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Article

Protocol for Membracidae inventory (Hemiptera, Auchenorrhyncha, Membracoidea): what are the ideal collection methods for the Atlantic Forest?

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Abstract: Membracidae are phytophagous insects that present different types of behavior, requiring a specific protocol for fast and efficient collection. This article evaluates the best methods for sampling these insects in Atlantic Forest areas. The protocol was applied in four areas of the Atlantic Forest in Paraíba state, Brazil, and involved a team of four people at a cost of US$180 per area. Each area contained 100 sampling units subdivided into 30 yellow sticky cards in the canopy and 30 yellow sticky cards in the lower stratum, 30 active collections and 10 light traps. In total, 2,678 specimens belonging to 91 species were sampled. The highest abundance and richness values were obtained using active collection (N = 1,517; S = 42) and cards in the canopy (N = 345; S = 53). All methods exhibited high complementarity, with more than half of the species (S = 50; 54.35%) recorded exclusively by only one of the methods applied. Similarity analysis revealed that active collection differs significantly from all other methods (R = 0.10, p = 0.0001) and that the sticky cards in the canopy differ from the collection in the lower stratum (p = 0.0001), whereas the other method pairs did not exhibit significant differences. In all areas, the active collection, the sticky cards in the canopy and the lower stratum had the best sample sufficiency, with at least 60% of the estimated values. To inventory Membracidae specimens in areas of the Atlantic Forest, a protocol that combines different collection methods is required, which in principle requires more time and expense. However, it is worth noting that it is possible to adjust this protocol according to the researcher's need. For a faster survey that includes the largest number of species, we suggest a combination of active collection and a light trap.
Keywords: *Biodiversity; Brazil; Estimators; List of species; Sampling standardization; Treehoppers.*

Protocolo para inventário de Membracidae (Hemiptera, Auchenorrhyncha, Membracoidea): quais os métodos de coleta ideais para Floresta Atlântica?

Resumo: Membracídeos são insetos fitófagos que apresentam diferentes tipos de comportamento, o que requer um protocolo específico para uma coleta rápida e eficiente. Este artigo avalia quais os melhores métodos para amostragem desses insetos em áreas de Floresta Atlântica. O protocolo foi aplicado em quatro áreas de Floresta Atlântica na Paraíba e envolveu uma equipe de quatro pessoas, ao custo de U$180 por área. Contém 100 unidades amostrais subdivididas em 30 cartões adesivos amarelos no dossel, e 30 no estrato inferior, 30 coletas ativas e 10 armadilhas luminosas. No total foram amostrados 2.678 espécimes pertencentes a 91 espécies. As maiores abundâncias e valores de riqueza foram obtidos usando a coleta ativa (N = 1.517; S = 42) e os cartões do dossel (N = 345; S = 53). Todos os métodos apresentaram alta complementaridade, com mais da metade das espécies (S = 50; 54,35%) registradas exclusivamente por apenas um dos métodos aplicados. A análise de similaridade mostrou que a coleta ativa difere significativamente de todos os outros métodos (R = 0,10; p = 0,0001), e que os cartões adesivos no dossel diferem da coleta no estrato inferior (p = 0,0001), enquanto os outros pares de métodos não apresentaram diferenças significativas. Em todas as áreas, a coleta ativa, os cartões adesivos no dossel e no estrato inferior, respectivamente, foram os que apresentaram melhor suficiência amostral, com valores de no mínimo 60% do estimado. Foi demonstrado que, para inventariar membracídeos em áreas de Floresta Atlântica, é necessário um protocolo que combine diferentes métodos de coleta, o que à priori, demanda mais tempo e custo. Contudo, vale ressaltar que é possível ajustar este protocolo de acordo com a necessidade do pesquisador. Indicamos que para um levantamento mais rápido e que contemple o maior número de espécies, o ideal é utilizar uma combinação de coleta ativa e armadilha luminosa.
Palavras-chave: *Biodiversidade; Brasil; Estimadores; Lista de espécies; Amostragem padronizada; Soldadinhos.*

Introduction

Extensive quantitative samplings are typically problematic because they require long periods of time, a large number of people and, consequently, significant resources (Cardoso 2009, Magurran 2011). Because increasingly fewer resources have been allocated for these purposes, rapid survey methods or protocols have become more popular (Oliver & Beattie 1996, Duelli 1997, Jones & Eggleton 2000, Muelelwa et al. 2010). In this context, rapid biodiversity assessments (RBA) have been increasingly implemented in inventory and monitoring studies, being used for diverse taxa in different habitats and ecosystems (ants/litter: Alonso & Agosti 2000, Agosti & Alonso 2000; spiders/Mediterranean oak forests: Cardoso et al. 2008; ants: Souza et al. 2012; scarab beetles/Amazon: Braga et al. 2013).

