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Diversity of flower visiting bees of *Eugenia uniflora* L. (Myrtaceae) in fragments of Atlantic Forest in South Brazil

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Abstract

Eugenia uniflora L. (Myrtaceae) is pollinated mostly by bees and there are no restrictions for pollen collection in their flowers. This stimulated us to study the bee diversity on its flowers in two forest fragments in southern Brazil, in August and September, 2012. A total of 826 bees belonging to 39 species and four subfamilies were captured with entomological nets. Halictinae presented the greatest richness, followed by Apinae, Colletinae and Andreninae. *Apis mellifera* was the only common species and the most abundant, followed by *Scaptotrigona bipunctata* and *Melipona obscurior*. Seven species were classified as intermediate and 31 as rare. Highest numbers of bees were collected from 10 to 11:30 h. The species richness of flower visiting bees was much higher than that of previous studies with first records of *Plebeia remota* and *Anthrenoides paolae*. *Eugenia uniflora* is a food source for exotic and native bees and possibly contributes to the conservation of these bees in forest fragments. In return, the bees probably act in maintaining this plant native of the Atlantic Forest.

Introduction

The Brazilian Atlantic Forest is one of the world's most diverse biomes, one of those most threatened by anthropic action (Myers et al., 2000), and one of 34 world hotspots and priority areas for conservation (Conservation International do Brasil et al., 2000). Within the Atlantic Forest biome the mixed ombrophilous forest formation (forest with *Araucaria*) is an area of great interest given its high biodiversity (Silveira et al., 2002).

Deforestation impacts, habitat fragmentation, exotic species introduction and irrational agriculture practices are the likely main causes of diminishing native populations of pollinators (Kevan & Phillips, 2001; Kremen et al., 2002; Steffan-Dewenter et al., 2006). This can affect reproduction of native plants and eventually lead to local extinction of plant populations, as well as of the animals which depend on them (Pinheiro Machado & Silveira, 2006). In tropical areas the bees are considered the main pollinators (Ramirez & Brito, 1992) and the animals best adapted to pollination (Faegri & Pijl, 1979). Bees and other pollinators seem to be declining globally (Potts et al., 2010) and habitat loss appears to be the most important factor driving the decline of bees (Brown & Paxton, 2009).

Myrtaceae is one of the largest plant families with thousands of species and approximately 140 genera, considered important in several Neotropical ecosystems (Johnson & Briggs, 1984; Kawasaki & Landrum, 1997; Wilson et al., 2001). It is one of the families most often found in the different vegetation formations of Brazil (Silva et al., 2001). *Eugenia*, a genus with approximately 1,000 species, is one of the largest genera, with distribution mostly in Central and South America (Merwe et al., 2004). In Brazil, *Eugenia* predominates with 388 species of which 302 are endemic (Sobral et al., 2015).

Eugenia uniflora, known commonly as "Pitangueira", is a native species from mixed ombrophilous forest, and a tree that can be used in landscaping or cultivated in home orchards (Lorenzi, 2000; Lorenzi, 2002). It has monoecious and generalist flowers, and pollen grains as the only floral reward to pollinators. It is therefore classified as a "pollen-flower", which is characteristic of many species in this genus (Romagnolo & Souza, 2006). It has a prominent role in the natural regeneration of mixed ombrophilous forest, through seed replacement and by providing food for birds (Aguiar et al., 2013). *Eugenia uniflora* grows under different shading conditions, and propagates easily by seeds, these being the determining factors in the regeneration process (Scalon et al., 2001; Callegaro et al., 2012). Silva & Pinheiro (2007) report that its flowers are visited by a wide variety of insects, including bees, which are the most common visitors.

During the anthesis period, which starts at the sunrise, remaining until the end of the day (Silva & Pinheiro, 2007), the pollen grains are totally exposed, without any restrictions to collection, a common trait of generalist flowers (Faegri & Pijl, 1979; Endress, 1994). The mass flowering, a common feature in Myrtaceae (Lughadha & Proença, 1996), makes the flowers stand out and gives a white aspect to the plants that can be considered as a strategy to attract pollinators (Gentry, 1974; O'Brien & Calder, 1993). The pollination in Myrtaceae is highly diversified and its species are visited by a large variety of animals, including bees, wasps, flies, birds and even mammals, but bees are the main pollinators (Beardsell et al., 1993; Proenca & Gibbs, 1994; Lughadha & Proença, 1996). In the Neotropical Region, the most common visitors of Myrtaceae are Apinae (Lughadha & Proenca & 1996). In a review on the flower visitors of Myrtaceae Gressler et al. (2006) reported bees the subfamilies Apinae and Halictinae only.

