Estimation of the species richness of hyperdiverse beetles (Coleoptera: Cerambycidae) in an area of Atlantic Forest, Minas Gerais, southeastern Brazil

Estimativa da riqueza de besouros hiperdiversos (Coleoptera: Cerambycidae) em uma área de Floresta Atlântica, Minas Gerais, sudeste do Brasil

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Abstract

Species are elementary units in community ecology studies. However, sample limitations obstruct the elaboration of accurate faunistic inventories, especially in biodiversity hotspots, such as tropical forests. In this way, the objective of this research was to describe the richness, using different non-parametric estimators of richness, in the family Cerambycidae, a group of hyperdiverse insects in the Atlantic Forest. Five hundred and eighty-one specimens belonging to 145 species and 3 subfamilies were collected. Among the species sampled, 46.2% were considered singletons, 13.8% doubletons, 48.95% unicates, 15.15% duplicates and 90.3% ecologically rare. The species accumulation curves did not stabilize, which was already expected considering that more species and individuals were sampled in the last months of collection. The estimated richness presented values much higher than the observed richness. This was a reflect of the high proportion of ecologically rare species present in the sample. This work showed that faunistic inventories of hyperdiverse groups, with only one year of collection and a single sampling methodology may underestimate the species richness of a region. Thus, larger time series associated with different collection methods are essential for a more accurate survey of biodiversity in the Atlantic Forest.
Resumo
Espécies são unidades elementares em estudos de ecologia de comunidades. Entretanto, limitações de amostragem impedem a elaboração de inventários faunísticos precisos, especialmente em hotspots de biodiversidade como as florestas tropicais. Desta forma, o objetivo desta pesquisa foi descrever a riqueza, utilizando diferentes estimadores não paramétricos de riqueza, da família Cerambycidae, um grupo de insetos hiperdiversos na Floresta Atlântica. Foram coletados quinhentos e oitenta espécimes pertencentes a 145 espécies e 3 subfamílias. Entre as espécies amostradas, 46,2% foram consideradas singletons, 13,8% doubletons, 48,95% unicates, 15,15% duplicates e 90,3% ecologicamente raras. As curvas de acumulação de espécies não se estabilizaram, o que era esperado considerando que um grande número de espécies e indivíduos de besouros foi amostrado nos últimos meses de coleta. A riqueza estimada apresentou valores muito superiores à riqueza observada. Isso foi reflexo da alta proporção de espécies ecologicamente raras presentes na amostra. Este trabalho mostrou que inventários faunísticos de grupos hiperdiversos, com apenas um ano de coleta e uma metodologia de amostragem, podem subestimar a riqueza de espécies de uma região. Assim, séries temporais maiores associadas a diferentes métodos de coleta são essenciais para um levantamento mais preciso da biodiversidade na Mata Atlântica.

Keywords
ACE, Chao 1, forest entomology, Jacknife 2, Neotropical Ecozone

Palavras-chave
ACE, Chao 1, entomologia florestal, Jaacknife 2, Região Neotropical

Introduction
Species richness is a key component in community ecology studies (Longino et al. 2002; Magurran 2004). However, due to sampling limitations, most biodiversity surveys underestimate the true richness of a region. This is a very common problem for biodiversity hotspots, such as tropical forests, where inventorying all species is impractical (Colwell and Coddington 1994; Basset et al. 2012; Chao et al. 2017).

Thus, biodiversity inventories need to be considered pondering effective sampling procedures and estimates, especially for hyperdiverse groups (Colwell and Coddington 1994; Basset et al. 2012). In this way, many richness estimators have been proposed to reduce the subsampling bias (Chao et al. 2017). Among them, there are non-parametric estimators that make mathematical assumptions of the abundance distributions and/or incidence of the species observed in the sampling to estimate richness (Chao 1984, 1987; Smith and van Belle 1984; Chao and Lee 1992; Chao and Yang 1993).

