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# Bee Diversity Associated with *Canavalia rosea* (Sw.) DC. (Fabaceae, Papilionoideae) in an Peri-Urban Patch of "Restinga"

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## Abstract

The Restinga is one of the physiognomies of the Atlantic Forest biome with the highest degree of anthropic impact, mainly due to disordered urbanization. The impacts generate habitat loss and threaten the animals and plants that inhabit this ecosystem. In the Restinga, the psammophilous vegetation have an important role in stabilizing the sandy substrate and maintaining the pollinator community. In this study, we analyzed the floral resources offered by *Canavalia rosea* to floral visitors between October 2019 and September 2020, in the Municipal Natural Park of *Barreto's Restinga*, in Macaé, Rio de Janeiro, Brazil. Sampling was carried out from 8:00 am to 4:00 pm, covering a 250-meter transect in the sandy cord, counting the total number of open flowers and collecting visiting bees. The morphology of *C. rosea* flowers was described to relate to the functional traits of visitors, related to pollen and nectar collection. The pollen load of visitors was analyzed to estimate the degree of specialization in relation to *C. rosea*. A total of 192 individuals of 14 bee species were captured. There was a significant relationship between the availability of floral resources, measured by the number of open flowers and the richness and abundance of bees. The greatest richness of bees was observed between January and March and the greatest abundance in January. The most frequent visiting genera in *C. rosea* were *Eufriesea*, *Centris* and *Xylocopa*. The greatest richness of pollen types was observed in *Eufriesea* species. Most *Xylocopa* individuals showed *C. rosea* pollen. In this study, the importance of *C. rosea* as a source of floral resources for fourteen species of solitary bees was demonstrated. The data generated increases the knowledge about the ecological patterns of *C. rosea* and its floral visitors and may contribute to the maintenance and expansion of *Restinga* areas in urban environments.

**Keywords:** Solitary Bees; Floral Ressources; Atlantic Forest; Urban Ecology; *Xylocopa*

## Introduction

The Atlantic Forest is a diverse biome, with a high rate of endemism and formed by different physiognomies [1,2]. Among them, the Restinga, a geologically recent environment, formed by processes of sandy sediment deposition during the Quaternary period [3,4]. Restinga areas, as they are adjacent to the coast, harbor vegetation constantly exposed to salinity, light and high temperatures, in addition to the scarcity of nutrients and water in the soil, mobility of the dunes and the

action of the winds [5].

The psammophilous vegetation in the Restinga occupies the sand area between the upper limit of high tide and the dunes, mitigating the action of erosive agents [6] and protecting the substrate from the action of the winds [4]. The genus *Canavalia*, one of the groups that compose the psammophilous vegetation, has a wide distribution on the coast of Brazil, with the species *Canavalia rosea* being one of the most abundant.

Individuals of *C. rosea* grow in a creeping way and form long mats along the sand cord of the beaches [7,8]. The long stolons, roots that attach to the substrate, and rapid growth contribute to soil stabilization, preventing erosion and reducing the direct impact of wind and wave action [9]. In addition, the flowers of *C. rosea* provide food resources such as pollen and nectar for several species of bees, mainly *Bombus*, *Epicharis*, *Centris* and *Xylocopa*, which are able to forage even under the strong winds [10-12].

Despite the importance of the ecosystem service of bees as pollinators of several plant species, several studies have reported worldwide declines in bee populations, the main causes being contamination by pesticides, fungi and habitats loss [13-19]. Habitat loss reduces the diversity and abundance of pollinating species [16], making resources such as nesting sites, food and other physical conditions scarce [20]. Estimates indicate that half of the Earth's surface has been altered by human actions, mainly agriculture and urbanization [21], causing loss of natural habitats for several species. Thus, natural habitats in urban environments are considered refuges [22] that can shelter part of the biodiversity, including communities of pollinators such as bees, butterflies and birds [23-25].

