



RESEARCH ARTICLE - ANTS

Diversity of Social Hymenoptera (Hymenoptera: Aculeata) and Reduction of Sampling Effort in a Dense “terra-firme” Rainforest in the Amazon

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Abstract

We investigated the richness and composition of social Hymenoptera collected with a Malaise trap in a rainforest in the Amazon. In addition, we studied the possibility of reducing the number of traps and the time the traps remain in the field to minimize the sampling effort. In reducing sampling effort, we seek to maintain taxonomic standards (similarity in composition) and reduce both the time the Malaise trap remains in the field and the number of traps per plot. We collected 63 species of social Hymenoptera, represented by 11 species of Apidae, 31 of Formicidae, and 21 of Vespidae, in 30 plots of 250 m distributed in 25 km² of rainforest in the Amazon. It was possible to reduce the permanence time of the Malaise trap in the field from 72 to 48 hours without significant loss of the maximum recorded diversity since there is high similarity (80%) to the maximum effort of 72 hours of sampling. The reduction from two to one trap directly impacted the sampled diversity. We estimate savings of 30.53% by reducing sampling effort and trap dwell time, allowing the resources saved to be invested more efficiently in biodiversity monitoring studies in the Amazon basin.

Introduction

The Amazon is considered one of the most biodiverse biomes in the world (Vieira et al., 2005; Hoorn & Wesselingh, 2011; Magalhães et al., 2011). However, constant anthropic disturbances and environmental changes have caused changes in ecosystems. It may affect the distribution pattern of living beings and species extinction, and in this sense, little is known about the resilience of the Amazon rainforest and the richness present in this biome (Borma & Nobre, 2013). Even with the advancement of management and conservation methods, areas considered hotspots still suffer from a lack of biological information (Hopkins, 2007; Schulman et al., 2007).

Hymenoptera is one of four insect orders known as megadiverse, along with Coleoptera, Lepidoptera, and Diptera (Mason et al., 2006). With more than 153,000 valid species

worldwide (Aguiar et al., 2013), Hymenopterans have diverse behaviors, habits, ecological niches, and biology (Fernández & Sharkey, 2006). Among the main representatives are bees, ants, and social wasps, which have social behavior and are among the best-known groups of the order (Hanson & Gauld, 2006). Social wasps (Vespidae: Polistinae), as well as bees (Apidae: Meliponini and some Apini) and ants (Formicidae), are organisms with predominantly eusocial species, as they have cooperative care for the offspring, division of labor, and overlap in the colony of generations in the same nest (Michener, 2007).

There is no single collection method capable of sampling all taxa. However, the Malaise trap (Malaise, 1937) has become popular for its ability to collect large and diverse samples with minimal effort. The Malaise trap is a flight interception device shaped like a tent, made of fine mesh



netting with an inclined roof leading to a collecting bottle at the upper end. Flying insects that hit the screen fly upward to bypass the barrier and fall into the bottle, usually filled with a preservative (Malaise, 1937).

Malaise traps are widely used in biodiversity research because they are easy to handle and represent one of the best mass collection methods available for terrestrial invertebrates, with Diptera and Hymenoptera being the most collected taxa (Skvarla et al., 2021; Hausmann et al., 2020). However, the large number of insects captured by Malaise traps makes sample processing labor-intensive (Karlsson et al., 2020). Additionally, there is a taxonomic impediment due to the few specialists available for species identification, making the process quite time-consuming. Reducing sampling effort can be a solution to the challenge of the high time required for taxonomic identification, allowing taxa to be identified in a more time and cost-efficient manner (Giangrande et al., 2003; deWaard et al., 2019).

Another difficulty in designing and implementing surveys is the high financial cost and the time required to carry out field and laboratory work (Gardner et al., 2008; Santos et al., 2008; Moraes et al., 2011), besides difficult access to sampling sites, especially in the Amazon region (Schulman et al., 2007). To optimize sampling efforts, some studies were conducted to verify if the information obtained with the reduction of the sampling effort would be statistically sufficient to represent the pattern found in the maximum effort. Aiming to reduce the sampling effort for collecting ants in the Amazon pristine forest, Souza et al. (2007) found that it was possible to reduce the sampling effort by combining pitfall traps and Winkler extractors while maintaining the reliability of the results. Research with oribatid mites (Santos et al., 2008), butterflies (Graça et al., 2017), and ants (Souza et al., 2009; Souza et al., 2012) in the Amazon region was carried out to investigate the effectiveness of reducing sampling effort by reducing the number of subsamples. Such studies found the possibility of preserving ecological information by reducing subsamples. This reduction enabled a decrease in the effort of sorting and identification, in addition to reducing the financial costs associated with activities in the field and processing the material in the laboratory (Graça et al., 2017; Souza et al., 2009; Souza et al., 2012).

Thus, this work aimed to gather information about the richness and composition of the social Hymenoptera of Reserva Ducke, a protected area of the primary Amazon rainforest. In addition, the possibility of reducing the sampling effort by reducing the number of Malaise traps per plot and reducing the time the traps remain in the field and the impact on the financial cost of reducing the sampling effort is evaluated.

Material and Methods

The study was carried out at Reserva Ducke, which belongs to the Instituto Nacional de Pesquisas da Amazônia

(INPA), located 26 km north of the city of Manaus, Amazonas, Brazil (02°55' to 03°01'S and 59°53' to 59°59.5'W). The reserve covers an area of approximately 100 km², with predominant vegetation of upland tropical forest (Baccaro et al., 2008).

Regarding the climate, the dry period runs from June to November, and the rainy period from December to May. The year is considered hot and humid, with an average annual precipitation of 2,523 mm, an average relative humidity of 87%, and an average annual temperature of 26°C. Climatic data were obtained at the Reserva Ducke Climatological Station and released by INPA through the Large-Scale Program for the Biosphere-Atmosphere in the Amazon (LBA) (<http://lba2.inpa.gov.br/>).

