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

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, EVOLUÇÃO E BIODIVERSIDADE

PAPÉIS ECOLÓGICOS DO MICO-LEÃO-PRETO (*Leontopithecus chrysopygus)* EM UMA FLORESTA OMBRÓFILA DENSA SUBMONTANA

GUILHERME HENRIQUE MUGNAINI

Rio Claro – SP
2026

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

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, EVOLUÇÃO E BIODIVERSIDADE

PAPÉIS ECOLÓGICOS DO MICO-LEÃO-PRETO (*Leontopithecus chrysopygus)* EM UMA FLORESTA OMBRÓFILA DENSA SUBMONTANA

GUILHERME HENRIQUE MUGNAINI

Dissertação apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Ecologia, Evolução e Biodiversidade.
Orientadora: Dra. Laurence Marianne Vincianne Culot
Coorientadora: Dra. Claudia Pandolfo Paz

Rio Claro – SP
2026

M951p

Mugnaini, Guilherme Henrique
Papéis ecológicos do mico-leão-preto (Leontopithecus chrysopygus) em uma floresta ombrófila densa submontana / Guilherme Henrique Mugnaini. -- Rio Claro, 2026
65 p. : tabs., fotos, mapas

Dissertação (mestrado) - Universidade Estadual Paulista (UNESP), Instituto de Biociências, Rio Claro
Orientadora: Laurence Marianne Vincianne Culot
Coorientadora: Claudia Pandolfo Paz

1. Ecologia. 2. Interação animal-planta. 3. Mico-leão-preto. 4. Dispersão de sementes. 5. Fungo micorrízico arbuscular. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Dados fornecidos pelo autor(a).

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Rio Claro

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: Papéis ecológicos do mico-leão-preto (Leontopithecus chrysopygus) em uma floresta ombrófila densa submontana

AUTOR: GUILHERME HENRIQUE MUGNAINI
ORIENTADORA: LAURENCE MARIANNE VINCIANNE CULOT
COORIENTADORA: CLAUDIA PANDOLFO PAZ

Aprovado como parte das exigências para obtenção do Título de Mestre em Ecologia, Evolução e Biodiversidade, área: Biodiversidade pela Comissão Examinadora:

gov.br Documento assinado digitalmente
LAURENCE MARIANNE VINCIANNE CULOT
Data: 15/06/2026 13:22:43-0300
Verifique em https://validar.iti.gov.br

Profa. Dra. LAURENCE MARIANNE VINCIANNE CULOT (Participaçao Presencial)
Departamento de Biodiversidade / UNESP / Câmpus de Rio Claro - IB

gov.br Documento assinado digitalmente
KATIA MARIA PASCHOALETTO MICCHI DE BARR
Data: 15/06/2026 13:31:27-0300
Verifique em https://validar.iti.gov.br

Profa. Dra. KATIA MARIA PASCHOALETTO MICCHI DE BARROS FERRAZ (Participaçao Presencial)
Departamento de Ciências Florestais / USP

gov.br Documento assinado digitalmente
MARCO AURELIO PIZO FERREIRA
Data: 15/06/2026 14:51:52-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. MARCO AURELIO PIZO FERREIRA (Participaçao Presencial)
Departamento de Biodiversidade / UNESP / Câmpus de Rio Claro - IB

Rio Claro, 12 de junho de 2026.

Agradecimentos

Agradeço a minha orientadora, Laurence, que sempre esteve presente em todas as etapas desta dissertação, apoiando em todos os momentos. Mesmo com sua agenda sempre cheia, ela achava algum tempo para transmitir seus conhecimentos e discutir ideias. Foi uma pessoa com quem aprendi muito, tanto no âmbito acadêmico quanto no pessoal.

Aos meus colegas de laboratório, pelos campos feitos juntos, pelas trocas e momentos vividos. Pelo acolhimento desde meu primeiro dia, fazendo com que eu me sentisse parte desse laboratório desde o início.

Ao Michel, o melhor mateiro do mundo, pela parceria e pelos inúmeros dias que esteve comigo enquanto acompanhávamos os micos. Pelas risadas e momentos de descontração em meio ao caos que é seguir um grupo de primatas.

A toda a equipe do Parque Estadual Carlos Botelho, pelo apoio e disponibilidade. A toda equipe da RPPN Trápaga, em especial Hiago Ermenegildo, pelo apoio durante toda a coleta de dados e por sempre disponibilizar um alojamento espetacular, que foi essencial durante os campos.

A Claudia Paz, minha coorientadora, por acreditar na parte experimental do projeto e sempre se mostrar animada e disponível com o andamento do mesmo.

A UNESP, a todos os professores e funcionários, pelo ensino público de qualidade e por todas as oportunidades vividas nesses dois anos.

E, claro, aos meus pais, por todo o amor que me oferecem, por sempre me apoiarem em minhas decisões, mesmo que não as entendam completamente, e por estarem presentes durante todo esse processo.

O presente trabalho foi realizado com apoio da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brasil. Processo nº 2024/00855-0 e Processo nº 2021/06668-0. As opiniões, hipóteses e conclusões ou recomendações expressas neste material são de responsabilidade do(s) autor(es) e não necessariamente refletem a visão da FAPESP.

RESUMO

Primatas desempenham diversos papéis ecológicos nos ambientes onde vivem. Devido à abundância e plasticidade deste grupo, eles podem ser encontrados em diversos tipos de habitats. Por conta desta alta plasticidade, se faz necessário avaliar o papel dessas espécies em diferentes contextos ambientais. Nos trópicos, eles podem ser dispersores de sementes, polinizadores, predadores e até alterar a composição vegetal através da folivoria. No entanto, existem alguns papéis ecológicos que já foram observados em outros grupos de mamíferos, mas que ainda não foram estudados nos primatas. Neste estudo, avaliamos o papel do mico-leão-preto (*Leontopithecus chrysopygus*) como dispersor de sementes e como um possível dispersor de fungos micorrízicos arbusculares (FMA) no Parque Estadual Carlos Botelho e na RPPN Trápaga. Buscamos entender as características das sementes dispersadas em termos de espécies, tamanho e categoria sucessional. Também analisamos a distribuição espacial das sementes dispersadas em termos de distância de dispersão e o estágio sucessional do local de deposição. Além disso, analisamos o tempo de retenção intestinal destas sementes e como esse tempo se relaciona com a distância de dispersão e com o tamanho das sementes. Em seguida, determinamos a efetividade de dispersão do mico-leão-preto, avaliando o componente quantitativo, via focais de eventos de frugivoria, e qualitativo, via experimento de germinacão. Para a possível dispersão de FMA, buscamos entender se o mico-leão-preto dispersa propágulos viáveis através das fezes, se o tempo que eles passam no chão aumenta a probabilidade desta dispersão ocorrer, e por fim, se ocorre a co-dispersão entre propágulos de FMA e sementes viáveis. Realizamos a busca ativa dos propágulos nas fezes e também montamos um experimento para avaliar a existência de associação entre raízes de sorgo e hifas de FMA derivadas das fezes. Nossos resultados mostram que os micos-leões-pretos dispersam sementes de 73% dos frutos consumidos, e que as sementes dispersadas são em maioria de espécies sensíveis à distúrbios. Os locais de deposição são majoritariamente floresta secundária em estágio avançado de recuperação, a uma distância de 164 ± 134 metros da árvore-mãe. Existe uma relação positiva entre o tempo de retenção e a distância de dispersão, uma relação negativa entre o tempo de retenção e o tamanho das sementes e uma relação negativa entre a distância de dispersão e o tamanho das sementes. A efetividade de dispersão se mostrou variada entre as espécies, com diversas combinações de qualidade e quantidade, apontando para maior efetividade em certas espécies. Por fim, em relação à possível dispersão de FMA, não encontramos nenhuma evidência da presença de propágulos nas fezes dos micos-leões-pretos, nos dois métodos empregados. O mico se mostrou um dispersor efetivo e importante para as dinâmicas florestais da Mata Atlântica. Mais estudos serão necessários para avaliar a existência ou não do papel de primatas na dispersão de FMA.

Palavras-chave: Ecologia, interação animal-planta, mico-leão-preto, dispersão de sementes, fungo micorrízico arbuscular.

ABSTRACT

Primates play diverse ecological roles in the environments where they live, due to the abundance and plasticity of this group, they can be found in various types of habitats. Because of this high plasticity, it is necessary to evaluate the role of a species in different contexts. In the tropics, they can act as seed dispersers, pollinators, predators, and even alter plant composition through folivory. However, there are some ecological roles that have already been observed in other mammal groups but have not yet been addressed for primates. In this study, we evaluated the role of the black lion tamarin as a seed disperser and as a possible disperser of arbuscular mycorrhizal fungi (AMF) at Carlos Botelho State Park and PNHR Trápaga. We aimed to understand the traits of the dispersed seeds in terms of species, size, quantity, and successional category. We also analyzed the spatial distribution of the dispersed seeds, the distance from the parent tree, and the successional stage of the deposition site. Furthermore, we analyzed the gut retention time of these seeds and how this time relates to dispersal distance and seed size. Next, we determined the dispersal effectiveness that the BLT has on nine species through the analysis of qualitative component, generated by a germination experiment, and quantitative component, generated through frugivory focal observations conducted in the field.

Regarding the possible AMF dispersal, we aimed to understand if the BLTs disperses viable propagules through its feces, if the time they spend on the ground increases the probability of dispersal, and finally, if co-dispersal occurs between AMF propagules and viable seeds. We did active searches for propagules in the feces and also set up an experiment to evaluate the existence of association between sorghum roots and AMF hyphae derived from the feces. Our results show that the BLTs disperse seeds from 73% of the consumed fruits, that the dispersed seeds belong mostly to disturbance-sensitive species, and that the deposition sites are predominantly secondary forest in an advanced stage of recovery, at 164 ± 134 meters from the parent tree. There is a positive relationship between retention time and dispersal distance, a negative relationship between retention time and seed size, and a negative relationship between dispersal distance and seed size. Dispersal effectiveness exhibited variations, with several combinations of quality and quantity, pointing to greater effectiveness for certain species. Finally, regarding the possible dispersal of AMF, we found no evidence of propagules in the BLTs' feces across the two employed methods. The BLTs proved to be an effective and important disperser for the forest dynamics in the Atlantic Forest. Further studies are needed to assess whether or not primates play a role in the dispersal of AMF.

Keywords: Ecology, animal-plant interaction, black-lion-tamarin, seed dispersal, arbuscular mycorrhizal fungi.

Lista de figuras

Sumário

CONTEXTO

A Mata Atlântica, da qual restam apenas 12,4% da área de vegetação original (SOS Mata Atlântica e INPE, 2022) e onde a maioria dos fragmentos possui menos de 50 hectares (Ribeiro et al., 2009), é considerada um hotspot de biodiversidade e um dos locais mais importantes para esforços de conservação (Myers et al., 2000). O mico-leão-preto é endêmico desse bioma, e, em 2014, uma nova população foi descoberta em São Miguel Arcanjo (Rodrigues et al., 2014). A fitofisionomia na qual essa população se encontra, a floresta ombrófila densa, difere das demais já documentadas para a espécie, além disso, sua região de ocorrência representa uma área de futuro refúgio climático (Meyer et al., 2014). A dispersão endocórica de sementes por primatas é um tópico amplamente abordado na literatura científica, e a dispersão de sementes pelo mico-leão-preto já foi objeto de estudos prévios (Silva, 2022; Alcolea, 2016). Entretanto, essa população em específico permanece sem dados sobre esse papel ecológico. Além disso, existem outras funções ecológicas que são pouco ou raramente discutidas para os primatas.

Por isso, esta dissertação foi estruturada em dois capítulos. O primeiro se intitula **"Seed dispersal characteristics of an endangered small bodied primate in a future climate refuge area".** Neste capítulo buscamos entender o papel do mico-leão-preto como dispersor de sementes em um contexto específico e nunca antes estudado em relação à dispersão, no Parque Estadual Carlos Botelho e na vizinha RPPN Trápaga. Primeiro determinamos as características das sementes dispersadas em termos de espécie, tamanho e categoria sucessional. Em seguida, determinamos a distribuição espacial das sementes, o estágio de sucessão da floresta do local de deposição e a distância da árvore-mãe. Depois, determinamos o tempo de retenção intestinal e testamos se esse tempo é influenciado pelo tamanho das sementes, também testamos se a distância de dispersão é influenciada pelo tempo de retenção ou pelo tamanho das sementes. Após isso, testamos o efeito da passagem pelo tubo digestório no sucesso e tempo de germinação das sementes. Por fim, através do sucesso de germinação obtido pelo experimento e através do número de sementes ingeridas por minuto, obtido por focais feitos em campo, construímos a paisagem de efetividade de dispersão para nove espécies vegetais. Com esses resultados conseguimos discutir a contribuição do mico-leão-preto como dispersor de sementes.

Já no segundo capítulo, **"Possible dispersion of arbuscular mycorrhizal fungi (AMF) by the black-lion-tamarin *Leontopithecus chrysopygus*"**, exploramos a possibilidade de um papel ecológico bem menos estudado, e nunca antes explorado em primatas, o de dispersor de fungos micorrízicos arbusculares (FMA). Buscamos evidências através de dois métodos distintos, que já tinham sido empregados em conjunto antes, mas para outros grupos animais. O primeiro foi a busca ativa, que consiste no destrinchamento e peneiramento das amostras fecais e posterior observação em uma lupa. O segundo método foi a preparação de um experimento de associação em que inoculávamos amostras de fezes em potes com

mudas de sorgo e posteriormente analisávamos as raízes do sorgo em busca da associação com hifas, em um microscópio.

Chapter 1

Seed dispersal characteristics of an endangered small bodied primate in a future climate refuge area

ABSTRACT

Zoochorous seed dispersal is an essential ecological process for the maintenance of tropical forests, in which primates play a fundamental role. Primates compose a significant portion of frugivores in the Neotropics, yet many species are threatened with extinction. The black lion tamarin (*Leontopithecus chrysopygus,* BLT), endemic to the Atlantic Forest and classified as 'Endangered' by the IUCN, is a small-bodied primate that may play a distinct role in forest regeneration. Because this species can occupy habitats ranging from well preserved to degraded forest fragments varying sizes, understanding the ecological functions it performs across different habitat conditions is essential. This study aimed to: (1) characterize the seeds dispersed by the BLTs; (2) determine the spatial distribution of dispersed seeds; (3) determine gut retention time and test its relationship with dispersal distance and seed size; (4) evaluate the effect of gut passage on seed germination dynamics; and (5) determine the seed dispersal effectiveness the BLTs has on dispersed species. We followed a group over 13 months in the Carlos Botelho State Park and Trápaga Private Natural Heritage Reserve, collecting feces and recording feeding events and GPS coordinates, complemented by a germination experiment with three treatments. We classified dispersed plant species according to their ecological guild: disturbance-opportunist; disturbance-sensitive; disturbance-insensitive; and rare, to assess the functional profile of dispersal. Of 209 fecal samples, 178 (85%) contained whole seeds. Mean seed dimensions were 2.9 ± 3.05 mm in length, 1.9 ± 2.1 mm in

width, with seeds deposited 164 ± 134 meters from the parent tree after 85 ± 49 min of intestinal retention. Dispersal distance was positively associated with retention time, while retention time was negatively influenced by seed size. Overall, defecated seeds did not differ in germination success and time from the other treatments. Three species stood out, exhibiting higher dispersal effectiveness compared to the others through different combinations of quality and quantity. The group's home-range was dominated by late-successional secondary forest, and the most frequently dispersed species belongs to the disturbance-sensitive guild. These results highlight that BLTs are crucial, species-specific seed dispersers in the Atlantic Forest dynamics. By facilitating germination, dispersing seeds over long distances, and connecting different successional forest stages, they actively promote habitat regeneration and functional connectivity.

