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SHORT COMMUNICATION

Interaction between bees and the flowers of *Clethra scabra* Person in the Araucaria Forest in Rio Grande do Sul State, Brazil

Sídia Witter[1]*, Larissa Bueno Ambrosini[1], Mardiore Pinheiro[2], Patrícia Nunes-Silva[3], Bruna Barros Dias Santos[4]

Abstract - *Clethra scabra* is a plant native to the Araucaria Forest of the Rio Grande do Sul State and provides food resources to flower visitors, however, it is threatened with extinction, and little is known about this interaction. We aimed to characterize the floral biology of *C. scabra* and identify its potential pollinators. The study was conducted in a 250 ha fragment of predominately Mixed Ombrophilous Forest in Cambará do Sul, RS. The number of flowers in inflorescences, the anthesis stages, the frequency of flower visitors and their behavior was recorded. The flowers of *C. scabra* are grouped in panicle-type inflorescences measuring between seven and 27 cm in length. The mean number of flowers/inflorescence was 64. Three phases were characterized and, in all of them, the presence of nectar was registered. Stingless bees (Apidae: Meliponini) are the potential pollinators of *C. scabra*, representing 80 % of the flower visitors. The important interaction between Meliponini and *C. scabra* in areas of Araucaria Forest in Campos de Cima da Serra in Rio Grande do Sul, observed in this study, provides a knowledge base for the conservation of these species, development of strategies for the sustainable management of biodiversity in the region, especially for endangered species such as *Melipona* and *C. scabra*.

Keywords: Meliponini. Flower visitors. Carne-de-vaca. Floral biology. Araucaria Forest.

Interações entre abelhas e flores de *Clethra scabra* Person na Mata com Araucária no Rio Grande do Sul

Resumo - *Clethra scabra* é uma espécie nativa da Mata com Araucária, no Rio Grande do Sul, e disponibiliza recursos alimentares para visitantes florais, porém encontra-se ameaçada de extinção e pouco é conhecido sobre essas interações. Objetivou-se caracterizar a biologia floral de *C. scabra* e identificar seus potenciais polinizadores. O estudo foi conduzido em um fragmento de 250 ha com predomínio de Floresta Ombrófila Mista em Cambará do Sul. Foi registrado o número de flores nas inflorescências, os estágios da antese, a frequência dos visitantes florais e seu comportamento nas flores. As flores de *C. scabra* estão reunidas em inflorescências do tipo panícula com comprimento entre sete e 27 cm. O número médio de flores/inflorescência foi 64. Três fases de antese foram caracterizadas e, em todas elas, foi registrada a presença de néctar. Stingless bees (Apidae: Meliponini) são os potenciais polinizadores de *C. scabra* representando 80 % dos visitantes florais. A importante interação entre Meliponini e *C. scabra* em áreas de Floresta com Araucária nos Campos de Cima da Serra, no Rio Grande do Sul, observada neste estudo, fornece uma base de conhecimento para a conservação dessas espécies e o desenvolvimento de estratégias para o manejo sustentável da biodiversidade na região, especialmente para espécies ameaçadas de extinção como *Melipona* e *C. scabra*.

Palavras-chave: Meliponini. Visitantes florais. Carne-de-vaca. Biologia floral. Mata com Araucária.

[1]Departamento de Diagnóstico e Pesquisa Agropecuária, Secretaria de Agricultura, Pecuária, Desenvolvimento Sustentável e Irrigação, Porto Alegre, RS, Brasil. *Corresponding author: E-mail: sidia-witter@agricultura.rs.gov.br.
[2]Departamento de Botânica, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil.
[3]X-Reality and GeoInformatics Lab (Vizlab), Universidade do Vale do Rio dos Sinos, São Leopoldo, RS, Brasil.
[4]Graduanda do Bacharelado em Agronomia, Universidade Federal da Fronteira Sul, Cerro Largo, RS, Brasil.

