UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE GENÉTICA, ECOLOGIA E EVOLUÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA

Karina Aparecida Soares de Pádua

# GENETIC AND ECOLOGICAL EFFECTS OF CHRONIC FOREST FRAGMENTATION ON A CRITICALLY ENDANGERED TREE SPECIES

Belo Horizonte
2023

Karina Aparecida Soares de Pádua

# GENETIC AND ECOLOGICAL EFFECTS OF CHRONIC FOREST FRAGMENTATION ON A CRITICALLY ENDANGERED TREE SPECIES

Dissertação apresentada ao Programa de Pós-Graduação em Genética da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Mestre em Genética.

Orientador: Prof. Dr. Alison G Nazareno

Belo Horizonte
2023

043 Pádua, Karina Aparecida Soares de.
Genetic and ecological effects of chronic forest fragmentation on a critically endangered tree species [manuscrito] / Karina Aparecida Soares de Pádua. – 2023.
107 f. : il. ; 29,5 cm.

Orientador: Prof. Dr. Alison G Nazareno.
Dissertação (mestrado) – Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Genética.

1. Genética. 2. Fragmentação. 3. Destruição de Habitat. 4. Espécies em Perigo de Extinção. 5. Fluxo Gênico. I. Nazareno, Alison Gonçalves. II. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas. III. Título.

CDU: 575

Ficha catalográfica elaborada pela bibliotecária Fabiane C M Reis – CRB 6 – 2680

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

## ATA DE DEFESA DE DISSERTAÇÃO

| | |
|---|---|
| *ATA DA DEFESA DE DISSERTAÇÃO* | **344/2023**<br>**Entrada 1º/2021**<br>**CPF: 127.756.626-76** |
| **Karina Aparecida Soares de Pádua** | |

Às quatorze horas do dia **31 de julho de 2023**, reuniu-se, no Instituto de Ciências Biológicas da UFMG, a Comissão Examinadora de Dissertação, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho intitulado: **"GENETIC AND ECOLOGICAL EFFECTS OF CHRONIC FOREST FRAGMENTATION ON A CRITICALLY ENDANGERED TREE SPECIES"**, requisito para obtenção do grau de Mestre em **Genética.** Abrindo a sessão, o Presidente da Comissão, **Alison Gonçalves 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 Gonçalves Nazareno | UFMG | 220.542.388-64 | Aprovada |
| Loreta Brandão de Freitas | UFRGS | 449.213.220-15 | Aprovada |
| Marta Svartman | UFMG | 101.787.258-97 | 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 julho de 2023.**

Alison Gonçalves Nazareno
Loreta Brandão de Freitas
Marta Svartman

Assinatura dos membros da banca examinadora:

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Documento assinado eletronicamente por **Alison Goncalves Nazareno**, **Professor do Magistério Superior**, em 01/08/2023, às 17:41, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Marta Svartman**, **Professora do Magistério Superior**, em 02/08/2023, às 12:00, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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**Referência:** Processo nº 23072.245871/2023-15 SEI nº 2500805

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Instituto de Ciências Biológicas
Programa de Pós-Graduação em Genética

# FOLHA DE APROVAÇÃO

## "GENETIC AND ECOLOGICAL EFFECTS OF CHRONIC FOREST FRAGMENTATION ON A CRITICALLY ENDANGERED TREE SPECIES"

**Karina Aparecida Soares de Pádua**

Dissertação aprovada pela banca examinadora constituída pelos Professores:

Alison Gonçalves Nazareno
UFMG

Loreta Brandão de Freitas
UFRGS

Marta Svartman
UFMG

Belo Horizonte, 31 de julho de 2023.

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**Referência:** Processo nº 23072.245871/2023-15 SEI nº 2500826

# AGRADECIMENTOS

Agradeço a Deus por toda força, sabedoria, paz e proteção;

Ao Programa de Pós-graduação em Genética (PPG) da Universidade Federal de Minas Gerais (UFMG) e seus colaboradores;

Ao meu Orientador, Drº Alison Gonçalves Nazareno, pela oportunidade de fazer parte desse projeto e por todos os ensinamentos que me guiaram a finalizá-lo;

À minha amiga e companheira de laboratório, Thaís Martins, por todo apoio, ensinamentos e colaboração neste projeto;

Ao meu marido, Jefferson, por me acolher e motivar quando eu mais precisava. Obrigada por sempre estar ao meu lado;

Aos meus pais, João e Maria do Carmo, pelo apoio e por entenderem meus momentos de ausência;

Aos meus irmãos, Kanídio, Kennedy, João Marcos e Carlos Eduardo, por sempre torcerem por mim;

À minha Tia Alessandra, por sempre me ouvir e acreditar no meu potencial;

Aos docentes da UFMG com os quais tive a honra de aprender;

Aos membros da banca examinadora, pela disponibilidade e contribuições para este trabalho;

À agência de fomento CAPES pelos recursos que permitiram a realização deste trabalho;

Aos colegas discentes do PPG, pela troca de conhecimentos. Minha eterna gratidão.

# RESUMO

Pressões antrópicas, como o desmatamento e a expansão agrícola, têm levado à fragmentação e degradação de habitats, impactando negativamente as espécies que habitam esses remanescentes florestais. Neste estudo, investigamos os efeitos da fragmentação florestal em *Dinizia jueirana-facao*, uma espécie rara, endêmica da Mata Atlântica e criticamente ameaçada de extinção. Com base em teorias que apontam a redução do fluxo gênico devido à fragmentação florestal, resultando em efeitos negativos sobre a diversidade genética e os processos ecológicos, hipotetizamos que esses efeitos estão presentes em *D. jueirana-facao*, considerando a ocorrência de tamanhos populacionais reduzidos e a dispersão de sementes por barocoria. Também testamos se a estrutura demográfica das localidades remanescentes de *D. jueirana-facao* se ajustam ao modelo de distribuição J invertido. Utilizando conjuntos informativos de marcadores SNPs derivados do sequenciamento de fragmentos de DNA associado a sítios de restrição (*ddRADseq* - *double-digest restriction site-associated DNA sequencing*), investigamos as distâncias de dispersão, a conectividades entre os fragmentos, a diversidade genética e quantificamos o fluxo gênico histórico e contemporâneo. Nossos resultados indicaram que a dispersão do pólen ocorre a curtas distâncias, com a maioria dos eventos de polinização cruzada ocorrendo dentro das populações. Além disso, observamos uma redução no fluxo gênico contemporâneo em comparação ao histórico, indicando mudanças temporais no fluxo gênico relacionadas à fragmentação florestal recente. Nossos resultados também indicaram ausência de endogamia, nenhuma perda aparente de diversidade genética e estrutura demográfica sadia. Embora esses resultados já tenham implicações importantes para a conservação e manejo da *D. jueirana-facao*, enfatizamos que uma compreensão mais abrangente das dinâmicas ecológica e evolutiva é ainda necessária, haja vista o cenário atual da perda, degradação e fragmentação de habitats.

**Palavras-chave:** *ddRADseq*. *Dinizia jueirana-facao*. Fluxo gênico. Fragmentação florestal. Genética da conservação.

# ABSTRACT

Anthropogenic disturbances, such as deforestation and agricultural expansion, have led to habitat fragmentation and degradation, negatively impacting the species that inhabit these forest remnants. In this study, we investigated the effects of forest fragmentation on *Dinizia jueirana-facao*, a rare species endemic to the Atlantic Forest and critically endangered. Based on theories that point to reduced gene flow due to forest fragmentation, resulting in negative effects on genetic diversity and ecological processes, we hypothesized that these effects are present in *D. jueirana-facao*, considering the occurrence of reduced population sizes and seed dispersal by barochory. We also tested whether the demographic structure of the remaining localities of *D. jueirana-facao* fit the reverse J-shaped distribution model. Using informative sets of SNPs derived from double-digest restriction site-associated DNA sequencing (ddRADseq), we investigated dispersal distances, connectivity between fragments, genetic diversity and quantified historical and contemporary gene flow. Our results indicated that pollen dispersal occurs at short distances, with most outcrossed pollination events occurring within populations. In addition, we observed a reduction in contemporary gene flow compared to historical, indicating temporal changes in gene flow related to recent forest fragmentation. Our results also indicated an absence of inbreeding, no apparent loss of genetic diversity and a healthy demographic structure. Although these results already have important implications for the conservation and management of *D. jueirana-facao*, we emphasize that a more comprehensive understanding of ecological and evolutionary dynamics is still needed, given the current scenario of habitat loss, degradation and fragmentation.

**Keywords:** Conservation genetics. ddRADseq. *Dinizia jueirana-facao*. Forest fragmentation. Gene flow.

# LISTA DE ILUSTRAÇÕES

## INTRODUÇÃO

## CAPÍTULO I

and Sooretama (yellow point in C) – located in the Espírito Santo State, Southeast Brazil. The distance between the areas is approximately 12 kilometers. 92

**FIGURE S2** Box plot quality of reads. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend to 5th and 95th percentiles. (A) Number of retained reads with all samples, outliers are represented by dots. n = 211 sample points. (B) Number of retained reads by locality, outliers are represented by dots. n = 116 and 95 sample points. Samples with less than 500,000 reads were discarded from the dataset. 93

# LISTA DE TABELAS

# LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| $A$ | Allelic richness |
| CAPES | Coordenação de Aperfeiçoamento de Pessoal de Nível Superior |
| CFS | Candidate Father Sample |
| CI | Confidence Interval |
| CMS | Candidate Mother Sample |
| DBH | Diameter at Breast Height |
| ddRADseq | double-digest restriction site-associated DNA sequencing |
| ES | Espírito Santo |
| $F_{IS}$ | Inbreeding coefficients |
| $F_{L}$ | kinship coefficient estimators |
| HW | Hardy-Weinberg equilibrium |
| IBD | Isolation by Distance |
| LD | Linkage Disequilibrium |
| MAF | Minor Allele Frequency |
| MD | Missing Data |
| PCR | Reação em Cadeia da Polimerase |
| $R$ | Private allelic richness |
| RBS | Reserva Biológica de Sooretama |
| $R_{ij}$ | Relationship coefficients |
| RNV | Reserva Natural Vale |
| RPPNs | Reservas Particulares de Patrimônio Natural |
| RRS | Sequenciamento de Representação Reduzida do genoma |
| SGS | Fine-spatial genetic structure |
| SNP | Single Nucleotide Polymorphisms |
| SV | Structural Variant |

# SUMÁRIO

# 1 INTRODUÇÃO GERAL

## 1.1 *Degradação da Mata Atlântica Brasileira*

A degradação histórica da Mata Atlântica Brasileira resultou de vários fatores, incluindo a interação humana, a limpeza das terras para culturas e pastagens, e a exploração econômica de *commodities*. A localização do bioma, situado mais ao leste do país, permitiu sua ocupação pelos colonos europeus, cujas atividades coloniais transformaram uma grande parte da floresta em áreas não florestais, principalmente devido aos extensos sistemas de plantação de cana-de-açúcar e café, assim como às atividades pecuárias (Marques e Grelle, 2021; Solórzano et al., 2021). O desmatamento continuou ocorrendo com a urbanização, a industrialização e a crescente demanda por energia, resultando na conversão de vastas extensões de floresta em carvão vegetal (da Fonseca, 1985). Atualmente, a área remanescente da Mata Atlântica corresponde a 24% da floresta original, com apenas 12,4% das florestas consideradas maduras e bem preservadas (Fundação SOS Mata Atlântica e INPE, 2021). Esses fragmentos remanescentes são pequenos, isolados e por vezes desprotegidos (Ribeiro et al., 2009; De La Sancha et al., 2014; Rezende et al., 2018). A fragmentação e perda do habitat acaba resultando em um aumento do risco de extinção de espécies raras e endêmicas desse bioma (Brooks e Balmford, 1996; Metzger, 2009; Leal et al., 2021; Teixeira e Nazareno, 2021).

As atividades coloniais tiveram um impacto significativo na Mata Atlântica, assim como as práticas contemporâneas também continuam afetando negativamente esse bioma. No entanto, há diferenças em relação à percepção e abordagem atuais, onde o foco principal das pesquisas a respeito da Mata Atlântica é sobre sua conservação (Marques et al., 2021), e que há cerca de 50 anos, a importância e a necessidade de conservação das florestas tropicais não eram reconhecidas. Somente em 1982, quando as primeiras pesquisas sobre o mico-leão-dourado (*Leontopithecus rosalia*) foram realizadas, é que a situação da Mata Atlântica ganhou reconhecimento e interesse mundial. Em 1985, da Fonseca (1985) publicou um artigo sobre o desmatamento do bioma, que já possuía poucos remanescentes florestais, pequenos e isolados, e reivindicou a necessidade de sua conservação. Marques et al. (2021) identificaram três períodos que contribuíram para a formação do conhecimento sobre a Mata Atlântica: o século XVIII e XIX, marcado pela descrição da fauna e flora por viajantes europeus; o período de 1890 a 1985, quando ocorreu a formação de coleções da biodiversidade da Mata Atlântica; e o período de 1985 a 2020, no qual foram publicados diversos estudos abrangendo diferentes níveis hierárquicos desse bioma.

No momento, a Mata Atlântica encontra-se amplamente degradada. No entanto, existem diversos programas em andamento com o objetivo de monitorar, restaurar, conservar e preservar esse importante bioma. Dentre eles, destacam-se a Fundação SOS Mata Atlântica e o Pacto pela Restauração da Mata Atlântica, que têm desempenhado um papel fundamental na promoção de ações voltadas para a recuperação desse ecossistema. Além disso, leis como a LEI Nº 11.428, de 22 de dezembro de 2006, também contribuem para a proteção e conservação da Mata Atlântica, estabelecendo diretrizes e regulamentações específicas para sua preservação. Essas iniciativas e instrumentos legais são essenciais para reverter o processo de degradação e assegurar a sobrevivência desse importante patrimônio natural.

Apesar do reconhecimento e atitudes voltadas para conservação do bioma, foi relatado no Atlas dos Remanescentes Florestais da Mata Atlântica que, entre o período de 2021 e 2022, o desmatamento da Mata Atlântica foi de 20.075 hectares, o segundo maior registro nos últimos 6 anos (Fundação SOS Mata Atlântica e INPE, 2023). Entre os 17 estados avaliados, 8 registraram aumento no desmatamento, incluindo o estado do Espírito Santo (ES) - região de estudo deste trabalho. No relatório do período 2019 - 2020, o ES foi o estado que mais aumentou sua taxa de desmatamento (Fundação SOS Mata Atlântica e INPE, 2021). O estado abriga grandes reservas florestais da Mata Atlântica, como a Reserva Biológica de Sooretama (RBS), a Reserva Natural Vale (RNV) e outras Reservas Particulares de Patrimônio Natural (RPPNs), por exemplo, Refúgio da Anta e Mutum Preto. Juntas estas reservas formam um bloco florestal com uma grande diversidade de espécies de plantas (Peixoto e Silva, 1997). Entretanto, esses fragmentos são circundados por empreendimentos de pecuária, agricultura e silvicultura (Peixoto e Jesus, 2016), que representam uma ameaça para fauna e flora remanescentes. Por isso torna-se importante monitorar estas áreas e estudar a saúde das populações animais e vegetais que compõem esse ecossistema. Particularmente porque esse retrato de fragmentos florestais pode levar ao isolamento e redução do tamanho populacional dessas espécies (Fitz et al., 2022).