INTRODUCTION

Zoochorous seed dispersal is a strategy used by most woody species in the tropics (Howe, 2014). This mutualistic plant-animal interaction is essential for the processes of natural regeneration and maintenance of forest biodiversity (Terborgh et al., 2002). All neotropical primates have the habit of feeding on fruits (Gómez and Verdú, 2012), and in the tropics, they represent a significant portion, ranging from 25% to 40%, of the biomass of frugivores (Chapman, 1995). Although some primate species behave as seed predators, the majority are classified as dispersers (Gómez and Verdú, 2012). However, of the 217 primate species in the Neotropics, 92 (42%) are threatened with extinction (IUCN SSC Primate Specialist Group, 2021).

Seed dispersal by neotropical primates has already been the subject of studies in scientific literature (e.g. Culot et al., 2010; Bufalo et al., 2016; Fuzessy et

al., 2018; Heymann et al., 2022). Primates consume a large quantity of seeds when feeding on fruits, seeds that often remain viable after defecation (Chapman, 1989). Passage through the intestinal tract of these animals can increase the germination success and reduce the time required for a seed to germinate. However, the magnitude of this effect is linked to the type of feeding guild (Fuzessy et al., 2016), with predominantly larger frugivorous species having a significant positive impact on germination success and small-bodied frugivorous-faunivorous species having a neutral effect (Fuzessy et al., 2016). On the other hand, small-bodied primates usually disperse seeds in a scattered manner, with few seeds per defecation, which reduces competition and may increase recruitment success (Culot et al., 2010; Culot et al., 2015). In addition, despite an overall neutral effect of frugivorous-faunivorous primates' gut passage on seed germination, the sole removal of the pulp by the digestive system can induce benefits for the germination, since the pulp may be a germination inhibitor in various species (Cipollini and Levey, 1997). Finally, several small-bodied species frequent both primary and secondary forests, therefore playing a crucial role dispersing pioneer and non-pioneer seeds in regenerating environments (Buchanan-Smith, 2000; Culot et al., 2010). These seeds may subsequently recruit and establish themselves as seedlings in these areas (Heymann et al., 2019). Therefore, the importance of this group is invaluable when it comes to gene flow, colonization of new areas, and natural regeneration of forest environments.

In the Brazilian Atlantic Forest, the small-bodied black lion tamarin (hereafter referred as BLT), *Leontopithecus chrysopygus* (Mikan, 1823), is recognized as seed disperser (Passos, 1997), but its role remains relatively underexplored. For instance, the ecological role of BLT in the submontane dense ombrophilous forest of Carlos

Botelho State Park (CBSP) (Rodrigues et al., 2014), where it occurs with three other primate species, is unknown. As this area differs from those previously documented for the species and is characterized as a future climate refuge (Meyer et al., 2014), it becomes essential to understand the ecological roles played by this species in this specific context.

Studying seed dispersal by the BLT and comparing it with the other primates of the community may clarify whether their roles are complementary or redundant. Although the genus *Brachyteles* contributes most to the dispersal of plant species with large seeds in the Atlantic Forest, all primates of this biome for which information is available regarding their dispersal functions, demonstrate the capacity to disperse seeds of different sizes (Bufalo et al., 2016). However, the limited sampling effort, and the few details about the characteristics of dispersed seeds and the spatial patterns of dispersal provided by previous studies, hinder a better assessment of the role of each primate in the environment (Bufalo et al., 2016).

Therefore, quantifying the contribution of the BLT to seed dispersal in this specific context, is crucial for both the conservation of this endangered endemic primate and the management of the remaining Atlantic Forest landscapes.

OBJECTIVES

The goal of this research is to assess the ecological role of the black lion tamarin as seed disperser in the Carlos Botelho State Park and the neighboring Trápaga Private Natural Heritage Reserve (PNHR). The aim is to determine the seed dispersal effectiveness of BLT through the evaluation of quantitative and qualitative components.

Specific objectives

1.1. Determine the traits of the seed species dispersed by the BLT in terms of size and successional classes relative to disturbance.

1.2. Determine the spatial distribution of seeds dispersed by BLT in terms of forest successional stages of the sites of deposition and seed dispersal distances.

1.3. Determine the gut passage time of dispersed seeds and test (a) if gut retention time is influenced by seed size; (b) if seed dispersal distance is influenced by gut retention time and seed size.

1.4. Test the effect of gut passage on seed germination success and time.

1.5. Determine the seed dispersal effectiveness through the evaluation of the quantitative and qualitative components.

HYPOTHESES

1.1. Since BLTs are small callitrichids and that species belonging to this family are known to frequent secondary forests (Buchanan-Smith 2000; Culot et al. 2010), and that BLTs in Carlos Botelho State Park are concentrated more (but not exclusively) on the edge of the park and on its border with the Trápaga PNHR, our expectation is that the BLT will disperse small to medium-sized seeds and a greater number and proportion of disturbance-opportunist and disturbance-insensitive tree species.

1.2. Based on the same information described in hypotheses 1.1, we expect BLTs to have a home range largely composed of early and mid-successional forests

rather than late-successional forest areas, consequently having more seed dispersal events in areas of earlier stages of succession.

1.3. Considering that seed physical characteristics, such as weight and volume, negatively influences gut retention time in callitrichids (Garber, 1986) and that seed size negatively influences retention time (Stevenson, 2000), we expected that, in BLTs, smaller seeds will be retained for longer periods in the digestive tract. Since differences in retention time can result in differences in dispersal distance (Tsuji et al., 2010), we expect dispersal distance to increase with retention time, leading to longer dispersal distances for smaller seeds.

1.4. If frugivorous-faunivorous primates do not affect the final germination success and time of defecated seeds (Fuzessy et al., 2016), then we expect that the seeds collected in the feces of BLTs will have similar germination rate and time than seeds whose pulp has been manually removed.

1.5. Since dispersal effectiveness is calculated as the product of qualitative and quantitative components (Schupp, 1993; Schupp et al., 2010) and given that some species fruit for longer durations or more frequently than others, thereby increasing their temporal availability for BLT consumption, we expect that dispersal effectiveness will vary significantly among plant species.

METHODS

1. Study area

The present study was carried out in the Carlos Botelho State Park (CBSP) (24º 06’ 55’’ e 24º 14’ 41’’ S e 47º 47’ 18’’ e 48º 07’ 17’’ W) and in the Trápaga Private Natural Heritage Reserve (PNHR) (24° 02' 46.6" S e 47° 58' 50.8" W) (Fig. 1). Composed of Submontane Dense Ombrophilous Forest, the CBSP and the Trápaga PNHR share a geographic boundary, and the BLT group studied moves between these two areas. The CBSP and the Trápaga PNHR are located in southeastern São Paulo, specifically at Serra da Paranapiacaba, encompassing the municipalities of São Miguel Arcanjo, Sete Barras, Tapiraí and Capão Bonito. The CBSP covers an area of 37,644 ha, with terrain characterized by hills and small ridges, and, together with other parks, it is part of the Ecological Continuum of the Serra de Paranapiacaba.

Mean annual precipitation ranges from 1700 mm to 2400 mm, without a water deficit throughout the year. The climate is humid subtropical with a hot summer, with mean annual temperatures ranging from 17°C to 22°C. The warmest months are December, January, and February, with average temperatures exceeding 25°C, while the coldest months are June, July, and August, with averages around 18°C (Instituto Florestal, 2008).

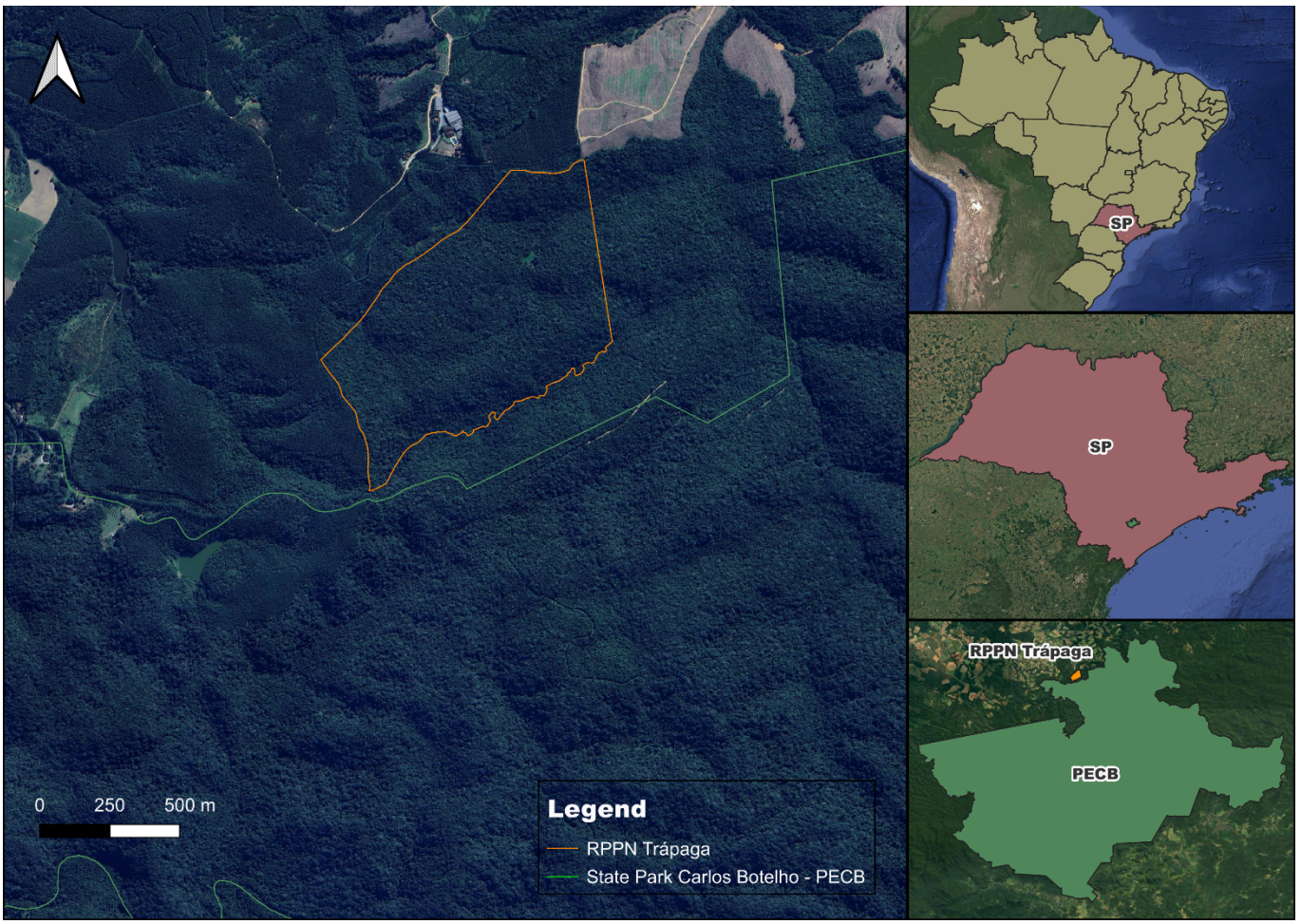

Figure. 1. Study area encompassing the Carlos Botelho State Park and the Trápaga PNHR. Both places are located in the municipality of São Miguel Arcanjo in the state of São Paulo.

2. Data Collection

We followed a fully habituated group of five BLTs from the time they woke up and left their sleeping site until the moment they went to sleep, for 7 full days per month over a period of 13 months, from April 2024 to April 2025. Group location was automatically registered via GPS every 5 minutes. Two individuals had VHF transmitter devices, which facilitated locating them the first day, via telemetry. In January 2025 a couple of twins were born.

We recorded the feeding events using the all-occurrences sampling method (Altmann, 1974). A feeding event was considered to have begun when two or more

individuals fed on the same tree and considered finished when the last individual left the tree. For each event, we recorded the time, the number of individuals feeding, and the duration of the event. We marked the feeding trees with aluminum plate, tape and a code, and recorded the life form, DBH (diameter at breast height), and the geographic coordinates with a GPS. We collected the fruits from the consumed species, either from the ground or directly from the tree; ideally, we tried to collect at least 15 seeds in total for each species, collecting from different plant individuals whenever possible. We used those seeds in the germination experiment to compare the germination success and time with the seeds dispersed through feces. Whenever feasible, we conducted focal observations during frugivory feeding events to estimate how many fruits were swallowed per minute (and thus infer the number of ingested seeds) and how many seeds were spat out. To measure seed dispersal distance from the parent tree, we used Heymann et al. (2012)'s criteria, which consists of considering only defecated seeds of a given species if, between the consumption of that species in a tree individual and the defecation of the seeds, no other feeding event involving the same species was recorded. We collected all the visible fresh fecal samples and their respective GPS coordinates. Fresh fecal samples were placed in dry vials, labeled with an identification code, the date, and time of collection. At the end of each day, we stored the samples in a refrigerator, about 4°C, until processing.

3. Sample processing

We counted the number of seeds of each fecal sample, measured the length and width using a caliper, and identified them to the species level, whenever possible. We compared the germination success and time among seeds defecated

by BLTs, seeds without pulp (manually depulped), and seeds with pulp (intact fruits). This approach allows us to assess whether the potential effects of gut passage are due to simple pulp removal or to scarification by the digestive tract (Samuels & Levey 2005).

Immediately after returning from each 7-day field data collection, we set up the germination experiment at the Jardim Experimental of São Paulo State University (UNESP), Rio Claro (Fig. 2). Initially, we washed the samples to remove fecal matter, separated the seeds according to species and treatment (feces, treatment with pulp and treatment without pulp) and subsequently sown them one centimeter deep in tubes filled with vermiculite. The seeds picked in the field were also stored in the refrigerator until the time of sow. For each species we divided the number of seeds and removed the pulp from one half, leaving the other half to be sown with the pulp. The experiment lasted for 59 weeks in total, each species was observed for at least 32 weeks. We tried to standardize the number of seeds per treatment, ideally 15 seeds, but due to field-related difficulties, it was not possible to reach that number for all species. The greenhouse where the experiment was conducted is equipped with an automatic irrigation system, operating two to three times per day depending on climatic conditions, with each irrigation lasting 5 minutes. We checked the experiment once a week to monitor germinated seeds. A seed was considered germinated when we could see some vegetative part emerging from the substrate.

Figure. 2. Germination experiment. Each seed is planted individually in a tube, and the labels indicate the sowing date, species, and treatment (feces, treatment with pulp and treatment without pulp).

