Phlebotomines (Diptera, Psychodidae) in the Speleological Province of the Ribeira Valley: 2. Parque Estadual do Alto Ribeira (PETAR), São Paulo State, Brazil

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ABSTRACT. Phlebotomines (Diptera, Psychodidae) in the Speleological Province of the Ribeira Valley: 2. Parque Estadual do Alto Ribeira (PETAR), São Paulo State, Brazil. The Parque Estadual do Alto Ribeira (PETAR) with about 250 caves, in an Atlantic forest reserve, is an important ecotourist attraction in the Ribeira Valley, an endemic area of American cutaneous leishmaniasis (ACL). With the purpose of investigating *Leishmania* vector species bothersome to humans the sandfly fauna was identified and some of its ecological aspects in the Santana nucleus, captures were undertaken monthly with automatic light traps in 11 ecotopes, including caves, forests, a camping site and domiciliary environments, and on black and white Shannon traps, from January/2001 to December/2002. A total of 2,449 sandflies representing 21 species were captured. The highest values of abundance obtained in the captures with automatic light traps were for *Psathyromyia pascalei* and *Psychodopygus ayrozai*. A total of 107 specimens representing 13 species were captured on black (12 species) and white (6 species) Shannon traps set simultaneously. *Psychodopygus geniculatus* females predominated on the black (43.75%), and *Psathyromyia lanei* and *Ps. ayrozai* equally (32.4%) on the white. *Nyssomyia intermedia* and *Nyssomyia neivai*, both implicated in the transmission of ACL in the Brazilian Southeastern region, were also captured. *Ny. intermedia* predominated in the open camping area. Low frequencies of phlebotomines were observed in the caves, where *Evandromyia edwardsi* predominated *Lutzomyia longipalpis*, the main vector of the American visceral leishmaniasis, was aslo present. This is its most southernly reported occurrence in the Atlantic forest.

KEYWORDS. Caves; ecology; leishmaniasis; sandfly; vectors.

RESUMO. Flebotomíneos (Diptera, Psychodidae) na província Espeleológica do Vale do Ribeira. 2: Parque Estadual Turístico do Alto Ribeira (PETAR), São Paulo, Brasil. O Parque Estadual Turístico do Alto Ribeira, com cerca de 250 cavernas, situado em reserva de floresta Atlântica, é uma importante atração turística na região do Vale do Ribeira, onde a leishmaniose tegumentar americana (LTA) é endêmica. Com o objetivo de investigar as espécies incômodas ao homem e/ou implicadas na transmissão de *Leishmania*, identificou-se a fauna flebotomínea e alguns aspectos ecológicos de suas populações no núcleo da caverna Santana. Mensalmente, de janeiro/2001 a dezembro/2002, foram realizadas capturas com armadilhas automáticas luminosas em 11 ecótopos, incluindo cavernas, florestas, área de camping e ambientes domiciliares, e com armadilhas de Shannon em mata. No total capturou-se 2.449 flebotomíneos, representando 21 espécies. Nas capturas com armadilhas automáticas luminosas, *Psathyromyia pascalei* e *Psychodopygus ayrozai* foram as mais abundantes. Com as armadilhas de Shannon branca e preta instaladas simultaneamente foram capturados 107 espécimes, representando 13 espécies, na preta (12 espécies) e na branca (6 espécies). Fêmeas de *Psychodopygus geniculatus* predominaram na preta (43,75%), e *Psathyromyia lanei* e *Ps. ayrozai,* igualmente (32,4%), na branca. *Nyssomyia intermedia* e *Nyssomyia neivai*, ambas implicadas na transmissão da LTA na região, também foram capturadas, *Ny. intermedia*, a 6ª mais abundante, predominou na área de camping aberto. Baixas freqüências de flebotomíneos foram observadas nas cavernas, onde *Evandromyia edwardsi* predominou. Destaca-se a captura de *Lutzomyia longipalpis,* principal vetor do agente da leishmaniose visceral americana, sendo o registro mais meridional da mata Alântica.

PALAVRAS-CHAVE. Cavernas; ecologia; flebotomíneos; leishmaniose; vetores.

A great concentration of carbonate karst areas, commonly favorable to the formation of caves, is to be found in the eastern half of Brazil, mainly in the states of Bahia, Minas Gerais and Goiás. Among the fourteen most extensive of them, with an area of about 11,900 km^2 and noteworthy as containing the largest number of caves, is the rock formation of the Açungui group, in the Speleological Province of the Ribeira Valley, in southeastern Brazil, largely in the south of São Paulo State (Auler 2002). This province, located on the left bank of the Ribeira de Iguape river, is in great part situated on the Serra de Paranapiacaba, mainly in two forest reserves: the Upper Ribeira State Tourist Park - Parque Estadual Turístico do Alto Ribeira (PETAR), with about 250 registered caves, and the Intervalleys State Park - Parque Estadual Intervales (PEI) (~80 caves) (T. Gonçalves, pers. comm.). This great number of caves and the remnant of Atlantic forest have attracted many people interested in ecological tourism to these parks.

