Membracidae diversity (Hemiptera: Auchenorrhyncha) in an Atlantic Forest area, Paraíba, Brazil, with preliminary comments on collection methods, spatial distribution and estimated species richness

Diversidade de Membracidae (Hemiptera: Auchenorrhyncha) em uma área da Mata Atlântica, Paraíba, Brasil, com comentários preliminares sobre métodos de coleta, distribuição espacial e riqueza estimada

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Abstract: In order to document the Membracidae of an area of the Brazilian Atlantic Forest in Paraíba, four collection methods were employed, distributed in 100 sample units during ten days in November 2014. A total of 1,182 membracids of 27 species were collected. The assemblages were marked with a high dominance, since six species accounted for 80.63% of the total number of individuals captured. Species with more than ten individuals were associated with the three environments of the forest: canopy, understory, and edge. The importance of the four sampling methods in the final membracid composition was evaluated through the Jaccard similarity index and its complement, where the values of complementarity were superior to the values of similarity, suggesting the dissimilarity of assemblages inventoried by each method. The observed richness corresponds to 90.0% and 79.4% of the richness estimated by Chao1 and Chao2. The richness estimated by Chao1 is within the upper limit of the confidence interval (95%) in the last plot of the species accumulation curve, while the richness estimated by Chao2 is above this limit. The additional sampling effort needed to collect 95% of the estimated richness is 969 individuals for Chao1 and 317 sample units for Chao2.

Keywords: Abundancy. Species diversity estimators. Faunistic inventory. Treehoppers.

Resumo: Para documentar os membracídeos em uma área de Mata Atlântica na Paraíba, quatro métodos de coleta, distribuídos em 100 unidades amostrais, foram utilizados durante dez dias, em novembro de 2014. Foram coletados 1.182 espécimes de 27 espécies, sendo essa taxocenose marcada por alta dominância, pois representantes de apenas seis espécies foram responsáveis por 80,63% do número total de indivíduos coletados. Espécies com mais de dez indivíduos foram associadas a três ambientes da floresta (dossel, sub-bosque e borda). A importância dos quatro métodos de amostragem na composição final da taxocenose foi avaliada através do índice de similaridade de Jacard e de seu complemento, onde os valores de complementaridade foram superiores aos de similaridade, sugerindo a dissimilaridade das taxocenoses inventariadas por cada método. A riqueza observada corresponde a 90% e 79,4% da riqueza estimada por Chao1 e Chao2. A riqueza estimada por Chao1 encontra-se dentro do limite superior do intervalo de confiança (95%) no último lançamento da curva de acumulação de espécies, enquanto a riqueza estimada por Chao2 encontra-se acima deste limite. O esforço adicional de amostragem, necessário para coletar 95% da riqueza estimada, é de 969 indivíduos para Chao1 e 317 unidades amostrais para Chao2.

Palavras-chave: Abundância. Estimadores de diversidade de espécies. Inventário faunístico. Membracídeos.

CREÃO-DUARTE, A. J., A. PEREIRA-COLAVITE, W. E. SANTOS, S. A. BARBOSA, V. A. CABRAL, A. LOURENÇO, I. S. BRAGA & W. R. SILVA, 2017. Membracidae diversity (Hemiptera, Auchenorrhyncha) in an Atlantic Forest area, Paraíba, Brazil, with preliminary comments on collection methods, spatial distribution and estimated species richness. **Boletim do Museu Paraense Emílio Goeldi. Ciências Naturais** 12(1): 11-21.

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Recebido em 13/04/2017
Aprovado em 11/06/2017
Responsabilidade editorial: Fernando da Silva Carvalho Filho

INTRODUCTION

The heightened degradation of tropical forests with irreversible reduction in the number of species has led us to reflect on the words of May (1988), that Biological Conservation is a science with limited time. The nonexistent consensus of an overall estimation of species richness (Mora *et al.*, 2011) has hindered our understanding of the magnitude of environmental changes in the Anthropocene (Caley *et al*., 2014). Thus, local species listings are required, since richness at a global scale is inferred from local richness data (Magurran, 2011).

The use of arthropods, and insects in particular, has been supported in monitoring the sustainable use of tropical forests, since they respond quickly to environmental changes (Alarape *et al*., 2015). Membracids are one of the most appropriate groups in this regard, mainly due to the diversity of plants that host them (Brown, 1997).

