Phlebotomines (Diptera, Psychodidae) in the Ribeira Valley Speleological Province – 1. Parque Estadual Intervales, state of São Paulo, Brazil

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ABSTRACT. Phlebotomines (Diptera, Psychodidae) in the Ribeira Valley Speleological Province - 1. Parque Estadual Intervales (PEI), São Paulo state, Brazil. The identification of the sandfly fauna and investigation of some ecological aspects of its populations in areas frequented by tourists of the PEI, an Atlantic forest reserve with many caves, were the objective of this study. Captures were undertaken monthly from January 2001 to December 2002, with automatic light traps installed in 13 ecotopes, including caves, forests, domiciliary and peridomiciliary environments, and by aspiration in armadillo burrows. Additionally, although not at regular intervals, Shannon traps were installed in forests and anthropic environments, aspirations were made on cave walls, among roots and fallen leaves, and some insects were captured while biting researchers. A total of 891 sandflies belonging to 21 species were captured. Six hundred specimens representing 19 species were captured with light traps, 215 in anthropic (2.24 insects/trap) and 385 in extra-domiciliary (1.46 insects/trap) environments. *Brumptomyia troglodytes* was the most abundant species (the Standardised Index of Species Abundance = 0.705). *Pintomyia monticola* predominated in the Shannon traps and showed anthropophilic and diurnal activity. *Psathyromyia pascalei* predominated in the aspirations; the largest number being in armadillo burrows. Eleven species were captured in caves; although some might be troglophiles, the majority used these ecotopes as resting places. *Nyssomyia intermedia, Nyssomyia neivai* and *Migonemyia migonei*, implicated in the transmission of cutaneous leishmaniasis in the Southeastern Brazilian region, were all found, though in such low densities as to suggest minimal risk of the disease in the PEI.

KEYWORDS. Atlantic forest; cave; leishmaniasis; vectors.

RESUMO. Phlebotominae (Diptera, Psychodidae) na Província Espeleológica do Vale do Ribeira - 1. Parque Estadual Intervales (PEI), estado de São Paulo, Brasil. A identificação da fauna flebotomínea e de alguns aspectos ecológicos de suas populações em áreas freqüentadas por turistas no PEI, situado em reserva de mata Atlântica, constituem-se nos objetivos deste estudo. As capturas foram mensais de janeiro/2001 a dezembro/2002, com armadilhas automáticas luminosas em 13 ecótopos, incluindo cavernas, matas e peridomicílio e aspiração em tocas de tatus. Sem intervalos regulares, foram realizadas capturas com armadilhas de Shannon na mata e ambientes antrópicos, aspiração em paredes de cavernas e entre raízes e folhedo e em membros da equipe quando picados por flebotomíneos. No total foram capturados 891 flebotomíneos pertencentes a 21 espécies. Com as armadilhas automáticas luminosas capturou-se 19 espécies e 600 espécimes, 215 em ambiente antrópico (2,24 insetos/armadilha) e 385 em ambiente natural (1,46 insetos/armadilha). *Brumptomyia troglodytes* foi a mais abundante, com o índice de abundância das espécies padronizado = 0,705. *Pintomyia monticola* predominou nas armadilhas de Shannon, mostrando-se antropofílica e com atividade hematofágica diurna e noturna. *Psathyromyia pascalei* predominou nas aspirações, com a maioria dos espécimes aspirados de tocas de tatu. Das 11 espécies capturadas em cavernas, embora algumas possam ser consideradas troglófilas, a maioria usa este ecótopo como local de abrigo. *Nyssomyia intermedia, Nyssomyia neivai* e *Migonemyia migonei*, implicados na transmissão da leishmaniose tegumentar na Região Sudeste do Brasil foram capturados, todavia em tão baixa densidade que sugere risco mínimo da doença no PEI.

PALAVRAS-CHAVES. Floresta Atlântica; caverna; leishmaniose; vetores.

One of the greatest concentrations of karst areas in the world, many of which offer conditions which favor the formation of caves, is found in Brazil (Auler 2002). The rock formations of the Açungui group, is the Speleological Province of the Ribeira Valley situated in southeastern Brazil, between the south of São Paulo and the north of Paraná states (Karmann & Ferrari 2002), being the best known Brazilian karst province from the biospeleological point of view (Trajano 1984). A great part of this province is situated on the Serra de Paranapiacaba, on the west bank of the Ribeira de Iguape River, mainly in two forest reserves: Parque Estadual do Alto Ribeira (PETAR), with more than 300 caves and the Parque Estadual Intervales (PEI) with about 50 caves (São Paulo 2008). This great concentration of caves and the extensive continuous remnant of Atlantic forest make this an important tourist attraction.

Among the inhabitants of caves and forests are the phlebotomines (Diptera, Psychodidae) (Aguiar & Medeiros 2003; Andrade Filho *et al*. 2001; Galati *et al*. 2003, 2006), the females of which are hematophagous, biting mammals, birds

and cold-blooded animals. Many species are anthropophilic and beyond bothering humans by their painful bites, which sometimes result in allergic manifestations, may be vectors of viruses, bacteria and Trypanosomatidae (Forattini 1973; Young & Duncan 1994). Among these latter, several species of the genus *Leishmania* are of particular interest as agents of leishmaniases which constitute a serious public heath problem due to the complexity of their clinical manifestations (visceral, muco-cutaneous, diffuse and cutaneous) and epidemiology. The leishmaniases occur in 88 countries with an estimated annual incidence of 1.5 million new cases of cutaneous forms and 500,000 new cases of the visceral form, with an overall prevalence of 12 million people infected and a total population of 350 million at risk worldwide. Brazil is among the five countries which together account for 90% of the visceral cases and the seven in which 90% of all cutaneous cases have been registered (Desjeux 2004).

The Ribeira Valley region, with 1,174 cases registered between 1998 and 2006 and an average annual coefficient of 47.7 new infections/100,000 inhabitants, has one of the highest incidences of human American cutaneous leishmaniasis cases in the State of São Paulo. The respective coefficients found in the municipalities in which the PETAR and the PEI are situated were, during this same period: Guapiara (1.6 new infections/100,000), Ribeirão Grande (2.74/100,000), Apiaí (5.6/100,000), Sete Barras (8.5/100,000), Eldorado (144.8/100,000) and Iporanga (152.9/100,000) (CVE 2007).

