

## ORCHID BEES ENHANCE SEED SET PRODUCTION OF AN UNDERSTORY HERB IN THE WESTERN BRAZILIAN AMAZON

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**Abstract**—Bee pollination is an important ecosystem service related to the maintenance of many flowering plants. We evaluated the relationship between orchid bee foraging time and the density of flowering plants and whether visitation varied according to the sex and size class of bees, using *Calathea mansonis* as a model species. We monitored 10 plots between December 2009 and November 2010 in a forest fragment in Senador Guimard, Acre, Brazil. We counted the number of flowering plants and flowers per plant and the behaviour of the observed bees. Additionally, we compared the bagged and exposed inflorescences for self-compatibility analysis. We sampled 173 orchid bees from 13 species, with *Eulaema cingulata* as the most abundant visitor. *Eulaema* (large bees) were more effective pollinators than *Euglossa* (small bees). We also found *Eulaema polyzona* individuals feeding on a Marantaceae species for the first time. The time spent by the bees visiting flowers did not differ with the density of flowering plants or the number of flowers per plant. However, flowers exposed to visitors produced 35% more seeds and 15% heavier seeds than bagged flowers. Considering plant–bee interactions, orchid bees may increase gene flow and compensate for the clonal reproduction of this herb.

**Keywords**—*Calathea*, *Eulaema*, Euglossini, floral ecology, inflorescences

### INTRODUCTION

Insect pollination, performed primarily by bees, provides vital pollination services to wild and crop plants (Ollerton et al. 2011). Some studies show that seed set increases with wild bee visitation in both forested and crop systems (Steffan-Dewenter & Tscharntke 1999; Garibaldi et al. 2013), and the loss of pollinators may lower plant fitness by reducing the production of seeds and, at a larger scale, by compromising gene flow between isolated plant populations (Steffan-Dewenter & Tscharntke 1999; Greenleaf & Kremen 2006; Garibaldi et al. 2013).

The presence of pollinators may significantly increase seed set, even for autogamous plant species that are self-pollinated and not directly dependent on pollinators to produce their seeds (e.g., coffee, soybeans) (Roubik 2002; Ricketts et al. 2004; Milfont et al. 2013). Additionally, factors such as the absence of spatial co-occurrence in fragmented environments, changes in plant phenology, decreases in abundance and changes in the behaviour of pollinators, and even changes in the interactions between plants and their pollinators affect the amount and quality of the pollen deposited at the stigmas of flowers (Aizen

et al. 2002; Wilcock & Neiland 2002; Burckle et al. 2013).

In Neotropical forests, orchid bees (Apidae, Euglossini) are essential pollinators of Amazonian native plants (Ramírez et al. 2002). These bees are especially known for pollinating orchids; males are attracted and rewarded mainly with scents produced by the flowers (Dressler 1968; Janzen 1971; Schiestl & Roubik 2002). Nevertheless, female bees visit other plant species to collect resin, nectar, and pollen along specific foraging routes (traplines) so that the same flowers may be visited repeatedly in a particular sequence (Janzen 1971). Similarly, males forage for nectar to meet their energetic needs partly from the same plants used by females, and they pollinate the plants (Kroodsmá 1975; Wikelski et al. 2010). However, the foraging strategies adopted by males and females may differ significantly. Female orchid bees present a narrower foraging area than males (Williams & Dodson 1972), while there is evidence that males have transient and nomadic foraging behaviour (Dodson 1970).

Understory herbs are good models for phenological studies of plant species and observations of how floral visitors affect seed production due to their small size and many individuals flowering adjacent to others in the Amazon (Bruna & Kress 2002). Plants from the herb genus *Calathea* Meyer (Zingiberales: Marantaceae) have a wide range of pollination mechanisms, mainly characterized by secondary pollen presentation and irreversible movement of the style during insect visitation. As the flowers do not open spontaneously, the first forager must force its entrance with its head and forelegs to open the buds and then feed on the nectar from flowers (Pischtschan & Claßen-Bockhoff 2008). Given this single and irreversible movement, each flower has only one chance of being cross-pollinated (Kennedy 1978; Yeo 1993). As pollen adheres to the body of the pollinator, not directly from the anther but from the style, reproduction of these herbs occurs with the participation of specific pollinator groups (Kennedy 1978; 2000).

Several studies register the occurrence of orchid bee species belonging to *Eulaema* Lepeletier, 1841 and *Euglossa* Latreille, 1802 (Apinae: Euglossina) as the main floral visitors of *Calathea* (Horvitz & Schemske 1986; Kennedy 1978; Barreto & Freitas

2007). However, there is no information on the most effective visitor or the relevance of bee body size variation for plant success. Furthermore, considering their different behaviours (e.g., Dressler 1982a), visits performed by males and females are expected to differ, with females being more frequent than males searching for pollen and nectar.

