UNIVERSIDADE FEDERAL DE MINAS GERAIS

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

DEPARTAMENTO DE BIOLOGIA GERAL

PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA

DISSERTAÇÃO DE MESTRADO

"ONE STEP AWAY FROM EXTINCTION: A POPULATION GENOMIC ANALYSIS OF A NARROW ENDEMIC, TROPICAL PLANT SPECIES"

ORIENTADO: Thais Martins Teixeira

ORIENTADOR: Alison Gonçalves Nazareno

BELO HORIZONTE

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Dissertação apresentada ao Programa de Pós-graduação em Genética da Universidade Federal de Minas Gerais, como requisito obrigatório para a obtenção do título de Mestre em Genética.

ORIENTADO: Thais Martins Teixeira

ORIENTADOR: Alison Gonçalves Nazareno

BELO HORIZONTE

2021

ATA DE DEFESA DE DISSERTAÇÃO / TESE

ATA DA DEFESA DE DISSERTAÇÃO	309/2021
	Entrada 1º/2019
Thais Martins Teixeira	CPF: 118.306.856-51

Às 14:00 (quatorze) horas do dia **31 de maio de 2021**, reuniu-se remotamente, devido a situação de pandemia decorrente do COVID-19, a Comissão Examinadora de Dissertação, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho intitulado: **"ONE STEP AWAY FROM EXTINCTION: A POPULATION GENOMIC ANALYSIS OF A NARROW ENDEMIC, TROPICAL PLANT SPECIES"**, requisito para obtenção do grau de Mestre em **Genética.** Abrindo a sessão, o Presidente da Comissão, **Alison Goncalves Nazareno**, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra à candidata, para apresentação de seu trabalho. Seguiu-se a arguição pelos Examinadores, com a respectiva defesa da candidata. Logo após, a Comissão se reuniu, sem a presença da candidata e do público, para julgamento e expedição de resultado final. Foram atribuídas as seguintes indicações:

Prof./Pesq.	Instituição	CPF	Indicação
Alison Goncalves Nazareno	UFMG	220.542.388-64	APROVADA
Fernanda Antunes Carvalho	UFMG	045.665.426-70	APROVADA
Dulcineia de Carvalho	UFLA	443.209.816-34	APROVADA

Pelas indicações, a candidata foi considerada: **APROVADA**

O resultado final foi comunicado publicamente à candidata pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ATA, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 31 de maio de 2021.

Alison Goncalves Nazareno – Orientador (UFMG)

Fernanda Antunes Carvalho (UFMG)

Dulcineia de Carvalho (UFLA)

Assinatura dos membros da banca:

UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Genética

FOLHA DE APROVAÇÃO

"ONE STEP AWAY FROM EXTINCTION: A POPULATION GENOMIC ANALYSIS OF A NARROW ENDEMIC, TROPICAL PLANT SPECIES"

Thais Martins Teixeira

Dissertação aprovada pela banca examinadora constituída pelos Professores:

Alison Goncalves Nazareno - Orientador

UFMG

Fernanda Antunes Carvalho

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Dulcineia de Carvalho

UFLA

Belo Horizonte, 31 de maio de 2021.

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RESUMO

Endemismo, distribuição restrita e pequeno tamanho populacional são características importantes na avaliação do risco de extinção de espécies. Espécies endêmicas e restritas que possuem tamanhos populacionais reduzidos podem estar mais propensas à extinção (local) em decorrência da ação de fatores demográficos e genéticos. Neste contexto, estratégias de conservação para espécies em risco iminente de extinção devem ser baseadas em estudos genéticos e/ou ecológicos. *Mimosa catharinensis* Burkart (Fabaceae) é uma espécie endêmica, extremamente restrita e criticamente ameaçada de extinção da Mata Atlântica, possuindo apenas uma população com 33 indivíduos. Com o intuito de gerar informações genéticas que possam subsidiar estratégias de conservação para *M. catharinensis*, parâmetros genéticos e demográficos foram obtidos com o uso de marcadores SNPs (128 − 1.497) derivados da técnica de representação reduzida do genoma *ddRADseq* (*double-digestion Restriction Site associated DNA*). Nossa análise populacional evidenciou que apesar do tamanho populacional extremamente pequeno, *M. catharinensis* mantém níveis moderados de diversidade genética [$uH_{E(0\% \text{ dados faltantes})}$=0,205; IC95% (0,160 − 0,250); $uH_{E(30\% \text{ dados faltantes})}$=0,233; IC95% (0,174 − 0,292)], ausência de endogamia [$F_{(0\% \text{ dados faltantes})}$=-0,145; IC95% (-0,189 − -0,101); $F_{(30\% \text{ dados faltantes})}$=-0,105; IC95% (-0,199 − -0,011)] e altas taxas de cruzamento ($t_{(0\% \text{ dados faltantes})}$=0,883 ± 0,0483 EP; $t_{(30\% \text{ dados faltantes})}$=0,909 ± 0,011 EP). Ademais, inferências demográficas sugerem a ocorrência de um gargalo populacional recente para *M. catharinensis*, seguido de um declínio constante no tamanho populacional. Enfatizamos que os resultados obtidos neste estudo devem ser considerados nos planos de conservação de *M. catharinensis*. No entanto, há ainda a necessidade de se investigar fatores ecológicos importantes como a biologia reprodutiva da espécie para que estratégias mais efetivas de conservação possam ser empregadas para preservar esse recurso genético *sui generis*.

PALAVRAS CHAVE: Genômica da Conservação, História demográfica, ddRADseq, *Mimosa catharinensis*, SNPs.

ABSTRACT

Endemism, restrict geographic distribution, and small population size are relevant features for the assessment of extinction risk of species. Narrow endemic species can often occur in small population sizes, which implies higher risk of (local) extinction due to demographic and genetic factors. Therefore, conservation efforts for species on the verge of extinction should be based on genetic and/or ecological studies. *Mimosa catharinensis* Burkart (Fabaceae) is a critically endangered and extremely narrow endemic species from the Brazilian Atlantic Rainforest, with only one population (N=33). To provide genetic information to support conservation plans for *M. catharinensis*, we used the reduced-representation sequencing approach ddRADseq (double-digestion Restriction Site associated DNA) to generate genome-wide high-throughput sequencing data. Our population genomic analysis showed that, regardless its extremely small population size, *M. catharinensis* is maintaining moderate levels of genetic diversity [$uH_{E(0\%missing\ data)}$=0.205, 95%CI (0.160, 0.250); $uH_{E(30\%missing\ data)}$=0.233, 95%CI (0.174, 0.292)], absence of inbreeding [$F_{(0\%missing\ data)}$=-0.145, 95%CI (-0.189, -0.101); $F_{(30\%missing\ data)}$=-0.105, 95%CI (-0.199, -0.011)] and high outcrossing rates ($t_{(0\%missing\ data)}$=0.883, SE ± 0.0483; $t_{(30\%missing\ data)}$=0.909, SE ± 0.011). Furthermore, demographic inferences suggest the occurrence of a recent bottleneck for the *M. catharinensis* population followed by a constant declining population. We emphasize that the results obtained in this study should be considered on conservation actions for *M. catharinensis*. Going forward, it is still needed to investigate important ecological factors such as the species' reproductive biology to support adequate conservation strategies in order to safeguard this unique genetic resource.

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1 INTRODUÇÃO GERAL

1.1 Espécies endêmicas restritas

Analisando toda a biodiversidade de plantas vasculares documentadas do planeta, estima-se que aproximadamente 36% são raras (Enquist et al. 2019). Para avaliar o *status* de raridade de uma espécie são observadas três características: distribuição geográfica, tamanho populacional local e especificidade de habitat (Rabinowitz 1981). De acordo com o conceito proposto por Rabinowitz (1981), espécies comuns são localmente abundantes e amplamente distribuídas em diversos habitats, dessa forma, todas as demais combinações das características supracitadas resultam em uma das sete formas de raridade existentes (i.e., endêmica, amplamente distribuída em populações pequenas; endêmica, com distribuição ampla em populações grandes; endêmica, restrita mas com populações grandes; cosmopolita amplamente distribuída em populações pequenas; cosmopolita, geograficamente restrita em populações pequenas; e cosmopolita, geograficamente restrita mas com populações grandes; e endêmica, geograficamente restrita em populações pequenas). Espécies endêmicas e restritas são consideradas raridades clássicas, e usualmente possuem como agravante o fato de ocorrerem em populações muito pequenas (López-Pujol et al. 2013).

Uma das principais preocupações em relação as espécies endêmicas e restritas refere-se a sua susceptibilidade à extinção em decorrência de efeitos genéticos, principalmente se tratando das espécies que ocorrem em populações pequenas e isoladas, as quais, de acordo com os preceitos teóricos da genética de populações, são mais propensas a sofrerem efeitos estocásticos (i.e., mutação, deriva genética e fluxo gênico) e determinísticos (i.e., seleção natural), ocasionando aumento da homozigosidade em razão da fixação de alelos, e a ocorrência de endogamia, possibilitando a redução do potencial adaptativo devido à perda de variabilidade genética (Holsinger e Gottlieb 1991, Barret e Kohn 1991, Ouborg et al. 2010).

Neste contexto, estratégias de conservação efetivas para promover a sobrevivência de espécies caracterizadas por populações pequenas e isoladas, como as endêmicas restritas, dependem de informações obtidas em estudos genéticos e/ou ecológicos e da implementação de planos de manejo que visem o crescimento ou a estabilidade demográfica populacional (Ralls et al. 2018).

