An efficient low-cost method for sampling floral visitors on down-facing flowers

Um método eficiente e de baixo custo para amostragem de visitantes florais em flores voltadas para baixo

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Abstract: Angiosperm flowers have morphological attributes that influence their interaction with pollinators, one of which is floral orientation. Pendent (down-facing) flowers are considered a protective mechanism against the loss of floral resources, such as pollen, nectar, and resin. This study proposes a new method for collecting floral visitors in *Clusia grandiflora* (Clusiaceae), a resinous species with downward-facing flowers. The Entomological Rod method consists of using a light stem (such as a buriti petiole or bamboo) pierced in the middle with a 50 ml Falcon tube attached, allowing direct capture of insects in the flowers without the need for pruning or sudden movements. Tested in Lençóis Maranhenses National Park (Maranhão, Brazil), the low-cost method demonstrated ease of application in difficult-to-access down-facing flowers.

Keywords: Pollination. Meliponini. Euglossini. Active search. Entomological rod.

Resumo: As flores de angiospermas possuem atributos morfológicos que influenciam sua interação com polinizadores, sendo um deles a orientação floral. Flores pendentes (voltadas para baixo) são consideradas um mecanismo de proteção contra a perda de recursos florais, como pólen, néctar e resina. Este estudo propõe um novo método para coleta de visitantes florais em *Clusia grandiflora* (Clusiaceae), uma espécie resinosa com flores voltadas para baixo. O método de haste entomológica consiste na utilização de uma haste leve (como pecíolo de buriti ou bambu) perfurada no meio com um tubo Falcon de 50 ml acoplado, permitindo a captura direta de insetos nas flores sem a necessidade de podas ou movimentos bruscos. Testado no Parque Nacional dos Lençóis Maranhenses (Maranhão, Brasil), o método de baixo custo demonstrou facilidade de aplicação em flores voltadas para baixo, de difícil acesso.

Palavras-chave: Polinização. Meliponini. Euglossini. Busca ativa. Haste entomológica.

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INTRODUCTION

Angiosperm flowers have various physical attributes, such as size, shape, texture, color patterns, spatial orientation, and volatile compounds. Initially, from an evolutionary point of view, these structures had the function of protecting the reproductive organs. Over time, however, they also acquired attractive functions, offering diverse floral resources for pollinators (Lima, 2000; Dafni et al., 2005), thereby becoming an important mechanism for reproductive isolation and the promotion of diversity (Grant, 1949).

Simple changes in floral morphology, such as flower orientation relative to the branch axis, can significantly influence reproductive success. Flowers oriented downward, known as down-facing flowers (Tadey & Aizen, 2001; Wang et al., 2014), represent an effective protective mechanism against the depletion of floral resources such as pollen and nectar (Ushimaru & Hyodo, 2005). Several angiosperm taxa have flowers with a pendulous orientation and offer varied features. Among them, *Clusia grandiflora* Splitg. (Clusiaceae, Figures 1A and 1B) stands out. This species is even more peculiar because it also produces resin, an uncommon feature among angiosperms (Figure 1C), which is restricted to only five genera within the order Malpighiales, *Clusia L.*, *Chrysochlamys Poepp.*, *Tovomitopsis Planch. & Triana* (Clusiaceae), *Clusiella* Planch. & Triana (Calophyllaceae), and

Figure 1. A) Branch of *Clusia grandiflora* with flower in anthesis seen from below; B) staminate flower of *C. grandiflora* seen from above; C) staminate flower of *C. grandiflora* see from below; D) the first author under a specimen of *C. grandiflora* in a shrubby restinga in the study area. Photos: Lucas C. Marinho (2023).

Dalechampia Plum. ex L. (Euphorbiaceae) (Armbruster, 1984; Langenheim, 1990; De Oliveira et al., 1999; Lokvam & Braddock, 1999; Gustafsson & Bittrich, 2003; Ribeiro et al., 2020).

A distinctive feature of many *Clusia* species is the production of floral resin. This resource, considered atypical among flowering plants, is widely used by several bee species as raw material for nest construction, waterproofing, and sanitation (Armbruster, 1984). It is assumed that resin use helps reduce the activity of pathogenic agents within nests. The bee genera most frequently observed visiting these plants include *Euglossa*, *Eulaema*, *Melipona*, and *Trigona* (Armbruster, 1984; Rech et al., 2014). Moreover, as proposed by Gustafsson and Bittrich (2003), one may speculate on the relationship between the remarkable floral morphological diversity observed in resiniferous species and resin production, regarded as key innovation driving morphological diversification in *Clusia* flowers.