On the Brazilian Atlantic coast, Restingas are mainly threatened by tourism and disorderly human occupation [26,27]. Works aimed at surveying the fauna and flora in these environments are important for understanding the communities that inhabit the Restingas and can support conservation actions or management plans. Very little is known about the invertebrate species that live in the Restingas, and even less about the interactions in which they participate [28]. In this sense, this work is the first developed in the newly created Municipal Natural Park of Barreto's Restinga, the second park in Brazil in extension of Restinga in urban area. The general objective was to survey the floral visitors of *C. rosea*, to answer the following questions: (1) do the plants provide floral resources throughout the year? (2) does the richness of visiting bees vary throughout the year? and (3) how is the foraging behavior of bee species visiting *C. rosea*? Are these bees generalists or specialists in resource collection?.

## Material and Methods

### Study Area

The study was carried out in the Municipal Natural Park of Barreto's Restinga (22º20'22"S and 41º44'28"W) located in the municipality of Macaé, State of Rio de Janeiro (Figure 1). The park has an area of approximately 32 hectares, composed of shrubby vegetation and a sandy cordon with psammophilous vegetation. The climate of the region is tropical, classified as Aw according to [29]. The average temperature is 22.9°C and the average annual precipitation is 1126 mm, with the highest concentration of rainfall during the summer [30].

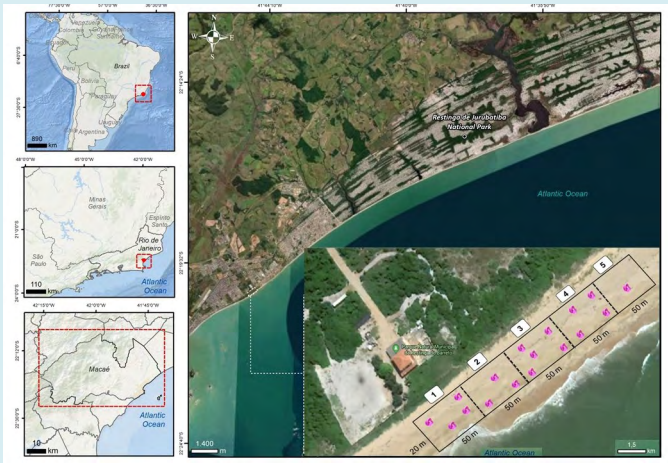


**Figure 1:** Geographic map of the study area showing the municipality of Macaé on the coast of Rio de Janeiro State, Brazil. The Municipal Natural Park of Barreto's Restinga (PNMRB) is separated from the Jurubatiba's Restinga National Park by an urban area. The white dashed indicates the sampling area and the plots within the 250 m transect in the sandy cord.

### Sampling

During one year, the study area was inspected fortnightly to assess whether *C. rosea* flowered. In the months with flowering, the collections were carried out monthly (from October 2019 to September 2020), between 08:00 am to 16:00 pm, along a 250 m transect divided into 5 plots (50 x 20 m) for better results in the flower count.

During the sampling, the total number of open flowers of *C. rosea* were quantified. Flowering duration was categorized according to [31] in short (< 1 month), intermediate (between 1 and 5 months) or long (> 5 consecutive months).

Floral visitors were actively collected with entomological nets by two collectors, who covered the entire transect in

the morning (8:00 to 12:00 h) and in the afternoon (13:00 to 16:00 h). The collectors remained for about 50 minutes in each sample plot, observing the behavior of the visitors in the flowers of *C. rosea* to describe the dynamics of insect-plant interaction. Floral visitors were classified as potential pollinators when their body size and behavior allowed the floral reproductive structures to come into contact with some part of the body, during visits to collect nectar or pollen, and as robbers when visitors did not contact the reproductive parts of the flower.

Captured bees were sacrificed in a killing chamber (ethyl acetate), mounted on entomological pins and identified to the highest possible taxonomic level based on the keys of [58] and [59]. Each individual was analyzed under an optical stereomicroscope to find the location of pollen grains. Subsequently, they were stored in the entomological collection of the Integrated Laboratory of Invertebrate Biology of the Institute of Biodiversity and Sustainability of the Federal University of Rio de Janeiro (NUPEM/UFRJ).