The Membracidae (Hemiptera: Auchenorrhyncha) is structured into nine subfamilies, 49 tribes, 441 genera, and more than 3,200 species (Deitz & Wallace, 2011). The species belonging to this family are distributed worldwide, with an increase in recorded diversity in the Neotropical Region (Wood, 1993). They are specific or generalist plant parasites (Creão-Duarte *et al*., 2012, 2016) that extract sap and consequently produce honeydew that attracts Hymenoptera, with which these Hemiptera establish biological associations (Olmstead & Wood, 1990; Wood, 1993; Fagundes *et al*., 2012). They are recognized by their well-developed pronotum that covers the abdomen and almost all of the wings in some species, and may assume a similar appearance to plant structures such as buds, leaves and thorns (Wallace & Deitz, 2007). The objectives of this study were to document the overall diversity of membracids in a remnant of the Atlantic Forest in Paraíba, Brazil, based on four collection methods. Additionally, we provide preliminary comments on the collection methods used, the spatial distribution of the species, and estimated richness of treehoppers.

MATERIAL AND METHODS

STUDY AREA

Fieldwork was conducted from 20 to 29 November 2014, in three large remnants of the Atlantic Forest, in *campus* I of the Federal University of Paraíba, in João Pessoa, Paraíba, Brazil, totaling 19.3 hectares. These three remnants are close together and were therefore considered as a single study area with the same meteorological conditions as the metropolitan region of João Pessoa: temperature of 25.2 °C, total annual precipitation of 1,888 mm, and relative humidity of 77.7% (averages from the last ten years) (INMET, 2016).

SAMPLING

The collections were based on 100 sample units distributed into four capture methods: 40 yellow pan traps (understory); 20 yellow adhesive cards (canopy); ten nocturnal collections with light traps, and 30 active collections (manual process).

The soil pitfall trays were randomly arranged 5 m from the forest edge, at least 20 m apart, and were composed of a rectangular plastic yellow container 30 x 22 x 7 cm, filled with water and detergent. The yellow adhesive cards Promip© (23 x 22 cm) were randomly placed in the tree canopy 40 m from the edge, at least 30 m apart. The sampling duration for these methods was ten days.

The light traps used mixed mercury vapor lightbulbs of 250 W and 220 V on a white cloth background (1.7 x 2 m) and each sample unit corresponded to 90 minutes of collection, each unit was at least 80 m apart. Active collection was conducted along a demarcated transect at the edge of forest remnants, and each sample unit corresponded to a visual inspection along 30 m of the transect near plants at the edge, up to 2 m in height, interspersed by 10 m, using killing jars and entomological nets for capture.

Species were identified by comparison using specimens from the reference collection, photos from a species database, and specialized bibliography. The specimens were incorporated into the *Coleção Entomológica* of the

Departamento de Sistemática e Ecologia of the Federal University of Paraíba (DSEC). The photographs were made using a stereomicroscope (Leica®/M205C) with a coupled high definition camera (Leica®/DFC295) and a microcomputer, and the software Leica® Application Suite.

DATA ANALYSIS

The species list was organized by absolute and relative abundance. Species with more than ten individuals were associated with three forest environments: canopy, understory and edge. Edge species were those whose representatives were collected only in active collections conducted at the edge. Canopy species were those individuals collected on the cards, and understory species were those collected in trays. The light trap collects both canopy and understory specimens; thus, the environment was determined through occurrences in cards and trays.

In order to verify the importance of sampling methods in the final composition of the membracid assemblage, the four sampling methods were evaluated in pairs for similarity and complementarity using the Jaccard index in the program PAST 3.13 (Hammer *et al*., 2001). The values for both similarity and complementarity vary from 0 to 1 and indicate none and total similarity, or total or no complementarity, that is, the smaller the similarity the more complementary the assemblages inventoried by the methods (Magurran, 2011).

Two non-parametric species richness estimators were used: Chao 1 (abundance data) and Chao2 (incidence data) in the program EstimateS, version 9.1.0 (Colwell, 2013). The data were subjected to 1,000 randomizations without replacement (*i.e.* the samples were added to the analysis in random order and each was selected only once) (Walther & Moore, 2005; Colwell, 2013). The observed species curve (S_{obs}), with a confidence interval of 95%, is equivalent to the species accumulation curve and was constructed by EstimateS. In terms of number of individuals (Chao1) and samples (Chao2), the complement required to reach 95% of the estimated richness was calculated using the procedures of Chao *et al*. (2009).

