

Orchid bee (Hymenoptera: Apidae) community from a gallery forest in the Brazilian Cerrado

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Abstract: The orchid bees are a very important group of pollinators distributed in the Neotropics. Although a lot of studies concerning male euglossine bees have been done in this region, few works have so far been carried out in the Cerrado biome. This manuscript has the main objective to present the orchid bee community from a Gallery Forest in the Northeastern Brazilian Cerrado landscape, taking account the species composition, abundance, seasonality and hourly distribution. Male euglossine bees were collected monthly from October 2007 to May 2009, in the Reserva Florestal da Itamaçoca belonging to the Companhia de Água e Esgoto do Maranhão, in Chapadinha municipality, Maranhão State. The scents eucalyptol, eugenol and vanillin were utilized, between 07:00 and 17:00hr, to attract the euglossine males. Cotton balls were dampened with the scents and suspended by a string on tree branches 1.5m above soil level, set 8m from one another. The specimens were captured with entomological nets, killed with ethyl acetate and transported to the laboratory to be identified. A total of 158 individuals and 14 species of bees were recorded. The genus *Eulaema* was the most representative group of euglossine bees in relation to the total number of the sampled individuals, accounting for 50.6% of bees followed by *Euglossa* (26.6%), *Eufriesea* (15.2%) and *Exaerete* (7.6%). The most frequent species were *Eulaema nigrita* (27.8%), *Eulaema cingulata* (19%) and *Euglossa cordata* (18.3%). Many species typical of forested environments were found in samples, like *Euglossa avicula*, *Euglossa violaceifrons* and *Eulaema meriana*, emphasizing the role played by the Gallery Forests as bridge sites to connect the two great biomes of Amazonia and Atlantic Forest. The occurrence of *Exaerete guaykuru* represents the second record of this species for the Neotropical region, and both records coming from the Gallery Forest zones. The male euglossine bees were sampled mainly in the dry season, where 62.5% of the individuals were collected in that period. *Eufriesea* species appeared at the baits only in the wet season. The hourly frequency of bees at scent baits showed a clear preference for the morning period, where 87.9% visited the baits from 07:00 to 12:00hr. The euglossine bee fauna found in the Northeastern Maranhão Cerrado is represented chiefly by species of large geographic distribution and by some forest bee species, where their occurrence is maybe related to the environmental conditions supported by the Gallery Forest ecosystem. Rev. Biol. Trop. 60 (2): 625-633. Epub 2012 June 01.

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Cerrado is the designation given to the Brazilian savanna vegetation. The Cerrado landscape is characterized by extensive savanna formations crossed by Gallery Forests and stream valleys. It occupies an area of two million km² accounting for 23% of the Brazilian territory, being exceeded only by the Amazonian forest (Ratter *et al.* 1997). Biologically, Cerrado is the richest savanna in the entire world (Castro *et al.* 1999).

This biome has been largely disturbed and such changes may truly have huge consequences to the biodiversity maintenance (Klink & Machado 2005). In the last 35 years, more than half of the Brazilian Cerrado has been transformed into pasture, agricultural crops and other uses, and, in Northeastern Maranhão, the deforestation process continues to be intense, mainly because of the increasing propagation of soy monoculture. It results in

the Cerrado fragmentation and in the disruption of the reproductive biology of many pollinator species, especially bees (Cane 2001).

Euglossini bees, also known as orchid bees, are an important group of Neotropical pollinators. This tribe is composed of five genera and about 200 species (Cameron 2004, Moure *et al.* 2007). They are characterized by their very long tongues and a conspicuous behavior displayed by males: they take up aromatic compounds from a variety of source as flowers, dead wood, feces and afterwards transfer the collected material to their enlarged hind tibia. However, the biological meaning of such behavior is still unclear (Eltz *et al.* 2005). Some Neotropical orchids attract euglossine bees which work as their exclusive pollinators in a co-evolutionary way (Dressler 1982).

Euglossine bees show close associations with a large number of plant species (Dodson *et al.* 1969, Williams & Dodson 1972), and may be extremely sensitive to the environmental fragmentation which can lead to a heavy loss of native flora (Brosi 2009). Generally, bees are good indicators of environmental quality and may be a useful tool in studies of impact of forest fragmentation (Brosi *et al.* 2008). Studying euglossine bees in a threatened landscape is very important for future research, where the impact on how the land-use changes affecting the abundance and community composition can be better evaluated.

