

Comparison of yellow pan, Malaise, and ethanolic traps for sampling parasitoid Vespoidea and other Hymenoptera in a semideciduous forest fragment of the Brazilian Atlantic Rainforest

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Abstract

Parasitoid wasps within the Vespoidea superfamily have been chronically under surveyed, thanks to the bigger attention given to the social species within that group or to the other parasitoids within the megadiverse Parasitica infraorder. To address that, we test a new sampling technique for the capture of parasitoid Vespoidea and other Hymenoptera in comparison to other two well established designs. Between the consecutive dry and wet seasons of 2014-2015 we placed sets of Malaise (MT), yellow pan (YPT) and ethanolic (ET) traps in a fragment of semideciduous Brazilian Atlantic Rainforest. We hypothesised that MT is the most efficient method for sampling Hymenoptera families and lower taxa of Vespoidea, while YPT and ET would be more efficient in attracting specific taxa, given their characteristic luring mechanisms. We calculated taxa accumulation curves to evaluate expected richness. Average Taxonomic Distinctiveness (Δ^+) was used as the continuous dependent variable in two-way ANOVAs. Faunal similarity was inspected through nonmetric multidimensional scaling (NMDS). All tests were performed taking season and trap design as explanatory variables. MT was the most efficient in sampling total richness and Δ^+ of Hymenoptera and Vespoidea lower taxa. It captured all 39 families recorded in the present study and 60 out of the 73 Vespoidea lower taxa. YPT tended to lure Diapriidae, Dryinidae, Ponerinae and Myrmicinae ants and a few taxa of Pompilidae and Mutillidae, proving its efficiency in sampling wingless and short-flying hymenopterans as well as the predators and parasites of other non-grass feeding insects. ET, although successful in estimating Hymenoptera Δ^+ , did not have a sufficient capture rate to give a precise estimate of total richness. It attracted, however, the social wasps *Polybia jurinei* Saussure and *Synoeca surinama* (L.), species traditionally captured by active search.

Synthesis and Applications: MT is confirmed as the most efficient capture method for Hymenoptera sampling and is recommended when a survey of the whole community is intended. ET was not successful in capturing any parasitoid Vespoidea as hypothesised but, as well as the YPT, it could be used as an alternative to active searches when the taxa listed above are the focus of the survey. These results provide a better understanding of passive capture methods for Hymenoptera sampling and can be considered in future surveys aiming to investigate their diversity, distribution and improve their conservation.

Key words: ethanolic trap, Hymenoptera, Malaise trap, passive capture, Vespoidea, yellow pan trap.

Introduction

Hymenoptera is one of the most diverse insect orders, if not the most diverse (Forbes *et al.*, 2018). It represents approximately 8% of the world's biodiversity (Davis *et al.*, 2010), counting with nearly 154000 species currently described, within 132 recognized families (Aguiar *et al.*, 2013). The estimate of the order's total diversity lays between 884000 and 1152000 species, most of it within the parasitoid families composing the paraphyletic infraorder Parasitica (Forbes *et al.*, 2018). With such amount of species yet to be described, collection methods for this group have been a matter of concern (Noyes, 1989; Marchiori and Penteado-Dias, 2002; Marchiori *et al.*, 2003; Fraser *et al.*, 2008). Given the wide range of behaviours and biology of Hymenoptera, all sampling techniques present a bias towards the capture of specific taxa, which requires diversification if a broad survey is intended (Noyes, 1989; Leong and Thorp, 1999; Russo *et al.*, 2011). However, the usage of multiple sampling techniques in the same study is impractical given time constraints, availability of labour force and financial resources, thereby requiring the adoption of the best cost-benefit option.

Aculeata, the monophyletic sister taxon of Parasitica, counts with more than 67000 of the currently described

hymenopteran species (Aguiar *et al.*, 2013). It also contains some parasitoid families that have been chronically under surveyed (i.e. Pompilidae, Tiphidae, Scoliidae, Bradynobaenidae, Rhopalosomatidae, Sapygidae, Sierolomorphidae and some Chrysididae families), given that studies within this group tend to focus on the more diverse families containing the social species (i.e. Formicidae, Vespidae and some Apoidea families) (Longino and Colwell, 1997; Lutinski *et al.*, 2008; Morato *et al.*, 2008). The only exception is the recent work by Vieira *et al.* (2017) that investigated the difference between the Malaise trap (MT) (Townes, 1972) and the yellow pan trap (YPT) on the sampling of velvet ants (Mutillidae), strict ectoparasitoids of other insects.

The MT works by flight interception and is the most generalist and commonly used passive capture technique for Hymenoptera sampling (Longino and Colwell, 1997; Campos *et al.*, 2000; Marchiori *et al.*, 2003; Lutinski *et al.*, 2008; Morato *et al.*, 2008; Noll *et al.*, 2012; Vieira *et al.*, 2017). However, it can be impractical due to its high price, weight or size (van Achterberg, 2009) and can be less efficient than active search (Silveira, 2002) or baiting (Noll and Gomes, 2009) for capturing social wasps (Polistinae). Moreover, parasitoid wasps from families like Tiphidae and Scoliidae are recognizably harder to

capture through MT (Skvarla *et al.*, 2021). Conversely, the YPT was first tested as an alternative for sampling the Hymenoptera community by Noyes (1989) in the tropical rainforest of Sulawesi and found to be efficient for capturing Proctotrupeoidea, Ceraphronoidea, Chalcidoidea and Aculeata. Further studies showed that pans are the recommended technique for monitoring Apidae, as they are affordable, easy to install and yield satisfactory results after 100 replicates (Lebuhn *et al.*, 2012). YPT, in particular, has successfully collected parasitoid wasps (Marchiori and Pentead-Dias, 2002; Marchiori *et al.*, 2003), velvet ants (Mutillidae) (Vieira *et al.*, 2017) and other Aculeata pollinators (Moreira *et al.*, 2016) in Cerrado grasslands and semideciduous forests of central Brazil. The attraction of Apidae, Mutillidae and parasitic families of Hymenoptera to YPT supports the hypothesis that the yellow colour attracts non-grass feeding insects and their respective predators and parasites (Kirk, 1984). This host/parasitoid hypothesis however has not been tested for the social and parasitic Vespoidea yet.

Following that same logic, and the high diversity of wasps that depend on beetles for their development (Forbes *et al.*, 2018), it can be hypothesised that the same attractive methods used to capture Coleoptera could also work for their Hymenopterans predators and parasites. Therefore, in this work we investigate the efficiency of the ethanolic trap (ET), used for sampling bark beetles, for the capture of their parasitoid Hymenoptera (e.g. Tiphidae and Scoliididae), in comparison to the well known methods describe above. The ET was proposed by Berti Filho and Flechtmann (1986) and an alternative design was tested by Murari *et al.* (2012) to capture bark beetles of the Scolytinae subfamily and related species in southern Brazil. ET works by mimicking the volatile components that attract these insects (Berti Filho and Flechtmann, 1986). Given that Scoliididae wasps are parasitoids of these beetles (Fernández and Sharkey, 2006) and that some Parasitica species are known to be attracted by these same components (Boone *et al.*, 2008), we hypothesised that these traps will also successfully attract the Vespoidea parasitoids.