### Floral Biology and Specialization of Floral Visitors

The floral morphology data of *C. rosea* were obtained from the analysis of fresh flowers in an optical stereomicroscope, using specialized literature as a reference [32,33]. The duration of anthesis was determined by monitoring flowers from pre-anthesis to petal fall.

To assess whether the floral visitors had pollen types other than *C. rosea* pollen, the pollen grains present in the floral visitors' bodies were removed with the aid of a sterile needle. The pollen present at each site of the body was recorded as an individualized sample, from which fresh slides were made using gelatin [34]. The identification of pollen grains was made by comparison with a reference slide made with *C. rosea* pollen. Pollens from other plants were categorized into morphotypes in order to quantify the number of plants used by floral visitors. For analysis purposes, the pollen present in the three most representative genera were evaluated.

### Statistical Analysis

Differences were compared using the Student-Newman-Keuls post-hoc test and correlations were made using Pearson's coefficient (r). Data were processed and analyzed using Sigma Plot v. 2.1.

## Results

The flowering period of *Canavalia rosea* was considered long, flowering for six consecutive months, from October 2019 to March 2020. Flowering occurred during spring and summer, with the highest number of open flowers observed in November (n = 3155), January (n = 4365) and March (n = 3578) (Table 1).

| Species/month | Oct-19 | Nov-19 | Dec-20 | Jan-20 | Feb-20 | Mar-20 | Total |
|---|---|---|---|---|---|---|---|
| Apidae | | | | | | | |
| *Centris sp.* 1 | 1 | 5 | 5 | 4 | 4 | 2 | 21 |
| *Centris sp.* 2 | - | - | - | - | 1 | - | 1 |
| *Centris sp.* 3 | - | - | - | 1 | - | - | 1 |
| *Centris sp.* 4 | - | - | 1 | 4 | 1 | 2 | 8 |
| *Centris sp.* 5 | - | - | - | - | 2 | - | 2 |
| *Centris sp.* 6 | - | - | - | - | 2 | 1 | 3 |
| *Centris sp.* 7 | - | - | 1 | 12 | 7 | 8 | 28 |
| *Epicharis sp.* 1 | - | - | 1 | - | - | - | 1 |
| *Epicharis sp.* 2 | - | - | - | 1 | 5 | 2 | 8 |
| *Eufriesea dentilabris* | 1 | 40 | 19 | 12 | 3 | - | 75 |
| *Eulaema nigrita* | - | 1 | 0 | 1 | - | 8 | 10 |
| *Mesoplia sp.*1 | - | 1 | - | 2 | 1 | - | 4 |
| *Xylocopa sp.* 1 | 1 | - | - | 12 | 1 | 4 | 18 |
| *Xylocopa truxali* | - | - | - | 1 | 2 | 9 | 12 |
| **Total abundance per month** | 3 | 47 | 27 | 50 | 29 | 36 | 192 |
| **Bee richness per month** | 3 | 4 | 6 | 10 | 11 | 8 | 14 |
| **Number of open flowers** | 478 | 3155 | 1938 | 4365 | 2078 | 3578 | 15592 |
| **Open flowers/m2** | 1,0 | 6,3 | 3,9 | 8,7 | 4,2 | 7,2 | - |

**Table 1:** Richness and abundance of bees and number of open flowers of *C. rosea* collected during the sampling months (2019/2020) in the Municipal Natural Park of Barreto's Restinga, Macaé/Rio de Janeiro.