In this vast tropical savanna biome, even with the continuous deforestation process, few works have been made on the euglossine bee fauna (Rebêlo & Cabral 1997, Nemésio & Faria 2004, Anjos-Silva *et al.* 2006, Carvalho *et al.* 2006, Alvarenga *et al.* 2007, Anjos-Silva 2007, 2010). So, the objectives of this work, was to study the species composition, abundance and the seasonal and hourly distribution of euglossine bees in a Gallery Forest in the Brazilian Cerrado.

MATERIAL AND METHODS

The study area is in the Northeastern part of Maranhão state, Brazil (3°44'17" S

- 43°20'29" W), about 300km far away from São Luís, the capital of the state. The Maranhão state Cerrado occupies a large transitional area positioned between three Brazilian regions: North, Northeast and Central-West. The research was undertaken in a Gallery Forest situated in Chapadinha, one of the 33 Maranhão municipalities found in the Cerrado zone, which occupies 30 percent of the geographical area of the state. The Gallery Forest grows along the Reserva Florestal da Itamacaoca water course, which belongs to the Companhia de Água e Esgoto do Maranhão, in Chapadinha. The reserve has a total land area of 460ha and is composed of a variety of plant habitats, and the main feature is still covered by the original vegetation (Silva *et al.* 2008). It is bordered on the North-Northeast side by urbanized areas; on the North-Northwest side by the University campus and on the South-Southeast side by Cerrado vegetation. The reserve is composed of a mosaic of vegetation types, such as Riparian and Gallery Forests, "Campo Cerrado" (a savannic intermediate formation) and "cerradões" (a forest formation) (Silva *et al.* 2008).

In the region the climate is hot and semi-humid with an annual average temperature ranging from 28°C-30°C (Fig. 1). There is a precipitation regime of 1600-2000mm/year. The region undergoes a dry season, from July to December, and a wet season, from January to June, according to Instituto Brasileiro de Geografia e Estatística (IBGE 1984).

The bee captures were carried out monthly from September 2007 to May 2009, between 07:00 and 17:00hr, by means of odoriferous baits. The scent lures used to attract males were eucalyptol (1.8-cineole) (Biodinâmica®), eugenol (clove oil) (Biodinâmica®) and vanillin (4-hydroxy-3-methoxybenzaldehyde) (Reagen®). These above-mentioned aromatic compounds have effectively been used in field bioassays as attractant for most male euglossine bees (Rebêlo 2001). Eucalyptol and eugenol were used without any dilution and vanillin was dissolved in distilled water before use. Cotton balls were dampened with the

respective scent each hour and hanged on tree branches 1.5m above soil level. The baits were set 8m from one another, according to Rebêlo & Garófalo (1991).

The specimens were captured with entomological nets, killed with ethyl acetate vapor in killing jars and then placed in labeled plastic bags. In the laboratory, males were pinned and identified to species. All the captured specimens are deposited in the insect collection of the Universidade Federal do Maranhão, São Luis, Maranhão, Brazil. The Mann-Whitney test was used for statistical analysis using GraphPad (San Diego, CA) Prism Software.

RESULTS

Over this 21-month follow-up study, a total of 158 individuals and 14 species distributed in the four genera of euglossine bees were recorded. *Eulaema* Lepeletier was represented by 80 individuals and three species, *Euglossa* Latreille (42 individuals; seven species), *Eufriesea* Cockerell (24 individuals; two species) and *Exaerete* Hoffmannsegg (12 individuals; two species) (Table 1). The most frequent species at baits were *Eulaema nigrita* (Lepeletier, 1841), accounting for 27.8% of the total sampled individuals, followed by *Eulaema cingulata*

(Fabricius, 1804) (19%) and *Euglossa cordata* (Linnaeus, 1758) (18.3%).

The most attractive scent bait was eucalyptol (11 species; 86 individuals), followed by vanillin (seven species; 44 individuals) and eugenol (six species; 27 individuals). *E. nigrita* was the most frequent species collected at eucalyptol-scented baits, followed by *E. cordata* (39.5% and 34.8%, respectively). Vanillin was more visited by *E. cingulata* and *E. surinamensis* (Linnaeus, 1758), both accounting for 27.2% of the collected individuals at that scent. Eugenol was represented mainly by *E. cingulata* (55.5%).

