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High diversity of bees detected in guarana crop and natural habitat due to the use of combined sampling methods

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Abstract. Bees are the main pollinators of native and agricultural plants. Identifying and knowing these insects responsible for the environmental service of pollination is essential for the maintenance and management of pollination in agricultural systems, especially in a high diversity biome as the Amazon rainforest. Some crops in this region are dependent of benefited by wild pollinators, especially native plants like guarana. To address methodological aspects of monitoring bee diversity, samplings were carried out in an agricultural environment (guarana crop, *Paullinia cupana*) surrounded by Amazon natural habitat at Manaus, Amazonas State. We used three combined methods (two passive traps: Malaise and yellow pan-traps, and one active: hand nets) in different periods, with the same number of samplings (12 each). In total, 4,143 native bees belonging to 171 species were sampled; 117 species (1,926 individuals) were collected with Malaise trap, 15 (91 individuals) with pan-traps, and 114 (2,126 individuals) through active sampling. Only seven species were common to all methods, 60 species on two methods and 104 species were unique to one sampling method (50 with Malaise, two with pan-traps, and 52 with hand nets). We reinforce the need for complementary sampling to known bee diversity as the best strategy here was the joint use of active samples and Malaise traps. Recently the concern with Amazon preservation has aroused worldwide interest, a fact that shreds of evidence the need for studies of biodiversity and taxonomy in several areas, since very little is known of this vast region.

Keywords: Amazon; Apoidea; Malaise; diversity; hand-netting; pan-trap.

The Amazon basin includes the largest remaining area of tropical rainforest, been usually considered as one of the most important ecological systems on earth (Foley *et al.* 2007). Kress *et al.* (1998) stated that one of the major challenges for environmental conservation in the next century would be the preservation of habitats rich in species as the Amazon basin. More than twenty years after their publication, we are facing biodiversity loss (Ellwanger *et al.* 2020), at the entire planet (Corlett *et al.* 2020; Rondeau *et al.* 2020). Impacts for this biodiversity loss comprises threats to our way of life that includes food security through biodiversity loss. In this sense, we know that pollination is one of the key environmental services for the environment and for sustainable agriculture, as about 75% of cultivated plants rely on cross-pollination for fruit and seed production (Klein *et al.* 2007). The main group of pollinators is the bees, due to their dependence on floral resources such as pollen and nectar.

According to the Discover Life Bee Species Guide and World Checklist (Ascher & Pickering 2018) about 20,350 bee species are recognized in the world, of these the Taxonomic Catalog of the Brazilian Fauna (CTFB) recognizes at least 1,781 for Brazil (Oliveira 2015). However, it is believed that less than half of the diversity of bees in the Neotropical region has been described. Pinheiro-Machado *et al.* (2002) published a state of knowledge of Brazilian bee surveys, pointing out that not a single bee community survey has ever been conducted in the Amazon region until 20 years ago. According to them, studies on local bee diversity in this ecosystem are from early collections made by Ducke (1906), and other studies on specific groups such as *Centris* (Morato et al. 1999) Euglossini (Oliveira & Campos 1995; Oliveira 1999) and Meliponini (Camargo 1970; Oliveira et al. 1995). More recently, several articles have drawn attention to the rich biodiversity of pollinators of Amazonian crops, such as Brazilian nuts (*Bertholletia excelsa*) (e.g., Maués *et al.* 2015, 2018), guarana (*Paullinia cupan*) (e.g., Krug *et al.* 2015; Krug *et al.* 2018; Oliveira *et al.* 2020), açaí (*Euterpe* spp.) (e.g., Campbell *et al.* 2018; Bezerra *et al.* 2020), and in protected areas as in the Carajás Reserve (e.g., Borges *et al.* 2020; Giannini *et al.* 2020) in Pará State.

In faunistic surveys and pollination studies, bees are usually captured while collecting food on flowers (Silveira *et al.* 2002). However, in several Amazon Forest spots, this is practically impossible due to the scarcity of flowering plants in the understory or even the difficulty to walk through vegetation (due to high plant density). One alternative is the use of flight interception traps, such as Malaise, that always collect bees, some of which are very rare in the collections, such as parasitic bees and species restricted to forest environments (Silveira *et al.* 2002). Alternatively, sampling on flowers that occur at forest border, near crop areas, whenever possible, could be a good strategy, since naturally, this field will exert a great deal of appeal to local species. Therefore, the use of alternative sampling methods to study biodiversity in places not yet surveyed and with little access or even in places already surveyed but by only one method can be an important choice to evaluate the bee community.

