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Species diversity and community structure of fruit-feeding butterflies (Lepidoptera: Nymphalidae) in an eastern amazonian forest

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ABSTRACT

Deforestation has negative impacts on diversity and community patterns of several taxa. In the eastern Amazon, where much deforestation is predicted for the coming years, forests patches may be essential to maintain the local biodiversity. Despite increasing concerns about the conservation of threatened areas, few studies have been performed to analyze the communities of diversified groups, such as insects, in the eastern Amazon. Here, we investigated species diversity and community structure of fruit-feeding butterflies, a well-known bioindicator group, in a threatened remnant of an eastern Amazonian forest located on Maranhão Island, northeastern Brazil. Fruit-feeding butterflies were sampled monthly for one year. Diversity and evenness indices, richness estimators, rarefaction curve, and rank-abundance plot were used to describe community structure in the study area. We captured 529 fruit-feeding butterflies in four subfamilies, 23 genera and 34 species. The three most abundant species, Hamadryas februa, Hamadryas feronia, and Hermeuptychia cf. atalanta are indicators of disturbed habitats and represented more than half of the collected individuals. Richness estimators revealed that between 87 and 94% of the fruit-feeding butterfly species were sampled, suggesting few additional records would be made for the area. Our results indicate that human-caused disturbances have altered local community patterns and provide baseline data for future research in threatened regions of the eastern Amazon.

KEY-WORDS: Biodiversity; Butterfly assemblage; Frugivorous butterflies; Neotropical region; Tropical forest.

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INTRODUCTION

Studies of species diversity provide relevant insights about biological communities, especially in threatened regions (Purvis & Hector, 2000). Through community structure approach, it is possible to understand how communities assemble and the mechanisms generating and maintaining species diversity, which is an important step for conservation planning (Purvis & Hector, 2000). It is widely known that habitat loss is among the main causes of species extinctions worldwide (Brooks et al., 2002; Pimm et al., 2014). Consequently, habitat loss changes community patterns of several taxa and disrupts processes that maintain ecosystem integrity (Jonsson et al., 2002; Larsen et al., 2005). Therefore, communities' responses to anthropogenic disturbances provide valuable information for biological conservation.

The Amazon forest has been severely degraded in the past years, increasing concerns for the biological conservation of this biome (Laurance et al., 2004). Specifically, the eastern portion of the Brazilian Amazon is poorly studied and highly threatened (Vieira et al., 2008; Martins & Oliveira, 2011), still needing species inventories and community structure analysis to increase our understanding of its biota. A major concern is the most endangered endemism center in Brazil, the Belém Center of Endemism, located in the eastern Amazon between the states of Pará and Maranhão (Silva et al., 2005). There is a growing need for studies performed in habitat patches of the eastern Amazonian forest, since these remaining habitats may contribute to partially offset biodiversity losses (Barlow et al., 2007; Tulloch et al., 2016).

Butterflies are constantly used to understand how deforestation impacts species diversity and represent valuable flagship species for biological conservation (Brown Jr. & Freitas, 2000). Among this group, fruit-feeding butterflies have been widely used as bioindicators because of their sensitivity to environmental modifications (Brown Jr. & Freitas, 2000; Fermon et al., 2005; Barlow et al., 2007; Uehara-Prado et al., 2007). This guild feeds on rotten fruits, plant exudates, carcasses, and mammal excrement (Devries, 1987; Devries et al., 1997; Freitas et al., 2014), and it includes four Nymphalidae subfamilies (Biblidinae, Satyrinae, Charaxinae and Nymphalinae [only the tribe Coeini]) (Wahlberg et al., 2009). Despite fruitfeeding butterflies being a well-known group, knowledge of butterfly diversity varies greatly with locations in Brazil (Santos et al., 2008). Thus, although this guild has received a great attention in some biomes, such as the Atlantic forest (Uehara-Prado et al., 2007; Ribeiro *et al.*, 2010; Santos *et al.*, 2011; Ribeiro *et al.*, 2012), the same is not true for the eastern Amazon, where there are still major gaps of butterfly inventories (Santos *et al.*, 2008; Martins *et al.*, 2017).