In summary, this study aimed to evaluate the relative efficiency of ET, YPT, and MT for sampling parasitoid Vespoidea, and Hymenoptera in general. We hypothesised that (i) MT would be the most efficient method for sampling total richness and taxonomic distinctiveness, and that (ii) YPT and ET could indicate the occurrence of specific taxa through the host/parasitoid hypothesis, given their particular luring mechanisms: YPT for attracting terrestrial and parasitoid wasps of non-grass feeding insects and ET for attracting predators and parasites of bark beetles. We evaluated the efficiency of these sampling techniques at the family level and for lower taxa in the Vespoidea suborder in a fragment of semideciduous Atlantic Rainforest in São Paulo State, southeastern Brazil, with consideration of seasonal variation. We hope that the results presented here will improve the knowledge of passive capture alternatives for collecting specimens of Hymenoptera and assist with their effective monitoring and conservation.

Materials and methods

Survey area

This study took place at “Polo Regional do Desenvolvimento Tecnológico dos Agronegócios do Centro-Norte”, property of the São Paulo government, located at Pindorama municipality, São Paulo State, Brazil (21°13'12"S 48°55'04"W). Sampling was undertaken in a fragment of semideciduous Atlantic rainforest of approximately 108 hectares surrounded by plantations of sugarcane (*Saccharum* spp.), rubber trees (*Hevea brasiliensis*), and *Brachiaria* pasture (figure 1). Approximately 90% of the fragment's vegetation was at the intermediate succession stage by the time of the survey, while the last 10% was at the secondary or primary stages (Necchi Jr *et al.*, 2012; figure 1). The slope gradient is smooth and varies from 392 m to 438 m in the E-W direction (Necchi Jr *et al.*, 2012).

Climate is temperate with dry winters and hot summers (Cwa type, according to Peel *et al.*, 2007), with annual precipitation varying from 1100 to 1250 mm. There are two well-defined seasons. The wet spans from October to March, is responsible for 85% of the annual precipitation, with monthly temperature averages up to 30 °C; the dry extends from April to September, is responsible for the remaining 15% of rainfall, with monthly temperature averages down to 20 °C (Necchi Jr *et al.*, 2012).

Study design

Given the marked seasonality (Necchi Jr *et al.*, 2012), two sampling sessions were undertaken, one at the end of the dry season, in the month of October 2014, and another at the end of the following wet season, between March and April 2015. In both sessions, traps were left on site for four weeks, passing through weekly verifications and solution replacement. Three traps designs were tested: MT, YPT and ET (figure 2). Traps were distributed in modules of one MT surrounded by four YPT and two ET 20 to 50 m apart and placed at three different sampling sites trying to depict the diversity of micro-habitats within a semideciduous forest: advanced successional stage, ecotone, and riparian zone (respectively sites 1, 2, and 3 on figure 1). All sites were inspected for nests of social Hymenoptera prior to the placement of traps to reduce sample bias.

The MT design used in this study was an adaptation of the Townes model (1972), composed of a 6 m × 2 m fine weft net and a bottle at each of its upper corners containing 1 litre of 70% ethanol solution. Once intercepted, insects are attracted upwards by phototropism, falling into the bottle where they are preserved until the collector's visit. YPT consisted of yellow plastic plates of 10 cm diameter and 4 cm depth buried at ground level and filled with 320 mL of ethanol solution 50% and 10 mL of 1,2-propilenoglicol. Propilenoglicol reduces the evaporation of the ethanol solution, allowing preservation of the insects after capture (Marchiori *et al.*, 2003). Placing the traps at ground level was the original design proposed by Noyes (1989) and found to be efficient for the capture of mutillids (Vieira *et al.*, 2017), therefore this disposition was preferred over suspending them in the midstory. During the wet season, we added a flat yellow plate with

