An Acad Bras Cienc (2022) 94(4): e20210763 DOI 10.1590/0001-3765202220210763
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ECOSYSTEMS

Trap and bait efficiency for catching Calliphoridae and Mesembrinellidae (Insecta, Diptera) at different heights

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Abstract: The influence of food types, reproductive behaviour, and the existence of a possible stratification to the attractiveness of Calliphoridae and Mesembrinellidae flies may contribute to the definition of collection methodologies. Thus, we assessed the effectiveness of traps with two bait types exposed at two different heights in the forests for collecting the aforementioned families. Traps were exposed in the Amazon rainforest floodplain area, where 40 traps were installed in 20 trees, in three periods of the year. On each tree, one trap was installed at 0.6 m and the other at 10 m above the ground, with either bovine lung or fermented banana. A total of 1,173 individuals were collected, including 10 species of Calliphoridae (962 individuals) and four species of Mesembrinellidae (211 individuals). Of the total in each family, Calliphoridae was most abundant in 0.6m lung bait traps (41%) and Mesembrinellidae in the 0.6 m banana bait traps (29%). Calliphoridae showed greater species richness, abundance, and differentiation in composition in low traps with lung as bait. In conclusion, only dipteran species from Calliphoridae have been affected and responded the collection methodologies employed of we, which may highlight remarkable differences in collection and subsequent data interpretation of inventories and monitoring using these insects.

Key words: Attractiveness, Calliphorids, Food resources, Mesembrinellids, Stratification.

INTRODUCTION

Considering that the biological diversity of the Amazon biome is being modified at an accelerated rate, insects have potential for use in environmental monitoring and assessment (Noriega et al. 2018, Cardoso et al. 2020). Insects stand out as bioindicators because they have great specificity and sensitivity to environmental characteristics, high diversity, large biomass, and they are easy to sample. For this, studies that improve the collection and sampling methodologies are of the utmost importance.

Calliphoridae and Mesembrinellidae are relevant dipteran insects from an environmental conservation, public health, and forensic entomology point of view (Luz et al. 2020). These families include species that respond rapidly to environmental impacts in different ecosystems, act as indicators of human alterations, and are important for the decomposition process of organic matter of animal or vegetable origin (Sousa et al. 2020, 2021, Mendes et al. 2021). Their presence in diverse habits is mediated by the availability of resources for feeding adults and immature stages as well as for oviposition (Avancini & Prado 1986, D'Almeida & Lima 1994). For the development of these flies, abiotic factors such as plant cover also influence habitat attractiveness, where the vegetation type, tree height and density can generate different habitat conditions (Ulyshen et al. 2010, Sousa et al. 2016, 2021, Luz et al. 2020, De Souza Amorim et al. 2022).

Studies on the influence of food resources, reproduction, and the existence of a possible stratification (soil or forest canopy) on the attractiveness of habitats for flies at different heights are essential for understanding the demands and patterns of these groups with direct application in entomological sciences. These data can generate information on preventive measures, eradication measures, identification of new species, and provide data for environmental monitoring and assessment, as well as providing sets of species at sites to be used in estimating post-mortem (PMI) (Sanford 2017).

The potential of flies as bioindicators is often underused due to limited knowledge of their distribution and the lack of standardization for methodologies, as different collection methods can generate different results and responses. There are, for example, differences in guild composition between capture techniques (bait traps, active capture with entomological nets and Malaise traps), where bait traps are more effective in estimating the taxonomic composition of flies (Olea et al. 2018). Specifically, for flies, faunal surveys may be affected by the type of baits used for their collection (Gadelha et al. 2015, Harvey et al. 2019) and the positioning of traps (Mendes 1991, Maguire et al. 2014). Currently, it is common for researchers to use traps up to two meters from the ground (Moretti & Godoy 2013, Sousa et al. 2020) and baits with different animal protein, such as: bovine lung (Esposito et al. 2010), sardine (Ferraz et al. 2010), bovine liver (Oliveira et al. 2016), chicken liver (Soares & Vasconcelos 2016), and pig (De Faria et al. 2018) or rat (Oliveira & Vasconcelos 2018) carcasses. This variation in bait types aims to attract species with different olfactory preferences, as different bait substrates and decomposition stages of the substrate may produce a specific odour profile (Harvey et al. 2019).

Studies have shown differences between faunas collected at different heights from ground level (Tanabe 2002, Birtele & Hardersen 2012, Maguire et al. 2014, De Souza Amorim et al. 2022), due to the vertical distribution of resources, microclimatic preferences by species and predator avoidance (Tanabe 2002, Stork & Grimbacher 2006). Although the animal protein baits are more common for collecting, these flies can also be attracted to other resources, such as excrements, decaying fruits, and cane molasses (D'Almeida & Lopes 1983, Cabrini et al. 2013, Moretti & Godoy 2013). Some species, for example, seem better adapted to substrates with higher protein content, which may be important for oviposition and the development of immature stages, while others look for substances containing high carbohydrate content to store as energy reserves (D'Almeida & Lima 1994).

In view of this, studies that aim to understand the implications of specificities in attracting flies for measuring the biodiversity of this group are of key importance. Specifically, assessing the efficiencies or limitations of using different methodologies can increase the effectiveness of sampling since the bait types and heights can determine the faunal composition collected (Stork & Grimbacher 2006, Harvey et al. 2019, De Souza Amorim et al. 2022). The objective of this work was to assess how the attractiveness for different types of baits (banana and bovine lung) and trap heights (0.6m and 10m) influence the abundance, species richness, and composition of Calliphoridae and Mesembrinellidae flies in forest areas of the Amazon. For this, we tested three hypotheses: (i) There is a greater abundance of flies (Calliphoridae and Mesembrinellidae) in bovine lung bait and in the lower forest stratum, as flies need to feed on protein resources to complete their development (Chapman 2013) and it is in the soil that these resources decompose (Stork & Grimbacher 2006); (ii) There is greater richness in the higher forest stratum, independent of bait type, due to the great diversity of species found in this environment (Ozanne et al. 2003); (iii) The species composition is distinct between types bait and forest strata (heights), as the food attractiveness reflects the distribution of species (Stork & Grimbacher 2006, Harvey et al. 2019).