1.2 *Mimosa catharinensis* - Um endemismo extremamente restrito da Mata Atlântica

Considerado um *hotspot* mundial de biodiversidade (Myers et al. 2000), a Mata Atlântica é a segunda maior floresta pluvial tropical do continente americano e um dos biomas mais ameaçados do planeta (e.g., Myers et al. 2000, Tambarelli et al. 2005, Ribeiro et al. 2009, Ribeiro et al. 2011, Scarano e Ceotto 2015). Ocupando aproximadamente 7% de sua área original, a distribuição atual do bioma é caracterizada por áreas fragmentadas, pequenas e geograficamente isoladas (Ranta et al, 1998, Gascon et al. 2000, Myers et al. 2000, Ribeiro et al. 2009). No entanto, apesar de possuir um longo histórico de degradação, resultante em perdas relevantes de sua biota, a Mata Atlântica ainda abriga cerca de 20.000 espécies de plantas, das quais, mais de 8.000 são endêmicas (Mittermeier et al. 2005, Joly et al. 2014). Apesar dos esforços para a conservação dos remanescentes de Mata Atlântica, evidenciados pelo elevado número de áreas legalmente protegidas no bioma, a efetividade de unidades de conservação em reduzir as ameaças à extinção de espécies é questionada, tendo em vista que áreas protegidas podem ser vulneráveis a invasões antrópicas, incêndios e outros estresses ambientais (Bruner et al. 2001, Joppa et al. 2008, Laurance et al. 2012).

Incluída na diversidade de plantas endêmicas da Mata Atlântica, *Mimosa catharinensis* (Fabaceae) é uma espécie arbustiva lianosa que possui, como agravantes ao seu risco de extinção, tamanho populacional reduzido e distribuição extremamente restrita, apresentando ocorrência confirmada apenas em uma área de Restinga (>1 km²), na região litorânea do Município de Florianópolis, SC (Burkart 1979). Por ser uma espécie rara e endêmica, *M. catharinensis* é avaliada como Criticamente Ameaçada (CR) na lista de espécies ameaçadas do estado de Santa Catarina (Resolução CONSEMA 51/2014).

Descrita em 1964, a única população conhecida da espécie *M. catharinensis* está localizada no Parque Estadual do Rio Vermelho - PAERVE, reconhecido como unidade de proteção integral em 2007 (Burkart 1979, Ferreira 2010). O histórico da área de abrangência do PAERVE envolve longos períodos de exploração da vegetação e uso do solo e mais recentemente descaracterização da vegetação nativa remanescente para o plantio de espécies exóticas potencialmente invasivas, cuja ocupação perdura até os dias atuais, constituindo uma das principais ameaças a biodiversidade nativa da restinga da Ilha de Santa Catarina (Ferreira 2010). Assim como os demais ecossistemas incluídos no domínio da Mata Atlântica, a Restinga tem sofrido fragmentação massiva devido a ocupação antrópica em áreas costeiras, e em decorrência de sua heterogeneidade característica, seu grau de ameaça pode ser ainda maior se avaliada isoladamente (Neves et al. 2017).

Pesquisas elaboradas para subsidiar o plano de manejo do PAERVE (Ferreira 2010) chamam atênção para evidências de desmatamento dentro e nas regiões adjacentes a área do parque, despejo de resíduos em locais frequentados por visitantes e impactos na vegetação resultantes da prática de esportes e acesso por veículos automotores dentro dos limites da unidade de conservação, inclusive na estrada de acesso à praia de Moçambique, onde a população de *M. catharinensis* está localizada.

Em face do iminente risco de extinção associado ao alto grau de endemismo e ao tamanho populacional reduzido, medidas efetivas para assegurar a conservação dos indivíduos remanescentes de *M. catharinensis* são extremamente necessárias, e devem ser pautadas em estudos genéticos e/ou ecológicos.

1.3 Genômica da conservação

A redução dos custos das tecnologias de Sequenciamento de Alto Rendimento (*High-throughput DNA sequencing*) e o avanço nas ferramentas para lidar com dados obtidos por tais tecnologias propiciaram um aumento significativo na utilização de dados genômicos em estudos populacionais, dando início a era da genômica populacional (Allendorf et al. 2010, Ralls et al. 2018, Wright et al. 2019).

Em um sentido amplo, o termo genômica populacional refere-se ao estudo que incorpora uma grande quantidade de *loci* ou regiões genômicas com o objetivo de entender com maior precisão a forma com que os processos microevolutivos (i.e., mutação, fluxo gênico, deriva genética e seleção natural) influenciam a estrutura genética de populações naturais (Garner et al. 2016). Estudos de genômica populacional permitem o aumento da compreensão dos impactos genéticos e evolutivos das mudanças climáticas e ambientais em populações naturais, fornecendo também informações importantes sobre as bases genômicas relacionadas à adaptação local, bem como de parâmetros genéticos populacionais (e.g., diversidade e estrutura genética, tamanho efetivo populacional, taxas de fluxo gênico, endogamia), possuindo enorme potencial de utilização no desenvolvimento de estratégias mais efetivas para a conservação de espécies (Rajora 2019, Hohenlohe et al. 2021).

Ademais, embora técnicas tradicionais de genética de populações permitam estimativas de diversidade genética, estrutura populacional e demografia histórica, dados genômicos proporcionam um aumento significativo na densidade de marcadores distribuídos ao longo de todo o genoma, aumentando o poder estatístico das inferências e fornecendo estimativas mais

acuradas (Allendorf et al. 2010, Shafer et al. 2015) podendo em alguns casos resultar em diferentes recomendações para a conservação (Supple e Shapiro 2018).

1.4 Marcadores moleculares em estudos genômicos populacionais

Técnicas baseadas no Sequenciamento de Alto Rendimento têm possibilitado a obtenção de milhares de marcadores SNPs (*Single Nucleotide Polymorphisms*) em escala populacional, oferecendo diversas vantagens em relação aos marcadores comumente empregados nos estudos em genética de populações, como os marcadores microssatélites (Davey et al. 2011). Marcadores SNPs são polimorfismos de base única, e constituem o marcador mais abundante no genoma das espécies, sendo o seu uso muito vantajoso nos estudos genéticos (Brooks 1999).

Dentre as estratégias mais utilizadas em estudos de genômica populacional, destacam-se as metodologias de sequenciamento de representação reduzida do genoma - RRS, tendo em vista que as principais questões biológicas a serem acessadas dentro e entre populações podem ser respondidas com informações de polimorfismos ao longo do genoma, evitando custos excessivos com sequenciamentos de genomas completos (Davey et al. 2011).

As metodologias de RRS mais utilizadas para à identificação de marcadores SNPs aplicados nos estudos populacionais pertencem à família de protocolos de sequenciamento de DNA associado a sítios de restrição - *RADseq* (Andrews et al. 2016). Em geral, protocolos de *RADseq* apresentam algumas características comuns: fragmentação do DNA genômico utilizando endonucleases; ligação dos fragmentos de DNA a adaptadores e a sequências de identificação (i.e., *barcodes*); seleção de fragmentos de DNA de tamanho específico (Andrews et al. 2016). As principais diferenças técnicas entre os protocolos de *RADseq* estão relacionadas à quantidade e tipo de enzimas de restrição utilizadas e a metodologia empregada para a seleção dos fragmentos (i.e., manual ou automática). Ademais, os custos para a preparação da biblioteca genômica e os dados produzidos por cada método podem variar (Andrews et al. 2016). A maior vantagem da utilização de metodologias *RADseq* consiste no fato de que não requerem informações genômicas prévias da espécie a ser estudada. Dessa forma, permitindo a aplicação da técnica mesmo em espécies não-modelos (Andrews et al. 2016).

Dentre as metodologias *RADseq*, o protocolo *ddRADseq* (*double-digestion Restriction Site associated DNA*) desenvolvido por Peterson et al. (2012), um dos mais utilizados atualmente, é caracterizado pela utilização de duas enzimas de restrição e tem se mostrado uma metodologia muito vantajosa, tendo em vista a alta eficiência na descoberta e genotipagem de

SNPs a um custo mais baixo em comparação com outras técnicas de representação reduzida do genoma (e.g., GBS, CRoPS, ezRAD, e CUTseq; Andrews et al. 2016, Zhang et al. 2019).

2 OBJETIVO

2.1 Objetivo geral

Gerar informações genéticas com o intuito de subsidiar a conservação e a redução do risco de extinção de *Mimosa catharinensis.*

2.2 Objetivos específicos

a) Estimar os níveis de diversidade genética intrapopulacional;

b) Avaliar o efeito de dados faltantes nos parâmetros genéticos;

c) Determinar os parâmetros do sistema reprodutivo da espécie, como as taxas de cruzamento e autofecundação;

d) Investigar a ocorrência de reprodução clonal;

e) Investigar a história demográfica de *M. catharinensis.*

3 CAPÍTULO I

One step away from extinction: a population genomic analysis of a narrow endemic, tropical plant species

TITLE: One step away from extinction: a population genomic analysis of a narrow endemic, tropical plant species

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KEYWORDS: Conservation genomics, demographic history, ddRADseq, *Mimosa catharinensis*, SNPs.