The phlebotomine fauna of the Ribeira Valley has been relatively well investigated and the behavior of the probable vectors of cutaneous leishmaniasis has been the most extensively studied, mainly in peridomiciliary and cultivated areas (Forattini *et al*. 1976; Gomes *et al*. 1980, 1982, 1983, 1986; Gomes & Galati 1987, 1989; Domingos *et al*. 1998; Taniguchi *et al*. 2002). However, no information relating to the phlebotomines in the Speleological Province of the Ribeira Valley has been found in the literature by these present authors, despite its having been investigated for a great number of organisms (Pinto-da-Rocha 1994; Trajano 2000). Certain human activities such as ecological tourism, research, mining, surveillance by forest wardens, colonization and others, undertaken in this province in forested and/or speleological areas, increase the chances of people's coming into contact with phlebotomine populations. So the objective adopted for this present project, in 2001 and 2002, was to identify the phlebotomine fauna and some aspects of its behavior in some of the caves situated in the PEI as well as in the forest and anthropic environments in the province, to investigate the exposure of the human population to the sandfly species in the area.

MATERIAL AND METHODS

Study area. The Parque Estadual Intervales (Fig. 1), situated between the Ribeira Valley and the southwest of São Paulo state (24º 12' – 24º 25' S and 48º 03' – 48º 30' W), occupies an area, at the present time, of 41,704 hectares, forming part of the Paranapiacaba massif. Together with three other reserves: the Estação Ecológica Xitué to the north, the Parque Estadual Carlos Botelho to the east, and the Parque Estadual do Alto Ribeira (PETAR) to the southwest, it forms a continuous protected forest with a total area of approximately 120,000 ha. Its altitude varies from 60 m in the extreme east to 1,095m on the northwestern limit (Carvalho *et al*. 2002).

The predominant climate is temperate without any dry season: Cfb according to Koeppen's International System (Köppen 1948). The annual average temperature varies from 17ºC – 19ºC, but the minimum may come close to zero in the coldest period (Gnaspini-Netto & Trajano 1992). The annual average rainfall is of approximately 1,600 mm in the upland areas (800 m) (SIGRH 2007), but in the lower areas may attain 4,000 mm (Morellato *et al*. 2000).

Atlantic rain forest, a transitional domain between dense ombrophilous mountain forest and mixed ombrophilous forest, constitutes the predominant vegetation. The dense ombrophilous submountain forest is present also in the lower areas.

The calcareous stratum, in which the approximately eighty known caves of the Parque Estadual Intervales are located, extends in a north-westerly direction to the PETAR. The area covered by these calcareous rocks may be divided, according to Gnaspini-Netto & Trajano (1992), into the following regions: Sede, Pedra do Fogo, Bocaina, Capoava, Figueira and Bulha d'Água (Fig. 1). In the present study three regions: Bocaina and Pedra do Fogo, about four km from each other, and Sede, very close to the latter, were sampled.

In the Bocaina region two caves: Minotauro and Barra Bonita, as well as the vegetation surrounding this latter, were sampled regularly. The Minotauro cave (24º 16' 2200" S, 48º 27' 2200" W, 890 m above sea level) presents a horizontal projection of 400 m, a discontinuous linear development of 560 m, a 25 m drop in level and a large entrance, and is accessible to tourists. The Barra Bonita cave (24º 16' 0300" S, 48º 27' 2400" W, 855 m a.s.l.), with a horizontal projection of 135 m, linear development of 162 m and a 14 m drop in level, is closed to tourists. The local vegetation is well preserved secondary forest with many epiphytes and also some bamboos (*Merostachys* sp.) (Carvalho *et al*. 2002). Close to the trail which crosses the Bocaina river there is an area of deteriorated pasture, approximately 200 m from the caves. These caves are accessible on foot from this pasture.

In this region, three other caves were visited sporadically: Paivas: (24º 16' 11" S, 48º 26' 32" W; 780 m a.s.l.) the largest in the region, with a delineated linear development of 2,880 m, presents one main entrance and two others of more difficult access and a skylight situated in one of the upper level halls. Fendão cave (24º 16' 11" S, 48º, 26' 55" W, 825 m a.s.l.), with a development of 1,120 m, of which 400 m are galleries of the Bocaina river, has several accesses to the exterior environment. Mãozinha cave (24º 16' 10" S, 48º 26' 55" W; 835 m a.s.l.) consists of a gallery of 62 m, with a small upper opening.

In the Pedra do Fogo region, only the Colorida cave and surrounding vegetation were sampled regularly. The Colorida cave (24º 16' 1300" S, 48º 25' 0900" W, 825 m a.s.l.) presents a horizontal projection of 765 m, linear development of 987

