UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre – PPG-ECMVS

Lorena Bueno Valadão Mendes

POLLINATION SYSTEMS ACROSS THE BRAZILIAN BIOMES WITH EMPHASIS ON BEE-BUZZ POLLINATED FLOWERS INTERACTIONS

Belo Horizonte
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Orientador: Prof. Dr. Pietro Kiyoshi Maruyama Mendonça

Co-orientador: Prof. Dr. Vinícius Lourenço Garcia De Brito

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Lorena Bueno Valadão Mendes

No dia 15 de dezembro de 2023, às 08:30 horas, por videoconferência, teve lugar a defesa de doutorado no Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, de autoria do(a) doutorando(a) Lorena Bueno Valadão Mendes, orientando(a) do Professor Pietro Kiyoshi Maruyama Mendonça, intitulada: **"Pollination systems across the Brazilian biomes with emphasis on bee-pollen flower interactions".** Abrindo a sessão, o(a) Presidente da Comissão, Doutor(a)Pietro Kiyoshi Maruyama Mendonça , após dar a conhecer aos presentes o teor das normas regulamentares do trabalho final, passou a palavra para o(a) candidato(a) para apresentação de seu trabalho. Estiveram presentes a Banca Examinadora composta pelos Doutores: Kayna Agostini (UFSCAR), Isabela Galarda Varassin (UFPR), Natalia Costa Soares (Universidad del Comahue), Paulo Eugenio Oliveira (UFU) e demais convidados. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do(a) candidato(a). Após a arguição, apenas os senhores examinadores permaneceram no recinto para avaliação e deliberação acerca do resultado final, sendo a decisão da banca pela:

(X) Aprovação da tese, com eventuais correções mínimas e entrega de versão final pelo orientador diretamente à Secretaria do Programa, no prazo máximo de 30 dias;

( ) Reprovação da tese - (marcar se é a primeira ou segunda reprovação): ( )*primeira reprovação ( ) segunda reprovação

*Conforme o disposto no Artigo 80 da Resolução Complementar do CEPE/UFMG Nº 02/2017, de 04 de julho de 2017, caso seja a primeira reprovação, poderá ser concedido, a critério do Colegiado de Curso, um prazo para a realização de nova defesa de tese.

A banca indica esta tese aos Prêmios CAPES e UFMG de teses? (X) SIM ( ) NÃO

Nada mais havendo a tratar, o Presidente da Comissão encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 15 de dezembro de 2023**.**

Assinaturas dos Membros da Banca Examinadora

Documento assinado eletronicamente por **Kayna Agostini**, **Usuária Externa**, em 09/01/2024, às 15:36, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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Dedico essa tese a vocês, Gislane e Elizeu, por terem feito dos meus sonhos os seus. E assim, possibilitado que este, hoje, fosse realizado.

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Há travessias que a gente só completa com o vento soprando ao contrário... mas é preciso ter força, é preciso ter raça, é preciso ter gana sempre, quem traz no corpo a marca, Maria, Maria mistura a dor e a alegria... (MILTON NASCIMENTO, 1999)

RESUMO

A interação entre plantas e polinizadores é um componente fundamental da biota terrestre, já que a maioria das plantas depende de animais para sua reprodução. Essa interação também é compreendida por impulsionar a grande diversidade das angiospermas, principalmente na forma, tamanho e cor dos elementos florais. Na maioria das vezes, tais elementos florais evoluíram para otimizar a transferência e recepção de pólen por meio de mutualismo. Compreender a significância funcional do conjunto de variações nas estruturas florais das plantas associadas aos visitantes florais é relevante no contexto ecológico e evolutivo. Portanto, as interações planta-polinizador compreendem uma enorme biodiversidade, embora pouco compreendida, especialmente em regiões tropicais. Esta tese investigou os diferentes padrões de sistemas de polinização em biomas brasileiros e, em seguida, focou em estudos de caso com associações especializadas envolvendo abelhas e flores de pólen. Mais especificamente, no capítulo I, investigamos a distribuição de sistemas de polinização nos diferentes biomas brasileiros, analisando tendências em interações planta-polinizador, padrões e vieses. No capítulo II, avaliamos a relação entre especialização morfológica e ecológica em espécies de plantas na família Melastomataceae, a maior radiação de flores de pólen, na Mata Atlântica. No capítulo III, utilizamos flores de pólen de *Chamaecrista* (Fabaceae) como modelo de estudo que necessitam para sua reprodução abelhas capazes de realizar vibração. Contrastamos componentes masculino e feminino do desempenho de polinização em uma rede de interação planta-abelha, para revelar como a combinação de medidas quantitativas e qualitativas das visitações florais podem aprimorar nossa compreensão das redes de interação. Em conjunto, esses estudos adotaram uma abordagem holística, incluindo metodologias padronizadas, dados comportamentais aprofundados e foco na eficácia da polinização, sendo essenciais para avançar nossa compreensão de diversos sistemas de polinização.

Palavras-chave: polinização por vibração; polinização por abelhas; Brasil; *Chamaecrista*; Fabaceae; redes de interação; Melastomataceae; flores de pólen.

ABSTRACT

The interaction between plants and pollinators is a fundamental component of terrestrial biota, as most plants depend on animals for their reproduction. This interaction is also understood as generating great diversity in angiosperms, mainly in the shape, size, and colour of the floral elements. Most of the time, such floral elements evolved by optimise pollen transfer and receipt by mutualism. Understanding the functional significance of the set of variations in plant floral structures associated to floral visitors is relevant in the ecological and evolutionary context. Therefore, plant-pollinator interactions comprise huge biodiversity, albeit poorly understood, especially in the tropical regions. This thesis investigated the different patterns of pollination systems throughout Brazilian biomes, and then focused on example studies with specialised associations involving bees and their pollen flowers. In chapter I, we investigate the distribution of pollination systems across the different Brazilian biomes, analysing trends on plant-pollinator interactions, patterns, and biases. In chapter I, we investigate the distribution of pollination systems across the different Brazilian biomes, analysing trends on plant-pollinator interactions, patterns, and biases. In chapter II, we evaluated the relationship between morphological and ecological specialisation of plant species in Melastomataceae family, the most speciose family of pollen flowers, from the Atlantic Forest. In chapter III, using as model systems flowers from *Chamaecrista* (Fabaceae), which require buzz pollination bees for their pollination, we contrasted male and female components of pollination performance in a plant-bee interaction network, to reveal how combining quantitative and qualitative measures of floral visitation may further our understanding of pollination networks. Taken together, these studies gave a holistic approach, including standardised methodologies, in-depth behavioural data, and a focus on pollination effectiveness, it is essential to advance our comprehension of diverse pollination systems.

Keywords: buzz-pollination; bee pollination; Brazil; *Chamaecrista*; Fabaceae; interaction networks; Melastomataceae; pollen flowers.

SUMÁRIO

INTRODUÇÃO GERAL

A polinização é um processo ecológico vital para a manutenção das comunidades de angiospermas e representa um importante serviço ecossistêmico (Ashman et al., 2004; Aguilar et al., 2006; Potts et al., 2010). Apesar de muitos estudos sobre interações planta-polinizador publicados nas últimas décadas, a pesquisa ainda está centrada em regiões temperadas (Vizentin-Bugoni et al., 2018; Teixido et al., 2022). No entanto, as regiões tropicais abrigam uma grande diversidade de espécies e ecossistemas, além de altos níveis de endemismo (Robbin and Opler, 1997; Raven, 1979; Raven et al., 2020; Brummit et al., 2021). O Brasil é um dos países tropicais mais biodiversos do mundo (Lewinsohn e Prado, 2005; Forzza et al., 2015), com fauna e flora muito distintas em diferentes paisagens. Além disso, o território brasileiro possui um vasto gradiente intertropical-subtropical, com variações distintas nos biomas quanto ao clima, propriedades do solo e topografia (IBGE, 2019). Essas características distintas influenciam a dinâmica ecológica e a adaptação de organismos, modulando as interações planta-polinizador em diferentes escalas ecológicas. Além disso, nossa compreensão das interações de polinização pode estar enviesada para certas plantas e grupos de polinizadores, levando a vieses taxonômicos e funcionais (Ollerton et al., 2015, Dellinger, 2020). Portanto, é necessário caracterizar tais vieses e levá-los em consideração ao avaliar padrões em interações planta-polinizador. Preencher essas lacunas ajudará a entender os mecanismos de manutenção das angiospermas em diferentes regiões.

As angiospermas exibem uma grande diversidade floral, principalmente em relação à variação na forma, tamanho e cor dos elementos florais (Lloyd e Barret, 1996; Marazzi et al., 2007; Vallejo-Marín et al., 2010). O número, disposição e forma dessas estruturas florais têm grande importância para a reprodução sexual das plantas (Endress, 1994; Teixeira et al., 2014). Além disso, a disposição floral também cria mecanismos que podem otimizar a polinização, assim como selecionam grupos de visitantes florais, incluindo tanto mutualistas quanto antagonistas (Vallejo-Marín et al., 2010; Oliveira e Maruyama, 2014). Por exemplo, sabe-se que a distância entre as estruturas reprodutivas femininas e masculinas, assim como a disposição e morfologia das pétalas, pode influenciar a deposição de pólen no corpo dos polinizadores, além de interferir diretamente na direção do fluxo polínico (Solís Monteiro e Vallejo-Marín, 2017; Oliveira e Maruyama, 2014). Assim, o estudo da morfologia floral é importante para entender o significado funcional do conjunto de variações das estruturas florais das plantas no contexto ecológico e evolutivo (Barret, 1992; Lloyd e Barret, 1996; Barret, 2010; Vallejo-Marín et al., 2010).

Aproximadamente 20.000 espécies de plantas possuem flores que liberam grãos de pólen por pequenas aberturas apicais das anteras (i. e., anteras poricidas; Buchmann, 1983; Luo et al., 2008; De Luca e Vallejo-Marin, 2013). Visitantes dessas espécies são geralmente insetos capazes de realizar vibrações para extrair os grãos de pólen (Buchmann e Hurley, 1978; Buchmann, 1983; De Luca e Vallejo-Marín, 2013). Além dos poros apicais, algumas outras características florais evoluíram de forma correlacionada a essas flores, incluindo a ausência de néctar ou outros recursos que possam alimentar os polinizadores (i. e., flores de pólen) e a produção de uma grande quantidade de grãos de pólen pequenos por antera (Buchmann, 1983; De Luca e Vallejo-Marín, 2013; Vogel, 1978; Faegri, 1986; De Luca e Vallejo-Marín, 2013; Hao et al., 2020). Esse conjunto de características florais associadas tem sido denominado síndrome de polinização por vibração (Buchmann and Hurley, 1978; De Luca and Vallejo-Marín, 2013).

A polinização por vibração ocorre em aproximadamente 60 famílias de plantas (e. g., Melastomataceae e Fabaceae; Buchmann, 1983; Vallejo-Marín et al., 2010; De Luca e Vallejo-Marín, 2013). Hipóteses sugerem que o surgimento dessa síndrome de polinização foi desencadeado pela necessidade dessas plantas, com apenas pólen como recurso, de restringir o acesso ao pólen dentro das anteras poricidas (Hargreaves et al., 2009; De Luca e Vallejo-Marín, 2013). Esse acesso restrito ao pólen pode reduzir a perda de gametófitos masculinos para visitantes que removem o pólen com menos ou nenhuma eficiência na deposição no estigma (Hargreaves et al., 2009; De Luca e Vallejo-Marín, 2013). Diferenças na eficiência de remoção do pólen são provavelmente a razão pela qual essas plantas são visitadas principalmente por abelhas capazes de realizar vibrações, indicando que são "funcionalmente especializadas" (Macior, 1964; Larson e Barrett, 1999). A relação entre polinizador e flor também pode ter uma grande influência na evolução de adaptações complexas (Luo et al., 2008; Vallejo-Marín et al., 2010; De Luca e Vallejo-Marín, 2013), como a especialização funcional de estames (i. e., heteranteria; Luo et al., 2008; Vallejo-Marín et al., 2010; De Luca e Vallejo-Marín, 2013). Embora já existam estudos que apontem a relação entre características florais associadas à síndrome de polinização por vibração (e. g., Vallejo-Marín et al., 2019), a compreensão de como tais características evoluíram dentro de grupos específicos, bem como a relação entre morfologia floral e aptidão dentro de populações e suas consequências na estimativa de redes de interação, permanece escassa.

Estudos recentes têm utilizado abordagens de redes para investigar sistemas de polinização e a relação entre plantas e seus polinizadores, incluindo a associação entre flores polinizadas por vibração e abelhas capazes de realizar vibrações (Mesquita-Neto et al., 2018). Tipicamente, as redes de interação entre plantas e polinizadores, bem como as métricas derivadas delas, são construídas utilizando dados de interação qualitativos ou quantitativos (Vizentin-Bugoni et al., 2018). No último caso, a intensidade da interação muitas vezes é considerada com base apenas na frequência de visitas, deixando de lado a eficácia total de cada visitante, incluindo sua eficiência na remoção e deposição de pólen (Ballantyne et al., 2015). Tentativas anteriores de construir redes mais informativas que incorporam a eficácia da polinização são limitadas (e. g., Ballantyne et al., 2015), contudo, elas incluem apenas medidas de sucesso feminino. Portanto, dada a presença de anteras poricidas na maioria das flores polinizadas por vibração, estimar a quantidade de pólen extraído por cada polinizador e sua deposição é mais precisa em comparação com outros sistemas florais (Vallejo-Marín et al., 2010; Solis-Monteiro and Vallejo-Marín, 2015; Papaj et al., 2017). Como resultado, as redes de interação entre plantas com flores polinizadas por vibração e visitantes florais podem ser estimadas considerando a eficácia de cada visitante, e como consequência, os componentes masculinos e femininos da aptidão da planta. Esta abordagem permanece pouco explorada no contexto de redes.

Nesta tese, nosso objetivo foi investigar os padrões de distribuição de sistemas de polinização em biomas brasileiros e apresentar dois estudos de caso enfatizando as interações entre flores de pólen e abelhas. No capítulo I, investigamos a distribuição de sistemas de polinização nos diferentes biomas brasileiros, analisando tendências em interações planta-polinizador, padrões e vieses. No capítulo II, avaliamos a relação entre especialização morfológica e ecológica de espécies de plantas na família Melastomataceae, a maior radiação de flores de pólen, da Mata Atlântica. No capítulo III, utilizamos flores de pólen de *Chamaecrista* (Fabaceae) como modelo de estudo que necessitam para sua reprodução abelhas capazes de realizar vibração. Contrastamos os componentes masculino e feminino do desempenho de polinização em uma rede de interação planta-abelha, para revelar como a combinação de medidas quantitativas e qualitativas de visitação floral pode aprimorar nossa compreensão de redes de polinização.

GENERAL INTRODUCTION

Pollination is a vital ecological process for the maintenance of angiosperm communities and represents an important ecosystem service (Ashman et al., 2004; Aguilar et al., 2006; Potts et al., 2010). Despite many studies on plant-pollinator interactions published in recent decades, research is still centred toward temperate regions (Vizentin-Bugoni et al., 2018; Teixido et al., 2022). However, tropical regions harbour a large diversity of species and ecosystems, in addition to high levels of endemism (Robbin and Opler, 1997; Raven, 1979; Raven et al., 2020; Brummit et al., 2021). Brazil is one of the most biodiverse tropical countries in the world (Lewinsohn and Prado, 2005; Forzza et al., 2015), with very distinct fauna and flora on different landscapes. Besides that, Brazilian territory has a vast intertropical-subtropical gradient, with distinct biomes variations in climate, soil properties, and topography (IBGE, 2019). These distinct characteristics influence the ecological dynamics and adaptation of organisms, modulating plant-pollinator interactions at different ecological scales. Moreover, our understating of pollination interactions may be biased to certain plants and pollinators groups, leading to taxonomic and functional biases (Ollerton et al., 2015, Dellinger, 2020). Therefore, it is necessary to characterise such biases and take these into account when assessing patterns in plant-pollinator interactions. Filling in these gaps will help to understand the mechanisms of angiosperm maintenance in different region.

Angiosperms exhibit great floral diversity, mainly regarding the variation in shape, size, and colour of floral elements (Lloyd and Barret, 1996; Marazzi et al., 2007; Vallejo-Marín et al., 2010). The number, arrangement, and shape of these floral structures have great importance for the sexual reproduction of plants (Endress, 1994; Teixeira et al., 2014). Furthermore, the floral arrangement also creates mechanisms that optimise pollination, as well as select groups of floral visitors, including both mutualists and antagonists (Vallejo-Marín et al., 2010; Oliveira and Maruyama, 2014). For example, it is known that the distance between the female and male reproductive structures, as well as the arrangement and morphology of the petals, can influence the deposition of pollen on the body of pollinators, in addition to directly interfering in the direction of pollen flow (Solís Monteiro and Vallejo-Marín, 2017; Oliveira and Maruyama, 2014). Thus, the study of floral morphology is important to understand the functional significance of the set of variations in the floral structures of plants in the ecological and evolutionary context (Barret, 1992; Lloyd and Barret, 1996; Barret, 2010; Vallejo-Marín et al., 2010).

Approximately 20,000 plant species have flowers that release pollen grains through small apical openings of the anthers (i.e. poricidal anthers; Buchmann, 1983; Luo et al., 2008; De Luca and Vallejo-Marin, 2013). Visitors to these species are generally insects capable of performing vibrations to extract pollen grains (Buchmann and Hurley, 1978; Buchmann, 1983; De Luca and Vallejo-Marín, 2013). Besides apical pores, some other floral traits are correlatedly evolved in these flowers, including the absence of nectar or other resources that can feed pollinators (i.e. pollen flowers) and production of a large number of small pollen grains per anther (Buchmann, 1983; De Luca and Vallejo-Marín, 2013; Vogel, 1978; Faegri, 1986; De Luca and Vallejo-Marín, 2013; Hao et al., 2020). This set of floral characteristics associated has been referred to as buzz-pollination syndrome (Buchmann and Hurley, 1978; De Luca and Vallejo-Marín, 2013).

Buzz pollination occurs in approximately 60 families of plants (e. g. Melastomataceae and Fabaceae; Buchmann, 1983; Vallejo-Marín et al., 2010; De Luca and Vallejo-Marín, 2013). It is hypothesised that the emergence of this pollination syndrome was instigated by the necessity for these plants, with just pollen as a resource, to restrict access to pollen within poricidal anthers (Hargreaves et al., 2009; De Luca and Vallejo-Marín, 2013). This restricted pollen access can reduce male gametophyte loss to visitors who remove the pollen with less or no efficiency in deposition on the stigma (Hargreaves et al., 2009; De Luca and Vallejo-Marín, 2013). Differences in pollen removal efficiency are probably the reason why these plants are visited mainly by bees capable of performing vibrations indicating that they are "functionally specialised" (Macior, 1964; Larson and Barrett, 1999). The relationship between pollinator and flower can also have a great influence on the evolution of complex adaptations (Luo et al., 2008; Vallejo-Marín et al., 2010; De Luca and Vallejo-Marín, 2013), such as specialisation functional stamens (i.e. heteranthery; Luo et al., 2008; Vallejo-Marín et al., 2010; De Luca and Vallejo-Marín, 2013). Although there are already studies that point out the relationship between floral characteristics associated with buzz-pollination syndrome (e.g. Vallejo-Marín et al., 2019), the understanding of how such characteristics evolved within specific groups as well as the relationship between floral morphology and *fitness* within populations and its consequences in estimating interaction networks remains scarce.

Recent studies have used network approaches to investigate pollination systems and the relationship between plants and their pollinators, including the association between buzz pollinated flowers and vibratory bees (Mesquita-Neto et al., 2018). Typically, interaction networks between plants and their pollinators, and also the through metrics derived from them, are constructed using qualitative or quantitative interaction data (Vizentin-Bugoni et al., 2018). In the latter case, interaction intensity is often considered solely based on visit frequency, neglecting the total effectiveness of each visitor, comprising their efficiency in pollen removal and deposition (Ballantyne et al., 2015). Prior attempts to construct more informative networks incorporating pollination effectiveness are limited (e.g., Ballantyne et al., 2015), yet they solely include measures of female success. So, given the presence of poricidal anthers in most buzz pollinated - flower, estimating the amount of pollen extracted by each pollinator and its deposition is more accurate compared to other floral systems (Vallejo-Marín et al., 2010; Solis-Monteiro and Vallejo-Marín, 2015; Papaj et al., 2017). Consequently, interaction networks between plants with buzz pollinated - flowers and floral visitors could be estimated by considering the effectiveness of each visitor and hence the male and female components of plant fitness. This approach remains underexplored within network contexts.

In this thesis, we aimed to investigate the patterns of distribution of pollination systems throughout Brazilian biomes, and then present two case studies emphasizing pollen flowers-bee interactions. In chapter I, we investigate the distribution of pollination systems across the different Brazilian biomes, analysing trends on plant-pollinator interactions, patterns, and biases. In chapter II, we evaluated the relationship between morphological and ecological specialisation of plant species in Melastomataceae family, the largest radiation of pollen flowers, from the Atlantic Forest. In chapter III, using pollen flowers from *Chamaecrista* (Fabaceae) which require buzz pollinating bees for their reproduction as a model system, we contrasted male and female components of pollination performance in a plant-bee interaction network, to reveal how combining quantitative and qualitative measures of floral visitation may further our understanding of pollination networks.

CHAPTER I

To bee or not to bee: biome-dependent pollination systems in a megadiverse tropical country

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ABSTRACT

Background and Aims About 90% of flowering plant species depend on animals for pollination, making plant-pollinator mutualisms crucial in terrestrial ecosystems. Despite many studies on plant-pollinator interactions published in recent decades, research is still skewed toward temperate regions. Additionally, some families of plants and pollinators may be over-represented, leading to subsequent taxonomic and functional biases. Here, we systematically reviewed the distribution of pollination systems across the different Brazilian biomes. We chose Brazil for its high biodiversity and considerable tradition in pollination studies to illustrate the diversity of plant-pollinator interactions and biome-based patterns, as well as the potential biases and knowledge shortfalls.

Methods We searched for articles in both the Web of Science and the Scielo Brazil databases using specific search terms. Subsequently, we applied specific criteria to refine our database, selecting only papers or records that focused on native plants and biotic pollination in Brazil.

Key Results Our sampling comprised 5,280 different records of plant-pollinator interactions, which included 119 plant families, 1,193 plant species, and 1,499 pollinator species. The results revealed relevant biases and knowledge gaps, from taxonomic and biogeographical perspectives. We found a significant relationship between the pollinator functional groups and the distribution of their interactions across biomes in the interaction network. Finally, our results indicated the predominance of functional specialisation in plant-pollinator interactions.

Conclusions According to our results, there is a huge diversity of interactions in a tropical country. However, we revealed different taxonomic and biogeographic biases in the studies carried out on pollination. So, our results can help to clarify where efforts need to be directed to reduce the biases found in plant-pollinator studies. Additionally, these results reveal that need more data on the natural history of pollination, with detailed characterisation of the interaction outcome between plants and their animal visitors in future publications. Furthermore, we suggest that researchers standardise methodologies for approaching plant-pollinator interaction studies. This also can contribute significantly towards filling knowledge gaps and improving our understanding of different pollination systems.

Keywords: bee pollination, Brazil, geographic bias, hummingbirds, plant-pollinator interactions, pollination network, taxonomic bias.

INTRODUCTION

About 90% of flowering plant species depend on animals for pollination (Ollerton *et al.*, 2011; Tong *et al.*, 2023), meaning that many plants cannot reproduce without flower visitors that transfer pollen among individuals within populations. Similarly, a great number of floral visitors depend mostly or exclusively on resources such as pollen and nectar (Ollerton, 2017). Thus, plant-pollinator mutualisms are one of the most ecologically important interactions in terrestrial ecosystems. From an evolutionary perspective, pollination has been regarded as a mutualistic interaction promoting the coevolution of plants and animals (Stebbins, 1970; Willmer, 2011). In this way, through reciprocal selection, pollinators are essential agents in the evolution of floral traits (Caruso, 2019), and plants are important agents of pollinators' trait selection (Pauw *et al.*, 2009). Therefore, plant-pollinator interactions play an important role in promoting diversity and maintenance of zoophilous plant communities and their pollinators (Dodd *et al.*, 1999; Aguilar *et al.*, 2006; Potts *et al.*, 2010; Ollerton *et al.*, 2011; Wei *et al.*, 2021).

