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Research article

Photoperiod Differences in Sand Fly (Diptera: Psychodidae) Species Richness and Abundance in Caves in Minas Gerais State, Brazil

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Abstract

Caves are unique habitats that are inhabited by a diverse and singular biota. Among these inhabitants are sand flies, which are of great epidemiological interest in the Neotropical region because they are vectors of *Leishmania*. The period of activity of these insects is usually crepuscular and nocturnal, but there are reports of diurnal activity of sand flies in caves. Thus, the aim of this study was to evaluate the periodicity of daily activity of sand flies in cave environments in the municipality of Pains, Minas Gerais. Sand flies were collected with light traps, which were operated for 5 consecutive days in the rainy season and in the dry season. Samples were collected every 12 h and separated between photophase and scotophase periods. In total, 1,777 sand flies of 23 species were collected. The most abundant species was *Lutzomyia renei* (Martins, Falcão, and Silva) (44%), followed by *Lutzomyia longipalpis* (Lutz and Neiva) (15%), *Evandromyia edwardsi* (Mangabeira) (11%), and *Micropygomyia quinquefer* (Costa Lima) (6%). The richness and abundance of total sand flies and the abundance of male and female sand flies in the aphotic zone of the caves did not differ between the photophase and scotophase, but differed between photoperiods at the entrance and at sites surrounding the caves. From our study of the daily activity of these insects in this ecotope, it will be possible to know which period of the day is of greatest risk of exposure of vertebrates who visit or live in these environments, including the human population.

Key words: aphotic zone, period of activity, photophase, scotophase, vector insect

Caves are stable environments, with little variation in the temperature and humidity of the interior microclimate, which is important for their ecosystems (Poulson and White 1969). In addition, cave environments lack light in areas distant from the entrance and present oligotrophic conditions (Trajano and Gnaspini-Netto 1991).

Among the animals that frequent cave environments are sand flies (Galati et al. 2003a, 2003b, 2003c; Alves et al. 2008; Carvalho et al. 2011, 2012, 2013; Polseela et al. 2011; De Oca-Aguilar et al. 2013; Saraiva et al. 2015). These insects are natural vectors of several microorganisms, especially protozoa of the genus *Leishmania* Ross (Forattini 1973, Young and Duncan 1994). Generally, sand flies are crepuscular and/or nocturnal, resting in natural shelters during the day, although some species may exhibit different patterns of daily activity under certain conditions (Teodoro et al. 2003, Young and Duncan 1994, Sherlock 2003).

In caves, some species of sand flies are active during the day (Carvalho et al. 2012). Some studies have shown that the period of activity of sand flies in the aphotic zones of caves, as well as in anthropic environments, is greater in the scotophase than in the photophase, indicating that they exhibit a nocturnal circadian rhythm (Guernaoui et al. 2006, Galati et al. 2010, Fuenzalida et al. 2011, Polseela et al. 2011). However, a study conducted in a cave in the municipality of Manaus, Amazonas, found different results, with the daily activity of a species of sand fly in the interior of the cave not differing between nocturnal and diurnal periods (Alves et al. 2011). Sand flies in caves may modulate their daily activity pattern in response to bats occupancy. Bats use caves as a shelter during the

day and leave during the night to search for food (Lampo et al. 2000) and may be a source of blood for sand flies.

Little is known of the behavior of sand flies in the caves. Knowledge of the daily activity of these insects in this ecotope will describe the period of the day when there is the greatest exposure of vertebrates who visit or live in these environments to blood-feeding sand flies, including the human population. Although the studied caves are not tourist attractions, the results found in our study may be extrapolated to tourist caves where there is a large flow of people and thus greater contact with these insects, which may be important for understanding the risk of transmission of *Leishmania*.