In the present study, we analyzed species diversity and community structure of fruit-feeding butterflies in a remnant of eastern Amazonian forest located on Maranhão Island, northeastern Brazil. Our aim is to understand how the studied community is structured and expand knowledge of the local fruit-feeding butterfly fauna. Ultimately, we aim to provide baseline data for future studies in a region highly threatened by human occupation and land-use changes.

MATERIAL AND METHODS

Study area

This study was conducted between April 2012 and May 2013 in an eastern Amazonian forest area of approximately 600 ha called "Sítio Aguahy" (hereafter SAG), owned by the Companhia Farmacêutica Quercegen Agronegócios (Quercegen S.A.) and located in the municipality of São José de Ribamar, state of Maranhão, northeastern Brazil (02.65°S; 44.14°W) (Fig. 1). Temperatures are high throughout the year, and the mean annual temperature is approximately 26°C. Rainy season occurs usually from January to July, accumulating about 94% of the total annual rainfall. The abiotic data were obtained through the meteorological station of the Universidade Estadual do Maranhão (UEMA), located at a distance of 10 km from the study area.

SAG is one of the remaining patches of Amazon forest on Maranhão Island, where the state capital, São Luís, is located. The study area was originally covered with pristine Amazon forest, mangrove swamps, and coastal vegetation, but urban expansion caused extensive habitat modifications, including the establishment of monocultures and secondary forests. In its most preserved sites, SAG possesses predominantly dense rainforests containing several species of palms, while there is a predominance of crops, coastal vegetation, and secondary forests in its remaining areas (Fig. 1). These secondary forests result from the abandonment of forest lands cleared for agriculture and vary in ages from 15 to 40 years old. Despite being a private propriety owned by Quercegen S.A., SAG has been constantly threatened by the expansion of the surrounding villages, highlighting the necessity of conservation measures to preserve this Amazon remnant.

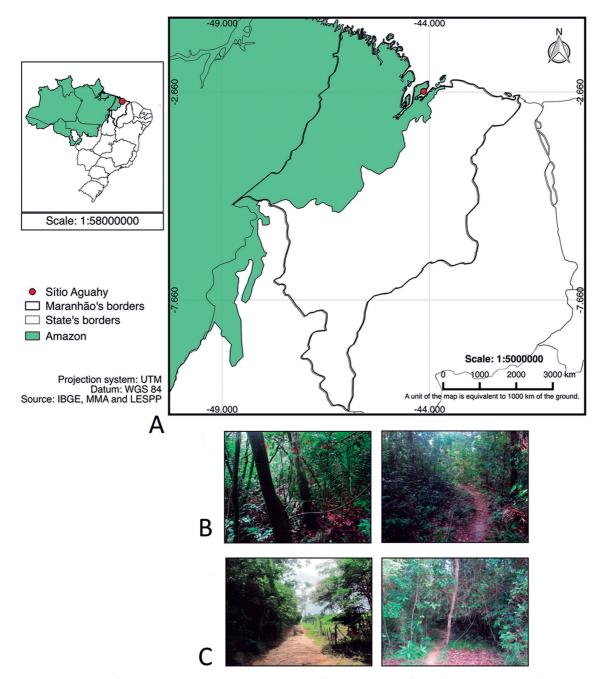
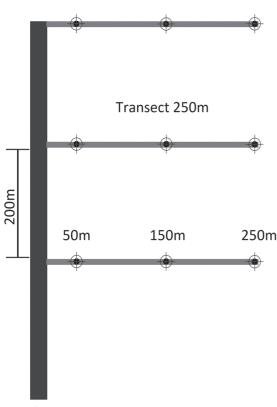


FIGURE 1: Location of Sítio Aguahy, in the eastern Amazon. (A) Maps of Brazil and the state of Maranhão, demonstrating the distribution of the Brazilian Amazon forest. (B) Dense rainforest (C) Secondary forest.

