

Host morphophysiological conditions and environment abiotic factors correlate with bat flies (*Streblidae*) prevalence and intensity in *Artibeus* Leach, 1821 (*Phyllostomidae*)

Correlação entre as condições morfofisiológicas do hospedeiro e fatores abióticos ambientais com a prevalência e intensidade de moscas (*Streblidae*) em *Artibeus* Leach, 1821 (*Phyllostomidae*)

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ABSTRACT

The aim of this study was to correlate *Streblidae* parasitism rates with temperature and humidity as well as sex, age and reproductive condition of *Artibeus* bats. *Streblidae* specimens were collected during two years in the Tinguá Biological Reserve and in two areas inside buffer zone, located in Nova Iguaçu, Rio de Janeiro State and preserved as wet specimens. The abundance of *Streblidae* species parasitizing *Artibeus* was analyzed, and no differences were found between them. However, the number of females parasitizing *Artibeus fimbriatus* was higher. Moreover, regarding sex, *Artibeus* females were more parasitized, particularly *A. fimbriatus* and *A. lituratus*. There was no correlation between mean intensity of infestation and body mass index of *Artibeus* species, even when correlated to abiotic data. This study contributes to better understand the parasitism on *Artibeus* by *Streblidae*, and based on results, it is clear that *Streblidae* show no preferences in terms of reproductive condition, body size, age, sex, temperature and humidity in parasitizing *Artibeus* species.

Key words: parasitism, bat flies, *Paratrichobius longicrus*, bats.

RESUMO

O objetivo deste estudo foi de correlacionar as taxas de parasitismo de *Streblidae* com a temperatura e umidade, bem como sexo, idade e condução reprodutiva dos morcegos *Artibeus*. Os espécimes de *Streblidae* foram coletados durante dois anos na Reserva Biológica do Tinguá e em duas áreas dentro da zona de amortecimento, localizadas em Nova Iguaçu, estado do Rio de Janeiro, e preservados em coleção úmida. A abundância de espécies de *Streblidae* que parasitaram *Artibeus* foi analisada e não foram encontradas diferenças entre elas. No entanto, o número de fêmeas que parasitaram *Artibeus fimbriatus* foi maior. Além disso, em relação ao sexo, as fêmeas de *Artibeus* foram mais parasitadas, principalmente, *A. fimbriatus* e *A. lituratus*. Não houve correlação

entre a intensidade média de infestação e o índice de corpóreo das espécies de *Artibeus*, mesmo quando correlacionados com dados abióticos. Este estudo contribui para uma melhor compreensão do parasitismo em *Artibeus* por *Streblidae* e, com base nos resultados, as moscas *Streblidae* não demonstraram preferências em termos de condição reprodutiva, tamanho corporal, idade, sexo, temperatura e umidade ao parasitar as espécies de *Artibeus*.

Palavras-chave: parasitismo, streblídeos, *Paratrichobius longicrus*, morcegos.

INTRODUCTION

Among bat ectoparasites, *Streblidae* (Diptera) stand out. Exclusive to bats, these flies establish a relationship of dependency with their hosts considering their blood-sucking habit and the fact that a large part of their life cycle occurs on these bats (OVERAL, 1980). Bats can harbor more than one *Streblidae* species, which generally are found on the body and/or wing membranes (LINHARES & KOMENO, 2000).

Many aspects can influence the abundance of *Streblidae*. Among the factors linked to the host are type of refuge, behavior, condition stage and body size (LINHARES & KOMENO, 2000), while temperature and humidity are abiotic attributes that can also affect the population of these parasites (RUI & GRACIOLLI, 2005; ESBÉRARD et al., 2012). In the Neotropics, parasitism by *Streblidae* on *Phyllostomidae* bats is well known. Nevertheless,

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there are still few studies about the host-parasite relationship between *Artibeus* and *Streblidae* species (GRACIOLLI & DICK, 2004).

In order to complement knowledge about how host or environmental factors can influence the relationship between *Streblidae* flies and *Artibeus* bats, it was analyzed data on sex, body size, reproductive condition and host age, as well as environmental temperature and humidity.