Given the complexity of ecological interactions between invertebrates and plants, it is es sential to standardize collection protocols that combine representativeness, efficiency, and low cost (Araújo, 2007). For adequate faunal sampling, the simultaneous use of complementary methods capable of encompassing the diversity of habits of the species involved is recommended (Reis & Kraemer, 2013). Faunistic sampling methods can be classified as active, when the researcher actively searches for animals, or passive, when data are collected using traps or other field devices (Krug & Alves-dos-Santos, 2008). Although passive methods have already been used in studies aimed at identifying and characterizing pollinators and flower visitors (Vrdoljak & Samways, 2012), active searching remains the most common procedure, especially through sensing in flowers using entomological nets (Sakagami et al., 1967; McCravy, 2018).

Although widely recommended, active search with nets, when applied to bee surveys, requires the combination of different capture methods. This combination increases the reliability of data analysis and improves the determination of the ecological importance of the collected fauna (Krug & Alves-dos-Santos, 2008). However, specific methods for capturing bees in downward-facing flowers are still lacking, representing a gap in studies of the reproductive biology of these plants. In this context, and seeking to expand the diversity of methods for collecting invertebrates in plant reproductive biology studies, we propose a new method for capturing bees in flowers with a descending floral orientation.

MATERIAL AND METHODS

SPECIES CHARACTERIZATION

In Brazil, the genus *Clusia* is predominantly found in the Amazon and Atlantic Forest (Nascimento Jr. & Alencar, 2025). Among the morphological traits of these plants, variation in size, the arrangement of floral parts, and coloration are notable (Marinho et al., 2020). Some species have an androecium with stamens fused in different forms, as well as the presence of staminodes, which are usually responsible for providing resin as a resource to pollinators. In most cases, it is possible to observe a division between resiniferous staminodes and fertile stamens, in which either pollen or resin can be produced (Gustafsson et al., 2007; Marinho et al., 2020).

Clusia grandiflora flowers have a rotund corolla composed of five large, free, white petals with pink bases, arranged radially in an actinomorphic pattern (Figures 1B and 1C). Staminate plants have stamens connected at the base, forming a crown-like structure around the resin-secreting staminodes. Pistillate flowers are characterized by a ring of staminodes surrounding the ovary, where resin production is lower than that of staminate plants. In addition, they emit a sweet and mild fragrance, perceptible when the flowers are open (Bittrich & Amaral, 1996; Bittrich, 1997; Fernandes, 2007). Regarding size, flowers of staminate plants have an average diameter of 6.46 cm, while flowers of pistillate plants average 5.94 cm — data obtained along this project for the local plants.

OBSERVATION AREA AND CONTEXT

The entomological rod method is proposed based on a study carried out in shrubby *restinga* vegetation (*sensu* Rodrigues et al., 2019) in a natural state of conservation (Figure 1D), located in the buffer zone of the Lençóis Maranhenses National Park (PNLM, acronym in Portuguese), in the state of Maranhão, Brazil (Figure 2).

We carried out three field expeditions to the study area in May, July, and August 2023 to monitor the flowering period of *C. grandiflora*. The populations had