Here, we evaluated the contribution of floral visitors to plant breeding using *Calathea mansonis* Körn (Zingiberales: Marantaceae), an understory herb that occurs from central America to northern South America (Forzza 2007). We hypothesized that bee behaviour is conditioned by sex, body size, and floral display present in each plot. The following predictions emerge from this hypothesis: 1) male bees will spend more time on flowers than females, 2) larger bees will be more effective than smaller ones, and 3) patches with more flowers will receive more visits and bees will spend more time than patches with fewer flowers. Additionally, we tested the contribution of pollinators to seed set of *C. mansonis* by comparing bagged and exposed flowers. Also, we tested variation in the effectiveness of visits based on body size (large vs. small) of bee visitors. We considered an effective visit when the bee touched the style and activated the trigger mechanism.

## MATERIALS AND METHODS

The study was conducted in a forest fragment at Catuaba Experimental Farm (CEF) in the municipality of Senador Guimard, state of Acre, Brazil (10°4'50.28 "S; 67°37'44.22 "W, DMS). The forest fragment sampled is a forest remnant of approximately 1,116 ha with a dense understory and a predominance of lianas and bamboo. The canopy is open and ranges from 20-40 m. Moreover, CEF also has secondary forests ("capoeiras") at different successional stages and pastures, mainly in the surrounding matrix of the remaining primary forest. The climate in the region is hot and humid, with two well-defined seasons: a rainy season between December and May, when the observations were conducted, and a dry season between June and November. The mean annual temperature varies from 22 to 24°C, and in the dry season (especially in July–August), it varies from 12 to 14°C. The mean annual precipitation is 1,973 mm, relative humidity ranges from 80 to 86%, and

climate classification according to the Köppen is defined as *Am* (tropical rainforest climate, Alvares et al. 2014; Instituto Nacional de Meteorologia-INMET 2013).

We observed visitor bees of *C. mansonis* flowers in 10 125 m<sup>2</sup> plots, separated by 90 to 800 m, all located within a forest area. Our choice of plot locations was guided by the high density of *C. mansonis* (> 10 plants per plot). Although it is difficult to determine whether plant clumps are separate organisms (i.e., genets) or genetically identical individuals (i.e., ramets) within the Marantaceae, here we considered a ramet as a plant (following Hoffman 2001).

We monitored the plots from December 2009 to November 2010. We made phenological observations from December 2009 to May 2010, and fruit and seed sets were observed from June to November 2010. We chose 10 individuals at random to make a morphological characterization of the species. We counted the number of flowering plants and inflorescences within each plot and the number of open flowers in each plant. We observed each plot for two non-consecutive days per month, from 7:00 to 16:30h.

We tested the contribution of flower visitors to fruit and seed sets by marking and bagging 20 inflorescences with flowers at pre-anthesis stages in a plot with a high abundance of flowering plants and exposing 20 plants within the same plot to visiting insects. We performed this process before anthesis to ensure that bagged flowers were not visited. Given the low frequency of flowering plants in some plots at the beginning of the experiment, we were not able to perform this test in the other nine plots. At the end of the flowering season (May 2010), we examined bagged and exposed inflorescences to determine whether fruit formation occurred. The fruits were collected, and the seeds were removed and weighed (fresh weight, 0.001 g precision). Here, we used the number of seeds per inflorescence to indicate reproductive success and effective pollination.

We observed bee visits in 73 plants, considering an arbitrary observation sequence of the plots, in 9 h observations in each plot per month (totalling 360 hours). We observed the behaviour of each visitor by registering (i) the time of its arrival on the flower, (ii) the duration of each visit (in

seconds), (iii) the type of resource collected, (iv) the number of flowers visited, and (v) whether the style was released during the visit. Here, we considered a “visit” when the bee landed on the flower petals. After leaving the flower and before collecting the bee, we followed the visitor within the same plot to check its visits to other nearby plants.

We considered the visits of the bees as “effective” when the bees could trigger the style of the plants and as “ineffective” when the bees landed on the flowers but did not trigger the style. Therefore, we classified the flowers we found with triggered style as “displayed.” We assumed that they were not available to transfer pollen to insect visitors.