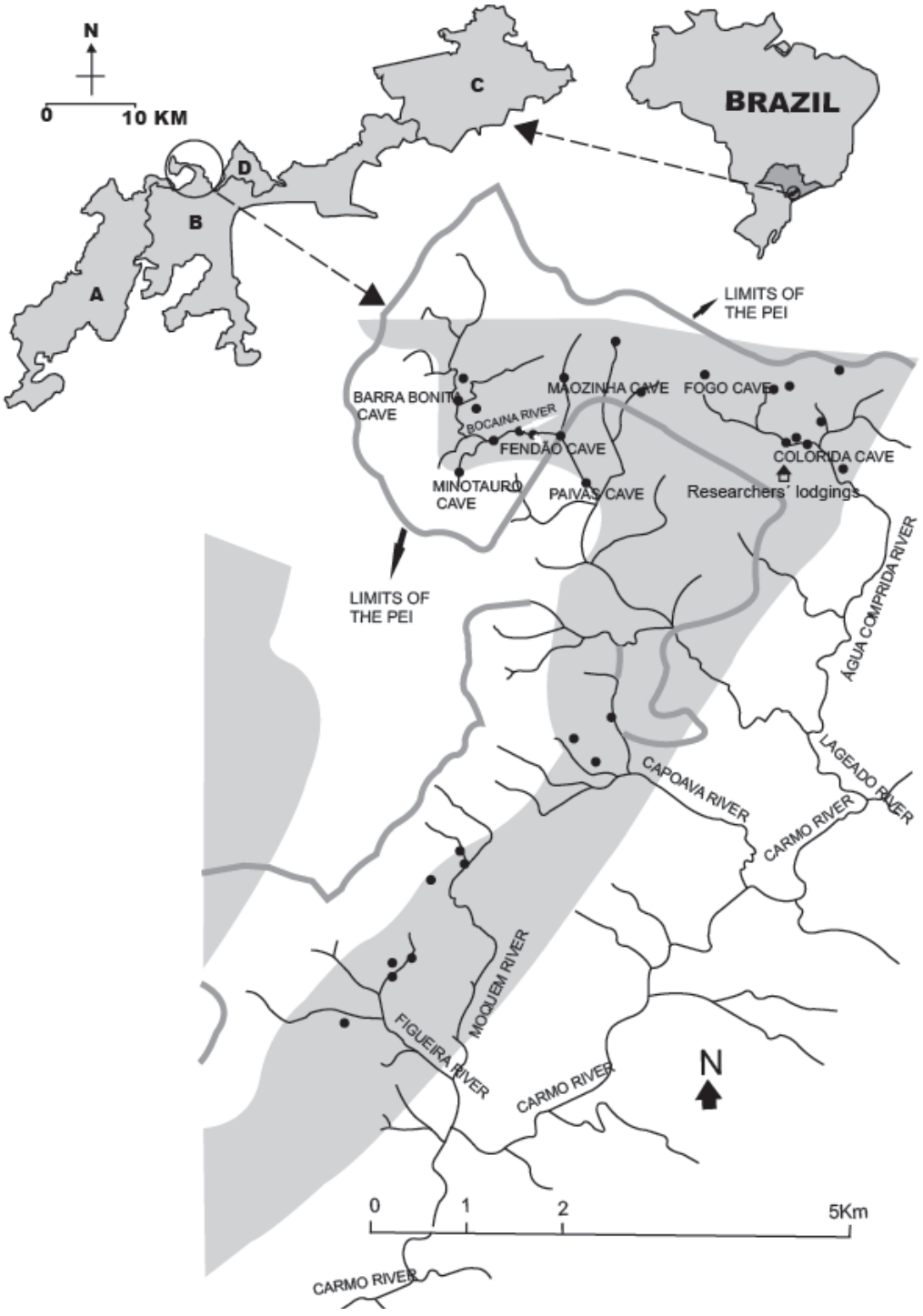

Fig. 1. Area of the Ecological Continuum (A-D). A, Parque Estadual Turístico do Alto Ribeira (PETAR). B, Parque Estadual Intervales (PEI). C, Parque Estadual Carlos Botelho and D, Estação Ecológica Xitué, and localization of some caves and the anthropic environment (researchers' lodging) studied in the PEI; grey area represents the calcareous stratum. Source: adapted from Gnaspini-Netto & Trajano (1992).

m and a drop in level of 25 m. Its principal entrance is small. It is often visited by tourists. The vegetation of this region consists of young secondary forest areas in which bamboos and lianas are abundant, there being many pioneer species and very dense undergrowth. The maximum height of the forest is 25 m. (Carvalho *et al*. 2002). Some trails traverse this vegetation. The Fogo cave (24º 15' 4900" S, 48º 25', 4800" W, 950 m a.s.l.), having a horizontal projection of 126 m, linear development of 163 m and a drop in level of 15 m, with a large entrance, and the Cipó cave (24º 16' 12.82193" S, 48º 24' 59.76557" W, 783 m a.s.l.) with 60 m of linear development and a small entrance were visited sporadically.

The Sede (headquarters) is situated in the NW region of the park at 860 m a.s.l. It is in this area that the reception

office, tourist lodgings, restaurant, center for recreational activities and conferences, areas for visitors, with bathrooms and kiosks and also the research headquarters, are installed. The area in which the headquarters is located is relatively flat, but is surrounded by hills, the height of which varies between 100 and 200 m above this plain. Apart from several streams crossing the area, there are also artificial lagoons and small reservoirs. Among the vegetation around the Sede, young secondary forest in which bamboo, "embaúba" (*Cecropia* spp) and "manacá-da serra" (*Tibouchina* spp) are abundant is predominant. Preserved old secondary forest may be found in some areas, mainly at the bottom of some valleys (Carvalho *et al.* 2002).

Methodology. Three techniques were employed for the captures, undertaken from January 2001 to December 2002: automatic light traps (Natal *et al*. 1991), modified by having a no-break battery (6 volts and 12 amperes) as energy source and an external collection chamber linked to the body of the trap by a sleeve, modified black or white Shannon traps (Galati *et al*. 2001), and aspiration.

The automatic light traps were installed monthly, in a total of 13 ecotopes, with the following distribution:

Bocaina region (six ecotopes): *i)* in the Minotauro cave-mouth, in a twilight area; *ii)* in Barra Bonita cave-mouth and *iii)* its interior; *iv)* in forest canopy (at ~15 m height); *v)* in forest, above ground (1.0 m) and *vi)* at edge of forest (at ~2 m height), around the Barra Bonita cave.

Pedra do Fogo region (five ecotopes): *i)* in Colorida cave (in a dark zone); *ii)* in a cavity of the external wall of the Colorida cave, approximately 5.0 m from its main entrance; *iii)* in forest canopy (at ~15.0 m height); *iv)* in forest, above ground (1.0 m) and *v)* at the edge of the forest (at 2.0 m height), around the Colorida cave.

Sede (two ecotopes related to the researchers' lodgings, with constant presence of hosts): *i)* on the veranda (at 3.0 m height) and *ii)* in the peridomicile (the trap was installed at 2.0 m height in a bush situated *ca*. 100 m from the house).

Several sites were sampled with modified white and/or black Shannon traps, at irregular time intervals. Members of the team made the captures monthly with the help of a guide, in two distinct areas, between 6.00 p.m. and 10.00 p.m. Two members of the team captured the insects in each area for the investigation of natural infection by flagellates as described by Galati *et al.* (2003). Occasionally, the guide, during his activities in the PEI, undertook captures with a black trap. The sites sampled were: forest, surrounding the Colorida, Minotauro, Barra Bonita, Cipó, Fogo and Paivas caves, and in the Sede region: São Inácio's church, another small church, the craftsman's house, the stone house, Tower trail, Morro do Espia trail, on the edge of the old lagoon, at the lake-side, in the peridomiciles of the researchers' lodgings and on Paiva's small-holding, about 3 km from the Sede, outside the park (Fig. 2).

Aspiration was carried out monthly with an electrical aspirator (6 volts) for one hour between 9.00 and 11.00 a.m. to investigate the presence of phlebotomines in armadillo burrows in a forested area round the researchers' lodgings.