The genus *Eulaema* was the most representative group of euglossine bees in relation to the total number of the sampled individuals, accounting for 50.6% of bees followed by *Euglossa* (26.6%), *Eufriesea* (15.2%) and *Exaerete* (7.6%) (Table 1).

The euglossine bees were sampled mainly in the dry season, where 62.5% of the individuals were captured in that period (Table 2). However, these results are not statistically significant ($p < 0.05$). *E. cordata* occurred exclusively in the dry period and the *Eulaema* species were mostly found in the dry season. *E. cordata* and the two more frequent *Eulaema* species *E. nigrita* and *E. cingulata* accounted

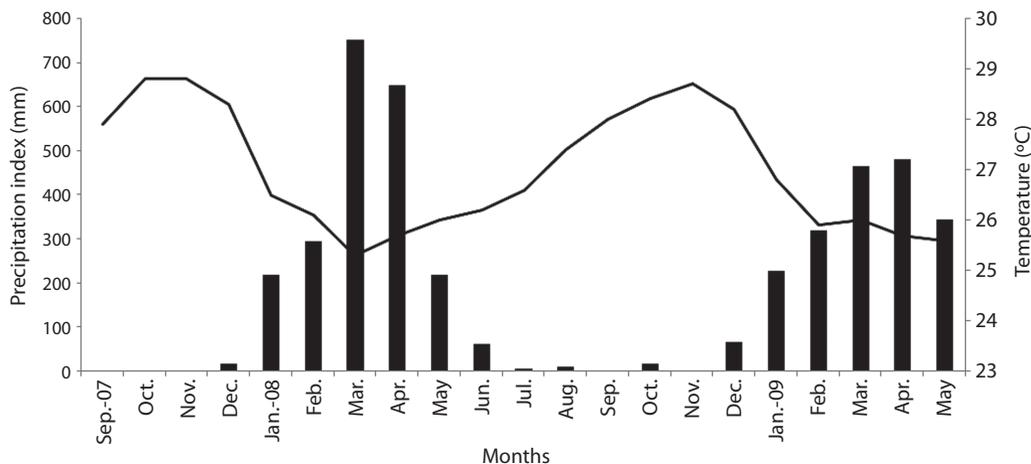


Fig. 1. Climatological data collected during the study period in the Northeastern Cerrado area of Maranhão state, Brazil, from 2007 to 2009. Source: Principal Climatological Station of Chapadinha via INMET (National Institute of Meteorology).

TABLE 1

Euglossine bee species captured at scent baits in the Northeastern Maranhão Cerrado, from September 2007 to May 200

Bees	Scent baits			Total	Individuals (%)	Genus (%)
	Eucalyptol	Eugenol	Vanillin			
<i>Eufriesea nigrescens</i> (Friese, 1925)	1	6	5	12	7.6	15.2
<i>Eufriesea surinamensis</i> (Linnaeus, 1758)			12	12	7.6	
<i>Euglossa avicula</i> Dressler, 1982			3	3	1.8	
<i>Euglossa cordata</i> (Linnaeus, 1758)	30			30	18.9	
<i>Euglossa modestior</i> Dressler, 1982	2			2	1.2	
<i>Euglossa pleosticta</i> Dressler, 1982	2			2	1.2	26.6
<i>Euglossa securigera</i> Dressler, 1982	1	2		3	1.8	
<i>Euglossa townsendi</i> Cockerell, 1904	1			1	0.6	
<i>Euglossa violaceifrons</i> Rebêlo & Moure, 1995	1			1	0.6	
<i>Eulaema cingulata</i> (Fabricius, 1804)	3	15	12	30	19.0	
<i>Eulaema meriana</i> (Oliver, 1789)	5		1	6	3.8	50.6
<i>Eulaema nigrita</i> Lepeletier, 1841	34	2	8	44	27.8	
<i>Exaerete guaykuru</i> Anjos-Silva & Rebêlo, 2006		1		1	0.6	
<i>Exaerete smaragdina</i> (Guérin-Ménéville, 1845)	7	1	3	11	6.9	7.6
Individuals	86	27	44	158		
Species	11	6	7	14		

TABLE 2

Seasonal distribution of euglossine bees collected at scent baits in a gallery forest of Maranhão Cerrado area from September 2007 to May 2009