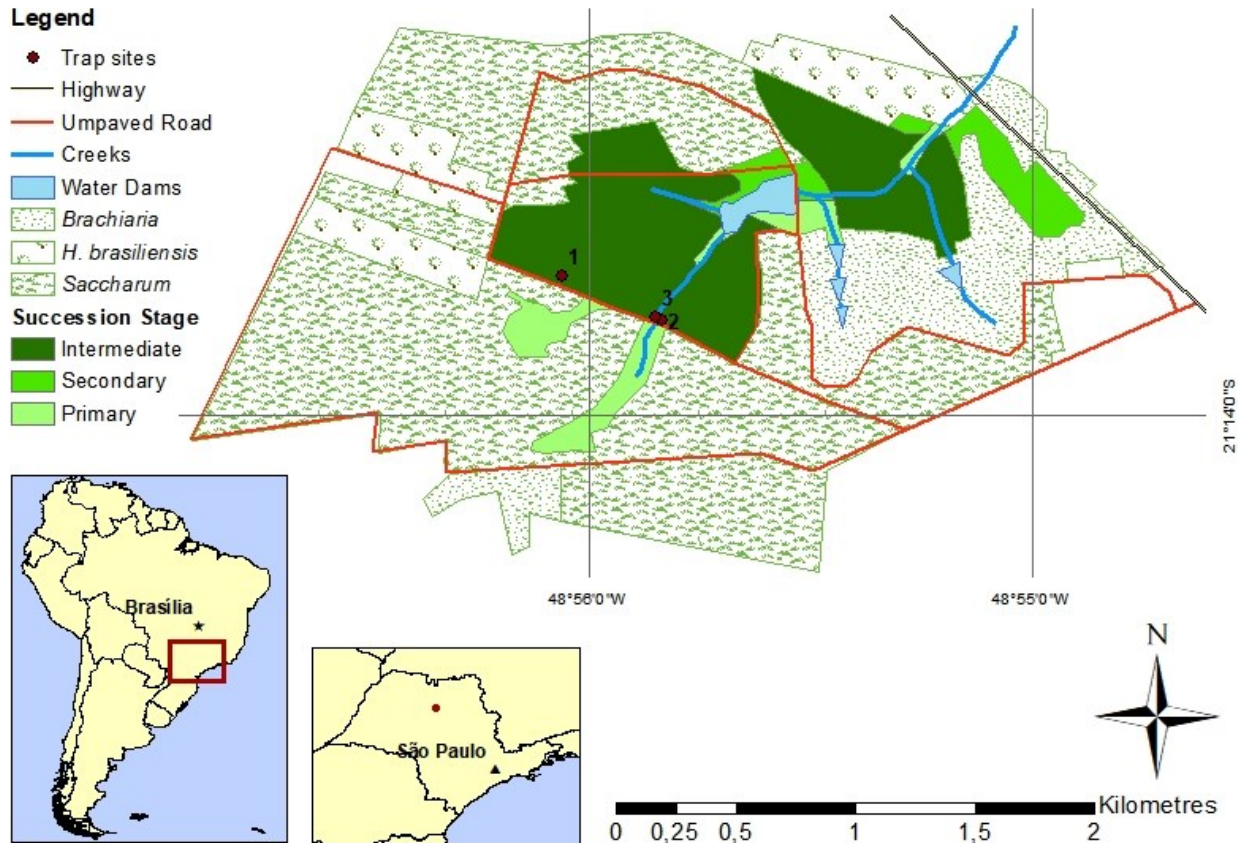


Figure 1: Polo Regional do Desenvolvimento Tecnológico dos Agronegócios do Centro-Norte, fragment of semideciduous Atlantic rainforest area highlighting the location of trapping sites and its surrounding matrix [Succession categories after Necchi Jr *et al.* (2012) classification, adapted to 2014–2015 composite satellite imagery].

a slightly wider diameter, sustained by two wooden sticks held 20 cm above the pan, preventing an inflow of rainwater. In areas of wind exposure, a rock was placed inside the pan to increase stability. There was no clear influence on the trap's efficiency due to these changes in design.

The ET used in this study followed the design of Berti Filho and Flechtmann (1986), being composed of two criss-crossed flight interception panels of 16 cm × 10 cm with a bottle in the centre filled with 35 mL of ethanol solution 96%. After being attracted by the solution and colliding with the panels, insects fall into a funnel and are directed to a lower bottle containing 350 mL ethanol solution 70% for preservation.

Storage and identification

Collected insects were brought to the Aculeata Laboratory of the “Instituto de Biociências, Letras e Ciências Exatas” (IBILCE) of São Paulo State University (UNESP) for triage and identification. Specimens were inventoried by date, trap design, trap site and stored in plastic vials containing 70% ethanol solution. Morphological keys were used to identify the Hymenoptera families (Rafael *et al.*, 2012), the genera of Mutillidae (Fernández and Sharkey, 2006) and Formicidae (Bolton, 1994; Fernández, 2003), the sub-genera and species of Pompilidae (Banks, 1946; 1947; Evans, 1961; 1965; 1966; 1973; Colomo de Correa, 1998; Vardy, 2005) and

the species of Tiphiidae (Allen, 1972), Scoliidae (Bradley, 1945; 1957) and Vespidae (Bohart and Stange, 1965; Richards, 1978; Cooper, 2000; Grandinete *et al.*, 2015). Sex and/or caste of Vespoidea specimens were recorded but not considered for statistical analyses. Qualitative considerations of sex and caste are presented in the discussion for the relevant species.

Statistical analysis

Sample units were considered as the weekly content of each trap and survey site. All analyses were performed twice, for the identified Hymenoptera families and for Vespoidea lower taxa alone. We calculated taxa accumulation curves using Mao's Tao sample-based rarefaction (Colwell *et al.*, 2012) to compare relative capture rates and expected richness of each trap design and sampling season. We calculated Average Taxonomic Distinctiveness (Δ^+) (Clarke and Warwick, 1998; 2001) for Hellinger transformed matrices of Hymenoptera families and Vespoidea lower taxa with varying length of steps between adjacent classes, to account for distinctiveness at different taxonomic levels. Δ^+ was then used as the continuous dependent variable in two-way ANOVA with sampling season and trap design as categorical independent variables and a Tukey test was applied to evaluate significance of the pairwise combinations of sampling season and trap designs (i.e. MT × YPT, MT × ET, and YPT × ET). Lastly, nonmetric multidimensional scaling



Figure 2: Photos of the three trap designs placed in the study area: (a) Malaise trap, (b) yellow pan trap, (c) ethanolic trap.

(NMDS) was applied to the Hellinger transformed community matrices, taking season and trap design as explanatory variables of faunal similarity. NMDS was calculated over Bray-Curtis dissimilarity matrices using three dimensions ($k = 3$). The best ordinations were chosen after reaching two convergent solutions or by the smallest stress after 100 random starts. Statistical analyses were performed in R 4.0.0 (R Core Team, 2018) using the 'vegan' package (Oksanen *et al.*, 2018).

Results

Trapping effort summed 12096 hours, capturing a total of 5089 Hymenoptera specimens belonging to 39 families (table 1), of which 38 were captured at the end of the dry season and 30 at the end of the wet (figure 3a). Trichogrammatidae was the only family not recorded at the end of the dry season (table 1). Δ^+ at the family level was significantly higher at the end of the dry season ($q = 6.64, p = 0.0097$). MT alone was responsible for capturing specimens of all 39 families compared to 24 and

Table 1: Relative abundance of Hymenoptera families captured in a fragment of semideciduous Atlantic Rainforest in southeastern Brazil, between 2014 and 2015, sorted by sampling season and trap designs.

Total	End of dry season			Families	End of wet season			Total
	ET	YPT	MT		MT	YPT	ET	
1973	22	727	1224	Formicidae	330	360	30	720
181	1	7	173	Ichneumonidae	199	7	1	207
136	1	27	108	Braconidae	165	9	1	175
85	1	32	52	Mymaridae	167	2	-	169
63	-	8	55	Platygastridae*	188	-	1	189
182	-	4	178	Pompilidae	13	1	-	14
98	1	27	70	Scelionidae	58	1	-	59
86	2	74	10	Diapriidae	33	16	-	49
16	1	2	13	Eulophidae	63	1	29	93
31	-	22	9	Ceraphronidae*	42	2	-	44
44	-	14	30	Figitidae	15	1	1	17
48	1	26	21	Pteromalidae	10	1	1	12
53	-	7	46	Crabronidae	4	-	-	4
41	2	15	24	Bethylidae	9	-	-	9
10	-	3	7	Chalcididae	32	2	2	36
28	2	2	24	Apidae	4	1	3	8
18	-	16	2	Dryinidae	5	8	1	14
12	-	2	10	Torymidae*	10	-	1	11
-	-	-	-	Trichogrammatidae*	20	-	-	20
17	3	-	14	Vespidae	2	-	-	2
17	-	4	13	Eupelmidae	1	-	-	1
5	-	1	4	Encyrtidae	7	1	-	8
11	-	1	10	Mutillidae*	-	-	-	-
1	-	-	1	Aphelinidae*	7	-	-	7
8	-	-	8	Perilampidae*	-	-	-	-
8	-	-	8	Sphecidae	-	-	-	-
6	1	1	4	Eurytomidae*	1	-	-	1
5	-	1	4	Chrysididae	1	-	-	1
1	1	-	-	Eucharitidae*	5	-	-	5
6	-	1	5	Evaniidae*	-	-	-	-
4	1	-	3	Aulacidae*	-	-	-	-
4	-	-	4	Embolemididae*	-	-	-	-
4	-	-	4	Megaspilidae*	-	-	-	-
2	-	-	2	Pergidae	1	-	-	1
1	1	-	-	Cimbicidae*	1	-	-	1
2	-	-	2	Proctotrupidae	-	-	-	-
1	-	-	1	Scoliidae*	1	-	-	1
1	-	-	1	Tiphiidae	1	-	-	1
1	-	-	1	Gasteruptiidae*	-	-	-	-
3210	41	1024	2145	Total	1395	413	71	1879

ET: ethanolic trap; YPT: yellow pan trap; MT: Malaise trap.