Caves are natural cavities in the subsoil that may be interconnected by subterranean spaces and most of the Açungui rock formations contain active drainage (Auler

2002). Their communication with the external environment may occur through sinkholes where the water disappears underground, or openings caused by caving-in (Dessen *et al*. 1980). The cave environments present smaller daily variation of temperature and relative humidity than the external ones; the annual average of the latter being almost 100% (Boggiani *et al.* 2007). Depending on the size of the cave, three zones may be distinguished: a twilight zone, close to the entrance, an intermediate one of complete darkness and variable temperature and that in the deepest areas, completely dark and of constant temperature (Poulson & White 1969). The foods present in the cave soil originate from animal droppings, vegetal debris, remains of arthropods and of other organisms found in aqueous solutions and moist or dry soil. The floor material consists of fragmented sediments, sand, silt and clay, such as may result from the fall of rock fragments from the cave roof and walls (Poulson & White 1969) and in some areas from the influx of material brought in by the rain.

Forests and caves are natural habitats of the phlebotomine sand flies (Diptera, Psychodidae) (Aguiar & Medeiros 2003; Cipa Group 1999), females of which are haematophagous, biting mammals, birds and cold-blooded animals. Many of their species are also anthropophilic and beyond bothering humans by their painful bites, may transmit pathogens such as viruses, bacteria and Trypanosomatidae (Shaw *et al.* 2003) to people who enter these environments. Among the Trypanosomatidae, species of the genus *Leishmania,* having wild or synanthropic mammals as primary hosts and causing visceral, cutaneous or muco-cutaneous leishmaniases in humans, have received the special attention of public health organizations due to the increase in the incidence and expansion of these infections throughout Brazilian territory and the severity of some of the clinical forms of these diseases.

The region of the Ribeira Valley registered a total of 1,174 cutaneous leishmaniasis cases between 1998 and 2006, which gives an average annual coefficient of incidence of 47.71/100,000 inhabitants, one of the highest of the São Paulo state. This coefficient for Iporanga municipality where the greater part of the PETAR is situated is 152.9/100,000 inhabitants (Cve 2007), one of the highest in the Ribeira Valley region.

Several studies on phlebotomines in forested and/or anthropic environments have been undertaken in the Ribeira Valley to investigate the vectors of cutaneous leishmaniasis (Forattini *et al*. 1976; Gomes *et al*. 1980, 1982, 1983, 1986, 1990; Gomes & Galati 1987, 1989; Domingos *et al*. 1998; Taniguchi *et al*. 2002); but, as far as we know, no research has yet been published on the speleological province of this region. Thus, this project was carried out to investigate leishmaniasis agent vectors bothersome to humans in view of the use of this area for ecotourism, which may bring many susceptible people into contact with them, and to identify the phlebotomine fauna and describe some aspects of its behavior in some of the caves and also in the forested and anthropic environments of PEI (Galati *et al*. 2010) and PETAR. The present paper relates specifically to the sandfly fauna of the PETAR.

MATERIAL AND METHODS

Study area. The Parque Estadual do Alto Ribeira (PETAR), together with three other reserves: the Parque Estadual Intervales (PEI) – to the northeast, the Estação Ecológica Xitué and Parque Estadual Carlos Botelho – to the north and east, respectively, of the PEI, form a continuous ecological area of ca. 120,000 ha. (Galati *et al.* 2010).

The PETAR is situated in a transitional area between the Atlantic Plateau and the coastal plain on the left margin of the middle and upper course of the Ribeira river, in the southwest of São Paulo state (24º 12' – 24º 25' S and 48º 03' – 48º 30' W), in Iporanga and Apiaí municipalities, on the Paranapiacaba range, at altitudes varying between 80 and 980 m. It is drained by the basin of the Betari, Iporanga and Pilões rivers that have their watersheds on the edge of the Atlantic Plateau, locally denominated "Planalto de Guapiara". Its climate, according to Nimer (1977), represents a transition between a tropical low altitude zone and a temperate zone of intermediate altitude and is classified as sub-tropical and highly humid with no dry season. The average annual rainfall for 1970-1996 was 1,963.3 mm, of which 681.8 mm fell in the dry season (April – September) and 1,281.5 mm in the rainy season. The predominant vegetation is ombrophylous dense sub-mountain forest, with trees of up to 25-30 m and many lianas, ferns, epiphytes and palm trees (Karmann & Ferrari 2002).

The PETAR has been divided into four logistic areas, denominated "nuclei": Santana, Caboclos, Ouro Grosso and Casa de Pedra, the majority of the tourist activities being concentrated in these areas. Some of these areas are exclusively used for recreation, others for environmental education and/or research (Carvalho *et al.* 2002). The PETAR received 43,324; 48,693 and 42,477 visitors, respectively, during 2001, 2002 and 2003, and the Santana nucleus accounted for 70-80% of them.

