

UNIVERSIDADE FEDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA EVOLUTIVA E BIOLOGIA
MOLECULAR

FERNANDA MIZUGUCHI LEITE

**DIVERSIFICAÇÃO EM FORMAÇÕES XEROMÓRFICAS DA MATA
ATLÂNTICA - UM ESTUDO DE CASO COM ESPÉCIES DO GÊNERO
PILOSCEREUS BYLES & ROWLEY (CACTACEAE).**

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Dissertação apresentada ao Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular, como parte dos requisitos para obtenção do título de mestre em Genética Evolutiva e Biologia Molecular.

Orientação: Prof. Dr. Evandro Marsola Moraes.

Co-orientação: Profa. Dra. Isabel Aparecida da Silva Bonatelli.

São Carlos - SP

2022

Mizuguchi Leite, Fernanda

Diversificação em formações xeromórficas da Mata Atlântica - Um estudo de caso com espécies do gênero *Pilosocereus* Byles & Rowley (Cactaceae) / Fernanda Mizuguchi Leite -- 2022. 57f.

Dissertação (Mestrado) - Universidade Federal de São Carlos, campus São Carlos, São Carlos
Orientador (a): Evandro Marsola de Moraes
Banca Examinadora: Danilo Trabuço do Amaral,
Fernando Faria Franco
Bibliografia

1. Diversificação da Mata Atlântica. 2. Filogeografia. 3. Modelagem de distribuição de espécies. I. Mizuguchi Leite, Fernanda. II. Título.

Ficha catalográfica desenvolvida pela Secretaria Geral de Informática (SIn)

DADOS FORNECIDOS PELO AUTOR

Bibliotecário responsável: Ronildo Santos Prado - CRB/8 7325



UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular

Folha de Aprovação

Defesa de Dissertação de Mestrado da candidata Fernanda Mizuguchi Leite, realizada em 02/09/2022.

Comissão Julgadora:

Prof. Dr. Evandro Marsola de Moraes (UFSCar)

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O Relatório de Defesa assinado pelos membros da Comissão Julgadora encontra-se arquivado junto ao Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular.

Dedico esse trabalho aos meus filhos Mateus e Benício.

“Diante da vastidão do tempo e da imensidão do universo, é um imenso prazer para mim dividir um planeta e uma época com você”.

(Carl Sagan)

“Se vi mais longe, foi por estar de pé sobre ombros de gigantes”.

(Isaac Newton)

AGRADECIMENTOS

Á minha família, meus pais Eliana e Jonas, meus avós Alice e Kazuo, pelo incondicional amor e apoio que ajudou a construir a pessoa que sou hoje. Todos os passos que dei e darei em toda a minha vida, sempre será dedicado e em gratidão a vocês.

Aos meus filhos Mateus e Benício, por ressignificarem a minha vida e me darem a honra de ser mãe. Agradeço por me mostrarem o amor da forma mais genuína que existe e por me escolherem para compartilhar esse sentimento. Cada passo e cada conquista sempre será para vocês e por vocês.

Ao meu marido Jefferson, por todo companheirismo, apoio, amor, dedicação e compreensão em todos os aspectos de nossas vidas. É um privilégio poder caminhar e crescer ao seu lado.

Ao Prof. Dr. Evandro Marsola de Moraes pela orientação desde a iniciação científica, por toda ajuda, aprendizado, paciência, pela confiança e persistência em desenvolver esse projeto de mestrado, por ser esse professor e pesquisador competente e dedicado, no qual eu tenho enorme gratidão e inspiração.

Á Profa. Dra. Isabel Aparecida da Silva Bonatelli pela co-orientação no projeto de mestrado, por ter me apresentado ao mundo acadêmico e científico durante a graduação, pelo apoio, pela calma e dedicação ao ensinar, pelos conselhos e por todo o incentivo. Todos seus ensinamentos guiaram meus passos até aqui. Serei eternamente grata pela sua confiança e amizade.

Ao Prof. Dr. Danilo Trabuco do Amaral pelo apoio e auxílio na construção do projeto, pelos ensinamentos sobre diversas análises e por todo o estímulo em me ajudar a finalizar esse trabalho.

Ao Prof. Dr. Fernando de Faria Franco por toda ajuda nas discussões do projeto e pelo apoio na coleta de campo.

Aos meus amigos Milena, Monique, Juliana, Thais, Leonardo, Neto e Mateus pela amizade, pelo enorme amparo e carinho em todos os momentos, pelo

companheirismo e por terem se tornado nossa rede de apoio e família de Sorocaba.

Á todos os colegas e funcionários que já passaram pelo Laboratório de Diversidade Genética e Evolução, por tanto aprendizado, pelas discussões enriquecedoras e pela prazerosa convivência.

Á todos os professores, colegas e a secretaria Ivanildes do PPGGEV por todo aprendizado, apoio e por complementarem a minha formação acadêmica e científica.

Á CAPES pela concessão da bolsa no mestrado.

Á todos os funcionários da UFSCar: colaboradores da biblioteca, secretaria, laboratórios didáticos, restaurante universitário, limpeza, segurança e manutenção. O trabalho de vocês é essencial para a permanência de cada estudante na universidade.

RESUMO

A Mata Atlântica Brasileira (BAF) é considerada um dos cinco principais *hotspots* do planeta. Ela estende-se pelas regiões litorâneas do leste do Brasil, do Paraguai e da Argentina, apresentando grande extensão geográfica, amplo gradiente de altitude, latitude e condições climáticas. Embora as suas paisagens sejam predominantemente compostas por florestas tropicais perenes, também existem áreas com vegetação xerofítica ou arbustiva aberta nas planícies costeiras arenosas e em afloramentos rochosos. Estas características abióticas promovem uma grande heterogeneidade de habitats, permitindo a formação de diferentes fisionomias e comunidades vegetais. As principais hipóteses propostas para explicar a origem da alta diversidade e endemismo nesta área baseiam-se principalmente em modelos de especiação alopatrica, como os Refúgios do Pleistoceno e modelos de diversificação de barreiras fluviais. Aqui, empregamos dados moleculares, modelagem de distribuição de espécies (SDM) e testes de equivalência e similaridade de nicho (NES) usando o complexo *Pilosocereus arrabidae* (*P. arrabidae*, *P. ulei*, *P. brasiliensis* sbsp. *brasiliensis* e *P. brasiliensis* subsp. *ruschianus*) como modelo biológico para abordar questões relacionadas à história evolutiva, barreiras de dispersão e refúgios das áreas secas dentro da BAF. Sequenciamos quatro marcadores nucleares (1.514 pb) para 97 indivíduos. No entanto, a baixa variação observada nesses marcadores não foi suficiente para resolver as relações mais estreitas entre espécies e indivíduos, bem como realizar análises genéticas populacionais. As análises de NES e SDM mostraram que as subespécies de *P. brasiliensis* ocupam nichos distintos, enquanto *P. arrabidae* e *P. ulei* apresentaram baixa sobreposição de nicho durante os últimos 130 ka. Os eventos de oscilação climática impuseram uma distribuição centro-sul a este grupo na atualidade. Os modelos de SDM também mostraram uma quebra na região das bacias dos rios Jequitinhonha e Contas, região que pode funcionar como barreira ecológica, afetando a distribuição das espécies. No geral, o complexo *P. arrabidae* parece ser um importante modelo biológico para entender a demografia e diversificação de táxons de áreas secas do BAF. A prospecção e utilização de abordagens de Sequenciamento de Nova Geração (NGS) pode ser uma estratégia promissora para avaliar hipóteses biogeográficas neste modelo.

Palavras-chave: Cactaceae, *Pilosocereus*, Mata Atlântica, Filogeografia, Modelagem de Distribuição de espécies.

ABSTRACT

The Brazilian Atlantic Forest (BAF) is considered one of the five main hotspots on the planet. It extends in coastal regions of eastern Brazil, Paraguay and Argentina, displaying a sizeable geographic extension and a wide gradient of altitude, latitude, and climate conditions. Although BAF landscapes are predominantly composed of evergreen rainforests, there are also areas with xerophytic or open shrubby vegetation in the sandy coastal plains and rocky outcrops. These abiotic features promote a considerable heterogeneity of habitats in the BAF, allowing the formation of different physiognomies and plant communities. Most of the hypotheses proposed to explain the origins of the high diversity and endemism in this area are based on allopatric speciation models, such as those built on Pleistocene Refuge Hypothesis (PRH) and riverine hypothesis. Here, we employed molecular data, species distribution modeling (SDM), and tests of niche equivalency and similarity (NES) using *Pilosocereus arrabidae* complex (*P. arrabidae*, *P. ulei*, *P. brasiliensis* subsp. *brasiliensis*, and *P. brasiliensis* subsp. *ruschianus*) as a biological model to address questions related to evolutionary history, dispersal barriers, and refuges of the dry areas within the BAF. We sequenced four molecular markers (1,514 bp) for 97 individuals. However, we failed to solve the shallow relationships between species and individuals, as well as to conduct population genetic analyses, due to the high level of saturation among the aligned loci. The NES and SDM analyses showed distinct niches for the *P. brasiliensis* subsp., despite the presence of a contact area between Rio de Janeiro and Espírito Santos states, while *P. arrabidae* and *P. ulei* displayed low niche overlap during the last 130 ka. The climatic oscillation events imposed a central-south distribution to this species in the present time. Here, we also observed a break in the region of Jequitinhonha and Contas river basins, a region that may work as an ecological barrier, affecting the species distribution. Overall, the *P. arrabidae* complex seems to be an important biological model for understanding the demography and diversification of taxa from dry areas of the BAF. The prospecting and use of Next Generation Sequencing (NGS) approaches, may be a promising strategy to evaluate biogeographic hypotheses in this study model.

Key-words: Cactaceae, *Pilosocereus*, Brazilian Atlantic Forest, Phylogeography, Species Distribution Modeling.

