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Crepuscular and nocturnal hawkmoths (Lepidoptera: Sphingidae) from a fragment of Atlantic rainforest in the state of São Paulo, southeastern Brazil

Kely Cristina Rocha Vieira¹, Simeão de Souza Moraes², Pedro Ivo Chiquetto-Machado² and Marcelo Duarte^{2,3*}

Abstract

A survey of the richness, abundance and species composition of Sphingidae (Lepidoptera) was performed in a fragment of Atlantic Rainforest at the Serra do Japi Biological Reserve, Municipality of Jundiaí, state of São Paulo, Brazil. Sampling was done monthly, on 2 consecutive nights, from Dec 2011 to Oct 2012, totaling 264 hours of sampling effort. The hawkmoths were attracted by a light trap. Our results were compared with data from a survey of Sphingidae carried out in another fragment of Atlantic Rainforest, i.e., at the Boraceia Biological Station, Municipality of Salesópolis, São Paulo. Richness estimators, rarefaction curves and rank/abundance plots were used to describe the assemblage sampled in this study and to compare it with the data set from Boraceia. We collected 462 specimens, members of 3 subfamilies, 5 tribes, 18 genera and 39 species. *Callionima parce* (Fabricius) was the most abundant species, comprising 33% of the individuals. *Orecta lycidas* (Boisduval), an under-sampled species in the Atlantic Rainforest, was recorded for the first time in the area of Serra do Japi. The assemblage showed lower species richness and evenness than the assemblage at Boraceia, probably due to differences in climate, vegetation and size of the forest fragment in which the reserves are situated. Nevertheless, our results emphasize the importance of the Serra do Japi Biological Reserve for the fauna of Lepidoptera of the Brazilian Atlantic Rainforest.

Key Words: Atlantic Rainforest; conservation; diversity; richness; scientific collection

Resumo

Um estudo sobre a riqueza, abundância e composição de espécies de Sphingidae (Lepidoptera) foi realizada em um fragmento de Mata Atlântica na Reserva Biológica da Serra do Japi, Município de Jundiaí, São Paulo, Brasil. As amostragens foram feitas mensalmente, por duas noites consecutivas, de dezembro de 2011 a outubro de 2012, totalizando 264 horas de esforço amostral. As mariposas foram atraídas por armadilha luminosa. Nossos resultados foram comparados com dados de uma amostragem de Sphingidae realizada em outro fragmento de Mata Atlântica, a Estação Biológica de Boraceia, Salesópolis, São Paulo. Estimadores de riqueza, curvas de rarefação e diagramas de rank-abundância foram utilizados para a descrição da comunidade amostrada neste estudo e para comparações com os dados de Boraceia. Foram coletados 462 espécimes, distribuídos em 3 subfamílias, 5 tribos, 18 gêneros e 39 espécies. *Callionima parce* (Fabricius) foi a espécie mais abundante, representando 33% dos indivíduos. *Orecta lycidas* (Boisduval), uma espécie pouco amostrada na Mata Atlântica, foi registrada pela primeira vez na Serra do Japi. A comunidade estudada apresentou riqueza de espécies e equabilidade inferiores às de Boraceia, provavelmente devido a diferenças de clima, vegetação e tamanho do fragmento florestal em que as reservas estão situadas. Ainda assim, nossos resultados enfatizam a importância da Reserva Biológica da Serra do Japi para a fauna de Lepidoptera da Mata Atlântica.

Palavras-Chave: Mata Atlântica; conservação; diversidade; riqueza; coleção científica

The moths of the family Sphingidae, usually known as hawkmoths, are important pollinators and a main component in the dynamic equilibrium of many ecosystems (Haber & Frankie 1989; Bawa 1990). Many species have high population densities and breed continuously throughout the year (Kitching & Cadiou 2000), and can be easily collected and identified, making them potential models in diversity studies (e.g., Léon-Cortés et al. 1998) and indicators in biodiversity monitoring. Although hawkmoths

have been evaluated as indicators of anthropogenic disturbance (Schulze & Fiedler 2003; Beck et al. 2006), the results were somewhat inconclusive, and they are apparently not widely used for this purpose.