Species	Rainy season	%	Dry season	%	Total
<i>Eufriesea nigrescens</i>	11	91.7	1	8.3	12
<i>Eufriesea surinamensis</i>	12	100			12
<i>Euglossa avicula</i>	3	100			3
<i>Euglossa cordata</i>			30	100	30
<i>Euglossa modestior</i>	1	50	1	50	2
<i>Euglossa pleosticta</i>	2	100			2
<i>Euglossa securigera</i>	3	100			3
<i>Euglossa townsendi</i>			1	100	1
<i>Euglossa violaceifrons</i>			1	100	1
<i>Eulaema cingulata</i>	10	33.3	20	66.7	30
<i>Eulaema meriana</i>	1	16.7	5	83.3	6
<i>Eulaema nigrita</i>	11	25	33	75	44
<i>Exaerete guaykuru</i>	1	100			1
<i>Exaerete smaragdina</i>	4	36.4	7	63.6	11
Total	59		99		158

for 83.8% of the sampled bees, being responsible for the seasonality configuration observed herein. *Eufriesea* species appeared at the baits only in the wet season, with one individual of *E. nigrescens* (Friese, 1925) sampled in the dry period.

The hourly frequency of bees at scent baits showed a clear preference for the morning period, where 87.9% visited the baits from 7:00 to 12:00hr (Table 3). The statistical analysis showed significant difference ($p < 0.05$) between the morning and afternoon samples,

TABLE 3
Hourly distribution of euglossine bees at the scent baits in the gallery Forest of Maranhão Cerrado, from September 2007 to May 2009

Species/hours	Morning period					Afternoon period					Total
	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	
<i>Eufriesea nigrescens</i>	1	2	3	3	3						12
<i>Eufriesea surinamensis</i>		1	1	2	3	1	4				12
<i>Euglossa avicula</i>				2	1						3
<i>Euglossa cordata</i>	1	2	6	12	6	1	1			1	30
<i>Euglossa modestior</i>				1		1					2
<i>Euglossa pleosticta</i>		1		1							2
<i>Euglossa securigera</i>	1		1				1				3
<i>Euglossa townsendi</i>				1							1
<i>Euglossa violaceifrons</i>					1						1
<i>Eulaema cingulata</i>	9	5	9	2	4			1			30
<i>Eulaema meriana</i>	1	1		2	1	1					6
<i>Eulaema nigrita</i>	11	11	9	6	2	1	1	2	1		44
<i>Exaerete guaykuru</i>						1					1
<i>Exaerete smaragdina</i>		7			3			1			11
Total of individuals	24	30	29	32	24	6	7	4	1	1	158
Total of species	6	8	6	10	9	6	4	3	1	1	14

regarding the number of individuals and species. *Euglossa cordata* males were present during all morning period while *E. nigrita* and *E. cingulata* appeared more frequently at the baits early in the morning. The males of both *Eufriesea* species visited the baits all morning and reaching two more hour intervals at the beginning of the afternoon period, particularly in the rainy days.

DISCUSSION

The three most frequent species surveyed in the Itamaçoca Reserve Gallery Forest are commonly found in open and dry Neotropical areas. *E. nigrita* is generally found in several Brazilian ecosystems, from Amazonian areas (Silva & Rebêlo 1999) to Southern Brazil (Sofia *et al.* 2004) and it is one of the fewer euglossine species found in the caatinga, a semi-arid Brazilian ecosystem characterized by scrub vegetation and presenting a low and highly seasonal rainfall (Lopes *et al.* 2007). The orchid bee *E. cordata* is well represented in open, disturbed and dry areas (Silva &

Rebêlo 2002), and also was recorded in the caatinga ecosystem, as well as *E. securigera* (Dressler, 1982) (Lopes *et al.* 2007). *Eulaema cingulata*, like *Eulaema nigrita* and *Euglossa cordata*, are a species commonly found in forested areas (Silva & Rebêlo 1999, Nemésio & Silveira 2007) and in the Brazilian Cerrado (Silva & Rebêlo 1999, Rebêlo & Cabral 1997, Mendes *et al.* 2008, Carvalho *et al.* 2006).

