



**UNIVERSIDADE FEDERAL DOS VALES DO JEQUITINHONHA E MUCURI**  
**Programa de Pós-Graduação em Biologia Animal**

**Marsal Danrlei de Amorim**

**O PAPEL DE VERTEBRADOS EM FLORES COM SISTEMA MISTOS DE  
POLINIZAÇÃO**

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**O PAPEL DE VERTEBRADOS EM FLORES COM SISTEMA MISTOS DE  
POLINIZAÇÃO**

Dissertação apresentada ao Programa de Pós-Graduação em Biologia Animal da Universidade Federal dos Vales do Jequitinhonha e Mucuri como parte dos requisitos para obtenção do Título de Mestre em Biologia Animal.

Orientador: André Rodrigo Rech

Co-orientador: Gudryan Jackson Barônio

Co-orientador: Cristiano Schetini de Azevedo

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**Dissertação** apresentada ao programa de Pós-Graduação em **Biologia Animal** da Universidade Federal dos Vales do Jequitinhonha e Mucuri, **nível de Mestrado**, como requisito parcial para obtenção do título de **Mestre em Biologia Animal**.

Orientador: **André Rodrigo Rech**

Co-Orientadores: **Cristiano Schetini de Azevedo,**  
**Gudryan Barônio**

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**Prof. André Rodrigo Rech - Presidente**

**Universidade Federal dos Vales do Jequitinhonha e Mucuri**

**Dr. Pedro Joaquim Bergamo - Membro**

**Jardim Botânico do Rio de Janeiro**

**Dr. Jeferson Bugoni - Membro**

**The University of Illinois at Urbana-Champaign**



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## RESUMO

As interações plantas-polinizador são mediadas por características complexas, essas estão relacionadas aos aspectos da biologia do animal, das plantas, de interações de terceiros e por fatores abióticos. Todas essas características influenciam na eficiência de um grupo de animais, ou seja, a adequação animal-planta, que se refere a junção entre as características das plantas, como tamanho da corola e quantidade de néctar e aos polinizadores. Uma questão muito discutida no contexto da adequação é se esses sistemas de polinização são generalistas ou especialistas e qual a força da adequação animal-planta nessas interações. Essa dissertação busca contribuir com estas questões testando: No capítulo 1, qual a diferença na contribuição de abelhas e beija-flores em 10 espécies de planta com características florais diversas nas quais se excluiu os polinizadores vertebrados com uso de gaiolas. No capítulo 2, também testamos a eficiência de dois grupos de visitantes florais, isolando flores com acesso exclusivo dos visitantes noturnos e diurnos. Ambos os estudos foram realizados em ambientes mega diversos, o primeiro no Campo Rupestre e o segundo na Amazônia central. No capítulo 1, a contribuição dos beija-flores variou com relação às características das plantas, com maiores contribuições nas plantas com características mais ligadas à ornitofilia. Porém os beija-flores contribuíram na polinização de diversas espécies, mesmo naquelas plantas mais generalistas, sugerindo que possuem grande importância na manutenção das comunidades de plantas nos Campos Rupestres. No capítulo 2, nosso estudo mostrou que os morcegos são os principais polinizadores da *Gongylolepis martiana* conforme sugerem os atributos morfológicos, configurando o primeiro registro de polinização por morcegos em Asteraceae. Também foi demonstrado que a sequência de ocorrência das interações (efeitos de prioridade), com as abelhas chegando antes dos beija-flores e retirando grande quantidade de pólen faz com que os beija-flores não atuem como polinizadores efetivos mesmo apresentando os requisitos compatíveis com essa função. Nossos resultados sugerem que tanto interação dos visitantes com os atributos florais, quanto entre si e com o contexto ambiental da interação, influenciam a evolução dos sistemas de polinização.

**Palavras-chave:** Experimento de Exclusão. Abelhas. Beija-flores. Morcegos. Polinização. Asteraceae. Campo Rupestre. Amazônia.



## ABSTRACT

Plant-pollinators interactions are mediated by complex features. These features are related to animals and plants biology, third-party interactions and abiotic factors. All these features determine the effectiveness of a pollinator group, or plant pollinator adequacy. One of the highly discussed questions about adequacy relates to its level of generalization or specialization and to the strength of adequacy drivers on interactions. This Master dissertation adds to this debate developing two search chapters. In chapter 1, we asked what is the difference in the contribution of hummingbirds and bees in the pollination of 10 plant species with diverse floral traits. In this study, vertebrates were excluded from flower visitation using wired cages. In chapter 2, we also isolated different pollinator groups bagging night and daylight flowers of *Gongylolepis martiana*, Asteraceae. We then counted pollen grains removal and deposition revealing a higher importance of bats (a novel pollinator group in Asteraceae) compared to hummingbirds in pollinating *G. martiana*. Both studies were conducted in different megadiverse environments, the *Campo Rupestre* and the Amazon forest. In chapter 1, the hummingbirds pollination importance varied with plant features, being greater in ornithophilous flowers. However, hummingbirds increased pollination in all species, even in non-ornithophilous flowers, suggesting a major importance of hummingbirds to the maintenance of plant communities in the *Campo Rupestre*. Beyond bat pollination, chapter 2 also revealed a priority effect where night visitors and especially early morning stingless bees reduced the potential of hummingbirds in pollinating *G. martiana* during daylight regardless of presenting apparent adequate pollinator behavior. Our data show plant pollination systems evolution being influenced by plant traits, pollinator-pollinator interactions and the environmental context where pollination takes place.

**Keywords:** Exclusion experiment. Bees. Hummingbirds. Bats. Pollination. Asteraceae. *Campo Rupestre*. Amazônia.

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<sup>1</sup> Formatado para a submissão na Journal of ecology

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## Introdução Geral

A polinização é parte do processo pelo qual as plantas se reproduzem e ocorre nas flores consistindo em levar o pólen da antera até o estigma de outra flor (RECH *et al.*, 2014). Em geral quando inclui um agente biológico, polinizadores, a literatura descreve esse processo como mutualista (BRONSTEIN, 2001; CHAMBERLAIN; BRONSTEIN; RUDGERS, 2014). Os agentes podem ser abelhas, beija-flores, morcegos, entre outros (OLLERTON; WINFREE; TARRANT, 2011; ROSAS-GUERRERO *et al.*, 2014). O benefício para as plantas é o transporte dos grãos de pólen para fertilizar os óvulos (ERBAR, 2003; MINNAAR *et al.*, 2019) e para os animais seriam recompensas como néctar, óleos, entre outras (BARÔNIO *et al.*, 2018). Mesmo que descritos como mutuamente benéficas para ambos os interagentes essa relação tem um custo e, portanto, ambos buscam maiores benefícios reduzindo ao máximo os custos (BRONSTEIN, 2001; CHAMBERLAIN; BRONSTEIN; RUDGERS, 2014). Extremos de maximização dos benefícios podem ser vistos nas plantas que atraem os polinizadores sem oferecem recursos, como ocorre em algumas orquídeas (COZZOLINO; WIDMER, 2005). Isso também ocorre com os animais que podem visitar a planta, sem transportar o pólen, sendo por exemplo pilhadores ou ladrões de néctar (MARUYAMA; CUSTÓDIO; OLIVEIRA, 2012) ou ladrões de pólen (KING; BALLANTYNE; WILLMER, 2013).

A definição do que é um polinizador está diretamente relacionada com a adequação entre suas características e as das flores (SAHLI; CONNER, 2007; KOSKI *et al.*, 2018). Mesmo quando há mais de um polinizador, suas características podem definir qual deles faz o melhor serviço de polinização (SAHLI; CONNER, 2007; KOSKI *et al.*, 2018). Algumas dessas características são por exemplo, tamanho (e.g. SAHLI; CONNER, 2007), local de deposição de pólen (e.g. KOSKI *et al.*, 2018) e comportamento do animal (e.g. FERREIRA; MARUYAMA; OLIVEIRA, 2016; SCHMIDT-LEBUHN *et al.*, 2019). As síndromes de polinização se referem justamente ao conjunto dessas características florais como cor, odor e tamanho da flor, que supostamente resulta da interação planta-animal (FAEGRI; PIJL, 1979; ROSAS-GUERRERO *et al.*, 2014). Em geral usamos o termo síndromes para predição ocorrência das interações, quando já foram feitos estudos com relação a biologia da polinização da planta e o polinizador é conhecido costuma se referir a interação como sistemas de polinização (RECH *et al.*, 2014).

As interações moduladas pelas características das flores compõem um continuum de especialização-generalização no qual um animal ou planta pode interagir com poucos (especialização) ou diversos parceiros (generalização - WASER *et al.*, 1996; DELLINGER, 2020). Em geral as interações tendem a serem generalistas em alguma medida (OLLERTON *et*

*al.*, 2007), enquanto a especialização estrita seria a exceção (WASER *et al.*, 1996). É possível pensar nos sistemas de polinização com hierarquias de importância, ou polinizadores principais e secundários (ROSAS-GUERRERO *et al.*, 2014). Neste caso, na ausência do principal polinizador, os polinizadores secundários poderiam substituí-los na manutenção desse processo (SUN *et al.*, 2017; LEHMANN *et al.*, 2019) ou mesmo em sistemas bimodais, nos quais ambos os grupos atuam de forma equivalente como polinizadores, por exemplo sistemas bimodais com abelhas e beija-flores (SCHMID *et al.*, 2011) ou com beija-flores e morcegos (QUEIROZ *et al.*, 2016; DELLINGER *et al.*, 2019). Sistema bimodais podem, em determinados contextos ecológicos, evoluir na direção de trocas de sistemas de polinização (ROSAS-GUERRERO *et al.*, 2014).

Em plantas que interagem com mais de um grupo de polinizadores (BOTES; JOHNSON; COWLING, 2009; WESTER; JOHNSON, 2017), o uso de medidas de eficiência de cada grupo é importante para determinar o principal polinizador (FREITAS, 2013). Essa eficiência seria a capacidade de fertilizar o maior número de flores, com o menor número de grãos de pólen necessário (FREITAS, 2013). Para medirmos o sucesso de um polinizador, ou a eficiência, com relação a planta, podemos usar as medidas de deposição de pólen coespecífico e heteroespecífico e a frutificação (FREITAS, 2013). Por exemplo, com visitas únicas pode-se medir a contribuição de uma espécie ou grupo restrito de animais, tanto para deposição de pólen, quanto para a formação de sementes ou frutos (FREITAS, 2013; KING; BALLANTYNE; WILLMER, 2013). Esses experimentos, no entanto, não avaliam o efeito cumulativo das visitas, a complementaridade funcional entre os diferentes visitantes ou a dinâmica estigmática resultante da mistura polínica. Outra possibilidade são os experimentos que excluem um grupo de polinizadores, como os que utilizam gaiolas para a exclusão de vertebrados (SUN *et al.*, 2017; PAUW, 2019). Além deste outro experimento de exclusão é a exclusão temporal, nela nós excluimos um determinado grupo de polinizadores pelo momento no tempo em que esses animais interagem, por exemplo, polinizadores noturnos e diurnos. A polinização em períodos diferentes é importante porque entre os turnos de visitantes, o visitante que chegar primeiro, noturno ou diurno, pode reduzir o recurso ofertado pela planta, reduzindo assim a viabilidade da flor reduzindo a capacidade do visitante, diurno ou noturno (AGUILAR-RODRÍGUEZ *et al.*, 2014). Estes experimentos permitem avaliar um cenário mais realista das consequências das interações-planta polinizador, mas não permite diferenciar polinizadores com tamanho corporal e comportamentos similares.

Além dos organismos que interagem com as flores, os ambientes são importantes moduladores das interações entre os polinizadores e as plantas (WASER; PRICE, 2016;

LAWSON; RANDS, 2019; MARUYAMA *et al.*, 2019). Por exemplo, em altitudes elevadas onde temperaturas são mais baixas os insetos tendem a diminuir o forrageamento e por conta disso os beija-flores tendem a se tornar polinizadores mais importantes (LEHMANN *et al.*, 2019). Alguns padrões globais de interação ave-flor, por exemplo, mostram que a especialização dos polinizadores é maior no novo mundo, onde os beija-flores são os principais polinizadores dentre os vertebrados (ZANATA *et al.*, 2017), e que é maior quanto mais próximos da linha do equador (SONNE *et al.*, 2020). Também mostra que em locais com maior variação ambiental (distúrbios, sazonalidade) as interações podem tender a menor especialização (SONNE *et al.*, 2016), como ocorre no Cerrado, onde os beija-flores tendem a interagir com maior frequência com flores não-ornitófilas (RODRIGUES; RODRIGUES, 2014; ARAÚJO *et al.*, 2018) ou em ambientes urbanizados (MARUYAMA *et al.*, 2019).

Nesse sentido, esta dissertação busca avançar na compreensão da diversidade de interações de polinização e das características que influenciam as consequências sobre a reprodução das plantas, principalmente aqueles relacionados às síndromes de polinização e ao conceito de generalização-especialização. Esse estudo foi feito em dois capítulos, no primeiro investigamos plantas visitadas tanto por beija-flores quanto por abelhas em um ambiente com sazonalidade de recursos por conta de perturbações ambientais, onde ambos os grupos animais teriam a capacidade de atuar como polinizadores. Neste capítulo esperávamos que a) os beija-flores pudessem ter maior importância como polinizadores para as plantas com flores com maior número de características ligadas a ornitofilia; e b) que essa importância poderia ser mais pronunciada na frutificação que na deposição de pólen, pois os beija-flores transportam pólen a maiores distâncias e de melhor qualidade o que geraria mais frutos. O segundo capítulo conta com o estudo de uma interação inusitada, onde foi demonstramos pela primeira vez que morcegos polinizam uma espécie de Asteraceae. Apesar de haver muitos relatos de possíveis espécies polinizadas por morcegos nesta que é a maior família de (Vogel 2015), não havia até o momento estudos que comprovem a polinização. A espécie *Gongylolepis martiana* apresenta características associadas à polinização por morcegos e, portanto, nossa hipótese era de essa espécie poderia de fato ser polinizadas por esses vertebrados. Com experimentos de exclusão avaliamos a importância de visitantes noturnos e diurnos na polinização de *G. martiana* a partir do impacto de cada grupo sobre a remoção e deposição de pólen.

### **Referências Bibliográficas**

AGUILAR-RODRÍGUEZ P. A. et al. First record of bat-pollination in the species-rich genus

Tillandsia (Bromeliaceae). **Annals of Botany**. [S. l.], v. 113, p. 1047–1055, 2014. Disponível em: <https://academic.oup.com/aob/article-lookup/doi/10.1093/aob/mcu031>

ARAUJO, Andréa Cardoso *et al.* Spatial distance and climate determine modularity in a cross-biomes plant-hummingbird interaction network in Brazil. **Journal of Biogeography**, [S. l.], v. 45, n. 8, p. 1846–1858, 2018. Disponível em: <https://doi.org/10.1111/jbi.13367>.

BARÔNIO, Gudryan Jackson *et al.* Entre Flores e Visitantes: Estratégias de Disponibilização e Coleta de Recursos Florais. **Oecologia Australis**, [S. l.], v. 22, n. 04, p. 390–409, 2018. Disponível em: <https://doi.org/10.4257/oeco.2018.2204.04>.

BOTES, Christo; JOHNSON, Steven D.; COWLING, Richard M. The Birds and the Bees: Using Selective Exclusion to Identify Effective Pollinators of African Tree Aloes. **International Journal of Plant Sciences**, [S. l.], v. 170, n. 2, p. 151–156, 2009. Disponível em: <https://doi.org/10.1086/595291>.

BRONSTEIN, Judith L. The Costs of Mutualism. **American Zoologist**, [S. l.], v. 41, n. 4, p. 825–839, 2001. Disponível em: <https://doi.org/10.1093/icb/41.4.825>.

CHAMBERLAIN, Scott A.; BRONSTEIN, Judith L.; RUDGERS, Jennifer A. How context dependent are species interactions? **Ecology Letters**, [S. l.], v. 17, n. 7, p. 881–890, 2014. Disponível em: <https://doi.org/10.1111/ele.12279>. Acesso em: 17 abr. 2020.

COZZOLINO, Salvatore; WIDMER, Alex. Orchid diversity: an evolutionary consequence of deception? **Trends in Ecology & Evolution**, [S. l.], v. 20, n. 9, p. 487–494, 2005. Disponível em: <https://doi.org/10.1016/j.tree.2005.06.004>.