With more than 1,400 described species (van Nieukerken et al. 2011), the hawkmoths have a worldwide distribution, but their greatest diversity is found in the tropics (Kitching & Cadiou 2000). The family is represented by 230 species in Brazil (Martin et al. 2011).

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Sphingids are primarily defined by their medium to large size; filiform antennae that may be clavate or pectinate, usually ending in a pronounced apical hook; and thick body with long narrow forewings (Tuttle 2007). Many species have an extremely elongated proboscis, occasionally exceeding the length of the body (Krenn 2010). Many species have nocturnal or crepuscular habits, though some are exclusively diurnal. When in flight, the hawkmoths are among the fastest insects, and some species are long-distance migrants (Lemaire & Minet 1998).

The sphingofaunas of several localities in the southeastern Brazilian Atlantic Rainforest have been investigated, in the states of São Paulo (Coelho et al. 1979; Duarte et al. 2008), Minas Gerais (Ferreira et al. 1986), Rio de Janeiro (Zikán & Zikán 1968; Martin et al. 2011) and Espírito Santo (Brown & Freitas 2000). Most of these studies are compilations of data obtained from the work of various collectors during several years of sampling, thus resulting in high values of abundance and richness. However, the lack of a standardized sampling effort (e.g., differences in the number of hours per night with the light trap in operation) is a significant problem impeding the use of these studies in conservation planning.

The objective of this study was to survey the richness, abundance and composition of the Sphingidae assemblage from the Serra do Japi Biological Reserve (SJBR), which is considered a priority area for preservation because it is one of the last areas of continuous forest in the state of São Paulo, containing a rich flora and fauna that remained after the colonization of the southeastern Brazil (Morellato 1992). We also present a comparison with data available from another preserved area in the state of São Paulo, the Boraceia Biological Station (BBS) (Duarte et al. 2008). With a standardized sampling procedure, the intent of this study is to provide potentially applicable contributions to the knowledge of the distribution of Sphingidae in the Atlantic Rainforest.

Materials and Methods

STUDY AREA

Located in a highly urbanized region between the cities of São Paulo, Jundiaí and Campinas, the SJBR is one of the few areas of preserved forests in the state of São Paulo (\$23°14' W 46°56', 1,049 m asl) (Fig. 1). The SJBR was established by Municipal Law 3,672 on 10 Jan 1991, covering an area of 20,712 km², with the objectives of conserving the genetic resources of local fauna and flora and contributing to the development of scientific research. In 1994 this protected area was declared an Atlantic Rainforest Biosphere Reserve (UNESCO 1994). The data on the hawkmoth fauna of the SJBR was compared with data previously collected at the BBS (\$23°39' W 45°53', 850 m asl), which is situated within a broad forest fragment in the Serra do Mar of the state of São Paulo. For information on its founding and research activities, see Travassos & Camargo (1958) and Duarte et al. (2008).

SAMPLING AND IDENTIFICATION

Sampling was performed monthly, from Dec 2011 to Oct 2012, for 2 consecutive nights, always during the new-moon phase. We used a 500-W mixed light bulb and a white 2 m x 2 m sheet to attract the moths. The light trap was supervised from 18:00 to 06:00 h, totaling 24 h of sampling effort per month and a total of 264 h of sampling. Specimens were manually collected and killed with an injection of ammonia (10% aqueous solution) in the ventral region, between the thorax and the abdomen.