In the early hours of the morning, the flowers exhale a smooth and sweet odor (Silva & Pinheiro, 2007), a strategy directly related to the attraction of bees (Faegri & Pijl, 1979, Endress, 1994; Proença & Gibbs 1994; Maués & Couturier, 2002). *Eugenia uniflora* produces fruit after cross-pollination and self-fertilization (Franzon et al., 2011) however, self-fertilization in a natural population may result in inbreeding depression (Keller & Waller, 2002), which reduces fertility, viability of the seeds, vigor, among others (Mettler & Gregg, 1973; Falconer & Mackay, 1996). According to Franzon et al. (2010) for the preservation of the *E. uniflora* is necessary to preserve them in the original forest fragments to ensure sufficient variability.

This study sought to document the diversity of the flower visiting bees of *E. uniflora* in fragments of mixed ombrophilous forest in south Brazil.

Materials and methods

Research Area

This research was conducted in two areas, located in the Guarapuava municipality, Paraná state (25°23'36"S; 51°27'19"W) in the central-south region of the state. According to the Köppen Geiger classification the climate in this region is humid subtropical, without a dry season, with the occurrence of severe frosts, average annual temperature around 22°C and average annual precipitation of 1961 mm.

The two study areas are fragments of mixed ombrophilous forest with similar plant physiognomy.The areas are located around 25°22'05''S/51°32'27,16''W, with 8 ha, and 25°23'43''S/51°25'29''W, with 60 ha. The areas are 12 km distant from each other and are surrounded by other fragments of this same formation and crop fields.

Sampling design

The visits for the monitoring of flowering phenology and the collection of flower visitors were performed between the end of August and during September 2012. In total we selected 51 trees to monitor the flowering and to collect the bees. During the whole flowering period bees were captured on *E. uniflora* flowers daily. The collection days alternated between the two study areas.

In 30-minute intervals per hour, the bees that visited *E. uniflora* flowers were captured using entomological nets, by one collector. All collected individuals were sorted into morphospecies. Some specimens were sent for identification to Gabriel A. R. Melo, at Universidade Federal do Paraná (UFPR). The rest of the obtained specimens were deposited at the Entomological Collection of Bees and Wasps of the Biology and Ecology Laboratory, of Universidade Estadual do Centro-Oeste (UNICENTRO).

Data analysis

The indices of Shannon-Wiener, Margalef and Pielou were used to assess the diversity, richness and evenness of the bees. Those indices were obtained and calculated using the PAST® software (Paleontological Statics), version 1.98 (1999 – 2010) (Hammer et al., 2001).

The frequency of occurrence (FO) and the species dominance were calculated for each bee species obtained. Frequency of occurrence is the percentage of the number of collections with a given species and was calculated as FO= (F/N) x100 (Silveira Neto et al., 1976), where "F" is the number of collections with the species and "N" is the total number of collections performed. The bee species were classified as primary (FO > 50%), secondary (FO = 25% - 50%), or accidental (FO < 25%).

The species dominance of bees (D) was calculated as D= (d/n) x100 (Palma, 1975), where "d" is the abundance of a specific species and "n" the total abundance. The species were classified as dominant (D > 5%), accessory (D = 2.5% - 5%), or accidental (D < 2.5%). According to Palma (1975), the FO and D indices when used together group and determine the species as common, intermediary or rare.

Results

The flowering occurred from August 25 to September 14, 2012. During this period, 17 collections were performed for a total of 168 hours. Due to the similarity of the collection areas the bees obtained at both locations were grouped in only one sample.

A total of 826 bees belonging to 39 species and the four subfamilies Andreninae, Colletinae, Apinae and Halictinae were captured. The most abundant species was *Apis mellifera* (337 individuals), followed by *Scaptotrigona bipunctata* (293) and *Melipona obscurior* (74). Fifteen species were recorded by one specimen only (Table 1). The subfamily with the highest number of species was Halictinae (23 species); Andreninae was represented by just one species (*Anthrenoides paolae*). The bees were captured from 9:00 to 16:30, with highest numbers collected in the intervals between 10:00 and 10:30, and 11:00 and 11:30 and with highest number of species in the interval from 11h to 11:30 (Table 2).