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Brazil is the most biodiverse country of the world and is home to more than 20 % of the extant species. Its rich biodiversity is a source of resources for the country, not only through the ecosystem services provided, but also by the opportunities that its conservation, sustainable use and genetic heritage represent (Brasil, s.d.).

In the Southern region of Brazil, characterized by a temperate climate, the Mixed Ombrophilous Forest, also known as the Araucaria Forest, is found only in the Brazilian Southern Plateau and occurs in an ombrophilous climate, which mean temperature is 18°C, and during three to six months it is below 15 °C (IBGE, 2004).

Among the native plant species of the Araucaria Forest is *Clethra scabra* Person, Clethraceae which common name varies with geographic region, examples are "guaperê", "vassourão", among others, but in Rio Grande do Sul it is often known as "carne-de-vaca" (Carvalho, 2006). In Brazil, this species occurs in Goiás, Bahia, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina e Rio Grande do Sul States; in the domains of Caatinga (in high-altitude forests in Chapada Diamantina), Atlantic Forest (Dense Ombrophilous Forests, Mixed Ombrophilous Forests, Semideciduous State Forest, Restinga) and Cerrado (Forested Savannah and Cerradão) (Rossi, 1992; Perdiz; Giulietti; Oliveira, 2015; Romão; Kinoshita, s.d.), with the largest number of specimens recorded in São Paulo and Paraná (CRIA, s.d.).

Clethra scabra has a shrubby to arboreal habit, is evergreen to semi-deciduous, and can reach 30 m in height and 60 cm in DBH (diameter at breast height) (Rossi, 1992; Perdiz; Giulietti; Oliveira, 2015). The species blooms between December and February, and its flowers provide pollen and nectar to floral visitors (Backes; Irgang, 2002; Ramalho, 2004; Lopes, 2012).

The nectar from *C. scabra* flowers is used by stingless bees (Meliponini) and honeybees (*Apis mellifera* L.) to produce a honey of light color and unique flavor, known in the Araucaria Forest region of Rio Grande do Sul (RS) as "white honey" ("mel branco" in Portuguese; Radaeski; Bauermann, 2021; Witter *et al*., 2021). This product is associated with environment conservation, because the three stingless bee species (*Melipona schencki* Gribodo, 1893, *Melipona torrida* Friese, 1916 e *Melipona quadrifasciata quadrifasciata* Lepeletier, 1836) that produce it, as well as *C. scabra*, are threatened with extinction in the State (Witter *et al*., 2021; Rio Grande do Sul, 2014a, 2014b). Considering the importance of the interaction between bees and *C. scabra*, especially the threatened Meliponini species of RS, the inventory of floral-visiting bees and their interactions with this plant constitutes the first step to define strategies for the conservation of both, as well as of the ecosystem where they are native to. Thus, this study aimed to characterize the floral biology of *C. scabra* and identify potential pollinators of the species in an area of Araucaria Forest.

The study was conducted in a 250 ha fragment with a predominance of Araucaria Forest, located in the municipality of Cambará do Sul, in the Northeastern region of RS (29°02' S 50°08' W) called Campos de Cima da Serra, with altitudes between 980 and 1047 m. According to the revised Koppen Climate Classification (Alvares *et al*., 2013), the region presents a Cfb climate, without dry season and with mild summer temperatures. According to climate data collected at the National Institute of Meteorology - INMET station installed in the municipality (29°02'56.6" S 50°08'59.5" W), the average temperature ranges between 16 °C and 18 °C in the warmest months, with averages close to 11 °C in the winter months. Annual precipitation exceeds 1800 mm. The study area was located on a family farm where

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dairy cattle farming, beekeeping and agriculture were carried out. It consisted of small agricultural areas, forested areas with araucaria and high-altitude fields.