DELLINGER, Agnes S. *et al.* Bimodal Pollination Systems in Andean Melastomataceae Involving Birds, Bats, and Rodents. **The American Naturalist**, [S. l.], v. 194, n. 1, p. 104–116, 2019. Disponível em: <https://doi.org/10.1086/703517>.

DELLINGER, Agnes S. Pollination syndromes in the 21 st century: where do we stand and where may we go? **New Phytologist**, [S. l.], p. nph.16793, 2020. Disponível em: <https://doi.org/10.1111/nph.16793>.



ERBAR, Claudia. Pollen tube transmitting tissue: Place of competition of male gametophytes. *In*: 2003, **International Journal of Plant Sciences.**: The University of Chicago Press, 2003. Disponível em: <https://doi.org/10.1086/377061>.

FAEGRI, K.; PIJL, L. V. D.. The principles of pollination ecology. **New York: Pergamon Press**, [S. l.], v. 64, p. 6–7, 1979.

FERREIRA, C.; MARUYAMA, P. K.; OLIVEIRA, P. E. Convergence beyond flower morphology? Reproductive biology of hummingbird-pollinated plants in the Brazilian Cerrado. **Plant Biology**, [S. l.], v. 18, n. 2, p. 316–324, 2016. Disponível em: <https://doi.org/10.1111/plb.12395>.

FREITAS, Leandro. Concepts of pollinator performance: is a simple approach necessary to achieve a standardized terminology? **Brazilian Journal of Botany**, [S. l.], v. 36, n. 1, p. 3–8, 2013. Disponível em: <https://doi.org/10.1007/s40415-013-0005-6>.

KING, Caroline; BALLANTYNE, Gavin; WILLMER, Pat G. Why flower visitation is a poor proxy for pollination: Measuring single-visit pollen deposition, with implications for pollination networks and conservation. **Methods in Ecology and Evolution**, [S. l.], v. 4, n. 9, p. 811–818, 2013. Disponível em: <https://doi.org/10.1111/2041-210X.12074>. Acesso em: 21 jul. 2020.

KOSKI, Matthew H. *et al.* Linking pollinator efficiency to patterns of pollen limitation: small bees exploit the plant–pollinator mutualism. **Proceedings of the Royal Society B: Biological Sciences**, [S. l.], v. 285, n. 1880, p. 20180635, 2018. Disponível em: <https://doi.org/10.1098/rspb.2018.0635>. Acesso em: 9 jun. 2020.

LAWSON, David A.; RANDS, Sean A. The effects of rainfall on plant–pollinator interactions. **Arthropod-Plant Interactions**, [S. l.], v. 13, n. 4, p. 561–569, 2019. Disponível em: <https://doi.org/10.1007/s11829-019-09686-z>.

LEHMANN, L. J. *et al.* Relative effectiveness of insects versus hummingbirds as pollinators of Rubiaceae plants across elevation in Dominica, Caribbean. **Plant Biology**, [S. l.], v. 21, n.

4, p. 738–744, 2019. Disponível em: <https://doi.org/10.1111/plb.12976>.

MARUYAMA, Pietro Kiyoshi *et al.* Plant-hummingbird interaction networks in urban areas: Generalization and the importance of trees with specialized flowers as a nectar resource for pollinator conservation. **Biological Conservation**, [S. l.], v. 230, p. 187–194, 2019. Disponível em: <https://doi.org/10.1016/j.biocon.2018.12.012>.

MARUYAMA, Pietro Kiyoshi; CUSTÓDIO, Luciana Nascimento; OLIVEIRA, Paulo Eugênio. When hummingbirds are the thieves: visitation effect on the reproduction of Neotropical snowbell *Styrax ferrugineus* Nees & Mart (Styracaceae). **Acta Botanica Brasilica**, [S. l.], v. 26, n. 1, p. 58–64, 2012. Disponível em: <https://doi.org/10.1590/S0102-33062012000100007>. Acesso em: 28 out. 2019.

MINNAAR, Corneile *et al.* Plant–pollinator interactions along the pathway to paternity. **Annals of Botany**, [S. l.], v. 123, n. 2, p. 225–245, 2019. Disponível em: <https://doi.org/10.1093/aob/mcy167>.

OLLERTON, J.; KILLICK, A.; LAMBORN, E.; WATTS, S.; WHISTON M. Multiple Meanings and Modes: On the Many Ways to Be a Generalist Flower. **Taxon** [S. l.], v. 56, n. 3, p.717-728, 2007.

OLLERTON, Jeff; WINFREE, Rachael; TARRANT, Sam. How many flowering plants are pollinated by animals? **Oikos**, [S. l.], v. 120, n. 3, p. 321–326, 2011. Disponível em: <https://doi.org/10.1111/j.1600-0706.2010.18644.x>.

PAUW, Anton. A Bird’s-Eye View of Pollination: Biotic Interactions as Drivers of Adaptation and Community Change. **Annual Review of Ecology, Evolution, and Systematics**, [S. l.], v. 50, n. 1, p. 477–502, 2019. Disponível em: <https://doi.org/10.1146/annurev-ecolsys-110218-024845>.

QUEIROZ, J. A. *et al.* Vertebrate mixed pollination system in *Encholirium spectabile*: A bromeliad pollinated by bats, opossum and hummingbirds in a tropical dry forest. **Journal of Arid Environments**, [S. l.], v. 125, p. 21–30, 2016. Disponível em: <https://doi.org/10.1016/J.JARIDENV.2015.09.015>. Acesso em: 15 dez. 2019.

RECH, André Rodrigo *et al.* **Biologia da Polinização**. Primeira ed. Rio de Janeiro: Projeto Cultural, 2014. *E-book*.

RODRIGUES, LC; RODRIGUES, M. Flowers visited by hummingbirds in the open habitats of the southeastern Brazilian mountaintops: species composition and seasonality. **Brazilian Journal of Biology**, [S. l.], v. 74, n. 3, p. 659–676, 2014. Disponível em: <https://doi.org/10.1590/bjb.2014.0097>.

ROSAS-GUERRERO, Víctor *et al.* **A quantitative review of pollination syndromes: Do floral traits predict effective pollinators?**. [S. l.]: John Wiley & Sons, Ltd (10.1111), 2014. Disponível em: <https://doi.org/10.1111/ele.12224>. Acesso em: 9 dez. 2019.

SAHLI, Heather F.; CONNER, Jeffrey K. Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). **American Journal of Botany**, [S. l.], v. 94, n. 2, p. 203–209, 2007. Disponível em: <https://doi.org/10.3732/ajb.94.2.203>.

SCHMID, S. *et al.* Bimodal pollination system of the bromeliad *Aechmea nudicaulis* involving hummingbirds and bees. **Plant Biology**, [S. l.], v. 13, n. SUPPL. 1, p. 41–50, 2011. Disponível em: <https://doi.org/10.1111/j.1438-8677.2010.00348.x>.

SCHMIDT-LEBUHN, Alexander N. *et al.* Pollen analogues are transported across greater distances in bee-pollinated than in hummingbird-pollinated species of *Justicia* (Acanthaceae). **Biotropica**, [S. l.], v. 51, n. 2, p. 99–103, 2019. Disponível em: <https://doi.org/10.1111/btp.12633>.

SONNE, J. *et al.* High proportion of smaller ranged hummingbird species coincides with ecological specialization across the Americas. **Proceedings of the Royal Society B: Biological Sciences**, [S.l.], v. 283, n. 1824, p. 20152512. 2016. Disponível em: [10.1098/rspb.2015.2512](https://doi.org/10.1098/rspb.2015.2512)

SONNE, Jesper *et al.* Ecological mechanisms explaining interactions within plant–hummingbird networks: morphological matching increases towards lower latitudes. **Proceedings of the Royal Society B: Biological Sciences**, [S. l.], v. 287, n. 1922, p. 20192873,

2020. Disponível em: <https://doi.org/10.1098/rspb.2019.2873>.

SUN, Shi Guo *et al.* Nectar properties and the role of sunbirds as pollinators of the golden-flowered tea (*Camellia petelotii*). **American Journal of Botany**, [S. l.], v. 104, n. 3, p. 468–476, 2017. Disponível em: <https://doi.org/10.3732/ajb.1600428>. Acesso em: 28 out. 2019.

VOGEL, Sefan. Vertebrate pollination in Compositae: Floral syndromes and field observations. **Stapfia**, [S. l.], v. 103, p. 5–26, 2015.

WASER, Nickolas M. *et al.* Generalization in pollination systems, and why it matters. **Ecology**, [S. l.], v. 77, n. 4, p. 1043–1060, 1996. Disponível em: <https://doi.org/10.2307/2265575>. Acesso em: 17 mar. 2020.

WASER, Nickolas M.; PRICE, Mary V. Drought, pollen and nectar availability, and pollination success. **Ecology**, [S. l.], v. 97, n. 6, p. 1400–1409, 2016. Disponível em: <https://doi.org/10.1890/15-1423.1>.

WESTER, Petra; JOHNSON, Steven D. Importance of birds versus insects as pollinators of the African shrub *Syncolostemon densiflorus* (Lamiaceae). **Botanical Journal of the Linnean Society**, [S. l.], v. 185, n. 2, p. 225–239, 2017. Disponível em: <https://doi.org/10.1093/botlinnean/box054>.

ZANATA, Thais B. *et al.* Global patterns of interaction specialization in bird–flower networks. **Journal of Biogeography**, [S. l.], v. 44, n. 8, p. 1891–1910, 2017. Disponível em: <https://doi.org/10.1111/jbi.13045>. Acesso em: 4 abr. 2020.

**CAPÍTULO 1 – Hummingbirds contribute to plant reproduction regardless of pollination syndrome in *Campo Rupestre* grassland**

Marsal D. Amorim<sup>1\*</sup>, Pietro K. Maruyama<sup>2</sup>, Gudryan J. Baronio<sup>3</sup>, Cristiano S. Azevedo<sup>4</sup>, André, R. Rech<sup>5</sup>

<sup>1</sup> Programa de Pós-Graduação em Biologia Animal, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG 39100-000 Brazil.

<sup>2</sup> Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG 31270-901, Brazil.

<sup>3</sup> Programa de Pós-Graduação em Ciência Florestal, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Campus JK, Rodovia MGT 367 - Km 583, nº 5.000, Alto da Jacuba, CEP 39100-000, Diamantina, MG, Brazil.

<sup>4</sup> Departamento de Evolução, Biodiversidade e Meio Ambiente, Instituto de Ciências Exatas e Biológicas, Campus Morro do Cruzeiro, s/n, Bauxita, CEP: 35400-000, Ouro Preto, MG, Brasil. Phone: +55 31 3559-1598. ORCID: 0000-0003-0256-901

<sup>5</sup> Faculdade Interdisciplinar de Humanidades, Universidade Federal dos Vales do Jequitinhonha e Mucuri, 39100-000 Diamantina, Minas Gerais, Brasil

\* Corresponding author: marsal.amorim@gmail.com

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## Resumo

1. Traços florais modulam o papel de animais distintos como polinizadores eficazes ao longo do continuum de generalização / especialização. Muitas espécies de plantas são visitadas por mais de um grupo funcional de polinizadores, que podem variar em sua contribuição para a reprodução das plantas. Caracterizar como diferentes grupos de polinizadores contribuem para a polinização de plantas generalistas pode ajudar a elucidar a importância de características florais distintas para as interações planta-polinizador.
2. Aqui nós investigamos a contribuição dos beija-flores para a polinização de dez espécies de plantas, com variados graus de adaptação presumida à polinização de pássaros, que são frequentemente visitadas por beija-flores e insetos (principalmente abelhas) no Campo Rupestre brasileiro. Excluímos experimentalmente beija-flores das flores e avaliamos sua contribuição relativa na deposição de pólen e na frutificação coespecífico / heteroespecífico. Em seguida, perguntamos se a importância dos colibris estava relacionada ao ajuste dos traços florais à síndrome da polinização ornitófila.
3. Os beija-flores aumentaram a deposição geral de pólen coespecífico e heteroespecífico, bem como a frutificação em todas as plantas, mas a magnitude desse efeito variou entre as espécies. Especificamente, características florais tipicamente associadas à polinização de pássaros foram associadas à deposição de pólen aumentada, mas não à frutificação, na presença de beija-flores. Portanto, o efeito relativo de beija-flores em associação com características florais depende de qual estágio o sucesso reprodutivo é avaliado, ou seja, deposição de pólen ou frutificação.
4. Síntese. Nós mostramos que os beija-flores melhoram a reprodução das plantas, mesmo para plantas não claramente adaptadas à síndrome da polinização das aves. Além disso, em qual fase reprodutiva a contribuição dos polinizadores é medida

influencia a avaliação da importância dos polinizadores em plantas com sistemas de polinização mistos e generalizados.

**Palavras chaves:** sistema de polinização mista, síndrome floral, ornitofilia, deposição de pólen, sucesso reprodutivo.

### **Abstract**

1. Floral traits modulate the role of distinct animals as effective pollinators along the generalization/specialization continuum. Many plant species are visited by more than one functional group of pollinators, which may vary in their contribution to plant reproduction. Characterizing how different pollinator groups contribute to the pollination of generalist plants can help in elucidating the importance of distinct floral characteristics for plant-pollinator interactions.
2. Here we investigated the contribution of hummingbirds to the pollination of ten plant species, with varying degree of presumed adaptation to bird pollination, which are frequently visited by both hummingbirds and insects (mainly bees) in the Brazilian *Campo Rupestre*. We experimentally excluded hummingbirds from the flowers and evaluated their relative contribution on conspecific/heterospecific pollen deposition and fruit set. Then, we asked if the importance of hummingbirds was related to the fit of floral traits to the ornithophilous pollination syndrome.
3. Hummingbirds increased overall conspecific and heterospecific pollen deposition, as well as fruit set across all plants, but the magnitude of this effect varied between species. Specifically, floral traits typically associated with bird pollination were associated with enhanced pollen deposition, but not fruit set, in the presence of hummingbirds. Therefore, the relative effect of hummingbirds in association with floral traits is dependent on which stage the reproductive success is evaluated, i.e. pollen deposition or fruit set.

4. *Synthesis*. We showed that hummingbirds improve plant reproduction even for plants not clearly adapted to bird pollination syndrome. Moreover, at which reproductive phase the contribution of pollinators is measured influences the assessment of pollinator importance in plants with mixed and generalized pollination systems.

**Keywords:** mixed-pollination system, floral syndrome, ornithophily, pollen deposition, reproductive success.

## 1| Introduction

The great diversity of Angiosperms is thought to be closely linked to the diversity of biotic pollinators (Ollerton, Winfree, & Tarrant, 2011; Ollerton, 2017). The diversity of floral forms was traditionally classified according to the concept of pollination syndromes, which is defined by sets of floral traits seemingly associated with specific groups of pollinators (Faegri & Pijl, 1979; Rosas-Guerrero et al., 2014). However, plant-pollinator interactions show varying degrees of specialization, with many plant species presenting generalized interactions (Waser, Chittka, Price, Williams, & Ollerton, 1996; Dellinger, 2020), where the role of distinct pollinator groups for plant reproduction is modulated by their morphologies and behavior, impacting pollen transfer and, consequently, plant fitness (Castellanos, Wilson, & Thomson, 2004; Koski, Ison, Padilla, Pham, & Galloway, 2018). Generalist plants, i.e. associated with more than one functional group of pollinators, are good models to test the contribution of distinct pollinator groups for plant reproduction, which underlie the understanding of ecological and evolutionary processes acting on pollination interactions (Dellinger, 2020). Specifically, insect to bird transition in pollination mode is recurrent across many plant lineages but not always complete (Rosas-Guerrero et al., 2014; Krauss et al., 2017), making plants associated to both groups excellent models for investigation.