Guarana is an important and traditional Amazon crop that is exclusively grown in Brazil, with great economic and social importance, especially in the Amazon region, where it is cultivated by large and small producers (Tavares *et al.* 2005). Guarana is widely visited by insects, especially bees, the main pollinators (Schultz & Valois 1974; Escobar *et al.* 1984; Krug *et al.* 2015). Thus, this crop can be an excellent model to be sampled by different sampling methods to study its pollinator fauna. Here we evaluated the influence of different sampling methods on recording native bee diversity in an guarana crop area.

MATERIAL AND METHODS

Study area. We sampled the local community of native bees in an experimental guarana crop field and surrounding area at Embrapa Amazônia Ocidental. The crop field area had approximately 10 hectares and was located at km 29 of the AM 010 highway (2°53’29.19” S / 59°58’40.58” W), in Manaus, Amazonas State, Brazil (Figure 1). The immediate surroundings of the guarana plantation were composed of diverse ruderal plants; these plants also grew inside the crop, between the rows. The plantation was distant from the native vegetation about 30-50 meters, being characterized as native vegetation in an advanced stage of regeneration. The clime in the region is tropical humid, type AM, with an average annual temperature of 26.5 ºC (Köppen 1936). According to monthly historical series of 40 years of precipitation evaluated by Antonio (2017) the rainy season generally occurs between the months of January and June, when 65% of the total annual precipitation in the region occurs, with a monthly average of 279.05 mm in these months. The same authors also cite a notable reduction in rainfall between July and September, with a monthly average rainfall of 159.15 mm between July and December.

We used three methods of sampling, two passive and one active methodology, in different periods, however with the same number of samples (twelve) each, described below. As previously works with this crop indicates that native bees are the main pollinators (Schultz & Valois 1974; Escobar *et al.* 1984; Krug *et al.* 2015), we focused only on those bees and thus did not record the exotic bee, *Apis mellifera* Linnaeus on our samplings.

Passive samplings. Malaise type Townes (1972) and yellow pan-traps (Möerick type) (Figure 2A-B), were arranged in two distinct sampling lines, each one with three sampling points (see Figure 1B, in Schoeninger *et al*. 2019). The first sampling point was established outside the crop, more precisely within 50 meters inside the adjacent native vegetation, the second point at the edge of the crop, and the third within the crop. The distance between each collection point was 60 m. At each sampling point, a Malaise trap and four pan-traps were set up, which were arranged around Malaise at a distance of five meters, in a total of six Malaise traps and 24 pan-traps per collection point (Schoeninger *et al*. 2019).

Samples were taken every two weeks between September 2012 and February 2013, totaling 12 samplings. Each sampling remained in the field for four days before the samples were collected. The sampling period covered the flowering period of guarana, as well as the transition and beginning of the rainy season and less rainy season.