The association between plants and pollinators can be broadly organised into pollination systems, represented within a *continuum* from specialised to generalised interactions (Waser *et al.*, 1996; Johnson and Steiner, 2000). For instance, pollinators from different taxa can exert equivalent selective pressures on floral traits by presenting similar behaviours during pollination. Thus, a generalist plant attracting a diverse number of pollinator species that are functionally equivalent can still be classified as a functional specialist in pollination (Ollerton *et al.*, 2007; Dellinger, 2020). At the same time, it is expected that floral phenotype reflects the adaptation to the most effective pollinators, tending to specialise in a specific pollination system (Stebbins, 1970; Faegri and Van Der Pijl, 1979; Fenster *et al.*, 2004; Rosas-Guerrero *et al.*, 2014). Specialised pollination systems have often been recognised within the framework of pollination syndromes, i.e. a set of floral traits expected to reflect convergent evolution to a specific group of pollinators (Fenster *et al.*, 2004; Ollerton *et al.*, 2007). For example, flowers with reddish tubular corolla and a high volume of nectar commonly attract birds and are classified as ornithophiles species, showing traits reflecting an expected adaptation to bird pollination (Faegri and Van Der Pijil, 1979; Smith *et al.*, 2008).

Nevertheless, many plants interact with different pollinators that vary in effectiveness and abundance that, furthermore, belong to different functional groups (e.g. Waser *et al.*, 1996; Johnson and Steiner, 2000; Ollerton *et al.*, 2009; Gómez *et al.*, 2020). In addition, the local composition of pollinators can vary in time and space, promoting a fluctuating landscape of selective pressures on floral traits, thereby limiting specialisation (Waser *et al.*, 1996; Aigner, 2001; Gómez *et al.*, 2007; Gómez *et al.*, 2015). In fact, populations of the same plant species can each be specialists and interact with specific groups of distinct pollinators across their area of occurrence or change pollinators along their flowering period, thus showing a more generalist pollination system as a species (Gómez *et al.*, 2007; Chacoff *et al.*, 2012; Gómez *et al.*, 2020). Hence, pollination interactions may be driven by environmental conditions that modulate the selective pressures on floral traits associated with specific pollinator functional groups, making certain regions more likely to have plants showing specialised pollination systems (Fenster *et al.*, 2004; Schemske *et al.*, 2009; Schleuning *et al.*, 2012; Rosas-Guerrero *et al.*, 2014; Gómez *et al.*, 2015). However, we still lack general assessments on specialisation of plant-pollinator interactions over broad geographical scales.

Despite many studies on plant-pollinator interactions published in recent decades, research is still skewed toward temperate regions, mostly of Europe and North America (Vizentin-Bugoni *et al.*, 2018; Teixido *et al.*, 2022). In addition, pollination studies can be biased to plants that have showier flowers, thus some families of plants and pollinators may be over-represented, leading to subsequent taxonomic and functional biases (Ollerton *et al.*, 2015, Dellinger, 2020). Therefore, when assessing patterns in plant-pollinator interactions, it is necessary to take these factors into account. Tropical regions harbour a large diversity of species and ecosystems, in addition to high levels of endemism (Robbin and Opler, 1997; Raven, 1979; Raven *et al.*, 2020; Brummit *et al.*, 2021). Brazil is one of the most biodiverse tropical countries in the world (Lewinsohn and Prado, 2005; BFG - The Brazil Flora Group, 2015), and has a vast territory along an intertropical-subtropical gradient, with six distinct biomes: Amazon, which occupies 49.5% of the national territory, Cerrado (23.3%), Atlantic Forest (13.0%), Caatinga (10.1%), Pampa (2.3%), and Pantanal (1.8%) (IBGE, 2019). These Brazilian biomes support a wide variety of vegetation landscapes, often with very distinct fauna and flora from one biome to another. Moreover, these biomes are subjected to great variations in climate, soil properties, and topography (IBGE, 2019), which influence ecological dynamics and organisms' adaptations to a particular environment, interfering with plant-pollinator interactions.

In this study, we systematically reviewed the distribution of pollination systems across the different Brazilian biomes. We use Brazil, a highly biodiverse country with a considerable tradition in pollination studies (Oliveira and Rech, 2018), to illustrate the diversity of plant-pollinator interactions and biome-based patterns, as well as the potential biases and knowledge shortfalls to steer pollination studies in the future. First, we asked the following questions to characterise the current knowledge of this interaction in the country: (1) are there geographic trends in research of plant-pollinator studies across the biomes?; (2) how are plant-pollinator studies distributed among plant families?; (3) is there a bias in the representation of functional groups of pollinators in plant-pollinator studies? and (4) how are the plant-pollinator interactions structured within biomes? Then, based on the data assembled we discuss the specialisation of pollination interactions, considering the different meaning of this concept. Our study provides evidence that the known diversity and specialisation degrees in pollination systems in the tropics are highly dependent on ecological context and research trends. Nevertheless, once such biases are acknowledged and taken in consideration the insights from pollination in Brazil can be contrasted to other tropical regions worldwide to understand biogeographic, ecological, and evolutionary patterns to the complex interactions among plants and their pollinators.

MATERIALS AND METHODS

Data collection

We searched for articles in both the Web of Science and the Scielo Brazil databases on 31 July 2021 using the following search terms: pollinat* AND (Bra?il* OR cerrado OR amazon* OR pampa* OR pantanal OR "atlantic forest*" OR caatinga OR neotropic* OR savan*) in the title, abstract and keywords in the full record by using the “Topic” (TS) field tag. Searches using terms in Portuguese were also included in both platforms: poliniz* AND (Brasil* OR cerrado OR amazon* OR pampa* OR pantanal OR "mata atlântica" OR caatinga OR neotropic* OR savan*). We considered all articles provided at the time of the survey and followed the PRISMA guideline (O’Dea *et al.*, 2021) to build our database and show eligibility and transparency of our survey.

From the resulting articles, we subsequently applied specific selection criteria to refine our database. The data review process was performed by all the co-authors, who followed the selection criteria based on the reading of each paper (title, then abstract, and then body text). We selected only the papers or records considering native plants and biotic pollination in Brazil. For pollinators, we decided to include the non-native species owed to their high prevalence as pollinators of many native plants, especially the introduced honeybee (*Apis mellifera*). Studies of theoretical nature, bibliographical review, experimental/manipulative studies in the field/laboratory without recording interactions with pollinators under natural conditions, and purely mating system-related studies (e.g. self-incompatibility, floral biology, reproductive assurance) were not considered. Moreover, papers that did not categorise the interaction between a given plant and an animal as a pollination event were not included (e.g. floral visitation, florivory, nectar robbing). Additionally, most articles did not record the frequency, efficiency, or relevance of each pollinator species (e.g. as primary and/or secondary pollinators). Because of that, we standardised all recorded floral visitors deemed as effective or legitimate pollinators in original studies as “pollinator”.

A record was considered as an interaction between a single plant species and a single pollinator species. Thus, if a given plant species was included in more than one article within a particular biome, we considered all the pollinators recorded among the different articles for that plant. Likewise, if a given plant was included in more than one article between different biomes, we considered the pollinators recorded per biome. From the selected articles, we extracted the following information: (1) year of the publication, (2) studied biome(s), (3) geographic coordinates, (4) plant species, (5) plant family, (6) pollinator species, and (7) pollinator functional group (e.g. bee, bat, butterfly).

Nationwide pollination systems and plant-pollination network

To assess the frequency of interactions recorded by each pollinator functional group across the different biomes, we created a matrix with biomes as rows and pollinator functional groups as columns. Each cell in the matrix corresponded to the number of interactions provided by each functional group per biome. We also elaborated a matrix to quantify the percentage of specialisation in plant-pollinator interactions (i.e., pollination systems) recorded across biomes. For this, biomes and pollinator functional groups were also linked as rows and columns, respectively, wherein each cell corresponded to the number of plant species that were exclusively visited by only one functional group. Lastly, we determined the interaction frequency of pollinator species in each functional group and the occurrence among different biomes, following the same procedure with biomes as rows and pollinator functional groups as columns. In this case, each cell in the matrix corresponded to the number of species in each functional group per biome. For each matrix construction, pollinators were classified as ants, bats, bees, beetles, birds, butterflies, flies, hawkmoths, hummingbirds, lizards, moths, and wasps. Bugs, cockroaches, crickets, grasshoppers, green lacewings, thrips, and non-flying mammals (marsupials, primates, and rodents) were grouped into “others” category, as these groups were not numerous and performed few interactions. Although non-flying mammals can substantially contribute to pollination and represent a markedly specialised pollination system

(Carthew and Goldingay, 1997; Ratto *et al.*, 2018, Amorim *et al.*, 2020), only a few articles included this group as pollinators (three articles, three different species and five interactions). The biomes in the matrix were classified into Amazon, Atlantic Forest, Caatinga, Cerrado, Pampa, and Pantanal. To visualize the matrix of interaction frequency of pollinator species within each functional group in each biome, we used a quantitative bipartite network illustration built on the Pajek 5.17 program (Mrvar and Batagelj, 2016).

While screening the selected studies, we also identified and separated the studies that presented data on community-level estimates of the proportion of different pollination systems. These studies were considered less biased by the active choice of the researchers when selecting which plants to study and were used to evaluate the distribution of pollination systems. We only included studies that did not focus on a specific taxonomic or functional group of plants and/or pollinators, i.e. they aimed at sampling all plants in a specific locality. However, we did not require these studies to have specific data on each plant-pollinator interaction with the identification of pollinators at the genera/species level, but that each plant was classified to a specific pollination system following field observations, not only categorised based on pollination syndromes. We also searched for additional community-level studies by checking the reference list in the studies found. Finally, we only focused on biotic pollination, so for studies that also reported wind pollination when characterising the communities, we recalculated the proportion of pollination systems by using only the percentage/number of plant species associated with animals.

Geographic bias

To characterise the geographical research bias, we extracted the geographical coordinates included in the articles. We used QGIS 3.18 Zürich (QGIS.org, 2021, QGIS Geographic Information System, QGIS Association, http://www.qgis.org) to assess the density of studies by elaborating density raster layers by kernel interpolation with a resolution of 0.0083° (~ 1 km). To estimate biogeographic bias in the number of plant species studied, we analysed a contingency table (number of rows and columns, respectively, in brackets) considering biomes (6) × studied/non-studied species in each biome (2). We obtained the number of species per biome using the most updated List of Species of the Brazilian Flora (Brazilian Flora, 2020). We assessed the interrelations between categorical variables with the Fisher exact test in R. Then, we analysed significant departures from expected frequencies for each table cell with the Fisher exact approach post-hoc test proposed by Shan and Gerstenberger

(2017). We used the web-based program provided in this reference (https://gshan.i2.unlv.edu/ZPostHoc/; accessed in April 2023) and set a significance level of 5%, adjusted with Simes' (1986) method.

Taxonomic bias

To determine biases across plant taxa (i.e. the disproportion of studied plant species per animal-pollinated family according to the richness of each family in each biome), we recorded the total number of animal-pollinated families and their total richness in each biome using the most updated List of Species of the Brazilian Flora (Brazilian Flora, 2020). Then, we calculated the percentage of studied species per family over the total number of studied species (observed percentage) and the percentage of species richness of each family over the total number of species (expected percentage) per biome. Both percentages were square-root transformed to standardise their variances and improve normality (Zar, 2010). For each biome, the observed percentage was regressed against the expected percentage. Subsequently, we tested for significant departures of the slope from 1 in the observed relationships with t-tests (Ribeiro *et al.*, 2016; Teixido *et al.*, 2021). A slope >1 indicates an over-representation of studied species in the richest families (positive bias), whereas a slope <1 indicates an under-representation of studied species in the richest families (negative bias).

For each studied family in each of the six biomes, we also calculated a relative bias rate (BR_i; Nemésio *et al.*, 2013; Ribeiro *et al.*, 2016; Teixido *et al.*, 2020) through the formulae:

$$\mathrm{BR}_i = a_i / b_i \qquad \text{(Eq.1)}$$

$$\mathrm{BR}_i = (b_i / a_i) \times (-1) \qquad \text{(Eq.2)}$$

where a_i represents the percentage of studied richness and b_i the percentage of the total current richness of family *i*. Equation (1) was used when a_i values were greater than b_i and equation (2) when b_i values were greater than a_i. These calculations generated positive and negative values, indicating an overrepresentation and an underrepresentation of the different families, respectively. That is families with a high or low representativeness of studied species, respectively, in relation to their representativeness over the total current species richness in each biome.

RESULTS

Nationwide patterns in plant-pollinator studies

Following our selection criteria, our final database comprised 408 articles and comprised 5,280 different records of plant-pollinator interactions, which included 119 plant families, 1,193 plant species and 1,499 pollinator species. The introduced honeybee (*Apis mellifera*) was the pollinator associated with the highest number of plant species and interactions (14.1% and 3.2%, respectively), followed by the hummingbird *Chlorostilbon lucidus* (9.2% and 2.1%), the bee *Trigona spinipes* (8.7% and 2.0%), and the bumblebee *Bombus morio* (6.6% and 1.5%). In terms of pollinator functional groups, bees were the pollinator functional group associated with the highest number of plant species (n = 747, 62.6 %) and interactions (n = 2,772, 52.5 %), followed by hummingbirds (n = 346 plant species, 29.0%; n = 950 interactions, 18.0 %), flies (n = 154, 12.9%; n = 423, 8.0%), wasps (n = 118, 9.9%; n = 265, 5.0%), butterflies (n = 96, 8.0%; n = 277, 5.2%). The others pollinator functional group was the group associated with the lowest number of plant species n = 30, 2.5%) and interactions (n = 49, 11.3%).

Among the plants, *Miconia theaezans* (Melastomataceae) was the species with the highest richness of pollinators (n = 106 and 7.1% of pollinator species; 2.0% of interaction records), followed by *Ipomoea carnea* (Convolvulaceae; n = 56, 1.1 % and 3.7%), *Triplaris gardneriana* (Polygonaceae; n = 43, 0.8 % and 2.8%), and *Combretum lanceolatum* (Combretaceae; n = 40, 0.8 % and 2.7%; Fig. 1). , *Miconia theaezans* received visits predominantly from flies (n = 35, 33.0%), bees (n = 27, 25.5%), and wasps (n = 24, 22.6%). I. carnea showed a predominant association with bees (n = 51, 91.1%), with a smaller proportion of interactions with hawkmoths (n = 5, 8.9%). For *T. gardneriana,* flies were the most frequent visitors (n = 16, 37.2%), followed by bees (n = 9, 20.9%), butterflies (n = 9, 20.9%), and wasps (n= 5, 11.6%). Finally, *C. lanceolatum* was almost exclusively visited by non-hummingbird birds (n = 39, 97.5%). Four plant families (Fabaceae, Asteraceae, Rubiaceae and Melastomataceae) were associated with >50% of pollinator assemblage recorded in our database and comprised about 25% of the interactions (Fig. 2). Particularly, Fabaceae showed the highest number of pollinators and records (17.2% of pollinator species and 7.3% of interactions), followed by Asteraceae (13.7% and 6.0%), Rubiaceae (13.3% and 5.8%), and Melastomataceae (11.5% and 4.9%). Bees were the main functional group visiting Fabaceae (n = 133 species, 51.4%), but vertebrates were also important (Fig. 2): hummingbirds (n = 34, 13.1%), other birds (n = 24, 9.3%) and bats (n = 11, 4.2%). Asteraceae mostly attracted bees (n

= 80 species, 37.7%), together with flies, wasps, and butterflies (n = 34, 16.0%, n = 27, 12.7%; n = 26, 12.3%, respectively). Bees were also the most abundant group in Rubiaceae (n = 82 species, 40.2%), followed by butterflies (n = 28, 13.7%), hummingbirds (n = 27, 13.2%) and wasps (n = 22, 10.8%). Melastomataceae had bees (n = 73, 42.4%), flies (n = 43, 25.0%) and wasps (n = 33, 19.2%) as the main visitors. Two notable exceptions to the predominance of bees and other insects among the families with the highest pollinator richness were Bromeliaceae and Malvaceae, which were mostly associated with hummingbirds (n = 41 species, 39.8% and n = 37 species 35.6%, respectively; Fig. 2).

Considering community-level studies, bee pollination was the dominant pollination system across Brazilian biomes, varying from 21.3% to 83.2% of plant species in each community (mean ± SD = 52.9 ± 18.1%; Fig. 3). The second most common pollination system involved small diverse insects (18.3 ± 16.3%), followed by flies (7.1 ± 8.9%) and hummingbirds (4.7 ± 0.1%; Fig. 3).

Nationwide pollination systems and plant-pollination network

We found a significant relationship between the pollinator functional groups and the distribution of their interactions across biomes in the interaction network ($\chi 2 = 446.7$, df = 60, $p < 0.001$; Fig.4). Bees (606 species), flies (182 species), wasps (112 species) and hummingbirds (60 species) were the groups recorded in all the biomes, followed by butterflies (152 species), beetles (102 species), birds (65 species), hawkmoths (40 species), others (30 species), ants (21 species), and bats (20 species) that occurred in five biomes. Moths (51 species) occurred in four biomes and one species of lizard was recorded in the Atlantic Forest biome (Table 1, Fig. 4). Considering other pollinator functional groups, ants and birds were more related to the Pantanal biome, bats and bees to the Amazon, butterflies to the Caatinga, flies to the Atlantic Forest, and other functional groups of pollinators (bug, cockroach, cricket, grasshoppers, green lacewing, thrips, and non-flying mammals) to the Cerrado biome (Fig. 4).

Table 1 shows both the frequency of interactions recorded by each pollinator functional group and the frequency of plant species exclusively pollinated by each functional group across biomes. Considering the total records of pollinator interactions by biomes, bees were the most recorded in all. The Amazon had wasps as the second most registered functional group. In the Atlantic Forest, the second most recorded group was hummingbirds, as well as the Cerrado and Caatinga. Finally, in Pampa and Pantanal the fly functional group ranked as the second most registered The Amazon comprised the highest proportion of studied plant species visited only by one pollinator functional group (96.4%), followed by the Pampa (90.0%), the Caatinga (89.7%), the Atlantic Forest (86.5%), the Cerrado (73.0%), and the Pantanal (55.4%). In all biomes, bee pollination was dominant, mainly in the Pampa, wherein nine out of ten studied species were exclusively pollinated by bees. Following bee pollination, Hummingbird-pollinated species were frequent in most biomes. Altogether, bee and hummingbird pollination comprised >85% of pollination systems in the Atlantic Forest, Caatinga, and Cerrado. In the Amazon, plant species only pollinated by bats and beetles were also prevalent. The Pantanal comprised the lowest proportion of specialisation in plant-pollinator interactions and the frequency of species exclusively pollinated by hummingbirds was proportionally low.

Geographic bias

The Atlantic Forest comprised 54% of the studies, followed by the Cerrado (17%), the Caatinga (13%), the Amazon and the Pantanal (7.5% each), and the Pampa (1%). Therefore, the Atlantic Forest concentrated the highest density of studies (Fig.5). The Pantanal was the biome with the highest proportion of studied plant families (49%), followed by the Cerrado (38%), the Atlantic Forest (32%), and the Caatinga (30%; Fig.6). Altogether, these four biomes comprised 95% of the total number of studied families in Brazil (Fig. 6A). In contrast, the Amazon (7%) and the Pampa (2%) were significantly underrepresented (Fig.6A). Considering the proportion of studied plant species (Fig. 6B), the Pantanal was oversampled (15%), while the Atlantic Forest, Caatinga, and Cerrado comprised similar proportions (ca. 3% each). However, the Atlantic Forest and the Cerrado together represented about 65% of the total number of studied species. Conversely, the Amazon and the Pampa were underrepresented (<0.5%; Fig.6B). Studies conducted in the Pampa only considered three families and ten species.

Taxonomic bias

Arecaceae (n = 12 species) and Fabaceae (n = 10) were the most studied families in the Amazon, accounting for 43% of the considered species in the biome. Following the representativeness of studied species in relation to the richness per family in this biome (Fig. 7), Smilacaceae was the most oversampled family, followed by Arecaceae, Cactaceae and Passifloraceae, while Euphorbiaceae, Apocynaceae, and Rubiaceae were underrepresented. Some important families, comprising more than 2% of known richness (>250 species in this biome), were neglected: Annonaceae, Araceae, Malvaceae, Melastomataceae, and Myrtaceae.

In the Atlantic Forest, Fabaceae (77 species) and Bromeliaceae (62) were clearly the most studied families, comprising about 30% of the studied species. Some families with very low representativeness (<20 species) were oversampled (e.g. Heliconiaceae, Talinaceae, Scrophulariaceae, Krameriaceae), while other families with more than 100 species (e.g. Lauraceae) were underrepresented (Fig. 7B). Nevertheless, all families comprising more than 2% of richness (>300 species) were considered in the surveyed literature.

Across non-forest biomes, Fabaceae (22 species) and Cactaceae (11) were the most studied families in the Caatinga, followed by Asteraceae and Malvaceae (9). Overall, these four families comprised 35% of all species considered in this biome. In the Cerrado, Asteraceae (36), Fabaceae (29), and Rubiaceae (20) were the most frequently studied families, comprising about 30% of the studied species. Several of the families with very low representativeness in these biomes were overrepresented (e.g., Caatinga: Plumbaginaceae and Burseraceae; Fig. 7C; Cerrado: Chloranthaceae and Krameriaceae; Fig. 7D). Conversely, some species-rich families such as Asteraceae and Melastomataceae in the Caatinga and Apocynaceae and Eriocaulaceae in the Cerrado were underrepresented (Fig. 7C, D). In both biomes, the families comprising more than 2% of the representativeness of the total current richness (>100 and >250 species, respectively) were studied at least once.

The Pampa grasslands only included studies for Cactaceae, Orchidaceae and, above all, Iridaceae (Fig. 7E). Thus, no studies were conducted for Asteraceae and Fabaceae, which together account for 30% of the known plant diversity in this biome. In the Pantanal, Fabaceae (26 species) and Malvaceae (15) were the most studied families, which represent ca. 25% of both the richness and the species considered in the surveyed literature within this wetland. Plant families with very low representativeness were overrepresented (e.g. Erythroxylaceae and Polygalaceae), while some of the richest families showed the lowest relative bias rates (e.g. Bignoniaceae and Sapindaceae; Fig. 7F).

Finally, we detected a significantly disproportionate decrease in the percentage of studied species in relation to the percentage of representativeness of each family (i.e. more diverse families were disproportionately underrepresented) in both the Amazon and the Pampa (Fig. S1). For the other biomes, the percentage of studied species was proportional to the percentage of known species across families (i.e. observed slope not significantly different from 1; Fig. S1).

DISCUSSION

We systematically reviewed the distribution of pollination systems across the Brazilian biomes, among which the biodiversity hotspots (i.e. Atlantic Forest and Cerrado) were the two most studied ones. Furthermore, bee pollination was the most reported pollination system across the majority of biomes. Our findings provide valuable insights into the diversity and distribution of plant-pollinator interactions in a large and biodiverse tropical country. Furthermore, indicate the predominance of functional specialisation in plant-pollinator interactions. Additionally, our results revealed relevant biases and knowledge gaps, from taxonomic and biogeographical perspectives. These results provide a basis for future studies on plant-pollinator interactions by encouraging new research to fill in the identified knowledge gaps.

Nationwide patterns in plant-pollinator studies

Studies on plant-pollinator interactions in Brazil have focused on a restricted number of plant families and species when considering the overall diversity described for the country. Although 269 plant families have been described for Brazil (Forza *et al.*, 2012), only 119 families (c. 44%) were recorded in our database. Most studies surveyed focused on plant families with the highest species diversity in Brazil, such as Fabaceae and Asteraceae. Additionally, a prominent proportion of plant families in some biomes with considerable biodiversity, such as the Amazon, was neglected. These biases partially limit our comprehension of the distribution of plant-pollinator interactions in Brazil, essential for the conservation of biodiversity and ecosystem services on interactions.

Considering the pollination systems, about 60% of the compiled studies recorded plant-bee interactions. This result potentially reflects both the greater representativeness of bee pollination worldwide (Ollerton *et al.*, 2017) and a growing interest in studying bees and their ecosystem services, including pollination, due to the accelerated global decline of bee species (Decourtye *et al.*, 2019). Furthermore, the introduced *Apis mellifera* was the pollinator that most interacted with plant species in our database. The extensive history of honeybee domestication and transportation has resulted in a mostly cosmopolitan current distribution (Hung *et al.*, 2018), including the Brazilian territory. Although several studies have shown that honeybees are important for pollination of crops and native plants (Morse, 1981; Aebi and Neumann, 2011; Hung *et al.*, 2018; Maruyama *et al.*, 2018), honeybees have also been identified as a dominant competitor for floral resources, which can have a negative impact on native bee pollinators across ecosystems and plant lineages (Roubik et al., 1986; Roubik and Villanueva-Gutierrez, 2009; Thomson, 2016; Valido *et al.*, 2019; Page and Williams, 2023). Since the presence of native bees is essential for pollination and reproductive success of many plant species (Ollerton *et al.*, 2011), the dominance of *A. mellifera* as the main pollinator in Brazil suggests that its introduction may potentially generate a set of ecological impacts and evolutionary processes on plant-pollinator interactions (Maruyama *et al.*, 2018). For native bees, *Bombus* species comprised the highest number of plant-pollinator interactions in our database. Bumblebees are a group of social bees that show generalist foraging behaviour and are associated with the pollination of many plant species (Theodorou *et al.*, 2020), so species from the genus are important pollinators for the Brazilian flora.