Estudos com plantas têm demonstrado que o aumento das distâncias entre os fragmentos restringe o fluxo de pólen e sementes (e.g., Jump and Peñuelas, 2006; Barbeta et al., 2011; Kitamura and Nakanishi, 2021; Chiriboga‑Arroyo et al., 2021; Jiang et al., 2022; Wang et al., 2023). Esse processo pode levar à perda de variação genética e à depressão endogâmica, uma vez que a probabilidade de acasalamento entre indivíduos parentes aumenta. Essa cascata de eventos, pode reduzir o potencial evolutivo e adaptativo das espécies (e.g., Fisher, 1930; Saunders et al., 1991; Young et al., 1996; Hughes et al., 2008; Łabiszak et al., 2021; Allendorf et al., 2022).

No cenário apresentado, a implementação de planos de conservação eficazes é crucial para garantir a sobrevivência de espécies com populações pequenas e isoladas. Especialmente aquelas que são endêmicas e restritas a uma determinada área. Essas estratégias dependem de informações obtidas por meio de estudos genéticos e ecológicos, além da aplicação de planos de manejo que buscam promover o crescimento ou a estabilidade demográfica dessas populações (e.g., Ralls et al., 2018; Theissinger et al. 2023).

## 1.2 *Fragmentação florestal e seus efeitos na estrutura demográfica de plantas*

A fragmentação florestal é um fenômeno global que tem ocorrido em diferentes biomas ao redor do mundo (Saravia et al., 2018; Jacobson et al., 2019). Sua ocorrência tem implicações significativas nos processos ecológicos das populações de plantas, assim como nos fatores genéticos e demográficos, que são os principais determinantes da viabilidade populacional e da sobrevivência desses organismos (Spielman et al., 2004). Estudos demonstraram que a fragmentação pode resultar em uma redução na disponibilidade de parceiros reprodutivos, afetando a polinização e a reprodução das plantas (e.g., Honnay et al., 2005; Traveset et al., 2018; Cristóbal-Pérez et al., 2021). Além disso, pode alterar a distribuição das faixas etárias das populações, influenciada pela taxa de recrutamento de novos indivíduos e pela a remoção de árvores adultas para os mais diversos fins (e.g., Tomimatsu and Ohara, 2006; Atsbha et al., 2019; Yemata and Haregewoien, 2022).

No contexto da conservação de espécies de vida longa, em que o monitoramento demográfico a longo prazo pode ser desafiador, a análise da distribuição de diferentes faixas etárias em uma população pode ser uma ferramenta valiosa. A estrutura demográfica fornece tais informações e pode ser utilizada para embasar decisões de manejo imediatas (Bruna and Kress, 2002; Nazareno and Reis 2014; Atsbha et al., 2019; Yemata and Haregewoien, 2022). Particularmente nas populações vegetais da Mata Atlântica, um bioma altamente degradado, monitorar a saúde dessas populações permite esforços direcionados para proteger e sustentar as populações diante das mudanças ambientais e pressões antropogênicas.

## 1.3 *Dinizia jueirana-facao*

A conservação da biodiversidade é uma preocupação global, impulsionada pela crescente perda de habitats naturais e pela ameaça iminente de extinção de muitas espécies (Hoban et al., 2023). No contexto da Mata Atlântica, um bioma altamente degradado, a espécie *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira (Fabaceae: Caesalpinioideae) destaca-se como uma espécie rara, endêmica e altamente vulnerável aos processos de perda, degradação e fragmentação de habitats. Atualmente, a espécie é classificada como criticamente ameaçada, na Lista Vermelha de Espécies Ameaçadas divulgada pela União Internacional para a Conservação da Natureza (IUCN, 2017), e na Lista Nacional de Espécies Ameaçadas de Extinção publicada pela PORTARIA MMA Nº. 148 (BRASIL, 2022).

Recentemente descoberta (Lewis et al., 2017), a espécie ocorre em apenas duas localidades conhecidas no Sudeste Brasileiro: uma dentro da Reserva Natural Vale (RNV), localizada no município de Linhares; e a outra em uma reserva de propriedade particular, distante 12,0 km da RNV, no município de Sooretama, ambas no Estado do Espírito Santo. Menos de 25 indivíduos foram relatados nestas duas localidades (Lewis et al., 2017). É importante ressaltar que, apesar dessas áreas estarem localizadas em reservas, elas estão cercadas por uma matriz de atividades agropecuárias, como pastagens, terras agrícolas com plantio de eucalipto, café e mamão, e também contornadas por estradas (Rolim et al., 2016). Além disso, os municípios de Linhares e Sooretama perderam grande parte da sua cobertura vegetal (Fundação SOS Mata Atlântica e INPE, 2021). Também há registros de que o histórico da região envolve coleta ilegal de espécimes de plantas e animais, caça predatória, incêndios florestais e períodos de seca (Kierulff et al., 2014). A fragmentação do habitat e o reduzido número de indivíduos podem representar sérios desafios para a sobrevivência da espécie. Esses fatores limitam o fluxo gênico e podem levar à perda de diversidade genética (Schlaepfer et al., 2018; Leigh et al., 2019; Exposito-Alonso et al., 2022).

*D. jueirana-facao* é uma árvore de dossel impressionante (19 - 40 m), com um tronco que varia entre 10 a 22 m e um casca de cor cinza (Figura 1B e 1C). Seus frutos são robustos, lenhosos, deiscentes e apresentam uma cor marrom escuro a preto quando maduros, contendo de 13 a 15 sementes (Figura 1A). As sementes são pretas e duras, 25 – 30 × 16 – 19 mm (Lewis et al., 2017). Sua dispersão primária é atribuída à gravidade, conforme sugerido por observações de campo (Figura 2A e 2B). A espécie apresenta uma inflorescência amarela brilhante, flores hermafroditas que medem de 8,5 a 10 mm (Lewis et al., 2017). Raramente, são observados casos de flores funcionalmente masculinas, devido à supressão do desenvolvimento do gineceu (Lewis et al., 2017). As épocas de floração e frutificação desta espécie são pouco conhecidas e consideradas imprevisíveis (Lewis et al., 2017). Coletas de flores foram relatadas em julho e outubro, e frutos em março, julho e setembro (Lewis et al., 2017).

O gênero *Dinizia* é constituído apenas por duas espécies conhecidas, *Dinizia jueirana-facao* e *Dinizia excelsa*, com uma característica marcante: o dimorfismo polínico. Enquanto *D. jueirana-facao* apresenta o pólen em mônadas, sua congênere, *D. excelsa*, possui pólen em tétrades (Lewis et al., 2017). Essa caracterização morfológica do pólen, combinada a estudos que indicam que o grau de agregação das unidades de dispersão de pólen está relacionado com o agente dispersor (Pacini, 2000; Timerman et al., 2014; Pacini e Franchi, 2020), sugere uma potencial dispersão mediada pelo vento em *D. jueirana-facao*. Esses estudos relatam que o pólen agregado (e.g., tétrade) ocorre principalmente em táxons polinizados por animais. De fato, *D. excelsa* apresenta dispersão de pólen mediada por insetos (Dick et al., 2003).

## 1.4 *Genômica da conservação*

A Genômica da Conservação combina conceitos e técnicas genômicas com a conservação da biodiversidade. Seu objetivo é compreender a genética e a diversidade genômica de populações de espécies ameaçadas ou em risco de extinção, a fim de fornecer informações valiosas para o desenvolvimento de estratégias eficazes de conservação (Theissinger et al., 2023). Ela envolve o uso ferramentas genéticas (i.e., marcadores moleculares), obtidos por meio de metodologias de sequenciamento genômico para avaliar a diversidade genética, estrutura genética populacional, parentesco entre indivíduos e populações, entender os padrões de fluxo gênico, hibridização, isolamento genético e história demográfica (Yoder et al., 2018; Hohenlohe et al., 2021; Kardos et al., 2021; Schweizer et al., 2021; Galla et al., 2022; Willi et al., 2022; Theissinger et al., 2023). A genômica da conservação foi aplicada a várias espécies, incluindo anfíbios (Nunziata et al., 2017), peixes (Naito et al., 2023), répteis (Hartley et al., 2023), mamíferos (Mclennan et al., 2018; Robinson et al., 2019), aves (Chen et al., 2019; Rabier et al., 2020), e plantas (e.g., Teixeira e Nazareno, 2021; Pfenninger et al., 2021; Jiang et al., 2022; Mercier et al., 2023; Jordan et al., 2023; Wang et al., 2023).

Estudos em genômica da conservação costumavam empregar, em sua maioria, metodologia de sequenciamento tradicional como Sanger. Entretanto, foram se diversificando com o surgimento das metodologias de sequenciamento de alto rendimento, ou seja, *high-throughput genomic sequencing* (Alvarado-Cerón et al., 2022; Olah et al., 2021). Essa evolução propiciou aumento no número de sequências de DNA com milhares de marcadores SNPs (*Single Nucleotide Polymorphisms*) e a investigação de questões biológicas com maior precisão, como a identificação de regiões genômicas diferenciadas entre populações e a análises filogeográficas de várias populações selvagens (Allendorf et al., 2022; Davey et al., 2011). Além disso, a caracterização de outros tipos de variações, como as variantes estruturais (SVs) tem sido usada para melhorar a compreensão sobre a variação genômica funcional e seu impacto no potencial adaptativo e populacional (Wellenreuther et al., 2019; Wold et al., 2021). Os avanços técnicos tornaram o sequenciamento de genomas mais rápido e eficiente e menos custoso. Entretanto, apesar da redução dos custos, seu valor ainda é substancialmente alto, principalmente para pesquisadores de países com alta diversidade de espécies e que, em muitos casos, têm recursos econômicos limitados para pesquisas. O que ocasiona a busca por métodos moleculares que possam responder questões biológicas com eficácia e economia (Luca et al., 2011; Eguiarte et al., 2022).

Uma abordagem que busca menores gastos e permite o estudo de diversos indivíduos (populações) é o sequenciamento parcial do genoma, denominado sequenciamento de representação reduzida - RRS. Existem diversos métodos derivados do RRS que têm evoluído e ganhado nomes exclusivos (see Campbell et al., 2018). Um exemplo é o protocolo de sequenciamento de DNA associado a sítios de restrição - RADseq (Miller et al., 2007; Baird et al., 2008; Andrews et al., 2016; Campbell et al., 2018). O RADseq é uma das metodologias mais amplamente utilizadas dentro do RRS, especialmente em estudos relacionados à genômica da conservação (e.g., Andrews et al., 2018; Chaves et al., 2019; Stojanova et al., 2020; Teixeira e Nazareno, 2021; Gladstone et al., 2022; Mercier et al., 2023).

RADseq abrange diferentes técnicas que objetivam diminuir custos e esforços dos estudos genômicos. Dentre estas, se destaca o método *ddRADse*q (*double-digestion Restriction Site associated DNA*), elaborado por Peterson et al. (2012). Neste protocolo, o DNA é fragmentado com duas enzimas de restrição e ligados a adaptadores, depois adiciona-se sequências exclusivas, denominadas DNA *barcodes*, que permitem multiplexar diferentes indivíduos em uma biblioteca. Então os fragmentos são selecionados por tamanho, amplificados com PCR (Reação em Cadeia da Polimerase) e sequenciados para identificação dos SNPs. Assim como as demais metodologias RADseq, o método *ddRADseq* não requer nenhuma informação genômica prévia sobre o táxon em estudo, sendo vantajoso para o estudo de espécies não-modelo, como *D. jueirana-facao*.

Mesmo diante dos atuais avanços nas tecnologias de sequenciamento, a escolha de qual técnica de marcador molecular aplicar, dependerá do tipo de questão de pesquisa a ser respondida, da sua complexidade, do tempo e também dos recursos financeiros disponíveis (Anne, 2006; Hodel et al., 2016; Amiteye, 2021).

## 1.5 *Objetivos*

**Objetivo Geral:**

Integrar dados genéticos e ecológicos para entender como espécies raras, endêmicas e criticamente ameaçadas de extinção respondem à fragmentação florestal.

**Objetivos específicos:**

(i) Analisar a estrutura demográfica de *D. jueirana-facao*.
(ii) Investigar a conectividade entre os fragmentos de *D. jueirana-facao*.
(iii) Quantificar o fluxo gênico contemporâneo.
(iv) Avaliar padrões de estrutura genética espacial e quantificar o fluxo gênico histórico.
(v) Comparar estimativas históricas e contemporâneas do fluxo gênico.
(vi) Inferir os efeitos da fragmentação do habitat na diversidade genética.
(vii) Disponibilizar os dados gerados neste estudo para balizar políticas públicas de conservação e manejo.

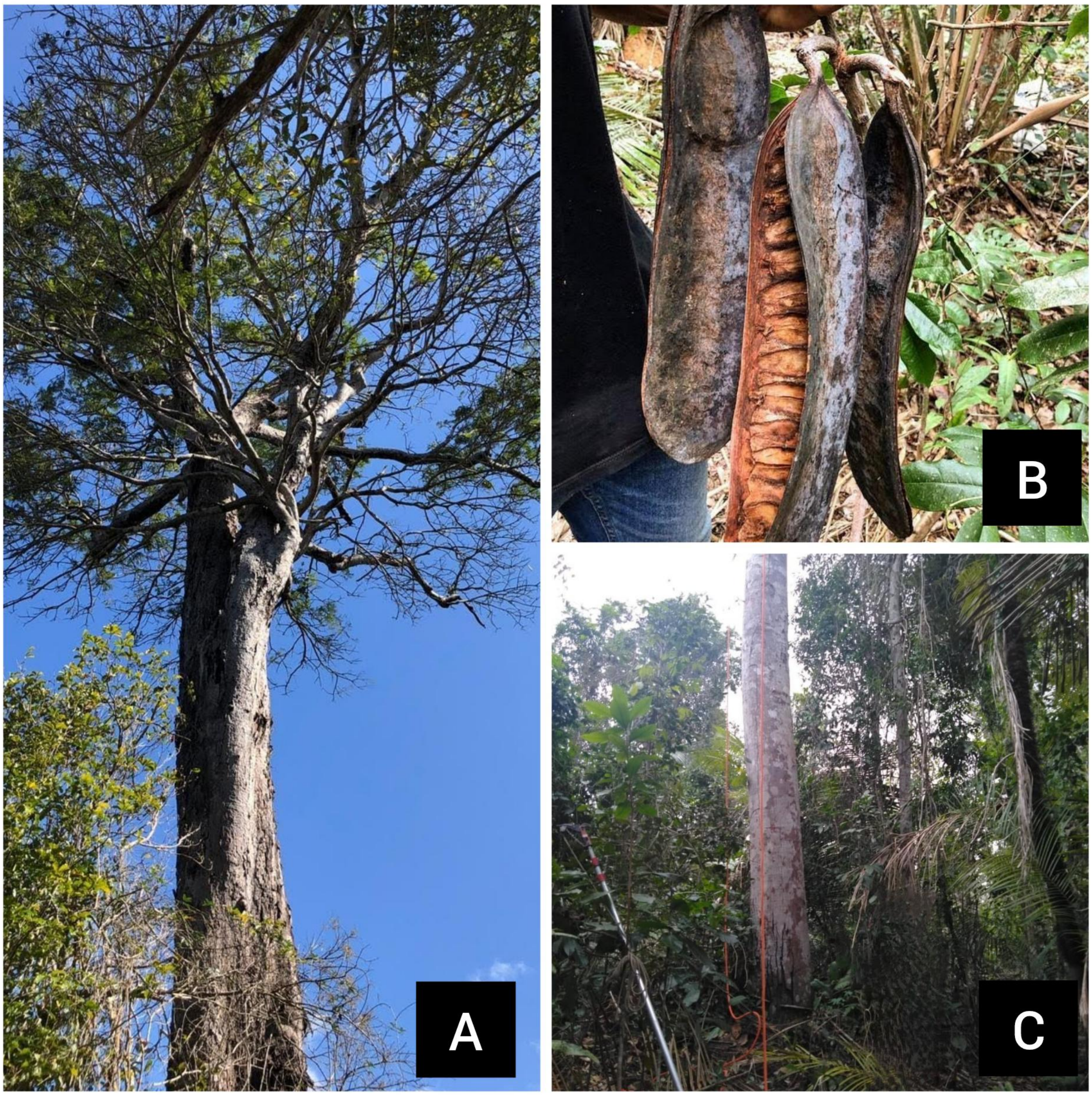

**FIGURA 1** *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira (A) com detalhes dos seus frutos (B) e do tronco (C).