4. Data Analysis

4.1 Traits of dispersed seeds

To determine the successional classes of each plant species dispersed by the BLTs, we used Toledo et al. (2025)' s classification. This approach differs from the more traditional and widely used categories of pioneer, early secondary, late

secondary, and climax species, by classifying the species based on their sensitivity to disturbance along the successional gradient. Four categories are defined: 1) disturbance-opportunist: early-stage indicators; 2) disturbance-sensitive: old-growth indicators; 3) disturbance-insensitive: species abundant across successional stages; and rare: for species that could not be assigned to a category due to low sample size. We chose Toledo et al' s classification because this classification is specific to the Atlantic Forest species and it includes almost all species the BLTs consumed in our data collection. Also, for the few species for which we were able to find information regarding the traditional approach and categories, most fell into more than one category.

4.2 Spatial distribution of dispersed seeds

We calculated the seed dispersal distance as the Euclidean distance between the coordinates of the parent tree and the seed deposition site, after converting latitude and longitude from decimal degrees to UTM coordinates. We did it in excel using the formula 'd = √((x_feces - x_tree)² + (y_feces - y_tree)²)'.

For the purposes of our analysis, a 'dispersal event' was considered the displacement of seeds from the parent tree to the dispersal site. Therefore, fecal samples containing seeds from multiple plant species were treated as multiple, independent dispersal events.

To analyze the forest successional stages of the home range and of seed deposition sites, we first estimated the group's home range using two different methods, Minimum Convex Polygon (MCP) and Kernel Density Estimation (KDE). For the Kernel method, different levels of spatial use probability (95%, 70%, and 50%) were considered, allowing the identification of intensive use areas (70% was

the core area) as well as the total extent of the home range. We estimated the home range size using GPS coordinates points collected every 5 minutes during group monitoring, considering only full-day trajectories (from sleeping site to sleeping site). We calculated the Kernel Density Estimator (KDE) for 95%, 70%, and 50% of the points using the ‘kernelUD’ function from the ‘adehabitatHR’ package (Calenge, 2024), and the Minimum Convex Polygon (MCP) using the ‘mcp’ function from the same package.

We determined the forest successional stages of the home range and of seed deposition sites using the most recent year (2024) of the ‘land use and land cover’ and ‘secondary vegetation’ maps from Collection 10 of the MapBiomas. In R, we used the ‘crop’ and ‘mask’ functions from the ‘terra package’ (Hijmans, 2025) to clip the raster to the extent of the area (Kernel 95%) and to retain only the pixels within it, respectively. We then filtered only the pixels classified as forest using the ‘land use and land cover’ raster and extracted the age values of each pixel within the Kernel using the ‘values’ function from the ‘terra package’ (Hijmans, 2025). This was followed by calculating class frequencies with ‘table’ and subsequently grouping them into successional categories using the ‘mutate’ and ‘group_by’ functions from the ‘dplyr’ package (Wickham et al., 2026).

4.3 Gut retention time, seed size, and seed dispersal distance

To test the relationships among dispersal distance, retention time, and seed size, we performed linear regressions between pairs of variables using the ‘lm’ function (R Core Team, 2024). The response variable was transformed whenever its distribution deviated from normality according to the Shapiro–Wilk test. To determine the most appropriate transformation to achieve normality, we used the ‘boxcox’

function from the ‘MASS’ package (Venables & Ripley, 2002) or applied a ‘log’ transformation. Subsequently, another Shapiro-Wilk test was performed on the models’ residuals.

4.4 Seed dispersal effectiveness

We performed germination experiments to estimate the seed dispersal quality of BLTs. We used three types of treatments: seeds with pulp, seeds without pulp and seeds found in feces. Only species that had all three treatments and at least one germination in any treatment were included in the statistical analyses.

To test the effect of seed handling on germination success and time, we first explored temporal dynamics using Kaplan-Meier estimators stratified by treatment with the function ‘survfit’ from the ‘survival’ package (Therneau, 2024). Cumulative germination probability over time was visualized for each treatment. Differences between groups were assessed using the log-rank test, implemented via ‘survdiff’ from the ‘survival’ package (Therneau, 2024) which tests the null hypothesis of equal survival functions across treatments over the entire observation period, and observed that the proportional hazards (PH) were not equal throughout the experiment, something that was confirmed later by the Schoenfeld residuals test.

We assessed the validity of the Cox model, which estimates the effect of covariates on the risk of germination over time while accommodating censored observations, through two main diagnostic steps. First, we checked possible collinearity between covariates (treatment and species) using the Generalized Variance Inflation Factor (GVIF), “vif” function from the ‘car’ package (Fox and Weisberg, 2019) calculated from an auxiliary linear model; GVIF values were 1.01 for treatment and 1 for species. Second, we tested the proportional hazards (PH)

assumption using Schoenfeld residuals implemented in the ‘cox.zph’ function of the ‘survival’ package (Therneau, 2024).

The initial Cox model with both covariates as fixed effects (Treatment + Species) was discarded due to PH violation for both variables (Treatment: $p = 0.015$; Species: $p < 0.001$). The final survival model combined a time-dependent treatment effect, implemented via ‘tt()’, with between-species heterogeneity modelled by a gamma frailty term ‘(frailty(Species, distribution = 'gamma'))’. The functional form of the time-varying treatment effect was selected by comparing three specifications via AIC: $x \times \log(t + 1)$, $x \times \sqrt{t}$, and $x \times t$, where ‘x’ corresponds to the treatment indicator and ‘t’ represents the time to germination. All three models yielded equivalent AIC values ($\Delta AIC < 2$), and the logarithmic form was adopted. The ‘tt()’ term allows the hazard ratio (HR) for treatment to vary over time, while the gamma frailty captures intra-species correlation and baseline germination heterogeneity among species. This model was preferred over species stratification because it explicitly quantifies between-species variance rather than absorbing it, also due to the unbalanced sample sizes (N) across species and treatments. As a complementary analysis, Cox models were fitted individually per species for exploratory purposes.

Final germination success was analyzed using generalized linear mixed models (GLMM) with a binomial distribution and logit link, fitted with the ‘glmer’ function from the ‘lme4’ package (Bates et al., 2015). The final model included treatment as a fixed effect, species as a random intercept with a random slope structure for treatment, allowing the treatment effect to vary across species ‘glmer(event ~ Treatment + (Treatment | Specie)’. The GLMM was preferred over a fixed-effects GLM due to pronounced sample size imbalance across species and the presence of complete separation in species lacking germination events under certain

treatments. Two models were compared via AIC: random intercept only versus random intercept and slope.

As a species-specific complementary analysis, a binomial GLM with ‘Treatment × Species’ interaction was fitted to the subset of species with n ≥ 5 observations per treatment and at least one germination event in each of the three treatments, excluding *Ficus luschnathiana* due to complete separation in the treatment with pulp. We performed pairwise treatment comparisons within each species using estimated marginal means ‘emmeans’ from the ‘emmeans’ package (Lenth, 2020). Species with no germination events in any treatment (*Miconia cinnamomifolia* and *Tapirira guianensis*) were excluded from all final analyses.

We used the ‘Seed dispersal effectiveness’ (SDE) framework proposed by Schupp (1993) and Schupp et al. (2010) for nine plant species for which we had focal data on their consumption by the BLTs. To plot the SDE landscape we used the function ‘effectiveness_plot’ from the package ‘effect.lndscp’ (Jordano and Sanchez, 2026). The qualitative component was the performance of the plant species in the feces treatment during the germination experiment. The quantitative component was calculated as the number of seeds ingested per minute **X** average visit time **X** total number of visits. To obtain the number of seeds ingested per minute we conducted frugivory focal observations on individuals of the group, the focal time was only recorded when we could see the individual eating, handling or actively searching for fruits in the tree. In scenarios where an individual ate some fruits, took a considerable break (engaging in another behavior that was not considered foraging, but still in the same tree) and resumed eating, the duration of this break was not included in the total time.

All graphs were created in R (R Core Team, 2024) using the ‘ggplot’ function from the ‘ggplot2’ package (Wickham, 2016), except for the histogram of dispersal distance, which was generated using the ‘truehist’ function from the ‘MASS’ package (Venables & Ripley, 2002) and the plots of the time-to-event analyses which were generated using ‘ggsurvplot’ from ‘survminer’ package (Kassambara et al., 2026).

All analyses were conducted in R software (R Core Team, 2024).

RESULTS

Traits of dispersed seeds

Across a total of 802 observation hours, we collected 209 fecal samples of which 85% (178) had whole seeds, 9,5% (20/209) of feces contained no seeds, and 5,2% (11/209) contained fragmented seeds. The BLTs consumed 37 fruit morphospecies, of which 31 were identified to species level and six to genus level (Table 1). The BLTs dispersed 73% of the fruit species consumed, with 15.7 ± 37.8 seeds per fecal sample (range: 1-376) of 1.1 ± 0.7 species (range: 0-3). Dispersed seeds were 2.9 ± 3.05 mm long (range: 0.6-18.5) and 1.9 ± 2.1 mm width (range: 0.4-13.5) (Fig. 3).

Among 243 dispersal events, 32.9% were disturbance-sensitive species (old-growth indicators), 18.9% were disturbance-insensitive species (abundant across successional stages), 18.5% were rare species, and 16.5% were disturbance-opportunist species (early-stage indicators) (Fig. 4).

Family	Species	Dispersion	Category
Anacardiaceae	*Tapirira guianensis*	1	Insensitive
Annonaceae	*Annona sylvatica*	1	Insensitive
Arecaceae	*Attalea dubia*	0	Opportunist
Arecaceae	*Euterpe edulis*	0	Insensitive
Bromeliaceae	*Aechmea nudicaulis*	0	NA
Burseraceae	*Protium kleinii*	0	Insensitive
Cactaceae	*Rhipsalis sp.*	0	NA
Cordiaceae	*Cordia sellowiana*	1	Opportunist
Fabaceae	*Inga marginata*	0	Insensitive
Lamiaceae	*Vitex polygama*	1	Opportunist
Melastomataceae	*Miconia cinnamomifolia*	1	Opportunist
Melastomataceae	*Miconia formosa*	1	Rare
Moraceae	*Ficus luschnathiana*	1	Sensitive
Moraceae	*Sorocea bonplandii*	0	Sensitive
Myrtaceae	*Campomanesia* sp.	1	NA
Myrtaceae	*Campomanesia xanthocarpa*	1	Insensitive
Myrtaceae	*Campomanesia guaviroba*	1	Sensitive
Myrtaceae	*Eugenia monosperma*	1	Rare
Myrtaceae	*Myrcia vellozoi*	1	Sensitive
Myrtaceae	*Myrcia* sp.	1	NA
Myrtaceae	*Eugenia* sp. 3	1	NA
Myrtaceae	*Eugenia cerasiflora*	1	Sensitive
Myrtaceae	*Eugenia sp. 1*	0	NA
Myrtaceae	*Eugenia sp. 2*	0	NA
Myrtaceae	*Psidium cattleyanum*	1	Opportunist
Nyctaginaceae	*Guapira opposita*	1	Insensitive
Phyllanthaceae	*Hyeronima alchorneoides*	0	Insensitive
Rubiaceae	*Cordiera myrciifolia*	1	Sensitive
Rubiaceae	*Randia armata*	1	Sensitive
Rubiaceae	*Posoqueria latifolia*	1	Insensitive
Salicaceae	*Casearia decandra*	1	Opportunist
Sapindaceae	*Allophylus edulis*	1	Insensitive
Solanaceae	*Solanum cinnamomeum*	1	Sensitive
Symplocaceae	*Symplocos laxiflora*	1	Opportunist
Urticaceae	*Coussapoa microcarpa*	1	Insensitive
Menispermaceae	*Hyperbaena oblongifolia*	1	NA
Sapotaceae	*Diploon cuspidatum*	1	Sensitive

Table 1. Species consumed and dispersed (1: dispersed; 0: not dispersed) by the black lion tamarins in Carlos Botelho State Park and Trápaga PNHR.

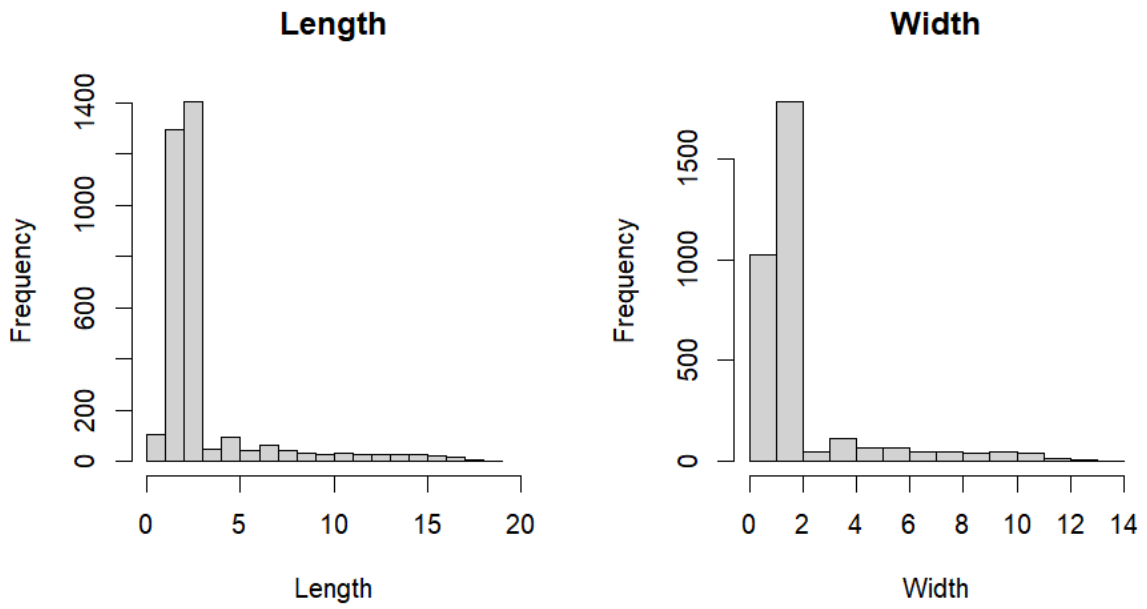

Figure 3: Histogram of seed sizes (millimeters) from dispersed seeds by one group of BLTs at Carlos Botelho State Park and Trápaga PNHR.