Table 1 - Species of flower visiting bees collected on *Eugenia uniflora* L. in fragments of mixed ombrophilous forest (Atlantic Forest) in south Brazil. Indices of frequency of occurrence (FO), dominance (D) and the denomination for each species of floral visitor bee.

Subfamily	Tribe	Genus/species	Number of Individuals	FO (%)	D (%)	Denomination
Andreninae	Protandrenini	*Anthrenoides paolae* Urban, 2005	15	47.05	1.82	Intermediary
Colletinae	Hylaeini	*Hylaeus* aff. *geminus* (Vachal, 1910)	1	5.8	0.12	Rare
	Hylaeini	*Hylaeus* aff. *vachali* Meade-Waldo, 1923	3	17.64	0.36	Rare
	Hylaeini	*Hylaeus* cf. *asper* (Vachal, 1909)	1	5.8	0.12	Rare
	Colletini	*Rhynchocolletes albicinctus* Moure, 1943	1	5.8	0.12	Rare
Halictinae	Augochlorini	*Augochlora* sp. Smith, 1853	5	29.42	0.61	Rare
	Augochlorini	*Augochloropsis* cf. *cognata* Moure, 1944	6	29.41	0.73	Intermediary
	Augochlorini	*Augochloropsis chloera* (Moure, 1940)	1	5.8	0.12	Rare
	Augochlorini	*Augochloropsis cupreola* (Cockerell, 1900)	2	11.76	0.24	Rare
	Augochlorini	*Augochloropsis imperialis* (Vachal, 1903)	5	23.52	0.61	Rare
	Augochlorini	*Augochloropsis notophos* (Vachal, 1903)	3	17.64	0.36	Rare
	Augochlorini	*Augochloropsis sparsilis* (Vachal, 1903)	1	5.8	0.12	Rare
	Augochlorini	*Augochloropsis sympleres* (Vachal, 1903)	3	17.64	0.36	Rare
	Augochlorini	*Ceratalictus psoraspis* (Vachal, 1911)	1	5.8	0.12	Rare
	Augochlorini	*Halictillus loureiroi* (Moure, 1941)	3	11.76	0.36	Rare
	Augochlorini	*Neocorynura* cf. *chapadicola* (Cockerell, 1901)	2	5.8	0.24	Rare
	Augochlorini	*Paroxystoglossa andromache* (Schrottky, 1909)	2	11.76	0.24	Rare
	Augochlorini	*Paroxystoglossa* cf. *barbata* Moure, 1960	1	5.8	0.12	Rare
	Augochlorini	*Paroxystoglossa* sp. Moure, 1941	4	17.64	0.48	Rare
	Halictini (Caenohalictina)	*Caenohalictus tesselattus* (Moure, 1940)	1	5.8	0.12	Rare
	Halictini (Halictina)	*Dialictus bruneriellus* (Cockerell, 1918)	2	11.76	0.24	Rare
	Halictini (Halictina)	*Dialictus pabulator* (Schrottky, 1910)	1	5.8	0.12	Rare
	Halictini (Halictina)	*Dialictus picadensis* (Strand, 1910)	2	11.76	0.24	Rare
	Halictini (Halictina)	*Dialictus* sp.1Robertson, 1902	1	5.8	0.12	Rare
	Halictini (Halictina)	*Dialictus* sp.2 Robertson, 1902	2	11.76	0.24	Rare
	Halictini (Halictina)	*Dialictus* sp.3 Robertson, 1902	1	5.8	0.12	Rare
	Halictini (Halictina)	*Dialictus* sp.4 Robertson, 1902	1	5.8	0.12	Rare
	Halictini (Halictina)	*Dialictus* sp.5Robertson, 1902	1	5.8	0.12	Rare

Table 1. Species of flower visiting bees collected on *Eugenia uniflora* L. in fragments of mixed ombrophilous forest (Atlantic Forest) in south Brazil. Indices of frequency of occurrence (FO), dominance (D) and the denomination for each species of floral visitor bee. (Continuation)

