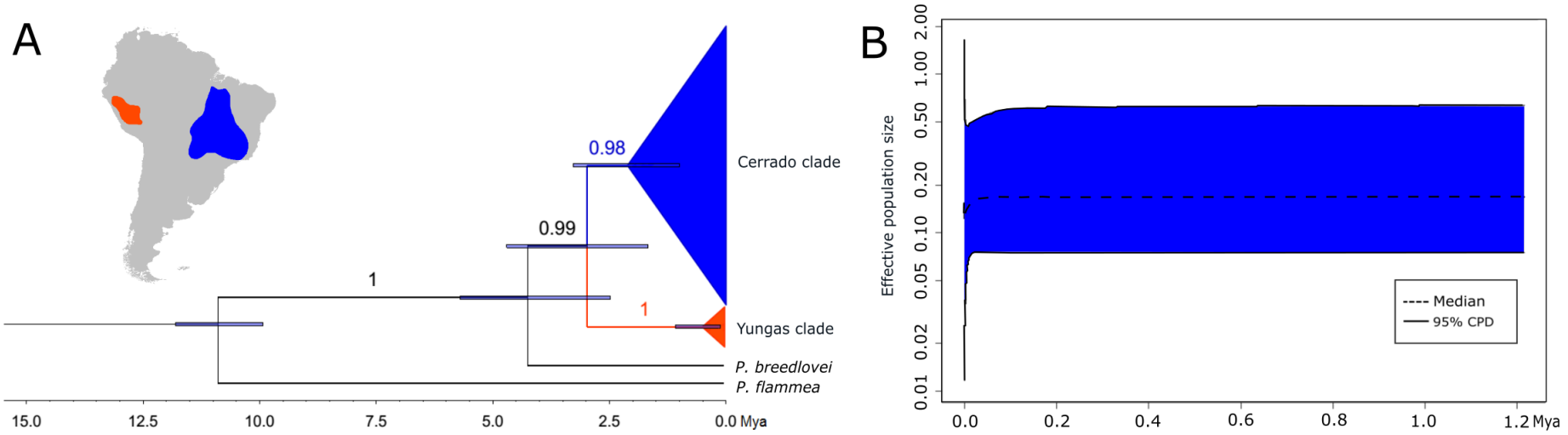

Figure 3. BEAST analyses: (a) Species-tree resulting from the BEAST analysis of three cpDNA (rps16-trnK) and nDNA (*PHYC* and *cpN*GS) regions. Posterior probabilities are shown above the branches and estimated divergence times are shown with 95% HPD. (b) Extended Bayesian skyline plot (EBSPs) for the Cerrado clade based on nDNA regions (*PHYC* and *cpN*GS). The *y*-axis indicates the effective population size in coalescence time units, with 95% HPD intervals shown in blue; the *x*-axis indicates the time before present in millions of years.

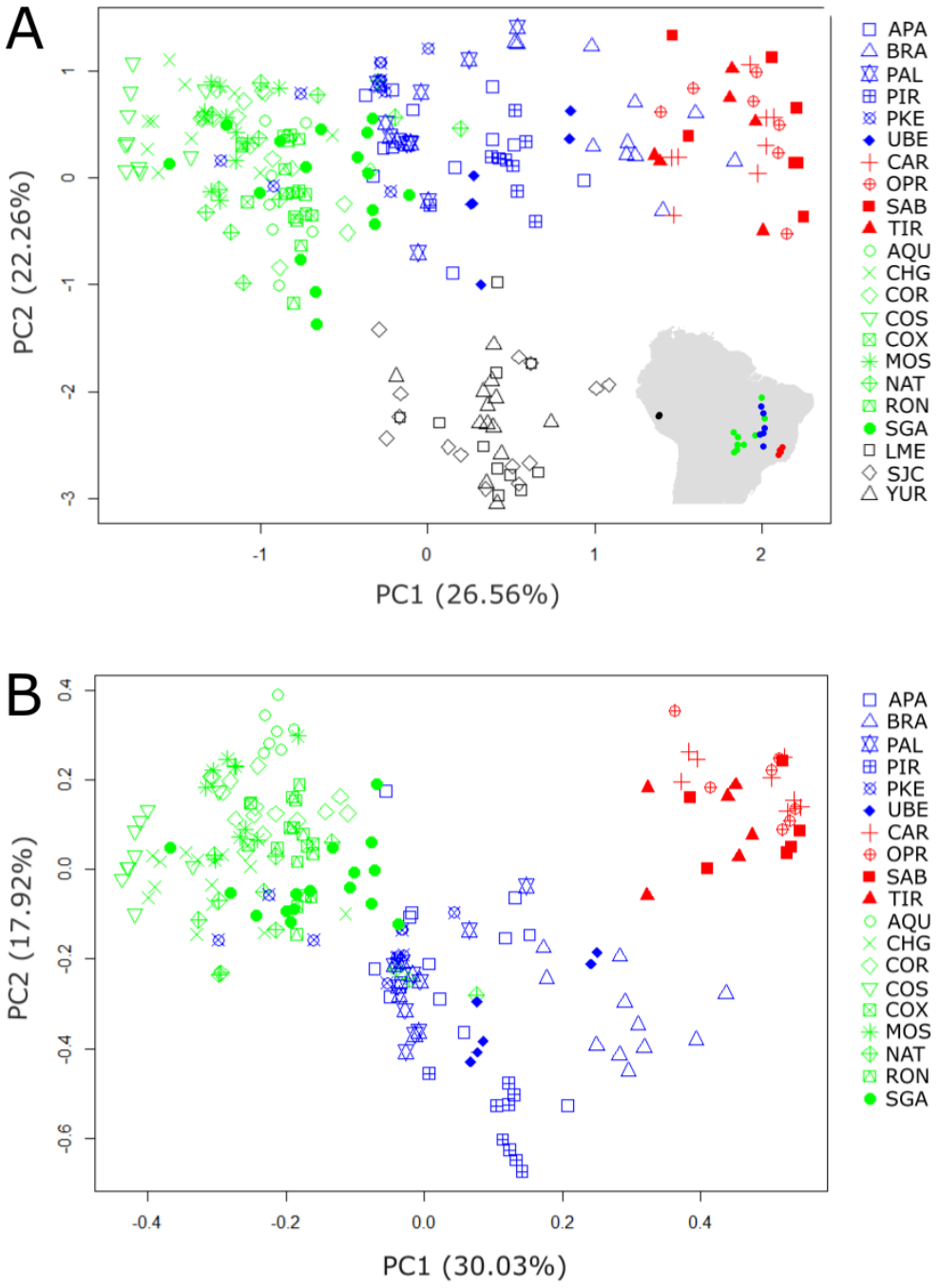

Figure 4. Principal coordinates analysis plot (PC1 x PC2) of (a) 318 individuals from 22 Yungas and Cerrado populations of *P. lanuginosa*, and (b) 274 individuals from 19 Cerrado populations based on eight microsatellite markers. Distinct symbols represent each population and distinct colors represent groups of populations (black = Yungas group; red = eastern Cerrado group; blue = central Cerrado group; green = central-western Cerrado group).

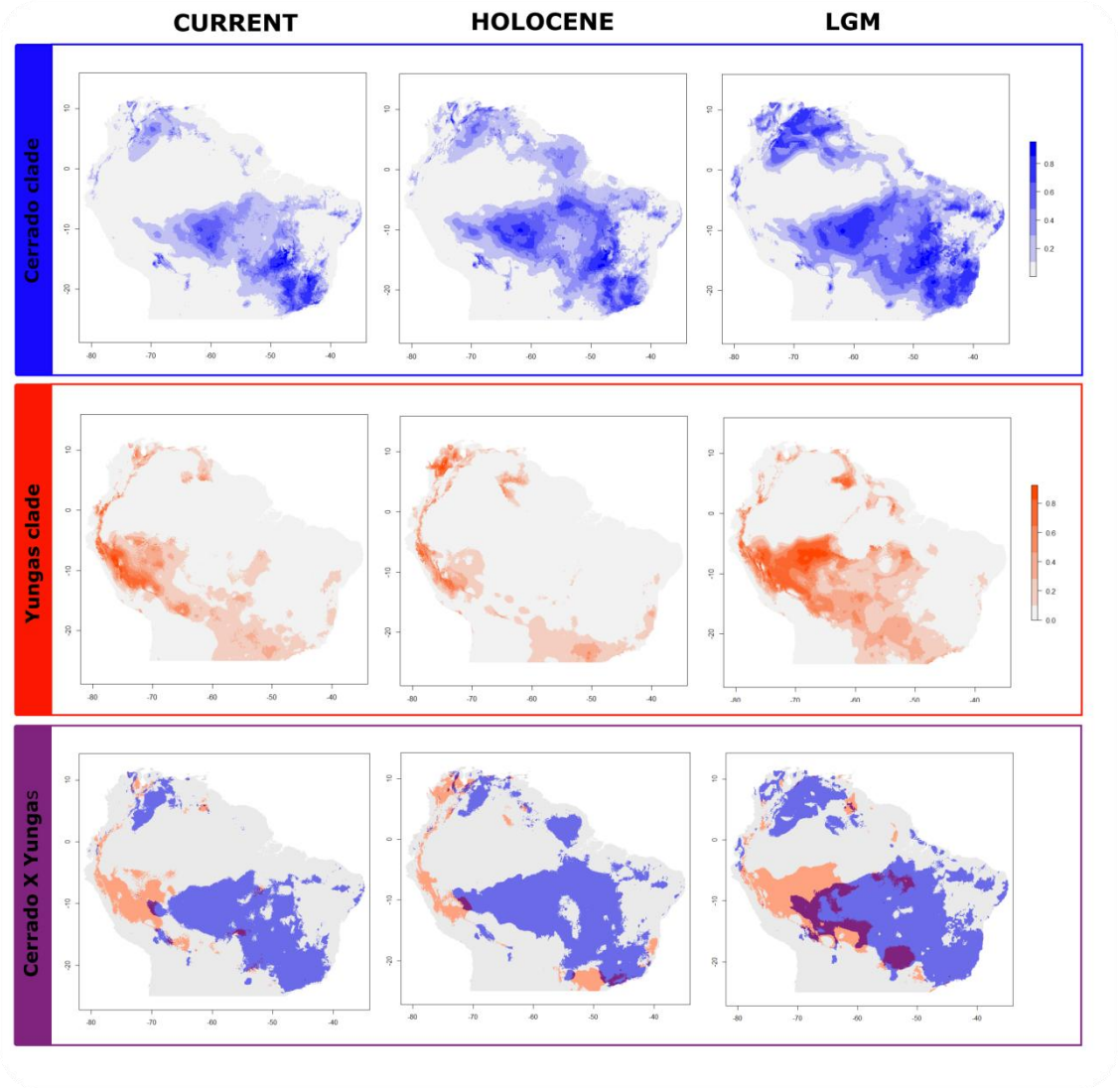

Figure 5. Predictions of suitable areas for occurrence of Cerrado (blue) and Yungas (orange) lineages of *P. lanuginosa*, under current (0 Kya pre-industrial) and past (Holocene: 6 Kya; LGM: 21 Kya) climatic conditions as inferred by the MAXENT maximum entropy algorithm. Predicted overlap areas are shown in purple, based on the minimum presence threshold.

Table 1. Sample information on 22 populations of *Pitcairnia lanuginosa* (Bromeliaceae).

Population	Locality/Country	Coordinates	Sample numbers (cpDNA/nDNA/SSR)
APA	Alto Paraíso/BR	47W 47' 26" , 14S 11' 04"	8/7/13
AQU	Aquidauana/BR	55W 29' 44" , 20S 27' 35"	7/7/16
BRA	Brasília/BR	48W 02' 55" , 15S 28' 55"	8/8/12
CAR	Carmésia/BR	43W 11' 52" , 19S 06' 40"	7/8/16
CHG	Chapada dos Guimarães/BR	55W 42' 55" , 15S 25' 45"	7/6/13
COR	Corguinho/BR	55W 09' 17" , 19S 54' 11"	7/7/16
COS	Costa Rica/BR	53W 03' 44" , 18S 35' 09"	9/9/17
COX	Coxim/BR	54W 45' 38" , 18S 28' 59"	9/7/16
MOS	Mossâmedes/BR	50W 11' 31" , 16S 04' 44"	8/7/15
NAT	Natividade/BR	47W 41' 58" , 11S 39' 24"	8/8/15
OPR	Ouro Preto/BR	43W 32' 33" , 20s 29' 36"	8/8/13
PAL	Palmas/BR	48W 08' 25" , 10S 18' 11"	8/8/15
PIR	Pirenópolis/BR	48W 54' 25" , 15S 47' 36"	8/8/12
PKE	Presidente Kennedy/BR	48W 34' 31" , 08S 29' 06"	8/7/14
RON	Rondonópolis/BR	54W 47' 43" , 16S 31' 05"	8/7/16
SAB	Sabará/BR	43W 49' 37" , 19S 54' 32"	9/9/15
SGA	São Geraldo do Araguaia/BR	48W 27' 58" , 06S 13' 58"	7/7/16
TIR	Tiradentes/BR	44W 09' 55" , 21S 06' 01"	9/8/16
UBE	Uberlândia/BR	48W 00' 19" , 18S 58' 05"	8/8/8
LME	La Merced/PE	75W 19' 04" , 11S 02' 08"	7/8/13
YUR	Yurinaki/PE	75W 06' 29" , 10S 51' 11"	7/8/16
SJC	San Juan de Cacazu/PE	75W 07' 01" , 10S 40' 24"	7/8/15

Table 2. Genetic diversity indices and neutrality tests for *Pitcairnia lanuginosa* lineages based on nDNA and cpDNA data. nDNA refers to the combined nuclear DNA regions cpNGS and PhyC.

	S	***h***	***H*d**	***p***	**Neutrality tests**		
					D	**Fs**	**R2**
Species							
cpNGS	14	8	0.550	0.013	0.766	4.351	0.103
PhyC	12	11	0.745	0.002	-0.251	-0.704	0.069
nDNA	26	18	0.796	0.003	0.207	0.828	0.085
cpDNA	9	6	0.677	0.003	0.908	5.170	0.140
Cerrado clade							
cpNGS	11	5	0.488	0.011	0.951	6.162	0.108
PhyC	9	9	0.678	0.001	-0.169	-0.592	0.0734
nDNA	20	14	0.744	0.003	0.257	1.189	0.088
cpDNA	3	5	0.590	0.001	0.795	2.252	0.1981
Yungas clade							
cpNGS	2	3	0.298	0.001	-0.611	-0.640	0.078
PhyC	1	2	0.156	<0.001	-0.418	-0.039	0.078
nDNA	3	4	0.443	<0.001	-0.643	-1.005	0.082
cpDNA	0	1	0.000	0.000	-	-	-

S = Number of polymorphic sites, *h* = number of haplotypes, *H*d = haplotype diversity, *p* = nucleotide diversity, D = Tajima's D statistic, Fs = Fu's FS statistic and R2 = Ramos-Onsins and Rozas' R2 statistic.

Table 3. Genetic diversity in 22 populations of *Pitcairnia lanuginosa* based on eight microsatellite markers.

Population	*A*	*PA*	H_O	H_E	F_{IS}	*M*
APA	38	3	0.382	0.530	0.317*	0.386
AQU	10	1	0.010	0.037	0.478*	0.424
BRA	21	0	0.158	0.320	0.382*	0.446
CAR	11	0	0.009	0.086	0.818*	0.443
CHG	21	4	0.159	0.352	0.308*	0.429
COR	23	5	0.183	0.298	0.344*	0.325
COS	11	0	0.031	0.052	0.396*	0.571
COX	11	2	0.000	0.116	1.000*	0.578
LME	15	1	0.031	0.194	0.680*	0.600
MOS	16	3	0.043	0.155	0.618*	0.289
NAT	25	1	0.085	0.346	0.752*	0.289
OPR	10	0	0.021	0.020	-0.067	0.309
PAL	16	0	0.088	0.164	0.250*	0.451
PIR	14	0	0.033	0.186	0.819*	0.552
PKE	16	3	0.130	0.172	0.317*	0.378
RON	14	1	0.087	0.173	0.495*	0.600
SAB	10	1	0.000	0.032	1.000*	0.455
SGA	43	5	0.445	0.601	0.253*	0.333
SJC	16	1	0.115	0.331	0.671*	0.400
TIR	10	0	0.008	0.023	0.639*	0.371
UBE	11	1	0.036	0.107	0.682*	0.363
YUR	15	1	0.082	0.231	0.660*	0.396

A = number of alleles, *PA* = number of private alleles, H_O = mean observed heterozygosity, H_E = mean expected heterozygosity, F_{IS} = inbreeding coefficient, and *M* = Garza-Williamson index. *Deviations from Hardy-Weinberg equilibrium ($p<0.05$).