MATERIALS AND METHODS

Study area

The experiment was carried out in the district of Mazagão Velho, municipality of Mazagão, located in the south of Amapá State, Brazil. The climate is tropical, with an average temperature of 27° C and an average annual rainfall of 2,410 mm (Instituto Nacional de Metereologia e Climatologia 2020). The region has two characteristic climatic periods: a rainfall season from December to May, and a dry period from June to November. The study area is close to the Mutuacá River and the forest, a flattened land with a slight slope on the riverbanks, which flows to the Amazon River (Pinto et al. 2008). The study area is part of a floodplain forest (0°13’16.6 “S 51°26’13.3 “W) located approximately 800 m from the community (Figure 1-I).

Sample design

The collecting effort of the fly species was carried out in three collections in 2019 (January, April, and July). To avoid possible edge effects, all the samples were installed with a minimum distance of 300 meters from the forest edge. From this limit, four trails with a minimum

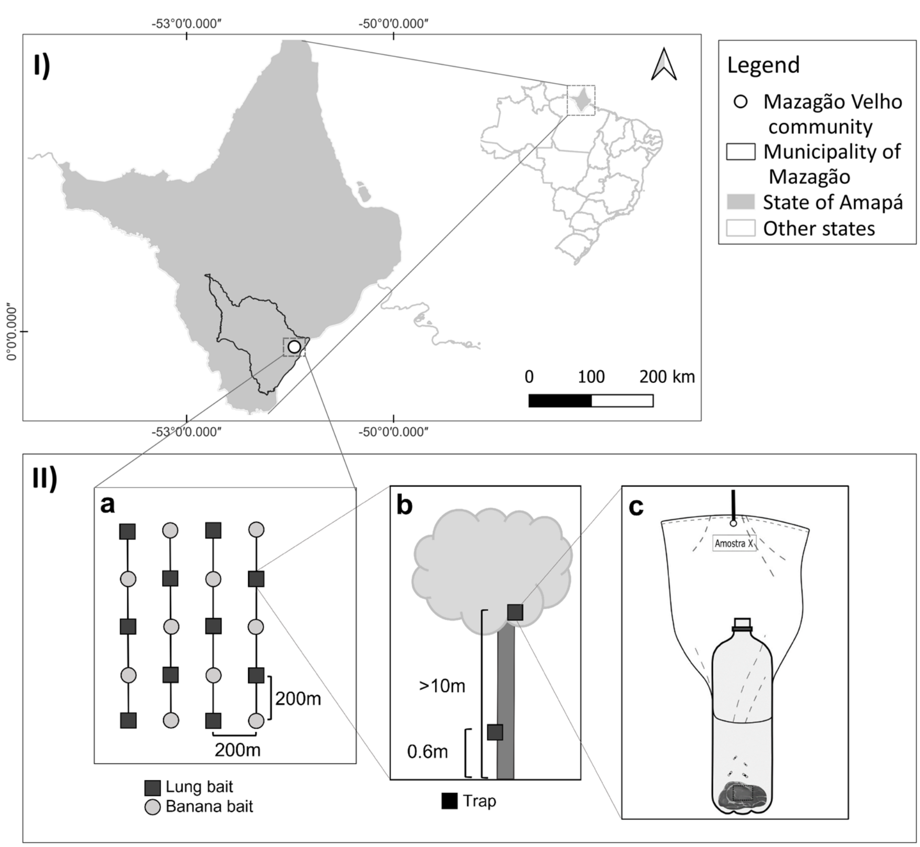

Figure 1. Map of the study area with the sampling point in the community of Mazagão Velho in the municipality of Mazagão, Amapá, Brazil (I) where the sample design was carried out (II): (a) For the experimental design, we organized four trails with a minimum distance of 200m from each other, alternating two types of baits: banana and bovine lung; (b) The tree has two traps: one at 0.6m from the ground (low) and another at 10m from the ground (high), each containing the same type of bait in the traps; (c) Schematic drawing of the trap used for collecting flies made from 2L plastic bottles and adapted from Ferreira (1978).

distance of 200m from each other were drawn. In each trail, five trees were systematically selected, with distances of 200m between them, to reduce the autocorrelation effect and ensure sample independence (Figueiredo et al. 2018, Dufek et al. 2020). None of the selected trees were fruit trees or were in bloom, thus avoiding interferences from other fly attractants (Figure 1-2a).

The three collections in 2019 were carried out using the same trees and alternating the type of bait with each collection, totalling a sampling effort of 120 traps (5 trees x 4 trails x 3 months x 2 heights = 120). For standardization, along the manuscript the trap placed 0.6m from the ground is called “low” and the one placed at 10 m is referred to as “high”.

Fly collection

The traps were installed at two heights: 0.6 m and 10 m above the ground. Two types of bait were used: bovine lung and banana. These traps were placed alternately in the trees, where each tree received two traps with the same type of bait: one low and one high. We used both traps in the same tree to mitigate the effects of the choice of tree species and microclimate variations in the fly community (Ulyshen et al. 2010, Luz et al. 2020), in addition to ensuring that the traps were exposed to the same set of flies. The traps were exposed for 48 hours in the forest (Figure 1-2b).

Calliphorids and mesembrinellids were captured with traps like those described by Ferreira (1978), adapted using 2L plastic bottles (Figure 1-2c). This trap consists of a plastic bottle cut horizontally in half, dividing it into the lower part (bottom) and upper part (neck). A hole has been opened at the bottom, which serves as an entrance for the insects; and the upper part is covered by a transparent plastic bag, which contains a string on its upper surface for attachment to tree branches. The parts have been joined with adhesive tape (Figure 1-2c).

The low traps were installed 0.6m above the ground on tree branches. The tall traps were hoisted using a slingshot, which threw a stone of about 60g at the top of the trees. This stone was tied to a thin and strong rope so that it would pass between the chosen branches and fall to the ground. The trap could then be attached to the tip of the fallen rope and lifted to the desired height.

To collect flies, the bait had 50g of each attractive material: protein (bovine lung) and fruit (banana), kept for 24 hours at room temperature before using. The bovine lung was exposed to room temperature 24 hours before including the bait in traps, so that it began the decomposition process to become more attractive to flies. The banana was kneaded and mixed with beer (to speed up the fermentation process) and was also exposed to room temperature. Both baits were protected during this process to avoid contact with insects before being used in the traps. The baits were then allocated individually to each trap. This methodology is the most frequent used in studies with Calliphoridae and Mesembrinellidae (Sousa et al. 2010, 2020). The types of baits used were also based on the frequency of use described in the literature (D’Almeida & Lima 1994, Sousa et al. 2010, Cabrini et al. 2013, Dufek et al. 2016).