Therefore, the aim of this study was to determine the period of daily activity of sand flies in five cave environments in the municipality of Pains, state of Minas Gerais. We tested the hypothesis that cave interiors (aphotic zone) possess greater species richness and abundance of sand flies during the nocturnal period compared with the diurnal period. This hypothesis was based on the fact that the sand flies are nocturnal, and despite the constant darkness in the aphotic zone, they are expected to respond to a circadian cycle with a greater number of individuals and species searching for food during the nocturnal period.

Materials and Methods

Study Area

The municipality of Pains forms part of a complex that possesses a large number of natural caves (CECAV 2015). The caves used in this study are localized in limestone outcroppings, contain water bodies, and receive water from outside the cave from dripping, which can create temporary ponds and even cause flooding during the rainy season. This region has the phytogeographic features of "Mata de Pains" or Deciduous *Seasonal* Forest and Cerrado (Ururahy et al. 1983). The area suffers from anthropic disturbance from mining, calcining, and agriculture.

The study took place in five cave environments and their surroundings in the municipality of Pains, state of Minas Gerais, Brazil: Gruta Santuário (20° 25′14″ S and 45° 46′26″ W), which is located at an altitude of 708 m above sea level (a.s.l.); Gruta Brega (20° 25′05″ S and 45° 46′19″ W) – 712 m a.s.l.; Gruta Camilo (20° 20′46″ S and 45° 40′58″ W) – 702 m a.s.l.; Gruta Isaias (20° 22′05″ S and 45° 39′28″ W) – 732 m a.s.l.; and Gruta Morrinho das Pedras (20° 21′25″ S and 45° 39′53″ W) – 678 m a.s.l. Gruta Camilo, Gruta Isaias, and Gruta Morrinho das Pedras have an aphotic zone about 20 m distance interior from the entrance, whereas in Gruta Santuário and Gruta Brega, the aphotic zone is at 40 and 100 m, respectively. All the caves were named by Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV). In these caves, we observed vertebrates including lizards, snakes, rodents, and bats (Fig. 1).

Collection of Sand Flies

The collection of sand flies was performed under the license N°45636-1 of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis.

Sand flies were collected at each site using paired model HP automatic light traps (Pugedo et al. 2005), with one trap set from 0600 to 1800 h and a second trap set from 1800 to 0600 h. Traps were operated uninterruptedly for two consecutive 120-h periods over 5 d of sampling in the rainy season (November 2014) and in the dry season (April 2015), for a total of 240 h sampling effort for the two traps.

At each cave and its surroundings, pairs of traps were set at three sampling points: one located at the cave entrance (the photic zone), one in the interior of the cave (aphotic zone), and one in the surrounding area (about 10 m from the cave entrance), for a total of 15 trapping points at the five caves and their surroundings in each season. The traps were installed approximately 1 m above the ground in all cave environments.

After sampling, the traps were removed, the sand flies killed in glycerinated alcohol, sorted, and sexed. Males were then placed in test tubes containing 70% alcohol and females were placed in test tubes containing 6% DMSO.

Sand Fly Identification

Male specimens were mounted in Canada Balsam and identified following the classification of Galati (2003). For female identification, the head and the last three segments of the abdomen were separated and mounted in Berlese medium, with the head positioned ventrally. All specimens were deposited in the Coleção de Flebotomíneos of the Centro de Pesquisas René Rachou/Fiocruz (FIOCRUZ/COLFLEB).

Due to the morphological similarity of the genitalia of female sand flies of the genus *Brumptomyia* França and Parrot, and the occurrence of males of three species of this genus in the studied cave environments, species identification of females of this genus was difficult. Thus, males of *Brumptomyia* were identified to species and females only to genus (i.e., *Brumptomyia* sp.).