Malaise traps allows the capture of insects through flight

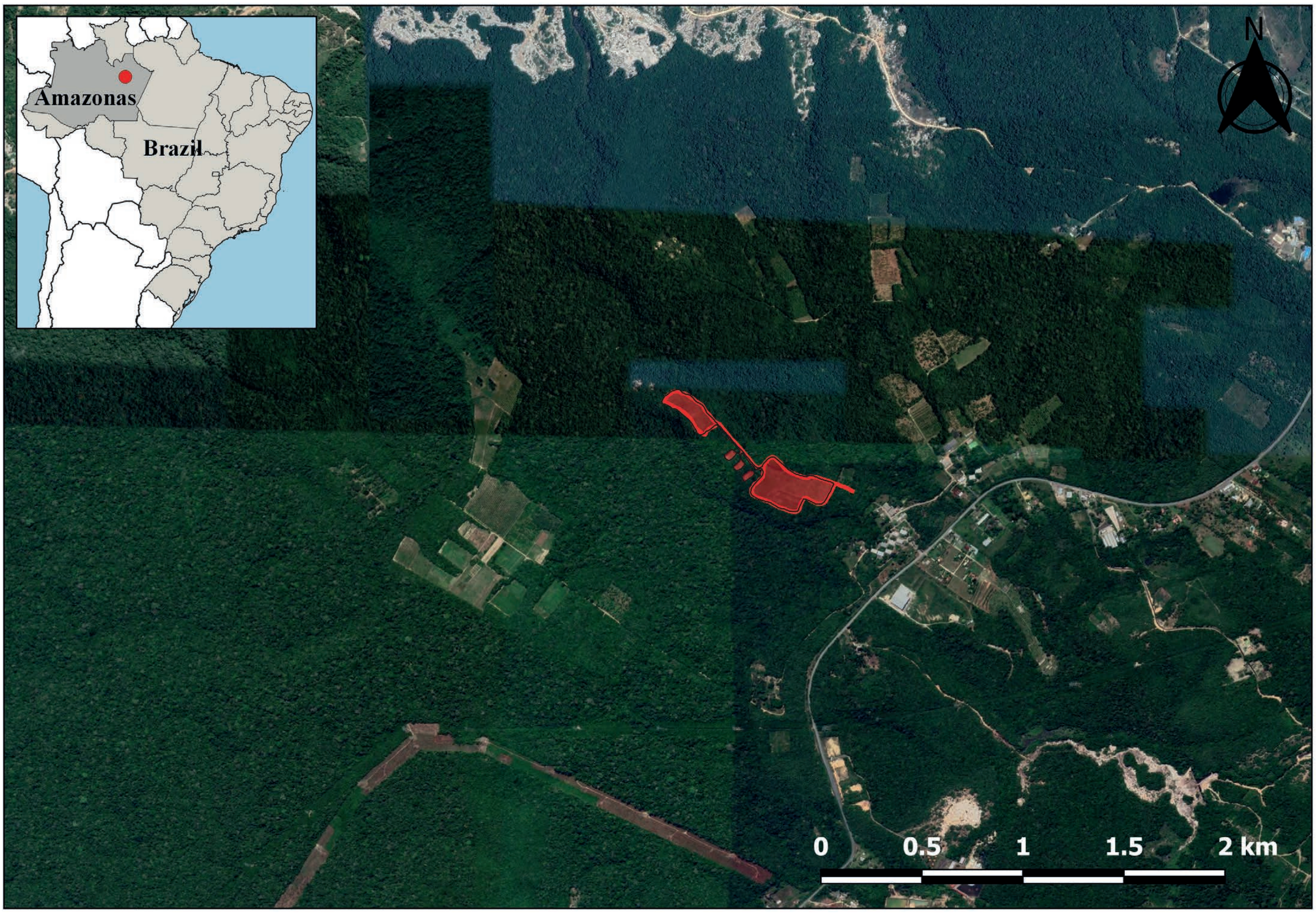

Figure 1. Location of the experimental area of guarana plantation (in red) (scientific name) at Embrapa Amazônia Ocidental and bee samplings site in Manaus/AM.

interception, besides being a permanent technique, works independently of attractiveness to obtain satisfactory results (Lewis & Whitfield 1999). Pan-traps (original white plastic trays) had the interior painted with yellow spray, once this attract insects as a function of the color, they measured 20 cm x 10 cm x 5 cm and were disposed on the ground, containing a solution of water and detergent, which traps the insects inside the solution.

Active samplings. Active samplings were carried out by two experienced collectors with entomological nets (Figure 2C), walking side by side. In the studied area, a collection transect of approximately 3.5 kilometers long was defined into the forest and in the edge, as well as into the guarana crop field. Throughout the transect, all flowering herbaceous-shrub plants were observed in search of floral visiting bees. Samplings were carried out monthly (2 days/month), always in the same transect performed completed each day, between June/2016 and May/2017, on two consecutive days, on the first day from 11 to 17h and the second day from 5 to 11h, totaling 12 hours of monthly collections. We use entomological nets up to 3 meters in length to reach flowers at different heights.

Identification and data analysis. All the captured bees were properly prepared and labeled before identification. The identification was carried out through specialized bibliographic material and comparison with species identified in the Invertebrate Collection of the National Institute of Amazonian Research (INPA). All individuals were identified to the lowest possible level and deposited at INPA Collection.

The diversity index of Shannon-Wiener (H') was used to quantitatively estimate diversity in the community by assigning greater weight to the rare species was calculated using the PASt program (Paleontological Statistics 1.32, Hammer *et al.* 2001). We also estimate the species accumulation curves (or collector's curves) for each sampling method through the 'BAT' package in R software (R Core Team 2019), using 1000 random permutations to the sampling order with the function *alpha.accum* (this function estimate the alpha diversity of a single site with accumulation of sampling units by returning a matrix of sampling units x diversity values) (Cardoso *et al.* 2015).

RESULTS

The combined results of all three methods revealed 171 native bee species in an agricultural environment (guarana crop) and close to the crop field, at the forest border. The 4,143 specimens of bees sampled belong to 55 genera and the five families present in Brazil (Table 1 and 2). Active samplings were responsible for 51% and 66% of the collected bee specimens and species, respectively, and malaise trap samplings were responsible for 46% and 68% of the collected bee specimens and species, and pan-traps were only responsible for 2% and 9%, respectively.