The hummingbirds were the second most recorded pollinator functional group in Brazil. Hummingbirds are associated with the pollination of thousands of plant species across the Americas (Rodriguez-Flores *et al.*, 2019; Dalsgaard *et al.*, 2021), with varying degrees of specialisation (Stiles, 1975; Maruyama *et al.*, 2013). However, a recent experimental study suggests that even for flowers not fitting the classical concept of ornithophily, hummingbirds may still contribute to plant reproduction (Amorim *et al.*, 2023). Therefore, hummingbirds' importance as pollinators may still be underestimated. Other groups, such as flies and butterflies represent diverse and relevant groups of pollinators (Ollerton, 2017), but were scarcely considered in the literature, requiring further studies assessing their importance for pollination of tropical plants.

A preconceived idea of pollination syndromes may lead researchers to record pollinators that correspond to the floral phenotype of a given syndrome, while the occurrence of other pollinators unrelated to this interaction is ignored (Waser *et al.*, 1996; Ollerton *et al.*, 2015). The differences between overall proportions found in the survey and in the community-level studies may be relevant to inform such potential bias. Mostly, the patterns found for the survey mirrored what was found for community studies, for instance when considering the predominance of bee pollination. However, the second and third most representative groups, i.e., small diverse insects and flies, were not represented in the same proportion, even when considering that small diverse insects lump together many different insect groups. At the same time, the slight overrepresentation of hummingbirds in the survey shows some preferences of researchers toward bird-pollinated plants.

Nationwide pollination systems and plant-pollination network

The distribution of pollinator functional groups across biomes mostly reflected general trends found above, where bees were the most representative group. A high proportion of plant species were exclusively visited by one pollinator functional group, demonstrating a tendency for functionally specialised pollination systems throughout Brazil, but with some differences among biomes, suggesting influences of the great environmental heterogeneity. The higher diversity and intense competition for resources are suggested to favour narrower niche breadths and specialisation in the tropics (Vamosi *et al.*, 2006; Ollerton *et al.*, 2011; Ollerton, 2017). Furthermore, it has been shown that tropical plants exhibit a higher average proportion of animal-pollinated species and more predictability of specialised phenotypic adaptations (syndromes) to specific pollinator groups (Ollerton *et al.*, 2011; Rosas-Guerrero *et al.*, 2014). The observed predominance of plants associated with one specific pollinator functional group may reflect the establishment and maintenance of relationships with a select but effective group of pollinators, enhancing the probability of reproductive success (Vamosi *et al.*, 2006). However, there is still much discussion on whether mutualistic interactions are indeed more specialised in the tropics (Ollerton *et al.*, 2009; Schleuning *et al.*, 2012).

Nevertheless, it is important to note that survey biases can result in both overestimation and underestimation of plant-pollinator interactions. Scientists may have been more attracted to studying specialised pollination systems to assess traits related to pollination syndromes (Ollerton *et al.*, 2015; Dellinger, 2020), influencing the pattern of specialisation observed here in the biomes studied. According to this pattern, in biomes such as the Amazon and Pampa, which had the highest rates of specialised pollination, the recorded studies focused on plant families with specialised pollination systems (e.g. Orchidaceae and Iridaceae). Otherwise, plant families with a large number of recorded species (e.g. Asteraceae and Fabaceae) and/or known for generalised pollination systems (e.g. Apiaceae) were studied in biomes with higher rates of generalisation, such as the Caatinga, Cerrado, and Pantanal.

Interestingly, the frequency of specialisation across biomes indicates that the higher is the number of studies the lower is the number of plants exclusively pollinated by only a pollinator functional group. Thus, the most underrepresented biomes, the Amazon and Pampa, showed the highest proportion of specialisation. This pattern suggests that the inclusion of more plant species and families increases the diversity in plant-pollinator interactions, reducing biases toward more specialised pollination systems and subsequent knowledge gaps. Reduction in specialisation is particularly relevant in the Pantanal, the biome with the least diversity of flowering plants and the smallest area among the Brazilian biomes (IBGE, 2019). In this wetland, exhaustive pollination network studies including many plant species and families comprise a high pollinator assemblage, seasonal and phenological variation, and floral, functional, and taxonomic diversity (Souza *et al.*, 2018; 2021). Altogether, our results suggest that the degree of specialisation in plant-pollinator interactions may not be only influenced by the great environmental heterogeneity across biomes, but also by the research trends and the approaches of studies conducted.

Geographic bias

Our review showed that the number of articles on pollination was overrepresented in the Atlantic Forest, in agreement with other studies on biological and ecological research in Brazil (Ribeiro *et al.*, 2016; Teixido *et al.*, 2020). Nearly 60% of the Brazilian population lives within the Atlantic Forest, where the largest cities in the country are found (Rio de Janeiro and São Paulo), meaning more research institutions and easy accessibility to sampling areas (Oliveira *et al.*, 2016). However, even in this most frequently studied biome, vast areas have still been neglected. In addition, the Atlantic Forest represents a global biodiversity hotspot that has been long-suffering forest conversion, which currently translates to 72% of native vegetation loss (Rezende *et al.*, 2018). Therefore, the large number of studies in this biome is relevant.

In contrast to the Atlantic Forest, the Amazon was mostly under sampled, which generates relevant knowledge gaps in pollination research in this large rainforest with relatively high biodiversity and undisturbed vegetation cover. The Amazon is the world's largest tropical forest which sustains many ecosystem services and is one of the most important regions for conserving biodiversity globally (Laurance *et al.*, 2001). The inaccessibility and limited infrastructure, together with concerns related to environmental impacts such as deforestation, mining, and conservation of indigenous lands that drive research focus to other subjects may be challenging factors in promoting more pollination studies in the region (Malhado *et al.*, 2013). Nevertheless, studies related to crop pollination and the economic value of these services are found in the Amazonian territory (Klein *et al.*, 2006; Borges *et al.*, 2020; Campbell *et al.*, 2022), and an additional effort focused on native plants would be desirable.

Within non-forest biomes, the Cerrado and the Caatinga were relatively well represented in relation to the Pampa and the Pantanal. Still, most of the studies on Cerrado pollination were conducted near large cities where research centres are located (Belo Horizonte – UFMG, Campinas – Unicamp and Uberlândia – UFU), while vast areas are overlooked. The Cerrado is a priority conservation hotspot with a high value of ecosystem services, including pollination (Strassburg *et al.*, 2017). Likewise, the Caatinga is a xeric ecosystem that encompasses a heterogeneous mosaic of dry woodlands, sand dunes, and mountain-isolated thorn forests with high endemicity levels of flowering plants (Fernandes *et al.*, 2020). Overall, research on biodiversity and conservation in the non-forest biomes of Brazil has been traditionally neglected (Overbeck *et al.*, 2007; Santos *et al.*, 2011; Overbeck *et al.*, 2015; Teixido *et al.*, 2020), which also reflects in our results.

Relative to the proportion of studied plant taxa, the Atlantic Forest and the Cerrado comprised most of the species (65%) included in pollination studies in Brazil. These biomes together comprise more than 55% of the flowering plant species in the Brazilian territory (Brazilian Flora, 2023). However, the representativeness of plant species included in the studies did not reach even 5% of the total in each biome, so even in these biomes more studies are necessary to better disentangle the complexity of plant-pollinator interactions. Conversely, the Pantanal was clearly the most studied biome in terms of the representativeness of plant families and species. As pointed out above, particular studies on plant-pollinator networks increased the number of plant families analysed. Relative to the Pampa, it only covers 2% of the Brazilian territory, being the second smallest biome in the country (IBGE, 2019). However, the Pampa comprises a diverse grassland ecosystem with a more temperate climate, which has been traditionally disturbed by the expansion of agriculture and livestock and neglected in terms of protection (Overbeck *et al.*, 2007; 2015; Andrade *et al.*, 2023). To rapidly gain at least the basic information on the pollination system in these neglected biomes sampling coverage, community-wide studies and applying network approaches may be useful.

Taxonomic bias

As a rule, families with high number of species had higher number of studied species. For example, Fabaceae, one of the richest families in Brazil, was the most studied family in most biomes. The notable abundance and diversity of legumes, including contrasting pollination systems (Souza *et al.*, 2017; Gélvez-Zúñiga *et al.*, 2018) together with their economic interest (Saikia *et al.*, 2020) may attract research funding and stimulate activity in plant-pollinator studies. The economic importance of palms in the Amazon (Smith, 2015) may also be a relevant factor to account for the predominance of studies with Arecaceae in this biome. In the Pampa, the most diverse families were in contrast mostly neglected, with studies focusing on highly specialised pollination systems associated with oil-collecting bees and Iridaceae (Báez-Lizarazo *et al.*, 2021).

Research on bromeliad pollination was mainly concentrated in the Atlantic Forest, where more than 60% of the Brazilian species occur (Forzza *et al.*, 2020). These plants are economically relevant for ornamentation and exhibit different pollinator groups, including bats and insects, but mostly hummingbirds (Sazima *et al.*, 1999; Canela and Sazima, 2003; Dalsgaard *et al.*, 2021), which may explain the high number of studies for this family. Interestingly, Cactaceae was the second most studied family in the Caatinga, despite representing only 2% of the total diversity of this dry tropical forest. The high representation of Cactaceae, extensively studied in this biome (de Lucena *et al.*, 2013), may stem from their importance as keystone resources for bat pollinators, an iconic and well-studied pollination system in this biome (Machado and Lopes 2004; Cordero-Schmidt *et al.*, 2021; Domingos-Melo *et al.*, 2023). The Cerrado was the only biome that had a prevalence of studies on Asteraceae, one of the most diverse families in Brazil. Families less explored in other biomes, such as Melastomataceae, were also frequently recorded in this biome. Both Asteraceae and Melastomataceae species frequently show bee pollination systems (Oliveira and Gibbs, 2002; Monteiro *et al.*, 2021), including for Melastomataceae specialised buzz-pollination system here and in other biomes (Valadão-Mendes *et al.*, 2022).

Still, a set of highly diverse families have been under sampled across biomes, such as Apocynaceae, Euphorbiaceae, Myrtaceae, and Piperaceae in the Amazon and the Atlantic Forest, or Convolvulaceae, Eriocaulaceae, Lamiaceae, and Malpighiaceae in the Caatinga and Cerrado. Future studies on pollination ecology should consider these groups for a more comprehensive understanding of the pollination systems across the country.

CONCLUSION AND FUTURE PERSPECTIVES

According to our results, there is a huge diversity of interactions in a tropical country. However, we revealed different taxonomic and biogeographic biases in the studies carried out on pollination. The difference we found between survey and community studies may indicate where efforts need to be directed to reduce the biases found in plant-pollinator studies. Furthermore, our study found a tendency for specialists to associate pollination systems in Brazil. Therefore, we suggest that new studies be developed to further investigate this trend. Not neglecting the importance of other groups, bees constitute the most important group of pollinators, even if other groups are even more diverse (Ollerton, 2017), emphasizing the importance of studying the different associations that this group of pollinators establishes with numerous groups of plants.

In several studies, the absence of information concerning flower visitors' behaviour made it difficult to distinguish the role of animals as pollinators in many cases. Although we found several records that probably reflect pollination interactions, the specific behaviour of the visitors was not described (e.g. only identified as floral visitors), which precluded their inclusion in our database. This makes it clear that we need more data on the natural history of pollination, with detailed characterisation of the interaction outcome between plants and their animal visitors in future publications. Therefore, we suggest that researchers standardise methodologies for approaching plant-pollinator interaction studies (Inouye, 1980; Salim *et al.*, 2022). This can contribute significantly towards filling knowledge gaps and improving our understanding of different pollination systems.

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TABLE

Table 1. Values of frequency of interactions recorded by each pollinator functional group and the frequency of plant species exclusively pollinated by each functional group across biomes. *<0.05%

	Biome											
	Amazon		**Atlantic Forest**		**Caatinga**		**Cerrado**		**Pampa**		**Pantanal**	
Functional group	Records (%)	Specialisation (%)	Records (%)	Specialisation (%)	Records (%)	Specialisation (%)	Records (%)	Specialisation (%)	Records (%)	Specialisation (%)	Records (%)	Specialisation (%)
Ants	0.0	0.0	0.2	0.0	0.2	0.6	0.8	0.3	1.0	0.0	1.7	0.0
Bats	1.6	16.1	3.0	3.6	1.4	2.6	0.9	3.7	0.0	0.0	0.2	0.5
Bees	44.4	32.0	50.1	50.4	56.7	44.9	55.5	33.9	68.0	90.0	74.3	43.0
Beetles	13.9	16.1	2.3	3.8	0.4	0.6	2.9	1.5	0.0	0.0	2.2	2.7
Birds	1.6	3.6	1.4	0.4	0.6	0.0	0.3	0.0	0.0	0.0	2.5	1.1
Butterflies	0.0	0.0	4.1	0.0	5.1	0.6	3.1	0.0	10.0	0.0	4.4	1.1
Flies	0.0*	0.0	7.1	1.9	1.8	0.6	8.5	1.7	11.0	0.0	7.2	2.2
Hawkmoths	0.5	1.8	2.0	0.9	0.8	1.3	0.4	0.3	0.0	0.0	0.2	0.0
Hummingbirds	5.3	16.1	21.1	23.6	29.8	36.6	20.6	28.9	4.0	0.0	1.0	1.6
Lizards	0.0	0.0	0.0*	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Moths	0.0	0.0	2.4	1.6	0.6	0.6	0.2	0.7	0.0	0.0	0.5	1.1
Wasps	31.7	7.1	3.2	0.2	2.3	1.3	6.6	2.0	4.0	0.0	5.0	1.6
Others	0.0	0.0	3.2	0.0	0.3	0.0	0.3	0.0	2.0	0.0	0.8	0.5
Total	100	96.4	100	86.5	100	89.7	100	73.0	100	90.0	100	55.4

FIGURE CAPTION

Fig. 1. Richness of pollinators interacting with the plant species reported in the compiled papers. The colours of the bars indicate the plant families to each species belongs.

Fig. 2. Richness of pollinators that interacted with the plant family reported in the compiled papers. The colours of the bars indicate the functional group of each pollinator species belongs.

Fig. 3. Proportion of pollinators interaction considering community-level studies. The colours of the bars indicate the functional group of each pollinator species belongs.

Fig. 4. Plant-pollinator bipartite network illustrating interactions between pollinator functional groups (right) and Brazilian biomes (left). The thickness of lines represents the number of species for each pollinator functional group (frequency).

Fig. 5. Mean kernel density (studies/km2) for pollination publications in the Brazilian biomes (each point refers to a single study). Areas from higher to lower density of studies are depicted by a red-to-blue (i.e. high-to-low) colour gradient.

Fig. 6. Biogeographic bias in the number of plants studied (A) proportion of plant families studied in each Brazilian biome and (B) proportion of plant species studied in each Brazilian biome. The arrow up or down above the bars indicates excess or deficit compared expected percentage. The number above the bars indicates the total number of species known in each biome.

Fig. 7. The relative bias of plant families with a high or low representativeness of studied species in relation to their representativeness over the total current species richness in each biome and represented in (A) Amazon (B) Atlantic Forest (C) Caatinga (D) Cerrado (E) Pampa and (F) Pantanal.

FIGURES

Fig.1

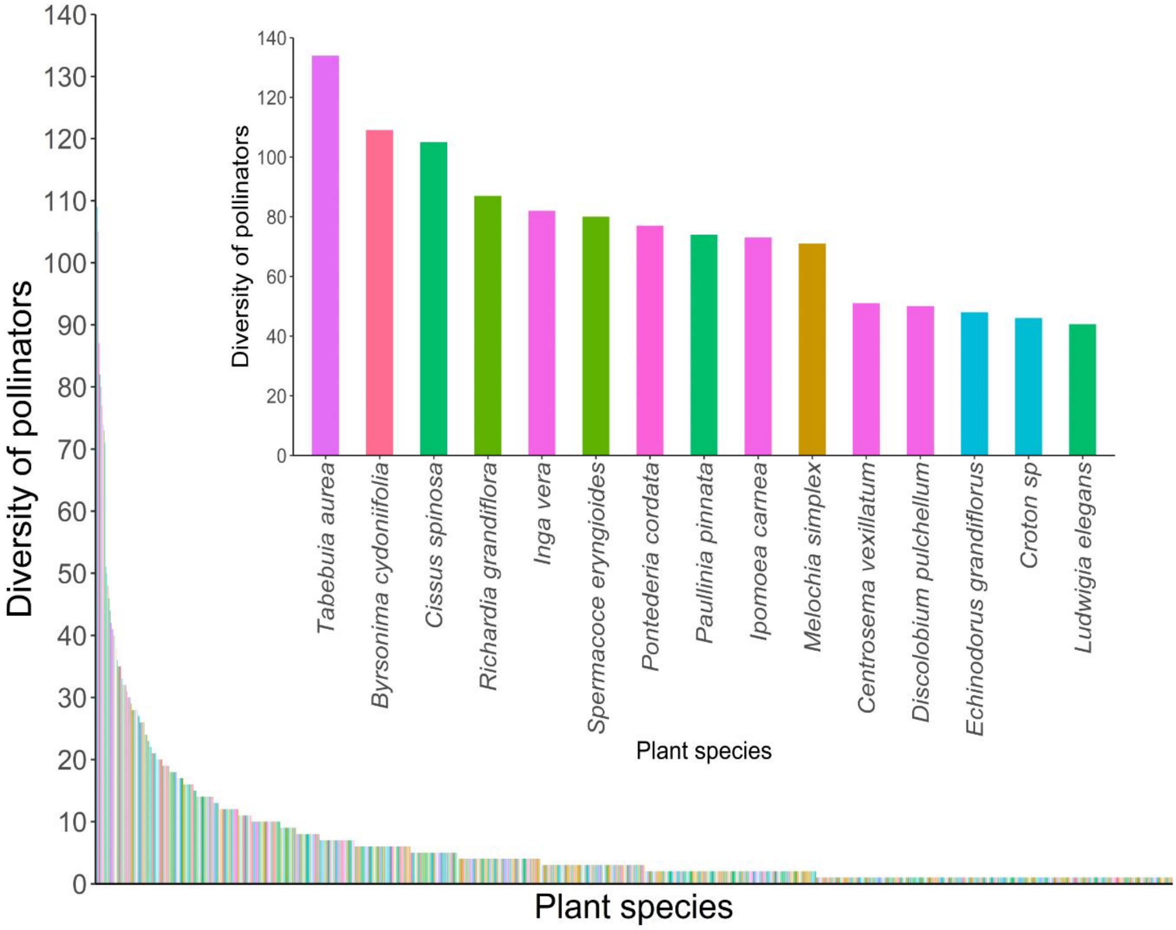


Fig. 2

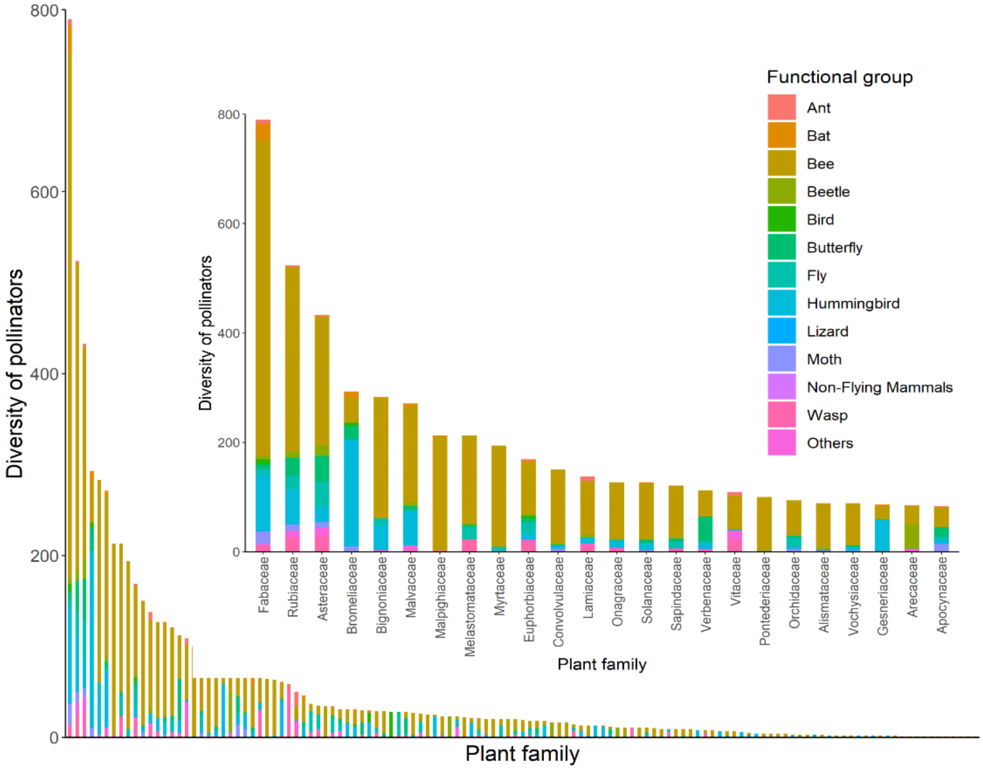

Diversity of pollinators
800
600
400
200
0
Plant family
Diversity of pollinators
800
600
400
200
0
Fabaceae
Rubiaceae
Asteraceae
Bromeliaceae
Bignoniaceae
Malvaceae
Malpighiaceae
Melastomataceae
Myrtaceae
Euphorbiaceae
Convolvulaceae
Lamiaceae
Onagraceae
Solanaceae
Sapindaceae
Verbenaceae
Vitaceae
Pontederiaceae
Orchidaceae
Alismataceae
Vochysiaceae
Gesneriaceae
Arecaceae
Apocynaceae
Plant family
Functional group
Ant
Bat
Bee
Beetle
Bird
Butterfly
Fly
Hummingbird
Lizard
Moth
Non-Flying Mammals
Wasp
Others

Fig. 3

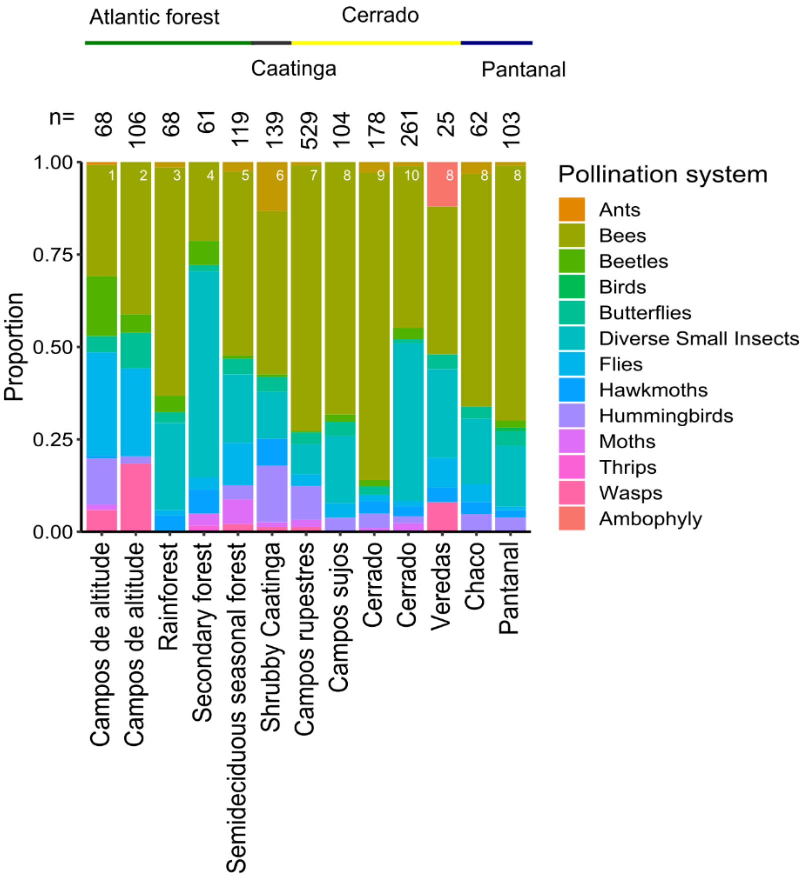

Atlantic forest
Cerrado
Caatinga
Pantanal
n=
68
106
68
61
119
139
529
104
178
261
25
62
103
1
2
3
4
5
6
7
8
9
10
8
8
8
Proportion
1.00
0.75
0.50
0.25
0.00
Campos de altitude
Campos de altitude
Rainforest
Secondary forest
Semideciduous seasonal forest
Shrubby Caatinga
Campos rupestres
Campos sujos
Cerrado
Cerrado
Veredas
Chaco
Pantanal
Pollination system
Ants
Bees
Beetles
Birds
Butterflies
Diverse Small Insects
Flies
Hawkmoths
Hummingbirds
Moths
Thrips
Wasps
Ambophyly