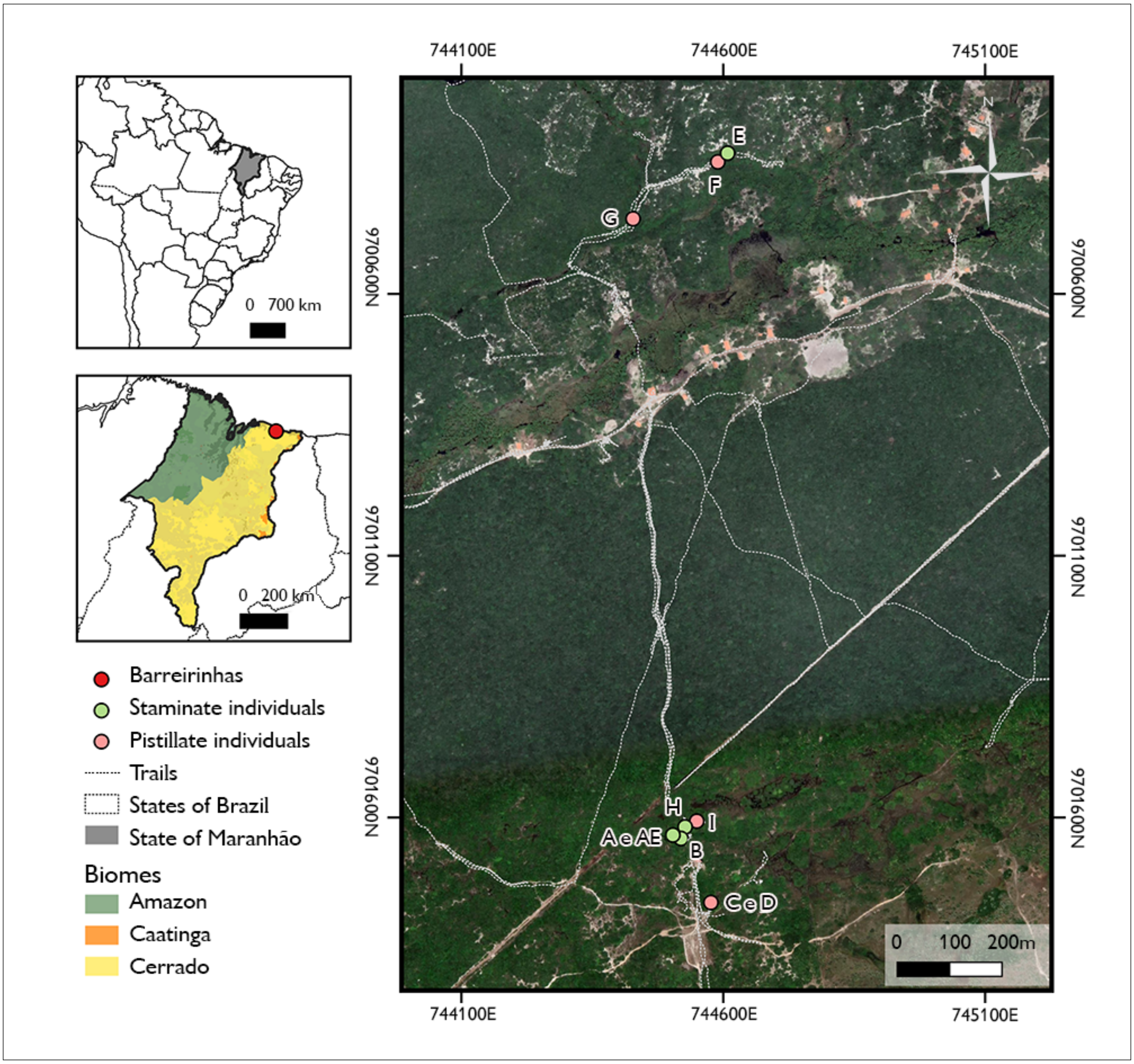

Figure 2. Map of the collection points in the natural restinga near the Atoleiro community in Barreirinhas, Maranhão, Brazil. The points indicate the locations of the monitored *Clusia grandiflora* individuals, identified by uppercase letters corresponding to the codes used on the labels. Green points represent staminate individuals, while pink points represent pistillate individuals. In the upper left corner, a map showing the location of the state of Maranhão in South America, along with a map indicating the municipality of Barreirinhas within the state (red point) and the distribution of biomes in Maranhão. Map: David B. Muniz (2023).

been previously monitored by collaborators living in the study area, who notified us of the beginning of flowering. Each field expedition consisted of two consecutive days of observations, where both techniques were used: entomological nets and rod. Each observation was conducted by four researchers (two per observed *Clusia* individual) from 5:40 AM to 5:40 PM, totaling 12 hours per day, in accordance with the region's solar cycle. To standardize data collection, each pair of observers recorded the time of each visit and the behavior of the flower visitor. Every three consecutive hours of observation, the pairs rotated the plants they were monitoring, ensuring that each pair of researchers observed all individuals for at least six hours. Pollen and resin collection behavior, the frequency of *C. grandiflora* flower visitors, and the phenological and morphological characteristics of the species were all recorded.