During the flowering period of *C. scabra* (January 2022), 43 inflorescences were randomly collected from five randomly selected branches (to collect the branches, the plant was divided into four parts called quadrants). In each inflorescence, the length of the floral axis was measured, the total number of flowers was counted, and the region of the inflorescence where floral anthesis begins was recorded. The stages of floral anthesis were characterized by describing the flowers (n = 80) collected in different inflorescences. The analysis of the flowers was performed with the aid of an *Olympus Tokio*® binocular stereomicroscope. Measurements of the corolla diameter of flowers of phases one and three (n = 40) were taken, 20 for each phase, with the aid of a digital caliper with a capacity of 0-150 mm/0-6. In addition, flower buds in pre-anthesis and all open flowers present in 30 inflorescences, fixed in 70 % alcohol, were analyzed.

Figure 1. Branch of *Clethra scabra* (Clethraceae) with panicle-type inflorescences. Note the variation in the length of the floral axis and in the number of flowers present in each inflorescence. Image: Fernando Dias.

Insect records were obtained through focal observation, over five consecutive days, from January 8 to 12, 2022. The records were made on the inner part of the canopy of one specimen of *C. scabra*, which was approximately six meters tall and located on the edge of the forest. Access to the flowers was made using a wooden ladder approximately seven meters long. The abundance of flower visitors was recorded throughout

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the day, from 8 am to 5 pm, for five minutes and with five-minute intervals between each new evaluation, totaling 15 h and 25 min of sampling. To minimize the counting of the same individual insect, at the end of each observation period, the branches of the plant were shaken. For the most abundant flower visitor species, behavioral aspects were recorded, such as the time spent on the inflorescence, the floral resource collected, the presence of pollen in the corbicula or scopa, and the anthesis stage of the flowers visited in each inflorescence. Behavior analysis was obtained on two consecutive days (January 11th and 12th 2022) by direct observation of the inflorescences between 9 am and 4 pm, totaling 84 bee records in 12 hours of evaluation.

The flowers of *C. scabra* are grouped in panicle-type inflorescences (compound raceme) with lengths between seven and 27 cm (15.96 ± 4.062; n = 43) (Fig. 1). The literature reports inflorescences with lengths between ten and 20 cm for this species (Ichaso; Guimarães, 1975; Rossi, 1989; Perdiz; Giulietti; Oliveira, 2015). The average number of flowers/inflorescence varied from 27 to 120 (64.44 ± 19.731; n = 43).

Of the total flowers analyzed in the inflorescences (n = 2,771), 93 % were in the bud or anthesis phase and only 7 % were senescent. The flowers in anthesis were in accordance with the description found in the literature, that is, they are hermaphrodites and have five white, concave petals with a fimbriated (fringed) apex, petals with adnate stamen, the stamens in number of ten are included (they do not exceed the apex of the corolla), the stigma is three-lobed and yellowish-white, and the ovary is tomentose and green (Rossi, 1989; Perdiz; Giulietti; Oliveira, 2015; Ramalho, 2004). In this study, it was observed that the flowers are fragrant and enter anthesis (opening of the corolla) from the base to the apex of the inflorescence axis, while in the same raceme it is possible to observe buds and flowers in different stages of anthesis, as well as senescent flowers. Thus, the first flowers to acquire characteristics of senescence (aging), that is, the wilted petals of brown coloration, are those at the base of the inflorescence. Of the senescent flowers examined, 38.46 % (n = 26) did not have anthers, while the rest had an incomplete number (less than ten) in a vertical arrangement, with dark, wrinkled and dry thecae. In 47.61 % of these flowers (n = 21), the apices of the stigmatic lobes acquired a dark coloration, and the remainder varied from light to dark.The characterization of floral structures in the bud stage and during anthesis are described below:

Flower buds – the buds in pre-anthesis have a corolla with white petals protrudes from the sepals in their upper half, closed or sometimes with a tiny opening at the apex, which is fimbriated by slightly golden trichomes (Fig. 2A). Ten stamens with indehiscent, light-yellow anthers, positioned between the ovary and the stigma and close to them. The anthers are extrorse (Fig. 3A), that is, with openings facing the external sides of the flower (Fig. 3A; Table 1). At this stage, the stigma is slightly wrinkled, dull and light in color. The presence of nectar was observed in 5 % of the buds (n = 20).