In this context, the set of floral traits that characterized specialized vertebrate pollination include higher energetic demand of pollinators when compared to insects,



implying a higher cost for plants due to a higher production of nectar to attract them (Cronk & Ojeda, 2008). This higher cost is potentially compensated by: (i) greater amount of pollen exportation, (ii) longer distances of pollen flow, and (iii) higher precision in pollen deposition (Krauss et al., 2017). Birds are the most diverse group of pollinators among the vertebrates, with estimated 500 genera of vascular plants pollinated by them (Cronk & Ojeda, 2008; Krauss et al., 2017). Hummingbirds, found only in the New World, are the most diverse and specialized group of bird pollinators (Krauss et al., 2017; Zanata et al., 2017). Specialized flowers pollinated by birds typically show long and tubular corollas, abundant nectar, bright colors and lack of scent, traits that characterize ornithophily (the bird pollination syndrome; Faegri & Pijl, 1979; Cronk & Ojeda, 2008; Krauss et al., 2017). Some of the traits that characterize bird pollination are in fact filters able to exclude other pollinators such as bees (Castellanos et al., 2004; Bergamo, Rech, Brito, & Sazima, 2016). On the other hand, hummingbirds are known to forage also on non-ornithophilous flowers (Maruyama et al. 2013; Waser, CaraDonna, Price 2018). Therefore, the degree to which plants rely only on birds as pollinators vary greatly and many plant species may be associated with both insect and bird pollinators, in mixed pollination systems (Schmid, Schmid, Zillikens, Harter-Marques, & Steiner, 2011; Bergamo et al., 2016).

According to the most effective pollinator principle (Stebbins 1970), plants should progressively adapt to the pollinator presenting the greatest positive impact on fitness. Following this rationale, flower morphologies adapted to bird pollination must present higher fitness output when visited by hummingbirds compared to insects. On the other hand, in flowers with generalized phenotypes, the fitness outcome of different pollinator visits should be fairly similar and vary across space and time (Gómez, 2002). Although the relative importance of pollinator groups is essential to understand the evolution of plant pollinator interactions, its measurement is not trivial (Zych, Goldstein, Roguz, & Stpiczyńska, 2013 and

references). In this context, pollinator importance is often evaluated through pollen deposition (Ne'eman, Jürgens, Newstrom-Lloyd, Potts, & Dafni, 2009), but this measure may not always translate to fruit or seed set (Sihag, 2018). Hence, it is important to compare pollen deposition and subsequent measures of reproductive success related to different pollinator groups and evaluate their variation over floral specialization gradients. Moreover, floral visitors usually carry both heterospecific and cospecific pollen, and the relationship between these loads may be an indication of facilitation among co-flowering plants (Ashman, Alonso, Parra-Tabla, & Arceo-Gómez, 2020). Facilitation occurs because plants that have similar characteristics attract more of the same pollinators and receive more pollen, but with the disadvantage of sharing them (Bergamo, Susin Streher, Traveset, Wolowski, & Sazima, 2020). If this balance is not positive, heterospecific deposition in itself creates disadvantage for plants, for example, by clogging of the pollen tube in the style (Ashman & Arceo-Gómez 2013), indicating competition instead of facilitation in these cases (Ashman et al., 2020).

Vertebrates involved in mixed pollination systems offer an interesting opportunity for experimental studies where efficiency measures of each group can determine the pollinator with the greatest contribution (Freitas, 2013). For example, by isolating flowers and allowing unique visits by each group, the contribution of a restricted group of animals can be measured, both for deposition and fruiting (Freitas, 2013; King; Ballantyne; Willmer, 2013). These experiments, however, do not evaluate the cumulative effect of the visits, the functional complementarity between the groups or the stigmatic dynamics resulting from the pollen mixture. Alternatively, cages may be used to allow insect visitation, excluding larger-sized flower visitors (Sun, Huang, Chen, & Huang, 2017; Magalhães, Maruyama, Tavares, & Martins, 2018). Although these experiments do not allow the differentiation of pollinators with similar body dimensions and behaviors, cage experiments can allow the evaluation of a more realistic scenario of the interactions. Past exclusion studies either evaluated a single

plant species (Hargreaves, Johnson, & Nol, 2004; Maruyama, Custódio, & Oliveira, 2012; Sun et al., 2017) or a few closely-related species (Botes, Johnson, & Cowling, 2009; Magalhães et al., 2018). Those studies lack comparisons of multiple unrelated coexisting species, encompassing different level of specialization. Since floral traits are expected to reflect adaptation to specific pollinators, plants showing floral traits associated with specialized bird pollination should present proportionally higher fitness outcome in the presence of hummingbirds than generalized plants (Hargreaves, Harder, & Johnson, 2012; Sun et al., 2017).

Here, we evaluated the hypothesis that hummingbirds are relatively more important as pollinators in plants showing traits typically associated with ornithophily, across an assemblage of flowers with varying degrees of floral specialization. We additionally tested the hypothesis that difference in the pollination service provided by hummingbirds vs. other pollinators would be more pronounced when considering the fruit set compared to pollen deposition, as birds are expected to transport pollen at greater distances, hence, providing higher quality pollen grains, that would generate more fruits.

## **2| Material and Methods**

### **2.1| Study Area**

We conducted this study in an area of *Campo Rupestre* (rocky outcrops) from *campus* Juscelino Kubitschek of the Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais/Brazil (18°11'48.23"S, 43°34'8.74" W). The *Campo Rupestre* in Brazil belongs to the Neotropical savanna domain (*Cerrado*) and is characterized by high endemism and diversity of plants. Despite covering less than 1% of Brazil, *Campo Rupestre* presents more than 10% of the plant diversity found in this country (Silveira et al., 2016). Interactions between plants and hummingbirds in this ecosystem show varying degrees of

specialization and overlapped interactions with other insect pollinator groups (Rodrigues & Rodrigues, 2014; Ferreira, Maruyama, & Oliveira, 2016; Camargo et al., 2019).

Our study site is located inside the University campus at an altitude of 1300m above sea level, in the Southern region of the Espinhaço Range. The 240 hectares of the University campus are protected and contiguous to the Biribiri State Park, a nature reserve of around 16000 ha. Climate is of mesothermic type (Cwb), temperate, with a cold and dry winter, and a hot and wet summer (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013). A previous study described the interaction network among nine hummingbird species and 50 plant species in the same location, with *Augastes scutatus*, endemic from the *Campo Rupestre*, as the most frequent hummingbird species visiting flowers in this area (Queiroz, 2018).

## **2.2| Study Species**

We selected ten abundant plant species visited by hummingbirds from the interaction network described by Queiroz (2018 - Table 1) which are known to also receive insect visitations. For each species selected, we measured corolla length, corolla opening size and nectar volume and concentration in two flowers per individual in five plants per species, to characterize specialization to bird pollination. Flowers used for nectar measurements were previously bagged while still buds and measured after flower opening. Morphological measures were taken with a manual caliper (error = 0.01). The functional corolla length was measured as the distance from the receptacle to the corolla opening (Wolf, Stiles, & Hainsworth, 1976) and the corolla opening was the largest diameter of the floral tube entrance. To measure nectar volume, we used micro syringes and microcapillaries and, for nectar concentration, we used a pocket refractometer

## **2.3| Visitant Observation and Hummingbird Exclusions**

To estimate the contribution of pollinators through vertebrate exclusion, we used hexagonal wire cages (holes of 12mm, 20-25 cm of diameter, Figure S1) on branches with flowers in

pre-anthesis (see Fang, Chen, & Huang, 2012; Maruyama et al., 2012; Sun et al., 2017). Cages were supported by an extra pole fixed on a concrete block to prevent branch and/or flower damage. From each of the 10 plant species studied, 10 individuals were selected, and branches with and without cages were observed for at least 20 hours according to visitors' behavior and visitation rate. For four days, between 7:00 am and 6:00 pm, we performed observations using three video cameras SONY® HDR-CX240 and visual recording carried out by the first author in periods of 15 min during the same period of camera shooting. These observations indicated that there is no difference in total visitation frequency per plant species between the treatment and control (paired Student t test;  $t = 0.999$ ;  $p = 0.422$ ;  $n = 240$ ), suggesting that cages do not interfere with pollinators behavior besides excluding vertebrates.

#### **2.4| Pollen Deposition and Fruit Set**

To measure the vertebrate and invertebrate contribution to pollination and plant reproduction, we compared pollen deposition (conspecific and heterospecific) and fruit set between the treatments [insects (vertebrate exclusion/cages) vs. insects + hummingbirds (control/free visitation)]. For pollen deposition, we marked and left flowers available to the pollinators for one day when the stigma was receptive. After one day, two stigmas per individual were collected (20 stigmas/treatment/species) and transported to the lab. Stigmas were then prepared on slides with glycerin gelatin and acetic carmine was used for pollen staining. Then, we observed slides under a light microscope (40x) to count conspecific and heterospecific (morphotypes) pollen grains. For fruit-set, we marked at least ten buds before anthesis inside and outside the cages per individual. After flower senescence, the cages were removed to avoid further effects on ovary development and the initial fruit set was counted one week later.

## 2.5| Data Analysis

We run a Principal Component Analysis (PCA) to reduce data variation into fewer dimensions and combined the four measured floral traits (corolla length, corolla opening, nectar volume and concentration) into a new variable reflecting specialization to bird pollination, using the package *factoextra* (Kassambara & Mundt, 2020). The first axis of PCA (PC1) encompassed 64.3% of the variability in the measured floral characteristics and was considered as a proxy for trait variation (see Results).

Even though invertebrate visitation frequency was not affected by the cages, we also tested for differences in the composition of floral visitors inside and outside the cages (with and without hummingbirds) building species composition matrices for both treatments. We then compared the matrices using a permutational analysis of variance (PERMANOVA) with the *adonis* function from the *vegan* package (Oksanen et al., 2019).

Then, we tested if hummingbird exclusion affected plant reproduction in the community by using a series of generalized linear mixed models (GLMMs), with Poisson distribution for conspecific and heterospecific pollen counts and binomial distribution for fruit set with the *lme4* package (Bates, Achler, Bolker, & Walker, 2015). The PC1 axis summarizing floral traits and the treatment (insects *vs.* insects + hummingbirds) were included as fixed factors and plant species as a random factor in the GLMMs. Response variables were conspecific and heterospecific pollen deposition, and fruit set, that were each included in separate models. We tested the significance of each fixed factor in the models using analysis of variance, with function *Anova*, and the Chi-square test, with the sum of squares (type 3), in the package *car* (Fox & Weisberg, 2011).

Finally, for each species we calculated Cohen's *d* effect sizes and its 95% Confidence Interval, contrasting the treatment (insects) and control (insects + hummingbirds) for pollen deposition (conspecific and heterospecific pollen) and fruit-set, in order to determine the

effect of hummingbirds on plant reproduction. In this calculation, positive values not overlapping zero indicate higher contribution of hummingbirds, while zero or negative values indicate that hummingbirds are equivalent to invertebrates (Nakagawa & Cuthill, 2007). To calculate Cohen's *d* effect sizes, we used the package *effsize* (Torchiano, 2020). All analyses were run in R (R Core Team, 2018).

### 3| Results

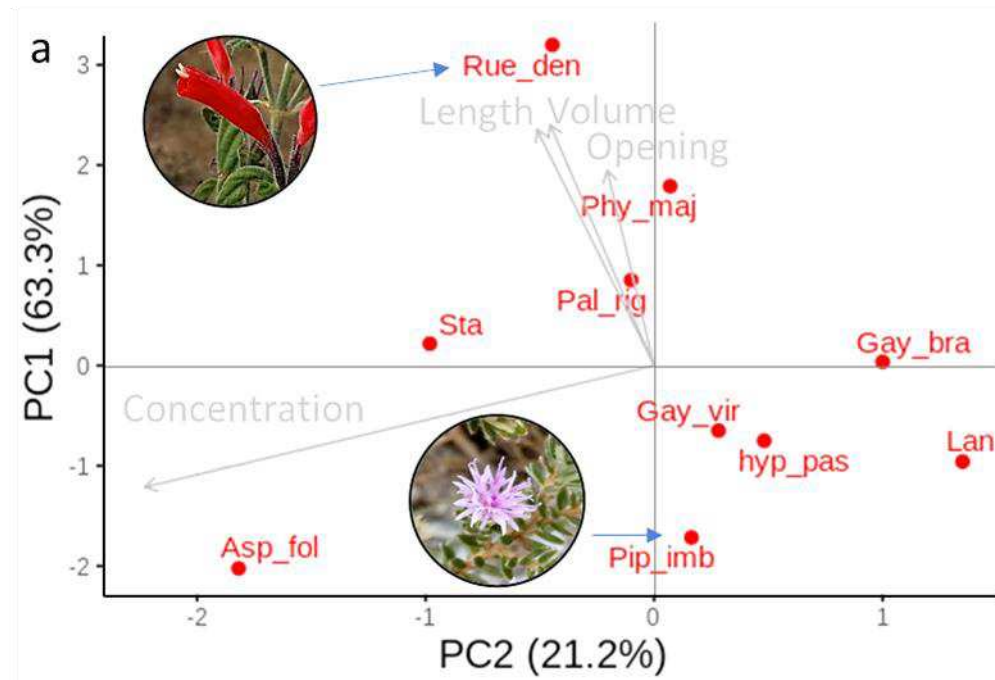
Three hummingbird species were observed visiting flowers in the control plants and, thus excluded by the cages (treatment): *Augastes scutatus*, *Phaethornis pretrei* and *Eupetomena macroura*. However, the exclusion of hummingbirds was not enough to change the overall composition of floral visitors between treatment and control flowers, indicating that overall insect visitation were similar and more abundant than hummingbird visitation (PERMANOVA,  $R^2 = 0.074$ ;  $F_{1,18} = 1.376$ ;  $p = 0.220$ ). Bees, including the exotic *Apis mellifera*, were the most frequent floral visitor group in treatments both excluding vertebrates (81% of interactions) and in the presence of vertebrates (71% of interactions), while the hummingbirds were responsible for about 21% of interactions, with *A. scutatus* being the most frequent hummingbird visiting uncaged flowers (Table S1).

The first axis of PCA (PC1) which explained 64.3% of floral trait variability was positively related to nectar volume ( $r = 0.58$ ), corolla length ( $r = 0.57$ ) and corolla opening ( $r = 0.47$ ; Figure 1; Table 1). That is, the greater the values of axis PC1, the more aligned to ornithophily the flowers were. The flowers with greater values of PC1 were *Ruellia densa*, *Physocalyx major* and *Palicourea rigida*, while *Aspilia foliosa* and *Piptolepsis imbricata*, both Asteraceae, had the smallest values of PC1. The second axis of PCA (PC2) expressed 21.2% of the variability in the floral traits, mostly driven by a negative association with nectar concentration ( $r = -0.95$ ; Figure 1).

**Table 1.** Plants used in this study. Code refers to the abbreviation of the species name. Values of corolla size (length and opening), nectar volume and concentration (Conc) for plant species.

Species	Code	Corolla		Nectar	
		Length (cm)	Opening (cm)	Volume (ul)	Conc. (Brix)
<b>Acantaceae</b>					
<i>Ruellia densa</i> (Nees) Hiern	Rue_den	3.35	0.41	10.92	23.92
<b>Asteraceae</b>					
<i>Aspilia foliosa</i> (Gardner) Baker	Asp_fol	0.70	0.18	0.45	39.37
<i>Piptolepsis imbricata</i> (Gardner) Sch.Bip.	Pip_imb	0.71	0.10	0.37	28.62
<b>Ericaceae</b>					
<i>Gaylussacia brasiliensis</i> (Spreng.) Meisn.	Gay_bra	1.16	0.26	3.22	21.83
<i>Gaylussacia virgnata</i> Mart. ex Meisn.	Gay_vir	0.79	0.33	1.03	26.83
<b>Lamiaceae</b>					
<i>Hyptis passerina</i> Mart. ex Benth.	Hyp_pas	0.56	0.36	0.31	26.16
<b>Orobanchaceae</b>					
<i>Physocalyx major</i> Mart	Phy_maj	2.49	0.47	4.96	23.94
<b>Rubiaceae</b>					
<i>Palicourea rigida</i> Kunth	Pal_rig	1.50	0.46	4.18	26.50
<b>Verbenaceae</b>					
<i>Lantana</i> sp.	Lan_sp	1.09	0.11	0.80	21.33
<i>Stachytarpheta</i> sp. Vahl.	Sta_sp	2.07	0.27	4.03	31.41





**Figure 1.** Flower morphospace of ten plant species with a mixed pollination system in the *Campo Rupestre* of Diamantina, Minas Gerais, Brazil. Position of plants in relation to flower characteristics: corolla length, corolla opening and nectar volume and concentration. Measured characteristics increase in the direction pointed by the respective grey arrow. Abbreviations refer to the species in table 1. Extremes of PC1 variation are illustrated by *Ruellia densa* (Rue\_den) and *Piptolepis imbricata* (Pip\_imb).