Sampling design and identification

We established two sampling sites located about two km from each other. Each sampling site was composed by a trail of 600 m linking three transects with distance of 200 m among them. These transects penetrated 250 m to the interior of the forest where cylindrical traps were disposed at distances of 50, 150 and 250 m from the beginning of the transect, resulting in a total of nine traps per sampling site (Fig. 2).

Each month, cylindrical butterfly traps of the Van Someren-Rydon type (Rydon, 1964) were baited with a mix of bananas and sugar cane juice and remained on the field for 33 hours (always being placed at 08:00 and hand removed at 17:00 h of the next day). Traps were suspended from low branches with



Trail 600m

FIGURE 2: Each sampling site was composed of one trail and three transects to the interior of the forest. Points represent cylindrical traps baited with a mix of banana and sugarcane juice.

trap bases at a distance of 1.0 to 1.5 m from the ground. We inspected traps twice a day and killed the specimens with an injection of ammonia in the ventral region or by thoracic compression. Nectar-feeding butterflies captured accidentally in traps were not considered in the analysis since the addition of these species might alter radically the community parameters (Freitas et al., 2014). Captured butterflies were taken to the Laboratório de Ecologia e Sistemática de Insetos Polinizadores e Predadores (LESPP/UFMA) and identified through comparison with specimens deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP), Museu de Zoologia da Universidade Estadual de Campinas (ZUEC), and through a specialized catalogue (Garwood et al., 2009). Vouchers are deposited in collections of three institutions: LESPP/UFMA, MZUSP, and ZUEC.

Data analysis

We used the Whittaker plot (rank-abundance distribution) and Pielou's index to investigate patterns of evenness in the studied community. The first measure is an important method for analyzing community evenness through the curve's inclination (Magurran, 2004; Melo, 2008), while the second is a direct measure of evenness based on Shannon's diversity index (Magurran, 2004). Species accumulation against sample effort was analyzed using sample-based rarefaction calculated through the Mao Tau method with 95% confidence intervals (Colwell & Coddington, 1994; Magurran, 2004). To estimate species richness of the entire sampled community, two richness estimators were used (Jackknife 1 and Chao 1) (Magurran, 2004).

Diversity indices have been used in ecological studies as an alternative to species richness (Magurran, 2004; Melo, 2008). One of the major advantages of diversity indices is their capacity of concentrating two community attributes (species richness and evenness) in a single measure (Melo, 2008). However, these indices have been constantly criticized for being abstract measures of difficult interpretation (Jost, 2006; Melo, 2008). One possible exception is Simpson's diversity index (1-D) (Magurran, 2004; Melo, 2008), which is here used as our diversity measure. This index calculates the probability that two individuals randomly selected from a sample will belong to the same species (Magurran, 2004). Data analyses were performed through R language (R Development Core Team, 2017), using the vegan (Oksanen et al., 2017) and Biodiversity R (Kindt & Coe, 2015) packages.

RESULTS

After 7,128 trap-hours of sampling, 529 fruitfeeding butterflies were collected, representing four subfamilies, 11 tribes, 23 genera, and 34 species (Table 1). The most abundant subfamily was Biblidinae, represented by 243 individuals (45.9% of the collected butterflies), followed by Satyrinae with 179 individuals (33.8%), Nymphalinae with 59 individuals (11.2%), and Charaxinae with 48 individuals (9.1%). On the other hand, the most speciose subfamilies were, respectively, Satyrinae with 14 species (41.2% of the species collected), Biblidinae with nine species (26.5%), Charaxinae with eight species (23.5%), and Nymphalinae with three species (8.8%).