Subfamily	Tribe	Genus/species	Number of Individuals	FO (%)	D (%)	Denomination
Apinae	Apini	*Apis mellifera* Linnaeus, 1758	337	100	40.80	Common
	Exomalopsini	*Exomalopsis (Diomalopsis) bicellularis* Michener & Moure, 1957	13	52.94	1.57	Intermediary
	Exomalopsini	*Exomalopsis (Phanomalopsis) aureosericea* Friese, 1899	2	11.76	0.24	Rare
	Meliponini	*Melipona (Eomelipona) obscurior* Moure, 1971	74	82.35	8.96	Intermediary
	Meliponini	*Plebeia emerina* (Friese, 1900)	2	11.76	0.24	Rare
	Meliponini	*Plebeia remota* (Holmberg, 1903)	21	70.58	2.54	Intermediary
	Meliponini	*Scaptotrigona bipunctata* (Lepeletier, 1836)	293	88.23	35.47	Intermediary
	Meliponini	*Schwarziana quadripunctata* (Lepeletier, 1836)	7	35.29	0.85	Intermediary
	Meliponini	*Tetragonisca angustula* (Latreille, 1811)	1	5.8	0.12	Rare
	Xylocopini (Ceratinina)	*Ceratina* cf. *(Ceratinula) biguttulata* (Moure, 1941)	3	11.76	0.36	Rare
	Xylocopini (Xylocopina)	*Xylocopa (Dasyxylocopa) bimaculata* Friese, 1903	1	5.8	0.12	Rare

The Margalef Richness Index (Dmg) for the bee sample collected in this study was 5.658, and the Shannon-Wiener Diversity Index (H') was 1.686.The Pielou Evenness Index (J) was 0.4601.

The obtained FO and Dvalues classified only *A. mellifera* as common visitors of *E. uniflora* flowers.Seven species were classified as intermediary: *M. obscurior, S. bipunctata, A. paolae, Schwarziana quadripunctata, Exomalopsis bicellularis, Plebeia remota* and *Augochloropsis* cf. *cognata*.The other 31 bee species were considered rare visitors of *E. uniflora* (Table 1).

Discussion

The richness and diversity of bee visitors of *E. uniflora* was much higher than the 11 species recorded by Silva and Pinheiro (2007) in the western part of the municipality of Rio de Janeiro (Rio de Janeiro state, Brazil).High values of richness and diversity indicate, in most cases, a well-structured community, with many rare species (Costa et al., 1993). According to Michener (1979), southern Brazil it is one of the regions in the world with the highest richness of Apoidea. Regarding the species richness per subfamilies, our sample from the Guarapuava (PR) region also had a higher numbers of species of Apinae visiting *E. uniflora* flowers than recorded in Rio de Janeiro state, but we must consider that both studies were conducted in totally different phytophysionomies.

The greatest activity of bees on *E. uniflora* flowers occurred during the hottest hours of the day. Bees' foraging activity is influenced by weather conditions, mainly temperature because maintaining high body temperature during the flight is energetically costly (Roubik, 1989).

Flower visitors of the subfamilies Colletinae and Andreninae are new records for *E. uniflora*.In the southern region of Brazil, the most diverse subfamilies are Apinae and Halictinae (Alves-dos-Santos, 2007), and we also found the greatest number of speciesfor Halictinae among the flower visitors or *E. uniflora*, demonstrating that this pattern is also reflected in our bee sample.

Silva and Pinheiro (2007) considered species of the genus *Xylocopa* to be among the pollinators of *E. uniflora*. It is therefore possible that *X. bimaculata*, even though it was a rare visitor, is also able to pollinate those flowers. Bees of the genus *Xylocopa* are also floral visitors of other Myrtaceae species (Schlindwein et al., 2003; Siqueira et al., 2012).

Out of all the species captured in this study, only *A. mellifera* was classified as common, which was also observed in *E. uniflora* flowers by both Pelacani et al. (2000) in São Paulo state (Brazil), and by Silva and Pinheiro (2007). Although *A. mellifera* is an exotic species in Brazil, it has become the most common floral visitor in the Neotropics (Roubik, 2000). *Apis mellifera* has considerable interference in the reproduction of many plant species and can facilitate or hamper their reproductive success, influencing directly or indirectly on the foraging of native pollinators (Paton, 1993; Vaughton, 1996; Villanueva-G, 2002). According to Silva and Pinheiro (2007) the high number of *A. mellifera* in the early anthesis can impair the pollen supply for native visitors. This may be one of the reasons for the high number of species considered rare visitors of *E. uniflora*, because there is a lower amount of pollen available when native bees start the foraging. Depletion of pollen is also indicated by the sharp decrease in the number of pollinators after 12:30 h.