Only seven species were common to all methods, 60 species on two methods and 104 species were unique to one sampling method (50 to Malaise traps, 02 to pan-traps, and 52 active samples) (Table 3). The diversity index H' for the whole area was 3.83 while active samples (3.59) and Malaise trap (3.42), obtained similar results and, pan-traps (1.90), the lowest diversity index.

Species accumulation curves also evidenced the difference among methods (Figure 3). Active and malaise samplings seem to approach an asymptote while the pan-traps were not as effective in reflecting local biodiversity.

Figure 2. Sampling methods used to collect bees on the guarana crop (scientific name): A- active sampling with entomological nets; B - the passive trap of Malaise type Townes; C - yellow pan-traps (Möerick type).

Table 1. Summary of bee sampling efforts in one guarana crop (scientific name) field and surrounding natural habitat in Brazilian Amazon.

Bee sampling	Number of collected	Total	Methodology		
			Malaise	Pan-traps	Active
Total	specimens	**4,143**	**1,926**	**91**	**2,126**
	species	**171**	**117**	**15**	**114**
Andrenidae	specimens	1	1	0	0
	species	1	1	0	0
Apidae	specimens	3,341	1,380	34	1,927
	species	115	73	9	88
Colletidae	specimens	34	3	0	31
	species	5	2	0	3
Halictidae	specimens	731	522	57	152
	species	38	35	6	16
Megachilidae	specimens	36	20	0	16
	species	12	6	0	7

**Apis mellifera scutellata* Lepeletier was not included in the analyzes.

Table 2. Bee species and the number of specimens sampled by method (M, Malaise; P, pan-traps; A, Active) in one guarana crop field (scientific name) and surrounding natural habitat in the Brazilian Amazon.

Nº	Family	Species	M	P	A	Total
	Andrenidae					
1		*Oxaea flavescens* Klug	1	0	0	1
	Apidae					
2		*Ancyloscelis apiformis* (Fabricius)	0	2	0	2
3		*Aparatrigona impunctata* (Ducke)	380	0	114	494
4		*Camargoia camargoi* Moure	4	0	0	4
5		*Centris* (*Centris*) *flavifrons* (Fabricius)	1	0	0	1
6		*Centris* (*Centris*) *nitens* Lepeletier	0	0	1	1
7		*Centris* (*Centris*) *varia* (Erichson)	1	0	0	1
8		*Cephalotrigona femorata* (Smith)	0	0	5	5
9		*Cephalotrigona* sp.1	0	0	1	1
10		*Ceratina* (*Calloceratina*) sp.n. Mahlmann (*in prep.*)	4	0	0	4
11		*Ceratina* (*Ceratinula*) sp.1	0	0	1	1
12		*Ceratina* (*Ceratinula*) sp.2	0	0	9	9
13		*Ceratina* (*Crewella*) sp.1	12	0	45	57
14		*Ceratina* (*Crewella*) sp.10	0	0	1	1
15		*Ceratina* (*Crewella*) sp.11	0	0	1	1
16		*Ceratina* (*Crewella*) sp.2	3	0	0	3
17		*Ceratina* (*Crewella*) sp.4	8	0	5	13
18		*Ceratina* (*Crewella*) sp.5	6	0	0	6
19		*Ceratina* (*Crewella*) sp.6	6	0	0	6
20		*Ceratina* (*Crewella*) sp.7	2	0	10	12
21		*Ceratina* (*Neoclavicera*) *rotundiceps* Smith	1	0	0	1
22		*Epicharis* (*Hoplepicharis*) aff. *fasciata* Lepeletier & Serville	0	0	1	1
23		*Euglossa* (*Glossura*) *ignita* Smith	0	0	1	1
24		*Euglossa* (*Glossura*) sp.1	4	0	1	5
25		*Euglossa* (*Glossura*) sp.2	1	0	0	1
26		*Euglossa* (*Glossuropoda*) *intersecta* Latreille	0	0	1	1
27		*Euglossa* sp.1	0	0	2	2
28		*Eulaema* (*Apeulaema*) *mocsaryi* (Friese)	1	0	0	1
29		*Eulaema* (*Eulaema*) *meriana* (Olivier)	0	0	1	1
30		*Exaerete frontalis* (Guérin)	0	0	3	3
31		*Exaerete smaragdina* (Guérin)	0	0	4	4

To be continue...