MATERIALS AND METHODS

Streblidae flies came from a previous study about bat ectoparasites conducted in Tinguá Biological Reserve and two areas inside buffer zone, in Nova Iguaçu, state of Rio de Janeiro, Brazil. The database (Microsoft Excel) built and partly published by LOURENÇO et al. (2014) was used to obtain data related to bat captures (number of bats and temperature).

Records on bat specimens included species name, capture location, morphometric data, reproductive condition and sex. Records also contained the host classification by age (infant, young or adult), and reproductive status (males with existing or abdominal scrotum; sexually inactive females, with palpable fetuses or secreting nipples) (COSTA et al., 2007).

Streblidae flies collected were stored in a wet collection (70% ethanol), organized in jars containing identification of the host species and date of each capture. They were identified, counted and sexed with the aid of a stereoscopic microscope. The identification was made using a dichotomous key (GRACIOLI & CARVALHO, 2001).

The abundance (number of specimens registered) of each sex of *Streblidae* on the host species and the abundance and prevalence (number of parasitized hosts divided by the total number of hosts) of the *Streblidae* species parasitizing males and females of different *Artibeus* species were compared using the Chi-square test with degree of freedom of one, with the R program (R DEVELOPMENT CORE TEAM, 2014). The mean intensity of *Streblidae* (number of *Streblidae* specimens divided by the number of parasitized bats) was compared with sex of *Artibeus* using the Mann-Whitney test with the Past 3.0 program (HAMMER et al., 2006).

The body mass index (BMI), resulting from the division of host weight by the length of forearm, was calculated to correlate parasite load of *Streblidae* with the host size.

The average minimum and maximum temperature and relative humidity were correlated with the mean intensity of *Streblidae* infestation

found in different *Artibeus* species by Spearman's r_s with the Past 3.0 program (HAMMER et al., 2006).

RESULTS

Five species of *Streblidae* - *Megistopoda aranea* (Coquillett, 1899), *Paratrichobius longicrus* (Miranda Ribeiro, 1907), *Aspidoptera falcata* Wenzel, 1976, *Aspidoptera phyllostomatis* (Perty, 1833) and *Metelasmus pseudopterus* Coquillett, 1907 - and four *Artibeus*, Leach, 1821 bat species - *Artibeus fimbriatus* Gray, 1838 *Artibeus lituratus* (Olfers, 1818), *Artibeus obscurus* (Schinz, 1821) and *Artibeus planirostris* (Spix, 1823) were correlated (Table 1).

Regarding the abundance of *Streblidae* males and females on host species, there was a higher number of female parasites (N=50) than males (N=27) only on *A. fimbriatus* ($\chi^2= 6.87$; P=0.008) (Table 1).

When considering hosts sex, the total number of females parasitized by flies (61.25%) ($\chi^2= 11.25$; P=0.0007) was higher than males, especially on *A. fimbriatus* ($\chi^2= 8.24$; P=0.003) and *A. lituratus* ($\chi^2=36.5$; P=0.000) (Table 2). The abundance of a few flies was too low to calculate or compare their prevalence and mean intensities. *Megistopoda aranea* and *A. phyllostomatis* were more prevalent on male *A. fimbriatus* specimens ($\delta P=30.00\%$; $\text{♀}P=20.75\%$; $\chi^2= 41.20$; P<0.005) and ($\delta P=30.00\%$; $\text{♀}P=26.42$; $\chi^2=19.6$; P<0.005), respectively. In contrast, *P. longicrus* was more prevalent on females of *A. lituratus* ($\delta P=24.05\%$; $\text{♀}P=38.43$; $\chi^2=45.11$; P<0.005).

The highest mean infestation intensities of *A. phyllostomatis* and *P. longicrus* were observed on female *A. fimbriatus* ($\delta MI=1.57$; $\text{♀}MI=1.68$; U=10; P=0.001) and *A. lituratus* ($\delta MI=1.71$; $\text{♀}MI=2$; U=416; P=0.005) respectively. There was no difference between *A. fimbriatus* sex and mean intensity of infestation by *M. aranea* ($\delta MI=1.12$; $\text{♀}MI=1.83$; U=30; P=0.15).