The three most abundant species were *Hama-dryas februa* (Hübner, [1823]) with 140 individuals (26.5% of total abundance), *Hamadryas feronia* (Linnaeus, 1758) with 73 individuals, and *Hermeuptychia* cf. *atalanta* (Butler, 1867) with 55 individuals. These species represented more than half of the collected specimens. Twenty-four species (70% of the collected

Species	Authorship	No. individuals	Proportion (%)
Charaxinae: Preponini			
Archaeoprepona demophon	(Linnaeus, 1758)	2	0.4
Prepona laertes	(Hübner, [1811])	7	1.3
Prepona pheridamas	(Cramer, 1777)	7	1.3
Charaxinae: Anaeini			
Fountainea ryphea	(Cramer, 1775)	5	0.9
Hypna clytemnestra	(Cramer, 1777)	1	0.2
Memphis acidalia	(Hübner, [1819])	7	1.3
Memphis leonida	(Stoll, 1782)	5	0.9
Zaretis isidora	(Cramer, 1779)	14	2.6
Nymphalinae: Coeini			
Historis acheronta	(Fabricius, 1775)	49	9.3
Historis odius	(Fabricius, 1775)	2	0.4
Nymphalinae: Nymphalini			
Colobura dirce	(Linnaeus, 1758)	8	1.5
Biblidinae: Biblidini		-	
Biblis hyperia	(Cramer, 1779)	3	0.6
Biblidinae: Catonephelini		-	
Catonephele acontius	(Linnaeus, 1771)	3	0.6
Eunica maja	(Fabricius, 1775)	2	0.4
Biblidinae: Ageroniini	(
Hamadryas amphinome	(Linnaeus, 1767)	13	2.5
Hamadryas chloe	(Stoll, 1787)	3	0.6
Hamadryas februa	(Hübner, [1823])	140	26.5
Hamadryas feronia	(Linnaeus, 1758)	73	13.8
Hamadryas laodamia	(Cramer, 1777)	1	0.2
Biblidinae: Callicorini		1	0.2
Callicore astarte	(Cramer, 1779)	5	0.9
Satyrinae: Morphini	(Granici, 1779))	0.9
Morpho helenor	(Cramer, 1776)	14	2.6
Morpho rhetenor	(Cramer, 1775)	2	0.4
Satyrinae: Brassolini	(Granici, 1779)	2	0.1
Caligo teucer	(Linnaeus, 1758)	1	0.2
Catoblepia berecynthia	(Cramer, 1777)	8	1.5
Opsiphanes invirae	(Hübner, [1808])	9	1.7
Opsiphanes quiteria	(Stoll, 1780)	3	0.6
Satyrinae: Satyrini	(3001, 1780)	J	0.0
	(Fabricius, 1775)	8	1.5
Cissia penelope Cissia tomotoin			
Cissia terrestris Homeocutatuoloin of atalanta	(Butler, 1867)	31	5.9
Hermeuptychia cf. atalanta Marmout tuchia lihuu	(Butler, 1867)	55	10.4
Magneuptychia libye Maserantishin and sta	(Linnaeus, 1767) (Echristica, 1777)	13	2.5
Magneuptychia ocypete	(Fabricius, 1776)	5	0.9
Pharneuptychia sp.	(E1	16	3
Taygetis laches	(Fabricius, 1793)	7	1.3
Yphthimoides renata	(Stoll, 1780)	7	1.3

TABLE 1: Fruit-feeding butterflies collected at Sítio Aguahy, separated by subfamily and tribe, with number of individuals (abundance) and the proportion of each species.

species) were represented by less than 10 individuals (Fig. 3). Of these, three species were represented by a single collected individual (singletons): *Caligo teucer* (Linnaeus, 1758), *Hamadryas laodamia* (Cramer, 1777), and *Hypna Clytemnestra* (Cramer, 1777); and four species were represented by two individuals (doubletons): Archaeoprepona demophon (Linnaeus, 1758), Eunica maja (Fabricius, 1775), Historis odius (Fabricius, 1775), and Morpho rhetenor (Cramer, 1775). Thus, the contribution of singletons plus doubletons to total richness was 20%. Furthermore, two species were collected in only one sampling month

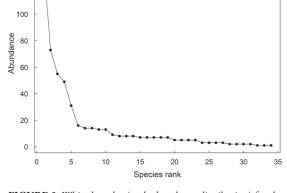


FIGURE 3: Whittaker plot (rank-abundance distribution) for the total sample of fruit-feeding butterflies in an eastern Amazonian forest. The y axis represents species abundance and the x axis ranks each species in order from most to least abundant.