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RUNNING TITLE: Population genomics of *Mimosa catharinensis*

3.1 ABSTRACT

Intraspecific genetic variation plays a fundamental role in maintaining the evolutionary potential of wild populations. Hence, the assessment of genetic diversity patterns becomes essential to guide biodiversity conservation policies, particularly for threatened species. To inform management strategies for conservation of *Mimosa catharinensis* – a narrow endemic, critically endangered plant species – we identified 1,497 unlinked SNP markers derived from a reduced representation sequencing method (i.e., double digest restriction site associated DNA sequencing, or ddRADseq). This set of molecular markers was employed to assess intrapopulation genetic parameters and the demographic history of one extremely small population of *M. catharinensis* (N=33) located in the Brazilian Atlantic Forest. Contrary to what is expected for narrow endemic and threatened species with small population sizes, we observed a moderate level of genetic diversity for *M. catharinensis* [$uH_{E(0\%\text{missing data})}$=0.205, 95%CI (0.160, 0.250); $uH_{E(30\%\text{missing data})}$=0.233, 95%CI (0.174, 0.292)]. Interestingly, *M. catharinensis*, which is a lianescent shrub with no indication of seed production for at least two decades, presented high levels of outcrossing ($t_{(0\%\text{missing data})}$=0.883, SE ± 0.0483; $t_{(30\%\text{missing data})}$=0.909, SE ± 0.011) and an apparent absence of inbreeding [$F_{IS(0\%\text{missing data})}$=-0.145, 95%CI (-0.189, -0.101); $F_{IS\ (30\%\text{missing data})}$=-0.105, 95%CI (-0.199, -0.011)]. However, the reconstruction of demographic history of *M. catharinensis* indicated that the population should be suffered a recent bottleneck. Our population genomic study tackles a central issue in evolution and conservation biology and we expect that it will be useful to help safeguard the remaining genetic diversity reported for this unique genetic resource.

3.2 INTRODUCTION

Differences in DNA sequences among individuals within a population represent its genetic diversity, a key component for the long-term survival of natural populations that is related to the maintenance of its evolutionary potential in the face of environmental change (e.g., Huenneke 1991, Reed and Frankham 2003, Spielman et al. 2004, Elegren and Galtier 2016, Frankham et al. 2017; but see Zimmermann et al. 2018). As a matter of fact, the impact of the loss of genetic variation on increasing risk of extinction for wild populations has been extensively debated in the past (e.g., Lande 1988, Caro and Laurenson 1994, Frankham 1995, Hedrick et al. 1995, Caughley and Gunn 1996, Frankham and Ralls 1998, Dobson 1999), suggesting that demographic parameters (e.g., population size, density, sex ratio, age structure, fecundity; see Tarsi and Tuff 2012) associated with stochastic and deterministic events should affect species on the verge of extinction before microevolutionary forces take effect (e.g., Frankham et al. 2010). Nonetheless, Spielman et al. (2004) reported that most threatened taxa, including plant species, present lower levels of genetic diversity than closely related non-threatened taxa, implying a higher risk of (local) extinction due to small population sizes, and as a consequence, a reduction in reproductive fitness.

Although the amount of genetic diversity in a population is commonly linked to its size and range (e.g., Frankham 1996, Hamrick and Godt 1996, Elegren and Galtier 2016), a better understanding is needed of the susceptibility of species that are rare, endemic, and with small population sizes to extinction due to reductions in genetic diversity. Species that present small population sizes and a restricted geographic range (i.e., narrow endemic) tend to present lower levels of genetic diversity than those with larger population sizes and wide distribution (Kimura and Crow 1964, Hamrick and Godt 1989, Honnay and Jacquemyn 2007; but see Ellis et al. 2006, Turchetto et al. 2016; Forrest et al. 2017, Martel et al. 2021). For instance, Honnay and Jacquemyn (2007) highlight significantly lower levels of genetic variation in small plant populations compared to large populations. The same pattern (i.e., low levels of genetic diversity) has been reported for threatened plant species with a history of fragmentation and/or population decline (e.g., Jiménez etal. 2014, Bupp et al. 2017, Simmons et al. 2018, Downey and Baskauf, 2020). In addition, high levels of inbreeding are expected for plant species with small and isolated populations (e.g., Falconer 1989, Barret and Kohn 1991, Allendorf and Luikart 2007, Frankham et al 2014, Oleas et al. 2014, Perrier et al. 2017, Rhoads et al. 2017, Toczydlowski and Waller 2019; but see Nazareno and Carvalho 2009, Guidugli et al. 2016), making the effects of genetic drift more pronounced. As a result, such populations are

particularly prone to (local) extinction, as they lose variability more readily when compared to populations in which drift is an unexpressive microevolutionary force (e.g., Barret and Kohn 1991, Bani et al. 2018, Toczydlowski and Waller 2019). In addition to genetic factors, small populations are subject to rapid decline and extinction due to demographic fluctuations and environmental changes (e.g., Primack and Rodrigues 2001, Frankham et al. 2010).

In this study, we aimed to assess the levels of genetic diversity and demographic history of a unique population of *Mimosa catharinensis* Burkart (Fabaceae), a rare, critically endangered (CONSEMA 2014), and narrow endemic species that occurs in a small area (~700 m^2) in the Brazilian Atlantic Forest (Burkart 1979). Considering the vulnerability of *M. catharinensis* to extinction and its unique reproductive biology (i.e., a plant species that is ecologically sterile), and based on the assumptions of population theory and the findings of empirical studies about genetic diversity in rare and endemic plant species occurring in small populations, we expected to find low levels of genetic diversity for *M. catharinensis*. Further, we expected to find indications of population retraction given the historic threats that have occurred in its biome (presented in the below section). To this end, and to contribute to *in situ* and *ex situ* conservation strategies, we used a high-throughput sequencing approach (i.e., double digest restriction site associated DNA sequencing, ddRADseq; Peterson et al. 2012) to identify unlinked and neutral SNP markers. This reduced representation sequencing method had been used extensively for species conservation studies (e.g., Nazareno et al. 2017, Chattopadhyay et al. 2019, Amor et al. 2020, Wright et al. 2020, Bard et al. 2021, Nazareno and Knowles 2021) mainly due to its robustness to generate thousands of neutral and non- neutral molecular markers at a relative low cost. Our population genomic study tackles a central issue in evolution and conservation biology and we expect that it will be useful to safeguard the genetic diversity reported for the only remaining population of *M. catharinensis*. The approach applied in this study can also be used to guide conservation efforts for other plant species on the brink of extinction.

3.3 MATERIALS AND METHODS

3.3.1 Species description

Mimosa catharinensis is a plant species endemic to the Brazilian Atlantic Rainforest with an extremely restricted distribution, limited to one population located in the Rio Vermelho State

Park (*Parque Estadual do Rio Vermelho* – PAERVE; Figure 1), Santa Catarina State, Southern Brazil (Burkart 1979, Ferreira 2010). The only documented population of *M. catharinensis* (~700m²) occurs in one of the most heavily impacted areas of the PAERVE (Figure 1) due to deforestation and environmental degradation for over 200 years (Berenhauser, n.d.). This narrow endemic and critically endangered plant species (CONSEMA 2014) is a lianescent shrub that produces masculine and hermaphrodite white, glabrous, and tetramerous flowers (Burkart 1979). Pollen dispersal occurs via zoophily (e.g., *Apis mellifera*; Silva et al. 2005), although nectar-dependent visitors are infrequent due to a lack of floral nectar (Silva et al. 2005). The pods are of the indehiscent craspedium type and are linear-oblong with curved prickles on the margins (Medeiros and Stefani 2018). Although the reproductive structures of *M. catharinensis* have been characterized morphologically, pods without seeds were observed during field collection in 2019. Based on herbarium records, the lack of seed production in *M. catharinensis* was first recorded in 1994 (Voucher 30,482; FLOR herbarium, UFSC). As no seedlings were found in its area of occurrence, *M. catharinensis* may be considered an ecologically sterile plant species (i.e., a plant species in which the recruitment rate over time is nil as a consequence of lack of sexual reproduction). As successful *in vitro* pollen germination had been reported for *M. catharinensis* (Silva et al. 2005), further pollination studies are needed to improve our understanding of its reproductive biology.

Although there are efforts by the Santa Catarina State government (Gasper et al. 2012) to generate genetic and ecological data to inform conservation efforts and to safeguard plant species threatened with extinction, no previous evolutionary studies – including phylogenetic analysis and genomic characterization – have been conducted on this critically endangered species.

3.3.2 History of the research area

Since 2002, the PAERVE has been recognized by UNESCO as one of the core areas of the Atlantic Forest Biosphere Reserve and consists of 1,530 hectares of Atlantic Rainforest biome (Ferreira 2010, Bechara et al. 2013). The introduction of pine and eucalyptus into the park has brought about significant changes to its *restinga* (sandy plains) vegetation and is the main cause of degradation of the coastal ecosystem of Santa Catarina Island (Ferreira 2010). Currently, it is estimated that 750ha of the park are covered by *Pinus* spp., of which about 33% are located in dunes and sandbanks that have been progressively invaded since pine was first introduced

(Bechara et al. 2013). Nevertheless, approximately 400ha of dense rainforest and 250ha of *restinga* vegetation fragments remain protected in the park (Bechara et al. 2013), the latter located mainly at the southern and northern tips of the conservation area (Ferreira 2010).

3.3.3 Population sampling

We collected leaf samples from all 33 identified adult individuals of unknown age that constitute the only remaining *M. catharinensis* population. Leaves were stored in silica gel for later extraction. One voucher (FLOR30482) is deposited at the FLOR herbarium at the Federal University of Santa Catarina – UFSC.