Fig. 4

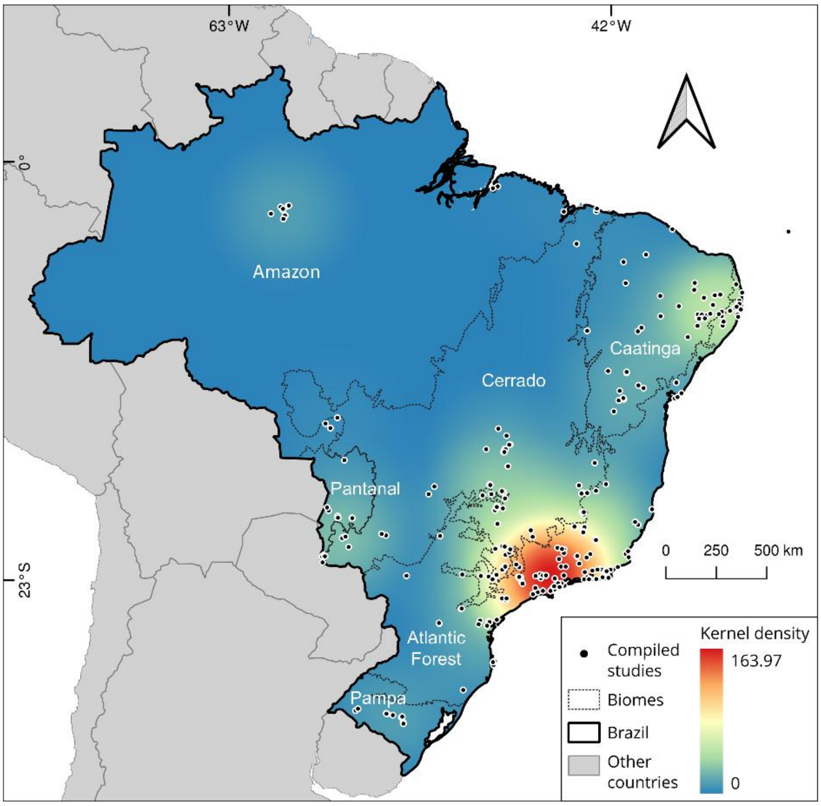

63° W
42° W
0°
23° S
Amazon
Cerrado
Caatinga
Pantanal
Atlantic Forest
Pampa
0
250
500 km
Compiled studies
Biomes
Brazil
Other countries
Kernel density
163.97
0

Fig. 5

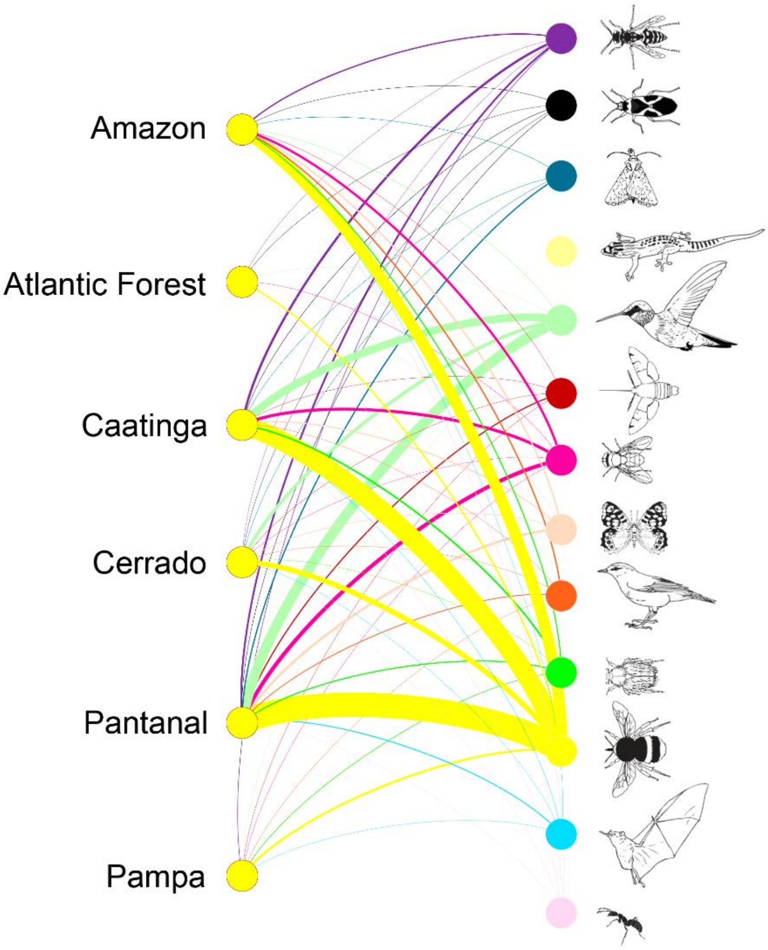


Fig. 6

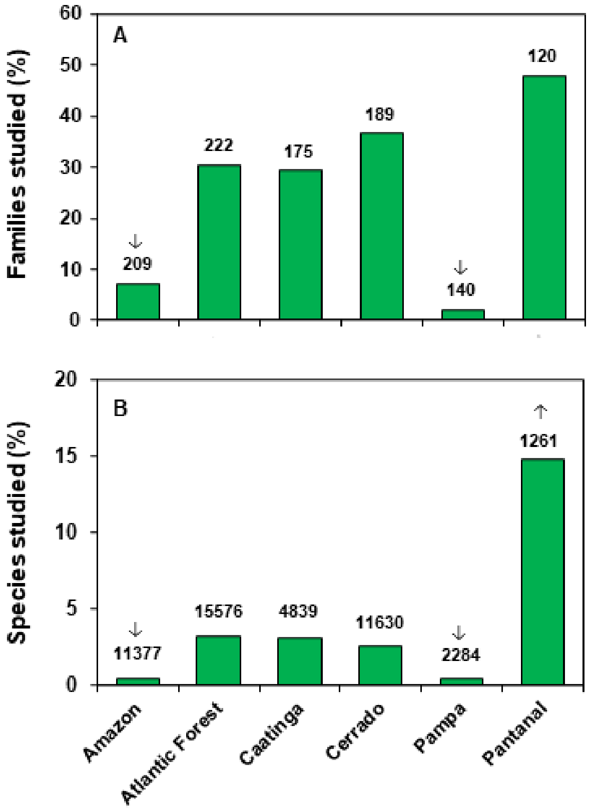
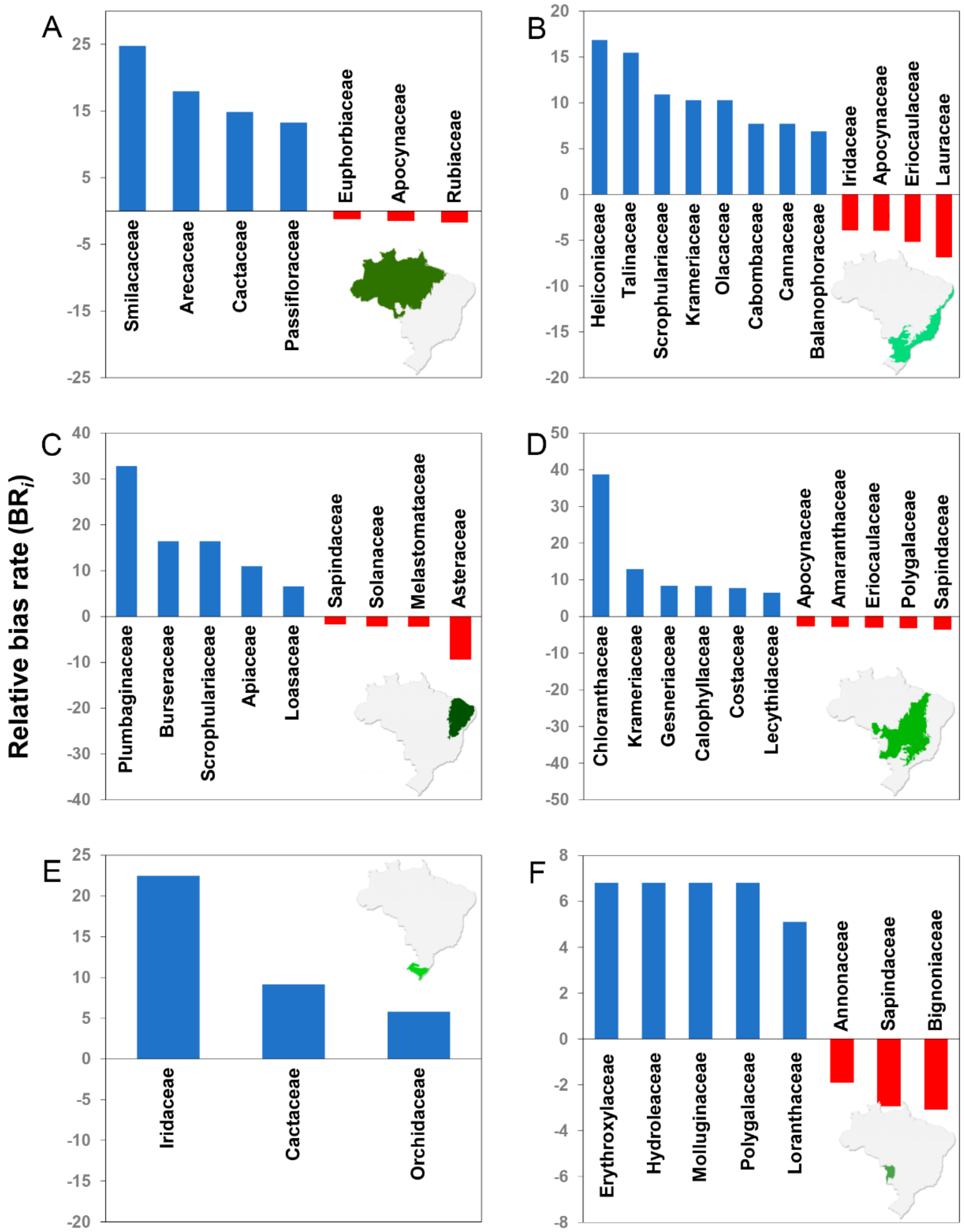

A
Families studied (%)
0
10
20
30
40
50
60
209
222
175
189
140
120
B
Species studied (%)
0
5
10
15
20
11377
15576
4839
11630
2284
1261
Amazon
Atlantic Forest
Caatinga
Cerrado
Pampa
Pantanal

Fig. 7


Relative bias rate (BR$_i$)
A
25
15
5
-5
-15
-25
Smilacaceae
Arecaceae
Cactaceae
Passifloraceae
Euphorbiaceae
Apocynaceae
Rubiaceae
B
20
15
10
5
0
-5
-10
-15
-20
Heliconiaceae
Talinaceae
Scrophulariaceae
Krameriaceae
Olacaceae
Cabombaceae
Cannaceae
Balanophoraceae
Iridaceae
Apocynaceae
Eriocaulaceae
Lauraceae
C
40
30
20
10
0
-10
-20
-30
-40
Plumbaginaceae
Burseraceae
Scrophulariaceae
Apiaceae
Loasaceae
Sapindaceae
Solanaceae
Melastomataceae
Asteraceae
D
50
40
30
20
10
0
-10
-20
-30
-40
-50
Chloranthaceae
Krameriaceae
Gesneriaceae
Calophyllaceae
Costaceae
Lecythidaceae
Apocynaceae
Amaranthaceae
Eriocaulaceae
Polygalaceae
Sapindaceae
E
25
20
15
10
5
0
-5
-10
-15
-20
Iridaceae
Cactaceae
Orchidaceae
F
8
6
4
2
0
-2
-4
-6
-8
Erythroxylaceae
Hydroleaceae
Molluginaceae
Polygalaceae
Loranthaceae
Annonaceae
Sapindaceae
Bignoniaceae

SUPPLEMENTARY DATA

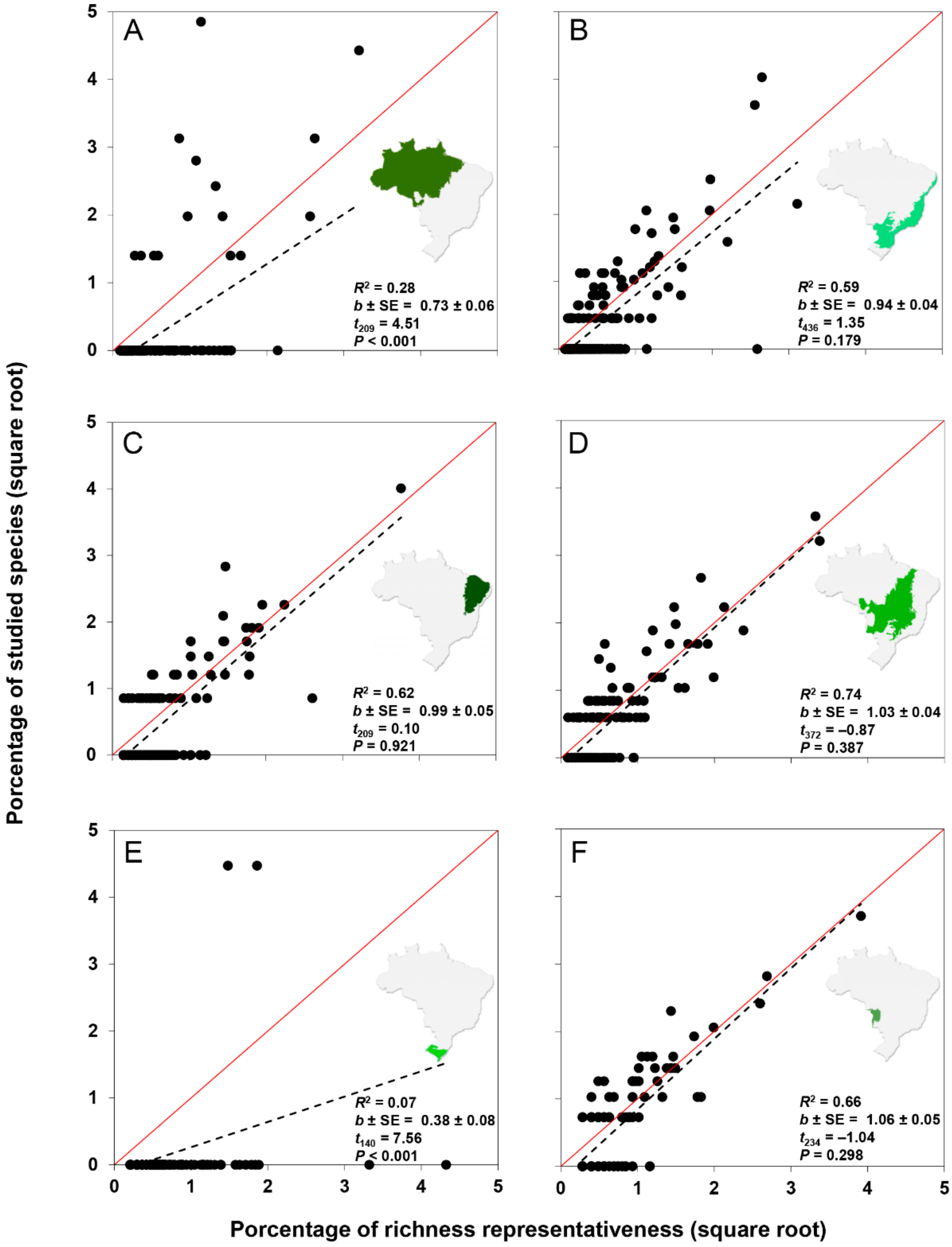


Fig. S1. The proportion of studied species per animal-pollinated family according to the richness of each family (taxonomic bias) in each biome represented in (A) Amazon (B) Atlantic Forest (C) Caatinga (D) Cerrado (E) Pampa and (F) Pantanal.

CHAPTER II

Flower morphology and plant–bee pollinator interactions are related to stamen dimorphism in Melastomataceae

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ABSTRACT

- Approximately 20,000 species of flowering plants offer mainly pollen to their pollinators, generally bees. Stamen dimorphism, a floral trait commonly present in some pollen flowers, are thought to be associated with exclusive pollen provision for highly effective bee pollinators. Notwithstanding, little is known about how stamen dimorphism is related to other floral morphological traits and, consequently, plant species interactions at the community scale. Here we investigated the relationship between stamen dimorphism and other floral morphological traits as well as the interactions with pollinators in plants of the family Melastomataceae.

- We characterized each plant species as stamen dimorphic or stamen isomorphic according to differences in size and shape between stamen sets. Data on interactions between the plants and their bee pollinators were analyzed as quantitative bipartite networks.

- We found that petal and style size and shape were correlated to stamen dimorphism. Stamen dimorphic species present larger flowers and less variable style shapes than stamen isomorphic species.

- Furthermore, stamen dimorphism is associated with greater richness of visiting bees, i.e., higher ecological generalization. During the evolutionary history of Melastomataceae, the dependence on pollinators for fruit set has possibly favoured the evolution of larger flowers with dimorphic stamens, which in turn are able to make use of a larger spectrum of pollen collecting bees, leading to ecological generalization.

Key words: floral traits, heteranthery, interactions networks, pollen flowers

INTRODUCTION

Plants and pollinators interact with each other in complex networks (Jordano, 1987; Bascompte *et al.*, 2006; Olesen *et al.*, 2007), and these interactions shape the morphological traits of one or even both interactants over the evolutionary time (Olesen *et al.*, 2007, 2008; Gervasi & Schiestl, 2017). Approximately 20.000 species of angiosperms have flowers that offer pollen as the main resource to their pollinators (Buchmann, 1983; Michener, 2007; Luo *et al.*, 2008; De Luca & Vallejo-Marin, 2013). These flowers, called “pollen flowers”, are usually pollinated exclusively by bees capable of vibrating their wing muscles during the flower visit in a phenomenon known as buzz pollination (Buchmann, 1983; Michener, 2007; Luo *et al.*, 2008; De Luca & Vallejo-Marin, 2013). There is a huge diversity of pollen flowers, and some of their floral traits are directly associated with exclusive pollen provision for highly effective bees, and, at the same time, exclusion of less effective visitors (Vogel, 1978; Buchmann, 1983;

Lloyd & Barret, 1996; Marazzi & Endress, 2007; Luo *et al.*, 2008; Vallejo-Marín *et al.*, 2010). Stamen dimorphism, also called heteranthery, which is characterized by the presence of two or more stamen sets that may differ in shape, position, size and/or color is one of such traits (Luo *et al.*, 2008; Vallejo-Marín *et al.*, 2010; Velloso *et al.*, 2018; Barrett 2021).

Despite some contradictory cases (*e.g.*, Peach & Mazer, 2019; Telles *et al.*, 2020; Dellinger *et al.*, 2021a; Kay et al. 2020), stamen dimorphism is often related to the "division of labor" in pollen flowers where smaller stamens, also called feeding stamens, provide pollen grains as food for bee larvae, while larger stamens, called pollination stamens, contribute to the safe transport of pollen grains between flowers (Luo *et al.*, 2008; Vallejo-Marín *et al.*, 2010). Considering each single floral visit, stamen dimorphism may restrict the access to high amounts of pollen grains only to a effective and morphologically matching bee (Solís-Montero & Vallejo-Marín, 2017). On the other hand, in flowers without stamen dimorphism pollen from all anthers is easily available to bees with different sizes during a single visit (Mesquita-Neto *et al*., 2017). Notwithstanding, little is known about how floral traits in pollen flowers such as stamen dimorphism are related to species interactions in the context of whole communities (but see Mesquita-Neto *et al*., 2018).

The study of interactions between plants and pollinators as ecological networks allows the investigation of the structure and dynamics of communities and facilitates the understanding of phenomena that cannot be inferred by isolatedly observing their components (Memmott, 1999; Bascompte, 2009; Vizentin-Bugoni *et al*., 2018). Moreover, interaction networks contribute to the understanding of generalization and specialization of pollination systems (Bascompte *et al.,* 2003; Olesen *et al*., 2007; Armbruster, 2017). Although the interaction between pollen flowers and buzzing bees is considered highly specialized in a broader context (Vallejo-Marín *et al*., 2010; Vallejo-Marín, 2019), the differences in specialization or generalization of species within networks can have implications on the evolution of floral traits in this system (Ollerton *et al*., 2007; Armbruster, 2017).

Pollinators are important selective agents that can drive the evolution of floral traits (Totland, 2001; Fenster *et al*., 2004; Gervasi & Schiestl, 2017). More specifically, different groups of pollinators with similar behaviour during flower visit, *e.g.,* bees that extract pollen from anthers by vibration, may promote similar selection pressures on flowers. However, despite the fact that stamen dimorphism has appeared in correlation with pollen flowers many times in the evolutionary history of angiosperms, this trait is considered evolutionarily labile (Vallejo-Marín *et al*., 2010; Melo *et al*., 2021). Furthermore, many pollen flowers do not have stamen dimorphism, but rather present a whole set of stamens with the same morphology (Melo *et al.*, 2021).

Relatively few studies address the patterns and causes of variation in floral morphological traits and the relationship between these traits and pollinators (Johnson, 1996; Waser, 1998; Johnson & Steiner, 2000). Since plants with generalized pollination systems must be under different selection pressures from different pollinator groups, a directional selection toward specific floral traits is unlikely. Therefore, it is expected that plants without stamen dimorphism will be more generalist in their interactions. Moreover, such flowers are also expected to show greater variation in other important floral traits related to pollination as the size and shape of petals and styles (Johnson & Steiner, 2000; Fenster *et al.*, 2004). On the other hand, plants with specialized pollination systems are subject to specific pollinator pressures, leading to the selection of specific floral traits (Nilsson, 1988; Galen, 1996; Johnson & Steiner, 2000; Fenster *et al.*, 2004). Thus, we expect a greater specialization in the interactions and consequently lower petal and style size and shape variation in flowers with stamen dimorphism.

In this study, we investigated the relationship between the presence of stamen dimorphism and other floral morphological traits in plants of the family Melastomataceae, the largest radiation of pollen flowers in angiosperms. We also investigated whether plant species specialization within the interaction network with buzzing bees can be inferred by the presence of stamen dimorphism. Specifically, we address the following questions: (i) is stamen dimorphism correlated with size and shape of the petals and styles? (ii) does stamen dimorphism explain the specialization of plant species in the interaction network?

MATERIAL AND METHODS

Study area and plant species

The study was carried out in two continuous areas of the Serra do Mar State Park, Núcleo Picinguaba (23°21'29.0" S 44°51'02.5" W) and Núcleo Santa Virgínia (23°20'09.4"S 45°08'45.7"W), in the state of São Paulo, Brazil, between September 2010 and August 2013. The predominant vegetation is Atlantic rainforest, a highly diverse perennial tropical rainforest that occurs mainly in the eastern region of the country (Joly *et al.*, 2012).

The Melastomataceae family is composed of approximately 5,160 species distributed in about 166 genera that occur more frequently in tropical and subtropical regions (Renner, 1993; Bacci *et al.*, 2019; Reginato *et al.*, 2020). The family is characterized by tree, shrub and sub-shrub species; leaves with acrodromous venation; bisexual flowers with radially symmetric corolla, most of them with anthers bearing small apical pores (i.e., poricidal anthers). Species within Melastomataceae present flowers with and without stamen dimorphism (Fig. 1, Melo *et al.*, 2021). Most species are pollinated by buzzing bees (Buchmann, 1983; Renner, 1989) but with varied level of specialization, and some species also interact with other groups of pollinators (Larson & Barrett, 1999; Varassin *et al.*, 2008; Brito *et al.*, 2016, 2017; Dellinger *et al.*, 2018). Plant vouchers were deposited in UEC Herbarium at Universidade Estadual de Campinas.

Shape and size of floral structures

We characterized each plant species as stamen dimorphic or stamen isomorphic by visually checking differences in size and shape between stamen sets within the same flower in the field. Petals and styles of the species were measured from digital photographs using geometric morphometric analyses. In this case, five individuals were selected from each species, and two flowers were collected from each of them. Subsequently, the flowers were dissected and floral structures were placed on a glass plate with graph paper (Fig. S1). This glass plate was then photographed with a camera (Canon EOS30D) mounted on a tripod and coupled to an interchangeable lens (EF-S 18-135mm f/3.5-5.6 IS STM). During photo shoots, the lens was arranged parallel to the plane of the glass plate and its focal length was adjusted to 50 mm to avoid parallax effects as much as possible (Oliveira *et al.*, 2020). Before the collection of digital landmarks and semi-landmarks, the photographs were organized using the TpsUtil software (http://life.bio.sunysb.edu/morph/). Morphometric data were then sampled by the Cartesian coordinates of the anatomical landmarks and semi-landmarks, using the TpsDig software (http://life.bio.sunysb.edu/morph/). Landmarks were selected based on the probable homology of the coordinates in the two-dimensional plane and the semi-landmarks were digitized as coordinates equally spaced between each landmark. Landmarks 1, 2 and 3 correspond to the top of the petal, the left end of the petal insertion on the floral receptacle, and the right end of the petal insertion on the floral receptacle, respectively (Fig. S1). On the other hand, landmarks 4 and 5 refer to the stigmatic surface and the insertion of the style on the top of the ovary, respectively (Fig. S1). The semi-landmarks were placed on the outline of the petals (10 semi-landmarks) and styles (8 semi-landmarks) (Fig. S1). After the definition of landmarks and semi-landmarks for the structures of all species, the coordinates from petals and styles were properly separated in two different data sets.