To facilitate the identification and to conform to the curatorial standards of the Museu de Zoologia, Universidade de São Paulo (MZUSP),

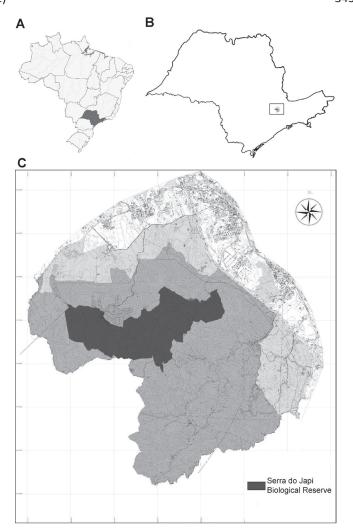


Fig. 1. Location of the Serra do Japi Biological Reserve. (A) Brazil with detail showing the state of São Paulo. (B) State of São Paulo with detail of the municipality of Jundiaí. (C) Municipality of Jundiaí and the Serra do Japi Biological Reserve.

the collected specimens were spread using entomological pins and spreading boards, and then left to dry for 14 days under natural conditions of temperature and humidity.

Collected specimens were identified with the aid of checklists and catalogues (D'Abrera 1986; Kitching & Cadiou 2000; Martin et al. 2011) and also using specimens previously identified and deposited in the Lepidoptera Collection of the MZUSP. In some cases, however, identification was not possible by these means; the specimens were then dissected to assess the structure of the genitalia, which in Lepidoptera is a valuable source of diagnostic and distinctive characters between closely related species (Hall & Harvey 2002; Willmott 2003; Moraes & Duarte 2009). Voucher specimens are deposited at MZUSP.

DATA ANALYSIS

Mean temperatures of the sampling nights per month were graphically compared with the monthly abundance and species richness of the hawkmoths. The software EstimateS, version 9.1 (Colwell 2013) was used

to construct a sample-based rarefaction curve (100 randomizations without replacement) (Gotelli & Colwell 2001) and to apply 4 different richness estimators (Jackknife 1, Jackknife 2, ICE and Chao 2), which are considered appropriate for studies with small-sized samples and with relatively low sampling effort (Hellmann & Fowler 1999; Hortal et al. 2006). Two individual-based rarefaction curves (Gotelli & Colwell 2001), one with data from the present study and another with data from a sampling effort of 27 consecutive months, conducted between 1948 and 1950 with light traps at the BBS (Travassos & Camargo 1958; the specimens are available at MZUSP), were also constructed with the aid of EstimateS and compared to check for differences in species richness between the Sphingidae assemblages of these 2 localities of the Atlantic Rainforest in southeastern Brazil. Rank/abundance plots (Magurran 2004) were also constructed for both assemblages, which allowed comparisons of richness and evenness. For comparisons with Boraceia in terms of species composition and relative abundance, we used the results presented by Duarte et al. (2008), with the complete data set from 1940 to 2004.

Results

In total, 462 individuals were collected, representing 3 subfamilies, 5 tribes, 18 genera and 39 species (Figs. 2 and 3; Table 1).

The most abundant subfamily was Macroglossinae, represented by 302 individuals (65% of the hawkmoths collected), followed by Sphinginae with 106 individuals (23%) and Smerinthinae with 54 individuals (12%). With respect to richness, Macroglossinae was the most numerous group, with 27 species (71% of the species collected), followed by Sphinginae with 7 species (16%) and Smerinthinae with 5 species (13%).

The predominance of Macroglossinae was practically constant in the samplings. Regarding species richness (Fig. 2A), Smerinthinae and Sphinginae showed very similar fluctuation patterns throughout the months, and none of them exceeded the number of Macroglossinae in any field trip. This subfamily was the only one recorded in the samples from May and June. The fluctuation of the subfamilies in terms of relative abundance (Fig. 2B) varied more widely during the months of sampling. In Jul, a subfamily different from Macroglossinae (i.e. Smerinthinae) was the most abundant.