RESULTS AND DISCUSSION

A total 1,182 individuals was collected of 27 membracid species (Figures 1A-1L and 2A-2O). However, four species could not be identified beyond genus (Table 1). Taxonomic issues involving uncertain identification were reported by Wallace (2008) and Wallace & Maloney (2010) in studies of membracid occurrence on oak in the Delaware Water Gap National Recreation Area and Pocono Till Barrens, Long Pond, Pennsylvania, respectively. This difficulty is expressed in other listings of Membracidae species richness (Lopes, 1995; Johnson & Freytag, 1997; Lencioni Neto, 2011; Creão-Duarte *et al*., 2012).

A total of 565 individuals of 19 species was collected in the forest interior, including 167 individuals of 11 species in 20 canopy cards, 360 of 13 species in 40 trays, and 38 individuals of eight species in ten collections with light traps. At the forest edge, 617 individuals of 21 species were collected during 30 active collections (Table 1). Several collection methods have been used in isolation or together in the capture of membracids, such as active collection (Lopes, 1995; Creão-Duarte *et al*., 2012, 2016), yellow adhesive cards (Kopp & Yonke, 1970; Mason & Loye, 1981; Johnson & Freytag, 1997; Wallace & Maloney, 2010), malaise and light trap (Godoy *et al*., 2006), and atomizers (Albertson & Dietrich, 2006).

Six species contributed 80.63% of the total abundance: *Enchenopa gladius* (Fabricius, 1775), 27.07% (Figure 1A); *Erechtia gibbosa* (DeGeer, 1773), 17.26% (Figure 1B); *Todea* sp., 12.35% (Figure 1C); *Membracis luizae* Evangelista & Sakakibara, 2010, 8.97% (Figure 1D); *Bolbonota melaena* (Germar, 1835), 7.53% (Figure 1E); and *Enchenopa squamigera* (Linnaeus, 1767), 7.45% (Figure 1F). The observed distribution pattern of species frequency (Figure 3) is represented by a few species with many individuals and many species with few individuals. This pattern repeats what Bartlett *et al*. (2008) found for membracids in Little Orleans, Maryland, where four species accounted for 85.2% of the total abundance. Studies involving other taxonomic groups report the same tendency of dominance by a few species of the assemblages, such as Tephritidae (Diniz *et al*., 2010) and Braconidae (Ruiz-Guerra *et al*., 2015).

Sixteen species were associated with one of the three forest environments. Those considered edge species were the following: *Membracis luizae*, *Bolbonota melaena*, *Enchenopa squamigera*, *Enchenopa concolor* (Fairmaire, 1846) (Figure 1H), *Enchophyllum ensatum* (Coquebert, 1801) (Figure 1J), *Cyphonia nordestina* Sakakibara, 1968 (Figure 1I), and *Leioscyta spiralis* (Haviland, 1925) (Figure 1L). With the exception of *B. melaena*, none of these species

Table 1. Number of individuals of membracid species captured using 100 sample units distributed into four collection methods: A (yellow adhesive cards); B (yellow pan traps); C (light traps); D (active collection); and AC (accumulated), in remnants of Atlantic Forest in João Pessoa, Paraíba. Highlighted species were not associated with a specific environment.