We collected the Social Hymenoptera using Malaise-type traps, with 70% alcohol used as a deadly and fixative substance (Figure 1). The collections were carried out between September and November 2014 in the 30 plots of the Biodiversity Research Program (PPBio) trail system, separated by 1 km of distance, distributed in an area of 25 km² in the Reserva Ducke.

In each plot, two Malaise traps were set up, one in the first 20 m and the other at 200 m of the plot, aiming for a comprehensive sampling and minimizing the interference between the two traps. Each trap had its material collected every 24 hours during the 72 hours in which the traps remained in the field, that is, after 24, 48, and 72 hours. Thus, 30 samples and 180 subsamples were obtained (30 plots x two traps per plots x three temporal efforts).

The collected material was taken to the laboratory, sorted, and identified at the family level with the help of Fernandez and Sharkey (2006) and Hanson and Gauld (2006). Later, the social Hymenoptera were mounted on pins, labeled, and identified by specialists in the respective taxonomic group. All identified material was deposited in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA).

Data analysis

1. Diversity of the social Hymenoptera community

We constructed rarefaction curves to evaluate the diversity of social Hymenoptera (bees, ants, and wasps), considering the presence and absence of each species in all 30 plots/samples in 25 km².

We performed Principal Coordinate Analysis (PCoA), with standardized presence and absence data using the “Decostand” function of the vegan package 2.6-2 (Oksanen et al., 2022) to determine whether the reduction in Malaise dwell time traps in the field would capture the community taxonomic patterns observed with the maximum effort of 72 hours. We used presence and absence data because they are social insects, and the proximity of nests to traps could cause bias in abundance metrics. We performed the Procrustes test (Peres-Neto & Jackson, 2001; Legendre et al., 2015) to verify



Fig 1. Malaise trap mounted perpendicular to a trail in Reserva Ducke (Image: Samuel S. Azevedo).

the degree of similarity between the composition of social Hymenoptera collected with maximum effort (72 hours) and reduced efforts of 24 and 48 hours.

To determine the maintenance of taxonomic standards, we adopted similarities in composition with a value of $r \geq 0.7$ (70%) as satisfactory for the Procrustes test, which guarantees that 30% or less dissimilarity will be accepted at levels of reduction of taxonomic information (Santos et al., 2008; Graça et al., 2017). We analyzed social Hymenoptera together so that the PCoA and Procrustes test would have less biased results.

We used the Procrustes test to verify whether reducing the number of traps per plot would capture taxonomic patterns similar to maximum effort. The test allows detecting the degree of similarity between communities sampled with just one trap per plot (traps at point 1 or point 2), using the maximum time (72 hours) compared to the maximum effort (two traps per plot, 72 hours). Since the biology of some species is unknown and little is known about their flight and movement capabilities, we were more cautious and did not consider the two samples in the same plot as independent. Therefore, we tested whether using only one trap per plot would capture a taxonomic pattern similar to using two traps per plot.

We divide the financial costs into field and laboratory costs. We estimated field costs based on the number of people and days for collection, which lasted approximately three months in the experiment (September to November 2014). Therefore, we estimated costs using values from 2014 (in US dollars), the sampling year. We consider laboratory costs, materials for storage, classification, assembly, and identification of insects.

All statistical analyses were performed using RStudio 3.6.1 software (free version 3.6.1) using the *vegan* 2.6-2 package (Oksanen et al., 2022).

Results

Richness and composition of social Hymenoptera (Hymenoptera: Aculeata) in a dense terra-firme rain forest in the Amazon

We collected 292 hymenopterans classified as social, belonging to 63 species/morphospecies, distributed in Apidae (109 specimens, five genera, and 11 species), Vespidae (128 specimens, five genera, and 21 species), and Formicidae (55 specimens, 16 genera, and 31 species/morphospecies). Due to difficulty in species identification, since the identification keys for ants are based on female workers, and in the present work, the majority are winged males, some ants were only morphotyped to the subfamily level (Table 1).

Vespidae was the most abundant family, representing 43.8% of the specimens collected, and *Angiopolybia pallens* (Lepelletier, 1836) was the most frequent species, being present in 24 of the 30 plots. The most diverse genera were *Polybia* and *Agelais*, both social wasps with nine and seven species, respectively. *Trigona guianae* Cockerell, 1910 (Apidae: Meliponini) was the most abundant species (28% of individuals overall and 76% of individuals within bees) and the second most frequent species in 17 plots. Ants were the least abundant but the richest group, with at least 21 species and 16 genera, including ten morphotypes.

According to the classification of rare species (singletons, doubletons, uniques, and duplicates), 45 species were considered rare, representing 71.4% of the total collected. Among these, 37 species were singletons and five doubletons, representing only one or two specimens collected. At the same time, 38 were unique, and seven were duplicates due to their limited distribution between one or two samples, respectively. Most rare species were ants, corresponding to

55.5% of species in general and 80.6% among ants. Of the bees, 63.6% were considered rare species, while social wasps had fewer rare species, 61.9% among wasps. The richness of social Hymenoptera ranged from two to nine species per plot,

with social wasps being the most common group in the plots, with 29 species in total and richness ranging from zero to six species per plot.

Table 1. Social Hymenoptera species collected with Malaise trap in the 30 plots of Reserva Ducke, with abundance and frequency (number of samples in which the species was recorded divided by the total number of samples).