Specimens of Calliphoridae and Mesembrinellidae were separated from other insect species collected, sexed and later identified to species using the dichotomous descriptions and keys of Guimarães (1977), Carvalho & Ribeiro (2000), Mello (2003), Kosmann et al. (2013) and Whitworth & Yusseff-vanegas (2019). After identification, the material was deposited in the wet Collections in the Zoological Collection of the Federal University of Pará.

Data analysis

To analyse the influence of baits (bovine lung and banana) and traps (low and high stratum of the forest) on the species richness, abundance, and composition, we analyse the results in two ways: the Calliphoridae and Mesembrinellidae families with their data added together and separately. This division was necessary because the ecophysiological requirements of each group are different or even antagonistic (Sousa et al. 2014, 2016, 2021). In addition, we evaluated family influence on the analyses.

Species richness and abundance by height and bait

To analyse whether the sampling effort was efficient in capturing the species of flies from the study site, we conducted three analyses of species accumulation curves: for the total number of individuals per sample (adding both families), for the sampling of individuals from Calliphoridae, and for the sampling of individuals from Mesembrinellidae. To do this, we use the "Inext" function in the *Inext* package (Hsieh & Chao 2016) with species incidence frequency data in the traps (as a sampling unit) to construct the curves. We use the argument "datatype = incidence_freq" which performs species accumulation curves based on frequency of occurrence, rarefaction, and species extrapolation.

To evaluate the hypothesis that there is a difference in fly richness and abundance between heights (low and high) and baits (banana and bovine lung), we used a Generalized Mixed Linear Model (GLMM). The species richness and abundance, respectively, were tested as response variables in different models for the summed data of both families and for Calliphoridae and Mesembrinellidae separately, with height and bait as explanatory variables and the three different collecting months as a random variable of the models. Therefore, six mixed models were performed. Both models were generated using the "*glmer*" function of the *lme4* package (Bates et al. 2014) with the "Poisson" distribution, suitable for counting data. The validation of the models was performed graphically following the protocol of (Zuur et al. 2010).

Species composition by baits and heights

To assess the hypothesis that there are differences in the species composition between baits and heights, a Permutational multivariate analysis of variance (PERMANOVA) was carried out. The species composition was the response variable in three different models, considering both families together and each one separately. We used the "*adonis*" function of the *vegan* package (Oksanen et al. 2019) with 9999 randomizations. The analysis was stratified within the collecting methods ("strata" argument), due to the block sampling design, i.e., the species composition within each collection. Once the significance was detected, we evaluated the difference in levels with simplified peer-to-peer (post-test) models. A Principal Coordinate Analysis (PcoA) was also performed, using the "*cmdscale*" function also from the vegan package, with Bray-Curtis index as similarity measure (Gotelli & Colwell 2011). For both the PERMANOVA and the PcoA, the abundance data were previously Log-transformed (x+1) to decrease effects from abundant species (Gotelli & Colwell 2011). Furthermore, to evaluate the specificity of fly species in the different methodologies used, we used the Indicator Value (IndVal) analyses to measure the association between a species and a methodology (Dufrêne & Legendre 1997). For the IndVal analyses, we used the "*multipatt*" function of the *indicspecies* package (Cáceres & Legendre 2009).

All the statistical analyses were performed using the R software (version 4.0.0; R Core Team 2020) with a 5% significance level. The figures were made using the *ggplot2* (Wickham 2016) and *gridExtra* (Auguie 2017) packages.

RESULTS

A total of 1,173 specimens were collected and identified as belonging to 14 species of flies. Calliphoridae showed the highest abundance, corresponding to 82% of the total flies collected in the study, including 962 individuals and 10 species, the most abundant of which was *Lucilia eximia* (Wiedemann 1819). The Mesembrinellidae presented an abundance of 211 individuals and four species, of which the most abundant was *Mesembrinella bellardiana* (Aldrich 1922). Considering the four groups studied (two bait types and two trap heights), there was a greater abundance of flies in low traps containing bovine lung baits, with 449 individuals (38.28%) and a richness of 12 species. The lowest abundance occurred in high traps containing banana bait, with 182 individuals (15.52%) and a richness of 13 species (Table I).

The species accumulation curves, based on the number of total samples, reached the asymptote, both when performed with the total sum of flies, as well as when analysed separately for calliphorids and mesembrinellids (Figure 2). The observed and estimated richness

Table I. Species richness and abundance of flies from the Calliphoridae and Mesembrinellidae families collected in two strata (high and low) by using two attractive baits (bovine lung and banana) in the municipality of Mazagão, Amapá State, Brazil.

Family / Species	Lung Low	Banana Low	Lung High	Banana High	Total
Calliphoridae					
Chrysomya albiceps (Wiedemann 1819)	54	30	34	25	143
Chrysomya megacephala (Fabricius 1794)	53	4	29	18	104
Chrysomya putoria (Wiedemann 1818)	13	13	8	6	40
Chloroprocta idioidea (Robineau-Desvoidy 1830)	74	27	36	11	148
Cochliomyia macellaria (Fabricius 1775)	37	22	32	9	100
Cochliomyia hominivorax Coquerel 1858	0	0	1	0	1
Hemilucilia semidiaphana (Rondani 1850)	56	26	32	14	128
Lucilia eximia (Wiedemann, 1819)	68	47	38	38	191
Lucilia cuprina (Wiedemann, 1830)	3	1	2	4	10
Paralucilia paraensis (Mello 1969)	46	21	23	7	97
Mesembrinellidae					
Mesembrinella bellardiana Aldrich, 1922	25	24	17	24	90
Mesembrinella bicolor (Fabricius, 1805)	12	22	23	19	76
Mesembrinella randa (Walker, 1849)	0	9	8	2	19
Mesembrinella quadrilineata (Fabricius, 1805)	8	8	5	5	26
Abundance	**449**	**254**	**288**	**182**	**1173**
Species richness	**12**	**13**	**14**	**13**	**14**

were close. When analysing total flies, the observed richness was 14 species, and the estimated richness was 15.347 species. When analysing Calliphoridae flies only, the observed richness was 10 species, and the estimated richness was 11.361. For Mesembrinellidae flies, the observed richness found was four and the estimated species richness was 4.008. When assessing sampling efficiency (observed richness/estimated richness) all curves were stabilized and resulted in sampling efficiency above 80% (Total 91%, Calliphoridae 88% and Mesembrinellidae 99%). These results confirm that the effort applied in the study was efficient in consistently measuring the richness of these species in the study area.