We captured bees observed in flowers with an entomological net. Then, we took them to the Entomology Laboratory of the Universidade Federal do Acre (UFAC), where we pinned, dried, and identified them based on taxonomic keys (Dressler 1982b; Nemésio 2009; Moure 2003). Later, we confirmed the species identification with Dr. Danielle Storck-Tonon (Universidade do Estado de Mato Grosso). Finally, we deposited the specimens in the Entomological Collection of UFAC and the *C. mansonis* voucher specimens in the Laboratory of Botany and the Plant Ecology of UFAC.

We used one-way ANOVA to evaluate the variation in the number of flowers visited, the number of flowers produced, and the rate of flower visitors during the four months of *C. mansonis* flowering. We calculated the rate of flower visitors by using the total number of visits divided by the number of flowers available to the visitors (opened) and the time of observation based on each month of observation [number of visits/(number of flowers × observation time)]. We transformed all values (log + 1) if they showed non-normality or non-homogeneity of variances. An *a posteriori* comparison of means (Tukey test) was performed for each significant ANOVA. In cases where data transformation did not result in normality, we used non-parametric Kruskal–Wallis tests.

We lumped bees into two groups, considering the most abundant and diverse genera sampled, to compare the effectiveness of visits performed by orchid bees. We used the visual body size

difference criteria to classify them into small bees (*Euglossa* species) and large bees (*Eulaema* species). Since *Exaerete* Hoffmannsegg, 1817 species are cleptoparasitic, we considered the occurrence of these species on the flowers of *C. mansonis* as ineffective, and therefore, they were disregarded in these analyses. We used a chi-square test to test whether the larger bee group (*Eulaema*) could be responsible for the most effective visits compared to smaller bees (*Euglossa*). In addition, we tested the variation in the number of flowers visited according to visitor sex (male vs. female) and the weight and number of seeds produced between treatments (bagged vs. exposed inflorescences) using t-tests. In these analyses, we used the total number of males and females sampled on each plot ( $N = 20$ ), the number of seeds from each treatment ( $N = 40$ ), and the seed weight from bagged and exposed flowers ( $N = 228$ ).

Finally, we performed a linear regression analysis using the mean duration of visits per plot as a dependent variable and the number of flowering plants per square meter in each plot to verify if the time spent by bees during the visitation was due to the density of flowering plants. We checked all the assumptions of the tests and performed analyses using R 3.4 (R Development Core Team 2017).

## RESULTS

The phenological reproductive events (i.e., flowering, fruit, and seed set) of *C. mansonis*

occurred in the wet season, and during the remainder of the year, individuals showed only vegetative features. The density of plants was  $0.67 \pm 0.39$  (mean  $\pm$  SD) ramets/m<sup>2</sup> within all 10 plots. The flowering season started in January 2010 and ended in April 2010, and the number of flowers peaked in March (Fig. 1). *Calathea mansonis* is a rhizomatous herb with yellow flowers arranged in pairs at an inflorescence (Fig. 2a). Each plant produced one to two inflorescences (Fig. 2b) originating directly from the rhizome and  $11.7 \pm 2.4$  (mean  $\pm$  SD) open flowers daily. The flowers were asymmetric, trimerous, and light yellowish (Fig. 2c), without a perceptible odor. Anthesis was diurnal, starting around 8:00 until 11:00 h when all the flowers were open. Flowers remained open until about 14:00 h when they began to wilt. Fruit maturation occurred in the middle of the wet season (May–June), forming dehiscent capsules approximately 1 cm long (Fig. 2d) with up to three seeds. The seeds had a brown colour and an aril (Fig. 2e).

During our sampling period, we collected 173 orchid bees from 13 species belonging to four genera (*Eulaema*, *Eufriesea*, *Euglossa*, and *Exaerete*) (Table 1). From the total number of orchid bees, we sampled 77 males and 96 females. The visitors collected most frequently were *Eulaema cingulata* (Fabricius, 1804) (51.1%), *Euglossa* sp. (16.1%), and *Eulaema mocsaryi* (Friese, 1899) (8.9%). The less frequent were *Euglossa chalybeata* Friese, 1925, *Euglossa orellana* Roubik, 2004, and *Exaerete*