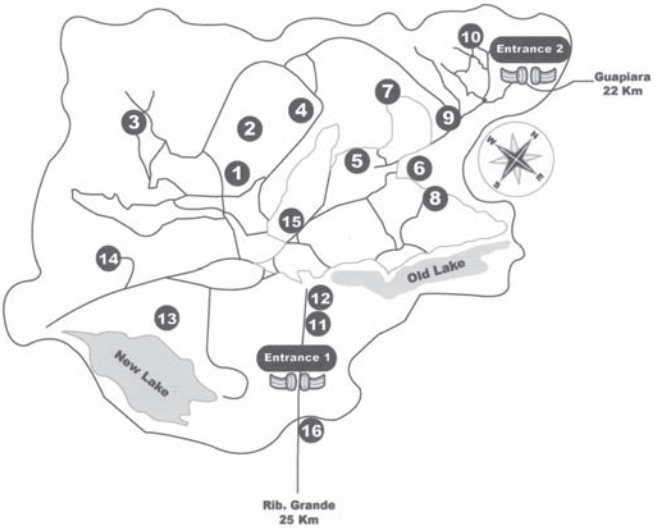

Fig. 2. Scheme of the principal administrative and tourist center (Sede) of the Parque Estadual Intervales and some touristic points and caves investigated. 1, researchers' lodging. 2, Colorida cave. 3, Água Comprida waterfall. 4, Cipó cave. 5, Artesão's house. 6, Santo Inácio church. 7, Morro do Espia tower. 8, Minotauro cave. 9, Fogo cave, 10, stone house. 11, tourist lodging. 12, administrative office, 13, reception office. 14 and 15, tourist lodgings. 16, small church and Paiva's small farm.

Some sporadic aspirations were made on the internal and/or external walls of caves and on the trunks and roots of trees, among fallen leaves and in holes in rocks.

Apart from the females dissected, the specimens captured with automatic light traps and those captured with the other techniques, after being killed in chloroform were conditioned in Petri dishes and kept under refrigeration until their clarification by the method described by Forattini (1973) and identification in accordance with Galati (2003). The abbreviation of the generic name of the species follows Marcondes (2007). A sample of these specimens is deposited in the collection of the Departamento de Epidemiologia of the Faculdade de Saúde Pública, USP.

The pluviometric data of the PEI were obtained from the Intervales meteorological station (810 m a.s.l.) (SIGRH 2007) and the temperature data of Capão Bonito municipality (900 m a.s.l.), which borders the PEI, from the Centro Integrado de Informações Agrometeorológicas do Instituto Agronômico de Campinas (CIIAGRO 2007).

Statistical analysis. The frequency of insects captured in armadillo burrows by seasonal variation was obtained by Williams' geometric average (Haddow 1960).

For the abundance and diversity calculations, the data used were those of the captures carried out with automatic light traps. The Standardised Index of Species Abundance (SISA) was calculated in accordance with Roberts & Hsi (1979) and Shannon's Diversity Index (H) and Pielou's Evenness Index (J) in accordance with Hayek and Buzas (1997).

The χ^2 test was used for the comparison between the numbers of insects captured during the same period at two different sites, as also between the numbers of insects captured in armadillo burrows in different seasons.

Table I. Numbers of specimens, by species and sex, captured with automatic light traps at the thirteen ecotopes sampled with respective Shannon diversity (H) and Pielou evenness (J) indexes in the regions of Pedra do Fogo, Sede and Bocaina in PEI, from January 2001 to December 2002.

Species	Region	Pedra do Fogo										Sede				Bocaina												Total	
	Ecotope	Colorida cave interior		Colorida external wall		forest canopy		forest ground		forest edge		Domicile		Peridomicile		Minotauro cave interior		Barra Bonita cave interior		Barra Bonita external wall		forest canopy		forest ground		forest edge			
	sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	MF	%
Br. bragai Sherlock		-	-	-	-	-	-	-	-	-		-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.4
Br. carvalheiroi Shimabukuro, Marassá & Galati		1	-	7	9	-	-	2	3	3	1	4	4	1	8	-	1	-	-	-	-	-	-	2	1	-	-	47	7.8
Br. cunhai (Mangabeira)		-	-	4	1	-	-	1	-	-	1	-	1	1	-	-		-	-	-	-	1	1	-	-	-	-	11	1.8
Br. troglodytes (Lutz)		-	-	3	7	3	1	1	2	-	3	86	52	28	17	-	-	-	-	9	3	50	20	16	2	10	8	321	53.4
Ev. correalimai (Martins, Coutinho & Luz)		-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	0.5
Ev. edwardsi (Mangabeira)		-	1	2	36	-	1	2	-	-	6	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	49	8.2
Ex. firmatoi (Barretto, Martins & Pellegrino)		-	3	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	5	0.8
Mg. rabelloi (Galati & Gomes)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	0.2
Mi. petari Galati, Marassá & Gonçalves-Andrade		-	3	-	4	-	-	-	-	-	-	-	-	-	-	1	3	-	-	-	-	-	-	-	-	-	-	11	1.8
Ny. intermedia (Lutz & Neiva)		-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	3	0.5
Ny. neivai (Pinto)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	6	-	-	-	-	-	-	-	1	8	1.3
Ny. sp*.		1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.2
Pa. lanei (Barretto & Coutinho)		3	2	14	13	1	3	2	12	8	12	-	3	1	-	-	-	-	-	-	-	-	1	-		3	3	81	13.5
Pa. pascalei (Coutinho & Barretto)		-	-	3	1	-	-	6	5	2	-	-	1	1	1	2	1	-	-	-	1	-	-	-	-	-	-	24	4.0
Pa. sp.		-	-	1	-	-	-	1	-	-	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	5	0.8
Pi. fischeri (Pinto)		-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.2
Pi. monticola (Costa Lima)		-	2	-	5	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	1	-	2	-	2	-	6	23	3.8
Ps. ayrozai (Barretto & Coutinho)		-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.2
Ps. geniculatus (Mangabeira)		-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0.4
Sc. microps (Mangabeira)		-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-		1	0.2
Total		5	12	36	79	4	6	15	23	13	25	90	63	35	27	4	10	1	8	9	5	51	25	18	5	13	18		1000
Total (MF)		17		115		10		38		38		153		62		14		9		14		76		23		31		600	
H		1.422		1.905		1.194		1.625		1.507		0.466		0.975		1.402		0.848		0.511		0.384		0.669		1.062		1.904	
J		0.684		0.794		0.861		0.781		0.725		0.239		0.501		0.871		0.772		0.465		0.239		0.609		0.766		0.636	

* specimen without genitalia. *Br.* = *Brumptomyia*; *Ev.* = *Evandromyia*; *Mg.* = *Migonemyia*; *Mi.* = *Micropygomyia*; *Ny.* = *Nyssomyia*; *Pa.* = *Psathyromyia*; *Pi.*= *Pintomyia*; *Ps.* = *Psychodopygus*; *Sc.* = *Sciopemyia*.

RESULTS

A total of 891 sandfly specimens belonging to 21 species were captured in the PEI, with the three techniques described. Nineteen species were obtained with the automatic light traps (Table I), 14 on the Shannon traps (Table II) and 7 by aspiration (Table III). Only two of the species were not captured with the automatic light traps: *Mg. migonei* (França) was only captured with the Shannon trap and *Sc. sordellii* (Shannon & Del Ponte) by aspiration.