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1. INTRODUÇÃO GERAL

A investigação de padrões e processos que moldaram a biodiversidade da região Neotropical tem sido um tópico de grande interesse na literatura de pesquisas na região. A complexidade de táxons e ecossistemas neotropicais é refletida no número de hipóteses já formuladas para explicar a distribuição e diversificação dos táxons (Antonelli et al. 2018; Rull, 2020). Embora o enfoque tenha sido dado aos eventos geológicos do Neógeno e às oscilações climáticas do Quaternário de forma independente, um entendimento mais abrangente que considere os efeitos desses eventos de forma combinada e contínua é necessário (Rull, 2020). Algumas evidências favorecem a diversificação de táxons durante as mudanças ambientais observadas no Neógeno, enquanto outras enfatizam a importância das mudanças de clima do Quaternário (Rull, 2008; Rull, 2020). Uma terceira linha de evidências destaca a importância combinada de ambos os períodos para o estabelecimento da biodiversidade da região (Pennington et al. 2004; Carnaval & Bates 2007; Hoorn et al., 2010). Apesar dos grandes avanços em conhecimentos sobre a biota neotropical nas últimas décadas (Thomé et al., 2021), muitas lacunas ainda estão em abertas e nosso entendimento está aquém do observado para táxons da América do Norte e Europa (Turchetto-Zolet et al., 2013; Antonelli et al., 2016; Cazé et al., 2016).

Localizada na região Neotropical, a Mata Atlântica brasileira (*Brazilian Atlantic Forest* - BAF) está entre os cinco principais *hotspots* do planeta, sendo reconhecida pela alta biodiversidade e pelos altos riscos de extinção dos táxons devido ao impacto humano (Guedes et al., 2005; Leite et al., 2016). A BAF está distribuída ao longo das regiões costeiras do leste do Brasil, do leste do Paraguai ao nordeste da Argentina (Oliveira-Filho & Fontes, 2000). Devido sua grande extensão geográfica, seu amplo gradiente de altitude e latitude, e os regimes de chuvas, a BAF se apresenta como um ambiente bastante heterogêneo, permitindo a formação de diferentes fitofisionomias e comunidades vegetais (Guedes et al., 2005; Ribeiro et al., 2009; Scarano, 2009). De acordo com estudos biogeográficos e paleoclimáticos na BAF (Moritz et al., 2000; Carnaval & Moritz, 2008; Thomé et al., 2010; Franco et al., 2017a), as principais hipóteses apresentadas para explicar a diversificação dos táxons enfatizam modelos de especiação alopátrica, como a teoria dos refúgios (Haffer, 1969) e os rios como barreiras (Wallace, 1852). Enquanto a primeira assume que as mudanças paleoclimáticas do Quaternário dispararam eventos recorrentes de isolamento e contato das linhagens, a segunda dispõe que alguns rios foram

barreiras importantes à distribuição de espécies, impedindo o fluxo gênico e promovendo a divergência entre populações em suas margens opostas.

Um dos ambientes menos explorados dentro das formações de BAF envolve a vegetação xerofítica ou arbustiva aberta, encontradas nas planícies costeiras arenosas, como na restinga, e em afloramentos rochosos chamados *inselbergs* (Scarano, 2002). Dentre os poucos trabalhos realizados na vegetação aberta da BAF, estão estudos com espécies de Cactaceae dos gêneros *Pilosocereus* (Menezes et al. 2016) e *Cereus* (Franco et al., 2017a). Se, por um lado, Menezes et al. (2016) sugerem a importância dos rios Doce, Jequitinhonha, Contas, São Francisco e Jaguaribe no estabelecimento da estrutura genética de espécies do complexo *Pilosocereus arrabidae*, Franco et al. (2017a) sugerem que a diversidade genética de três espécies do gênero *Cereus* foi determinada por um evento inicial de colonização do sul para o norte. Uma vez que a região norte de BAF parece ter sido mais estável durante as mudanças climáticas do Pleistoceno, enquanto a região sul da floresta foi coberta predominantemente por vegetação aberta durante os tempos glaciais (Carnaval & Moritz, 2008; Carnaval et al., 2014, Costa et al., 2018), é possível que a colonização da biota xérica possa mostrar o padrão oposto de táxons associados a áreas florestais (Franco et al., 2017a).

Pilosocereus é o gênero de Cactaceae com maior número de espécies e abrangência geográfica no leste do Brasil (Zappi, 1994; Hunt et al., 2006). O gênero tem distribuição disjunta ao longo da região leste do Brasil a noroeste da América do Sul, alcançando o México e o Caribe (Oliveira-Filho & Fontes, 2000). Esse padrão de distribuição faz com que o gênero seja um modelo para estudar a diversificação de linhagens em formações xeromórficas, como utilizado por Bonatelli et al. (2014) em seu estudo com um grupo de espécies restritas aos campos rupestres do leste da América do Sul. As espécies do gênero *Pilosocereus* são distribuídas em cinco subgrupos taxonômicos informais (ARRABIDAE, AURISSETUS, LEUCOCEPHALUS, PENTAEDROPHORUS e PIAUHYENSIS). Esses grupos foram definidos com base no habitat, variação da morfologia floral, distribuição geográfica, morfologia dos espinhos e padrão de ramificação (Zappi, 1994; Hunt et al., 2006). De acordo com a filogenia de Romeiro-Brito (2017), as espécies *P. arrabidae*, *P. ulei*, *P. brasiliensis* e *P. brasiliensis* subsp. *ruschianus* formam um clado bem suportado (chamado de complexo *P. arrabidae*), tendo sua divergência datada no Pleistoceno. Essas espécies possuem distribuição disjunta no litoral sudeste brasileiro (nos estados do Rio de Janeiro, Bahia e Espírito Santo) e em áreas de Mata Atlântica no estado de Minas Gerais (Taylor &

Zappi, 2004; Zappi et al., 2012). As relações filogenéticas e a distribuição geográfica das espécies deste complexo o tornam um bom modelo biológico para estudar as hipóteses sobre a diversificação da vegetação xerófila na Mata Atlântica. Esses dados, que ainda são escassos quando comparados a táxons da vegetação predominante do bioma (Franco et al., 2017a), podem trazer informações que auxiliem na compreensão de uma história mais completa sobre os padrões biogeográficos da Mata Atlântica (Calvente, 2010; Franco et al., 2017a; Munõz, 2017).

Levando-se em conta, a necessidade de complementar a história da BAF por uma perspectiva dos ambientes secos, o presente trabalho buscou investigar as seguintes hipóteses: (i) as espécies do complexo *Pilosocereus arrabidae* apresentam uma história evolutiva associada às alterações na distribuição das áreas secas da BAF; (ii) existem regiões com maior diversidade genética que sugerem a ocorrência de possíveis refúgios para as espécies xerófitas da região; (iii) a estrutura genética das espécies mostra sinais de barreiras ao fluxo gênico que podem ser representadas por rios, depressões ou montanhas, ou ainda por barreiras desconhecidas para a região.

No Capítulo I, utilizamos dados genéticos e climáticos para investigar relações filogenéticas, estrutura populacional e possíveis alterações da distribuição das espécies ao longo de diferentes períodos.

CAPÍTULO 1

Diversification in dry environments of the Brazilian Atlantic Forest - a case study with species from the genus *Pilosocereus* Byles & Rowley (Cactaceae)

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1. Introduction

The Neotropical region has long been recognized for its amazing biodiversity (Antonelli et al., 2016). Extending from central Mexico to southern Brazil (Morrone, 2014), the region comprises about 37% of the world's species of seed plants (Antonelli and Sanmartín, 2011). For that reason, this region has attracted efforts to explain the origin of the neotropical biodiversity and the underlying drivers of species diversification. The environmental and biogeographic effects of a complex process involving palaeogeographical reorganizations and palaeoclimatic changes have become increasingly evident (Hoorn et al., 2010; Rull, 2008, 2011). Although the knowledge about the Neotropical biota has increased in the last decades (Thomé et al, 2021), many gaps are still open and our understanding is far behind the well-studied taxa from North America and Europe (Turchetto-Zolet et al., 2013; Antonelli et al., 2016, 2018; Cazé et al., 2016).

The Brazilian Atlantic Forest (BAF) is considered one of the five main hotspots on the planet, recognized as a center of high biodiversity at the current risk of extinction due to human impact (Guedes et al., 2005; Leite et al., 2016). The BAF extends in coastal regions of eastern Brazil, comprising its western maximum extent in the east of Paraguay and in the northeast of Argentina (Oliveira-Filho & Fontes, 2000). The large geographic extension, the wide gradient of altitude and latitude, and the rain regimes promote a large

heterogeneity of habitats in the BAF, allowing the formation of different physiognomies and plant communities (Guedes et al., 2005; Ribeiro et al., 2009; Scarano, 2009).

The current knowledge about the main drivers to explain diversification in BAF is limited compared to other biotas of the Neotropical region. The main hypotheses proposed to explain the origin of the high diversity and endemism within BAF are mostly based on allopatric speciation models, such as the Pleistocene Refuge Hypothesis (PRH) (Haffer, 1969) and riverine hypothesis (Wallace, 1852). These findings are based on phylogeographic, biogeographic, and paleoclimatic studies that investigated which historical and ecological processes would be responsible for the origin and maintenance of biodiversity in this domain (Moritz et al., 2000; Carnaval & Moritz, 2008; Thomé et al., 2010; Franco et al., 2017a; Marques et al., 2021).

The Pleistocene Refuge Hypothesis (PRH) was proposed by Haffer (1969) for Amazonian species, but other studies using evidences from palaeoecology, modelling and phylogeography suggest the occurrence of PRH in other subtropical areas (Barrable et al., 2002; Hugall et al., 2002; Hughes et al., 2005; Graham et al., 2006). In BAF, the study of Carnaval and Moritz (2008) assumes that paleoclimatic changes would have promoted recurrent isolation events and reconnection of lineages, causing allopatric speciation and eventual secondary contact. Two regions with historically stable climates within BAF (north-south break *sensu* Carnaval et al., 2014) have been described and tested as possible refuges for some taxa during glacial periods (Carnaval & Moritz, 2008; Carnaval et al., 2009; 2014; DaSilva & Pinto-da-Rocha, 2011; Cazé et al., 2016).

The riverine hypothesis (Wallace, 1852) proposes that rivers could represent barriers to species distribution, preventing gene flow and promoting divergence between populations on opposite banks of the rivers. In BAF, the Doce River has been identified as an important barrier for mammals (Costa & Leite, 2000), birds (Silva et al., 2004), butterflies (Brown, 2005), lizards (Pellegrino et al., 2005), ants (Resende et al., 2010) and amphibians (Thomé et al., 2010; Fouquet et al., 2012).

Another explanation for the diversification of BAF biota is the ecological gradient hypothesis, which posits that gradual variation in temperature, precipitation, and latitude promote divergent selection with or without gene flow in parapatric or allopatric populations (Endler, 1977; Rice & Hostert, 1993; Smith et al., 1997). Examples of this model in the Atlantic Forest were observed in birds by Cabanne et al. (2011) and small

mammals by Pinheiro et al. (2003) and Lara et al. (2005), where rainfall gradient, large altitudinal, and corresponding habitat differences between lowland and upland forests can provide an ecological gradient across which divergence could occur.