The use of RBA should ensure that the diversity of the taxon sampled reflects its composition in the areas where it is applied (Jones & Eggleton 2000, Gillies et al. 2009). For this purpose, collection protocols are developed or adapted (Borisko et al. 2007, Buss & Borges 2008, Cardoso et al. 2008) using numerous collection methods to sample the largest possible number of representatives of the species that are part of a given assembly.

Well-structured protocols, in addition to facilitating inventory and monitoring studies, ensure the possibility of data sharing in comparative studies based on the use of these protocols (Gotelli & Colwell 2001). In the present study, we present a sampling protocol to inventory Membracidae in the Atlantic Forest. The family currently has about 3,500 described species and 428 genera, classified into nine subfamilies (Deitz & Wallace 2010). In Brazil, there are about 690 described species and 121 genera (Evangelista et al. 2019). Although membracids have a worldwide distribution, eight subfamilies are restricted to the New World.

Membracids exhibit a complex and unique variety of pronotal forms, with projections of various shapes and colors, including mimicry, camouflage, aposematism, and defense against predators (Evangelista et al. 2017). Treehoppers exhibit interaction with more than 100 herbaceous and woody host plant families, and they are considered pests in some due to damage caused by egg insertion into plant tissue (Deitz & Wallace 2010); these insects establish an intricate mutualistic network with ant species, receiving protection from predators and parasitoids while providing honeydew —a sugary product resulting from the metabolism of their carbohydrate-rich diet— to the formicids (Funkhouser 1950, Wood 1993). In many cases, these relationships overlap with a wide spectrum of social regimes, ranging from solitary individuals to gregarious species with offspring defense and maternal care behaviors (Lin et al. 2004, Lin 2006).

In addition, we highlight the fact that membracids were listed as good biological indicators of environmental changes, with broad possibilities of being employed in monitoring studies (Brown 1997). Recently, studies conducted in phytogeographical zones of rainforest (southern Brazil) on ecological networks involving these insects, their attendant ants (mutualistic interactions) and host plants (antagonistic interaction), were developed to better understand the role of these insects in the ecosystems (Gadelha et al. 2016, Gadelha et al. 2017).

The application of this protocol presupposes the following question: what is the best method for collecting Membracidae in the Atlantic Forest? In this context, considering that these insects inhabit different niches, such as the canopy, border and lower stratum of the forest, we aimed to evaluate the efficiency of different collection methods that allow capture of these insects in these locations, and to test the hypothesis that a combination of different methods is necessary to inventory the diversity of Membracidae in areas of the Atlantic Forest.

Material and Methods

1. Study areas

The protocol was applied from May 2015 to April 2016 in four areas of the Atlantic Forest of Paraíba, which are subject to a mean annual temperature of 25°C, 80% relative humidity, approximately 1,700 mm of rainfall and a warm humid tropical climate, type As' in the Köppen classification (Alvares et al. 2013): Area 1 – Refúgio da Vida Silvestre (Wildlife Refuge; RVS) *Mata do Buraquinho* (519.75 ha), located in the urban perimeter of the municipality of João Pessoa (07º08'38"S; 34º51'34"W); Area 2 – Reserva Particular do Patrimônio Natural (Private Natural Heritage Reserve; RPPN) *Engenho Gargaú* (1,058.6 ha), located in the municipality of Santa Rita, (07º01'52"S; 34º57'41"W), approximately 15 km from João Pessoa; Area 3 – Reserva Biológica (Biological Reserve; REBIO) *Guaribas* (SEMA 2 – 3,016.09 ha), located in the municipalities of Mamanguape (06º40'40"S; 41º12'47"W) and Rio Tinto (06º44'59"S; 41º07'11"W), 51 km from João Pessoa; and Area 4 – RPPN *Fazenda Pacatuba* (*Pacatuba* Farm; 266.53 ha) (7º02'33"S; 35º08'14"W), located in the district of Santa Helena, municipality of Sapé, 47 km from João Pessoa.

2. Collection methods

Samples were collected by four people for seven days, the first and last days being used to place and remove sticky cards, respectively. The sampling method was based on sampling units (Magurran 2011, p. 143) because the presence of gregarious species in Membracidae could cause distortions if the sampling was based on the number of individuals.

The samplings used 100 sampling units per area using the following capture methods: 60 double-sided yellow sticky cards (Promip ©) (23 x 11 cm/side), distributed in the canopy (30) and in the lower stratum (30); 10 nocturnal collections, on a white cloth background (2 x 2 m), with mixed mercury light (250W and 220v), fed by a por Table generator, featuring a two-cycle motor with a frequency of 60 Hz and ~ 700W; and 30 active collections (manual process) using capture nets or directly the killing jars.

Each sticky card corresponds to one sampling unit. The sticky cards in the canopy were distributed 50 m from the border, near the trails inside the forest, at least 30 m apart from each other, using a slingshot with metal support, high-speed throwing lines and yellow Durepox© spheres. The sticky cards of the lower stratum were arranged 1.5 m above ground level, beginning 50 m from the border, approximately 20 m apart. The sampling time for these collection methods was five days.