The flower visitors of *C. grandiflora* were initially captured with entomological nets and euthanized/stored in 50 ml Falcon tubes containing 70% alcohol. However, considering the morphology and orientation of the flowers (Figure 1B), which face the ground and are often arranged between branches (Figure 1A), this would require reverse movements (bottom-up), making handling the net, which is typically applied to upward-facing flowers, difficult. Furthermore, it would be necessary to prune branches to allow for closer contact, which would compromise the natural environment and potentially alter the number and behavior of flower visitors.

Due to the difficulty and low efficiency of using entomological nets to capture bee visitors of *C. grandiflora*, we developed a device consisting of a long, lightweight rod made from a buriti (*Mauritia flexuosa* L.f.) petiole, with a 50 ml Falcon tube attached to its upper end, previously perforated for easy attachment (Figures 3 and 4). The buriti petiole was chosen based on its local availability, as the species is abundant in the region. However, the method also allows for replacement with bamboo rods, as illustrated in Figure 3, or other lightweight materials. This setup allows safe access to the flowers and accurate collection of visitors.

RESULTS AND DISCUSSION

During the study, 84 flower visitors were collected, the vast majority (66 specimens) were captured using the entomological rod, while only 18 were collected using entomological nets. Of the 84 individuals recorded on *C. grandiflora* flowers, most were observed on staminate flowers and belonged to the order Hymenoptera. Other orders, such as Coleoptera and Hemiptera, were also recorded but in smaller numbers. At present, it is not possible to assess the efficiency of the method by comparing our results with other studies on floral visitors of *Clusia* — for example, Bittrich and Amaral (1996, 1997) and Kaminski and Absy (2006) — since those works did not report the abundance of individuals, but only species composition.

From the order Hymenoptera, 78 bees (Apidae) and one ant (Formicidae) were recorded visiting *C. grandiflora* flowers. From the other orders, five individuals were observed, two of which belonged to the subfamily Eumolpinae (Coleoptera: Chrysomelidae) and moved across all parts of the pistillate plant, including the flowers. Although the proposed method, entomological rod, has not been tested with insect orders other than Hymenoptera, Coleoptera, and Hemiptera, in principle it can be applied to any specimen that can be captured in a liquid medium, such as Diptera, Orthoptera, Araneae, among others. However, for certain groups, such as mosquitoes (Diptera: Culicidae), butterflies, and moths (Lepidoptera), the method is not suitable, since preservation in liquid medium is not recommended. These insects are quite fragile, bearing long setae and scales that are easily damaged or lost in such media. The absence of these structures often makes taxonomic identification unfeasible (Almeida et al., 2024).

Our results show that most bee species captured using the entomological rod method are small to medium in size (2-15 mm). This outcome is directly related to the

Figure 3. Graphic diagram showing the application of the entomological rod method in the capturing of floral visitors of *Clusia grandiflora*. Illustration: Victor E. Cantanheide (2025).

fact that the tribe Meliponini was the most abundant and diverse in our sampling. However, the method is not limited to bees of this size range, as a specimen of *Eulaema* (Euglossini), a genus whose species can exceed 30 mm in length (large-sized), was also collected with this method. If the researcher considers the opening diameter of a 50 ml Falcon tube (30 mm) insufficient for the target specimens of their study, the method can be easily adapted to larger diameter containers, thereby better accommodating the body size of the floral visitors in question.

The new method allowed for quick and accurate captures, directly on the stamens or resin pit of *C. grandiflora*, with minimal disturbance to the environment and avoiding sudden movements that could facilitate the insect's escape. Among the main advantages of the methods include its low cost, accessible materials, and efficiency in difficult-to-reach environments where nets are impractical. The method reduces specimen loss and optimizes collection time.

To prevent the dissolution of resin collected and transported in the corbiculae of bees, caused by the

Figure 4. Entomological rod developed for capturing floral visitors to *Clusia grandiflora* flowers. Photos: Lucas C. Marinho (2023).

alcohol present in the Falcon tube, a factor that may compromise chemical analyses or the quantification of resin transported by pollinators, especially in resiniferous species, we propose replacing alcohol with water containing a neutral detergent. This solution reduces the surface tension of the water and allows the insects to be retained without dissolving the resin.

CONCLUSION

The method proposed here proved to be effective for collecting floral visitors from down-facing flowers, especially in conditions where netting is difficult to access. Its application can complement traditional sampling methods, contributing to studies of pollination ecology and plant reproductive biology in natural environments.