It is important to consider that the diversification hypotheses proposed for the BAF biota are not mutually exclusive; that is, a single model cannot explain the complex pattern of the origin of the species biodiversity in this species-rich region. Refuges and geographical barriers could have interacted locally to isolate populations that later became divergent over time, as observed in studies with BAF frog species (Moritz, 2000; Thomé et al., 2010). Previous studies have highlighted the activity of geological faults in the BAF region during the Quaternary (Saadi et al., 2002; Ribeiro, 2006; Thomé et al., 2010; Franco et al., 2017a). These events likely have important implications for some biological systems but are commonly overlooked in biogeographic studies on this region. Indeed, Neotropical biodiversity should be faced as a product of many distinct and interacting evolutionary processes, involving complex scenarios of diversification which we are beginning to understand (Antonelli & Sanmartín, 2011, Antonelli et al. 2018).

Although BAF landscapes are predominantly composed of evergreen rainforests, there are also areas with xerophytic or open shrubby vegetation in the sandy coastal plains, such as in the *restinga*, and in rocky outcrops called inselbergs (Scarano, 2002). The *restinga* extends over most of the coastal region of Brazil and is characterized by sandy soils, and shrubby vegetation exposed to high levels of direct solar radiation and marine influence (Scarano, 2002). Inselbergs are dome-shaped granite outcrops that emerge in the coastal and inland landscapes, forming zones of microclimate that specialized shelter vegetation in adverse conditions of high temperature, low humidity, and poor soil (Porembski, 2007).

Studies with xerophytic vegetation within this region have already been done with species of the Cactaceae family, as in the *Pilosocereus* genus, with has a disjunct distribution between eastern Brazil and northwest South America, reaching Mexico and the Caribbean. This genus has the largest number of species and geographic coverage in eastern Brazil. This distribution pattern makes the genus *Pilosocereus* a model for the study of lineage diversification in xeromorphic formations, as used by Bonatelli et al. (2014) in their study with a group of species restricted to the rupestrian fields of eastern South America. In BAF and Caatinga, Menezes et al. (2016) highlights the importance of

the rivers in the genetic structure of the ARRABIDAE group - the informal morphological group proposed by Zappi (1994) - which showed that genetic variation is partitioned mainly among geographic regions delimited by the Doce, Jequitinhonha, Contas, São Francisco and Jaguaribe rivers. Franco et al. (2017a) investigated the biogeographic influences on xeric biota in BAF using two cactus species of the genus *Cereus* Mill. (Cactaceae, Cereaceae) as a model and suggest that the genetic diversity of populations was shaped by an initial colonization event from south to north in BAF. Considering the idea that the northern evergreen forest was more stable across Pleistocene climate changes, while the southern forest was covered predominantly by open vegetation during glacial times (Carnaval & Moritz, 2008; Carnaval et al., 2014), the xeric biota colonization might show the opposite pattern of taxa associated with forested areas (Franco et al., 2017a).

However, studies on taxa associated with BAF's dry environments still remain scarce compared to existing data on evergreen forests (Franco et al., 2017a; Mota et al, 2020; Amaral et al, 2021a; 2021b; Barrios-Leal et al, 2021.). This gap needs to be minimized for a better and complete understanding of the diversification processes of this region since species that grow outside the forests can substantially contribute to our knowledge about climate dynamics and diversification in different regions of BAF (Pinheiro et al., 2011).

In this study, we used *P. arrabidae* complex as a model to address the following questions: (i) the species of the complex presents a shared evolutionary history that is associated with changes in the distribution of dry areas of the BAF (ii) there are regions with high genetic diversity that suggest the occurrence of possible refugia for the xerophytic species in the region, (iii) the genetic structure of the species shows signs of possible barriers to gene flow that rivers, depressions or mountains can represent, or even by unknown barriers in the region. To answer these questions, we combined genetic, species distribution modeling (SDM) and tests of niche equivalency and similarity (NES).

2. Materials and methods

2.1. Species studied and sampling.

We sampled 97 individuals from 24 localities along the southeastern Brazilian coast (in the states of Rio de Janeiro, Bahia, and Espírito Santo) and BAF areas in Minas Gerais state (Taylor and Zappi, 2004; Zappi et al., 2012), covering the entire known geographic

distribution of the taxon under study (Fig. 1; Table S1). Voucher specimens were deposited in the SORO - Herbário do Centro de Ciências e Tecnologias para a Sustentabilidade (Universidade Federal de São Carlos - campus Sorocaba, UFSCar). Among the three investigated species, *P. arrabidae* has the largest distribution in BAF, inhabiting *restinga* areas from the Santa Cruz Cabrália region (south of Bahia state) to the west of the city of Rio de Janeiro (RJ). *Pilosocereus brasiliensis* is divided into two subspecies: *P. brasiliensis* subsp. *brasiliensis* and *P. brasiliensis* subsp. *ruschianus*. The subspecies *brasiliensis* occurs on the coast of Espírito Santo and Rio de Janeiro states, in *restingas* and inselbergs near the sea. The subspecies *ruschianus* occurs in inselbergs of BAF interior regions in the south of Bahia, Espírito Santo, and northwest Minas Gerais. *Pilosocereus ulei* has a more restricted distribution, being endemic to *restinga* areas from Cabo Frio, Armação de Búzios, and Arraial do Cabo in Rio de Janeiro state (Zappi, 1994).

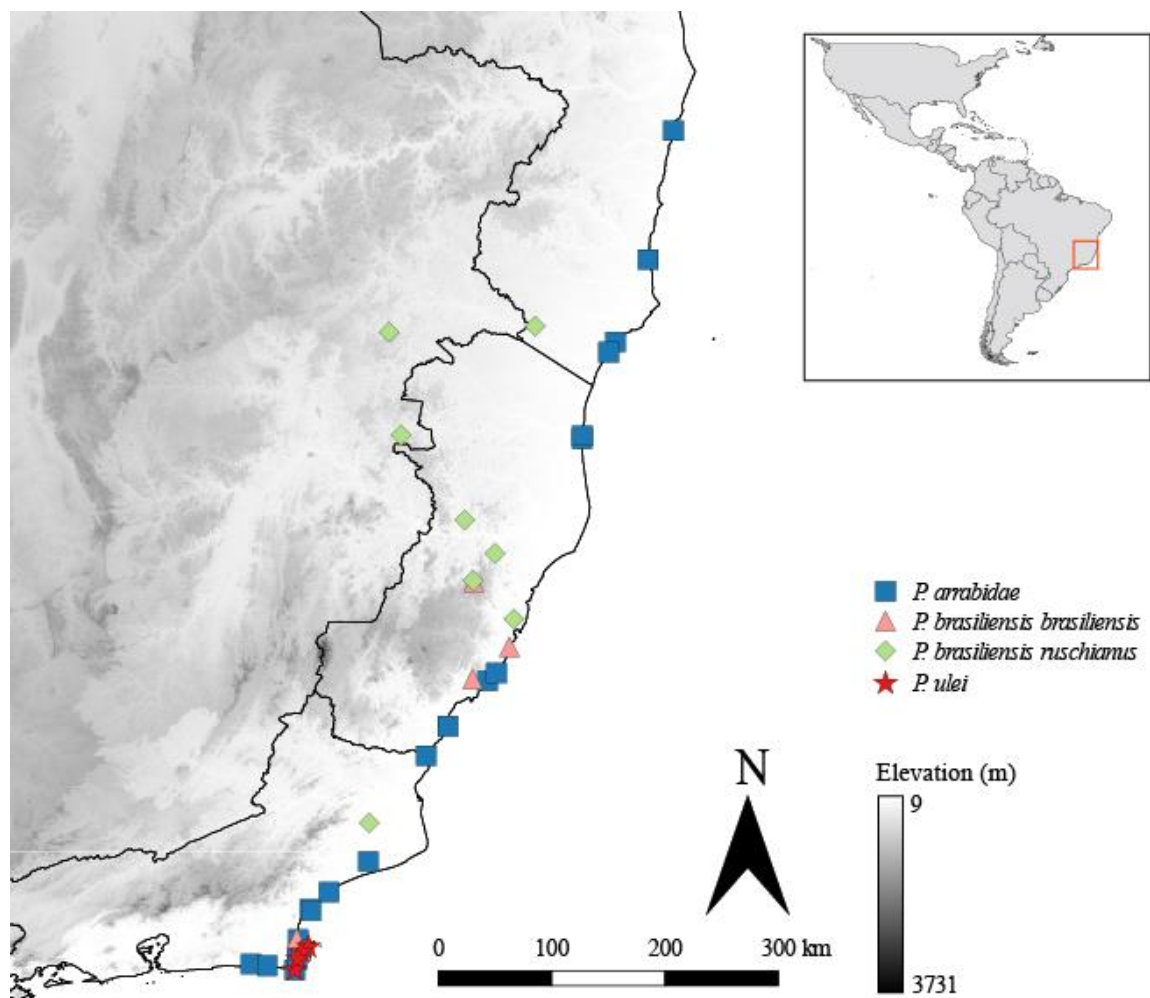


Figure 1. Geographical distribution of sampled localities for *Pilosocereus* along the southeastern Brazilian coast: *P. arrabidae* (blue square), *P. brasiliensis* subsp. *brasiliensis* (pink triangle), *P. brasiliensis* subsp. *ruschianus* (green diamond) and *P. ulei* (red star).

2.2. DNA extraction, amplification, and Sanger sequencing.

DNA was extracted from root tissue samples using the 3x CTAB method (cetyltrimethyl ammonium bromide, Doyle & Doyle, 1987). We selected four anonymous nuclear loci (ANLs, Perez et al. 2016) based on the polymorphism information for the studied species estimated in a previous study (Romeiro-Brito, 2017). ANLs are single-copy loci with no correspondence to known genes (Lee & Edwards, 2008). These markers are attractive for phylogenetic studies since they are chosen randomly in the genome and have high chances of being non-coding regions following the neutral evolution model (Thompson et al., 2010).