The area sampled belongs to the Santana nucleus (Fig. 1), situated on the right and left banks of the Betari River. On the right bank are the researchers' lodgings, the Santana cave and the camping site. A track beginning at the reception office leads to the main entrance of the Santana cave, about 800 m away. The researchers' lodgings are situated on this track, ca. 300 m from the main Santana cave mouth, the camping site, a grassy area 100 m long x 20 m wide, being situated some 120 m beyond the Santana cave entrance, on the right bank of the Betari River. This cave entrance is about 130 m from the right bank of the Betari. The Morro Preto and Couto caves, with an underground connection between them, are located on the left bank of the Betari River. A track which crosses the Betari gives access to the three caves. The distance between the principal entrances of the Santana and Couto caves is of about 180 m, that between the former and the Morro Preto cave is of approximately 300 m, and that between this latter and the Couto cave is of about 120 m. These areas are in preserved primary forest with many bromeliaceas.

Caves studied: the Santana cave (24º 31' 5100" S, 48º 42' 0600" W, 250 m a.s.l.) presents a horizontal projection with 6.3 km of mapped passages, discontinuous linear development and a drop in level of 61 m (Karmann & Ferrari 2002; Sociedade Brasileira de Espeleologia 2007). The main entrance is not very large (4.0 m wide x 2.5 m high) and is fenced off. There is a skylight near this entrance. The cave's galleries and saloons are richly ornamented with stalactites, stalagmites and curtains. The Roncador River, a tributary of the Betari, runs through the cave and drains several of its galleries. Only 490 m is used as a tourist circuit (T. Gonçalves, pers. comm.). The Morro Preto cave (24º 31' 1900" S, 48º 41' 5400" W, 305 m.a.s.l), has 832 m of horizontal projection, discontinuous linear development and a drop in level of 61 m (Sociedade Brasileira de Espeleologia 2007). It presents a great portico and giant stalactites. The Couto cave (24º 31' 1400" S, 48º 31' 1400", 290 m a.s.l), situated below the Morro Preto cave and linked to it, presents 471 m of horizontal projection, discontinuous linear development and a drop in level of 26 m. A small river runs through it and when it leaves it does so as a waterfall. It has a small entrance (~2 m wide x 1.5 high) about five metres from the waterfall on the track that gives access to the principal entrance of the Morro Preto cave.

Methodology. The captures were carried out monthly by two different techniques: i) automatic light traps (Natal *et al.* 1991) modified as follows: they have a no-break battery (6 V and 12 A) as energy source and an external collection chamber linked to the body of the trap by a sleeve, and ii) modified black and white Shannon traps (Galati *et al*. 2001).

A total of 11 ecotopes were sampled in the Santana nucleus with automatic light traps installed between 1.0 m and 2.0 m above ground level, from 18.00 to 07.00 hours, with the following distribution: a) on the right bank of the Betari River: 1) at the researchers' lodgings (veranda; 300 m a.s.l.); 2) on the rocks located 10 m from the principal entrance of the Santana cave, 262 m a.s.l. 3) at the principal Santana cave entrance; 260 m a.s.l. and 4) at the camp site, 260 m a.s.l.; b) on the left bank of the Betari river: 5) at the forest edge close to the river bank, 260 m a.s.l.; at three sites on the track that leads to the Morro Preto cave: 6) near a Ficus tree's roots among rocks, 280 m a.s.l., about 80 m from the river bank, 7) in a rocky wall at the beginning of the Morro Preto outcrop, 130 m from the river bank, 300 m a.s.l. and 8) at the end of the track, close to the principal Morro Preto cave entrance, 170 m from the river bank, 305 m a.s.l. 9) at the principal Couto cave entrance, situated about 50 m from the bank, 275 m a.s.l., 10), in the Morro Preto cave, 305 m a.s.l. (a shady zone, about 30 m from the main entrance) and 11) in the Morro Preto cave (dark zone about 110 m from the principal entrance).

The periods of capture at each site were different. The ecotopes 3, 5, 8, 10, and 11 were sampled from January 2001 to December 2002; 1, 4, 6, 9, from February 2001 to December 2002 and 2 from December 2001 to December 2002.

Diurnal captures were undertaken from 07.00 – 17.00 hours both in the Morro Preto cave- (entrance - shady zone) and in its interior (dark zone), from January 2001 to December 2002.

The Shannon traps were installed monthly (except in March 2001, due to the heavy rains) in forest close to the Betari River banks with modified white and/or black Shannon traps, from 18.00 – 22.00 hours. Two members of the team captured the insects for the investigation of natural infection by flagellates, as described by Galati *et al*. (2003). The insects were captured in flasks which were kept in a polystyrene box lined with humid plaster of Paris until the females could be dissected.

The specimens captured with automatic light traps and the males captured with Shannon traps, after being killed with chloroform, were conditioned in Petri dishes and kept under refrigeration until their clarification by the method described by Forattini (1973) and their identification in accordance with Galati (2003b). A sample of these specimens is deposited in the collection of the Departamento de Epidemiologia of the Faculdade de Saúde Pública, USP.

The nomenclature of the phlebotomines follows Galati (2003a) and the abbreviation of the generic names of the species follows Marcondes (2007).