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REFERENCES

Almeida, L. M., Marinoni, L., & Clarkson, B. (2024). Coleta, montagem, preservação e métodos para estudos. In J. A. Rafael, G. A. R. Melo, C. J. B. Carvalho, S. Casari, & R. Constantino (Eds.), *Insetos do Brasil: diversidade e taxonomia* (2. ed., pp. 120-136). Instituto Nacional de Pesquisas da Amazônia. https://doi.org/10.61818/56330464c07

Araújo, J. S. (2007). *Métodos de amostragem, influência dos fatores ambientais e guia de identificação dos escorpiões (Chelicerata, Scorpiones) da Reserva Ducke, Manaus, Amazonas, Brasil* [Master thesis, Instituto Nacional de Pesquisas da Amazônia]. https://repositorio.inpa.gov.br/handle/1/38817

Armbruster, W. S. (1984). The role of resin in angiosperm pollination: ecological and chemical considerations. *American Journal of Botany*, *71*(8), 1149-1160. https://doi.org/10.2307/2443391

Bittrich, V., & Amaral, M. C. E. (1996). Flower morphology and pollination biology of some *Clusia* species from the Gran Sabana (Venezuela). *Kew Bulletin*, *51*(4), 681-694. https://doi.org/10.2307/41119722

Bittrich, V. (1997). O gênero *Clusia* (Guttiferae) para a floricultura. *Revista Brasileira de Horticultura Ornamental*, *3*(1), 13-19. https://ornamentalhorticulture.com.br/rbho/article/view/131

Bittrich, V., & Amaral, M. C. E. (1997). Floral biology of some *Clusia* species from Central Amazonia. *Kew Bulletin*, *52*(3), 617-635. https://doi.org/10.2307/4110290

Dafni, A., Kevan, P. G., & Husband, B. C. (2005). *Practical pollination biology*. Enviroquest Ltd.

De Oliveira, C. M. A., Porto, A. L. M., Bittrich, V., & Marsaioli, A. J. (1999). Two polyisoprenylated benzophenones from the floral resins of three *Clusia* species. *Phytochemistry*, *50*(6), 1073-1079. https://doi.org/10.1016/S0031-9422(98)00476-2

Fernandes, S. D. C. (2007). *Morfologia, anatomia, histoquímica e aspectos fisiológicos da lâmina foliar de espécies de Clusia (Clusiaceae)* [Dissertação de mestrado, Universidade de Brasília]. https://repositorio.unb.br/handle/10482/1571

Grant, V. (1949). Pollination systems as isolating mechanisms in angiosperms. *Evolution*, *3*(1), 82-97. https://doi.org/10.2307/2405454

Gustafsson, M. H. G., & Bittrich, V. (2003). Evolution of morphological diversity and resin secretion in flowers of *Clusia* (Clusiaceae): insights from ITS sequence variation. *Nordic Journal of Botany*, *22*(2), 183-203. https://doi.org/10.1111/j.1756-1051.2002.tb01364.x

Gustafsson, M. H. G., Winter, K., & Bittrich, V. (2007). Diversity, phylogeny and classification of *Clusia*. In U. Lüttge (Ed.), *Clusia — A woody Neotropical genus of remarkable plasticity and diversity* (pp. 95–116). Springer. http://dx.doi.org/10.1007/978-3-540-37243-1_7

Kaminski, A. C., & Absy, M. L. (2006). Bees visitors of three species of *Clusia* (Clusiaceae) flowers in C;entral Amazonia. *Acta Amazonica*, *36*(2), 259-264. https://doi.org/10.1590/S0044-59672006000200016

Krug, C., & Alves-dos-Santos, I. (2008). O uso de diferentes métodos para amostragem da fauna de abelhas (Hymenoptera: Apoidea), um estudo em floresta ombrófila mista em Santa Catarina. *Neotropical Entomology*, *37*(3), 265-278. https://doi.org/10.1590/S1519-566X2008000300005

Langenheim, J. H. (1990). Plant resins. *Scientific American*, *78*(1), 16-24. https://www.jstor.org/stable/29773859

Lima, C. (2000). *Flores e insetos: A origem da entomofilia e o sucesso das angiospermas* [Trabalho de conclusão de curso, UniCEUB]. https://repositorio.uniceub.br/jspui/handle/123456789/2386