Effect of height difference and bait types on the richness and abundance

When assessing whether there are differences in the total abundance and richness of fly families (Calliphoridae and Mesembrinellidae) and each family separately between treatments, we observed that there was no interaction effect in either model (p= 0.599 and 0.386; p=0.456 and 0.232; p= 0.456 and 0.182 respectively) (Table II).

The hypothesis that the abundance of flies would be higher in bovine lung bait in low traps was corroborated, since there were differences

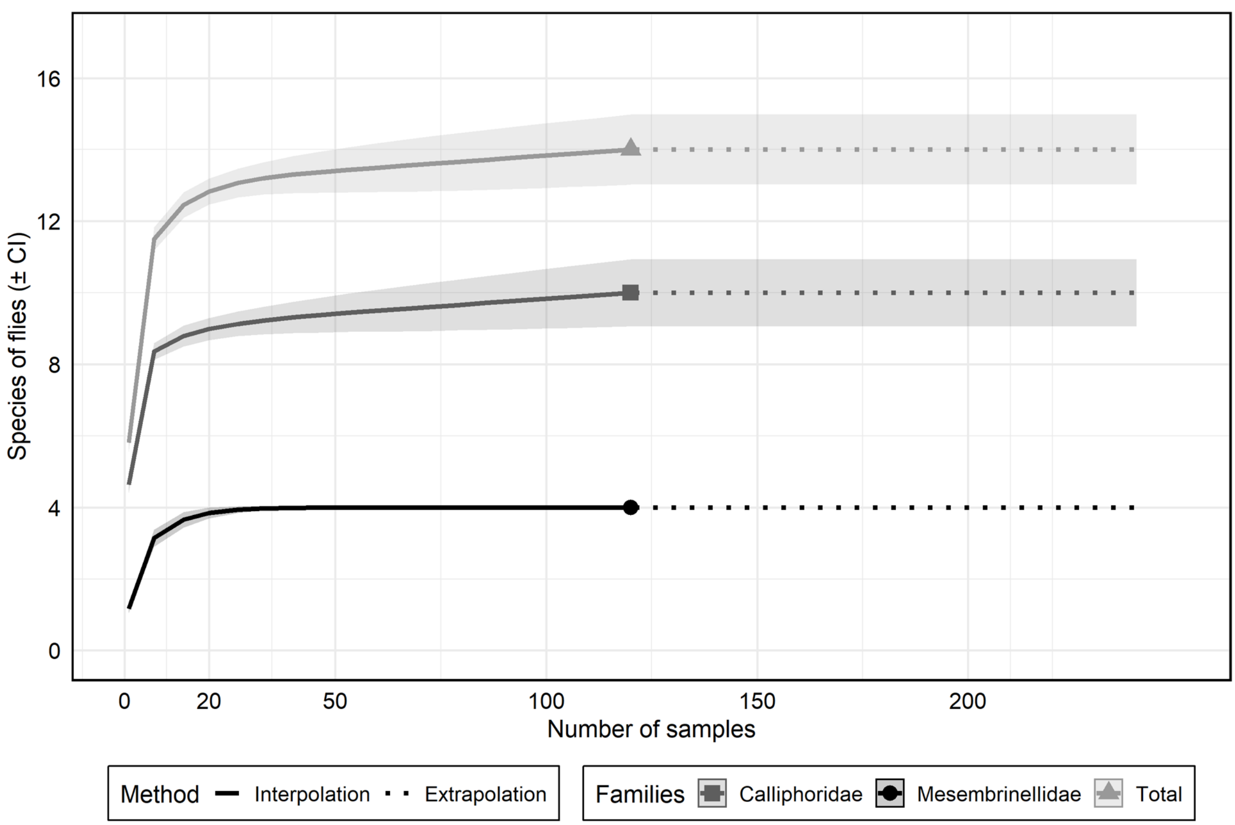

Figure 2. Species accumulation curve (based on rarefaction and extrapolation, CI = confidence interval) of total identified fly species (line with triangle), Calliphoridae species (line with square) and Mesembrinellidae species (line with circle) collected in Varzea Forest in Mazagão, Amapá State, Brazil. (The continuous line represents the observed species richness, and the dotted line represents the extrapolated richness of the samples; the polygons (square, circle, and triangle) represent the total number of samples in the study (120); and the thin lines above and below in a lighter tone represent the confidence interval of the analysis).

in the total abundance with effects separated from the bait type and heights (Table II; Figure 3). When analysing the abundance of each family separately, differences with separated effects were also identified between the bait type and heights for Calliphoridae (Table II; Figure 3), which represented 62% of the abundance in bovine lung baits and low traps (639 and 595 individuals respectively). The Mesembrinellidae showed no difference in abundance (Table II; Figure 3).

The hypothesis that the greatest species richness would be in high traps, regardless of the bait used, was refuted since differences were found between the type of bait and height, with the greatest richness was found in low height traps using bovine lung bait (Table II; Figure 3). When analysing the families separately, a difference was also found with isolated effects on the type of bait and trap height for Calliphoridae, where bovine lung baits and high traps collected the greatest richness (Table II; Figure 3). The Mesembrinellidae family did not differ in species richness by bait or height (Table II; Figure 3).

Effect of different heights and bait types on the species composition

The hypothesis that the composition of fly species is distinct between bait types and forest strata was corroborated as the total fly composition differed between heights (Pseudo-F = 3.18; $p<0.01$) and baits (Pseudo-F = 7.68; $p < 0.001$) (Table III). The PcoA explained in its first two

Table II. Parameters estimated by the Generalized Mixed Linear Model (GLMM) using Poisson distribution to assess if the richness and abundance of Calliphoridae and Mesembrinellidae species differ between baits and heights. The response variable was species richness and abundance, and the predictor variables are the fixed bait factors with two levels (bovine lung and banana) and height (low and high) and the random factor Collection with three levels (C1, C2 and C3). Estimative (Est.); Standard error (SE); Variance (VA); Standard deviation (SD).