*First record in the study area.

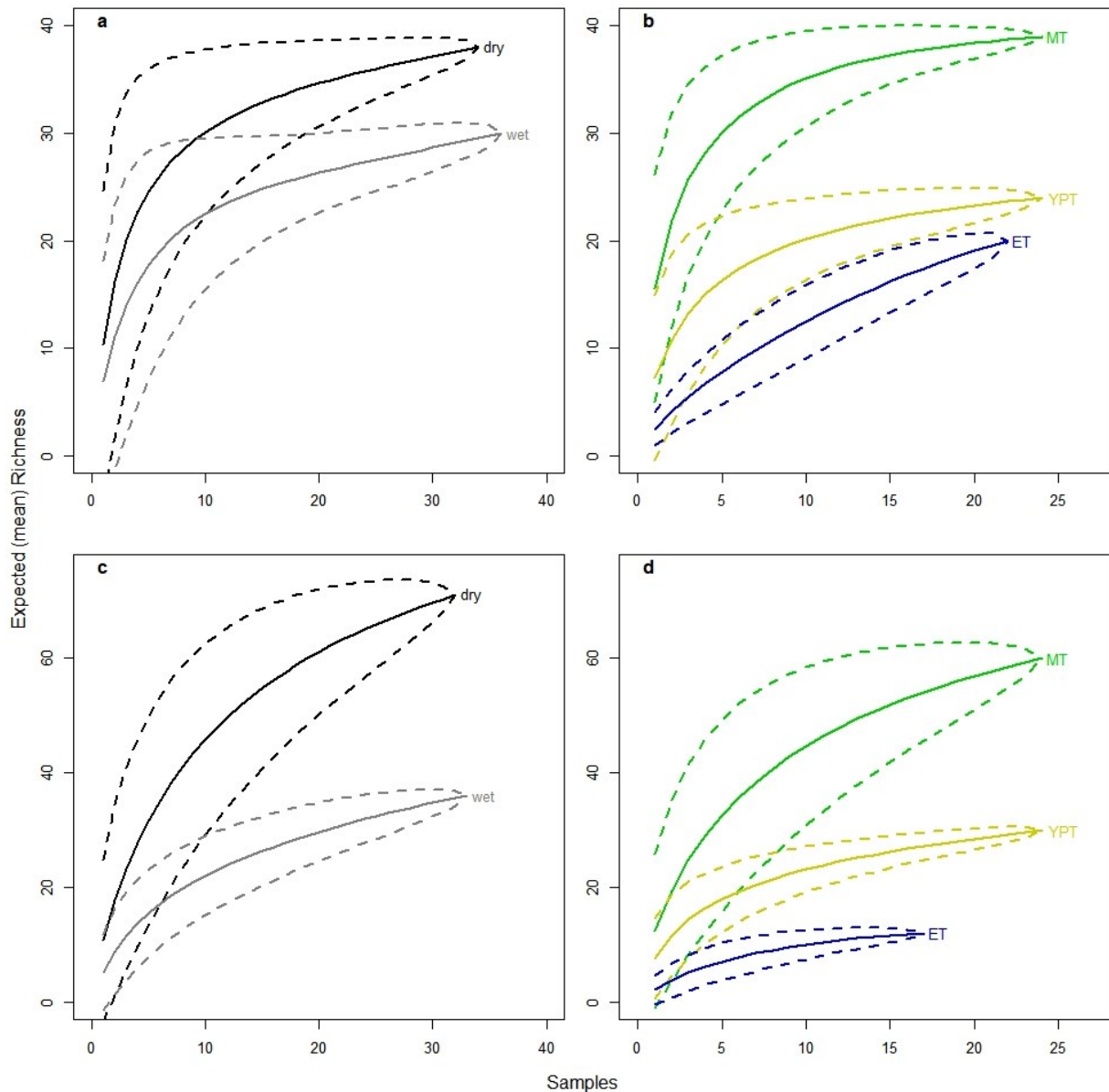


Figure 3. Accumulation curves of Hymenoptera families (a, b) and Vespoidea lower taxa (c, d) observed in a fragment of semideciduous Atlantic rainforest in southeastern Brazil, between 2014 and 2015, subdivided by sampling season (a, c) and trap design (b, d). Solid lines denote the expected mean richness, and dashed lines denote the $\pm 2\sigma$ confidence interval. Sample units correspond to one week of sampling effort per trap design and trap site.

20 families captured by YPT and ET, respectively (figures 3b, table 1). MT and ET were equally efficient in sampling family diversity independently of sampling season ($q = 2.06$, $p = 0.7832$). MT was significantly more efficient than YPT for sampling family diversity overall ($q = 7.24$, $p = 0.0469$), while ET was more efficient than YPT only when considering sampling season interaction ($q = 5.18$, $p = 0.0167$). Although MT and ET reached similar values of Δ^+ for Hymenoptera families, the low capture rate of ET resulted in high variance in comparison to both MT and YPT (figure 4a).

Among the Hymenoptera specimens captured, we identified 73 taxa belonging to six Vespoidea families: Formicidae, Pompilidae, Vespidae, Mutillidae, Tiphiidae and Scoliidae (table 2). Seventy-one Vespoidea taxa were captured at the end the dry season and 36 at the end of the

wet (figure 3c). *Anochetus* Mayr and *Pachycondyla* Smith were the only two genera not captured at the end of the dry season (table 2). Vespoidea Δ^+ was significantly higher at the end of the dry season ($q = 9.22$, $p < 0.0001$), and only YPT did not show changes in Δ^+ estimates between sampling seasons (figure 4b). MT captured 60 Vespoidea taxa, while YPT captured 30 and ET 12 (figure 3d, table 2). Overall, MT was significantly more efficient in sampling Vespoidea Δ^+ than YPT ($q = 13.54$, $p < 0.0001$) and ET ($q = 9.45$, $p = 0.0047$), while ET was more efficient than YPT only when considering sampling season interaction ($q = 4.10$, $p = 0.0446$).