Family/Subfamily/Tribe/Species	Abundance	Frequency
Apidae		
Apinae		
Meliponini		
<i>Melipona amazonica</i> Schulz, 1906	1	3.33
<i>Melipona brachychaeta</i> Moure, 1950	1	3.33
<i>Melipona fulva</i> Lepeletier, 1836	2	6.66
<i>Melipona lateralis</i> Erichson, 1840	4	13.33
<i>Oxytrigona obscura</i> (Friese, 1900)	1	3.33
<i>Partamona mourei</i> Camargo, 1980	3	10
<i>Partamona testacea</i> Klug, 1807	1	3.33
<i>Partamona vicina</i> Camargo, 1980	10	20
<i>Plebeia</i> sp.	1	3.33
<i>Trigona crassipes</i> (Fabricius, 1793)	2	6.66
<i>Trigona guianae</i> Cockerell, 1910	83	56.66
Formicidae		
Formicidae sp.1	1	3.33
Formicidae sp.2	1	3.33
Amblyoponinae		
Amblyoponini		
<i>Fulakora</i> sp.	1	3.33
Dolichoderinae		
Leptomyrmecini		
<i>Azteca</i> sp.	1	3.33
<i>Forelius maranhenses</i> Emery, 1888	1	3.33
Tapinomini		
<i>Tapinoma melanocephalum</i> (Fabricius, 1793)	5	10
Dorylinae		
<i>Leptanilloides</i> sp.	4	10
<i>Neivamyrmex</i> sp.1	4	10
<i>Neivamyrmex</i> sp.2	1	3.33
Formicinae		
Formicinae sp.	1	3.33
Camponotini		
<i>Camponotus senex</i> (Smith, 1858)	1	3.33
Lasiini		
<i>Nylanderia guatemalensis</i> (Forel, 1885)	1	3.33
<i>Nylanderia</i> sp.	1	3.33
Myrmelachistini		
<i>Brachymyrmex</i> sp.	1	3.33
Myrmicinae		
Myrmicinae sp.1	2	6.66
Myrmicinae sp.2	1	3.33

Table 1. Social Hymenoptera species collected with Malaise trap in the 30 plots of Reserva Ducke, with abundance and frequency (number of samples in which the species was recorded divided by the total number of samples). (Continuation)

Family/Subfamily/Tribe/Species	Abundance	Frequency
Myrmicinae		
Myrmicinae sp.3	1	3.33
Myrmicinae sp.4	1	3.33
Myrmicinae sp.5	3	6.66
Myrmicinae sp.6	1	3.33
Attini		
<i>Cyphomyrmex</i> sp.	2	6.66
Creumatogastini		
<i>Creumatogaster limata</i> Smith, 1858	1	3.33
<i>Creumatogaster tenuicula</i> Forel, 1904	5	16.66
Dacetini		
<i>Strumigenys</i> sp.	1	3.33
<i>Strumigenys zeteki</i> (Brown, 1959)	1	3.33
Pheidolini		
<i>Ochetomyrmex semipolitus</i> Mayr, 1878	2	3.33
Ponerinae		
Ponerinae sp.	1	3.33
Ponerini		
<i>Anochetus</i> sp.1	4	10
<i>Anochetus</i> sp.2	1	3.33
<i>Odontomachus</i> sp.	1	3.33
<i>Mayaponera</i> sp.	3	10
Vespidae		
Polistinae		
Epiponini		
<i>Agelaia angulata</i> (Fabricius, 1804)	13	30
<i>Agelaia brevistigma</i> (Richards, 1978)	1	3.33
<i>Agelaia centralis</i> (Cameron, 1907)	1	3.33
<i>Agelaia constructor</i> (de Saussure, 1854)	9	20
<i>Agelaia fulvofasciata</i> (DeGeer, 1773)	8	16.66
<i>Agelaia pallipes</i> (Olivier, 1791)	4	10
<i>Agelaia testacea</i> (Fabricius, 1804)	5	13.33
<i>Angiopolybia obidensis</i> (Ducke, 1904)	3	10
<i>Angiopolybia pallens</i> (Lepeletier, 1836)	49	80
<i>Chartergellus amazonicus</i> Richards, 1978	1	3.33
<i>Polybia crysothorax</i> (Lichtenstein, 1796)	1	3.33
<i>Polybia dimidiata</i> (Olivier, 1791)	1	3.33
<i>Polybia dimorpha</i> Richards, 1978	1	3.33
<i>Polybia ignobilis</i> (Haliday, 1936)	1	3.33
<i>Polybia minarum</i> Ducke, 1906	1	3.33
<i>Polybia occidentalis</i> (Olivier, 1791)	12	6.66
<i>Polybia parvulina</i> Richards, 1970	8	6.66
<i>Polybia rufitarsis</i> Ducke, 1904	1	3.33
<i>Polybia singularis</i> Ducke, 1905	6	13.33
Mischocyttarini		
<i>Mischocyttarus</i> sp.1	1	3.33
<i>Mischocyttarus</i> sp.2	1	3.33

The rarefaction curves did not reach the asymptote, and all indicated a strong tendency towards an increase in species as the sampling effort of the number of samples increases (Figure 2). Ants represent the least sampled group concerning the diversity available at the site, as the community richness curve is further from stabilizing (Figure 2C).

Reduction of sampling effort considering the time the traps remain in the field

With the reduction of the permanence time of Malaise traps in the field to 48 hours, there was no significant difference

in the total species richness of social Hymenoptera compared to the richness observed with the maximum effort of 72 hours, with 74.6% of the observed species being collected with maximum effort. The difference in species richness of bees and social wasps from the 48-hour effort is only one and three species, respectively. However, when comparing ant richness, 12 species (31.7%) would be excluded when reduced to 48 hours. With the reduction of the effort of all social Hymenoptera to 24 hours, 31 species were excluded, representing 49% of the sampled species, compared to 72 hours, the maximum effort (Table 2).