The light trap operated from 6:00 pm to 9:00 pm, with collection points spaced 100 m apart, and each sampling unit corresponded to 90 minutes of collection. In the active collection, each sampling unit corresponded to the inspection of the plants at the border, up to 2 m in height, along 30 m, interspersed by 20 m, for a total transect of 900 m. Once well represented in the active collection (more than 50 specimens), species were no longer captured, and only the abundance was recorded.

3. *Material preparation*

Insects collected with sticky cards were subjected to a glue removal procedure by immersion in Varsol® (24h) and acetone (C_3H_6O) (24h). It is important to note that the collection on sticky cards rarely causes damage that prevents the taxonomic identification or inclusion of specimens in the entomological collections, even because membracids have a hard and well sclerotized cuticle. However, our field experience suggests that as soon as the sticky cards are removed from the plants, specimens should be carefully transferred with forceps to a flask with the glue remover, and the sticky cards with insects still adhered to the glue should never be closed.

After being assembled and dried, the specimens were incorporated into the collection of the Entomological Collection of the Departamento de Sistemática e Ecologia (Department of Systematics and Ecology; DSEC) at the Federal University of Paraíba (UFPB).

4. *Data analysis*

Data on abundance, species richness and composition were analyzed according to area and collection method. Species with at least ten collected individuals were considered restricted to one area or method. The number of species shared and unique to each method was illustrated in a Venn diagram built using the Venny 2.1 program (Oliveros 2015).

The efficiency of each method was measured based on the mean accumulation of species per sampling unit. The relationship between species richness and abundance, per method, was calculated using a simple linear regression. The methods were compared by rarefaction, considering the accumulation of species according to abundance.

To test the similarity between the methods according to species composition, an analysis of similarity (ANOSIM) was carried out using the Bray-Curtis index (9,999 permutations) and Bonferroni sequential correction. The Jaccard similarity index was also calculated to analyze the complementarity between the collection methods used.

Regression, rarefaction, ANOSIM and Jaccard index analyses were performed using the program Past 3.21 (Hammer et al. 2001).

Nonparametric estimators of species richness were applied to each area when the four collection methods were used simultaneously and separately. To verify the sample sufficiency, the observed richness was compared to the mean estimate obtained from the abundance (ACE and Chao1) and species incidence estimators (ICE, Chao2 Jackknife 1 and 2, and Bootstrap). Estimates were obtained using the software EstimateS 9.1.0 (Colwell 2013).

Results

A total of 2,678 specimens belonging to 91 species of 44 genera (Table 1) were collected. The most abundant species was *Bolbonota melaena* (Germar, 1835) (N = 366), which, together with *Harmonides dispar* (Fabricius, 1803) (N = 317), *Enchenopa squamigera* (Linnaeus, 1758) (N = 258) and *Leioscyta spiralis* (Haviland, 1925) (N = 208), corresponded to 42.9% of all specimens collected. Among the four most abundant species, *H. dispar* was the species were collected (N = 26, 8,2%) by active collection, but. It was the most collected species using the light trap method (N = 87; 27.4%), although most of its specimens (64.4%) were recorded in sticky cards in both the canopy (N = 89; 28.1%) and in the lower stratum (N = 115; 36.3%).

The method that collected the greatest abundance was active collection (N = 1,517 or 56.65%), followed by the card in the canopy (N = 542 or 20.24%), light attraction (N = 345 or 12.88%), and card in the lower stratum (N = 274 or 10.23%) methods. The method that recorded the highest number of species was the one that used sticky cards in the canopy (S = 53), followed by the methods of active collection (S = 42), light trap (S = 42) and sticky cards in the lower stratum (S = 22). The accumulation of species revealed that the addition of new species is greater per sampling unit using the light trap (1.05 species added to each sampling unit). The use of sticky cards in the lower stratum was the least productive method and required an average of 5.5 cards for new records of species (0.18 species added per card) (Table 2).

Species richness exhibited a positive and significant relationship with abundance in all methods applied, with greater use of light traps. When comparing the methods by rarefaction (cutoff point of 261 individuals), the most efficient methods were those that used sticky cards in the canopy (S = 42.71 ± 2.31) and collection with light traps (S = 37.14 ± 1.69), with no significant difference between both. Active collection (S = 27.40 ± 2.12) and sticky cards in the lower stratum (S = 21.61 ± 0.60) are the methods with the lowest species richness in rarefaction (Figure 1).