Phase one - characterized by the beginning of the opening of the flower petals, so that there is an increase in the opening at the apex of the corolla (diameter of the corolla opening = 3 mm x 3 mm) (n = 20), allowing the visualization of the reproductive organs (Fig. 3B). The apex of the petals presents slightly golden fimbriae. In this phase, the stamens begin to move away from the center of the flower, showing a greater distance of the anthers in relation to the pistil (compared to the position of the stamens in the flower bud). Anthers of light yellow coloration, dehiscent, with a longitudinal slit in

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each theca, mostly in the introrsa position and with apex and base of the anthers inverted (compared to the bud phase, Fig. 3A), due to the folding of the apex of the filaments towards the center of the flower (Fig. 3B; Table 1); anthers located slightly below the stigma or at the same height as it. Wrinkled and shiny stigma of light color. Nectar, accumulated in droplets deposited at the internal base of the stamens and on the ovary, was recorded in all flowers (n = 20) analyzed (Fig. 2C).

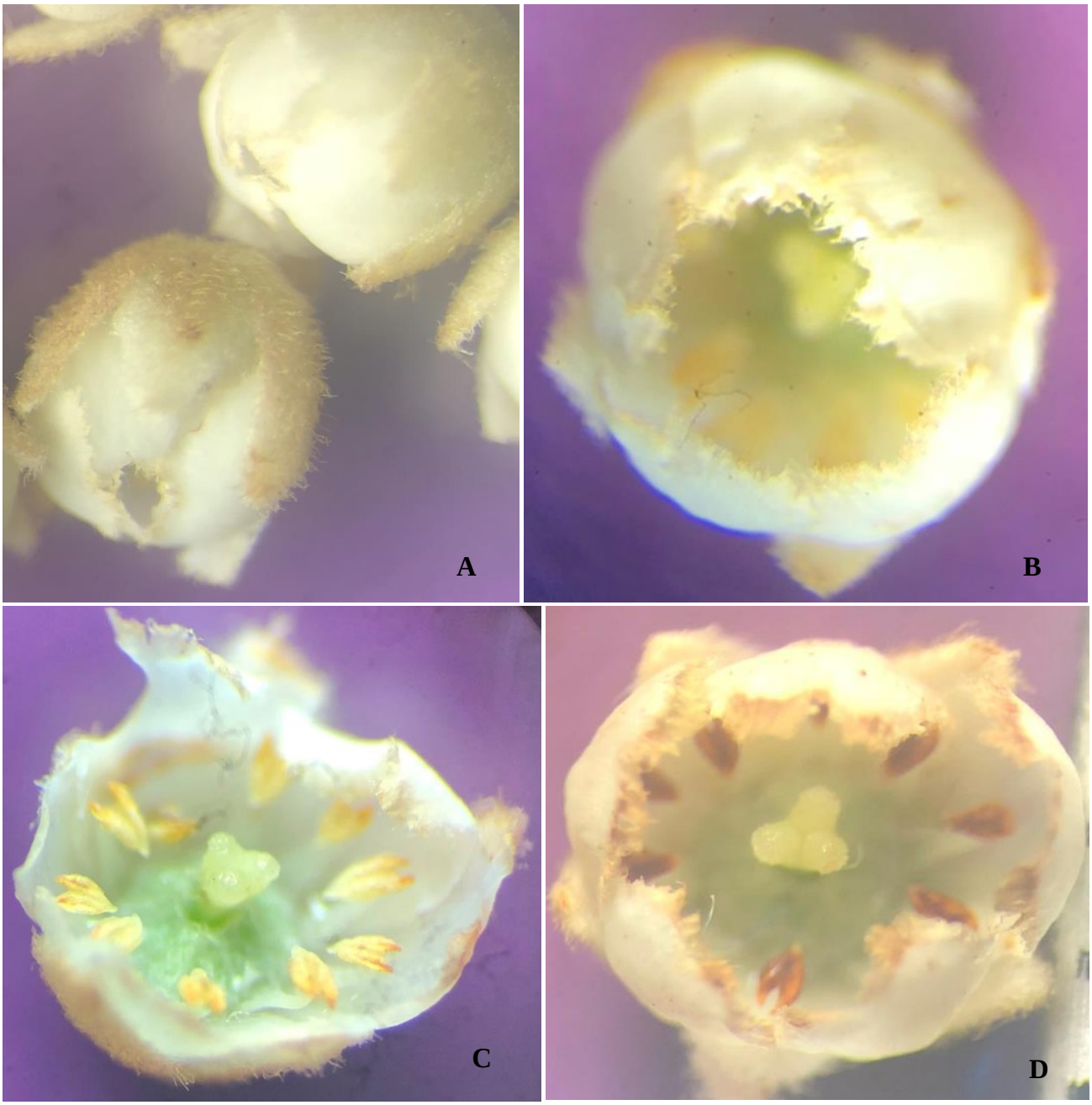

Figure 2. *Clethra scabra* (Clethraceae) flowers at different phases. A) Bud; B) Phase one; C) Phase two (petals are cut to expose the anthers and the pistil); D) Phase three. C and D: Dehiscence direction is introrse, that is toward to the pistil.