Euglossa avicula (Dressler, 1982) has been found in Amazonian forested areas (Oliveira & Campos 1995, Silva & Rebêlo 1999). In the Cerrado landscape it was recorded from a riparian forest in Northeastern Maranhão by Carvalho *et al.* (2006). *Euglossa pleosticta* (Dressler, 1982) is also well known in Northern forest environments (Silva & Rebêlo 1999, Silva & Rebêlo 2002) and in the Atlantic forest of Brazil (South of Bahia, Espírito Santo, Rio de Janeiro, Northeast of São Paulo) (Rebêlo 2001). It is also found in the Cerrado zone of Minas Gerais state, Southeastern Brazil (Alvarenga *et al.* 2007). *Euglossa modestior* (Dressler, 1982) is found in Amazonian basin and has already been described from the

Cerrado zones of Maranhão state (Rebêlo & Cabral 1997). *Euglossa violaceifrons* (Rebêlo & Moure, 1995) was recorded from Brazilian forests of South Maranhão (Silva & Rebêlo 1999) and Northeast of São Paulo state, Southeastern Brazil (Rebêlo & Moure 1996). *Eufriesea nigrescens* has been recorded from the Amazonian basin and Paraguay as well as the open areas of Maranhão state (Rebêlo 2001, Silva *et al.* 2009). *Eulaema meriana* (Olivier, 1789) was collected for the first time in the Cerrado landscape. It is a typical species found in forested areas (Silva & Rebêlo 1999, Oliveira & Campos 1995) avoiding urban environments (Parra-H & Nates-Parra 2007).

In this context, the occurrence of *Exaerete guaykuru* (Anjos-Silva & Rebêlo, 2006) in the Gallery Forest of Northeastern Cerrado represents the second record for the Neotropical region, since this species was recently described from a Gallery Forest environment of Mato Grosso state, Brazil (Anjos-Silva & Rebêlo 2006). However, it is extremely important to protect those kinds of habitats because so many species of animals are profoundly responsive to environmental disturbances and use the Gallery Forest zones as refuge sites inside the degradation context of the Cerrado vegetation (Moura & Schlindwein 2009).

Eulaema species were the predominant visitors to the odoriferous baits, different from the results obtained by other researches in the Neotropical region using a similar methodology, showing that *Euglossa* is the most expressive group at the scent baits, in both individuals and species (Janzen *et al.* 1982, Ackerman 1983, Oliveira & Campos 1995, Neves & Viana 1997, Rebêlo & Garófalo 1997, Silva & Rebêlo 1999, Silva & Rebêlo 2002, Carvalho *et al.* 2006). The two *Eulaema* species more sampled in the gallery forest, *E. nigrata* and *E. cingulata*, accounted together for 46.8% of the total number of individuals. These species have a large geographic distribution and one of them, *E. nigrata*, is frequently associated with open and dry areas (Rebêlo & Garófalo 1991, Silva & Rebêlo 2002).

Eulaema male activities are favored by satisfactory climate conditions and thus visit the scent baits early in the morning as already observed by Silva & Rebêlo (1999). *Euglossa* species arrive late at the scent baits and the hot and dry climate conditions appear to be the main limiting factors. *Eulaema* species are large, black and have densely pubescent thoraces and *Euglossa* species are smaller, lack external pubescence and are brightly colored, metallic blue or green (Rebêlo 2001). The morphological aspects and the pubescence have an effect on the flight activity of *Eulaema* species (Casey *et al.* 1985) where the thoracic temperature can be maintained high at low temperature conditions favoring them to visit the baits early.

The most frequent species captured in the gallery forest of Maranhão Cerrado depicted an unfamiliar seasonality curve that is divergent from the most researches undertaken in the Neotropics in which a high number of species and individuals have been found in the rainy season (Silva & Rebêlo 1999, 2002). The species *E. cordata*, *E. cingulata* and *E. nigrata* were responsible for that unusual pattern of seasonal distribution. But, in view of the statistical analysis, there is no significant difference between the two seasons. Silva *et al.* (2009) observed that *E. cingulata* and *E. cordata* were most frequently found in the dry period in the eastern Maranhão "restinga". The same pattern was observed by Silva & Rebêlo (1999) in Amazonian Forest areas, Aguiar & Gaglianone (2008), in remnants of lowland forest in the Rio de Janeiro State, and by Farias *et al.* (2008) in forest fragments of Paraíba State. Neves & Viana (1999) observed that most sampled euglossine males in riparian forest in areas of Bahia semi-arid region occurred after the rainy season and the increased flower production together with a moderate temperature and humidity favored that seasonal distribution pattern. In that research, 50% of the captured individuals were *E. cordata* (Neves & Viana 1999).