All methods exhibited high complementarity (at least 70%) (Table 3). More than half of the species (S = 50; 54.35%) were recorded exclusively by one of the methods applied (Active – 14 spp.; Canopy – 17 spp.; Lower – 2 spp.; Light – 16 spp.). However, most species were not considered restricted to the method because they had a small number of specimens (eight or fewer individuals). Species that were considered restricted were recorded only in the active collection (S = 9) and with sticky cards in the canopy (S = 2). Of the 91 species recorded, only 20 (21.98%) were shared by at least three of the four collection methods used, and of these, five species had individuals collected in all methods (*Enchenopa gladius* (Fabricius, 1803), *Enchenopa monoceros* (Germar, 1821), *Erechtia* sp. 1, *Harmonides dispar* and *Horiola picta* (Coquebert, 1801)) (Figure 2 and Table 1).

The ANOSIM revealed that there are significant differences in species composition according to the collection method (R = 0.10; p = 0.0001), and active collection differs from all other methods. The collection with sticky cards in the canopy differs from collection in the lower stratum (p = 0.0001) but has a composition similar to that of collection with a light trap (p = 0.98). Additionally, no significant differences in species composition were found between the collection methods with sticky cards in the lower stratum and the use of light traps (p = 0.15).

All areas were sufficiently well sampled when the four methods were used concomitantly (Table 4). By analyzing the methods separately in each area, the active collection and the sticky cards in the canopy and lower stratum were those that exhibited the best sampling sufficiency, with values that were at least 60% of estimated. The light trap, however, exhibited values below 50% of sample sufficiency for most areas (Table 5).

Application of the protocol required the presence of four people/area for seven days, and two days were used to place and remove cards. The 100 sampling units compose the number of samples that optimizes time, sample effort and cost, estimated at US$180 dollars/seven days of collection. This value meets the food requirements, purchase of sticky cards, throwing lines and fuel for the light trap; however, reducing the

Table 1. Membracidae collected in four areas of the Atlantic Forest of Paraíba: EGG, RPPN Engenho Gargaú; FZP, RPPN Fazenda Pacatuba; MTB, RVS Mata do Buraquinho; RBG, Reserva Biológica Guaribas, using the methods of active collection (A), sticky cards in the canopy (B), sticky cards in the lower stratum (C) and a light trap (D). * restricted to active collection; ** restricted to canopy collection.

Species	EGG				FZP				MTB				RBG				Total
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
Bolbonota melaena (Germar, 1835)	95				153	4			50				58	5	1		366
Harmonides dispar (Fabricius, 1803)		12	13	7	7	27	6	2		17	13	11	19	33	83	67	317
Enchenopa squamigera (Linnaeus, 1758)*	43				7				122				86				258
Leioscyta spiralis (Haviland, 1925)	19		1		14	1			171		2						208
Erechtia gibbosa (De Geer, 1773)	24	4	1		1	5			102	7			4	4			152
Colisicostata scutellaris (Buckton, 1902)					70	18		1	35	26		1					151
Procyrta pectoralis (Fabricius, 1803)		1				6		14		48		3		16	8	53	149
Enchenopa gladius (Fabricius, 1803)		3	24		4		8		1	11	33	10	8		10	34	146
Horiola picta (Coquebert, 1801)		1	1	1		1			4	6	2	1	54	11	16	7	105
Neotynelia martinsi Creão-Duarte & Sakakibara, 2000		7		1				1		4		18		18		18	67
Enchenopa concolor (Fairmaire, 1846)*	3				1				49				4				57
Membracis luizae Evangelista & Sakakibara, 2010*					1				54								55
Cyphonia clavata (Fabricius, 1787)					1				38		1					2	42
Ceresa ustulata Fairmaire, 1846					30												30
Enchenopa gracilis (Germar, 1821)	21				7												28
Pseuderechtia sp.2**														28			28
Erechtia sp.3		18				1	1							5	1		26
Enchenopa monoceros (Germar, 1821)		3		1	1				1					9	8	1	24
Heteronotus mourei Creão-Duarte & Sakakibara, 1992		1			1	1		7	7	7							24
Peltosticta yonkei Sakakibara, 1976**		12								9				3			24
Notocera camelina Sakakibara, 1977			2		5						10		3		3		23
Horiola ferruginea Fairmaire, 1846					21					1							22
Ceresa vitulus (Fabricius, 1775)	2	4			5	1			5	3				1			21
Todea sp.					4	2			1	6				1	5		19
Amastris rotheai Evangelista & Sakakibara, 2007		1						2				1		5		8	17
Tolania furcata-group sp.		3				8		1						4			16
Erechtia sp.1			1		6	4							3			1	15
Cyphonia nordestina Sakakibara, 1968*					14												14
Melusinella nervosa (Fairmaire, 1846)*	13																13
Pseuderechtia sp.1						5	7							1			13
Cymbomorpha olivacea (Fabricius, 1803)					1			2					1			8	12
Notocera cerviceps (Fowler, 1894)		1												9	2		12
Amblyophallus exaltatus (Fabricius, 1803)*	2				9												11
Ceresa atlantica Andrade, 2015*	10				1												11
Havilandia pruinosa (Haviland, 1925)	1	1								6		1	2				11
Stilbophora tripartita (Fairmaire, 1846)					9					1							10
Postanomus cornutulus (Stål, 1862)		1												6		3	10
Amastris elevata (Funkhouser, 1922)	1					4		2								1	8
Talipes appendiculatus (Fonseca, 1936)		7												1			8
Amastris sp.5					5		1	1									7
Darnis olivacea Fabricius, 1803		1								6							7
Enchophyllum ensatum (Coquebert, 1801)									7								7
Germariana terminalis (Walker, 1858)		4				3											7

Continue...