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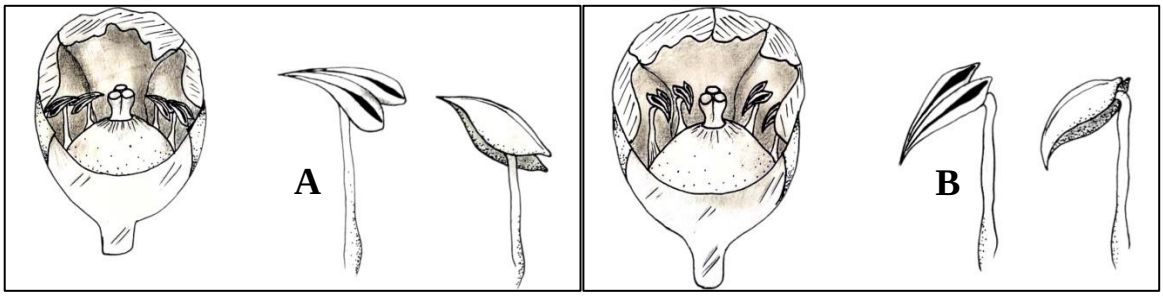

Figure 3. *Clethra scabra* (Clethraceae) flowers without some petals, showing the pistil and the position of the anthers. A) Filaments with extrorse horizontal anthers; B) Filaments with anthers tilted toward the center of the flower (introrse).

Phase two - in this phase, the corolla presented petals further away from the center in relation to phase one of anthesis, and directed towards the periphery, giving the flower a cup shape (corolla opening diameter = 4 mm x 3 mm) (n = 20). The petals have intense golden fimbriae. The stamens are further away from the pistil and at approximately the same height. The anthers become more yellowish and remain mostly in the introrse position, tilted with the apex downwards (Fig. 3B; Table 1). The stigma remains rough, shiny and with light color. The presence of nectar was also recorded in this phase.**Phase three** – flowers in this phase differ from phase two in that the petals have darker fimbriae, the stamens are even more separated from the pistil, and the anthers are dark and introrse (Fig. 3B). The beginning of the wrinkling of the thecae was observed in this phase (Fig. 2D; Table 1). The stigma remains with light color, wrinkled and shiny. The presence of nectar was also recorded at this stage.

