UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
Campus de Botucatu

RELAÇÃO ENTRE O TAMANHO FLORAL E AS ESTRATÉGIAS REPRODUTIVAS EM PLANTAS COM FLORES COM ANTERAS PORICIDAS

TAMIRIS DAIANE DELGADO DE LIMA

Tese apresentada ao Instituto de Biociências, Campus de Botucatu, UNESP, para obtenção do título de Doutora junto ao Programa de Pós-Graduação em Ciências Biológicas Botânica, Área de concentração Ecologia Vegetal.

Botucatu- SP
2023

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"Dedico este trabalho aos meus avós paternos e maternos, "In Memorian", por me ensinar a importância do estudo, pela existência de meus pais, pois sem eles este trabalho e muitos dos meus sonhos não se realizariam."

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Sumário

LIMA, T. D. D. RELAÇÃO ENTRE O TAMANHO FLORAL E AS ESTRATÉGIAS REPRODUTIVAS EM PLANTAS COM FLORES COM ANTERAS PORICIDAS, 2023. 100p. TESE (DOUTORADO) – INSTITUTO DE BIOCIÊNCIAS, UNESP – UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO", BOTUCATU.

Resumo - A interação planta-polinizador é uma troca mutualista em que as plantas oferecem recursos florais em troca de serviços de polinização. Essa relação envolve custos para as plantas, levando-as a desenvolver estratégias para maximizar a eficácia de suas ofertas, como a publicidade das vantagens da visita floral por meio de características morfológicas específicas. O tamanho floral é uma dessas características cruciais, influenciando a atratividade visual e a quantidade de recompensa disponível para os polinizadores. Esta tese visa ampliar o entendimento da relação entre o tamanho floral, atração de visitantes florais e estratégias reprodutivas em plantas com anteras poricidas. No Capítulo 1, fizemos uma revisão que buscar entender como o tamanho floral de espécies de plantas com flores com anteras poricidas influencia a diversidade e a composição dos visitantes florais, esperamos que flores maiores atraiam maior diversidade de visitante e maior número de grupos funcionais de polinizadores. Além disso, prevê-se que flores maiores possam atrair mais abelhas visitantes, tanto polinizadoras efetivas quanto antagonistas. No Capítulo 2, utilizando uma abordagem meta-analítica, examinamos como o tamanho e a morfologia floral afetam a estratégia reprodutiva, esperando que plantas com flores maiores dependam mais da polinização por vibração e enfrentem maior limitação polínica em comparação com plantas de flores menores. E esperamos também que espécies com mais de um recurso floral possam ter uma menor limitação polínica.

Palavras-chave: Evolução floral, polinização por vibração, flor de pólen, apomixia, polinização por abelhas, tamanho floral, sistemas de acasalamento, anteras poricidas

LIMA, T. D. D. RELATIONSHIP BETWEEN FLORAL SIZE AND REPRODUCTIVE STRATEGIES IN PLANTS WITH PORICIDAL ANTHERS, 2023. 100p. TESE (DOUTORADO) – INSTITUTO DE BIOCIÊNCIAS, UNESP – UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO", BOTUCATU.

Abstract - The plant-pollinator interaction is a mutualistic exchange where plants offer floral resources in exchange for pollination services. This relationship involves costs for the plants, leading them to develop strategies to maximize the effectiveness of their offerings, such as advertising the advantages of floral visits through specific morphological characteristics. Floral size is one of these crucial characteristics, influencing visual attractiveness and the amount of reward available to pollinators. This thesis aims to broaden the understanding of the relationship between floral size, attraction of floral visitors, and reproductive strategies in plants with poricidal anthers. In Chapter 1, we conducted a review to understand how the floral size of species with flowers with poricidal anthers influences the diversity and composition of floral visitors. We expect that larger flowers attract greater diversity of visitors and a larger number of functional groups of pollinators. Additionally, it is anticipated that larger flowers may attract more visiting bees, both effective pollinators and antagonists. In Chapter 2, using a meta-analytical approach, we examined how floral size and morphology affect the reproductive strategy, expecting that plants with larger flowers rely more on vibration pollination and face greater pollen limitation compared to plants with smaller flowers. We also expect that species with more than one floral resource may have lower pollen limitation.

Keywords: Flower evolution, buzz-pollination, pollen flower, apomixis, bee pollination, flower size, mating systems, poricidal anthers

Introdução geral

A interação planta-polinizador é uma relação mutualista que pode ser pensada como um mercado biológico de troca (Ollerton, 2007). Neste caso, as plantas ofertam os recursos florais como néctar, óleo, resina, odor e pólen aos seus parceiros polinizadores, que em troca, facilitam o transporte dos gametas masculino até o estigma para que a reprodução sexuada das plantas ocorram (Bronstein, 2001). Existem benefícios e custos associados a esse sistema de troca de produtos por serviços. A produção do recurso floral é custosa para as plantas, e estas utilizam diferentes estratégias para minimizar esse custo. Uma das estratégias que as plantas utilizam para minimizar o custo de produção do recurso floral é a propaganda das possíveis vantagens que o visitante pode ter ao realizar a visita floral (Latty and Trueblood, 2020). Essa propaganda se dá por características morfológica das flores como a forma, tamanho, simetria, cor e perfumes (Goulson, 1999; Fenster et al., 2006). Portanto, algumas características da flor podem sinalizar para o polinizador o quão vantajosa pode ser a realização da visita.

Neste contexto, o tamanho da flor é uma característica essencial pois está diretamente associando com o quão a flor pode ser atrativa aos visitantes florais. Em geral, flores grandes são mais atraentes visualmente ao polinizadores quando comparada a flores pequenas (Eckhart, 1999). Além disso, o tamanho da flor também pode refletir em grande parte a quantidade de recompensa disponível aos visitantes florais (Fenster et al., 2006). Outro aspecto bastante descrito na literatura é que o tamanho floral influencia diretamente a evolução do sistema de acasalamento e o sistema reprodutivo (Sargent et al., 2007; Goodwillie et al., 2010). Finalmente, variações do tamanho floral podem favorecer o isolamento reprodutivo de algumas plantas na população, até mesmo causando a especiação em alguns casos (Krizek and Anderson, 2013). Há estudo que relatam que a variação no tamanho da flor pode ser uma barreira reprodutiva mais importante do que a cor da flor (e.g. Orchidaceae; Schiestl and Schluter, 2009). Portanto o tamanho floral é um descritor essencial associado a diferentes estratégias reprodutivas das plantas, influenciando diretamente a atratividade aos polinizadores, e por consequência, no seu sucesso reprodutivo.

Nesse sentido, esta tese tem como objetivo ampliar o nosso sobre a relação entre o tamanho floral, atração de visitantes florais e a estratégia reprodutiva em plantas que produzem flores com anteras poricidas. No Capítulo 1, investigamos em um trabalho de revisão a relação entre o tamanho floral de espécies com flores de pólen com anteras poricidas e a assembleia de visitantes florais. Especificamente, respondemos três perguntas: como o tamanho floral influencia: (i) a diversidade e (ii) a composição de visitantes florais? e (iii) como o tamanho das flores está relacionado com o tamanho e comportamento da espécie de abelha mais frequente nas flores de cada espécie? De acordo com nossas perguntas, esperamos que as espécies de plantas com flores maiores sejam visitadas por uma maior diversidade de visitantes florais, com maior diversidade funcional quando comparadas com espécies de plantas com flores pequenas. De forma não excludente, esperamos também que quanto maior a flor, maior seja o número de abelhas visitantes, sejam elas polinizadoras efetivas (i.e. que vibram as anteras) ou antagonistas (i.e. que não vibram as flores). Finalmente, nós esperamos ainda que a espécie de abelha mais frequente tenha um tamanho corporal maior em espécies de plantas com flores maiores do que em flores menores. No capítulo 2, utilizamos uma abordagem meta-analítica para investigar como o tamanho e morfologia floral impactam a estratégia reprodutiva em espécies de plantas com flores com anteras poricidas na quais o pólen é o único ou principal recurso floral. Para isso, nós realizamos uma revisão quantitativa sistemática dos estudos de caso publicados que investigaram a estratégia reprodutiva das plantas com anteras poricidas por meio de experimentos de polinização. A partir desses estudos, nós respondemos duas perguntas: (i) Como o tamanho das flores, se relaciona (i) com o grau de dependência do serviço prestado pelos polinizadores e (ii) com a magnitude da limitação polínica em flores de pólen? Nossas hipóteses são que (i) plantas com flores maiores sejam mais dependentes da polinização por vibração por abelhas para sua reprodução, e (ii) tenham maior limitação polínica quando comparadas com espécies com flores menores, menos dependentes de polinizadores e menos limitadas polinicamente. De forma não excludente, esperamos também que espécies de plantas com mais de um recurso floral tenham menor limitação polínica.

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CAPÍTULO 1: **Flower size affects bee species visitation pattern on flowers with poricidal anthers across pollination studies**

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Abstract

Flower size is a crucial trait for pollinator attraction. In flowers with poricidal anthers, the plant constraints the pollinator's access to the flower's rewards by enclosing pollen grains in poricidal anthers. The pollen is only released by mechanical vibrations applied mainly by bee species during buzz pollination. Although buzz-pollinated flowers exhibit a large size variation across species, their influence over patterns of flower visitation by different pollinator species remains to be elucidated. To fill such gap, we asked how flower size influences flower visitor assemblage, especially bees, across plant species and how flower size is related to body size and vibrating behavior of the most frequent bee species. We hypothesized that the assemblage of animals, especially bees, visiting larger flowers have higher taxonomic and functional diversity than smaller flowers, and the most frequent bee species visiting larger flowers have larger body size and a mandatory vibrating behavior. To test this hypothesis, we conducted a systematic review of pollination studies that reported patterns of flower visitation in plant species with poricidal anthers, describing the relationship between flower size and flower visitor metrics. We found that large-flowered species were visited by three times more species than small-flowered species, including vibrating and non-vibrating bee species. Unlike our expectations, non-vibrating bees were largely absent from small-flowered species. The most frequent bee species visiting flowers, irrespective of flower size, exhibited vibrating behavior. The most frequent bee species visiting large flowers were twice the body size of those visiting small flowers, highlighting a morphological match between flowers and pollinators. Although larger flowers are more susceptible to interactions with exploiters, reproductive success should be favored owing to the higher probability of their interaction with vibrating, large-bodied bee species. Still, a reduction in flower size probably prevents interactions with non-vibrating bees, while favoring vibrating small-bodied bee species.

Keywords: Flower evolution, buzz-pollination, bee diversity, flower attraction, pollen flower, quantitative review.

1. Introduction

Flowers bear different synorganized structures that can protect gametes and flower rewards from antagonists, but attract potential pollinators (Armbruster, 2014; Diggle, 2014). The corolla, which can vary in size, shape, symmetry, color, and smell, commonly performs pollinator attraction (Fenster et al., 2006; Goulson, 1999). Other flower structures can also perform pollinator attraction, such as the calyx or the androecium (Bai et al., 2011; Gumbert et al., 1999). These organs alone, or together, can provide different cues indicating the location and quality of a pollinator's rewards, thus influencing the chances of flower visitation and, ultimately, effective pollination (Latty and Trueblood, 2020). Since most pollinators are visually oriented, pollination attraction is often based on the visual stimulus that also provides cues about flower rewards to pollinators (Chittka and Raine, 2006). Thus, a flower's visual attractiveness can indicate how rewarding a flower can be to a pollinator.

Flower size is positively associated with visual attractiveness to pollinators since it indicates the quantity of potential reward (Fenster et al., 2006), even though the flowers of some species are concatenated in inflorescence (e.g., Fisbein and Venable, 1996), so the unit of attraction is not always a single flower. Overall, plant species with large flowers are more attractive to pollinators, receiving higher visitation rates than plant species with smaller flowers (Eckhart, 1999). Numerous studies report this positive relationship between floral size and visitation rate, linking this increase in flower visitation with higher attractiveness and resources available in relatively large flowers (Ashman and Stanton, 1991; Campbell et al., 1991; Fausto et al., 2001). For instance, experimental studies have verified the preference of bees for artificial flowers with a higher nectar quantity commonly linked to flower size (e.g., Blarer et al., 2002). Also, a study with *Rosmarinus officinali* found that larger flowers were more attractive and offered more resources for bees than smaller flowers (Herrera, 2005). Therefore, it is expected that larger-flowered populations have higher visitation rates than smaller-flowered populations in the wild (e.g., Elle and Carney, 2003). Flower size associated with flower resources can play an essential role in pollen removal and deposition patterns by pollinators owing to its influence on pollinator attraction (Rademaker et al., 1997).

The relationship between flower size and pollinator attraction has been mostly recorded and quantitatively revised in flowers that produce nectar as the primary reward to pollinators (e.g., Ashman and Stanton, 1991; Elle and Carney, 2003; Fausto et al., 2001). However, this relationship is still poorly understood in plant species offering pollen grains as the primary reward to pollinators (hereinafter termed as poricidal flowers – Buchmann, 1983). Poricidal flowers are estimated to comprise 6 to 8% of all angiosperm species (Buchmann, 1983), including plant species bearing (i) traditional pollen flowers, defined as "flowers which offer a surplus of pollen as the only reward to their insect visitors, instead of nectar or other substances" (Vogel, 1978), and (ii) poricidal flowers that produce additional flower resources, such as nectar or oil (e.g., some Melastomataceae species; Brito et al., 2016; Kriebel and Zumbado, 2014). In these species, the consumption of pollen grains by flower visitors can directly compromise the pollination process and the reproductive outcome of these plants (De Luca and Vallejo-Marín, 2013; Vallejo-MarÍn et al., 2009). On the other hand, enclosing pollen grains in poricidal dehiscent anthers is an effective strategy for reducing conflict between flower visitors and plants by restricting the access of flower rewards to a narrower set of visitors able to vibrate the anthers and extract the pollen (i.e., buzz-pollination) (Goulson, 1999). These buzz-pollinators are mainly vibrating bee species that apply mechanical vibration indirectly with their flight muscles to poricidal anthers (De Luca and Vallejo-Marín, 2013; Vallejo-Marín, 2019). As a mechanical response to the bee's vibration, pollen grains are mostly immediately released from the flower through the terminal anther pores. Then, some pollen grains are actively captured by bees, while other pollen grains are dispersed on their bodies (Buchmann, 1983; Hrncir et al., 2008; Michener, 1962). Since poricidal flowers vary in flower size, reward quantity and visiting pollinator species, especially the bee assemblage, the relationship between such variables may be similar to that observed on nectar-producing flowers.

In *Solanum* species, small-flowered plants with poricidal anthers have reduced floral organs and resources, with lower spatial segregation between anthers and stigma and a lower pollen-ovule ratio than large-flowered species (Vallejo-Marín et al., 2014). Consequently, it has been suggested that smaller poricidal flowers, like nectar-producing species, are less attractive to pollinators and consequently attract a smaller bee assemblage than larger poricidal flowers (Vallejo-Marín et al., 2014). This pattern was also observed in Melastomataceae, in which species with larger flowers had a greater richness of visiting bees (Valadão-Mendes et al., 2022). An exciting aspect emerging from studies with poricidal flowers, in contrast to nectar-producing flowers, is the potential relationship between flower size and functional traits, especially vibrational features, of bee assemblages visiting such flowers. For instance, smaller- and larger-flowered poricidal species are more often visited by vibrating than non-vibrating bee species (Brito and Sazima, 2012; Souza et al., 2012; Valadão-Mendes et al., 2022), but more rarely by other functional groups (e.g., Fidalgo et al., 2018; Quakenbush, 2018; Souza et al., 2012). Furthermore, smaller poricidal Solanaceae flowers frequently interact with small-bodied bee species (Vallejo-Marín et al., 2014), which exhibit a lower ability for pollen extraction in comparison to the large-bodied bees (Stout, 2000). Also, bee species that cannot vibrate the poricidal anthers (e.g., *Trigona*) are often found on larger-flowered species (Figueiredo et al., 2009; Nogueira et al., 2018). This pattern is probably recurrent, given the greater number of resources in larger flowers, which allows a greater assemblage of visiting bees, including non-vibrating bees as antagonists (e.g., Nogueira et al., 2018; Saab et al., 2021). Therefore, flower size can be related to the general attractiveness of poricidal flowers to visitors and the patterns of flower visitation by animals differing in their capacity as pollinators.