The pluviometric data of the PETAR were obtained from the Serra dos Motas meteorological station (240 m a.s.l.), Iporanga municipality (Sigrh 2007) and the temperature data of the Eldorado municipality, which borders on the PETAR, from the Centro Integrado de Informações Agrometeorológicas do Instituto Agronômico de Campinas (Ciiagro 2007).

Statistical analysis. The frequencies of the insects captured in simultaneous periods were obtained by Williams' geometric average (Haddow 1960). The χ^2 test was used to assess the different frequencies of some species as between white and black Shannon traps.

The abundance and diversity of the species were obtained on the basis of the data 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).

Pearson's correlation coefficient was used in the search for possible correlations between the Williams' geometric average of the numbers of insects captured monthly in all the ecotopes and the rainfall of the previous 30 days before the monthly capture date, as also between the Williams' average and the average temperature for the month.

RESULTS

With the two techniques, automatic light and Shannon traps, used for the captures in the PETAR, a total of 2,449 specimens of phlebotomines, representing 21 species and four subtribes, were collected: Brumptomyiina - *Brumptomyia cunhai* (Mangabeira, 1942), *Brumptomyia nitzulescui* (Costa Lima, 1932), *Brumptomyia troglodytes* (Lutz, 1922) and *Brumptomyia carvalheiroi* Shimabukuro, Marassá & Galati, 2007; Lutzomyiina - *Evandromyia edwardsi* (Mangabeira, 1941), *Expapillata firmatoi* (Barretto, Martins & Pellegrino, 1956), *Lutzomyia amarali* (Barretto & Coutinho, 1940), *Lutzomyia longipalpis* (Lutz & Neiva, 1912), *Migonemyia* *migonei* (França, 1920), *Pintomyia fischeri* (Pinto, 1926), *Pintomyia misionensis* (Castro, 1959), *Pintomyia monticola* (Costa Lima, 1932) and *Sciopemyia microps* (Mangabeira, 1942); Psychodopygina - *Nyssomyia intermedia* (Lutz & Neiva, 1912), *Nyssomyia neivai* (Pinto, 1926), *Psathyromyia lanei* (Barretto & Coutinho, 1941), *Psathyromyia pascalei* (Coutinho & Barretto, 1940), *Psathyromyia* sp., *Psychodopygus ayrozai* (Barretto & Coutinho, 1940) and *Psychodopygus geniculatus* (Mangabeira, 1941) and Sergentomyiina - *Micropygomyia petari* Galati, Marassá & Gonçalves-Andrade, 2003. Twenty species were captured with the automatic light traps (Table I) and 13 on the Shannon traps, *Pi. misionensis* being captured exclusively by this latter technique (Table VI).

In the captures with automatic light traps undertaken in 11 ecotopes and which yielded a total of 249 samples 2,300 specimens were obtained (Table I), *Pa. pascalei* being the most frequent (26.2%) followed by *Pa. lanei* (25.0%), *Ps. ayrozai* (11.7%), *Pi. fischeri* (8.2%), *Ps. geniculatus* (7.7%), *Ny. intermedia* (4.2%) and *Ev. edwardsi* (3.3%). However, the abundance of the species does not correspond exactly to the order of frequency: *Pa. pascalei* and *Ps. ayrozai* were the only species captured in all the ecotopes, hence justifying their 1st and 2nd positions in the Standardized Index of Species Abundance (SISA), 0.922 and 0.848, respectively, in Table III.

Pa. lanei (SISA = 0.795), *Ps. geniculatus* (0.727) and *Ev. edwardsi* (0.558), which occupied the 3rd, 4th and 6th positions, respectively, in abundance were absent from only one ecotope. The first two from the dark zone of the Morro Preto cave and the last from the entrance of the Couto cave. *Pi. fischeri* (SISA = 0.679; 5th position) and *Ny. intermedia* (SISA = 0.489; 8th position) were not captured in two ecotopes, the first in the two of the Morro Preto cave and the second in the dark zone of the Morro Preto cave and in the entrance of the Couto cave. *Pi. monticola* though present in low frequency (1.8%), occupied the 7th position in abundance (SISA = 0.533) as it was not only captured in the dark zone of Morro Preto cave. At the other extreme was *Lu. longipalpis*, the least frequent and least abundant species, only one specimen being captured in the dark zone of the Morro Preto cave.

The greatest species richness (18) was obtained near the Ficus tree's roots and the rock wall of the Morro Preto outcrop, and the lowest (8) in the dark zone of the Morro Preto cave.

The highest values of Shannon's diversity (H) and evenness (J) indices occurred in the domicile, on the rocks near the entrance of the Santana cave and in the entrance of this same cave, H being 2.20, 2.056, 2.053 and J 0.858, 0.779, 0.856, respectively; the lowest values occurring in the two ecotopes of the Morro Preto cave: the dark zone (H = 1.145; J = 0.551) and the entrance (H = 1.342; J = 0.540).