Shape variables were generated in the R software using the *geomorf* package (through Generalized Procrustes Analysis - GPA). In this analysis, the shape of the structure can be represented by a point in a non-Euclidean space. “Relative warp” (RW) values were obtained from a principal component analysis (PCA) of the variance-covariance matrix resulting from the GPA. The values of first RW were used as descriptors of the shape of each analyzed structure (i.e., petals and styles). On the other hand, the size of the centroid, defined as the square root of the sum of the squares of the distances of the anatomical landmark or semi-landmark *i* in relation to the centroid of the points, was used as a descriptor of petal and style size.

Bee pollinators

The frequency of bee pollinators was collected by focal observation and/or video camera recording in approximately three individuals per species, and in approximately three to 70 flowers (an exception was *Miconia* sp. 1 in which we observed hundreds of flowers at the same time). Observations were made from September 2010 to August 2013, totaling 36 months. Bee pollinators were observed from 7 am to 2 pm, totaling approximately 20 hours per species and more than 500 hours of observations (Table S1). Bee visitors were classified as pollinators when they vibrated and touched the reproductive structures during their visit, respectively. One exception was *Apis mellifera* that, despite been not able to vibrate flowers, could extract the pollen from the large pored anthers of *Miconia biserrata* by walking through the flowers. We collected, dried, fixed and deposited samples of the bee pollinators in the Laboratório de Biologia Comparada de Hymenoptera of the Federal University of Paraná, UFPR, Paraná, Brazil, for later identification (see Acknowledgements).

Specialization of plant species within the network

Data on interactions between the plants and their bee pollinators were analyzed as quantitative bipartite networks, using the bipartite package in the R 3.4.3 software (Dormann *et al*., 2008; R Core Team, 2020). The frequency of interactions in the network represented the number of visits per hour and per flower of a given bee pollinator to a plant species. Two measures of specialization were estimated: 1) the degree, indicating the number of pollinator species with which a plant had interactions, and 2) the species specialization index (d'), which is calculated as the deviation of the distribution of plant interactions from what is expected based on the availability of pollinator species in the network (Blüthgen *et al*., 2006; Blüthgen *et al.*, 2008). We only included in the network analysis the plants and pollinators involved in legitimate visitations.

Statistical analysis

We used the function *cor.test* to test the correlation between the occurrence of stamen dimorphism and (a) the size of petals and styles, (b) the variation in the size of petals and styles, (c) the shape of petals and styles, and (d) the variation in the shape of petals and styles. Finally, we used a t-test to check whether the occurrence of stamen dimorphism explain the specialization of species within the network. All data analyses were performed using the R software (R Core Team, 2020).

RESULTS

Shape and size of floral structures

We characterized the flower morphology of 22 species of Melastomataceae found in our study area. Petal and style shapes were correlated to stamen dimorphism in Melastomataceae species. In general, dimorphic species presented more orbicular petals while petals of isomorphic species were more lanceolated (cor = 0.71; t = 3.46; df = 20; $p < 0.05$; Fig. 2A; Fig. S2A). The styles of dimorphic species tended to present a slightly curved apex while the style shape of isomorphic species was more linear (cor = 0.77; t = 5.48; df = 20; $p < 0.001$; Fig. 2C; Fig. S2B). Despite the lack of evidence for difference in intraspecific petal shape variance between dimorphic and isomorphic species (cor = 0.23; t = 1.08; df = 20; $p > 0.05$; Fig. 2B), styles shape varied more in isomorphic species (cor = 0.72; t = 4.60; df = 20; $p < 0.001$; Fig. 2D).

Petal and style sizes were also correlated to stamen dimorphism in Melastomataceae. Species presenting stamen dimorphism had larger petals (cor = 0.71; t = 4.54; df = 20; $p < 0.001$; Fig. 3A) and styles (cor = 0.74; t = 4.41; df = 20 ; $p < 0.05$; Fig. 3C) than isomorphic species. Petals and styles of stamen dimorphic species were on average, respectively, 8.1 and 2.8 times larger than those of isomorphic plants. Dimorphic species also had higher intraspecific variance in petal size (cor = 0.54; t = 2.85; df = 20; $p < 0.05$; Fig. 3B), but there was no evidence for difference in style size variance between dimorphic and isomofphic species (cor = 0.36; t = 1.76; df = 20; $p > 0.05$, Fig. 3D).

Melastomataceae–bee pollinators interactions

We observed 22 species of bee pollinators interacting with flowers of 23 species of Melastomataceae (Fig. 4; Table S1). *Bombus morio* was the bee species with the highest frequency of visits with about 72% of visits, followed by *Augochloropsis* sp. 2 with approximately 5% of all visits. The diversity of bee pollinators is related to the presence of stamen dimorphism (Fig. 5). There was more bee species visiting the stamen dimorphic plants than stamen isomorphic ones (degree, $t = 3.32$; $df = 13.51$; $p = 0.005$; Fig. 5A). Additionally, stamen dimorphic species were less specialized than stamen isomorphic species ($t = 2.08$; $df = 12.46$; $p = 0.05$; Fig 5B).

DISCUSSION

Our community level investigation on Melastomataceae floral morphology revealed that stamen dimorphism is linked to petals and style shape, size as well as the variation in these traits. Specifically, species with stamen dimorphism has larger flowers with more orbicular petals and hook-shaped styles. Stamen dimorphic flowers also had higher petal size variance. However, in contrast to what we expected, these flowers did not show higher variation in the size nor in the shape of their styles. Our results also showed that plants with stamen dimorphism have a greater richness of bee pollinators visiting their flowers and less specialized interactions, which suggests that they are ecologically more generalist.

Angiosperm flowers show a wide morphological diversity, and the size and the intraspecific variation in floral attractive structures such as petals can be determined by floral development (Armbruster, 2014). In this sense, larger flowers tend to have higher variation in their structures, following their larger dimensional scale (Murren, 2012; Armbruster *et al*., 2014; Pélabon *et al*., 2013; Armbruster & Wege, 2019). However, although flowers with larger petals are expected to show larger intraspecific variation in petal size and larger styles (Murren, 2012; Armbruster *et al.*, 2014; Pélabon *et al*., 2014), we did not observe a higher variation in size and shape of the styles in stamen dimorphic flowers of Melastomataceae. This smaller intraspecific variation in style traits of stamen dimorphic flowers may optimize a specific contact with particular parts of the pollinator's body (Webb & Lloyd, 1986; Barrett, 2002b; Jesson & Barrett, 2003; Solís-Monteiro & Vallejo-Marín, 2017; Tong & Huang, 2018; Konzmann *et al*., 2020). Such specific contact may target the pollen grains on ungroomed and safe sites of the bee's body, resulting in higher amounts as well as higher quality of conspecific pollen deposited onto the stigma (Koch *et al*., 2017). Moreover, specific stigma contact may also decrease heterospecific pollen deposition within the context of plant communities (Tong & Huang, 2018). These incongruent results for intraspecific variation in petals and styles suggest that attraction (petals) and efficiency (style) traits may form two different floral modules in stamen dimorphic species, similarly to what has been demonstrated for the tribe Merianieae in Melastomataceae (Dellinger *et al.*, 2019).

In our study, the morphology of petals and styles is clearly evolutionarily biased once the stamen isomorphic and dimorphic species occurring in the study area mostly belonging to two different large Melastomataceae lineages, i.e., the Miconieae and Melastomateae tribes, respectively (Goldenberg *et al.*, 2008; Michelangeli *et al.*, 2013). It is noteworthy that the very same lineages also present different reproductive strategies concerning their phenology and breeding systems, with many species in Miconie tribe reported as pollinator independent to set seeds (Santos *et al.*, 2012; Brito *et al.*, 2017). In Melastomataceae, pollen self-deposition may occur in some species in which individuals are self-compatible (e.g., *Trembleya parviflora*, *Tibouchina heteromalla*, *T. stenocarpa*; dos Santos *et al.*, 2012). However, most reported pollinator independent species are apomitic (dos Santos *et al.*, 2012; Brito *et al.*, 2017b; Caetano *et al.*, 2013, 2018). Thus, the occurrence of small flowers without any morphological differences in the stamens may be related to the "selfing syndrome", as in *Solanum* species (Vallejo-Marín *et al.*, 2014). In fact, some of the species studied here are apomitic and have small flowers without stamen dimophism, such as *Leandra xanthocoma, Miconia dodecandra, Miconia ibaguensis*, and *Miconia albicans* (dos Santos *et al.*, 2012; Brito *et al.*, 2017b). On the other hand, during the evolutionary history of Melastomataceae family, the dependence to pollinators for fruit set has possibly favoured the evolution of larger flowers with dimorphic stamens (Melo *et al.*, 2021). Whether the floral traits correlated to the presence or absence of stamen dimorphism reveals two major floral syndromes related to the breeding systems in the evolutionary history of Melastomataceae remains to be tested.

Flowers with complex morphologies, including the presence of stamen dimorphism, are considered phenotypically specialized, which could mean that they will be visited by fewer pollinator species, thus being also ecologically specialized (Ollerton *et al.*, 2007; Armbruster, 2017; Yoder *et al.*, 2020). In fact, flowers with more specialized symmetries, *i.e.*, zygomorphic flowers, are visited by fewer pollinator species than actinomorphic flowers (Yoder *et al.*, 2020). However, contrary to our expectations, we found that species with stamen dimorphism had a greater number of bee pollinator species as well as greater quantitative generalization. As flowers of stamen dimorphic species were generally larger, it may be that these flowers present greater amount of pollen grains per anther, thus offering more resources and attracting a higher number of pollinators. Melastomataceae species with poricidal anthers are visited by bees that vary in size, from small Halictidae and Meliponini bees to large *Xylocopa* and *Bombus* bees (Renner, 1989; Larson & Barrett, 1999; Brito & Sazima, 2012; Soares & Morellato, 2018). Bee body size influences both bee behavior and foraging activities in flowers (De Luca *et al.*, 2019), and large bees have the ability to remove large amounts of pollen in a short time interval by grabbing and vibrating many anthers simultaneously (Mesquita-Neto *et al.*, 2018). Small bees, on the other hand, grab one or a few anthers in each visit, spend more time on each vibration, and remove less pollen (Mesquita-Neto *et al.*, 2018). Plant species with larger flowers could therefore receive visits from different bee pollinator groups which differ in size and foraging behaviors (Mesquita-Neto *et al.*, 2018). On the other hand, plants with smaller flowers would be associated only with smaller floral visitors, reducing the amount and diversity of bees that forage in their flowers. This pattern could explain the greater specialization of species with smaller flowers and isomorphic stamens.

In summary, we show that floral traits such as petal and style size and shape as well as variations in these traits are correlated to the presence of stamen dimorphism in the species of Melastomataceae. Furthermore, stamen dimorphism in larger flowers is associated with greater richness of visiting bees and ecological generalization. Hence, floral traits may determine the specialization of plant species at the community level and, ultimately, the structure of the interaction networks between pollen flowers and bees. Nevertheless, how pollen flower floral traits function as filters determining the links between plant species and bee pollinators is still not well understood. Given that buzz pollination occurs in a complex mechanical interaction between buzzing bees and flowers, which in turn regulate the pollen release (Vallejo-Marín, 2019), future studies could investigate how floral and bee traits linked to their mechanical fit structure the interaction network as a whole.

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FIGURE CAPTION

Fig 1. Flowers of Melastomaceae species: A, *Miconia crenata* (Vahl) Michelang.; B, *Miconia neourceolata* Michelang.; C, *Miconia reversa* (DC.) Ocampo & Almeda; D, *Miconia dodecandra* Cogn.; E, *Miconia ibaguensis* (Bonpl.) Triana; F, *Miconia amygdaloides* (DC.) R. Goldenb.; G, *Pleroma oleifolium* (DC.) R. Romero & Versiane; H, *Pleroma clavatum* (Pers.) P.J.F. Guim. & Michelang.; I, *Chaetogastra clinopodifolia* DC.; J, *Pleroma fothergillae* (Schrank & Mart. ex DC.) Triana; K, *Pleroma granulosum* (Desr.) D. Don; L, *Pleroma heteromallum* (D. Don) D. Don; M, *Pleroma sp.* 1; N, *Pleroma stenocarpum* (Schrank & Mart. ex DC.) Triana; O, *Miconia xanthocoma* (Naudin) R. Goldenb.; P, *Miconia albicans* (Sw.) Steud.; Q, *Rhynchanthera brachyrhyncha* Cham.; R, *Trembleya parviflora* (D. Don) Cogn.

Fig 2. Size of petals and styles of Melastomataceae species occurring in Serra do Mar State Park, Núcleo Picinguaba and Núcleo Santa Virgínia. A, Petal size of species according to stamen dimorphism. B, Petal size variance according to stamen dimorphism. C, Style size of species according to stamen dimorphism. D, Style size variance according to stamen dimorphism. Blue dots represent dimorphic plant species while red dots represent isomorphic plant species. *Significance at the 0.05 level, t-test.

Fig 3. Shape of petals and style structures of Melastomataceae species. A, Shape of petals of species according to stamen dimorphism. B, Shape variation of species according to stamen dimorphism. C, Shape of style of species according to stamen dimorphism. D, Shape variation of species according to stamen dimorphism. Blue points represent dimorphic plant species and red points represent isomorphic plant species. *Significance at the 0.05 level, t-test.

Fig 4. Bipartite network representing the interactions among Melastomataceae plants and pollinator bees, occurring in Serra do Mar State Park, Núcleo Picinguaba and Núcleo Santa Virgínia in Brazil.

Fig 5. Specialization of Melastomataceae species within the interaction network with pollinator bees. A, plant species degree (number of partners) according to stamen dimorphism. B, Plant species specialization (d’) according to stamen dimorphism. Blue dots represent dimorphic plant species and red dot represent isomorphic plant species. *Significance at the 0.05 level, t-test.

FIGURES

Fig.1.

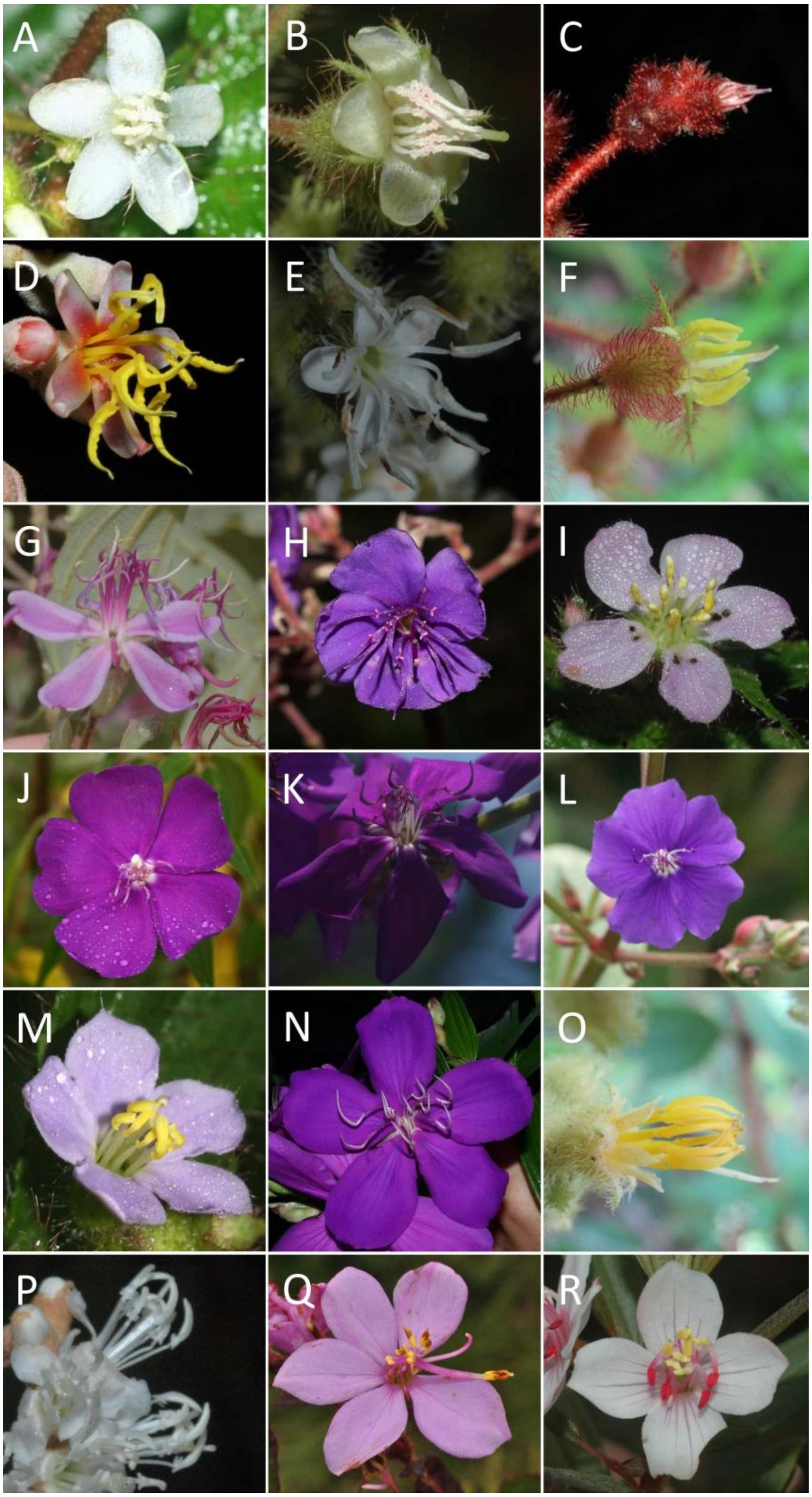


Fig. 2.

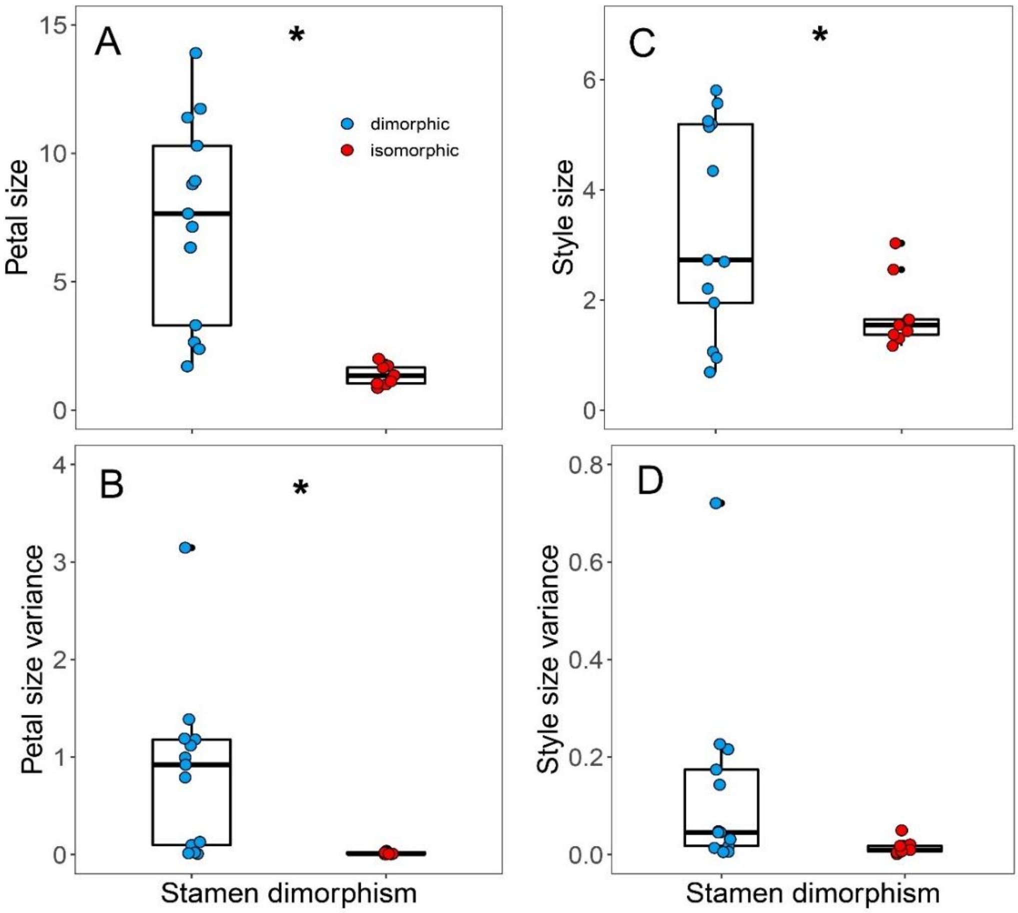

A
*
dimorphic
isomorphic
Petal size
15
10
5
0
C
*
Style size
6
4
2
0
B
*
Petal size variance
4
3
2
1
0
D
Style size variance
0.8
0.6
0.4
0.2
0.0
Stamen dimorphism
Stamen dimorphism

Fig. 3.

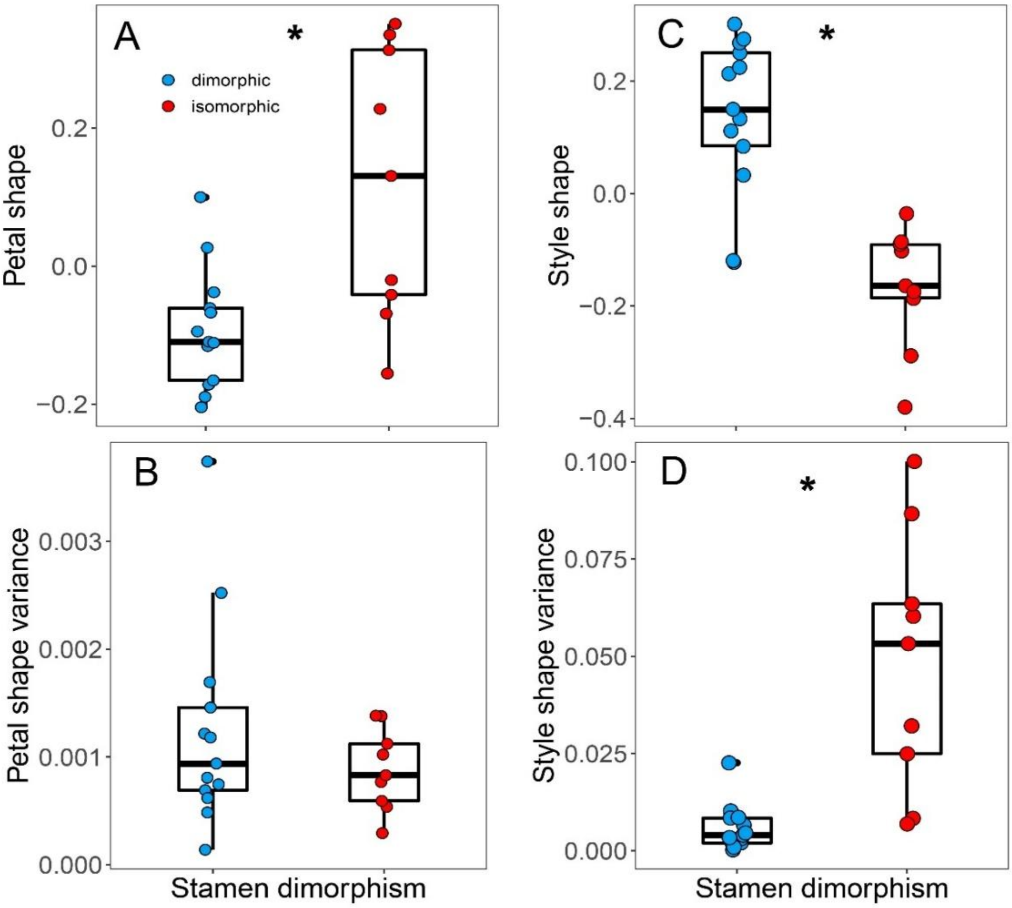

A
*
dimorphic
isomorphic
Petal shape
0.2
0.0
−0.2
B
Petal shape variance
0.003
0.002
0.001
0.000
Stamen dimorphism
C
*
Style shape
0.2
0.0
−0.2
−0.4
D
*
Style shape variance
0.100
0.075
0.050
0.025
0.000
Stamen dimorphism

Fig. 4.