Longhorn beetles (Coleoptera: Cerambycidae) are among the most diverse (with approximately 35,000 species) and important families of insects in the world (Monné et al. 2009, 2010; Wang 2017). The Americas have about 9,000 species (Monné and Bezark 2009), of which 4,315 occur in Brazil (Monné 2019). Here, we describe the richness of a hyperdiverse family of insects in the Atlantic Forest using three different non-parametric estimators of richness.
Material and methods

Study area

The samples were collected in three sites (Chá, Macacos and Repolheiro) of the Tripuí Ecological Station (TES) (Gatti et al. 2018), a fragment of the seasonal semi-deciduous Atlantic Forest, with an area of 337 ha, located in the city of Ouro Preto, a metallurgical area of the state of Minas Gerais, southeastern Brazil (20°23’45”S, 43°34’33”W) (Fig. 1). The station is located in the Tripuí sub-basin and has an altitudinal gradient ranging from 1,280 to 1,450 m. According to the Köppen classification, the climate is Cwb, with temperate summer and dry winter, precipitation index of about 1,600 mm, and average annual temperature of 18 °C (Werneck et al. 2000, 2001; Alvares et al. 2013).

Longhorn beetle collection

Beetles were collected using luminous traps set in the three study areas during one year, from February 2014 to January 2015. Two collections per month were performed, and each collection campaign was composed of three consecutive nights of collection, totaling six nights of collection per month. In this way, we have a sampling size of 216 (3 traps × 6 nights × 12 months). The luminous trap used for the collection was the Luiz de Queiroz model with an ultraviolet fluorescent light source of 15 W and 100 V, manufactured by Biocontrole. The operation of the traps is crepuscular, from dusk to dawn, between 6 p.m. and 6 a.m. The traps were placed 1.80 m above the ground to allow a greater scattering of light (De Paula and Ferreira 1998, 2000; Gatti et al. 2018). The beetles were killed still in the field, inside the trap, with ethyl acetate. In the laboratory, the collected specimens were organized and identified to the lowest possible taxonomic level by the authors of the study. Obviously, our sampling is limited to the collection methodology, and in this case, the great majority of collected beetles have nocturnal habit. All collected specimens are deposited in the Entomological Collection of the Ecological Entomology Laboratory of the Universidade Federal de Ouro Preto, under the care of Prof. Marco Antônio A. Carneiro.

Statistical analyses

To evaluate the sampling effort, two species accumulation curves were made: the rarefaction curve, which finds the mean of the accumulation of individuals; and the exact method, which finds the average expectation of species richness in each sample (Oksanen et al. 2015). To estimate the richness of longhorn beetles, three non-parametric estimators were used based on species abundance and/or incidence, Chao 1 (Chao 1984, 1987), ACE (Chao and Lee 1992; Chao and Yang 1993) and Jacknife 2 (Smith and van Belle 1984). These analyses were
performed using the packages ‘vegan’ (Oksanen et al. 2015) and ‘BiodiversityR’ (Kindt and Coe 2005) developed for the statistical program R (R Development Core Team 2014).

**Results**

A total of 581 specimens of longhorn beetles were collected, distributed in 145 species and 3 subfamilies (see Suppl. material 1, Table S1). Sixty-seven (46.2%) species were considered singletons (represented by only one individual in the sampling) and 20 (13.8%) doubletons (represented by only two individuals in the sampling). Seventy-one (48.95%) species occurred in a single sample and 22 (15.15%) species occurred in two samples. One hundred and thirty-one species, or 90.3% of the sample, had abundance between 1 and 10 specimens and were, therefore, considered ecologically rare (Chao and Lee 1992; Chao and Yang 1993; Mao and Colwell 2005). The estimates of richness were higher (ACE = 236 ± 2.4 SD; Chao 1 = 250 ± 6.2 SD; Jack 2 = 264 ± 2.2 SD) than the number of species observed (Sobs = 145 ± 8 SD) and their curves did not stabilize (Fig. 2).

Regardless of the adjustment method used, the species accumulation curves did not stabilize (Fig. 3). This result was expected, since the last months of collection comprised the summit of the rainy season, and more species (81 or 55.85% of the total sample) and specimens (428 or 73.7% of the total sample) were collected between October 2014 and January 2015 (Fig. 4).
Figure 2. Richness estimates of longhorn beetles (Coleoptera: Cerambycidae), sampled during 12 months of collection (February/2014 to January/2015), with luminous traps, at the Tripuí Ecological Station, Ouro Preto, Minas Gerais, Brazil. ACE = 236 ± 2.4 SD; Chao 1 = 250 ± 6.2 SD; Jack 2 = 264 ± 2.2 SD.