Lokvam, J., & Braddock, J. F. (1999). Anti-bacterial function in the sexually dimorphic pollinator rewards of *Clusia grandiflora* (Clusiaceae). *Oecologia*, *119*(4), 534-540. https://doi.org/10.1007/s004420050816

Marinho, L. C., Alencar, A. C., & Nascimento Jr., J. E. (2020). *Clusia*: Carnose leaves and beautiful flowers. *Cactus and Succulent Journal*, *92*(4), 271-279. http://dx.doi.org/10.2985/015.092.0402

McCravy, K. W. (2018). A review of sampling and monitoring methods for beneficial arthropods in agroecosystems. *Insects*, *9*(4), 170. https://doi.org/10.3390/insects9040170

Nascimento Jr., J. E., & Alencar, A. C. (2025). *Clusia*. In *Flora e Funga do Brasil.* Jardim Botânico do Rio de Janeiro. https://floradobrasil.jbrj.gov.br/FB6830

Rech, A. R., Agostini, K., Oliveira, P. E., & Machado, I. C. (2014). *Biologia da polinização*. Editora Projeto Cultural.

Reis, A. S., & Kraemer, B. M. (2013). Fauna cavernícola terrestre: revisão bibliográfica dos métodos de coleta de invertebrados e vertebrados. In M. A. Rasteiro, & L. Morato (Orgs.), *Anais do 32º Congresso Brasileiro de Espeleologia* (pp. 99-107). Sociedade Brasileira de Espeleologia. http://www.cavernas.org.br/anais32cbe/32cbe_099-107.pdf

Ribeiro, M. M. J., Silva, K. M. M., Palavecino, L. A., Pinto, L. C., Ferreira, B. L. A., Lobão, A. Q., Castro, H. C., Montenegro, R. C., Barros, C. F., Joffily, A., Valverde, A. L., & Paiva, S. R. (2020). Anatomical, histochemical and biological studies of *Clusia grandiflora* Splitg. (Clusiaceae). *Brazilian Archives of Biology and Technology*, *63*, e20190674. https://doi.org/10.1590/1678-4324-2020190674

Rodrigues, M. L., Mota, N. F. O., Viana, P. L., Koch, A. K., & Secco, R. S. (2019). Vascular flora of Lençóis Maranhenses National Park, Maranhão State, Brazil: checklist, floristic affinities and phytophysiognomies of restingas in the municipality of Barreirinhas. *Acta Botanica Brasilica*, *33*(3), 498-516. https://doi.org/10.1590/0102-33062018abb0421

Sakagami, S. F., Laroca, S., & Moure, J. S. M. (1967). Wild bee biocenoticas in São José dos Pinhais (PR), south Brazil, preliminary report. *Journal of the Faculty of Science, Hokkaido University Series VI Zoology*, *16*(2), 253-291. http://hdl.handle.net/2115/27447

Tadey, M., & Aizen, M. A. (2001). Why do flowers of a hummingbird pollinated mistletoe face down? *Functional Ecology*, *15*(6), 782-790. https://doi.org/10.1046/j.0269-8463.2001.00580.x

Ushimaru, A., & Hyodo, F. (2005). Why do bilaterally symmetrical flowers orient vertically? Flower orientation influences pollinator landing behavior. *Evolutionary Ecology Research*, *7*, 151-160.

Vrdoljak, S. M., & Samways, M. J. (2012). Optimising coloured pan traps to survey flower visiting insects. *Journal of Insect Conservation*, *16*, 345-354. https://doi.org/10.1007/s10841-011-9420-9

Wang, H., Xiao, C.-L., Gituru, R. W., Xiong, Z., Yu, D., Guo, Y.-H., & Yang, C.-F. (2014). Change of floral orientation affects pollinator diversity and their relative importance in an alpine plant with generalized pollination system, *Geranium refractum* (Geraniaceae). *Plant Ecology*, *215*, 1211-1219. https://doi.org/10.1007/s11258-014-0379-y

AUTHORS' CONTRIBUTION

H. C. Andrade contributed to project administration and writing (original draft, review, and editing), formal analysis, investigation, and data curation; M. E. S. David-Silva contributed to data collection and writing (review); D. B. Muniz contributed to data collection, formal analysis, investigation, project administration and writing (original draft, review, and editing); and L. C. Marinho contributed to supervision, funding acquisition, project administration and writing (original draft, review, and editing).