Among the 39 families identified in this study, 16 had never been observed in the study area (Noll *et al.*, 2012) (table 1). Of the identified Vespoidea taxa, the pompilids *Agenioideus lucanus* (Banks) and *Braunilla* Wasbauer *et*

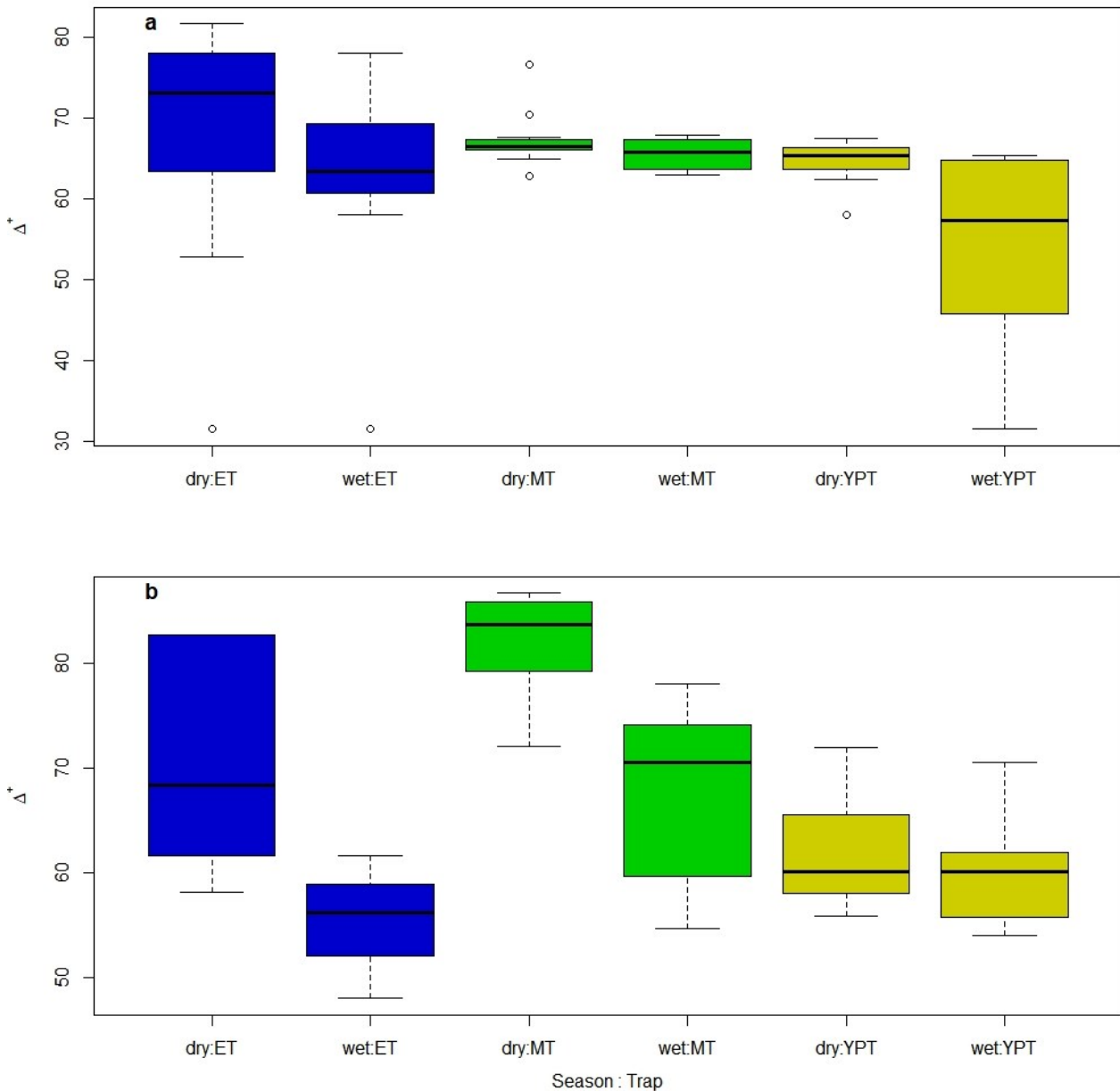


Figure 4. Boxplots of average taxonomic distinctiveness (Δ^+) of Hymenoptera families (a) and Vespoidea lower taxa (b) observed in a fragment of semideciduous Atlantic rainforest in southeastern Brazil, between 2014 and 2015, using season and trap design as explanatory variables. ET: ethanolic trap; MT: Malaise trap; YPT: yellow pan trap.

Kimsey had never been recorded in semideciduous Atlantic rainforests (Evans, 1965; Wasbauer and Kimsey, 2019). A further 38 Vespoidea taxa were also recorded for the first time in the study area (Noll *et al.*, 2012) (table 2).

Faunal similarity

The best-fitted ordination of Hymenoptera families highlights the bias of most families towards MT, while the YPT cluster shows a contribution to the capture of Formicidae, Diapriidae and Dryinidae, and ET corresponds to a few peripheral observations without clear tendency to any family (figure 5a). This same relation is observed for Vespoidea lower taxa (figure 5b). Although MT performed best in sampling Vespoidea taxonomic diversity (figure 4b), YPT was responsible for capturing most of

the Ponerinae ants, including every specimen of *Odonotomachus* Latreille, *Ectatomma* Smith, *Hypoconera* Santschi, *Anochetus* and 96% of the *Gnamptogenys* Roger specimens. It also captured every specimen of Myrmicinae ants of the genera *Atta* F., *Trachymyrmex* Forel, *Mycocepurus* Forel and *Apterostigma* Mayr, as well as all the adult females of *Sericomyrmex* Mayr, *Linepithema* Mayr, and *Myrmelachista* Roger, the only specimen of the mutillid genus *Traumatotutilla* Andre, the pompilid species *Anoplius apiculatus* (Smith) and *Auplopus* aff. *pratensis* Dreisbach. ETs, in turn, were responsible for the only captures of the vespids *Polybia jurinei* Saussure, a male, and *Synoeca surinama* (L.), two females (table 2). In both analyses, there was no clear cluster separation when grouping data by season or sampling site.

Table 2: Relative abundance of Vespoidea taxa identified in a fragment of semideciduous Atlantic rainforest in south-eastern Brazil, between 2014 and 2015, sorted by sampling season and trap designs.