Species	Collection method				Total	%	%AC
	A	B	C	D			
Enchenopa gladius (Fabricius, 1775)	12	305	3	0	320	27.07	27.07
Erechtia gibbosa (DeGeer, 1773)	29	4	0	171	204	17.26	44.33
Todea sp.	68	1	5	72	146	12.35	56.68
Membracis luizae Evangelista & Sakakibara, 2010	0	18	0	88	106	8.97	65.65
Bolbonota melaena (Germar, 1835)	1	1	0	87	89	7.53	73.18
Enchenopa squamigera (Linnaeus, 1767)	0	3	0	85	88	7.45	80.63
Harmonides dispar (Fabricius, 1803)	12	0	17	7	36	3.05	83.67
Enchenopa concolor (Fairmaire, 1846)	0	6	0	27	33	2.79	86.46
Cyphonia nordestina Sakakibara, 1968	0	0	0	22	22	1.86	88.32
Enchophyllum ensatum (Coquebert, 1801)	0	0	0	22	22	1.86	90.19
Tropidoscyta torva (Germar, 1835)	20	1	0	0	21	1.78	91.96
Leioscyta spiralis (Haviland, 1925)	0	2	0	18	20	1.69	93.65
Notocera camelina (Sakakibara, 1977)	0	16	0	1	17	1.44	95.09
Procyrta pectoralis (Fabricius, 1803)	9	1	2	0	12	1.02	96.11
Havilandia pruinosa (Haviland, 1925)	9	1	0	1	11	0.93	97.04
Neotynelia martinsi Creão-Duarte & Sakakibara, 2000	5	0	4	1	10	0.85	97.88
Notogonioides sinopae (Sakakibara, 1996)	0	0	3	4	7	0.59	98.48
Cyphonia trifida (Fabricius, 1775)	0	0	0	4	4	0.34	98.82
Tolania sp.	0	0	3	0	3	0.25	99.07
Ceresa ustulata Fairmaire, 1846	0	0	0	2	2	0.17	99.24
Cymbomorpha olivacea (Fabricius, 1803)	1	0	0	1	2	0.17	99.41
Tolania furcata-group sp.	1	1	0	0	2	0.17	99.58
Ceresa vitulus (Fabricius, 1775)	0	0	1	0	1	0.08	99.66
Enchenopa auridorsa Sakakibra & Marques, 2007	0	0	0	1	1	0.08	99.75
Enchenopa monoceros (Germar, 1833)	0	0	0	1	1	0.08	99.83
Erosne sp.	0	0	0	1	1	0.08	99.92
Anobilia splendida Tode, 1966	0	0	0	1	1	0.08	100.00
Total number of individuals	167	360	38	617	1,182	100	
Total number of species	11	13	8	20	26		

were registered in the canopy or light trap. *Membracis luizae* is found on many edge plants, exotic and native (Sakakibara & Evangelista, 2010), and some individuals of this species were collected in trays inside the forest which can be explained by the location of the trays close to the edge, an explanation that also holds for *E. squamigera*, *E. concolor*, and *L. spiralis*. *Enchenopa gladius* and *Notocera camelina* (Sakakibara, 1977) (Figure 2A) were considered understory species because they were captured mainly in trays and are the only species whose individuals were sighted inside the forest.

Procyrta pectoralis (Fabricius, 1803) (Figure 2B), *Tropidoscyta torva* (Germar, 1835) (Figure 1K), *Havilandia pruinosa* (Haviland, 1925) (Figure 2C), and *Neotynelia martinsi* Creão-Duarte & Sakakibara, 2000 (Figure 2D) were considered canopy species, since their representatives were mainly collected in the canopy and light traps. Furthermore, even though *H. pruinosa* was not registered on the edge, it is common on *Byrsonima sericea* DC. (Malpighiaceae) that can be found along the edge. *Harmonides dispar* (Fabricius, 1803) (Figure 1G) was registered in adhesive cards (canopy), light traps, and active collection, but was considered a canopy species since the species captured at the edge were on regrowth of *Pouteria grandiflora* (A. DC.) Baehni (Sapotaceae), that is a large tree found in the interior of the forest reaching the canopy.

Erechtia gibbosa and *Todea* sp. were mainly represented in canopy and edge collections, that is, in places with higher

Figure 1. Collected Membracidae: A) *Enchenopa gladius* (Fabricius, 1775); B) *Erechtia gibbosa* (DeGeer, 1773); C) *Todea* sp.; D) *Membracis luizae* Evangelista & Sakakibara, 2010; E) *Bolbonota melaena* (Germar, 1835); F) *Enchenopa squamigera* (Linnaeus, 1767); G) *Harmonides dispar* (Fabricius, 1803); H) *Enchenopa concolor* (Fairmaire, 1846); I) *Cyphonia nordestina* Sakakibara, 1968; J) *Enchophyllum ensatum* (Coquebert 1801); K) *Tropidoscyta torva* (Germar, 1835); L) *Leioscyta spiralis* (Haviland, 1925). Scale bar: 1 mm.

light incidence, possibly because they have host plants that are found in these two places, that may imply that they are generalist species. In a study of herbivorous insects in tropical forests, Basset (1999) concluded that generalists vary from 78-84% among sap-feeding insects (*sap-sucking*), and that compared to leaf-chewing insects (*leaf-chewing*) sap-sucking insects outnumber the latter more than twice. In a study of membracid niche overlap in the Caatinga, Creão-Duarte *et al*. (2012) observed that most species were generalists. The same low species specificity of Hemiptera and Auchenorrhyncha herbivores was also found by Dem *et al*. (2013) in a tropical forest study in New Guinea.