Table 4. Analysis of Molecular Variance (AMOVA) based on eight microsatellite markers in *Pitcairnia lanuginosa*. Groups of Cerrado populations are defined according to BEAST species-tree (Yungas versus Cerrado) and PCoA results (Yungas, Eastern Cerrado, Central Cerrado and Central-Western Cerrado).

Source of variation	% variation	Fixation index	p-value
Cerrado versus Yungas			
Among groups	17.01	F_{CT}: 0.17	<0.01
Among populations within groups	56.11	F_{SC}: 0.68	<0.0001
Within populations	26.88	F_{ST}: 0.73	<0.0001
Three PCoA groups within Cerrado			
Among groups	27.75	F_{CT}: 0.28	<0.0001
Among populations within groups	43.74	F_{SC}: 0.61	<0.0001
Within populations	28.51	F_{ST}: 0.71	<0.0001

Appendix S1

Table S1.1. Amplification primers for nDNA and cpDNA regions tested in this study. The selected regions are highlighted in bold.

	Region	Primer name	Primer sequence (5'-3')	Reference
nDNA	*agt1*	Pe4474III_F1	TTCTTTGAYTGGAATGAYTACTT	Naumann et al. (2011)
		Pe4474V_R3	GGATYAATGGWGTWGTGTTCTG	
		By2AGT1_F	ACTGGTGCATGGGAGAGTGCACT	Vasconcelos (personal communication)
		By2AGT1_R	AGATGCCCTATTCTGAAWACCTT	
	ncpGS	**By1ncpGS_F**	**CAGTGGGAGTACCAAGTTGGACC**	**Vasconcelos (personal communication)**
		By1ncpGS_R	**TGCACCGTTCCAATCACCCTGTA**	
	G3pdh	G3pdh-F	CATCTAGCAAGGACTGGAGAGG	Strand et al. (1997)
		G3pdh-R	GCTGAAGATACCTGCTGTCACC	
	leafy	LFY Br F	GTGGATAAACGTACGTACCCGTCACTAGCG	Versieux et al. (2012)
		LFY Br R	GAGATGATGAGCGTGTACAATACTCGCTCAGC	
	PHYC	**phyc515f-br**	**AAGCCCTTYTACGCTATCCTGCACCG**	**Barfuss (2012)**
		phyc1699r-br	**ATWGCATCCATTTCAACATCTTCCCA**	
cpDNA	*matK*	matk5_F	ATACCCTGTTCTGACCATA	Crayn et al. (2000)
		BROM1_R	GGTTCCAGAAGATGTTAATCG	Schulte et al. (2005)
	petG-trnP	petG-trnP_F	GGTCTAATTCCTATAACTTTGGC	Huang et al. (2002)
		petG-trnP_R	GGGATGTGGCGCAGCTTGG	
	trnC-petN	trnC	CCAGTTCAAATCTGGGTGTC	Demesure et al. (1995)
		petN1r	CCCAAGCAAGACTTACTATATCC	Lee and Wen (2004)
		trnC	CCAGTTCAAATCTGGGTGTC	Demesure et al. (1995)
		petN_BR	CCAATCTAATTCCAGACAGAGTCA	Versieux et al. (2012)
	rpl16 intron	rpl16_F	GCTATGCTTAGTGTGTGACTCGTTG	Small et al. (1998)
		rpl16_R	CCCTTCATTCTTCCTCTATGTTG	

rpl32-trnL	rpl32 (F)	CAGTTCCAAAAAAACGTACTTC	Shaw et al. (2007)
	trnL (R)	CTGCTTCCTAAGAGCAGCGT	
rps16-trnK	**rps16_x_2F2 (F)**	**AAAGTGGGTTTTTATGATCC**	**Shaw et al. (2007)**
	trnk_x1 (R)	**TTAAAAGCCGAGTACTCTACC**	
trnL	trnL-Ff (R)	ATTTGAACTGGTGACACGAG	Taberlet et al. (1991)
	trnL-Fc (F)	CGAAATCGGTAGACGCTACG	
trnC-ycf6	ycf6 (R)	GCCCAAGCRAGACTTACTATATCCAT	Shaw et al. (2005)
	trnC (F)	CCAGTTCRAATCYGGGTG	Demesure et al. (1995)

Table S1.2.. Amplification primers for all microsatellite loci tested in this study. Selected loci are highlighted in bold.

Locus	Primer name	Primer sequence (5'-3')	Motif	Species	Reference	Amplification	Polymorphism
E6B	E6B_F	CGTACGAAGGTAAGCACAA	$(CAA)_{12}$	*Tillandsia fasciculata*	Boneh et al. (2003)	Yes	Yes
	E6B_R	CCGTTGAAGAGGTTAGAGG					
E19	E19_F	TCTTACTGCTCTCCATTGGT	$(CT)_{15}$	*Tillandsia fasciculata*	Boneh et al. (2003)	Yes	Yes
	E19_R	ATTTTTGGTGTTGCAGATGT					
P2P19	P2P19_F	ATGCTGCCCACTGAAGATTT	$(GAA)_{13}$	*Tillandsia fasciculata*	Boneh et al. (2003)	No	-
	P2P19_R	TCCGTCCAAGGTTTATTTGC					
E6	E6_F	AAACTATGGATTCCCCAACT	$(CAA_{)14}$	*Tillandsia fasciculata*	Boneh et al. (2003)	Yes	No
	E6_R	CGGTTCCTCCTTAGTCTTTT					
CT5	**CT5_F**	**AATGAGTTTCAGTTTTAGAAGC**	**$(GA)_{25}$**	***Guzmania monostachya***	**Boneh et al. (2003)**	**Yes**	**Yes**
	CT5_R	**CCAAGAAAAGAACGGATCA**					
VgA04	**VGA04_F**	**CAAACCCTTCTCACCTCACC**	**$(TC)_6(CT)_{11}$ $(TC)_{16}$**	***Vriesea gigantea***	**Palma-Silva et al. (2007)**	**Yes**	**Yes**
	VGA04_R	**CGACTCACCTGGCCCTAAT**					
PaA05	**PAA05_F**	**ACCGGGTTCAGGGAAAATAC**	**$(TTC)_{10}NN$ $(CT)_{17}$**	***Pitcairnia albiflos***	**Paggi et al. (2008)**	**Yes**	**Yes**
	PAA05_R	**TTGAGGCTAAGAGCGAGGAG**					
PaA10	PAA10_F	AACCATTGACATCCGCTGTT	$(ATG)_{10}$	*Pitcairnia albiflos*	Paggi et al. (2008)	Yes	-
	PAA10_R	CTTCGGAAGCTCCTCTGGAT					
PaC05	PAC05_F	TCGATGTCGACGGTAGTGAG	$(AG)_{18}NN(GA)_7$	*Pitcairnia albiflos*	Paggi et al. (2008)	Yes	Yes
	PAC05_R	TCCTCTCGCTTTGATTCACC					
PaD07	PAD07_F	TCCATGTGCCTCATCATAGC	$(TG)_{10}$	*Pitcairnia albiflos*	Paggi et al. (2008)	Yes	No
	PAD07_R	TGCCCACAAAGCATATCAGT					
PaA09	**PAA09_F**	**AGAAGAGAACCCACCCCAAG**	**$(CT)_{25}$**	***Pitcairnia albiflos***	**Paggi et al. (2008)**	**Yes**	**Yes**
	PAA09_R	**GTGTTCCGCGACACTACAAA**					
PaB12	**PAB12_F**	**CCCGAGGGACATTCTCTCTT**	**$(CT)_{19}NN(CT)_4NN$ $(CT)_4NN(TG)_7(TC)_5$**	***Pitcairnia albiflos***	**Paggi et al. (2008)**	**Yes**	**Yes**
	PAB12_R	**CATGGCGCAGTAGTGTTTTC**					

PaZ01	PAZ01_F	TGACCAGATAGCACCATCCA	$(GAC)_9$	*Pitcairnia albiflos*	Paggi et al. (2008)	No	-
	PAZ01_R	TTGAGTGTTGGAGCCCACTT					
PaB11	PAB11_F	AGAGGCTGAGAGAGGTAAACCA	$(TTC)_8$	*Pitcairnia albiflos*	Paggi et al. (2008)	No	-
	PAB11_R	CGAGCCCTCTTTCTGAACC					
Op13	Op13_F	CACCCATTGAGAAGGAAACG	$(AG)_{20}$	*Orthophytum ophiuroides*	Aoki-Gonçalves et al. (2014)	No	-
	Op13_R	GATGAGTGGACTGGTAAAGACC					
Op77A	Op77A_F	CACAACAGAGGCTCGAAAGA	$(AG)_9$	*Orthophytum ophiuroides*	Aoki-Gonçalves et al. (2014)	No	-
	Op77A_R	GCCCGACTCCTCCAATAACC					
Op89	Op89_F	AACCCTAGTTCCACCGATCA	$(CA)_{12}$	*Orthophytum ophiuroides*	Aoki-Gonçalves et al. (2014)	No	-
	Op89_R	CTCTTCACCCCCACAAATTC					
Op93	Op93_F	TTATCGGGCAGGGGAAATTA	$(AG)_{10}$	*Orthophytum ophiuroides*	Aoki-Gonçalves et al. (2014)	Yes	No
	Op93_R	ACCTTGTCACACACGCAAAG					
Vs1	Vs1_F	ACCCCGAACCCTATTTGAAG	$(CT)_{11}$	*Vriesea simplex*	Neri et al. (2015)	Yes	Yes
	Vs1_R	CCCCCAGGAACCCATACT					
Vs2	Vs2_F	GCCCACTAGAAACAGGCAAG	$(CT)_{12}(GT)_8$	*Vriesea simplex*	Neri et al. (2015)	Yes	No
	Vs2_R	CCCCATTAAACTCTAGGCATCC					
Vs8	**Vs8_F**	**CACTGTCGGGTGTTATGTGA**	**$(CTT)_{10}$**	***Vriesea simplex***	**Neri et al. (2015)**	**Yes**	**Yes**
	Vs8_R	**GGCGAGTGACATACCGATTT**					
Vs9	Vs9_F	ATGCATCCAACCAAGCTCTC	$(TG)_{11}$	*Vriesea simplex*	Neri et al. (2015)	No	-
	Vs9_R	GAATCGAGTCGGTGTGACCT					
Vs10	Vs10_F	CCATACCTCAATTCCTCATTCG	$(CA)_8$	*Vriesea simplex*	Neri et al. (2015)	No	-
	Vs10_R	GTGCATGCAGAGCCTTATGA					
Ac01	Ac01_F	CCTGACAACAAAAGGAGTGG	$(GA)_{21}$	*Aechmea caudata*	Goetze et al. (2013)	Yes	-
	Ac01_R	TACGACGATTCCAAAAGAGG					
Ac11	Ac11_F	TACTGCCCTCCATTTCCAC	$(AC)_{10}(CT)_5$	*Aechmea caudata*	Goetze et al. (2013)	No	-
	Ac11_R	CGCGAATGTGTATGATCTTG					
Ac25	Ac25_F	ATACTGCCCTCCATTTCCAC	$(AC)_{13}$	*Aechmea caudata*	Goetze et al. (2013)	No	-
	Ac25_R	GCTGATCTCAAACTACGAGCA					

Ac55	Ac55_F	GTAGCTGAGGTTTCCAGATCC	$(CT)_{27}$	*Aechmea caudata*	Goetze et al. (2013)	No	-
	Ac55_R	CTTGTATGGGCCTTTTTGG					
Ac64	Ac64_F	CCGTGGTTTTGTTTGTCTCT	$(AG)_{29}$	*Aechmea caudata*	Goetze et al. (2013)	No	-
	Ac64_R	GGGGTCAGGAAAGGAGAATA					
Ac78	Ac78_F	GACTTGTTCTGAAACGCAAAA	$(CA)_3CG(CA)_4A$ $(AC)_8$	*Aechmea caudata*	Goetze et al. (2013)	No	-
	Ac78_R	TTGCCCTCTAAGAGAGACTGG					
ngFos_1	ngFos1_F	GCTTGACTCTCATTCATCC	$(CA)_{10}$	*Fosterella rusbyi*	Wöhrmann et al. (2012)	No	-
	ngFos1_R	AGTGACCGACCACTGTAAAC					
ngFos_4	ngFos4_F	TTCTCAGATCGTGGTCTTTAC	$(CA)_9$	*Fosterella rusbyi*	Wohrmann et al. (2012)	No	-
	ngFos4_R	TCGACTCTACTATCCATGACC					
ngFos_6	ngFos6_F	GATGCTCTCATGTGTGTCTCT	$(TGT)_8$	*Fosterella rusbyi*	Wohrmann et al. (2012)	No	-
	ngFos6_R	TATCCGAAGAAACCCTAATTC					
ngFos_11	ngFos11_F	AACTAGGGTTAGGGTTTCTGA	$(ACA)_{12}$	*Fosterella rusbyi*	Wohrmann et al. (2012)	No	-
	ngFos11_R	ACTTGTTAGAATCGTCGGAGT					
ngFos_12	FOS12_F	CCTGCACGATGCGTTGTC	$(TCT)_{19}$	*Fosterella rusbyi*	Wohrmann et al. (2012)	Yes	Yes
	FOS12_R	GTAAAATCCATATCCGAATCC					
ngFos_14	ngFos14_F	AGTAGCCGGAGATCTTGAA	$(GTC)_7$	*Fosterella rusbyi*	Wohrmann et al. (2012)	No	-
	ngFos14_R	CCTTCGAACTCAATAATACCC					
ngFos_16	FOS16_F	ATATTTGCGAAGAAGTCGAG	$(AGC)_8$	*Fosterella rusbyi*	Wohrmann et al. (2012)	Yes	-
	FOS16_R	ATTATCGTCGCCATGTTCT					
Acom12.12	Acom12.12_F	TAGAGGTCGGGAGAACGAAA	$(CGA)_9$	*Ananas comosus*	Wöhrmann and Weising (2011)	Yes	Yes
	Acom12.12_R	GCGGAGGCTACTGATGCTAC					
Acom67.2	Acom67.2_F	CATCCATCCATCCATCCAAT	$(AT)_{10}$	*Ananas comosus*	Wöhrmann and Weising (2011)	No	-
	Acom67.2_R	GTCGTTGATCATTCGCAAAA					
Acom78.4	Acom78.4_F	GCAAATGAGGCCACAAACTT	$(GT)_{14}$	*Ananas comosus*	Wöhrmann and Weising (2011)	Yes	No
	Acom78.4_R	GGGTGGTGTGGACTTTCTCT					
Acom82.8	Acom82.8_F	CCCTGAAGGTGGAGATTGTG	$(GT)_{10}$	*Ananas comosus*	Wöhrmann and Weising (2011)	Yes	No
	Acom82.8_R	AAAAACCAAAACCCTGGACA					