Of the species belonging to the genus *Scaptotrigona* only *S. xanthotricha* Moure, 1950 was registered visiting *E. uniflora* flowers in Rio de Janeiro state (Silva & Pinheiro, 2007). Other species, namely *S. depilis* (Moure, 1942) and

Table 2. Dial pattern of the number of flower visiting bees captured on *Eugenia uniflora L.,*in fragments of mixed ombrophilous forest.

Subfamily	Tribes	Genus/species	Hour of the day								Total of individuals
			9h	10h	11h	12h	13h	14h	15h	16h	
Andreninae	Protandrenini	*Anthrenoides paolae* Urban, 2005	-	2	7	2	3	1	-	-	**15**
Colletinae	Colletini	*Rhynchocolletes albicinctus* Moure, 1943	-	-	1	-		-	-	-	**1**
	Hylaeini	*Hylaeus* aff. *geminus* (Vachal, 1910)	-	-	-	-	1	-	-	-	**1**
	Hylaeini	*Hylaeus* aff. *vachali* Meade-Waldo, 1923	1	-	1	-	-	-	1	-	**3**
	Hylaeini	*Hylaeus* cf. *asper* (Vachal, 1909)	-	-	1	-	-	-	-	-	**1**
Halictinae	Augochlorini	*Augochlora* sp. Smith, 1853	-	1	3	-	1	-	-	-	**5**
	Augochlorini	*Augochloropsis* cf. *cognata* Moure, 1944	1	-	2	-	1	-	2	-	**6**
	Augochlorini	*Augochloropsis chloera* (Moure, 1940)	-	-	-	-	-	-	1	-	**1**
	Augochlorini	*Augochloropsis cupreola* (Cockerell, 1900)	-	1		1	-	-	-	-	**2**
	Augochlorini	*Augochloropsis imperialis* (Vachal, 1903)	-	2	1	1	-	-	1	-	**5**
	Augochlorini	*Augochloropsis notophos* (Vachal, 1903)	-	-	1	-	1	1	-	-	**3**
	Augochlorini	*Augochloropsis sparsilis* (Vachal, 1903)	-	-	1		-	-	-	-	**1**
	Augochlorini	*Augochloropsis sympleres* (Vachal, 1903)	-	-	1	2	-	-	-	-	**3**
	Augochlorini	*Ceratalictus psoraspis* (Vachal, 1911)	-		1	-	-	-	-	-	**1**
	Augochlorini	*Halictillus loureiroi* (Moure, 1941)	-	-	2	-	1	-	-	-	**3**
	Augochlorini	*Neocorynura* cf. *chapadicola* (Cockerell, 1901)	-	-	2	-	-	-	-	-	**2**
	Augochlorini	*Paroxystoglossa andromache* (Schrottky, 1909)	-	1	-	-	1	-	-	-	**2**
	Augochlorini	*Paroxystoglossa* cf. *barbata* Moure, 1960	-	-	1	-	-	-	-	-	**1**
	Augochlorini	*Paroxystoglossa* sp. Moure, 1941	2	2	-	-	-	-	-	-	**4**
	Halictini (Caenohalictina)	*Caenohalictus tesselattus* (Moure, 1940)	-	-		-	1	-	-	-	**1**
	Halictini (Halictina)	*Dialictus picadensis* (Strand, 1910)	-	-	1	-	1	-	-	-	**2**
	Halictini (Halictina)	*Dialictus bruneriellus* (Cockerell, 1918)	-	2	-	-	-	-	-	-	**2**
	Halictini (Halictina)	*Dialictus pabulator* (Schrottky, 1910)	-	-	-	1	-	-	-	-	**1**
	Halictini (Halictina)	*Dialictus* sp.1 Robertson, 1902	-	-	1	-	-	-	-	-	**1**
	Halictini (Halictina)	*Dialictus* sp.2Robertson, 1902	-	-	2	-	-	-	-	-	**2**
	Halictini (Halictina)	*Dialictus* sp.3Robertson, 1902	-	-	1	-	-	-	-	-	**1**
	Halictini (Halictina)	*Dialictus* sp.4Robertson, 1902	-	-	1	-	-	-	-	-	**1**
	Halictini (Halictina)	*Dialictus* sp.5Robertson, 1902	-	-	1	-	-	-	-	-	**1**
Apinae	Apini	*Apis mellifera* Linnaeus, 1758	96	86	62	43	19	15	12	4	**337**
	Exomalopsini	*Exomalopsis (Diomalopsis) bicellularis* Michener & Moure, 1957	2	3	4	1	2	1	-	-	**13**
	Exomalopsini	*Exomalopsis (Phanomalopsis) aureosericea* Friese, 1899	-	-	-	1	-	1	-	-	**2**