**FIGURA 2** Expedição de campo para coleta de material foliar para posterior extração de DNA. Em (A) é mostrado o processo de coleta em árvores adultas de *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira com o auxílio de profissional especializado em escalada de árvores. Detalhe da coleta de dados (e.g., circunferência na altura do peito) para os estudos de estrutura demográfica é apresentada em B.

# 2 CAPÍTULO I

## Genetic and ecological effects of chronic forest fragmentation on a critically endangered tree species

# GENETIC AND ECOLOGICAL EFFECTS OF CHRONIC FOREST FRAGMENTATION ON A CRITICALLY ENDANGERED TREE SPECIES

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**KEYWORDS:** conservation genetics, *Dinizia jueirana-facao*, gene flow, reduced representation sequence, Single Nucleotide Polymorphism (SNP)

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**RUNNING TITLE: THE IMPACT OF FOREST FRAGMENTATION ON *Dinizia jueirana-facao***

## ABSTRACT

Forest fragmentation can be detrimental effects on plant populations, leading to reduce population sizes and depleting genetic diversity as a consequence. As a matter of urgency, it is crucial to assess the consequences of forest fragmentation on ecological processes and patterns of genetic diversity, particularly for threatened plant species facing anthropogenic pressures. Here, we examined the response of *Dinizia jueirana-facao*, a rare and critically endangered tree species endemic to the Brazilian Atlantic Forest, to forest fragmentation. Based on theoretical predictions for species with small population sizes and plants with gravity-dispersed seeds, we hypothesized that forest fragmentation would reduce gene flow, resulting in decreased levels of genetic diversity. We also investigated whether population size structure of fragmented populations of *D. jueirana-facao* followed a typical reverse J-shaped distribution. Using informative SNP datasets derived from the ddRADSeq (double-digest restriction site-associated DNA sequencing), we found that pollen dispersal occurred within short distances, with the majority of the outcrossed pollination events occurring locally. Furthermore, contemporary estimates of gene dispersal distance were lower than historical estimates, indicating a seasonal shift in the scale of gene flow in both localities due to recent forest fragmentation. Surprisingly, our findings revealed no evidence of inbreeding, no apparent loss of genetic diversity, and a healthy demographic size structure for *D. jueirana-facao*. While our results already have important implications for the conservation and management of this critically endangered and rare tree species, we stress the need for a more comprehensive understanding of the ecological and evolutionary process for this unique genetic resource, in the face of ongoing habitat loss and fragmentation.

# INTRODUCTION

Continental-scale analyses of tropical forest fragmentation patterns reveal that continued deforestation leads to a predictable evolution, with an increase in the number and decrease in the size of fragments (Taubert et al., 2018; Fischer et al., 2021). This trend has been observed in the Brazilian Atlantic Forest, where remaining vegetation cover corresponds to 24% of native vegetation, with many forest patches smaller than 50 ha (Ribeiro et al., 2009; Rezende et al., 2018; Fundação SOS Mata Atlântica and INPE, 2023). This biome has been historically degraded by human action through agricultural expansion, urbanization, and industrialization (Fonseca, 1985; Diniz et al., 2022). Such a scenario is worrying for plant populations affected by the reduction of total forest area and increasing number of forest fragments, as it can lead to isolation (Fitz et al., 2022) and damage important processes such as pollen and seed dispersal mechanisms. It may also act at the genetic level by increasing genetic drift and leading to loss of genetic diversity (Saunders et al., 1991; Young et al., 1996; Honnay and Jacquemyn, 2007; Łabiszak et al., 2021).

The effects of forest fragmentation on plant populations have been studied frequently (e.g., Chiriboga-Arroyo et al., 2021; Cristóbal-Pérez et al., 2021; Obico et al., 2021; Waqar et al., 2021; de Santana et al., 2022; Jiang et al., 2022). Although diverse in terms of approaches and contexts, studies consistently converge on the impacts of forest fragmentation due to environmental change and anthropogenic pressures. They highlight the loss of standing genetic variation (Schlaepfer et al., 2018; Leigh et al., 2019; Exposito-Alonso et al., 2022) and the acceleration of biodiversity loss (Horváth et al., 2019), which significantly affect the species survival (Hansen et al., 2020). These results underscore the utility and importance of using genomic tools in efforts to develop conservation strategies (Theissinger et al. 2023).

This study focuses on the species *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira (Fabaceae: Caesalpinioideae), an endemic tree species that occurs in a restricted area of remnants of Brazilian Atlantic Forest in Espírito Santo state. The region has a flat to slightly sloping relief, which makes it attractive for agriculture and characterizes the surroundings of these forests with a sudden transition of landscape with pastures and farmland in addition to the presence of roads. Given their restricted area of occurrence and conservation status (i.e, critically endangered), the study aims to understand the effects of forest fragmentation on *D. jueirana-facao*, integrating ecological e genetics data. The approach allows us to understand the dispersion patterns of the species and investigate if there is connectivity between the two known fragments of *D. jueirana-facao*, analyzing the movement of pollen and seeds (i.e., contemporary gene flow) inferred by parentage analysis. It also provides access to historical gene flow by an indirect gene dispersal method linked to fine-spatial genetic structure (SGS), deduced through the relationship between the spatial distance that separates individuals with kinship coefficients (Vekemans and Hardy, 2004). Comparing historical and contemporary estimates of gene flow enables us to better understand the consequences of forest fragmentation on *D. jueirana-facao* (Nazareno and Knowles, 2021).

The study also examines the impact of forest fragmentation on the intrapopulation genetic diversity level. This factor is crucial for long-term ecosystem protection and its erosion poses a threat to ecological stability at various hierarchical levels. The level of genetic diversity is closely linked to the evolutionary potential of populations in the face of environmental changes, including fragmentation (Fisher, 1930; Young et al., 1996; Hughes et al., 2008; Allendorf et al., 2022). Habitat fragmentation leads to a series of consequences for populations, including isolation, increased crossing between related individuals, and restricted pollen flow. As a result, genetic drift intensifies, spatial genetic structure becomes pronounced, and genetic diversity is depleted (e.g., Aguilar et al., 2008; Vranckx et al., 2011; Tambarussi et al., 2017; Chiriboga‑Arroyo et al., 2021; Obico et al., 2021).

Our investigation of the effects of forest fragmentation on estimates of genetic diversity of *D. Jueirana-facao* localities aims to test the hypothesis of genetic diversity loss due to limited gene flow, as predicted by population theory in the context of habitat fragmentation (e.g., Young et al., 1996; Allendorf et al., 2022). Additionally, we examine the hypothesis of a recent reduction in gene flow scale, as reflected in dispersal distance. This pattern is expected due to the perturbation effect, which is primarily expressed in contemporary estimates, while historical estimates exhibit temporal inertia over a few generations (Oddou-muratorio and Klein, 2008; Nazareno and Knowles, 2021). Previous studies on one of the two known *D. jueirana-facao* localities have indicated a greater dispersion pattern for pollen than for seeds, as well as variations between historical and contemporary estimates of dispersal distance (Nazareno and Knowles, 2021).

In addition to conducting gene flow analyses and assessing genetic diversity, our study aims to investigate the demographic size structure and mating system of *D. jueirana-facao*. Overall, our study addresses crucial inquiries within the field of *D. jueirana-facao* management and conservation by integrating population genomics and ecological analyses. The primary objective is to provide valuable insights into the species' dynamics and viability, specifically identifying localities that are at a higher risk of experiencing inbreeding and loss of adaptive potential. We stress that the approach applied here serves to guide conservation and management efforts, such as habitat restoration and enhancing connectivity between localities. Furthermore, the results of our research can contribute to the development of seed collection strategies for *ex situ* conservation and reforestation, as previously discussed for other plant species (e.g., Bittencourt and Sebbenn, 2007; Ramos et al., 2018; Oliveira et al., 2020). Ultimately, this research plays a crucial role in help to maintain the evolutionary potential of *D. jueirana-facao* – a rare and unique genetic resource.

## MATERIALS AND METHODS

Here, we integrate both ecological and genetic data to try to understand how a rare, endemic, and critically endangered tree species responds to the effects of forest fragmentation. Our approach was divided into two categories: (i) ecological data, which focus on demographic size structure pattern and (ii) genomic data, which was obtained from reduced representation genome libraries to explore genetic variation, gene flow, and mating systems for *D. jueirana-facao*. The methods applied for each category are detailed in the following sections.

### *Species, studied region, and sampling*

For ages, *Dinizia* was considered a monotypic genus, represented solely by *D. excelsa* Ducke. However, with the description by Lewis et al. (2017), *Dinizia* now comprises two members, including *Dinizia jueirana-facao*, a rare and endemic canopy-emergent tree species. Despite being recently described, this tree species is listed as critically endangered (IUCN, 2017; BRASIL, 2022).

*Dinizia jueirana-facao* exhibits an emergent canopy ranging from 19 to 40 m and a trunk height of 10 to 22 m. It features bright yellow inflorescence, hermaphrodite flowers measuring 8.5 – 10 mm, with occasional instances of functionally male flowers preceding the suppression of gynoecium development. The dehiscent woody fruits are dark brown to black when ripe, measuring 40 – 46 × 8.5 – 10 cm and containing 13 – 15 black seeds that are hard and measure 25 – 30 × 16 – 19 mm (Lewis et al., 2017).

While there is a morphological characterization of the reproductive structures of *D. jueirana-facao*, no information regarding pollination syndrome and seed dispersal is currently available. Field observations suggest that this species primarily disperses its seeds through gravity. Based on morphological characterization of pollen monads in *D. jueirana-facao* (Lewis et al., 2017), there is a possibility of wind-mediated dispersal. However, bees and small beetles emerge as potential candidates for pollination, as these organisms have been reported as pollen dispersers in the congener species *Dinizia excelsa* – an Amazon tree species which presents similar floral traits (Dick et al., 2003; Lewis et al., 2017).

With only two known localities confined to the Atlantic Forest, the occurrence of *D. jueirana-facao* is limited to Southeast Brazil, specifically within the Reserva Natural Vale in the Linhares municipality and on a private property located 12.0 km away from the reserve in the Sooretama municipality, both situated in the Espírito Santo State. The species remains under threat due to habitat fragmentation resulting from its small population sizes and a history of anthropogenic activities. The Reserva Natural Vale has previously experienced challenges such as illegal collection of plant and animal specimens, predatory hunting, forest fires, and periods of drought (Kierulff et al., 2014). Although the Reserva Natural Vale shares borders with other reserves, such as the Reserva Biológica de Sooretama (RBS) and two other private reserves of natural heritage, forming a potential gene flow matrix, its boundaries are surrounded by contrasting environments such as pastures, farmland, and roads.

Reproductive adults, intermediates and seedlings samples of these localities were exhaustively collected in the forest patches in October of 2019. A total of 211 individuals were found, and 116 of them were in the RNV and the other 95 outside the reserve in Sooretama. Leaf samples were collected and stored in the freezer until DNA extraction and the geographic coordinates of each individual were recorded using a Garmin portable GPS (~30 cm error). We measured the stem circumference of plants at approximately 1.3 m from the ground using a tape measure. This measurement was then used to calculate the diameter at breast height (DBH) using the formula $d = \frac{p}{\pi}$, (Milios et al., 2016), where $p$ represents the circumference (the distance around the stem).

For the purpose of genetic analyses, *D. jueirana-facao* individuals were categorized into two ontogenetic stages based on DBH (diameter at breast height): seedlings **(**DBH < 5 cm) and reproductive adults (DBH > 14 cm). For the genetic analyses, we used two distinct sample strategies. For paternity assignments and SGS, we used samples from only two ontogenetic stages (i.e., reproductive adults and seedlings), totalizing 80 and 55 *D. jueirana-facao* individuals in RNV and Sooretama, respectively. To test the effects of forest fragmentation on the levels of genetic diversity for *D. jueirana-facao*, we included 33 and 42 individuals for RNV and Sooretama, respectively. For genetic estimates of contemporary effective population size ($N_E$), we included 114 and 70 *D. jueirana-facao* individuals for RNV and Sooretama, respectively.

For ecological analysis, we added a third ontogenetic stage: the intermediate individuals (5 cm > DBH > 14 cm). The threshold DBH for reproductive adults was determined by the lowest DBH observed among reproductive adults in the entire sample. Reproductive events were assessed during the field expedition, and additional events were inferred based on the presence of dried reproductive pods and/or seeds beneath the plants.

### *Population demographic size structure*

To analyze the demographic structure of *D. jueirana-facao*, we utilized a dataset containing information on the distribution of DBH and tree height. First, we tested the normality of these data, applying the Shapiro-Wilk Normality test. After that, we used the Kolmogorov-Smirnov test to verify if there are differences in demographic size structure between ontogenetic stages and localities. Diametric distribution and height graphs were plotted to examine the pattern of demographic size structure. All these statistical analyses were performed in R (R Core Team, 2018). Additionally, the density (ind/ha) of seedlings, intermediates, and reproductive adults was used to determine the regeneration status within each locality. The regeneration status was categorized as: (i) good = if seedlings > or < intermediates > reproductive adults; or (ii) fair = if seedlings > or ≤ intermediates ≤ reproductive adults (Tiwari, et al., 2010; Atsbha et al., 2019; Baidya et al., 2022; Yemata and Haregewoien, 2022).

### *DNA extraction, library preparation and sequencing*

Samples of genomic DNA were obtained from 211 individuals by extracting DNA from their leaves using the Macherey-Nagel kit (Macherey-Nagel GmbH & Co. KG), following the manufacturer's instructions. The library preparation for double-digest restriction site-associated DNA sequencing (ddRADseq) was performed according to the Peterson et al. (2012) protocol, with modifications from Nazareno et al. (2017) to minimize variability in the number of reads per individual.

After extraction, the DNA was quantified using the Qubit dsDNA Assay Kit (Invitrogen). Enzymatic digestion was carried out using EcoRI and MseI restriction enzymes (New England Biolabs) on each sample containing 0.5 µg of genomic DNA, to then be purified with elution in 40 µL water with the Agencourt AMPure XP system (Beckman Coulter) as instructed by the manufacturer.