Statistical Analysis

The effect of sampling period (diurnal and nocturnal) on the sex, richness, and abundance of sand flies collected in the interior, entrance, and surroundings of the caves was determined using a generalized linear mixed model (GLMM; Zuur et al. 2009). In these models, the sums of the richness and abundance of sand flies and the sums of the abundance of females and of males of sand flies for the 5 consecutive days of sampling of the interior, entrance, and surrounding of each cave in each season were used as response variables, while the sampling period (diurnal and nocturnal) was used as an explanatory variable and the caves and year seasons (repeated measures) as a random variable. All models were performed using the software R 3.2.4 (R Development Core Team 2016), followed by residual analysis to choose the best data distribution, which for our data was the negative binomial distribution.

Results

In total, 1,777 phlebotomine sand flies were collected, including 489 (27.5%) males and 1,288 (72.5%) females, of 23 species belonging to 11 genera (Table 1). The most abundant species was *Lutzomyia renei* (Martins, Falcão and Silva) (44%), followed by *Lutzomyia longipalpis* (Lutz and Neiva) (15%), *Evandromyia edwardsi* (Mangabeira) (11%), and *Micropygomyia quinquefer* (Costa Lima) (6%). All other species together amounted to 24% of the total (Table 1).

Cave entrances yielded 838 (47%) individuals, with the most abundant species being *Lu. renei* (39%) and *Lu. longipalpis* (26%), whereas 635 (36%) individuals were collected from the cave interiors, with *Lu. renei* (68%) also being the most abundant species. In the cave surroundings, 304 (17%) individuals were collected, with *Mi. quinquefer* (18%) being the most abundant. All species recorded in the cave interiors and surroundings were also found at the

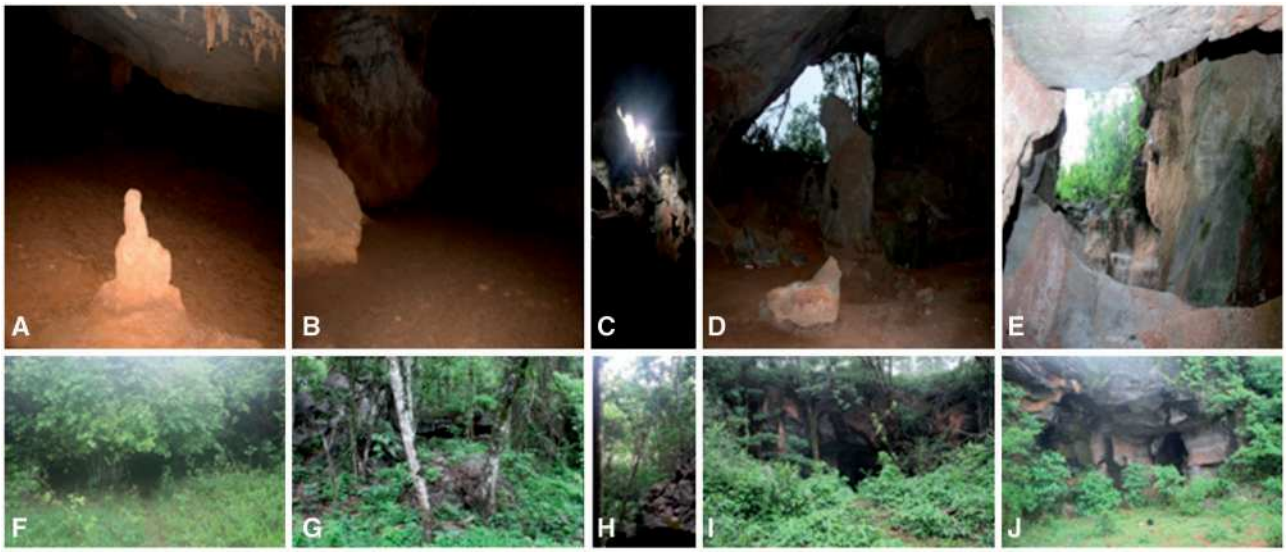

Fig. 1. The study caves and their surroundings in the municipality of Pains, Minas Gerais, Brazil. A and F, Gruta Santuário and surroundings; B and G, Gruta Brega and surroundings; C and H, Gruta Camilo and surroundings; D and I, Gruta Isaias and surroundings; and E and J, Gruta Morrinho das Pedras and surroundings.