*C. rosea* presents a racemose inflorescence (Figure 2A&2B), with specialized flowers that present differentiated petals in a vexillum (the lowest petal of the corolla), an external pair called wings and an internal pair of petals united by the margin forming the keel (Figure 2C-2E). At the base of the vexillum, an integumented region with a greenish-white color is observed and the rest of the petal has a different color pattern (Figures 2B-D). The petals of the wing and keel present a spatulate to falciform morphology and an acute auricle present in each petal, with a leathery and dilated texture (Figure 2C). The reproductive organs remain included in the keel and wing (Figure 2D-2I), being exposed only during contact with the floral visitor (Figure 2J-K).

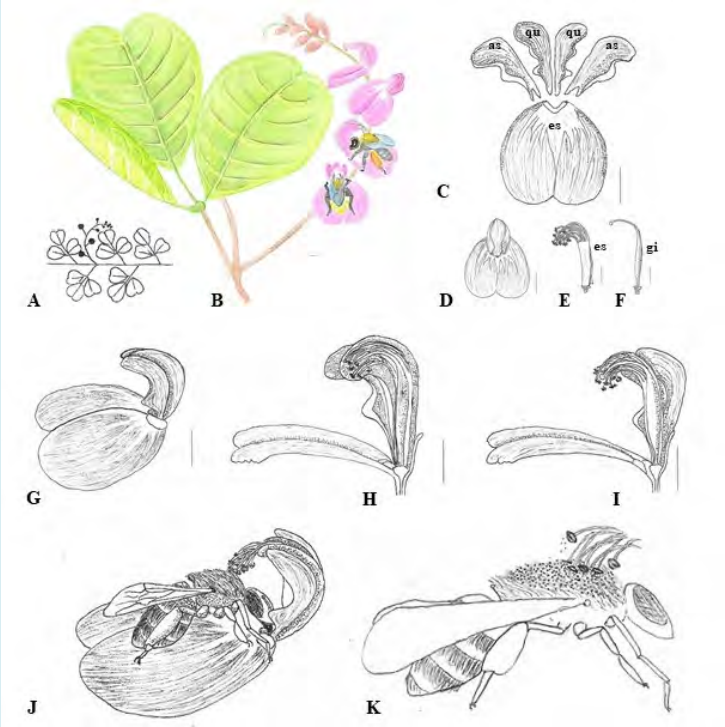


**Figure 2:** Floral morphology of *Canavalia rosea*. A-B. General characteristics of the plant: alternate phyllotaxis, trifoliate compound leaf, racemose inflorescence. *C. Corolla* petals: vexillum (es), wings (as), keel's petals (qu). D. Flower in front view. E. Monadelphic stamens(es) with free anthers. F. Gyneceus: ovary, style and stigma. G. Flower section in side view, wings and keel in natural position. H-I. Flower section in side view, wings and keel in displaced position with reproductive whorls exposed. J-K Out of scale diagram, in (J) flower in side view with floral visitor (model *Eulaema sp.*), wings and keel in displaced position due to bee visit and in (K) detail of pollen transfer from the anthers of *C. rosea* for the bee's dorsal scope. Bars: 1 cm.

Floral anthesis is diurnal, starting between 6 and 7 am, characterized by the distension and reflection of the vexillum. The duration of the flowers is short, approximately 9 hours. The floral senescence process is initially characterized by the drying of the vexillum, followed by the fall of the other petals.

During anthesis, bees were observed visiting the flowers to collect nectar. In field observations, the bees exhibited behavior of flying over the flower until they landed on the vexillum, from where they force the dilated region of the auricle of the wing and keel with their heads, pushing the petals back and guiding the tongue along the base of the vexillum until reach the nectary region (Figure 2J). The stamens and stigma, previously included and hidden, become apparent and touch the dorsal scopa of the bee, enabling the transfer of pollen (Figure 2K).

A total of 192 bees were collected, belonging to the Apidae family, distributed in 06 genera and 14 species (Table 1). The months with the highest richness of bee species were during the summer period, January (n = 10), February (n = 11) and March (n = 8) and the highest peaks of abundance were observed in spring, November (n = 47) and in summer, January (n = 50) (Table 1; Figure 3). It was observed from October to December a greater abundance of Eufriesea dentilabris and in the period from January to March an increase in the occurrence of Xylocopa sp (Figure 3). The *Centris* bees genus were observed throughout all flowering months (Figure 3).