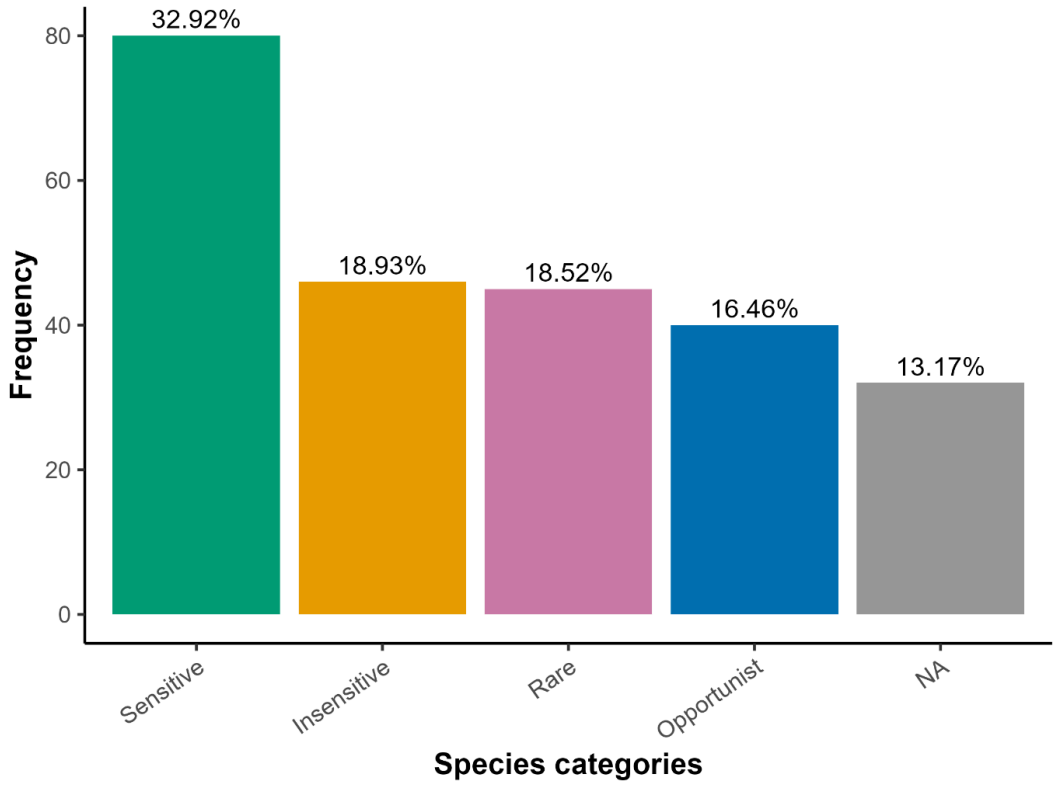

Figure 4. Frequency of the species category dispersed by a group of BLTs: 1) disturbance-sensitive: old-growth indicators; 2) disturbance-insensitive: species that are abundant across successional stages; 3) rare: for species that could not be assigned to a category due to low sample size; 4) disturbance-opportunist: early-stage indicators and 5) NA: species consumed by the study group but not included in the study by Toledo et al. (2025).

Spatial distribution of dispersed seeds

We determined the dispersal distances of 62 dispersal events for which the parent tree was confidently identified. BLTs dispersed seeds at a distance of 164 ± 134 m (range: 4-536 m) (Fig. 5).

The estimated MCP home range of BLTs was 459.2 ha (100%) and 380 ha (95%) (Fig. 6). On the other hand, the Kernel results were 337.8 ha when considering 95%, 155.8 ha using 70% and 91.7 ha using 50% of the records (Fig. 7).

The group's home range (Kernel 95%) was composed of 89.7% of late-successional forest (forest of 31 years old or older, hereafter referred as 'older forest'), followed by 8.4% of mid-successional forest (forest within the range of 11 to

30 years old, hereafter referred as ‘intermediate forest’), and 1.9% of early-successional forest (within the range of 1 to 10 years old, hereafter referred to ‘early forest’) (Fig. 8).

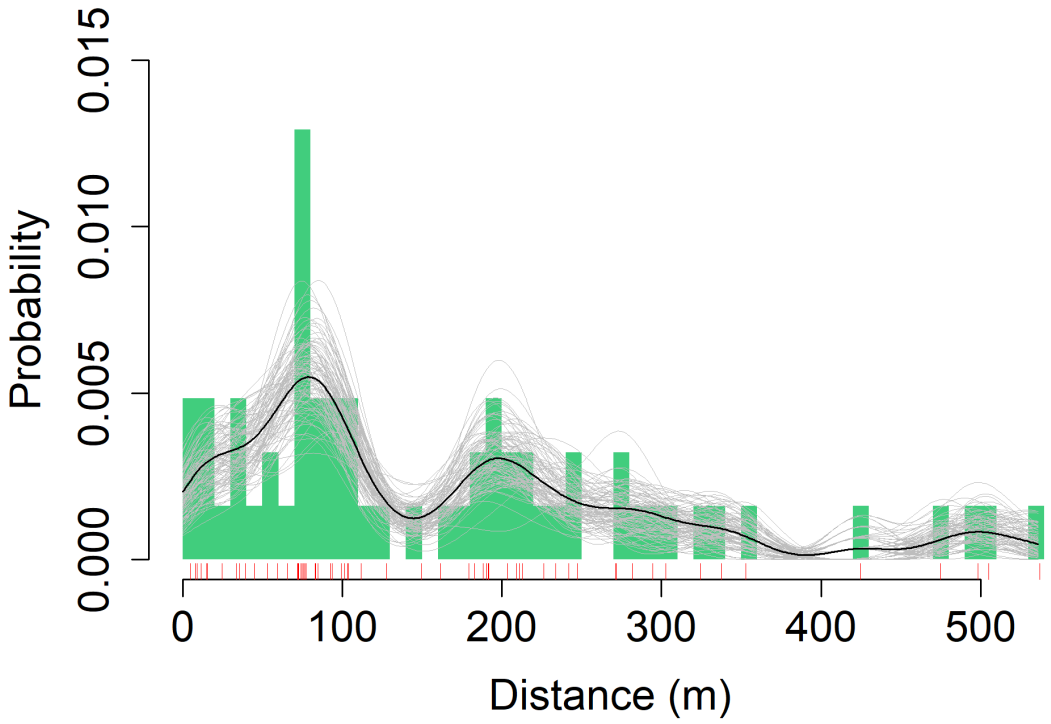

Figure 5. Histogram of seed dispersal distances observed in the field, from a group of BLTs at Carlos Botelho State Park and Trápaga PNHR. The green bars represent the observed events, the grey lines indicate the expected range for 100 simulations, the black line shows the mean of the simulations, and the red marks indicate each dispersal event.

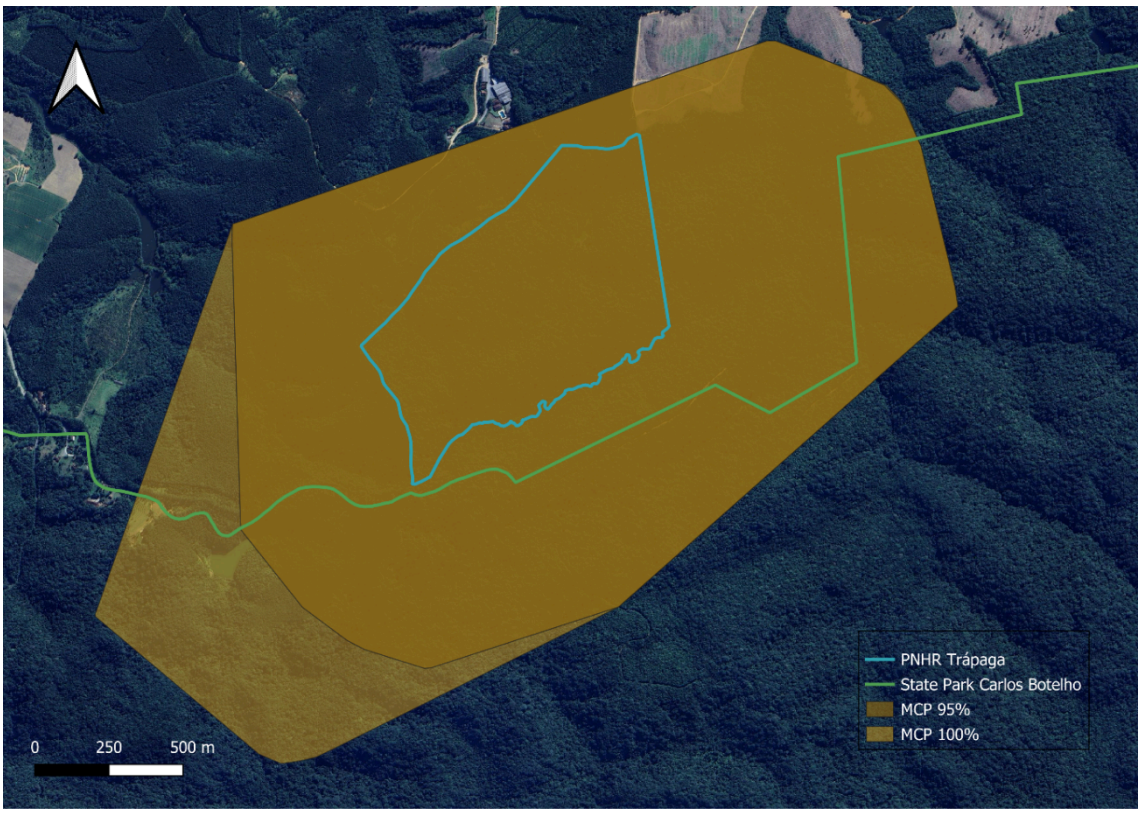

Figure 6. Minimum Convex Polygon (MCP) of the group's home range with 100% and 95% of the records. This group of BLTs was followed during 13 consecutive months from April 2024 to April 2025 and its home range includes Carlos Botelho State Park and Trápaga PNHR.

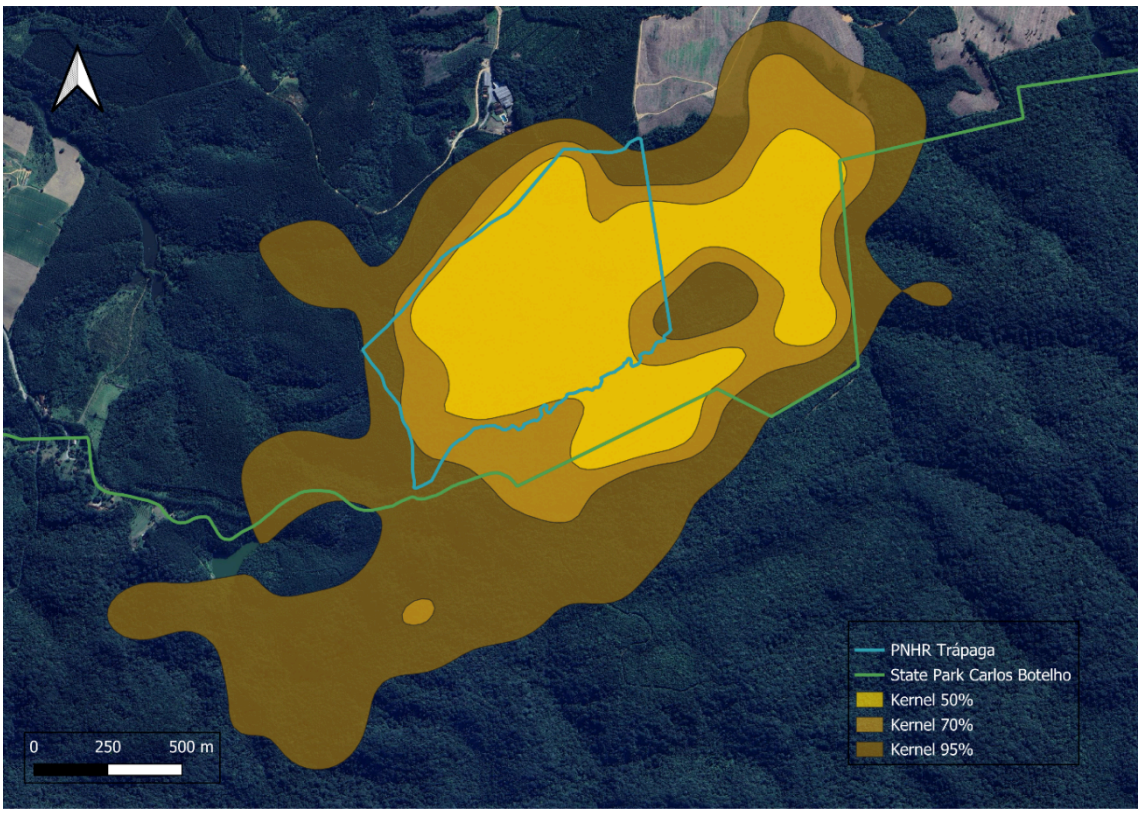

Figure 7. Kernel Density Estimation (KDE) of the group's home range with 95%, 70%, and 50% of the records. This group of BLTs was followed during 13 consecutive months from April 2024 to April 2025 and its home range includes Carlos Botelho State Park and Trápaga PNHR.

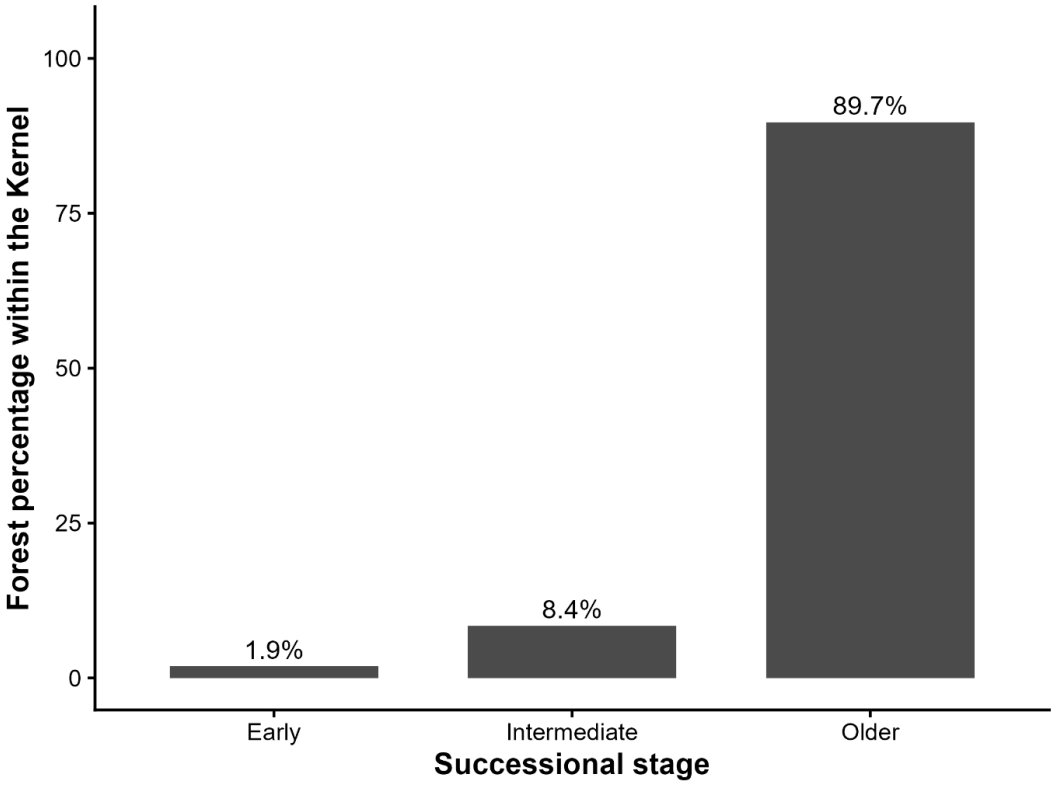

Figure 8. Distribution of forest successional stages within the Kernel 95% of the BLT group of Carlos Botelho State Park and Trápaga PNHR. 'Early forest' referred to a forest within the range of 1 to 10 years old, 'intermediate forest' to a forest within the range of 11 to 30 years old and 'older forest' referred to a forest of 31 years old or older.