Table 2. *Continue...*

Nº	Family	Species	M	P	A	Total
32		*Exomalopsis* (*Exomalopsis*) *analis* Spinola	0	0	1	1
33		*Exomalopsis* (*Exomalopsis*) *auropilosa* Spinola	159	17	48	224
34		*Exomalopsis* (*Exomalopsis*) *minor* Schrottky	97	9	31	137
35		*Exomalopsis* sp.1	3	0	1	4
36		*Exomalopsis* sp.2	0	0	1	1
37		*Florilegus* (*Euflorilegus*) *festivus* (Smith)	1	0	1	2
38		*Frieseomelitta paranigra* (Schwarz)	1	1	0	2
39		*Frieseomelitta portoi* (Friese)	1	0	2	3
40		*Frieseomelitta* sp.1	0	0	2	2
41		*Frieseomelitta* sp.n. F.F. de Oliveira (*in prep.*)	0	0	62	62
42		*Frieseomelitta trichocerata* Moure	0	0	52	52
43		*Geotrigona subnigra* (Schwarz)	0	0	18	18
44		*Lestrimelitta* aff. *glabrata* Camargo & Moure	6	0	0	6
45		*Lestrimelitta glaberrima* Oliveira & Marchi	3	0	0	3
46		*Lestrimelitta limao* (Smith)	2	0	0	2
47		*Lestrimelitta* sp.1	1	0	0	1
48		*Leurotrigona pusilla* Moure & Camargo	10	0	3	13
49		*Melipona (Eomelipona) amazonica* Schulz	8	0	3	11
50		*Melipona* (*Eomelipona*) *bradleyi* Schwarz	2	0	0	2
51		*Melipona* (*Eomelipona*) *illustris* Schwarz	2	0	0	2
52		*Melipona* (*Eomelipona*) *puncticollis* Friese	3	0	5	8
53		*Melipona* (*Michmelia*) *captiosa* Moure	1	0	1	2
54		*Melipona* (*Michmelia*) *fulva* Lepeletier	26	0	59	85
55		*Melipona* (*Michmelia*) *seminigra merrillae* Cockerell	0	0	22	22
56		*Melipona* (*Michmelia*) *seminigra seminigra* Friese	0	0	5	5
57		*Mesoplia rufipes* (Perty)	1	1	0	2
58		*Mesoplia* sp.1	0	0	1	1
59		*Nannotrigona melanocera* (Schwarz)	11	0	90	101
60		*Nannotrigona schultzei* (Friese)	5	0	11	16
61		*Nogueirapis minor* (Moure & Camargo)	0	0	2	2
62		*Nomada* sp.1	8	0	5	13
63		*Nomada* sp.2	17	0	0	17
64		*Osiris* sp.1	0	0	1	1
65		*Osiris* sp.2	0	0	5	5
66		*Oxytrigona obscura* (Friese)	3	0	0	3
67		*Paratetrapedia basilaris* Aguiar & Melo	1	0	36	37
68		*Paratetrapedia connexa* (Vachal)	0	0	8	8
69		*Paratetrapedia* sp.1	4	0	7	11
70		*Paratetrapedia* sp.2	4	0	0	4
71		*Paratetrapedia* sp.3	3	0	70	73
72		*Paratetrapedia* sp.4	0	0	38	38
73		*Paratetrapedia* sp.5	0	0	1	1
74		*Paratrigona euxanthospila* Camargo & Moure	5	0	4	9
75		*Paratrigona melanaspis* Camargo & Moure	17	0	11	28
76		*Paratrigona pannosa* Moure	14	0	34	48
77		*Paratrigona* sp.1	0	1	1	2
78		*Partamona auripennis* Pedro & Camargo	0	0	12	12
79		*Partamona mourei* Camargo	14	0	3	17
80		*Partamona pearsoni* (Schwarz)	6	0	0	6
81		*Partamona vicina* Camargo	26	0	18	44
82		*Plebeia alvarengai* Moure	10	0	3	13

To be continue...