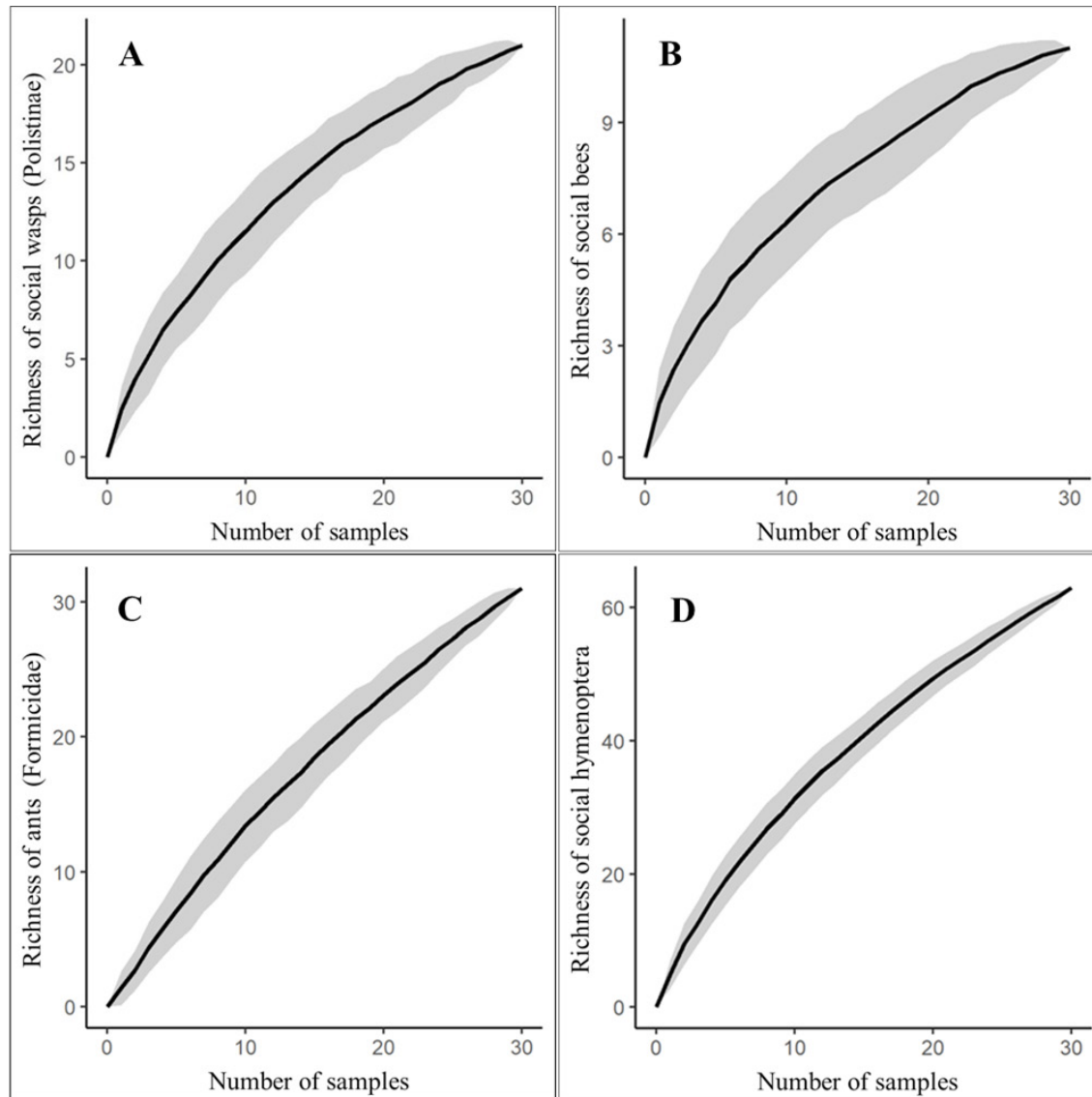


Fig 2. Rarefaction curve of social Hymenoptera based on the number of samples, (A) social wasps, (B) bees, (C) ants and (D) social Hymenoptera together. Gray area represents 95% confidence interval.

According to the Procrustes test, the composition of social Hymenoptera sampled at 48 hours had a similarity of 80.5% compared to traps lasting in the field for 72 hours ($r = 0.805$, $p < 0.001$). A number higher than the minimum

similarity accepted (70%) decided a priori. The composition obtained after 24 hours was 63.6%, similar to the composition sampled with maximum effort ($r = 0.636$, $p < 0.001$) (Figure 3).

Table 2. Abundance of social Hymenopteran species collected in the 30 plots of Reserva Ducke with Malaise trap in three sampling efforts.

Family/Subfamily/Tribe/Species	24h	48h	72h	Family/Subfamily/Tribe/Species	24h	48h	72h
Apidae				Attini			
Apinae				<i>Cyphomyrmex</i> sp.			
Meliponini				Crematogastini			
<i>Melipona amazonica</i> Schulz, 1906	1	1	1	<i>Crematogaster limata</i> Smith, 1858	1	1	1
<i>Melipona brachychaeta</i> Moure, 1950	-	-	1	<i>Crematogaster tenuicula</i> Forel, 1904	2	4	5
<i>Melipona fulva</i> Lepeletier, 1836	1	2	2	Dacetini			
<i>Melipona lateralis</i> Erichson, 1840	3	3	4	<i>Strumigenys</i> sp.			
<i>Oxytrigona obscura</i> (Friese, 1900)	1	1	1	<i>Strumigenys zeteki</i> (Brown, 1959)			
<i>Partamona mourei</i> Camargo, 1980	3	3	3	Pheidolini			
<i>Partamona testacea</i> Klug, 1807	-	1	1	<i>Ochetomyrmex semipolitus</i> Mayr, 1878			
<i>Partamona vicina</i> Camargo, 1980	8	9	10	Ponerinae			
<i>Plebeia</i> sp.	1	1	1	Ponerinae sp.			
<i>Trigona crassipes</i> (Fabricius, 1793)	-	1	2	Ponerini			
<i>Trigona guianae</i> Cockerell, 1910	32	51	83	<i>Anochetus</i> sp.1			
Formicidae				<i>Anochetus</i> sp.2			
Formicidae sp.1	-	-	1	<i>Odontomachus</i> sp.			
Formicidae sp.2	-	-	1	<i>Mayaponera</i> sp.			
Amblyoponinae				Vespidae			
Amblyoponini				Polistinae			
<i>Fulakora</i> sp.	-	-	1	Epiponini			
Dolichoderinae				<i>Agelaia angulata</i> (Fabricius, 1804)			
Leptomymecini				<i>Agelaia brevistigma</i> (Richards, 1978)			
<i>Azteca</i> sp.	-	1	1	<i>Agelaia centralis</i> (Cameron, 1907)			
<i>Forelius maranhenses</i> Emery, 1888	-	-	1	<i>Agelaia constructor</i> (de Saussure, 1854)			
Tapinomini				<i>Agelaia fulvofasciata</i> (DeGeer, 1773)			
<i>Tapinoma melanocephalum</i> (Fabricius, 1793)	4	5	5	<i>Agelaia pallipes</i> (Olivier, 1791)			
Dorylinae				<i>Agelaia testacea</i> (Fabricius, 1804)			
<i>Leptanilloides</i> sp.	1	3	4	<i>Angiopolybia obidensis</i> (Ducke, 1904)			
<i>Neivamyrmex</i> sp.1	2	4	4	<i>Angiopolybia pallens</i> (Lepeletier, 1836)			
<i>Neivamyrmex</i> sp.2	-	-	1	<i>Chartergellus amazonicus</i> Richards, 1978			
Formicinae				<i>Polybia crysothorax</i> (Lichtenstein, 1796)			
Formicinae sp.	-	1	1	<i>Polybia dimidiata</i> (Olivier, 1791)			
Camponotini				<i>Polybia dimorpha</i> Richards, 1978			
<i>Camponotus senex</i> (Smith, 1858)	-	-	1	<i>Polybia ignobilis</i> (Haliday, 1936)			
Lasiini				<i>Polybia minarum</i> Ducke, 1906			
<i>Nylanderia guatemalensis</i> (Forel, 1885)	1	1	1	<i>Polybia occidentalis</i> (Olivier, 1791)			
<i>Nylanderia</i> sp.	-	-	1	<i>Polybia parvulina</i> Richards, 1970			
Myrmelachistini				<i>Polybia rufitarsis</i> Ducke, 1904			
<i>Brachymyrmex</i> sp.	-	-	1	<i>Polybia singularis</i> Ducke, 1905			
Myrmicinae				Mischocyttarini			
Myrmicinae sp.1	-	2	2	<i>Mischocyttarus</i> sp.1			
Myrmicinae sp.2	-	1	1	<i>Mischocyttarus</i> sp.2			
Myrmicinae sp.3	-	1	1	Total			
Myrmicinae sp.4	-	-	1	138			
Myrmicinae sp.5	1	3	3	222			
Myrmicinae sp.6	-	1	1	292			