The numbers of specimens, by species and sex, captured in the Pedra do Fogo, Sede and Bocaina regions with automatic light traps in the thirteen ecotopes with the respective Shannon diversity (H) and Pielou evenness (J) indices, calculated for both sexes together, are presented in Table I. Of the 600 sandflies captured with automatic light traps, 215 (average of 4.48 insects/trap) were collected in anthropic environments (domiciliary and peridomiciliary) and 385 (1.46 insects/trap) in the extra-domiciliary environments (caves and forests). In the five ecotopes of the Pedra do Fogo region, including one cave, 218 sandflies (1.82 insects/trap) were captured, and in the six ecotopes of the Bocaina region, with two caves, 167 (1.16 insects/trap). In the extra-domiciliary environment of the Pedra do Fogo region, *Pa. lanei* predominated (32.0%), followed by *Ev. edwardsi* (22.0%). This latter species predominated in the cavity of the external rocky walls of the Colorida cave (33.0%) and *Pa. lanei* in all of the other ecotopes: Colorida cave (29.4%), canopy (40.0%), ground

(36.8%) and forest edge (52.6%). In the Bocaina region, in the Minotauro cave *Pi. monticola* predominated (35.7%), and in the Barra Bonita cave *Ny. neivai* (66.7%). In all the other ecotopes, *Br. troglodytes* was captured with the highest frequencies: at the entrance of the Barra Bonita cave (85.7%), in the canopy (92.1%), on the ground (78.3%) and at the forest edge (58.1%). In the domiciliary and peridomiciliary environments, *Br. troglodytes* represented 90.2% and 72.6%, respectively, of the total captured.

The highest species richness and Shannon diversity index were found in the Pedra do Fogo region. In the cavity of the external wall of the Colorida cave (11 species and H = 1.905). In all the ecotopes of this region, excepting the canopy with the presence of four species, the others had similar species richness (8), but on the forest ground the Shannon diversity index was the greater (H = 1.625) than in the others. In the Bocaina region, both the Minotauro cave and forest canopy had similar species richness (5), but the Shannon diversity index was highest in the Minotauro cave (H = 1.402) and lowest in the canopy (0.384).

The highest evenness Pielou indices were observed in the Minotauro cave (J = 0.871), in the Bocaina region, and canopy (J = 0.861) in the Pedra do Fogo region, whereas the lowest and equal indices (J = 0.239) were observed in the domicile of the Sede and canopy of the Bocaina region, both greatly influenced by the large numbers of *Br. troglodytes* (Table I).

The seasonal frequencies (insects/season) of both sexes of *Br. troglodytes*, calculated by the Williams' average, in the two ecotopes where the species was more numerous during the period January 2001 to December 2002 (Domicile - Sede region and canopy - Bocaina region), were, respectively: summer (2.83; 2.26), autumn (0.0; 0.0), winter (0.0; 0.0) and spring (3.93; 0.35). Thus this species was clearly associated with more humid and hotter periods, as may be observed in Figure 3.

The numbers of captures undertaken with black (B) and/or white (W) Shannon traps by site and those of insects captured, and the numbers of hours dedicated to the captures, were: in the Bocaina Region: in front of the cave entrances: Minotauro (1B, 2 h. – *Pi. monticola* 3♀ and 1W, 2 h. – *Mi. petari* 1♂, *Pi. monticola* 1♂, 2♀); Barra Bonita 1B, 3 h. – *Ps. geniculatus* 1♂, *Br. troglodytes* 1♀; Paivas 1B, 4 h. – negative; inside forest 3B, 12 h. 1W 4h - negative. Pedra do Fogo Region: in front of the cave entrances: Colorida (17 B, 64 h. – *Ev. edwardsi* 1♂ 7♀; *Ex. firmatoi* 2♂ 1♀*Pi. monticola* 2♀; *Pa. lanei* 4♂ 1♀; *Pa. pascalei* 1♂; and 11 W, 44h. – *Br. carvalheiroi* 1♂; *Ex. firmatoi* 2♀, *Pa. lanei* 3♀), Cipó (5B, 18 h. – *Ev. edwardsi* 3♀, *Pa. lanei* 3♀, *Ps. ayrozai* 2♀, *Ps. geniculatus* 1♀ and 2 W, 8 h. – *Ev. edwardsi* 1♀, *Pa. lanei* 2♀) and Fogo (3 B, 13 h. – *Ev. edwardsi* 3♀, *Pi. monticola* 2♀, *Ps. ayrozai* 1♀,); Sede Region: on the forest tracks: Tower (1B, 2h - negative, 1W 2h. - negative), Água Comprida waterfall (2B, 7 h. – *Pi. monticola* 1♀, *Pa.* sp. 1♀ and 2W 7 h. – *Pa. pascalei* 1♀) and Morro do Espia (1B, 4 h. - negative), on the veranda of the domiciles Artesão's house (3B, 12h. and 4 W 16h. - negative) and researchers' house (1B, 4 h. 1W, 4 h. - negative), beside the churches: Igrejinha (7B, 18h – *Br. bragai* 1♂ 1♀, *Br. troglodytes* 2♂ 1♀, *Br*. sp. 1♂, *Mg. migonei* 1♂ 1♀, *Ny. neivai* 1♀, *Pi. monticola* 71 ♀, *Pa. lanei* 1♀, *Pa.* sp. 1♀) and Santo Inácio (1B, 4 h. - negative), beside the Stone House (1W 4 h., negative), lake edge (1B, 1:30 h. –*Br*. sp. 1♀, *Pi. monticola* 2♀), edge of old lagoon (1B, 1:30 h. – *Pi. monticola* 6♀). Peridomicile of Paiva's small farm, 3 km outside the PEI (1B, 4 h., 1W, 4h. – negative). So modified black and/or white Shannon traps (Table II) yielded a total of 146 sandflies representing 14 species, giving an average of 1.95 insects/trap, 0.58 insects/hour (black 2.68 insects/trap or 0.85 insects/hour; white 0.48 insects/trap or 0.12 insects/hour). *Pi. monticola* clearly predominated on the black trap 87/134 (72.5%). However, of this total, 61/87 (70.1%) were captured in one single capture in the forest beside the small church (Igrejinha), in April 2002 (18.30 h.-19.30 h.). *Ev. edwardsi* with 14 specimens (10.4%) was the second most attracted by the black trap. The richness on the black trap was 13 species and on the white, 6. The females were more attracted to both traps than the males, the female/male ratio being 8.57:1:0, while on the white it was 3.0:1.0.