Acom117.15	Acom117.15_F	GCAACCCCAATACCCTAACC	$(CT)_{20}$	*Ananas comosus*	Wöhrmann and Weising (2011)	Yes	-
	Acom117.15_R	GTACTCCGCCATTGTTGGTG					
Acom109.6	Acom109.6_F	CTTTTGCTCAGAAAGCAGGTT	$(CT)_{25}$	*Ananas comosus*	Wöhrmann and Weising (2011)	Yes	No
	Acom109.6_R	TGCGTGCTTGACCTCTGTTA					
Pit4	Pit4_F	CCGACTCTATCGTCAAAGG	$(CT)_{16}$	*Pitcairnia geyskesii*	Sarthou et al. (2003)	No	-
	Pit4_R	TTATCACCTCCCATGTCTCC					
Pit5	Pit5_F	TTGAGCCATGAACAATAGGG	$(GA)_{20}$	*Pitcairnia geyskesii*	Sarthou et al. (2003)	Yes	No
	Pit5_R	AGAATTCTAGTGGCAGTCCTC					
Pit8	Pit8_F	GAGGATGAAGGATTTCCAAGG	$(CCTCT)_5(TC)_{35}$ $(TGC)_{11}$	*Pitcairnia geyskesii*	Sarthou et al. (2003)	No	-
	Pit8_R	ACCGTCCCACGATAAGAGC					
Pit9	Pit9_F	AACCATTACATGCACCCTCAC	$(TC)_{13}$	*Pitcairnia geyskesii*	Sarthou et al. (2003)	Yes	Yes
	Pit9_R	TCACTGGGGAAGCCATAGAG					
Dd07	Dd07_F	GATTCGGAAGGATGACAAGA	$(GA)_{25}$	*Dyckia distachya*	Zanella et al. (2012)	No	-
	Dd07_R	CGGCACAGGAATACAAAGAG					
Dd10	Dd10_F	CTATGGGACTGCTGGACACT	$(TG)_7$	*Dyckia distachya*	Zanella et al. (2012)	Yes	No
	Dd10_R	CTTGCTGGTAATCGTGTTCC					
Dd16	Dd16_F	AATTGCACCAAACAGGGATT	$(GT)_7$ $(GC)_4$	*Dyckia distachya*	Zanella et al. (2012)	No	-
	Dd10_R	GACACGACCCACATAGATGC					
Dd20	Dd20_F	GGTGGAAATGGTGGGTTACA	$(CA)_7$	*Dyckia distachya*	Zanella et al. (2012)	Yes	No
	Dd20_R	GGCAGGCAAGGTATGAAGAA					

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Appendix S2

Priors setting for ABC analysis

We kept demographic scenarios relatively simple, and chose prior distributions based on general assumptions or previous knowledge on the target species. The prior for theta ($\theta = 4N_0\mu$) followed an uniform distribution ranging from one to six, assuming a current effective population size (N_0) of 1000 and microsatellite mutation rate (μ) ranging from $2.5*10^{-4}$ to $1.5*10^{-3}$, within mutation rate intervals inferred for other plants (Udupa & Baum 2001; Thuillet et al. 2002; Marriage et al. 2009). The prior for time of divergence (T_2) between Cerrado and Yungas lineages followed an uniform distribution bounded to the 95% confidence time to the most recent common ancestor inferred by Beast analysis (1.4 to 4.4 mya; see Results), while the prior for the time of past migration (T_1) followed the interval spanning the Pleistocene climatic oscillations (6 to 120 kya). Both times are measured in units of $4N_0$ generations, given a generation time of five years (personal observation), which corresponds to values spanning from 70 to 220 and from 0.3 to 6, respectively. For scenarios including migration, we used uniform prior distributions ranging from 20 to 200 for both migration rates ($m_{c\text{-}y}$ *and* $m_{y\text{-}c}$), which corresponds to 0.5 to 5% of N_0. Finally, for dispersal scenarios, the prior for the magnitude of the founder event, measured as the ratio of N_0 (θ_r), ranged from 1% to 2%, while the growth rate prior followed a logarithmic distribution to assure a smooth population growth following the founder event.

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Appendix S3

Table S3.3. Records of *P. lanuginosa* used for species distribution models.

Lineage	Record	Country	Locality	Coordinates	
				Longitude	Latitude
Cerrado	B1	Brazil	Brasília - Distrito Federal	-48.0500	-16.0300
	B2	Brazil	Brasília - Distrito Federal	-48.0486	-15.4819
	B3	Brazil	Alto Paraíso de Goiás - Goiás	-47.8412	-14.1938
	B4	Brazil	Alto Paraíso de Goiás - Goiás	-47.7904	-14.1845
	B5	Brazil	Alto Paraíso de Goiás - Goiás	-47.5953	-14.0400
	B6	Brazil	Alto Paraíso de Goiás - Goiás	-47.4375	-13.9350
	B7	Brazil	Cavalcante - Goiás	-47.4553	-13.8103
	B8	Brazil	Cocalzinho de Goiás - Goiás	-48.8247	-15.8042
	B9	Brazil	Corumbá de Goiás - Goiás	-48.7820	-15.8500
	B10	Brazil	Corumbá de Goiás - Goiás	-48.7800	-15.8500
	B11	Brazil	Corumbá de Goiás - Goiás	-48.7681	-15.8417
	B12	Brazil	Formosa - Goiás	-47.4300	-15.3000
	B13	Brazil	Luziania - Goiás	-48.1864	-16.2675
	B14	Brazil	Mossâmedes - Goiás	-50.1920	-16.0788
	B15	Brazil	Mossâmedes - Goiás	-50.1894	-16.0783
	B16	Brazil	Mossâmedes - Goiás	-49.8531	-15.9333
	B17	Brazil	Niquelândia - Goiás	-48.3167	-14.0333
	B18	Brazil	Niquelândia - Goiás	-48.3875	-14.3706
	B19	Brazil	Pirenópolis - Goiás	-48.9021	-15.7955
	B20	Brazil	Pirenópolis - Goiás	-48.9068	-15.7934
	B21	Brazil	Teresina de Goiás - Goiás	-47.4719	-13.9569
	B22	Brazil	Barra dos Garças - Mato Grosso	-52.2300	-15.2800
	B23	Brazil	Chapada dos Guimarães - Mato Grosso	-55.8352	-15.4292
	B24	Brazil	Chapada dos Guimarães - Mato Grosso	-55.7155	-15.4255
	B25	Brazil	Chapada dos Guimarães - Mato Grosso	-55.6583	-15.4075
	B26	Brazil	Chapada dos Guimarães - Mato Grosso	-55.8241	-15.4062
	B27	Brazil	Itaúba - Mato Grosso	-55.6022	-10.9611
	B28	Brazil	Novo Mundo - Mato Grosso	-55.2156	-9.6672
	B29	Brazil	Rondonópolis - Mato Grosso	-54.7954	-16.5181
	B30	Brazil	Aquidauana - Mato Grosso do Sul	-55.4956	-20.4597
	B31	Brazil	Aquidauana - Mato Grosso do Sul	-55.6981	-20.3633
	B32	Brazil	Aquidauana - Mato Grosso do Sul	-55.6886	-20.3317
	B33	Brazil	Corguinho - Mato Grosso do Sul	-55.1550	-19.9014
	B34	Brazil	Corguinho - Mato Grosso do Sul	-55.3313	-19.6977
	B35	Brazil	Costa Rica - Mato Grosso do Sul	-53.0622	-18.5858
	B36	Brazil	Costa Rica - Mato Grosso do Sul	-52.9524	-18.4262
	B37	Brazil	Coxim - Mato Grosso do Sul	-54.7604	-18.4830
	B38	Brazil	Sonora - Mato Grosso do Sul	-54.8917	-17.6203
	B39	Brazil	Belo Horizonte - Minas Gerais	-44.0041	-20.0181

	B40	Brazil	Carmésia - Minas Gerais	-43.1996	-19.1127
	B41	Brazil	Cordisburgo - Minas Gerais	-44.3749	-19.1609
	B42	Brazil	Itambé do Mato Dentro - Minas Gerais	-43.3278	-19.4350
	B43	Brazil	Ouro Preto - Minas Gerais	-43.5427	-20.4934
	B44	Brazil	Ouro Preto - Minas Gerais	-43.5418	-20.4903
	B45	Brazil	Ouro Preto - Minas Gerais	-43.6514	-20.3908
	B46	Brazil	Sabará - Minas Gerais	-43.8269	-19.9089
	B47	Brazil	Sabará - Minas Gerais	-43.7511	-19.8468
	B48	Brazil	Tiradentes - Minas Gerais	-44.1654	-21.1006
	B49	Brazil	Uberlândia - Minas Gerais	-48.0046	-18.9716
	B50	Brazil	Buenópolis - Minas Gerais	-44.1956	-17.8641
	B51	Brazil	Canaã dos Carajás - Pará	-49.8894	-6.3100
	B52	Brazil	Conceição do Araguaia - Pará	-50.1700	-8.0500
	B53	Brazil	Parauabepas - Pará	-50.1700	-6.0700
	B54	Brazil	Parauapebas - Pará	-50.2992	-6.0151
	B55	Brazil	São Geraldo do Araguaia - Pará	-48.4660	-6.2329
	B56	Brazil	Itapuã do Oeste - Rondônia	-62.9038	-9.2724
	B57	Brazil	Itapuã do Oeste - Rondônia	-63.0814	-9.1981
	B58	Brazil	Ji-Paraná - Rondônia	-61.9001	-10.1177
	B59	Brazil	Porto Velho - Rondônia	-64.6692	-9.2731
	B60	Brazil	Pedregulho - São Paulo	-47.4565	-20.2537
	B61	Brazil	Natividade - Tocantins	-47.6902	-11.6614
	B62	Brazil	Natividade - Tocantins	-47.6992	-11.6564
	B63	Brazil	Palmas - Tocantins	-48.1404	-10.3030
	B64	Brazil	Presidente Kennedy - Tocantins	-48.6008	-8.5006
	B65	Brazil	Presidente Kennedy - Tocantins	-48.5752	-8.4852
	B66	Bolivia	Velasco - Santa Cruz	-60.3833	-14.8167
	B67	Bolivia	Velasco - Santa Cruz	-60.7697	-14.5617
	L1	Bolivia	Andrés Ibáñez - Santa Cruz	-63.4150	-17.6794
	L2	Bolivia	José Ballivián - Beni	-67.0667	-15.2667
	L3	Bolivia	Caranavi - La Paz	-67.6500	-16.0333
	L4	Peru	La Convención - Cusco	-72.7775	-11.9319
	L5	Peru	Huánuco - Huánuco	-76.0333	-9.5167
	L6	Peru	Pachitea - Huánuco	-75.5986	-10.0153
	L7	Peru	Chanchamayo - Junin	-75.3428	-11.1267
	L8	Peru	Chanchamayo - Junin	-75.3178	-11.0356
	L9	Peru	Chanchamayo - Junin	-75.1081	-10.8531
Yungas	L10	Peru	Chanchamayo - Junin	-75.1739	-10.6494
	L11	Peru	Huampal - Oxapampa	-75.5797	-10.1856
	L12	Peru	Huampal - Oxapampa	-75.5458	-10.0542
	L13	Peru	Oxapampa - Oxapampa	-75.1169	-10.6733
	L14	Peru	Oxapampa - Oxapampa	-75.1083	-10.4928
	L15	Peru	Tocache - San Martin	-76.6766	-7.8736
	L16	Peru	Purus - Uycali	-71.1000	-10.0667

Table S3.4. Variables contribution for species distribution models (SDMs).

Clade	Period	Variables contribution (%)							
		Bio2	**Bio3**	**Bio8**	**Bio13**	**Bio14**	**Bio15**	**Bio18**	**Bio19**
Cerrado clade	Current	8.4	17.5	**21.5**	**23.5**	**20.8**	0.2	3.2	4.8
	Holecene	9	**21.4**	**22.8**	**21.9**	16.8	0.3	2.4	5.4
	LGM	8.3	**19.9**	**22.4**	**23.9**	18	0.5	3.2	3.8
	LIG	8.3	**21.4**	**22.9**	**22.3**	17.8	0.3	2.8	4.2
Yungas clade	Current	8.4	11	0.3	0.2	0.9	23.9	16.5	**38.8**
	Holecene	8.4	11.7	0.4	0.3	1.2	22.9	16	**39.1**
	LGM	5.5	9.7	0.2	0.2	1.8	22.3	19.5	**40.8**
	LIG	7.2	10.7	0.3	0	1.3	24.3	16.5	**39.6**

Bio2 = Mean Diurnal Range (Mean of monthly (max temp - min temp)); Bio3 = Isothermality (Bio2/Bio7) (*100); Bio8 = Mean Temperature of Wettest Quarter; Bio13 = Precipitation of Wettest Month; Bio14 = Precipitation of Driest Month; Bio15 = Precipitation Seasonality (Coefficient of Variation); Bio18 = Precipitation of Warmest Quarter; and Bio19 = Precipitation of Coldest Quarter.

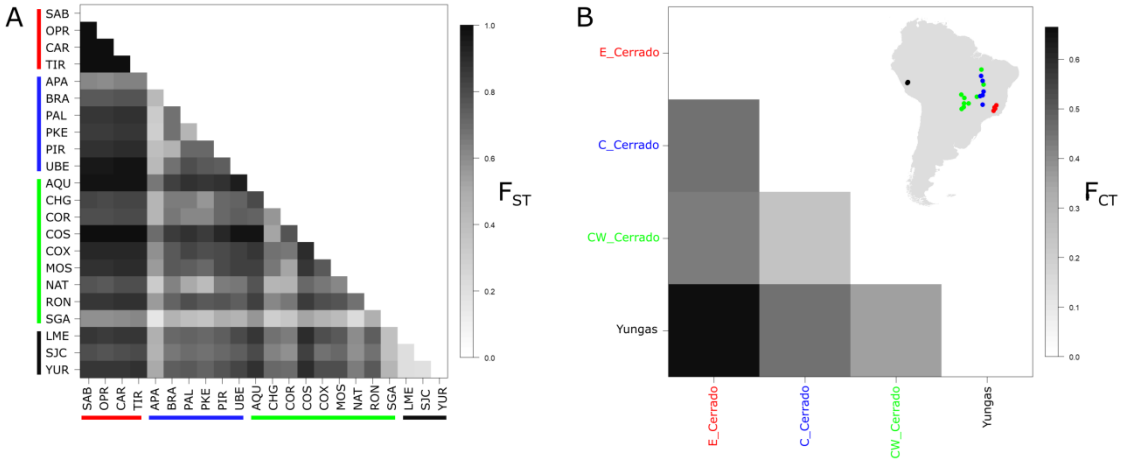
Appendix S4

Table S4.6. Genetic diversity by microsatellite marker based on 22 populations and 318 individuals of *Pitcairnia lanuginosa*.

SSR marker	N	A	H_E	H_O	F_{IS}
PaA05	13.955	1.955	0.188	0.075	0.603*
PaA09	14.136	1.818	0.148	0.077	0.479*
PaA10	13.955	2.045	0.154	0.100	0.351*
PaB12	14.227	2.864	0.314	0.122	0.610*
PaC05	13.864	2.500	0.304	0.130	0.573*
Vs8	11.773	2.682	0.272	0.147	0.459*
Ct5	7.500	2.091	0.239	0.111	0.536*
Ac1212	14.364	1.182	0.025	0.014	0.435*

N = mean number of samples per population, A = mean number of alleles per population, H_E = expected heterozygosity, H_O = observed heterozygosity, F_{IS} = inbreeding coefficient. *Deviations from Hardy-Weinberg equilibrium ($p<0.001$).

Figure S4. Matrices depicting (a) pairwise F_{ST} distances among 22 *Pitcairnia lanuginosa* populations and (b) F_{CT} among four genetic groups as defined by PCoA analysis (Eastern Cerrado, Central Cerrado, Central-western Cerrado and Yungas).



Appendix S5

Table S5.7. Posterior probabilities of scenarios I to VI (see Fig. 2) estimated by multinomial logistic regression (mnlogistic)and neural network (neuralnet) methods using either a full data set (274 samples from Cerrado and 44 samples from Yungas) or four distinct unbiased data sets (44 samples from each Cerrado and Yungas). Best model for each data set is highlighted in bold.

Data set	**Posterior probabilities (mnlogistic|neuralnet)**					
	Scenario I	**Scenario II**	**Scenario III**	**Scenario IV**	**Scenario V**	**Scenario VI**
Full	0.000|0.006	0.000|0.000	**1.000|0.993**	0.000|0.0002	0.000|0.0007	0.000|0.000
Subset 1	0.112|0.165	0.000|0.000	**0.796|0.723**	0.000|0.000	0.092|0.111	0.000|0.000
Subset 2	0.083|0.113	0.000|0.000	**0.880|0.861**	0.000|0.000	0.037|0.026	0.000|0.000
Subset 3	0.044|0.082	0.000|0.000	**0.945|0.910**	0.000|0.000	0.011|0.008	0.000|0.000
Subset 4	0.003|0.017	0.000|0.000	**0.996|0.979**	0.000|0.000	0.000|0.004	0.000|0.000

Subset 1= AQU, PAL and SAB populations from Cerrado.

Subset 2 = COX, PIR, and TIR populations from Cerrado.

Subset 3 = CAR, PKE and RON populations from Cerrado.

Subset 4 = APA, COS and OPR populations from Cerrado.

Table S5.8. Confusion matrix based on cross-validation tests using 100 simulated samples for each scenario. The best scenario is highlighted in gray and percentage of corrected classifications by scenario are in bold.