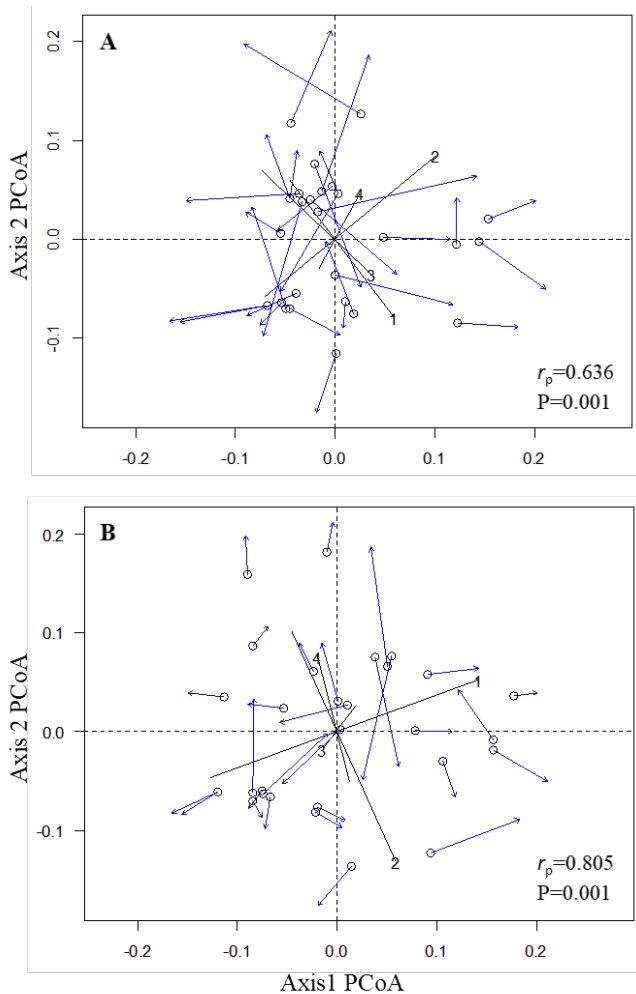


Fig 3. Values of similarities in Procrustes rotations using the first two axes of the PCoA for species composition of social Hymenoptera at maximum effort (72 hours) and at reduced effort. (A) reduction to 24 hours and (B) reduction to 48 hours. Each circle indicates the plot with maximum effort and at the other extreme the same plot with reduced effort. The distance between them indicates how much these plots varied in relation to species composition.

Richness and composition of social Hymenoptera in sampling with one trap per plot compared to maximum effort (two traps)

The richness of social Hymenoptera per plot ranged from two to nine species using two Malaise traps per plot (maximum effort). When only one trap was used per plot, the richness per plot ranged from one to eight for traps in point 1 and from zero to seven for traps in point 2. Of the 63 species, only 22 species (34.9%) are present at both points, eight species of wasps, which corresponds to 38% of the 21 species of wasps, seven (45.4%) of bees among bees, and nine (29%) of ants among ants (Table 3).

According to the Procrustes test, the composition of species of social Hymenoptera sampled with only one trap per plot was different from the composition verified with the maximum effort that uses two traps per plot. The traps from point 1 showed 63.7% ($r = 0.637$, $p < 0.001$), while the traps