Model	Explanatory variables	Fixed effects	Est.	SE	Z score	p-value	Random effect	VA	SD
Total	Richness	Intercept	1.510	0.133	11.317	**< 0.001**	Collection	0.038	0.195
		height	0.160	0.075	2.119	**0.034**			
		bait	0.276	0.076	3.625	**< 0.001**			
	Abundance	Intercept	1.709	0.195	8.737	**< 0.001**	Collection	0.103	0.322
		height	0.413	0.059	6.933	**< 0.001**			
		bait	0.521	0.060	8.637	**< 0.001**			
Calliphoridae	Richness	Intercept	1.223	0.130	9.408	**< 0.001**	Collection	0.031	0.176
		height	0.183	0.085	2.160	**0.031**			
		bait	0.366	0.086	4.260	**< 0.001**			
	Abundance	Intercept	1.365	0.201	6.770	**< 0.001**	Collection	0.107	0.327
		height	0.484	0.066	7.313	**< 0.001**			
		bait	0.679	0.068	9.966	**< 0.001**			
Mesembrinellidae	Richness	Intercept	1.138	0.190	0.729	0.466	Collection	0.043	0.209
		height	0.071	0.168	0.422	0.673			
		bait	0.071	0.168	0.422	0.673			
	Abundance	Intercept	0.551	0.193	2.852	**0.004**	Collection	0.069	0.268
		height	0.095	0.137	0.691	0.489			
		bait	0.153	0.138	1.106	0.268			

axes 41.448% of the total variation in data, with banana bait and high traps presenting distant points and occupying more of the negative part of the second axis, while bovine lung bait and low traps presented close points and occupy the positive part of the first and second axis (Figure 4a, b). So, both families' faunistic composition added together proved to be influenced by lung bait and the low stratum of the forest.

When we analyse the species composition of families separately, we detected an interaction in the composition of the Calliphoridae family between the treatments studied (Pseudo-F = 2.360; p= 0.046) (Table III). The PcoA explained in its first two axes 51.482% of the total variation of the data, with differences between high banana and low lung baits because they were less overlapping (Figure 4c). The Mesembrinellidae family showed no difference in species composition between baits (F= 0.370; p= 0.725) and by heights (F= 0.500; p= 0.633).

Therefore, Calliphoridae seems to be the one that most contributes to the differences between types of baits and strata of trees of the two families when considered together, since Mesembrinellidae did not present differences in species composition between the baits and heights when analyzed separately. Thus, species composition is more associated with families than with the whole community.

Considering the baits used, the species collected with banana (13) were like those

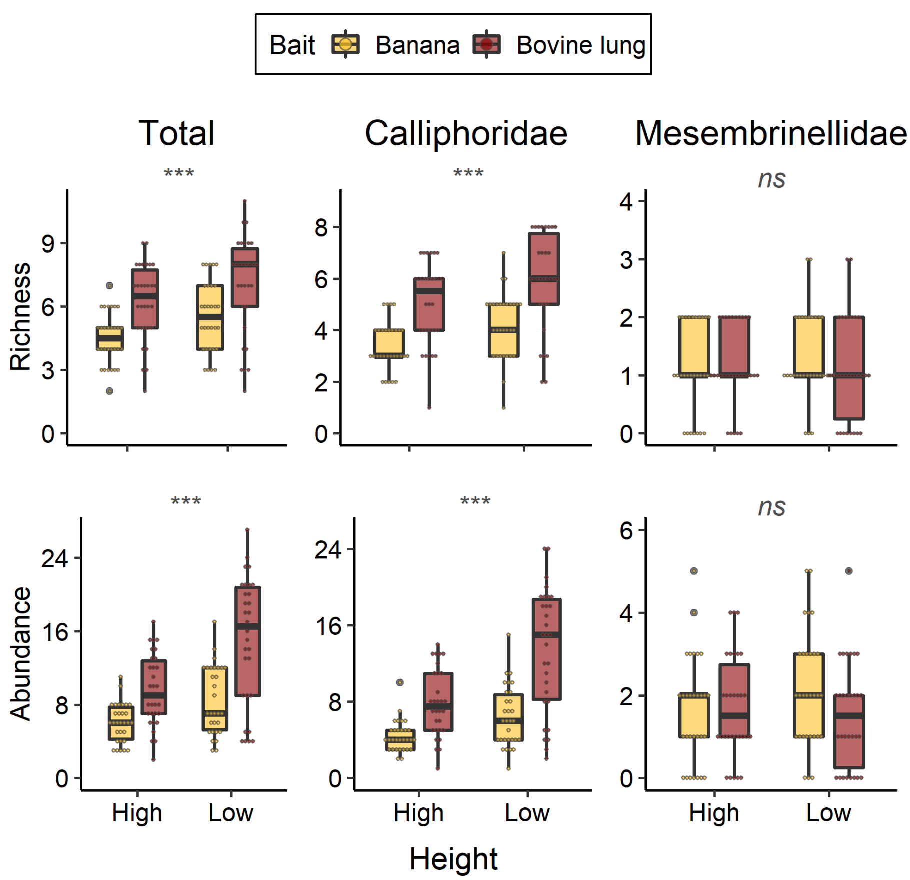

Figure 3. Analysis of species richness and abundance of flies collected in two strata (high and low) using two attractive baits (banana and bovine lung) in the Forest of Varzea in the municipality of Mazagão, Amapá State, Brazil. (Total = Calliphoridae + Mesembrinellidae; * = significant result; ns= non-significant result).**

collected with bovine lung (14). For the heights, it was observed that the high traps collected one more species than in low traps (14 and 13 respectively) (Figure 5). Only one fly species, *Mesembrinella quadrilineata* (Mesembrinellidae) was specifically associated with a single treatment, for the bovine lung baits in high traps (Supplementary Material - Table SI).

Since basically the same species were collected in all treatments, species accumulation curves were generated for the two baits and heights separately. For Calliphoridae and Mesembrinellidae, it was observed that lung baits may be used in low traps because they reached the asymptote first (Supplementary Material - Figure S1). With this method, the same biodiversity metrics can be measured with a smaller number of samples, since there is no need to use different types of baits and height, making it possible to better distribute the traps in the region and increase spatial variation. Additionally, lower sampling needs help alleviate collection and diagnostic burdens.