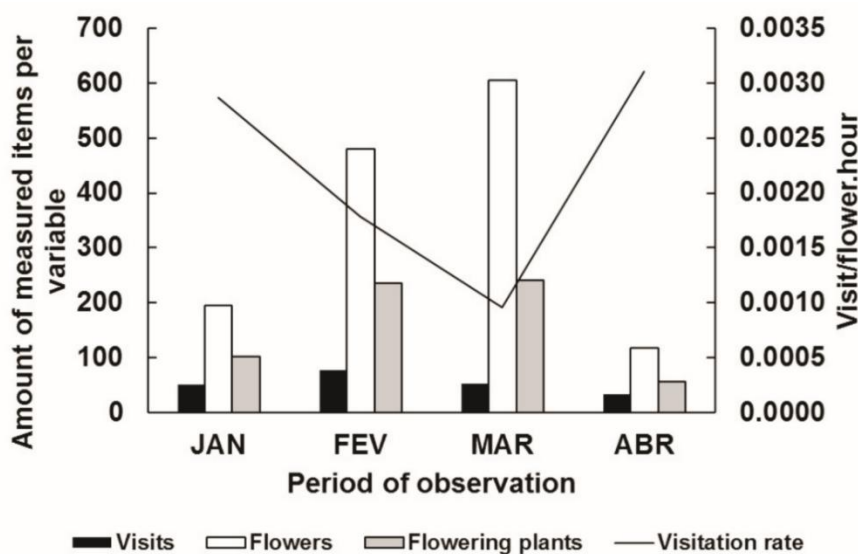
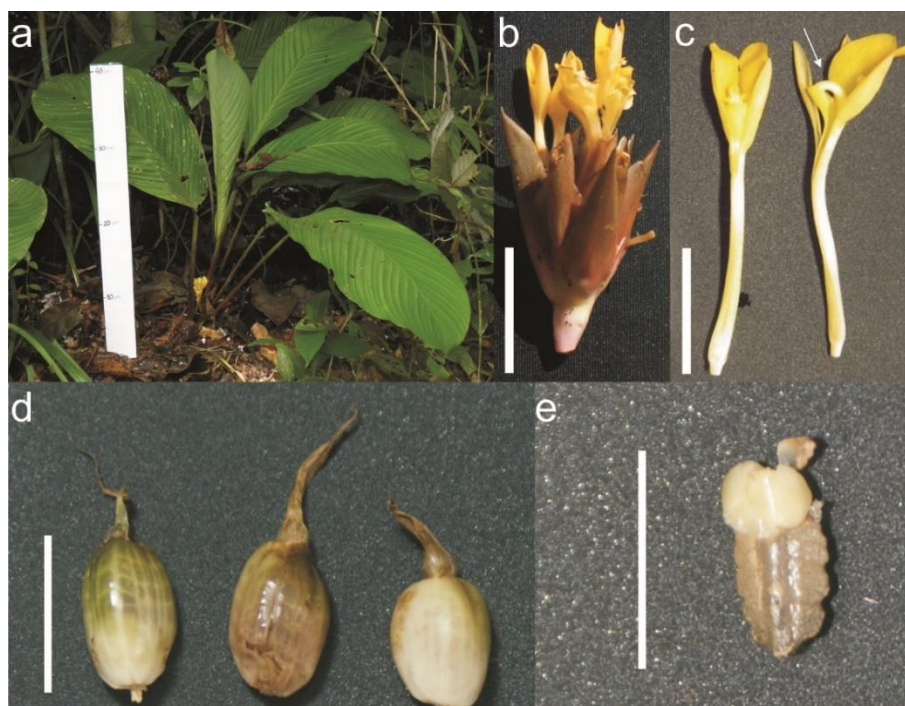


Figure 1. Variation in total visits, number of flowers, flowering plants (y-axis), and the rate of flower visitors (z-axis) during the observation period in *Calathea mansonis* plants



**Figure 2.** Morphological aspects of *Calathea mansonis*. a Plant. b Inflorescence. c Flowers in anthesis: nonvisited flower (left) and visited flower (right). d Fruits. e seed. Note the details of the moved style (arrow). Bars = 2 cm (b and c), Bars = 1 cm (d and e)

**Table 1.** The abundance of orchid bee species sampled in *Calathea mansonis*, the resource collected, and the mean time visitors visit 10 plots observed at Catuaba Experimental Farm, municipality of Senador Guimard, state of Acre, Brazil. “Resource” refers to the items collected by insects during the visits: N = nectar; P = pollen; N/P = nectar and pollen. “Time” is the mean duration of the visit of the insect to the flower, measured in seconds.