3.3.4 Genomic library preparation and sequencing

DNA was extracted from leaf samples of all collected individuals employing the NucleoSpin® kit (Machereney-Nagel GmbH & Co. KG) following manufacture's guidelines. After extraction, the quality of each sample was verified using Thermo Scientific NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific Inc.) and the concentration of double-strand DNA (dsDNA) was obtained by using Qubit dsDNA Assay Kit (Invitrogen). The genomic library was prepared using a double-digest restriction site-associated DNA sequencing (i.e., ddRADseq) protocol (Peterson et al. 2012) with modifications proposed by Nazareno et al. (2017). Digestions reactions were performed using 0.5µg of genomic DNA, 5,000 units of *MseI*, 5,000 units of HF-*EcoRI*, and 1x CutSmart buffer (New England Biolabs) in a total 20µl reaction volume for 3h at 37°C, ending with a 20min deactivation step at 65°C (Nazareno et al. 2017). Reactions products were cleaned using Agencourt AMPure XP system (Beckman Coulter) according to manufacturer's recommendations. The dsDNA was quantified using Qubit and the amount of DNA was standardized before to procedure with the ligation. We used 80ng DNA, 1M of *MseI* adaptor, 0.33M of *EcoRI* double-strand adaptor unique for each sample, 1U of T4 DNA ligase (New England Biolabs) and 1.40µl of T4 ligase buffer in 30µl ligation reaction that was stored at a room temperature (23°C) for 30min After, heat-killed the reaction at 65°C for 10min and then, slowly cooled until reach room temperature. Details of barcode sequences can be found in Nazareno et al. (2017). Products of the ligation reaction were purified using the Agencourt AMPure XP system and used in PCR reactions. PCR reactions were carried

out in a total volume of 20μl, containing 13.5μl of ligation product, 0.2M dNTPs, 1.0μM MgCl2 0.5U iProof™ High-Fidelity DNA Polymerase (Bio-Rad) and 2X of iProof buffer. The PCR protocol (98°C for 30s, 20 cycles of 98°C for 20s, 60°C for 30s and 72°C for 40s, followed by a final extension at 72°C for 10min) was performed in an Eppendorf System. The amplicons were cleaned with the Agencourt AMPure XP system and quantified using Qubit dsDNA assay Kit. We used an automated size-selection technology (i.e., Pippin Prep; Sage Science, Beverly, MA, United States) at 2% agarose cartridge to select DNA fragments at a target range size of 375–475bp. The library was sequenced (100-bp single-end) on a lane of Illumina HiSeq 2,500 flow cell (Illumina Inc., San Diego, CA, United States) at The Centre for Applied Genomics in Toronto, Canada.

3.3.5 Raw data processing and SNPs identification

Data quality was checked using the program FastQC version: 0.11.8 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The file containing raw sequence reads was analyzed in Stacks 2.41 (Catchen et al. 2011; Catchen et al. 2013, Rochette et al. 2019) using *de novo* assembly. Initially, we used the process_radtags program in Stacks to examine individual barcodes, enzyme cutsite integrity and to demultiplex the data. For barcodes rescue we admitted at most two mismatches (− barcode_dist 1 2). We filtered raw reads using the phred score 33 and used the option −t to trim all of it at 85 base pairs (−phred 33, −t85). With the process_radtags output, we run the Ustacks program, that uses a maximum likelihood framework to aligns short-read sequences and create putative alleles (stacks). The maximum distance allowed between "stacks" was two nucleotides. The minimum depth of coverage required to create a "stack" and the maximum number of stacks at a single de novo locus was set as three. We enabled the deleveraging algorithm (−d) to solve merged tags, and a bounded-error model to identify SNPs. An alpha value of 0.05 and an upper bound of 0.1 were used. In the next step, we performed Cstacks to merge alleles of all samples and create a catalog of consensus loci. Three mismatches were allowed between loci in catalog build. Each individual sample loci were then compared against the catalog through the Sstacks program. We ran tsv2bam program to organize the single-end reads by locus, instead by sample, creating a BAM file that was used as input to the Gstacks program. Gstacks uses the single-reads to build contigs and merges them into loci. It also aligns reads to the loci using Ukkonen's algorithm, identifies SNPs for each locus and each individual genotype and converts SNPs into phased haplotypes.

Finally, we used POPULATIONS in Stacks (Catchen et al. 2011, Catchen et al. 2013, Rochette et al. 2019) to generate and export the SNP data set, as well as FASTA files containing the per-locus consensus sequences, and individual loci sequences, that were applied in the further analyses. To assess the effects of missing data on genetic estimates, we run POPULATIONS several times with a Minor Allele Frequency (MAF) of 5% (−min_maf 0.05), a maximum heterozygosity (−max_obs_het) of 0.65, and the percentage of missing data varying from 0 to 30%. All data sets include one random SNP per locus. To avoid potential bias sources in the forward genetic analyses, Hardy–Weinberg (H–W) equilibrium tests was done using the adegenet and pegas packages (https://CRAN.R- project.org/package=adegenet; Jombart 2008, Paradis 2010, Jombart and Ahmed 2011) implemented in R. In addition, linkage disequilibrium (LD) between loci was tested using Arlequin 3.5.2 (Excoffier and Lischer 2010). Type I error rates for these tests were corrected for multiple comparisons using the sequential Bonferroni procedure (Rice 1989), and SNPs that failed the H–W equilibrium test and/or SNP pairs in LD were excluded from further analyses. To exclude non-nuclear loci, the per-locus consensus sequences were aligned against reference chloroplast and mitochondrial genomes using *Acacia dealbata* (NCBI accession number KX852435) and *Acacia ligulata* (NCBI accession number MH933866), respectively. We used the BLAST*n* program (http://blast.ncbi.nlm.nih.gov) removing loci that presented identity greater than or equal to 80%.

3.3.6 Genetic diversity

To assess the influence of missing data on genetic diversity estimates, we used the "BasicStats" function in the DiveRsity package in the R software environment (https://CRAN.R-project.org/package=diveRsity; Keenan et al. 2013). We estimated unbiased expected genetic diversity (uH_E; Nei and Roychoudhury 1974), observed heterozygosity *(H_O)*, and the inbreeding coefficient (Wright's Fixation Index F_{IS}; Wright 1943). Population genetics statistics were averaged across loci using the DiveRsity package in R (Keenan et al. 2013). Confidence intervals at 95% were obtained to evaluate differences among means of genetic parameters estimated for all data sets. We used the final data set to calculate minor allele frequencies using the adegenet package in R (Jombart 2008, Jombart and Ahmed 2011).

3.3.7 Mating system of *Mimosa catharinensis*

We used the kinship coefficient (Loiselle et al. 1995) implemented in the SPAGeDi program (version 1.5; Hardy and Vekemans 2002) to estimate random outcrossing rates (1-*s*, where *s* indicates the estimated selfing rate) based on standardized identity disequilibrium for all data sets. Significance for the identity disequilibrium coefficient was obtained with 1,000 permutations, and a jackknife over loci approach was applied to calculate the standard error of outcrossing estimates.

3.3.8 Clonality assessment

We evaluated the power of discrimination (PD) for the complete single nucleotide polymorphism (SNP) data set (i.e., the data set without missing data; MD) using the equation 1-*PI*, where PI represents the probability that two individuals drawn at random from a population will have the same genotype at multiple loci (Waits et al. 2001). This analysis was performed in GeneAlEx 6.5 program (Peakall and Smouse 2012). Taking into account that clonal reproduction has been reported for plant species of the Fabaceae family (e.g., Dev et al. 2010, Gui et al. 2013, Gaddis et al. 2014, Mori et al. 2018, Amor et al. 2020), including *M. catharinensis* (Rogalski et al. 2005), a clonality test was applied for *M. catharinensis* using the "poppr" package (Kamvar et al. 2014, Kamvar et al. 2015) in R version 3.5.3 (R development Core Team 2019). The "bitwise.dist" function was used to calculate a pairwise genetic distance matrix between individuals. Then, we ran the "mlg.filter" function to apply a threshold that defines the minimum distance to determine distinct multi-locus genotypes (MLGs; see Kamvar et al. 2015). Applying a threshold when using SNP markers is important in order to deal with the limited observable genetic differences between genets due to sporadic somatic mutations or issues associated with high-throughput sequencing technologies (e.g., genotyping errors and missing data; Kamvar et al. 2015, Mastretta-Yanes et al. 2015). We chose the average neighbor as the clustering algorithm with a threshold of 0.04, which has been shown to be sufficient to explain intra-clonal variation when SNPs derived from ddRADseq are used (Amor et al. 2020). In addition, a less conservative threshold of 0.06 was used to infer the numbers of MLGs in *M. catharinensis*. The threshold of 0.06 was chosen in order to minimize the limitation of our experimental design, which did not include replicates to estimate genotyping errors. To

visualize the number of putative lineages, we ran the "upgma" function (Phangorn package; Schliep 2011) to construct ultrametric trees.