This systematic review aimed to investigate the relationship between flower size and pollinator assemblage across plant species bearing poricidal flowers. We asked (i) how flower size influences the assemblage of bee species visiting the flowers of plant species bearing poricidal anthers; and (ii) how flower size is related to the size and vibrating behavior of the most frequent bee species visiting the flowers of different plant species with poricidal anthers. We hypothesized that large-flowered species are visited by more species than small-flowered species, leading to a more diverse visitor assemblage in species with large flowers, including both vibrating and non-vibrating species, pollinators and antagonists, respectively. We also hypothesized that large-flowered species are more likely to be visited by large-bodied bee species exhibiting a mandatory sonication than small-flowered species. To evaluate these hypotheses, we first conducted a systematic review of pollination studies describing the visitation patterns of flowers with poricidal anthers by different species. Second, we built a dataset describing variation in the flower size of plant species used as a model in the studies reviewed in our systematic search. Finally, we identified the most frequent bee species visiting the flowers in each study and extracted their body size from the literature.

2. Material and Methods

2.1. Study case dataset

We built a dataset based on a systematic search of published studies describing the visiting patterns of pollen flowers with poricidal anthers in different plant species. Our systematic search utilized a combination of keywords that were used in different literature search engines: Web of Science (www.webofknowledge.com), Life Science Scopus (www.scopus.com), and Google Scholar (www.scholar.google.com) (Supplementary Material S1), we utilized different search engines to enhance our chances of locating relevant studies. The database included pollination studies available up to August 2021, and after removing duplicates, our sampling effort returned 300 potentially appropriate studies.

After our search, we screened the studies by reading each paper's abstract and methods. We included in our database the studies that (i) used as models noncommercial plant species bearing flowers with poricidal anthers, (ii) recorded the pattern of flower visitation over an explicit period (hours), and (iii) were conducted in the field. When we could not assess the first criterion directly from the study case, we retrieved the required information from taxonomic literature related to the plant species or from ecological literature about species with poricidal anthers (e.g., Buchmann, 1983; Vallejo-Marín et al., 2010; Vogel, 1978). After the screening, we got 32 suitable pollination studies, which summed data on 56 plant species with poricidal anthers. Two studies not initially recovered in our literature review were included. They are studies co-authored by the authors of this study (Lima and Nogueira, 2019; Valadão-Mendes et al., 2022) and met our criteria. We surveyed the following information in each study for each poricidal flower species: scientific name and botanical classification at the family level, primary and secondary flower rewards, total observation time of flower visitors, and flower visitor species.

2.2. Flower size measurement

To measure the size of poricidal flowers of different plant species, we used digital images of each plant species available at online plant collection databases: speciesLink (http://inct.splink.org.br/), Reflora (https://floradobrasil.jbrj.gov.br), the

New York Botanical Garden, NYBG (http://sweetgum.nybg.org); the Royal Botanic Gardens Kew (https://www.kew.org), and the Global Plants – JSTOR (https://plants.jstor.org). We considered that the images were suitable for our measurements when (i) the plant specimen had visible, well-preserved flowers, i.e., with parts not folded or damaged; (ii) an image with high resolution and available scale bar; (iii) flowers maintained structures attractive to a pollinator, i.e., corolla and androecium; (iv) plant specimen was identified by a specialist of its botanical group or could be otherwise identified by comparison to other identified specimens. We excluded duplicates and kept the images of 198 specimens representing 56 plant species (100 % of model plant species used in the study cases). Specimen images were rare for some plant species, so the sampling size was variable across species (averaging 3.5 (range: 1-10) specimen images per species). However, it is essential to note that our focus is on describing the interspecific variation in floral size. This variation is expected to exceed intraspecific variation, meaning that any potential bias related to variation in our sampling size should play a minor role in our analysis.

To measure flower size of plant species included in our dataset, we used ImageJ 1.8.0. software (Rasband, 2022). For that, we selected five images among all the digital photos we retrieved for each plant species that met the selection criteria described above. Images that did not reach these criteria were discarded. Twenty-one plant species (37 % of our dataset) had less than five suitable images for measuring. In those cases, we measured more than one flower per specimen image until we got five measurements of flower size for each plant species. In botanical collections, tridimensional floral structures are flattened into two dimensions after herborization, leading to distortion of the original flower shape. To avoid distortion bias, we measured flowers with non-twisted parts in each image (see our inclusion criteria above on the quality of flowers to be measured). These non-twisted flowers could still exhibit some distortion levels. However, as we were interested in descriptors of flower size rather than flower shape and the relative variation in these measurements across species, we considered that such distortion would have negligible effect on our measurements. Before each measurement, we calibrated digital photos based on the scale bar attached to vouchers (flower size measurement protocol - Supplementary Material S2). We used flower diameter as a proxy of flower size. We defined flower diameter as the linear distance between the two edges formed by the tip of the corolla at opposite sides of the flower (i.e., the corolla opening diameter), perpendicular to the flower's central axis (Supplementary Material S3). When flowers were tubular (e.g., Ericaceae species), we described flower size based on two linear measurements: diameter at the middle of the flower tube and diameter at the flower tube opening (Supplementary Material S3). We then utilized the diameter at the middle of the flower tube as a proxy of flower size for these tubular flowers. The diameter was larger at this level in the species of our dataset, representing the visual pattern of flower size and not constraints to the access of flower resources (e.g., diameter at the flower tube opening).

Based on flower diameter values, we classified flowers with poricidal anthers into three discrete categories: small flowers (diameter ≤ 0.99 mm), medium flowers (1.00 mm ≤ diameter ≤ 2.99 mm), and large flowers (diameter ≥ 3.00 mm). We applied this classification for two main reasons. First, the relationship between flower size and flower visitor diversity is expected to follow nonlinear trajectories (Olesen et al., 2007; Kuppler et al., 2021). In this case, we adopted categories using logarithmic classes of flower sizes across plant species, with higher categories including a larger range of flower size variation, to better represent the fundamental nature of the data and improve the fit of linear models. Second, flower measurements on dried specimens are likely to have some fraction of error compared to measurements on fresh materials, especially on larger flowers, but broad categories tend to decrease the bias related to such error. The three-level categorical predictor (small, medium, and large flowers) was used in all statistical tests and was called flower size throughout the study. Slight variations in the range of the three categories of flower size did not change the patterns detected in our study.

2.3. Flower visitors: species diversity, functional groups, and most frequent species

To evaluate the role of flower size on the functional diversity of bee assemblages visiting pollen flowers, we first classified flower visitors into ten groups (hereinafter termed visitor group) based on the functional group the visitor belongs to (Fenster et al., 2004; Koski et al., 2015; Moretti et al., 2009): beetle, ladybug, bedbug, spider, ant, wasp, fly, butterfly, and bees. Since bees are the main species performing buzz-pollination, we classified them into two distinct functional groups based on the genus-wide capacity of bee species in producing vibrations, according to Cardinal et al. (2018): (i) vibrating bee species and (ii) non-vibrating bee species. The capacity to produce flower vibrations is essential in these pollination systems because vibrating bee species are expected to extract pollen efficiently from poricidal anthers and ultimately pollinate flowers (Vallejo-Marín 2019). We assumed vibrating bee species as potential pollinators and non-vibrating bee species as antagonists or neutral visitors (e.g., Nogueira et al. 2018).

For each poricidal flower species, we counted the number of groups visiting the flowers; the number of visitor species that touched the stigma; the number of visitor species that sporadically or never touched the stigma; the number of bee species recorded on flowers; the number of vibrating bee species; the number of non-vibrating bee species; and the number of bee species that cut or chew anthers to extract pollen grains (e.g., *Trigona* species) mentioned in each study.

We also obtained detailed information about the most frequent visitor species for each plant species with poricidal flowers. We focused on the main flower visitor because the frequency of flower interaction with a given mutualistic partner is a reliable indicator of its net benefit for the plant partner (Vázquez et al., 2005). We accessed the frequency of flower visitors in most selected studies (60.6%). Still, in 37.8% of cases, we only had access to the species name of the most frequent flower visitor without any quantitative estimation of visitation frequency. In sum, we recovered the most frequent flower visitor in 98.4% of plant species analyzed, in which the main species visiting poricidal flowers was always a bee species. From the identity of the main bee species visiting each plant, we obtained data on the vibration behavior and body size for each case. First, we focused on the role of poricidal flower size on the probability of plants having as the main visitor vibrating bee species, i.e., species with vibrational behavior on poricidal anthers. We also obtained body length data of each study's most frequent bee visitor species. When the authors in each study did not explicitly report bee body length, we searched for such information in the taxonomic literature (Abrahamovich et al., 2005; Bezerra and Machado, 2003; Eltz et al., 2011; Fracasso and Sazima, 2004; Freitas and Silveira, 2017; Gaglianone, 2001; Gibbs, 2011; Liu and Koptur, 2003; Marchi and Alves-dos-Santos, 2013; Mawdsley, 2018; Nadia and Machado, 2005; Oliveira et al., 2008; Pereira et al., 2011; Sigrist and Sazima, 2015; Vivallo, 2019; Vivallo and Zanella, 2012). Moreover, we used the PaDiL database to consult taxonomic synonyms of the most frequent visitor bee species (https://www.padil.gov.au/pollinators/pest/main/138578). By doing so, we could retrieve body length data based on synonym descriptions or records. When body length data were described as a range (e.g., 9-14 mm), we used the mean between the maximum and minimum body length values reported for each bee species.

Since body length data were derived mainly from taxonomic descriptions and identification keys providing range statistics (37.7% of the studies), we assumed a skeptical posture regarding body length values. Because of that, in addition to the evaluation of the role of flower size on mean body size of the main bee visitors, we also performed a categorical analysis investigating the role of flower size to assess the probability of poricidal flowers having a large-bodied bee species as the main flower visitor. In this case, we primarily adopted the classification of bee body size used in previous studies, grouping the bees into three categories: small bees with a body length < 10 mm, medium bees with a body length between ≤10 mm and ≤ 15 mm, and large bees with body length > 15 mm (Pereira et al., 2011; Stout, 2000). However, as a result of our relatively low sample size, we finally adopted only two categories to classify the most frequent visitor bee species: small-bodied (body length value < 15 mm) and large-bodied bee species (body length value ≥ 15 mm). We also considered body length continuously in the statistical analyses.

2.4. Statistical analysis

Across the 55 plant species of our dataset, three species offered pollen grains plus another reward to their floral visitors. Since the mechanisms driving the flower attractiveness to pollinators in these plants may differ from flowers offering only pollen (Parachnowitsch et al., 2019), we excluded these three species from our statistical analyses described below. Thus, we used information from 52 plant species offering only pollen to pollinators in our general analyses. Although unsuitable for formal statistical analysis, we also visually inspected the number of visitor species interacting with poricidal flowers in our complete dataset, including the three species with more than one flower resource (see Supplementary Material S4). The visual inspection can provide valuable descriptive information for future studies addressing this still-overlooked flower type.

We applied a generalized linear mixed model (GLMM) to test if flower size influences the characteristics of flower visitor assemblage, utilizing either a negative binomial or a Poisson distribution. We built one model for each of the following response variables: number of species visiting each flower species, number of groups of flower visitors, number of bee species visiting flowers, and number of vibrating and non-vibrating bee species visiting flowers. The predictor variable was always flower size described as a categorical variable with three levels: small, medium, and large flowers. We included the total period (hours) in which the flower visitors were observed in each study as an additional fixed factor and the study case as a random factor. The 'study case' factor sought to control the different methods applied in each study.

To evaluate if flower size influences mean body length of the most frequent bee species that visit the flower, we applied a generalized linear mixed model (GLMM) utilizing a negative binomial. Finally, we performed two binomial proportion tests to test if larger flowers are more likely to be visited by vibrating and large-bodied bees than smaller flowers. For these tests, we used the proportion of studies in which the main species visiting the poricidal flowers was a vibrating or large-bodied bee species. In each test, we used the size of the flowers (i.e., small, medium, or large) as the predictor variable.

All statistical analyses were conducted using the 'lme4' package (Bates et al., 2015) in the R environment version 4.1.2 (R Core Team, 2021).

3. Results

We sampled data on 55 plant species with poricidal flowers representing nine botanical families, summing 5124 hours of observation of flower visitors. Most plant species with poricidal flowers belonged to three plant families: Melastomataceae (41.4%; Fig. 1A), Fabaceae (22.9%; Fig. 1B), and Solanaceae (14.3%; Fig. 1C). Plant species in our dataset mainly had medium flower sizes (57.1%), followed by large flower sizes (25.7%) and then small flower sizes (17.1%). See the complete list of poricidal flower species in Supplementary Material S5. The three species offering more than one reward to visitors included three families: Bixaceae, Ericaceae (Fig. 1D), and Krameriaceae. The species *Bixa orellana* and *Vaccinium uliginosum* ssp. *uliginosum* offered nectar in addition to pollen to its visitors, while *Krameria tomentosa* provided oil. In these studies, large flowers of *Bixa orellana* were visited by 16 species in 7 functional groups of visitors (beetle, ladybug, bed bug, ant, wasp, fly, and vibrating and non-vibrating bee species). In contrast, the tiny flowers of *Vaccinium uliginosum* ssp. *uliginosum* were visited by 29 species distributed in 4 functional groups of visitors (wasp, fly, butterfly, and vibrating and non-vibrating bee species). The species *Krameria tomentosa* was visited by 21 species in 2 groups of visitors (fly and bees). The most frequent visitor species across these three plant species were bee species.

3.1. Flower size and the assemblage richness of floral visitors

The floral size of pollen-flower species explained the number of species visiting flowers across plant species (F = 4.85; p = 0.011). Plant species with large flowers were visited by 2.4 times more species than those with small flowers (Fig. 2A). Bees were the only visitors of most plant species in our dataset (82%). The flowers were visited by bees and other visitor groups in 18% of plant species belonging to the following groups of visitors: beetles, flies, wasps, ants, and butterflies. Plant species were generally visited by only a few visitor groups (on average, one or two), regardless of flower size (F = 1.78; p = 0.176; Fig. 2B).

3.2. Flower size and the patterns of flower visiting by bees

Floral size explained variation across species in the number of bee species visiting flowers (F = 4.09; p = 0.021). The number of visiting bee species was 0.4 and 1.2 times greater among large-flowered plant species than medium-flowered and small-flowered plant species, respectively (Fig. 3). Most bee species (87.9%) visiting flowers with poricidal anthers exhibited vibrating behavior. Large-flowered plant species were visited two times more by vibrating bee species than small-flowered plant species (F = 3.60; p = 0.033; Fig.4A). The number of vibrating bee species visiting large-flowered plant species was around 0.4 times greater than medium-flowered plant species and 1.1 times greater than small-flowered plant species. Floral size did not influence the number of non-vibrating bee species visiting flowers (F = 1.87; p = 0.163; Fig. 4B).

3.3. Flower size predicts the size and behavior of the most frequent visitor bee species

In all study cases, bees were the most frequent flower visitor species. We observed 35 species of bees acting as the main visiting species on flowers across plant species. The most frequent visitor species belonged to the genus *Bombus* (27.9%), *Xylocopa* (13.1%), *Augochloropsis* (13.1%), and *Centris* (11.5%).

The most frequent visiting bee species commonly had vibrating behavior (85.5% of cases). Large-flowered plant species were mainly visited by vibrating bee species (83.3 % of cases; Fig. 5A). Similarly, vibrating bee species were those mainly visiting small- and medium-flowered plant species (87.8 and 91.7% of cases, respectively; Fig. 5A).

Large-bodied bee species were the main visitor in 77% of the studies included in our dataset (Fig. 5B); In contrast, medium- (60 % of cases; Fig. 5B); and small-flowered plant species (85 % of cases; Fig. 5B) had a small-bodied bee species as the main visitor. In the quantitative evaluation, flower size predicted body length (mm) of the most frequent visitor bee species. The average size of the main bee species visiting the flowers was 14.4 mm ± 5.0. Bee species visiting large-flowered plant species were two times larger than those visiting small-flowered plant species (F = 5.93; p = 0.004; Fig. 6).

4. Discussion

We found that plant species with large poricidal flowers were visited by a larger and richer assemblage of floral visitor species than small-flowered plants. We also found that variation in flower size had different effects on the traits of visitor species. When looking at the whole assemblage of flower visitors, plant species with large and medium flowers interacted with both vibrating and non-vibrating bee species, while plant species with small flowers interacted almost exclusively with vibrating bee species. However, when focusing only on the most frequent visitor species, all flowers had a vibrating bee species as the main visitor, regardless of flower size. Still, large flowers were more likely to have a large-bodied bee species as the most frequent visitor than small flowers. The main species visiting large flowers had a body length two times larger than those visiting small flowers. Altogether, these results indicate that flower size affects the richness and functional diversity of the visitor's assemblage, as well as traits, mainly body size, of the main visitor species interacting with poricidal flowers.