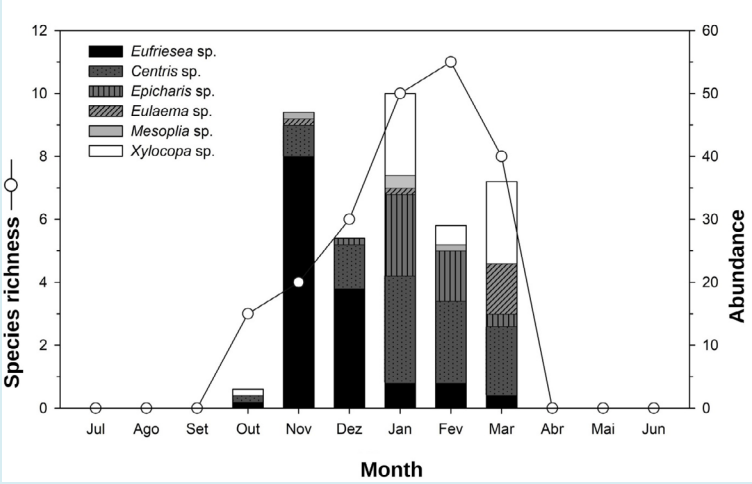


**Figure 3:** Richness and abundance of floral visiting bees of *Canavalia rosea* collected during the sampling months (2019/2020) in the Municipal Natural Park of Barreto's Restinga, Macaé, Rio de Janeiro State, Brazil.

Linear regression analysis showed that the richness ($F_{1;5} = 8.89$; $P < 0.031$; Figure 4) and the abundance ($F_{1;5} = 84.09$; $P < 0.0001$; Figure 4) of species of bees were positively related to the number of open flowers of *C. rosea*.

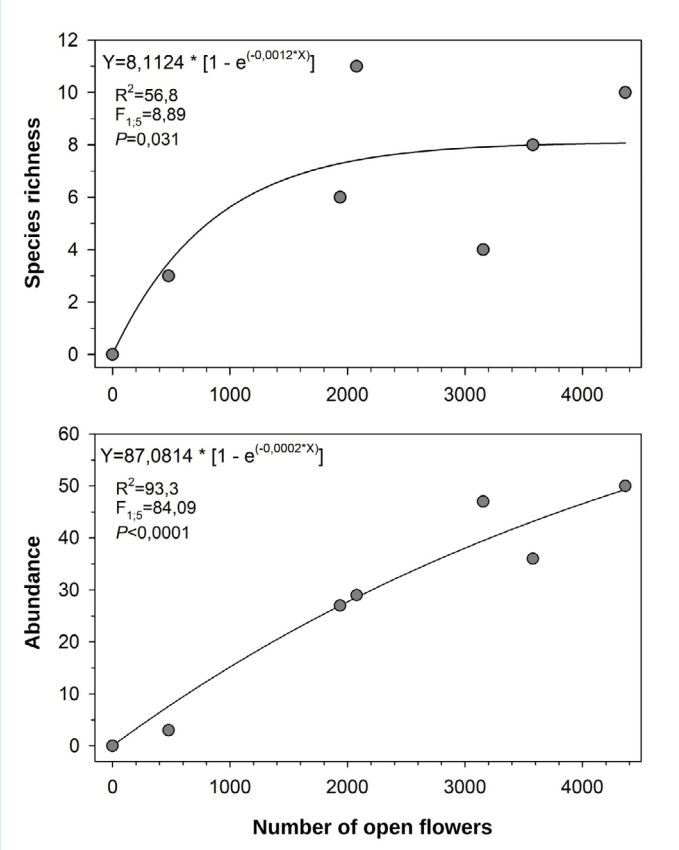


**Figure 4:** Linear regression analysis between the richness and abundance of bee species and availability of floral resources in *Canavalia rosea* (open flowers) collected during the sampling months (2019/2020) in the Municipal Natural Park of Barreto's Restinga, Macaé, Rio de Janeiro State, Brazil (Sigma Plot v. 2.1).