Table II. Number of phlebotomines, by sex, captured with black or white Shannon traps in forests of the PEI, from January 2001 to December 2002.

Species	Black		White		Total			
no. captures (nº hours)	50 (158 h.)		25 (95 h.)		75 (253 h.)			
sex	M	F	M	F	M	F	MF	%
Br. bragai	1	1	-	-	1	1	2	1.37
Br. carvalheiroi	1	1	1	-	2	1	3	2.06
Br. troglodytes	2	2	-	-	2	2	4	2.74
Ev. edwardsi	1	13	-	1	1	14	15	10.27
Ex. firmatoi	2	1	-	2	2	3	5	3.42
Mg. migonei	1	1	-	-	1	1	2	1.37
Mi. petari	-	-	1	-	1	-	1	0.69
Ny. neivai	-	1	-	-	-	1	1	0.69
Pa. lanei	4	6	-	4	4	10	14	9.59
Pa. pascalei	1	1	-	-	1	1	2	1.37
Pa. sp.	-	2	-	-	-	2	2	1.37
Pi. monticola	-	87	1	2	1	89	90	61.64
Ps. ayrozai	-	3	-	-	-	3	3	2.05
Ps. geniculatus	1	1	-	-	1	1	2	1.37
Total	14	120	3	9	17	129	146	100.0

Br. = Brumptomyia; *Ev. = Evandromyia*; *Ex. = Expapillata*; *Mg. = Migonemyia*; *Mi. = Micropygomyia*; *Ny. = Nyssomyia*; *Pa. = Psathyromyia*; *Pi. = Pintomyia*; *Ps. = Psychodopygus*.

A total of 56 females captured with automatic light and Shannon traps or biting the researchers were dissected for investigation of their digestive tracts for natural infection by flagellates with negative results, but one of *Pi. monticola*

was found to be infested with microfilariae. The species and respective numbers of specimens dissected were: *Brumptomyia* sp. (4), *Br. troglodytes* (18), *Ev. edwardsi* (3), *Ex. firmatoi* (1), *Mi. petari* (2), *Ny. neivai* (1), *Pa. lanei* (6), *Pa. pascalei* (1), *Pa.* sp. (2) and *Pi. monticola* (18).

A total of 67 hours were spent on aspiration in several ecotopes, resulting in the capture of 145 specimens representing 8 species (Table III). Of this total, 94.5% were collected in armadillo burrows, with the predominance of *Pa. pascalei* (54.8%), *Br. carvalheiroi* (32.8%), *Br. troglodytes* (10.2%) and *Pi. monticola* 2.2%, with a significant statistical difference between the two most frequent species ($\chi^2 = 10.65$; $p < 0.01$; f.d. = 1). In the total of specimens captured, the male/female ratio was 2.6:1.0. The results were negative on the internal walls of seven caves; however, five species: *Br. cunhai, Ev. edwardsi, Pi. monticola, Pa. lanei* and *Sc. sordellii* (the one specimen captured of this latter being the only one found in the PEI), were collected on the external walls of the Colorida and Fogo caves.

The seasonal distribution of the specimens captured monthly in the armadillo burrows during 2001 and 2002 in the forest surrounding the researchers' house is presented in Table IV. The Williams' averages by season were: autumn (3.28 insects), winter (2.34 insects), summer (0.0 insects) and spring (0.47 insects), thus showing a clear difference between the drier and colder periods (autumn and winter) and the humid and hot ones (spring and summer). This result contrasts with that related to *Br. troglodytes*, the most abundant species captured with light traps in the two ecotopes where it was most numerous (domicile in the Sede and canopy in the Bocaina region).

Table III. Numbers of phlebotomine specimens captured by aspiration, by site, number of hours, species and sex, in the PEI, during the period from January 2001 to December 2002.

Sites	Number of hours	Species	Male	Female
Pedra do Fogo region				
Colorida cave – internal walls	12	-	-	-
Colorida cave - external walls	12	*Br. cunhai*	1	1
		Ev. edwardsi	-	1
		Pa. lanei	3	-
Armadillo borrow close to Colorida cave's principal entrance	1	*Br. carvalheiroi*	8	5
		Br. troglodytes	7	6
		Pa. pascalei	1	-
Fogo cave – internal walls	1	-	-	-
Fogo cave – external walls	1	*Pi. monticola*	-	1
		Sc. sordellii	1	-
Bocaina region				
Fendão cave – external walls	1	-	-	-
Mãozinha cave – internal walls	1	-	-	-
Barra Bonita cave – internal walls	1	-	-	-
Minotauro cave – internal walls	1	-	-	-
Paivas cave – internal walls	1	-	-	-
Sede region				
Armadillo borrow in forest surrounding the researchers' house	24	*Br. carvalheiroi*	28	4
		Br. troglodytes	1	-
		Pi. monticola	-	3
		Pa. pascalei	55	19
Trunks or roots of trees, fallen leaves and holes in soil and rocks	11	-	-	-
Total	67		105	40

Br. = *Brumptomyia*; *Ev.* = *Evandromyia*; *Pa.* = *Psathyromyia*; *Pi.* = *Pintomyia*; *Sc.* = *Sciopemyia*.

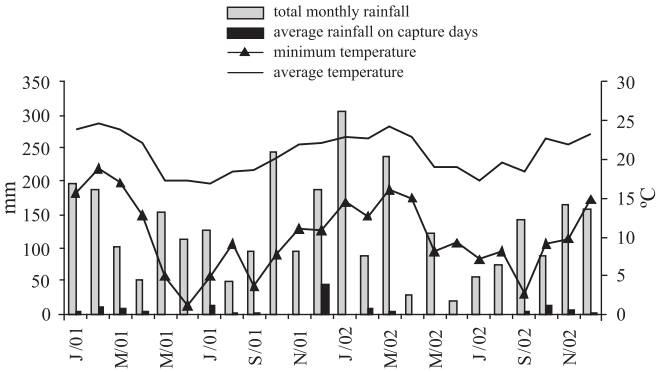

Fig. 3. Total monthly rainfall, average rainfall on capture days in the PEI (810 m a.s.l.), and minimum and average monthly temperatures in Capão Bonito municipality (900 m a.s.l.)

The standardized index of species abundance (SISA) (Table V) calculated only for the species captured with automatic light traps installed in the thirteen ecotopes distributed in the three regions shows *Br. troglodytes* as the most abundant species (SISA = 0.703), followed by *Pa. lanei* (SISA = 0.594), *Pi. monticola* (SISA= 0.455) and *Brumptomyia carvalheiroi* (SISA = 0.434), in that order.