Euglossine bees are usually found at baits in the morning period (Braga 1976, Neves & Viana 1999, Bezerra & Martins 2001, Silva & Rebêlo 2002), where the weather conditions

are favorable to bee activities. In the studied area, the weather conditions were represented chiefly by increasing temperature and decreasing humidity along the day, forcing bees to occur mostly in the morning period (from seven am to noon), where relative humidity was higher and temperature and light intensity were moderate. The wind speed at the collecting site was mostly low or not detectable, mainly in the afternoon periods (data not shown), influencing strongly on the scent dispersal and the frequency of bees at the scent baits. It was not feasible to measure the real impact of weather changes on the bee activity during the day, which should become a potential target for further researches in the Cerrado area. In general, bees visit flowers mostly at the morning period due to the good climate conditions and floral resources availability (Melo *et al.* 2009, Oliveira *et al.* 2011) and this may explain the male euglossine bee activity at the baits.

In conclusion, the euglossine bee fauna found in the Northeastern Maranhão Cerrado is represented chiefly by species of large geographic distribution. Some forest bee species in a less extent maybe require particular environmental conditions found in the Gallery Forest ecosystem. The most frequent bees were sampled at the baits after the rainy season, in a different way of the most researches carried out in the Neotropical areas. The bee frequency at the scent baits was most pronounced in the morning period, possibly controlled by the peculiar climate variables found in the Cerrado areas.

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RESUMEN

Las abejas de las orquídeas son un grupo muy importante de polinizadores distribuido en el Neotrópico, incluyendo Brasil. Aunque una gran cantidad de estudios sobre las abejas euglosinas se han hecho en esa región,

pocos trabajos han sido hasta ahora llevado a cabo en el bioma del Cerrado. Este manuscrito tiene como objetivo principal mostrar la comunidad de abejas de orquídeas de un bosque de galería en el Cerrado brasileño, así como la composición de las especies, abundancia, estacionalidad y distribución horaria. Abejas euglosinas fueron capturadas mensualmente desde octubre 2007 hasta mayo 2009 en la Reserva Forestal da Itamaçoca perteneciente a la Companhia de Água e Esgoto do Maranhão, en el municipio de Chapadinha, Maranhão, Brasil. Eucalipto, eugenol y vainillina se utilizaron para atraer las abejas, entre las 07:00 y 17:00hr. Bolas de algodón fueron impregnadas con los respectivos compuestos aromáticos y suspendidas por un cordel en ramas de los árboles alrededor de 1.5m sobre el nivel del suelo, separados 8m entre sí. Los especímenes fueron capturados con redes entomológicas, fijados con acetato de etilo y transportados al laboratorio para su identificación. Un total de 158 individuos y 14 especies de abejas fueron registrados. El género *Eulaema* fue el grupo más representativo de las abejas euglosinas, lo que representa el 50.6% de las abejas seguido por *Euglossa* (26.6%), *Eufriesea* (15.2%) y *Exaerete* (7.6%). Las especies más frecuentes fueron: *Eulaema nigrita* (27.8%), *cingulata Eulaema* (19%) y *Euglossa cordata* (18.3%). Muchas especies típicas de los ambientes forestales fueron capturadas, como *Euglossa avicula*, *Euglossa violaceifrons* y *Eulaema meriana*. La aparición de *Exaerete guaykuru* representa el segundo registro de esta especie para la región Neotropical, y ambos registros procedentes de las zonas de bosque de galería. Las abejas euglosinas fueron recolectadas principalmente en la estación seca (62.5%), pero el género *Eufriesea* apareció sobre todo en la estación lluviosa. Las abejas mostraron una clara preferencia por el período de la mañana, donde el 87.9% visitó los cebos aromáticos entre 07:00 y las 12:00hr. La fauna de abejas euglosinas está representada principalmente por especies de distribución geográfica amplia y por algunas especies forestales. La aparición de estas especies puede deberse a las condiciones ambientales presentes en el bosque de galería.

Palabras clave: diversidad, Euglossini, *Exaerete guaykuru*, Cerrado, cebos aromáticos.

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