3.3.9 Demographic history

The demographic history of *M. catharinensis* was inferred using a composite likelihood-based approach implemented in FastSimCoal v. 2.6 (Excoffier et al. 2013). VCF files were converted into the *.arp format using the program PGDSpider 2.1.1.5 (Lischer and Excoffier 2012). Assuming genetic distances of 0.04 and 0.06, we obtained two observed folded Site Frequency Spectrums (SFS based on minor allele frequency) for our filtered SNP data set using the Arlequin 3.5 program (Excoffier and Lischer, 2010). We tested four evolutionary scenarios: constant population size, bottleneck, population decline, and population expansion (Figure 2). We performed 100 replicates for each tested model and folded SFS. The parameters for each run were estimated based on 100,000 simulations and 40 ECM optimization cycles. We used an overall substitution rate of 7×10^{-9} per site/generation as reported for *Arabdopsis thaliana* (Ossowski et al. 2010). The maximum estimated likelihood for each demographic scenario and SFS were used to identify the model with the best fit, which was chosen based on Akaike's Information Criterion (AIC; Akaike 1973, 1974) estimated as ΔAIC. All models were ranked, with the model with the lowest ΔAIC being considered the most plausible. It is important to note that models with ΔAIC ≤2 have substantial support (Burnham and Anderson, 2001). We also assessed the probability that each model is the best fit by estimating Akaike weights (w_i). Once the best model was selected, its estimated parameters were used to simulate 100 SFS data sets to built confidence intervals based on bootstrap distribution.

3.4 RESULTS

3.4.1 Raw data processing and SNP calling

The Illumina sequencing generated a total of 79,759,227 raw reads, of which less than 1% was discarded due to low quality. The average (±SE), minimum, and maximum retained reads were 2,384,161 (±115,118 SE), 1,470,849, and 4,011,918. The total number of genotyped RADtag loci was 274,113 with a mean coverage depth of 12.7 (±2.7 SD). After Bonferroni adjustment, significant deviations from Hardy–Weinberg equilibrium were observed for all data sets

(Table 1). No linkage disequilibrium was observed between loci after Bonferroni correction for k tests (Table 1). After alignment, one locus matched the plastome of *A. dealbata* (96.47% identity) and it was excluded from the data set. The final numbers of unlinked SNPs used in each data set are shown in Table 1.

3.4.2 Genetic diversity parameters, inbreeding coefficient, and outcrossing rate

Genetic diversity parameters (uH_E and H_O) and Wright's Fixation Index (F_{IS}) for data sets with different percentages of missing data are shown in Table 1. For all genetic indices (uH_E, H_O, and F_{IS}) obtained for the *M. catharinensis* population, no statistical differences were observed among the data sets (Table 1). Further analyses were performed using the data set without missing data, which presented a minor allele frequency (MAF) averaged at 0.125 (±0.093 SD). Based on mating system analysis for the data sets with different percentages of missing data (Table 1), the outcrossing rates varied from 0.883 (SE±0.0483) to 0.909 (SE±0.011). Although the outcrossing rate was inflated with an increase in MD, no statistical differences were observed among the data sets (Supplementary Table S1).

3.4.3 Clonality Assessment

The results of the PD analysis showed that at least 13 loci are enough to discriminate closely related individuals with high accuracy, indicating that the data set with 0% missing data (i.e., 128 SNPs) has high discriminatory power to identify *M. catharinensis* individuals. The defined threshold of 0.04 did not cluster MLGs into multilocus lineages (MLLs; Figure 3A). While the genetic distance of 0.04 did not indicate the presence of clonal genotypes for *M. catharinensis*, the threshold of 0.06 indicates 23 putative MLLs (Figure 3B).

3.4.4 Demographic history

The highest likelihoods are summarized in Table 2, as well as the results for the Akaike estimates (ΔAIC and w_i). The model with the best fit for the complete (N=33) and the reduced (n=23) data sets was the bottleneck (Table 2). The inferred parameters for the bottleneck model and the 95% CIs obtained for the data sets with different numbers of individuals are shown in Supplementary Table S2. The parameter estimates under the empirical SFS suggests that

population contraction occurred only a few generations ago ($TBOT_{compl}$= 82; $TBOT_{33}$=10), with a continuous decline in population size after the bottleneck. However, the 95% CIs were wide and overlapping.

3.5 DISCUSSION

Genomic approaches are valuable as they can provide new insights and a better understanding of the amount, distribution, and functional significance of genetic variation in natural populations (Allendorf et al. 2010). As predicted by Funk et al. (2012), the use of genomic data is on the rise for species in relation to discussions around conservation (Hawkins et al. 2018, Huy et al. 2018, Lanes et al. 2018, Ball et al. 2020, Bao et al. 2020, Chen et al. 2020, Liu et al. 2020, Zang et al. 2020, Zimmerman et al. 2020, Bradbury et al. 2021). Thus, such data can play a critical role in informing management strategies and policy for species on the verge of extinction, many of which are narrow endemics and prime targets for conservation genomic assessments (Silva et al. 2020). Considering that estimates of genetic parameters have a direct influence on conservation decision-making, we applied a reduced representation library approach (i.e., ddRADseq) to generate genomic data for *M. catharinensis*. This data was used to assess intrapopulation genetic variation, as well as the demographic history for this critically endangered, narrow endemic species of the Atlantic Rainforest.

Our findings show that, contrary to what is expected for narrow endemics (Kimura and Crow 1964, Hamrick and Godt 1989, Spielman et al. 2004, Honnay and Jacquemyn 2007), and despite its extremely small population size, *M. catharinensis* has moderate intrapopulation genetic diversity, expressed here by HE, with an apparent absence of inbreeding. Although the use of only one metric to assess genetic diversity is not an optimal condition, our results bring to light several factors that can influence the observed patterns of genetic diversity and inbreeding in relictual populations of threatened species. Our results underscore the importance of assessing the genetic patterns of each species in order to critically evaluate their genetic variability and the microevolutionary forces by which they are shaped (Amos and Harwood 1998, Turchetto et al. 2016). Below, we discuss how our findings can inform conservation and management strategies for *M. catharinensis*.

3.5.1 Contradicting patterns of genetic diversity in small populations

Despite the general expectation of low intrapopulation genetic variability and high susceptibility to inbreeding depression associated with pronounced effects of genetic drift in small populations (e.g., Kimura and Crow 1964, Hamrick and Godt 1989, Barret and Kohn 1991, Ellstrand and Elam 1993, Honnay and Jacquemyn 2007), the results for *M. catharinensis* do not support the hypothesized loss of genetic diversity, which should reflect high levels of inbreeding, as a consequence of reduced population size and narrow endemism. Although contradictory to theoretical predictions, unexpectedly high levels of genetic diversity are not unusual for narrow endemics (e.g., Fernández-Mazuecos et al. 2014, Jiménez-Mejías et al. 2015, Turchetto et al. 2016, Forrest et al. 2017, Goetze et al. 2018, Sękiewicz et al. 2020, Bard et al. 2021, Garcia-Jacas et al. 2021), creating an indistinct pattern for this kind of plant species. For instance, in the studies by Fernández-Mazuecos et al. (2014) and Jiménez-Mejías et al. (2015), the authors highlight the paradox of genetic diversity levels in narrow and extremely narrow endemic plant species from the Mediterranean. While Fernández-Mazuecos et al. (2014) found moderate levels of genetic diversity for *Naufraga balearica*, Jiménez-Mejías et al. (2015) reported high levels of genetic diversity for *Pseudomisopates rivas-martinezii.* In comparison to previous genetic assessments for narrow endemics in the same regions, the authors (Fernández-Mazuecos et al., 2014, Jiménez-Mejías et al. 2015) found that, although some species have low genetic variability, most species showed moderate to high levels of genetic diversity (e.g., Sales et al. 2001, Coppi et al.2008, Mameli et al. 2008, Mayol et al. 2012, De Castro et al. 2013, Forrest et al. 2017, Sękiewicz et al. 2020, Garcia-Jacas et al. 2021). Thus, narrow endemism does not always imply limited genetic diversity. However, such contrasting results may be due to differences in population size, distribution range, ecological traits, and evolutionary history (Fernández-Mazuecos et al. 2014).

In addition, several studies have indicated that life-history traits (e.g., lifecycle, growth form, mating and breeding systems) strongly influence the amount and distribution of genetic variation in natural populations (e.g., Hamrick and Godt 1996, Ellis et al 2006, Elegren and Galtier 2016, Goetze et al. 2018, Bard et al. 2021, De Kort et al. 2021). For *Aechmea kertesziae*, a narrow endemic species of Brazilian Coastal *restingas* (sandy vegetation), traits such as self-incompatibility, long-term persistence, clonal reproduction, and consistent population size may explain the high levels of genetic diversity observed for the species (Goetze et al. 2018). Specifically for *M. catharinensis*, the predominance of historical outcrossing, as shown in our analysis, seems to be an important factor that contributes to the moderate levels of

intrapopulation genetic diversity. It is important to note that outcrossing plants are likely to present higher levels of intrapopulation genetic diversity than self-compatible species (Hamrick and Godt 1996, Glémin et al. 2006, Ball et al. 2020). Moreover, a lack of successful sexual reproduction in *M. catharinensis* (i.e., production of pods without seeds) suggests that the level of intrapopulation genetic diversity has remained static for at least two decades, even though our demographic inferences indicated a recent decline in population size.

As life-history traits play a significant role in shaping the patterns of genetic diversity, it is important to compare the levels of genetic diversity among congeneric species in order to mitigate phylogenetic effects (Bevill and Louda 1999, Simon and Hay 2003). This is essential to better understand how features such as population sizes and geographic distribution ranges affect reproductive biology and, as a consequence, the genetic variability of closely related plant species. For example, population genetics studies indicate that *Petunia secreta* and *Petunia exserta*, both of which are narrow endemic species, have high genetic diversity indices; however, these indices are lower than those reported for its congener *Petunia axillaris*, which has a wider geographic distribution range (Turchetto et al. 2016). Beyond dissimilarities in the area of occurrence of these *Petunia* species, different floral syndromes and reproductive systems have been described as the probable causes of the observed genetic variation (Kokubun et al. 2006, Turchetto et al. 2016).