The most abundant tribe was Dilophonotini with 219 specimens (47%), followed by Sphingini with 106 (23%), Macroglossini with 74 (16%), Ambulycini with 54 (12%) and Philampelini with 9 (2%). Dilophonotini was the best-represented tribe with 9 genera and 15 species, followed by Macroglossini with 1 genus and 9 species, Sphingini with 3 genera and 7 species, Ambulycini with 3 genera and 5 species, and Philampelini with 1 genus and 3 species.

Callionima Lucas was the most abundant genus, with 183 individuals (40%), followed by Xylophanes Hübner with 74 (16%). The least abundant genera were Erinnyis Hübner, Orecta Rothschild & Jordan, Pachylia Walker and Pseudosphinx Burmeister, each with a single individual and comprising less than 0.2% of the collected specimens. Xylophanes, the genus with the highest richness, was represented by 9 species (24% of the species collected). Nine genera (Erinnyis, Hemeroplanes Hübner, Neococytius Hodges, Orecta, Pachylia, Pachylioides Hodges, Perigonia Herrich-Schäffer, Protambulyx Rothschild & Jordan and Pseudosphinx) were represented by only 1 species each. The abundance and richness of all genera are shown in Fig. 2.

Our data showed that the most abundant species at the SJBR is *Callionima parce* (Fabricius), which comprised 33% of the individuals collected, followed by *Cocytius lucifer* Rothschild & Jordan with 9% and *Manduca florestan* (Cramer) with 8%. Fourteen species were represented by a single collected specimen. One of them, *Orecta I. lycidas* (Boisduval), which has seldom been collected in other areas of rainforest, was recorded at the SJBR for the first time.

A clear relationship was apparent between the monthly mean temperatures and sphingid richness (Fig. 4A). The decrease in the mean

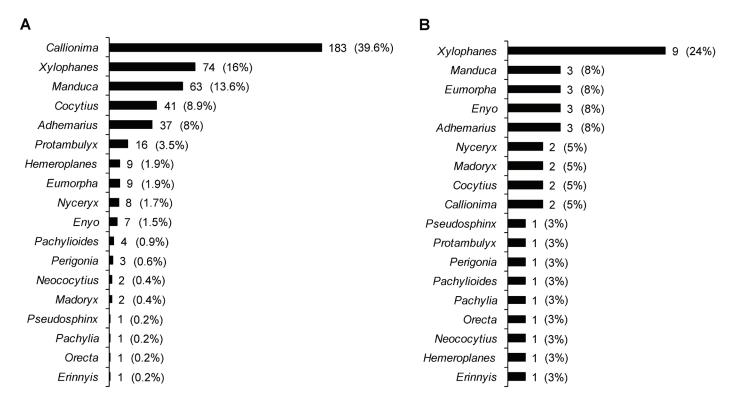
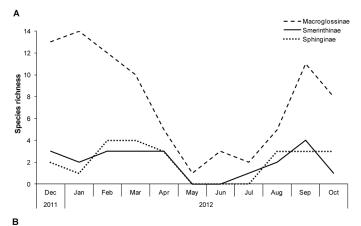


Fig. 2. Absolute and relative abundance (A) and richness (B) of the genera collected.



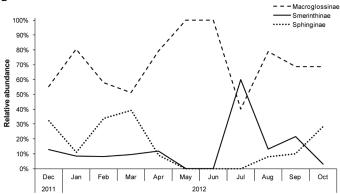


Fig. 3. Fluctuation of richness (A) and relative abundance (B) of the subfamilies during the sampling period.

temperatures from Mar to May was accompanied by a large decrease in the number of collected species; by Jul-Aug, the richness started to increase with the rising temperatures. Although a similar relationship is apparent between abundance and temperature (Fig. 4B), it is less clear. Despite the high mean temperatures in summer, the abundance levels were considerably lower in Jan and Feb.