Table 2. Dial pattern of the number of flower visiting bees captured on *Eugenia uniflora L.,* in fragments of mixed ombrophilous forest. (Cont.)

Subfamily	Tribes	Genus/species	Hour of the day								Total of individuals
			9h	10h	11h	12h	13h	14h	15h	16h	
Apinae	Meliponini	*Melipona (Eomelipona) obscurior* Moure, 1971	2	13	18	17	14	9	1	-	**74**
	Meliponini	*Plebeia emerina* (Friese, 1900)	-	-	1	1	-	-	-	-	**2**
	Meliponini	*Plebeia remota* (Holmberg, 1903)	2	2	3	7	5	1	1	-	**21**
	Meliponini	*Scaptotrigona bipunctata* (Lepeletier, 1836)	44	91	85	46	19	7	1	-	**293**
	Meliponini	*Schwarziana quadripunctata* (Lepeletier, 1836)	1	2	3	1	-	-	-	-	**7**
	Meliponini	*Tetragonisca angustula* (Latreille, 1811)	-	-	-	-	1	-	-	-	**1**
	Xylocopini (Ceratinina)	*Ceratina* cf. *(Ceratinula) biguttulata* (Moure, 1941)	-	-	-	2	1	-	-	-	**3**
	Xylocopini (Xylocopina)	*Xylocopa (Dasyxylocopa) bimaculata Friese, 1903*	1	-	-	-	-	-	-	-	**1**
	Total No of specimens		**152**	**208**	**208**	**126**	**72**	**36**	**20**	**4**	**826**
	Total No of species		**10**	**13**	**27**	**14**	**16**	**8**	**8**	**1**	-

S. fulvicutis (Moure, 1964) were found as abundantflower visitors of Myrtaceae in Mato Grosso do Sul and Amazonas states in Brazil (Marques-Souza et al., 2007; Ferreira et al., 2010). *Scaptotrigona bipunctata* is among the most common species of Meliponini found in Paraná state (Paraná, 2009), which explains the large number of individuals collected in this work. This species displays highly defensive behaviour (Nogueira-Neto, 1970), which could explainits abundance and its frequency found here, when compared to the presence of other species.

Melipona obscurior bees are polylectic foragers (Kleinert-Giovannini & Imperatriz Fonseca, 1987) and there are records of their occurrence in Bahia state, as well as in southern and southwestern Brazil (Roubik, 1989; Nogueira-Neto, 1997; Silveira et al., 2002). Bees in this genus (*Melipona scutellaris* Latreille, 1811) were reported to pollinate *Psidium guajava* L. (Myrtaceae) (Castro & Araújo, 1998).

The findings that deserve highlighting in this work is the unprecedented recording of *Plebeia remota* in *E. uniflora* flowers. *Plebeia remota* is not commonly observed as a floral visitor or pollinator of other Myrtaceae species. Another unprecedented record was the presence of *A. paolae* visiting *E. uniflora* flowers, given that no floral visitation or pollination of this species to any Myrtaceae has been observed before.

Apis mellifera is possibly the most important potential pollinator of *E. uniflora* because it was the most frequent floral visitor, followed by *S. bipunctata* and *M. obscurior*. This study reveals that *E. uniflora* is an easily obtainable pollen source for exotic and native bees, and thus contributes to the conservation of these bee species in forest fragments helping in the maintaining the biodiversity of the bees in these fragments. At the same time, these bees are probably acting in the preservation of this native plant and assist in the regeneration of the highly fragmented Atlantic Forest.

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