The annual rainfall in the PEI (at the meteorological station) in 2000, before the beginning of the project, was of 1,031.8 mm, during 2001 of 1,600.7 mm and in 2002 of 1,488.8 mm. The distribution of the total monthly rainfall, the average of the daily rains and the monthly mean and minimum temperatures in Capão Bonito municipality, close to the PEI, are shown in Figure 3. The driest and coldest period extends from April/May to September. Only in December 2001 did high levels of rainfall occur on capture days and in 2001 the minimum temperatures were lower than those of 2002.

DISCUSSION

During the two years of captures in the PEI, the total number of insects collected in the three areas sampled was very small. Several of the species captured, such as those of the genus *Brumptomyia* and *Pa. pascalei* which present a clear association with armadillo burrows (Aguiar & Medeiros 2003), are known not to bite humans. For several species captured, there are evidences of their attraction to humans, *Ex. firmatoi* (Gomes *et al.* 1989), *Mg. migonei*, *Ny. neivai* and *Pi. fischeri* (Aguiar *et al.* 1989; Gomes *et al.* 1989), *Ny. intermedia (*Aguiar *et al.* 1987, Afonso *et al.* 2005, 2007), *Pa.*

lanei (Gomes & Galati, 1989), *Pi. monticola* (Hermeto *et al*. 1994; Afonso *et al*. 2007), *Ps. ayrozai* (Gomes & Galati, 1989; Aguiar *et al.* 1993, Afonso *et al*. 2007) and *Ps. geniculatus* (Aguiar *et al.* 1993). Nonetheless, of these species, only *Mg. migonei, Ny. intermedia* and *Ny. neivai* have been implicated as vectors of *Leishmania* (*Viannia*) *braziliensis* Vianna, 1911, the agent of human cutaneous leishmaniasis in the Brazilian Southeast (Rangel & Lainson 2003; Pita-Pereira *et al.* 2005; Andrade Filho *et al.* 2007). But as in the PEI they presented very low frequencies, the risk of transmission of this parasite, whether in caves or in forests, seems also to be very small.

The capture of *Ny. intermedia* and *Ny. neivai*, this latter resuscitated from the synonymy of the former by Marcondes (1996), with allopatry, respectively on the coast and in the interior of São Paulo state, corroborates their occurrences in sympatry in the Ribeira Valley as registered by Marcondes *et al*. (1998) and Andrade Filho *et al*. (2007); however, for *Ny. intermedia* this finding represents its the most western and highest limits. The presence of *Ny. neivai* exclusively in the Bocaina region is perhaps due to its proximity to the open area of degraded pasture, since this species predominated in anthropized areas of the interior of São Paulo (Condino *et al*. 1998; Odorizzi & Galati 2007).

Pi. monticola, with the highest frequencies on the Shannon traps and captured biting the guide and researcher workers of the team, presents some probability of bothering the humans who frequent the PEI. Further, this species has been suspected of being a vector of *Leishmania* (*Leishmania*) *enriettii* Muniz & Medina, 1948, a parasite of the guinea-pig, on the outskirts of Curitiba city in the Paraná state, and has been shown to be susceptible to experimental infection by this agent captured in the same region, but the flagellates seem to have failed to produce infection when inoculated into clean laboratory guinea-pigs (Luz *et al*. 1967). The infection of a guinea-pig was also identified on a farm in Capão Bonito municipality (Machado *et al*. 1994; Lainson 1997) situated very close to the PEI. Thus perhaps, if more females of *Pi. monticola* had been dissected, more information about the vector of this parasite and possibly other leishmanias would have been obtained, since only 18 of its females were dissected and in foci of cutaneous leishmaniasis the natural infection by flagellates rate is only about 0.2% (Galati *et al*. 1996). Some other aspects of its behaviour observed in the PEI had also been registered in other areas: attraction to light in the Rio Doce Valley in Minas Gerais state, a focus of cutaneous leishmaniasis (Hermeto *et al*. 1994), and in areas of Atlantic forest in Rio de Janeiro state (Afonso *et al*. 2007); predominance in high areas (850-950 m) in the Espírito Santo state (Ferreira *et al*. 2001); its capture in greater numbers in April in another fragment of Atlantic forest in Morretes, state of Paraná (Marcondes *et al.* 2001).

Ex. firmatoi, *Pa. lanei*, *Pa.* sp. *Pi. fischeri*, *Ps. ayrozai* and *Ps. geniculatus*, species with some degree of anthropophily may, despite their low frequencies, sporadically cause bother to visitors to and inhabitants of the PEI. *Pa.* sp., a new species close to *Pa. pestanai*, was captured in small numbers biting the researchers in the PEI.

Ev. edwardsi, the sixth most abundant in the automatic light trap captures, the second with Shannon traps and the most frequently captured in areas associated with the caves of the Pedra do Fogo Region, has often been captured in Atlantic forest areas. It has been found to be naturally infected by *Le. braziliensis* in Cotia municipality, in the metropolitan region of Greater São Paulo, in a focus of visceral canine leishmaniasis and human cutaneous leishmaniasis (Sucen 2005).

Table IV. Phlebotomines captured in armadillo burrows situated in the surroundings of Sede of the PEI, by species, sex and season of 2001 and 2002.

Species Season/year	*Brumptomyia carvalheiroi*		*Brumptomyia troglodytes*		*Pintomyia monticola*		*Psathyromyia pascalei*		Total		
	M	F	M	F	M	F	M	F	M	F	T
2001											
Summer	-	-	-	-	-	-	-	-	-	-	-
Autumn	15	1	-	-	-	1	17	8	32	10	42
Winter	9	2	-	-	-	2	22	9	31	13	44
Spring	-	-	-	-	-	-	-	-	-	-	-
Subtotal	24	3	-	-	-	3	39	17	63	23	86
2002											
Summer	-	-	-	-	-	-	-	-	-	-	-
Autumn	-	-	-	-	-	-	5	2	5	2	7
Winter	-	-	1	-		-	11	-	12	-	12
Spring	4	1	-	-	-	-	-	-	-	-	-
Subtotal	4	1	1	-	-	-	16	2	21	3	24
Total	28	4	1	-	-	3	55	19	84	26	110

Br. troglodytes was the most abundant species captured with automatic light traps in the PEI (the anthropic environments included), differently from other areas of the Ribeira Valley (Gomes & Galati 1989; Gomes *et al.* 1990; Domingos *et al.* 1998). As the activity of the species of *Brumptomyia* is essentially in forests and closely associated with armadillo burrows (Aguiar & Medeiros 2003), the predominance of this species in the forested areas is to be expected, though not in an anthropic environment, such as was the veranda of the domicile and peridomicile of the Sede. Probably the dominance of *Brumptomyia* in the domicile and peridomicile of this region may be attributed to the attraction of the lights and to the fact that in the cold, dry season, the armadillo females breast-feed their young in their burrows. Hence, a greater number of sandflies were aspirated in this period. At the beginning of the hot and humid season, the armadillos together with their young abandon their burrows to look for food. The humid area with very dense undergrowth, situated around the domicile sampled (Sede region), provided a favorable environment for this purpose and armadillo tracks are very common there. Thus are the highest frequencies of this group of phlebotomines in the domicile and peridomicile in more humid and hotter periods to be explained. On the other hand, *Pa. pascalei*, which predominated in armadillo burrows, is probably less attracted to light, as may be deduced by the small numbers of specimens captured with the automatic light traps.