Species	Resource			Time (s)	SD	Total abundance
	N	P	N/P			
<i>Eufriesea flaviventris</i> (Friese, 1899)	X		X	15.7	0.69	4
<i>Euglossa chalybeata</i> Friese, 1925	X			22.0	0.31	1
<i>Euglossa ignita</i> Smith, 1854	X			7.0	0.42	2
<i>Euglossa imperialis</i> Cockerell, 1922	X			11.3	1.25	13
<i>Euglossa orellana</i> Roubik, 2004	X			8.0	0.31	1
<i>Euglossa piliventris</i> Guérin, 1845	X			4.5	0.42	2
<i>Euglossa</i> sp.	X	X	X	7.8	3.90	29
<i>Eulaema bombiformis</i> (Packard, 1869)	X			7.7	0.51	4
<i>Eulaema cingulata</i> (Fabricius, 1804)	X	X	X	12.0	4.87	92
<i>Eulaema meriana</i> (Olivier, 1789)	X			7.5	0.42	2
<i>Eulaema mocsaryi</i> (Friese, 1899)	X		X	8.3	1.65	16
<i>Eulaema polyzona</i> (Mócsary, 1897)	X			10.7	0.70	6
<i>Exaerete smaragdina</i> (Guérin, 1844)				4.0	0.31	1
Abundance						173
Richness						13

*smaragdina* Guérin, 1844 (0.5% for each species). Visitors observed in the flowers of *C. mansonis* collected nectar (in 89.5% of the total visits), pollen (1.4%), or both (2.7%). About 6.4% of the total visits were ineffective. The insect was present in the flower, but no resource was collected. We also observed visits from two other butterfly species

from the Pieridae ( $N = 1$ ) and Hesperidae ( $N = 6$ ) families. Still, since our focus was on the orchid bees and the number of visits we observed was low, we disregarded these visits in our analyses.

An average of  $6.7 \pm 0.6$  (mean  $\pm$  SD) flowers was visited per day in each plot, and a single insect



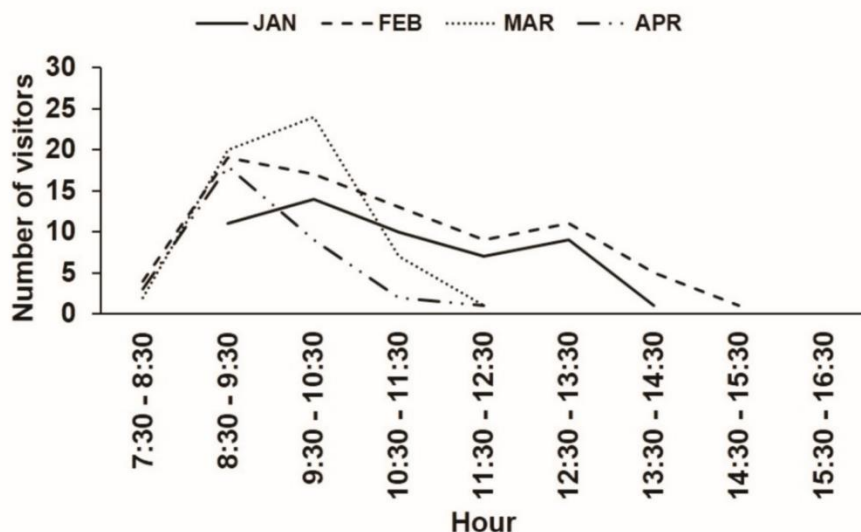


Figure 3. Variation in the number of insects that visit *Calathea mansonis* flowers during the day in a forest remnant, Senador Guiomard municipality, state of Acre, Brazil.

landed in 1–2 flowers during our observations ( $1.32 \pm 0.59$ ; mean  $\pm$  SD). During the visits, all the bee species of both sexes showed similar behavioural patterns, with a time of overflight of about  $10 \pm 3.36$  (mean  $\pm$  SD) seconds. Initially, the bee alighted, holding itself up with the front legs, and inserted the glossa into the corolla tube to collect nectar. During this movement, both male and female orchid bees move the style forward, taking the pollen grains deposited before in the stigma and sticking them to its glossa. In this case, pollen-gathering behaviour was incidental (or passive), which is pollen that accumulates on bees as they forage for nectar (Portman et al. 2019). As a result, the pollen was deposited on the stigma, and the pollinator was considered effective when the bee inserted its glossa loaded with pollen into other flowers. Bee visits were generally more intense in the early hours, concentrating in the morning, with a peak between 8:30 and 10:30 a.m., although we also recorded visits until 3:30 p.m. In the first month of observation, the bees visited the flowers for long periods during the day, with visits in the afternoon (Fig. 3).

There was no statistically significant difference in the number of *C. mansonis* flowers visited by bees ( $F_{(3,36)} = 1.865$ ,  $P = 0.153$ ), nor in the rate of flower visitors (Kruskal-Wallis test:  $H_{(3, N = 40)} = 4.570$ ;  $P = 0.206$ ) across months. However, we found an influence of the month on the variation in the number of flowers ( $F_{(3,36)} = 7.015$ ,  $P < 0.001$ ). Although flower number was higher in March (Fig. 4), the differences were only significant

compared to January and April (Tukey's HSD,  $P = 0.023$  and Tukey's HSD,  $P = 0.003$ , respectively) and between February and April (Tukey's HSD,  $P = 0.009$ ) (Fig. 4). The other comparisons were not statistically significant.