Scenario	I	II	III	IV	V	VI	Total
I	**42**	0	22	0	36	0	100
II	1	**37**	0	26	0	36	100
III	25	1	**67**	0	7	0	100
IV	0	21	0	**63**	0	16	100
V	18	1	12	0	**69**	0	100
VI	0	20	0	12	0	**68**	100

Table S5.9. Parameters estimated from 0.5% retrieved simulations under chosen scenario in an approximate Bayesian computation framework (Scenario III; dispersal from Cerrado to the Yungas).

Parameters	Prior	Median	Posterior (95% CI)
θ	1-6	3.89	3.4 –4.40
T_1	0.3-6	0.26	0.24–0.30
T_2	70-220	252.87	215.69–274.69
θ_r	0.01 - 0.02	0.015	0.014-0.017

CI=confidence interval; θ =theta; T_2 = divergence time between Cerrado and Yungas lineages (in coalescence time units, i.e., in generations/$4N_0$); T_1 = time bounded by Pleistocene events (in coalescence time units). θ_r = ratio of theta moved from ancestral population N_0 to the Yungas clade.

CAPÍTULO III

Drift-selection interactions drive the diversification of *Pitcairnia lanuginosa* (Bromeliaceae) across its broad but patchy Neotropical distribution

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Drift-selection interactions drive the diversification of *Pitcairnia lanuginosa* (Bromeliaceae) across its broad but patchy Neotropical distribution

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ABSTRACT

The relative roles of genetic drift and natural selection on the diversification of intra and inter-specific lineages is a major question in evolutionary biology. Both genetic drift and divergent selection are predicted to be important drivers on the species diversification within patchy habitats, but the extent to which these joint forces act on natural populations are strongly affected by species' ecological features. In this study, we infer the evolutionary history and genomic structure of *Pitcairnia lanuginosa*, a widespread, patchy distributed species, occurring across Central Tropical South America. We sampled populations in the Brazilian Cerrado and Central Andean Yungas, and genotyped hundreds of SNP markers defined through double-digest restriction-site associated DNA sequencing (ddRAD-Seq). In addition, we measured physiological traits and compared patterns of phenotypic (P_{ST}) and genetic (F_{ST}) divergence (P_{ST}-F_{ST} comparisons) in a subset of Cerrado populations. Our results from molecular analyses showed an extremely low genetic diversity and remarkable differentiation among populations, supporting an important role of genetic drift for population divergence. In agreement, F_{ST} outliers loci tests and most P_{ST}-F_{ST} comparisons suggested limited effect of selection as a force driving *P. lanuginosa* local adaptation over its range-wide distribution into mesic habitats. However, P_{ST}-F_{ST} comparisons suggested divergent selection on few physiological traits linked to drought tolerance and Bayesian generalized linear mixed models (GLMMs) revealed that genetic variation within the Cerrado biome may be better explained by isolation by environment (IBE) of climatic conditions than by isolation by distance (IBD). Our study has important implication to improve our knowledge of the joint roles of genetic drift and divergent selection in generating divergence and diversity in species with naturally patchy populations.

Keywords: Genetic drift, divergent selection, local adaptation, ddRAD sequencing, environmental stress, P_{ST}-F_{ST} comparisons.

INTRODUCTION

Contrasting neutral and adaptive genomic variation has been a keystone of studies on evolutionary processes driving genetic structure in the last decade (Orsini et al. 2013, Andrews et al. 2016). Non-continuously distributed populations represent a particularly interesting system to infer the relative role of genetic drift and natural selection for species diversification. Although both evolutionary forces are predicted to be important, the extent to which these joint forces act on patchy populations are strongly affected by species' ecological features. Genetic studies on such systems have long shown that restricted gene flow among populations often result in strong genetic drift (e.g. Ortego et al. 2010; Palma-Silva et al. 2011; Nistelberger et al. 2015), but the importance of drift–selection interactions for diversification dynamics and local adaptation of patchy distributed species have only recently been tested using molecular markers (e.g., Funk et al. 2016; Perrier et al. 2017; Wang et al. 2017; Prentice et al. 2017). Furthermore, we lack empirical evidence on genetic drift acting as a sole driver of speciation processes (Coyne and Orr 2004; Sobel et al. 2010), despite its putative role of providing the initial divergence on which natural selection subsequently acts or speeding diversification processes (Ueyda et al. 2009; Piqt et al. 2016). Identification of the underlying mechanisms responsible for genetic structure within patchy habitats may, therefore, shed light on the potential but still controversial role of drift on speciation processes.

Species ability to disperse and establish in habitats under distinct climate conditions is directly linked to drift-selection interactions. While many species show evidence of ecological shifts (i.e., niche differentiation), other might have restrict ecological requirements and only establish in regions with similar environmental conditions (i.e., niche conservatism) (McCommark et al. 2010; Bacon et al. 2017; Baranzelli et al. 2018). Organisms ability to adapt to new environmental conditions is determined by either *de novo* mutations or by standing genetic variation, but adaptation from standing variation is likely to be faster (Barret and Schluter 2008). Because of strong drift effects, small founded populations can lack standing genetic variation on which selection acts to shape species morphological and physiological traits. Furthermore, pronounced drift may overwhelm divergent selection arising from new beneficial mutations, precluding adaptive divergence in small populations colonizing new habitats (Wright 1951; Leimu and Fischer 2008). Therefore, understanding the

evolutionary strategy that allow or not non-continuously distributed species to increase their ecological range toward distinct ecological conditions require coupling genetic and ecological information.

For the highly heterogeneous Neotropics, an increasing number of genetic studies with widespread species has improved our understanding of diversification across biomes (Antonelli et al. 2018), but whether differentiation within species is accompanied or not by local adaptation has been less explored (but see Bacon et al. 2017). The application of next generation sequencing (NGS) to Neotropical systems promises to allow novel insights into diversification events and biomes histories (e.g., Nadeau et al. 2013, Harvey and Brumfield 2013, Ebel et al. 2015). In addition, these recent sequencing technologies can help to uncover adaptive processes underlying ecological patterns. Indeed, the increasing number of loci generated by NGS and constant development of computational methods has allowed for more powerful inferences regarding genetic structure in non-model species (Davey et al. 2011; Narum et al. 2013), besides having greatly improved our ability to distinguish neutral from adaptive processes (e.g. Andrews et al. 2016). Approaches for identifying locally adaptive candidate loci rely on the identification of markers with unusually high genetic differentiation among populations or searching for correlations between allele frequencies and environmental information (Hoban et al. 2016). Moreover, when phenotypic information is available at population level, phenotypic divergence (P_{ST}) can be compared to differentiation of neutral alleles (F_{ST}) to give insights into the degree of differentiation caused by selective *versus* neutral processes (P_{ST}–F_{ST} comparisons; Brommer 2011; Leinonen et al. 2013). Nevertheless, there are still challenges associated with identifying sufficient genetic variation to detect loci under selection and to perform genotype-environment or genotype-phenotype associations (Leinonen et al. 2013; Lowry et al. 2017), particularly in species with restricted gene flow (Perrier et al. 2017).

Here, we used *Pitcairnia lanuginosa* Ruiz and Pav. as a model to infer the relative role of genetic drift and natural selection on the diversification within patchy neotropical habitats. *P. lanuginosa* has a widespread distribution in tropical regions of South America, with small populations typically scattered across the space and associated to riparian forests in the Central Andean Yungas and Brazilian Cerrado (Figure 1). A previous phylogeographic study have suggested that diversification between lineages occupying the Cerrado and Yungas are likely caused by past dispersal

rather than vicariance (Leal et al. Capítulo 2). In this former study we found low levels of genetic diversity and high population structure, likely resulting from the lack of traits for seed mediated gene flow, and extremely high rates of spontaneous self-fertilization (Leal et al. Capítulo 2). Despite the strong effect of drift pointed by these findings, adaptive processes may also be important to explain diversification patterns of *P. lanuginosa*, as predicted for naturally fragmented species experiencing low gene flow. In addition, the large-scale latitudinal and longitudinal distribution of *P. lanuginosa* have potentially submitted populations to contrasting climatic conditions. Indeed, *P. lanuginosa* (considered synonymous of *Pitcairnia burchellii* Mez; Smith and Downs 1974) exhibit physiological and anatomical characteristics to cope with severe drought stress (Vieira et al. 2017a,b), which is important for local adaptation to the greater seasonality in the Cerrado biome (wet summer and dry winter) when compared to Central Andean Yungas. Nevertheless, the extent to which high genetic structure could be interpreted in terms of adaptive processes can be strongly affected by mating system (Glémin 2007; Wright et al. 2013). In fact, the increased self-fertilization in *P. lanuginosa*, for instance, may reduce populations effective population sizes, thereby enhancing genetic drift. Under the increased effect of drift, adaptive alleles can be lost, limiting its local adaptation (Charlesworth and Charlesworth 1987; Wright et al. 2013).

Here we take advantage of hundreds of SNP markers derived from double-digest restriction-site associated DNA sequencing (ddRAD-seq, Peterson et al. 2012), and information on physiological traits associated with environmental stress, to investigate the relative role of neutral and adaptive processes in causing the high genetic divergence across the patchy distribution of *P. lanuginosa*. Specifically, we wanted to: (1) investigate whether high genetic drift linked to low levels of gene flow and high inbreeding may preclude local adaptation in this species; (2) access the relative importance of Isolation by Distance (IBD) and Isolation by Environment (IBE) on the patterns of genetic structure of the species; and (3) evaluate whether the consistent association of *P. lanuginosa* to mesic habitats result from niche conservatism within the species. The wide yet patchy distribution in the Neotropics, make *P. lanuginosa* a particularly interesting model to investigate how ecological processes determine the adaptive potential. The information on genomic variation of this species may thereby shed light on general mechanisms acting on non-continuously distributed populations.

MATERIAL AND METHODS

Population sampling

We sampled 19 populations of the *Pitcairnia lanuginosa* complex across most of its geographic range from May 2014 to May 2015 (Table 1, Figure 1). Specimens from eight populations were transported from field to a green house (see Table 1), and planted into pots to grown under controlled conditions in the experimental green house of the São Paulo State University, UNESP, Rio Claro, Brazil. Populations sampling covers two distinct ecorregions located in the Brazilian Cerrado (a savanna like biome) and Central Andean Yungas (in the Amazonia-Andes transition) (Figure 1). Herbarium specimens were deposited within the herbarium HBRC, Universidade Estadual Paulista–UNESP, Rio Claro, São Paulo, Brazil.

Physiological traits measurements

We carried out physiological measurements in a total of 24 individuals from eight out of 19 sampled populations of *P. lanuginosa* (Table 1), which represent the extent of geographic distribution of the species in the Cerrado (Figure 1). We acclimated these individuals for one year into a greenhouse at the São Paulo State University, UNESP, Rio Claro, Brazil. We set apart two ramets of each individual to grow into distinct pots with the same substrate composition for an additional acclimation period of three months prior to collecting physiological data. We then submitted each ramet per individual to the following treatments (24 samples per treatment): (A) control, consisting in watering pots every two days (about 80 mL of water, which corresponded to field capacity); and (B) water shortage, consisting in no watering for 30 days. We measured physiological traits based on photosynthetic response of leaf samples from each ramet growing under these contrasting water conditions on August 2016. By measuring those traits we aim at detecting phenotypic signal related to light, temperature and drought stress.

We selected a sample of ca. 2 cm^2 from a leaf of each ramet to measure the photosynthetic responses to light (i.e. rapid light curve) and to heat stresses under the control and water shortage treatments using a modulate fluorometer (MINI-PAM-II, Walz). We then measured the following parameters: maximal photochemical efficiency (F_v/F_m), maximum relative electron transport rate (ETR_{max}), the initial slope of the curve of light-response (α), the light saturation coefficient (Ik) the upper critical thermal

limit (CT_{max}), and the sensitivity to temperature change (*z*). To calculate heat tolerance parameters CT_{max} and *z*, we followed the framework described by Rezende et al. (2014), which consider the intensity and duration of thermal stress to draw the individual 'thermal tolerance landscape', or individual capacity to withstand heat at any given temperature. We followed procedures described by Godoy et al. (2011), and modified by Chaves et al. (2018), to measure the critical time duration under a static stressful temperature that promotes a 50% decay of the initial F_v/F_m (D_{50}) in each leaf sample. To draw the 'thermal tolerance landscape' of each sample, we calculated D_{50} under four distinct temperatures (40, 45, 50, and 60°C) and performed a linear regression to measure CT_{max} and *z* parameters according to Rezende et al. (2014). Measures of F_v/F_m at each temperature were simultaneously carried out using a thermal-gradient block designed by Labouriau (1977) and adapted by Cardoso (2010). Finally, we used the decay of each parameter for ramets under water shortage in relation to ramets under controlled conditions for each individual as proxies of drought stress.

DNA extraction and ddRAD sequencing

Total genomic DNA from 122 individuals (3-8 specimens from 19 populations, Table 1) was extracted from leaf samples using the Qiagen DNA plant minikit (Qiagen, Finland). Sample quality and concentration were checked on 1% agarose gels and NanoDrop 2000 spectrophotometer (Thermo Scientific) and normalized to 60 ng/ul as quantified by the Qubit dsDNA BR assay (Invitrogen). The ddRAD libraries were prepared and sequenced on the Ion Torrent Proton platform using a modified version of Peterson et al.'s (2012) protocol at the Genome Transcriptome Facility of Bordeaux (INRA, Cestas, France). In brief, we digested 50ul (300 ng total) of genomic DNA using two rare-cutter restriction enzymes (PstI and MspI) at 37° C for two hours, and inactivated them at 80°C for 20 minutes. After bead-purification, a common P1 and unique barcode adaptors were added to each sample, and ligation was performed at 22°C for 2 hours followed by inactivation at 65°C for 20 minutes. We then used a qPCR to quantify each sample before normalizing and pooling them in a 48-plex. We employed an automated size-selection technology (Pippin, Sage Science) to select fragments with expected size (~180 base pairs). Each library was then amplified by PCR, quantified at the Agilent 2100 Bioanalyzer and sequenced on the Ion Torrent Proton P1v2 chip.

***De novo* assembly**

We analyzed raw read data using PYRAD version 3.0.5 software (Eaton 2014). This program assembles short read RAD-seq data using a clustering algorithm that allows for indel variation within and between samples and incomplete overlap among reads (Eaton 2014). This particularity makes the method suitable for highly structured populations. Following preliminary testing, we settled the parameters as follows: maximum number of sites with quality score <20 (NQual): 5, clustering threshold (Wclust): 0.85, minimum coverage for a cluster (Mindepth): 6, and maximum individuals with a shared heterozygous site (MaxSH): 3. All other parameters were kept at default values. PYRAD was run on the GenoToul bioinformatics facility (INRA, Toulouse, France). We then employed VCFTOOLS (Danecek et al. 2011) to filter pre-filtered loci with a minimum of 30% taxon coverage, as studies have shown that even lower missing data cutoffs lead to correct inferences (Chattopadhyay et al. 2014; Huang and Knowles 2016; Hodel et al. 2017). We also used VCFTOOLS to remove any individuals with genotypes for less than 30% of loci. Because of the known long-term divergence between Cerrado and Central Yungas populations (Leal et al., Capítulo 2), two other data matrices including only samples from the Cerrado or from the Yungas were separately build using PYRAD and then filtered by VCFTOOLS according to the same criteria. VCF files were converted to various formats using PGDSPIDER 2.0.7.2 (Lischer and Excoffier 2012) to perform further analyses.

Genomic diversity and differentiation

We calculated diversity indices, such as proportion of polymorphic loci; mean frequency of the most frequent allele per loci and nucleotide diversity (π), using the 'populations' module of Stacks v1.35 (Catchen et al. 2013). We also estimated pairwise F_{ST} among sampled populations using ARLEQUIN 3.5 (Excoffier and Lischer 2010).