The armadillo *Dasypus novemcinctus* has been identified as the wild host of *Leishmania (Viannia*) *naiffi* Lainson & Shaw, 1989 in the Brazilian states of Pará and Amazonas, and

Table V. Index of Species Abundance (ISA) and Standardised Index of Species Abundance (SISA) of phlebotomines captured monthly with automatic light traps at 13 ecotopes in the PEI, from January 2001 to December 2002.

Species	ISA	SISA	Position
Br. bragai	11.385	0.056	15th
Br. carvalheiroi	7.231	0.434	4th
Br. cunhai	9.077	0.266	7th
Br. troglodytes	4.269	0.703	1st
Ev. correalimai	11.769	0.021	18th
Ev. edwardsi	8.115	0.353	6th
Ex. firmatoi	10.385	0.147	11th
Mg. rabelloi	11.423	0.052	16th
Mi. petari	9.243	0.251	8th
Ny. intermedia	10,192	0.164	10th
Ny. neivai	10.038	0.178	9th
Pa. lanei	5.462	0.594	2nd
Pa. pascalei	7.808	0.381	5th
Pa. sp.	10.885	0.101	12th
Pi. fischeri	11.269	0.066	15th
Pi. monticola	7.000	0.455	3rd
Ps. ayrozai	11.615	0.035	17th
Ps. geniculatus	11.115	0.080	13th
Sc. microps	11.269	0.066	14th

Br. = Brumptomyia; *Ev. = Evandromyia*; *Ex.= Expapillata*; *Mg. = Migonemyia*; *Mi. = Micropygomyia*; *Ny. =Nyssomyia*; *Pa. = Psathyromyia*; *Pi. = Pintomyia*; *Ps. = Psychodopygus*; *Sc. = Sciopemyia.*

in French Guyana, and *Ps. ayrozai* has been suspected as the vector, mainly among populations of this mammal. However, thus far this parasite has not been found in the Brazilian Southeastern Region where cutaneous leishmaniasis cases have mainly been associated with *Le. braziliensis* (Lainson & Shaw 2005). Thus, infection by this parasite and possibly others in armadillos calls for further investigation, as well as that in the sandflies associated with their burrows, since it is they which may maintain the natural enzootic foci.

Despite the large number of Brazilian speleological provinces (Auler 2000), very few studies of their phlebotomine fauna exist. Of the species cited by the CIPA group (1999), none was found in the PEI. Comparing the fauna of the PEI with that of the calcareous speleological province of the Serra da Bodoquena (Galati *et al.* 2003) in the Brazilian Mid-West, where savannah is the predominant vegetation (Galati *et al*. 2006), only the species *Brumptomyia cunhai* and *Sc. sordellii* are common to both areas. Though inhabited by distinct species, both provinces share some common groups: *Evandromyia*: *cortelezzii* series, *Pintomyia* (*Pintomyia*), *Micropygomyia* (*Sauromyia*), *Nyssomyia*, *Psathyromyia* (*Forattiniella*) and *Psathyromyia* (*Psathyromyia*), *shannoni* series. However, no species of the dominant *Lutzomyia* (*Lutzomyia*) group present in the caves or forests of the Serra da Bodoquena was found in the PEI as, for example, *Lu. almerioi* Galati & Nunes 1999, an anthropophilic, troglophilic species of high density, and probable vector of agents of cutaneous and visceral leishmaniases (Savani *et al*. 2009).

As regards the forest sandfly fauna of the Bodoquena, neither *Nyssomyia whitmani* (Antunes & Coutinho, 1939), one of the main vectors of *Le. braziliensis*, the most widespread Brazilian agent of cutaneous leishmaniasis, nor *Lutzomyia longipalpis*, the main vector of *Leishmania* (*Leishmania*) *infantum chagasi* Cunha & Chagas, 1937, the two most abundant species on the Serra da Bodoquena, was found in the PEI. However, two species of *Nyssomyia*, *Ny. intermedia* and *Ny. neivai*, also considered to be vectors of cutaneous leishmaniasis, were captured in the PEI, but their density was very low.

Another clear difference observed between these two provinces is the very low density of phlebotomines in caves of the PEI, as well as in their surrounding forests. Thus it seems that the risk of transmission of leishmaniasis in the PEI is very small, if any.

It is possible that the greater altitude, lower temperatures and higher relative humidity of the PEI do not afford an adequate environment for the development of the immature stages of the phlebotomines and that the microhabitat represented by the armadillo burrow, being more appropriate for the sandfly, probably explains the predominance of *Brumptomyia spp* and *Pa. pascalei*, closely associated with this mammal (Aguiar & Medeiros 2003).

Of the few specimens (40 insects/72 samples) representing the 10 species captured in caves: *Br. carvalheiroi*, *Ev. edwardsi*, *Ex. firmatoi, Mi. petari, Ny. intermedia*, *Ny. neivai*, *Pi. monticola*, *Pa. lanei*, *Pa. pascalei* and *Sc. microps* none seems to present any high degree of cave dependency, since all the species have been captured in environments without caves. Some of these species use these ecotopes as resting sites. However, *Ev. edwardsi*, *Sc. microps* and *Mi. petari* may also have their breeding places in caves, because beyond having been captured in this project exclusively (or in the highest frequencies) in caves or on the external walls of the Colorida cave, they belong to groups found in close association with the caves of the karst Corumbá formation in the Brazilian Mid-West region (Galati *et al.* 1997, 2003) and some of their populations may therefore be identified as troglophiles. Though there is no information as to the occurrence of *Ex. firmatoi* in other caves, in the PEI it was captured exclusively in the Colorida cave, on its external walls or on the Shannon traps in front of it. So it is possible that it is also a troglophile.

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