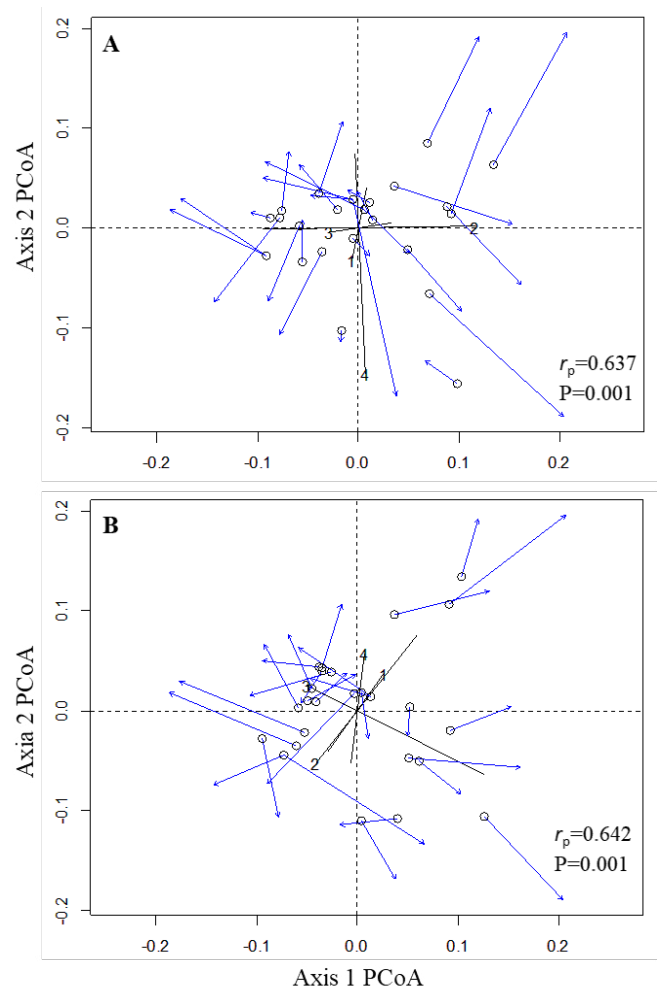


Fig 4. Similarity values in Procrustes rotations using the first two PCoA axes for species composition of social Hymenoptera at maximum effort (two traps per plot) and at reduced effort (one trap per plot). (A) point 1 trap and (B) point 2 trap. Each circle indicates the plot with maximum effort and at the other extreme the same plot with reduced effort. The distance between them indicates how much these plots varied in relation to species composition.

from point 2 showed 64.2% ($r = 0.642$, $p < 0.001$) of similarity with the maximum effort (Figure 4).

Financial costs of the study

According to the expenses for carrying out collections in the field and expenses in the laboratory (Table 4), the estimated savings with the reduction in the time of permanence of the traps in the field was 30.53% when reduced to 48 hours of collection using Malaise traps (Table 5). Of the abovementioned expenses, the costs of traps, collection bottles, 70% alcohol, and transportation by car to the collection site remained fixed. The other values were dependent (variables) on the sample time in the field, being reduced by up to a third in the case of 24 hours.

The reduction in costs with the reduction of traps in the field was not calculated since the results of the statistical analyses showed that, in this reduction, there would be a substantial loss of information.

Table 3. Abundance of species of social Hymenoptera collected with Malaise trap in Point 1 and Point 2.

Family/Subfamily/Tribe/Species	Point 1	Point 2	Family/Subfamily/Tribe/Species	Point 1	Point 2
Apidae			Attini		
Apinae			<i>Cyphomyrmex</i> sp.	1	1
Meliponini			Crematogastini		
<i>Melipona amazonica</i> Schulz, 1906	1	-	<i>Crematogaster limata</i> Smith, 1858	-	1
<i>Melipona brachychaeta</i> Moure, 1950	-	1	<i>Crematogaster tenuicula</i> Forel, 1904	3	2
<i>Melipona fulva</i> Lepeletier, 1836	1	1	Dacetini		
<i>Melipona lateralis</i> Erichson, 1840	1	3	<i>Strumigenys</i> sp.	1	-
<i>Oxytrigona obscura</i> (Friese, 1900)	1	-	<i>Strumigenys zeteki</i> (Brown, 1959)	-	1
<i>Partamona mourei</i> Camargo, 1980	1	2	Pheidolini		
<i>Partamona testacea</i> Klug, 1807	1	-	<i>Ochetomyrmex semipolitus</i> Mayr, 1878	1	1
<i>Partamona vicina</i> Camargo, 1980	2	8	Ponerinae		
<i>Plebeia</i> sp.	-	1	Ponerinae sp.	-	1
<i>Trigona crassipes</i> (Fabricius, 1793)	2	-	Ponerini		
<i>Trigona guianae</i> Cockerell, 1910	62	21	<i>Anochetus</i> sp.1	3	1
Formicidae			<i>Anochetus</i> sp.2	-	1
Formicidae sp.1	1	-	<i>Odontomachus</i> sp.	1	-
Formicidae sp.2	-	1	<i>Mayaponera</i> sp.	2	1
Amblyoponinae			Vespidae		
Amblyoponini			Polistinae		
<i>Fulakora</i> sp.	1	-	Epiponini		
Dolichoderinae			<i>Agelaia angulata</i> (Fabricius, 1804)	3	10
Leptomymecini			<i>Agelaia brevistigma</i> (Richards, 1978)	-	1
<i>Azteca</i> sp.	-	1	<i>Agelaia centralis</i> (Cameron, 1907)	-	1
<i>Forelius maranhenses</i> Emery, 1888	-	1	<i>Agelaia constructor</i> (de Saussure, 1854)	5	4
Tapinomini			<i>Agelaia fulvofasciata</i> (DeGeer, 1773)	6	2
<i>Tapinoma melanocephalum</i> (F. 1793)	3	2	<i>Agelaia pallipes</i> (Olivier, 1791)	2	2
Dorylinae			<i>Agelaia testacea</i> (Fabricius, 1804)	5	-
<i>Leptanilloides</i> sp.	1	3	<i>Angiopolybia obidensis</i> (Ducke, 1904)	1	2
<i>Neivamyrmex</i> sp.1	2	2	<i>Angiopolybia pallens</i> (Lepeletier, 1836)	23	26
<i>Neivamyrmex</i> sp.2	1	-	<i>Chartergellus amazonicus</i> Richards, 1978	1	-
Formicinae			<i>Polybia crysothorax</i> (Lichtenstein, 1796)	-	1
Formicinae sp.	1	-	<i>Polybia dimidiata</i> (Olivier, 1791)	1	-
Camponotini			<i>Polybia dimorpha</i> Richards, 1978	1	-
<i>Camponotus senex</i> (Smith, 1858)	-	1	<i>Polybia ignobilis</i> (Haliday, 1936)	1	-
Lasiini			<i>Polybia minarum</i> Ducke, 1906	1	-
<i>Nylanderia guatemalensis</i> (Forel, 1885)	-	1	<i>Polybia occidentalis</i> (Olivier, 1791)	12	-
<i>Nylanderia</i> sp.	1	-	<i>Polybia parvulina</i> Richards, 1970	1	7
Myrmelachistini			<i>Polybia rufitarsis</i> Ducke, 1904	-	1
<i>Brachymyrmex</i> sp.	-	1	<i>Polybia singularis</i> Ducke, 1905	4	2
Myrmicinae			Mischocyttarini		
Myrmicinae sp.1	1	1	<i>Mischocyttarus</i> sp.1	1	-
Myrmicinae sp.2	-	1	<i>Mischocyttarus</i> sp.2	-	1
Myrmicinae sp.3	-	1	Total	168	124
Myrmicinae sp.4	1	-			
Myrmicinae sp.5	3	-			
Myrmicinae sp.6	-	1			