Subsequently, adapter ligations were performed by combining 80 ng of DNA, 0.35 µM of a MseI adaptor (common for all samples), 0.50 µM of a sample-specific EcoRI double-strand adaptor, 1U of T4 DNA ligase (New England Biolabs), and 1.5 µL of T4 ligase buffer. It was performed at 23°C for 30 minutes, heated at 65°C for 10 minutes, and slowly cooled to 23°C.

The ligation products were purified using the Agencourt AMPure XP system and amplified following the PCR protocol described by Nazareno et al. (2017). The Pippin Prep system and a 2% agarose cartridge (Sage Science, Beverly, MA) were used in size-selected DNA fragments, selecting a target range size of 375-475 bp. The library was sequenced with 100 bp single-end reads on four lanes of an Illumina HiSeq 2500 flowcell (Illumina Inc., San Diego, CA) at The Centre for Applied Genomics in Toronto, Canada.

### *SNPs identification and data filtering*

Data quality verification was conducted using the FastQC program, version 0.11.8 (Andrews, 2010). The raw sequence reads files were analyzed using Stacks 2.41 version (Catchen et al., 2011, 2013; Rochette et al., 2019) using *de novo* assembly. The process_radtags program in Stacks was utilized to assign reads to individuals and demultiplex the data. This process allowed a maximum of two mismatches in the redemption of barcodes (option -barcode-dist-1 2). Reads of low quality and those lacking the expected EcoRI were filtered out, while all reads were trimmed to 85 base pairs. Samples with less than 500,000 quality reads were excluded from further analysis.

Ustacks was employed to align short-read sequences from the output of process_radtags and generate "stacks" (or putative alleles) using a maximum-likelihood framework (Hohenlohe et al., 2010). The stacks were built with a minimum depth of coverage of three sequences, a maximum number of stacks at a single *de novo* locus, and a maximum distance of two nucleotides between stacks. The -d and -r parameters in Ustacks were used to address over-merged tags and remove highly repetitive stacks, respectively. SNPs were identified using an error rate (option bounded --bound_high 0.1), and the alpha value for the SNP model was set at 0.05.

Cstacks was utilized to create a catalog, or set of consensus loci, containing all loci from all individuals with three mismatches allowed between sample loci. The SStacks program was then employed to search the set of stacks in the catalog. In the locus clustering stage, tsv2bam in Stacks was used to establish a data orientation by locus rather than by sample, storing them in standard BAM files. The next step involved using the tsv2Bam outputs in Gstacks to build contigs with single reads, merge the contig with the single-end locus, and align reads from individual samples to the locus. SNPs were identified for each locus and each individual in Gstacks, and genotyped SNPs were converted into a haplotype set. Finally, the POPULATIONS program in Stacks (Catchen et al., 2011, 2013; Rochette et al., 2019) was executed to filter and export the SNP dataset from the Stacks standard format to GENEPOP format. Population statistics were computed using the exported SNP dataset. The FASTA files containing the per-locus consensus sequences, and individual loci sequences were also exported for further analysis.

### *Prior genomic analyses*

The sensibility of missing data (MD) on genetic diversity parameters and the number of SNPs was examined by running POPULATIONS program in Stacks (Catchen et al., 2011, 2013; Rochette et al., 2019) multiple times, varying the MD percentage from 0% to 30% with a fixed minor allele frequency (MAF) higher than 0.004, allowing the percentage at which the second most common allele occurs in the sample (n=135 diploid individuals) to be less than

99.9%. Additionally, the effect of MAF on the number of SNPs was tested by running POPULATIONS with a range of 0.05 to 0.5 (options -p 4 --min-maf 0.50 --max-obs-het 0.70).

To mitigate potential bias, the datasets underwent tests for Hardy-Weinberg equilibrium (HW) and linkage disequilibrium (LD) using the R package Pegas version 1.1 (Paradis, 2010) and the software Genepop version 4.7.5 (Rousset, 2008) respectively. Type I error rates for these tests were corrected for multiple comparisons using the sequential Bonferroni procedure (Rice, 1989).

Further two filter analyses were performed. First, to exclude plastid loci, the per-locus consensus sequences were aligned against reference chloroplast and mitochondrial genomes of three closely related species: *Haematoxylum brasiletto* (NCBI accession number NC_026679.1; NC_045040.1; MN709823.1), *Arapatiella psilophylla* (NCBI accession number MN709845.1) and *Schizolobium parahyba* (NCBI accession number MN709795.1). We used the BLASTn program (Altschul et al., 1990) to identify loci that presented identity greater than or equal to 80%. Second, to identify and remove SNPs potentially under selection, the software Bayescan version 2.0 was utilized (Foll and Gaggiotti, 2008). Default settings were maintained, including 20 pilot runs of 10,000 iterations, a burn-in of 50,000 iterations, and a final run of 100,000 iterations. The prior odds of the neutral model were set to 10,000 to minimize false positives (Foll and Gaggiotti 2008).

Different datasets were subjected to the aforementioned preliminary tests to meet the assumptions of each analysis. For paternity, SGS, and mating system analyses, a dataset with 702 SNPs (10% MD and 0.4 MAF) was used. For genetic diversity and inbreeding estimates, a dataset with 4,738 SNPs (10% MD and 0.01 MAF) was employed. For the estimates of $N_E$, a dataset with 5,100 SNPs (10% MD and 0.01 MAF) was used.

### *Parentage analysis*

The software COLONY version 2.0.6.8 (Wang, 2004) was utilized for the analysis of paternity in cases where there is known maternal kinship but unknown paternal kinship. The dataset was divided into three classes: Seedling Sample, CFS (Candidate Father Sample), and CMS (Candidate Mother Sample) based on the aforementioned ontogenetic stages. All adult trees were considered as CFS and/or CMS and the known maternal kinship option in Colony was added as putative mothers.

Putative mothers were determined by analyzing distance matrices between reproductive adults and seedlings based on their geographic coordinates. It was assumed that the putative mother is the breeding tree closest to the progeny, as trees farther away are more likely to contribute to pollen dispersal distance (Dow and Ashley, 1996; Nazareno and Knowles, 2021). Paternity reconstruction was conducted separately for seedlings from RNV (n=21) and Sooretama (n=29), using CFS and CMS from both locations (n=85).

The software COLONY was utilized with the following configurations: polygamy for both males and females, inbreeding mating, no clones, monoecious, full likelihood estimates, and medium likelihood precision. The Run Specifications and Sibship Prior parameters were left at their default values. Initially, mistyping error rates (genotyping errors including mutations) and allelic dropout rates were set to 0.001 and 0, respectively. COLONY then re-estimated these rates for each marker locus based on the best reconstructed pedigree.

To calculate seed and pollen dispersal distances, the following criteria were applied: (I) only parents with a paternity or maternity attribution probability greater than 95% were considered; (II) seedlings with only one putative parent assigned were assumed to be the mother, and this parent was used to infer the seed dispersal distance assuming that the putative mother is the adult reproductive tree closest to the seedling, since those more distant tend to contribute with pollen dispersal distance (e.g., Dow and Ashley, 1996; Guidugli et al., 2016;

Nazareno and Knowles, 2021); (III) for seedlings with two assigned parents, the closest candidate to the seedling was considered the maternal parent, while the other candidate was the paternal parent (e.g., Dow and Ashley, 1996; Guidugli et al., 2016).

Using the obtained paternity results, the direct gene flow ($\sigma^2 rt$) was calculated using the equation $\sigma^2 rt = ⅕\, \sigma^2 p\text{-}rt + \sigma^2 s\text{-}rt$ (Crawford, 1984), where $\sigma^2 p\text{-}rt$ represents the pollen dispersal variance and $\sigma^2 s\text{-}rt$ represents the seed dispersal variance. Seed (*ms*) and pollen (*mp*) immigration rates were calculated as the percentage of genotypes not assigned to a candidate parent within the population (with probability > 0.95). In this case, *ms* represents the percentage of seedlings without any assigned parent in relation to the total, while *mp* represents seedlings with only one assigned parent (Smouse and Sork, 2004; Burczyk et al., 1996; Sebbenn et al., 2011).

### *Fine-Spatial Genetic Structure*

The SGS was evaluated using the SPAGeDi program v. 1.5d (Hardy and Vekemans, 2002) through spatial autocorrelation analysis between kinship coefficients and distance intervals among individuals and the *Sp* statistic. Relationship coefficients ($R_{ij}$) were computed for each ontogenetic stage of the two localities. The kinship coefficient estimators $F_L$ (Loiselle et al., 1995) and six distance intervals were employed, with the jackknife method across all the loci used to calculate the 95% confidence interval (CI) of the standard error of the relatedness coefficients.

The intensity of SGS was assessed using the *Sp*-statistic defined by Vekemans and Hardy (2004). The Sp-statistic was used to compare the extent of SGS between localities. The equation $Sp = -b/(1 - F_1)$, was used to obtain the *Sp*-statistic, where *b* is the regression slope of $F_{ij}$ on log spatial distance, and $F_1$ is the mean $F_{ij}$ between individuals for the first distance class (Vekemans and Hardy, 2004). Significance of the regression was determined by 10,000 permutations of multilocus genotypes. The observed $R_{ij}$ was considered statistically significant if it deviated from the 95% confidence interval established by data permutations for the null hypothesis of no spatial genetic structure, $R_{ij}$=0 (Peakall et al., 2003; Shchipanov et al., 2022). Significant positive or negative structure was inferred if the CIs of standard error did not overlap.

The gene dispersal distance will also be estimated to compare with the direct gene flow obtained by paternity analysis (Crawford, 1984; Vekemans and Hardy, 2004). The root-mean-squared dispersal distance ($\sigma$) was calculated using the equation$\sigma^2 = Nb/4\pi De$, where the Wright's neighborhood size (*Nb*) equals *1/Sp*, and effective population density (*De*) equals product of the census density $D$ and *Ne/N* (Vekemans and Hardy, 2004). To calculate *D* we use *D=N/area*, where *N* represents the total number of sampled plants of each locality of *D. jueirana-facao* (RNV= 116; Sooretama= 95) divided by their distribution area (RNV= 42.99 hectares; Sooretama= 64.81 hectares) reported by Lewis et al. (2017). After, to calculate the values of effective population size (*Ne*) we use the number of reproductive trees sampled (RNV= 17; Sooretama= 13). *De* RNV values were 0.2 ind.ha for adult trees and 0.48 ind.ha for seedlings, while for Sooretama were 0.4 ind.ha for adult trees and 0.44 ind.ha for seedlings.

### *Genetic diversity and inbreeding parameters*

To evaluate the impact of forest fragmentation on the genetic variation of *D. jueirana-facao* over distinct generations, we employed a rarefaction method implemented in ADZE 1.0 (Szpiech et al., 2008) to estimate standardized allelic richness (*A*) and standardized private allelic richness (*R*). This method accounted for differences in sample sizes between the two ontogenetic stages (i.e., reproductive adults and seedlings) of *D. jueirana-facao* in both localities. ADZE was run using a maximum standardized sample size of 29, with a missing data tolerance of 0.15 per locus. In addition, genetic estimates of $N_E$ for each sampling location were obtained using the linkage disequilibrium method in the program NeEstimator Version 2.1 (Do et al., 2014). To include all alleles in the $N_E$ calculation, we used an allele frequency critical value equal 0 (Doet al., 2014). The 95% CI were calculated using the jackknife resampling method.

To represent individuals before (≥120 years old) and after the period of forest fragmentation, we selected reproductive adults and seedlings from RNV (n= 12 and n= 21, respectively) and Sooretama (n= 29 and n= 13), based on tree age estimates and historical reports of anthropogenic activities in the study region (Kierulff et al., 2014; Rolim et al., 2016). Tree ages were estimated using DBH data and a growth rate of 0.36 cm/year SE±0.19 (DBH > 20 cm) and 0.62 cm/year SE±0.19 (DBH < 20 cm). These estimates were based on the growth rate of the congener species *D. excelsa*, which is 0.53 cm/year (DBH > 20 cm) and 0.91 cm/year (DBH < 20 cm) (Schwartz et al., 2016). Considering that tree species in the Amazon biome, such as *D. excelsa*, exhibit higher growth rates compared to those in the Atlantic Forest (Locosselli et al., 2017), we used a conservative growth rate that was 32% lower than that of *D. excelsa*.

To assess the level of inbreeding for the two generations in both localities, we calculated the inbreeding coefficient (Wright's Fixation Index $F_{IS}$; Wright, 1943) using the BasicStats function in the R package DiveRsity (Keenan et al., 2013).

### *Mating system analysis*

The kinship coefficient, as described by Loiselle et al. (1995) and implemented in the SPAGeDi program (Hardy and Vekemans, 2002), was utilized to estimate random outcrossing rates (1- $s$, where $s$ represents the selfing rate). These rates were determined based on standardized identity disequilibrium for adult individuals from both localities of *D. jueirana-facao*. Significance for the identity disequilibrium coefficient was obtained through 1,000 permutations, and a jackknife over loci was employed to calculate the standard error of outcrossing estimates.

## RESULTS

### *Demographic size structure*

A total of 211 individuals were surveyed in the *D. jueirana-facao* localities, with 116 individuals in RNV and 95 individuals in Sooretama. The population density was 2.7 ind.ha in RNV and 1.46 ind.ha in Sooretama. Using the previously defined ontogenetic stages (see materials and methods), we estimated that in RNV, 18.1% (21) of the individuals were seedlings, and 52.5% (61) were reproductive adults. In Sooretama, 38.9% (37) were seedlings, and 33.6% (32) were reproductive adults.

The data on the distribution of DBH and tree height did not exhibit a normal distribution (results on Table S1). When comparing these distribution data between localities for each ontogenetic stage, we found no significant differences in both diameter and height distributions ( $p > 0.05$, Table S1), except when comparing the diameter distribution of Sooretama adults with RNV adults, which showed significant differences ( $p < 0.05$, Table S1).

Graphical analysis of these distributions revealed a higher proportion of individuals in the smaller height and DBH classes in both localities (see Figure 1). In RNV, 49.1% of trees had a DBH between 0.3 and 14 cm, while in Sooretama, this proportion was 68.4%. The height distribution showed that 73.2% of individuals in RNV were between 0.08 and 15 m in height, compared to 81% in Sooretama. Furthermore, an inverted J-shaped distribution pattern was observed for the DBH classes, which is commonly used in population size analyses (e.g., Boz and Maryo, 2020; Muluneh et al., 2021; Yemata and Haregewoien, 2022; Xie et al., 2023). Finally, in terms of regeneration status, RNV exhibited a fair status, while Sooretama showed a good status (Table S2).

### *Sequencing, SNPs identification, data filtering and prior genomic analyses*

Illumina sequencing yielded approximately 144 million single-end raw reads per lane. The mean number of retained reads that met the default quality filters, including a Phred quality score > 33, were as follows: 2,319,619.1 ± 1,076,166.86 SE for the first lane, 2,099,300.824 ± 1,299,075.296 for the second lane, 2,030,593.393 ± 1,702,835.731 for the third lane, and 2,026,080.63 ± 1,357,852.272 for the fourth lane. A total of 12% of individuals (n=27) were excluded from the dataset for having less than 500,000 retained reads (Figure S2).