Amplification reactions were conducted in a total volume of 25 μ L containing 1x reaction Sigma buffer, 1.5 mM MgCl₂, 10 mM dNTP mix, 10 μ L forward and reverse primers, 0.2 U Sigma Taq polymerase, and 1 μ L of DNA, and run using the following parameters: initial denaturation at 94°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, specific melting temperature (T_m) for each molecular marker (Perez et al., 2016; Supplementary Table S2) for 30 seconds, 72°C for 30 seconds and a final extension of 2 minutes at 72°C. PCR protocol and annealing temperatures followed the conditions of Perez et al. (2016). Amplified DNA fragments were purified by ExoSAP-IT (USB Corporation) and were sent for both forward and reverse sequencing in Macrogen Inc. (Seoul, Korea). The sequences were visualized, edited and manually verified using the software Chromas Pro v.1.49 (Technelysium Pty., Queensland, Australia). Alignments were made with the MUSCLE algorithm (Edgar, 2004) with default parameters in software MEGA X (Kumar et al., 2018). We then used the PHASE v.2.1.1 software (Stephens & Donnelly, 2003) implemented in DnaSP 5.1. (Librado & Rozas, 2009) to solve the haplotype gametic phases of nuclear markers. DAMBE v.7.3.11 software (Xia, 2018) was used to calculate the substitution saturation of the aligned sequences.

2.3. Genetic diversity, structure analysis, and phylogenetic analyses.

The genetic structure of the studied species was investigated using a Spatial Bayesian clustering method (BAPS) and a non-Bayesian analysis (DAPC; Jombart et al., 2010). A Spatial Bayesian was implemented in the BAPS v6.0 program (Corander & Marttinen, 2006) from a population model without mixing. The best K value was estimated according

to the highest marginal likelihood value. Ten repetitions were performed for each K value (1 to 10) to determine the optimal number of groups genetically homogeneous. The population structure was also investigated from discriminant analysis of principal components (DAPC) implemented with the *adegenet* package of the R program v3.3.1 (Jombart, 2008; R Development Core Team 2011). This analysis is based on data transformation from a principal component analysis (PCA) and subsequent analysis discriminant that seeks to maximize the separation between groups. The number of likely groups was obtained using the grouping algorithm by K-means. The optimal number of groups was inferred according to the lowest value of the Bayesian information criterion (BIC). DAPC analysis does not require EHW or linkage disequilibrium as premises, in contrast to the framework of BAPS. The phylogenetic reconstruction was performed in IQTree v.2.1 software (Nguyen et al., 2015) with 10,000 ultrafast bootstrap replicates and *P. aureispinus* as an outgroup (Romeiro-Brito et al., in prep.).

2.4. Species distribution modeling and niche equivalency and similarity tests.

We used in this analysis the recent field collection records complemented with the record occurrence deposited in the GBIF database (Global Biodiversity Information Facility; <https://www.gbif.org/>). The species distribution models (SDM) were conducted for each species and subspecies independently at several distinct epochs, current (WorldClim data-set; 1970– 2000), Late Holocene (LH; c. 0.3-4.2 ka), Mid-Holocene (MH; c. 4.2-8.3 ka), Early-Holocene (EH; c. 8.3-11.7 ka), Last Glacial Maximum (LGM; c. 21 ka), and Last Interglacial (LIG; c. 131 ka). We used the bioclimatic variables (precipitation and temperature) from each epoch at 2.5' spatial resolution (Brown et al., 2018; Fick and Hijmans, 2017). The dimensionality of the variables was reduced using the Pearson Correlation analysis at the RStoolbox R package (Leutner and Horning, 2017), testing the 19 bioclimatic variables in WorldClim. We carried out the SDM using three algorithms present in the biomod2 R package (Thuiller et al., 2016): Artificial Neural Network (ANN), Random Forest (RF), and MaxEnt. The model performance was evaluated using the area under the curve (AUC) and true skill statistic (TSS).

The niche equivalency and similarity tests (NES) were performed using the *ecospat* R package (Di Cola et al., 2017), calculating the Schoener's *D* statistics (Schoener, 1968) and the Hellinger distance-based metric (I) (Warren et al., 2008) for niche overlap. Values of both metrics range from 0 (no overlap) to 1 (indistinguishable) and are used to calculate

equivalency and similarity p-values with simulated niches. As suggested by Rödder and Engler (2011), the value intervals can be interpreted as: *0 to 0.2 = no or very limited overlap, 0.2 to 0.4 = low overlap, 0.4 to 0.6 = moderate overlap, 0.6 to 0.8 = high overlap, and 0.8 to 1.0 = very high overlap.*

The equivalency tests assume that niches need to be indistinguishable to be equivalent, thus p-values greater than 0.95 represent that niches are more equivalent than expected by chance. The similarity tests assume that niche conservatism is expected due to phylogenetic relationships and consider p-values lower than 0.05 to represent more similar niches than expected by chance (Kohler et al., 2020).

3. Results

3.1. Sequence characterization, phylogenetic, and genetic population analyses

In all, 392, 312, 434, and 376 base pairs (bp) were obtained from the alignment of the *PaANL80*, *PaANL87*, *PaANL126*, and *PaANL205* nuclear anonymous regions, respectively (Table S3). Overall, the markers with the highest genetic variability for the species were *PaANL87* and *PaANL126*, presenting the highest values for the number of segregating sites, number of parsimoniously informative sites and average number of nucleotide differences. For each species, for *P. brasiliensis* and *P. ulei*, the most variable marker was *PaANL80* and *PaANL126* for *P. arrabidae*. The number of haplotypes observed ranging from 7 to 40 haplotypes in distinct loci.

We checked the phylogenetic signal of the sequences and, based on Xia's metrics calculated in DAMBE software, all the loci of *P. arrabidae* complex displayed high levels of saturation (Table S4), which suggest a poor phylogenetic signal. However, we proceed with the downstream analyses. The phylogenetic analyses using Bayesian and Maximum Likelihood (ML) approaches displayed the close relationship between *P. arrabidae*, *P. brasiliensis*, and *P. ulei* (Fig. 2a) with high branch support. However, the relationships between the species and individuals were barely resolved and supported, yielding a large polytomy clustering the lineages in a species complex.

The DAPC and BAPS analyses (Fig. 2b-c) also failed to recover patterns between loci and concatenated data, which seems to be an artifact due to the high divergence and

polymorphism among sequences. However, we can cite general patterns that emerged from these analyses. For example, the genetic populations identified by BAPS do not agree with the geographic distribution and classification of *Pilosocereus* species. However, when comparing BAPS results with the DAPC and the phylogenetic tree, some concordant patterns are observed. Both analyses classified individuals of *P. brasiliensis ruschianus* from the inselbergs of Santa Teresa (ES), Colatina (ES) and São João da Manteninha (MG) as part of the same genetic group. Another clear pattern was the clustering of some individuals of *P. arrabidae* and *P. ulei* that occur in sympatry in Cabo Frio, Arraial do Cabo and Armação de Búzios. The concordant patterns among analyses also showed other groups of individuals such as *P. arrabidae* with *P. brasiliensis*, but they do not match the geography and taxonomy of the species.

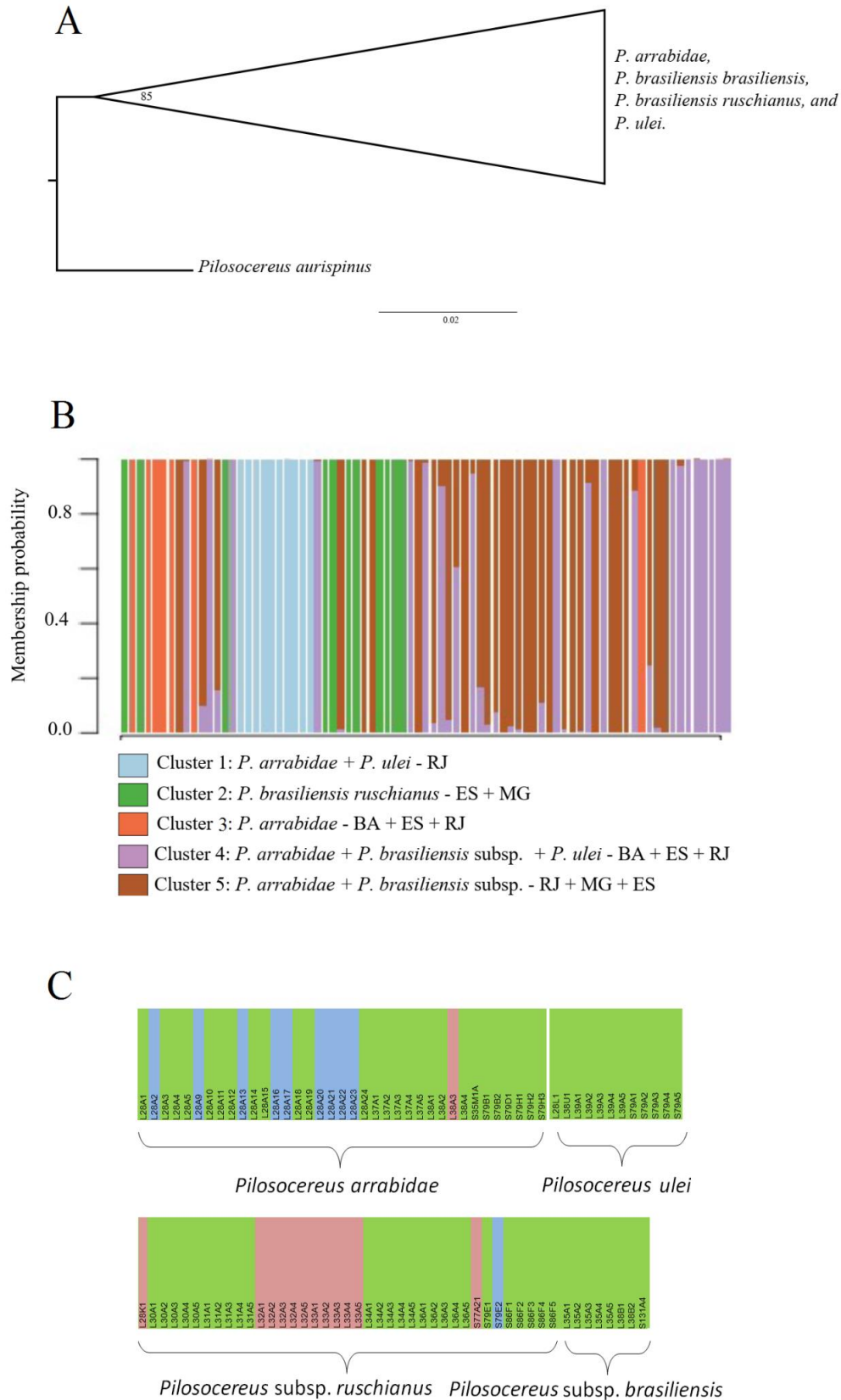


Figure 2. Phylogenetic and genetic structure analyses performed for the *P. arrabidae* complex with the concatenated sequences. (a) Phylogenetic analysis performed with IQtree, (b) bar graph showing DAPC results (K=5), (c) bar graph showing results of BAPs (K=3).