A total of 2,973 flower visitors of *C. scabra* from the orders Hymenoptera, Diptera and small insects difficult to identify by focal observation, were counted (Table 2). Bees (Apiformes) of the families Halictidae and Apidae represented 93 % of this total. Other studies showed that bees from different families (Colletidae, Halictidae and Apidae) visit the flowers of *C. scabra* (Ramalho, 2004; Freitas; Sazima, 2006). In the present study, Meliponini corresponded to 80 % of the visiting bees, followed by *A. mellifera* L. (20 %) and only one individual of *Bombus pauloensis* Friese, 1912 and one specimen from the family Halictidae (Table 2). In the Atlantic Forest, where *C. scabra* is an higher vegetation strata species, the flowers were visited by ten species of Meliponini, which accounted for approximately 80 % of all foraging on the flowers (Ramalho, 2004). These bees, although present in both the lower and upper strata of the forest, are associated with the upper stratum (Ramalho, 2004).

The most abundant (highest number of individuals) Meliponini species observed in *C. scabra* flowers in Cambará do Sul was *Schwarziana quadripunctata* Lepeletier, 1836 (42 %), followed by *Melipona schencki* (27 %), *Plebeia* spp. (24 %) and *Melipona torrida* (7 %) (Table 2). In 2011, in the same study area, 144 native Meliponini nests were recorded on the ground and in different tree species. These nests belonged to seven Meliponini species, with 97 % of them belonging to *M. schencki* (in hollow tree trunks), *S. quadripunctata* (underground nests) and *Plebeia* spp. (in hollow tree trunks) (Lopes, 2012). This could explain the high percentage of Meliponini recorded as floral visitors of *C. scabra*. However, further studies are

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needed to understand this interaction, since other factors could explain the results obtained, such as the quantity and quality of available floral resources, floral attractants, such as smells, among others.

Table 1. Description of the flower structures' aspect of *Clethra scabra* in the bud phase and during anthesis.

	Bud	Phase 1	Phase 2	Phase 3
Petals	Closed or with a small aperture at the apex; fimbriae slightly golden.	Partially open, fimbriae at the apex slightly golden.	Open, fimbriae de brightly golden.	Open, fimbriae of dark color.
Stamens	Close to the ovary and pistil.	Further away from the ovary and pistil.	Far from the ovary and pistil.	Far from the ovary and pistil.
Anthers	Indehiscent, light-yellow, extrorse, below the stigma.	Dehiscent, light-yellow, introrse, below the stigma or almost at the same height.	Dehiscent, yellow, introrse, approximate height of the stigma.	Dehiscent, dark, beginning of wrinkling of thecae, introrse, approximate height of the stigma.
Stigma	Dull, light color and slightly wrinkled.	Bright, light color and wrinkled.	Bright, light color and wrinkled.	Bright, light color and wrinkled.
Nectar	Present in 5% of the flowers.	Present at the base of the stamens.	Present at the base of the stamens.	Present at the base of the stamens.

Table 2. Taxa and number of individuals of flower visitors observed on *Clethra scabra* flowers from 01/08/2022 to 01/12/2022 in Cambará do Sul, Rio Grande do Sul.

Order	Family	Tribe	Species	Number of individuals
Hymenoptera				
Apiformes				
	Halictidae			1
	Apidae	Apini	*Apis mellifera*	556
		Bombini	*Bombus pauloensis*	1
		Meliponini	*Melipona schencki*	589
			Melipona torrida	169
			Schwarziana quadripunctata	918
			Plebeia spp.	521
Espheciformes				93
Diptera				80
Other insects				45
Total				**2,973**

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Behavior of flower visitors – All observed bees visiting *C. scabra* always explored flowers of the same inflorescence before flying to another one, and only once alternating visits to two inflorescences was observed. Since each inflorescence of *C. scabra* has flowers at different stages of development, it was found that the flowers in phase one of anthesis, which corresponds to the beginning of the corolla opening, were least explored by the bees (13 %) and, of this percentage, visits by *M. schencki* accounted for 64 %. The low attractiveness of flowers in this phase of anthesis for bees could be attributed to their characteristics, because despite presenting nectar and dehiscent anthers, the petals are not yet fully open, making access to the flower's resources difficult. Flowers in phase three were the most visited by the bees (55 %), followed by flowers in phases two and three together. The flowers in these phases are characterized by the full opening of petals, which resulted in greater visibility and availability of nectar and pollen to flower visitors.