As expected, large poricidal flowers were visited by a higher number of visitor species than medium and small flowers in our study. These results indicate that plant species with larger pollen-flowers can be more attractive to visitor species available in the habitat, especially bee species. In nectar flowers, the higher attractiveness of large flowers has been explained by the correlation between flower size and reward quantity offered to visitors (Totland and Matthews, 1998). Such correlation is expected to favor the cognitive association between flower size and nectar reward, driving visitors' preference towards larger nectar flowers (Kennedy and Elle, 2008). In the case of poricidal flowers, a body of evidence shows that flower size is related to the quantity of pollen offered to visitors (e.g., Solanaceae species - Vallejo-Marín et al., 2014). Also, floral size is correlated with other morphological traits, and such correlation could modify the flower's signaling to visitors. However, no evidence suggests that the spatial disposition of the reproductive structures can be cognitively associated with floral quality by floral visitors. Therefore, it is likely that the positive relationship between the size of poricidal flowers and the richness of visitors is explained by the same mechanisms driving this association in plant species with nectar flowers.

In our study, bee species were the most frequent visitors across plant species with poricidal flowers, regardless of their size. This observation highlights the high ecological specialization and dependence between this flower type and bees. However, not all bee species interacting with poricidal flowers exhibit vibrating behavior (Mesquita-Neto et al., 2018). In our dataset, non-vibrating bee species were observed visiting medium and large flowers, while they were largely absent in species with small poricidal flowers. These non-vibrating bees may act as antagonists, removing pollen grains without performing pollination (Buchmann, 1983; Nogueira et al., 2018; Vallejo-Marín, 2019). In the absence of benefits, non-vibrating bees would impose pollen costs for plants depending on the time of interaction with the flowers (before or after the pollinator visit – Barker and Bronstein, 2016) and the quantity of pollen removed from the poricidal anthers. Also, non-vibrating bees exploit pollen with different behaviors, such as 1) "milking" the anther using their mandibles, plus buzzing (Roubik, 1989); 2) gleaning pollen leftovers that remain on flower organs after another bee visit (e.g., *Apis melifera* L.; Mesquita-Neto et al., 2018; Michener et al., 1978; Thorp and Estes, 1975, A. Nogueira pers. obs.); and 3) destroying the anthers with their mouth parts (Mesquita-Neto et al., 2018), such as Trigonini bees (e.g., *Trigona*). These behaviors (except the second one) were described across the studies reviewed here. While the first behavior potentially results in pollination, the second is unlikely to do so, as gleaning bees are considered commensals that do not impact reproductive plant success (Roubik, 1989). Differently, the third behavior is typically exploitative. Then, the positive association between flower size and reward quantity may make large flowers more attractive to pollinators and pollen larcenists.

We detected the existence of a morphological matching between flower size and body size of the most frequent bee species visitor across plant species. Plant species with larger flowers had a higher probability of interacting more frequently with large-bodied bee species, just as similarly found for small flowers and their small-bodied bee partners. The phenotypic matching between flowers and their visitors forecasts a greater rate of effective pollination (Whalen, 1978) since flower visitors are more likely to touch and deliver pollen grains to flower stigma when its body size has a better fit to floral morphology (Solís-Montero and Vallejo-Marín, 2017). However, the cause of phenotypic matching in poricidal plant species and their buzzing bee partners may not be as straightforward as in other pollination systems. Heavier bees from the same colony can have a greater vibration amplitude, increasing the amount of pollen ejected by the poricidal anthers (De Luca et al., 2013). Other studies evaluating insect vibration predicted this same pattern of vibrational amplitude by heavier bees (Cocroft et al., 2000; Cocroft and De Luca, 2005). Assuming that the size and weight of bees are correlated in our dataset, it is expected that large-bodied bees will remove more pollen from large-anthered flowers than small-bodied ones. A similar hypothesis can be made for small bees and small-flowered plant species. Complementarily, phenotypic matching can also result from the morphological adjustment of the bee's body in relation to a flower's organs if we consider floral morphology of the poricidal flowers of the most representative plant families within our dataset – Fabaceae, Melastomataceae, and Solanaceae. Poricidal flowers of these plant groups usually have a suite of floral features, such as enantiostyly and hetheranthery (Buchmann, 1983), in which the proper size and positioning of pollinator in relation to the flower's reproductive organs (stigma and anthers) are important for successful pollination, especially in medium to large flowers where the distance of such organs is larger (Vallejo-Marín et al., 2014). These aspects deserve more attention in future studies exploring phenotypic matching of vibrating bee species and their pollen flower partners.

Since the most frequent partners are the main species that drive the net benefit in mutualistic interactions (Vazquez et al., 2015), it is possible that the benefits of having a high-quality matching partner as the main visitor offset the costs of facing a higher probability of exploitation by occasional non-vibrating visitors for plant species with larger poricidal flowers. Meanwhile, smaller poricidal flowers naturally face a lower chance of interacting with exploiters, while interacting more often with vibrating small-bodied bees that also phenotypically match their flower size. Therefore, interspecific variation in the size of poricidal flowers may reflect different reproductive strategies maximizing the reproductive success within different ecological contexts. In the case of small-flowered plants producing less pollen, the costs associated with pollen limitation should be offset by increased pollen transfer efficiency owing to the constraint of flower visitation by non-effective pollinators. In this case, legitimate visits of vibrating bees, morphologically coupled with the flower, would extract most of the pollen available in the flowers, ensuring both male and female plant reproductive success. Regarding large-flowered plants, the costs of being more attractive to ineffective pollinators can be offset by a higher probability of flower visitation by effective pollinators.

Our results on the patterns of flower visitation are described at the community level, reflecting the composition of the bee community occurring at each sampling site. More refined information about flower visitors, such as the flower visitation rates by different animal species, was largely absent. Although it does not invalidate our findings, investigations into the relationship between flower size and flower visitation rate by each visitor species should be approached in future studies and systematic reviews. Such research could provide a more accurate description of animal responses to flower traits, improving our ability to propose general mechanisms about the role of floral traits in attracting visitors to nectar and poricidal flowers. Another exciting avenue of investigation is to evaluate the influence of multiple flower rewards on the relationship between the size of poricidal flowers and patterns of flower visitation. In our systematic search, we found only three studies exploring poricidal species offering more than one reward type to its visitors (Carneiro et al., 2015; Mayer et al., 2012; Nascimento and Pereira, 2017). Since reward type is one trait that can also be cognitively associated with flower quality to visitors (Parachnowitsch et al., 2019), it is possible that the offering of multiple resources would lead to changes in the relationship between flower size and its relative value to visitors, consequently influencing the patterns of flower visitation by different species. For instance, the small-flowered plant species *Miconia brevitheca* and *Miconia tonduzii* var. *tonduzii* produce nectar and pollen rewards and are less commonly visited by vibrating bee species (Kriebel and Zumbado, 2014). Instead, the flowers of these species are often visited by syrphid and tachinid flies, which collect pollen and nectar from the flowers (Kriebel and Zumbado, 2014). This result is entirely different from the patterns we described here, in which bee species were the main flower visitor of species with poricidal flowers (93%).

In plants with poricidal flowers, variation in flower size is positively correlated with the distance between the anther and stigma floral organs (Vallejo-Marín et al., 2014). Therefore, small-flowered species could represent a modification of the plant's reproductive system, favoring the transference of pollen grains within flowers and decreasing plant pollinator dependence on reproduction ('selfing syndrome' – Sicard and Lenhard, 2011). However, the reduced separation of reproductive organs in small pollen-flowers is not consistently associated with changes in the poricidal nature of anthers, preventing pollen access to bee species exhibiting no vibrating behavior. This pattern could be changed only if small pollen-flowers, for instance, had a large anther pore opening, allowing non-vibrating bee species and other visitor types to be effective pollinators (Goldenberg et al., 2008). This is not the general pattern for species with small poricidal flowers included in our review. Some exceptions to this pattern were recorded among some Ericaceae that also offered nectar as a reward to its pollinating hummingbird species (de Araújo et al., 2011; Freitas, 2018; Luteyn, 2002; Navarro et al., 2008). In this plant group, different species with poricidal anthers with large anther pore openings also have large nectar disks near the flower ovary, thus increasing reward types and facilitating access to pollen grains, consequently increasing the functional diversity of flower visitors (Brito et al., 2016; Kriebel and Zumbado, 2014; Luteyn, 2002; Navarro et al., 2008).

5. Conclusions

Here, we showed that the size of poricidal flowers plays a significant role in shaping the size-traits bee species assemblages visiting these flowers and the behavioral traits of the most frequent species visiting these flowers. Despite such effect on flower visitation patterns, we advocate that the positive correlation between floral size and the amount of pollen offered by poricidal flowers leads to a situation in which the effect of flower size on the visitation patterns probably has a low impact on pollination outcome. Although larger flowers are more susceptible to interactions with exploiters attracted by the relatively large amount of pollen, reproductive success should be favored by the higher probability of having vibrating large-bodied bee species as the main visitor species; both traits are crucial for a high-quality pollinator. On the other hand, the reduction in flower size and the morphological and resource amount changes deriving from it probably prevent these flowers from interacting with non-vibrating bees, while favoring their visitation by vibrating small-bodied bee species as pollinators.

6. References

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Figure text

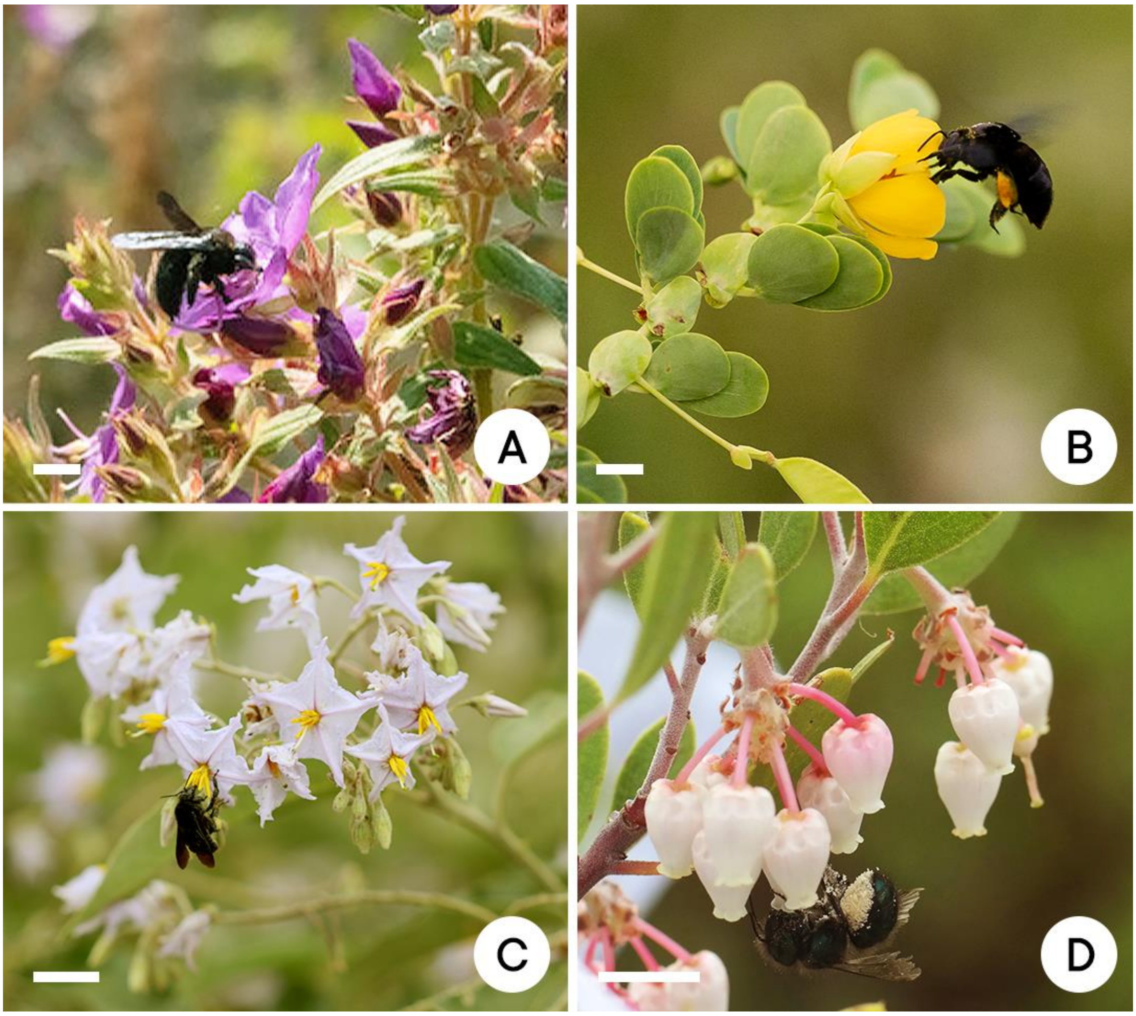


Figure 1 – Poricidal flowers visited by female bees collecting pollen grains. The first three images include representatives of the three families with the highest number of species available in our database. At the same time, the fourth image represents a poricidal flower that also produces nectar. A – Poricidal flower of *Rhynchanthera grandiflora* (Melastomataceae) receiving a visit of *Xylocopa* sp. (Photo by César Augusto Arvelos); B – Poricidal flower of *Chamaecrista latistipula* (Fabaceae) receiving a visit of *Eulaema nigrita* (Photo by Tamiris Delgado); C – Poricidal flower of *Solanum paniculatum* (Solanaceae) receiving a visit of *Bombus pauloensis* (Photo by César Augusto Arvelos) and D – Poricidal flower of *Arctostaphylos pungens* (Ericaceae) receiving a visit of *Andrena* sp. with white pollen grains accumulated in the bee's abdominal region (Photo by Anselmo Nogueira). Scale bars: 1 cm.

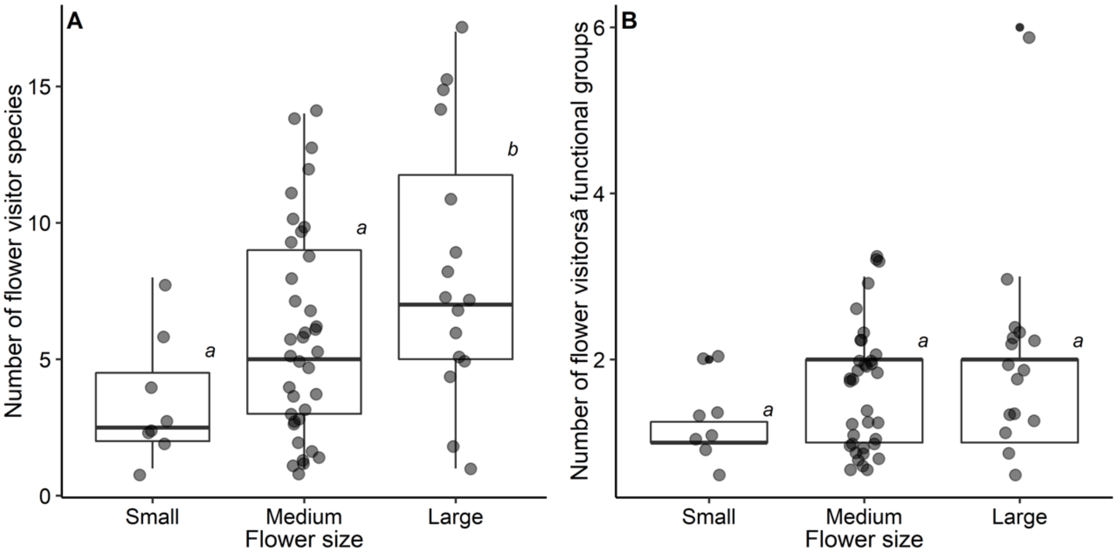


Figure 2 – The number of species and functional groups of floral visitors for different flower size categories. A) The number of floral visitor species recorded for different flower size categories; B) The number of floral visitor functional groups recorded for different flower size categories. Throughout, flower size categories reflect flower diameter values: 'small', flower diameter < 0.99 cm; 'medium', 1 cm ≤ flower diameter ≤ 2.99 cm; 'large', flower diameter > 3 cm. Boxes represent the 50% interquartile range, horizontal lines indicate the median, whiskers represent the 1.5*interquartile range, and points represent plant species recorded in the study case dataset. Different letters indicate significant differences among flower categories in the posthoc Tukey test ($p < 0.05$)

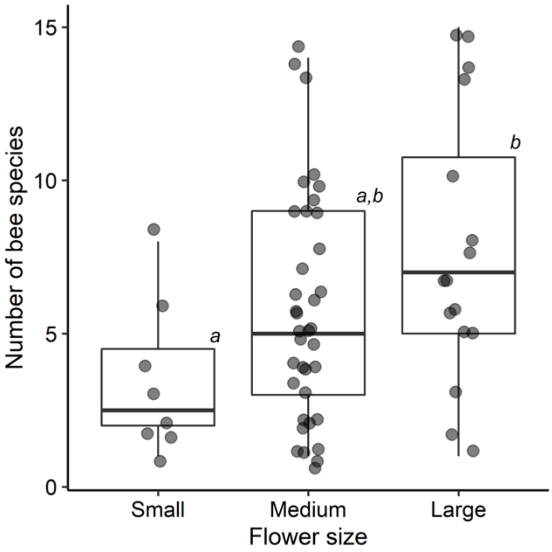


Figure 3 – The number of bee species visiting different flower size categories. Flower size categories reflect flower diameter values: 'small', flower diameter < 0.99 cm; 'medium', 1 cm ≤ flower diameter ≤ 2.99 cm; 'large', flower diameter > 3 cm. Boxes represent the 50% interquartile range, horizontal lines indicate the median, whiskers represent the 1.5*interquartile range, and points represent plant species recorded in the study case dataset. Different letters indicate significant differences among flower categories in the posthoc Tukey test ($p < 0.05$).