The greatest species predominance by ecotope occurred with *Ev. edwardsi* in the dark zone of the Morro Preto cave (70.6%). *Pa. pascalei* predominated over the other species in the Morro Preto cave entrance (62.1%), at the top of the track (31.3%), in the rocky wall at the beginning of the Morro Preto outcrop (32.8%) and on the veranda of the domicile (30.9%); *Pa. lanei,* among the rocks near the Santana cave entrance (38.9%), in the Santana cave mouth itself (38.5%), in the Couto cave entrance (37.2%) and near the Ficus tree's roots (28.2%). *Pi. fischeri*, on the edge of the forest on the left bank of the Betari River (34.8%) and *Ny. intermedia* on the camping site (49.3%).

Fig. 1. Localization of the Santana and Morro Preto caves in the PETAR. Source: adapted from Karmann & Ferrari 2002.

The average of sandflies captured per trap in all the ecotopes during the common intervals (December 2001 to December 2002), obtained by the arithmetical and Williams' geometric means (Table I), presented the highest values among the rocks close to the Santana cave mouth (26.08 and 8.59 insects/capture, respectively). The second greatest arithmetical mean (21.00 insects/capture) was found in the traps installed near the Ficus tree's roots, but Williams' geometric mean was smaller (4.86) than those of the rock walls of the Morro Preto outcrop (8.26), the camping site

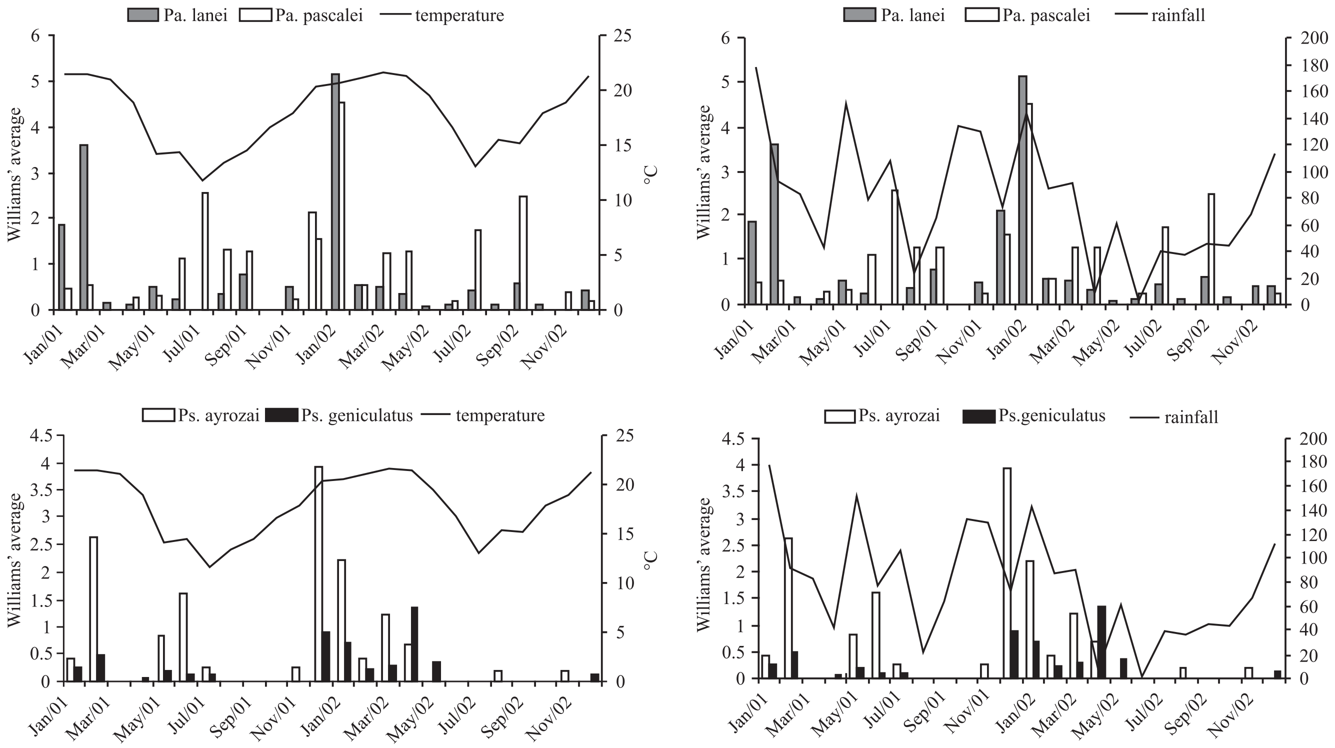

Fig. 2. Monthly distribution of the four most frequent sand fly species: *Pa. lanei*, *Pa. pascalei*, *Ps. ayrozai* and *Ps. geniculatus* in the PETAR from January 2001 to December 2002, rainfall and minimum average temperature of the 30 days before the day of capture.

(5.77) and at the top of the track to the Morro Preto cave (5.32). The smallest values were observed in the Santana cave mouth (arithmetical and geometric means being 1.0 and 0.79 insects/capture, respectively) and in the dark zone of the Morro Preto cave (1.85 and 1.21). In the majority of the ecotopes the values of the arithmetical averages of the period before the common one were close to each other, but were highest in the common period on the camping site and in the entrance of the Morro Preto cave.