Although *Mimosa* is one of the largest genera from Mimosoideae (Barneby 1991), there are few population genetics studies for species of this genus (Moreira et al. 2011, Pramual et al. 2011, Arruda et al. 2019, Araújo et al. 2020, Morales et al. 2020), and none of these previous studies used SNP markers. Nevertheless, genetic diversity has been estimated using markers such as RAPD (*Mimosa pigra*; Pramual et al. 2011), AFLP (*Mimosa* subser. Dolentes–Brevipedes; Morales et al. 2020), ISSR (*M. caesalpiniifolia* Benth.; Araújo et al. 2020), and allozyme (*Mimosa scabrella*; Moreira et al. 2011, Arruda et al. 2019). In this context, *M. catharinensis* presented lower levels of intrapopulation genetic diversity than that reported for its widespread congener *M. scabrella* (H_E=0.362 to 0.469; Moreira et al. 2011, Arruda et al. 2019).

When comparing studies on narrow endemics assessed using SNPs, and considering the theoretical maximum heterozygosity for biallelic markers (H_E=0.5), we are unable to make generalizations about the genetic diversity of narrow endemics. Indeed, *M. catharinensis* displays higher levels of genetic diversity than self-compatible (e.g., Ball et al. 2020) and clonal and functional sterile species (e.g., Amor et al. 2020). However, some tropical plant species

(e.g., Lanes et al. 2018, Ball et al. 2020) showed the most similar genetic diversity to *M. catharinensis.*

3.5.2 Inbreeding and mating system of *Mimosa catharinensis*

Our findings indicate an excess of heterozygosity for this plant species, counteracting the theoretical expectation that reductions in population size will result in high levels of inbreeding. In terms of genetic variability, the levels of inbreeding in narrow endemic plant species with small population sizes vary according to individual features of each species/population (Angeloni et al. 2011). Reports for plant species with very small populations vary from high levels of inbreeding (Jiménez et al 2014, Finlay et al. 2017, Rodrigues et al. 2019, Wang 2020), to no evidence of inbreeding (Edwards et al. 2014, 2021, Spoladore et al. 2017), to an excess of heterozygotes (Cabrera-Toledo et al. 2008, Radosavljević et al. 2015, Amor et al. 2020) as found herein. Barret and Kohn (1991) emphasize that the mating pattern is a prime determinant of inbreeding levels in natural populations regardless of their size. In fact, self-compatible species can be more susceptible to inbreeding considering that self-incompatibility is likely to have evolved to prevent inbreeding depression (Charlesworth and Charlesworth, 1987). On the other hand, predominantly outcrossing species can suffer from inbreeding depression due to mating between relatives (Husband and Schemske 1996).

The (historical) outcrossing rate observed in *M. catharinensis* suggests that it has a mixed mating system, which is characterized as ranging from 5 to 95% depending on environmental conditions and pollination frequency (Karasawa 2015). Such a wide range in outcrossing rates for mixed-mating species are common and can change over time and space due to environmental conditions and intrinsic population features, such density and demographic structure (e.g., Wright et al. 2013). As with *M. catharinensis*, high outcrossing rates have been reported for many Neotropical plant species (e.g., Ward et al. 2005, Feres et al. 2012, 2021; Nazareno and Reis 2012, Spoladore et al. 2017, Godoy et al. 2018, Montagna et al. 2018, Sujii et al. 2021), including two congener *Mimosa* species (Moreira et al 2011, Arruda et al. 2020). Studies on populations of *M. scabrella* (Arruda et al. 2020), for instance, showed a similar reproductive pattern with high outcrossing rates (t=0.925/0.845) and low estimates of selfing (s=0.075/0.155). For *M. catharinensis*, the interpretation of negative F_{IS} values coupled with high *t* values should be viewed with caution due to the distinct biology of the species (i.e., production of seedless pods and absence of seedlings in the study area). Such biological

characteristics observed for *M. catharinensis* suggest that a significant selective pressure against homozygotes must have occurred in previous generations, especially when taking into account the excesses of heterozygosity observed in its small population. As *M. catharinensis* population is constituted only by adult trees, a further study investigating inbreeding depression seems not to be a feasible task as a consequence of the lack of different ontogenic stages in its population.

Although pollen grains have been reported as viable for *M. catharinensis* (Silva et al. 2005), the apparent reproductive failure seems to be due to an extremely low frequency of floral visitors (Silva et al. 2005), implying low pollination efficiency. This phenomenon has been also described for other *Mimosa* species, such as *Mimosa bimucronata* (Seijo and Neffa, 2004). As with *M. catharinensis, M. bimucronata* does not produce nectar, however, its newly opened flowers exude a slight fruit odor to attract pollinators (Silva et al. 2011). Despite poor fruit set due to inefficient pollination, *M. bimucronata* produces viable fruits and seeds (Seijo and Neffa 2004). In contrast, *Mimosa pudica* showed high pollination efficiency, mediated by *A. mellifera,* resulting in high fruit and seed yields and quality (Taimanga and Fohouo 2018). Further pollination biology studies are needed to clarify the causes of reproductive failure in *M. catharinensis*.

3.5.3 Reconstruction of the demographic history of *Mimosa catharinensis*

The selection of the best demographic model was the main focus of the coalescent-based inference for *M. catharinensis*, considering a comparison of two data sets with different numbers of individuals and excluding putative clonal individuals based on their genetic distance. The fact that confidence intervals were wide and overlapping did not affect our results and can be related to the uncertainty of the estimated parameters and/ or the small SFS (Rödin-Mörch et al. 2019). Nonetheless, the values presented in Supplementary Table S2 should be interpreted with caution. The generated SFS for both data sets suggest that a recent bottleneck is the best explanation for the current levels of genetic diversity in *M. catharinensis*. This corroborates our findings of excess heterozygosity for the species, which is a characteristic of populations that have experienced a recent bottleneck (Luikart et al. 1998). Of the factors that may have resulted in a drastic decrease in species population sizes in the *restinga*, the fragmentation of Santa Catarina Island's native vegetation is particularly relevant, especially the degradation that occurred within the PAERVE before it was deemed a protected area. The

implementation of reforestation projects in the park using exotic, invasive species also resulted in fires, changes to the soil, and deforestation of native vegetation, all of which accentuated the damage occurring to such a heterogeneous vegetal formation (Heberle 2011).

In contrast to the evidence of population contraction for *M. catharinensis*, the majority of studies on past demographic changes in tropical plant species from South America have reported indications of expansion during the Quaternary period (e.g., Turchetto-Zolet et al. 2013). On the other hand, demographic stability has been suggested for the period for species from the Southern Brazil coastal *restinga* (Goetze et al. 2018). Therefore, further studies are needed to infer historical changes in population sizes of plant species exclusive to Santa Catarina Island (Hassemer et al. 2015), whose recent demographic changes may be similar to that reported herein for *M. catharinensis*.

3.5.4 Implications for conservation

The extremely small number of individuals (N = 33) in the only known *M. catharinensis* population, coupled with strong evidence for a lack of sexual reproduction, suggests that the conservation perspectives for this critically endangered species are concerning. Although no inbreeding was detected, the species is facing imminent risk of extinction because of its reduced population size, making it more susceptible to stochastic events. As a matter of fact, ex situ conservation activities must be implemented in order to safeguard the remaining genetic diversity of *M. catharinensis*. One potential strategy suggested in studies on biodiversity conservation of threatened species consists of the preservation of tissue culture, an *in vitro* cultivation of isolated live tissue, enabling the propagation of species facing difficulties with natural reproduction (Paiva and Paiva 2001, Santos et al. 2019). Protocols to apply tissue culture techniques for conservation of endangered species have been successfully developed (e.g., Sherif et al. 2018, Choudary et al. 2020, Lerin et al. 2021; Mishra et al. 2020), and such an approach has been used to conserve the narrow endemic species *Styphelia longissima* (Thomas et al. 2021).

In addition, as *in vitro* germination tests show pollen grain viability (Silva et al. 2005), additional pollination biology studies (e.g., pollination treatments such as manual cross-pollination and self-pollination) should be performed to test for viable seed production. The information available through pollination biology studies can help us to solve the puzzle of *M. catharinensis* reproduction, allowing us to better plan effective conservation measures for this

rare plant species. In terms of *in situ* conservation, the fact that the species is found in a protected area is a significant starting point, although to date this fact has been insufficient for its conservation. Today, the management of invasive, exotic species (e.g., *Pinus* spp. and *Eucalyptus* spp.), one of the main issues threatening the flora of PAERVE, should be prioritized. Furthermore, it is widely reported that adequate restoration of the contaminated areas of the park are urgently needed (Ferreira 2010, Bechara et al. 2013), and efforts to achieve this goal have been implemented according to the park's Management Plan (IMA 2020). Moreover, although a robust floristic survey of Santa Catarina State has been conducted (Vibrans et al. 2012), not all the biodiversity present in the state was sampled. As such, biodiversity inventories within Brazil's protected areas are crucial (Oliveira et al. 2017), and efforts on field surveys must be undertaken to verify the narrow occurrence of this critically endangered plant species. Likewise, we are committed to monitoring the *M. catharinensis* population size over time to avoid further losses in genetic diversity.

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3.7 FUNDING

3.8 DATA ACCESSIBILITY

SNP data set for *Mimosa catharinensis* is available for download from the Dryad Digital Repository (https://doi.org/10.5061/dryad.j9kd51ccz).