A neighbour-net tree was inferred using the software SPLITSTREE4 using uncorrelated p-distances and bootstrap procedures (Huson and Bryant 2006). We further assessed patterns of genomic structure employing two distinct Bayesian approaches implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003) and INSTRUCT (Gao et al. 2007) softwares. The software STRUCTURE was run under the admixture model allowing for correlated allele frequencies, using a burn-in of 200,000 followed by 1,000,000 Markov Chain Monte Carlo (MCMC) iterations. Twenty replicate runs were performed with values of K ranging from 1 to 20. We also

run STRUCTURE for each cluster evidenced in the first run separately to reveal a putative hierarchical substructuring. We inferred the most likely K as described by Evanno et al. (2005) using STRUCTURE HARVESTER (Earl 2012) and admixture proportions for the best K were averaged among runs using the main pipeline of the CLUMPAK server (Kopelman et al. 2015). The clustering method INSTRUCT was further used to validate STRUCTURE results, once *P. lanuginosa* has high rates of inbreeding that violate STRUCTURE assumptions of Hardy-Weinberg equilibrium (Pritchard et al. 2000). INSTRUCT was designed to quantify the contribution of non-random mating caused by either inbreeding or population substructure (Gao et al. 2007), which is suitable for species performing selfing fertilization. INSTRUCT analysis was run for K=1 to K=20, for five independent chains, each with 200,000 iteration steps and 50,000 burn-ins, with a thinning interval of ten steps, using the joint inference of population selfing rate and population sub-structure mode. We chose best K based on the deviance information criterion (DIC), and used OBSTRUCT (Gayevskiy et al. 2014) to plot averaged results among chains. We then used ARLEQUIN 3.5 to test the partition of genetic diversity within and between congruent groups revealed by STRUCTURE and INSTRUCT by an analysis of molecular variance (AMOVA).

Outliers detection

We employed two different approaches to test for signatures of selection across the entire distribution of *P. lanuginosa* and within populations sampled either in the Cerrado or Andean Yungas, using independently assembled data sets. First, we detected outliers based on locus-specific F_{ST} using the program BAYESCAN v2.1 (Foll and Gaggiotti 2008) with a prior odd of 10 and a false discovery rate (FDR) of 0.05. After 20 pilot runs of 5000 iterations each and a burn-in of 50000 iterations, 5000 MCMC samples were taken with a thinning interval of 10. BAYESCAN examines populations pairwise F_{ST} for each locus and decompose the coefficients into a population-specific component (β), and a locus-specific component (α) using a logistic regression (Foll and Gaggiotti 2008). From detected outliers loci, those with positive values of α suggest diversifying selection, while negative values suggest balancing or purifying selection. To complement this previous analysis, we explored loci associated with environmental variation using the software BAYESCENV (Villemereuil and Gaggiotti 2015). We searched for associations between SNP allele frequencies and the first axis of a PCA

using 11 temperature variables (TEMP) or 8 precipitation variables (PREC) extracted from WorldClim (Hijmans et al. 2005). BAYESCENV was run for both environmental variables using a prior probability of 0.5 and a prior preference of 0.5. After 20 pilot runs of 5000 iterations each and a burn-in of 50000 iterations, 5000 MCMC samples were taken with a thinning interval of 10 steps. Diagnostics of the log likelihoods and F_{ST} values for both analyses were checked using the 'CODA' R-package.

To determine if outlier loci identified by these distinct approaches were in genic regions, we blasted reference sequences against *P. flammea* annotated transcriptomes (Palma-Silva et al. 2016). We then used the assembled longer sequence contigs (ca. 5000 pb) to conduct BLAST searches against protein databases using the Full-Lenghter Tool (Lara et al. 2007).

Environment–genotype associations

We used a bayesian generalized linear mixed modeling (GLMM) approach to test whether genome-wide genetic differentiation among Cerrado populations was driven by environmental differences among populations, as determined by temperature and precipitation variation. We used two distinct pairwise F_{ST} matrices from multi-locus SNPs as response variable: one including all 618 SNPs assembled for the Cerrado populations, and a sub set of 17 outliers loci inferred by BAYESCAN). As predictor variables, we tested four different matrices: (1) euclidean distances among populations to assess the effect of isolation by distance (IBD) alone (GEO matrix), (2) 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 (ENV matrix); (3) euclidean distances along the first and second axis of a PCA of 11 WorldClim temperature variables (ENV_{temp} matrix); and (4) euclidean distances along the first and second axis of a PCA of eight WorldClim precipitation variables (ENV_{precip} matrix). The predictor ENV captured mainly temperature of coldest quarter and precipitation of the wettest quarter, while ENV_{temp} corresponded mainly to annual mean temperature and isothermality, and ENV_{precip} to precipitation of driest and coldest quarter (Table S1).

We confronted models including a single predictor variable or a combination of variables against a null model (see Table 4). The analysis was performed with R package MCMCGLMM (Hadfield 2010), following scripts available in Lexer et al. (2014). MCMCGLMM was run with a burn-in of 500,000 followed by 2,000,000

MCMC iterations with a thinning interval of 750, under standard priors. Chain convergences were checked using the CODA R package. We used the Deviance Information Criterion (DIC) to compare models and draw conclusions on the relative roles of IBD *versus* IBE in the divergence of *P. lanuginosa* within the Cerrado.

P_{ST}-F_{ST} comparisons

We also evaluated the relative importance of selective and neutral processes behind *P. lanuginosa* evolution by comparing differentiation in physiological quantitative traits (as measured by P_{ST} index) and neutral markers derived from ddRAD sequencing (as measured by F_{ST} pairwise index) among a sub set of eight *P. lanuginosa* populations (Table 1, Figure 1). To represent the degree of phenotypic differentiation among populations, we employed an approximation of Q_{ST} called P_{ST} (Merilä and Crnokrak 2001), once the estimation of the additive genetic variance component of Q_{ST} would be impossible without a breeding design. P_{ST} is directly calculated from the total phenotypic variance components with no distinction between the relative contribution of genetic and environmental variations (Leinonen et al. 2006; Silva and Silva 2018) and has been applied to distinct biological systems as an alternative of Q_{ST} calculations (e.g., He et al. 2013; Shinn et al. 2015). The logic of P_{FT}-F_{ST} comparison relies on the assumption that the F_{ST} obtained from molecular neutral markers reflects the divergence induced by genetic drift without selection (Merilä and Crnokrak, 2001). Thus, $P_{ST}>F_{ST}$ or $P_{ST}<F_{ST}$ mean that quantitative traits show a higher or lower level of differentiation than expected under the influence of genetic drift alone, as induced by divergent or balancing selection, respectively; while $P_{ST}=F_{ST}$ means that no departure from neutral expectations can be detected.

Population pairwise P_{ST} was calculated from between-population (σ^2_B) and within population (σ^2w) components of variance for each of the 12 physiological traits following Brommer's (2011) formula $P_{ST}=c\sigma^2_B/(c\sigma^2_B+2h^2\sigma^2_W)$ in R package 'Pstat' (Silva and Silva 2018). We assumed that the proportion of the total phenotypic variance which is due to additive genetic effects is equal to the narrow-sense heritability, as we have no previous data on contributions of additive genetic variance to among (c) and within (h^2) population differences in physiological traits for our sampled populations. Because of the known problem associated with the lack of accurate estimation of the parameters c and h^2 for a set of traits (see Brommer 2011), we assessed the strength of P_{ST}–F_{ST} comparisons exploring the variation range of $0<c/h^2\leq1$ using 'Pstat'. We also

calculated P_{ST} values and their confidence intervals for the first and second principal component (PC1 and PC2) from PCAs grouping all traits or grouping separately those traits related to heat, light and drought stress. In its turn, pairwise F_{ST} values were calculated using populations module of Stacks for a total of 584 putatively neutral SNPs, excluding those 34 outliers detected by BAYESCAN and/or BAYESCENV approaches. Confidence intervals of P_{ST} and F_{ST} values were determined with the bootstrap method (1,000 x) under a confidence level of 0.95%. P_{ST} was considered to significantly differ from F_{ST} when their confidence intervals did not overlap. Correlations between P_{ST} and F_{ST} population pairwise matrices were further examined with a mantel test as implemented in 'ade4' R package (Dray and Dufour 2007). The significance of the tests was obtained with 10,000 permutations.

RESULTS

Genetic diversity and structure patterns

Three lanes of ddRAD-sequencing (40-48 samples per lane) using ION Torrent technology resulted in ~1,992,450 SE reads per *P. lanuginosa* sample, with an average of 73% reads per sample passing quality control (Table S2). PYRAD assembled a total of 13,858 prefiltered RAD loci. After VCFTOOLS filtering for the maximum amount of missing data within loci and individual, our final matrix included 115 individuals and 688 SNPs. Finals matrices for the Cerrado and Yungas sub sets had a total of 618 and 714 SNPs, and 95 and 21 individuals, respectively.

P. lanuginosa populations had extremely low within-population genetic variation (Table 2). The proportion of polymorphic sites per population ranged from 1.93 to 18.52, while mean frequency of the most common allele are >0.9 in all populations (Table 2). Observed and expected heterozigozities per population varied from zero to 0.0009 and 0.0092 to 0.0753, respectively (Table 2). Although levels of genetic diversity are non-homogenously distributed across space, all eastern populations demonstrated reduced levels of genetic diversity when compared to the other populations (Figure 1).

We detected generally high pairwise F_{ST} values (Figure S1) ranging from zero (SJC-YUR) to 0.91 (SJC-PIR). Independent analyses consistently recovered the same genetic structure. Neighbour-net analysis revealed only two major splits corresponding to Cerrado and Yungas populations with bootstrap support of 100% (Figure 2), despite

Eastern Cerrado populations are slightly distinguished from those belonging to the Central-Western distribution (Figure 2). The first level of the STRUCTURE Bayesian clustering analysis showed two genetically well-differentiated groups formed by Cerrado and Yungas populations, respectively (Figure 3B). STRUCTURE analysis depicting population divergence within each of these clusters found K=2 and K=3 as the best respective K numbers. Within the Cerrado, Eastern populations and Central-western populations formed two distinct clusters, suggesting an east-west gradient of genetic variance. Populations from Yungas are more admixed, but revealed some level of incipient differentiation (Figure 3B). INSTRUCT recovered the same pattern clustering populations into three groups corresponding to Eastern Cerrado, Central-Western Cerrado and Yungas with lower levels of admixture between both Cerrado clusters, and high inbreeding coefficients (0.956, 0.961 and 0.961, for each respective cluster; Figure 3A and 3C). AMOVA evidenced that the majority of the genomic variance is significantly partitioned among groups, either when contrasting Cerrado and Yungas or Eastern Cerrado, Central-western Cerrado and Yungas (F_{CT}=53.38 and 52.89, respectively), suggesting a historical genetic isolation among these groups (Table 3). About 24% and 19% of the variation accounted for differences among populations nested within each of these groups, respectively, while 22% and 27% accounted for within populations variance (Table 3).

Drift-selection interactions

Only few loci had higher or lower F_{ST} values than expected under neutrality, suggesting that most of genetic structure among populations can be explained by stochastic processes (Figure 4). BAYESCAN method detected a total of 34 F_{ST} outliers with a signature of divergent or balancing selection among populations (Figure 4A), while BAYESCENV using precipitation and temperature variables detected 24 and 23 outliers loci with common signature of balancing selection (Figure 4D,G). Except for two F_{ST} outliers putatively under divergent selection detected by BAYESCAN, outlier loci had lower F_{ST} estimates and negative α values which suggest balancing selection (Figure 4); with 22 of them being consistently identified by both analyses (Figure S2). When searching for F_{ST} outliers within the Cerrado, BAYESCAN pointed to a total of 17 outliers, being four of them putatively under divergent selection (Figure 4B). For this data set, BAYESCENV analysis revealed four and three common outliers, using precipitation and temperature information, respectively (Figure 4E,H), and two loci

consistently detected by all analyses (Figure S2). No F_{ST} outliers were detected among populations from the Yungas (Figure 4). BLAST search failed to recover information for most of the outliers loci, except for two loci under divergent selection associated with ion channel activity or transmembrane transport (GO: 0005216, GO:0016021 and GO:0034220) and activating signalization, and a locus under balancing selection evolved with intracellular protein transport (GO:0000943, GO:0003676, GO:0008270 and GO:0015074).

GLMMs suggest a clear effect of environmental distance on the genetic structure within the Cerrado distribution. When contrasted to other predictors, the model including environmental distance (ENV) as the single explanatory variable achieved the greatest DIC support (Table 4), indicating that genetic variation across the species distribution in Cerrado is better explained by IBE than by IBD. Such model is mostly associated with temperature of coldest quarter and precipitation of the wettest quarter (Table S1). Nevertheless, when using a matrix derived from outliers loci, models combining environmental and geographic pairwise distances as predictors of genomic variance fit better than the other models (Table 4).

Most P_{ST} estimates for 12 physiological traits overlapped with the confidence interval of F_{ST} among Cerrado populations (mean=0.562, CI=0.481-0.618; Figure 5), thus fitting the neutral expectation. However, for two traits, the decay of *Fv*/*Fm* and ik under drought stress, P_{ST} were significantly higher than F_{ST} (mean=0.943 and 0.847; CI=0.898-0.982 and 0.738-0.932, respectively; Figure 5), which have been likely caused by directional selection in these two traits. Accordingly, most physiological traits related to drought stress presented increased values (although not significant) of P_{ST} when compared to the other traits related to light or heat stresses, and the principal component that groups variables related to drought stress ($PC2_{drought}$) was also significantly higher than F_{ST} (mean=0.831, CI=0.698-0.941). Sensitivity analyses indicate that these results are robust to c/h^2 variation, as except for low values of c/h^2 (Figure S3). In agreement to P_{ST}-F_{ST} comparisons, mantel tests pointed to significant associations between inter-population phenotypic variation (P_{ST}) and neutral genetic variation (F_{ST}) for CT_{max}, (r=0.404; p=0.016), Ik (r=0.361; p=0.036), and α decay under drought stress (r=0.496; p=0.031), suggesting that neutral processes may underlie phenotypic differentiation of these specific traits.

DISCUSSION

The application of high throughput sequencing refined our understanding on the genetic mechanisms underlying the diversification of the widespread but patchily distributed tropical species *Pitcairnia lanuginosa*. The analysis of hundreds of loci revealed a low genetic variation within-populations and pronounced genetic differentiation among populations of this highly endogamic species, likely resulting from strong genetic drift. Data also revealed few outliers loci and few phenotypical traits putatively under divergent selection, which suggest an additional role of local adaptation, despite strong genetic drift, in populations under similar microclimatic conditions. Below we discuss these main findings based on information about drift-selection interactions for a species with naturally fragmented populations, and highlight which species' ecological features may be particularly linked to the genomic patterns recovered here.

We found a remarkably low genetic diversity within *P. lanuginosa* populations, confirming previous results achieved by traditional markers for this species (Leal et al. Capítulo 2). The low levels of variability likely resulting from strong genetic drift may decrease the adaptive potential of *P. lanuginosa* to novel environment conditions, because of the lack of standing genetic variation on which natural selection could act (Messer and Petrov 2013). Accordingly to the hypothesis of strong drift, Bayesian outlier analyses detected a limited number of loci under putative divergent selection in *P. lanuginosa* (two loci when for the entire distribution or four loci for the Cerrado distribution). Moreover, P_{ST} values among Cerrado populations for most physiological traits are within the expected under neutral conditions. *P. lanuginosa* populations occur within riparian forests, and may therefore be exposed to similar microclimatic conditions across its distribution. In fact, local adaptation is expected to be less frequent between populations experiencing similar environmental conditions (reviewed by Hereford 2009). Uniform selection pressure resulting from similar environmental conditions (such as those in riparian forests) could also explain the low morphological variation of *P. lanuginosa* (personal observation). Although we expect contrasting macroclimatic conditions across the wide distribution in the Tropical South America for this species, the fact of occurring in riparian forest, an specialized microhabit, suggests that niche conservatism may be the major process leading to the diversification of this species.

On the other hand, GLMM analysis evidenced a wide association between genomic and environmental matrices (temperature and precipitation), suggesting the existence of isolation by environment (IBE) within the Cerrado. Furthermore, three traits related to drought stress (i.e., decay of *Fv*/*Fm* and ik under drought stress) showed an increased phenotypic variation in our P_{ST}-F_{ST} comparisons among Cerrado populations. Because these populations are submitted to seasonal variations of precipitation, such traits could be important for local adaptation in the species. Together, these evidences suggest that divergent natural selection may have an additional role on the genomic differentiation of *P. lanuginosa*. Because genetic drift lead to increased F_{ST} indices for neutral loci, we cannot reject that outlier methods employed here may lack enough power to detect positive selection among populations (De Mita et al. 2013; Wang et al. 2017). Also, more candidates to selection could arise from outliers tests for bigger data sets (thousand of markers), giving further insights on the relative importance of local adaptation for the diversification of *P. lanuginosa*.