In the collections undertaken in the diurnal period in the Morro Preto cave (mouth and interior) only two females of *Pa. pascalei* were captured, and this in January 2002.

The distribution by sex in the total of specimens captured shows more females than males (female/male ratio =1.52:1.0). Only females of *Lu. amarali, Lu. longipalpis*, *Psathyromyia* sp., *Sc. microps* and *Pi. monticola* were captured, this latter species being one the most abundant. Males and females of *Ny. neivai* were captured in approximately equal numbers. Males predominated among the species of *Brumptomyia,* excepting *Br. carvalheiroi*, and among those of *Pa. pascalei* (the male/female ratio varying from 1.5:1.0 to 2.1:1.0), and in the 11 remaining species the females predominated (the female/male ratio varying from 1.15:1.0 to 11.0: 1.0).

The monthly distribution is only presented for the most abundant species: *Pa. lanei*, *Pa. pascalei*, *Ps. ayrozai* and *Ps. geniculatus*, in Fig. 2. When Pearson's Correlation coefficients were calculated as between the Williams' geometrical average of both sexes captured monthly with automatic light traps in all the ecotopes of PETAR in relation to the monthly rains during the 30 days before the capture day and the monthly minimum average temperature (n = 24), a positive statistically significant correlation was obtained in relation to the rains for *Pa. lanei* (r = 0.50, p = 0.012) and *Ps. ayrozai* (r = 0.57, p = 0.004), but low positive correlation and no statistical significance for *Pa. pascalei* (r = 0.09, p = 0.678) and *Ps. geniculatus* (r = 0.16, p = 0.462). As regards temperature, a low negative correlation was found for *Pa. pascalei* (r = - 0.176, p = 0.411) and a positive one for *Pa. lanei* (r = 0.378, p = 0.069), *Ps ayrozai* (r = 0.324, p = 0.122) and *Ps. geniculatus* (r = 0.502, p = 0.012), only this latter, however, being of statistical significance.

In 21 collections undertaken simultaneously with black and white Shannon traps a total of 107 specimens belonging to 13 species were captured, twelve of them on the black and six on the white trap (Table IV). A greater number of specimens was captured on the black traps (67) than on the white (40), the difference being statistically significant (χ^2 = 6.81; p < 0.01). Traps of both colors captured more females than males, but the difference between the number of specimens of each sex collected was greater on the white (white female/male ratio = 12.33:1.0; χ^2 = 28.9; p < 0.001) than on the black (female /male ratio = 2.53: 1.0; χ^2 = 12.55; p < 0.001. On the black trap, both sexes of *Ps. geniculatus* predominated (male, 42.1% and females, 43.75%) in relation to those of the other species, but only the females presented a statistically significant difference over those of the other species, *Ps. geniculatus* being captured 2.63 times more than those of *Pi. fischeri*, the second most frequent (χ^2 = 5.83; p < 0.02). On the white traps, females of two species, *Pa. lanei* and *Ps. ayrozai*, predominated equally (32.4%); the difference

Table I. Number of both sexes of phlebotomines captured in the PETAR with automatic light traps by species and ecotope, between January 2001 and December 2002, and the averages of the total and simultaneous periods of capture, the Williams' geometric mean of the simultaneous period of capture, the Shannon diversity (H) and the Pielou evenness (J) indices by ecotope.

Species / Ecotope	Santana cave entrance	Rocks close to Santana entrance	Camp site	Forest edge	Ficus tree roots/ rocks	Rocky wall - M. Preto outcrop	Couto cave entrance	Top of the track	Morro Preto cave entrance	M. Preto cave dark zone	Domicile veranda	Total	%
N. captures	24	13	23	24	23	24	23	24	24	24	23	249	
Br. carvalheiroi	-	1	-	2	2	3	-	3	1	-	-	12	0.522
Br. cunhai	-	22	12	-	12	18	1	2	-	1	1	69	3.000
Br. nitzulescui	-	10	1	-	5	6	3	8	-	1	4	38	1.653
Br. troglodytes	-	2	-	-	2	1	2	3	-	-	5	15	0.652
Ev. edwardsi	2	12	1	1	5	10	-	2	18	24	2	77	3.348
Ex. firmatoi	-	-	-	-	3	2	-	4	2	1	-	12	0.522
Lu. amarali	1	-	-	-	3	1	-	-	1	-	-	6	0.261
Lu. longipalpis	-	-	-	-	-	-	-	-	-	1	-	1	0.044
Mi. petari	1	10	-	-	1	24	-	5	2	1	-	44	1.913
Mg. migonei	-	6	-	1	17	1	-	8	-	-	5	38	1.652
Ny. intermedia	2	12	67	2	3	2	-	3	3	-	3	97	4.217
Ny. neivai	1	-	10	9	2	1	-	1	-	-	-	24	1.044
Pa. lanei	10	132	1	19	137	144	32	92	2	-	6	575	25.000
Pa. pascalei	2	35	9	12	105	161	27	110	113	3	25	602	26.174
Pa. sp.	-	-	-	-	3	1	-	4	3	-	-	11	0.478
Pi. fischeri	1	24	18	31	42	20	11	31	-	-	11	189	8.217
Pi. monticola	2	9	3	3	6	9	1	6	2	-	1	42	1.826
Ps. ayrozai	2	45	4	6	61	58	4	51	26	2	9	268	11.652
Ps. geniculatus	2	19	10	3	76	29	4	18	9	-	8	178	7.739
Sc.microps	-	-	-	-	-	-	1	-	-	-	1	2	0.087
Total	26	339	136	89	485	491	86	351	182	34	81	2300	100.000
Average (total period)	1.08	26.08	5.91	3.71	21.09	20.46	3.74	14.63	7.58	1.42	3.52	9.24	
Average (simult. period)	1.00	26.08	9.23	3.31	21.00	19.69	5.08	14.00	11.69	1.85	3.08	9.62	
Williams' geom. mean (simultaneous period)	0.79	8.59	5.77	2.05	4.86	8.26	1.91	5.32	3.49	1.21	1.50	3.16	
H	2.053	2.056	2.015	1.881	2.016	1.970	1.503	1.964	1.342	1.145	2.200	2.174	
J	0.856	0.779	0.841	0.784	0.697	0.682	0.653	0.693	0.540	0.551	0.858	0.779	