Table 1. Phlebotomines collected from caves and their surroundings in the municipality of Pains-MG, in November 2014 and April 2015

Cave environments/Periods																						
Species	Entrance						Total	Interior						Total	Surroundings						Total	Total
	Diurnal		Total	Nocturnal		Total		Diurnal		Total	Nocturnal		Total		Diurnal		Total	Nocturnal		Total		
	♀	♂		♀	♂			♀	♂		♀	♂			♀	♂		♀	♂			
Brumptomyia avellari	–	1	1	–	1	1	2	–	1	1	–	3	3	4	–	–	–	–	16	16	16	22
Brumptomyia brumpti	–	–	–	–	2	2	2	–	–	–	–	3	3	3	–	–	–	–	14	14	14	19
Brumptomyia nitzulescui	–	–	–	–	7	7	7	–	–	–	–	1	1	1	–	–	–	–	1	1	1	9
Brumptomyia sp.	2	–	2	7	–	7	9	1	–	1	2	–	2	3	–	–	–	39	–	39	39	51
Evandromyia bacula	–	–	–	1	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Evandromyia cortelezzii	4	–	4	15	5	20	24	13	2	15	8	2	10	25	1	2	3	16	7	23	26	75
Evandromyia edwardsi	2	–	2	43	10	53	55	37		37	62	18	80	117	2	–	2	25	5	30	32	204
Evandromyia lenti	–	–	–	2	1	3	3	–	–	–	–	–	–	–	–	–	–	9	1	10	10	13
Evandromyia termitophila	–	–	–	1	–	1	1	–	1	1	–	–	–	1	–	–	–	2	1	3	3	5
Expapillata firmatoi	–	–	–	3	6	9	9	–	–	–	1	1	2	2	–	1	1	1	–	1	2	13
Lutzomyia longipalpis	2	3	5	49	168	217	222	2	13	15	4	6	10	25	–	–	–	9	21	30	30	277
Lutzomyia renei	159	8	167	155	8	163	330	250	19	269	132	17	149	418	3	–	3	27	5	32	35	783
Micropygomyia quinquefer	1	9	10	10	17	27	37	7	1	8	3	3	6	14	7	3	10	18	28	46	56	107
Migonemyia migonei	3	–	3	26	9	35	38	2	–	2	2	1	3	5	2	–	2	1	2	3	5	48
Nyssomyia neivai	–	–	–	1	–	1	1	–	–	–	–	1	1	1	–	–	–	3	2	5	5	7
Nyssomyia whitmani	–	–	–	1	–	1	1	–	–	–	–	–	–	–	–	–	–	4	1	5	5	6
Pintomyia christenseni	–	–	–	1	–	1	1	–	–	–	–	–	–	–	–	–	–	1	–	1	1	2
Pintomyia fischeri	–	–	–	–	1	1	1	–	–	–	–	–	–	–	–	–	–		1	1	1	2
Pintomyia monticola	1	–	1	6	–	6	7	–	–	–	–	–	–	–	–	–	–	6	1	7	7	14
Pintomyia pessoai	1	–	1	7	6	13	14	–	–	–	–	–	–	–	1	–	1	5	–	5	6	20
Psathyromyia aragaoi	–	–	–	–	1	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Psathyromyia lutziana	–	–	–	2	1	3	3	3	2	5	–	1	1	6	–	–	–	–	–	–	–	9
Sciopemyia microps	5	–	5	5	–	5	10	2	–	2	–	–	–	2	1	–	1	–	–	–	1	13
Sciopemyia sordellii	6	5	11	38	10	48	59	4	–	4	4	–	4	8	2	–	2	5	2	7	9	76
Total specimens per sex	186	26	**212**	373	253	**626**	**838**	321	39	**360**	218	57	**275**	**635**	19	6	**25**	171	108	**279**	**304**	**1,777**
Total species	11	5	**12**	19	16	**24**	**24**	10	7	**12**	9	12	**14**	**16**	8	3	**9**	16	16	**20**	**21**	**23**

entrance of the caves, but some species were exclusively found at the entrance.