The analyzed pollen grains were collected from species of the genera *Centris*, *Eufriesea* and *Xylocopa*. The genus with the greatest range of pollen types collected was *Eufriesea* (n = 9; Figure 5). For the genus *Xylocopa*, pollen grains of *C. rosea* were found in 90% of the specimens and located in the dorsal scopa, with variation of one and two pollen types among the individuals sampled (Figure 5).

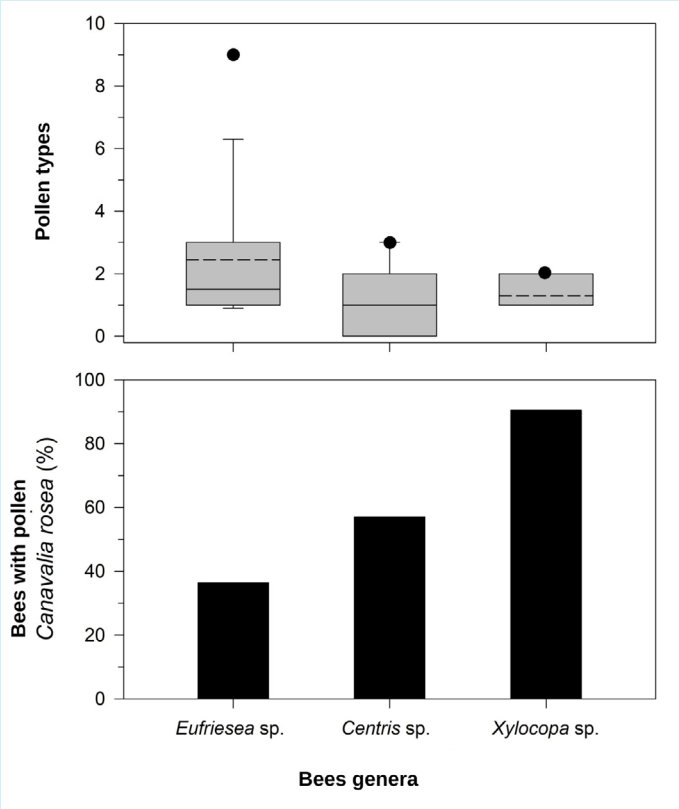


**Figure 5:** Linear regression analysis between the richness and abundance of bee species and availability of floral resources in *Canavalia rosea* (open flowers) collected during the sampling months (2019/2020) in the Municipal Natural Park of Barreto's Restinga, Macaé, Rio de Janeiro State, Brazil (Sigma Plot v. 2.1).

## Discussion

The flowering pattern of *C. rosea* is long and continuous, as described in other studies [33,35,36]. Flowering occurred in spring/summer, months with the highest averages of temperature and rainfall. In the Restinga of Maricá (RJ) a short flowering period was observed between the months of March and May [37], while in the state of Maranhão, despite a continuous flowering, a flowering peak was observed in the months August and September [11]. The different flowering patterns of *C. rosea* may be associated with regional and annual climatic differences, since precipitation and temperature can directly affect the phenology of plants [38-40].

The floral morphology of *C. rosea* has already been described in the literature, highlighting the presence of floral structures such as the vexillum that favors interaction with the visitor and maximizes pollination, thus configuring a specialized pollination system [33,35]. Also, the color pattern from the base to the apex of the vexillum possibly signals the location for the bees to land and serves as a guide to direct the visitor's tongue to the flower nectary [41,42].