Considerable variation in the number of SNPs was observed, ranging from 0% to 30% MD, for both RNV (SNP range = 450 to 9,869) and Sooretama (SNP range = 506 to 9,481). Genetic diversity parameters showed no sensitivity to MD variation, with overlapping 95% confidence intervals (CI) for each ontogenetic stage (see Table S3). To test the effect of MAF on the number of polymorphic sites, a fixed value of 10% MD was used, resulting in approximately 4,300 SNPs. The number of SNPs due to MAF variation ranged from 11 SNPs (MAF = 0.5) to 4,194 SNPs (MAF = 0.05) (Table S4).

Prior to genomic analyses, steps were taken to avoid possible sources of bias. For the paternity, SGS, and mating system analyses, an initial dataset consisting of 702 SNPs was identified. After a Bonferroni adjustment, 44 SNPs deviated from the Hardy-Weinberg equilibrium ($p > 7.12 \times 10^{-5}$). No SNPs showed LD after Bonferroni adjustment ($k = 2.46 \times 10^{5}$, $p < 2.03 \times 10^{-7}$), and 14 potential loci under selection were detected, with no plastid loci found. Following filtering, 58 loci were removed, leaving a total of 644 SNPs for analyses. For the genetic diversity and inbreeding parameter estimates, a total of 4,738 SNPs was identified. However, 166 SNPs deviating from HW after a Bonferroni adjustment ($p > 1.05 \times 10^{-5}$) were removed, along with 61 potential loci under selection. No SNPs showed LD after Bonferroni adjustment ($k = 1.12 \times 10^{7}$, $p < 4.46 \times 10^{-9}$), and three plastid loci were found. Following filtering, a total of 4,508 SNPs remained for analysis. For $N_E$, our initial dataset contained 5,100 SNPs, it was also filtered and 352 SNPs deviating from HW after a Bonferroni adjustment ($p > 9.8 \times 10^{-6}$) were removed. No SNPs showed potential loci under selection and also no SNPs showed LD after Bonferroni adjustment ($k = 1.3 \times 10^{7}$, $p < 3.84 \times 10^{-9}$). Seven plastidial loci were found. After filtering, a total of 4,741 SNPs remained for analysis.

### *Paternity assignment*

Based on 644 SNPs, sixteen seedlings were assigned to 11 parents within the RNV locality, with some resulting from selfing. However, the parents of five seedlings could not be identified. In Sooretama, 18 seedlings were assigned to 12 parents within the same locality, but 11 seedlings either had unidentified parents or the inferred probability was less than 95%.

Figure 2 illustrates the dispersal distances of seeds and pollen. In both localities, the majority of dispersal occurred over short distances. For RNV, the average seed dispersal distance was 13.2 meters (m) ± 12.7 SE (range 2.9 - 55 m), while the average pollen dispersal distance was 44.7 m ± 46.4 SE (range 4.25 - 149.1 m). Conversely, in Sooretama, the average seed dispersal distance was 8.39 m ± 5.7 SE (range 2.3 - 23.6 m), and the average pollen dispersal distance was 33.4 m ± 10.3 SE (range 21.4 - 48 m).

The immigration rates of RNV were determined to be 23.80% for seeds (*ms*) and 19.04% for pollen (*mp*), whereas in Sooretama, the rates were 20.68% *ms* and 44.82% *mp*. Taking into account the variances in pollen and seed dispersal, the total gene flow was estimated to be 34.04 m and 8.38 m for RNV and Sooretama, respectively.

### *SGS and indirect dispersal distance*

A significant spatial genetic structure, as indicated by the 95% CI, was observed in the RNV locality, with a maximum distance of 134.5 m for seedlings and 53.6 m for reproductive adults. Similarly, significant values were found in Sooretama, with distances of up to 32.1 m for seedlings and 37.1 m for reproductive adults. Overall, the $F_L$ values decrease and become non-significant in the upper distance classes (Figure 3).

Both *D. jueirana-facao* reproductive adults and seedlings exhibited variable kinship coefficients across distance classes for both localities, with the highest Loiselle's kinship coefficient observed in the first distance class (0-50 m). In RNV, the highest kinship for seedlings ($F_L = 0.112$, $p < 0.05$) corresponded within theoretical expectations to half-siblings ( $F_L = 0.125$), while in Sooretama this coefficient ($F_L = 0.0614$, $p < 0.05$) corresponded to first cousins ($F_L = 0.0625$). Among *D. jueirana-facao* reproductive adults, the highest kinship ($F_L =$ 0.0248, $p < 0.05$) corresponded within theoretical expectations to full-siblings ($F_L = 0.250$), while in Sooretama this coefficient represents half-siblings ($F_L = 0.125$) for the highest observed estimate ($F_L = 0.0150$, $p < 0.05$).

The magnitude of SGS, as measured by the *Sp*-statistic, was stronger in the RNV locality compared to Sooretama. For the former locality, the *Sp* values were 0.05088 (CI = 0.05085 - 0.05091) and 0.01664 (CI = 0.01664 - 0.01664) for seedlings and reproductive adults, respectively. For Sooretama, the Sp values were 0.03158 (CI = 0.03157 - 0.03159) and 0.01162 (CI = 0.0116 - 0.01162) for seedlings and reproductive adults, respectively. Additionally, the SGS was found to be stronger in seedlings compared to reproductive adults (Table 1).

Considering the variances in pollen and seed dispersal, the total indirect gene flow distance was higher than the contemporary estimates in both localities (Table 1).

### *Mating system, genetic diversity, and inbreeding parameters*

Based on the analysis of the mating system, the outcrossing rate was found to be 0.977 (SE±0.004) for RNV and 0.942 (SE± 0.009) for Sooretama.

In terms of genetic variation, the mean allelic richness (*A*) values differed across generations for RNV, with reproductive adults [*A*= 1.711 (95% CI= 1.705 - 1.717)] presenting higher values compared to seedlings [*A*= 1.661 (95% CI= 1.655 - 1.667)]. However, for Sooretama, there were no significant differences between reproductive adults [*A*= 1.690 (95% CI= 1.684 - 1.696)] and seedlings [*A*= 1.694 (95% CI= 1.689 - 1.700)]. When we compare allelic richness between different localities, we observe that reproductive adults in RNV had higher values than Sooretama (see Table S5). Nonetheless, seedlings in RNV showed lower values than those observed in Sooretama.

In the RNV locality, the comparison of average private allelic richness (*R*) between ontogenetic stages showed higher values for reproductive adults [*R*= 0.197 (95% CI= 0.192 – 0.202)] than seedlings [*R*= 0.164 (95% CI= 0.160 – 0.168)]. In contrast, Sooretama displayed smaller *R* values for reproductive adults [*R*= 0.1766 (95% CI= 0.172 - 0.181)] compared to seedlings [*R*= 0.197 (95% CI= 0.193 - 0.201)]. The results also indicated that reproductive adults in RNV had lower *R* values than those observed in Sooretama, and the same pattern was observed for seedlings. (see Table S5). Lastly, estimates of contemporary $N_E$ were 40.0 (95%CI = 33.5 - 44.1) for RNV locality and 31.3 (95%CI = 24.6 - 37.7) for Sooretama.

Regarding the average inbreeding coefficients $F_{IS}$, both localities showed negative values for reproductive adults (RNV= -0.051, Sooretama= -0.049). However, seedlings exhibited positive values (RNV= 0.044, Sooretama= 0.006). Nevertheless, no statistically significant differences ($p$-value > 0.05) were found when comparing the values between generations. Furthermore, the mean $F_{IS}$ between loci was not significantly different from zero for all generations.

## DISCUSSION

This study aimed to investigate the genetic and ecological consequences of forest fragmentation on *D. jueirana-facao*, a rare and critically endangered tree species endemic to the Brazilian Atlantic Forest. Our hypothesis was that forest fragmentation would result in reduced genetic diversity, limited gene flow, and altered demographic size structure, as commonly observed for plant populations in fragmented and degraded habitats (e.g., Young et al., 1996; Lowe et al., 2005; Aguilar et al., 2008; Nazareno and Reis, 2014; Rother et al., 2016; Frankham et al., 2017; Schlaepfer et al., 2018; Allendorf et al., 2022; Khosa et al., 2023; Wu et al., 2023). Our findings indicate that *D. jueirana-facao* seems to be resilient to forest fragmentation and habitat loss, as evidenced by a healthy demographic size structure, absence of inbreeding, no apparent reduction in genetic diversity, and high levels of gene flow in the studied areas. We will now discuss the implications of these results, exploring potential mechanisms behind the species' resilience and factors contributing to genetic diversity and connectivity in the face of forest fragmentation. Additionally, we will highlight the implications for conservation and management of *D. jueirana-facao*, emphasizing the need for a comprehensive understanding of its ecological and evolutionary dynamics in the context of ongoing habitat loss and fragmentation.

### ***Small populations, but not as small as previously believed***

Our discovery of a significantly larger number of *D. jueirana-facao* individuals (n > 211) in comparison to a previous study (Lewis et al., 2017) which reported less than 25 trees is highly relevant. Overall, our findings provide a more accurate understanding of the population abundance of *D. jueirana-facao*. Although the forest fragments where *D. jueirana-facao* occurs are surrounded by roads and an agricultural matrix, the potential existence of *D. jueirana-facao* individuals or populations outside the study area remains unknown and should be prioritized in future field expeditions. Such survey efforts would enhance our comprehensive understanding of the species' geographic distribution and aid in identifying priority areas for conservation.

Contrary to expectations for a threatened plant species (e.g., Carvalho and Nascimento, 2009; Helm and Witkowski, 2012; Cousins et al., 2014; Nazareno and Reis, 2014; Rother et al., 2016; Khosa et al., 2023), the population size structure of *D. jueirana-facao* exhibited a typical reverse J-shaped distribution (Meyer, 1952). This pattern indicates a higher number of individuals in smaller diameter classes (Atsbha et al., 2019; Balemlay and Siraj, 2021) and suggests healthy populations that naturally regenerate through recruitment (Boz and Maryo, 2020; Balemlay and Siraj, 2021). Similar findings have been reported in various plant species, even in disturbed environments (Muluneh et al., 2021; Yemata and Haregewoien, 2022; Atsbha et al., 2019). However, it is crucial to consider that factors such as resource availability, competition among individuals, natural or anthropogenic disturbances, natural selection against inbreeding, and habitat traits can influence the diameter-height size distribution, as well as seedling recruitment and survival (Nazareno and Reis, 2014; Takahashi et al., 2018; Moraes et al., 2018; West, 2023).

When comparing the demographic structure between *D. jueirana-facao* localities, their regeneration patterns were distinct, with RNV showing fair regeneration, while Sooretama exhibited good regeneration. This is a positive aspect for the species, as the presence of seedlings in populations may be attributed to unlimited abiotic factors and a positive trade-off between predation, selection and/or competition and survival rate (Balemlay and Siraj, 2021; Yemata and Haregewoien, 2022). Overall, our results demonstrate a healthy demographic size structure for *D. jueirana-facao*, indicating its resilience to environmental disturbances. However, long-term monitoring of *D. jueirana-facao* localities, collecting annual demographic data, is imperative to protect the ecological integrity of their populations.

### ***Dinizia jueirana-facao populations seem not to be genetically isolated***

Contemporary estimates of gene flow in both investigated localities were lower than historical estimates, highlighting the threat posed by forest fragmentation in the species *D. jueirana-facao*. This pattern was particularly evident in Sooretama, where even lower values were observed. These findings are consistent with previous studies involving trees (Monthe et al., 2017; Lompo et al., 2020; Nazareno and Knowles, 2021; Jiang et al., 2022), as predicted by the theory suggesting that recent disturbances primarily affect contemporary estimates of gene dispersal (Oddou-Muratorio & Klein, 2008). However, despite the occurrence of disturbances, gene dispersal can persist at significant levels within populations (Oddou-Muratorio et al., 2010; Sola et al., 2020), indicating the existence of mechanisms promoting genetic connectivity at restricted geographic scales. Therefore, the persistence and variations in the magnitude of contemporary gene flow can be attributed to a range of factors, such as population density, species' mating system, and specific responses to anthropogenic disturbances due to the range of distinct plant life-history traits (Bacles and Jump, 2011; Hamrick and Trapnell, 2011; Hardy et al., 2019; Goncalves et al., 2022).

Regarding the gene dispersal distances of *D. jueirana-facao*, contemporary estimates reveal that gene flow by pollen is remarkably more efficient, with distances approximately four times greater than seed dispersal in the Sooretama locality, as previously observed for RNV (Nazareno and Knowles, 2021). This trend is consistent with previous studies in tree species (e.g., Guidugli et al., 2016; Hardy et al., 2019; Angbonda et al., 2021; Sujii et al., 2021), highlighting the importance of pollen flow to prevent inbreeding and self-pollination in tree species with both hermaphrodite and functional male flowers, such as *D. jueirana-facao*. In fact, the pattern of pollen flow observed for *D. jueirana-facao* seems to be directly linked to the lack of inbreeding (i.e., inbreeding rates were close to zero) and very low historical selfing rates.

The effectiveness of pollen or seed dispersal is one of the biological aspects contributing to the resistance of plant species to the detrimental effects of forest fragmentation (Sen and Ravikanth, 2022). This process is closely related to plant density, landscape features, disperser behavior, floral and fruit traits, breeding and mating systems, and even associated with pollination systems (Dick et al., 2003; Thavornkanlapachai and Byrne, 2018; Solís-Hernández et al., 2019; Johnson et al., 2019; Cruzan and Hendrickson, 2020). For instance, in fragmented landscapes, gene flow through pollen can increase, as observed in tropical species with specialized pollination systems (Nason & Hamrick, 1997; Nazareno and Carvalho, 2009) or even for plant species with generalized pollination systems such *as Dinizia excelsa*, where fragmentation led to changes in pollinators allowing pollen transport over very long distances (Dick et al., 2003). Other studies have shown the positive effect of secondary vegetation in reducing the average isolation of forest fragments, enabling greater landscape connectivity, improving the mobility of potential dispersers, and maintaining gene flow (Molin et al., 2018; Ribeiro et al., 2021; Alencar et al., 2023). Nevertheless, some studies have reported that even wind-pollinated tree species can be negatively affected by fragmentation and habitat loss (Jump and Peñuelas, 2006; Barbeta et al., 2011; Kitamura and Nakanishi, 2021), indicating that knowledge of the species' life-history traits is the main, but not the only factor in predicting the genetic consequences of habitat fragmentation.

The primary means of seed dispersal for *D. jueirana-facao* is gravity, while pollen may potentially be dispersed by wind. This mode of pollen dispersal may contribute to its efficient long-distance distribution. Our findings demonstrate that deforestation and habitat loss, leading to an open landscape, contribute to long-distance pollen flow, which mitigates the potential negative genetic effects of forest fragmentation. We observed gene immigration beyond the forest fragment edges, indicating long-distance pollen movement. However, it is important to consider the estimated immigration rates with caution due to uncontrolled factors in the sampling, such as missing genotypes caused by tree mortality and illegal timber activities in the past years (Oddou-Muratorio et al., 2010; Sola et al., 2020).

Despite the presence of gene immigration, we did not identify any parentage assignments between the two studied localities, as the assignments were exclusive to each locality. While the population census was realized in RNV locality, we covered approximately 80% of the Sooretama locality area. This suggests that the parent candidates in the Sooretama locality may not have been sampled or may have been missing due to the presence of dead and decaying trees. Nevertheless, our results indicate that the RNV locality is not genetically isolated, as there is evidence of long-distance pollen dispersal between the forest fragment and the nearest pollen source, as previously observed by Nazareno and Knowles (2021).