3.9 AUTHOR CONTRIBUTIONS

AN designed the study, collected the samples, and conducted molecular work. TT performed analyses, and led the writing of the manuscript with input from AN, who also provided analytical support. All authors contributed to the article and approved the submitted version.

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TABLE 1 Variation of genetic parameters (H_O, observed heterozygosity; uH_E, unbiased expected heterozygosity; F, Wright's Fixation Index) according to changes in percent of missing data for the only known population of *Mimosa catharinensis*.

% MD	SNPs	HWE[1]	LD[2]	Blast[3]	Filtered SNPs	H_O	95%CI	uH_E	95%CI	F	95%CI
0	139	(10)$; p < 3.6 \times 10^{-5}$	(00); $k=9.59 \times 10^{4}$, $p<5.2 \times 10^{-7}$	01 (cp)	128	0.245	0.183, 0.307	0.205	0.160, 0.250	-0.145	-0.189, -0.101
5	283	(19); $p < 1.8 \times 10^{-5}$	(00); $k=3.99 \times 10^{5}$, $p<1.2 \times 10^{-7}$	01 (cp)	263	0.269	0.201, 0.337	0.224	0.174, 0.274	-0.147	-0.207, -0.087
10	591	(50); $p < 8.5 \times 10^{-6}$	(00); $k=1.74 \times 10^{6}$, $p<2.9 \times 10^{-8}$	01 (cp)	540	0.283	0.210, 0.356	0.233	0.181, 0.285	-0.152	-0.221, -0.083
15	733	(65); $p < 6.8 \times 10^{-6}$	(00); $k=2.68 \times 10^{6}$, $p<1.9 \times 10^{-8}$	01 (cp)	667	0.287	0.213, 0.361	0.234	0.185, 0.291	-0.148	-0.221, -0.075
20	1,019	(97); $p < 4.9 \times 10^{-6}$	(00); $k=5.18 \times 10^{6}$, $p<9.6 \times 10^{-9}$	01 (cp)	921	0.293	0.217, 0.369	0.246	0.192, 0.300	-0.138	-0.223, -0.053
25	1,404	(118); $p < 3.6 \times 10^{-6}$	(00); $k=9.84 \times 10^{6}$, $p<5.1 \times 10^{-9}$	01 (cp)	1,285	0.277	0.199, 0.355	0.238	0.181, 0.295	-0.115	-0.206, -0.024
30	1,634	(136); $p < 3.1 \times 10^{-6}$	(00); k=1.33×10^{7} $p<3.74 \times 10^{-9}$	01 (cp)	1,497	0.268	0.188, 0.348	0.233	0.174, 0.292	-0.105	-0.199, -0.011

[1]Number of SNPs with significant departures from Hardy-Weinberg equilibrium (HWE) after a Bonferroni adjustment.

[2]Number of SNPs with significant departures from Linkage Disequilibrium (LD) after a Bonferroni adjustment.

[3]Number of SNPs that matched some regions of the chloroplast (cp) and/or mitochondrial (mt) reference genomes.

TABLE 2 Comparison among demographic models for the *Mimosa catharinensis* population. The most plausible demographic scenario for each dataset is in bold.

I[1]/Model	k[2]	Max Est Lhood[3]	Max Obs Lhood[4]	AIC[5]	ΔAIC[6]	w_i[7]
33						
Neutral	1	-179.30	-148.16	823.72	9.50	8.0×10^{-4}
Bottleneck	7	-175.06	-148.16	814.21	0.00	1.0×10^{0}
Expansion	4	-179.21	-148.16	833.31	19.09	7.1×10^{-6}
Decline	4	-179.23	-148.16	833.38	19.17	6.8×10^{-6}
23						
Neutral	1	-579.40	-143.06	1160.81	754.98	1.1×10^{-165}
Bottleneck	7	-195.91	-143.06	405.82	0.00	1.0×10^{0}
Expansion	4	-518.76	-143.06	1045.53	639.70	1.2×10^{-140}
Decline	4	-296.43	-143.06	600.876	195.05	4.4×10^{-44}

[1]Number of individuals used in the SFS (Site Frequency Spectrum) estimates.
[2]Number of independently adjusted parameters within the model.
[3]Maximum estimated likelihood.
[4]Maximum observed likelihood.
[5]Akaike's information criterion ($2k$-2lnLhood).
[6]Difference between the calculated AIC and the minimum AIC ($AIC_i - AIC_{min}$).
[7]Akaike's weight.

TABLE S1 Selfing (*s*) and outcrossing (1-*s*) rates estimated for *Mimosa catharinensis* according to changes in percent of missing data (MD).

MD %	*s*	1-*s*	95% CI*
0	0.116	0.883	0.789 - 0.977
5	0.115	0.885	0.820 - 0.949
10	0.100	0.900	0.862 - 0.937
15	0.094	0.906	0.872 - 0.939
20	0.102	0.898	0.870 - 0.925
25	0.098	0.902	0.879 - 0.925
30	0.091	0.909	0.887 - 0.930

*95% confidence intervals calculated for 1-*s*.

TABLE S2 Inferred parameters for the bottleneck model for *Mimosa catharinensis* considering the complete (N=33) and the reduced (n=23) data sets. The 95% confidence intervals were obtained based on 100 parametric bootstraps.

Parameter	Complete	95%CI	Reduced	95%CI
NCUR[1]	2157.0	168.0 - 11082.5	530.0	13.0 - 18941.0
NANC[2]	108210.0	49.0 - 105906.0	1045457.0	25.5 - 1045457.0
NBOT[3]	13934.0	9198.5 - 97765.5	97872.0	10124.0 - 104697.5
TBOT[4]	82.0	16.0 - 8106.0	10.0	13.5 - 9113.5

[1] Current population size.
[2] Ancestral population size.
[3] Population size at the end of the bottleneck.
[4] Number of generations since the bottleneck occurred.

3.11 FIGURE CAPTIONS

FIGURE 1 Location of the extremely small and unique population of *Mimosa catharinensis* in Rio Vermelho State Park (*Parque Estadual do Rio Vermelho*; PAERVE), Santa Catarina State, Southern Brazil.

FIGURE 2 Representation of the demographic scenarios tested for *Mimosa catharinensis* in FastSimCoal 2.6. Estimates include coalescent-based current (NCUR) and ancestral (NANC) population size, number of generations since the bottleneck occurred (TBOT), and population size at the end of the bottleneck (NBOT).