In a meta-analysis with data from 31 studies conducted between 1994-2010, Guimarães *et al*. (2014)

Figure 2. Collected Membracidae: A) *Notocera camelina* (Sakakibara, 1977); B) *Procyrta pectoralis* (Fabricius, 1803); C) *Havilandia pruinosa* (Haviland, 1925); D) *Neotynelia martinsi* Creão-Duarte & Sakakibara, 2000; E) *Notogonioides sinopae* (Sakakibara, 1996); F) *Cyphonia trifida* (Fabricius, 1775); G) *Tolania peltacauda*-group sp.; H) *Ceresa ustulata* Fairmaire, 1846; I) *Cymbomorpha olivacea* (Fabricius, 1803); J) *Tolania furcata*-group sp.; K) *Ceresa vitulus* (Fabricius, 1775); L) *Enchenopa auridorsa* Sakakibra & Marques, 2007; M) *Enchenopa monoceros* (Germar, 1833); N) *Erosne* sp.; O) *Anobilia splendida* Tode, 1966. Scale bar: 1 mm.

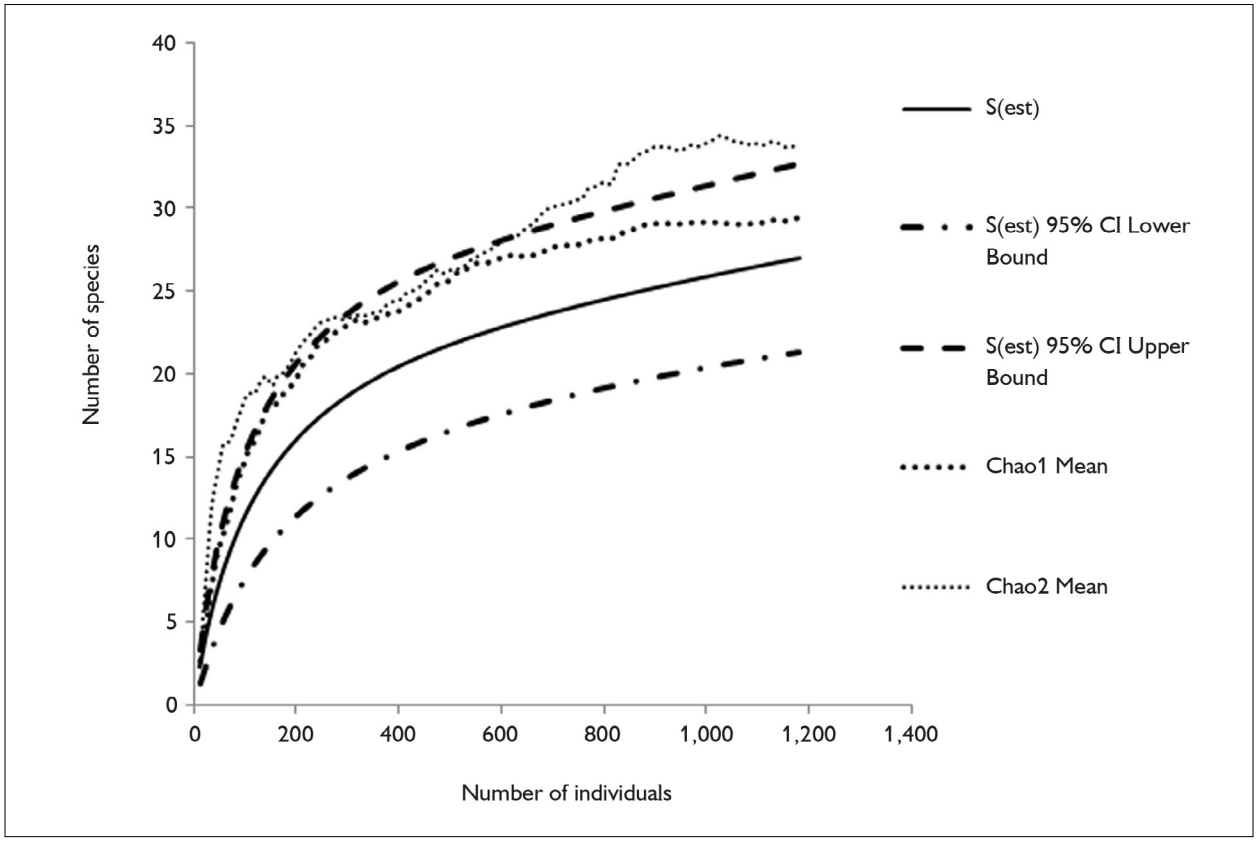

Figure 3. Frequency distribution of membracid species collected in Atlantic Forest remnants, Paraíba, using four collection methods.