Jackknife 1, Jackknife 2, ICE and Chao 2, respectively, estimated the existence of 53.6, 65.2, 54.3 and 75.4 species at the SJBR, in contrast to the 39 species that were actually collected. All the values provided by the estimators are above the upper bound of the 95% confidence interval obtained for the sample-based rarefaction curve (Fig. 5), strongly suggesting the existence of hawkmoth species that were not sampled in this study. The comparison between the individual-based rarefaction curves for the hawkmoth assemblages of Serra do Japi and Boraceia (Fig. 6) indicated significantly higher species richness in the latter locality. At the mark of 462 individuals (total abundance obtained at SJBR), the assemblage of the Serra do Japi included 39 ± 8.3 hawkmoth species, while the assemblage of Boraceia showed a species richness of 56.9 ± 5.4 .

Finally, marked differences were observed between the rank/abundance plots of the 2 assemblages (Fig. 7). The curve for the SJBR was shorter, as a result of the lower species richness, and more steeply sloping, indicating intense dominance, in terms of abundance, of a single species (i.e., *Callionima parce*) over the others.

Discussion

Besides the results from the richness estimators (Fig. 5), which suggest that the hawkmoth species composition of the SJBR was not fully sampled, another evidence for this possibility comes from the fact

that 36% of the collected species (14 out of 39) were represented by only a single specimen. This is a very high proportion, as clearly demonstrated by comparison with other standardized sphingid surveys in the Brazilian Atlantic Rainforest, e.g., by Coelho et al. (1979), Laroca & Mielke (1975), Duarte & Schlindwein (2008), Primo et al. (2013) and Cruz-Neto et al. (2011), which obtained respectively 0%, 13%, 16%, 26% and 29% of species represented by a single individual. Moreover, a simple visual inspection of Fig. 5 suggests that the rarefaction curve is not stabilized, which is additional evidence in favor of the existence of still-uncollected sphingid species at Serra do Japi.

The comparison between the individual-based rarefaction curves for the hawkmoth assemblages of the Serra do Japi and Boraceia showed that, for an equal number of individuals, the latter locality showed significantly greater species richness (Fig. 6). As previously discussed, however, the species composition of the Serra do Japi was probably not fully sampled, and estimates of its richness are expected to increase with additional field trips. New samplings would be especially profitable if performed in the warmest months, when richness is likely to be substantially higher (Fig. 4A).

Another important difference between the 2 assemblages is shown in Fig. 7. The hawkmoth community of Boraceia showed greater evenness, while in the Serra do Japi one species was strongly predominant over the others. In fact, *C. parce* comprised exactly 1/3 of the individuals collected in the Serra do Japi, whereas in Boraceia the most common species (*Xylophanes thyelia* (Linnaeus)) had a lower (10%) relative abundance.

In both surveys, the 3 tribes with the highest levels of abundance and richness were the same. However, the hierarchical order for these tribes was different. For the Serra do Japi, Dilophonotini was the most abundant; while for Boraceia, Macroglossini was the most abundant. This resulted from the fact that in the Serra do Japi the genus *Callionima* was the largest contributor to the abundance index, with 40% of the collected individuals; in Boraceia, on the other hand, a lower proportion of 4% represented the same genus. In Boraceia the genus that contributed most was *Xylophanes*, with 29% of the individuals, while in Serra do Japi this genus had a relative abundance of 16%.

Dilophonotini was the richest tribe in both localities. However, in Serra do Japi the second-richest tribe was Macroglossini, followed by Sphingini, whereas in Boraceia the inverse relationship was found, with Sphingini as the second-richest tribe, followed by Macroglossini. These results can be explained mainly by the difference in the sample of genera and species of Sphingini between the 2 localities. For example, only 4 species of *Manduca* were collected at the SJBR, while 11 species of this genus were recorded at the BBS. Furthermore, 2 genera recorded for Boraceia (*Amphimoea* Rothschild & Jordan and *Lintneria* Butler) have not been collected in Serra do Japi.