(uniques), disregarding the singletons: *H. odius* and *M. rhetenor*; and six species were collected in two sampling months (duplicates): *A. demophon, Biblis hyperia* (Cramer, 1779), *E. maja, Hamadryas chloe* (Stoll, 1787), *Opsiphanes quiteria* (Stoll, 1780), and *Yphthimoides renata* (Stoll, 1780).

Simpson's diversity index was calculated in 0.88 and Pielou's evenness in 0.76. The sample-based rarefaction curve appears to achieve an asymptote around the 12th sample (Fig. 4), suggesting few additional records are to be expected for the study area. Jackknife 1 and Chao 1, respectively, estimated around 39 and 36 species at SAG, in contrast to the 34 species actually collected. In other words, we estimate that between 87 and 94% of the fruit-feeding butterfly species of SAG were sampled.

DISCUSSION

The most representative subfamilies of fruitfeeding butterflies at SAG were Biblidinae and Satyrinae, two of the richest and most abundant subfamilies of Nymphalidae in different regions of Brazil (Ramos, 2000; Uehara-Prado *et al.*, 2007; Santos *et al.*, 2011; Ribeiro *et al.*, 2012). In fact, two species of Biblidinae (*H. februa* and *H. feronia*) and one of Satyrinae (*H. cf. atalanta*) were the most abundant species in the study area. These species are common in forest edges, disturbed tropical forests, plantations, and urban environments, probably due their low biological constraints (Brown Jr., 1992; Ramos, 2000; Uehara-Prado *et al.*, 2007); Cosmo *et al.*, 2014). Therefore, their high abundances are an indication that human disturbances

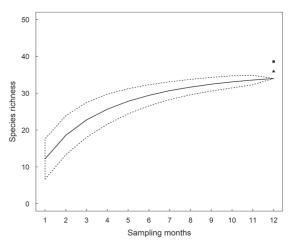


FIGURE 4: Sample-based rarefaction curve (species accumulation) with 95% confidence intervals (dashed lines) to indicate the degree of completeness of the samples (square: Jackknife 1; triangle: Chao 1).

have favored population increases of tolerant and opportunist organisms. In accordance with these results, the vegetation of SAG has been dominated in several sites by secondary forests and crops, which probably contributed to these species' success. The Whittaker plot and the results from Simpson's diversity and Pielou's evenness reinforce that the studied community is dominated by few abundant species. Similar patterns have been observed in numerous insect communities of tropical forests (DeVries & Walla, 2001; Tonhasca Jr. *et al.*, 2002; Silva & Di Mare, 2012) and appear to be even more prominent in disturbed environments and fragmented landscapes where opportunistic species thrive and sensitive species suffer population declines (Schmidt *et al.*, 2013; Cajaiba *et al.*, 2017).

Richness estimators suggest that the fruit-feeding butterfly fauna of SAG was nearly completely sampled around the 12th sampling month, indicating that sampling was adequate to provide an accurate representation of the local community. However, the observed richness is considerably lower than the richness found in other areas of the Brazilian Amazon. For example, Barlow et al. (2007) recorded 128 species in a study area between the states of Pará and Amapá, and Ribeiro et al. (2012) collected 68 species in the state of Amazonas. Respecting the eastern Amazon, little information on fruit-feeding butterfly diversity have been published, especially for the state of Maranhão (Martins et al., 2017). To date, the only published study so far analyzing community structure of fruit-feeding butterflies in the state was performed by Ramos (2000), in which 90 species were collected.

Differences in species richness among studies may be caused by multiple factors, such as sample

140

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biases, human disturbances, and the size of the study areas. Regarding sample biases, it has been long recognized that is almost impossible to detect all species in a given area (Colwell & Coddington, 1994; Magurran, 2004). This is especially true for tropical insect communities due their high species richness and large fraction of rare species (Novotny & Basset, 2000). For this reason, methods of extrapolation are essential to help us estimate species richness more accurately, since they are supposedly independent of sample sizes (Magurran, 2004). Considering that our observed richness was similar to the estimated richness values, we believe that sample biases were not the main drivers of the differences recorded between our study and those from Barlow et al. (2007), Ribeiro et al. (2012), and Ramos (2000), for example. Also, we consider that other factors that could potentially increase our observed richness, such as sampling in the forest canopy (Devries & Walla, 2001; Fermon et al., 2005), would not significantly change the number of sampled species, since canopy height at SAG rarely exceeds 15 m even in the most preserved sites.