Around 91.3% of seed dispersal events (222/243) occurred within the older forest. The species group that was most frequently dispersed in older forest was sensitive species (34%). Opportunist species were the group species most frequently dispersed in intermediate forest (35.3%). However, our results indicate that BLTs also transport seeds from older forests into younger stands since sensitive species were the group species most frequently dispersed in early forest (50%) (Fig. 9).

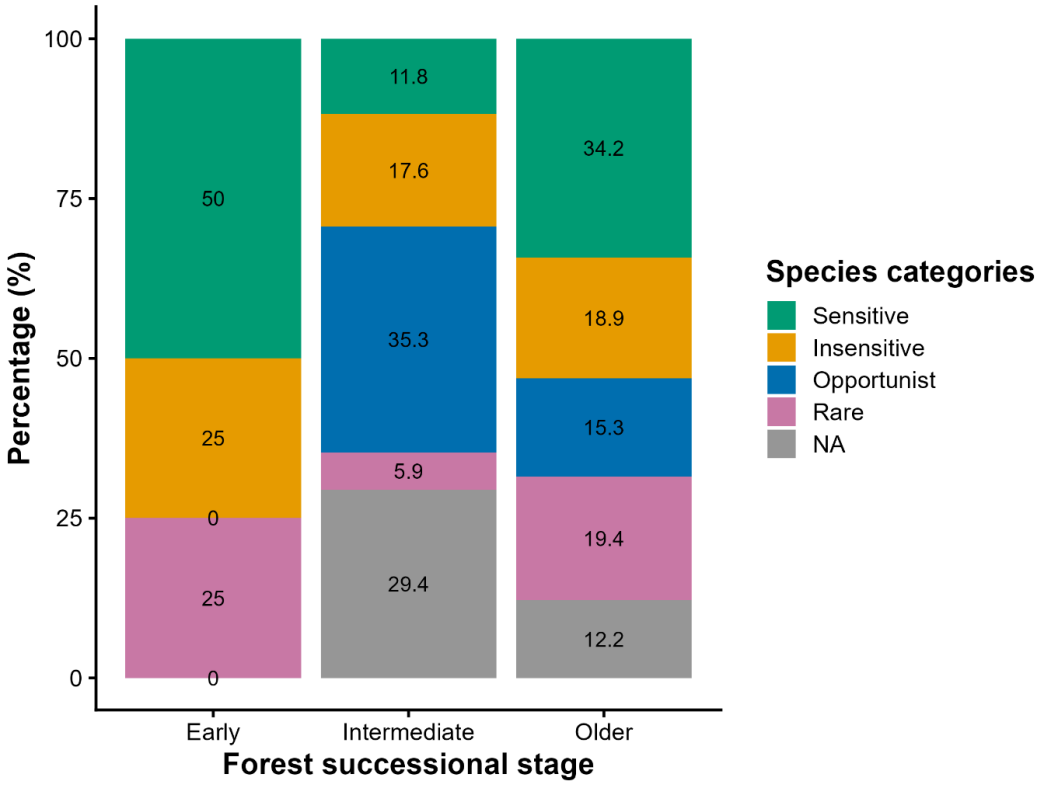

Figure 9. Frequency of each category of dispersed species in different forest age group. Species categories are defined as: 1) disturbance-sensitive: old-growth indicators; 2) disturbance-insensitive: species that are abundant across successional stages; 3) rare: for species that could not be assigned to a category due to low sample size; 4) disturbance-opportunist: early-stage indicators and 5) NA: species consumed by the study group but not included in the study by Toledo et al. (2025). 'Older' is a forest of 31 years old or older, 'Intermediate' is a forest within the range of 11 to 30 years old and 'Early' is a forest within the range of 1 to 10 years old.

Gut retention time, seed size, and seed dispersal distance

The seeds of the 62 dispersal events for which a dispersal distance could have been estimated were 10.5 ± 4.7 mm long and 7.3 ± 3.2 mm width (N=53). We estimated a gut retention time of 85 ± 49 minutes (N=62). Dispersal distance was positively influenced by retention time (N=62, R^2=0.24, p<0.001) (Fig. 10A). Retention time was negatively influenced by seed width (N=53, R^2=0.16, p<0.01) (Fig. 10B). Although bigger seeds were associated with shorter dispersal distances, seed size explained only a small fraction of the variation (N=53; R^2=0.08; p=0.036) (Fig. 10C).

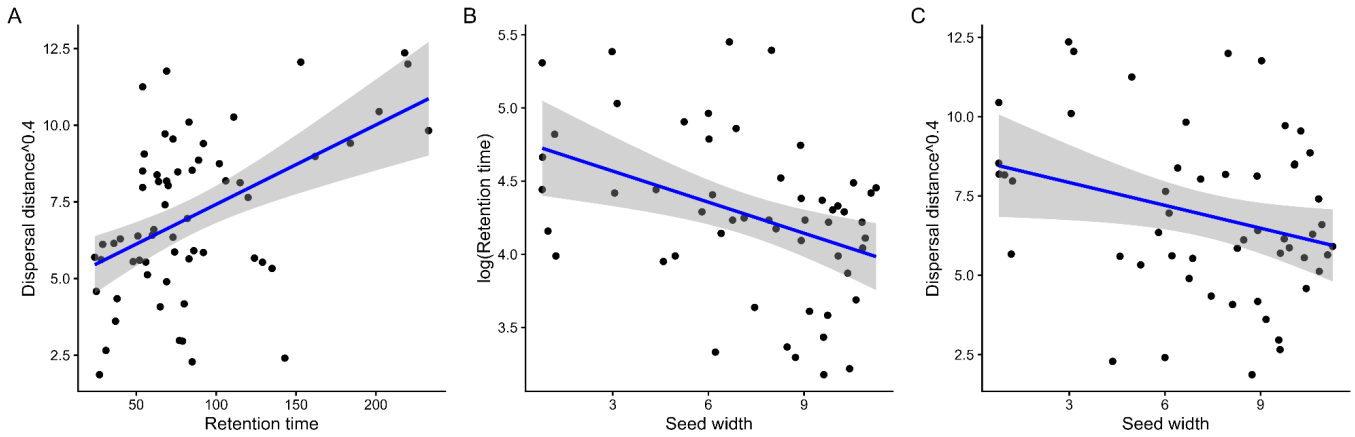

Figure 10. (A) Linear regression between dispersal distance (meters) and retention time (minutes). N=62; R^2=0.24; p<0.001. (B) Linear regression between retention time ((log)minutes) and seed width (millimeters). N=53, R^2=0.16, p=0.002. (C) Linear regression between dispersal distance (meters) and seed width (millimeters). N=53; R^2=0.08; p=0.036.

Seed dispersal effectiveness

Effect of gut passage on germination success:

Overall, although the probability to germinate tended to be higher in the feces and in the treatment without pulp compared to the treatment with pulp (Table 2), the differences were not statistically significant (Table 3).

Treatment	Prob. of germination (mean)	Standard error	Confidence interval
With pulp	0.246	0.12	0.0839 - 0.538
Without pulp	0.348	0.12	0.1530 - 0.612
Feces	0.372	0.07	0.2357 - 0.532

Table 2: Model-based estimates of germination probability for three treatments: with pulp, without pulp, and feces. Probabilities (mean ± Standard Error) and their respective 95% confidence intervals were calculated using a binomial GLMM.

Contrasts	Odds ratios	Standard error	z-ratio	p-value
With pulp vs. without pulp	0.611	0.404	-0.745	0.7366
With pulp vs. feces	0.551	0.339	-0.970	0.5959
Without pulp vs. feces	0.902	0.438	-0.213	0.9752

Table 3: Pairwise comparisons of seed germination probabilities among treatments. Results are presented as Odds Ratios, which indicates the relative likelihood of germination between groups, with respective Standard Errors (SE) and z-ratios derived from the binomial GLMM. P-values were adjusted for multiple comparisons using the Tukey method.

Responses to pulp removal and gut passage varied between species. Seven out of 9 species did not show any significant difference between treatments (Table 4). *C. xanthocarpa*, *Guapira opposita*, and *Solanum cinnamomeum* exhibited higher germination success in the treatment with pulp, although these differences were not statistically significant. Conversely, *Casearia decandra*, *Myrcia* sp., and *Myrcia vellozoi* showed higher germination in the treatment without pulp, though also without significance. Only *Coussapoa microcarpa* yielded identical germination rates between the treatments without pulp and feces. In contrast, *Campomanesia sp.* presented a significantly higher germination success in the treatment without pulp when compared to feces (OR = 21; p = 0.02), and *Eugenia cerasiflora* had a higher germination success both in treatments without pulp and feces when compared to treatment with pulp (with pulp vs. without pulp OR = 0.074; p = 0.002; with pulp vs. feces OR = 0.043; p = 0.001).

For species where generalized linear models (GLM) could not be performed, results were based strictly on observed germination success. *Diploon cuspidatum* showed higher germination in the without pulp treatment, while *Ficus luschtaniana* achieved 100% germination in the pulp treatment. Additionally, *Hyperbaena*

oblongifolia, *Allophylus edulis*, *Symplocos laxiflora*, and *Vitex polygama* exhibited higher germination rates in the feces treatment.

Species	WP total/ germinated	WP percentage	NP total/germinated	NP percentage	Feces total/germinated	Feces percentage	odds.ratio WP vs NP	odds.ratio WP vs Feces	odds.ratio NP vs Feces	p.value WP vs NP	p.value WP vs Feces	p.value NP vs Feces
Campomanesia sp.	7/6	85.70%	15/14	93.30%	15/6	40%	0.429	9	21	0.838	0.1604	**0.0238**
Campomanesia xanthocarpa *	11/7	63.60%	15/3	20%	15/4	26.60%	7	4.812	0.688	0.0777	0.1585	0.9029
Casearia decandra *	15/5	33.30%	15/10	66.60%	15/6	40%	0.25	0.75	3	0.173	0.9241	0.3177
Coussapoa microcarpa	5/3	60%	15/11	73.30%	15/11	73.30%	0.545	0.545	1	0.8417	0.8417	1
Eugenia cerasiflora	15/5	33.30%	31/27	87%	25/23	92%	0.074	0.043	0.587	**0.002**	**0.0019**	0.8284
Guapira opposita	9/1	11.10%	12/1	8.30%	28/3	10.70%	1.375	1.042	0.758	0.9751	0.9994	0.9714
Myrcia sp.	15/4	26.60%	15/7	46.60%	15/2	13.30%	0.416	2.364	5.687	0.4984	0.6416	0.1412
Myrcia vellozoi *	10/3	30%	10/8	80%	7/3	42.80%	0.107	0.571	5.333	0.0841	0.8497	0.2801
Solanum cinnamomeum	20/12	60%	30/15	50%	15/4	26.60%	1.5	4.125	2.75	0.7672	0.1352	0.3059
Hyperbaena oblongifolia	2/0	0%	2/0	0%	7/5	71.40%	-	-	-	-	-	-
Allophylus edulis	10/0	0%	11/0	0%	15/2	13.30%	-	-	-	-	-	-
Diploon cuspidatum *	6/0	0%	7/4	57.10%	7/2	28.50%	-	-	-	-	-	-
Ficus luschnathiana	15/15	100%	14/1	7.14%	34/20	58.80%	-	-	-	-	-	-
Symplocos laxiflora	14/0	0%	15/0	0%	17/2	11.70%	-	-	-	-	-	-
Vitex polygama	5/0	0%	6/1	16.60%	6/3	50%	-	-	-	-	-	-

Table 4: Seed germination by species in the three treatments: with pulp (WP), without pulp (NP) and feces. Values represent germination success (n total/ n germinated and %), odds ratios and p values for comparisons between treatments. Missing values (-) indicate cases where absence of germination in one or more treatments prevented the calculation of the model parameters. Species with * have seeds in the treatments with- and without-pulp from only one tree individual.

Effect of gut passage on germination success across time:

Although the germination probability was higher in treatment without pulp, the Log-rank test showed no statistical difference between curves (p = 0.23) (Fig. 11). The hazard ratio (HR) plot (Fig. 12) shows that, initially, the treatments without pulp and feces reduce germination probability (HR < 1), but after a few weeks, it increased and passed the HR of the treatment with pulp.

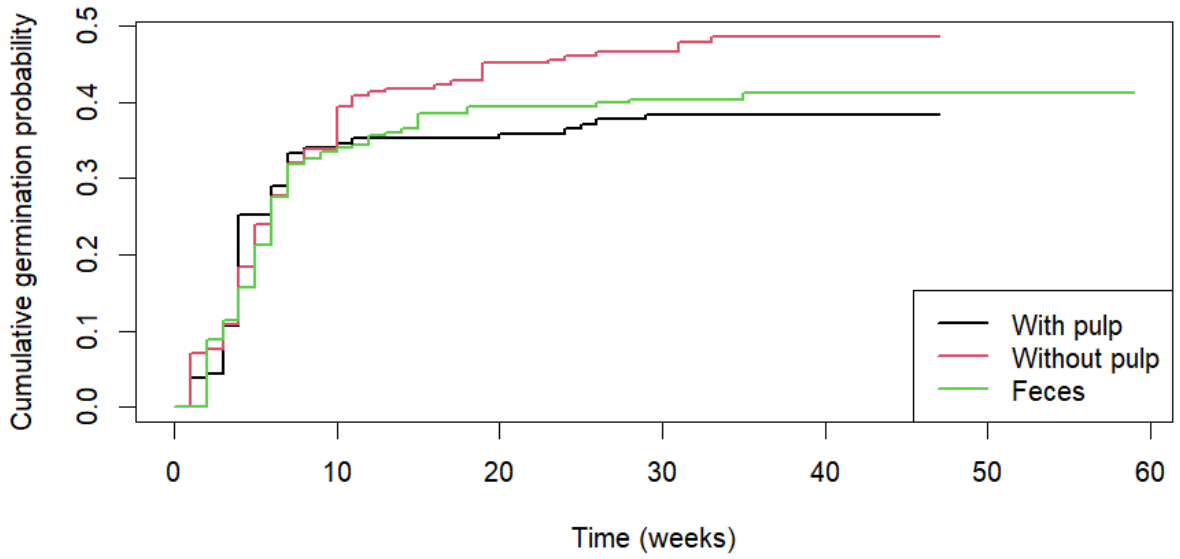

Figure 11: Cumulative germination probability over a 59-week period for three treatments. The log-rank test (p = 0.23) indicates no statistically significant difference between the germination curves over time.