3.2. Species distribution modeling (SDM) and niche equivalency and similarity tests (NES).

We performed the species distribution modeling for the *P. arrabidae*, *P. ulei*, *P. brasiliensis* subsp. *brasiliensis* and *P. brasiliensis* subsp. *ruschianus* separately. The distribution patterns displayed by the SDM analysis developed here were highly predictive of actual patterns of occurrence with a high AUC and TSS value (AUC > 0.95; TSS > 0.92), indicating a good predictive model performance. The Pearson Correlation analysis showed that the climatic variables most associated with the distribution of species in the periods tested were BIO16 (Precipitation of Wettest Quarter) and BIO18 (Precipitation of Warmest Quarter).

The SDM analyses for the present period obtained here had high performance, validated by the sampling records. For the current period (Fig. 3), the predicted area for *P. brasiliensis* subspecies and *P. arrabidae* includes a great part of the Brazilian Atlantic Coast, from the south portion of Rio de Janeiro to Bahia. The *P. ulei* displays a microendemic occurrence in Rio de Janeiro (lakes region of RJ), which was also recovered in our models. The SDM of the two subspecies of *P. brasiliensis* for the present recovered the same occurrence that we found the species at the current period, whereas the *P. brasiliensis* subsp. *brasiliensis* shows a central-north distribution and *P. brasiliensis* subsp. *ruschianus* has a central south distribution. However, both species displayed a contact area between Rio de Janeiro, Minas Gerais, and the Espírito Santo States.

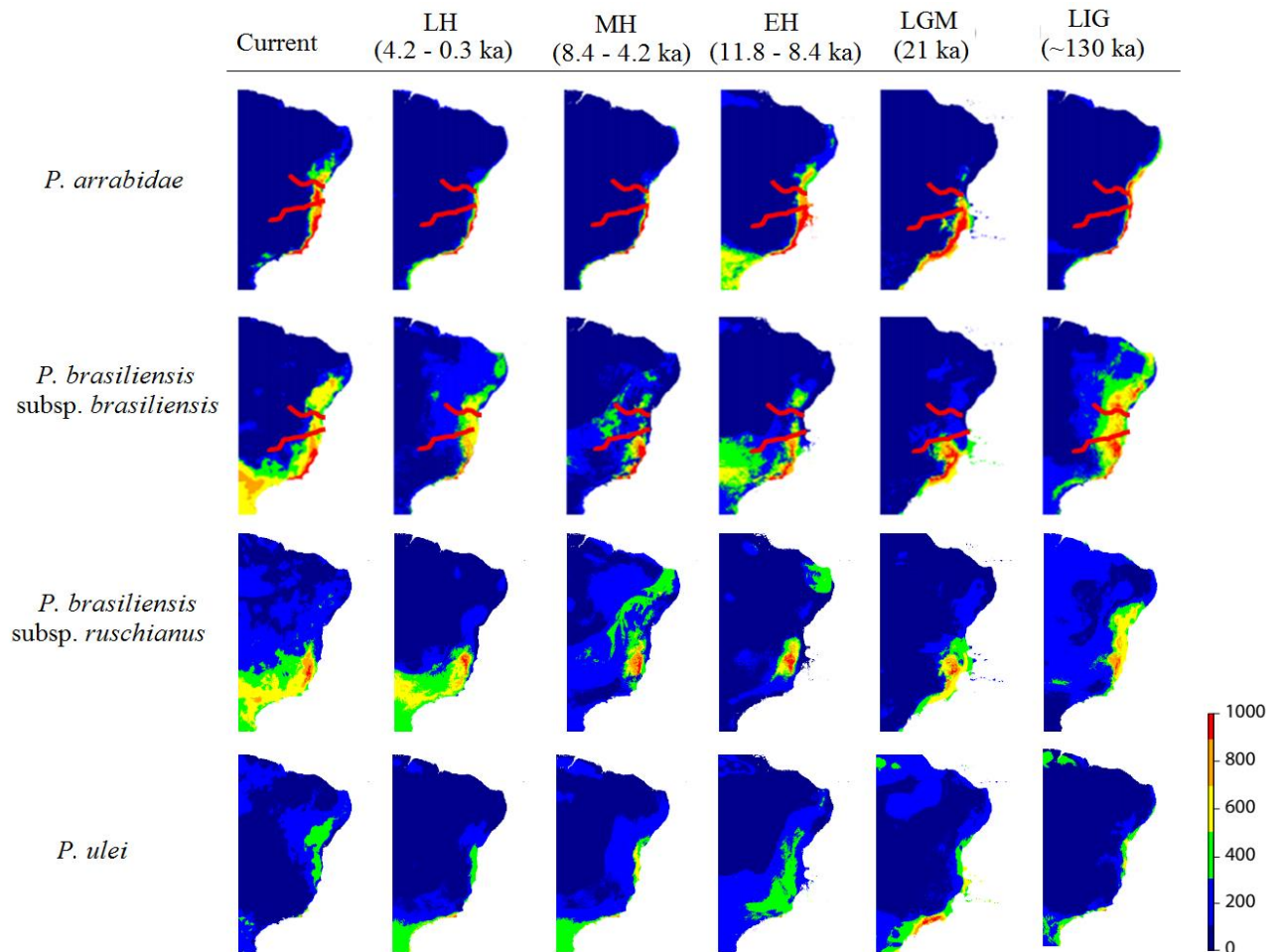


Figure 3. Species distribution modeling showing suitable areas for species from *P. arrabidae* complex over different periods. The red lines in *P. arrabidae* and *P. brasiliensis* subsp. *brasiliensis* maps represented the distribution of the Contas river (northern) and Jequitinhonha river (southern). The warmest colors show the most suitable areas for the species. From left to right: current, LH (4.2 - 0.3 ka), MH (8.4 - 4.2 ka), EH (11.8 - 8.4 ka), LGM (21 ka), LIG (131 ka).

The past modeling (from 130 ka to 0.3 ka) showed an enlargement of the areas compared to the current predicted areas for *P. arrabidae* and *P. ulei*. However, our models showed stability in *P. brasiliensis* subsp. *ruschianus* and a considerable area reduction in *P. brasiliensis* subsp. *brasiliensis* from the Last Interglacial to the LGM period, with a narrowing distribution through the coast.

The niche equivalency and similarity (NES) tests were conducted to evaluate the niche overlap between the *P. brasiliensis* subspecies and among the species during the last 130 ka. *P. arrabidae* and *P. ulei* showed higher p-values for the niche equivalency test ($p > 0.79$ in all modeled periods) and lower p-values for the niche similarity test ($p < 0.002$ in

all modeled periods), this means niches are more similar and equivalent than expected by chance (Table S5).

The niche equivalency and similarity were also statistically supported when comparing *P. arrabidae* and *P. brasiliensis* subsp. *brasiliensis*, and *P. arrabidae* and *P. brasiliensis* subsp. *ruschianus*. However, the *D* and *I* metrics values in these comparisons changed in distinct periods (Table S4). The results showed that *P. ulei* and *P. brasiliensis* subspecies displayed lower niche equivalency and similarity among the evaluated period. In contrast, the niche equivalency and similarity between *P. arrabidae* and *P. brasiliensis* subsp. *brasiliensis* decrease in LGM and increase in the last 4 ka.

4. Discussion

BAF is one of the richest and largest biodiversity hotspots in the world, however, is also one of the most threatened (Leite et al., 2016; Marques et al., 2021). It is a largely shaped region on the Atlantic Coast and South of Brazil that encompasses distinct topographic and geographic conditions, including patches of dry areas and inselbergs (Franco et al., 2017a), *restinga*, moist forest patchy (Dinnersteins et al., 2017) and mountainous landscapes (Silva et al., 2020). Besides, the combination of distinct altitudinal levels and orography resulted in a complex interaction between abiotic and biotic features. These interactions provide the occurrence of numerous refugia (historical refugia) and shelter to several adapted species of usually relict origin (Carnaval & Moritz, 2008; Carnaval et al., 2009), culminating in high endemism areas (Paz et al., 2019). The geographical nuances observed in the BAF (depressions and mountain ranges) created natural barriers for some species, such as the Doce, Jequitinhonha, Pardos, de Contas, and São Francisco rivers (Menezes et al., 2017; Barbo et al., 2021; Padial et al., 2021). These barriers hide paleogeographic and biogeographic histories associated with the dynamics of species diversification in different regions of BAF (Pinheiro et al., 2011).

In this study, we report the attempt to evaluate the diversification and possible barriers between the species of the *P. arrabidae* complex within BAF, using phylogenetic and population studies and species distribution modeling. However, our data failed to resolve the relationships of the tips. The accuracy of the phylogenetic reconstruction is highly dependent on sequence quality, the correct alignment, and the regularity and consistency

of the nucleotide substitution process (Xia & Xie, 2001). Due to the high level of sequence saturation and the complex phylogenetic relationships of species, we were unable to test our phylogeographic hypotheses using approaches based on genetic data.

The identification, development, and application of molecular markers in phylogenetic, phylogeographic, and population studies are essential and significant steps for the accuracy of the downstream analyses (Waltier et al., 2006; Oliveira de Oliveira et al., 2021). Here, we employed four molecular markers proposed by Perez et al. (2016), which were annotated as anonymous nuclear regions. The preliminary results suggested that these markers have successfully recovered an important phylogenetic signal to the genus *Pilosocereus*. However, our findings demonstrated that the sequence data from these four loci are useless for population genetic application, even including a large number of individuals (N = 97). Choosing a molecular marker for plant evolutionary studies is a tough task (Parker et al., 1998; Garant & Kruuk, 2005; Shaw et al., 2014; Romeiro-Brito et al., in prep.). The polymorphism, the site invariance, and the heterogeneity rates are challenges that we have to lead in genetic studies with Cactaceae (Bonatelli et al., 2012; Franco et al., 2022). Menezes et al. (2016) tested hypotheses about the phylogeography of the informal group ARRABIDAE (*P. arrabidae*, *P. azulensis*, *P. catingicola*, *P. catingicola* subsp. *salvadorensis* and *P. splendidus*), with distribution in the Caatinga and Atlantic Forest, using chloroplast DNA markers and observed that the group is not monophyletic. Although the study by Menezes et al. (2016) is informative, the approach in a non-monophyletic group and the limitation of genetic variation of the chosen markers hampered the test of their hypotheses. Some studies suggested and provided a list of primers from chloroplast that may be useful for plants, such as *rbcL*, *matK*, and *rpl13* (Shaw et al., 2005; Romeiro-Brito et al., 2016). However, we attempt to seek new strategies using nuclear anonymous regions with less success.