Our data also revealed high levels of genetic differentiation among *P. lanuginosa* populations (F_{ST} > 0.70, p-value 0.0001). This result is in agreement with our previous findings based on microsatellites markers (F_{ST} = 0.71, p-value 0.0001 - Leal et al., Capítulo 2), indicating that even a relatively small number of highly informative markers may accurately infer genetic structure (Funk et al. 2016; Hodel et al. 2017). Patterns of genetic structure recovered here is also similar to what has been described for organisms distributed across islands or island-like terrestrial habitats with RAD markers (e.g. Funk et al. 2016; Wang et al. 2017), highlighting the important role of drift in promoting diversification within non-continuous distributed organisms. *Pitcairnia lanuginosa* occurs as small and isolated populations, and presents a combined set of ecological characteristics that likely limit gene flow. The species show limited seed dispersal, owing to the tiny seeds without any specific dispersal mechanism (Smith and Downs 1974), reduced flowering phenological overlap among populations and high rates of self-fertilization, which decrease opportunities for both seed and pollen-mediated gene flow thereby promoting differentiation. Although populations pairwise F_{ST} values are generally high, Bayesian analyses consistently assign *P. lanuginosa* populations to three clusters occurring in the Eastern Cerrado, Central-western Cerrado and Yungas. Such divergence within the species was also previously detected using DNA sequencing and microsatellite markers (Leal et al. Capítulo 2) and

this pattern of genetic variation has been commonly reported for other Cerrado plant species (revised by Leal et al. 2016; Resende-Moreira et al. 2017; Buzzati et al. 2018). Because there is no obvious historical or geographical barriers to gene flow explaining the concordance among ecologically distinct species, this pattern deserves an special attention and could be better investigated under a comparative framework. For riparian plants, such as *P. lanuginosa,* genetic structure could also be linked to historical drainage re-organization events, as has been shown for other species in Asian mountains valleys (Yue et al. 2002; Zhang et al. 2011), an hypothesis to be further investigated.

Patterns of genomic diversity and structure recovered for *P. lanuginosa* are likely under strong influence of the high rates of self-fertilization. Despite the predicted benefits of selfing for reproductive assurance when pollinators and/or mates are rare, particularly during colonization events, high rates of self-fertilization may limit the species' evolutionary potential (Herlihy and Eckert 2002; Wright et al. 2013). Because the strength of selection scales to the effective population size (Kimura 1983), natural selection may be less efficient in selfing populations and, to an even greater extent, in recently founded populations experiencing selfing (Laenen et al. 2018). Indeed, studies have found that small populations are less likely to display local adaptation, but the influence of mating system on limiting adaptation is still controversial (see Leimu and Fischer 2008; Hereford 2010). Selfing could theoretically promote local adaptation if selfing populations experience less gene flow than outcrossing populations (Linhart and Grant, 1996; Hereford 2010). In addition, theoretical analysis have shown that selfing tends to increase the effect of selection by exposing alleles to homozigosity that can be more easily removed or selected (Charlesworth and Charlesworth 1987; De Mita et al. 2013). The influence of self fertilization on the probability of selection seem therefore unpredictable, and further empirical studies on organisms performing predominantly selfing may help testing some of these theoretical predictions.

In summary, genomic analyses with a set of hundreds of loci generated by ddRAD sequencing, and additional data on physiological variation at the population level, found support for genetic drift as the main evolutionary mechanism driving divergence across the wide yet patchy distribution of *P. lanuginosa*. Natural selection may have an important role, particularly at maintaining highly advantageous variants or eliminating deleterious mutations within populations, and thus allowing the persistence into small patches of riparian forests. Determining to what degree differentiation among

populations is caused by adaptive *versus* neutral processes provide important insights about diversification processes for wide-range distributed species. Adaptive divergence within species has been increasingly investigated over the past years, revealing the prominent role of ecology on species diversification (Nosil 2012), as have been predicted since Darwin's book 'On the origins of species'. As demonstrated by the increasing literature employing genome-wide markers, patterns of genetic diversity and structure are rarely explained by a single evolutionary force (e.g, Orsini et al. 2013, Moura et al. 2014; Gaither et al. 2015). Rather, complex patterns commonly arise when employing a wide genome coverage and natural selection is often assumed as a relevant force, even for populations experiencing strong drift (e.g. Prentice et al. 2017; Wang et al. 2017). The balance between genetic drift and selection, and the relative contribution of plasticity to environmental adaptation, seem to be strictly dependent on the ecological context. For patchy distributed species inhabiting isolated but relatively stable habitats, such as riparian forests, drift may be stronger than selection for majority of loci. But even these relatively stable habitats can be subject to disturbance due to water flooding or intermittent drought, imposing conditions in which plant must adapt to the environment either through genetic adaptation or phenotypic plasticity.

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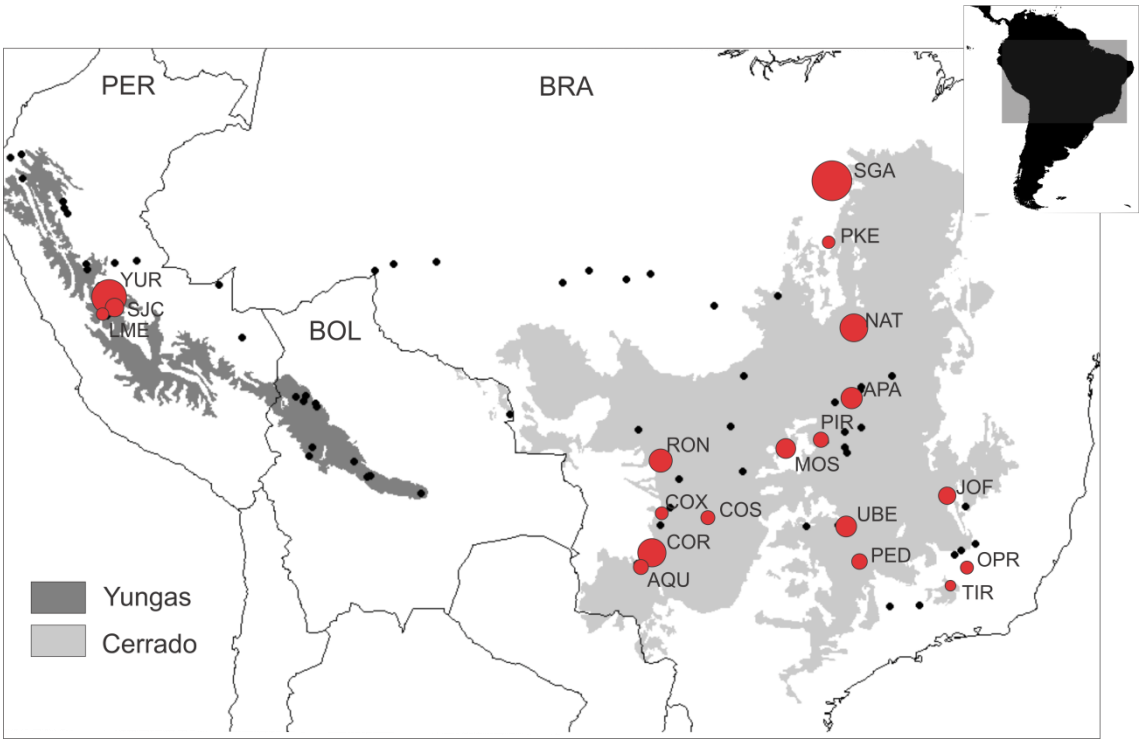

Figure 1. Population sampling and patterns of genetic diversity in 19 populations of *Pitcairnia lanuginosa*, as measured by the proportion of polymorphic loci per population (red circles).

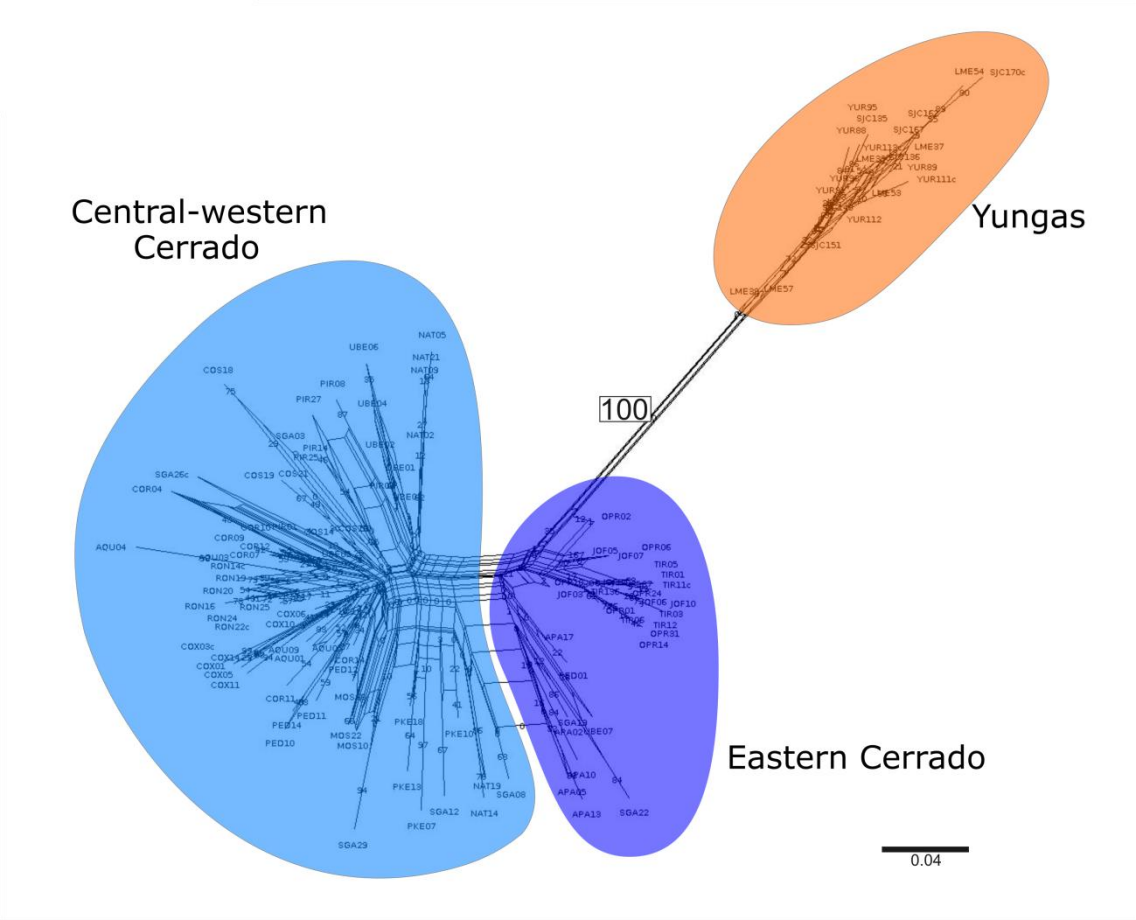

Figure 2. Neighbour network tree for 115 individuals of *Pitcairnia lanuginosa* based on 688 SNPs as infered by SplitsTree software using the neighbour-net model and uncorrected p-distances.

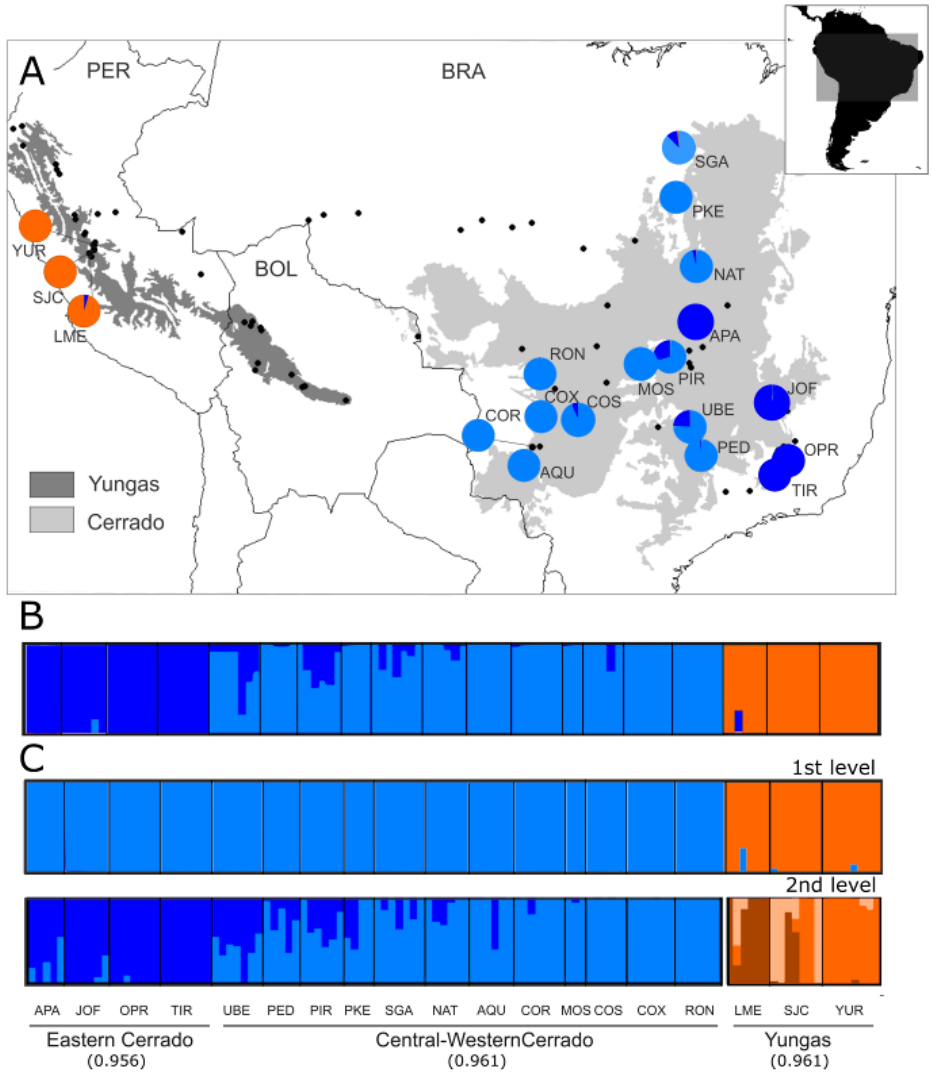

Figure 3. Genetic structure of 19 populations of the *Pitcairnia lanuginosa* as inferred by STRUCTURE and INSTRUCT analyses. (A) Geographic distribution of individuals belonging to each genetic cluster as detected by INSTRUCT. (B) Bar plot depicting the INSTRUCT clustering for the whole data set, and highlighting inbreeding rates for each group, as calculated by INSTRUCT. Inbreeding coefficients per cluster is shown below the barplot. (C) Bar plots depicting the STRUCTURE clustering for all populations (first level) and for the Brazilian and Peruvian clusters separately (second level).