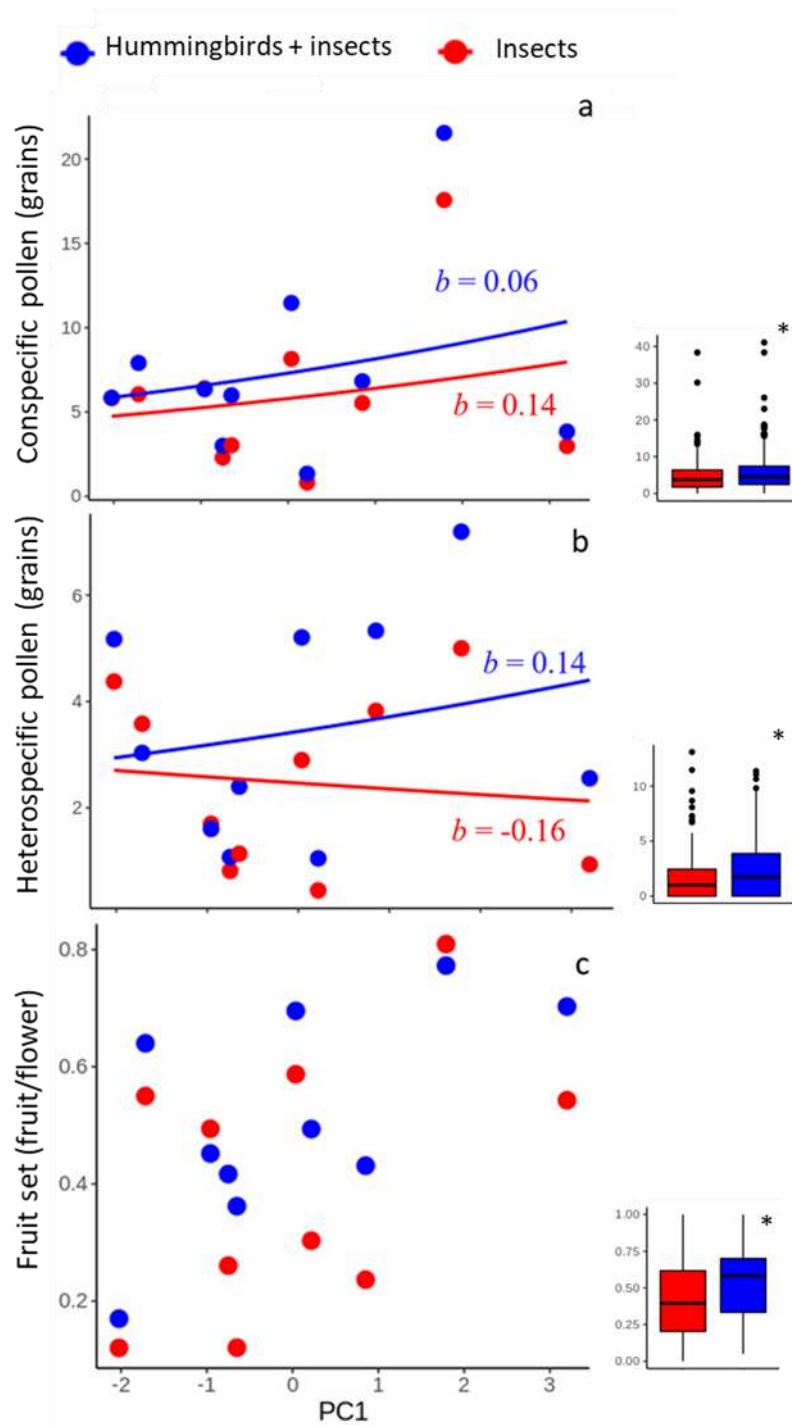
Both conspecific and heterospecific pollen deposition were positively related to ornithophilous traits (PC1), i.e., more specialized flowers received more pollen (Table 2). This effect on conspecific pollen was stronger in the presence of hummingbirds (Figure 2a). For heterospecific pollen deposition, this effect was positive in the presence of hummingbirds and negative when they were excluded (Figure 2b). Finally, the presence of hummingbirds increased fruit set independently of floral characteristics (Table 2). Considering species-specific effect sizes results were idiosyncratic, and also dependent on the stage at which the contribution of hummingbirds was evaluated (Figure 3, Figure S3). For instance, the presence

of hummingbirds was higher than bees for conspecific pollen in *R. densa* and *Gaylussacia brasiliensis*. The presence of hummingbirds was higher for heterospecific pollen only in *G. brasiliensis*. Finally, strongest hummingbird contributions to fruit set were observed for *Stachytarpheta* sp., *P. rigida* and *Gaylussacia virgnata*.

**Table 2.** Results of the GLMs for pollen deposition (conspecific and heterospecific) and fruit set according to treatment (insects and insects + hummingbirds) and floral characteristics (PC1).

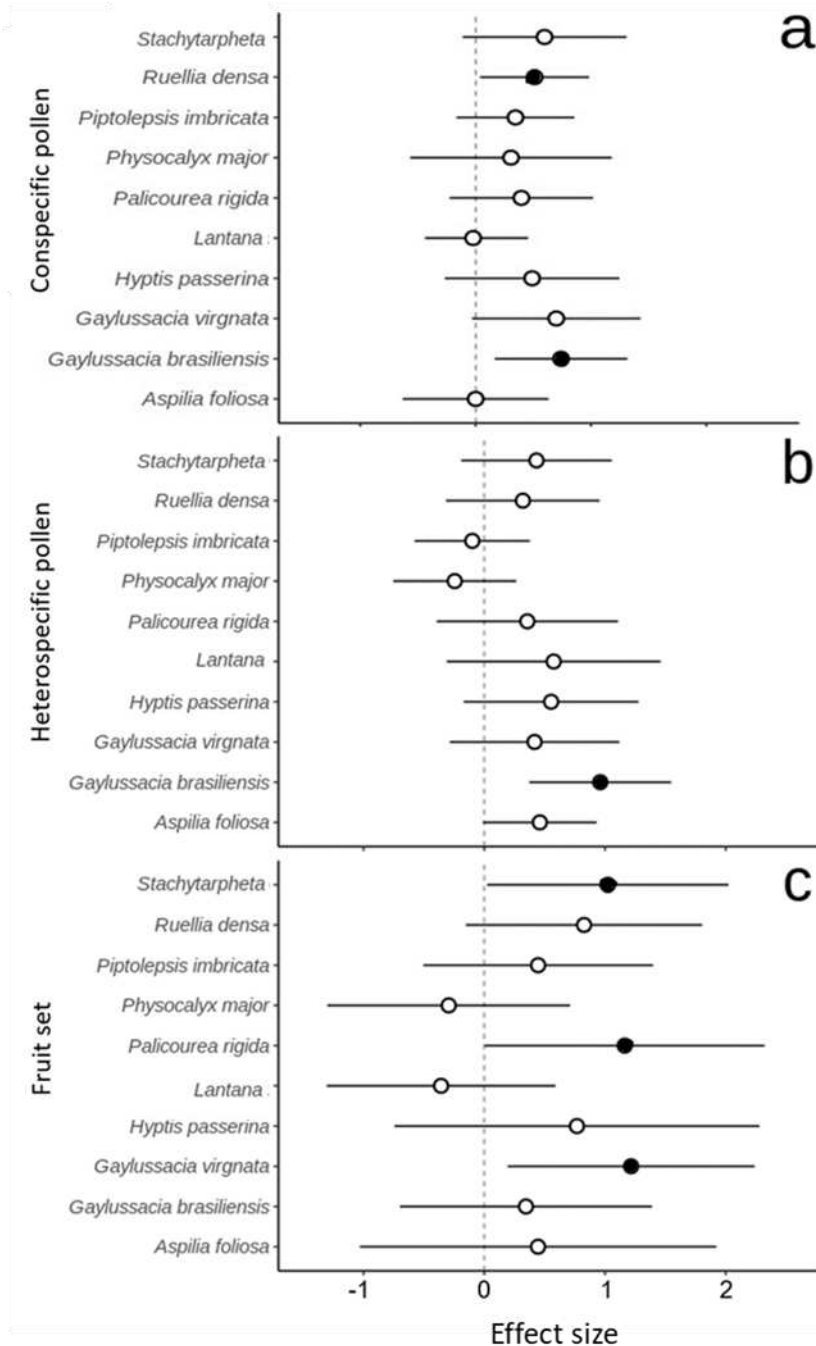
Response variable	Predictors	$\chi^2$	<i>p</i> -value
Conspecific pollen	PC1 * Treatment	9.177	0.002*
	PC1	0.008	0.928
	Treatment	1.026.36	0.002*
Heterospecific pollen	PC1 * Treatment	157.76	0.002*
	PC1	0.178	0.672
	Treatment	417.272	0.002*
Fruit set	PC1 * Treatment	3.081	0.079
	PC1	3.419	0.064
	Treatment	73.025	0.002*

\* Significant results.



\* Significant results.

**Figure 2.** Relationship between floral characteristics (PC1) and reproduction of 10 plant species with mixed pollination systems from the *Campo Rupestre*. a) Conspecific pollen deposition, b) Heterospecific pollen deposition and c) Fruit set. Lines inclination indicated as b. Box plots illustrates the differences between treatment and control, with \* indicating significant results.



**Figure 3.** Cohen's d effect size and 95% confidence interval for each variable analyzed for 10 plant species. a) Conspecific pollen deposition, b) Heterospecific pollen deposition and c) Fruit set. Hummingbirds are more relevant for plant reproduction than insects alone when confidence intervals do not overlap zero (black dots).

#### 4| Discussion

We showed that hummingbirds increase the deposition of conspecific and heterospecific pollen, and the fruit set in plants with mixed pollination systems from the *Campo Rupestre*. Conspecific pollen deposition was higher in more specialized flowers in both control and treatment flowers, albeit with a stronger trend when hummingbirds were present. On the other hand, heterospecific deposition showed an opposing trend with and without hummingbirds. While hummingbirds increased the overall fruit set, this was not related to floral traits. Finally, the magnitude of how much hummingbird contribute to plant reproduction is dependent on plant identity, i.e. only three species showed strong indication of increased fruit set when hummingbirds visited flowers in addition to insects, as indicated by effect sizes.

Hummingbirds are known to rely on non-ornithophilous flowers, especially in more seasonal and open vegetation areas such as the *Campo Rupestre* (Rodrigues & Rodrigues 2014; Araujo et al., 2018). Because hummingbirds frequently visit non-ornithophilous flowers even in the presence of more specialized and “private” resources, non-specialized flowers may constitute a relevant part of their diet (Maruyama et al., 2013; Waser et al., 2018). Although how much hummingbirds contribute to the reproduction of such non-specialized flowers is still debated (Maruyama et al. 2012, 2013), we show that they increase fruit set overall. One important point to consider for plants frequently visited by both hummingbirds and insects, i.e., plants with mixed-pollination systems, is that the relative importance of each group may change according to the environment (e.g. Lehmann et al. 2019). Although our study did not evaluate the relative contribution across multiple sites or years, the general enhancement of deposition and fruitset are an indication of the positive effects of hummingbirds in the *Campo Rupestre*.

Hummingbirds deposited proportionally more conspecific pollen in flowers traditionally associated with higher specialization on hummingbird pollination (Castellanos et

al., 2004; Cronk & Ojeda 2008; Ferreira et al., 2016), which corroborates the previous evidence linking floral traits and effectiveness of pollination services (Castellanos et al., 2004; Santiago-Hernández et al., 2019). Nevertheless, hummingbirds increased conspecific pollen deposition but also heterospecific pollen deposition, as a result of plants sharing the same pollinators. Previous studies indicated that higher rates of heterospecific pollen deposition can reduce fruit set (Arceo-Gómez & Ashman, 2011; Coetzee, Spottiswoode, & Seymour, 2020). However, heterospecific pollen deposition found here apparently has a minor impact compared to the benefits of increased conspecific pollen deposition, since fruit set increased overall with bird visitation. This positive correlation on conspecific and heterospecific pollen deposition was previously reported for the larger community of hummingbird pollinated plants in the same community, including 18 more plant species besides the ones studied here (Lopes et al., 2020, submitted). These overall positive associations potentially indicate the occurrence of facilitation between hummingbirds visiting co-flowering species in the *Campo Rupestre* (Ashman et al., 2020).

The differences on the magnitude of effects observed among plant species illustrate how hummingbirds may distinctively contribute to plant reproduction. *Palicourea rigida*, for example, was previously described as mainly pollinated by hummingbirds (Machado, Silva, Consolaro, Barros, & Oliveira, 2010). However, in *P. rigida* and *Gaylussacia virgnata*, increased fruit set without higher conspecific pollen deposition suggested that hummingbirds enhanced plant reproductive success through higher quality of the pollen they deposit (Peuker et al., 2020). This, and the fact that hummingbird visits are considerably less frequent than insect visits, suggest that the role of hummingbirds in enhancing plant reproduction is not only an additive effect due to more visits in the control plants (insects + hummingbirds). Pollination effectiveness is determined by pollinator handling behavior, body size and shape, as well as trait matching with the flower which determines pollen deposition (Castellanos et

al., 2004). However, even if a pollinator deposits less pollen, it may still be a more effective pollinator if its visits lead to higher fruit set because it brings better quality pollen (Sahli & Conner, 2007; Freitas, 2013). Such effects may be especially common when considering birds, in relation to insects as pollinators (Krauss et al. 2017). In contrast, some species such as *R. densa* and *G. brasiliensis* experienced strong higher conspecific pollen deposition when hummingbirds had access to the flowers, which did not translate to higher fruit set, probably due to saturation effects related to ovule fertilization (Erbar, 2003). There is evidence that the quality component of the pollen can take longer to saturate when compared to the pollen quantity (Alzen & Harder, 2007). Also, higher conspecific pollen deposition could have saturated the number of fruits and not the number of seeds, which we did not measure. However, stigma saturation may also help to understand the distinct results observed when pollen deposition or fruit set were measured, as higher deposition is not indefinitely translated into ovule fertilization: the number of ovules is often lower than the number of pollen grains deposited if there is no pollen limitation (Erbar, 2003; Minnaar, Anderson, de Jager, & Karron, 2019). Our study reinforces that the stage at which the success of pollinator visits is estimated is highly relevant when evaluating the importance of different pollinators groups (Ollerton, Rech, Waser, & Price, 2015; Santiago - Hernández et al., 2019).

Even though our results showed that isolation treatment produced different results on plant fitness measures, a generalized pollination system including both birds and insects may increase reproductive assurance (Waser et al. 1996; Martén-Rodríguez, Fenster, Agnarsson, Skog, & Zimmer, 2010). This reproductive assurance should be even more important in places such as the *Campo Rupestre* with environmental disturbances such as periodic fires, strong seasonality and local climate variation which may influences pollinator availability (Fang et al., 2012; Lehmann et al., 2019; Leal et al., 2020). In contrast to strong specialization, generalist plants with mixed pollination systems may show an advantage, if the

cost of specialization outweighs the benefit of keeping a wider array of potential pollinators, including fewer effective ones (Aigner, 2001).

In sum, we showed that hummingbirds contribute to plant reproduction, even for more generalized and non-oritophilous plants. For some species, this higher relative importance of hummingbirds seems to be related to the higher quality of the pollen they deposit. Precise detection of the relative contribution of distinct pollinator groups may require more than simple measures of conspecific pollen deposition, especially in outcrossing plants. We emphasize that experimental studies quantifying the relative contribution of pollinators at distinct stages of reproduction are important to advance our understanding of the patterns across the generalization/specialization continuum in plant-pollinator interactions.