Continuation...

Heteronotus albospinosus Haviland, 1925				1	3		1		1				1			7
Lycoderides capixaba Sakakibara, 2013		3										1	3			7
Enchophyllum nigrocupreum (Walker, 1858)		4				1									1	6
Erechtia sp.2							1				1		2		2	6
Pseuderechtia neivai (Fonseca, 1941)		2	4													6
Tolania peltacauda-group sp.1								2				1			3	6
Anobilia splendida Tode, 1966							1			3		1				5
Enchenopa sp.					2			1		1		1				5
Tropidoscyta torva (Germar, 1835)								1							4	5
Amastris sp.													1		3	4
Euwalkeria sp.															4	4
Sundarion sp.								1					1		2	4
Amastris guttata Fonseca, 1942												1			2	3
Amastris sp.1		1													2	3
Calloconophora sp.	1														2	3
Eumela fornicata (Germar, 1821)								1							2	3
Micrutalis sp.1		2											1			3
Amastris funkhouseri Haviland, 1925	1								1							2
Amastris sp.6													2			2
Amastris sp.7													2			2
Bocydium sp.		2														2
Ceresa sp.				2												2
Cymbomorpha sp.2									2							2
Cymbomorpha vaginata (Germar, 1835)								2								2
Enchenopa auridorsa Sakakibara & Marques, 2007	2															2
Membracis sp.1					2											2
Micrutalis binaria (Fairmaire, 1846)															2	2
Neotynelia pubescens (Fabricius, 1803)				1	1											2
Notogonioides sinopae Sakakibara, 1996								2								2
Potnia diringshofeni Creão-Duarte & Sakakibara, 1997		2														2
Smiliorachis sp.1						2										2
Stictopelta sp.									1	1						2
Tolania peltacauda-group sp.2								2								2
Amastris sp.2								1								1
Amastris sp.4								1								1
Anobilia nigra Tode, 1966													1			1
Cladonota apicalis (Stål, 1869)				1												1
Cymbomorpha sp.1											1					1
Harmonides sp.												1				1
Membracis sp.2								1								1
Membracis tectigera Olivier, 1792					1											1
Micrutalis sp.2															1	1
Micrutalis sp.3					1											1
Micrutalis tripunctata (Fairmaire, 1846)													1			1
Neotynelia nigra (Funkhouser, 1940)		1														1
Neotynelia vertebralis (Fairmaire, 1846)								1								1
Paraceresa brasiliensis Remes Lenicov, 1971				1												1
Smiliorachis sp.2															1	1

Table 2. Sampling efficiency and regression among species abundance and richness of Membracidae for the methods of active collection, sticky cards in the canopy, sticky cards in the lower stratum and a light trap used in four areas of the Atlantic Forest of Paraíba from May 2015 to April 2016. N, sampling units.

Method	Abundance	Richness	N	Efficiency
Active	1517	42	120	0.35±0.38
Canopy	542	53	120	0.44±0.42
Lower	274	22	120	0.18±0.16
Light	345	42	40	1.05±0.66

Table 3. Jaccard similarity index and complementarity (in bold) of four collection methods (active collection, sticky cards in the canopy, sticky cards in the lower stratum, and a light trap) of Membracidae applied in four areas of the Atlantic Forest of Paraíba from May 2015 to April 2016.

	Active	Canopy	Lower	Light
Active		**0.6986**	**0.7451**	**0.7612**
Canopy	0.3014		**0.7500**	**0.7297**
Lower	0.2549	0.2500		**0.8113**
Light	0.2388	0.2703	0.1887	

permanence in the field to five days should not adversely impact the final result of the inventory and will reduce the total cost of the protocol.

Discussion

The combination of the different methods used in the present study for the collection of Membracidae is ideal for the efficient sampling of a given area, which was confirmed by our results and corroborates the proposed hypothesis. However, depending on the goals to be achieved and/or available logistics, some methods may be considered more appropriate and combined in different ways. The active collection, sticky cards in the canopy and light traps are the most indicated methods for collecting a larger number of Membracidae species in the Atlantic Forest. If it is impossible to use a light trap, it is necessary to combine active collection and sticky cards in the canopy and lower stratum, and if it is impossible to combine methods, active collection is the most preferred because of its low cost. However, it should be noted that the efficacy of this method is directly linked to the experience and ability of collectors.