4.1. *P. arrabidae* complex distribution modeling.

Here, we have identified some climatic constraints for the distribution of *P. arrabidae* complex using species distribution modeling and niche equivalency and similarity tests. The subspecies of *P. brasiliensis* showed distinct distribution and abiotic conditions in the past and the present. In this analysis, we rejected the null hypothesis of niche equivalence and similarity, demonstrating how the niches of *P. brasiliensis* subspecies are not transposable. Because of these results, it is inaccurate to imply common niches to them, as

well as modelling their distribution as a unique species (Elith & Leathwick, 2009; Dias Tarli et al., 2018). Thus, closely-related species (in this case, subspecies) do not necessarily share the same environmental niche spaces, e.g., *Cereus jamacaru* subsp. *jamacaru* and *C. jamacaru* subsp. *calcirupicola* (Cactaceae; Amaral et al., 2021a), a common mechanism observed among insects, vertebrates, and plants to alleviate competition and to better explore environmental resources (Silvertown et al., 2001; Warren et al., 2008; Namyatova, 2020; Maltseva et al., 2021).

Based on the NES and PCA approaches, the niches of *P. arrabidae* and *P. ulei* are the most similar and equivalent, when compared to the other species, in the last 130 ka. The continuum niche overlap between these two species may explain their common occurrence in the field and the pattern found in BAPS, DAPC and phylogenetic tree, with was the clustering of some individuals of *P. arrabidae* and *P. ulei* that occur in sympatry in Cabo Frio, Arraial do Cabo and Armação de Búzios. The modeling (from 130 ka to 0.3 ka) showed an enlargement of the areas compared to the current predicted areas for this species, probably associated with the decrease of sea level, which made the expansion to the east viable. Thus, the isolated *P. ulei* species reached the part of the Island of Ilha Grande and Dois Rios (south coast of Rio de Janeiro state).

However, a question remains. Why do *P. ulei* displays a microendemic occurrence and *P. arrabidae* does not? A quite simple answer is that the large distribution of *P. arrabidae* and *P. brasiliensis* subspecies in the BAF against the microendemic distribution of *P. ulei* may rely on the physiological and morphological differences between them. Similar patterns were observed among insects, vertebrates, and plants (Silva et al., 2014; Rodrigues et al., 2016; Rueda-M et al., 2021; Torrez-Martínez et al., 2021). In the NES tests, the climatic variables related to the precipitation of the hottest and humid quarter of the last 130 ka (BIO 16 and 18) were the most associated with the distribution of the species in the tested periods. That is, these climatic variables of precipitation can play a critical role in assessing the current distribution of species. (Amaral et al., 2021, pers. comm.; Aquino et al., 2021; Barrios et al., 2021).

Here, we were not able to test the effect of the BAF river as a dispersal barrier using molecular data. However, for *P. arrabidae* and *P. brasiliensis* subsp. *brasiliensis*, the SDM showed a similar break in both species in the region of Jequitinhonha and Contas river basins (Fig. 3). Dominguez et al. (2006) observed oscillations of sediment dispersal

during the Holocene, associated with an increase in the intensity of the NE-E waves. The alterations in the wave intensity were caused by an overall decrease in the frequency of cold fronts during the winter and summer, which also changed the precipitation pattern in the Jequitinhonha basin (Dominguez et al., 2009). The changes in atmospheric circulation and precipitation levels may act as environmental barriers to these species, creating an ecological instead of physical barrier to dispersal, affecting the species distribution.

The climatic niche overlap between the studied species was not constant from the LIG to the present (Table S4), except for *P. arrabidae* and *P. ulei*. The modeling showed a considerable area reduction in *P. brasiliensis* subsp. *brasiliensis* from the LIG to the LGM period. We usually observed in Cactaceae the opposite pattern, with area expansion during the LGM (drier and colder period). Despite that, the BAF always works as a refuge for these species, mainly in those areas between Rio de Janeiro, Minas Gerais, and the Espírito Santo States. These areas also allowed constant contact between the three species during the last 150 ka. The oscillation of NES values also suggested climate fluctuations through time, which was observed mainly in the last 20 ka (Rull, 2020). Thus, distinct events of secondary contact followed by fragmentation and the presence of common refugia during the last 150 ka may have occurred in the *P. arrabidae* and *P. brasiliensis* subspecies.

Comparing the modeling results among the species and distinct periods, we observed a moderate stability of BAF formation in the Atlantic Coast during the last 130 ka, which corroborates with other niche modeling studies (Carnaval & Moritz, 2008; Sobral et al., 2015). However, from the LGM (20 ka) to the present, the northern areas of BAF (from Alagoas to central-south of Bahia states) seem less suitable for *P. arrabidae* complex. These areas encompass the Bahia and Pernambuco Coastal Forests ecoregions (Dinnerstein et al., 2017). It is note to worth a break in *P. arrabidae* distribution in the central Bahia, regions of Jequitinhonha and Contas rivers (Figure 3). Overall our findings suggested that the fragmentation and expansion of BAF during the climatic fluctuation within the last 20 ka (Carnaval & Moritz 2008; Carnaval et al. 2009; Sobral et al; 2015) and the abiotic distinction between the south and north of BAF have a direct influence on the *P. arrabidae* and *P. brasiliensis* subsp. *brasiliensis* distribution. After those climatic oscillation events, the climate conditions imposed a central-south distribution to this species in the present time.

Our distribution modeling suggested that the Pleistocene events related to climatic oscillations drive the current distribution of the species, indicating a continuum forest distribution of the BAF from the Central-south of Bahia to São Paulo States (Serra do Mar corridor; Franco et al., 2017a, 2017b; Silva et al., 2018; Amaral et al., 2021a). Besides, the sustenance of xeric and dry areas, whereas the sea-level decrease, provide refuge areas, such as the lakes region of RJ (Cabo Frio, Arraial do Cabo, and Armação de Búzios) and new habitats/areas for the expansion of the *P. arrabidae* complex, especially the expansion of *P. ulei* through the Brazilian shield (Leite et al., 2016; Amaral et al., 2021a). An intriguing result of our modeling is the drastic reduction of the *P. brasiliensis* subsp. *brasiliensis* distribution during the LGM, similar results obtained by Menezes (2016) using species of genus *Pilosocereus* as biological models. The glaciation events may have affected some BAF species differently than predicted by the Pleistocene Refuge Hypothesis (PRH). In this case, the standard mechanisms related to habitat retraction due to harsh climate conditions may explain this pattern in *P. brasiliensis* subsp. *brasiliensis*. New molecular analyses should bring new insights into the expansion and retraction of the *P. arrabidae* complex during the Pleistocene.

5. SUPPLEMENTARY MATERIAL

TABLE S1. Sample information of populations of *Pilosocereus* species and population coordinates.

Species	Code	Locality - State	Coordinates (S, W)	
<i>P. arrabidae</i>	S35M1	Nova Viçosa - BA	17°59'19.0"S	39°29'07.9"W
<i>P. arrabidae</i>	S79B1	Arraial do Cabo - RJ	22°57'48.0"S	42°01'39.6"W
	S79B2			
<i>P. arrabidae</i>	S79D1	Guarapari - ES	20°40'26.8"S	40°29'59.0"W
<i>P. arrabidae</i>	S79H1	São Mateus - ES	18°45'18.2"S	39°44'50.8"W
	S79H2			
	S79H3			
<i>P. arrabidae</i>	L28A1	Prado – BA	17°20'08.6"S	39°13'31.1"W
	L28A2			
	L28A3			
	L28A4			
	L28A5			
<i>P. arrabidae</i>	L28A9	Araruama - RJ	22°54'57.6"S	42°22'26.4"W
<i>P. arrabidae</i>	L28A10	Arraial do Cabo - RJ	22°55'44.4"S	42°14'49.2"W
<i>P. arrabidae</i>	L28A11	Araruama - RJ	22°52'51.6"S	42°00'36.0"W
<i>P. arrabidae</i>	L28A12	Cabo Frio - RJ	22°43'19.2"S	41°59'56.4"W
<i>P. arrabidae</i>	L28A13	Rio das Ostras - RJ	22°28'55.2"S	41°54'18.0"W
<i>P. arrabidae</i>	L28A14	Macaé - RJ	22°20'49.2"S	41°45'21.6"W
	L28A15			
<i>P. arrabidae</i>	L28A16	Quissamã - RJ	22°06'14.4"S	41°26'38.4"W
<i>P. arrabidae</i>	L28A17	Presidente Kennedy - ES	21°16'04.8"S	40°59'09.6"W
<i>P. arrabidae</i>	L28A18	Marataízes - ES	21°01'58.8"S	40°48'46.8"W
<i>P. arrabidae</i>	L28A19	Guarapari - ES	20°36'39.6"S	40°25'40.8"W
	L28A20			
<i>P. arrabidae</i>	L28A21	São Mateus - ES	18°43'51.6"S	39°44'49.2"W
<i>P. arrabidae</i>	L28A22	Mucuri - BA	18°04'12.0"S	39°32'16.8"W
<i>P. arrabidae</i>	L28A23	Santa Cruz Cabralia - BA	16°18'54.0"S	39°01'26.4"W
	L28A24			

	L37A1			
	L37A2			
<i>P. arrabidae</i>	L37A3	Rio das Ostras - RJ	22°29'36.8"S	41°53'57.5"W
	L37A4			
	L37A5			
	L38A1			
	L38A2			
<i>P. arrabidae</i>	L38A3	Armação de Búzios - RJ	22°48'25.8"S	41°58'51.7"W
	L38A4			
	L38A5			
<i>P. brasiliensis ruschianus</i>	S77A21	São João de Manteninha– MG	18°43'34.8"S	41°11'07.8"W
<i>P. brasiliensis ruschianus</i>	S79E1	Colatina - ES	19°39'46.2"S	40°26'26.4"W
	S79E2			
<i>P. brasiliensis ruschianus</i>	S86F1	Guarapari – ES	20°39'33.1"S	40°36'59"W
	S86F2			
	S86F3			
	S86F4			
	S86F5			
<i>P. brasiliensis brasiliensis</i>	S131A4	Armação de Búzios - RJ	22°43'7"S	42°00'40"W
<i>P. brasiliensis ruschianus</i>	L28K1	Santa Teresa - ES	19°53'13.2"S	40°36'28.8"W
	L30A1			
	L30A2			
<i>P. brasiliensis ruschianus</i>	L30A3	Teófilo Otoni - MG	17°54'32.9"S	41°16'41.8"W
	L30A4			
	L30A5			
	L31A1			
	L31A2			
<i>P. brasiliensis ruschianus</i>	L31A3	Argolô - BA	17°51'46.7"S	40°07'18.2"W
	L31A4			
	L31A5			