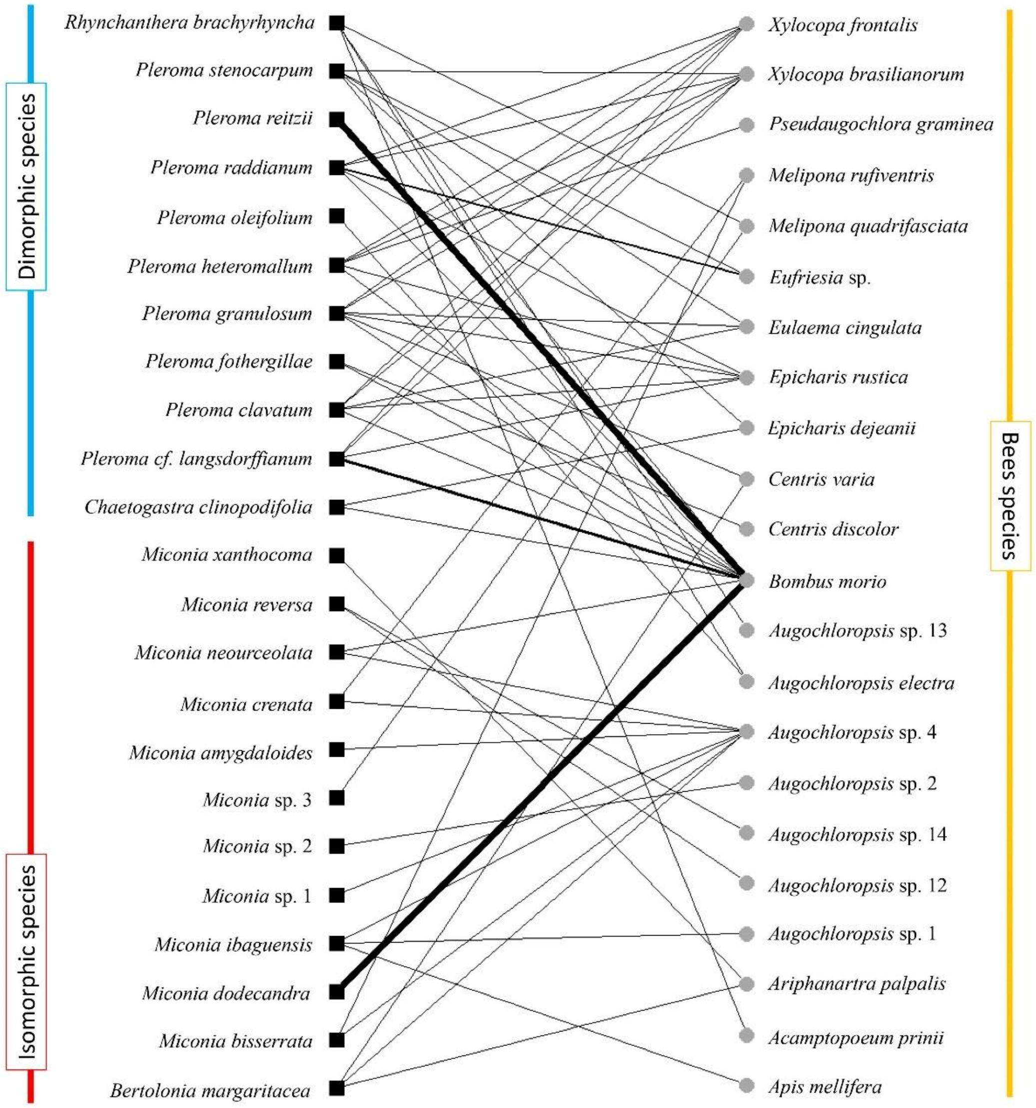
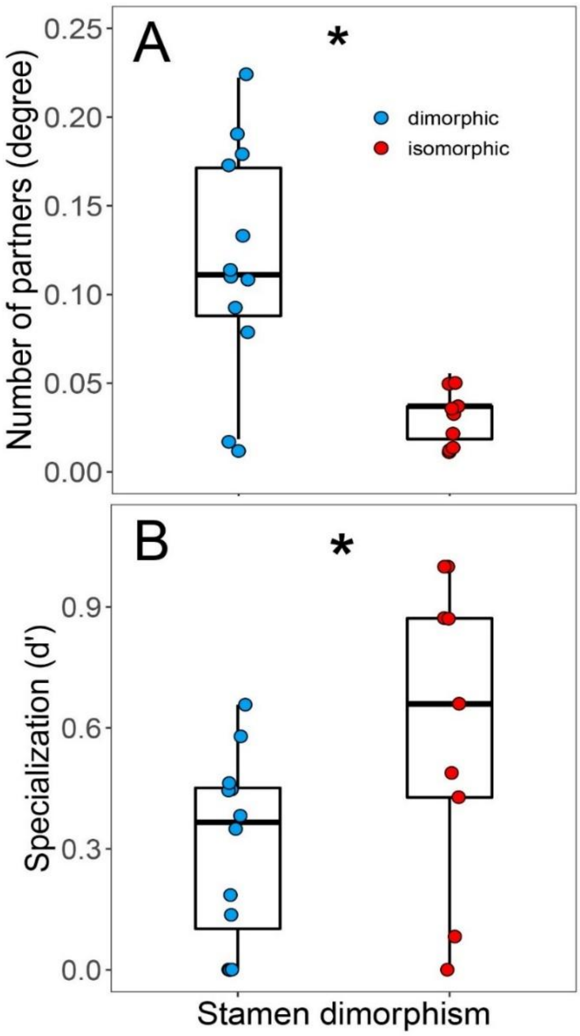

Dimorphic species
Isomorphic species
Bees species
Rhynchanthera brachyrhyncha
Pleroma stenocarpum
Pleroma reitzii
Pleroma raddianum
Pleroma oleifolium
Pleroma heteromallum
Pleroma granulosum
Pleroma fothergillae
Pleroma clavatum
Pleroma cf. langsdorffianum
Chaetogastra clinopodifolia
Miconia xanthocoma
Miconia reversa
Miconia neourceolata
Miconia crenata
Miconia amygdaloides
Miconia sp. 3
Miconia sp. 2
Miconia sp. 1
Miconia ibaguensis
Miconia dodecandra
Miconia bisserrata
Bertolonia margaritacea
Xylocopa frontalis
Xylocopa brasilianorum
Pseudaugochlora graminea
Melipona rufiventris
Melipona quadrifasciata
Eufriesia sp.
Eulaema cingulata
Epicharis rustica
Epicharis dejeanii
Centris varia
Centris discolor
Bombus morio
Augochloropsis sp. 13
Augochloropsis electra
Augochloropsis sp. 4
Augochloropsis sp. 2
Augochloropsis sp. 14
Augochloropsis sp. 12
Augochloropsis sp. 1
Ariphanartra palpalis
Acamptopoeum prinii
Apis mellifera

Fig. 5.


A
*
● dimorphic
● isomorphic
Number of partners (degree)
0.25
0.20
0.15
0.10
0.05
0.00
B
*
Specialization (d')
0.9
0.6
0.3
0.0
Stamen dimorphism

SUPPLEMENTARY DATA

Table S1. Sampling information for each species of Melastomataceae in Serra do Mar State Park, Núcleo Picinguaba and Núcleo Santa Virgínia.

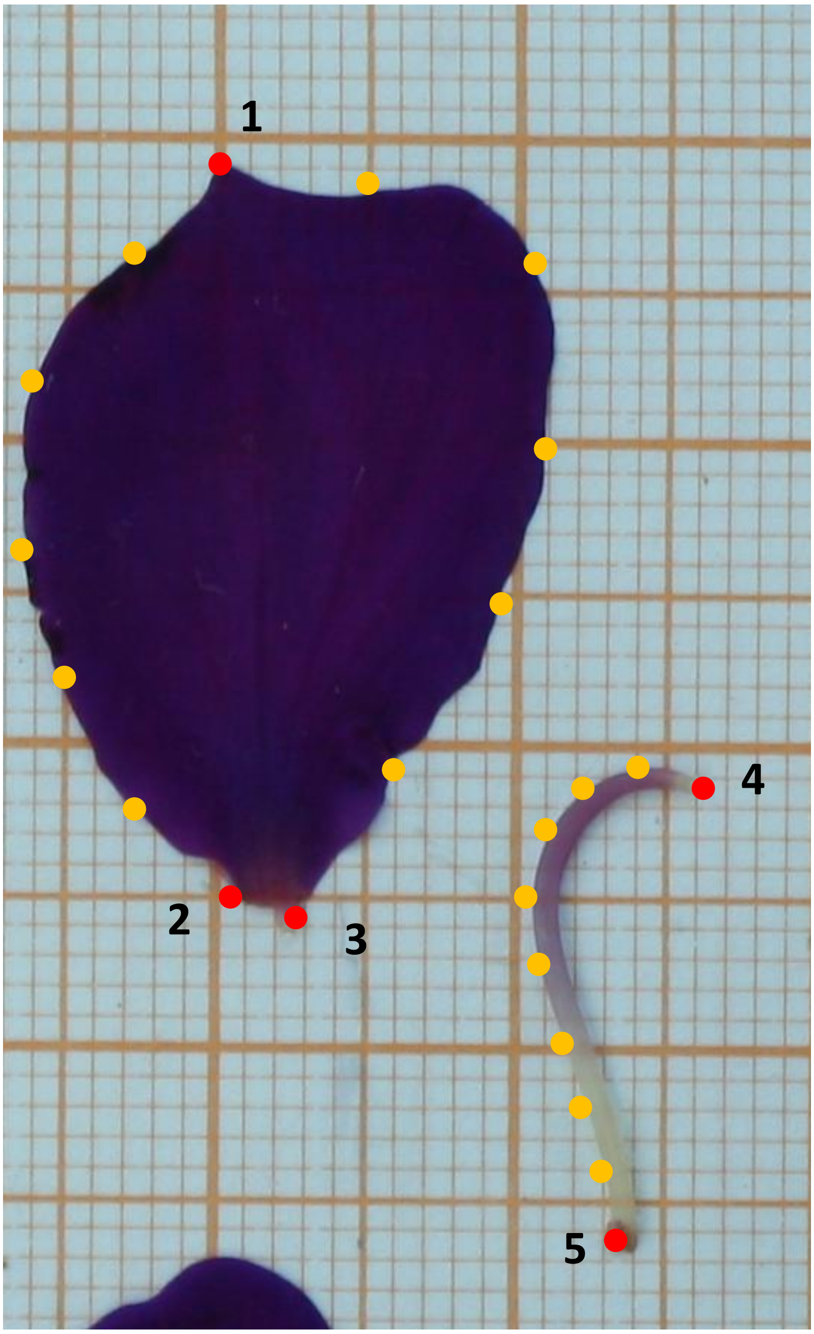
Plant species	Stamen dimorphism	Observation type	Region	Number of observed flowers	Sampling period (hours)	Frequency of bee visits (%)	Morphometric data
Bertolonia margaritacea Naudin	isomorphic	F + V	NP	3 to 9	13.55	0.123	N
Chaetogastra clinopodifolia DC.	dimorphic	F + V	NSV	2 to 5	14.92	0.389	Y
Miconia albicans (Sw.) Steud.	isomorphic	F + V	NP	10 to 30	14.75	0.000	N
Miconia amygdaloides (DC.) R. Goldenb.	isomorphic	F + V	NP	5 to 10	17.68	0.002	Y
Miconia biserrata (DC.) Michelang.	isomorphic	F + V	NP	3 to 20	20.65	0.016	Y
Miconia crenata (Vahl) Michelang.	isomorphic	F + V	NP	3 to 12	15.35	0.069	Y

Miconia dodecandra Cogn.	isomorphic	F	NP	10 to 30	20.33	0.001	Y
Miconia ibaguensis (Bonpl.) Triana	isomorphic	F + V	NP	20 to 50	9,55	0.186	Y
Miconia neourceolata Michelang.	isomorphic	F + V	NP	5 to 20	20.50	0.004	Y
Miconia reversa (DC.) Ocampo & Almeda	isomorphic	F + V	NP	15 to 20	20.50	0.095	Y
Miconia sp. 1	isomorphic	F	NP	400 to 3000	18.95	0.00002	N
Miconia sp. 2	isomorphic	F	NSV	13	7.00	0.242	N
Miconia sp. 3	isomorphic	F + V	NSV	5 to 10	26.08	0.002	Y
Miconia xanthocoma (Naudin) R. Goldenb.	isomorphic	F + V	NSV	3 to 10	19.52	0.002	Y
Pleroma clavatum (Pers.) P.J.F. Guim. & Michelang.	dimorphic	F + V	NP	5 to 29	19.33	0.259	Y
Pleroma fothergillae (Schrank & Mart. ex DC.) Triana	dimorphic	F + V	NSV	3 to 25	18.92	0.003	Y

Pleroma granulosum (Desr.) D. Don	dimorphic	F	NP	50 to 110	20.00	0.408	Y
Pleroma heteromallum (D. Don) D. Don	dimorphic	F + V	NP	4 to 82	28.08	0,010	Y
Pleroma cf. langsdorffianum (Bonpl.) Triana	dimorphic	F	NP	40	6.58	2.787	Y
Pleroma oleifolium (DC.) R. Romero & Versiane	dimorphic	F	NSV	10 to 15	13.50	0.006	Y
Pleroma raddianum (DC.) Gardner	dimorphic	F	NP	6 to 12	60.00	0.142	Y
Pleroma reitzii (Brade) P.J.F. Guim. & Michelang.	dimorphic	F + V	NSV	7 to 57	26.58	0.001	Y
Pleroma sp. 1	dimorphic	F	NSV	5 to 10	20.75	0.000	Y
Pleroma stenocarpum (Schrank & Mart. ex DC.) Triana	dimorphic	F + V	NP	10 to 15	18.15	0.085	Y
Rhynchanthera brachyrhyncha Cham.	dimorphic	F	NSV	10 to 15	20.50	0.158	Y

Trembleya parviflora (D. Don) Cogn.	dimorphic	F	NSV	7 to 10	13.75	0.000	Y

F = Focal observation; V = Video observation; NP = Núcleo Picinguaba; NSV = Núcleo Santa Virgínia; N = No, Y = Yes.



1
2
3
4
5

Fig. S1: Set of landmarks and semi-landmarks used to describe the size and shape of petals and styles of Melastomataceae species in Serra do Mar State Park, Núcleo Picinguaba and Núcleo Santa Virgínia. Red landmarks 1, 2 and 3 correspond to the top of the petal, the left end of the petal insertion on the floral receptacle, and the right end of the petal insertion on the floral receptacle, respectively. On the other hand, red landmarks 4 and 5 refer to the stigmatic surface and the insertion of the style on the top of the ovary, respectively. Yellow semi-landmarks were defined as coordinates equally spaced on the outline of the petals (10 semi-landmarks) and styles (8 semi-landmarks).

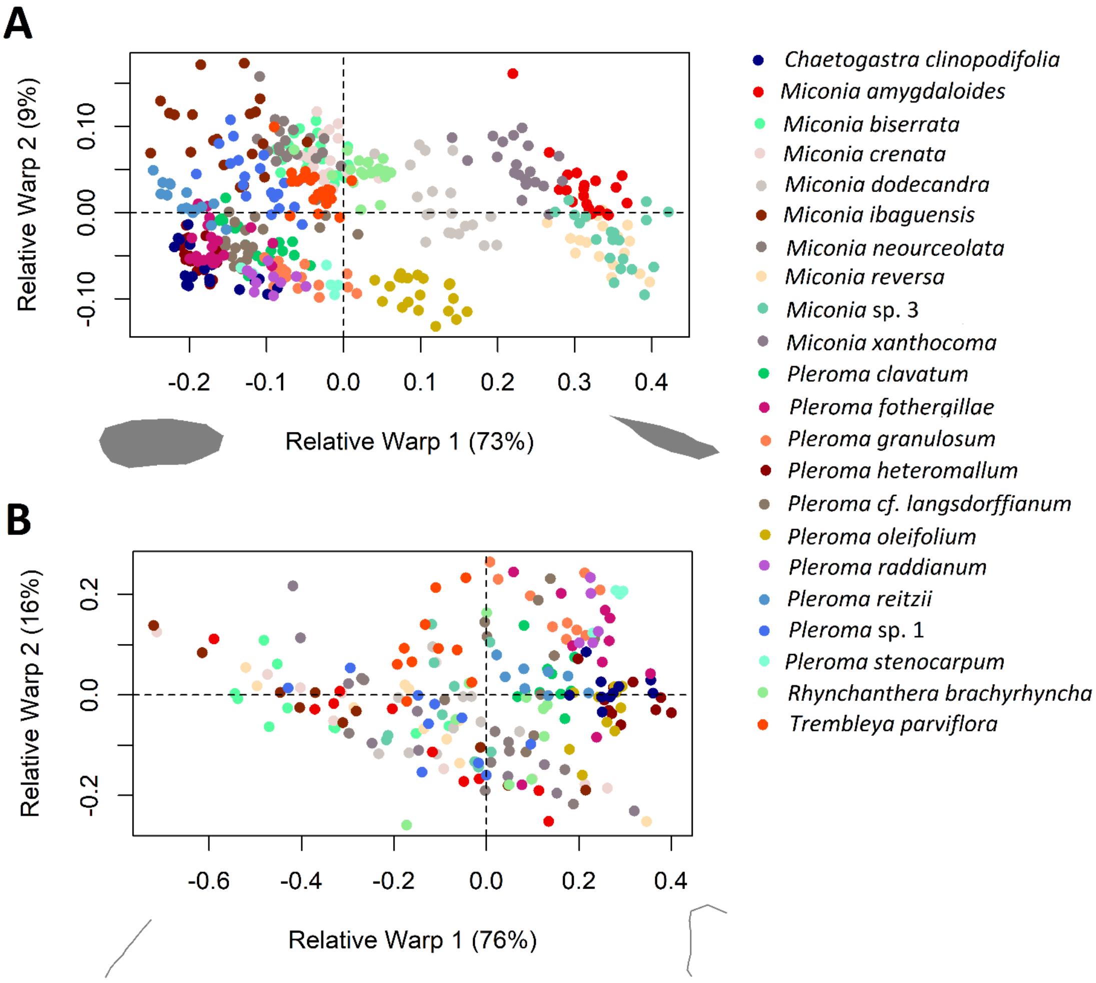


Fig. S2: Morphospace representing the shape variation of (A) petals and (B) styles of Melastomataceae species in Serra do Mar State Park, Núcleo Picinguaba and Núcleo Santa

Virgínia. Shaded shapes below the plots represents the extreme values in the first relative warp for floral structures.

CHAPTER III

Adding the “buzz” in plant-pollinator networks: contrasting frequency visitation and effectiveness as interaction currencies

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Em fase de preparação para submissão na revista *New Phytologist*

ABSTRACT

1. Community-level analyses using ecological network approaches have provided new insights into how plant-pollinator interactions function in ecosystems. However, they often ignore the critical quality component that make-up the effectiveness of each partner. Here, we aimed to evaluate the pollination performance components of plant-bee interactions in a buzz-pollination system, combining quantitative and qualitative components into ecological networks.

2. To investigate effectiveness patterns of plant-bee interactions we recorded the frequency of bee visitation and conducted single visits experiments to estimate pollen removal and deposition in ten buzz-pollinated species of *Chamaecrista* (Fabaceae) at the campos rupestres vegetation, Brazil. We incorporated the quantitative and qualitative data of interaction on networks, extracted their metrics, and compared the results.

3. The different components of plant-pollinator interaction can influence our interpretation of species roles and network structure. Qualitative components, especially varied widely among pairwise interactions and when considering the bee functional groups. The predominant pollination partners were bees capable of vibrating all the anthers, and they conducted a notably higher number of legitimate visits. Moreover, network-level metrics differed between the networks evaluated. The male performance network produced more specialised and modular networks, while female performance and frequency networks produced more generalised networks.

4. The assessment of pollination effectiveness by a floral visitor is essential to comprehend its impact on the reproductive success of plants. Obtaining a precise measure of this effectiveness is crucial for understanding the ecology and evolution of interactions between plants and pollinators. This understanding is important for determining which pollinators primarily contribute to the fitness of plants and thereby enhancing the reliability and predictive power of network analyses in studies of plant-pollinator interactions.

KEYWORDS

interaction, pollonation, *Chamaecrista*, bee, campo rupestre, buzz-pollination

INTRODUCTION

Pollination is an essential interaction within the life cycle of most flowering plants and plays an important role in shaping the dynamics of ecological and evolutionary processes in most ecosystems (Faegri & Van Der Pijl, 2013; Tong et al., 2023). Community-level analyses have provided new insights into how interactions between plants and pollinators occurs in ecosystems, especially using ecological network approaches (Bascompte & Jordano, 2007; Thebault & Fontaine; 2006; Vázquez et al., 2009; Magrach et al., 2021). In general, mutualistic networks are built qualitative or quantitative data on observed visitation and/or indirect evidence, such as pollen load in the body of pollinator (Vázquez et al., 2005; Vizentin-Bugoni et al., 2018; Souza et al., 2021). The study of the structure of these networks have contributed to elucidate patterns of community organisation, stability, and functioning (Pimm et al., 1991; Thompson, 1982; Magrach et al., 2021). However, quantification of plant-pollination interaction through the number of visits the plant receives fails to guarantee that pollination indeed occurred, as more than bee visited, and effective pollination requires the transference of pollen grains (i.e. quality component of the mutualism; Ballantyne et al., 2015; González-Castro et al., 2022). Actually, because most networks are built using only the frequency of visits as the currency for interaction strength/intensity it rises the question about how consistent are species the roles in the networks (Vázquez et al. 2005; King et al., 2013; Ballantyne et al., 2015; González-Castro et al., 2022). A deeper understanding of species contribution to ecosystem dynamics needs, therefore, to add measures of the effective transfer of pollen grains weighting the visitations (King et al., 2013).

The investigation of the effectiveness of plant-pollinator interactions is fundamental to understanding the ecological and evolutionary functions performed by the species involved (Schupp, 1993; Schupp et al., 2017). Highly effective species in mutualistic interactions are the ones that contribute significantly to the reproductive success and population dynamics of their partners (Schupp, 1993; Santiago-Hernandez et al., 2019; Gomez et al., 2021). Consequently, they may impose strong selective pressures on the mediating traits involved in the interactions, affecting phenotypic evolution and coevolution in significant ways (Valverde et al., 2019). For pollination interactions, the effectiveness is directly connected with pollen transfer, split in male and female efficacy. The plant male effectiveness refers to the transfer of pollen among flowers, generally mediated by pollinators, such as bees (Thompson, 1986; Øystein et al., 2023). In contrast, female effectiveness relates to the reception of pollen on stigmatic surfaces and the subsequent formation of fruits and seeds (Schupp et al., 2017; Fontúrbel et al., 2017; Gomez et al., 2021). The balance between these two components is essential for plant reproductive success (King et al., 2013; Cortés-Rivas et al., 2022). Understanding these measures of effectiveness allows us to assess the contributions of different interacting organisms to the fitness of many plants and identify which processes, related to quantity or quality, mediate this function (Schupp, 1993; Schupp et al., 2017; Gomez et al., 2021).

Previous studies that incorporated different measures of quality for each plant-pollinator interaction in networks had considered data on flower handling (e.g. Mesquita-Neto et al., 2018), pollen deposition on stigmas (King et al., 2013; Ballantyne et al., 2015) and subsequent measures such as fruit and seed set (Santiago-Hernandez et al., 2019). They demonstrated that the type of data used to build interaction networks can change the network structure and, consequently, the understanding of the interaction between partners. Other studies at the species level have demonstrated the importance of considering variables such as pollen deposition to determine the pollination efficiency of different floral visitors (e.g., Barrios et al. 2016). Nevertheless, all these focused on the female performance of pollination. The male performance of the flowers, which is related to pollen removal from the anthers, may operate in ways substantially different from the female one (Bergamo et al., 2023). For instance, some pollinators may be more efficient in removing pollen from the anthers than in delivering it to the stigmas because pollen transport faces different barriers, such as e.g. pollen theft (Mesquita-Neto et al., 2018) and bee grooming behaviour (Koch et al. 2017; Tong and Huang, 2018). Furthermore, it is known that the female component of pollination saturates more quickly than the male component (Janzen, 1977; Bergamo et al., 2023). Therefore, it is important to consider both the male and the female performances of the flowers to have a more complete picture of the quality of the plant-pollinator interactions, mainly in specialised systems of interactions between plants and pollinators (e.g. buzz-pollination).

Buzz-pollination is a specialised interaction involving plants with distinct floral morphologies and a unique behaviour displayed by certain bees during pollen collection (Vallejo-Marín, 2019). Considering plant species, floral morphologies/adaptations seem to have evolved specifically in concert, characterising a buzz pollination syndrome (Vallejo-Marín, 2019), which includes pollen released through tiny pores or apical slits (i.e., poricidal anthers) (Buchmann, 1983; De Luca & Vallejo-Marín, 2010), functional specialisation of stamens (i.e., heteranthery; Luo et al., 2008; Vallejo-Marín et al., 2010) and pollen grains as the main floral resources (i.e. pollen flowers; Vogel, 1978). On the other hand, the pollinators of these flowers are generally insects, mostly bees, capable of performing vibrations to extract pollen grains which are used to feed their larvae (Buchmann e Hurley, 1978; Buchmann, 1983; Vallejo-Marín, 2022). However, the fact that pollen is needed for both the reproduction of the plants and as a resource for pollinators, generates a "pollen dilemma" where plant reproduction is affected by the two conflicting processes (Luo et al., 2008). Therefore, floral characteristics may act directly on differences in the performance of each floral visitor in the pollination of species as well as in fitness within populations.