Average intensities of *Streblidae* were compared between the hosts considering their reproductive condition. The highest values were observed for *M. aranea* parasitizing *A. lituratus* inactive females (MI=4.0). The second highest values were noted for *A. phyllostomatis* parasitizing *A. obscurus* post-lactation females (MI=3.0), followed by *P. longicrus* parasitizing *A. lituratus* (MI=2.1) and *A. planirostris* (MI=2.0) males with existing scrotum (Table 3). Concerning flies' preference according to host age, the highest mean parasitism intensity was observed for *P. longicrus* (MI=2.7) on young individuals of *A. lituratus* (Table 3).

No relationship was found between the abundance of *Streblidae* and BMI when considering

Table 1 - The abundance of *Streblidae* of sexes associated with species *Artibeus* in the region of Tinguá, Nova Iguaçu, state of Rio de Janeiro.

<i>Streblidae</i>	----- <i>A. fimbriatus</i> -----					----- <i>A. lituratus</i> -----				
	♀	♂	i	χ ²	P	♀	♂	i	χ ²	P
<i>M. aranea</i>	17.00	10.00	2.00	1.81	0.17	3.00	6.00	0.00	-	-
<i>M. proxima</i>	0.00	0.00	0.00	-	-	1.00	0.00	0.00	-	-
<i>P. longicrus</i>	0.00	1.00	1.00	-	-	109.00	132.00	23.00	2.19	0.13
<i>A. phyllostomatis</i>	23.00	15.00	0.00	1.68	0.19	4.00	1.00	0.00	1.80	0.17
<i>Me. pseudopterus</i>	4.00	1.00	0.00	-	-	3.00	0.00	0.00	-	-
Total	50.00	27.00	3.00	6.81	0.01*	121.00	139.00	23.00	1.20	0.26
<i>Streblidae</i>	----- <i>A. obscurus</i> -----					----- <i>A. planirostris</i> -----				
	♀	♂	i	χ ²	P	♀	♂	i	χ ²	P
<i>M. aranea</i>	4.00	0.00	1.00	-	-	4.00	0.00	2.00	-	-
<i>M. proxima</i>	0.00	0.00	0.00	-	-	0.00	0.00	0.00	-	-
<i>P. longicrus</i>	0.00	0.00	0.00	-	-	0.00	2.00	0.00	-	-
<i>A. phyllostomatis</i>	3.00	2.00	0.00	0.20	0.65	7.00	2.00	0.00	2.77	0.09
<i>Me. pseudopterus</i>	1.00	0.00	0.00	-	-	0.00	0.00	0.00	-	-
Total	8.00	2.00	1.00	3.60	0.05*	12.00	5.00	2.00	2.80	0.08

(♀- female; ♂- male; i- undetermined), *There was a significant difference.

all individuals of *Artibeus* (N=188; R=-0.02; P=0.74), even when considering *A. fimbriatus* (N=36; R=0.16; P=0.33), *A. lituratus* (N=132; R=0.002; P=0.75), *A. obscurus* (N=5; R=0.78; P=0.2) and *A. planirostris* (N=15; R=0.4; P=0.2). Also, no correlation was found between the abundance of *Streblidae* and relative humidity (N=50; R=-0.16584; P=0.2487), minimum temperature (N=50; R=-0.16584; P=0.2487), mean temperature (N=48; R=-0.2432; P=0.095) and maximum temperature (N=48; R=0.2485; P=0.093).

DISCUSSION

Data about the sex of *Streblidae* species that parasitize *Artibeus* in Atlantic Forest fragments in Rio de Janeiro were added by this study. We did not expect a higher abundance of *Streblidae* females parasitizing *A. fimbriatus* since previous reports in the literature suggest a greater abundance of males (LINHARES & KOMENO, 2000; RUI & GRACIOLLI, 2005; BERTOLA et al., 2005; DICK & PATERSON, 2008).

Table 2 - The abundance of *Artibeus* sexes of parasitized by *Streblidae* in the region of Tinguá, Nova Iguaçu, Rio de Janeiro.