Visits of large *Eulaema* bees resulted in more triggered flower styles than small *Euglossa* bees ( $\chi^2 = 8.650$ ;  $df = 1$ ;  $P = 0.003$ ) (Table 2). We found *Eulaema cingulata*, *E. mocsaryi*, and *Euglossa* sp. in the flowers of *C. mansonis* throughout the flowering season. We observed *E. chalybeata*, *E. orellana*, and *E. smaragdina* only in February (Fig. 5). There was no variation in the number of flowers visited by Euglossini males and females ( $t = -0.786$ ,  $df = 18$ ,  $P = 0.441$ ), and the mean time spent by bees during visitation was not related to the density of flowering plants in the plots ( $r^2 = -0.041$ ,  $F_{(1,8)} = 0.643$ ,  $P = 0.445$ ).

On average, non-bagged flowers produced 35% more seeds than bagged ones (non-bagged:  $9.7 \pm 10.97$  seeds vs. bagged:  $1.7 \pm 2.39$  seeds (mean  $\pm$  SD);  $t_{\text{sep variance}} = 3.190$ ;  $df = 20.800$ ;  $P = 0.004$ ). Similarly, seeds from non-bagged flowers were about 15% heavier than those from bagged flowers (non-bagged:  $0.045 \pm 0.009$  g vs. bagged:  $0.039 \pm 0.009$  g;  $t = 3.024$ ,  $df = 226$ ,  $P = 0.003$ ; mean  $\pm$  SD).

## DISCUSSION

This study found that *C. mansonis* exhibited an improvement in seed yield (increased quantity and weight) due to visits of species of Euglossini bees. This plant species has only one flowering period

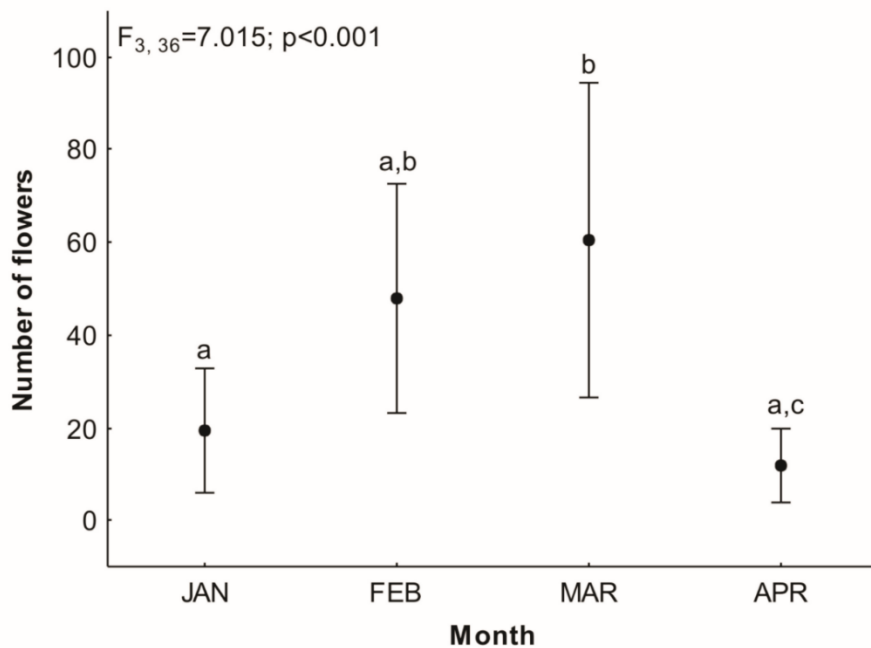


Figure 4. ANOVA results for the number of flowers observed during the flowering period of *Calathea mansonis*, Senador Guimard municipality, state of Acre, Brazil. Equal letters designate that they are not statistically different according to a post-hoc Tukey test. The variable was transformed (log +1), but the graph presents untransformed data. Vertical bars denote 95% of confidence intervals with means weighted.

during the wet season, lasting four months (from January to April). Its anthesis starts in the morning, during which we observed most flower visits. However, we recorded some visits in the afternoon. Orchid bees consumed nectar from the flowers and frequently visited more than one flower in the same resource patch. Contrary to what we expected, the time spent by orchid bees during each visit was not related to the number of floral resources available in the environment since we found a higher rate of flower visitors in the period with a lower number of flowers. Furthermore, males and females did not show a preference pattern for visiting flowers of this species, and both sexes collected food in the flowers of *C. mansonis* with the same frequency.