Considering the uncertainty surrounding the genetic connectivity of the Sooretama locality, and even though there are no apparent negative effects of forest fragmentation on genetic diversity, it is crucial to promote connectivity among the remaining fragments. This will help avoid the negative genetic effects observed in small populations (Schlaepfer et al., 2018; Toczydlowski and Waller et al., 2019; Naaf et al., 2021; Yıldız et al., 2022). Strategies such as the implementation of ecological corridors could be an effective action to minimize further detrimental genetic and ecological effects in these small localities (e.g., Damschen et al., 2014; Rosot et al., 2018).

In terms of historical gene flow estimates, we observed SGS in both reproductive adults and seedlings of both localities. This indicates a high probability of relatedness between individuals (e.g., up to 134.5 m in RNV seedlings), with a decline as geographic distances increase. Typically, plant species with long-distance dispersal of pollen and seeds exhibit low or no SGS (Hardy et al., 2006). Our study reveals a significant SGS, with higher *Sp* values (*Sp* range = 0.01162 - 0.05088) compared to other tree species [e.g., *Parkia biglobosa* (*Sp* range = 0.0002 to 0.003; Lompo et al., 2020), *Entandrophragma cylindricum* (*Sp* = 0.0058; Monthe et al., 2017)]. This suggests that the observed SGS in *D. jueirana-facao* may be a result of seed dispersal in close proximity to mother trees, with pollen dispersal playing a secondary role. Previous studies have shown that the strength of SGS is influenced by pollination and seed dispersal vectors (Vekemans and Hardy, 2004; Hardy et al., 2006; Dick et al., 2008; Gelmi-Candusso et al., 2017; Goncalves et al., 2022; Gamba and Muchhala et al., 2023). For instance, Vekemans and Hardy (2004) and Dick et al. (2008) demonstrated that plants dispersed by gravity tend to have higher *Sp* values compared to species dispersed by animals, even if pollen movement is random and long-distance (Dick et al., 2008).

### *Is Dinizia jueirana-facao resilient to the negative effects of forest fragmentation?*

Despite the expected loss of genetic variation and reduced gene flow in fragmented and small plant populations (e.g., Young et al., 1996; Lowe et al., 2005; Aguilar et al., 2008; Frankham et al., 2017; Schlaepfer et al., 2018; Allendorf et al., 2022), our results seemingly support the hypothesis that *D. jueirana-facao* is capable of mitigating the adverse effects of forest fragmentation. Consistent with the findings for *D. jueirana-facao*, numerous studies, including meta-analyses, have reported a perceived resilience of tree species to the population genetic effects of habitat loss and fragmentation (e.g., Browne et al., 2015; Solís-Hernández et al., 2019; Braun et al., 2020; González et al., 2020; Lompo et al., 2020; Ony et al., 2020; De Santana et al., 2023). This unexpected scenario arises from the long lifespan of trees, the presence of overlapping generations that help prevent the loss of genetic diversity, the flexible mating systems in some species that can bypass self-incompatibility and produce self-pollinated offspring, and the prevalence of extensive gene flow. Indeed, the extensive pollen flow over long distances and migration events, coupled with our contrasting findings of genetic diversity between the two *D. jueirana-facao* localities, indicate that forest fragmentation has not yet had a negative impact on this tree species. In fact, due to temporal inertia, the population genetic consequences of fragmentation, manifested by increased inbreeding between generations, may not be immediately apparent in long-lived plant species such as *D. jueirana-facao*, requiring consideration of the interaction between the time elapsed after the fragmentation process and the species' life cycle (e.g., Hamrick 2004; Kramer et al., 2008; Lowe et al., 2015; Garcia et al., 2019; González et al., 2020; Delnevo et al., 2021; de Santana et al., 2023).

Although we did not find evidence of inbreeding in either locality or for the two ontogenetic stages of *D. jueirana-facao*, our results reveal a slight reduction in allelic and private allelic richness only for RNV. Undoubtedly, allelic richness is the genetic parameter most sensitive to the effects of forest fragmentation compared to other commonly used metrics, making it suitable for tracking recent effects of habitat fragmentation (Jump and Peñuelas 2006, Nazareno and Jump 2012, Hoban et al., 2023). While reduced genetic diversity associated with forest fragmentation has been well-documented in many other species (e.g., Moraes et al., 2018; Lauterjung et al., 2019; Hernández et al., 2020; Waqar et al., 2021; Kumara et al., 2022), it is also common for genetic diversity to be maintained (Solís-Hernández and Fuchs, 2019; Braun et al., 2020; De Santana et al., 2023). This contrast reveals how life-history traits (e.g., life cycle, growth form, mating system) strongly influence the amount and distribution of genetic variation in natural populations (Goetze et al., 2018; González et al., 2020; Bard et al., 2021; De Kort et al., 2021).

While historical outcrossing rates suggest an allogamous mating system for *D. jueirana-facao*, paternity assignments have revealed a higher incidence of contemporary selfing events in the RNV locality compared to Sooretama (see Table S6). Indeed, it was in the RNV locality that we found a slight loss on genetic variation. It is worth noting that outcrossing rates can vary spatially and temporally, and the prevalence of different mating systems can be influenced by abiotic factors that affect plant fitness (Wright et al., 2013; Barrett and Harder, 2017). For instance, despite expectations of autogamous and apomictic systems in anthropized environments, Silva et al. (2022) found a higher occurrence of allogamous species. Landscape anthropization (Eckert et al., 2010), environmental changes, plant density, and demographic size structure can all impact mating rates (Wright et al., 2013). Therefore, conducting long-term studies related to genetic, phenology and pollination biology are crucial to better understand the factors that affect reproduction in *D. jueirana-facao*.

### ***Caveats, limitations, and future studies***

While our study aimed to assess the genetic and ecological effects of forest fragmentation in a rare, endemic, and critically endangered tree species, there are limitations and caveats to consider regarding the contemporary gene flow analyses, particularly related to sampling design (e.g., incomplete sampling in the Sooretama locality) and limited knowledge about the presence of *D. jueirana-facao* outside the two known localities. Although the isolation of the Sooretama locality remains uncertain due to unsampled or missing candidate parents (e.g., decayed or removed trees due to illegal timber activity), our study provides evidence that this locality does not exhibit the expected characteristics of isolated and small populations affected by forest fragmentation (e.g., Waqar et al., 2021; Kumara et al., 2022; Jiang et al., 2022). The fragmented populations of *D. jueirana-facao* in Sooretama demonstrates remarkable resilience to cope with the effects of forest fragmentation. Although our results are promising, long-term ecological and genetic studies are still needed. In addition to our SNP-based approach, deeper genomic analyses such as the identification and characterization of structural variants – which has been proposed for threatened species (Wold et al., 2021) – would provide an excellent opportunity to enhance the conservation of *D. jueirana-facao*. These complementary approaches should be prioritized as a foundation for conservation and management programs for *D. jueirana-facao*.

### ***Practical conservation recommendations***

This study offers valuable insights for the conservation of *D. jueirana-facao*. Specifically, our estimates of indirect dispersal indicate the need to collect seeds from reproductive trees separated by at least 134.5 m for RNV and 37.1 m for Sooretama. This information should be considered in ex-situ conservation and restoration programs (Bittencourt and Sebbenn, 2007; Ramos et al., 2018; De Oliveira et al., 2020; Nazareno and Knowles 2021; Wang et al., 2023). It is important to note that, while the individuals in RNV and Sooretama are located in protected areas, with an apparent lacking of inbreeding and showing a healthy demographic size structure, the Sooretama locality is threatened by ongoing habitat loss due to deforestation associated with human activities such as agriculture, livestock farming, and mining. Therefore, continuous monitoring of the localities where *D. jueirana-facao* occurs is urgently needed as part of conservation programs. Particularly, agriculture in and around these forest fragments and ecotourism activities should be addressed in collaboration with stakeholders (e.g., local communities and important national companies such as Vale do Rio Doce) to ensure the maintenance and conservation of this rare genetic resource. Additionally, based on estimates of contemporary $N_E$, the findings indicate that *D. jueirana-facao* have $N_E$ values below the recommended threshold of 50 set by the "50/500 rule" for short-term inbreeding concerns. The species also does not meet the minimum Ne threshold of 500 (Franklin, 1980; Allendorf et al., 2022), or as proposed by Frankham (2022), a $N_E$ value greater than 1000, for maintaining long-term evolutionary potential. This suggests that both localities may be at risk of reduced genetic diversity and increased susceptibility to genetic problems in the future. Therefore, keeping the areas where *D. jueirana-facao* occurs without further degradation and habitat loss would help to maintain more than 97% of *D. jueirana-facao*'s genetic diversity – a threshold above that recommended for the CBD draft post-2020 global biodiversity framework (Frankham, 2022; Hoban et al., 2023).

## ACKNOWLEDGEMENTS

We thank Thaís Martins and Domingos A. Folli for assistance during fieldwork. We also thank Reserva Natural Vale for their field support of this research.

## FUNDING

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for funding a one-year visit of AGN to the University of Michigan, Ann Arbor (88887.369570/2019-00) and for funding through a grant to KASP. Additional funds were provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through a Pq-2 grant to AGN (306182/2020-3). This research was also sponsored by the Mohamed bin Zayed Species Conservation Fund (project number: 202525142). This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

## DATA ACCESSIBILITY

SNP data sets for *D. jueirana-facao* are available for download from the Dryad Digital Repository (https://doi.org/10.5061/dryad.0vt4b8h01). The raw data generated for *D. jueirana-facao* are available to download from the ENA (European Nucleotide Archive) under accession number ERP129560.

## AUTHOR CONTRIBUTIONS

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

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## TABLES

**TABLE 1** Estimates of fine-scale spatial genetic structure (SGS) and of historical dispersal distance for seedlings and reproductive adult trees of *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira in two Atlantic forest fragments – Reserva Natural da Vale (RNV) and Sooretama – located in the Espírito Santo State, Southeast Brazil. The number of individuals and the parameters such as inbreeding coefficient (*F*), average kinship coefficient ($F_1$) between individuals for the first distance class (i.e., the smallest distance class, which includes distances < 50 m) and its standard error (SE), the *Sp*-statistic, the neighborhood size (*Nb*) and its 95% CI, and the root-mean-squared dispersal distance (σ) and its 95% CI, are shown considering the effective densities of 0.2 for reproductive adult trees and 0.48 for seedlings in RNV, and 0.4 for reproductive adult trees and 0.44 for seedlings in Sooretama.

| | | | **SGS parameters** | | | | **Gene dispersal estimates** | |
|---|---|---|---|---|---|---|---|---|
| | | ***N*** | ***F*** | F1 | ***SE*** | ***Sp*** | ***Nb*** | **σ (m)** |
| RNV | Seedlings | 21 | 0.1077 | 0.1123 | 0.0051 | 0.05088 | 19.65<br>(19.64 - 19.67) | 105.12<br>(105.09 - 105.15) |
| | Adults | 59 | 0.0054 | 0.0248 | 0.0017 | 0.01664 | 60.09<br>(60.08 - 60.1) | 308.09<br>(308.08 - 308.12) |
| Sooretama | Seedlings | 29 | 0.0222 | 0.0614 | 0.0035 | 0.03158 | 31.66<br>(31.65 - 31.68) | 123.4<br>(123.38 - 123.43) |
| | Adults | 26 | -0.0408 | 0.0150 | 0.0026 | 0.01162 | 86.09<br>(86.06 - 86.13) | 192.67<br>(192.63 - 192.7) |

## FIGURE CAPTIONS

**FIGURE 1** Histograms of the diameter at breast height (DBH) and height distribution for different ontogenetic stages in *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira in two Atlantic forest fragments – Reserva Natural da Vale (RNV) and Sooretama – located in the Espírito Santo State, Southeast Brazil.

**FIGURE 2** Histogram of gene flow distances for pollen (black bars) and seeds (gray bars) of *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira in two Atlantic forest fragments – Reserva Natural da Vale (RNV - A) and Sooretama (B) – located in the Espírito Santo State, Southeast Brazil.

**FIGURE 3** Average Loiselle's kinship coefficient $F_L$ plotted against geographical distance (black dashed line) for seedlings and adults of *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira in two Atlantic forest fragments – Reserva Natural da Vale (RNV) and Sooretama. The error bars (gray dashed lines) were generated by jackknifing over loci to approximate SE, and 95% CI were generated through 10,000 data permutations and are indicated by gray solid lines.

**FIGURE 1**

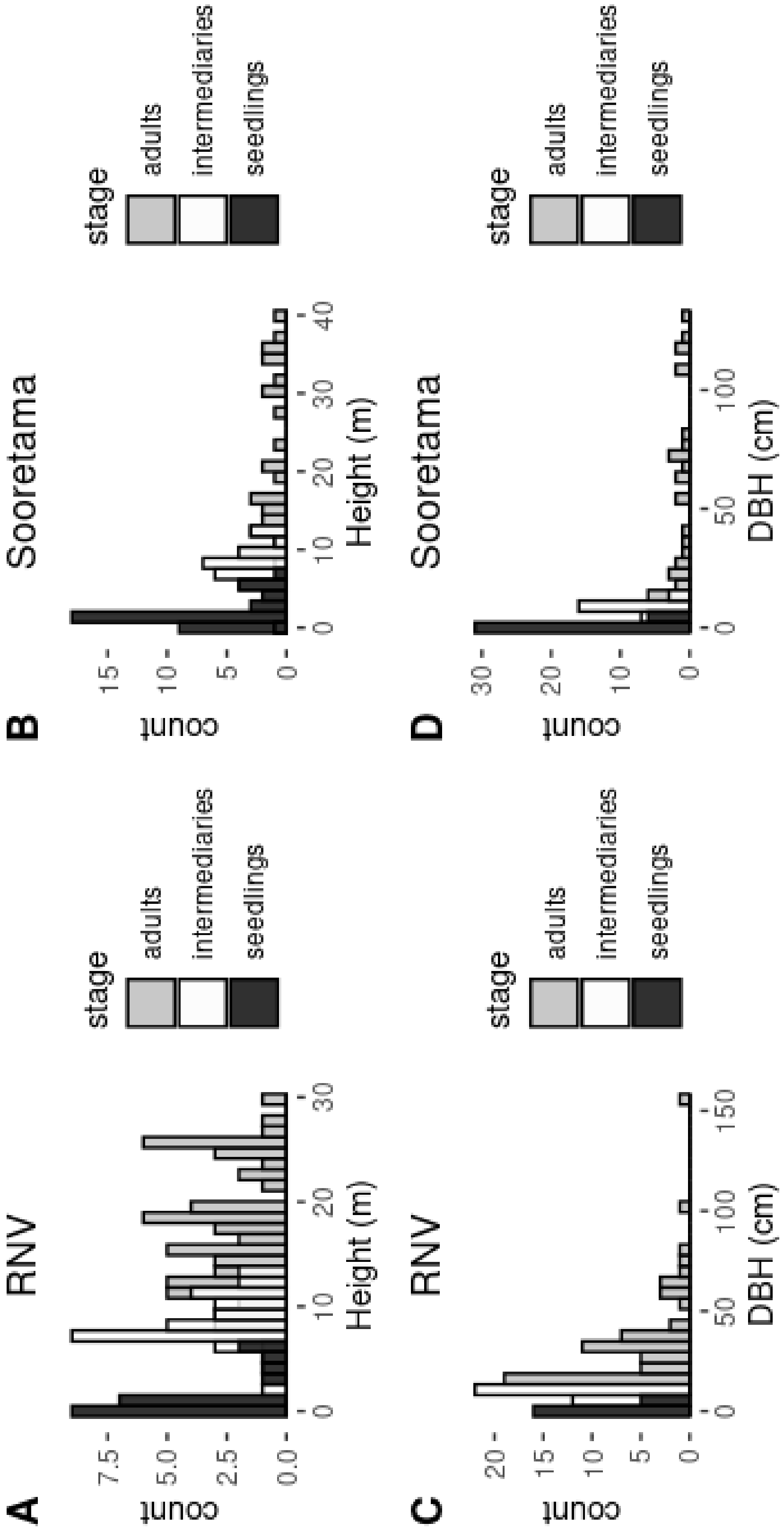

A
RNV
count
7.5
5.0
2.5
0.0
0
10
20
30
Height (m)
stage
adults
intermediaries
seedlings
B
Sooretama
count
15
10
5
0
0
10
20
30
40
Height (m)
stage
adults
intermediaries
seedlings
C
RNV
count
20
15
10
5
0
0
50
100
150
DBH (cm)
stage
adults
intermediaries
seedlings
D
Sooretama
count
30
20
10
0
0
50
100
DBH (cm)
stage
adults
intermediaries
seedlings