Total	End of Dry Season			Taxa	End of Wet Season			Total
	ET	YPT	MT		Formicidae	MT	YPT	
454	8	110	336	<i>Camponotus</i> sp.	69	10	7	86
337	1	10	326	<i>Pseudomyrmex</i> sp.	51	-	-	51
324	1	256	67	<i>Pheidole</i> sp.	17	78	1	96
205	9	27	169	<i>Solenopsis</i> sp.	58	4	7	69
135	5	108	22	<i>Brachymyrmex</i> * sp.	32	13	6	51
94	-	86	8	<i>Wasmannia</i> * sp.	27	8	-	35
75	-	2	73	<i>Cephalotes</i> sp.	4	-	-	4
66	2	7	57	<i>Myrmelachista</i> * sp.	13	-	-	13
59	-	59	-	<i>Atta</i> * sp.	-	171	-	171
44	-	10	34	<i>Linepithema</i> * sp.	38	2	3	43
42	-	40	2	<i>Gnamptogenys</i> * sp.	1	27	-	28
27	-	-	27	<i>Nesomyrmex spininodis</i> *	3	-	-	3
15	2	2	11	Formicinae Male	9	1	-	10
12	-	12	-	<i>Mycocephurus</i> * sp.	-	-	-	-
10	-	3	7	Myrmicinae Male	4	-	1	5
10	-	9	1	<i>Sericomyrmex</i> sp.	-	3	-	3
9	-	9	-	<i>Cyphomyrmex</i> * sp.	2	2	-	4
9	-	9	-	<i>Nylanderia</i> * sp.	1	4	-	5
7	-	7	-	<i>Odontomachus</i> sp.	-	4	-	4
7	-	7	-	<i>Trachymyrmex</i> sp.	-	3	-	3
6	-	3	3	<i>Acromyrmex</i> * sp.	-	2	-	2
6	-	-	6	<i>Crematogaster</i> * sp.	-	-	8	8
3	-	3	-	<i>Ectatomma</i> sp.	-	1	-	1
2	-	2	-	<i>Apterostigma</i> * sp.	-	-	-	-
2	-	2	-	<i>Hypoponera</i> * sp.	-	1	-	1
2	-	-	2	Ponerinae Male	10	-	-	10
2	-	-	2	<i>Tapinoma</i> * sp.	1	-	-	1
-	-	-	-	<i>Anochetus</i> sp.	-	2	-	2
-	-	-	-	<i>Pachycondyla</i> * sp.	2	-	-	2
Pompilidae								
35	-	-	35	<i>Notoplaniceps fenestralis</i> *	-	-	-	-
22	-	-	22	<i>Notocyphus</i> m.s.2	-	-	-	-
16	-	1	15	<i>Epipompilus quinquenotatus</i> *	1	-	-	1
13	-	-	13	<i>Notocyphus</i> m.s.1	-	-	-	-
12	-	-	12	<i>Poecilopompilus familiaris</i> *	-	-	-	-
10	-	-	10	<i>Epsiron conterminus</i>	-	-	-	-
9	-	-	9	<i>Priochilus</i> aff. <i>gracile</i>	-	-	-	-
7	-	-	7	<i>Ageniella</i> (<i>Ageniella</i>) m.s.1	-	-	-	-
7	-	-	7	<i>Pepsis montezuma</i> *	-	-	-	-
6	-	-	6	<i>Ageniella</i> (<i>Ageniella</i>) m.s.2	-	-	-	-
5	-	-	5	<i>Priochilus</i> aff. <i>fustiferum</i>	7	-	-	7
5	-	-	5	<i>Auplopus basalis</i>	-	-	-	-
5	-	-	5	<i>Epipompilus</i> * sp.	-	-	-	-
4	-	-	4	<i>Ageniella</i> (<i>Priophanes</i>)	1	-	-	1
4	-	-	4	<i>Poecilopompilus mixtus</i> *	-	-	-	-
3	-	1	2	<i>Auplopus</i> aff. <i>pratens</i>	-	-	-	-
3	-	-	3	<i>Ageniella fallax</i>	-	-	-	-
3	-	-	3	<i>Aimatocare</i> * sp.	-	-	-	-
2	-	-	2	<i>Priochilus scrupulum</i>	3	-	-	3
2	-	-	2	<i>Agenioideus accoleus</i>	-	-	-	-
2	-	-	2	<i>Aplochaes adraustes</i> *	-	-	-	-
2	-	-	2	<i>Pepsis viridis</i> *	1	-	-	1
1	-	1	-	<i>Anoplius apiculatus</i> *	-	-	-	-
1	-	-	1	<i>Anoplius bolli</i> *	-	1	-	1
1	-	-	1	<i>Agenioideus lucanus</i> †	-	-	-	-
1	-	-	1	<i>Braunilla</i> † sp.	-	-	-	-

(Continued)

(Table 2 continued)

Total	End of Dry Season			Taxa	End of Wet Season			Total
	ET	YPT	MT	Pompilidae	MT	YPT	ET	
1	-	1	-	<i>Notocyphus</i> m.s.3	-	-	-	-
Vespidae								
6	-	-	6	<i>Agelaia pallipes</i>	-	-	-	-
3	-	-	3	<i>Pachymenes ghilliani</i> *	2	-	-	2
2	-	-	2	<i>Agelaia multipicta</i>	-	-	-	-
2	-	-	2	<i>Polybia fastidiosuscula buyssoni</i> *	-	-	-	-
2	2	-	-	<i>Synoeca surinama</i> *	-	-	-	-
1	1	-	-	<i>Polybia jurinei</i>	-	-	-	-
1	-	-	1	<i>Zethus strigosus</i> *	-	-	-	-
Mutillidae								
2	-	-	2	<i>Tallium</i> * sp.	-	-	-	-
2	-	-	2	<i>Ephuta</i> * m.s.2	-	-	-	-
2	-	-	2	<i>Ephuta</i> * m.s.3	-	-	-	-
1	-	-	1	<i>Ephuta</i> * m.s.1	-	-	-	-
1	-	-	1	<i>Darditilla</i> * sp.	-	-	-	-
1	-	-	1	<i>Lophomutilla</i> * sp.	-	-	-	-
1	-	-	1	<i>Timulla</i> * sp.	-	-	-	-
1	-	1	-	<i>Traumatomutilla</i> * sp.	-	-	-	-
Tiphidae								
1	-	-	1	<i>Tiphia campanula</i> *	1	-	-	1
Scoliidae								
1	-	-	1	<i>Campsomeris ianthina</i> *	1	-	-	1
2176	31	788	1357	Total	359	337	33	729

ET: ethanolic trap; YPT: yellow pan trap; MT: Malaise trap; m.s.: morphospecies; aff.: species affinis.

*First record in the study area.

†First record in semideciduous Atlantic Rainforest.

Discussion

The present study identified 39 Hymenoptera families and 73 Vespoidea lower taxa in a semideciduous fragment of Atlantic rainforest with the use of three trap designs: MT, YPT, and ET. Of the techniques evaluated, MT presented the highest capture rate, sampled the highest richness and taxonomic distinctiveness of Hymenoptera families and Vespoidea lower taxa. YPT scored second regarding capture rate and richness. ET performed well in the dry season when predicting the average taxonomic distinctiveness of Hymenoptera families, although its low capture rate resulted in high variance. For every trap design there was an overall tendency for a higher capture rate and richness at the end of the dry season (except for a few Parasitica families and Formicidae genera). The lower performance of the three types of collection techniques during the wet season was probably determined by the intense precipitation, since rainfall affects hymenopterans perception and sensory intake (Lawson and Rands, 2019), reducing their activity.