Table 2. *Continue...*

Nº	Family	Species	M	P	A	Total
83		*Plebeia minima* (Gribodo)	45	0	1	46
84		*Plebeia* sp.1	36	1	0	37
85		*Ptilotrigona lurida* (Smith)	2	0	348	350
86		*Scaptotrigona bipunctata* (Lepeletier)	5	0	4	9
87		*Scaptotrigona* sp.n. Oliveira (*in prep.*)	34	0	1	35
88		*Scaura amazonica* Nogueira, Oliveira & Oliveira	8	0	43	51
88		*Scaura latitarsis* (Friese)	1	0	4	5
89		*Scaura longula* (Lepeletier)	7	0	1	8
91		*Tetragona dorsalis* (Smith)	18	0	9	27
92		*Tetragona handlirschii* (Friese)	3	0	16	19
93		*Tetragona kaieteurensis* (Schwarz)	0	0	14	14
94		*Tetragona essequiboensis* (Schwarz)	1	0	1	2
95		*Tetragona goettei* (Friese)	16	0	13	29
96		*Tetragona kaieteurensis* (Schwarz)	21	0	11	32
97		*Tetragonisca angustula* (Latreille)	55	0	53	108
98		*Tetrapedia* sp.1	0	0	1	1
99		*Trigona branneri* Cockerell	5	0	79	84
100		*Trigona cilipes* (Fabricius)	5	0	77	82
101		*Trigona* gr. *fuscipennis*	6	0	34	40
102		*Trigona guianae* Cockerell	169	1	203	373
103		*Trigona hypogea* Silvestri	0	0	76	76
104		*Trigona williana* Friese	9	0	21	30
105		*Trigonisca* cf. *dobzhanskyi* (Moure)	0	0	1	1
106		*Trigonisca* cf. *vitrifrons* Albuquerque & Camargo	17	1	1	19
107		*Trigonisca* sp.1	2	0	0	2
108		*Trigonisca* sp.2	1	0	0	1
109		*Tropidopedia duckeana* Aguiar & Melo	0	0	4	4
110		*Tropidopedia eliasi* Aguiar & Melo	0	0	11	11
111		*Tropidopedia* gr. *pallidipennis*	0	0	1	1
112		*Tropidopedia guaranae* Mahlmann & Oliveira	3	0	0	3
113		*Xylocopa* (*Neoxylocopa*) *aurulenta* (Fabricius)	2	0	2	4
114		*Xylocopa* (*Neoxylocopa*) *frontalis* (Olivier)	0	0	1	1
115		*Xylocopa* (*Neoxylocopa*) *tegulata* Friese	0	0	10	10
116		*Xylocopa* (*Schonnherria*) sp.1	0	0	1	1
	Colletidae					
117		*Colletes* sp.1	2	0	0	2
118		*Hylaeus* (*Gongyloprosopis*) *orbicus* (Vachal)*	0	0	12	12
119		*Hylaeus* (*Hylaeopsis*) sp.1	1	0	0	1
120		*Hylaeus* sp.1	0	0	18	18
121		*Ptiloglossa* sp.1	0	0	1	1
	Halictidae					
122		*Augochlora* sp.1	15	0	53	68
123		*Augochlora* sp.10	1	0	0	1
124		*Augochlora* sp.2	8	0	7	15
125		*Augochlora* sp.3	4	0	7	11
126		*Augochlora* sp.4	4	0	3	7
127		*Augochlora* sp.5	112	1	0	113
128		*Augochlora* sp.6	3	0	0	3
129		*Augochlora* sp.7	1	0	0	1
130		*Augochlora* sp.8	1	0	0	1

To be continue...

Table 2. *Continue...*

Nº	Family	Species	M	P	A	Total
131		*Augochlora* sp.9	4	0	0	4
132		*Augochloropsis cupreola* (Cockerell)	81	4	31	116
133		*Augochloropsis hebescens* (Smith)	0	0	7	7
134		*Augochloropsis* sp.1	4	0	1	5
135		*Augochloropsis* sp.2	21	0	0	21
136		*Augochloropsis* sp.3	11	0	1	12
137		*Augochloropsis* sp.4	91	0	11	102
138		*Augochloropsis* sp.5	1	0	0	1
139		*Augochloropsis* sp.6	5	0	0	5
140		*Augochloropsis* sp.7	2	0	0	2
141		*Habralictus* sp.1	2	0	0	2
142		*Lasioglossum* (*Dialictus*) sp.1	80	34	13	127
143		*Lasioglossum* (*Dialictus*) sp.2	2	2	0	4
144		*Lasioglossum* (*Dialictus*) sp.3	2	0	1	3
145		*Lasioglossum* (*Dialictus*) sp.4	1	0	0	1
146		*Megalopta amoena* (Spinola)	1	0	5	6
147		*Megalopta genalis* Meade-Waldo	2	0	0	2
148		*Megalopta mura* Santos & Melo	1	0	0	1
149		*Megalopta sodalis* (Vachal)	0	0	7	7
150		*Megaloptidia nocturna* (Friese)	1	0	2	3
151		*Neocorynura* sp.1	2	0	0	2
152		*Neocorynura* sp.2	1	0	0	1
153		*Neocorynura* sp.3	1	0	0	1
154		*Pereirapis* cf. *semiaurata* (Spinola)	3	15	2	20
155		*Pereirapis* sp.2	0	1	0	1
156		*Pseudaugochlora flammula* Almeida	1	0	0	1
157		*Pseudaugochlora graminea* (Fabricius)	47	0	1	48
158		*Stilbochlora* aff. *eickworti* (Engel, Brooks & Yanega)	3	0	0	3
159		*Temnosoma* sp.1	3	0	0	3
	Megachilidae					
160		*Anthodioctes* cf. *santosi* Urban	0	0	1	1
161		*Anthodioctes* sp.1	1	0	0	1
162		*Coelioxys* sp.1	0	0	1	1
163		*Megachile* (*Pseudocentron*) *curvipes* Smith	0	0	2	2
164		*Megachile* sp.1	14	0	0	14
165		*Megachile* sp.2	1	0	7	8
166		*Megachile* sp.3	2	0	0	2
167		*Megachile* sp.4	1	0	0	1
168		*Megachile* sp.5	1	0	0	1
169		*Megachile* sp.6	0	0	3	3
170		*Megachile* sp.7	0	0	1	1
171		*Rhynostelis plesiognatha* Parizotto & Melo	0	0	1	1
	Total		**1926**	**91**	**2126**	**4143**