<i>Streblidae</i>	----- <i>A. fimbriatus</i> -----				----- <i>A. lituratus</i> -----			
	♀	♂	χ ²	P	♀	♂	χ ²	P
<i>M. aranea</i>	12.00	6.00	2.00	0.15	2.00	1.00	0.00	0.00
<i>P. longicrus</i>	1.00	1.00	-	-	97.00	33.00	31.50	0.00*
<i>A. phyllostomatis</i>	15.00	7.00	2.90	0.08	3.00	1.00	-	-
<i>Me. pseudopterus</i>	3.00	1.00	-	-	3.00	0.00	-	-
Total	31.00	15.00	8.64	0.003*	105.00	35.00	36.05	0.00*
<i>Streblidae</i>	----- <i>A. obscurus</i> -----				----- <i>A. planirostris</i> -----			
	♀	♂	χ ²	P	♀	♂	χ ²	P
<i>M. aranea</i>	3.00	0.00	-	-	3.00	2.00	-	-
<i>P. longicrus</i>	0.00	0.00	-	-	0.00	2.00	-	-
<i>A. phyllostomatis</i>	3.00	1.00	-	-	3.00	1.00	-	-
<i>Me. pseudopterus</i>	0.00	1.00	-	-	0.00	0.00	-	-
Total	6.00	2.00	2.00	0.00	6.00	5.00	0.07	0.78

(♀-female; ♂- male; i- undetermined), *There was a significant difference.

Table 3 - Average intensity of Streblidae in different stages of development, gender and reproductive stages of *Artibeus*.

Host	Development Stage							
	JV	Male			Female			
Ectoparasite		TA	TE	IN	GV	LAC	PLAC	PL/GV
<i>A. fimbriatus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>M. aranea</i>	0.00	1.10	1.00	1.60	0.00	0.00	2.20	0.00
<i>P. longicrus</i>	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00
<i>A. phyllostomatis</i>	0.00	1.50	0.60	1.50	1.50	0.00	1.50	3.00
<i>Me. pseudopterus</i>	0.00	1.00	0.00	1.50	0.00	0.00	1.00	0.00
<i>A. lituratus</i>	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>M. aranea</i>	0.00	1.00	0.00	4.00	0.00	0.00	0.00	0.00
<i>M. proxima</i>	2.70	0.00	0.00	0.00	1.00	0.00	0.00	0.00
<i>P. longicrus</i>	0.00	1.50	2.10	2.30	2.30	2.00	1.90	1.00
<i>A. phyllostomatis</i>	1.00	1.00	0.00	1.50	0.00	0.00	0.00	1.00
<i>Me. pseudopterus</i>	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
<i>A. obscurus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>M. aranea</i>	0.00	0.00	0.00	2.00	0.00	0.00	1.00	0.00
<i>A. phyllostomatis</i>	0.00	0.00	1.00	1.00	0.00	0.00	2.00	0.00
<i>Me. pseudopterus</i>	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
<i>A. planirostris</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>M. aranea</i>	0.00	1.00	1.00	1.00	0.00	0.00	1.50	0.00
<i>P. longicrus</i>	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00
<i>A. phyllostomatis</i>	0.00	1.00	0.00	2.50	0.00	0.00	3.00	0.00

TA- Abdominal scrotum, TE- testicle scrotum, IN- Inactive, GV- Pregnant, LAC- Lactating, PLAC- Post- Lactating e PL/GV- Post-Lactating and Pregnant.

The predominance of *Streblidae* males is explained by the fact they remain on the host body throughout their life cycle, including during mating (OVERAL, 1980), while females leave the host for larviposition. According to Fritz (1983), larviposition occurs mainly during foraging, when most capture methods are applied, which explains the greater number of male parasites (DITTMAR et al., 2009). When captures are directed to bat shelters, the probability of finding a greater number of *Streblidae* females on the host increases (DITTMAR et al., 2011), since males are more susceptible to predation by another host through grooming.

It is well known that in dioic animals the birth probability of males and females is equal. However, studies with some ectoparasites such as ticks, fleas, lice and flies have shown that females are more abundant than males (DICK & PETERSON, 2008). For *Streblidae* in Brazil, there are few studies that report a greater abundance of females (RUI & GRACIOLLI, 2005).