The abundance of specimens collected by the methods used indicates that active collection is the most promising, and this method is very different from the other methods used. Sticky cards and light traps are attractive methods and, as such, have little effect on gregarious species, such as *Bolbonota melaena* and *Enchenopa squamigera*, and subsocial species as *Leioscyta spiralis* and *Erechtia gibbosa* (De Geer, 1773) (for notes on the nomenclature used for behaviors see Lin 2006, tab. 1). Species that exhibit this behavior are reluctant to abandon eggs and nymphs (Tallamy & Wood 1986, Godoy et al. 2006), which greatly facilitates the capture of these insects in active collection. This is the reason why so many individuals from the same species are collected by this method and the reason why these four species contribute to more than one-third of the total abundance.

When the richness is compared according to method used, sticky cards in the canopy is the best method, and this result is also maintained when the collection option is limited to a certain number of individuals per method, as shown by rarefaction. The Membracidae inhabit the parts of plants that are more exposed to light, such as apical branches and inflorescences (Creão-Duarte et al. 2017), and therefore were recognized as sun loving insects (Funkhouser 1950). As the forest canopy is the habitat where this condition is higher, these insects naturally occur in this location in greater diversity, and sticky cards are one of the best methods to access this fauna (Kopp & Yonke 1970, Johnson & Freytag 1997, Wallace & Troyano 2006).

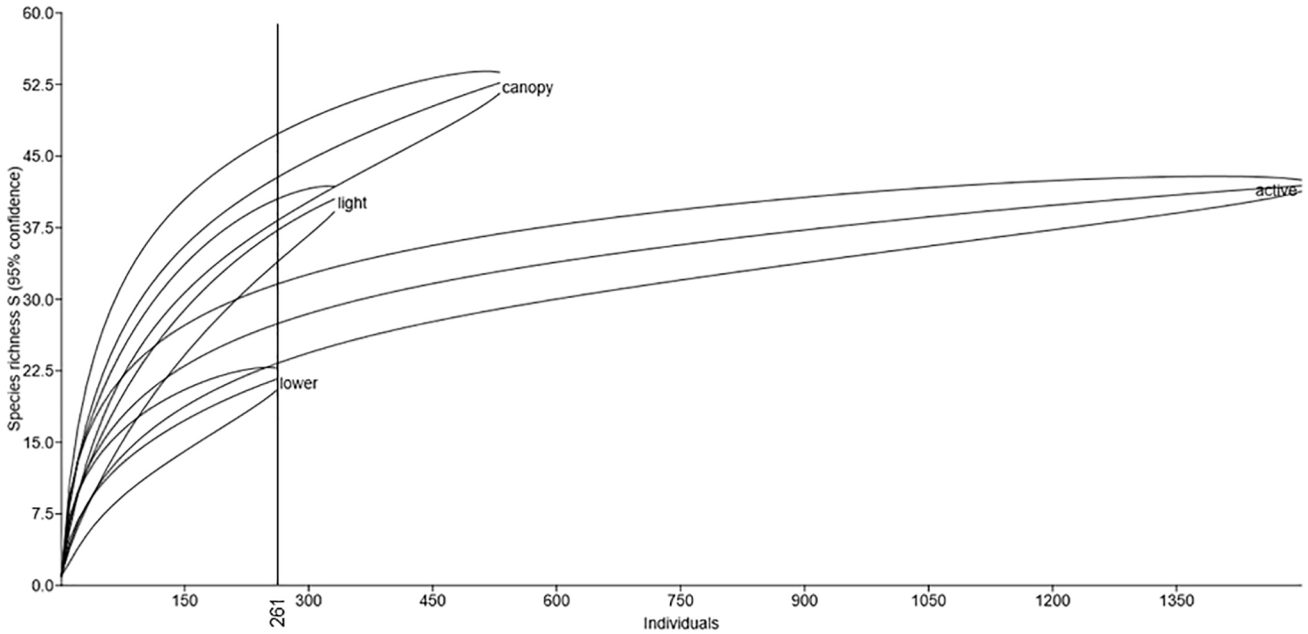

Figure 1. Rarefaction curve (95% confidence interval) among methods of active collection, sticky cards in the canopy, sticky cards in the lower stratum and a light trap for Membracidae in four areas of the Atlantic Forest of Paraíba, collected from May 2015 to April 2016.