DISCUSSION

As far as we know, this is one of the first great native bee lists using more than one sampling methodology associated with a native crop in an area with a crop and surrounding forest and open secondary vegetation in the Brazilian Amazonia and probably the first broad survey of a local bee fauna in the Amazon region since Ducke (1906). In spite of differences in the collection periods and epoch for passive and active methods, it is assumed that the results, especially the high number of species recorded exclusively by malaise and by active methods, stress the importance of using different sampling methods to get highest possible diversity. Increasing occurrence records for places where we lack species occurrence and distribution may increase our reliability on conservation studies (Hortal *et al.* 2015).

Gonçalves & Brandão (2008) using the same three sampling methods that we use in Atlantic Forest, found out that the most effective technique in capturing bees was the trap Malaise (84 species collected), followed by hand net sampling (39 species) and yellow pan-traps (Moericke) captured only five species. Though Krug & Alves-dos-Santos (2008) discovered that yellow pan traps were useful in bees sampling in an Araucaria forest (72 species were collected in pantraps and 130 species were sampled with hand net), they did not use Malaise traps. In this work we found a close number of species being collected with Malaise and hand net, while pantraps capture few species when compared to the two previous methodologies.

Table 3. The proportion of common and unique species in each method of sampling expressed in percentage.

	Malaise	Pan-traps	Active	Unique	H’
Malaise	0	2.3	32.2	29.2	3.42
Pan-traps	2.3	0	0.5	1.2	1.9
Active	32.2	0.5	0	30.5	3.59

As stated by Michener (1979, 2007) that tropics present the lowest diversity when compared to other regions, a scenario also described by Orr *et al.* (2021), but both studies are limited by the scary knowledge of bee diversity in tropical forests, for example the really limited number of local bees diversity surveys in the Brazilian Amazonia (see above and Pinheiro-Machado *et al.* 2002) provide those records can be more complex.

Until now, 773 species of bees are currently recognized for the entire northern region of Brazil (seven states of the Federation: Acre, Amapá, Amazonas, Pará, Rondônia, Roraima, and the Tocantins), which correspond to 43.4% of the species registered for the country. Considering those numbers, we could say that we sampled approximately 20% of the species richness known for this region (even considering possible new species or new records among the undetermined species recorded here). This relatively expressive representation becomes even more evident if we take into account that the study was carried out in a small sampling site with the anthropic influence of agriculture and near a large urban center and for a restricted period. Legal Amazon has an approximate surface area of 5,015,067.75 km², corresponding to about 58.9% of the Brazilian territory (IBGE 2021).