Table 4. Field and laboratory costs for carrying out the collection of specimens used in this study (in dollars USD).

Field expenses	Cost	Units	24 hours	48 hours	72 hours	% of the cost	24 hours	48 hours	72 hours
Student (performer)	variable	days	1/3	2/3	3/3	40.7	937.50	1,875.00	2,812.50
Woodsman one	variable	days	1/3	2/3	3/3	14.5	333.33	666.66	1,000.00
Woodsman two	variable	days	1/3	2/3	3/3	14.5	333.33	666.66	1,000.00
Food (for 3 people)	variable	days	1/3	2/3	3/3	16.3	375.00	750.00	1,125.00
Alcohol 70% (liter)	variable	liters	20	20	20	1.2	83.33	83.33	83.33
Collection flasks	variable	units	60	60	60	1.4	100.00	100.00	100.00
Diesel for displacement (liter)	fixed	liters	20	20	20	0.6	41.66	41.66	41.66
Malaise traps	fixed	units	6	6	6	10.8	750.00	750.00	750.00
Total (US\$)						100%	2,954.15	4,933.31	6,912.49
Laboratory expenses									
Student (performer) - sorting order	variable	days	1/3	2/3	3/3	36.33	1,875.00	3,750.00	5,625.00
Student (performer) - family screening	variable	days	1/3	2/3	3/3	36.33	1,875.00	3,750.00	5,625.00
Student (performer) - family screening	variable	days	1/3	2/3	3/3	6.05	312.50	625.00	937.50
Storage bottles	variable	units	1/3	2/3	3/3	1.94	100.00	200.00	300.00
Alcohol 96% (liter)	variable	liters	1/3	2/3	2/3	1.61	83.33	166.66	250.00
Entomological pins	variable	units	138	222	292	0.78	57.50	92.50	121.66
Apidae Identification	variable	morphospecies	8	10	11	2.96	333.33	416.66	458.33
Formicidae Identification	variable	morphospecies	12	21	31	8.35	500.00	875.00	1,291.66
Vespidae Identification	variable	morphospecies	12	18	21	5.65	500.00	750.00	875.00
Total (US\$)						100%	5,636.66	10,625.82	15,484.15

Table 5. Total costs for carrying out the present study, and percentage of total expenses (in dollars USD).

Expenses	24 hours	48 hours	72 hours
Field	2,954.15	4,933.31	6,912.49
Laboratory	5,636.66	1,025.82	15,484.15
Spending	38.35%	69.47%	100%
Total (US\$)	8,590.81	15,559.13	22,396.64

Discussion

Richness and composition of social Hymenoptera (Hymenoptera: Aculeata) in a dense "terra-firme" rain forest in the Amazon

The presence of many rare species (singletons, doubletons, uniques, or duplicates) in the three sampled groups, bees 71.4%, ants 80.6% and social wasps 61.9%, is considered a common phenomenon in works carried out in tropical forests (Franklin et al., 2013), especially in megadiverse groups such as Hymenoptera that occupy a broad ecological niche.

Another possible cause of so many rare species could be insufficient collection in time and space in small habitats (Coddington et al., 2009). Also, the high number of specialist

and ephemeral species with low density makes it difficult to collect many specimens of the same species (Novotny & Basset, 2000).

Studies in the Amazon rainforest with vespids demonstrate that the biome has a great diversity of social wasps (Somavilla & Oliveira, 2017). Among the 21 species recorded in the present study, *Agelaia brevistigma* (Richards, 1978) and *Polybia minarum* Ducke, 1906 had yet to be recorded for Reserva Ducke. Thus, the richness of social wasps for Reserva Ducke increased to 105 species, reinforcing the reserve's potential as a rich repository of social wasp biodiversity in the Brazilian Amazon.

The richest genera were *Polybia*, *Agelaia*, and *Mischocyttarus*, in agreement with other works carried out in the Amazon (Silveira et al., 2008; Silva & Silveira, 2009; Somavilla et al., 2014; Somavilla et al., 2019). The three genera correspond to 85.7% of all wasps collected in the present study.

The success in collecting *Polybia* and *Agelaia* can be explained by the fact that *Agelaia* usually has a greater abundance of individuals per colony (Zucchi et al., 1995). Consequently, this facilitates its capture and may have directly reflected on the results since of the common wasp species, 62.5% are *Agelaia*. Because they are very active social wasps when foraging, *Polybia* species are more likely

to be collected in traps such as Malaise (Carpenter & Marques, 2001). The abundance and high frequency of *Angiopolybia pallens*, occurring in 80% of the samples, was also observed in other studies carried out in the Amazon (Silveira, 2002; Silveira et al., 2005; Silveira et al., 2008; Silva & Silveira, 2009; Somavilla et al., 2014; Graça & Somavilla, 2018). The combination of two or more collection methods in the same sampling is more efficient for collecting social wasps (Silveira, 2002; Somavilla et al., 2019) because, instead of allocating an extensive sampling effort to just one method, we could break them down into smaller efforts with different sampling methods that would better explore the environment.