The time (seconds) spent on the flowers of an inflorescence varied among species: *Plebeia saiqui* (Friese, 1900) (66 ± 46.747, min = 21, max = 142) was the bee that spent the longest time foraging on the flowers, followed by *S. quadripunctata* (47.47 ± 36.624, min = 9, max = 124), *M. schencki* (22.28 ± 20.377, min = 8, max = 84), *A. mellifera* (19 ± 9.503, min = 6, max = 36) and *M. torrida* (17 ± 12.858, min = 5, max = 50).

The nectar was collected by bees during their visits to flowers through the opening of the corolla, where they inserted the proboscis into the droplets accumulated and deposited at the internal base of the stamens and on the ovary, thus making contact with the anthers and stigmas. However, smaller bee species (*Plebeia* spp.), often did not accessed the flower through the opening of the corolla, but rather through the external part of the apex of the petals. In this case, the bees leaned over and circled the flower to collect the nectar at the base of the stamens without contacting the stigma. In only three of the visits by all the bees to the flowers of *C. scabra*, grooming behavior of the body, legs and wings was observed, and no pollen was observed on the corbiculae of any of the species whose behavior was analyzed, thus, active pollen collection on this plant species by the bees was not observed.

The results of this study show that the flowers of *C. scabra* are very attractive to Meliponini, its main floral visitors and potential pollinators. Furthermore, it is one of the 16 species used by *M. schecki* as a nesting substrate in the Araucaria Forest (Lopes, 2012). *Clethra scabra* is among the four most abundant plant species in the study area and during the flowering period, between January and February, it offers a large supply of food for bees (Lopes, 2012). Nine species of meliponines with native nests were identified in the study area, and most of these species have great potential for honey production (Lopes, 2012). However, despite the great tradition of beekeeping in the region and the great potential for the development of meliponiculture, this activity is still little developed. According to Ambrosini *et al*. (2023) only 5 % of the 16,209 families involved in meliponiculture in Rio Grande do Sul, considering own consumption, commercial exploitation or just the presence of stingless bee nests on rural properties, are located in the study region.

In Cambará do Sul and neighboring municipalities, Meliponini have great potential for regional honey production, both the “white honey” of *C. scabra* (Witter *et al*., 2021) and the yellow honey of the native forest, and the practice of meliponiculture with native species of the region is possible both in rural and urban areas.

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The important interaction between Meliponini and *C. scabra* in areas of Araucaria Forest in Campos de Cima da Serra in Rio Grande do Sul, observed in this study, provides a knowledge base for the conservation of these species, development of strategies for the sustainable management of biodiversity in the region, especially for endangered species such as *Melipona* and *C. scabra*. Furthermore, it provides subsidies that allow the implementation of friendly practices and public policies to prevent the decline of their populations and their sustainable use in areas of natural occurrence.

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Conflict of Interests

The authors declare that the research was conducted in the absence of any potential conflicts of interest.

Ethical Statements

The authors confirm that the ethical guidelines adopted by the journal were followed by this work, and all authors agree with the submission, content and transfer of the publication rights of the article to the journal. They also declare that the work has not been previously published nor is it being considered for publication in another journal.

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ORCID

Sídia Witter
https://orcid.org/0000-0003-0621-9014

Larissa Bueno Ambrosini
https://orcid.org/0000-0002-1115-4748

Mardiore Pinheiro
https://orcid.org/0000-0001-8698-5856

Patrícia Nunes-Silva
https://orcid.org/0000-0002-9215-9822

Bruna Barros Dias Santos
https://orcid.org/0009-0006-5336-2955

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