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### **Authors' Contributions**

MDA, CSA and ARR conceived the ideas and design methodology; MDA and GJB collected the data; MDA, PKM and GJB analyzed the data; MDA wrote the first draft of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

### **References Bibliographic**

- Aigner, P. A. (2001). Optimality modeling and fitness trade-offs: when should plants become pollinator specialists? *Oikos*, 95(1), 177–184. doi: 10.1034/j.1600-0706.2001.950121.x
- Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M., & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22(6), 711–728. doi: 10.1127/0941-2948/2013/0507



- Aizen, M. A., & Harder, L. D. (2007). Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology*, 88(2), 271–281. doi: 10.1890/06-1017
- Araujo, A. C., Martín González, A. M., Sandel, B., Maruyama, P. K., Fischer, E., Vizentin-Bugoni, J., ... Svenning, J.-C. (2018). Spatial distance and climate determine modularity in a cross-biomes plant-hummingbird interaction network in Brazil. *Journal of Biogeography*, 45(8), 1846–1858. doi: 10.1111/jbi.13367
- Arceo-Gómez, G., & Ashman, T.-L. (2011). Heterospecific pollen deposition: does diversity alter the consequences? *New Phytologist*, 192(3), 738–746. doi: 10.1111/j.1469-8137.2011.03831.x
- Ashman, T.-L., Alonso, C., Parra-Tabla, V., & Arceo-Gómez, G. (2020). Pollen on stigmas as proxies of pollinator competition and facilitation: complexities, caveats and future directions. *Annals of Botany*, 125(7), 1003–1012. doi: 10.1093/aob/mcaa012
- Ashman, T.-L., & Arceo-Gómez, G. (2013). Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. *American Journal of Botany*, 100(6), 1061–1070. doi: 10.3732/ajb.1200496
- Bates, D., Achler, M. M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1–48. doi: 10.18637/jss.v067.i01
- Bergamo, P. J., Rech, A. R., Brito, V. L. G., & Sazima, M. (2016). Flower colour and visitation rates of *Costus arabicus* support the ‘bee avoidance’ hypothesis for red-reflecting hummingbird-pollinated flowers. *Functional Ecology*, 30(5), 710–720. doi: 10.1111/1365-2435.12537
- Bergamo, P. J., Streher, N. S., Wolowski, M., & Sazima, M. (2020). Pollinator-mediated facilitation is associated with floral abundance, trait similarity and enhanced community-level fitness. *Journal of Ecology*, 108(4), 1334–1346. doi: 10.1111/1365-2745.13348
- Botes, C., Johnson, S. D., & Cowling, R. M. (2009). The Birds and the Bees: Using Selective Exclusion to Identify Effective Pollinators of African Tree Aloes. *International Journal of Plant Sciences*, 170(2), 151–156. doi: 10.1086/595291
- Camargo, M. G. G., Lunau, K., Batalha, M. A., Brings, S., Brito, V. L. G., & Morellato, L. P. C. (2019). How flower colour signals allure bees and hummingbirds: a community-level test of the bee avoidance hypothesis. *New Phytologist*, 222(2), 1112–1122. doi: 10.1111/nph.15594
- Castellanos, M. C., Wilson, P., & Thomson, J. D. (2004). “Anti-bee” and “pro-bird” changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology*, 17(4), 876–885. doi: 10.1111/j.1420-9101.2004.00729.x
- Coetzee, A., Spottiswoode, C. N., & Seymour, C. L. (2020). Post-pollination barriers enable coexistence of pollinator-sharing ornithophilous *Erica* species. *Journal of Plant Research*. doi: 10.1007/s10265-020-01226-8
- Cronk, Q., & Ojeda, I. (2008). Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, 59(4), 715–727. doi: 10.1093/jxb/ern009
- Dellinger, A. S. (2020). Pollination syndromes in the 21 st century: where do we stand and where may we go? *New Phytologist*, nph.16793. doi: 10.1111/nph.16793
- Erbar, C. (2003). Pollen tube transmitting tissue: Place of competition of male gametophytes. *International Journal of Plant Sciences*, 164(5 SUPPL.). doi: 10.1086/377061

- Faegri, K., & Pijl, L. V. D. (1979). *The principles of pollination ecology*. New York: Pergamon Press, 64, 6–7.
- Fang, Q., Chen, Y.-Z., & Huang, S.-Q. (2012). Generalist passerine pollination of a winter-flowering fruit tree in central China. *Annals of Botany*, 109(2), 379–384. doi: 10.1093/aob/mcr293
- Ferreira, C., Maruyama, P. K., & Oliveira, P. E. (2016). Convergence beyond flower morphology? Reproductive biology of hummingbird-pollinated plants in the Brazilian Cerrado. *Plant Biology*, 18(2), 316–324. doi: 10.1111/plb.12395
- Fox, J., & Weisberg, S. (2011). *An R Companion to Applied Regression* (Second). Retrieved from <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>
- Freitas, L. (2013). Concepts of pollinator performance: is a simple approach necessary to achieve a standardized terminology? *Brazilian Journal of Botany*, 36(1), 3–8. doi: 10.1007/s40415-013-0005-6
- Gómez, J. M. (2002). Generalización en las interacciones entre plantas y polinizadores. *Revista Chilena de Historia Natural*, 75(1). doi: 10.4067/S0716-078X2002000100010
- Hargreaves, A. L., Harder, L. D., & Johnson, S. D. (2012). Floral traits mediate the vulnerability of aloes to pollen theft and inefficient pollination by bees. *Annals of Botany*, 109(4), 761–772. doi: 10.1093/aob/mcr324
- Hargreaves, A. L., Johnson, S. D., & Nol, E. (2004). Do floral syndromes predict specialization in plant pollination systems? An experimental test in an “ornithophilous” African Protea. *Oecologia*, 140(2), 295–301. doi: 10.1007/s00442-004-1495-5
- Kassambara, A., & Mundt, F. (2020). *factoextra: Extract and Visualize the Results of Multivariate Data Analyses*. Retrieved from R package version 1.0.7
- King, C., Ballantyne, G., & Willmer, P. G. (2013). Why flower visitation is a poor proxy for pollination: Measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution*, 4(9), 811–818. doi: 10.1111/2041-210X.12074
- Koski, M. H., Ison, J. L., Padilla, A., Pham, A. Q., & Galloway, L. F. (2018). Linking pollinator efficiency to patterns of pollen limitation: small bees exploit the plant–pollinator mutualism. *Proceedings of the Royal Society B: Biological Sciences*, 285(1880), 20180635. doi: 10.1098/rspb.2018.0635
- Krauss, S. L., Phillips, R. D., Karron, J. D., Johnson, S. D., Roberts, D. G., & Hopper, S. D. (2017, May 1). Novel Consequences of Bird Pollination for Plant Mating. *Trends in Plant Science*, Vol. 22, pp. 395–410. doi: 10.1016/j.tplants.2017.03.005
- Leal, R. L. B., Moreira, M. M., Pinto, A. R., de Oliveira Ferreira, J., Rodriguez-Girones, M., & Freitas, L. (2020). Temporal changes in the most effective pollinator of a bromeliad pollinated by bees and hummingbirds. *PeerJ*, 8, e8836. doi: 10.7717/peerj.8836
- Lehmann, L. J., Maruyama, P. K., Joaquim Bergamo, P., Maglianesi, M. A., Rahbek, C., & Dalsgaard, B. (2019). Relative effectiveness of insects versus hummingbirds as pollinators of Rubiaceae plants across elevation in Dominica, Caribbean. *Plant Biology*, 21(4), 738–744. doi: 10.1111/plb.12976

- Lopes S. A. (2019). Deposição de polen heteroespecífico em plantas visitadas por beija-flores no Campo Rupestre em Diamantina-MG. *Minas Gerais*. Trabalho de conclusão de curso. Universidade Federal dos Vales do Jequitinhonha e Mucuri.
- Machado, A. de O., Silva, A. P., Consolaro, H., Barros, M. A. G. e, & Oliveira, P. E. (2010). Breeding biology and distyly in *Palicourea rigida* H. B. & K. (Rubiaceae) in the Cerrados of Central Brazil. *Acta Botanica Brasilica*, 24(3), 686–696. doi: 10.1590/S0102-33062010000300012
- Martén-Rodríguez, S., Fenster, C. B., Agnarsson, I., Skog, L. E., & Zimmer, E. A. (2010). Evolutionary breakdown of pollination specialization in a Caribbean plant radiation. *New Phytologist*, 188(2), 403–417. doi: 10.1111/j.1469-8137.2010.03330.x
- Maruyama, Pietro K., Oliveira, G. M., Ferreira, C., Dalsgaard, B., & Oliveira, P. E. (2013). Pollination syndromes ignored: importance of non-ornithophilous flowers to Neotropical savanna hummingbirds. *Naturwissenschaften*, 100(11), 1061–1068. doi: 10.1007/s00114-013-1111-9
- Maruyama, Pietro Kiyoshi, Custódio, L. N., & Oliveira, P. E. (2012). When hummingbirds are the thieves: visitation effect on the reproduction of Neotropical snowbell *Styrax ferrugineus* Nees & Mart (Styracaceae). *Acta Botanica Brasilica*, 26(1), 58–64. doi: 10.1590/S0102-33062012000100007
- Minnaar, C., Anderson, B., de Jager, M. L., & Karron, J. D. (2019). Plant–pollinator interactions along the pathway to paternity. *Annals of Botany*, 123(2), 225–245. doi: 10.1093/aob/mcy167
- Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews*, 82(4), 591–605. doi: 10.1111/j.1469-185X.2007.00027.x
- Ne’eman, G., Jürgens, A., Newstrom-Lloyd, L., Potts, S. G., & Dafni, A. (2009). A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Reviews*, no-no. doi: 10.1111/j.1469-185X.2009.00108.x
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., ... Wagner, H. (2019). *vegan: Community Ecology Package*. Retrieved from <https://cran.r-project.org/package=vegan>
- Ollerton, J. (2017). Pollinator Diversity: Distribution, Ecological Function, and Conservation. *Annual Review of Ecology, Evolution, and Systematics*, 48(1), 353–376. doi: 10.1146/annurev-ecolsys-110316-022919
- Ollerton, J., Rech, A. R., Waser, N. M., & Price, M. V. (2015). Using the literature to test pollination syndromes—Some methodological cautions. *Journal of Pollination Ecology*, 16. doi: 10.26786/1920-7603(2015)17
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321–326. doi: 10.1111/j.1600-0706.2010.18644.x
- Peuker, M. A., Burger, H., Krausch, S., Neumüller, U., Ayasse, M., & Kuppler, J. (2020). Floral traits are associated with the quality but not quantity of heterospecific stigmatic pollen loads. *BMC Ecology*, 20(1), 54. doi: 10.1186/s12898-020-00323-5
- Queiroz, S. N. D. P. (2018). *Dinâmica temporal e funcional da rede de interações entre plantas e beija-flores no Campo Rupestre da Serra do Espinhaço, Minas Gerais*. Universidade Federal dos Vales do Jequitinhonha e Mucuri.

- R Core Team. (2018). *R: A Language and Environment for Statistical Computing*. Retrieved from <https://www.r-project.org/>
- Rodrigues, L., & Rodrigues, M. (2014). Flowers visited by hummingbirds in the open habitats of the southeastern Brazilian mountaintops: species composition and seasonality. *Brazilian Journal of Biology*, 74(3), 659–676. doi: 10.1590/bjb.2014.0097
- Rosas-Guerrero, V., Aguilar, R., Martén-Rodríguez, S., Ashworth, L., Lopezaraiza-Mikel, M., Bastida, J. M., & Quesada, M. (2014). A quantitative review of pollination syndromes: Do floral traits predict effective pollinators? (R. Irwin, Ed.). *Ecology Letters*, Vol. 17, pp. 388–400. doi: 10.1111/ele.12224
- Schmid, S., Schmid, V. S., Zillikens, A., Harter-Marques, B., & Steiner, J. (2011). Bimodal pollination system of the bromeliad *Aechmea nudicaulis* involving hummingbirds and bees. *Plant Biology*, 13(SUPPL. 1), 41–50. doi: 10.1111/j.1438-8677.2010.00348.x
- Sahli, H. F., & Conner, J. K. (2007). Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany*, 94(2), 203–209. doi: 10.3732/ajb.94.2.203
- Sihag, R. C. (2018). Some Unresolved Issues of Measuring the Efficiency of Pollinators: Experimentally Testing and Assessing the Predictive Power of Different Methods. *International Journal of Ecology*, 2018, 1–13. doi: 10.1155/2018/3904973
- Silveira, F. A. O., Negreiros, D., Barbosa, N. P. U., Buisson, E., Carmo, F. F., Carstensen, D. W., ... Lambers, H. (2016). Ecology and evolution of plant diversity in the endangered campo rupestre: a neglected conservation priority. *Plant and Soil*, Vol. 403, pp. 129–152. doi: 10.1007/s11104-015-2637-8
- Santiago-Hernández, M. H., Martén-Rodríguez, S., Lopezaraiza-Mikel, M., Oyama, K., González-Rodríguez, A., & Quesada, M. (2019). The role of pollination effectiveness on the attributes of interaction networks: from floral visitation to plant fitness. *Ecology*, 100(10). doi: 10.1002/ecy.2803
- Stebbins, G. L. (1970). Adaptive Radiation of Reproductive Characteristics in Angiosperms, I: Pollination Mechanisms. *Annual Review of Ecology and Systematics*, 1(1), 307–326. doi: 10.1146/annurev.es.01.110170.001515
- Sun, S. G., Huang, Z. H., Chen, Z. B., & Huang, S. Q. (2017). Nectar properties and the role of sunbirds as pollinators of the golden-flowered tea (*Camellia petelotii*). *American Journal of Botany*, 104(3), 468–476. doi: 10.3732/ajb.1600428
- Torchiano, M. (2020). *effsize: Efficient Effect Size Computation*. doi: 10.5281/zenodo.1480624
- Waser, N. M., CaraDonna, P. J., & Price, M. V. (2018). Atypical Flowers Can Be as Profitable as Typical Hummingbird Flowers. *The American Naturalist*, 192(5), 644–653. doi: 10.1086/699836
- Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77(4), 1043–1060. doi: 10.2307/2265575
- Wolf, L. L., Stiles, F. G., & Hainsworth, F. R. (1976). Ecological Organization of a Tropical, Highland Hummingbird Community. *The Journal of Animal Ecology*, 45(2), 349. doi: 10.2307/3879

- Zanata, T. B., Dalsgaard, B., Passos, F. C., Cotton, P. A., Roper, J. J., Maruyama, P. K., ... Varassin, I. G. (2017). Global patterns of interaction specialization in bird–flower networks. *Journal of Biogeography*, 44(8), 1891–1910. doi: 10.1111/jbi.13045
- Zych, M., Goldstein, J., Roguz, K., & Stpiczyńska, M. (2013). The most effective pollinator revisited: pollen dynamics in a spring-flowering herb. *Arthropod-Plant Interactions*, 7(3), 315–322. doi: 10.1007/s11829-013-9246-3

### **Apêndice A - Animais Observados Visitando as Plantas.**

**Table S1.** Animals observed interacting with the studied plant species in the *Campo Rupestre* of Diamantina, Minas Gerais state, Brazil.