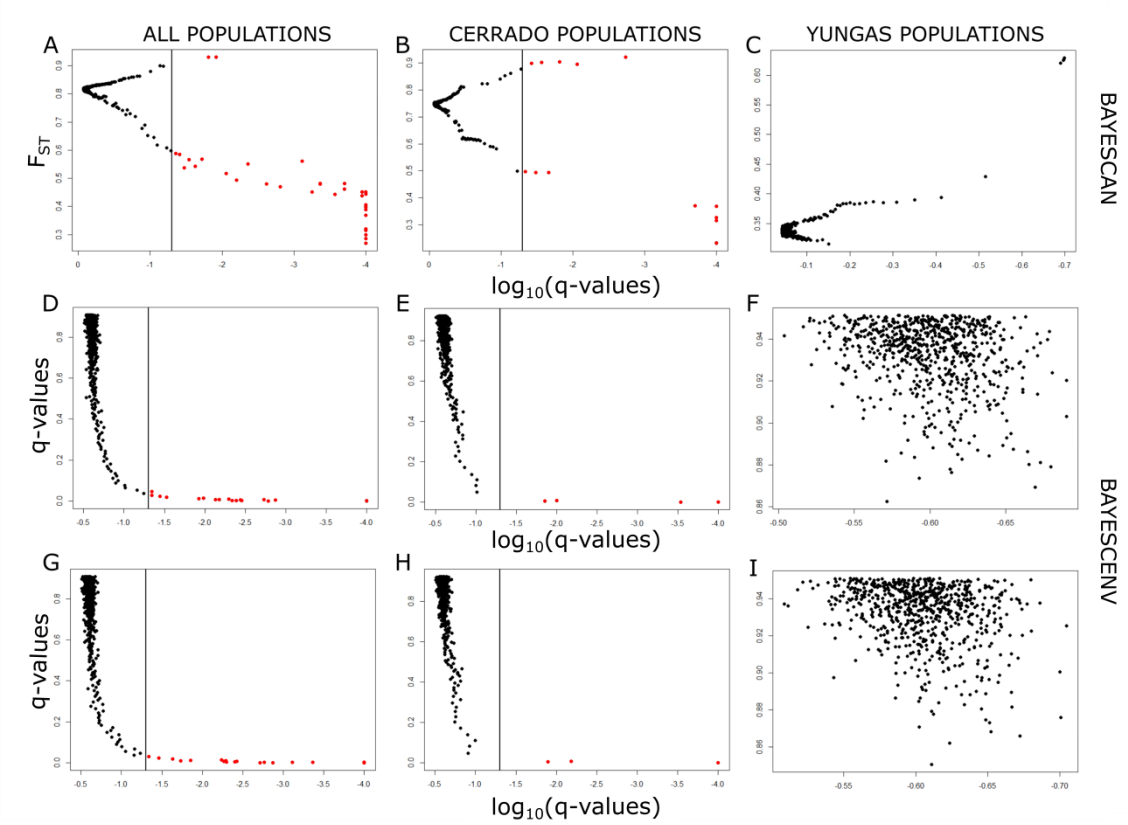

Figure 4. F_{ST} outliers among populations of *Pitcairnia lanuginosa*. Results from BAYESCAN analysis (A) among 19 sampled populations for 688 SNPs, (B) among 16 populations from the Cerrado for 618 SNPs, and (C) among three populations from the Yungas for 714 SNPs. Results for BAYESCENV analysis based on precipitation and temperature bioclimatic variables (D and G) among 19 sampled populations for 688 SNPs, (E and H) among 16 populations from the Cerrado for 618 SNPs, and (F and I) among three populations from the Yungas for 714 SNPs.

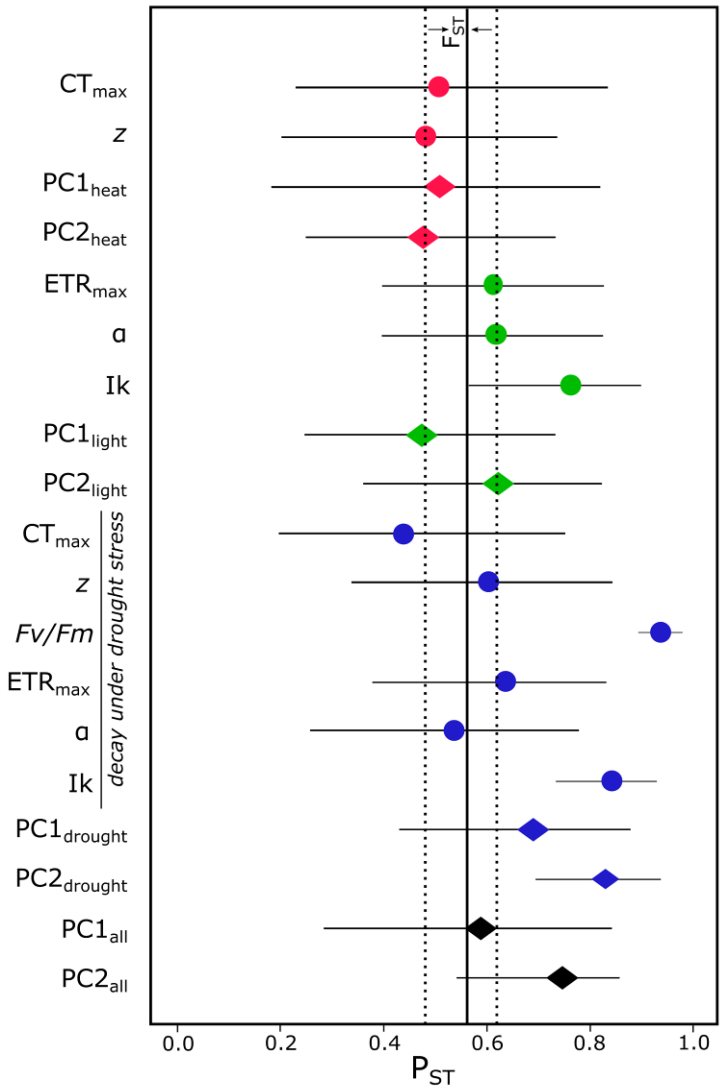

Figure 5. Forest plot comparing means and confidence intervals of P_{ST} for 12 phenotypic traits and neutral F_{ST} among eight populations of *Pitcairnia lanuginosa* based on 1,000 bootstraps. Traits related to heat, light and drought stresses are showed in red, green and blue, respectively. P_{ST} based on principal components (PC) grouping traits related to each stress and for all 12 traits are showed above them.

Table 1. Population sampling of *Picairnia lanuginosa*, with geographical coordinates and sample sizes for genetic analyses and for physiological traits measurements.

Population	N_G	N_P	Locality	Coordinates	Ecorregion
APA	5	3	Alto Paraíso - Brazil	47W 47' 26" , 14S 11' 04"	Cerrado
AQU	6	-	Aquidauana - Brazil	55W 29' 44" , 20S 27' 35"	Cerrado
COR	7	3	Corguinho - Brazil	55W 09' 17" , 19S 54' 11"	Cerrado
COS	5	3	Costa Rica - Brazil	53W 03' 44" , 18S 35' 09"	Cerrado
COX	7	-	Coxim - Brazil	54W 45' 38" , 18S 28' 59"	Cerrado
JOF	6	-	Joaquim Felício - Brazil	44W 11' 03" , 17S 51' 58"	Cerrado
MOS	3	-	Mossâmedes - Brazil	50W 11' 31" , 16S 04' 44"	Cerrado
NAT	6	3	Natividade - Brazil	47W 41' 58" , 11S 39' 24"	Cerrado
OPR	7	-	Ouro Preto - Brazil	43W 32' 33" , 20s 29' 36"	Cerrado
PED	5	-	Pedregulho - Brazil	47W 27' 21" , 20S 15' 05"	Cerrado
PIR	6	3	Pirenópolis - Brazil	48W 54' 25" , 15S 47' 36"	Cerrado
PKE	4	-	Presidente Kennedy - Brazil	48W 34' 31" , 08S 29' 06"	Cerrado
RON	7	3	Rondonópolis - Brazil	54W 47' 43" , 16S 31' 05"	Cerrado
SGA	7	3	São Geraldo do Araguaia - Brazil	48W 27' 58" , 06S 13' 58"	Cerrado
TIR	7	3	Tiradentes - Brazil	44W 09' 55" , 21S 06' 01"	Cerrado
UBE	7	-	Uberlândia - Brazil	48W 00' 19" , 18S 58' 05"	Cerrado
LME	6	-	La Merced - Peru	75W 19' 04" , 11S 02' 08"	Yungas
SJC	8	-	San Juan de Cacazu - Peru	75W 07' 01" , 10S 40' 24"	Yungas
YUR	7	-	Yurinaki - Peru	75W 06' 29" , 10S 51' 11"	Yungas

N_G = number of samples per population for genetic analyses; N_P number of samples per population for physiological traits measurements.

Table 2. Genetic diversity parameters for 19 sampled populations of *Pitcairnia lanuginosa*.

Population	Mean N	S	PA	PL (%)	P	H_O	H_E	π
APA	2.58	527	18	7.78	0.98	0.0000	0.0318	0.0375
AQU	2.46	534	11	6.37	0.98	0.0000	0.0254	0.0296
COR	3.18	613	36	10.28	0.97	0.0000	0.0416	0.0490
COS	2.28	485	14	3.71	0.98	0.0000	0.0176	0.0227
COX	3.44	617	28	8.59	0.97	0.0006	0.0326	0.0387
JOF	2.92	613	11	4.89	0.98	0.0000	0.0213	0.0253
LME	2.63	552	4	3.62	0.99	0.0000	0.0157	0.0194
MOS	1.75	362	11	1.93	0.99	0.0000	0.0092	0.0118
NAT	2.79	636	25	11.16	0.96	0.0000	0.0494	0.0579
OPR	3.13	622	7	4.98	0.99	0.0000	0.0190	0.0222
PED	2.00	566	13	4.42	0.98	0.0000	0.0202	0.0252
PIR	2.87	614	12	5.70	0.98	0.0000	0.0244	0.0301
PKE	2.26	464	8	6.03	0.98	0.0000	0.0257	0.0307
RON	3.88	565	17	5.31	0.98	0.0000	0.0213	0.0251
SGA	3.52	637	58	18.52	0.94	0.0000	0.0753	0.0885
SJC	3.18	563	11	4.44	0.99	0.0009	0.0170	0.0201
TIR	3.68	553	8	5.06	0.98	0.0000	0.0218	0.0264
UBE	3.44	525	9	6.86	0.98	0.0000	0.0263	0.0307
YUR	5.23	487	31	13.96	0.97	0.0000	0.0433	0.0479

N = number of individuals per population; Mean N = mean number of individuals per locus within populations; S = number of sites; PL = proportion of polymorphic loci; P = Mean frequency of the most frequent allele per loci within population and π = nucleotide diversity.

Table 3. Analysis of Molecular Variance (AMOVA) for 19 populations of *Pitcairnia lanuginosa* based on 688 SNPs markers. Populations are grouped into two groups of Cerrado and Andean Yungas populations (according to Structure) or three groups of Eastern Cerrado, Central-Western Cerrado and Yungas populations (according to Instruct).

Source of variation	df	Variance components	% variation	F statistics	p
Among groups (Cerrado *versus* Yungas)	1	5.930	53.58	F_{CT}=0.54	<0.001
Among populations within groups	17	2.674	24.16	F_{SC}=0.52	<0.001
Within populations	211	2.462	22.25	F_{ST}=0.78	<0.001
Among groups (Eastern Cerrado, Central-Western Cerrado *versus* Yungas)	2	4.728	52.89	F_{CT}=0.53	<0.001
Among populations within groups	16	1.749	19.56	F_{SC}=0.41	<0.001
Within populations	211	2.462	27.55	F_{ST}=0.72	<0.001

Table 4. Contrasted results of generalized linear mixed models (GLMM) testing the influence of geographic distance (GEO), environmental (ENV) distance based on 19 bioclimatic variables, and environmental distance based on either temperature (ENV_{temp}) or precipitation (ENV_{precip}) bioclimatic variables on the genetic divergence among 16 populations of *Pitcairnia lanuginosa* from the Cerrado. Analysis were performed for all 618 loci, or a sub set of 34 outliers loci or 584 putatively neutral loci.

Model	All loci			Outliers loci		
	DIC	ΔDIC	DICweight	DIC	ΔDIC	DICweight
null	-186.501	3.570	0.066	-107.795	9.238	0.005
GEO	-187.728	2.343	0.122	-114.162	2.871	0.119
ENV	-190.072	**0.000**	0.392	-107.768	9.265	0.005
ENV_{temp}	-186.952	3.120	0.082	-106.268	10.764	0.002
ENV_{precip}	-186.743	3.329	0.074	-111.708	5.325	0.035
GEO+ENV	-187.962	2.109	0.137	-117.033	**0.000**	0.501
GEO+ENV_{temp}	-186.644	3.428	0.071	-115.492	**1.541**	0.232
GEO+ENV_{precip}	-186.197	3.874	0.057	-113.836	3.197	0.101

Supplementary Figures

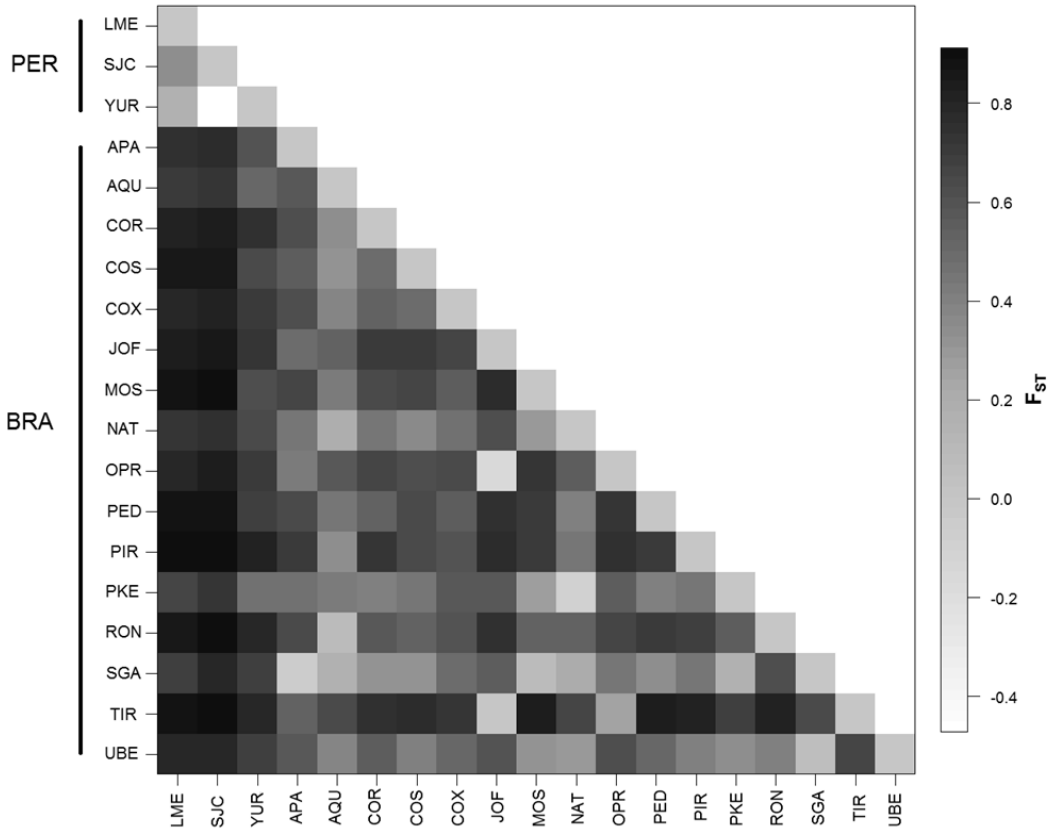

Figure S1. Matrix of pairwise F_{ST} among 19 populations of *Pitcairnia lanuginosa* based on 688 SNPs defined through ddRAD sequencing.

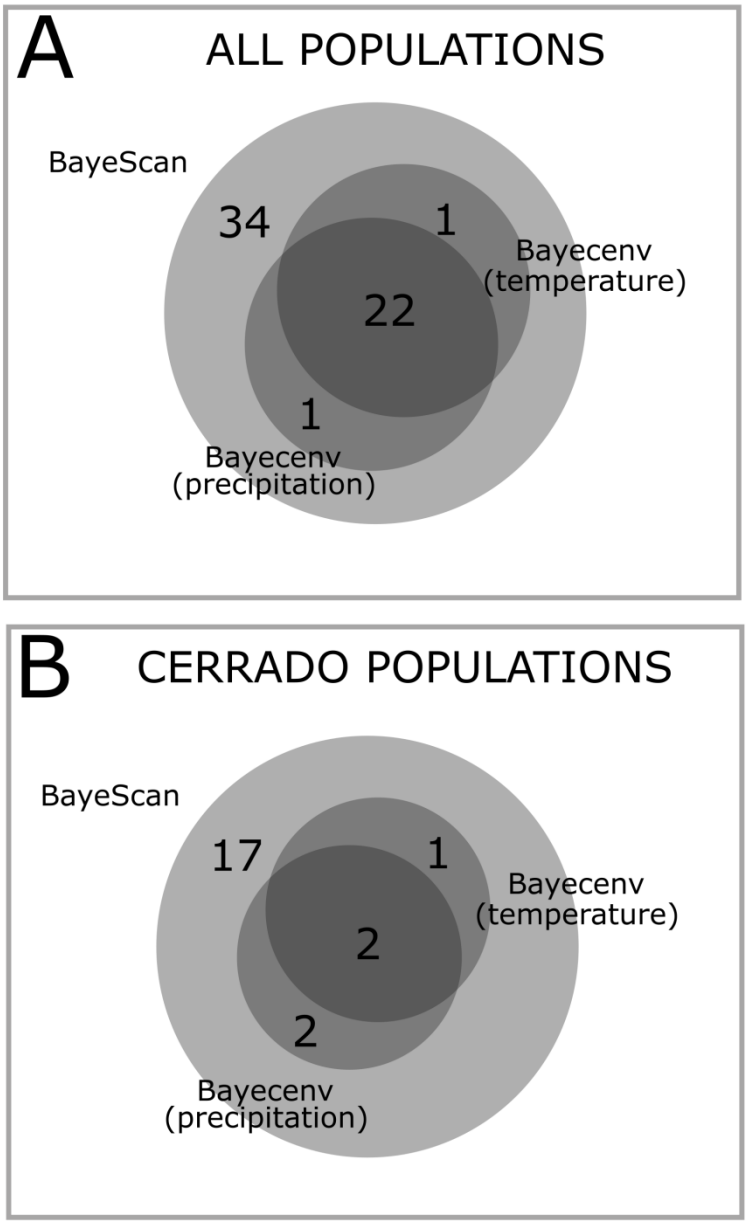

Figure S2. Venn diagrams of shared outlier loci detected by BayeScan and Bayescenv analyses employing temperature and precipitation variables based on (A) 688 SNPs assembled among 19 *Pitcairnia lanuginosa* populations or (B) 618 SNPs assembled for a sub set of 16 Cerrado populations.