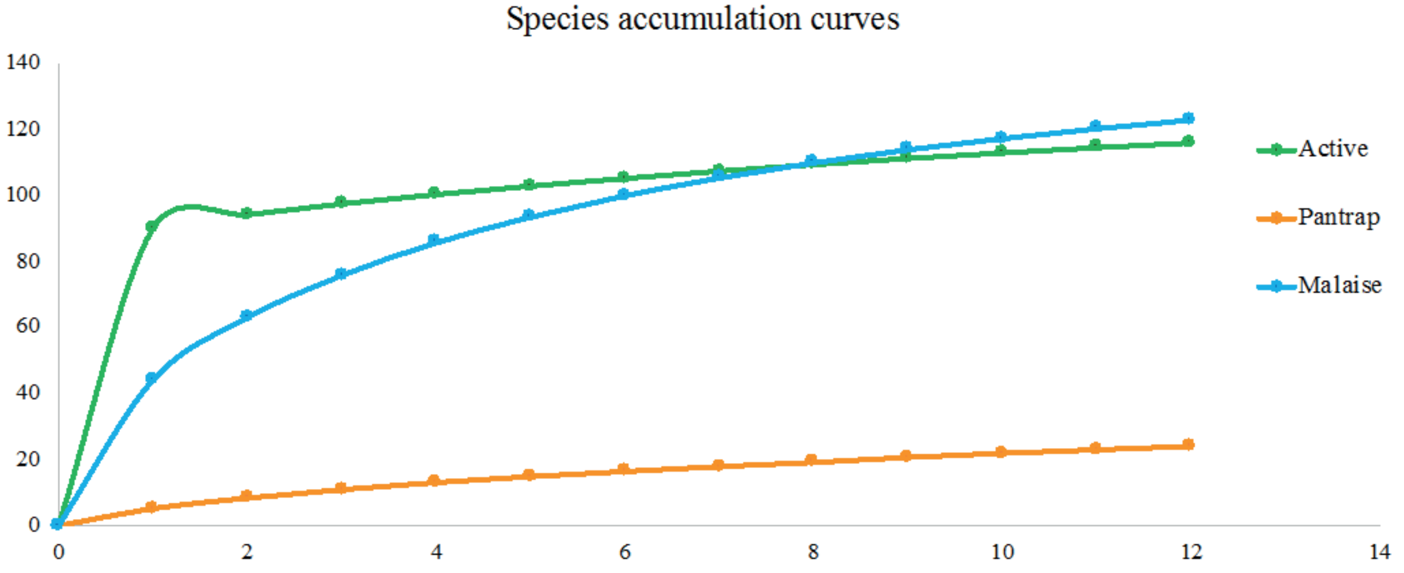

Figure 3. Species accumulation curves for each of the sampled methods used in one guarana crop field and surrounding natural habitat in the Brazilian Amazon.

Therefore, when we think on the classical biogeographic distribution pattern of bee species on a global scale, the regions with the highest bee diversity are the temperate dry ones (Michener 1979, 2007; Silveira *et al.* 2002). Even though the same authors pointed out that the bee fauna in the Amazon region remains essentially unknown. Intensive inventories of local bee faunas in the amazon region, especially if representing different regions and landscapes, should contribute to minimize this limitation. Moreover, we sampled 171 bee species and morphospecies, probably some of these not yet described, and a new record for Brazil (*Hylaeus orbicus*, Mahlmann *et al.* 2020). The hardship of access to many parts of the Amazon forest, high costs of mobility, and distance from research centers are the main factors that contribute to this lack of knowledge. Borges *et al.* (2020) recorded a total of 222 species in Carajás, representing nearly 80% of the bee fauna in Pará State and 33% of these bees (at least at the generic level) have been identified as crop pollinators. Absy *et al.* (2018) made a review of studies previously conducted in the Brazilian Amazon using pollen analyses of 48 stingless bees, revealing a wide diversity of this species group. The same fact was also found in our study, where we sampled 61 stingless bee species.

Silveira *et al.* (2002) and Krug & Alves-dos-Santos (2008) states that the best way to survey bee communities may vary according to location and logistics, but better results in the number of species are achieved when several methods are employed. In our study, we observed that the pan-traps did not present satisfactory results for the Amazon region, however, the active sampling and Malaise traps together obtained excellent results. Thus, the choice of bee sampling methodology for the Amazon region can be best defined in terms of logistics of location. Despite the temporal and methodological differences, a large number of specimens and species were sampled and the results indicate that the best strategy to collect bee species in a broader way for the Amazon region is to jointly use active samples and Malaise traps. Therefore, other native bee surveys deserve attention in Amazon crops, especially considering different contexts (management and landscape).

Although many long-term insects and bee monitoring articles aiming to assess population declines are being published worldwide (Biesmeijer *et al.* 2006; Meiners *et al.* 2019; Sánchez-

Bayo & Wyckhuys 2019), there are still numerous regions with an absence of information. These gaps can be also aggravated by the lack of Brazilian policies to protect natural pollinators populations (Hipólito *et al.* 2021). In Brazil, pollinators contribute at least US$12 billion to total annual agricultural economics (Giannini *et al.* 2015), and due to many anthropic harms to biodiversity especially in the Amazon region (e.g., fires, loss of natural landscapes), we risk losing a lot of our biodiversity before we even know it. Our guarana fields were close to natural areas, probably supporting a higher bee diversity which in turn benefit guarana crops (Klein *et al.* 2007).

We found out that combined surveys with Malaise and hand net contribute to produce a better representativeness of local bee faunas in the Amazon region, as already found for other regions.

We observed a relatively high diversity of wild bees in a local bee fauna adjacent to an agricultural area of guarana, even considering only an annual survey based on hand netting or malaise traps, demonstrate that if more similar collection efforts are undertaken in several sites in this huge region, especially in areas and phytophysiognomies not well studied until now, we probably will have a more realistic view of the Amazon bee diversity.

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