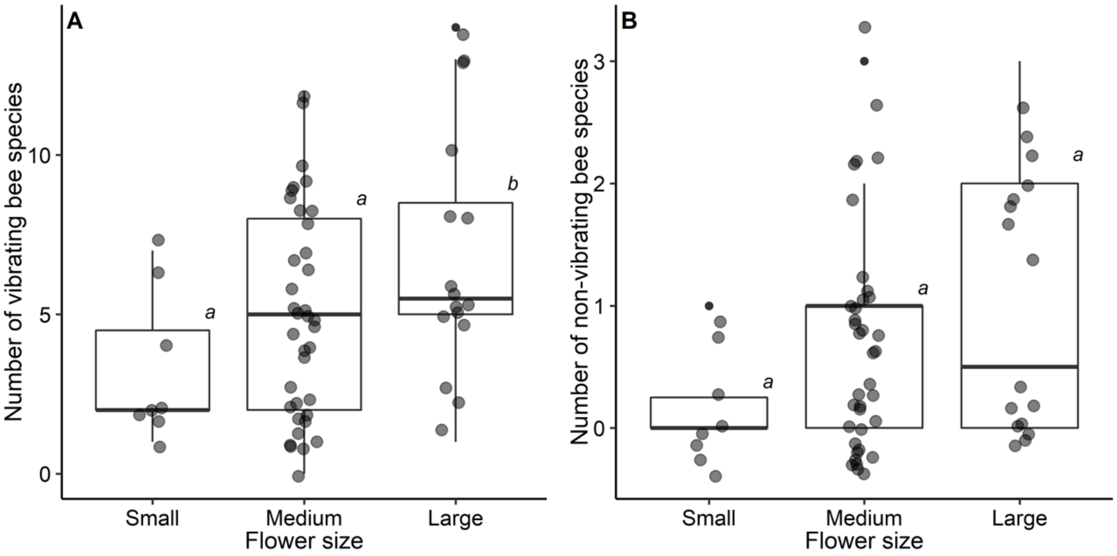


Figure 4 – The number of vibrating and non-vibrating bee species visiting different flower size categories. A) The number of vibrating bee species visiting different flower size categories; B) The number of non-vibrating bee species visiting different flower size categories. Throughout, flower size categories reflect flower diameter values: ‘small’, flower diameter < 0.99 cm; ‘medium’, 1 cm ≤ flower diameter ≤ 2.99 cm; ‘large’, flower diameter > 3 cm. Boxes represent the 50% interquartile range, horizontal lines indicate the median, whiskers represent the 1.5*interquartile range, and points represent plant species recorded in the study case dataset. Different letters indicate significant differences among flower categories in the posthoc Tukey test ($p < 0.05$).

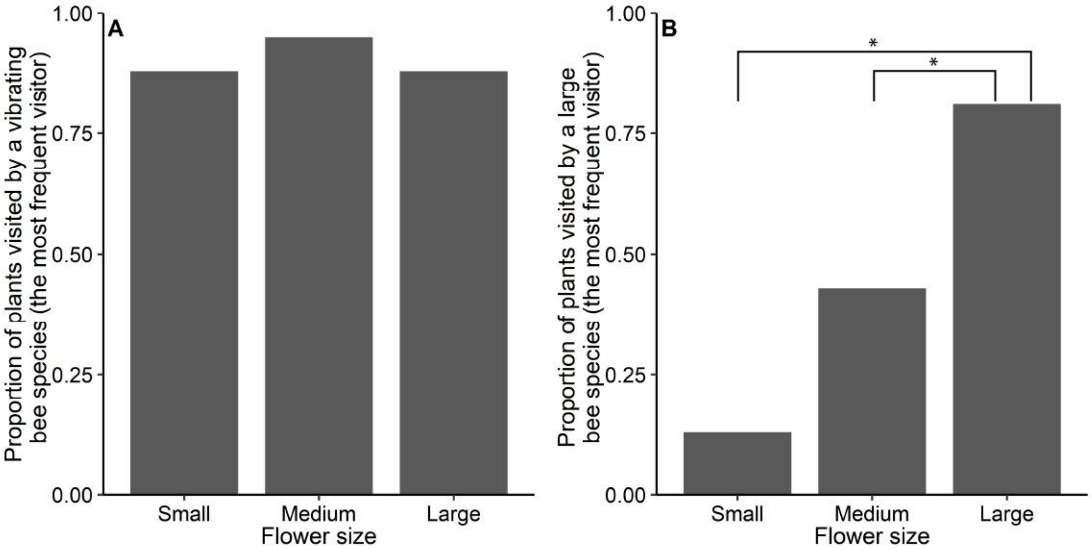


Figure 5 – Proportion of species visited by large-bodied and non-vibrating bee species into different flower size categories. A) Proportion of species visited by large-bodied bee species into different flower size categories. B) Proportion of species visited by non-vibrating bee species into different flower size categories. Throughout, flower size categories reflect flower diameter values: ‘small’, flower diameter < 0.99 cm; ‘medium’, 1 cm ≤ flower diameter ≤ 2.99 cm; ‘large’, flower diameter > 3 cm. Asterisks (*) indicate statistically significant differences ($p \leq 0.05$).

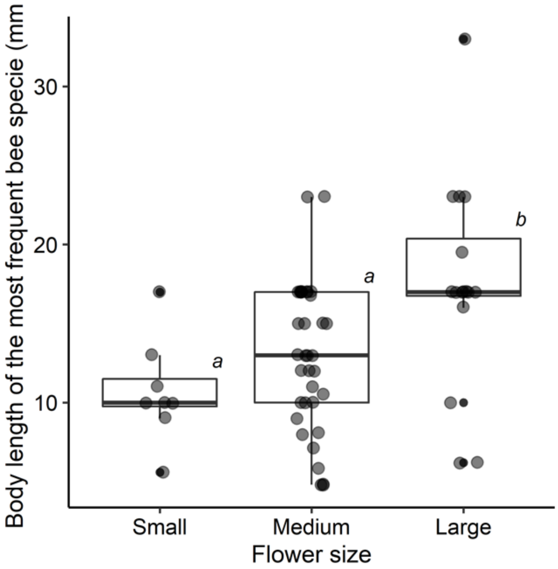


Figure 6 – Body length of the most frequent bee species visiting different flower size categories. Flower size categories reflect flower diameter values: 'small', flower diameter < 0.99 cm; 'medium', 1 cm ≤ flower diameter ≤ 2.99 cm; 'large', flower diameter > 3 cm. Boxes represent the 50% interquartile range, horizontal lines indicate the median, whiskers represent the 1.5*interquartile range, and points represent plant species recorded in the study case dataset. Different letters indicate significant differences among flower categories in the posthoc Tukey test ($p < 0.05$).

Supplementary material

Supplementary material S1. The systematic quantitative review used search engines, search strategies, and keyword combinations. The systematic review targeted experimental pollination studies that use plant species with poricidal anthers as plant models.

Search engine	Search strategy	Key-word combinations
Web of Science and Scopus	Advanced search	TS= (("pollinator dependency" OR "pollinator* exclusion" OR "pollen manipulation" OR "open-pollinated" OR "open-pollination" OR "self-pollination" OR"self pollinate"OR "pollination manipulation*" OR "reproductive strateg*" OR "controlled pollination*" OR "pollinator limitation" OR "pollen limitation" OR "limitação polínica" OR "pollination experiment*" OR "floral biology" OR "biologia floral" OR "sistema reprodutivo" OR "reproductive system" OR "breeding system" OR "mating system" OR "mating" OR "autogamy" OR "selfing" OR "sexual reproduction" OR "self pollen" OR "natural pollination" OR "hand-pollinated" OR "supplementary pollination" OR "caging" OR "selfed flowers" OR "spontaneously selfed" OR "autopolinização" OR "self-pollination" OR "autogamic" OR "autogamy" OR "emasculation" OR "pollinator exclusion" OR "enclosed plants" OR "manual pollination" OR "system of reproduction") AND ("flores com anteras poricidas" OR "flowers with poricidal anther*" OR "anteras poricidas" OR "poricidal anthers" OR "flores de pólen" OR "pollen flower*" OR "anther pore apical" OR "buzz pollination" OR "buzz-pollination" OR "flor* com anter* poricid*" OR "antera* poricida*" OR "poricid* anthe*" OR "buzz pollinat*" OR "buzz-pollinat*" OR "anthe* with apical por*" OR "heteranther*" OR "heteromorphic androecium" OR "heterostemony" OR "mirror-image flower*" OR "mirror image flower*" OR "enantiostyly" OR "nectarless flower*" OR "pollen as the only reward*" OR "vibrated the anther*"OR "absence of nectar" OR "vibratory mechanism*" OR "buzz anther*" OR "polinização por vibração"))
Google scholar	Regular Search – each key-word	Sistema reprodutivo flores com anteras poricidas

	combination separately	anther poricidal reproductive system reproductive system in pollen flower reproductive system, flower anthers poricidal sistema reprodutivo & flores com anteras poricidas poricidal anthers & reproductive system poricidal anthers reproductive system “sistema reprodutivo” & “flores com anteras poricidas” “poricidal anthers” & “reproductive system” “pollen flowers” & “reproductive system” “flower” & “poricidal anthers” & “reproductive system” “breeding system” & “pollen flowers” “breeding system” & “poricidal anthers” “breeding system” & “buzz pollination” “breeding system” & “buzz-pollination” “reproductive system” & “poricidal anthers” “reproductive system” & “buzz pollination” “reproductive system” & “buzz-pollination” “mating system” & “poricidal anthers” “mating system” & “pollen flowers” “mating system” & “buzz pollination” “mating system” & “buzz-pollination” “sexual reproduction” & “pollen flowers” “sexual reproduction” & “poricidal anthers” “sexual reproduction” & “buzz pollination” “sexual reproduction” & “buzz-pollination”

Supplementary material s2. Flower size measurement protocol. Flower size is based on flower diameter measured on virtual herbarium specimens. Protocol developed by the *Laboratório de Interações Planta-Animal* (Nogueira's Lab) in the Universidade Federal do ABC, São Bernardo do Campo - SP, Brazil.

FLOWER SIZE MEASUREMENT PROTOCOL

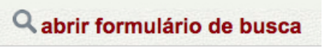
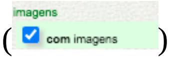
Searching for virtual herbarium specimens

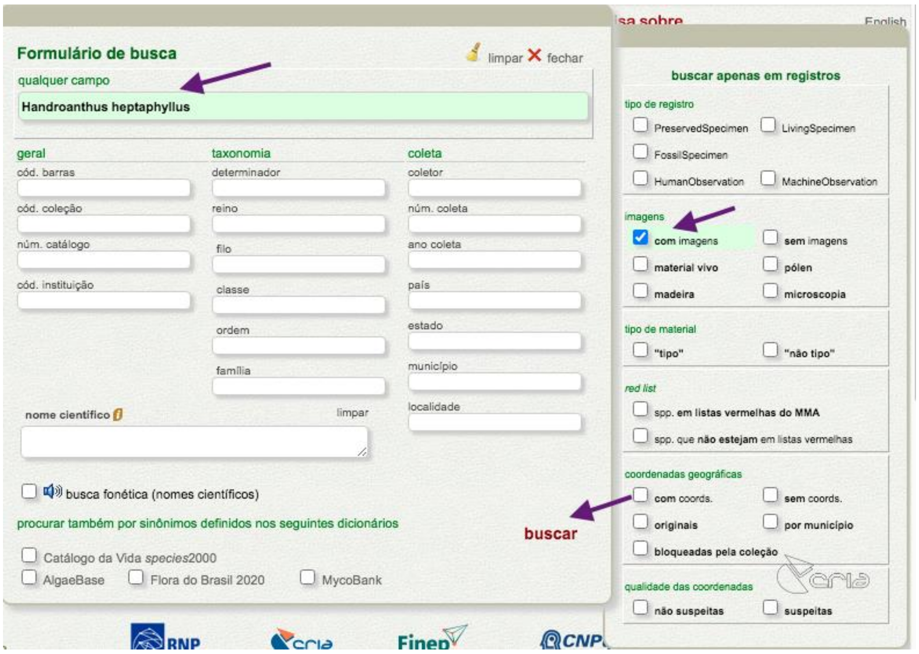
Steps:

1 Select one digital herbarium database: speciesLink (http://inct.splink.org.br); Reflora (https://floradobrasil.jbrj.gov.br); the New York Botanical Garden – NYBG (http://sweetgum.nybg.org); the Royal Botanic Gardens Kew (https://www.kew.org/); Global Plants – JSTOR (https://plants.jstor.org).

2 Further steps are based on the speciesLink database, but they were also applied to other digital databases.

3 Open the chosen URL, http://inct.splink.org.br

4 Click on the a*brir formulário de busca* [Open search form] button (). Write the species name in the *qualquer campo* field [Any field]. Narrow down your search to results with images by checking the *com imagens* [with images] box () on the right side. Click on the *Buscar* [Search] button and wait for the results. See the following example:



qualquer campo: Handroanthus heptaphyllus

imagens: ☑ com imagens

5. The search result is a list of herbarium vouchers with their respective digital images.

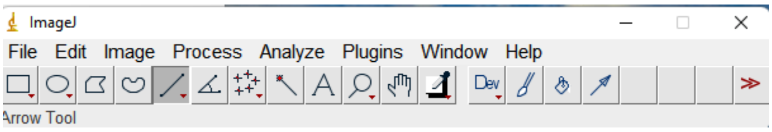
Click on any image to open it (). Procedure to inclusion criteria

6. Inclusion criteria:

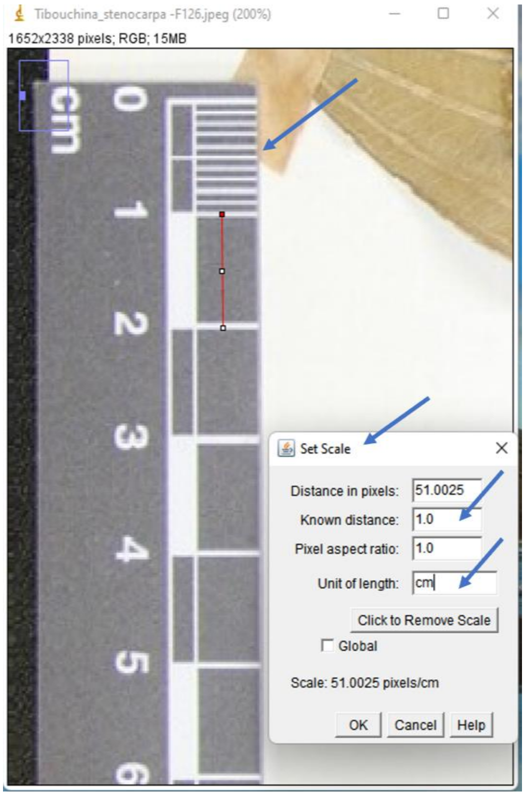
1 The specimen has at least one visible flower.
2 Flowers have their petals or other pollinator attraction organs under a good preservation state, e.g., no length reduction due to florivory, fully visible without breaks, and fully expanded parts (not folded or overlapping).