SAG has been constantly threatened by human activities due the urban expansion of the state capital, São Luís, which probably diminished species richness in this area during the last decades. Indeed, decreases in fruit-feeding butterfly richness after human disturbances have been demonstrated by different studies (Vedeller *et al.*, 2005; Barlow *et al.*, 2007), although the type of disturbance appears to be important for determining this pattern. For example, Fermon *et al.* (2005) and Uehara-Prado *et al.* (2007) demonstrated that secondary forests and fragmented landscapes may harbor as many or more butterfly species than continuous primary forests, while plantations and urban forests apparently harbor fewer and generalist species (Brown Jr. & Freitas, 2000; Barlow *et al.*, 2007).

Additionally, butterfly richness has been suggested as being positively correlated with habitat area, following the classic species-area relationship (Ricklefs & Lovette, 1999; Benedick et al., 2006). An empirical example is provided by Uehara-Prado et al. (2007), where a positive relationship was found between fragment area and species richness of fruit-feeding butterflies in the Atlantic forest. Considering that SAG is a small Amazon forest remnant inserted in a matrix of villages and crops, it is possible that sensitive butterfly species have already become locally extinct due the reduced size and habitat heterogeneity of this forest patch, impacting the observed species richness. Interestingly, despite presenting low species richness, small forest fragments can still contribute substantially to regional diversity (Benedick et al., 2006), since these

areas can maintain species that are no longer present in disturbed habitats nearby.

CONCLUSION

The low species richness and high abundance of generalist and opportunistic species, associated with the results from diversity and evenness indices, are signals that land-use and other human disturbances have altered the diversity and community structure of fruit-feeding butterflies at SAG. This is particularly worrisome considering that SAG is one of the few remaining areas of Amazon forest on Maranhão Island. For this reason, we reinforce the importance of conservation measures to protect this Amazon remnant and recommend that researchers perform community structure analyses of different taxa to guide conservation planning at SAG and other regions of the eastern Amazon forest.

RESUMO

O desmatamento tem impactos negativos na diversidade e padrões de comunidades de diversos taxa. Na Amazônia Oriental, onde um grande desmatamento é previsto para os próximos anos, manchas florestais podem ser essenciais para manter a biodiversidade local. Apesar de crescente preocupação quanto à conservação de áreas ameaçadas, poucos estudos foram realizados a fim de analisar comunidades de grupos diversificados, como insetos, na Amazônia Oriental. Aqui, nós investigamos a diversidade de espécies e a estrutura da comunidade de borboletas frugívoras, um reconhecido grupo de bioindicadores, em um remanescente de floresta Amazônica Oriental localizada na Ilha do Maranhão, Nordeste do Brasil. As borboletas frugívoras foram amostradas mensalmente por um ano. Índices de diversidade e equitabilidade, estimadores de riqueza, curva de rarefação e um gráfico de rank/abundância foram utilizados para descrever a estrutura da comunidade na área de estudo. Nós capturamos 529 borboletas frugívoras de quatro subfamílias, 23 gêneros e 34 espécies. As três espécies mais abundantes, Hamadryas februa, Hamadryas feronia e Hermeuptychia cf. atalanta são indicadores de habitats perturbados e representaram mais da metade dos indivíduos coletados. Os estimadores de riqueza revelaram que entre 87 e 94% das espécies de borboletas frugívoras foram amostradas, sugerindo que poucos registros adicionais seriam feitos para a área. Nossos resultados indicam que distúrbios antrópicos alteraram padrões da comunidade local e fornecem dados para pesquisas futuras em regiões ameaçadas da Amazônia Oriental.

PALAVRAS-CHAVE: Biodiversidade; Assembleia de borboletas; Borboletas frugívoras; Região Neotropical; Floresta tropical.

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