The diversity of Meliponini bees in the present study was similar to that found by Almeida et al. (2019) in a study also in the Amazon region, but using another type of trap, the attractive one. They recorded a diversity ranging from nine to 20 species, depending on the attractant used in the collection. In our study, *Trigona guianae* was the only common species among the 29 stingless bees collected by Almeida et al. (2019).

Melipona and *Partamona* were the most diverse genera in the bee samples, with four and three species, respectively. This result is superior to that found by Almeida et al. (2019), who collected two and one species of *Melipona* and *Partamona*, respectively. In contrast to Oliveira et al. (1995) in a study also in a terra firme forest in the Amazon that used several collection methods, in which Malaise was not included, only the richness of *Partamona* was superior in our work, while for *Melipona* there was a record of seven species, in contrast to four species in the present study.

As in the present study, most studies that evaluate ant sampling methods tend to observe biodiversity aspects, such as species richness or composition (Lopes & Vasconcelos, 2008; Tista & Fiedler, 2011; Gomes et al., 2018). However, few works use the Malaise trap as a sampling technique for ants (Delabie & Reis, 2000; Souza et al., 2024), making it challenging to establish comparative bases for the group concerning the sampling method.

In our study, 55.7% of captured ants are winged. However, most identification keys are based on the morphology of apterous forms (workers), making it challenging to identify the sampled material. There is a significant lack of information about winged ants, with more than 50% of males not described (Boudinot, 2015). Thus, most of the alates obtained in this study were only morphotyped to possibly represent different taxonomic identities.

The sampling method and collection period can explain ants' low abundance and richness compared to other studies in the Amazon region. Since the Malaise trap tends to capture more winged ants than wingless forms, and the abundance and release of winged ants occur more during the rainy season (Kaspari et al., 2001), mainly to facilitate soil excavation for nesting (Hölldobler & Wilson, 1990). In this sampling, the collections were carried out in months of low precipitation, which may also explain the low capture.

Using flight mainly to disperse species to find a new colony, winged ants can also be essential for conservation work through dispersion and distribution dynamics during flight, in addition to influencing other animals that feed on these ants during flight (Helms et al., 2016). In this regard, the Malaise trap can become an essential mechanism for studies with ants that live beyond the soil, mainly for stratification studies and arboreal ants.

Unlike other works carried out with ant diversity in Reserva Ducke, which used pitfall traps and a Winkler extractor (Souza et al., 2012; Souza et al., 2018), our study recorded one specimen of *Fulakora* and four of *Leptanilloides*, all winged males. This highlights the importance of collecting winged ants using a Malaise trap since the trap increases the possibility of collecting rare groups and those with an underground habit through winged ants, which would be challenging to collect in traditional traps (Delabie & Reis, 2000; Delsinne et al., 2015; Martins et al., 2020; Souza et al., 2024).

Reduction of sampling effort in the collection of social Hymenoptera

Ecologists use estimates to know the diversity of an area. Therefore, it is necessary to find a methodological pattern of sufficient sampling effort that can give us efficient and reliable estimates. Furthermore, the extrapolation of samples to estimate the species richness of an environment is considered complicated, mainly due to the large number of singletons and doubletons present in the samples (Santos et al., 2008).

In a study with frugivorous butterflies in the same locality, Graça et al. (2017) verified that reducing the sampling effort from four to just two collections would capture the ecological information found in the maximum effort. While for taxonomic information, three collections would be necessary for sufficient sampling.

In the present study, where only taxonomic approaches were used, it was seen that it would be possible to reduce the collection effort from 72 hours to 48 hours due to the high similarity of richness and composition concerning the maximum effort (72 h). Especially for social bees and wasps, when using the reduced effort (48h), only one and three species would not be sampled, respectively.

In this case, it is recommended to use other complementary methods in conjunction with the Malaise trap for a more comprehensive sampling of diversity. Additional sampling methods, such as active collection and the use of attractants, would allow exploring other environments, making sampling the diversity of wasps and bees faster and more efficient, even if there is a greater financial or time cost since the main point in sampling is the cost-benefit. As much as several sampling methods are known, few studies have tried to standardize these methods and create sampling protocols for using these combined methods.

As expected, using only one Malaise trap per plot was insufficient for collections of social Hymenoptera, mainly because 57.5% of the species were singletons. Thus, excluding 50% of the traps eliminated several species, with considerable losses in biological information.

Other works carried out in the Amazon using subsample reduction in the collection of ants (Souza et al., 2009; Souza et al., 2012), oribatid mites (Santos et al., 2008), and frugivorous butterflies (Graça et al., 2017), obtained significant results in the reduction of subsamples with a direct effect in the reduction of the collection and sorting time of the material in the laboratory and, consequently, in the reduction of financial costs.

The results of sampling social Hymenoptera with Malaise traps can be used as a complementary method for studies inside and outside the Brazilian Amazon. However, it is interesting that more studies are carried out using combined collection methods and developing new collection protocols using the necessary collection effort of each method to survey these groups.

Financial costs for obtaining results

The savings in financial costs efficiently reduced the time the traps remained in the field. It is possible to reduce the dwell time of the traps by up to a third of the time without losing much taxonomic information concerning the maximum effort (72 h). For social Hymenoptera, exclusively, the total savings with this reduction was around 30.53% compared to the total value. Although some costs are fixed, as new groups are being studied, the costs tend to increase in value, as it will be necessary to calculate the costs of each group (assembly, labeling, identification, and time worked).

Authors' Contributions

ABS: Conceptualization, formal analysis, methodology, investigation, resources, writing-original draft, writing-review & editing.

BRT: Conceptualization, methodology, investigation, resources, writing-review & editing.

JLPS: Conceptualization, methodology, investigation, resources, writing-review & editing.

FSPG: Conceptualization, methodology, investigation, resources, writing-review & editing.

DRRF: Conceptualization, methodology, investigation, resources, writing-original draft, writing-review & editing.

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