During the diurnal period, 597 (34%) phlebotomine sand flies were collected, consisting of 360 (60%) individuals in the cave interiors, 212 (36%) in the cave entrances, and 25 (4%) in the cave surroundings. The most abundant species during the diurnal period was *Lu. renei* (73%). The nocturnal period yielded 1,180 (66%) individuals, with 275 (23%) in the cave interiors, 626 (53%) in the

cave entrances, and 279 (24%) in the cave surroundings, with *Lu. renei* (29%), *Lu. longipalpis* (22%), and *Ev. edwardsi* (11%) being the most abundant species in these three cave environments.

All species recorded during the diurnal period were also found during the nocturnal period, but some species such as *Brumptomyia brumpti* (Larrousse), *Brumptomyia nitzulescui* (Costa Lima), *Evandromyia bacula* (Martins, Falcão and Silva), *Psathyromyia aragaoi* (Costa Lima), *Evandromyia lenti* (Mangabeira), *Nyssomyia whitmani* (Antunes and Coutinho), *Nyssomyia neivai* (Pinto), *Pintomyia christenseni* (Young and Duncan), and *Pintomyia fischeri* (Pinto) were exclusively collected during the nocturnal period.

At cave entrances during the diurnal period, the most abundant species was *Lu. renei* (79%), whereas during the nocturnal period, *Lu. longipalpis* (35%) was most common, followed by *Lu. renei* (26%). In cave interiors (aphotic area), *Lu. renei* was the most abundant species during both the diurnal and nocturnal periods (75 and 54%, respectively). In cave surroundings, *Mi. quinquefer* was always the most abundant species (40% diurnal and 16% nocturnal).

Comparing the richness and abundance of sand flies between diurnal and nocturnal periods of the interior, entrance, and surrounding environments of the caves, we observed that the richness and abundance in the cave interiors did not differ between diurnal and nocturnal periods ($z = 1.19$, $P = 0.23$; $z = 1.13$, $P = 0.19$, respectively), but differed between diurnal and nocturnal periods in the entrance and surrounding of the caves, where there was a greater richness and abundance of sand flies during the nocturnal period (Abundance: $z = 3.30$, $P = 0.009$ and Richness: $z = 4.42$, $P < 0.001$; Abundance: $z = 5.19$, $P < 0.001$ and Richness: $z = 5.51$, $P < 0.001$, respectively; Fig. 2).

The abundance of female and male sand flies in the cave interiors did not differ between diurnal and nocturnal periods ($z = 1.46$, $P = 0.143$; $z = 1.93$, $P = 0.054$, respectively), while for entrance and surrounding of the caves, there were significant differences between the periods, with a greater abundance of females and males during the nocturnal period (Females: $z = 3.33$, $P = 0.008$ and $z = 4.49$, $P < 0.001$; Males: $z = 3.47$, $P = 0.0005$ and $z = 4.48$, $P < 0.001$, respectively; Fig. 2).

Discussion

The original hypothesis of this study was refuted, because we did not find a significant difference in richness and abundance of sand flies between nocturnal and diurnal periods in the aphotic region of