7. A new page will open. Click on the printer button (🖨) to download the image in the PDF extension. Convert the PDF image to either the JPEG or TIFF extension. As a free option, use the PDF converter https://pdftoimage.com. Download the open access ImageJ software (https://imagej.nih.gov/ij/download.html). Procedure to measure digital images with ImageJ.

8. Measuring digital images with ImageJ:



0 Scale setting. We suggest leaving the whole specimen visible in the software frame. Select the zoom tool (🔍) to magnify any part of the image if useful. Click on the measure tool (↗) and select a 1-centimeter distance over the rule. Then, click on the analyze function (Analyze) and select the set scale option (Set Scale...). A new window will open. Fill the ‘Known distance’ field with the distance measured over the rule, here 1.0. Fill the ‘Unit of length’ field with the ruler scale in centimeters (cm). Read all information carefully, check if the filled values are correct, and click on the ‘Save’ button. If the scale setting was correct, measure a known distance over the ruler to double check. Scale setting must be repeated for each image.



1652x2338 pixels; RGB; 15MB

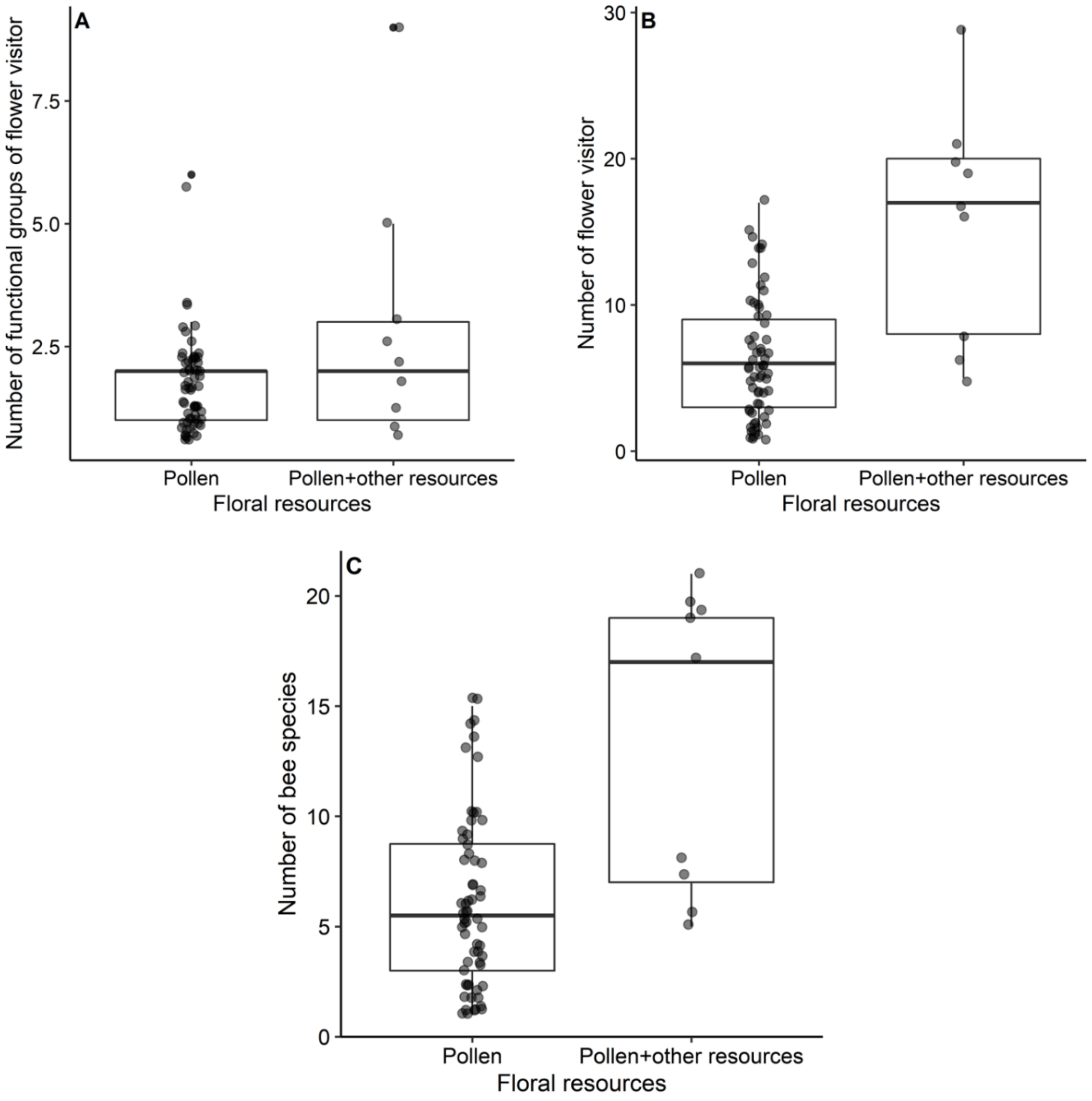
Distance in pixels: 51.0025
Known distance: 1.0
Pixel aspect ratio: 1.0
Unit of length: cm
☐ Global
Scale: 51.0025 pixels/cm

1 Select the zoom tool (🔍) to magnify a flower. Before measuring, set the following standards:
   a) Identify the main pollinator attraction organ (e.g., corolla or androecium in poricidal flowers flowers).
   b) Set the flower axis as a line passing through the flower, commonly on the flower pedicel, dividing the flower base image into two parts.
   c) Set a line passing orthogonally to the flower axis and reaching the tip of the corolla (or androecium if this is the attraction organ) at opposite sides of the flower.
   d) The flower diameter is the distance between the tip (boundary) of petals (or stamens) at opposite sides of the flower.
   e) If the flower is tubular (e.g., Ericaceae species), set three lines, two orthogonal and one parallel to the flower axis, respectively, and measure: (i) the diameter at the flower tube opening, (ii) the diameter at mid length of the flower tube.

2 Measure the flower diameter with the measure tool (📏).

3 After that, follow the next image.

Supplementary material s3. Flower size measurements across plant species with poricidal flowers. The black dotted line represents the flower axis. The continuous red line represents the flower diameter and passes orthogonally by the flower axis, connecting the corolla tips at opposite sides of the flower. A - *Tibouchina hatschbachii* (Melastomataceae); B – *Vaccinium uliginosum* (Ericaceae); C - *Solanum Carolinense* (Solanaceae) and D - *Chamaecrista desvauxii* (Fabaceae).


A
Number of functional groups of flower visitor
7.5
5.0
2.5
Pollen
Pollen+other resources
Floral resources
B
Number of flower visitor
30
20
10
0
Pollen
Pollen+other resources
Floral resources
C
Number of bee species
20
15
10
5
0
Pollen
Pollen+other resources
Floral resources

Supplementary material s4 – Relationship between the flower visitor assemblage and flower reward type across plant species with poricidal flowers. A – On average, the number of flower visitor species recorded in pollen flowers and flowers with pollen and additional reward types (e.g., oil, nectar, or resin). Plant species with pollen flowers had 6.2 ± 4.1 flower visitor species, while plant species with flowers with pollen and additional reward type had 15.7 ± 7.5 flower visitor species. B – The number of functional groups of flower visitors recorded in plant species with pollen flowers and flowers with pollen and additional reward type. On average, plant species with pollen flowers had 1.7 ± 0.9 functional groups, while plant species with flowers with pollen and other reward types had 3 ± 2 functional groups. C – The number of bee species recorded in pollen flowers and flowers with pollen and additional reward type. The same pattern occurs in the number of bee species. On average, plant species with pollen flowers had 6 ± 4 bee species, while plants with flowers with pollen and additional reward types had 13.5 ± 6.4 bee species. The low sampling of plant species bearing flowers with pollen and additional reward types prevented statistical analyses and generalizations, highlighting the need for further investigations on plant species with flowers with poricidal anthers and multiple reward types.

Supplementary material s5. Data extracted from plant species with poricidal flowers recovered in each case study analyzed. Species - species name in the original publication. Flower size reflects flower diameter categories: 'small', flower diameter < 0.99 cm; ‘medium’, 1 cm ≤ flower diameter ≤ 2.99 cm; ‘large’, flower diameter > 3 cm. Flower visitors – the total number of visitors, recorded in the original publication. Most frequent bee species flower visitor – the bee species name cited as the main visitor among other species. Bee size reflects bee body length categories of the most frequent bee species - small-bodied bee species with body length value < 15 mm, and a large-bodied species (L), those with body length value ≥ 15 mm.

Species name	Botanical family	Flower size	Number of flower visitors	Most frequent bee species flower visitor	Bee size
Tibouchina pulchra Cogn.	Melastomataceae	Large	7	*Bombus morio*	Large
Senna silvestris (Vell.) H.S.Irwin & Barneby	Fabaceae	Large	9	*Xylocopa brasilianorum*	Large
Senna silvestris (Vell.) H.S.Irwin & Barneby	Fabaceae	Large	7	*Xylocopa brasilianorum*	Large
Cochlospermum orinocense (Kunth) Steud.	Bixaceae	Large	7	*Centris caxiensis*	Small
Cambessedesia hilariana (Kunth) DC.	Melastomataceae	Medium	8	*Centris sp.*	Small
Senna corymbosa (Lam.) H.S.Irwin & Barneby	Fabaceae	Medium	3	*Bombus atratus*	Large
Dichorisandra hexandra (Aubl.) C.B.Clarke	Commelinaceae	Medium	14	*Augochloropsis cupreola*	Small

Dichorisandra incurva Mart. ex Schult.f.	Commelinaceae	Medium	14	*Pseudaugochloropsis graminea*	Small
Solanum rostratum Dunal	Solanaceae	Medium	6	*Thygater analis*	Small
Solanum rostratum Dunal	Solanaceae	Medium	5	*Apis mellifera*	Small
Solanum rostratum Dunal	Solanaceae	Medium	10	*Augochlora sp.*	Small
Senna multijuga (Rich.) H.S.Irwin & Barneby	Fabaceae	Large	14	*Bombus morio*	Large
Senna cana (Nees & Mart.) H.S.Irwin & Barneby	Fabaceae	Large	17	*Xylocopa frontalis*	Large
Melastoma affine D. Don	Melastomataceae	Large	8	*Lestis bombylans*	Large
Solanum melissarum Bohs	Solanaceae	Medium	3	*Euglossa cordata*	Small
Sauvagesia erecta L.	Ochnaceae	Small	4	*Augochloropsis sp*	Small
Sauvagesia sprengelii A.St.-Hil.	Ochnaceae	Small	6	*Florilegus simili*	Small
Cassia spectabilis DC.	Fabaceae	Medium	10	*Centris similis*	Large
Cassia spectabilis DC.	Fabaceae	Medium	12	*Centris similis*	Large
Cassia spectabilis DC.	Fabaceae	Medium	11	*Centris scopipes*	Small
Ouratea spectabilis (Mart.) Engl.	Ochnaceae	Medium	3	*Centris sp.*	Small
Solanum luridifuscescens Bitter	Solanaceae	Medium	7	*Melipona bicolor*	Small

Chamaecrista ramosa (Vogel) H.S.Irwin & Barneby	Fabaceae	Medium	9	*Xylocopa carbonaria*	Large
Chamaecrista keyensis Pennell	Fabaceae	Medium	6	*Megachile georgica*	Small
Tibouchina cerastifolia Cogn.	Melastomataceae	Medium	9	*Bombus pauloensis*	Large
Tibouchina clinopodifolia (DC.) Cogniaux	Melastomataceae	Medium	5	*Bombus pauloensis*	Large
Tibouchina gracilis (Bonpl.) Cogn.	Melastomataceae	Medium	6	*Bombus pauloensis*	Large
Paraboea rufescens (Franch.) BLBurtt	Gesneriaceae	Medium	6	*Bombus sp.*	Large
Tibouchina hatschbachii Wurdack	Melastomataceae	Large	15	*Trigona spinipes*	Small
Tibouchina hatschbachii Wurdack	Melastomataceae	Large	15	*Trigona spinipes*	Small
Chamaecrista desvauxii (Collad.) Killip.	Fabaceae	Large	4	*Bombus morio*	Large
Chamaecrista flexuosa (L.) Greene	Fabaceae	Medium	6	*Xylocopa cearensis*	Large
Medinilla multiflora Mansf.	Melastomataceae	Medium	7	*Halictus sp.*	Small
Bertolonia margaritacea Naudin	Melastomataceae	Medium	3	*Centris sp.*	Small
Chaetogastra clinopodifolia DC.	Melastomataceae	Medium	2	*Bombus morio*	Large
Miconia biserrata (DC.) Michelang	Melastomataceae	Small	2	*Augochloropsis sp.*	Small
Miconia dodecandra Cogn.	Melastomataceae	Medium	1	*Bombus morio*	Large
Miconia ibaguensis (Bonpl.) Triana	Melastomataceae	Small	3	*Apis mellifera*	Small
Miconia amygdaloides (DC.) R.Goldenb.	Melastomataceae	Small	1	*Augochloropsis sp.*	Small

Miconia crenata (Vahl.) Michelang.	Melastomataceae	Medium	2	*Augochloropsis sp.*	Small
Miconia neourceolata Michelang	Melastomataceae	Small	2	*Bombus morio*	Large
Miconia reversa (DC.) Ocampo & Almeda	Melastomataceae	Small	2	*Augochloropsis sp.*	Small
Miconia xanthocoma (Naudin) R.Goldenb.	Melastomataceae	Medium	1	*Ariphanartra palpalis*	NA
Pleroma cf. langsdorffianum	Melastomataceae	Medium	4	*Bombus morio*	Large
Pleroma clavatum (Pers.) P.J.F.Guim. & Michelang.	Melastomataceae	Medium	5	*Bombus morio*	Large
Pleroma fothergillii (Schrank et Mat. ex DC.) Triana	Melastomataceae	Large	2	*Bombus morio*	Large
Pleroma granulosum (Desr.) D. Don	Melastomataceae	Large	6	*Xylocopa brasilianorum*	Large
Pleroma heteromallum (D.Don) D.Don	Melastomataceae	Medium	5	*Xylocopa brasilianorum*	Large
Pleroma oleifolium (DC.) R. Romero & Versiane	Melastomataceae	Medium	1	*Augochloropsis electra*	Small
Pleroma raddianum (DC.) Gardner	Melastomataceae	Large	5	*Bombus morio*	Large
Pleroma reitzii (Brade) P.J.F.Guim. & Michelang.	Melastomataceae	Large	1	*Bombus morio*	Large
Pleroma stenocarpum (Schrank et Mart. ex DC.) Triana	Melastomataceae	Large	5	*Eulaema cingulata*	Large
Rhynchanthera brachyrhyncha Cham.	Melastomataceae	Medium	4	*Augochloropsis electra*	Small

Chamaecrista desvauxii var. *latistipula* (Benth.) G.P.Lewis	Fabaceae	Large	11	*Bombus morio*	Large
Chamaecrista nictitans (L.) Moench	Fabaceae	Small	8	*Dialictus sp.*	Small
Chamaecrista chamaecristoides (Collad.) Greene	Fabaceae	Medium	10	*Eulaema sp.*	Large
Solanum torvum Sw.	Solanaceae	Medium	4	*Euglossa viridissima*	Small
Solanum torvum Sw.	Solanaceae	Medium	3	*Euglossa viridissima*	Small
Solanum torvum Sw.	Solanaceae	Medium	1	*Euglossa viridissima*	Small
Solanum torvum Sw.	Solanaceae	Medium	1	*Xylocopa micans*	Large
Solanum stramonifolium Jacq.	Solanaceae	Medium	13	*Melipona scutellaris*	Small

CAPÍTULO 2: When size does not matter: poricidal flowers exhibit high bee dependence and low pollen limitation regardless of flower size

* Este manuscrito será submetido para o jornal New Phytologist

Abstract

- The transition from cross-fertilization to self-fertilization in plants involves a reduction in flower size, leading to closer reproductive organs, promoting self-pollination. Large-flowered species rely more on pollinators, but poricidal flowers in buzz-pollinated plants have unique features, accessed only by buzzing bees. Here, we investigated how the size of flowers with poricidal anthers relates to the degree of dependence on bee pollinator service and the magnitude of pollen limitation. We hypothesized that plant species with larger flowers, non-apomictic, and with additional rewards than pollen (i) depend more on vibrating bees for plant reproduction and (ii) are more pollen limited than plant species with smaller flowers, apomictic and without other rewards.
- We conducted a systematic quantitative review and used a meta-analytic approach to investigate both hypotheses. We recovered from the literature effect sizes describing the reproductive dependence of poricidal flowers on pollinators and the pollen limitation in 150 plant species with poricidal flowers in 89 studies that performed pollination experiments. We obtained flower size measurements from herbarium materials in online databases and digital plant collections.
- In contrast to most angiosperms and our initial hypotheses, flower size in plants with poricidal flowers does not affect their dependence on pollinators or the level of pollen limitation in natural populations. Interestingly, non-autogamous poricidal anthers heavily rely on bee pollinators, while autogamous apomixis shows lower dependence, irrespective of flower size. Flowers with poricidal anthers have minimal pollen limitation, indicating efficient buzz pollination. This efficiency remains consistent, even when the flower offers additional resources like nectar.