Here, we recorded a higher number of orchid bee species than in other surveys carried out with *Calathea* species in Brazil (Barreto & Freitas 2007) and other species of the genus with similar flowers in Central America (Kennedy 1978, 2000; Kress & Beach 1994). For example, for *Calathea ovandensis* Matuda, *Eulaema cingulata* and *Euglossa* spp. were the most representative pollinators, besides *Eulaema polychroma* Mocsáry, 1899, *Exaerete smaragdina*, and *Rhathymus* sp. Lepeletier & Serville, 1828 (Apinae: Rhathymini) (Schemske & Horvitz 1988). It is important to note that although we recorded *E. smaragdina* in the flowers of *C. mansonis*, this species has parasitic behaviour in the nests of *Eulaema nigrata* Lepeletier, 1841 (Garófalo & Rozen Jr 2001) and *Eufriesea surinamensis*

Table 2. Visitation types based on incidental pollen collection realized by orchid bees on *Calathea mansonis* flowers (YES = visits resulted in the release of the style; NO = visits did not result in the release of the style; DISPLAYED = visits in flowers with style already released). The bees are grouped by genus.

	<i>Eulaema</i>		<i>Euglossa</i>		<i>Eufriesea</i>		<i>Exaerete</i>	
	Visits	% of total visits	Visits	% of total visits	Visits	% of total visits	Visits	% of total visits
Release of style								
YES	52	31.32	10	6.02	2	1.2	0	0
NO	35	21.08	23	13.86	1	0.6	0	0
DISPLAYED	29	17.47	13	7.83	0	0	1	0.6
Total visits	116		46		3		1	

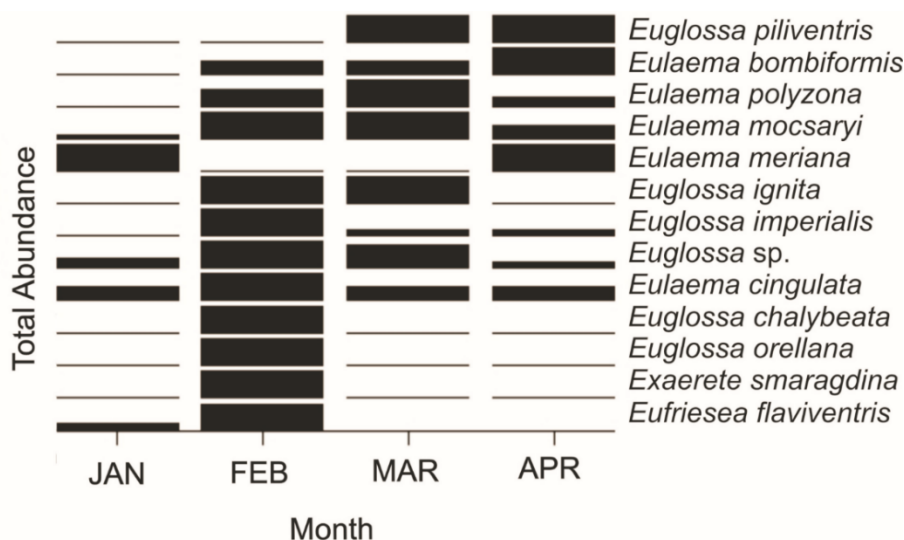


Figure 5. The abundance of *Calathea mansonis* visitors during its flowering months in the municipality of Senador Guimard, state of Acre, Brazil

Linnaeus, 1758 (Dodson & Frymire 1961). Thus, here we considered the visits from this bee casual since we recorded only a single individual and did not observe it collecting floral resources. Even so, this is the first time *Eulaema polyzona* Mocsáry, 1897, was recorded feeding on a Marantaceae species. Females of this species have been recorded as flower visitors of at least five plant species in America (Ramírez et al. 2002). Here, we found both males and females (three individuals of each sex) collecting nectar in *C. mansonis* flowers.

Our results show that orchid bees feeding in flowers behave according to the usual pattern of activity found for the group, with a high number of activities during the mornings, even though most studies addressing the patterns of orchid bee activity usually consider only individuals sampled with scent baits (Oliveira 1999; Farias et al. 2007; Storck-Tonon et al. 2009). A possible explanation for this pattern of activity is that a high amount of nectar is produced at the beginning of the day, with the highest mean volume found between 7:00 and 9:00 a.m. in the flowers of *Calathea cylindrica* (Roscoe) K. Schum., for example (Barreto & Freitas 2007). Orchid bees tend to visit flowers with less concentrated nectar compared to other long-tongued bee species. Although both quantity and concentration of nectar were not measured in this study, it is possible that the frequency of visits made by male and female orchid bees is related to the different energy requirements that bees may have (Roubik et al. 1995).