With regard to species composition, one last particularity of the sphingid assemblage of Serra do Japi is noteworthy. Among the 462 hawkmoths collected, the genus *Erinnyis* was represented by a single individual of *E. alope* (Drury). This is a surprising result, because this genus is frequently one of the commonest and richest found in surveys in the Atlantic Rainforest, especially in open degraded environments where host plants from the family Euphorbiaceae are usually abundant (these plants are considered pioneers growing after deforestation; 10 species are know to occur in SJBR – see Leitão-Filho 1992). In fact, *Erinnyis* was by far the most abundant genus in the studies of Laroca & Mielke (1975), Coelho et al. (1979) and Duarte & Schlindwein (2008), and was represented by 5, 3 and 5 species, respectively. In Boraceia it was the third most abundant genus (12% of the individuals collected) and was represented by 6 species.

Taking into account that the Atlantic Rainforest originally covered around 1.5 million km² (150 million ha; Ribeiro et al. 2009), the SJBR

Table 1. Sphingidae species collected at the Serra do Japi Biological Reserve, separated by subfamily and tribe, with number of individuals and months in which each species was collected.

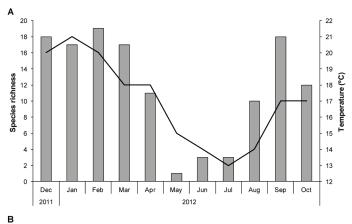
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Xylophanes titana (Druce, 1878)	17	Χ	Χ	Χ	Χ					Χ	X 2	Χ
	1		Χ									
Yulonhanes yulohates (Rurmeister 1979)	14	Χ			Χ				Χ	Χ	Χ	
Aylobotes (Bullicister, 1070)	9		Χ		Χ	Χ		Χ			Χ	
Macroglossinae: Philampelini												
Eumorpha analis (Rothschild & Jordan, 1903	3) 7		Χ	Χ							X X	Χ
Eumorpha anchemolus (Cramer, 1779)	1											Х
Eumorpha labruscae labruscae (Linnaeus, 1758)	1		Χ									
Smerinthinae: Ambulycini												
Adhemarius daphne daphne (Boisduval, [1875])	3										Χ	
Adhemarius eurysthenes (R. Felder, [1874])	20	Х			Х	Χ			Χ	Χ		Х
Adhemarius gannascus (Stoll, 1790)	14	Х	Х	Χ	Х	Х					Х	
Orecta lycidas lycidas (Boisduval, [1875])	1			Х								
Protambulyx strigilis (Linnaeus, 1771)	16	Χ	Χ	Х	Χ	Χ				Χ	Χ	
Sphinginae: Sphingini												
Cocytius antaeus (Drury, 1773)	1									Х		
Cocytius lucifer Rothschild & Jordan, 1903				Х	Χ	Χ					X X	Χ
Manduca brasiliensis (Jordan, 1911)	11			Х	Х	Х						Х
Manduca diffissa petuniae (Boisduval, [1875])	12	Х		Х	Х						,	•
Manduca florestan (Stoll, 1782)	38	Х	Х		Х	Χ					X X	х
Manduca janira (Jordan, 1911)	2		^	Х	^						,	•
Neococytius cluentius (Cramer, 1775)	2									Х	X	

and the BBS, which are separated by less than 120 km, may be considered very close localities within the biome for comparisons of the fauna of migrant organisms (e.g., most sphingid moths). Nevertheless, the hawkmoth assemblages differed markedly in terms of richness, species composition and relative abundance of the subgroups, as discussed above. These differences are probably related to environmental particularities of the localities, such as climate and phytophysiognomy.

The Serra do Japi is one of the few remnants of the Atlantic Rainforest in the interior of the state of São Paulo (Morellato 1992), and is located 100 km from the Atlantic coast. Its vegetation is affected by the

montane relief and shows heterogeneous physiognomy. In the lower parts of the Serra do Japi, up to approximately 870 m above sea level, the trees are tall, large and well spaced, with typical characteristics of rainforest. In the upper parts, at altitudes around 1,100 m, the vegetation presents a very distinct physiognomy, with shorter and smaller, densely packed sclerophyllous trees, typical of semi-deciduous high-altitude forests (Cardoso-Leite et al. 2005).