**FIGURE 2**

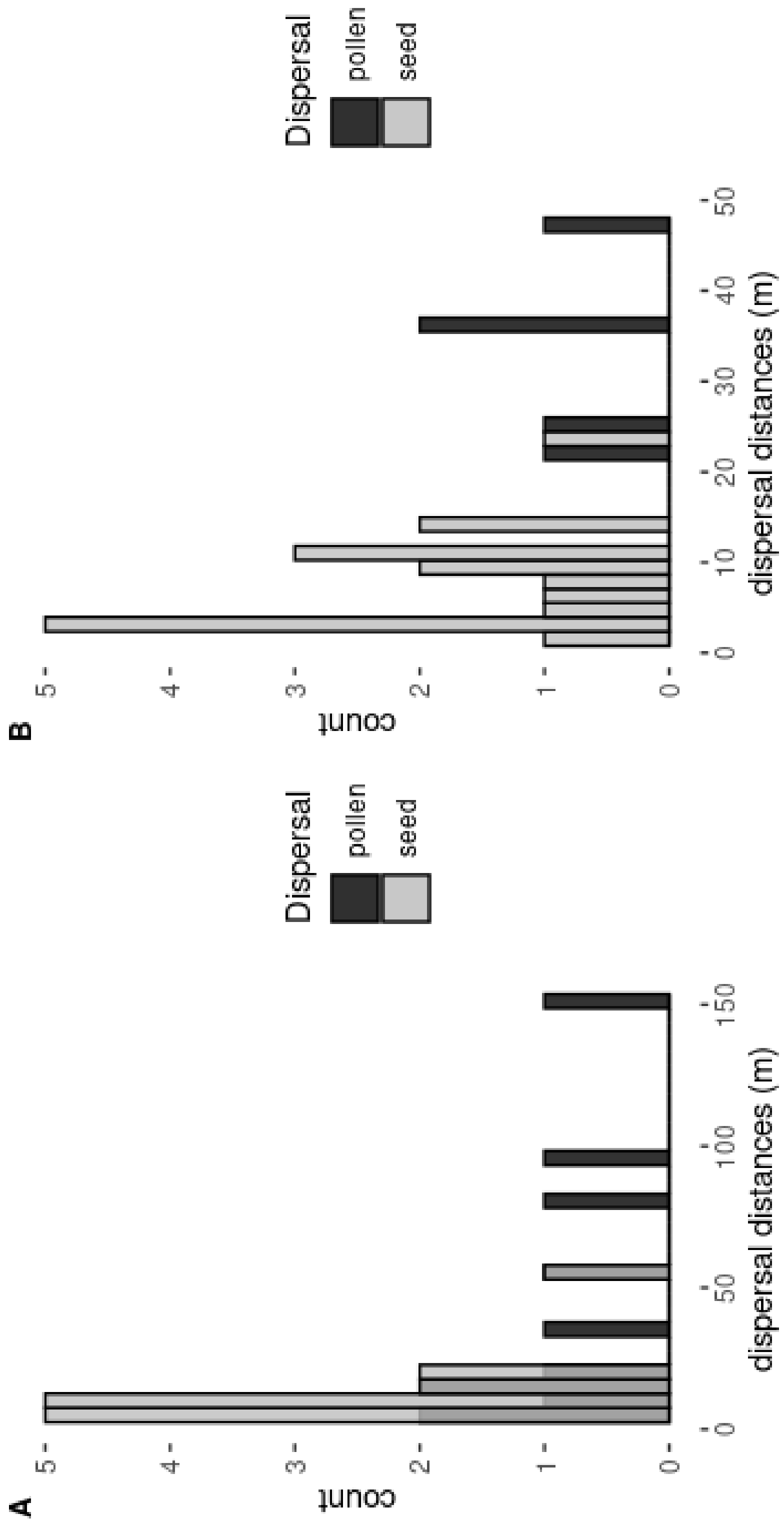
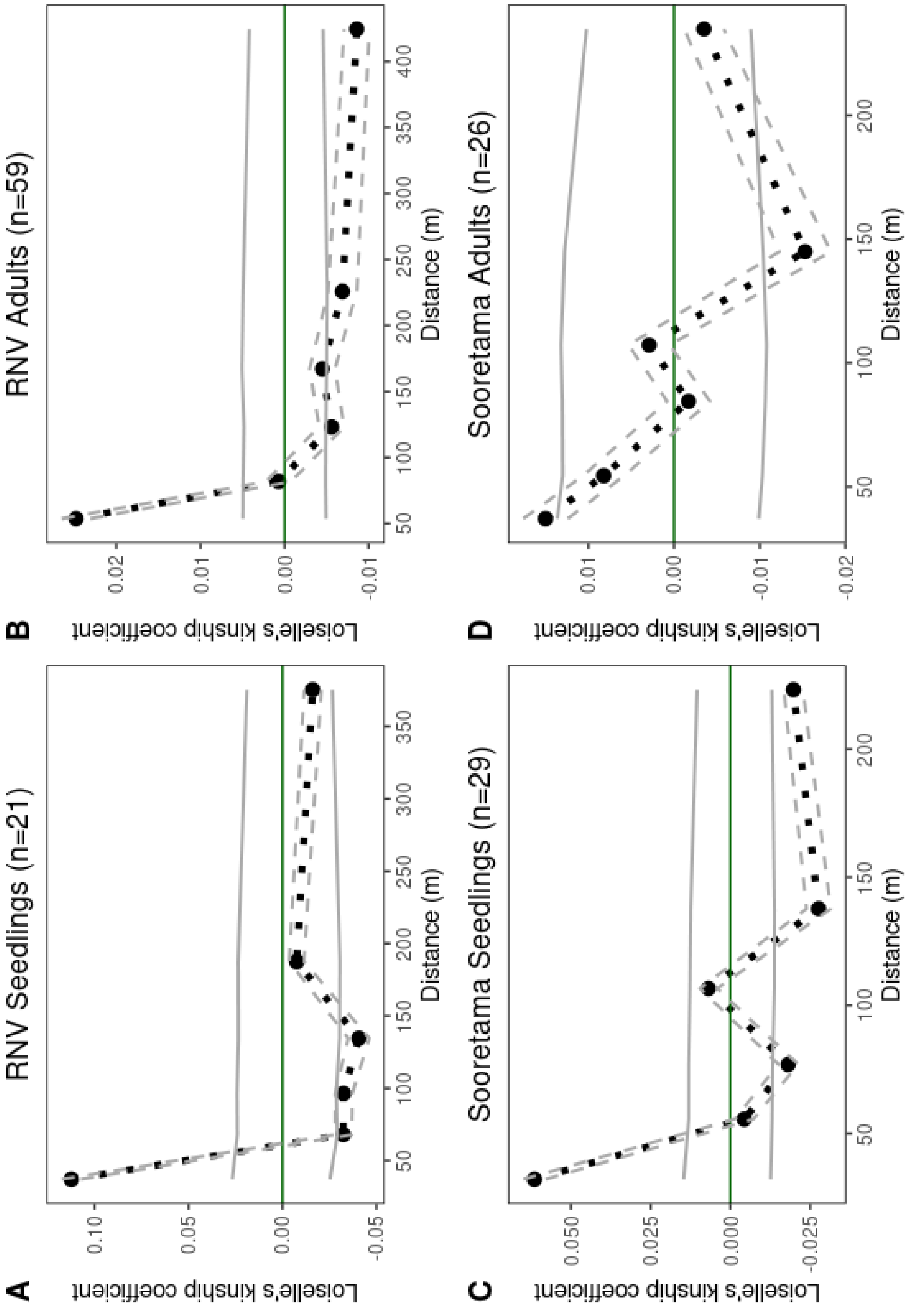

A
count
5
4
3
2
1
0
0
50
100
150
dispersal distances (m)
Dispersal
pollen
seed
B
count
5
4
3
2
1
0
0
10
20
30
40
50
dispersal distances (m)
Dispersal
pollen
seed

**FIGURE 3**


A
RNV Seedlings (n=21)
Loiselle's kinship coefficient
0.10
0.05
0.00
-0.05
Distance (m)
50
100
150
200
250
300
350
B
RNV Adults (n=59)
Loiselle's kinship coefficient
0.02
0.01
0.00
-0.01
Distance (m)
50
100
150
200
250
300
350
400
C
Sooretama Seedlings (n=29)
Loiselle's kinship coefficient
0.050
0.025
0.000
-0.025
Distance (m)
50
100
150
200
D
Sooretama Adults (n=26)
Loiselle's kinship coefficient
0.01
0.00
-0.01
-0.02
Distance (m)
50
100
150
200

## SUPPLEMENTARY MATERIAL

# GENETIC AND ECOLOGICAL EFFECTS OF CHRONIC FOREST FRAGMENTATION ON A CRITICALLY ENDANGERED TREE SPECIES

## Supporting Information

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**TABLE S1** Results of Shapiro-Wilk Normality and Kolmogorov-Smirnov tests applied to the variables DBH (Diameter at Breast Height) and Height for each ontogenetic stage of *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira in two Atlantic forest fragments – Reserva Natural da Vale (RNV) and Sooretama – located in the Espírito Santo State, Southeast Brazil. *p*-value > 0.05 are in bold.

| | | Shapiro-Wilk Normality Test | | | |
|---|---|---|---|---|---|
| | | DBH | | Height | |
| | | W | *p-value* | W | *p-value* |
| RNV | seedlings | 0.62775 | 3.924e-06 | 0.6934 | 2.281e-05 |
| | adults | 0.75325 | 9.013e-09 | 0.94848 | 0.01221 |
| Sooretama | seedlings | 0.78015 | 5.324e-06 | 0.79315 | 9.518e-06 |
| | adults | 0.87798 | 0.001784 | 0.92285 | 0.02811 |
| | | Kolmogorov-Smirnov Test | | | |
| | | KS test | *p-value* | KS test | *p-value* |
| Seedlings | RNV vs Sooretama | 0.24067 | **0.4197** | 0.30116 | **0.1759** |
| Adults | RNV vs Sooretama | 0.30943 | 0.03593 | 0.27393 | **0.09148** |

**TABLE S2** Regeneration status of *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira in two Atlantic forest fragments – Reserva Natural da Vale (RNV) and Sooretama – located in the Espírito Santo State, Southeast Brazil.

| | **Area (ha)** | **Seedlings** | **Intermediates** | **Adults** | **Regeneration status** |
|---|---|---|---|---|---|
| RNV | 42.99 | 0.49 | 0.79 | 1.42 | Fair |
| Sooretama | 64.81 | 0.57 | 0.40 | 0.49 | Good |

**TABLE S3** The effect of missing data (MD) variation on genetic parameters ($H_O$ - observed heterozygosity, $H_E$ - expected heterozygosity, $F_{IS}$ - inbreeding coefficient) for each ontogenetic stage of *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira in two Atlantic forest fragments – Reserva Natural da Vale (RNV) and Sooretama – located in the Espírito Santo State, Southeast Brazil.

| **Seedlings – RNV** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **%MD** | **SNPs** | $H_O$ | **95% CI** | $H_E$ | **95% CI** | $F_{IS}$ | **95% CI** |
| 0% | 450 | 0.180 | 0.102 - 0.258 | 0.186 | 0.11 - 0.262 | 0.020 | -0.076 - 0.116 |
| 5% | 2,674 | 0.203 | 0.124 - 0.282 | 0.214 | 0.136 - 0.292 | 0.041 | -0.06 - 0.142 |
| 10% | 4,057 | 0.194 | 0.113 - 0.275 | 0.208 | 0.127 - 0.289 | 0.053 | -0.06 - 0.166 |
| 15% | 5,195 | 0.186 | 0.105 - 0.267 | 0.202 | 0.121 - 0.283 | 0.064 | -0.057 - 0.185 |
| 20% | 6,268 | 0.180 | 0.097 - 0.263 | 0.198 | 0.115 - 0.281 | 0.073 | -0.058 - 0.204 |
| 25% | 7,206 | 0.177 | 0.094 - 0.26 | 0.196 | 0.113 - 0.279 | 0.078 | -0.058 - 0.214 |
| 30% | 8,408 | 0.173 | 0.088 - 0.258 | 0.194 | 0.109 - 0.279 | 0.083 | -0.062 - 0.228 |
| **Reproductive adults – RNV** | | | | | | | |
| **%MD** | **SNPs** | $H_O$ | **95% CI** | $H_E$ | **95% CI** | $F_{IS}$ | **95% CI** |
| 0% | 517 | 0.199 | 0.153 - 0.245 | 0.191 | 0.148 - 0.234 | -0.031 | -0.065 - 0.003 |
| 5% | 2,985 | 0.230 | 0.182 - 0.278 | 0.225 | 0.18 - 0.27 | -0.011 | -0.052 - 0.03 |
| 10% | 4,579 | 0.220 | 0.173 - 0.267 | 0.221 | 0.176 - 0.266 | 0.007 | -0.039 - 0.053 |
| 15% | 5,917 | 0.211 | 0.164 - 0.258 | 0.216 | 0.17 - 0.262 | 0.021 | -0.031 - 0.073 |
| 20% | 7,243 | 0.205 | 0.157 - 0.253 | 0.212 | 0.166 - 0.258 | 0.036 | -0.022 - 0.094 |
| 25% | 8,365 | 0.201 | 0.153 - 0.249 | 0.211 | 0.164 - 0.258 | 0.043 | -0.018 - 0.104 |
| 30% | 9,869 | 0.197 | 0.148 - 0.246 | 0.209 | 0.161 - 0.257 | 0.053 | -0.012 - 0.118 |