	L32A1			
	L32A2			
<i>P. brasiliensis ruschianus</i>	L32A3	Colatina - ES	19°23'50.9"S	40°40'44.8"W
	L32A4			
	L32A5			
	L33A1			
	L33A2			
<i>P. brasiliensis ruschianus</i>	L33A3	Santa Teresa - ES	19°5'41.7"S	40°36'44.9"W
	L33A4			
	L33A5			
	L34A1			
	L34A2			
<i>P. brasiliensis ruschianus</i>	L34A3	Serra - ES	20°10'58.3"S	40°17'27.6"W
	L34A4			
	L34A5			
	L35A1			
	L35A2			
<i>P. brasiliensis brasiliensis</i>	L35A3	Vila Velha - ES	20°24'12.4"S	40°19'33.8"W
	L35A4			
	L35A5			
	L36A1			
	L36A2			
<i>P. brasiliensis ruschianus</i>	L36A3	Campos dos Goytacazes - RJ	21°47'56.8"S	41°26'10.3"W
	L36A4			
	L36A5			
	L38B1			
<i>P. brasiliensis brasiliensis</i>	L38B2	Armação de Búzios - RJ	22°48'25.5"S	41°58'51.7"W
	S79A1			
	S79A2			
<i>P. ulei</i>	S79A3	Arraial do Cabo – RJ	22°57'48.0"S	42°01'39.6"W
	S79A4			

<i>P. ulei</i>	S79A5	Arraial do Cabo – RJ	22°57'48.0"S	42°01'39.6"W
<i>P. ulei</i>	L28L1 (669)	Armação de Búzios - RJ	22°46'58.8"S	41°55'01.2"W
<i>P. ulei</i>	L38U1	Armação de Búzios - RJ	22°49'28.9"S	41°58'25.9"W
	L39A1			
	L39A2			
<i>P. ulei</i>	L39A3	Cabo Frio - RJ	22°52'36.9"S	42°00'14.6"W
	L39A4			
	L39A5			

TABLE S2. Annealing conditions for ANLs markers used in study, their primers forward and reverse, length in base pairs (L) and specific melting temperature (Tm) for each pair of primers.

<i>Loci</i>	<i>Primer Foward</i> (5' – 3')	<i>Primer Reverse</i> (5' – 3')	L	Tm (°C)
<i>PaANL_80</i>	AAGAAGAACGGGCGAGTTG	AGGAGGTGGCAATGCAGTAG	477	58
<i>PaANL_87</i>	TCTTTATGGCGTTATTCACCTCG	CGAAGGCCTAACTTGACAGG	395	58
<i>PaANL_126</i>	TCCTAAACAAGGGCTACGAAG	TGTACCAATGGGCAGCAC	451	60
<i>PaANL_205</i>	AAATCGGAGTCACAACAGAGA	TACCGAGATCTTGCGATGC	382	54

TABLE S3. Characterization of the genetic diversity of anonymous nuclear molecular markers for the sequences obtained. N: number of samples sequenced; S: number of segregating sites; PICs: number of parsimony-informative sites; Nh: number of haplotypes; Hd: haplotypic diversity; π : nucleotide diversity; k: mean number of nucleotide differences; θ_w : Watson's theta by sequence.

<i>Loci</i>	<i>Species</i>	N	bp	S	PICs	Nh	Hd	π	k	θ_w
<i>PaANL_80</i>	<i>P. arrabidae</i>	37	392	40	17	33	0,852 ($\pm 0,040$)	0,00728 ($\pm 0,00114$)	2,846	8,206
	<i>P. brasiliensis</i>	45	392	108	21	38	0,944 ($\pm 0,014$)	0,01369 ($\pm 0,00434$)	5,257	21,296
	<i>P. ulei</i>	12	392	12	7	14	0,938 ($\pm 0,028$)	0,00802 ($\pm 0,00097$)	3,138	3,213
<i>PaANL_87</i>	<i>P. arrabidae</i>	34	312	46	32	24	0,795 ($\pm 0,049$)	0,01105 ($\pm 0,00262$)	3,435	9,605
	<i>P. brasiliensis</i>	45	312	57	39	35	0,884 ($\pm 0,025$)	0,02925 ($\pm 0,00245$)	9,098	11,239
	<i>P. ulei</i>	12	312	18	8	9	0,844 ($\pm 0,046$)	0,00825 ($\pm 0,00224$)	2,565	4,820
<i>PaANL_126</i>	<i>P. arrabidae</i>	31	434	72	66	40	0,979 ($\pm 0,009$)	0,03311 ($\pm 0,00335$)	14,27 0	15,331
	<i>P. brasiliensis</i>	45	434	55	40	39	0,954 ($\pm 0,012$)	0,01443 ($\pm 0,00175$)	6,246	10,845
	<i>P. ulei</i>	8	434	13	6	7	0,692 ($\pm 0,124$)	0,00525 ($\pm 0,00138$)	2,275	3,918
<i>PaANL_205</i>	<i>P. arrabidae</i>	37	376	16	10	19	0,847 ($\pm 0,027$)	0,00965 ($\pm 0,00044$)	3,628	3,282
	<i>P. brasiliensis</i>	43	376	27	16	29	0,874 ($\pm 0,029$)	0,00893 ($\pm 0,00069$)	3,359	5,372
	<i>P. ulei</i>	12	376	15	13	12	0,880 ($\pm 0,045$)	0,00863 ($\pm 0,00178$)	3,246	4,017

TABLE S4. Test of substitution saturation performed in DAMBE. The test assumes that if the value of $I_{ss} > I_{ss.c}$ means high levels of saturation, which suggests a poor phylogenetic signal and a useless sequence. If $I_{ss} < I_{ss.c}$ there is a low saturation in the sequences.

Alignment	I_{ss}	I_{ss.c}	<i>p</i>	Interpretation (Xia et al. 2003)
<i>PaANL_80</i>	1.19	0.71	0.000	Useless sequence
<i>PaANL_87</i>	1.19	0.7	0.000	Useless sequence
<i>PaANL_126</i>	1.23	0.72	0.000	Useless sequence
<i>PaANL_205</i>	0.95	0.7	0.000	Useless sequence

TABLE S5. Niche similarity and equivalence tests. Each line indicates the dataset pair, the results of D metric and I metric for equivalency and similarity tests with respective p-values. NA indicates lower niche overlap; no statistical support.

Current				
Dataset pair	Niche equivalency		Niche similarity	
	D	I	D	I
<i>P. arrabidae</i> and <i>P. ulei</i>	0.16 (p=0.95)	0.4 (p=0.92)	0.16 (p=0.001)	0.4 (p=0.002)
<i>P. brasiliensis brasiliensis</i> and <i>P. ulei</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)
<i>P. brasiliensis brasiliensis</i> and <i>P. arrabidae</i>	0.05 (p=1)	0.2 (p=1)	0.05 (p=0.01)	0.2 (p=0.012)
<i>P. brasiliensis ruschianus</i> and <i>P. arrabidae</i>	0.04 (p=0.99)	0.055 (p=1)	0.04 (p=0.19)	0.055 (p=0.19)
<i>P. brasiliensis ruschianus</i> and <i>P. ulei</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)
<i>P. brasiliensis ruschianus</i> and <i>P. brasiliensis brasiliensis</i>	0.002 (p=1)	0.018 (p=1)	0.002 (p=0.16)	0.018 (p=0.16)
LH (4.2 - 0.3ka)				
Dataset pair	Niche equivalency		Niche similarity	
	D	I	D	I
<i>P. arrabidae</i> and <i>P. ulei</i>	0.14 (p=0.95)	0.37 (p=0.88)	0.14 (p=0.0009)	0.37 (p=0.0009)
<i>P. brasiliensis brasiliensis</i> and <i>P. ulei</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)
<i>P. brasiliensis brasiliensis</i> and <i>P. arrabidae</i>	0.03 (p=1)	0.15 (p=1)	0.03 (p=0.007)	0.15 (p=0.008)
<i>P. brasiliensis ruschianus</i> and <i>P. arrabidae</i>	0.014 (p=0.99)	0.018 (p=1)	0.014 (p=0.3)	0.018 (p=0.3)
<i>P. brasiliensis ruschianus</i> and <i>P. ulei</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)
<i>P. brasiliensis ruschianus</i> and <i>P. brasiliensis brasiliensis</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)

MH (8.4 - 4.2ka)				
Dataset pair	Niche equivalency		Niche similarity	
	D	I	D	I
<i>P. arrabidae</i> and <i>P. ulei</i>	0.17 (p=0.93)	0.4 (p=0.86)	0.17 (p=0.0009)	0.4 (p=0.0009)
<i>P. brasiliensis brasiliensis</i> and <i>P. ulei</i>	NA	NA	NA	NA
<i>P. brasiliensis brasiliensis</i> and <i>P. arrabidae</i>	NA	NA	NA	NA
<i>P. brasiliensis ruschianus</i> and <i>P. arrabidae</i>	0.002 (p=0.99)	0.002 (p=0.99)	0.002 (p=0.002)	0.002 (p=0.002)
<i>P. brasiliensis ruschianus</i> and <i>P. ulei</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)
<i>P. brasiliensis ruschianus</i> and <i>P. brasiliensis</i> <i>brasiliensis</i>	NA	NA	NA	NA
EH (11.8 - 8.4ka)				
Dataset pair	Niche equivalency		Niche similarity	
	D	I	D	I
<i>P. arrabidae</i> and <i>P. ulei</i>	0.13 (p=0.96)	0.36 (p=0.93)	0.13 (p=0.0009)	0.36 (p=0.0009)
<i>P. brasiliensis brasiliensis</i> and <i>P. ulei</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)
<i>P. brasiliensis brasiliensis</i> and <i>P. arrabidae</i>	0.003 (p=1)	0.055 (p=1)	0.003 (p=0.01)	0.055 (p=0.01)
<i>P. brasiliensis ruschianus</i> and <i>P. arrabidae</i>	0.01 (p=1)	0.014 (p=1)	0.01 (p=0.35)	0.014 (p=0.35)
<i>P. brasiliensis ruschianus</i> and <i>P. ulei</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)
<i>P. brasiliensis ruschianus</i> and <i>P. brasiliensis</i> <i>brasiliensis</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)