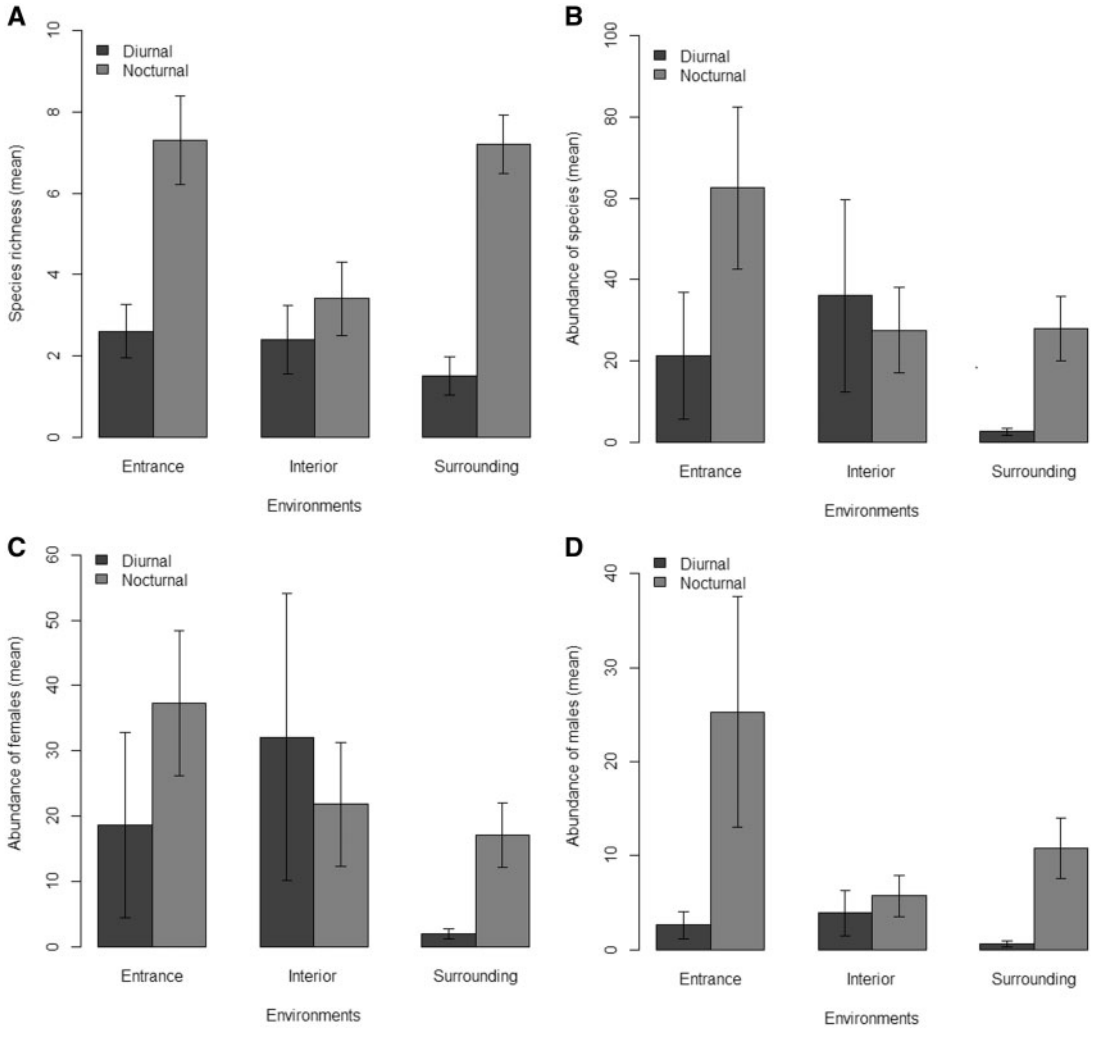

Fig. 2. Effect of sampling period (diurnal and nocturnal) on the sex, richness, and abundance of sand flies collected in the interior, entrance, and surrounding environments of the caves. A, Mean (± standard error) species richness of sand flies; B, mean (± standard error) abundance of species of sand flies; C, mean (± standard error) abundance of females of sand flies; D, mean (± standard error) abundance of males of sand flies.

the caves. This result indicated that in the cave interiors, these insects are active during both periods, which may be due to continuous resource availability. We also have not found a difference in the abundance of females and males between the photoperiods which would support that these insects are active during both periods.