  The phenotypic coupling between poricidal flowers and vibrating bee species enables a highly efficient pollination process, promoting sexual reproduction and exhibiting similarities to patterns observed in other specialized pollination systems.

Keywords: apomixis, bee pollination, flower size, sonication, mating systems, melittophily, meta-analyses.

1. Introduction

Throughout the evolutionary history of flowering plants, reproductive strategies have undergone multiple changes, favoring the transition from allogamous to autogamous systems (Barrett, 2002). Multiple hypotheses have been proposed to explain these recurrent transitions between mating systems (Sicard and Lenhard, 2011). Among those, the hypothesis of resource allocation shift due to reduced pollinator availability has greater empirical support in explaining the emergence of autogamous systems. According to this hypothesis, reduced pollinator availability in populations would favor the selection of smaller, less attractive flower phenotypes with alternative reproductive safety mechanisms such as autonomous autogamy and apomixis (Sargent et al., 2007; Sicard and Lenhard, 2011; Paterno et al., 2020). Therefore, in general, under scenarios of pollinator scarcity, increased pollen limitation in populations could create the necessary ecological conditions for the selection of smaller flower phenotypes that depend less on pollinators for plant sexual reproduction (Sicard and Lenhard, 2011). Consequently, it is expected that plant species with smaller flowers exhibit lower dependence on pollinators than species with larger flowers to mitigate pollen limitation.

The transition from obligatory cross-fertilization mating systems to those allowing self-fertilization, accompanied by a reduction in flower size, has occurred multiple times throughout evolution (Barrett, 2002). In fact, flowers capable of self-fertilization are often smaller than their cross-fertilized ancestors (Stebbins, 1970). The reduction in flower size is commonly linked to a decrease in spatial separation between the male and female reproductive organs within the flower (hereafter referred to as herkogamy) (Barrett et al., 1996; Vallejo-Marín et al., 2014). The reduction in herkogamy has also been identified as a crucial functional trait for increasing self-fertilization rates in self-compatible plants (Opedal, 2018). Thus, larger flowers in angiosperm species are expected to rely more heavily on pollination services from flower visitors. This increased dependence of plants with larger flowers on pollinators is balanced by the higher attractiveness of these flowers, leading to a greater number of floral visitors (e.g., Delgado et al., 2023). However, the pollination service is not constant over time and space. Consequently, in the absence or scarcity of flower visitors, larger flowers in angiosperm species may experience more pronounced pollen limitation within local populations when compared to plant species with smaller flowers.

Hypotheses explaining the parallel evolution of self-fertilization mating systems with a reduction in flower size have been frequently described for self-compatible flowers that offer nectar as the primary flower resource to pollinators (e.g., flowers of *Curatella americana*, Rech et al., 2018). Similar expectations have also been proposed for species with flowers that primarily offer pollen as the flower resource (hereafter referred to as poricidal flowers – Buchmann, 1983; Fig. 1) (e.g., Solanaceae, Vallejo-Marín et al., 2014). However, there is limited empirical evidence regarding these processes in poricidal flowers, which represent 8% of all angiosperm species (Buchmann, 1983). Some of these plant species exhibit (i) traditional pollen flowers, providing an abundance of pollen as the primary reward for visiting insects, instead of nectar or other substances (Vogel, 1978). Other species with poricidal flowers (ii) also offer additional flower resources, such as nectar or oil (see also Delgado et al., 2023 for this definition of poricidal flowers). Regardless the type of flower resource to flower visitors, pollen extraction from the poricidal anthers is almost exclusively performed by bee species capable of vibrating flowers. These vibrating bees actively collect pollen to feed their larvae in the nests while passively transferring pollen to the flower's stigma (Vallejo-MarÍn et al., 2009; De Luca and Vallejo-Marín, 2013). In the absence of vibrating bees, buzz-pollination becomes unviable for these plants since, regardless of flower size, the pollen grains remain trapped within the poricidal anthers. Even in plants with smaller poricidal flowers, autonomous autogamy would still be very rare, except in tiny poricidal flowers with large anther pores that release pollen grains without bee vibration (e.g., some *Miconia* species; Maia et al., 2016). Therefore, contrary to expectations for flowers where nectar is the primary flower resource, it would be expected that plants with poricidal flowers would be highly dependent on buzzing bees as pollinators. Thus, poricidal flowers could easily experience high pollen limitation when vibrating bees are absent or when they excessively exploit pollen grains offering a low-quality pollination service to the plants.

Despite self-fertilization in flowers with poricidal anthers typically relies on the presence of a vector to remove pollen from the poricidal anthers, these plants may have evolved alternative strategies to minimize their pollinator dependence. One of these strategies is apomixis (Hörandl, 2009), i.e., the asexual formation of seeds, with the disruption of the sexual process due to hybridization events followed by polyploidization (Koltunow and Grossniklaus, 2003; Hand and Koltunow, 2014; Cardoso et al., 2018).

This reproductive strategy can reduce the reproductive bottleneck imposed by pollen limitation in small populations or compensate for the lack of pollinators (Rambuda and Johnson, 2004; Cardoso et al., 2018), although reducing the populational genetic diversity (Hand and Koltunow, 2014). There are different types of apomixis, but most apomictic plants depend on the fertilization of the polar nuclei for endosperm development and then only produce viable seeds when some pollen is deposited to the stigma, a process known as pseudogamous apomixis (Hörandl, 2009; Sampaio et al., 2013b, a). However, a small portion of apomictic species, including species with poricidal anthers, develop endosperm autonomously (Hörandl, 2009; Sampaio et al., 2013a). Autonomous apomixis and self-fertilization are reproductive strategies that provide reproductive assurance for plants regardless of pollinators (Hörandl, 2009) and, through different mechanisms, they reduce the plants' dependence on pollinators and the pollen limitation in natural populations.

This study used a meta-analytic approach to investigate the relationship between flower size and reproductive strategies in plant species with poricidal flowers. Our research consisted of a systematic review of pollination studies performing manipulative experiments to assess the impact of pollinator exclusion on plant reproductive success and/or the level of pollen limitation within and between natural populations. Our study addressed two key questions: (i) How do flower size, the occurrence of apomixis, and the presence of additional flower resources influence the reliance of plants on pollinator services for reproduction? (ii) How do flower size, the occurrence of apomixis, and the presence of additional flower resources determine the extent of pollen limitation in these plant species? We first hypothesized that non-apomictic plant species with larger flowers and additional flower rewards besides pollen would exhibit a higher dependence on pollinators than apomictic plant species with smaller poricidal flowers lacking additional rewards such as nectar or oil. This pattern would occur because larger flowers have higher herkogamy, reducing the likelihood of self-pollination without vector assistance. Consequently, the plants become more reliant on these vectors, especially if they are non-apomictic and lack additional floral rewards other than pollen. Furthermore, we also hypothesized that non-apomitic species with larger poricidal flowers bearing only pollen as flower rewards to pollinators would face greater pollen limitation.

2. Materials e methods

2.1. Data search, screening, and eligibility

To conduct our meta-analysis, we first applied the PICO framework (Population, Intervention, Comparison, Outcome protocol) (Nakagawa et al., 2017) to guide the systematic review process by helping to create comprehensive search strategies, facilitating study selection, and aiding data extraction for outcome comparisons across studies with plant species with poricidal flowers – see Fig. 1 (PICO model components available in Table S1). We established our systematic search strategy for studies on the Web of Science (www.webofknowledge.com - core library) and Scopus (www.scopus.com, subject area: Life Science) platforms, using combinations of different keywords (see Table 1). With the search, we gathered 261 potential suitable studies. In addition to this main search, we performed an additional search on Google Scholar and the *GloPL* database to maximize the breadth of our searches and the number of studies found. The *GloPL* database is a global database that compiles pollen limitation data on angiosperm species (Burns et al., 2019). This additional search resulted in the location of 111 studies not located in the first search. Our search resulted in 372 studies published until June 26, 2023.

We analyzed the titles, abstracts, and/or methods of each of the 372 case studies retrieved in the searches described above. We selected all studies that (i) used as model plant species bearing flowers with poricidal anthers; (ii) carried out pollination experiments including at least two of three experimental treatments: bagged flowers avoiding pollinator visits, unbagged flowers that were exposed to natural pollinator visits, and flowers that were cross-supplemented with pollen from one or more plant donors; (iii) provided complete descriptive statistics of any proxy of plant reproductive success in each treatment, including mean, standard deviation or error, and sample size; and (iv) were conducted in the field rather than in laboratory or greenhouse.

In some studies, the descriptor of plant reproductive success was presented as proportion (e.g., the proportion of flowers converted to fruits) without describing deviance or standard error associated with pollination treatments. In these cases, we estimated the expected standard deviation using a binomial model based on the formula $\sqrt{p}.\frac{1-p}{n}$, in which p represents the proportion of fruits formed and n, the sample size in the treatment (Gelman and Hill, 2007). In eight studies, the authors did not inform the type of anther dehiscence in the plant species used as a model. In these cases, we searched for this information in taxonomic studies describing the species, genera and botanical families to which the species belonged and in publications describing the diversity of pollen flowers and/or with poricidal anthers (e.g., Vogel, 1978; Buchmann, 1983; Vallejo-Marín et al., 2010). In the end, we found 89 ecological studies, including 150 plant species with poricidal flowers, that met our inclusion criteria and which, consequently, were included in our final database used for extracting the effect sizes used in our analyses.

In addition to descriptive statistics for each treatment, we extracted from the ecological studies the following information related to the plant species used as models in the studies: whether the species were self-compatible or self-incompatible, whether they were apomictic autogamous or not, and what resources these species offer to pollinators (pollen, nectar, oil, and/or resin). All this information was available in the studies included in our database.

2.2 Calculation of effect sizes

To evaluate each our two hypotheses, we created two different effect size metrics using the *Hedge's g* (Gurevitch and Hedges, 2001), the first one describing the degree of dependence of flowers with poricidal anthers on pollinators, and the second one describing the magnitude of their pollen limitation in each plant population. In our case, the *Hedge's g* is a standardized difference between means of plant reproductive success descriptors in the different experimental pollination treatments used in the studies. We calculated this metric based on the means, standard deviations, and sample size reported by the studies included in our database using the *escalc* function from the *metafor* package in R (Viechtbauer, 2010).

We calculated the effect size describing the pollinator dependency contrasting the mean female reproductive success of plants in the treatment in which flowers were supplemented with pollen from a donor plant (cross-pollination) with the mean of treatment in which flower visitors were experimentally prevented from visiting flowers (pollinator exclusion treatment). For the most comprehensive variable of female reproductive success, seeds per fruit, the higher seed production in the supplement compared with the bagged treatment, indicated a positive and significant dependence on pollinators as applied by Koch et al. (2020). Therefore, the greater and more positive the value of *Hedge's g*, the greater the dependence of plants on the cross-deposition of pollen by pollinators and, consequently, the lower the self-fertilization capacity of the species.

Similarly, we calculated the effect size of pollen limitation by contrasting the average female reproductive success of plants in the treatment in which flowers were supplemented with pollen from a donor plant (cross-pollination) with the treatment in which flowers were naturally exposed to flower visitors available in the study area (natural pollination). In this case, the higher the *Hedges' g* values, the lower the effect of pollen supplementation and, consequently, the higher the pollen limitation on the reproductive success of the plants in the populations used in the studies.

2.3 Extraction of flower size from botanical collections

To assess how flower size may affect the degree of dependence of poricidal flowers on pollinators and the magnitude of their pollen limitation, we measured the flower size of the plant species included in our database using photographs of herborized materials deposited in online databases and herbaria with digital collections: speciesLink (http://inct.splink.org.br/); New York Botanical Garden - NYBG (http://sweetgum.nybg.org/); Royal Botanic Gardens Kew (https://www.kew.org/) and the Global Plants database - JSTOR (https://plants.jstor.org/). For select the sampling herborized materials, we used four criteria according to Delgado et al., (2023): i) the specimen should be herborized with visible flowers and with parts not bent or damaged; ii) digital images had to be in high resolution and with scale; iii) the corolla and androecium of the flowers intact; (iv) the specimen must be identified by a taxonomic specialist or to have information that would allow its identification by comparison with another specimen already identified by a specialist. We excluded duplicates and obtained 665 specimen images of the 150 plant species included in our database (i.e., 100% of the plant species used as models in our database studies), with a mean of 5.0 ± 3.6 (range: 1-25) images (specimens) per species.

To estimate the average flower size, we selected five flowers from images of different exsiccates for each species in our database. In the case of species for which we found more than five images (exsiccates), we measured one flower per exsiccate and discarded the spare ones. For 41 plant species, we found less than five images in digital collections.

In these cases, we measured more than one flower per exsiccate to obtain measurements of at least five flowers for these species. Before each measurement, we calibrated the digital photos based on the scale attached to the voucher specimens and measured the diameter of the flower. We defined the flower diameter as the diameter of the corolla opening measured by the linear distance between the two edges formed by the tip of the corolla on opposite sides, perpendicular to the central axis of the flower. When flowers were tubular (e.g., *Ericaceae* species), we used the diameter in the middle of the flower tube to measure flower size. Following the standardization performed by Delgado et al. (2023), we also classified flower diameter measurements into three discrete size categories: small flowers (diameter ≤ 0.99 mm), medium flowers (1.00 mm ≤ diameter ≤ 2.99 mm), and large flowers (diameter ≥ 3.00 mm) (see Delgado et al., 2023 for more details).

2.4 Meta-analytical models

To evaluate our hypotheses, we built six multilevel meta-regression models using the *metafor* package (Viechtbauer, 2010) in R software (R Core Team, 2022). The three first models explored the effect sizes describing the degree of dependence of poricidal flowers on pollinators, and the other three models explored the effect sizes describing the magnitude of pollen limitation.

To evaluate the overall mean reproductive dependence of poricidal flowers on pollinators, we built a random-effect meta-analytical model with a multilevel structure only including the set of random variables. Subsequently, we also constructed two meta-analytical models to assess how variations in flower size, occurrence of apomixis, and flower resources other than pollen affect the dependence of plants with poricidal flowers on pollinators. We expect that larger flowers with higher herkogamy rely more on the presence of a vector for pollen transfer, thus being more dependent on vectors for sexual reproduction. In the first model, we used the effect sizes of pollinator dependence (*Hedges' g*) as the response variable and the three categories of flower size and the presence/absence of autonomous apomixis as the two moderators in the additive model. In this case, autonomous apomixis would minimize the plant reproductive dependence on bee pollinators. In the second model, we used the effect sizes of pollinator dependence (*Hedges' g*) as the response variable, and the flower size categories and the presence/absence of additional flower resources other than pollen as the two moderators in the additive model. In this case, the presence of an additional flower reward other than pollen, would minimize the plant dependence on pollinators for sexual reproduction.

To evaluate the overall mean magnitude of pollen limitation in plant species with poricidal flowers, we built a random-effect meta-analytical model with a multilevel structure only including the set of random variables. Subsequently, we also constructed two meta-analytical models to assess how variations in flower size, occurrence of apomixis, and flower resources other than pollen affect the effect size of pollen limitation on the female reproductive success of plants with poricidal flowers. In the first model, we used the effect sizes of pollen limitation (*Hedges' g*) as the response variable, and the three categories of flower size and the presence/absence of autonomous apomixis as the two moderators in the additive model. We incorporated apomixis into this model since autonomous apomictic plants produce seeds without pollen transfer (asexual reproduction), making them naturally non-pollen limited (Eckert et al., 2007). This feature sets them apart from non-apomictic plant species. In the second model, we used the effect sizes of pollen limitation (*Hedges' g*) as the response variable, and the flower size categories and the presence/absence of additional flower resources other than pollen as the two moderators in the additive model. We included other flower resources in this model, as additional resources could increase the frequency and diversity of flower visitors, reducing pollen limitation in these plants.

In the six models, we included the inverse of the variances of *Hedges' d* values. We also included five random factors in all models described above: incompatibility system (self-compatible or self-incompatible), study identity, phylogenetic relatedness between plant species, taxonomic name (plant identity), and the type of female plant reproductive success descriptor measured in the studies. The type of reproductive success descriptor was grouped into descriptors of fruit formation from flowers, number of seeds per fruit, number of seeds per ovule, and the number of fruits formed per inflorescence.