between the frequency of these species and that of the second most frequent, *Pi. fischeri* (16.2%) being without significance ($\chi^2 = 2.0$; $p > 0.05$), but statistically significant in relation to that of the third most frequent *Ps. ayrozai* (10.8%); ($\chi^2 = 4.0$; $p < 0.05$).

Beyond these captures, two others (in January and February 2001) were undertaken with black traps only; during the former month heavy rain fell and no insect was captured, in the latter month a total of 42 specimens of two species, *Ps. ayrozai* (2 males and 26 females) and *Ps. geniculatus* (1 male and 13 females) were collected. Thus with this color a total of 149 sand flies were captured and the numbers of females of *Ps. geniculatus* (34) and *Ps. ayrozai* (32) presented no statistically significant difference ($\chi^2 = 0.25$; $p > 0.90$), the two species being practically equally attracted to the black Shannon traps.

Another 36 specimens (*Pa. lanei* 1 F, *Ps. ayrozai* 25 F and *Ps. geniculatus* 1 M, 9 F) were captured landing on the researchers while these were positioned in the Shannon traps. Thus *Ps. ayrozai* was 2.8 times more attracted to humans than was *Ps. geniculatus* ($\chi^2 = 7.53$; $p < 0.01$).

A total of 66 females belonging to the following species: *Br. cunhai* (5), *Br. nitzulescui* (4), *Br. carvalheiroi* (1), *Ev. edwardsi* (7), *Mi. petari* (1), *Mg. migonei* (1), *Ny. neivai* (1), *Pi. fischeri* (8), *Pi. monticola* (3), *Pa. lanei* (13), *Pa. pascalei* (7), *Psathyromyia* sp. (1), *Ps. ayrozai* (10) and *Ps. geniculatus* (4) were captured with automatic light or Shannon traps and dissected for investigation of flagellates in their digestive tracts, with negative results.

DISCUSSION

Among the sand fly fauna captured in the PETAR, apart from *Br. cunhai*, *Lu. longipalpis* and *Pi. misionensis* - not previously registered in the Ribeira Valley, all the other species had been reported in one or other of the series of ecological studies undertaken in this region of the São Paulo state (Forattini *et al*. 1976; Gomes *et al.* 1978, 1980, 1982, 1983, 1986, 1990; Gomes & Galati 1987, 1989; Domingos *et* al. 1998; Taniguchi *et al.* 2002; Andrade-Filho *et al.* 2007; Galati *et al.* 2003). Although neither of the two last species had been found in the Atlantic forest on the north coast of São Paulo state, *Br. cunhai* had (Brito *et al.* 2002).

On the other hand, some species found in other areas of the Ribeira Valley, such as *Brumptomyia bragai* Mangabeira & Sherlock, 1961, *Bichomomyia flaviscutellata*

Table II. Number of phlebotomines captured in the PETAR with automatic light traps by species, sex and ecotope, between January 2001 and December 2002.