Our data also suggest that in the cave interiors, entrances, and surrounding areas, sand flies exhibit differences in their activity rhythms due to the presence of sunlight that suppressed the flight activity of these insects which are mainly nocturnal.

The most abundant species in our study, *Lu. renei*, *Lu. longipalpis*, *Ev. edwardsi*, and *Mi. quinquefer*, are commonly found in cave environments (Galati et al. 2003c, 2010a; Carvalho et al. 2012, 2013). We observed that some species were collected only during the nocturnal period, some species only during the diurnal period, and some species in both periods. It is possible that these species use the caves as places for rest, protection, shelter, breeding, and feeding, as reported by Carvalho et al. (2013).

Some studies have reported similar results. Alves et al. (2011) did not find significant differences between diurnal and nocturnal periods in the number of individuals of the sand fly *Deanemyia maruaga* (Alves.Freitas & Barrett) inside a cave in the municipality of Manaus, Amazonas. Likewise, Carvalho et al. (2012) found a similar result for the distribution of total sand flies captured in the aphotic zone of a cave in the municipality of Lassance, Minas Gerais, but these authors detected peak of abundance at certain hours of both photophase and scotophase periods. Yet a study performed by Polseela et al. (2011) in caves in Thailand reported results that differed from our study, with a greater number of sand flies during the nocturnal period.

Our study also showed that all species present in the cave interiors and surroundings were also recorded at the cave entrance, which suggests that sand flies were circulating throughout these environments. A similar result was reported by Carvalho et al. (2013), who found that all the species recorded inside a cave in the municipality of Lassance, Minas Gerais, were also present in the surroundings.

One interesting and relevant finding of the present study was the high number of females captured in the caves (interior and entrance). This is significant because they feed on vertebrates and therefore can transmit *Leishmania*, the causative agent of leishmaniasis (Morrison et al. 1993, Colmenares et al. 1995, Killick-Kendrick 1999). This finding may be due to a greater opportunity for females to feed on the blood of the variety of species of vertebrates present in caves during the diurnal and nocturnal periods (Matavelli et al. 2015). Of the five caves sampled in our study, bats and rodents were captured during the nocturnal period at the entrance and interior of four of these caves (unpublished data) and probably during the diurnal period, these small mammals also used the caves as shelters. According to Gomes and Galati (1987), the predominance of females can also be explained by their attraction to both the light of traps and the availability of a blood supply near the traps.

The presence of species incriminated in *Leishmania* transmission, such as *Lu. longipalpis*, *Migonemyia migonei* (França), *Ny. neivai*, and *Ny. whitmani*, deserves special attention. The presence of these species inside and around the caves can be crucial to the sylvatic transmission cycle of *Leishmania* between reservoirs present in these environments and humans, who can become infected accidentally when visiting the caves.

Understanding the daily activity of sand flies in cave environments is important for determining the period of risk of *Leishmania* transmission between the sand flies and the vertebrate hosts who visit or live in these caves. All studied cave environments are close to human habitation, and therefore, there is the possibility of rural transmission cycle with spillover to humans in this region. The female sand flies feed on blood of many vertebrates (Brazil and Brazil 2003), including bats (Tesh et al. 1971, Christensen and Herrer 1980, Lampo et al. 2000). During the night, bats exit the caves in search of food, and many species are close to human habitations. In these habitations, the sand flies that have more nocturnal habits may become infected when they feed on these bats (De Lima et al. 2008, Savani et al. 2010, Shapiro et al. 2013) and may then transmit *Leishmania* to synanthropic animals, domestic (dog), and/or man in their next bloodmeal and, therefore, the *Leishmania* transmission cycle may be completed. The study of the habits of the sand flies, such as the present study, facilitates proper planning for human avoidance of contact with these insects in these environments.

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