Figure 3. Accumulation curves of species of longhorn beetles (Coleoptera: Cerambycidae) sampled during 12 months of collection (February/2014 to January/2015), with luminous trap, at the Tripuí Ecological Station, Ouro Preto, Minas Gerais, Brazil. A) Rarefaction curve; B) Exact method curve. Vertical bars represent ± 2 SD.

Discussion

The high values found for the richness estimates (Fig. 2) are a reflection of the high proportion of rare species (mainly singletons and doubletons) with low sampling incidence. It is important to make it clear that the fact that these species are rare in our sample does not necessarily mean that they are rare in nature. They may be specialist species with low population levels, poorly sampled generalists or tourist species (Novotný and Basset 2000; Longino et al. 2002). The fact is that rare species
are important components of herbivorous insect communities in tropical forests, and should never be excluded or marginalized in the studies of these communities (Novotný and Basset 2000).

The richness value estimated by ACE is lower than that of Jack 2 and Chao 1 (Fig. 2). As the value given by Chao 1 is a minimum estimate of richness (Chao 1984), the ACE seems to be underestimating the richness of longhorn beetles at the TES. Since different estimators will estimate different species richness, it is essential and safer to always use more than one estimator to make inferences about the richness of an area (O’Hara 2005; Hortal et al. 2006). Estimators are a very useful tool for performing ecological studies, but it is important to make it clear that they also have their mathematical and methodological biases and one needs to be cautious when using them. None of them can infer with certainty how many species may exist in an area, and it is advisable to treat these estimates as minimum richness (Longino et al. 2002; O’Hara 2005).

Considering that the species accumulation curves did not stabilize (Fig. 3), due to the increase of species sampled in the final months of collection (Figure 4A), and that the results of the richness estimators showed that there may be more than one hundred additional species to the ones collected, new species of longhorn beetles must be sampled in the TES with the continuation of sampling. Thus, more accurate faunal inventories require larger time series in long-term projects with the combinations of different collection methods (Wolda 1988; Longino et al. 2002; Basset et al. 2012). Longhorn beetles belong to a hyperdiverse group, consisting of taxa that inhabit the most varied forest habitats (Monné et al. 2009) and that present great differences in the period of larval development, varying from 1 to 3 years (Lieutier et al. 2004). Hence, it is expected that the community composition of these beetles will vary not only seasonally but also annually. Thus, new species would always be present year after year of collection. This study demonstrates that inventories of hyperdiverse groups carried out during one year of collection and with a single sampling method underestimate species richness and composition, reducing the effectiveness of practical actions aimed at the conservation and maintenance of biodiversity.
The idea that a good sampling should cause a stabilization in the accumulation curves, understood as the point at which the inclusion of sample units will not culminate in the significant addition of new species, is a point of debate for the work done with groups of hyperdiverse organisms that inhabit tropical forests (Schilling and Batista 2008). These forests have the greatest biodiversity in the world, making the complete stabilization of curves somewhat unrealistic (Longino et al. 2002).

The months of November and December, which correspond to spring in the southern hemisphere and comprise the apex of the rainy season, with naturally very high temperature and rainfall, were the months when more species and specimens of beetles were sampled (Fig. 4). This synchrony of the longhorn beetles with the rainy season probably is related to the period of greater availability of resources. In this period, the great majority of plants has new leaves and flowers that coincides with increased amount of dead branches in the forest (Bullock and Solís-Magallanes 1990; Martínez-Yrízar 1995). Thus, the rainy season is marked by the emergence of adults in search of food, host plants, dead trunks and sexual partners for mating and nesting (Toledo et al. 2002; Makino et al. 2007; Rodrigues et al. 2010).

The Atlantic Forest has a high, not yet known, diversity of longhorn beetles, and studies with consecutive years of collection and implementation of other sampling methods are essential for obtaining new records of species. Only then we will have clearer information to better understand the temporal fluctuations of the hyperdiverse beetle communities.

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Supplementary material 1

Table S1. List of species of the longhorn beetles (Coleoptera: Cerambycidae), and their respective abundances, sampled with luminous trap at the Ecological Station of the Tripuí, Ouro Preto, Minas Gerais, Brazil
Authors: Felipe Donateli Gatti, Marco Antonio Alves Carneiro
Data type: species data
Explanation note: Morphospecies numbered following Gatti et al. 2018 and entomological collection of the Laboratório de Entomologia Ecológica, UFOP.
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