Among the floral visitors of *C. rosea*, species of the genus *Xylocopa* are identified as the most abundant [33,43-46]. In the present study, of the 14 species sampled, the *Xylocopa* species were the most representative as possible pollinators, since 90% of the analyzed individuals had *C. rosea* pollen grains in the dorsal scopa. The high frequency of *Xylocopa* visiting *C. rosea* has already been demonstrated in Brazilian Restingas in Maranhão [44] and in Grumari, Rio de Janeiro [46]. This pattern has also been reported in other countries such as Costa Rica [43], Indonesia [33] and Puerto Rico [45]. The high frequency of *Xylocopa* was also observed visiting other species of *Canavalia*, such as *Canavalia brasiliensis* [47,48], *Canavalia ensiformis* [49], *Canavalia lineata* [50] and *Canavalia paraguariensis* [51].

The body size of *Xylocopa* species allows them to fly even in strong winds and withstand high radiation and temperatures in the Restinga [10-12]. In addition, the body of these bees is robust, which makes it possible to efficiently push the specialized petals of the flower, leading to the precise deposition of pollen in the bee's dorsal scope, while in smaller bees the pollen is dispersed throughout the body, which can cause a smaller pollination efficiency [44].

Some studies have already described the species of floral visitors, aspects of pollination and nutritional value of *C. rosea* fruits [9,52-54]. However, these studies did not evaluate the richness and abundance during the flowering months of *C. rosea*, and it was not possible to establish a comparison between the Restinga areas, considering the size of the fragments or the degree of human threats. Specimens of bees of the genera *Xylocopa*, *Centris* and *Eufriesea*, described in this study, are also among the frequent floral visitors in plant communities associated with the Restinga [55-57].

The results showed that the richness and abundance of bees associated with *C. rosea* responded positively to the availability of floral resources (open flowers). Bees are sensitive to environmental responses associated with resource offerings [58,62]. The effects of seasonality influence the availability of floral resources, such as the amount of flowers and the reward to visitors (nectar and pollen), changing the structure of the bee community [62-64].

Bees associated with *C. rosea*, in general, are solitary and their populations fluctuate throughout the year according to the species of flowering plants, with a positive relationship between the richness of plant and bee species [62,64-66]. Times of the year with a greater variety of resources can support species with different ecological needs [67], which may explain the greater richness in the summer months, where higher rainfall and temperature can result in a greater amount of available floral resources. The supply of nectar, for example, is considered an important factor in structuring the composition of the pollinator community [62]. The decline in the abundance of *Eufriesea dentilabris* in the flowering period of *C. rosea* from January to March may be related to the increase in the supply of floral resources by other plant species during the summer, which would explain the lower abundance of *C. rosea* in this period.

In the sandy cord *C. rosea* forms resource patches associated with other plant species, such as *Ipomoea* sp. [43; 44, 33), which offer attractive resources for bees. Analyzes of pollen types showed that bees visiting *C. rosea* are generalists [35]. However, medium to large pollinators are characteristic of the Canavalia genus, since floral biology selects visitors who are able to reach the nectaries, which also restricts the occurrence of a greater diversity of associated bees.

The floral structure of *Canavalia* species is similar to each other [35,60] which explains this pattern of bees associated with the genus. The floral visitors of *C. rosea* by pushing the wing and keel petals with their head to reach the nectary, consequently, promote the exposure of the flower's reproductive organs, causing the deposition of pollen grains in the bee's body, allowing further pollination to occur [44]. This pattern of flower behavior explains the fact that bees collected in *C. rosea* have pollen grains deposited mainly on the dorsal scopa [33]. In this study, we observed that the pollen grains of *C. rosea* were adhered to the dorsal scopa of the species of *Centris*, *Eufriesea* and *Xylocopa*, different from the pattern observed in Japan for Megachile bees, where the pollen was mainly in the scopa of the hind legs [61].

## Conclusion

The present study is pioneering research in the Restinga area of the recently created Municipal Natural Park of Barreto's Restinga and the data generated are relevant for future management plans for the area, since they contribute with ecological data to the understanding of the dynamics of flowering, availability of floral resources of *C. rosea* and the associated bee fauna.

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