Additionally, the characteristics of pollen flowers, as pollen enclosed in specialised poricidal anthers, makes it easier to measure the removal of pollen grains (Solís-Monteiro & Vallejo-Marín, 2017; Papaj et al., 2017, Brito et al., 2021), allowing simultaneous investigation of both male and female performance components. In this way, the interaction network between plants with pollen flowers and flower visitors could be estimated taking into account the effectiveness of each visitor as well as the male and female components of plant fitness. Within this context, we aim to evaluate the pollination performance components of plant-bee interactions, combine quantitative and qualitative components of these interactions, and compare the resulting plant-pollinator networks. To do so, we used buzz-pollinated flowers from the highly diverse genus *Chamaecrista* (Fabaceae), known for considerable variation on floral morphological traits while keeping a specialised association with bee pollinators (Gottsberger & Silberbauer-Gottsberger, 1988; Tucker, 1996). More specifically, we address the following questions: (i) how do pollen removal and deposition vary between different floral visitors? (ii) does the plant pollination performance change when mediated by different bee species? (iii) are networks considering only frequency components different from the ones including male and female performances? (iv) are bee species levels of specialisation on quantitative networks different from qualitative networks? We hypothesised that different bee species remove and deposit different quantities of the pollen grain and, consequently, mediate different plant pollination performances. By incorporating plant male and female performance components mediated by floral visits, we expect to find more specialised networks, consistent with the nature of the studied pollination system.

MATERIALS AND METHODS

Study area and plant species

We conducted this study from February/2021 to April/2022 at the surroundings of the Federal University of the Vales do Jequitinhonha e Mucuri (UFJVM - 18° 11S 43° 34W). The university is located at Diamantina municipality, Minas Gerais, Brazil. The study area is characterised as *campos rupestres* vegetation, a mosaic of open vegetation where herbs and shrubs species predominate, between stretches of rocky outcrops (Silveira et al., 2016; Monteiro et al., 2021). Furthermore, it is a vegetation that combines high biodiversity and high endemism

(Silveira et al., 2016; Monteiro et al., 2021), comprising great richness of co-flowering plant species with poricidal anthers throughout the year.

We used plants from the genus *Chamaecrista* Moench (Fabaceae), as a study model. *Chamaecrista* genus comprises about 230 species in Brazil and is widely distributed in campos rupestres vegetation. Minas Gerais is one of the main centres of diversity for the group, with 105 species restricted to it, and there is a high degree of endemism in the campos rupestres. Characterised by under-shrub and shrubby species, flowers from *Chamaecrista* genus exhibit an asymmetric corolla composed of five petals (Irwin & Barneby, 1982). The reproductive structures are usually displaying enantiostyly and dimorphic anthers (Irwin & Barneby, 1982). Flowers offer only pollen as a floral resource (called pollen flowers), have anthers with poricidal dehiscence, and pollen grains are released only when bees vibrate the anthers (e.g. buzz pollination). Anthesis is usually diurnal and flowers last just one day. The fruit is linear, dry, and legume-type (Irwin & Barneby, 1982). Plant species investigated were deposited in the HUFABC Herbarium at Federal University of ABC, São Bernardo do Campo, SP, Brazil and all the specimens were identified by a specialist (*see* Acknowledgements).

Frequency of bee visitation and selected species

The total frequency of bee visitation was sampled on 70 sunny days in 2021 (during March to December) and 21 sunny days in 2022 (January to Mach) for each plant species. We selected 10 co-flowering plant species and tagged 5 to 10 plant individuals per species. We obtained the flower visitation data through focal observations, between 6:00 h to 14:00 h, peak activity times of bee visits. Thus, to register the bee visitation pattern, we remained and observed for 30 min at a single individual plant of a species and sampled all flower visitors' behaviour. After 30 min, we moved to another individual of plant species, and so on throughout the day. We recorded on each floral visit: the bee species, bee behaviour during pollen collection, and which floral structure the bee touched. We performed a total of 528 h of sampling effort with approximately 55 h per plant species. We collected all bee species and deposited them at the laboratory Centro de sínteses ecológicas at Federal University of Minas Gerais, UFMG, Belo Horizonte, Brazil, and all the specimens were identified by a specialist (see Acknowledgements).

Estimation of pollen grain removal and pollen deposition on the stigma

To measure pollen removal from the anthers and deposition from the stigmatic surface we performed an experiment of single visits from October 2021 to April 2022, between 07:00 h and 11:00 h. We used the same 10 plant species that were used to describe visit frequency and tagged 5 to 10 plant individuals per species. The experiment consisted of estimating the number of pollen grains that a bee removes and deposits during a visit to a flower. To perform the experiment, we bagged flower buds of all plant species each afternoon, and the day after, once the flowers opened, the bags were removed and exposed to floral visitors. We collected flowers as soon as they received the first bee visit. Subsequently, we transferred each flower to 50 ml Falcon tubes and recorded the visitor and plant identities. We performed the single visits experiment every day until there were no more bagged flowers left to sample. We performed, considering all the experiment, a total of 908 flowers and around 90 flowers per plant species.

To estimate the quantity of pollen grain removal by each bee, we brought the flowers from the single visit experiments to the laboratory, removed the anthers, and transferred them to 1.5 ml Eppendorf tubes. After that, we macerated the anthers with a plastic rod and added 240 µl of a solution of acetic carmine and glycerine (3:1). Later, we stirred the tubes using the vortex and for 60 s, transferred 10 µl of the solution to a Neubauer chamber, and the pollen grains were counted under light microscopy (×100 or ×400). Furthermore, we collected virgin bagged flowers before any visit and used them as control samples (n = 10). We estimated the number of total pollen grains on the anthers using the same technique explained above. Finally, we subtracted the average of the total number of pollen estimated in the control samples from the total number of pollen remaining in the single-visit samples. Resulting on a proxy for the total amount of pollen removed per bee during its floral visit. We adjust the protocols of Barbosa et al., (2023) and Trevizan et al., (2023) for collection, fixation, dilution, and pollen grain estimation.

The flowers used to perform the single visits experiments also were used to count the total number of pollen grains deposited by the bee. To estimate the quantity of pollen grains deposited by each bee we brought the flowers from the single visit's experiments to the laboratory and removed the stigma. After that, they were arranged on microscope slides under coverslips with fuchsin gel. Later, all pollen grains deposited were counted under light microscopy (×100 or ×400) with the help of a hand counter. To evaluate whether the studied *Chamaecrista* species have self-deposition events, we collected flowers without bee visits and performed the same steps described above allowing us to use them as control samples (n = 10).

Characterisation of visitor functional group

Visitors may perform differently in their ability to remove and deposit pollen grains, and therefore in their efficiency in the pollination of the studied plant species. In order to facilitate their roles, we grouped bees into four functional groups. We delimited these functional groups based on their pollen collection and flower manipulated behaviour, according to Mesquita-Neto et al., (2018), and our observations: (a) *theft* - bees that collect pollen with their mouthparts from the apical pore; and/or collect pollen squeezing the anthers and causing pressure stimulating pollen grain removal; and/or glean residual pollen that adhered on floral attributes after pollen extraction by vibrating bees. These bees generally do not cause floral damage. (b) *robber* - bees that collect pollen cutting and perforating anthers and/or petals of flowers. These bees always cause floral damage; (c) *anther-buzzing* - bees that collect pollen by vibrating single anthers or a set of anthers. These bees alter their position within the flower between vibrating actions. (d) *flower-buzzing*: bees that collect pollen by vibrating all the anthers (or almost all) and applying one or more vibration. These bees generally don't change their position in the flower.

Data analysis

To analyse the effect of the visitor functional group on pollen removal and deposition, and the performance of each functional group on male and female components we used a generalised linear model implemented (using *lme4* R-package). The fixed factors were treatment (functional group - categorical), *Chamaecrista* species (categorical) and their interaction and to performance landscape we use pollen deposition was the response variable. We used *bonferroni* tests to determine whether different bee functional groups differ on pollen removal and deposition.

We built five different bipartite networks (using *bipartite* R-package) according to our data: (a) *frequency network* (VN) - using the frequency of interaction between visitor groups and plant species; (b) *deposition network* (DN) - considering the mean of conspecific pollen deposited in a virgin stigma surface during a single visit by bee species; (c) *female performance network* (FPN) - combined data of visiting frequency and pollen deposition by bees on plant species; (d) *release network* (RN) - the mean of anthers pollen removal during a single visit by bee species of each plant species; (e) *male performance network* (MPN) - combined data of visiting frequency and pollen removal by visitors of each plant species. After that, we calculated the metrics of networks to understand the structure and patterns of interactions of the resulting networks and compare them to each other: *nestedness* (NODF) - assesses the likelihood of the network displaying a nested pattern in which specialists interact with proper subsets of the species interacting with generalists (Blüthgen et al. 2008); *modularity* (Q_w) - measures the frequency of interactions occurring within modules (subunits within the community) in comparison to interactions between modules, and *specialisation* (H_2') - established based on the count of interactions a species engaged in relative to the total potential interactions within the system. Furthermore, quantifies the degree of dissimilarity between the interactions of each species compared to those of other species in the network. Finally, H_2' is unaffected by the network's size or the number of observations per species (Blüthgen et al., 2008). The significance of the modularity (Qw), nestedness (NODF), and specialisation (H_2') was assessed by comparing the observed values to those obtained by the null model (*bipartite* R-package). The significance of metric values was determined based on non-overlapping with the 95% confidence intervals of randomized values. While no formal statistical tests were executed to compare metric values across the different networks, these methodologies align with those employed in prior research studies (Genini et al., 2010; Maruyama et al., 2015; Ballantyne et al., 2015; Mesquita-Neto et al., 2018). Thus, we calculated the species strength and species-level specialisation (d') within networks. We conducted a *t-test* to compare d' and species strength metrics between the VN, FPN, and MPN networks. It is important to highlight that statistical comparisons of d' and species strength within the DN and RN network were not undertaken, as this network lacked a measure of visitor interaction frequency. It is noteworthy that the generality of visitor species, in conjunction with the generality of plant species, quantifies the average number of species with which a plant or visitor group engages in direct interactions. All analyses were performed using the R software (in R Core Team v. 4.2.1, 2023).

RESULTS

Plant-bee interaction and bee pollination effectiveness

We recorded a total of 1,838 interactions between 33 bee species and 10 plant species from the *Chamaecrista* genus during the sampling period. *Bombus pauloensis* was the most frequent bee (25% of the total bee visitation), however, the number of visits varied between plant species. When focusing on each functional group separately, we observed that the most frequent bee behaviour of flower manipulation was vibration. More specifically, *flower-buzzing* and *anther-buzzing* functional group corresponded to 79% of the total visitation frequency. The functional groups presented different patterns of pollen removal and depositionfor

Chamaecrista species (Figure 2). Bees categorised as *flower-buzzing* deposited the highest quantities of pollen on the stigma surface (24.4 ± 14.8; mean and s.d. respectively; Figure 2a). While bees categorised as *thieves* and *robbers* deposited the smallest quantity of pollen grain (3.6 ± 3.3). On the other hand, for pollen removal, *robber* bees were the group that removed the highest pollen amount from the anthers (25671.2 ± 2418.3) whereas *thieves* bees removed smaller quantities (141.22 ± 320.4) (Figure 2b).

Different species within the same functional groups cluster together and exhibit similar efficiencies in mediating both female and male plant performances (Figure 3). Species belonging to the *robber* functional group were more efficient in removing pollen from the anthers of *Chamaecrista* species within the studied community. Species in this functional group cut the anthers and can remove nearly all the pollen. Bees in the *theft* functional group mediated less plant male efficiency. However, when assessing female performance, we observed that species in the *flower-buzzing* group were the most efficient, following by *anther-buzzing*. Conversely, *robber* and *thieves* bees were the groups with lower efficiency in depositing pollen on the flowers of the evaluated species. Furthermore, the results indicate that despite significant differences between functional groups in pollen removal and deposition, species overall show limited efficiency in transporting pollen grains within this system.

Plant-bee networks

The network metrics differed among the interaction networks according to the different currencies (Table 1; Figure 4 and 5). The VN network (visitation frequency data) exhibited a moderate level of specialisation and modularity (Figure 4a and 5). In contrast, the DN network (pollen grain deposition data) demonstrated slightly more generalisation and nestedness than others (Figure 4b and 5). The RN network (pollen grain removal data; Figure 4c) presented a marginally greater specialisation degree compared with DN network but, on the other hand, exhibited slightly more generalisation than VN network (Figure 5). Further, the FPN network (visitation frequency and deposition data combined; Figure 4d and 5) demonstrated a reasonable level of specialisation and nestedness compared to RN and DN networks. Nevertheless, FPN network was more generalist than the VN network. Finally, notably, when visitation frequency and pollen release data were combined, the resulting MPN network (Figure 4e), exhibited slightly more specialisation and modularity, but less nestedness than all the other networks (Figure 5). Additionally, when we compare the individual visitor performance throughout the networks, it is possible to notice that it changes between networks. For example, *Bombus* *pauloensis* deposits low average numbers of pollen grains, but their high frequency of visitation results in much higher female performance values. Similarly, this pattern can be observed in the male components' networks (RN and MPN). The generality of bees did not differ significantly among visitor groups. Plant generality was higher in the DN network compared to the others. Most visitors deposited similar amounts of pollen per visit thus contributing equally to the metric. Finally, plant generality was lower in the FPN and MPN network compared to VN, DN and RN networks.

Regarding species-level specialisation, the VN, MPN, and FPN networks did not differ significantly for plants or bees. This result was also observed concerning the strength of interactions between the VN and MPN networks for plants and between the VN and FPN or VN and MPN networks for bees. However, the strength of interactions was higher in the female performance network than visitation frequency network for plants ($t = 6.35$; $p<0.001$), as well as between female and male performance networks for plants ($t = 7.25$; $p<0.001$) and for bees ($t = -2.35$; $p<0.05$).

DISCUSSION

Here we demonstrate that different components of plant-pollinator interaction affects our interpretation of species roles and network structure, as hypothesised. More specifically, plant male and female performances varied widely among pairwise interactions and when considering the bee functional groups. The predominant pollination partners were bees belonging to the *flower-buzzing* functional group, and they conducted a notably higher number of legitimate visits. Moreover, network-level metrics differed between the networks built using distinct currencies. The pollen removal data combined with visitation frequency, i.e. male performance network, produced more specialised and modularity networks. On the other hand, female performance and frequency networks produced more generalised networks. Thereby, by evaluating different components of interactions we most clearly illustrated the different contributions of different bee species to the *Chamaecrista* species.

Bee pollination effectiveness

Floral visitors to *Chamaecrista* were highly dissimilar considering the frequency of visitation, and the capacity of pollen deposition and removal. A similar pattern was found in other studies that evaluated different species of buzz-pollinated flowers and the performances of bees (Mesquita-Neto et al., 2018, 2021; Barbosa et al., 2023). It is known that distinctive characteristics such as differences in behaviour, vibration characteristics, flower handling time, or bee size can influence interactions and impact the performance of bee species on male and female components of pollination (De Luca et al., 2013; Solís-Montero & Vallejo-Marín, 2017; Mesquita-Neto et al., 2018, 2021; Barbosa et al., 2023).

Species from the *flower-buzzing* functional group were the most recorded, deposited the highest number of pollen grains, and were capable of removing a high number of pollen grains in a single visit. These bees, such as *Bombus* and *Xylocopa* species, are large, spend less time at the flower manipulating the anthers, and make shorter vibrational pulses (Mesquita-Neto et al., 2019). These bee species are capable of effectively adjusting pollen-collecting behaviour to quickly extract large amounts of pollen from poricidal anthers (Buchmann, 1983; Burkart et al., 2014; De Luca et al., 2013, Mesquita-Neto et al., 2021). Besides that, by vibrating the flowers, these bees touches with the stigma on most visits, as their body size usually exceeds the gap between the anthers and the stigma, thus contacting both reproductive structures simultaneously (e.g. Solís-Montero & Vallejo-Marín, 2017; Barbosa et al., 2023). Considering that the vibratory behaviour of bees is functionally specialised (Vallejo-Marín, 2019), bees that vibrate flowers while embracing all or most of the anthers are more likely to promote pollination. Additionally, it has been demonstrated that large bees manipulate poricidal anthers much faster and are capable of visiting more flowers than small bees (Mesquita-Neto et al., 2021). Thereby, they promote the important cross-pollination among plant individuals. Thus, body size and behaviour can explain the higher performance of these bees. This also means that the absence of these bees would certainly impact negatively on the reproductive success of *Chamaecrista,* making these species the key-pollinators in the buzz pollination system.

The *anther-buzzing* functional group was the second most prevalent and capable group in depositing and removing pollen from the flowers. These bees are typically smaller, produce longer vibrational pulses on anthers separately each time, and spend more time overall on flowers (e.g. Barbosa et al., 2023). Additionally, they were not able to vibrate all anthers together therefore removing less pollen (e.g. Mesquita-Neto et al., 2018). Because they are smaller, they have less contact with the stigma, making them less efficient in pollinating poricidal anther plants (Renner, 1983; Luo et al., 2009; Liu & Pemberton, 2009; Wanigasekara & Karunaratne, 2012; Mesquita-Neto et al., 2018). Because of these characteristics on removal and deposition of pollen, they exhibit lower efficiency in pollination compared to *buzzing-flower* species. However, other studies suggested that some smaller bees show behaviours that potentially compensate for poor fit with the flower and achieve levels of pollen release and deposition like larger flower-buzzing bees (De Luca et al., 2013; Rosi-Denadai et al., 2020; Barbosa et al., 2023). For instance, it is known smaller bees, such as *Augochloropsis* and *Melipona* species, spend more time manipulating the anthers and changing their position during the vibration (see Mesquita-Neto et al., 2021). Thus, this makes it possible for these bees to deposit and remove pollen to levels almost similar to bumblebees (see De Luca et al., 2013; Rosi-Denadai et al., 2020). Moreover, just by vibrating, combined with the specialised floral morphologies in *Chamaecrista*, pollen deposition on the stigma can occur even without the bee directly contacting the stigma. Therefore, anther vibrating bees are still more efficient than non-vibratory bees, acting as secondary pollinators.

On the other hand, the non-vibratory bees are described as antagonistic in buzz-pollination systems and often damage floral structures (De Luca & Vallejo-Marín, 2013; Mesquita-Neto, 2021). Particularly, species from the *robber* functional group always exhibited the behaviour of cutting the petals, decreasing the visual attractiveness of flowers (Hargreaves et al., 2009; Rego et al., 2018), as well as chewing the anthers to collect pollen. Because of this non-specifically behaviour of the buzz-pollination system, this pollen collected rarely is exported to stigmas (Mesquita-Neto et al., 2018, 2021). So, this destructive behaviour of anthers leads to the loss of available floral resources (i.e. pollen grain) and impacts male fitness. Moreover, it demonstrated that damage of modified petal, known as the cucullus, can result in the loss of the specialised pollination mechanisms mediated by floral traits in the buzz-pollination (Gottsberger & Silberbauer-Gottsberger, 1988; Costa et al., 2013; Nogueira et al., 2018). Species from *Trigona* bees, included in this group, are widely distributed and frequently observed in various species with pollen flowers (Renner, 1983; Gross, 1993; Biesmeijer & Slaa, 2006; Schlindwein et al., 2014; Rego et al., 2018; Mesquita-Neto et al., 2018). Despite apparent lower efficiency in removing pollen, these bees are capable of cutting all the anthers in a single visit while rarely touching the stigma. Considering that they are also very frequent visitors, robber bees can act as significant antagonists in the *Chamaecrista* system.

Finally, bees belonging to the pollen *theft* group generally do not cause damage to flower structures and are considered opportunists in this pollination system. Pollen thieves species typically exhibited the behaviour of collecting pollen from apical pores or pollen remaining on the petals. Thus, these bees are less efficient in extracting pollen and rarely they deposit pollen on the stigma, making them less efficient for pollination. Despite collecting pollen and acting as potential competitors for resources, their effect on the reproductive success of plant species is low or neutral.

The varied performance of different bee groups, ranging from key vibratory bees to opportunistic and even destructive ones, directly influences plant reproductive success in the community. These results underscore the importance of considering not only interaction frequency but also specific bee characteristics and behaviours to fully comprehend the impact of pollinators on these plants.

Plant-bee networks

Previous studies suggest that networks based on effectiveness components are more specialised than the ones considering only visitation frequency, as not all floral visitors are truly effective pollinators even when apparently contacting the reproductive structures (King et al., 2013; Ballantyne et al., 2015; Santiago-Hernández et al., 2019). However, our results contradict these findings, as networks constructed from female components of pollination (i.e., pollen deposition and pollination performance) varied little on overall metrics and were even slightly more generalist than the one based on visitation. Such small differences can be explained by a similar quantity of pollen grains deposited on the stigmas and similar behaviour of most bee floral visitor's species. First, the *Chamaecrista* species have a highly specialised pollination system (i.e. buzz-pollination) and specific morphological adaptations, even though there are considerable variation in size and the morphology of the petals/anthers, so there is some uniformity in the interactions across species (Gottsberger & Silberbauer-Gottsberger, 1988; Arceo-Gómez et al. 2011; Almeida et al. 2013a, 2013b; Nogueira et al. 2018). Such intimate interaction, subject to strong selective pressures on the traits of both bees and flowers to optimise pollination may imply a small variation in the female performance across pairwise interactions (Solís-Monteiro et al., 2017; Mesquita-Neto et al., 2021). For example, in *Senna* (Fabaceae) asymmetrical petals direct pollen to specific regions of the bee's body and increase the probability of successful pollen deposition (Amorin et al., 2017). Moreover, other buzz-pollinated species also have floral structures (i.e. bracteoles, sepals, and connectives) that direct the streams of pollen cloud towards the pollinator's body and optimise pollination (Endress, 1994; Houston & Ladd, 2002; Huang and Shi, 2013). Such structures are examples of the specialised morphological adaptation found in this system. Second, most bee species position themselves enclosed in the flowers during anther vibration, providing the necessary matching to ensure contact with the reproductive structures of the flowers (Vallejo-Marín, 2022; Barbosa et al., 2023). 80% of the recorded bees interacting with *Chamaecrista* exhibited vibratory behaviour during flower manipulation. This behaviour may be sufficient to ensure pollen deposition on stigmas during flower manipulation by different species of bees. Therefore, the vibratory behaviour observed in most bee visits may have conferred similarity in the amount of pollen deposition in plant species and consequently led to little change in the overall network metric.

On the other hand, the male components of pollination (i.e. pollen release and pollination performance) reinforce that networks based on qualitative components are more specialised than frequency networks. When plant species are visited by multiple pollinators or functional groups, performance between floral visitors will likely differ (Pearson et al., 2023; Weinman et al., 2023), leading to variations in interaction network metrics (King et al., 2013; Ballantyne et al., 2015; Mesquita-Neto et al., 2018; Santiago-Hernández et al., 2019). Thus, male performance varies more than female performance in the studied system. Additionally, when pollen is the only floral resource, plants confront the challenge of mitigating excessive pollen loss while ensuring continued attractiveness to pollinators (Opedal et al., 2023). Consequently, floral strategies have evolved to limit pollen access, making it potentially available only to specific pollinator groups likely to ensure successful species pollination (Ashman and Morgan, 2004; Ashman et al. 2004). Restricting pollen in poricidal anthers is reported as one of these plant species' strategies to prevent pollen loss (Buchmann, 1983). In this scenario, the pollen stored within anthers can only be released, in theory, by bees capable of performing specific vibrations (Vallejo-Marín, 2022). This may limit and/or result in low pollen removal by some groups of floral visitors who can not access this pollen through vibrations. The intimate association between buzz-pollinated flowers and some vibratory bees (Buchmann, 1983) may clarify the observed variations in metrics and the increased specialisation, even low, observed compared to other networks.

Despite showing some different patterns considering the type of data used, our results demonstrate that visitation networks may underestimate levels of specialisation between plants and their pollinators, especially by considering seldom-used male performance measures. Furthermore, the networks including qualitative components bring more accurate estimates of the quality of plant-pollinator interactions and bring a more realistic approach to pollination systems. Other studies show that frequent visitors may even have a negative impact (Vázquez et al., 2012), while less common visitors may play a more effective role in reproductive success (Sakamoto et al., 2012). Our results reinforce that analysing plant-pollinator interactions based only on visitation frequency may not provide an accurate understanding of the pollinator's importance in pollination systems. Thus, the incorporation of data on pollinator effectiveness into network construction will contribute to understanding the quality of interactions between plants and pollinators, and the complexity of different pollination systems.