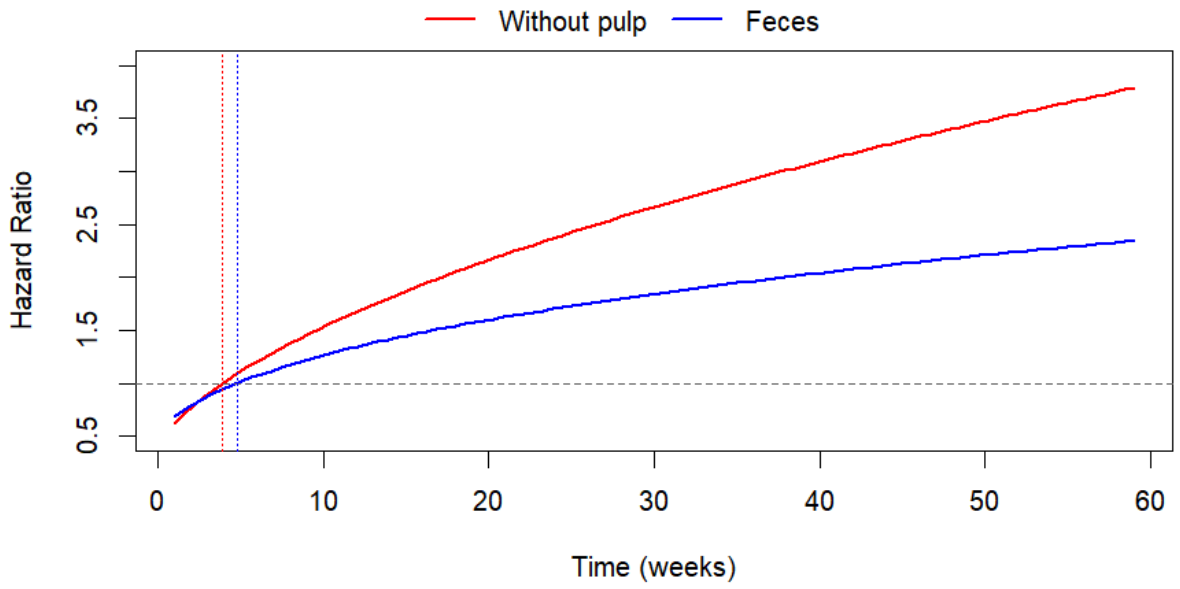

Figure 12: Hazard ratios over time for seeds from feces and treatment without pulp relative to the treatment with pulp, estimated using the final Cox model. The horizontal dashed line indicates HR = 1 and vertical dashed lines indicate the time at which effects change over time.

Based on the results of the final Cox proportional hazards model, we identified certain trends regarding germination dynamics. Both the treatment without pulp (HR = 0.42; p = 0.08) and the feces treatment (HR = 0.52; p = 0.19) exhibited lower hazard ratios (HR) compared to the treatment with pulp in the first weeks. In practical terms, this indicates a tendency toward slower germination or lower daily germination rates in these groups compared to seeds with pulp until the fourth and fifth weeks, although these differences did not reach statistical significance. Nevertheless, the time-dependent terms showed higher HR and statistical significance for tt(treatment without pulp) (treatment without pulp HR = 1.70; p = 0.03), suggesting that the effect of treatments on germination success varied over time. Seeds without pulp initially showed lower germination success compared to the ones with pulp, however, this effect exhibited significant temporal shifts, over the course of the experiment the treatment without pulp eventually showed higher

germination success. In contrast, seeds from feces showed a similar but non-significant temporal trend (Feces HR = 1.44; p = 0.15). A high heterogeneity in germination among species was observed (variance of random effects = 0.77; p < 0.001). This Cox model provided a significantly better fit than the null model and good predictive power (likelihood ratio test, p < 0.001; concordance = 0.722).

Variable	Coeficient	Hazard Ratio	P value
With pulp	-	1.00	-
Without pulp (Initial)	-0.853	0.426	0.080
Feces (Initial)	-0.640	0.527	0.190
Without pulp tt()	0.534	1.705	**0.037**
Feces tt()	0.364	1.440	0.150

Table 5: Results of the time-dependent Cox proportional hazards model with frailty factor evaluating the effects of treatments on germination rate through time. Coefficients and hazard ratios are shown relative to the reference treatment (with pulp). “Initial” terms represent the effect of treatments at the beginning of the experiment, while *tt()* terms indicate time-dependent effects modeled as interactions with log (time + 1). Positive coefficients in *tt()* indicate that treatment effects increase germination over time.

We also run an individual cox model for each species. The proportional hazards assumption was tested individually for each one. Species that do not violate PH are shown in Table 6.

Species	Contrast	HR	CI	p-value
Campomanesia xanthocarpa	NP vs WP	0.235	0.06 – 0.91	**0.0363**
Campomanesia xanthocarpa	Feces vs WP	0.34	0.09 – 1.16	0.086
Campomanesia xanthocarpa	Feces vs NP	1.447	0.32 – 6.46	0.6283
Casearia decandra	NP vs WP	2.756	0.94 – 8.07	0.0647
Casearia decandra	Feces vs WP	1.357	0.41 – 4.45	0.6141
Casearia decandra	Feces vs NP	0.493	0.17 – 1.35	0.1708
Diploon cuspidatum	NP vs WP	5.88E+08	0 - Inf	0.9986
Diploon cuspidatum	Feces vs WP	2.72E+08	0 - Inf	0.9987
Diploon cuspidatum	Feces vs NP	0.462	0.08 – 2.53	0.3739
Ficus luschnathiana	NP vs WP	0.029	0.004 – 0.22	**0.0007**
Ficus luschnathiana	Feces vs WP	0.321	0.15 – 0.64	**0.0016**
Ficus luschnathiana	Feces vs NP	10.916	1.45 – 81.6	**0.0199**
Guapira opposita	NP vs WP	0.703	0.04 – 11.2	0.8032
Guapira opposita	Feces vs WP	0.925	0.09 – 8.89	0.9464
Guapira opposita	Feces vs NP	1.316	0.13 – 12.6	0.8119
Myrcia sp.	NP vs WP	1.965	0.57 – 6.71	0.2814
Myrcia sp.	Feces vs WP	0.464	0.08 – 2.53	0.3757
Myrcia sp.	Feces vs NP	0.236	0.04 – 1.13	0.0721
Myrcia vellozoi	NP vs WP	4.481	1.18 – 16.9	**0.0274**
Myrcia vellozoi	Feces vs WP	1.564	0.31 – 7.75	0.584
Myrcia vellozoi	Feces vs NP	0.349	0.09 – 1.32	0.121
Solanum cinnamomeum	NP vs WP	0.772	0.36 – 1.65	0.5047
Solanum cinnamomeum	Feces vs WP	0.352	0.11 – 1.09	0.0705
Solanum cinnamomeum	Feces vs NP	0.455	0.15 – 1.37	0.1626

Table 6: Hazard ratios (HR), 95% confidence intervals (CI), and p-values generated by individual Cox proportional hazards models comparing the effect of treatments on germination success through time for eight plant species. The contrasts compare the following treatments: NP (without pulp), WP (with pulp), and Feces.

In *Ficus,* seeds with pulp germinated quicker than seeds without pulp and seeds found in feces. For *Campomanesia xanthocarpa* seeds with pulp also germinated faster than without pulp. On the other hand, seeds of *Myrcia vellozoi* germinated quicker without pulp when compared to treatment with pulp.

When we used the tt() function on species that violated proportional hazards, *Eugenia cerasiflora* generated infinite coefficients. Models of *Coussapoa microcarpa* and *Vitex polygama* did not converge. For *Campomanesia* sp., seeds from feces exhibited a higher initial germination success, but this effect declined over time (time-dependent effect: $p = 0.054$), no significant effect was observed for seeds

without pulp, the overall model was significant (p = 0.01). *Allophylus edulis, Symplocos laxiflora* and *Hyperbaena oblongifolia* were not included in individual analysis due to low sample size.

SDE:

The effectiveness of dispersal varied among species, forming four distinct groups (Fig. 13). The first group, characterized by low quality and low quantity, exhibited SDE values ranging from 22 to 153, and is represented by *Symplocos laxiflora* (SDE: 22), *Allophylus edulis* (SDE: 46), *Guapira opposita* (SDE: 99), *Solanum cinnamomeum* (SDE: 137) and *Posoqueria latifolia* (SDE: 153). The second group consists of a single species, *Eugenia cerasiflora* (SDE: 614), which exhibited a very high dispersal quality (0.92) but low quantity. The third group included *Miconia formosa* (SDE: 2829*)* and *Ficus luschnathiana* (SDE: 125433), showed intermediate dispersal quality (0.58 and 0.53 respectively) and high quantity. The final group, containing only *Miconia cinnamomifolia* (SDE: 0.00), exhibited the poorest quality of dispersal (0.00) despite a very high quantity of seeds.

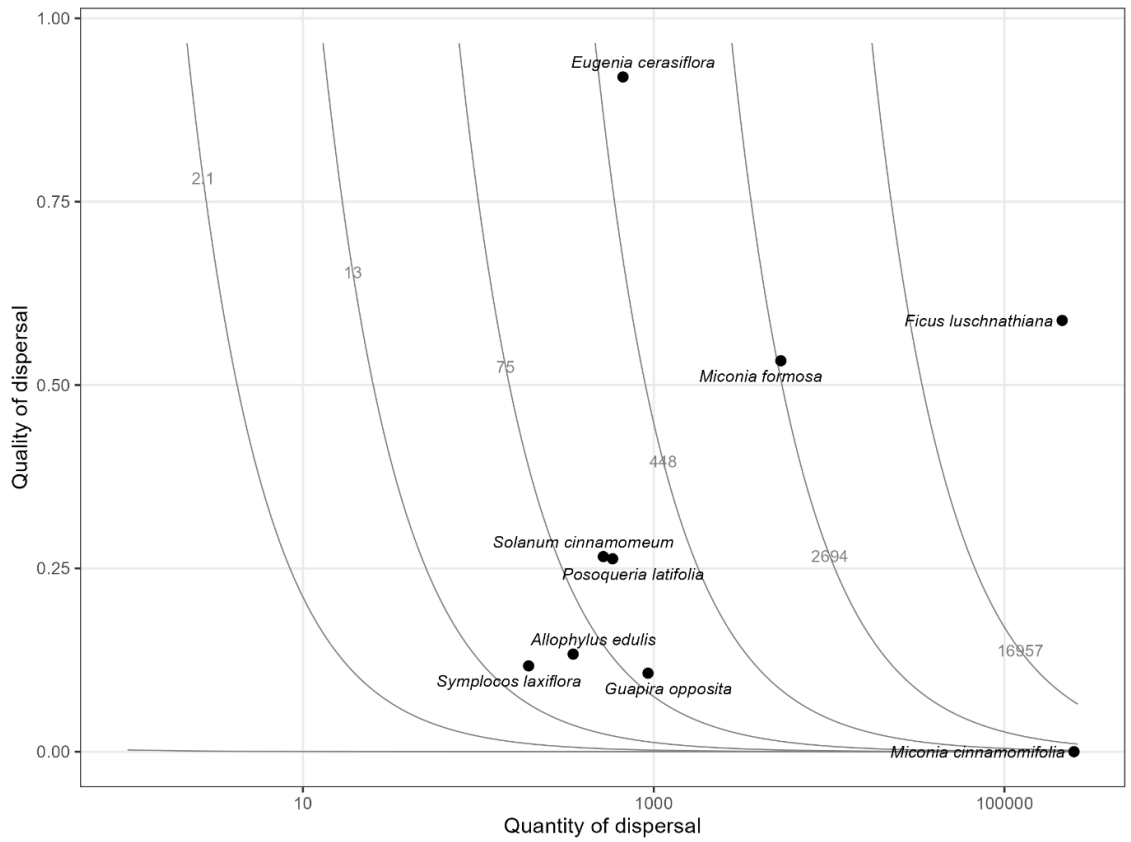

Figure 13: Seed Dispersal Effectiveness (SDE) landscape for species consumed by BLTs. The horizontal axis represents the (log)quantitative component (seeds ingested per minute x average visit time x n total visits), while the vertical axis shows the qualitative component (germination success of the feces treatment). Isoclines indicate combinations of quantity and quality that yield the same overall effectiveness.

DISCUSSION

Traits of dispersed seeds, dispersal distance and their relationship with gut retention time

Here we highlight the established role of BLT as an effective seed disperser. We demonstrate that most of the consumed plant species had their seeds dispersed in BLTs' feces, encompassing a wide range of sizes.

However, the mean seed size was skewed downwards due to the high abundance of *Ficus* and *Miconia* seeds, which are very small, but the presence of large seeds (>12 mm), whose dispersal relies heavily on large frugivores (Fuzessy et

al., 2018), can indicate the BLTs' capacity to partially fulfill this disperser role in areas where large frugivores are no longer present.

In our study, BLTs dispersed seeds at a lower mean dispersal distance compared to other studies on the same species. For example, at Fazenda Santa Maria, a mean distance of 343,8 ± 225,8 meters was recorded within a home range of 82,9 ha (Alcolea, 2016). At the Morro do Diabo State Park, the mean dispersal distance was 263 ± 13 meters in a 383 ha home range (continuous forest group) and 210 ± 9 meters in a 230 ha home range (continuous forest with edge effect group). At Fazenda Rio Claro, the mean distance was 127 ± 7 meters in a 35 hectares home range (Silva, 2022). Nevertheless, the distances exhibited here are sufficient for seeds to escape high mortality rates associated with areas closer to the parent tree (Howe et al., 1985; Augspurger, 1983).

The negative influence of seed size on gut retention time aligns with previous studies (Garber, 1986; Stevenson, 2000), supporting the expectation that smaller seeds are dispersed farther from the parent tree. Although retention time and seed size partially explained dispersal distance, their explanatory power was relatively low. This suggests that other variables, such as movement patterns, may influence dispersal distance. These movement patterns also affect the density and spatial distribution of dispersed seeds (Côrtes and Uriarte, 2013; Razafindratsima et al., 2014) and can, in turn, be driven by resource availability, such as the fruiting status of trees (Janmaat et al., 2006).

Home range successional stages and seed successional categories

Contrary to our hypothesis, 'older forest' comprised most of the BLTs' home range and BLTs did not use early and intermediate areas more intensively. This could

be happening due to the early and intermediate areas being patchily distributed throughout the home range and sometimes located at the forest edges. Some of these edges are separating the native forest from the pine plantations, which the BLTs only use occasionally to reach another native forest patch.

Alternatively, while the BLTs might be passing through newer forest patches more frequently, they may spend relatively little time there due to their high mobility (mean daily path length: 2,275 meters in this study; 3,346 and 2,301 meters in Silva, 2022). Consequently, because older forest covers a much larger proportion of their home range (Fig. 8), seeds are disproportionately dispersed in the older areas.

Similarly, our expectation that BLTs would predominantly disperse opportunistic and insensitive species, was not fully supported. In contrast, we found that sensitive species constituted the most frequently dispersed category overall, followed by insensitive species. Furthermore, sensitive species were the most dispersed group across both older and early forests, highlighting the capacity of BLTs to link different successional stages, as previously observed in other small primate species (Culot et al., 2010). This may be happening due to the high percentage of older forest, resulting in a high concentration of sensitive species individuals in the home range. The older a secondary forest is, the more similar it becomes to an old-growth forest in terms of species richness (Guariguata and Ostertag, 2001; Rozendaal et al., 2019) and proportion of non-pioneer species (Liebsch et al., 2008). This behavior may potentially enhance forest resilience and natural regeneration in Atlantic Forest landscapes.