We consider the style trigger to be a characteristic of pollination success. However, the number of pollen grains deposited on a stigma from a single visit is the most robust measure of pollinator effectiveness for a particular plant species and visitor pairing (Ne'eman et al. 2010). Here, we found that large *Eulaema* bees are more effective pollinators of *C. mansonis*; notably, *E. cingulata* was the most abundant visitor to our plant. Similar results were also found for other species of Marantaceae, such as *Ischinosiphon gracilis* Koern and *C. cylindrica* flowers, with *E. cingulata* as the most effective pollinator (Barreto & Freitas 2007; Leite & Machado 2007). Despite the superior efficiency of large bees for other plant species, less efficient pollinators may be essential to plant reproduction, especially if they are the most frequent visitors (Sahli & Connor 2007). However, further studies involving bee measuring and pollination behaviour s should be performed to guarantee they can effectively pollinate the addressed species.

There is no information distinguishing the number of flowers that males and females of orchid bees can visit while gathering food resources. On average, *C. cylindrica*, an understory herb from southeastern Brazil, had a higher flower visitation rate of about nine times that found in this study (Barreto & Freitas 2007). We attribute these differences to dealing with different *Calathea* species and bee species with other metabolic requirements. However, these patterns are still to be studied further. Due to the morphological



characteristics presented by the flowers of *Calathea* species, which do not open spontaneously and need to be forced open, the bees will generally spend two to three times their usual opening time working on a flower before abandoning it (Kennedy 1978). Furthermore, the energy requirements of bees can vary between males and females (Armbruster & McCormick 1990). Females search more frequently for resources to provision nests and, therefore, can make longer visits compared to males. However, the results found in this study do not allow us to confirm that orchid bees visit *C. mansonis* flowers only to collect nectar.

Contrary to our predictions that the number of visited flowers may increase with floral display, with more bees visiting patches with more flowers (Klinkhamer & Metz 1994), we found periods of low flower display responding to the highest rate of flower visitors. Alternatively, in the respective populations analysed, *C. mansonis* flowers could represent a free niche (due to floral complexity for other visitors), and orchid bees show a stronger preference for *C. mansonis* individuals, regardless of floral display. (Goulson 1999).

Although *C. mansonis* has vegetative propagation and forms its fruits spontaneously, we found that the presence of floral visitors increased the yield and seed weight compared to bagged flowers, which may favor the genetic diversity of floral patches (Horvitz et al. 2010). The difference in weight we found between seeds produced by flowers visited by orchid bees compared to isolated flowers indicates the importance of pollinators for *C. mansonis* for seed production. Past studies have already shown the importance of natural pollinators for seed production. For tomatoes, coffee, and sunflower, for example, the presence of wild bees and the co-occurrence of wild and honeybees substantially increase fruit production (Greenleaf & Kremen 2006; De Marco & Coelho 2004; Klein et al. 2003).

Our study adds information on the high diversity of visitors found in *C. mansonis*, which contrasts with other Marantaceae species pollinated by two to five insect species (Kennedy 1978; Barreto & Freitas 2007; Leite & Machado 2007). At the same time, considering the plant-bee interactions, orchid bees may increase gene flow, acting as a compensatory mechanism for clonal reproduction. Therefore, in a scenario of pollinator

loss, plant populations of *C. mansonis* may be directly affected by final reproductive output. This study also highlights the findings of previous work showing the importance of bee body size as a predictor of successful pollen transfer, considering the complexity of the flower structure presented by Marantaceae flowers. However, we used a simple grouping of bees based on the visual size variation between them. Finally, since Marantaceae species have broad distributions in the Neotropics, a region where orchid bee assemblages are being negatively affected by deforestation and habitat fragmentation (Brosi 2009; Nemésio 2013; Tonhasca Jr et al. 2002), new studies may shed more light on the importance of orchid bees for the pollination of another Amazonian herb species.

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#### AUTHOR CONTRIBUTION

Concept and design TFB & EFM, data collection TFB, data analysis TFB & DPS, writing TFB, edits and approval for publication DPS, FALC, MMM & EFM.

#### DISCLOSURE STATEMENT

The authors declare no conflict of interest.

#### DATA AVAILABILITY STATEMENT

The data used to write this article are not available online. Please contact the corresponding author for data requests.

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