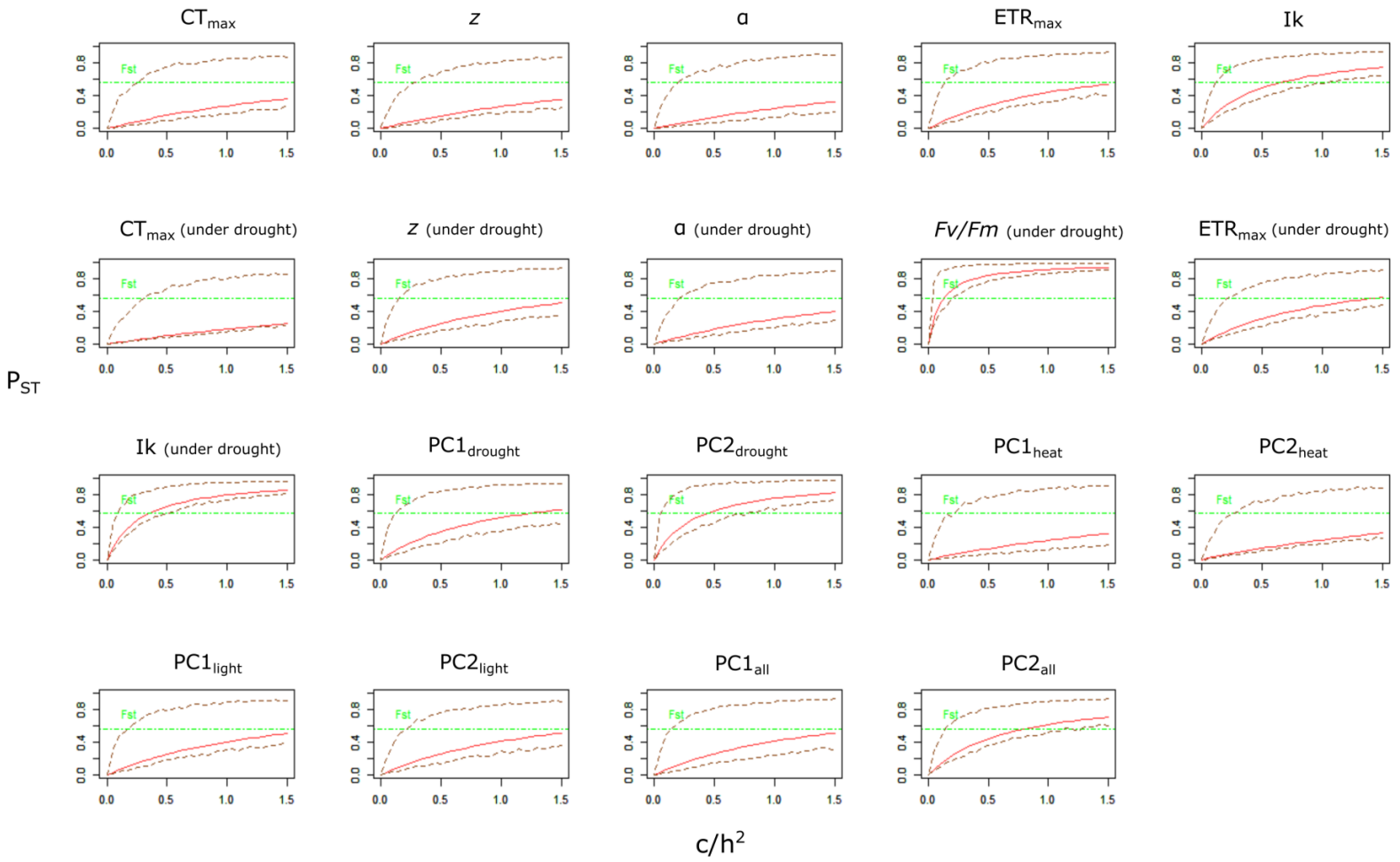

Figure S3. Sensitivity analysis of the P_{ST}-F_{ST} comparison for 12 physiological traits and or principal components summarizing drought, heat and light stresses traits among a sub set of eight populations of *Pitcairnia lanuginosa*. F_{ST} is shown as a green line, and varying P_{ST} values are shown as a red line for $c/h^2 \leq 1$, with 95% confident intervals marked as dotted lines.

Supplementary tables

Table S1. Correlation between Bioclim variables and two principal components of the PCAs using 19 bioclimatic variables extract from WorldClim (ENV), or a sub set of 11 temperature variables (ENV_{temp})and eight precipitation variables (ENV_{precip}).

Variable	ENV		ENV_{temp}		ENV_{precip}	
	PC1	PC2	PC1	PC2	PC1	PC2
Annual Mean Temperature	-0.337	0.047	0.372	-0.066	-	-
Mean Diurnal Range	-0.095	0.245	0.133	-0.467	-	-
Isothermality	-0.212	-0.218	0.215	0.369	-	-
Temperature Seasonality	0.205	0.317	-0.212	-0.406	-	-
Max Temperature of Warmest Month	-0.319	0.085	0.357	-0.169	-	-
Min Temperature of Coldest Month	-0.329	-0.105	0.349	0.185	-	-
Temperature Annual Range	0.019	0.303	0.011	-0.553	-	-
Mean Temperature of Wettest Quarter	-0.299	0.187	0.332	-0.231	-	-
Mean Temperature of Driest Quarter	-0.335	-0.094	0.365	0.106	-	-
Mean Temperature of Warmest Quarter	-0.314	0.139	0.349	-0.195	-	-
Mean Temperature of Coldest Quarter	-0.340	-0.070	0.371	0.078	-	-
Annual Precipitation	-0.123	-0.280	-	-	-0.099	0.602
Precipitation of Wettest Month	0.115	-0.350	-	-	-0.418	0.183
Precipitation of Driest Month	-0.057	0.216	-	-	0.421	0.208
Precipitation Seasonality	0.184	-0.250	-	-	-0.447	-0.187
Precipitation of Wettest Quarter	0.034	-0.385	-	-	-0.367	0.378
Precipitation of Driest Quarter	-0.085	0.275	-	-	0.460	0.144
Precipitation of Warmest Quarter	0.207	0.280	-	-	0.200	-0.362
Precipitation of Coldest Quarter	-0.218	-0.006	-	-	0.229	0.481

Table S2. Ion-Torrent data quality for 115 sequenced individuals of *Pitcairnia lanuginosa*.

Sample ID	Number of Bases	Bases >Q20	Number of Reads	Mean Read Length (pb)
APA02	398,483,577	330,581,663	2,116,974	188
APA05	155,996,776	128,897,546	846,457	184
APA10	313,921,547	259,120,557	1,693,132	185
APA13	138,425,800	114,999,512	737,800	188
APA17	490,734,694	407,766,303	2,635,311	186
AQU01	153,865,694	131,125,888	853,982	180
AQU03	555,316,022	476,860,286	3,070,090	181
AQU04	228,676,354	196,808,003	1,261,094	181
AQU05	225,224,582	187,669,444	1,193,354	189
AQU09	249,980,103	214,662,768	1,373,506	182
COR04	71,902,655	61,785,605	401,813	179
COR07	331,705,426	281,709,782	1,949,967	170
COR09	459,433,980	388,192,654	2,593,297	177
COR10	383,168,213	322,929,836	2,157,374	178
COR11	312,613,851	268,776,409	1,825,856	171
COR12	302,096,772	251,089,842	1,611,051	188
COR14	389,575,089	322,454,486	2,091,485	186
COS14	230,236,814	197,582,299	1,271,710	181
COS16	254,274,533	217,728,312	1,409,348	180
COS18	252,838,402	216,844,734	1,395,604	181
COS19	193,609,297	164,158,065	1,128,667	172
COS21	243,788,911	207,352,046	1,410,469	173
COX01	305,791,089	259,756,148	1,799,564	170
COX03	838,788,849	716,493,177	4,944,458	170
COX05	333,649,721	277,742,899	1,762,798	189
COX06	216,188,270	185,799,161	1,191,786	181
COX10	231,553,047	198,309,488	1,281,084	181
COX11	235,530,317	203,382,560	1,295,940	182
COX14	428,579,688	353,920,428	2,288,577	187
JOF03	634,423,756	535,132,103	3,742,344	170
JOF04	428,953,571	365,370,180	2,505,501	171
JOF05	317,799,810	270,832,992	1,866,824	170
JOF06	470,473,493	396,510,856	2,801,037	168
JOF07	444,336,965	367,472,959	2,377,164	187
JOF10	733,707,062	603,512,900	3,944,926	186
LME33	199,993,305	170,385,578	1,108,971	180
LME37	131,386,712	112,211,945	774,473	170
LME39	205,242,509	175,207,454	1,142,828	180
LME53	314,155,989	267,034,022	1,864,920	168

LME54	210,221,372	179,842,562	1,229,961	171
LME57	255,821,390	212,710,011	1,370,043	187
MOS10	97,095,507	83,112,652	567,978	171
MOS22	152,668,111	126,020,421	829,619	184
MOS26	279,395,582	241,408,444	1,540,402	181
NAT02	539,529,486	459,342,500	3,179,763	170
NAT05	281,037,825	239,907,197	1,632,809	172
NAT09	730,089,169	621,162,898	4,245,545	172
NAT14	655,968,691	540,008,289	3,535,863	186
NAT19	532,611,832	454,676,828	3,079,924	173
NAT21	155,455,495	132,850,239	851,421	183
OPR01	329,860,326	281,747,043	1,809,428	182
OPR02	498,216,388	423,622,412	2,927,065	170
OPR06	423,467,110	357,592,235	2,370,680	179
OPR14	195,868,983	166,643,474	1,149,259	170
OPR18	113,557,071	96,820,980	662,621	171
OPR24	404,373,118	333,480,379	2,165,237	187
OPR31	345,958,876	295,128,438	1,912,120	181
PED01	139,375,294	118,470,146	775,097	180
PED10	135,927,442	116,511,295	795,247	171
PED11	117,276,496	100,619,183	652,228	180
PED12	256,186,998	219,063,697	1,498,575	171
PED14	185,990,829	159,576,707	1,029,672	181
PIR01	80,184,537	68,224,895	474,084	169
PIR03	377,514,482	324,500,785	2,101,368	180
PIR08	295,459,298	253,504,139	1,643,947	180
PIR14	208,359,950	175,642,418	1,239,701	168
PIR25	410,785,031	347,238,614	2,319,414	177
PIR27	226,014,763	192,957,159	1,326,034	170
PKE07	300,112,389	249,339,624	1,592,493	188
PKE10	184,270,860	152,616,720	989,845	186
PKE13	97,040,204	80,741,415	517,281	188
PKE18	315,217,330	259,142,256	1,684,200	187
RON14	311,159,315	267,446,248	1,729,151	180
RON16	450,985,280	383,785,743	2,642,614	171
RON19	420,586,894	357,812,714	2,336,203	180
RON20	549,503,075	463,405,280	3,258,711	169
RON22	483,634,922	396,594,366	2,629,161	184
RON24	395,069,477	336,134,758	2,315,486	171
RON25	360,575,756	307,273,146	1,992,732	181
SGA03	788,575,034	665,736,787	4,686,485	168
SGA08	312,417,789	264,259,392	1,755,786	178
SGA12	470,118,165	396,886,561	2,668,679	176

SGA19	590,591,816	500,418,507	3,340,546	177
SGA22	958,863,153	811,044,477	5,696,990	168
SGA26	281,243,682	230,979,478	1,520,438	185
SGA29	288,597,879	238,231,160	1,548,439	186
SJC35	297,330,717	251,335,761	1,688,293	176
SJC36	579,021,666	491,106,307	3,463,906	167
SJC48	404,485,058	330,914,106	2,200,454	184
SJC51	296,114,540	250,241,887	1,773,047	167
SJC62	517,728,551	436,648,980	3,094,535	167
SJC167	260,431,441	220,877,894	1,446,194	180
SJC170	207,936,428	172,018,983	1,115,854	186
TIR01	296,853,922	253,536,913	1,639,097	181
TIR03	124,178,440	105,792,855	718,803	173
TIR05	238,431,280	202,647,928	1,397,684	171
TIR06	299,839,258	255,939,156	1,652,630	181
TIR11	306,720,994	250,967,403	1,662,625	184
TIR12	329,376,995	278,403,378	1,842,991	179
TIR13	386,685,678	321,492,518	2,059,751	188
UBE01	300,505,377	252,875,426	1,771,339	170
UBE02	199,790,988	171,556,764	1,104,113	181
UBE03	357,445,641	305,513,646	1,985,637	180
UBE04	234,457,218	198,325,293	1,372,889	171
UBE05	372,516,853	308,536,779	1,971,049	189
UBE06	205,159,738	176,670,080	1,135,341	181
UBE07	142,961,512	118,359,803	772,414	185
YUR11	358,509,696	304,766,258	2,116,503	169
YUR12	248,966,142	209,508,594	1,487,509	167
YUR13	3,571,041,740	2,932,345,063	19,460,976	183
YUR88	533,247,117	450,561,487	2,985,076	179
YUR89	234,198,421	199,293,870	1,370,010	171
YUR91	363,119,612	311,008,080	2,019,668	180
YUR95	590,967,068	502,007,026	3,342,040	177
YUR98	324,704,326	266,094,002	1,771,375	183

CONSIDERAÇÕES FINAIS

O conhecimento sobre os processos envolvidos na diversificação da biota neotropical tem se beneficiado pelo número crescente de estudos microevolutivos (a nível populacional) com espécies ecologicamente diversas e pela emergência de novas tecnologias de sequenciamento e de métodos que estenderam seu uso à organismos não-modelo. Esta tese reúne três manuscritos que agregam informações sobre fatores históricos e ecológicos que afetam a distribuição geográfica e estrutura genética de populações ao revisar estudos filogeográficos de plantas no Brasil e gerar novos dados empíricos sobre uma espécie herbácea de ampla distribuição nos Neotrópicos, com populações pequenas e isoladas, *Pitcairnia lanuginosa* (Bromeliaceae). Em conjunto com estudos de genética/genômica de populações e filogeografia de outros organismos, as informações aqui geradas ajudam a esclarecer os processos complexos responsáveis pela origem e manutenção da biodiversidade dos Neotrópicos.

Muitos obstáculos a pesquisa da biodiversidade ainda limitam nosso conhecimento sobre a evolução da flora e fauna neotropical. Embora o estudo dos mecanismos e processos de diversificação nos Neotrópicos seja desafiador, nós argumentamos que esforços futuros deverão ser direcionados de forma a: (a) aumentar os estudos com grupos poucos estudados, ou com características ecológicas distintas, (b) expandir o uso de dados genômicos, (c) integrar informações moleculares com outros tipos de dados, como morfologia, fisiologia e ecologia, e (d) cobrir áreas geográficas pouco amostradas. Ainda, argumentamos que o acúmulo de dados empíricos independentes e a síntese das informações acumuladas por meio de revisões e meta-análises tem o potencial de ressaltar padrões biogeográficos gerais em meio ao aparente cenário de complexidade que caracteriza a evolução nos Neotrópicos.