FIGURE 3 The ultrametric topology trees for the *Mimosa catharinensis* population based on genetic distances (horizontal axis) and using a threshold of (A) 0.04 and (B) 0.06.
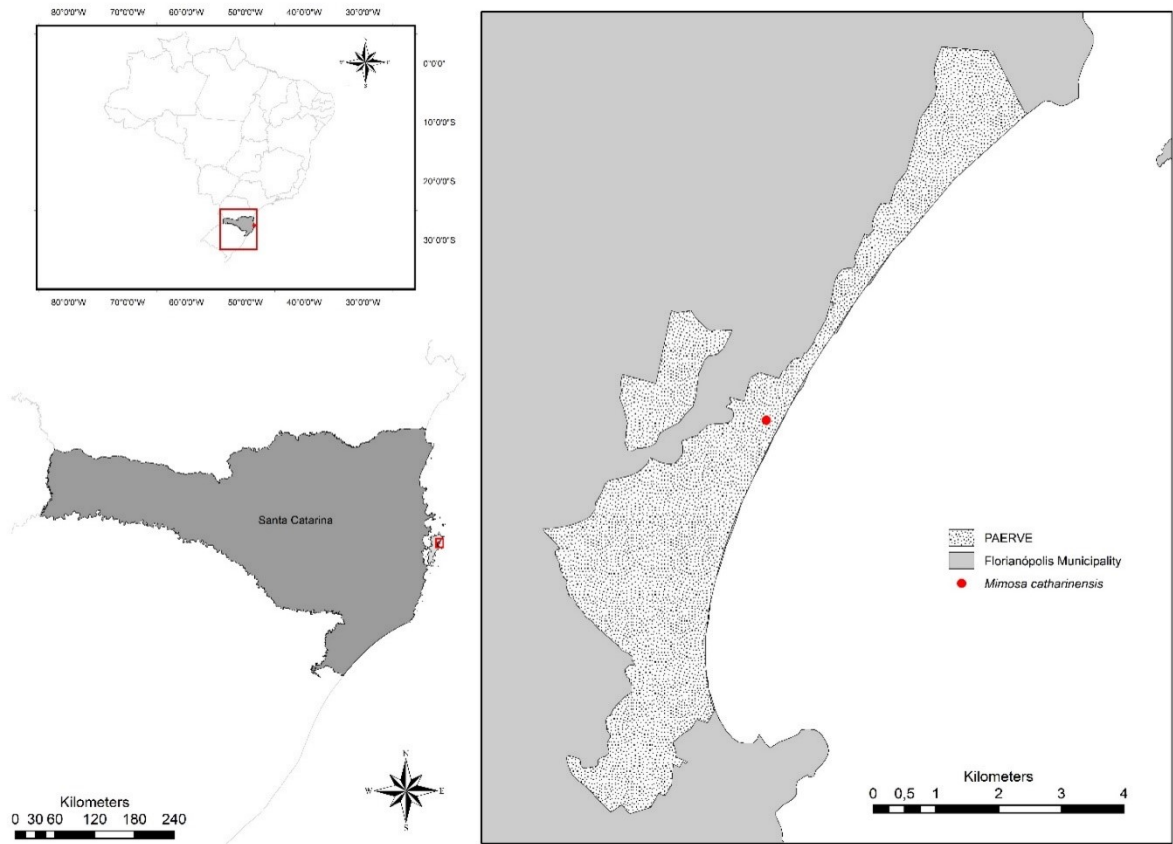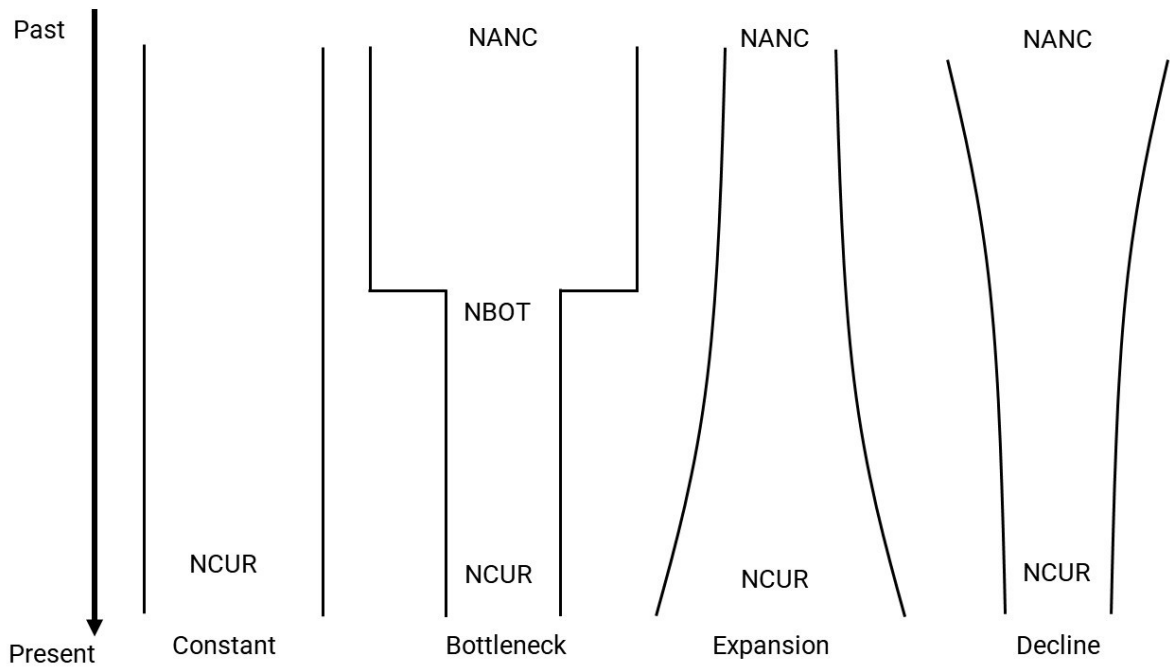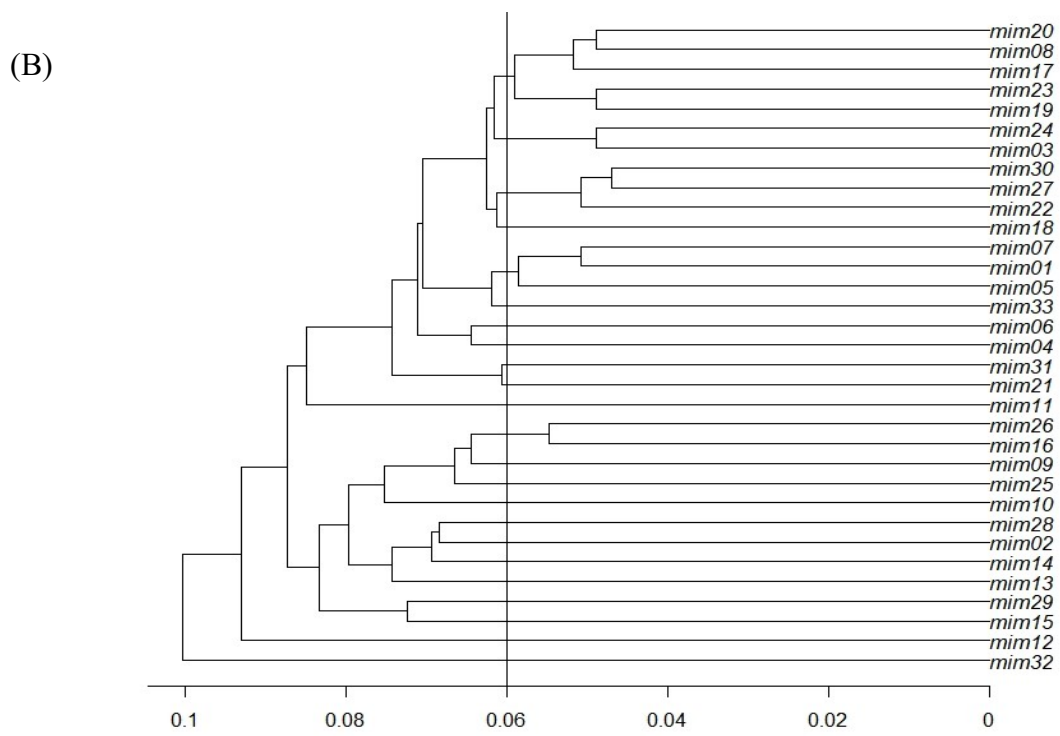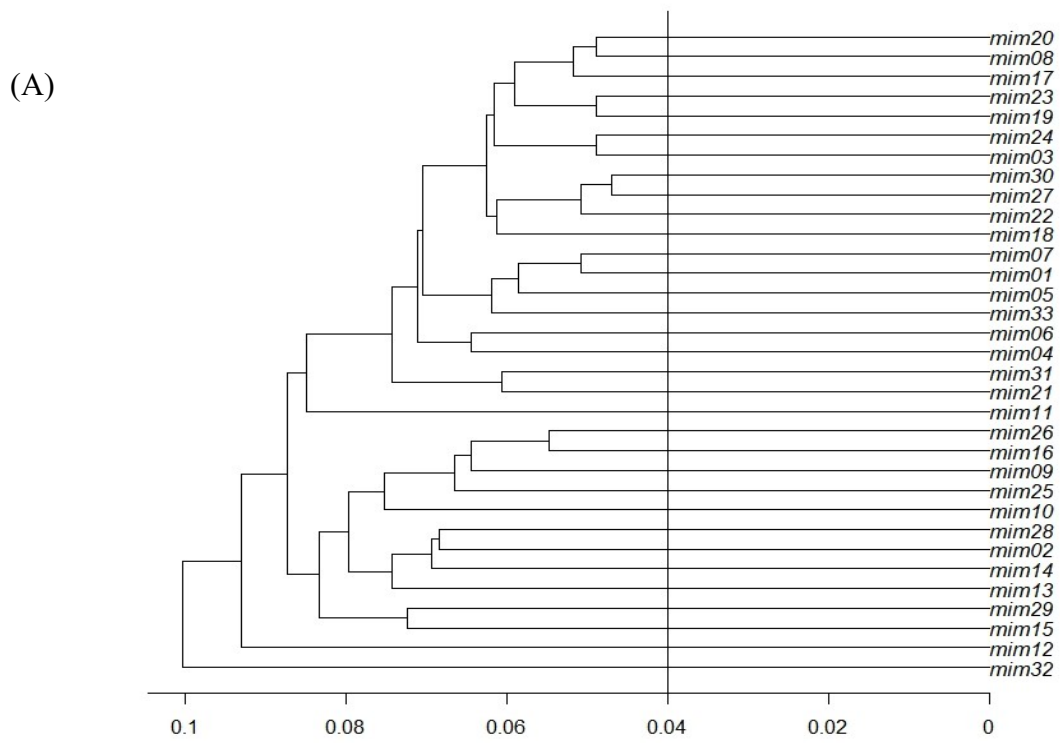
FIGURE 1

FIGURE 2

FIGURE 3

(A)

(B)

4 CONSIDERAÇÕES FINAIS

Avaliar as consequências genéticas e ecológicas de espécies com tamanhos populacionais reduzidos é de fundamental importância para o delineamento de políticas públicas de conservação eficientes, tanto *in situ* quanto *ex situ*. Para tanto, a utilização de metodologias genômicas tem se tornado cada vez mais frequente, devido ao alto poder estatístico e resolução para estimar parâmetros genéticos e demográficos relevantes para nortear o real posicionamento de ameaça de uma espécie e traçar estratégias para minimizar o seu risco de extinção.

Os resultados obtidos neste trabalho, embora extremamente relevantes para o aumento do conhecimento dos níveis de diversidade genética e história demográfica da espécie, constituem um ponto inicial para o desenvolvimento de estratégias efetivas para a conservação da espécie *M. catharinensis*. Tendo em vista que fatores ecológicos e genéticos estão intimamente relacionados, estudos robustos a respeito da biologia reprodutiva da espécie devem ser conduzidos e priorizados para elucidar questões que não puderam ser esclarecidas com os dados obtidos neste trabalho.

Enquanto planos efetivos de conservação para a espécie não forem elaborados, a espécie continuará apresentando um elevado risco de extinção. Apesar de não estar geneticamente depauperada, a ausência de reprodução sexual documentada para a espécie implica na impossibilidade desta espécie expandir sua variabilidade genética. Dessa forma, cabe à Administração do PAERVE elaborar, com base nas informações geradas, estratégias que busquem minimizar o declínio populacional, e consequentemente, a diversidade genética existente nos indivíduos remanescentes da espécie.

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