Given that we estimated effect sizes of pollinator dependency and effect sizes of pollen limitation across multiple plant species, we included phylogenetic relatedness between plant species in our models due to historical non-independence between species. To this end, we constructed an ultrametric phylogeny comprising all 113 plant species that comprise the pollinator dependency effect database. We also built an ultrametric phylogeny comprising 129 plant species that comprise the pollen limitation effect database. In both cases, we used the V. PHYLOMAKER R package (Jin and Qian, 2019) to construct the plant phylogeny. We then transformed the ultrametric tree into a standardized variance-covariance matrix that reflects the phylogenetic relatedness between plant species. The standardized variance-covariance matrix was then input into our six meta-analytic models.

2.4 Publication bias and data heterogeneity

We analyzed possible publication biases in the studies in our database based on a modification of Egger's regression (Egger et al., 1997) proposed by Nakagawa and Santos (2012). In this modification, we use the residuals from each meta-analytic model as the response variable and the square root of the variance of the effect sizes as the moderator in a meta-regression. If the regression intercept differs from zero, the relationship between the effect sizes and their respective variances is asymmetric and, consequently, biased (Sterne and Egger, 2005)

We used the I^2 index to estimate the heterogeneity of our models. I^2 allows the breakdown of the overall heterogeneity of the models, indicating the percentage of variation explained by each of the random variables included in the meta-analytic models (Higgins, 2003). The sum of all I^2 represents the total heterogeneity explained by the models.

3. Results

3.1 - Impact of flower size, apomixis, and additional flower reward on pollinator dependency effect size

To test our first hypothesis on pollinator dependency, we obtained 176 effect sizes from 99 plant species with poricidal flowers belonging to 13 families (Fig. 1). The most common families in our database were *Melastomataceae* (43.2% of effect sizes) and *Fabaceae* (25.2%). Additionally, 19.3% of the plant species included in our database are autonomous apomictic, and 80.7% are autonomous non-apomitic. Regarding the flower resource, 75.5% of the plant species contain only pollen as a flower reward for bee pollinators, while 24.5% provide pollen and other flower resource, especially nectar.

Regarding flower size, 29.5% of the species included in our database have small flowers (N = 52 effect sizes), 44.3% have medium flowers (N = 78 effect sizes), and 26.1% have large flowers (N = 46 effect sizes).

Overall, plant species with poricidal flowers had a high mean degree of pollinator dependence (*Hedges' g* + SE = 3.6 ± 1.4; Table 2, first line; Fig. 2-A). A large proportion of the effect sizes in our database were positive above 1 standard deviation unit (Fig. 2-A), indicating that pollinator exclusion is negative for plant reproduction in most cases, highlighting a high degree of dependence of these plants on the pollinator for female reproductive success. On the other hand, we did not observe differences in the mean degree of pollinator dependency between species with different flower sizes (Fig. 3). Differently, we detected a high degree of pollinator dependency in non-apomictic plant species than in species with autonomous apomictic flowers that not differed from zero (Table 2, second line, Fig. 3-A). In sum, we found that non-apomictic plant species rely heavily on pollinators for reproduction, while species with autonomous apomictic flowers do not show a significant dependence on pollinators for their female reproductive success. Finally, the high mean degree of pollinator dependency in poricidal flowers was not related to the offering of an alternative reward besides pollen to pollinators (Table 2, third line, Fig. 3-B).

3.2 - Impact of Flower Size, apomixis and additional floral reward on the magnitude of pollen limitation

To test our second hypothesis on the magnitude of pollen limitation on poricidal flowers, we obtained 258 effect sizes describing pollen limitation in 129 species with poricidal flowers belonging to 16 families. The most common families in our database are *Melastomataceae* (37.6% of species), *Solanaceae* (18.8%), and *Fabaceae* (17.8%). Additionally, only 12.4% of plant species are autonomous apomictic and 87.6 % are autonomous non-apomitic flowers. Regarding the floral resource, 71.4% of the plant species contain only pollen as floral reward to pollinators, while 28.6% have pollen and other floral resource, especially nectar. Regarding flower size, 25.2% of the species included in our database have small flowers (N = 69 effect sizes), 52.9% have medium flowers (N = 145 effect sizes), and 21.9% have large flowers (N = 60 effect sizes).

Overall, plant species with poricidal flowers had low levels of pollen limitation, slightly positive and significantly different from zero (*Hedges' g* + SE = 0.5 ± 0.2; Table 2, fourth line; Fig. 2-B). Also, we did not observe differences in the magnitude of pollen limitation between plant species with different poricidal flower sizes (Fig. 4). We detected a very low levels of pollen limitation in non-apomictic plant species than in species with autonomous apomictic flowers that not differed from zero (Table 2, fifth line, Fig. 4-A). Additionally, the existence of an additional floral resource did not correlate with the magnitude of pollen limitation, which was very low in all cases (Table 2, sixth line, Fig. 4-B).

3.3 - Publication bias and data heterogeneity

We found evidence of publication bias in our dataset of size effects since the intercepts of all Egger's regressions for the rma.mv models were different from zero (Table 3). Also, all the models described above exhibited an intermediate level of heterogeneity, explained mainly by the type of reproductive success descriptor, study ID and the taxonomic name of plant species (Table 4).

4. Discussion

Here, we conducted a quantitative review of pollination studies and used a meta-analytical approach to investigate how flower size impacts the reproductive strategy of flowering plant species with poricidal anthers that are involved in a specialized buzz-pollination system. According to our expectations, a decline in flower size would be linked to self-fertilization strategies and a decrease in the plants' reliance on flower visitors for sexual reproduction, finally reducing pollen limitation locally. We also expected that pollinator dependence and pollen limitation would be reduced due to other mechanisms or traits, such as autogamous apomixis (asexual reproduction) and/or the presence of other floral resources besides pollen. However, contrary to what has been described for most angiosperms and to our initial hypotheses, our results showed that flower size in plants with poricidal flowers (~8% of angiosperms) does not influence the reproductive dependence of these plants on pollinators or even the magnitude of pollen limitation in natural populations. Interesting, non-apomictic buzz-pollinated plants with poricidal flowers (the majority in our dataset) exhibited a high dependence on bee pollinators for the female reproductive success, although they are slightly pollen limited. In contrast, autonomous apomictic plants with poricidal flowers demonstrated more reproductive independence from pollinators, and consequently, no pollen limitation was observed. The presence of an additional resource, such as nectar, in the poricidal flowers had no effect on these general patterns, similar to the lack of significant impact shown by floral size.

Our result demonstrates no direct relationship between flower size in species with poricidal anthers and the rate of spontaneous self-pollination, as species with smaller flowers exhibited the same degree of pollinator dependence as species with larger poricidal flowers. This result was different from what was expected for some lineages of plants with poricidal anthers (e.g., Vallejo-Marín et al., 2014), and it is more commonly observed in nectar flowers. In fact, this pattern differs from what has been described in plant species with nectar flowers bearing non-poricidal anthers, where the transition from relatively large to small flowers is accompanied by a change in flower organ allometry and a subsequent increase in self-fertilization rates.

Our result demonstrates no direct relationship between flower size in species with poricidal anthers and the rate of spontaneous self-pollination, as species with smaller flowers had the same degree of pollinator dependence as species with larger poricidal flowers. This result was different from what was expected for some lineages of plants with poricidal flowers (e.g. (Vallejo-Marín et al., 2014), and more commonly observed in nectar flowers with non-poricidal anthers, where the transition from relatively large to small flowers is accompanied by a change in flower organ allometry and a subsequent increase in self-fertilization rates (Harder and Aizen, 2010). Therefore, the reliance of poricidal flowers on vibrating bees to perform the buzz-pollination does not vary as a function of flower size, something distinct from the most common pattern in angiosperms. Such difference may be due to the tightly locked nature of pollen grains within the poricidal anthers. Although plants with poricidal anthers may evolve strategies for self-fertilization, they still depend on vectors, primarily vibrating bees (but also some flies), to release pollen grains and deposit them on their stigmas (Vallejo-Marín, 2019). Therefore, in this specialized pollination systems, the rate of spontaneous self-pollination did not commonly evolve in response to changes in flower size and herkogamy as expected in other pollination systems.

In our study, autonomous apomitic plant species were much less dependent on pollinators for reproduction than non-apomitic plants, regardless its flower size. It indicates that the autonomous apomixis, a kind of asexual reproduction (Eckert et al., 2007), can be an effective reproductive security strategy in plants with poricidal flowers. Apomixis is frequently reported within the Melastomaceae family (Goldenberg and Shepherd, 1998; Santos et al., 2012; Maia et al., 2016), and in our database, 63% of the apomictic species were from this plant lineage. Some apomictic Melastomataceae plants have low pollen viability and even complete sterility in some populations (Goldenberg and Shepherd, 1998; Goldenberg and Varassin, 2001; Maia et al., 2016; Caetano and Oliveira, 2022). Also, flower visitation on these plants is extremely low, and in some cases, flowers did not receive visitors during anthesis (Renner, 1989; Goldenberg and Shepherd, 1998). Therefore, the larger effect size only in non-apomictic species is easily explained in our study, further corroborated by the fact that autonomous autogamy is common in *Melastomataceae* species (but see exceptions such as *Miconia angelana*, *M. sintenisii*, and *Tibouchina papyrus*; Renner, 1989; Santos et al., 2010, 2012). Still, it is important to consider that our study focused only on autonomous apomixis and not on other cases of pseudogamic apomixis. In the case of pseudogamous apomixis, the species remain dependent on pollinators, as fertilization is fundamental for endosperm production and full seed development. As a result, in such cases, plants are expected to remain dependent on pollinators regardless its flower size, aligning with the general patterns revealed in our analyses for plants with poricidal anthers.

Pollinator behavior during flower visits exerts strong directional selection on flower morphology (Knight et al., 2005; Glaettli and Barrett, 2008). In the case of poricidal flowers, restricting access to the floral resource to a select group of pollinators would promote the evolution of floral adaptations to these specific pollinators (phenotypic specialization *sensu* Ollerton, 2007). Such adaptations optimize the positive balance of interactions between both species involved (Buchmann, 1983; Anderson et al., 2009; Vallejo-Marín et al., 2010; Newman et al., 2014). For example, in a study conducted by Vallejo-Marín and collaborators (2010), the authors observed correlations between various morphological features of buzz-pollinated flowers, such as heteranthery, enantiostyly, among others, across 16 botanic families not closely related within angiosperms. In addition, many species with poricidal flowers have more than one type of anther within each flower (heterantery) with differentiated functions that favor pollen grains distribution for pollination and bee feeding (Saab et al., 2021). This phenomenon can be illustrated by *Melastoma malabathricum* (*Melastomataceae*), where the experimental removal of large stamens does not impact flower visitation, unlike the removal of short stamens, highlighting the distinct functions of stamens within flowers (Luo et al., 2008). These features, among others, could increase the reproductive benefits and decrease the pollen costs, explaining the pattern of very low pollen limitation in these plant populations. We also highlighted that these flower features reducing pollen limitation were not necessarily associated with changes in flower size. If these unique features of flowers with poricidal anthers indicate greater phenotypic specialization, the high efficiency of buzz-pollination could be explained by the morphological and behavioral coupling between plants and vibrating bees that visit flowers and effectively buzz poricidal anthers (Delgado et al., 2023). Indeed, in more specialized pollination systems, pollinators tend to be more efficient in transferring pollen to the stigma (Fenster et al., 2004; Almeida et al., 2013; Rosas-Guerrero et al., 2014; Amorim et al., 2017, 2021). In sum, buzz pollination emerges as a highly bee-dependent system regardless its flower size, with vibrating bees playing a crucial role in effectively promoting plant sexual reproduction, leading to a really low levels of pollen limitation in natural populations.

Contrary to what we expected, plant species with more than one floral resource do not have lower pollen limitation than species that offer only pollen to flower visitors. Vallejo-Marín et al. (2010) reported nectar production in poricidal flowers in three plant families. In the literature, we encountered five additional families with poricidal flowers, some of which also produce floral nectar. In these instances, nectar enhances flower attractiveness, providing additional rewards to pollinators and potentially improving pollination success (e.g., Moquet et al., 2015). Including nectar in the pollination system can lead to less specialization (or more generalization), attracting a wider range of floral visitors and ensuring successful sexual reproduction of the plants. However, in more specialized pollination system, attracting a more diverse assemblage of flower visitors is not necessarily advantageous since only a subset of them can be effective pollinators. In the case of poricidal flowers, the unique morphological features of poricidal flowers linked with vibrating bees may already be optimized to mitigate pollen limitation, reducing the relative importance of flower attractiveness to pollen transfer efficiency (Knight et al., 2005). Therefore, regardless the flower size or resource, this highly efficient pollination system experiences little or no pollen limitation, likely attributed to the adaptive nature of specific phenotypic features of the interacting species (Delgado et al., 2023).

In conclusion, our findings demonstrate that flower size in poricidal flowers across angiosperms is not related to variation in bee pollinator dependence and the magnitude of pollen limitation. Flowering plants with non-autogamous poricidal anthers depend highly on bee pollinators for reproduction, while lower pollinator dependence comes from autogamous apomixis, mainly in *Melastomaceae*, independent of flower size. Flowers with poricidal anthers, irrespective of their size, exhibit a very low pollen limitation, highlighting the high efficiency of buzz pollination in transferring pollen from poricidal anthers to stigmas. This efficiency holds whether the flower offers only pollen or provides an additional floral resource like nectar. This pattern emerges because poricidal flowers and vibrating bees are phenotypically coupled, and just a few visits of larger or smaller bee species that can vibrate and release pollen grains from poricidal flowers with different sizes (Delgado et al., 2023) could be enough to facilitate sexual reproduction, a phenotypic coupling similarly observed in other specialized pollination systems.

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Figures

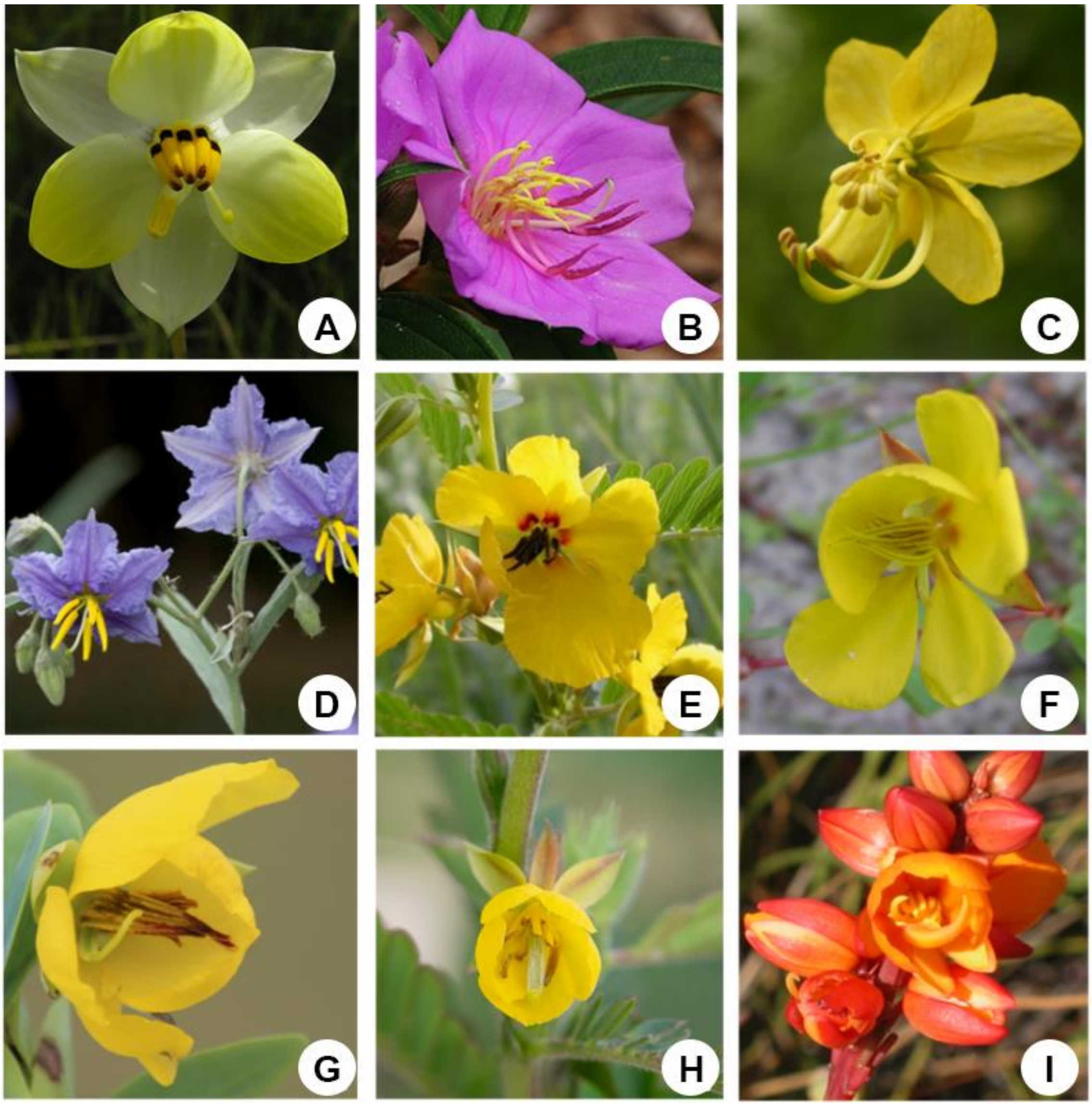

Figure 1: Flowers of different angiosperm species with poricidal anthers: A- *Cynella alba* (Photo: Barrett); B- *Melastoma malabathricum*; C- *Cassia fistula* (Photo: Sidhu S.); D- *Solanum sp.* (Photo: Patrick A.) E- *Chamaecrista fasciculata* (Photo: Schroeder J.); F- *Chamaecrista ramosa* (Photo: Queiroz. R); G- *Chamaecrista desvauxii* (Photo: Tamiris Delgado); H- *Chamaecrista nictitans* (Photo: Tamiris Delgado) e I- *Chamaecrista ochnacea* (Photo: Shimizu G.).