<b>Species</b>
<b>Apidae</b>
<i>Apis mellifera</i>
<i>Bombus</i> sp.
<i>Trigona</i>
<b>Halictidae</b>
Halictidae sp. 1
Halictidae sp. 2
<b>Megachilidae</b>
Megachilidae sp. 1
Megachilidae sp. 2
<b>Vespidae</b>
Vespidae sp. 1
Vespidae sp. 2
Vespidae (Parasitoid)
Diptera
<b>Lepidoptera</b>
Lepidoptera sp. 1
Lepidoptera sp. 2
Lepidoptera sp. 3
Hesperiidae
Coleoptera
<b>Trochilidae</b>
<i>Augastes scutatus</i>
<i>Eupetomena macroura</i>
<i>Phethornis pretrei</i>

**Apêndice B – Taxa por grupos de visitantes para cada espécie de planta**

**Table S2.** Rate of floral visitors by group for each plant species.

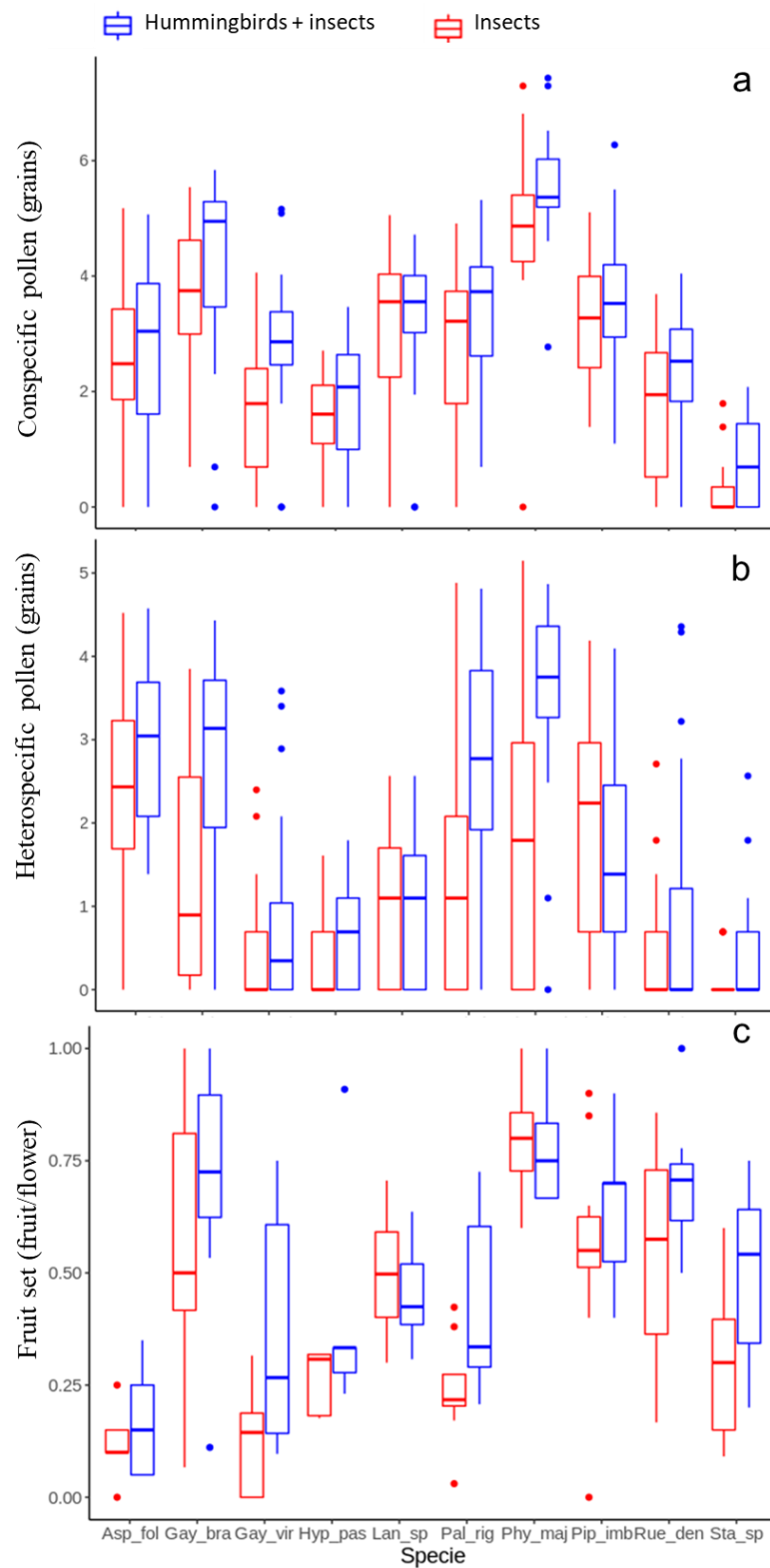
Plant Species	Hummingbirds + Insects				Insects		
	Hummingbirds	Bees	Others	n	Bees	Others	n
<i>Ruellia densa</i>	0.46	0.38	0.15	13	0.87	0.12	8
<i>Aspilia foliosa</i>	0.04	0.93	0.02	74	1.0	0.0	32
<i>Piptolepsis imbricata</i>	0.09	0.77	0.13	22	0.5	0.5	22
<i>Gaylussacia brasiliensis</i>	0.21	0.78	0.0	14	1.0	0.0	26
<i>Gaylussacia virgnata</i>	0.26	0.47	0.26	19	0.81	0.18	27
<i>Hyptis passerina</i>	0.05	0.94	0.0	19	1.0	0.0	3
<i>Physocalyx major</i>	0.77	0.22	0.0	9	0.0	0.0	0
<i>Palicourea rigida</i>	0.30	0.61	0.08	13	0.66	0.33	6
<i>Lantana sp.</i>	0.05	0.82	0.11	17	0.93	0.06	15
<i>Stachytarpheta sp.</i>	0.76	0.23	0.0	21	1.0	0.0	13
Total	0.21	0.71	0.06	221	0.86	0.13	152

Apêndice C - Gaiolas para a Exclusão de Vertebrados.



**Figure S1.** Pictures of flowers on the cages with vertebrate exclusion. a) *Gaylussasia brasiliensis*; b) *Physocalyx major*; c) *Palicourea rigida*; d) *Aspilia foliosa*.

### Apêndice D - Médias da Deposição e Frutificação para as 10 Espécies de Plantas.



**Figure S3.** Mean and standard deviation of pollen deposition and fruitification for plant species. a) Conspecific pollen deposition, b) Heterospecific pollen deposition, c) Fruit set.



## CAPÍTULO 2 - *Gongylolepis martiana*, an Asteraceae pollinated by bats in the Amazon

Running head: Bat-pollination in Asteraceae

Marsal Danrlei Amorim<sup>1</sup>, Daniel da Silva Costa<sup>2</sup>, Dayse Raiane Passos Krahl<sup>2</sup>, Erich Fischer<sup>2,3</sup>,  
André Rodrigo Rech<sup>4</sup>

<sup>1</sup>Programa de Pós-Graduação em Biologia Animal, Universidade Federal dos Vales de Jequitinhonha e Mucuri, 39100-000 Diamantina, Minas Gerais, Brasil

<sup>2</sup>Programa de Pós-Graduação em Botânica, Instituto Nacional de Pesquisa da Amazônia, 69067-375 Manaus, Amazonas, Brasil

<sup>3</sup>Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, 79070-900 Campo Grande, Mato Grosso do Sul, Brasil

<sup>4</sup>Faculdade Interdisciplinar de Humanidades, Universidade Federal dos Vales do Jequitinhonha e Mucuri, 39100-000 Diamantina, Minas Gerais, Brasil

Correspondence

M. D. Amorim

Programa de Pós-Graduação em Biologia Animal, Universidade Federal dos Vales de Jequitinhonha e Mucuri, 39100-000 Diamantina, Minas Gerais, Brasil.

E-mail: marsal.amorim@gmail.com

Phone: 55-(38)99994-3726

**Keywords:** Amazônia, Asterales, campina, hummingbirds, nectar-feeding bats, vertebrate pollination, white-sand forest.

### Summary sentence

Floral traits and pollen removal from flowers and deposition on stigmas indicate the pollination by nectar-feeding bats for *Gongylolepis martiana* and confirm the occurrence of chiropterophily in Asteraceae.

## Resumo

- As espécies da maior família de plantas, Asteraceae, geralmente apresentam flores polinizadas por abelhas, embora a polinização por pássaros tenha sido documentada e a polinização por morcegos tenha sido sugerida para algumas espécies. Aqui, nós investigamos a polinização de *Gongylolepis martiana*, uma planta cujas flores são consideradas polinizadas por morcegos.
- Avaliamos características florais e visitantes diurnos e noturnos em uma população de *G. martiana* em uma floresta de areia branca na Amazônia brasileira, medindo a remoção de pólen das flores e a deposição em estigmas por visitantes diurnos e noturnos.
- Os floretes abriram ao entardecer e duraram quatro dias, com a fase masculina iniciando na primeira noite e a fase feminina na terceira noite. O néctar acumulado noturno por capítulo foi de 69,6 µl e a concentração de açúcar foi de 15%. Morcegos e colibris que se alimentam de néctar contataram as partes florais sexuais, mas a remoção e deposição de pólen foram muito maiores durante a noite do que à luz do dia, quando as abelhas Meliponini reduziram notavelmente a disponibilidade de pólen para transporte através dos colibris. Outros visitantes noturnos de *G. martiana* eram raros e incluíam abelhas noturnas e mariposas, que buscavam pólen e néctar, respectivamente.
- Nossos resultados apoiam que os morcegos nectarívoros são os principais polinizadores de *G. martiana*, confirmando a hipótese de Vogel para a polinização de morcegos em Asteraceae, e particularmente no gênero *Gongylolepis*. O início da antese e de cada fase floral sexual no início da noite definiu um efeito prioritário de morcegos nectarívoros e abelhas diurnas que impedem os beija-flores de serem polinizadores eficientes. A alta densidade de indivíduos floridos de *G. martiana* em

manchas de florestas de areia branca provavelmente aumenta a atração de morcegos, enquanto a baixa quantidade de néctar por planta favorece a polinização cruzada.

**Palavras chaves:** Amazônia, Asterales, Campina, Beija-flores, Morcegos nectívoros, Polinização por vertebrados, Floresta de areia-branca

## Abstract

- The largest family Asteraceae presents mainly generalist systems main pollination, with more common bees pollination, although bird-pollination has been documented and bat-pollination has been suggested for some species. Here, we investigated the pollination of *Gongylolepis martiana*, a plant whose flowers are thought to be pollinated by bats.
- We assessed floral traits and visitors (diurnal and nocturnal) in a population of *G. martiana* on a white-sand forest in the Brazilian Amazon, measuring pollen removal from anthers and deposition on stigmas by diurnal and nocturnal visitors.
- Florets opened at dusk and lasted for four days, with the male phase starting on the first night and the female phase on the third night. Accumulated nectar per capitulum was 69.6  $\mu\text{l}$  per night and sugar concentration was 15%. Nectar-feeding bats and hummingbirds contacted sexual floral parts, but pollen removal and deposition were greater throughout the night than during the day, when Meliponini bees reduced considerably pollen availability and compromising pollen transportation by hummingbirds. Other nocturnal visitors of *G. martiana* were rare, including nocturnal bees and moths that foraged for pollen and nectar, respectively.
- Our results support that nectarivorous bats are the main pollinators of *G. martiana*, confirming Vogel's hypothesis of bat pollination in Asteraceae, particularly in the genus *Gongylolepis*. Since anthesis and each sexual floral phase started in the evening, nectarivorous bats and diurnal bees caused priority effects that prevented hummingbirds from being efficient pollinators. The high density of flowering individuals of *G. martiana* in patches from white-sand forests likely increases bat attraction, while the small amount of nectar per plant favours cross-pollination.

## Introduction

Pollination by animals was likely one of the major drivers of diversification acting on the evolution of many floral traits, e.g., corolla length, colour, and odour (Van der Niet & Johnson 2012). However, Asteraceae, the largest family of flowering plants, intriguingly presents little variation in pollination systems among species (Torres & Galetto 2002; Vogel 2015). Most pollinator species associated with this family are bees, although other invertebrates are common in generalist Asteraceae flowers (Vogel 2015). Currently, birds are the only vertebrate group confirmed as pollinators of a few Asteraceae species, e.g., *Cirsium coulteriherv*, *Vernonia fulva*, and *Mutisia campanulata* (Buzato *et al.* 2000; Torres & Galetto 2002; Hipólito *et al.* 2013; Vogel 2015). Nonetheless, Vogel (2015) suggested that pollination by bats is likely for 17 Asteraceae species based on their floral traits and observation of occasional visits of bat on *Cirsium subcoriaceum* and *Gongylolepis jauaensis* flowers. However, the role of bats as pollinators remains inconclusive. Although unusual, bat pollination has been recorded for 528 species in 67 angiosperm families, so such a system would indeed be expected in the large Asteraceae family (Fleming *et al.* 2009).

The architecture of Asteraceae flowers, i.e. open capitula, with nectar-producing florets and relatively short tube corollas, has been associated with a generalist pollination system, which contributes to the high invasive ability of many species (Dargas *et al.* 2016; Martín-Forés *et al.* 2018). Generalized pollination systems ordinarily involve sharing floral rewards among a variety of phylogenetically unrelated visitors (Torres & Galetto 2002; Vogel 2015), which may cause exploitative competition among those visitors sharing the same rewards (Missagia *et al.* 2014; Dargas *et al.* 2016; Ye *et al.* 2017). However, some plant species have long-lived flowers that are open for an entire circadian period, which may reduce temporal overlap among different floral visitors. Still nocturnal visitors may affect resource availability for diurnal visitors in the case when flowers open first at night, and vice-versa (Prieto-Benítez *et al.* 2016; but see Ye *et al.* 2017 and references therein for nectar resource replenishment).

Such “priority effect” is known as the effect that a given visitors species has on subsequent interactions due to its prior arrival at resources (Hoverman *et al.* 2013; Rasmussen *et al.* 2014). A clear example of priority effect in pollination systems may be resource removal by nocturnal visitants that become unavailable for daylight flower visitors (Aguilar-Rodríguez *et al.* 2014). These priority effects can go beyond simple competition as the behavior of the first flower visitors may change possible outcomes of the following visitors. This is especially important in flowers on which nectar, for example, can be replenished but pollen not.

We studied the pollination biology of *Gongylolepis martiana* (Asteraceae), a shrub or treelet (1.5-6 m tall) species that is endemic to northern South America and presumably associated with leached sandy soils in the Amazon rainforest. This is one of the two Amazonian species of *Gongylolepis* whose pollination systems are unknown. *Gongylolepis martiana* presents vinaceous capitula (heads), a rare trait in the family, with relatively large individual flowers (florets), and our nectar measurements showed amount compatible with pollination by vertebrates, more specifically by bats (Faegri & Pijl 1979; Vogel 2015). According to Vogel’s (2015) prediction, these features of *G. martiana* flowers lead to hypothesize pollination by vertebrates in this species, and considering the flower opening at dusk, nectar-feeding bats became the main candidates. Therefore, we evaluated whether the floral biology and effectiveness of floral visitors in *G. martiana* confirm its pollination by bats.

### **Material and Methods**

We studied a population of *G. martiana* from August 17 to 22, 2019, in a 10-ha pristine campina (60°44’ 27.2” W, 03° 04’ 14.5” S) in the Reserva de Desenvolvimento Sustentável do Rio Negro (RDS Rio Negro; 103,086 ha), Amazonas state, Brazil. The climate is tropical rainforest (Af) according to Köppen, with mean annual temperature of 26 °C, ranging from 22

to 32 °C, and mean annual rainfall of 2,300 mm, varying monthly from 60 mm (August) to 300 mm (March). The RDS encompasses mainly dense ombrophilous forest (40 m tall canopy) with small patches of white-sand forests (2-8 m tall canopy) called Campina (short open-canopy) or Campinarana (tall closed-canopy), composed of sclerophyllous vegetation on highly leached soils (Adeney *et al.* 2016; Demarchi *et al.* 2019).

To assess the floral biology, we recorded the floral longevity, registering the period when buds started to open to florets wilting in five individuals. The observed stages were anthesis, male phase, female phase and post-anthesis. We selected another six individuals in different flowering phases to record the number of buds and male and female florets per head. We recorded floral visitors through focal observations and photos for three consecutive days, between 0600 and 2200 h, totaling 48 hours. We measured nectar volume ( $\mu\text{l}$ ) and sugar concentration (brix) from florets of four heads that were previously bagged at bud stage (accumulated nectar) and two heads that were exposed to the visitors. Nectar volume was measured with glass microcapillaries (to  $\pm 5 \mu\text{l}$ ), and sugar concentration was measured with a portable refractometer (to  $\pm 1\%$  brix). Measurements were taken twice from each head, at dusk and then at dawn the next day. To estimate the total nectar and sugar mass that was offered nightly to the visitors by the entire population of *G. martiana*, we recorded the number of open florets per head and the total number of functional heads per plant on all 98 individuals flowering in the campina studied. We then multiplied the total number of florets by the mean nectar volume accumulated per floret, and used the mean sugar concentration to estimate total sugar mass. Exsiccates of *G. martiana* were deposited in the herbarium at the Instituto Nacional de Pesquisas da Amazônia (INPA Herbarium).