| Seedlings – Sooretama | | | | | | | |
|---|---|---|---|---|---|---|---|
| **%MD** | **SNPs** | $H_O$ | **95% CI** | $H_E$ | **95% CI** | $F_{IS}$ | **95% CI** |
| 0% | 517 | 0.204 | 0.139 - 0.269 | 0.200 | 0.14 - 0.26 | -0.015 | -0.08 - 0.05 |
| 5% | 2,917 | 0.224 | 0.156 - 0.292 | 0.225 | 0.161 - 0.289 | 0.008 | -0.069 - 0.085 |
| 10% | 4,433 | 0.215 | 0.146 - 0.284 | 0.221 | 0.155 - 0.287 | 0.023 | -0.063 - 0.109 |
| 15% | 5,761 | 0.209 | 0.138 - 0.28 | 0.216 | 0.149 - 0.283 | 0.034 | -0.06 - 0.128 |
| 20% | 7,032 | 0.201 | 0.131 - 0.271 | 0.212 | 0.145 - 0.279 | 0.045 | -0.054 - 0.144 |
| 25% | 8,100 | 0.197 | 0.126 - 0.268 | 0.210 | 0.141 - 0.279 | 0.052 | -0.053 - 0.157 |
| 30% | 9,481 | 0.193 | 0.121 - 0.265 | 0.208 | 0.138 - 0.278 | 0.059 | -0.052 - 0.17 |
| **Reproductive adults – Sooretama** | | | | | | | |
| **%MD** | **SNPs** | $H_O$ | **95% CI** | $H_E$ | **95% CI** | $F_{IS}$ | **95% CI** |
| 0% | 506 | 0.218 | 0.143 - 0.293 | 0.201 | 0.136 - 0.266 | -0.065 | -0.126 - -0.004 |
| 5% | 2,847 | 0.238 | 0.16 - 0.316 | 0.224 | 0.155 - 0.293 | -0.046 | -0.12 - 0.028 |
| 10% | 4,299 | 0.228 | 0.149 - 0.307 | 0.219 | 0.148 - 0.29 | -0.029 | -0.114 - 0.056 |
| 15% | 5,543 | 0.220 | 0.141 - 0.299 | 0.214 | 0.143 - 0.285 | -0.016 | -0.108 - 0.076 |
| 20% | 6,747 | 0.211 | 0.131 - 0.291 | 0.209 | 0.136 - 0.282 | -0.004 | -0.104 - 0.096 |
| 25% | 7,781 | 0.207 | 0.127 - 0.287 | 0.207 | 0.134 - 0.28 | 0.005 | -0.1 - 0.11 |
| 30% | 9,119 | 0.203 | 0.122 - 0.284 | 0.205 | 0.131 - 0.279 | 0.013 | -0.1 - 0.126 |

**TABLE S4** The effect of minor allele frequency (MAF) variation on the number of single nucleotide polymorphisms (SNPs) for *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira. The percentage of missing data was set at 10%.

| **MAF** | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.35 | 0.40 | 0.45 | 0.50 |
|---|---|---|---|---|---|---|---|---|---|---|
| **SNPs** | 4,194 | 3,421 | 2,834 | 2,289 | 1,856 | 1,406 | 1,030 | 702 | 330 | 11 |

**TABLE S5** Mean values of allelic richness ($A$), private allelic richness ($R$), and inbreeding coefficients ($F_{IS}$) presented for each ontogenetic stage of *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira in two Atlantic forest fragments – Reserva Natural da Vale (RNV) and Sooretama – located in the Espírito Santo State, Southeast Brazil. 95% confidence intervals for each genetic parameter are also shown.

| | **Site** | ***A*** | **95% CI** | ***R*** | **95% CI** | $F_{\mathrm{IS}}$ | **95% CI** |
|---|---|---|---|---|---|---|---|
| Adults | RNV | 1.711 | 1.705 - 1.717 | 0.197 | 0.192 - 0.202 | -0.051 | -0.21 - 0.108 |
| | Sooretama | 1.690 | 1.684 - 1.696 | 0.176 | 0.172 - 0.181 | -0.049 | -0.186 - 0.088 |
| Seedlings | RNV | 1.661 | 1.655 - 1.667 | 0.164 | 0.160 - 0.168 | 0.044 | -0.061 - 0.149 |
| | Sooretama | 1.694 | 1.689 - 1.700 | 0.197 | 0.193 - 0.201 | 0.006 | -0.068 - 0.08 |

**TABLE S6** Paternity analysis for *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira in two Atlantic forest fragments – Reserva Natural da Vale (RNV) and Sooretama – located in the Espírito Santo State, Southeast Brazil. Only parent pairs inferred with a probability higher than 95% were considered. # represents the candidate parents that were not identified in the sample. Selfing events are shown in bold.

| **RNV** | | | |
|---|---|---|---|
| **ID - Seedlings** | **ID - Inferred Father** | **ID - Inferred Mother** | **Probability** |
| pop1_102 | pop1_86 | pop1_100 | 1.0000 |
| pop1_113 | pop1_114 | pop1_111 | 1.0000 |
| pop1_12 | pop1_1 | pop1_8 | 1.0000 |
| pop1_17 | #1 | #9 | 1.0000 |
| pop1_18 | #2 | #9 | 1.0000 |
| pop1_25 | pop1_1 | pop1_26 | 1.0000 |
| pop1_27 | #3 | pop1_26 | 1.0000 |
| **pop1_28** | **pop1_26** | **pop1_26** | **1.0000** |
| pop1_32 | pop1_26 | pop1_37 | 1.0000 |
| **pop1_34** | **pop1_37** | **pop1_37** | **1.0000** |
| **pop1_35** | **pop1_37** | **pop1_37** | **1.0000** |
| **pop1_36** | **pop1_37** | **pop1_37** | **1.0000** |
| **pop1_38** | **pop1_37** | **pop1_37** | **1.0000** |
| **pop1_39** | **pop1_1** | **pop1_1** | **1.0000** |
| pop1_54 | #4 | pop1_51 | 1.0000 |
| pop1_55 | #5 | #9 | 1.0000 |
| pop1_59 | #5 | pop1_60 | 1.0000 |
| pop1_7 | #6 | #10 | 1.0000 |
| pop1_72 | #7 | #11 | 1.0000 |
| pop1_85 | #8 | pop1_78 | 1.0000 |
| pop1_87 | pop1_78 | pop1_86 | 1.0000 |

| **Sooretama** | | | |
|---|---|---|---|
| **ID - Seedlings** | **Inferred Father** | **Inferred Mother** | **Probability** |
| pop2_209 | #1 | pop2_217 | 1.0000 |
| pop2_210 | pop2_231 | pop2_217 | 1.0000 |
| pop2_212 | #2 | pop2_217 | 1.0000 |
| pop2_213 | #3 | pop2_217 | 1.0000 |
| pop2_218 | pop2_231 | pop2_217 | 1.0000 |
| pop2_223 | pop2_220 | #17 | 0.6224 |
| pop2_223 | #17 | pop2_220 | 0.3776 |
| pop2_226 | #4 | pop2_247 | 0.9059 |
| pop2_226 | pop2_247 | #4 | 0.0941 |
| pop2_230 | pop2_217 | pop2_231 | 0.9093 |
| pop2_230 | pop2_231 | pop2_217 | 0.0907 |
| pop2_237 | #5 | pop2_232 | 1.0000 |
| pop2_245 | #6 | pop2_250 | 1.0000 |
| pop2_254 | pop2_264 | pop2_257 | 0.9966 |
| pop2_260 | pop2_264 | pop2_259 | 1.0000 |
| pop2_261 | pop2_217 | pop2_257 | 0.7131 |
| pop2_261 | pop2_257 | pop2_217 | 0.2869 |
| **pop2_263** | **#7** | **#7** | **1.0000** |
| pop2_265 | #8 | pop2_264 | 1.0000 |
| pop2_266 | #9 | pop2_264 | 1.0000 |
| pop2_268 | #10 | #18 | 1.0000 |
| pop2_272 | #10 | pop2_264 | 1.0000 |
| pop2_273 | #11 | pop2_264 | 1.0000 |
| pop2_274 | pop2_257 | pop2_275 | 1.0000 |
| pop2_277 | #7 | pop2_275 | 1.0000 |
| **pop2_279** | **#7** | **#7** | **1.0000** |
| pop2_280 | pop2_287 | #7 | 0.9059 |
| pop2_280 | #7 | pop2_287 | 0.0941 |
| pop2_285 | #12 | pop2_291 | 1.0000 |
| pop2_286 | #13 | #12 | 1.0000 |
| pop2_288 | #5 | pop2_287 | 1.0000 |

| | | | |
|---|---|---|---|
| pop2_289 | #14 | #12 | 1.0000 |
| pop2_290 | #15 | pop2_291 | 1.0000 |
| pop2_294 | #16 | #19 | 1.0000 |

**FIGURE S1** Map showing the occurrence area (A-B) of *Dinizia jueirana-facao* G. P. Lewis & G. S. Siqueira in two Atlantic forest fragments – Reserva Natural da Vale (red point in C) and Sooretama (yellow point in C) – located in the Espírito Santo State, Southeast Brazil. The distance between the areas is approximately 12 kilometers.

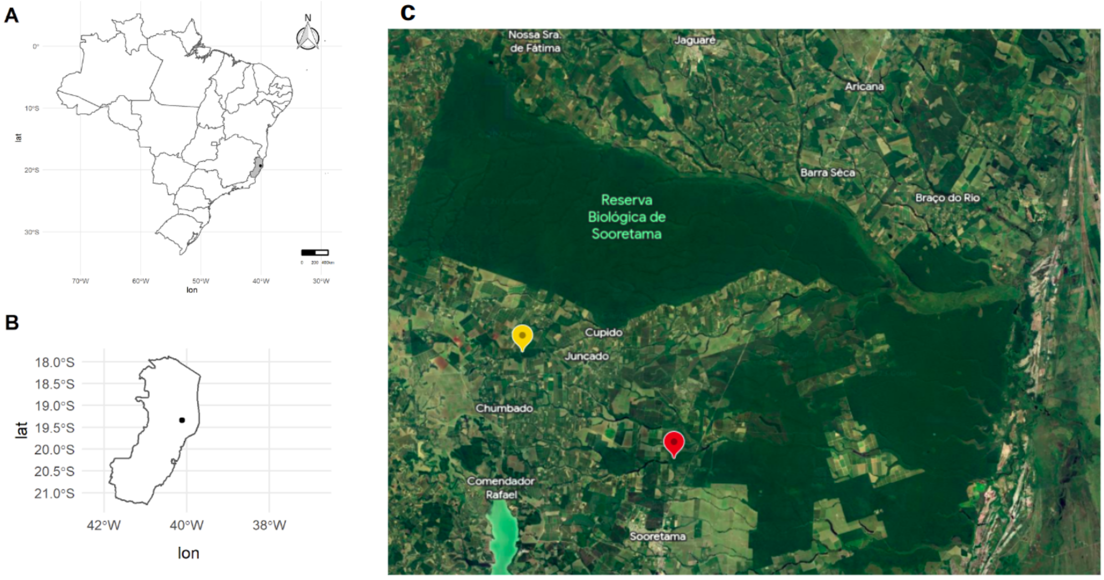
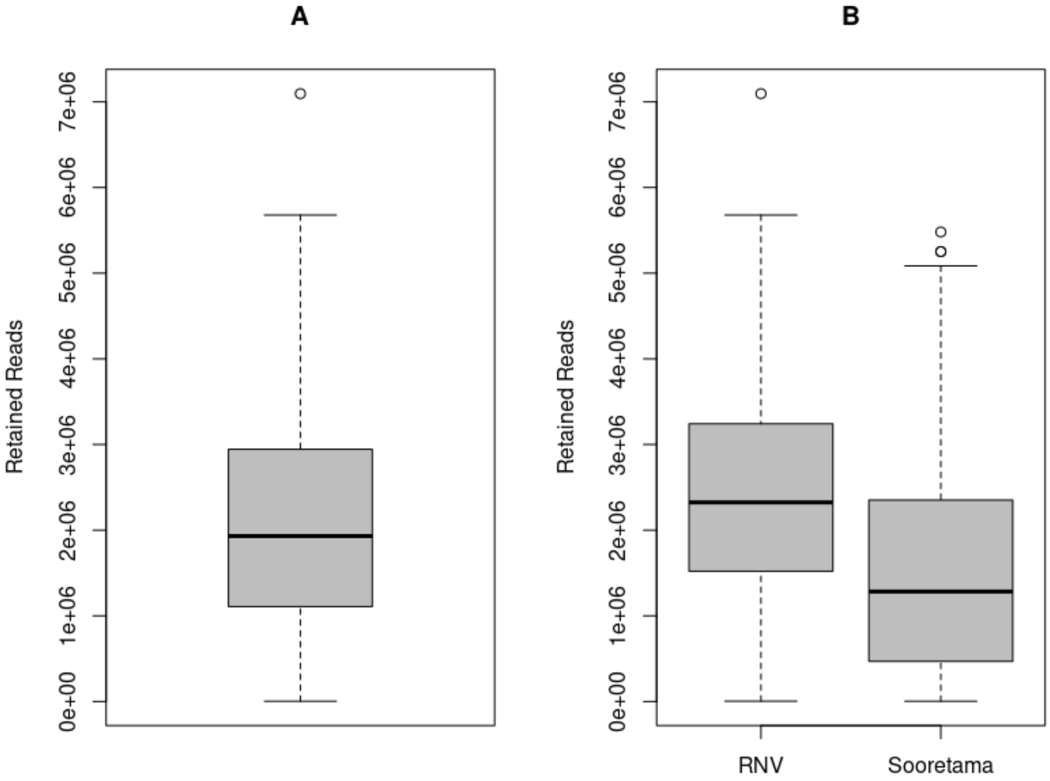


**FIGURE S2** Box plot quality of reads. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend to 5th and 95th percentiles. (A) Number of retained reads with all samples, outliers are represented by dots. n = 211 sample points. (B) Number of retained reads by locality, outliers are represented by dots. n = 116 and 95 sample points. Samples with less than 500,000 reads were discarded from the dataset.



# 3 CONSIDERAÇÕES FINAIS

A conservação e o manejo adequado de espécies raras e ameaçadas de extinção contribuem para a preservação da biodiversidade na Mata Atlântica, que se encontra depauperada com poucos e pequenos remanescentes florestais. Neste contexto, utilizamos abordagens genéticas e ecológicas para investigar as consequências da fragmentação florestal em *Dinizia jueirana-facao*. Nossos resultados sugerem que essa espécie parece ser resiliente aos efeitos negativos da fragmentação florestal. Contrariando as expectativas, observamos uma estrutura demográfica saudável, ausência de endogamia, diversidade genética aparentemente preservada e altos níveis de fluxo gênico nas localidades estudadas.

Em estudos futuros, planejamos investigar as características genômicas e ecológicas que podem contribuir para essa aparente resiliência. Para isso, serão necessários estudos a longo prazo, abrangendo a estrutura demográfica, a biologia da polinização e a fenologia da espécie. Além disso, realizaremos análises genômicas mais aprofundadas, identificando variantes estruturais que possam estar relacionadas a características genômicas funcionais.

Por fim, enfatizamos a importância de monitorar continuamente as localidades onde essa espécie ocorre, especialmente em face da perda contínua de habitat e fragmentação florestal. Também recomendamos a implementação de medidas de conservação, como a promoção da conectividade entre fragmentos florestais e a conscientização das comunidades locais e empresas relevantes. Essas ações visam garantir a conservação e o manejo adequado desse recurso genético único.

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