LGM (21ka)				
Dataset pair	Niche equivalency		Niche similarity	
	D	I	D	I
<i>P. arrabidae</i> and <i>P. ulei</i>	0.15 (p=0.92)	0.38 (p=0.79)	0.15 (p=0.007)	0.38 (p=0.009)
<i>P. brasiliensis brasiliensis</i> and <i>P. ulei</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)
<i>P. brasiliensis brasiliensis</i> and <i>P. arrabidae</i>	0.02 (p=1)	0.12 (p=0.99)	0.05 (p=0.03)	0.2 (p=0.032)
<i>P. brasiliensis ruschianus</i> and <i>P. arrabidae</i>	0.12 (p=0.98)	0.2 (p=0.99)	0.12 (p=0.33)	0.2 (p=0.3)
<i>P. brasiliensis ruschianus</i> and <i>P. ulei</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)
<i>P. brasiliensis ruschianus</i> and <i>P. brasiliensis</i> <i>brasiliensis</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=1)	0.0002 (p=1)
LIG (131ka)				
Dataset pair	Niche equivalency		Niche similarity	
	D	I	D	I
<i>P. arrabidae</i> and <i>P. ulei</i>	0.13 (p=0.97)	0.36 (p=0.94)	0.13 (p=0.002)	0.36 (p=0.0009)
<i>P. brasiliensis brasiliensis</i> and <i>P. ulei</i>	0.0 (p=1)	0.0002 (p=1)	0.0 (p=NA)	0.0002 (p=NA)
<i>P. brasiliensis brasiliensis</i> and <i>P. arrabidae</i>	0.05 (p=1)	0.2 (p=0.99)	0.05 (p=0.01)	0.2 (p=0.0099)
<i>P. brasiliensis ruschianus</i> and <i>P. arrabidae</i>	0.005 (p=1)	0.007 (p=1)	0.005 (p=0.33)	0.007 (p=0.31)
<i>P. brasiliensis ruschianus</i> and <i>P. ulei</i>	0.0 (p=NA)	0.0002 (p=NA)	0.0 (p=NA)	0.0002 (p=NA)
<i>P. brasiliensis ruschianus</i> and <i>P. brasiliensis</i> <i>brasiliensis</i>	0.0 (p=NA)	0.0002 (p=NA)	0.0 (p=NA)	0.0002 (p=NA)

6. CONSIDERAÇÕES FINAIS

Este trabalho é um dos poucos que exploraram formações xeromórficas na BAF. Nele, investigamos as relações filogenéticas, estrutura populacional e alterações históricas na distribuição de espécies de cactos do complexo *P. arrabidae* na tentativa de contribuir com o cenário de alterações históricas em áreas secas da BAF. Infelizmente, os dados genéticos utilizados se mostraram insuficientes para resolver relações filogenéticas mais estreitas entre as espécies, bem como as relações de fluxo gênico. No entanto, um padrão geral que emerge das análises realizadas é que a história evolutiva desses táxons deve ser bastante interligada e o estudo em conjunto dessas espécies parece ser importante para acessar as questões levantadas neste trabalho.

Devido à dificuldade encontrada no uso dos dados genéticos, nossa discussão se apoiou nas análises de modelagem de distribuição de espécies e sobreposição de nicho. Neste sentido, podemos afirmar que a alteração da vegetação xerófitas não parece ter seguido o esperado pela Teoria dos Refúgios, uma vez que observamos uma retração acentuada na distribuição das espécies durante o LGM. Ao mesmo tempo fomos capazes de observar quebras na distribuição das espécies na região dos rios Jequitinhonha e Contas que podem indicar barreiras históricas ao fluxo gênico.

De forma geral, o complexo *P. arrabidae* parece ser um bom modelo de estudo para o entendimento da diversificação e demografia de táxons de áreas secas da BAF. Maiores esforços na prospecção de marcadores moleculares com o uso de técnicas de sequenciamento de Nova Geração (*Next Generation Sequencing* - NGS), por exemplo, podem ser um caminho promissor para responder hipóteses biogeográficas mais específicas envolvendo este modelo de estudo.

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Anexo 1. Informações sobre as espécies e seus respectivos códigos, localidades de coleta, coordenadas geográficas, habitat e vouchers.

Species	Code	Locality - State	Coordinates	Habitat	Voucher
<i>P. arrabidae</i>	S35M1	Nova Viçosa - BA	-17.988611, -39.485528	Restinga	SORO 4488
<i>P. arrabidae</i>	S79B1	Arraial do Cabo - RJ	-22.963333, -42.027667	Restinga	SORO 2656
	S79B2				
<i>P. arrabidae</i>	S79D1	Guarapari - ES	-20.674111, -40.499722	Restinga	N/A
<i>P. arrabidae</i>	S79H1	São Mateus - ES	-18.755056, -39.747444	Restinga	SORO 4903
	S79H2				
	S79H3				
<i>P. arrabidae</i>	L28A1	Prado – BA	-17.335722, -39.225306	Restinga	N/A
	L28A2				
	L28A3				
	L28A4				
	L28A5				
<i>P. arrabidae</i>	L28A9	Araruama - RJ	-22.916000, -42.374000	Restinga	EAC 56055
<i>P. arrabidae</i>	L28A10	Arraial do Cabo - RJ	-22.929000, -42.247000	Restinga	N/A
<i>P. arrabidae</i>	L28A11	Araruama - RJ	-22.881000, -42.010000	Restinga	EAC 58008
<i>P. arrabidae</i>	L28A12	Cabo Frio - RJ	-22.722000, -41.999000	Restinga	EAC 56052

<i>P. arrabidae</i>	L28A13	Rio das Ostras - RJ	-22.482000, -41.905000	Restinga	EAC 56050
<i>P. arrabidae</i>	L28A14	Macaé - RJ	-22.347000, -41.756000	Restinga	EAC 56054
	L28A15				
<i>P. arrabidae</i>	L28A16	Quissamã - RJ	-22.104000, -41.444000	Restinga	N/A
<i>P. arrabidae</i>	L28A17	Presidente Kennedy - ES	-21.268000, -40.986000	Restinga	EAC 58007
<i>P. arrabidae</i>	L28A18	Marataízes - ES	-21.033000, -40.813000	Restinga	EAC 58035
<i>P. arrabidae</i>	L28A19	Guarapari - ES	-20.611000, -40.428000	Restinga	EAC 56051
	L28A20				
<i>P. arrabidae</i>	L28A21	São Mateus - ES	-18.731000, -39.747000	Restinga	N/A
<i>P. arrabidae</i>	L28A22	Mucuri - BA	-18.070000, -39.538000	Restinga	EAC 56058
<i>P. arrabidae</i>	L28A23	Santa Cruz Cabrália - BA	-16.315000, -39.024000	Restinga	EAC 56056
	L28A24				
<i>P. arrabidae</i>	L37A1	Rio das Ostras - RJ	-22.493556, -41.899306	Restinga	SORO 6295
	L37A2				
	L37A3				
	L37A4				
	L37A5				

	L38A1				
	L38A2				
<i>P. arrabidae</i>	L38A3	Armação de Búzios - RJ	-22.807167, -41.981028	Restinga	SORO 6300
	L38A4				
	L38A5				
<i>P. brasiliensis ruschianus</i>	S79E1	Colatina - ES	-19.662833, -40.440667	Inselberg	SORO 2654
	S79E2				
	S86F1				
	S86F2				
<i>P. brasiliensis ruschianus</i>	S86F3	Guarapari – ES	-20.659194, -40.616389	Inselberg	SORO 4568
	S86F4				
	S86F5				
<i>P. brasiliensis brasiliensis</i>	S131A4	Armação de Búzios - RJ	-22.718611, -42.011111	Inselberg	SORO 4912
<i>P. brasiliensis ruschianus</i>	L28K1	Santa Teresa - ES	-19.887000, -40.608000	Inselberg	EAC 56048
	L30A1				
	L30A2				
<i>P. brasiliensis ruschianus</i>	L30A3	Teófilo Otoni - MG	-17.90913, -41.27827	Inselberg	SORO 6306
	L30A4				
	L30A5				

	L31A1				
	L31A2				
<i>P. brasiliensis ruschianus</i>	L31A3	Argolô - BA	-17.86297, -40.12172	Inselberg	SORO 6304
	L31A4				
	L31A5				

	L32A1				
	L32A2				
<i>P. brasiliensis ruschianus</i>	L32A3	Colatina - ES	-19.39747, -40.67911	Inselberg	SORO 6301
	L32A4				
	L32A5				

	L33A1				
	L33A2				
<i>P. brasiliensis ruschianus</i>	L33A3	Santa Teresa - ES	-19.878250, -40.612472	Inselberg	SORO 6302
	L33A4				
	L33A5				

	L34A1				
	L34A2				
<i>P. brasiliensis ruschianus</i>	L34A3	Serra - ES	-20.18286, -40.29099	Inselberg	SORO 6305
	L34A4				
	L34A5				

<i>P. brasiliensis ruschianus</i>	S77A21	São João de Manteninha– MG	-18.726333, -41.185500	Inselberg	SORO 4540
	L35A1				
	L35A2				
<i>P. brasiliensis brasiliensis</i>	L35A3	Vila Velha - ES	-20.403444, -40.326056	Inselberg	SORO 6303
	L35A4				
	L35A5				
	L36A1				
	L36A2				
<i>P. brasiliensis ruschianus</i>	L36A3	Campos dos Goytacazes - RJ	-21.79911, -41.43619	Inselberg	SORO 6310
	L36A4				
	L36A5				
<i>P. brasiliensis brasiliensis</i>	L38B1	Armação de Búzios - RJ	-22.807083, -41.981028	Restinga	SORO 6308
	L38B2				
	S79A1				
	S79A2				
<i>P. ulei</i>	S79A3	Arraial do Cabo – RJ	-22.963333, -42.027667	Restinga	SORO 4557
	S79A4				
	S79A5				

<i>P. ulei</i>	L28L1	Armação de Búzios - RJ	-22.783000, -41.917000	Restinga	EAC 56053
<i>P. ulei</i>	L38U1	Armação de Búzios - RJ	-22.824694, -41.973861	Restinga	SORO 6296
	L39A1				
	L39A2				
<i>P. ulei</i>	L39A3	Cabo Frio - RJ	-22.876917, -42.004056	Restinga	SORO 6298
	L39A4				
	L39A5				