Effect of gut passage on germination

Our results showed no significant differences among the three treatment groups regarding overall seed germination success and time, although a trend toward higher germination probabilities in the feces and without-pulp treatments was observed. These findings regarding the effect of gut passage align with the existing literature (Fuzessy et al., 2016). Although predominantly frugivorous, BLTs are more accurately classified as frugivorous-faunivorous and include insects in their diet (Passos,1999; Keuroghlian and Passos, 2001; Raskin et al., 2024). This specific feeding guild has been shown to have a generally neutral effect on seed germination processes.

The temporal variation in the germination time of the without-pulp treatment was particularly interesting. In the first weeks, this treatment exhibited a lower germination success than the treatment with-pulp, but, in the posterior weeks, the without-pulp treatment showed a higher germination rate. It is important to note that the high germination rate observed in the with-pulp treatment for certain species may be biased by our experimental design. For *Campomanesia* sp., *Campomanesia xanthocarpa*, *Ficus luschnathiana*, and *Coussapoa microcarpa*, a single whole ‘‘fruit’’ was planted per tube. Consequently, the tubes in this specific treatment contained multiple seeds, increasing the probability of at least one seed germinating and potentially inflating germination success and speed estimates for the with-pulp treatment in these species.

A similar trend in temporal variation was observed for the feces treatment, however, without statistical significance. This aligns with the fact that primates that include a significant amount of insects in their diets can actually delay germination (Fuzessy et al 2016). Although fruits are the preferred resource to BLTs, the

proportion of arthropods in their diets varies depending on fruit availability and can represent up to 60% of their assimilated diets (Raskin et al., 2024).

When analyzing the species individually, it became evident that some benefit more from gut passage than others. For instance, in *Campomanesia* sp., gut-passed seeds exhibited a faster initial germination rate, even though the germination success was higher in the without-pulp treatment compared to the feces treatment. Although the final germination success was higher in the without-pulp treatment, the more rapid germination promoted by gut passage can be highly advantageous, as earlier germination often contributes to improved plant fitness (Verdú and Traveset, 2005). *Eugenia cerasiflora* showed an inhibitory effect from the pulp, as both treatment without-pulp and feces exhibited similar germination success, but statistically significant higher germination success than seeds with pulp. Therefore, we can infer, for this species, that the gut passage enhances germination through the removal of the pulp but not through the digestive scarification (Samuels & Levey 2005).

Seed dispersal effectiveness

The SDE framework reinforced what we observed in the other results: the dispersal service provided by the BLTs is species-specific. For species with a massive number of seeds per fruit, such as *Miconia formosa* and *Ficus luschnathiana,* effectiveness is compensated for intermediate germination quality with a high input of seeds per fecal sample. Conversely, for *Eugenia cerasiflora*, they provided a high-quality dispersal service despite low quantitative values. This may indicate that, for some species, the role of BLTs consists more in depulping seeds, and therefore removing this potential germination inhibitor (Cipollini and Levey,

1997), than in dispersing a high number of seeds. The case of *Miconia cinnamomifolia* highligths that frequent consumption and high ingestion of seeds does not guarantee effective dispersal, however the absence of germination among the three different treatments may indicate that the conditions imposed by the experiment, such as temperature and humidity, were not ideal for germination.

Consequently, by effectively dispersing seeds from different successional categories with multiple quality-quantity combinations, into distinct forest successional stages, BLTs play a crucial role in forest dynamics.

CONCLUSION

Our study demonstrates that the BLT is an effective seed disperser, playing an important role in the dynamics of Atlantic Forest. Rather than providing a uniform service, BLTs exhibit species-specific dispersal effectiveness. They act as massive seed vectors for species with high seed quantities per fruit, while functioning as germination facilitators for other species through the removal of pulp. Moreover, their extensive daily paths allow dispersion to occur far enough from the parent tree, and, by frequently dispersing seeds of disturbance-sensitive (non-pioneer) species into both older and early secondary successional forests, BLTs actively drive functional connectivity and habitat regeneration.

REFERENCES

Augspurger, C. K. (1983). Seed Dispersal of the Tropical Tree, Platypodium Elegans, and the Escape of its Seedlings from Fungal Pathogens. *Journal of Ecology*, *71*(3), 759–771.

Albert, A., Hambuckers, A., Culot, L. et al. Frugivory and Seed Dispersal by Northern Pigtailed Macaques (Macaca leonina), in Thailand. International Journal of Primatology. 34, 170–193 (2013).

Alcolea, M. (2016). Dispersão de sementes pelo mico-leão-preto, *Leontopithecus chrysopygus* (Primates, Callitrichidae) em um fragmento de Mata Atlântica [Unpublished undergraduate thesis]. Primatology Laboratory, Universidade Estadual Paulista “Júlio de Mesquita Filho”.

Andresen, E. (2000). Ecological roles of mammals: the case of seed dispersal. In A. Entwistle & N. Dunstone (Eds.), Priorities for the conservation of mammalian diversity (pp. 2–26). Cambridge: Cambridge University Press.

Bates, D., Maechler, M., Bolker, B., Walker, S., (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48.

Buchanan-Smith, H. M., Hardie, S. M., Caceres, C. & Prescott, M. J. Distribution and forest utilization of Saguinus and other primates of the Pando Department, Northern Bolivia. International Journal of Primatology. 21, 353–379 (2000)

Bufalo, S.F., Galetti, M., Culot, L., 2016. Seed dispersal by primates and implications for the conservation of a biodiversity hotspot, the Atlantic Forest of South America. International Journal of Primatology. 37, 333–349.

Calenge C., (2024). _adehabitatHR: Home Range Estimation_. R package version 0.4.22, <https://CRAN.R-project.org/package=adehabitatHR>.

Chapman, C. A. (1995). Primate seed dispersal: Coevolution and conservation implications. Evolutionary Anthropology: Issues, News, and Reviews, 4, 74–82.

Chapman, C. A. (1989). Primate Seed Dispersal: The Fate of Dispersed Seeds. Biotropica, 21(2), 148–154.

Cipollini ML, Levey DJ. Secondary metabolites of fleshy vertebrate-dispersed fruits: adaptive hypotheses and implications for seed dispersal. Am Nat. 1997 Sep;150(3):346-72.

Culot, L., Huynen, M. C. & Heymann, E. W. Partitioning the relative contribution of one-phase and two-phase seed dispersal when evaluating seed dispersal effectiveness. Methods in Ecology and Evolution. 6, 178–186 (2015).

Culot, L., Muñoz Lazo, F.J.J., Huynen, MC. et al. Seasonal Variation in Seed Dispersal by Tamarins Alters Seed Rain in a Secondary Rain Forest. International Journal of Primatology. 31, 553–569 (2010).

Côrtes, M.C. and Uriarte, M. (2013), Integrating frugivory and animal movement: a review of the evidence and implications for scaling seed dispersal. Biol Rev, 88: 255-272.

Danaë M. A. Rozendaal *et al.*, Biodiversity recovery of Neotropical secondary forests.*Sci. Adv.*5,eaau3114(2019).

deToledo, R. M., Pivello, V. R., Vangansbeke, P., Jakovac, C., Verheyen, K., Verdade, L. M., & deLima, R. A. F. (2025). Reassembly dynamics of tropical secondary succession: Evidence from Atlantic forests. Journal of Ecology, 113, 2106–2119.

FUNDAÇÃO SOS MATA ATLÂNTICA; INSTITUTO NACIONAL DE PESQUISAS ESPACIAIS. Atlas dos remanescentes florestais da Mata Atlântica período 2020-2021. São José dos Campos: INPE, 2022.

Fuzessy, L.F., Cornelissen, T.G., Janson, C. and Silveira, F.A.O. (2016), How do primates affect seed germination? A meta-analysis of gut passage effects on neotropical plants. Oikos, 125: 1069-1080.

Fuzessy, L.F., Janson, C., Silveira, F.A.O. Effects of seed size and frugivory degree on dispersal by Neotropical frugivores. Acta Oecologica 93, 2018. 41-47.

Fuzessy, L.F., Janson, C., Silveira, F.A.O., Effects of seed size and frugivory degree on dispersal by Neotropical frugivores, Acta Oecologica, Volume 93, 2018, Pages 41-47, ISSN 1146-609X.

Fox J, Weisberg S (2019). _An R Companion to Applied Regression_, Third edition. Sage, Thousand Oaks CA. <https://www.john-fox.ca/Companion/>.

Garber PA. The ecology of seed dispersal in two species of callitrichid primates (Saguinus mystax and Saguinus fuscicollis). Am J Primatol. 1986;10(2):155-170.

Gómez, J. M., & Verdú, M. (2012). Mutualism with plants drives primate diversification. Systematic Biology, 4, 567–577.

Guariguata, M.R., Ostertag, R., Neotropical secondary forest succession: changes in structural and functional characteristics, Forest Ecology and Management, Volume 148, Issues 1–3, 2001, Pages 185-206, ISSN 0378-1127.

Heymann, E.W., Culot, L., Knogge, C. et al. Small Neotropical primates promote the natural regeneration of anthropogenically disturbed areas. Sci Rep 9, 10356 (2019).

Heymann, E.W.; Fuzessy, L.; Culot, L. Small but Nice–Seed Dispersal by Tamarins Compared to Large Neotropical Primates. Diversity 2022, 14, 1033.

Heymann, E. W., Luettmann, K., Michalczyk, I. M., Pinedo Saboya, P. P., Ziegenhagen, B., & Bialozyt, R. (2012). DNA fingerprinting validates seed dispersal curves from observational studies in the Neotropical legume Parkia. PLoS One, 7, e35480.

Hijmans R (2025). _terra: Spatial Data Analysis_. R package version 1.8-21, <https://CRAN.R-project.org/package=terra>.

Howe, H. F., Schupp, E. W., & Westley, L. C. (1985). Early Consequences of Seed Dispersal for a Neotropical Tree (Virola surinamensis). *Ecology*, *66*(3), 781–791.

Howe, H.F., 2014. Diversity Storage: Implications for tropical conservation and restoration. Global Ecology and Conservation. 2, 349-358

Instituto Florestal. (2008). Parque Estadual Carlos Botelho: plano de manejo. São Paulo: Instituto Florestal.

IUCN SSC PRIMATE SPECIALIST GROUP. Neotropical Primates. [S. l.]: IUCN, 2021. Disponível em: http://www.primate-sg.org/red_list_threat_status/. Acesso em: 6 jun. 2026.

Janmaat, K.R.L., Byrne, R.W. Zuberbühler, K., Evidence for a spatial memory of fruiting states of rainforest trees in wild mangabeys, Animal Behaviour, Volume 72, Issue 4, 2006, Pages 797-807, ISSN 0003-3472.

Jordano P, Rodriguez-Sanchez F., (2026). _effect.lndscp: Effectiveness landscapes_. R package version 0.2.8, commit 72a73918abe997d19f1333fa5e596649231c7aab, <https://github.com/pedroj/effectiveness_pckg>.

Kaplin, B. A., and Lambert, J. E. (2002). Effectiveness of seed dispersal by Cercopithecus monkeys: Implications for seed input into degraded areas. Seed dispersal and frugivory: ecology, evolution and conservation. Third International Symposium-Workshop on Frugivores and Seed Dispersal, São Pedro, Brazil, 6-11 August 2000.

Kassambara A, Kosinski M, Biecek P (2026). _survminer: Drawing Survival Curves using 'ggplot2'_. R package version 0.5.2, <https://CRAN.R-project.org/package=survminer>.

Keuroghlian, A., & Passos, F. C. (2001). Prey foraging behavior, seasonality and time-budgets in black lion tamarins, Leontopithecus chrysopygus (Mikan, 1823) (Mammalia, Callitrichidae). Brazilian Journal of Biology, 61(3), 455-459.

Levey, W. R. Silva, e M. Galetti (Eds.), Seed dispersal and frugivory: Ecology, evolution and conservation (pp. 351–364). New York: CABI Publishing.

Lenth R (2025). _emmeans: Estimated Marginal Means, aka Least-Squares Means_. R package version 1.10.7, <https://CRAN.R-project.org/package=emmeans>.

Liebsch, D., Marques, M.C.M., Goldenberg, R., How long does the Atlantic Rain Forest take to recover after a disturbance? Changes in species composition and ecological features during secondary succession, Biological Conservation, Volume 141, Issue 6, 2008, Pages 1717-1725, ISSN 0006-3207.

Meyer, A.L.S., Pie, M.R. and Passos, F.C. (2014), Assessing the exposure of lion tamarins (Leontopithecus spp.) to future climate change. Am. J. Primatol., 76: 551-562.

Myers, N., Mittermeier, R., Mittermeier, C. et al. Biodiversity hotspots for conservation priorities. Nature 403, 853–858 (2000).

Passos, F. D. C. Seed dispersal by black lion tamarin, Leontopithecus chrysopygus (Primates, Callitrichidae), in southeastern Brazil "NOTES" Mammalia, vol. 61, no. 1, 1997, pp. 109-140.

Passos, F. C. (1999). Dieta de um grupo de mico-leão-preto, Leontopithecus chrysopygus (Mikan) (Cebidae, Primates), no Parque Estadual de Vassununga, São Paulo. Revista Brasileira de Zoologia, 16(1), 269-278.

Ribeiro, M.C., Metzger, J.P., Martensen, A.C., Ponzoni, F.J., Hirota, M.M., The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation, Biological Conservation, Volume 142, Issue 6, 2009, Pages 1141-1153, ISSN 0006-3207.

Raskin, A., Kaisin, O., Michel, L.N., Lejeune, B., Lepoint, G., Amaral, R.G., Bufalo, F., Sabino, G.P., Araújo, M.S., Rezende, G.C., Brotcorne, F. and Culot, L. (2025), Stable Isotopes Analysis of Black Lion Tamarins Reveals Increasing Arthropod Consumption When Fruit Productivity Decreases in Forest Fragments. American Journal of Primatology, 87: e23698.

Razafindratsima, O.H., Jones, T.A. and Dunham, A.E. (2014), Patterns of movement and seed dispersal by three lemur species. Am. J. Primatol., 76: 84-96.

Rodrigues, S.B.M., Gagetti, B.L., e Piratelli, A.J. "First record of *Leontopithecus chrysopygus* (Primates: Callitrichidae) in Carlos Botelho State Park, São Miguel Arcanjo, São Paulo, Brazil" Mammalia, vol. 80, no. 1, 2016, pp. 121-124.

Rezende, G.C., Knogge, C., Passos, F.C., Ludwig, G., Oliveira, L.C., Jerusalinsky, L. & Mittermeier, R.A. 2020. Leontopithecus chrysopygus. The IUCN Red List of Threatened Species 2020: e.T11505A17935400.

R Core Team (2024). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Samuels, I. A., and D. J. Levey. “Effects of Gut Passage on Seed Germination: Do Experiments Answer the Questions They Ask?” Functional Ecology, vol. 19, no. 2, 2005, pp. 365–68. JSTOR.

Schupp, E.W. Quantity, quality and the effectiveness of seed dispersal by animals. Vegetatio 107, 15–29 (1993).