A previous survey (Noll *et al.*, 2012) identified 27 families in the same forest fragment, of which four (Colletidae, Cynipidae, Leucospidae and Tenthredinidae) were not collected in the present survey. Thus, a total of 43 Hymenoptera families in that area were recorded. This study also adds more information on the distribution of the spider-hunting wasps of the Pompilini tribe, *A. lucanus* and *Braunilla*, which have never been observed in semideciduous Atlantic rainforest. *A. lucanus* was previously

known from the *Araucaria* moist forests of southern Brazil (Evans, 1965), and its occurrence in the study site extends its range approximately 750 km north. *Braunilla* is widespread in the neotropics (Wasbauer and Kimsey, 2019), and although never recorded in semideciduous Atlantic rainforests, its occurrence in the study site is in accordance with its range. The number of new records registered in this study is due to the more extensive use of MT in the study area. In their survey, Noll *et al.* (2012) placed two 4 m² MT for a total of eight months, while the present study placed three 12 m² MT for a total of two months, which corresponds to a 12.5% higher survey effort. This highlights the efficiency of MT in sampling Hymenoptera diversity in comparison to the active capture designs tested in this study, as fractionally increasing the use of MT has shown to be more efficient in detailing community richness than diversifying trap designs, contrary to what has been proposed to other pollinator groups (Potts *et al.*, 2021).

Nevertheless, the importance of other designs must be noted. The main reason YPT failed to predict the mean taxonomic distinctiveness of Hymenoptera families was its bias towards Diapriidae, Dryinidae and Formicidae, as demonstrated by NMDS (figure 5a). Indeed, the lowest Δ^+ registered for YPT in comparison to MT and ET (figure 4a) highlights the stricter faunal assembly collected by this method in comparison to the other two (figure 5). Among the Formicidae YPT was efficient to collect the majority of the Ponerinae ants, except for the specimens of *Pachycondyla* and the males of this subfamily which

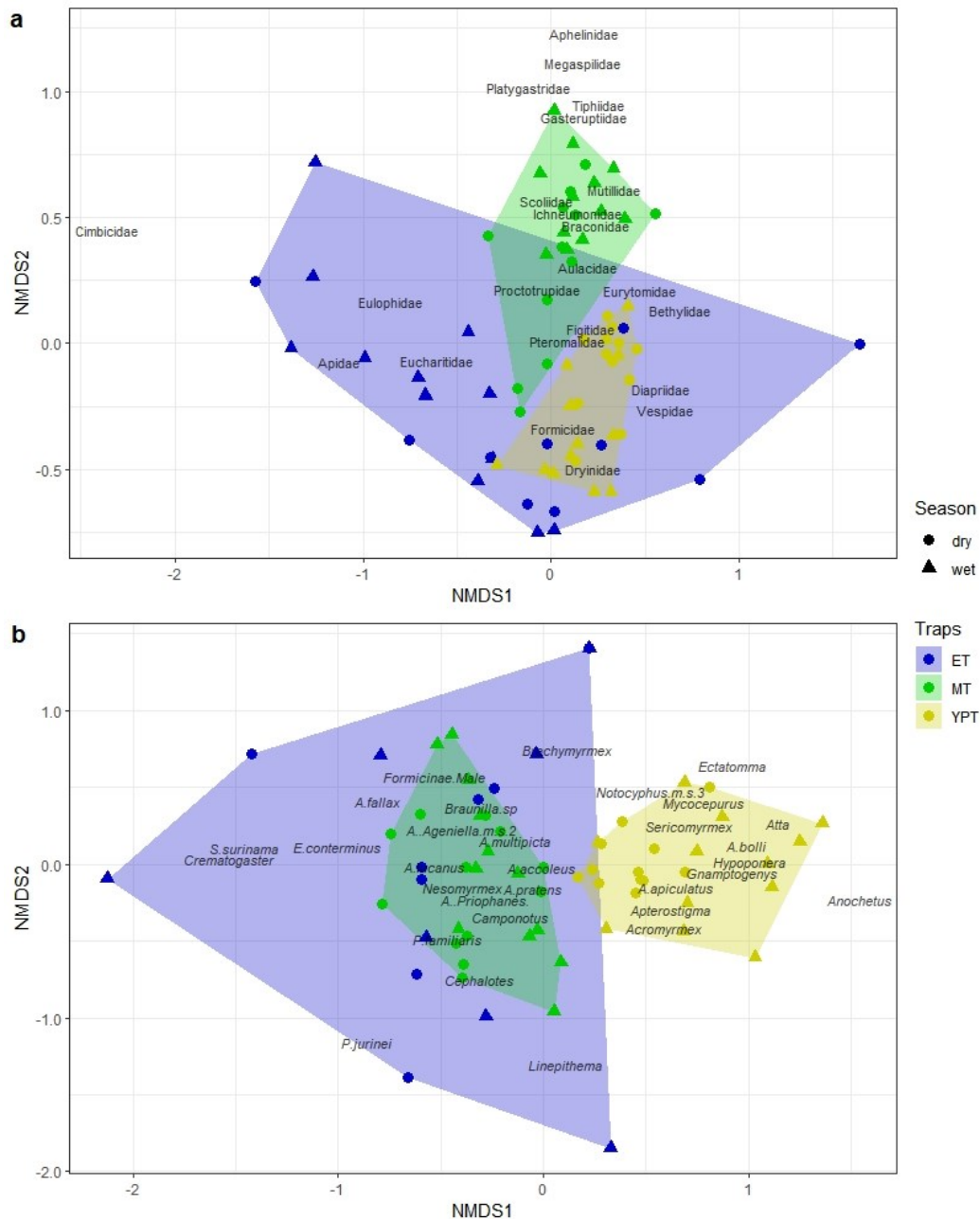


Figure 5. Nonmetric multidimensional scaling (NMDS) plots of Hymenoptera families ($stress = 0.112$; $k = 3$) (a) and Vespoidea lower taxa ($stress = 0.111$, $k = 3$) (b) observed in a fragment of semideciduous Atlantic Rainforest in southeastern Brazil, between 2014 and 2015, using season and trap design as explanatory variables. To avoid overlapping, some taxon labels were removed. ET: ethanolic trap; MT: Malaise trap; YPT: yellow pan trap.

were captured by the MT. YPT also showed a tendency for the capture of the Myrmicinae ants, being responsible for the attraction of all the specimens of the genera *Atta*, *Trachymyrmex*, *Mycocepurus*, and *Apterostigma* and the majority of the *Pheidole* Westwood, *Wasmannia* Forel, *Cyphomyrmex* Mayr, *Sericomyrmex*, and *Acromyrmex* Mayr. YPT efficiency in capturing diapriids has also been documented in the Cerrado grasslands (Marchiori and Pentead-Dias, 2002) and fragments of deciduous forests (Marchiori *et al.*, 2003) in central Brazil and may be linked to their parasitoid habits. Diapriids tend to inhabit moist shady environments, never far from the ground and water, where they are mostly known to parasitize Diptera

(Fernández and Sharkey, 2006). A few diapriids species however parasitize Myrmicinae ants of the genera *Cyphomyrmex*, *Trachymyrmex*, and *Acromyrmex* (Lachaud and Pérez-Lachaud, 2012), therefore their shared tendency to be captured by YPT (figure 5). Dryinids on the other hand normally feed upon the sweet fluids produced by their Auchenorrhyncha hosts (Fernández and Sharkey, 2006) and could likely have been attracted by the ethanol volatiles produced by the YPT, and not by its colour.