reported that the edge exhibits a strong effect on plants and insects compared to the interior of the forest. In other words, edge plants have a higher rate of herbivory (73.7%) and herbivorous insects (*chewers*) are more abundant (14%) and have higher richness (65%) when compared to other masticators in the forest interior. Iannuzzi *et al*. (2005) studied distribution patterns of families from five insect orders in three forest habitats (edge, trails, and interior) in a remnant of 390 hectares of Atlantic Forest in Pernambuco, Brazil, where the greatest abundance and species richness were observed at the edge compared to the interior for Hemiptera, including membracids.

The results (Table 2) show that the complementarity values exceed the similarity values of observed richness between five of the six possible pairs of collection methods, suggesting that the assemblages from each method are dissimilar to each other, reinforcing the necessity of the four different methods for inventorying membracids. Sørensen *et al*. (2002) used six sampling methods to estimate the diversity of spiders in the understory of a forest in Tanzania. The complementarity values obtained in the latter study indicated that of the possible 15 pairs formed by the six methods, only two had complementarity values below 50%, and the two

Table 2. Similarity and complementarity indexes between pairs of membracid sampling methods in the Atlantic Forest (Paraíba). Complementary values are highlighted.

	Card	Tray	Light	Active
Card		0.5	0.36	0.28
Tray	0.5		0.17	0.36
Light	0.64	0.83		0.16
Active	0.72	0.64	0.84	

methods generated consistently different assemblages from those produced by the other four methods.

The values of species richness estimated by the Chao1 (30) and Chao2 (34) exceed the observed richness (27) (Table 3). The estimated richness with Chao1 is within the upper limit of the confidence interval (95%) in the last plot of species accumulation curve, whereas the richness estimated by Chao2 is above this limit (Figure 4). Values of estimated richness by Chao2 surpassed the values estimated by Chao1, which is expected when uniques are

Table 3. Estimation of sampling effort for abundance (Chao1) and occurrence (Chao2) data in order to obtain 95% of the estimated richness (g). Legends: F_1 = singletons; F_2 = doubletons; Q_1 = unique; Q_2 = duplicates; N = individuals; S_{obs} = observed species; S_{est} = estimated species.

Habitat, taxon and location	N	S_{obs}	Chao1				Chao2			
			S_{est}	F_1	F_2	g = 95	S_{est}	Q1	Q_2	g = 95
Atlantic Forest, Membracidae, João Pessoa, Paraíba	1,182	27	30	5	3	969	34	7	2	317