Preference of hematophagous arthropods according to host sex sometimes follows a pattern. For example, fleas have preference for male rodents (KOWALSKI et al., 2014; KIFFNER et al., 2014.).

This is explained by the fact that high testosterone rates of males during the breeding season reduce their immune response, and they become more susceptible to parasitism when compared to healthy females (ZUK & MCKEAN, 1996).

The *Streblidae* preference for female hosts have a behavioral explanation, since females spend more time in refuges due to parental care (ESBERÁRD et al., 2012). It should be recalled that larviposition, development of pupae and emergence of new *Streblidae* individuals occurs in the refuge.

Although young bats were only slightly parasitized by *Streblidae* (1.42% prevalence), it is known that young bats usually host a large number of parasites (RUI & GRACIOLLI, 2005). However, the mean intensity of *P. longicrus* parasitizing young specimens of *A. lituratus* was considered high. High parasite loads in young individuals may be an ectoparasite strategy for the dispersion of new colonies, as these individuals, especially males, will form new colonies (LINHARES & KOMENO, 2000; BERTOLA et al., 2005).

About *Streblidae* preference regarding reproductive condition of the host, while the highest mean intensity was inactive on females parasitized

by *M. aranea* on *A. lituratus* (MI=4.0), this result needs to be carefully analyzed due to the high worm burden of a single individual. Other studies report that inactive females are less parasitized, because they spend less time in refuges, and are more active than pregnant and lactating females, making them less attractive to ectoparasites (MUNOZ-ROMO, 2006; ESBERÁRD et al., 2012).

Lactating females have closer contact with their young, increasing the surface area for fly infestation, since both can share ectoparasites. Additionally, both spend more time in refuges (ESBERÁRD et al., 2012). Although RUI & GRACIOLLI (2005) concluded that there are no behavioral differences related to sex and age of the host that favor or impair infestation by *P. longicrus* of *A. lituratus*. BERTOLA et al. (2005) reported higher prevalence of *P. longicrus* on *A. lituratus* in adult females, especially post-lactating specimens, and the greatest abundance and mean intensity were on pregnant females, corroborating with data presented in this study.

Among reproductive condition of *Artibeus* species, males with existing scrotum had the highest parasite load. This result can be explained by the fact that these animals had contact with females at mating time. In addition, they spend less time grooming, which decreases ectoparasite load. Another factor that may contribute is the immunosuppressive effect exerted by high levels of testosterone, making them more susceptible to parasitism (KLEIN, 2004).

Although we did not observe any relationship between the *Streblidae* parasite load and *Artibeus* species' BMI, LINHARES & KOMENO (2000) reported smaller bats to be more parasitized. When analyzing the relationship between the *Streblidae* intensity and BMI of *A. planirostris*, the smallest species among the four analyzed, we noted a tendency for smaller animals to be more parasitized. *Artibeus planirostris* was parasitized preferably by *M. aranea* and *A. phyllostomatis*, which have specific feeding behaviors on different anatomical regions of the same host (LINHARES & KOMENO, 2000).

Although there was no correlation between *Streblidae* parasite load and abiotic factors (temperature and air humidity), on days with lower minimum and average temperatures and lower mean relative humidity, there was a trend of hosts being more parasitized. It is known that the temperature and relative humidity are important factors, since they directly affect the reproduction and mortality of ectoparasites (RUI & GRACIOLLI, 2005). These effects may occur because abiotic factors also directly

influence the hosts' behavior, causing more active warmer weather, as reported by MORRISON & McNAB, (1967). Due to host activity increase, they perform more often (MUÑOZ-ROMO, 2006) which reduce parasite load (GIORGI et al., 2001).

CONCLUSION

The parasitism rates by *Streblidae* were not influenced by morphophysiological factors such as BMI and the reproductive age of the host, only influenced by host sex, mainly at *A. fimbriatus* and *A. lituratus*. The abiotic factors showed no association with *Streblidae* parasitism in different species of *Artibeus*.

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