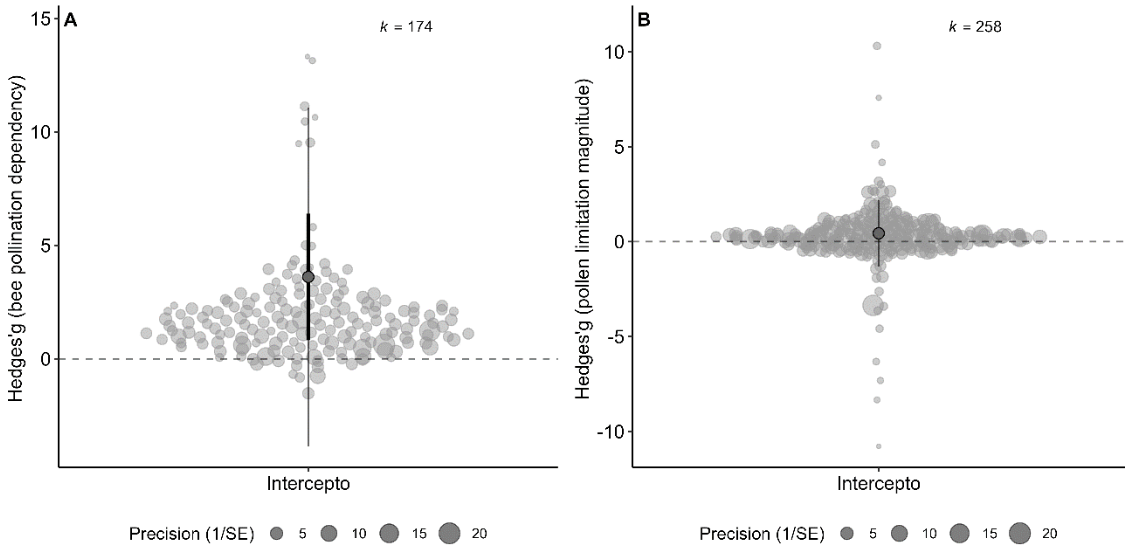

Figure 2 - Variation in overall effect sizes without the addition of moderators. A- Variation in effect sizes describing the pollinator dependency to the reproductive success of plants with poricidal anthers. B- Magnitude of pollen limitation on the reproductive success of plants with poricidal anthers. The grayscale dots are the effect sizes (*Hedges' g*) extracted from each case study. The dot size varies according to the precision of effect sizes (*Hedges 'g*). The light gray dot is the average effect size the statistical model estimates. The vertical bold lines are the 95% confidence intervals. The horizontal dashed line is the reference value for no effect (*Hedges' g* = 0). K = number of effect sizes (number of plant species).

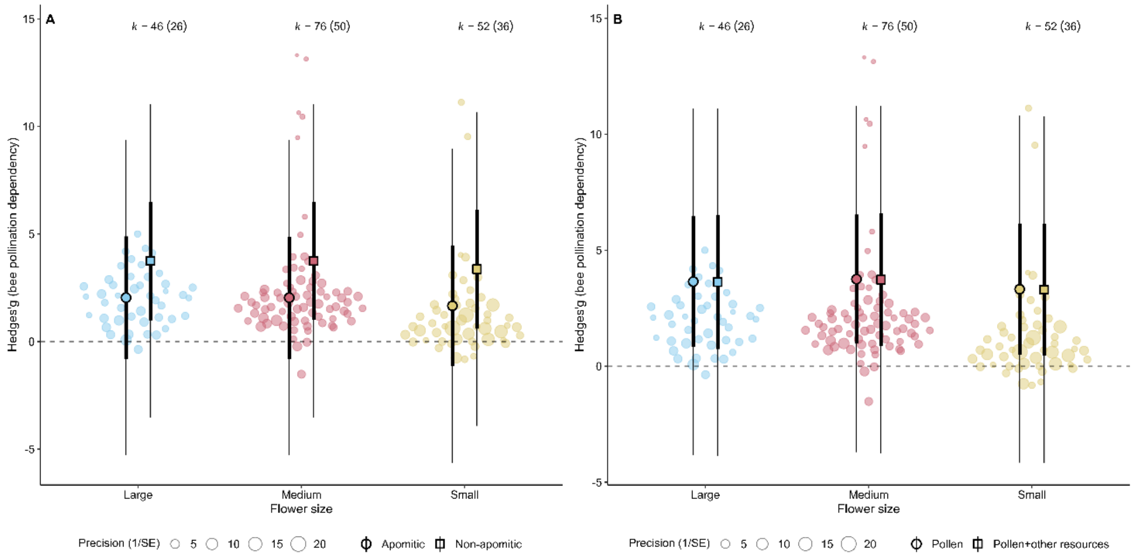

Figure 3 – Variation of effect sizes describing the degree of dependence of poricidal flowers on pollinators as a function of floral size, occurrence of autonomous apomixis and type of resource offered to floral visitors. Positive values represent a higher degree of dependency on pollinators, and the flower size categories are: 'small', flower diameter < 0.99 cm; 'medium', 1 cm ≤ flower diameter ≤ 2.99 cm; 'large', flower diameter > 3 cm. **A** – Impact of flower size and apomixis on pollinator dependency, including autonomous apomictic plants (circle) and non-apomictic plants (square). **B** - Impact of flower size and rewards on pollinator dependency, including flowers with only pollen (circle) and flowers with pollen plus additional resources (square). The dots represent the effect sizes (*Hedges' g*) extracted from each ecological study, and the dot size varies according to the precision of the effect size. The central circles are the estimated mean effect sizes. The vertical bold lines are the 95% confidence intervals. The horizontal dashed line is the reference value for the absence of the effect of pollinator exclusion (*Hedges' g* = 0). K = number of effect sizes (number of plant species).

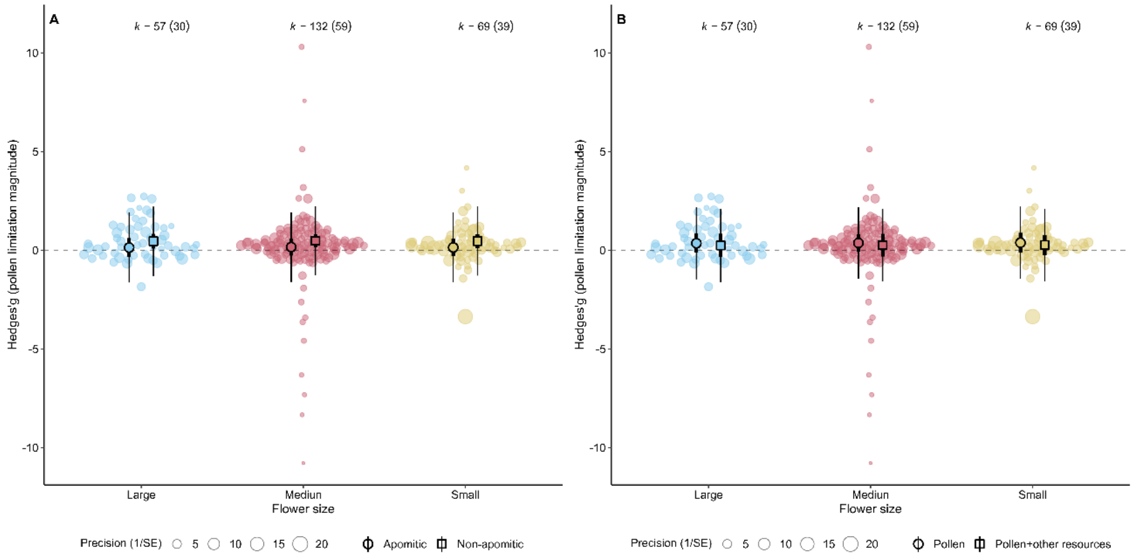

Figure 4 - Variation of effect sizes describing the pollen limitation magnitude in plant species with poricidal flowers as a function of flower size, occurrence of autonomous apomixis and type of resource offered to floral visitors. Positive values represent a higher pollen limitation on plant populations, and the flower size categories are: 'small', flower diameter < 0.99 cm; 'medium', 1 cm ≤ flower diameter ≤ 2.99 cm; 'large', flower diameter > 3 cm. **A** – Impact of flower size and apomixis on the magnitude of pollen limitation, including autonomous apomictic plants (circle) and non-apomictic plants (square). **B** - Impact of flower size and rewards on the magnitude of pollen limitation, including flowers with only pollen (circle) and flowers with pollen plus additional resources (square). The dots represent the effect sizes (*Hedges' g*) extracted from each study, and the dot size varies according to the precision of the effect size. The circles are the estimated mean effect sizes. The vertical bold lines are 95% confidence intervals. The horizontal dashed line is the reference for the absence of the pollen limitation effect (*Hedges' g* = 0). K = number of effect sizes (number of plant species).

Tables

Table 1: The systematic quantitative review used search engines, search strategies, and keyword combinations. The systematic review targeted experimental pollination studies that use plant species with poricidal flowers as plant models.

Search engine	Search strategy	Key-word combinations
Web of Science and Scopus	Advanced search	TS= (("pollinator dependency" OR "pollinator* exclusion" OR "pollen manipulation" OR "open-pollinated" OR "open-pollination" OR "self-pollination" OR"self pollinate"OR "pollination manipulation*" OR "reproductive strateg*" OR "controlled pollination*" OR "pollinator limitation" OR "pollen limitation" OR "limitação polínica" OR "pollination experiment*" OR "floral biology" OR "biologia floral" OR "sistema reprodutivo" OR "reproductive system" OR "breeding system" OR "mating system" OR "mating" OR "autogamy" OR "selfing" OR "sexual reproduction" OR "self pollen" OR "natural pollination" OR "hand-pollinated" OR "supplementary pollination" OR "caging" OR "selfed flowers" OR "spontaneously selfed" OR "autopolinização" OR "self-pollination" OR "autogamic" OR "autogamy" OR "emasculation" OR "pollinator exclusion" OR "enclosed plants" OR "manual pollination" OR "system of reproduction") AND ("flores com anteras poricidas" OR "flowers with poricidal anther*" OR "anteras poricidas" OR "poricidal anthers" OR "flores de pólen" OR "pollen flower*" OR "anther pore apical" OR "buzz pollination" OR "buzz-pollination" OR "flor* com anter* poricid*" OR "antera* poricida*" OR "poricid* anthe*" OR "buzz pollinat*" OR "buzz-pollinat*" OR "anthe* with apical por*" OR "heteranther*" OR "heteromorphic androecium" OR "heterostemony" OR "mirror-image flower*" OR "mirror image flower*" OR "enantiostyly" OR "nectarless flower*" OR "pollen as the only reward*" OR "vibrated the anther*"OR "absence of nectar" OR "vibratory mechanism*" OR "buzz anther*" OR "polinização por vibração"))

Google scholar	Regular Search – each key-word combination separately	Sistema reprodutivo flor com antera poricida anther poricidal reproductive system reproductive system in pollen flower reproductive system, flower anthers poricidal sistema reprodutivo & flores com anteras poricidas poricidal anthers & reproductive system poricidal anthers reproductive system “sistema reprodutivo” & “flores com anteras poricidas” “poricidal anthers” & “reproductive system” “pollen flowers” & “reproductive system” “flower” & “poricidal anthers” & “reproductive system” “breeding system” & “pollen flowers” “breeding system” & “poricidal anthers” “breeding system” & “buzz pollination” “breeding system” & “buzz-pollination” “reproductive system” & “poricidal anthers” “reproductive system” & “buzz pollination” “reproductive system” & “buzz-pollination” “mating system” & “poricidal anthers” “mating system” & “pollen flowers” “mating system” & “buzz pollination” “mating system” & “buzz-pollination” “sexual reproduction” & “pollen flowers” “sexual reproduction” & “poricidal anthers” “sexual reproduction” & “buzz pollination” “sexual reproduction” & “buzz-pollination”

Table 2: Summary of the six meta-analytical models used in this study.

Models			Number of size effects (number of species)	Estimates (± SE)	Z statistic	Q statistic	P-value	Graphs
	Hedges'g	Moderators						
1	Pollinator dependency	None	174 (112)	3.6 (1.4)	2.5	-	0.011	Figure 2A
2	Pollinator dependency	Flower size + apomixis		-	-	25.4	< 0.01	Figure 3A
3	Pollinator dependency	Flower size + floral resource		-	-	1.4	0.705	Figure 3B
4	Pollen limitation	None	258 (128)	0.4 (0.1)	2.5	-	0.010	Figure 2B
5	Pollen limitation	Flower size + apomixis		-	-	4.1	0.258	Figure 4A
6	Pollen limitation	Flower size + floral resource		-	-	0.3	0.951	Figure 4B

Table 3: Summary of the Egger's test for each meta-analytical model (see the complete models in the Table 2).

Models	Estimates	T-value	P value
1	- 3.67	-18.19	<0.001
2	-3.46	-17.14	<0.001
3	-3.62	-18.14	< 0.001
4	0.28	1.58	0.114
5	0.31	1.76	0.078
6	0.27	1.53	0.126

Table 4: Heterogeneity estimates of the six meta-analytical models constructed in this study. The first three model explored the size effects describing the degree of pollinator dependency, and the last three models explored the effect sizes describing the magnitude of pollen limitation (see the complete models in the Table 2). Bold estimates represent the random variables that explained most of each model variation.

Models	**I^2 for each random variable**					**Total**
	Study ID	**Taxonomic name**	**Plant phylogeny**	**Plant reproductive sucess descriptor type**	**Incompatibility system**	
1	13.0	10.3	2.5	**71.9**	<0.1	97.9
2	10.9	11.2	<0.1	**75.6**	<0.1	97.8
3	10.4	10.8	1.4	**71.9**	<0.1	97.9
4	**32.4**	**40.4**	<0.1	0.2	3.7	76.8
5	**31.0**	**43.1**	<0.1	0.1	2.0	76.4
6	**32.1**	**41.3**	<0.1	0.2	3.5	77.2

Supplementary material

Table S1. –PICO framework (Population, Intervention, Comparison, Outcome protocol) used to guide the systematic review process by helping to create comprehensive search strategies, facilitating study selection, and aiding data extraction for outcome comparisons across studies (Nakagawa et al., 2017).

PICO components	
Population	Angiosperm species with poricidal flowers (flowers bearing poricidal anthers)
Intervention/Exposure	Flower size (flower diameter or flower tube section)
Comparison	Experimental evaluation of the influence of bee visitation on focal plant species with poricidal flowers in contrasting groups, including a control group with bees allowed to visit unmanipulated flowers (natural pollination) and two manipulative treatment groups: (i) bees prevented from visiting flowers (bagged flowers in a pollinator exclusion treatment); and (ii) flowers hand-pollinated by researchers (pollen supplemented group of plants with poricidal flowers).

Outcome	Fruit set; seed set; number of pollen grains per stigma; number of pollen tubes per stigma/style.