DISCUSSION

This study shows that the species richness, abundance, and composition of tropical Calliphorid flies is influenced by bait type and

Table III. PERMANOVA result for the response of the Calliphoridae and Mesembrinellidae families fly composition in relation to the heights (high and low) and baits (lung and banana) collected in the Forest in Mazagão municipality, Amapá State, Brazil. Degrees of freedom (d.f.); Sum of squares (SS); Average of squares (MS).

Model	Explanatory variables	d.f.	SS	MS	*Pseudo F*	R^2	*p**
Total	Height	1	0.49	0.48	3.18	0.02	**<0.01**
	Bait	1	1.18	1.18	7.68	0.06	**<0.001**
	Residuals	117	17.97	0.15		0.91	
	Total	119	19.64			1	
Calliphoridae	Height	1	0.57	0.57	4.12	0.03	**<0.01**
	Bait	1	1.21	1.21	8.70	0.06	**<0.001**
	Height: Bait	1	0.33	0.33	2.36	0.01	**0.046**
	Residuals	116	16.18	0.13		0.88	
	Total	119	18.30			1	
	peer-to-peer						
	High Banana vs Low Banana				3.555	0.057	**0.042**
	High Banana vs High Lung				4.555	0.078	**0.018**
	High Banana vs Low Lung				9.984	0.145	**0.006**
	Low Banana vs High Lung				9.933	0.048	0.150
	Low Banana vs Low Lung				6.758	0.104	**0.006**
	High Lung vs Low Lung				2.748	0.045	0.168
Mesembrinellidae	Height	1	0.12	0.12	0.50	0	0.63
	Bait	1	0.09	0.09	0.37	0	0.72
	Residuals	94	23.42	0.24		0.99	
	Total	96	23.64			1	

***p* determined by permutation.**

the height of trap exposure. Still, the abundance and species richness of Mesembrinellidae were not affected by bait type and height. These findings highlight the importance of bait type and exposure height as predictors of fly community sampling in environments of the Amazon rainforest and serve as possible metrics for the standardization and optimization of collection protocols.

Patterns of species richness and abundance

The species richness found in this work is like that found in other studies in the Amazon forest (Esposito et al. 2010, Sousa et al. 2016). In general, the richness and abundance of

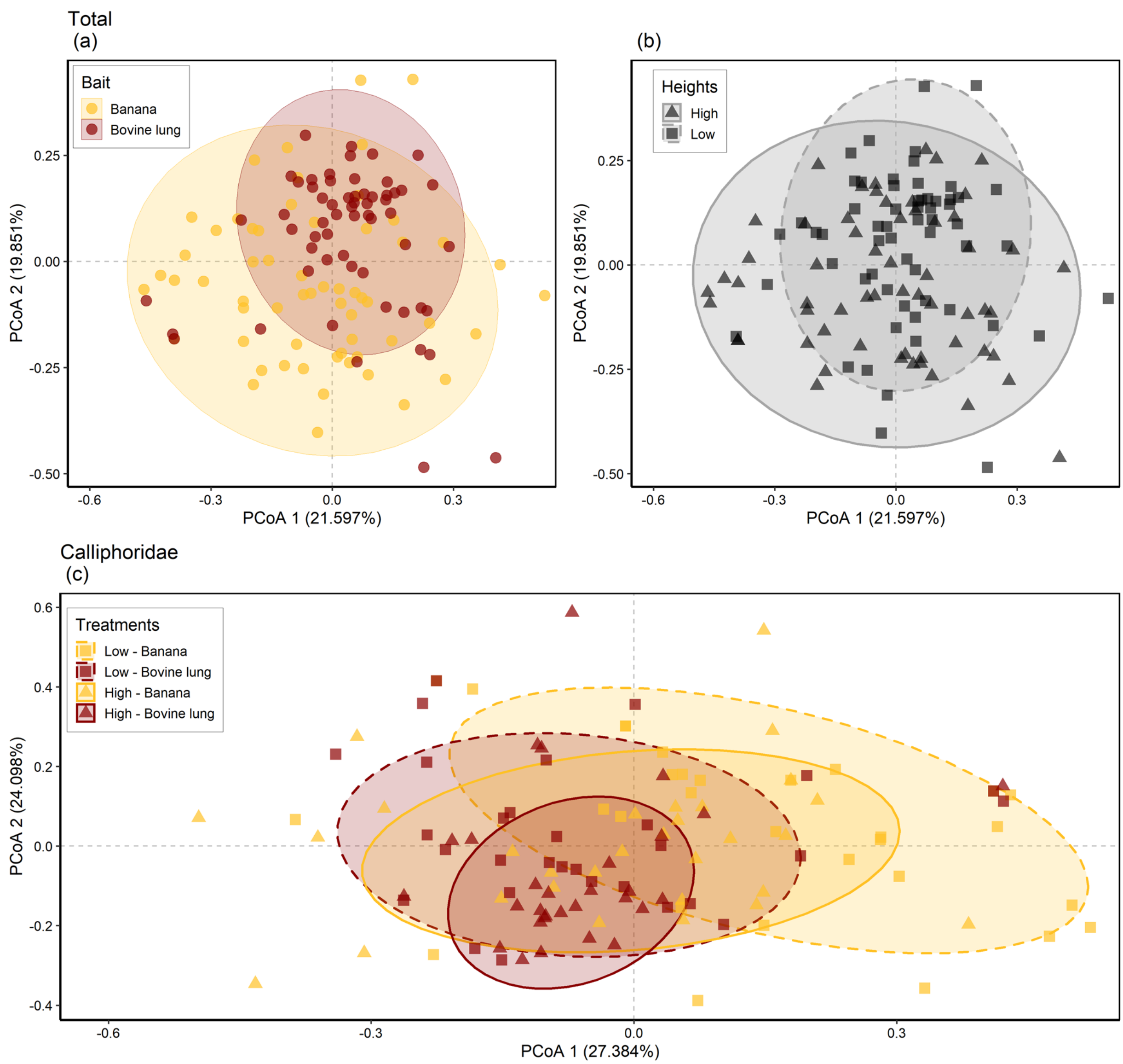

Figure 4. Principal Coordinate Analysis (PCoA) of the composition Total flies Calliphoridae and Mesembrinellidae collected in (a) two baits (banana and bovine lung) and (b) two heights (high and low); and Analysis of the Main Coordinates (PCoA) of the composition only of the flies Calliphoridae (c) showing interaction between the four treatments (high banana, high lung, low banana, low lung) collected in the forest in Mazagão, State of Amapá, Brazil.

flies were affected by baits and the placement (height) of where the traps were exposed. Bovine lung bait at the low stratum attracted a greater abundance of specimens. Although the greatest richness was detected in low traps, the high traps also collected all species in this study, with the high stratum and lung bait collecting one additional species. Calliphorid flies were the most abundant family in the collections and contributed most to the analysis when data of both families were used, since calliphorids presented the same pattern when considered separately. However, Mesembrinellidae diversity did not present differences in richness or abundance in the samplings.