Besides its contribution to most of the Formicidae taxa, YPT also captured the only specimens of the pompilid *A. apiculatus* and the mutillid *Traumatomutilla* sp. This shows a clear advantage of this method over pitfall, the

traditional method for surveying ants, since its colour luring mechanism also attracts parasitoid wasps. The capture of a female of *Traumatotutilla* corroborates the preference of this genus to YPT over MT, as previously observed in the central Brazil Cerrado (Vieira *et al.*, 2017). However, other genera that presented a preference for YPT according to Vieira *et al.* (2017), namely, *Talium* Andre and *Darditilla* Casal, were only captured by MT in the present study. YPT also captured all the females of the spider-hunting wasp *Notocyphus* Smith and of the ants *Sericomyrmex*, *Linepithema*, and *Myrmelachista*, whereas the great majority of male ants were captured by the MT. For being placed on ground level, YPT ease the attraction of wingless specimens, as is the case of the worker ants and the females of *Traumatotutilla*, or the ones that move by short and low flights, as most of the Pompilidae females (Noyes, 1989). The common bias towards male dispersal in *Linepithema* and other Formicidae (Passera and Keller, 1993; Hakala *et al.*, 2019) also helps explain their higher capture rate by MT, as opposed to YPT, which on its turn captured the less mobile females.

Yellow coloured pans and trays are known for efficiently attracting a wide range of parasitoid Aculeata and other Parasitica wasps (Noyes, 1989; Campos *et al.*, 2000; Moreira *et al.*, 2016; Viera *et al.*, 2017). Other colours however, such as blue or white, have a significant influence on the attraction of other groups, specially Apoidea and other Hymenoptera pollinators (Leong and Thorp, 1999; Moreira *et al.*, 2016; Potts *et al.*, 2021). These were not the focus of this study thus diversification of pan colours was not judged necessary.

Although presenting the lowest capture rates among the investigated traps (figure 3b, 3d), ET was especially efficient in estimating taxonomic diversity (figure 4a), capturing representatives of ten superfamilies, and the only individuals of Eucharitidae and Cimbicidae at the end of the dry season (table 1). Eucharitid wasps are parasitoids of ant larvae, some species parasitizing *Camponotus* Mayr and *Solenopsis* Westwood (Torréns, 2013), which were also captured by ET. However, the number of Eucharitid wasps and their ant hosts species was too small to suggest a similar bias towards the host/parasitoid hypothesis, as identified for YPT. The limited number of Cimbicids captured in this study also prevents any conclusive remark over their attraction by ET.

ET did not attract any of the Vespoidea parasitoids of bark beetles as hypothesised but it was successful in capturing the social wasps *S. surinama* and *P. jurinei*. The attraction of *P. jurinei* by the odour of mango (*Mangifera indica*) fruits (Barbosa *et al.*, 2014) and *S. surinama* by honey (Fernandes *et al.*, 2010) provides evidence to hypothesise that ET is successful in capturing these species because it mimics the volatile components produced by the carbohydrates they forage. In fact, the active capture of *P. jurinei* and other Polistinae wasps in semideciduous Atlantic rainforests in southeastern Brazil was significantly enhanced by spraying an attractive solution of sucrose and sodium chloride in the surveyed area, resulting in a higher capture rate and richness of Polistinae species and Hymenoptera families than MT sampling (Noll and Gomes, 2009). In the eastern Amazon rainforest, active

capture was also more efficient than MT in capturing Polistinae wasps, and both *S. surinama* and *P. jurinei* could only be caught after active search or with the help of locals to find their hives (Silveira, 2002). Therefore, ET could be a reliable alternative to passive capture design in surveys focusing these social wasps. Nevertheless, it is important to note that other Polistinae wasps were only captured by MT in this study, including *Agelaia pallipes* (Olivier), which also had its capture rate increased by the use of sucrose and sodium chloride solution prior to active capture (Gomes and Noll, 2008; Noll and Gomes, 2009).

There was no clear difference in faunal assembly between the sampling sites. Although diversification of micro-habitats was considered, the present study might have profited from a higher diversity if sampling sites were distributed farther apart from each other (specially sites 2 and 3, figure 1), exploring the gradient of habitats between the border and interior of the fragment. Even tough ecotones normally present higher species richness and abundance of Hymenoptera if compared to the adjacent habitats (Coelho and Ribeiro, 2006; da Rocha-Filho *et al.*, 2017), in the UK woodlands variation in microhabitats, such as changes in vegetation structure, yielded very dissimilar samples of Ichneumonidae wasps (Fraser *et al.*, 2007), and placing MT in both the edge and core of the forest fragment increased sampling efficiency (Fraser *et al.*, 2008).

Conclusion

Among the capture technique investigated, MT has proven to be the most efficient for sampling parasitoid Vespoidea and Hymenoptera in general. It is therefore recommended for studies aiming to estimate communities' total richness, taxonomic distinctiveness and other biodiversity parameters. YPT presented a bias towards terrestrial and short-flying wasps, capturing the great majority of the Ponerinae ants and the only female specimens of the spider-hunting wasp *Notocyphus* and the velvet ant *Traumatotutilla*. It also showed a tendency for the capture of Myrmicinae ants and their Diapriid parasitoids, corroborating the host/parasitoid hypothesis, besides also attracting Dryinids, parasitoids of phytophagous Hemiptera. Although efficient in estimating Hymenopteran taxonomic diversity, ET was not successful in attracting predator and parasitic Vespoidea as hypothesised. It did attract however the only specimens of *S. surinama* and *P. jurinei* in this study, showing its potential for sampling some species of Polistinae social wasps and presenting itself as an economical alternative to MT for sampling the Hymenoptera community in higher taxonomic levels. Furthermore, it was observed that in this study area, sampling was more successful at the end of the dry season. This study also registered the first records of the pompilid taxa *A. lucanus* and *Braunilla* in semideciduous Atlantic rainforests of southeastern Brazil. These results provide a reliable background for planning Hymenoptera surveys and could be considered beyond the neotropics, especially in studies where time and resources are a limiting factor.

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