CONCLUSION

Considering the multifaceted role of bee pollination effectiveness within *Chamaecrista* species and the diverse functional groups of bees involved in these pollination systems, our comprehensive analysis highlights the nuanced impact of various bee species on plant reproductive success. Specifically, the assessment of pollination effectiveness by a floral visitor is essential to comprehend its impact on the reproductive success of plants. Obtaining a precise measure of this effectiveness is crucial for understanding the ecology and evolution of interactions between plants and pollinators. By examining the variation in pollination effectiveness, it is possible to identify which floral visitors play the role of true pollinators, while others provide minimal benefits or act as nectar thieves. This understanding is crucial for determining which pollinators genuinely contribute to the fitness of plants, including both male and female components, thereby enhancing the reliability and predictive power of network analyses in studies of plant-pollinator interactions. Furthermore, this information can provide valuable insights into the intensity of selective pressures exerted by each floral visitor, fundamental for understanding the evolutionary aspects of specialised pollination systems, such as buzz-pollination. Therefore, we suggest that future studies consider integrating distinct qualitative measures of interactions, along with quantitative measures, to assess the structure and functioning of pollination systems characterised as ecological networks.

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TABLE

Table 1. Values of network metrics for visitation frequency, pollen deposition, pollen release, and female and male performance networks.

	Network				
Network metric	Visitation frequency network (**VN**)	Deposition network (**DN**)	Release network (**RN**)	Female performance network (**FPN**)	Male performance network (**MPN**)
Specialisation (H_2')	0.40	0.31	0.35	0.38	0.45*
Nestedness (NODF)	53.70	50.49	54.35	46.86	54.39
Modularity (Qw)	0.40	0.31	0.35	0.39	0.44*
Generality					
Plant	8.89	11.04*	8.64	6.42	4.75*
Bees	4.78	5.16	5.45	4.76	4.58

FIGURE CAPTION

Fig. 1. Flowers and floral visitors. (A - A1) *Chamaecrista ramosa* and its' floral visitor *Melipona quinquefasciata*. (B - B1) *Ch. rotundifolia* and its' floral visitor *Dialictus* sp. 13. (C - C1) *Ch. flexuosa* and its' floral visitor *Augochlora* sp. 05. (D - D1) *Ch. ramosa* var. 1 and its' floral visitor *Augochloropsis* sp. 50. (E - E1) *Ch. debilis* and its' floral visitor *Bombus pauloensis*. (F - F1) *Ch. hedysaroides* and its' floral visitor *B. morio*. (G - G1) *Ch. bracteolata* and its' floral visitor *Eufriesea nigrohirta*. (H - H1) *Ch. nictitans* and its' floral visitor *Paratrigona subnuda*. (I - I1) *Ch. desvauxii* and its' floral visitor *Augochloropsis* sp. 50. (J - J1) *Ch. distichoclada* and its' floral visitor *Trigona spinipes*.

Fig. 2. Box plots showing the bee functional groups considering. (A) The quantity of single-visit pollen deposition of plant species. (B) The quantity of single-visit pollen removal of plant species. Bars are coloured according to the bee functional group on *Chamaecrista* species: thieving bees (orange), robber bees (green), anther-buzzing bees (purple), and flower-buzzing bees (pink). Different letters indicate significant differences ($p \leq 0.01$).

Fig. 3. The effectiveness landscape representation considering the male and female performance of pollination of bee visitors to *Chamaecrista* species. The colours represent the functional groups for bees, as indicated within the plot.

Fig. 4. Bipartite networks illustrating the interactions among *Chamaecrista* species and bee visitors. (A) Frequency network. (B) Pollen deposition network. (C) Pollen release network. (D) Female performance network; and (E) Male performance network. Gray lines represent species interaction and line thickness indicates the strength of interaction. The colours represent the bee functional group. Key code: visitor group (1) *Apis mellifera*. (2) *Augochlora* sp. 05. (3) *Augochloropsis* sp. 49. (4) *Augochloropsis* sp. 50. (5) *Augochloropsis* sp. 51. (6) *Augochloropsis* sp. 52. (7) *Augochloropsis* sp. 53. (8) *Bombus morio*. (9) *Bombus pauloensis*. (10) *Caenohalictus* sp. 01. (11) *Centris fuscata*. (12) *Centris tarsata*. (13) *Centris trigonoides*. (14) *Ceratina minima*. (15) *Dialictus* sp. 13. (16) *Eufriesea nigrohirta*. (17) *Exomalopsis fulvofasciata*. (18) *Exomalopsis tomentosa*. (19) *Melipona quinquefasciata*. (20) *Oxaea* sp. 01. (21) *Paratetrapedia lineata*. (22) *Paratrigona subnuda*. (23) *Pseudaugochlora flammula*. (24) *Pseudaugochlora graminea*. (25) *Ptiloglossa* sp. 05. (26) *Tetragonisca angustula*. (27) *Thectochlora alaris*. (28) *Trigona braueri*. (29) *Trigona spinipes*. (30) *Xylocopa fortuita*. (31) *Xylocopa hirsutíssima*. (32) *Xylocopa subcyanea*. (33) *Xylocopa truxali*. Plant species (A) *Chamaecrista hedysaroides*. (B) *Ch. bracteolata*. (C) *Ch. nictitans*. (D) *Ch. ramosa* var. 1. (E)

Ch. debilis. (F) *Ch. desvauxii*. (G) *Ch. flexuosa*. (H) *Ch. ramosa* var. 2. (I) *Ch. distichoclada*. (J) *Ch. rotundifolia*.

Fig. 5. Box plots of metrics of each network. (A) Level of specialisation (H_2'). (B) Level of nestedness (NODF). (C) Level of modularity (Qw). (D) Generality of plant and bee species.

FIGURES

Fig. 1.

Fig. 2.

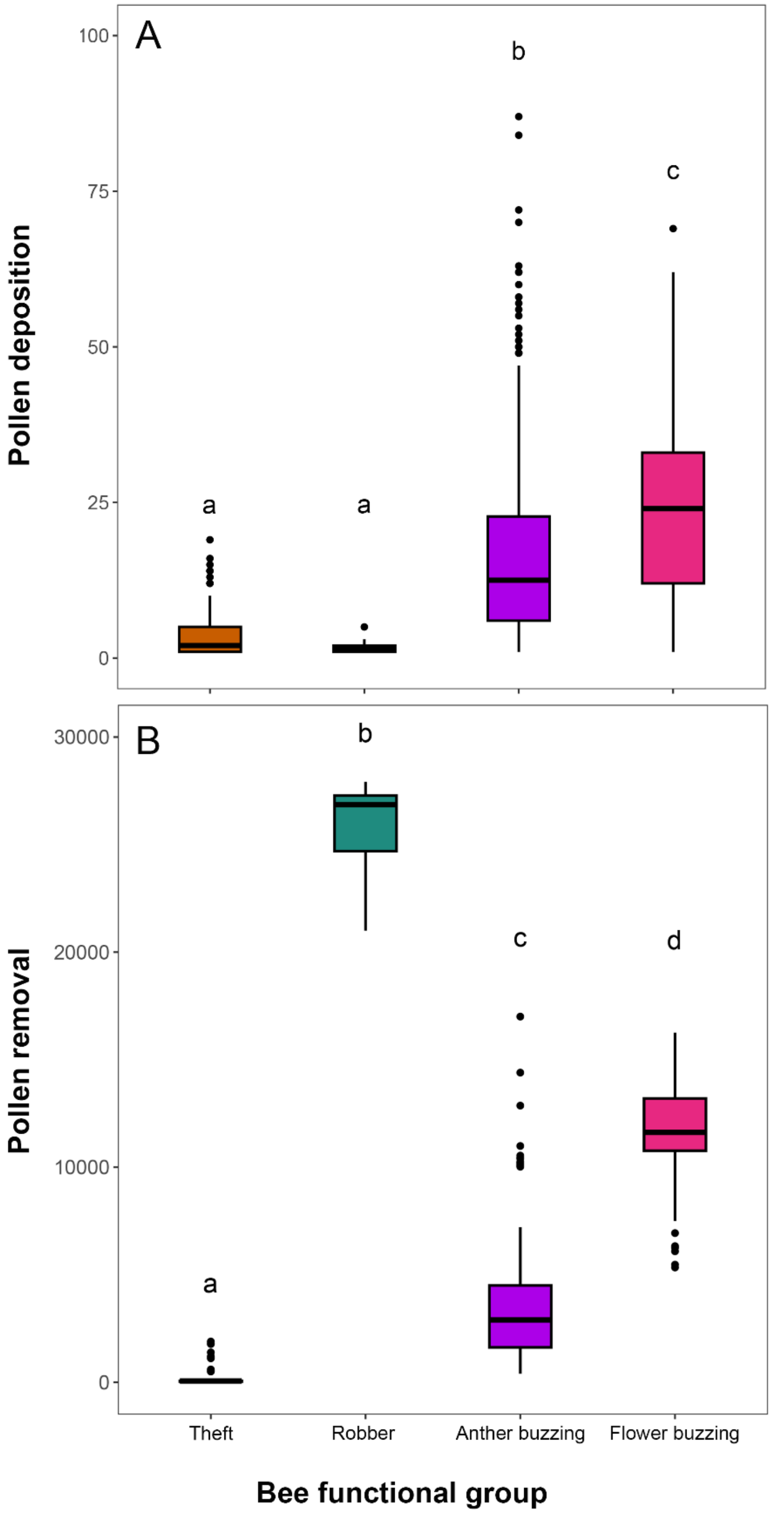

A
100
75
50
25
0
Pollen deposition
a
a
b
c
B
30000
20000
10000
0
Pollen removal
a
b
c
d
Theft
Robber
Anther buzzing
Flower buzzing
Bee functional group

Fig. 3.

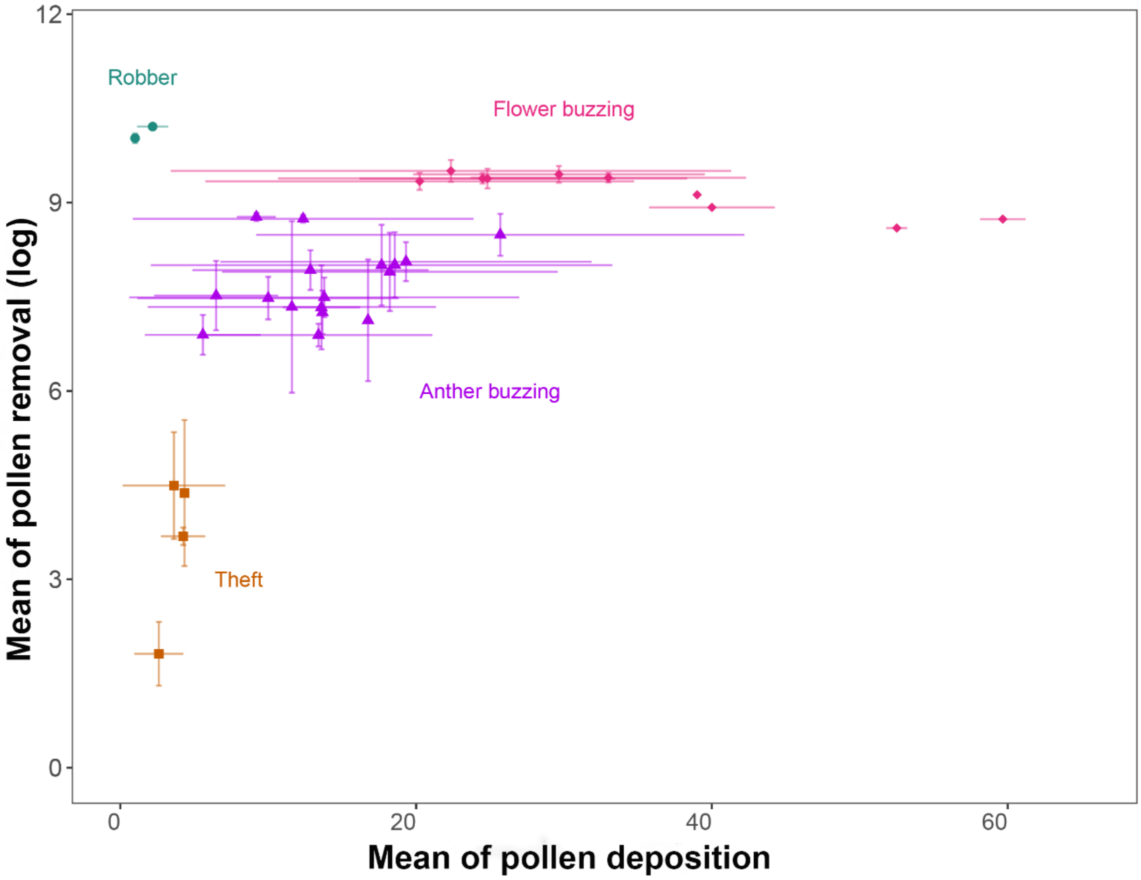

Robber
Flower buzzing
Anther buzzing
Theft
Mean of pollen removal (log)
Mean of pollen deposition
12
9
6
3
0
0
20
40
60

Fig. 4.

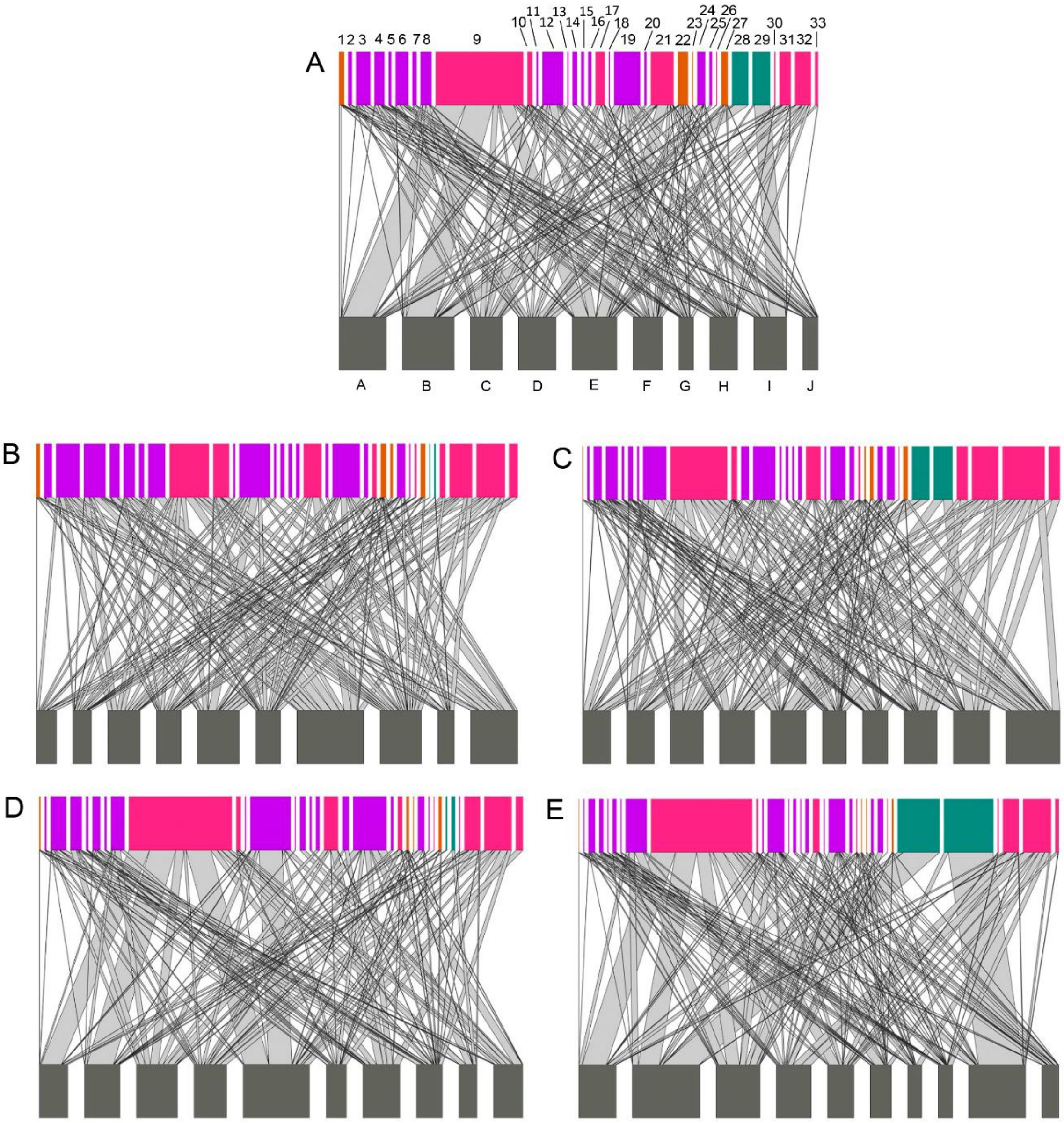
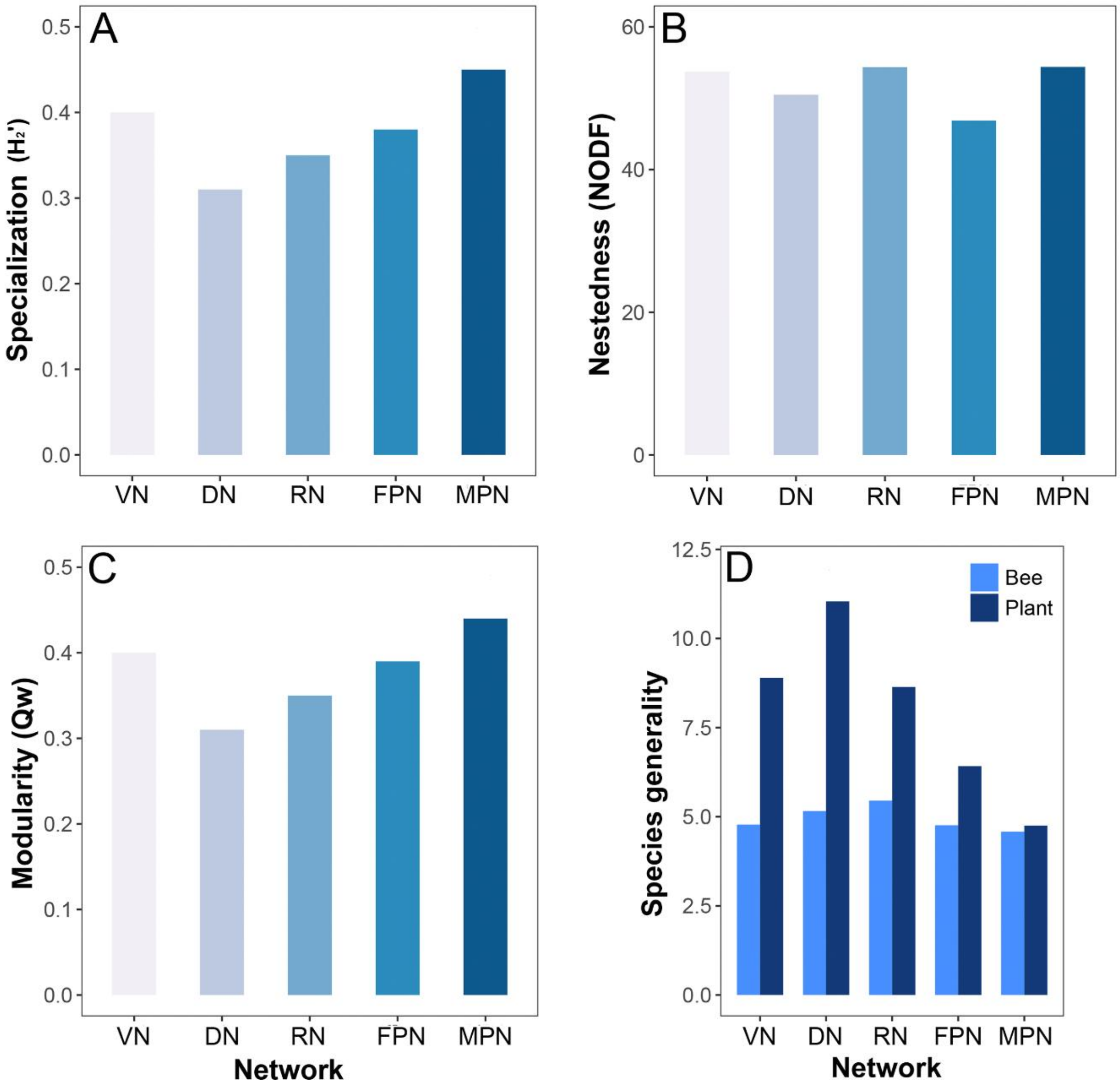

A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33
A B C D E F G H I J
B
C
D
E

Fig. 5.


A
Specialization (H2')
0.0
0.1
0.2
0.3
0.4
0.5
VN
DN
RN
FPN
MPN
B
Nestedness (NODF)
0
20
40
60
VN
DN
RN
FPN
MPN
C
Modularity (Qw)
0.0
0.1
0.2
0.3
0.4
0.5
VN
DN
RN
FPN
MPN
Network
D
Species generality
0.0
2.5
5.0
7.5
10.0
12.5
Bee
Plant
VN
DN
RN
FPN
MPN
Network

CONCLUSÕES GERAIS

As conclusões dos estudos da tese destacam coletivamente as diversas facetas das interações entre plantas e polinizadores e as complexidades subjacentes que influenciam esses relacionamentos. As análises do primeiro capítulo revelam uma enorme diversidade de interações dentro de um país tropical, além de esclarecer os vieses taxonômicos e biogeográficos em estudos de polinização. De acordo com nossos resultados, as interações entre plantas e polinizadores foram mais estudadas nos biomas da Mata Atlântica e do Cerrado. Além disso, e não surpreendentemente, a polinização por abelhas foi o sistema de polinização mais relatado na maioria dos biomas. Assim, essas descobertas enfatizam a importância de alguns grupos específicos de polinizadores e plantas dentro da imensa biodiversidade do Brasil, revelando um continuum de associações generalizadas a especializadas. Além disso, elas revelam a necessidade de direcionar os esforços de pesquisa para mitigar vieses e aprofundar a compreensão das tendências observadas nos sistemas de polinização. No segundo capítulo, nossa investigação demonstrou que em espécies de Melastomataceae, o dimorfismo dos estames está relacionado com a forma, tamanho e variação desses traços nas pétalas e no estilete. Além disso, descobrimos que esses traços florais podem determinar a especialização das espécies de plantas no nível da comunidade. Assim, esses resultados destacam o papel potencial da morfologia floral no estabelecimento das interações, especialmente entre flores polinizadas por vibração e abelhas capazes de vibrar. Por fim, o terceiro capítulo, com a análise abrangente da eficácia da polinização por abelhas, enfatiza a importância de avaliar o impacto dos visitantes florais no sucesso reprodutivo das plantas. Demonstramos que diferentes componentes da interação entre plantas e polinizadores podem influenciar nossa interpretação dos papéis das espécies e da estrutura da rede. Além disso, o desempenho masculino e feminino das plantas varia amplamente entre interações de pares. Essa compreensão é vital para distinguir os polinizadores mais importantes de outros visitantes com eficácia variável, contribuindo para o aprimoramento da confiabilidade e do poder preditivo da rede. Em última análise, uma abordagem geral, para preencher lacunas de conhecimento e avançar nossa compreensão de diversos sistemas de polinização é essencial combinar observações detalhadas da história natural dor organismos, incluindo metodologias padronizadas, dados comportamentais aprofundados e um foco na eficácia dos mutualismos.

GENERAL CONCLUSIONS

The conclusions from the studies in the thesis collectively underscores the diverse facets of plan-pollinator interactions and the underlying complexities influencing these relationships. The first chapter analyses reveal a huge diversity in interactions within a tropical country, as well as shedding light on taxonomic and biogeographic biases in pollination studies. According to our results, the plant-pollinator interactions were most studied in the Atlantic Forest and Cerrado biomes. Besides that, and, not surprisingly bee pollination was the most reported pollination system across the majority of biomes. Thus, these findings emphasize the importance of some specific pollinator and plant groups within the huge biodiversity in Brazil, revealing a continuum from generalised to specialised associations. Moreover, these reveal the need to redirect research efforts to mitigate biases and delve deeper into understanding the trends observed in pollination systems. In the second chapter, our investigation demonstrated that in Melastomataceae species the stamen dimorphism is linked to petals and style shape, size as well as the variation in these traits. Moreover, we found that these floral traits may determine the specialisation of plant species at the community level. Thus, these results highlight the potential role of floral morphology on establishment of interactions, especially between buzz-pollinated flowers and vibratory bees. Finally, the third chapter with the comprehensive analysis of bee pollination effectiveness emphasizes the importance of assessing the impact of floral visitors on plant reproductive success. We demonstrate that different components of plant-pollinator interaction can influence our interpretation of species roles and network structure. Besides, plant male and female performances vary widely among pairwise interactions. This understanding is vital for discerning the most important pollinators from other visitors with varying effectiveness, contributing to the refinement of network reliability and predictive power. Ultimately, a more detailed approach, combining detailed natural history observations, including standardised methodologies, in-depth behavioural data, and a focus on pollination effectiveness, is essential to fill knowledge gaps and advance our comprehension of diverse pollination systems.

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