The importance of knowing the canopy fly fauna lies in the fact that there are differences in the insect community at different tree strata, so it is possible to know how the different groups exploit the available resources in the vertical structure of the forest (Tanabe 2002, Birtele & Hardersen 2012, Puker et al. 2020, De Souza Amorim et al. 2022). However, many current studies expose traps at heights up to two meters from the ground and use decaying animal bait to attract dipterans (Esposito et al. 2010, Sousa et al. 2020, 2021). Calliphoridae, for example, can be collected in greater numbers in traps installed at different heights of the ground. Differences in the abundance of *Lucilia cuprina* (Wiedemann, 1830) in traps installed at different heights (0.5; 1.0; 1.5; 2.0 metres above ground) have already been reported (Vogt et al. 1995), and other calliphorids have been collected at 8, 16, 24, and 32 meters from the ground (De Souza Amorim et al. 2022).

A greater number of species in the canopy, compared to those in soil, can be a consequence of many rare species in this environment, as has been reported for beetles (Stork & Grimbacher 2006). Sex differences in strata preference have also been reported for Syrphidae flies by Birtele & Hardersen (2012). The differences in sexual relations between the canopy and the soil were also observed in this study. Females were captured in greater numbers at ground level, and

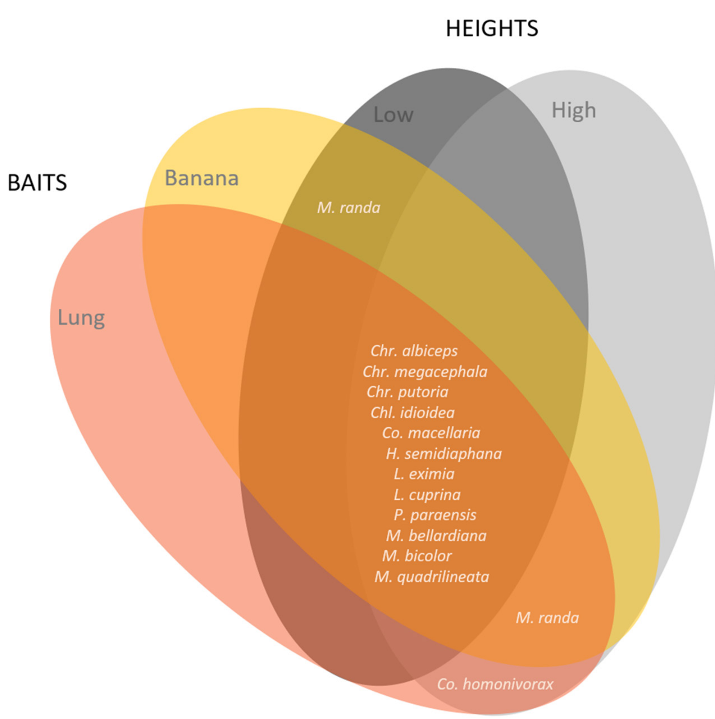

Figure 5. **Venn diagram containing the variation of Calliphoridae and Mesembrinellidae species collected in two baits (bovine lung and banana) and two heights (low and high) in the municipality of Mazagão, Amapá State, Brazil.**

males were captured more often in the canopy. Studies on the differences in male and female Diptera distribution are scarce, but similar sex differences in preferences between soil and canopy have been reported for Tachinidae (Stireman et al. 2012). After mating, the females need specific nutrients for egg maturation (D'Almeida & Lima 1994), and for that, they must fly close to the ground, where there is a greater abundance and diversity of food. Thus, it is likely that the greater abundance of flies in low traps is related to behavioural differences. The greater abundance of males in high traps is less clear because the presence of females and trophic resources in this stratum probably does not explain this behaviour and is more likely due to microclimatic preferences and predator avoidance (Stork & Grimbacher 2006).

The vegetation type, height, density, and distribution of trees in these environments can interfere or favour the entry of sunlight and can create new habitat conditions for fly development, feeding, and oviposition (Ulyshen et al. 2010, Luz et al. 2020). There was not a significant increase in species richness in the canopy in our results, but it was recorded that the diversity of species in this layer comparable to the biodiversity in the soil. The forest biodiversity cannot be understood completely without investigating the canopy region (Birtele & Hardersen 2012, De Souza Amorim et al. 2022). The fact that the flies arrive in the trap shows the effectiveness of the method. Niche sharing is very common, also valid for flies (Stork & Grimbacher 2006, Ulyshen et al. 2010, Birtele & Hardersen 2012, Maguire et al. 2014, Giovanni et al. 2015). In addition, the canopy may offer resources such as fruits and carcasses of animals that die and become trapped in the trunk or branches of trees. These results also emphasise that vertical gradient sampling, considering canopies, should be counted in surveys of fly communities to advance knowledge on ecological monitoring of calliphorids and mesembrinellids.

Traps containing protein baits are commonly used to catch insect populations and may in turn be more attractive than other baits due to the available nutrients required for the reproduction of some species. Different baits produce specific odours and the level of attractiveness of this substrate varies according to the species and its physiological status, such as sex, age, or reproductive status. Bovine lung baits are more attractive because they are favoured by developing and reproductively active flies (Avancini & Prado 1986). Though flies were less abundant in banana bait traps, these traps still presented a considerable number of specimens, as flies are attracted by the fruit fermentation process and still need the carbohydrates found in this substrate to develop (D'Almeida & Lima 1994). This highlights the importance of this type of substrate for fly collection.