Schupp, E.W., Jordano, P. and Gómez, J.M. (2010), Seed dispersal effectiveness revisited: a conceptual review. New Phytologist, 188: 333-353.

Silva, A. S. de A. e. (2022). Efetividade do mico-leão-preto Leontopithecus chrysopygus como dispersor de sementes e seu papel na regeneração florestal [Universidade Estadual Paulista (Unesp)].

Stevenson PR. Seed dispersal by woolly monkeys (Lagothrix lagothricha) at Tinigua National Park, Colombia: dispersal distance, germination rates, and dispersal quantity. Am J Primatol. 2000 Apr;50(4):275-89.

Terborgh, J.; Pitman, N.; Silman, M.; Schichter, H.; Núñez, P. Maintenance of tree diversity in tropical forests. In Seed Dispersal and Frugivory: Ecology, Evolution and Conservation; Levey, D.J., Silva, W.R., Galetti, M., Eds.; CABI: Wallingford, UK, 2002; pp. 1–17.

Therneau T (2024). _A Package for Survival Analysis in R_. R package version 3.7-0, <https://CRAN.R-project.org/package=survival>.

Tsuji, Y., Morimoto, M. and Matsubayashi, K. (2010), Effects of the physical characteristics of seeds on gastrointestinal passage time in captive Japanese macaques. Journal of Zoology, 280: 171-176.

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0.

Verdú, M. & Traveset, Anna. (2005). Early emergence enhances plant fitness: A phylogenetically controlled meta-analysis. Ecology. 86. 1385-1394.

Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

Wickham H, François R, Henry L, Müller K, Vaughan D (2026). _dplyr: A Grammar of Data Manipulation_. R package version 1.2.0, <https://CRAN.R-project.org/package=dplyr>.

Chapter 2

Possible dispersion of arbuscular mycorrhizal fungi (AMF) by the black-lion-tamarin *Leontopithecus chrysopygus*

Primates play diverse ecological roles in the habitats where they live. Among these roles, seed dispersal is the most studied function (Chapman, 1995; Culot et al. 2010; Gómez and Verdú, 2012; Bufalo et al. 2016), followed by pollination carried out by primates that feed on nectar (Heymann, 2011). However, it is very likely that primates play a crucial role in the organization of forest ecosystems due to their abundance and biomass, influencing not only the structure of plant communities via seed dispersal, but also other aspects of this ecosystem. For example, it is known, though little studied, that primates affect plant production and survival through leaf consumption (Chapman et al., 2013).

Low degrees of folivory can potentially bring advantages to certain tree species (Chapman et al., 2013). On the other hand, leaf consumption can lead to the death of individual trees through excessive foraging or can slow down tree growth compared to species that have not been excessively foraged, thus altering the forest composition and nutrient cycling of the site (Chapman et al., 2013). Furthermore, there are other potential ecological roles of primates that have not yet been investigated, such as soil fertilization via defecation and the dispersal of arbuscular mycorrhizal fungal spores (AMF), which are functions already evidenced in other mammals (McKendrigk et al., 1980; Mangan and Adler, 2000; Mangan and Adler, 2002).

AMF form mutualistic associations with approximately 80% of vascular plants, facilitating plant access to certain nutrients (Smith and Read, 2008) and are essential for the survival and development of tropical trees (Siqueira et al., 1998). This type of mycorrhizal fungi connects plants through a network of hyphae, allowing the exchange of resources between coexisting plants and is likely to play a significant role in global nutrient cycling (van der Heijden et al., 2015). Therefore, it plays an important role in structuring ecosystems, influencing the composition of plant communities (Klironomos et al., 2011; van der Heijden et al., 2015) and the processes of ecological succession (Francis and Read, 1994).

Species of small arboreal and terrestrial mammals, such as rodents and marsupials from Panama, have been recorded dispersing AMF in their feces, with some of them acting as possible vertical dispersers, carrying spores from the ground up to epiphytic plants (Mangan and Adler, 2000). These plants are capable of hosting these mutualistic fungi at their roots (Lesica and Antibus, 1990; Rabatin et al., 1993). Although seed dispersal by primates is frequently addressed, AMF dispersal by this group has never been studied.

Considering that some bird species have the capacity to co-disperse viable seeds and AMF spores, and that the probable moment of ingestion of these spores occurs during their search for seeds, fallen fruits, and invertebrates on the ground (Correia et al., 2019), it is likely that frugivore-faunivorous primates such as the black lion tamarin (BLT) may also fulfill this function. Although the BLT spends most of its time in the trees, it occasionally descends to the ground to capture prey and collect fruits (Carvalho and Carvalho, 1989; Passos and Keuroghlian, 1999). Therefore, it becomes essential to investigate whether there is any dispersal or co-dispersal behavior among these primates, even if the consumption of AMF is involuntary on

their part. Therefore, the objectives of this study were (1) to examine if the BLT disperses AMF propagules through its feces, and if there are seed co-dispersal events with these propagules and (2) if the BLT disperses AMF propagules, test if terrestriality, i.e., the time spent on the ground, is related to the probability of dispersal. Here we hypothesize that during the act of foraging on the ground, BLTs will involuntarily ingest AMF propagules present in the soil, fallen fruits, and consumed animals. We also expect that some seeds will be dispersed together with AMF propagules, and that the time BLTs spend on the ground will be positively correlated with the probability of dispersion.

We conducted this study in two areas that share a geographic boundary, the Carlos Botelho State Park (CBSP) (24º 06’ 55’’ e 24º 14’ 41’’ S e 47º 47’ 18’’ e 48º 07’ 17’’ W) and Trápaga Private Natural Heritage Reserve (PNHR) (24° 02' 46.6" S e 47° 58' 50.8" W). Located in the state of São Paulo both places are composed by submontane dense ombrophilous forest and have a terrain characterized by hills and small ridges and are part of the Ecological Continuum of the Serra de Paranapiacaba.

We collected data over 7 days per month, during 13 months, ranging from April 2024 to April 2025. Following a habituated group of BLTs through the whole day, we collected all fresh visible fecal samples and recorded their GPS coordinates. If feces touched the ground, we scraped off and collected only the part that did not come into contact. Samples were stored in dry vials with an identification code, time of collection, date and surface of collection. Every day when we returned to the base, the samples were stored in a refrigerator at about 4ºC.

Behavioral and vertical stratification data were also collected to assess how often individuals descended to the ground, allowing a better discussion of the

presence or absence of spores as a function of ground-use frequency. For this, we used the scan sampling method (Altmann, 1974) on the entire group at 5-minute intervals, recording the behavior (Table 1) and the vertical stratum of each visible individual, if it was on the ground or above it.

Scan sampling	
Behaviors	**Description**
Frugivory	Individual searching or ingesting fruits.
Gummivory	Individual feeding on gum.
Foraging	Individual feeding or searching for animal prey
Playing	Individuals chasing each other without aggression.

Table 1: Categories of behaviors for scan sampling records.

At the Laboratory of Ecology and Systematics of Fungi (UNESP Rio Claro – Prof. Dr. André Rodrigues), fecal samples were disaggregated and transferred with deionized water to a falcon tube, each tube was manually shaken for one minute, after that all content was poured through a granulometric sieve of 53 μm, into a Petri dish. All samples in Petri dishes were thoroughly observed in a stereo microscope of 40X (Mangan e Adler, 2002).

An association experiment among sorghum roots and AMF hyphae was also applied to search for propagules (Fig. 1). In this experiment, sorghum seeds were washed three times in deionized water and subsequently sown in pots. The pots, which were previously disinfected by immersion in a 10% sodium hypochlorite solution for 10 minutes, contained sterile sand that had been autoclaved for 20

minutes as the substrate. The experiment was conducted in the Jardim Experimental of São Paulo State University (UNESP), Rio Claro. Automatic irrigation happened two to three time per day. After seedlings reached a proper development, about 10 centimeters, BLT fecal samples were disaggregated in deionized water and inoculated individually in each pot.

The method used for the treatment of the sorghum roots was adapted from INVAM (The International Collection of Vesicular Arbuscular Mycorrhizal Fungi; Koske e Gemma, 1989). After at least four months after the inoculation, seedlings were removed from the pots, and its roots were carefully washed with water. After that, we cut the roots into pieces of approximately one centimeter, then putted the pieces in a KOH 10% solution in water bath (90º C) for 20 minutes.

Afterwards, samples were taken of the water bath and washed with deionized water. Subsequently, we transferred it to an HCL 1% solution in room temperature for 4 minutes. After this period, samples were removed from this solution and transferred into another solution composed of lactoglycerol and blue ink (0.5g of ink per 1 liter of colorless lactoglycerol) for 4 minutes in a water bath (90º C). After this step, the excess of blue lactoglycerol was removed, samples were washed in deionized water and finally transferred into a tube with colorless lactoglycerol (water, lactic acid and glycerin in equal parts, 1x1x1) for preservation.

The search for a mycorrhizal association among sorghum roots and AMF hyphae was made in an optical microscope with a 40x objective (Aguirre, Nouhra and Urcelay, 2021). Experimental design also included control pots that were not inoculated with anything, to each 10 pots inoculated with fecal matter, two control pots were added. This experiment lasted from August 2024 until September 2025.

Figure. 1. Association experiment between sorghum roots and AMF. Each pot contained one sorghum seed and one fecal sample collected from the BLTs. Labels indicate the identification code of the sample and date of inoculation.

Overall, we conducted active searches across 38 samples and analyzed microscope slides from 75 different inoculated pots of the association experiment. No evidence of AMF propagule dispersal was found by either method. Although

hyphae associated with sorghum roots were observed on several microscopical slides, these hyphae were septate, indicating they belonged to another fungal group rather than AMF. Furthermore, we also observed this type of hyphae is some slides derived from control pots, indicating potential contamination in the experiment. Correia et al. (2019) employed both methods and found no evidence of dispersal in the active searches, however, evidence of dispersal was detected in their association experiment. This may happen due to the probably low quantity of AMF propagules ingested, thereby hindering the detection.

In our study the lack of evidence may indicate the absence of dispersal by the BLTs. This might be due to the low terrestriality presented by the BLTs during data collection, leading to the low probability of propagules ingestion.

More studies need to be carried out in order to disprove this ecological role in primates, potentially by employing methods such as DNA metabarcoding to definitively identify the presence or absence of this fungal group in feces. Perhaps future research could involve collecting feces from other primate species to search for this role, such as *Sapajus libidinosus* or baboons (*Papio sp.)* which have a higher rate of terrestriality.

REFERENCES

Aguirre, F., Nouhra, E., Urcelay, C. Native and non-native mammals disperse exotic ectomycorrhizal fungi at long distances from pine plantations, Fungal Ecology. 49, 2021.

Bufalo, S.F., Galetti, M., Culot, L., 2016. Seed dispersal by primates and implications for the conservation of a biodiversity hotspot, the Atlantic Forest of South America. International Journal of Primatology. 37, 333–349.

Carvalho, C.T. & C.F. De Carvalho. 1989. A organização social dos sauis-pretos. (Leontopithecus chrysopygus Mikan), na reserva em Teodoro Sampaio, São Paulo (Primates, Callitrichidae). Revista brasileira de Zoologia. 6: 707-717.

Chapman, C.A., Bonnell, T.R., Gogarten, J.F. et al. Are Primates Ecosystem Engineers? International Journal of Primatology. 34, 1–14 (2013).

Chapman, C. A. (1995). Primate seed dispersal: Coevolution and conservation implications. Evolutionary Anthropology: Issues, News, and Reviews, 4, 74–82.

Culot, L., Muñoz Lazo, F.J.J., Huynen, MC. et al. Seasonal Variation in Seed Dispersal by Tamarins Alters Seed Rain in a Secondary Rain Forest. International Journal of Primatology. 31, 553–569 (2010).

Correia, M., Heleno, R., da Silva, L.P., Costa, J.M. and Rodríguez-Echeverría, S. (2019), First evidence for the joint dispersal of mycorrhizal fungi and plant diaspores by birds. New Phytologist, 222: 1054-1060.

Francis R, Read D.J., 1994. The contributions of mycorrhizal fungi to the determination of plant community structure. Plant and Soil 159:11–25.

Gómez, J. M., & Verdú, M. (2012). Mutualism with plants drives primate diversification. Systematic Biology, 4, 567–577.

Heymann E.W. (2011) Florivory, nectarivory and pollination - a review of primate-flower interactions. Ecotropica 17: 41-52.

McKendrigk, J.D., Batzli, G.O., Everett, K. R., & Swanson, J.C., (1980) Some Effects of Mammalian Herbivores and Fertilization on Tundra Soils and Vegetation, Arctic and Alpine Research, 12:4, 565-578.

Klironomos J, Zobel M, Tibbett M, Stock WD, Rillig MC, Parrent JL, Moora M, Koch AM, Facelli JM, Facelli E et al. 2011. Forces that structure plant communities: quantifying the importance of the mycorrhizal symbiosis. New Phytologist 189: 366–370.

Lesica, P and R. K. Antibus. 1990. The occurrence of mycorrhizae in vascular epiphytes of two Costa Rican rain forests. Biotropica 22:250–258.

Mangan, S.A., Adler, G.H. Seasonal dispersal of arbuscular mycorrhizal fungi by spiny rats in a neotropical forest. Oecologia 131, 587–597 (2002).

Passos, F. D. C., e Keuroghlian, A. (1999). Foraging behavior and microhabitats used by black lion tamarins, Leontopithecus chrysopygus (Mikan)(Primates, Callitrichidae). Revista Brasileira de Zoologia, 16, 219-222.

Rabatin, S.C., Stinner B.R. e Paoletti M.G. 1993. Vesicular-arbuscular mycorrhizal fungi, particularly Glomus tenue, in Venezuelan bromeliad epiphytes. Mycorrhiza 4:17–20

R.E. Koske, J.N. Gemma, A modified procedure for staining roots to detect VA mycorrhizas, Mycological Research, Volume 92, Issue 4, 1989, Pages 486-488, ISSN 0953-7562.

Mangan, S.A., Adler, G.H., Consumption of Arbuscular Mycorrhizal Fungi by Terrestrial and Arboreal Small Mammals in a Panamanian Cloud Forest, Journal of Mammalogy, Volume 81, Issue 2, May 2000, Pages 563–570.

Siqueira, J. O., M. A. C. Carneiro, N.Curi, S.C.S. Rosado, And A. C. Davide. 1998. Mycorrhizal colonization and mycotrophic growth of native woody species as related to successional groups in Southeastern Brazil. Forest Ecology and Management 107:241–252.

Smith S.E., Read D.J., (2008) Mycorrhizal Symbiosis. 3rd edition. Academic Press, London, p 800.

van der Heijden M.G.A., Martin F.M., Selosse M-A, Sanders IR. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist 205: 1406–1423.

FINAL CONSIDERATIONS

Ultimately, the findings regarding seed dispersal emphasize that protecting BLTs populations is crucial for maintaining plant diversity, promoting natural regeneration, and ensuring the long-term resilience of Atlantic forest landscapes.

About the FMAs, we encourage further studies addressing this topic in primates, using methodologies different from or complementary to those employed in this study. This ecological role, if performed by primates, would serve as yet another piece of evidence, among many others, to foster and strengthen the conservation appeal for this highly threatened group.