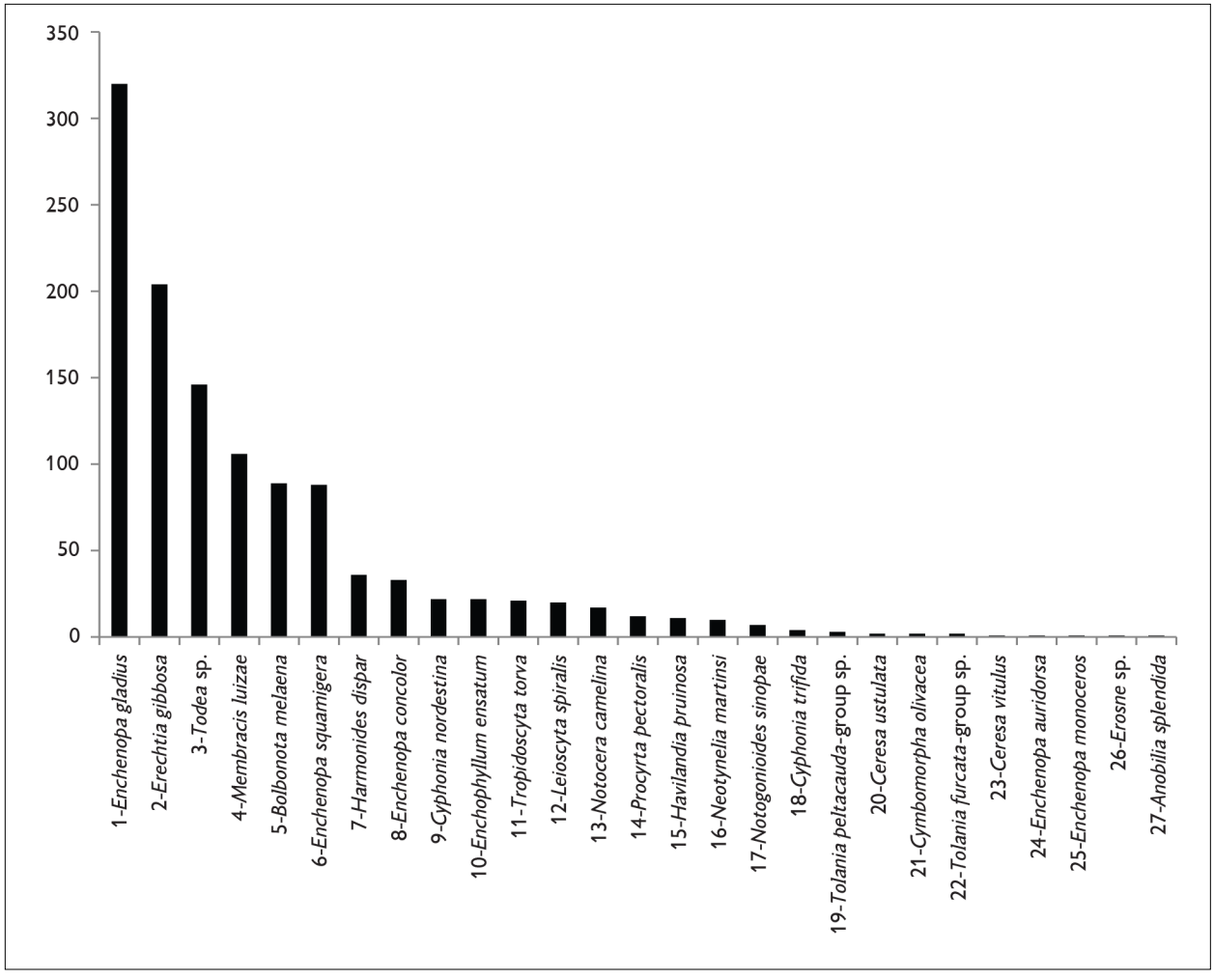

Figure 4. Species accumulation and estimated richness curve using Chao1 and Chao2.

superior to singletons (Table 3). Other studies developed with other zoological groups presented similar comparative results between these estimators to those found in this study: Opiliones (Bragagnolo & Pinto-da-Rocha, 2003), Arctiidae (Teston *et al*., 2012), and Scarabaeinae (Silva *et al*., 2012).

The observed richness corresponds to 90.0% and 79.4% of the richness estimated by Chao1 and Chao2, respectively. Chao *et al*. (2009) proposed a method to estimate the increase in effort required to obtain 95% of the estimated richness when these estimators are employed. The results indicate the increase of individuals (Chao1) and samples (Chao2) to be used to reach 95% of the estimated richness (Table 3). The values of sampling effort for Chao2 require greater effort, since it implies more than tripling the original effort, that corresponds to 100 sample units. In a study of longitudinal variation in termites in 15 Atlantic Forest locations, Cancello *et al*. (2014) observed the need to increase the sampling effort in 11 sites, whose limits ranged from 50% to 1,400%, in order to reach 95% of the estimated richness.

In summary, the use of the four collection methods during the ten days may be the basis of a collection protocol for membracids, bearing in mind the observed species richness (27) and abundance (1,182). Considering the values estimated by Chao1 (28) and Chao2 (31), the 27 species represent a significant portion of the estimated richness for the 19 hectares of Atlantic Forest. However, to reach 95% of the estimated richness by Chao2 would imply tripling the effort, that may not be feasible considering time and costs restraints. The methods used are appropriate and complementary. However, some adjustments are necessary, such as: 1) increase the number of canopy cards, since there is a high diversity in this location; 2) observe a greater distance between the trays with respect to the forest edge, in order to exclude the possibility of catching representatives of edge species with this method; and 3) since the edge of the remnant is not natural but anthropized, the active collection should be restricted to exotic plants and those naturally found at the edge.

ACKNOWLEGEMENTS

WES, VAC, AL and ISB would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the student scholarships. APC is grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process #350052/2014-0) and Fundação de Apoio à Pesquisa do Estado da Paraíba (FAPESQ) for the research grant.

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