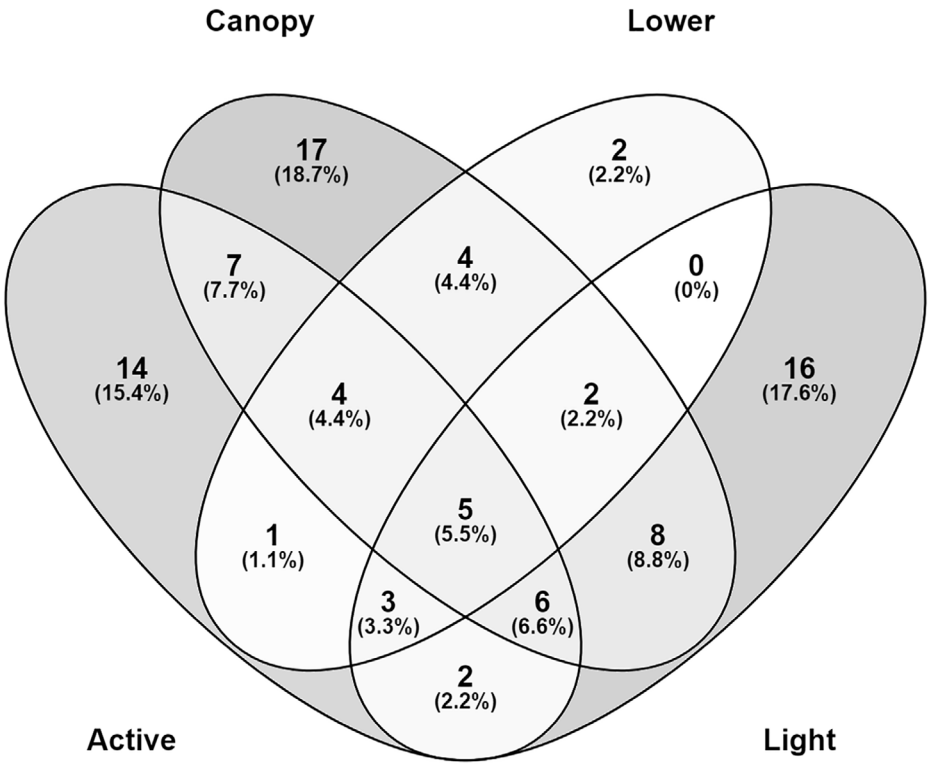

Figure 2. Venn diagram produced from shared and unique species of Membracidae collected in four areas of the Atlantic Forest of Paraíba from May 2015 to April 2016, using methods of active collection, sticky cards in the canopy, sticky cards in the lower stratum and a light trap.

Table 4. Sampling sufficiency of Membracidae collected in four areas of the Atlantic Forest of Paraíba from May 2015 to April 2016, using methods of active collection, sticky cards in the canopy, sticky cards in the lower stratum and a light trap. EGG, RPPN Engenho Gargaú; FZP, RPPN Fazenda Pacatuba; MTB, RVS Mata do Buraquinho; RBG, REBIO Guaribas. N, abundance; S, species richness.

	RBG	MTB	FZP	EGG
N	789	929	563	397
S	48	36	58	41
Singletons	11	11	21	12
Doubletons	9	3	10	7
Uniques	15	13	26	16
Duplicates	11	4	9	10
1. Estimators of abundance				
ACE	56.11	50.36	83.51	53.58
Chao 1	53.49	49.73	77.06	49.23
Mean richness estimate	54.80	50.05	80.29	51.41
Sampling sufficiency (%)	87.59	71.93	72.24	79.76
2. Estimators of incidence				
ICE	62.51	49.95	90.8	61.33
Chao 2	56.66	51.44	90.18	51.8
Jackknife 1	62.85	48.87	83.74	56.84
Jackknife 2	66.88	57.72	100.49	62.82
Bootstrap	55.22	41.29	68.97	48.33
Mean richness estimate	60.82	49.85	86.84	56.22
Sampling sufficiency (%)	78.92	72.22	66.79	72.92

The collection methods exhibited high complementarity, which explains the need for combining different methods. The species recorded in the light trap and sticky cards of the lower stratum exhibited greater complementarity and, consequently, lower fauna similarity. These data suggest the stratification of the treehopper fauna's composition in the studied areas, where species such as *Enchenopa gladius* and *Notocera camelina* Sakakibara, 1977 are most collected on the sticky cards placed in the lower strata, compared to the upper strata (Lourenço 2017). The vertical variation in arthropod fauna from different forest strata of tropical forests (Campos et al. 2006, Grimbacher & Stork 2007) it was also registered for Membracidae by Mason & Loye (1981) and Johnson & Freytag (1997).

Lower complementarity and, consequently, greater fauna similarity occurred among sticky cards placed in the canopy and active collection at the border (Table 3), which are places where habitat conditions (tender parts of plants exposed to the sun) are similar; therefore, a more similar Membracidae fauna is expected (Creão-Duarte et al. 2017). The results of Davis & Sutton (1998), who indicated that invertebrate communities typical of the forest canopy (dorsal border) can move, in whole or in part, from the canopy to areas near the border, contribute to explaining this similarity. Even considering that the border effects resulting from forest fragmentation have marked effects on the floristic and faunal composition of the fragments, especially when the latter are small, some groups of insects may increase at the border (Laurance et al. 2002), including Membracidae, which rely on a large community of attendant ants (Dejean & Giberneau 2000).