To evaluate the pollination contribution of diurnal and nocturnal flower visitors, we counted pollen grains exposed on polliniferous styles (thus available for deposition on a visitors' body) four times and the pollen grains deposited on the stigmas three times in the 24-

hour period following the beginning of male and female phases, respectively. Each head presented a number of open florets, allowing different florets from the same heads to be sampled throughout time. The pollen available in polliniferous styles was collected from 11 heads of 11 male phase individuals. From these heads, we sampled three different florets at 1800 h, as well as the next day at 0600 h (exposed only to nocturnal visitors), 0800 h (after peak visiting of dawn stingless bees) and 1800 h (exposed only to diurnal visits). To calculate pollen removal, we subtracted the pollen in the anthers at the start time (1800h) and the pollen present in the anthers at each of the sampled times (0600, 0800 and 1800) and this difference was considered the pollen removed. To sample pollen loads deposited on the stigmas, we used 10 heads in the female phase (third day) from 10 individuals. Pollen on the stigmas was collected three times: at 1800 h (beginning of stigma receptivity), the next day at 0600 h (exposed only to nocturnal visitors) and 1800 h (exposed only to diurnal visitors). Heads used to measure pollen removal and pollen deposition during the daytime (collected at 0800h and 1800 h from the next day, respectively) were previously enclosed in paper bags throughout the night (from 1800 to 0600 h) to assure that only diurnal visitors interacted with these flowers. We collected one floret per head and counted the pollen grains on polliniferous styles (for male phase florets) or deposited on the stigmas (for female phase florets). All pollen samples were collected with small cubes of glycerin-carmin jelly, and then mounted on slides to count pollen under a microscope (Kearns & Inouye 1993). We also tested stigma receptivity in isolated florets using a drop of hydrogen peroxide 6% on the stigmatic surfaces (Dafni & Maués 1998). To evaluate if pollen removal and if pollen loads deposited on the stigmas varied between treatments (flowers exposed to nocturnal visitors vs. flowers exposed to diurnal visitors), we tested for differences in the number of pollen grains among the sampling times using Generalized Linear Models with Poisson distribution (which is adequate for count data) with the lme4 package in software R (R Core Team 2018). Significance was

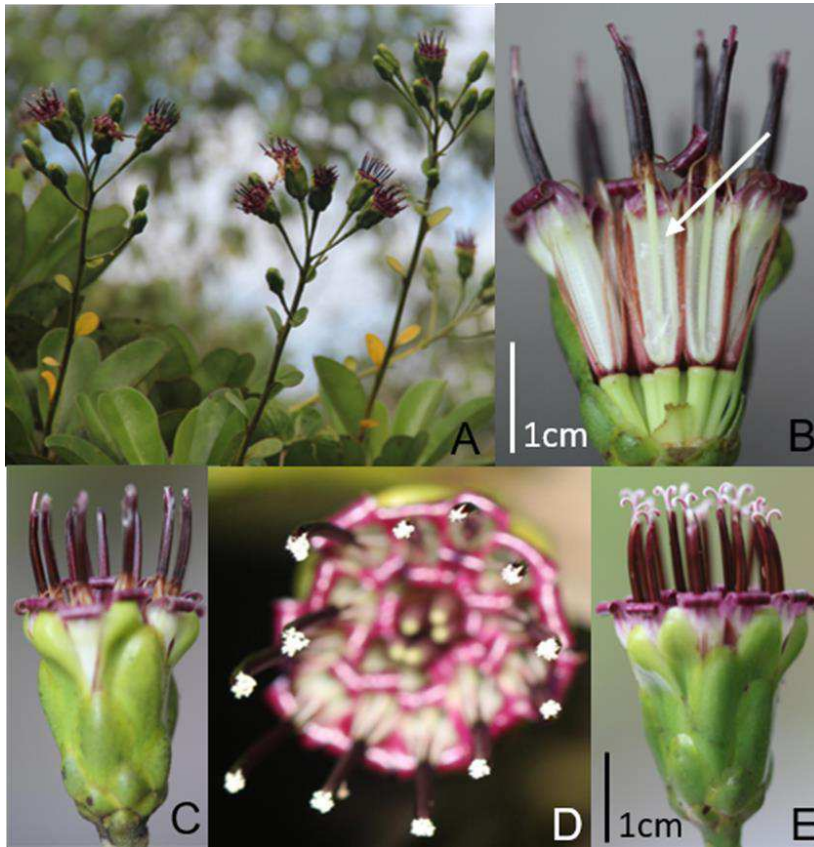


tested using a type 2 ANOVA using the package car and post-hoc Tukey in software R (R Core Team 2018).

## Results

*Gongylolepis martiana* individuals presented 1-15 flowering branches, each with 5-10 terminal and solitary heads on erect to inclined peduncles (Figure 1). The rayless scented heads displayed green 27-30 mm long involucre and approximately 16 zygomorphic and perfect disc florets with curled vinaceous corolla lobes, rigid dark anther tubes, and styles exerted 10-12 mm above the lobes. Florets in the same head opened sequentially from peripheral to central rings and each individual floret lasted for three to four days. Flower opening started at dusk, around 1800 h, and pollen was exposed 30 min after on the top of polliniferous styles (Figure 1). Pollen was available during the first day of anthesis (male phase), and stigma surfaces were only exposed on the third day (female phase) (Figure 1). Stigmatic receptivity started around 2100 h and stigmas remained receptive until the end of the following day when florets wilted. Around 80% of the heads presented all open florets in the same sexual phase, but 20% of them opened new florets (i.e. male phase florets) when the other florets were already at the female phase. Individual plants concomitantly presented male and female heads. Among flowers that were bagged overnight, mean nectar volume accumulated per floret was 8.7  $\mu\text{l}$  (SD = 3.98, N = 30) and mean sugar concentration was 15% (SD = 4.25, N = 30). Among florets exposed to the visitors, mean nectar was 3.3  $\mu\text{l}$  (SD = 3.09, N = 30) with sugar concentration of 10% (SD = 2.03, N = 30). We recorded 98 flowering individuals in the study area, and 788 heads with open florets in one night (mean = 8.04 heads  $\text{ind}^{-1}$ , SD = 5.0899). Each head showed nearly eight open florets, which provided an estimated volume of 69.6  $\mu\text{l}$  of nectar accumulated per night  $\text{plant}^{-1}$ , and at least 109,690  $\mu\text{l}$

of nectar or 16.45 g of sugar from the population of *G. martiana* in one night. This estimate is conservative as we did not account for nectar replenishment.



**Figure 1.** Flowers of *Gongylolepis martiana* (Asteraceae) in the Amazon, Brazil. (A) Panicles; (B) Flower tubes with nectar (arrow); (C) Closed stigmas during the male phase; (D) Pollen exposition on polliniferous styles; (E) Opened stigmas during the female phase.

Visitors of *G. martiana* flowers included bats, hummingbirds, bees, moths, wasps, and cockroaches (Table 1). Nocturnal visitors were mainly nectar-feeding bats, which contacted polliniferous styles and stigmas with their heads or necks (Figure 2A). Based on the known regional bat fauna, morphology and visiting behavior, the visitors were presumably *Glossophaga soricina* or *Lonchophylla thomasi* (Phyllostomidae). Bats visited one or two heads per plant through fast approaches (1-2 s) and returned to the same plants in 20-30 min intervals. In general, all pollen was removed after the first visit by bats. Other nocturnal visitors were Sphingidae and Erebidae moths and the bee *Megalopta* sp. (Halictidae), but they

were very rare ( $\leq 4$  visits each) and visibly removed very small amounts of pollen. *Ascalapha odorata* (Erebidae) remained on the same head for more than 15 min, probing every floret. It is unlikely that the moths contacted polliniferous styles or stigmas when visiting *G. martiana* flowers. Among diurnal visitors, hummingbirds visited the flowers for nectar and all bees visited flowers exclusively for pollen; bees were never recorded visiting female phase florets. Hummingbirds contacted the sexual floral parts with their bills or eventually with their chins, and moved among different plants, but some individuals defended territories in patches with abundant *G. martiana* flowers.

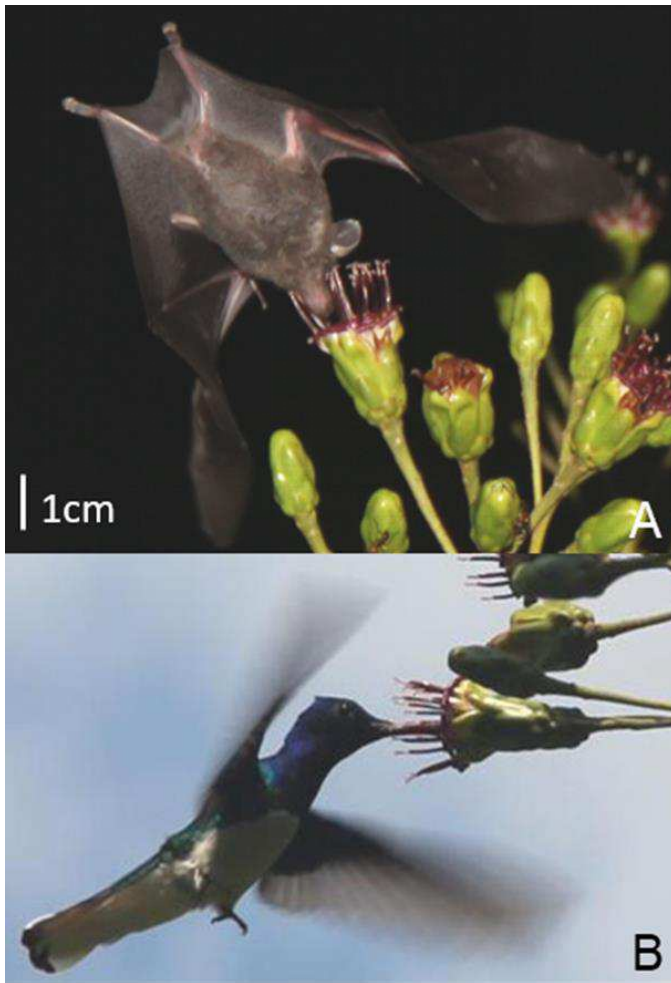
**Table 1.** Floral visitors of *Gongylolepis martiana* (Asteraceae) recorded through direct observations in an Amazonian white-sand forest, Brazil.

Daytime period	Visitor	Resource explored	Observation time (h)	Visitation frequency <sup>1</sup> (N head <sup>-1</sup> h <sup>-1</sup> )
Nocturnal	Nectarivorous bats <sup>2</sup> (Chiroptera, Phyllostomidae)	Nectar	4	5
	<i>Ascalapha odorata</i> (Lepidoptera, Erebidae)	Nectar	4	2
	<i>Perigonia</i> sp. (Lepidoptera, Sphingidae)	Nectar	4	1
	<i>Megalopta</i> sp. (Hymenoptera, Halictidae)	Pollen	4	2
	Unidentified cockroach (Blattaria, Blattidae)	Nectar	4	2
Diurnal	Hummingbirds <sup>3</sup> (Apodiformes, Trochilidae)	Nectar	12	21
	<i>Tetragona</i> sp. (Hymenoptera, Apidae)	Pollen	12	>50
	Unidentified wasp (Hymenoptera, Vespidae)	?	12	3

<sup>1</sup> Based on the period of records for nocturnal (4 h, from 1800 to 2200 h) and diurnal (12 h, from 0600 to 1800 h) visitors.

<sup>2</sup> *Glossophaga soricina* or *Lonchophylla thomasi*.

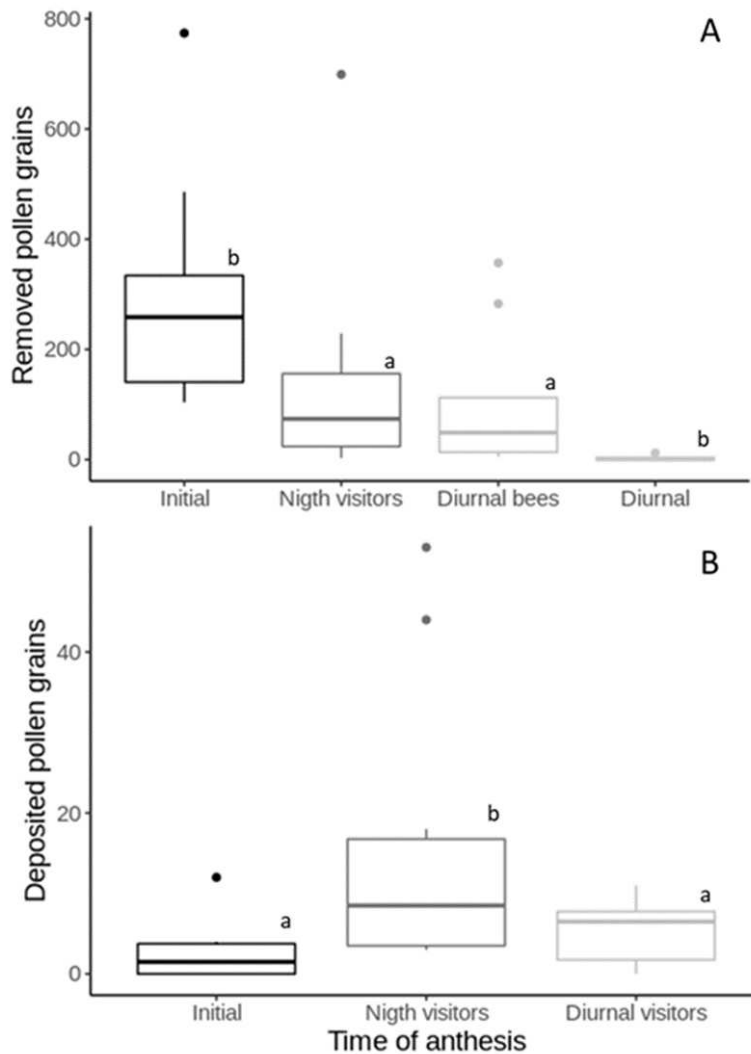
<sup>3</sup> *Florisuga mellivora* and one unidentified species.



**Figure 2.** Visitors of *Gongylolepis martiana* (Asteraceae) in the Amazon, Brazil. (A) nectar-feeding bat (likely a *Lonchophylla thomasi* or *Glossophaga soricina* individual, based on morphology and the local bat fauna); (B) *Florisuga mellivora* (Trochilidae).

All florets had their pollen removed within 24 hours after exposition at the beginning of the night (Figure 3a). Most of the male phase flowers exposed only to nocturnal visitors (presumably bats) had all pollen removed during the night, although some had large amounts of pollen the following morning represented by the large standard deviation ( $\bar{x} = 133$ ,  $SD = 211,7$ ). In male phase flowers exposed only to diurnal visitors, we recorded a reduction of pollen availability shortly after removing bags at dawn, when diurnal bees activity peaked collecting pollen ( $\bar{x} = 92,8$   $SD = 119, 8$  pollen grains left). These bees remained collecting up to around 1000h in the morning. At the end of the day there were virtually no pollen grains left on the polliniferous stigma ( $\bar{x} = 3,5$   $SD = 4,2$ ). In addition, the number of pollen grains

deposited on the stigmas was four times greater for female phase flowers exposed only to nocturnal visitors ( $\bar{x} = 23,5$  SD = 30,07) versus flowers exposed only do diurnal visitors ( $\bar{x} = 5,2$  SD = 3,5) ( $F_{2:27} = 6.370$ ;  $p = 0.02$ , Figure 3B).



**Figure 3.** Number of pollen grains removed from polliniferous styles (A) and deposited on the stigmatic surfaces (B) in *Gongylolepis martiana* (Asteraceae) flowers at different times throughout the anthesis in the Amazon, Brazil. Distinct letters indicate statistically significant differences. The only significant difference was in pollen deposition (B) with was higher at 12h than at 0h or 24h ( $p = 0.02$ , post-hoc Tukey).

## Discussion

Visitors and floral traits of *Gongylolepis martiana*, along with patterns of pollen removal from flowers and deposition on stigmas, strongly support that nectarivorous bats are the main pollinators of this species, corroborating Vogel's (2015) hypothesis of bat pollination in Asteraceae and for the genus *Gongylolepis*. This finding increases the diversity of pollinators documented in this family. Moreover, our results suggest that even cases of highly conserved architecture flowers, such as those of Asteraceae flowers, can accommodate diverse pollination systems. Compared to entomophilous Asteraceae (Torres & Galetto 2002; Hipólito *et al.* 2013), *G. martiana* presents larger tubular corollas with higher volumes of more diluted nectar, as expected for vertebrate pollination systems (Vogel 2015). In addition, the presence of odour and discreet green involucres with white and dark lilac flowers in *G. martiana* contrast the odour less and bright red/yellow heads reported for bird-pollinated Asteraceae (Buzato *et al.* 2000; Vogel 2015).