Calliphoridae showed high abundance in low traps with bovine lung baits and lesser abundance in high traps with banana baits. Other studies have observed similar patterns showing that lung and other protein baits are more attractive when compared to ripe banana and pineapple (Mendes 1991, D'Almeida & Mello 1996). Because the type of bait can affect the species composition attracted to the traps, comparisons between studies with different types of baits may be limited (Vilte et al. 2019). *Chloroprocta idioidea*, for example, is considered to be associated with preserved environments in the Brazilian Amazon (Esposito et al. 2010, Sousa et al. 2020, Mendes et al. 2021). It was the second most abundant species in this study, and according to Batista-da-Silva et al. (2010) it prefers decaying fruits or even animal faeces, as these substrates can be found in greater abundance in the forest.

Mesembrinellidae was more abundant in banana baits, regardless of height, despite not showing great differences in abundance between treatments. Mesembrinellids are more attracted to bird faeces or plant material, feeding mainly on rotten fruit (Guimarães 1977). They are also commonly collected with animal baits (Esposito et al. 2010, Sousa et al. 2010, 2021), which corroborates the results of this study. On the other hand, adults of the same family rarely fly more than a few meters above the forest ground (Guimarães 1977, Gadelha et al. 2009), which is different from what was observed here. This family was very representative in high traps, demonstrating their ability to fly and use other strata, such as the understory. Observations of this family in the high stratum may be explained by resource searching behaviours and conditions, as well as being attracted by banana fermentation. Although Mesembrinellidae species live inside the forests, they can also be observed in open habitats (such as high trees) in the early mornings, late afternoons, or cloudy days, purportedly when searching for food resources (Guimarães 1977). These collections were performed during rainy months, which may also explain the abundance of these specimens in the higher traps, as the temperature conditions become milder with height. In our study, we found few species and low abundance of Mesembrinellidae, which may have affected our ability to detect statistical patterns associated with different the experimental treatments.

Patterns in species composition

We identified that the differences in species composition between the baits and the trap heights were more associated with the subgroups than with the entire community. Thus, when studying Calliphoridae and Mesembrinellidae, these families should be interpreted separately to avoid bias in the data.

The collection methods using protein baits and traps closer to the forest ground are effective for the collection of both families. However, when there is interest in collecting Calliphoridae flies separately, banana baits can also be used closer to the soil or higher up on the trees. This is due to the ability of this family to feed on both animal and carbohydrate-rich substrates (Avancini & Prado 1986, D'Almeida & Lima 1994). For sampling of Mesembrinellidae, the most effective method may be to focus on protein-rich baits and traps closer to the soil (Figure S1), because these flies are largely collected in traps with animal carcass or decomposing vegetable bait (Guimarães 1977).

The species *L. eximia* and *Chl. idioidea* were the most abundant in Calliphoridae and *M. bellardiana* in Mesembrinellidae. These species were being attracted by both banana baits and bovine lung baits at both trap heights. These same species were also the most abundant found in prior studies by Esposito et al. (2010) and Sousa et al. (2015) in environments similar to those sampled in our study, and they were strongly associated with preserved environments (Sousa et al. 2010, 2014, 2020, Cabrini et al. 2013, Mendes et al. 2021). Therefore, this pattern may be indicative of the conservation status of the sampled area.

The least abundant species was *Cochliomyia hominivorax*. It has a biophagous habit and is the cause of primary myiasis (Guimarães et al. 1983, Batista-da-Silva et al. 2012). It, is not commonly collected by bait traps with decomposing bovine lung, suggesting its collection record in this study is unique and perhaps a could be considered a random collection.

Of all the species identified in this study, only *Mesembrinella quadrilineata* had a specific association with bovine lung bait and the high forest stratum. This is an asynanthropic species, i.e., associated with preserved environments and, like the other mesembrinellids, is found in forested environments (Guimarães 1977, Sousa et al. 2010, 2020, Whitworth & Yusseff-vanegas 2019). There are no studies on the biology of this species in the literature, but it is known that the family can be attracted to both animal carcasses and rotting fruits (Guimarães 1977).

In view of this, the distribution of Calliphoridae and Mesembrinellidae species in the environment is influenced by the conditions that the habitat present for individuals, where the community can vary according to the attractive resources prevalent in the locations closest to the ground and on top of the trees. In addition, the degree of attractiveness of the set of traps (baits and stratum) to calliphorid and mesembrinellids flies may be related to the biologically-based preferences of each family and each species, either for food and/or for reproduction.

In conclusion, the flies from the Mesembrinellidae family were not differentially attracted to either of the bait types (bovine lung and banana), or trap heights (high and low) used in this study. However, these methodologies were important for Calliphoridae flies, which were predominantly collected in traps containing protein-rich baits placed near the ground. They should be analyzed separately for a better interpretation of their community structure. Besides, considering the total individuals collected, calliphorids were more abundant in bovine lung baits and mesembrinellids were more abundant in banana baits. Lastly, the traps closest to the forest ground were most visited by both calliphorids and mesembrinellids.

Acknowledgments

BLBF thanks Any Gomes, Camila Gama, Camila Mendes, Vivian Abreu and Josué Videira for their help with the field work and Solange Barbosa, Augusto Barbosa, Cristiano Barreto for the field logistics. Rony Almeida for helping with the analysis. This study was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) through a post-graduate scholarship to BLBF. Leandro Juen (process: (304710/2019-9) and Maria Cristina Esposito (process: 309090/2019-9) thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for productivity scholarships.

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SUPPLEMENTARY MATERIAL

Figure S1.
Table SI.

How to cite
FAÇANHA BLB, ESPOSITO MC & JUEN L. 2022. Trap and bait efficiency for catching Calliphoridae and Mesembrinellidae (Insecta, Diptera) at different heights. An Acad Bras Cienc 94: e20210763. DOI 10.1590/0001-3765202220210763.

Manuscript received on May 25, 2021;
accepted for publication on May 9, 2022

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All authors contributed to the design of the study. The preparation of the material, data collection and analysis were performed by BLBF, MCE and LJ. The first manuscript version was written by BLBF and all authors commented on previous manuscript versions. BLBF and MCE collected the samples and identified the material. BLBF and LJ analyzed the data and wrote the analyses. BLBF, MCE and LJ wrote the article. All authors read and approved the final manuscript.