As expected, the species composition resulting from active collection differed from that resulting from all other methods due to the very nature of the method, which is subject to the experience and

Table 5. Sampling sufficiency of Membracidae collected in four areas of the Atlantic Forest of Paraíba from May 2015 to April 2016, using methods of active collection, sticky cards in the canopy, sticky cards in the lower stratum and a light trap. Abundance estimators: ACE and Chao 1; Incidence estimators: ICE, Chao 2, Jackknife 1, Jackknife 2 and Bootstrap. EGG, RPPN Engenho Gargaú; FZP, RPPN Fazenda Pacatuba; MTB, RVS Mata do Buraquinho; RBG, REBIO Guaribas. N, abundance; S, species richness.

	N	S	Mean abundance estimate	Sampling sufficiency (abundance)%	Mean incidence estimate	Sampling sufficiency (incidence)%
RBG Active	245	13	13.76	94.48	19.09	68.08
RBG Canopy	173	27	39.93	67.63	39.45	68.45
RBG Lower	137	10	10.88	91.95	12.33	81.10
RBG Light	234	26	29.22	88.98	33.23	78.25
MTB Active	648	16	20.99	76.24	20.84	76.78
MTB Canopy	167	21	26.79	78.40	33.96	61.84
MTB Lower	63	8	21.26	42.34	15.01	59.96
MTB Light	51	13	46.20	25.98	26.78	44.81
FZP Active	386	31	48.42	64.03	46.10	67.25
FZP Canopy	100	22	32.23	68.27	31.34	70.20
FZP Lower	27	8	11.88	67.37	11.99	66.73
FZP Light	50	23	39.42	58.35	48.64	47.29
EGG Active	238	15	19.59	76.57	19.16	78.29
EGG Canopy	102	27	35.86	75.29	42.66	63.30
EGG Lower	47	8	13.32	60.06	12.64	63.29
EGG Light	10	4	9.78	40.90	8.05	49.70

ability of the collectors, whereas the other methods are methods that attract a species. The composition of divergent species such as those inventoried by sticky cards in the canopy and in the lower stratum are due to the differences in fauna that naturally exist in the vertical strata of the rainforest (Charles & Basset 2005, Brehm 2007). Compositions of similar species—such as those inventoried using sticky cards in the canopy and by use of the light trap—result from these methods accessing species that inhabit the same sites, predominantly the canopy.

The lack of a list of species of Membracidae for the areas where the protocol was applied seems to prevent any comparison to estimate the reliability of the diversity sampled. Similar previous situations have determined the need to know stop rules, i.e., indicators that the sampling performed is sufficient (Magurran 2011). The representation of at least two specimens per species collected was the stop rule suggested by Colwell & Coddington (1994); when species accumulation curves reach the asymptote is also recognized as an indication of sample sufficiency, although large-scale collecting efforts do not ensure this (Longino et al. 2002). Coddington et al. (1991) suggest that a sampling intensity of 10:1 (specimens:species) for tropical rainforest conditions would be sufficient for a reliable richness estimate. Sørensen et al. (2002) suggested 30–50:1 for the assemblages of spiders in a montane forest. Cardoso (2009) considers that an inventory can be considered "reasonable" when approximately 50% of the estimated species are sampled, "comprehensive" when 70–80% of the estimated species are sampled and "exhaustive" when it reaches 90% of species.

When we consider our results, by area, regarding these stop rules, we conclude that they are satisfactory and meet the expectations of a protocol. Sampling sufficiency by area may be classified as comprehensive according to Cardoso (2009); we observed that our results exhibit values of specimens/species better than those suggested by Sørensen et al. (2002); and even when we consider the proposal by Coddington et al. (1991), which is more rigorous, we observed that the application of the protocol in two of the four areas are within the rigors of the proposals of these authors.

Considering the sampling sufficiency by method, the lowest values were observed in the light traps in three of the four areas and resulted from a high number of *singletons* and *uniques* in relation to the number of *doubletons* and *duplicates*, which refutes the need for a greater number of samplings using this method; however, expense and logistic difficulties should be weighed against initiatives different from those proposed here.

The combination of collection methods to inventory Membracidae in the Atlantic Forest presented here is the most appropriate. However, for an expeditious survey that includes the largest number of species, ideally, one would use a combination of active collection with a light trap.

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Author contributions

Valberta Alves Cabral: conceptualization and design of the study; field work; material identification and data curation; contribution to manuscript preparation.

Antonio José Creão-Duarte: conceptualization and design of the study; material identification and data curation; original manuscript preparation.

Aline Lourenço: material identification and data curation; field work; contribution to manuscript preparation.

Carolina Nunes Liberal: contribution to manuscript preparation; contribution to critical revision.

Alessandre Pereira-Colavite: field work; contribution to manuscript preparation, review and editing.

Conflicts of interest

The authors declare no conflict of interest.

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