Nocturnal visitors (mainly nectar-feeding bats) removed and deposited higher amounts of pollen in comparison with the other visitors of *G. martiana*, and they returned to flowers at regular intervals, indicating their typical trap-lining foraging strategy (Sazima *et al.* 1999; Fleming *et al.* 2009). Hummingbirds frequently visited *G. martiana* flowers, however, pollen loads on stigmas of diurnal visitors were significantly lower in comparison with nocturnal visitors. We hypothesize that the weak contribution of diurnal visitors may be related to the fact that bees removed massive amounts of pollen at dawn. Thus, bees may exhibit two antagonistic roles: pollen theft (as they did not visit female phase flowers) and reduced pollen available for effective transportation by hummingbirds. The initial pollen exposure and stigma receptivity starting in the early evening is behind a priority effect: bat visits markedly reduced pollen availability, which could have diminished the potential pollination role of diurnal visitors. A similar "night-to-day" priority effect was also reported for the bat-pollinated bromeliad *Tillandsia macropetala* (Aguilar-Rodríguez *et al.* 2014). Furthermore, diurnal bees

arrived in the flowers before hummingbirds, which may have aggravated the priority effects in *G. martiana* at our study site. The behavior of small bees collecting high quantities of pollen with negative effect on plant reproduction has already been demonstrated in other pollination systems (Koski *et al.* 2018). This scenario of low pollen quantity combined with high removal by diurnal bees suggests that escaping daytime pollen thieves could have been an evolutionary force toward pollination by nocturnal animals (Botes *et al.* 2009; Hargreaves *et al.* 2012). *Gongylolepis martiana* flower traits do not prevent hummingbirds from being potential pollinators, and the long anthesis duration with nectar by day and night resembles some cases of pollination systems that are truly adjusted for both hummingbirds and nectar-feeding bats (Buzato *et al.* 1994; Muchhala *et al.* 2009). Our findings for *G. martiana* could represent an evolutionary shift from bird to bat pollination, with the time when each sexual phase begins being a crucial trait toward bat pollination, along with floral colors and the presence of odour (Aguilar-Rodríguez *et al.* 2014; Vogel 2015).

The long duration of anthesis with pronounced dichogamy, as we found for *G. martiana* flowers, has been reported for other Asteraceae (e.g., Hipólito *et al.* 2013), although is unusual among bat-pollinated plants (Fleming *et al.* 2009). The marked separation of sexual phases seems to be important for an Asteraceae species pollinated by bats, because bats would likely promote self-pollination in small and clustered florets in *G. martiana* if those heads presented both sexual phases concomitantly. Bats are costly pollinators in comparison with anthophilous insects because they require higher rewards and more robust flowers (Faegri & van der Pijl 1979). On the other hand, bats can travel longer distances between conspecific plants, promoting outcrossing (Cronk & Ojeda 2008; Fleming *et al.* 2009). Although individual plants of *G. martiana* offer little resources compared to other bat-pollinated plants, the species is densely concentrated in the campina white-sand forest, creating islands of habitat scattered throughout the vast Amazon rainforest. Patchy and dense populations of *G.*

*martiana* can compensate for the low nectar offered by individual plants and favour bat foraging over different flowering individuals, likely increasing the attraction for bats and cross-pollination among *G. martiana* flowers. Based on the daily energy intake of *Glossophaga soricina* (~ 50 kJ d<sup>-1</sup>; Voigt, 2003), the accumulated nectar estimated from our population of *G. martiana* could supply the total daily demand of 5.5 bat individuals, *i.e.*, about 18 flowering *G. martiana* plants could supply all the energy demanded by one bat overnight.

Overall, our results confirm pollination by bats in Asteraceae and support the chiropterophilous traits described by Vogel (2015) for this family. For instance, *Gongylolepis oblancoolata*, *G. benthamiana*, and *G. huachamacari* have floral traits similar to *G. martiana* and occur in dense patches in sclerophyllous and shrubby highland vegetation (Pruski 1989; Vogel 2015). The hypothetical evolutionary scenarios of bat pollination in Asteraceae require phylogenetic studies that consider the characteristics of flowers and the lineages with apparently chiropterophilous species. Finally, our findings support the use of floral traits to form testable hypotheses to study the diversity of pollinators in Asteraceae.

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### **References**

Adeney J.M., Christensen N.L., Vicentini A., Cohn-Haft M. (2016) White-sand Ecosystems in Amazonia. *Biotropica* **48**:7–23. [online] URL: <http://doi.wiley.com/10.1111/btp.12293>



- Aguilar-Rodríguez P.A., MacSwiney G. M.C., Krömer T., García-Franco J.G., Knauer A., Kessler M. (2014) First record of bat-pollination in the species-rich genus *Tillandsia* (Bromeliaceae). *Annals of Botany* **113**:1047–1055. [online] URL: <https://academic.oup.com/aob/article-lookup/doi/10.1093/aob/mcu031>
- Botes C., Johnson S.D., Cowling R.M. (2009) The Birds and the Bees: Using Selective Exclusion to Identify Effective Pollinators of African Tree Aloes. *International Journal of Plant Sciences* **170**:151–156. [online] URL: <http://www.journals.uchicago.edu/doi/10.1086/595291>
- Buzato S., Sazima M., Sazama I. (1994) Pollination of three species of *Abutilon* (Malvaceae) intermediate between bat and hummingbird flower syndromes. *Flora* **189**:327–334. [online] URL: <https://www.sciencedirect.com/science/article/pii/S0367253017306138> (accessed 19 December 2019).
- Buzato S., Sazima M., Sazima I. (2000) Hummingbird-Pollinated Floras at Three Atlantic Forest Sites1. *Biotropica* **32**:824–841. [online] URL: <http://doi.wiley.com/10.1111/j.1744-7429.2000.tb00621.x>
- Cronk Q., Ojeda I. (2008) Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany* **59**:715–727. [online] URL: <https://academic.oup.com/jxb/article-lookup/doi/10.1093/jxb/ern009>
- Dafni A., Maués M.M. (1998) A rapid and simple procedure to determine stigma receptivity. *Sexual Plant Reproduction* **11**:177–180. [online] URL: <http://link.springer.com/10.1007/s004970050138>
- Dargas J.H.F., Chaves S.R., Fischer E. (2016) Pollination of lark daisy on roadsides declines as traffic speed increases along an Amazonian highway (N. Vereecken, Ed.). *Plant Biology* **18**:542–544. [online] URL: <http://doi.wiley.com/10.1111/plb.12437>
- Demarchi L.O., Scudeller V.V., Moura L.C., Lopes A., Piedade M.T.F. (2019) Logging impact on Amazonian white-sand forests: perspectives from a sustainable development reserve. *Acta Amazonica* **49**:316–323. [online] URL: [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S0044-59672019000400316&tlng=en](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0044-59672019000400316&tlng=en)
- Faegri K., Pijl L.V.D.. (1979) *The principles of pollination ecology*. New York: Pergamon Press **64**:6–7.
- Fleming T.H., Geiselman C., Kress W.J. (2009) The evolution of bat pollination: A phylogenetic perspective. *Annals of Botany* **104**:1017–1043. [online] URL: <https://academic.oup.com/aob/article-lookup/doi/10.1093/aob/mcp197> (accessed 9

- December 2019).
- Hargreaves A.L., Harder L.D., Johnson S.D. (2012) Floral traits mediate the vulnerability of aloes to pollen theft and inefficient pollination by bees. *Annals of Botany* **109**:761–772. [online] URL: <https://academic.oup.com/aob/article-lookup/doi/10.1093/aob/mcr324> (accessed 21 December 2019).
- Hipólito J., Roque N., Galetto L., Viana B.F., Kevan P.G. (2013) The pollination biology of *Pseudostiffia kingii* H. Rob. (Asteraceae), a rare endemic Brazilian species with uniflorous capitula. *Brazilian Journal of Botany* **36**:247–254. [online] URL: <http://link.springer.com/10.1007/s40415-013-0023-4>
- Hoverman J.T., Hoyer B.J., Johnson P.T.J. (2013) Does timing matter? How priority effects influence the outcome of parasite interactions within hosts. *Oecologia* **173**:1471–1480. [online] URL: <http://link.springer.com/10.1007/s00442-013-2692-x> (accessed 6 November 2019).
- Kearns C.A., Inoué D.W. (1993) Techniques for pollination biologists. *University press of Colorado*.
- Koski M.H., Ison J.L., Padilla A., Pham A.Q., Galloway L.F. (2018) Linking pollinator efficiency to patterns of pollen limitation: small bees exploit the plant–pollinator mutualism. *Proceedings of the Royal Society B: Biological Sciences* **285**:20180635. [online] URL: <https://royalsocietypublishing.org/doi/10.1098/rspb.2018.0635> (accessed 9 June 2020).
- Martín-Forés I., Acosta-Gallo B., Castro I., de Miguel J.M., del Pozo A., Casado M.A. (2018) The invasiveness of *Hypochoeris glabra* (Asteraceae): Responses in morphological and reproductive traits for exotic populations (H. Auge, Ed.). *PLOS ONE* **13**:e0198849. [online] URL: <https://dx.plos.org/10.1371/journal.pone.0198849>
- Missagia C.C.C., Verçoza F.C., Alves M.A. (2014) Reproductive phenology and sharing of floral resource among hummingbirds (Trochilidae) in inflorescences of *Dahlstedtia pinnata* (Benth.) Malme. (Fabaceae) in the Atlantic forest. *Anais da Academia Brasileira de Ciências* **86**:1693–1702. [online] URL: [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S0001-37652014000401693&lng=en&tlng=en](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0001-37652014000401693&lng=en&tlng=en)
- Muchhala N., Caiza A., Vizuete J.C., Thomson J.D. (2009) A generalized pollination system in the tropics: bats, birds and *Aphelandra acanthus*. *Annals of Botany* **103**:1481–1487. [online] URL: <https://academic.oup.com/aob/article-lookup/doi/10.1093/aob/mcn260> (accessed 19 December 2019).

- Prieto-Benítez S., Dötterl S., Giménez-Benavides L. (2016) Circadian rhythm of a *Silene* species favours nocturnal pollination and constrains diurnal visitation. *Annals of Botany* **118**:907–918. [online] URL: <https://academic.oup.com/aob/article-lookup/doi/10.1093/aob/mcw136>
- Pruski J.F. (1989) Compositae of the Guayana Highland-II. Novelties in *Gongylolepis* and *Stenopadus* (Mutisieae). *Annals of the Missouri Botanical Garden* **76**:993. [online] URL: <https://www.jstor.org/stable/2399688?origin=crossref>
- R Core Team (2018) R: A Language and Environment for Statistical Computing. [online] URL: <https://www.r-project.org/>
- Rasmussen N.L., Van Allen B.G., Rudolf V.H.W. (2014) Linking phenological shifts to species interactions through size-mediated priority effects (L. Persson, Ed.). *Journal of Animal Ecology* **83**:1206–1215. [online] URL: <http://doi.wiley.com/10.1111/1365-2656.12203> (accessed 6 November 2019).
- Rosas-Guerrero V., Aguilar R., Martén-Rodríguez S., Ashworth L., Lopezaraiza-Mikel M., Bastida J.M., Quesada M. (2014) A quantitative review of pollination syndromes: Do floral traits predict effective pollinators? (R. Irwin, Ed.). *Ecology Letters* **17**:388–400. [online] URL: <http://doi.wiley.com/10.1111/ele.12224> (accessed 9 December 2019).
- Sazima M., Buzato S., Sazima I. (1999) Bat-pollinated Flower Assemblages and Bat Visitors at Two Atlantic Forest Sites in Brazil. *Annals of Botany* **83**:705–712. [online] URL: <https://academic.oup.com/aob/article-lookup/doi/10.1006/anbo.1999.0876>
- Torres C., Galetto L. (2002) Are Nectar Sugar Composition and Corolla Tube Length Related to the Diversity of Insects that Visit Asteraceae Flowers? *Plant Biology* **4**:360–366. [online] URL: <http://doi.wiley.com/10.1055/s-2002-32326>.
- Van der Niet T., Johnson S.D. (2012) Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology & Evolution* **27**:353–361.
- Voigt, C.C. (2003) Reproductive energetics of the nectar-feeding bat *Glossophaga soricina* (Phyllostomidae). *J Comp Physiol B* **173**, 79–85. <https://doi.org/10.1007/s00360-002-0316-6>
- Vogel S. (2015) Vertebrate pollination in Compositae: Floral syndromes and field observations. *Stapfia* **103**:5–26.
- Ye Z.-M., Jin X.-F., Wang Q.-F., Yang C.-F., Inouye D.W. (2017) Nectar replenishment maintains the neutral effects of nectar robbing on female reproductive success of *Salvia przewalskii* (Lamiaceae), a plant pollinated and robbed by bumble bees. *Annals of Botany* **119**:1053–1059. [online] URL: <https://academic.oup.com/aob/article->

lookup/doi/10.1093/aob/mcw285

### **Considerações Finais**

Estudar a biologia da polinização é forma importante de compreender os processos evolutivos e ecológicos que produziram e mantêm a biodiversidade. Tanto as Angiospermas quanto os polinizadores que interagem com elas, têm papéis que vão além dessas interações. Portanto, no capítulo 1 demonstramos que as interações entre planta-polinizador são flexíveis com relação a perspectiva da adequação entre as características das flores e a importância de um polinizador. Além disso, os beija-flores são importantes mesmo para plantas que não têm características típicas de plantas visitadas preferencialmente por eles. Em ambientes como os Campos Rupestres essa flexibilidade pode ser maior por conta das variações sazonais. Portanto, mesmo a importância dos beija-flores é contexto-depende, portanto pode ser maior ou menor dependendo das condições do momento do estudo.

No capítulo 2 foi registrada pela primeira vez a ocorrência de morcegos polinizando flores de Asteraceae. Também demonstramos a importância da ocorrência das interações com relação ao espaço, mas principalmente ao tempo. Dada a ordem de chegada nas flores - as abelhas chegam antes dos beija-flores - os beija-flores não foram capazes de atuar como polinizadores mesmo tendo comportamento e morfologia compatíveis com essa função. As Asteraceae têm uma série de características que as distinguem das demais plantas, como a deposição secundária de pólen e a organização floral em capítulos. É também a família de plantas com maior número de espécies descobertas, por si só essa informação já torna essa família de plantas importante para estudos evolutivos. Ao considerar que essa interação ocorreu na Amazônia, também ressaltamos que um dos cartões postais da América do Sul têm muito espaço para pesquisas e descobertas que podem vir a ser importantes para a humanidade.

Aqui reforçamos que a aplicabilidade do conceito de síndromes de polinização depende do contexto. Ao prever os polinizadores com base neste conceito, tornam-se importante estudos de biologia reprodutiva para verificar como os sistemas funcionam efetivamente. Uma ressalva importante é que os sistemas de polinização são sistemas complexos que envolvem mais que apenas os interagentes, e que as características das flores influenciam não apenas na existência da interação, mais também a qualidade. Ambos Amazônia e os Campos Rupestre do Cerrado, embora mega diversos, são pouco estudados quanto a polinização, sendo este um dos poucos trabalhos com exclusão de polinizadores nessas áreas. Estudos como este que envolve a exclusão de visitantes são experimentos importantes para conhecimento da contribuição dos

visitantes florais e dos polinizadores. Essa importância aumenta em flores com sistemas mistos ou generalistas onde, a priori, a contribuição de um grupo de polinizadores possa parecer menor ou inexistente. Espera-se que mais estudos possam ser feitos nessas áreas, baseados nesse presente trabalho.

Finalmente, é importante ressaltar que a existência de uma universidade como a Universidade Federal dos Vales do Jequitinhonha e Mucuri em uma região distante dos grandes centros urbanos incentiva o desenvolvimento de pesquisas voltadas ao entendimento de ecossistemas locais como os Campo Rupestre e a história natural e ecologia das espécies que habitam este ambiente. Dessa forma, essas universidades constituem uma importante fonte de avanços para o campo da biologia teórica, além de também pode ser importante para o desenvolvimento da região. Contudo, não podemos deixar de colocar que ambos os ambientes, amazônico e Campos Rupestre (Cerrado), assim como qualquer outro bioma ou ambiente de vegetação brasileiro vem sendo depredado. O Brasil passa por um momento de intensa destruição da natureza, onde muitas vezes colocamos a natureza na posição de inimiga do desenvolvimento, fato exacerbado com as queimadas que ocorreram e ocorrem no Brasil durante o ano de 2020. Além de dever, também deveríamos querer proteger esses ambientes que são essenciais para a existência da humanidade e uma imensa fonte de informação sobre o planeta terra e as transformações que nele ocorreram durante os mais 4 bilhões de anos de existência, conforme pode ser visto nos resultados que aqui apresentamos.