The BBS, on the other hand, is situated only 12 km from the coast, on the crest of the Serra do Mar, in a large fragment of the Atlantic Rainforest that extends along the coast of the state of São Paulo.



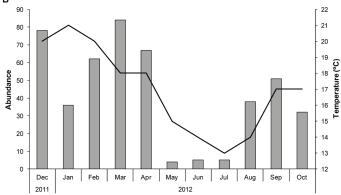


Fig. 4. Sphingidae richness (A) and abundance (B) by month of sampling. Line represents monthly mean temperatures.

Boraceia is among the wettest areas in Brazil, due to the frequent winds from the ocean; despite the low altitude (850 m), its vegetation is cloud forest, with very high humidity and frequent fog (Heyer et al. 1990). These authors described the phytophysiognomy of Boraceia as having a "low, continuous canopy", "one tree and one bush layer or stratum" not clearly marked, tree trunks usually very bent and branched, and an enormous abundance of mosses, lichens and epiphytes.

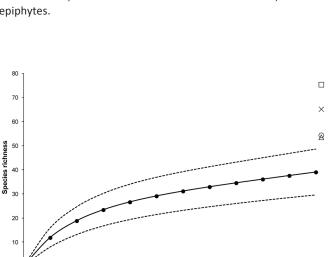


Fig. 5. Sample-based rarefaction curve with 95% confidence interval and results of the richness estimators (triangle: Jackknife 1; X: Jackknife 2; open circle: ICE; square: Chao 2).

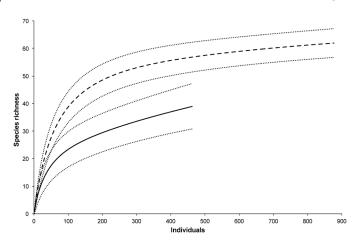


Fig. 6. Individual-based rarefaction curves, with 95% confidence intervals, of the samplings at the Serra do Japi Biological Reserve (this study; solid line) and at the Boraceia Biological Station (Duarte et al. 2008; dashed line).

Despite the closeness of the 2 localities, the SJBR and the BBS have relatively little in common in terms of climate and vegetation. The higher humidity, particularities of vegetation and the situation of Boraceia within the largest remnant of Atlantic Rainforest (Ribeiro et al. 2009), presumably providing more protection for its species, are probably determinant factors for the higher species richness. Though the sphingid richness of Serra do Japi is very likely to increase with additional sampling effort, it seems very improbable that it will achieve such a high richness as the 75 hawkmoth species recorded at the BBS from 1940 to 2004 (Duarte et al. 2008).

Regardless of the isolation and the small size of the forest fragment in which the SJBR is located, our results can be considered positive indicators of the state of conservation of the area. The richness of 39 species obtained in this study represents 17% of the Brazilian sphingofauna and includes some species that seldom appear in inventories of hawkmoths in southern and southeastern Brazil (e.g., Hemeroplanes longistriga (Rothschild & Jordan), Madoryx bubastus (Cramer), Orecta lycidas lycidas (Boisduval)), emphasizing the importance of Serra do Japi to the fauna of Lepidoptera of the Atlantic Rainforest.

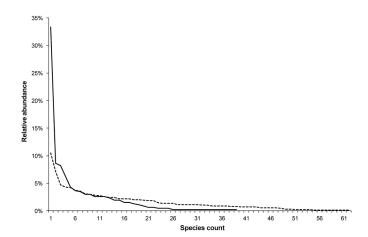


Fig. 7. Rank/abundance plots of the samplings at the Serra do Japi Biological Reserve (this study; solid line) and at the Boraceia Biological Station (Duarte et al. 2008; dashed line).

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