Ecotope / Species	Santana cave entrance		Rocks close to Santana entrance		Camp site		Forest edge		Ficus tree roots/ rocks		Rocky wall of the M. Preto outcrop		Couto cave entrance		Top of the track		Morro Preto cave entrance		M. Preto cave dark zone		Domicile veranda		Total	
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
Br. carvalheiroi	-	-	-	1	-	-	-	2	-	2	-	3	-	-	1	2	-	1	-	-	-	-	1	11
Br. cunhai	-	-	21	1	8	4	-	-	8	4	7	11	-	1	1	1	-	-	1	-	1	-	47	22
Br. nitzulescui	-	-	6	4	1	-	-	-	1	4	4	2	2	1	5	3	-	-	1	-	4	-	24	14
Br. troglodytes	-	-	2	-	-	-	-	-	2	-	1	-	1	1	1	2	-	-	-	-	2	3	9	6
Ev. edwardsi	-	2	1	11	-	1	1	-	-	5	2	8	-	-	-	2	5	13	7	17	1	1	17	60
Ex. firmatoi	-	-	-	-	-	-	-	-	-	3	-	2	-	-	1	3	-	2	-	1	-	-	1	11
Lu. amarai	-	1	-	-	-	-	-	-	-	3	-	1	-	-	-	-	-	1	-	-	-	-	-	6
Lu. longipalpis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Mi. petari	-	1	-	10	-	-	-	-	-	1	3	21	-	-	2	3	1	1	-	1	-	-	6	38
Mg. migonei	-	-	1	5	-	-	-	1	3	14	-	1	-	-	-	8	-	-	-	-	1	4	5	33
Ny. intermedia	1	1	9	3	24	43	-	2	2	1	1	1	-	-	3	-	2	1	-	-	3	-	45	52
Ny. neivai	1	-	-		4	6	4	5	2	-	1	-	-	-	-	1	-	-	-	-	-	-	12	12
Pa. lanei	2	8	52	80	-	1	2	17	37	100	42	102	4	28	22	70	-	2	-	-	2	4	163	412
Pa. pascalei	2	-	32	3	4	5	7	5	79	26	105	56	19	8	73	37	58	55	3	-	16	9	398	204
Pa. sp.	-	-	-	-	-	-	-	-	-	3	-	1	-	-	-	4	-	3	-	-	-	-	-	11
Pi. fischeri	-	1	-	24	3	15	2	29	9	33	-	20	2	9	4	27	-	-	-	-	1	10	21	168
Pi. monticola	-	2	-	9	-	3	-	3	-	6	-	9	-	1	-	6	-	2	-	-	-	1	-	42
Ps. ayrozai	1	1	11	34	-	4	1	5	19	42	26	32	2	2	13	38	12	14	1	1	7	2	93	175
Ps. geniculatus	1	1	12	7	7	3	1	2	30	46	9	20	1	3	5	13	2	7	-	-	1	7	69	109
Sc. microps	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	2
Total	8	18	147	192	51	85	18	71	192	293	201	290	31	55	131	220	80	102	13	21	39	42	911	1389

(Manganeira, 1942), *Micropygomyia ferreirana* (Barretto, Martins & Pellegrino, 1956 (= *Mi. borgmeieri*; Galati *et al.* 2003); *Evandromyia petropolitana* (Martins & Silva, 1968), *Migonemyia rabelloi* (Galati & Gomes, 1992) and *Migonemyia vaniae* Galati, Fonseca & Marassá, 2007, this latter described from the Serra district, Iporanga municipality (Galati *et al.* 2007), were not captured in the PETAR.

Two other species had been reported for the Ribeira Valley region: *Pa. shannoni* (Dyar, 1929) and *Ps. guyanensis* (Floch & Abonnenc, 1941). The former was wrongly identified, being in fact *Pa. pestanai.* The second was considered as senior synonym of *Ps. geniculata* by Forattini (1973), but in accordance with Young & Duncan (1994), *Ps. guyanensis* is a valid species and has its distribution restricted to French Guyana (Domingos *et al.* 1998).

Comparing the phlebotomine fauna and its behavior in PETAR with those of the Parque Estadual Intervales (PEI), both parks belonging to the same ecological continuum, but whose sites were sampled at different altitudes, 260–305 m and 780–950 m, respectively, several differences and some similarities were observed: i) though in both of the areas a total of 21 species were captured, only 17 were common to both: *Br. cunhai*, *Br. troglodytes*, *Br. carvalheiroi*, *Ev. edwardsi*, *Ex. firmatoi, Mg. migonei*, *Mi. petari*, *Ny. intermedia*, *Ny. neivai*, *Pi. fischeri*, *Pi. monticola*, *Pa. lanei*, *Pa. pascalei*, *Psathyromyia* sp., *Ps. ayrozai*, *Ps. geniculatus* and *Sc. microps*. Four species were captured exclusively in the PETAR: *Br. nitzulescui*, *Lu. amarali*, *Lu. longipalpis* and *Pi. misionensis* and four others in the PEI: *Br. bragai*, *Ev. correalimai*, *Mg. rabelloi* and *Sc. sordellii*; ii) the mean number of insects per trap during the same period of capture (with automatic light traps) in the PETAR was 9.24, while in the PEI it was 1.92 ($\chi^2 = 4.8$; $p < 0.05$), these difference being most evident in the extradomiciliary areas, since on the veranda of the respective domicile sampled in the PETAR and the PEI this number is quite similar (3.19 and 3.52, respectively); iii) although the species richness is not greatly different, 20 species in PETAR and 18 in PEI, the most abundant species in the PETAR is *Pa. pascalei* and that in the PEI, *Br. troglodytes*. The 2nd and 4th most abundant in the PETAR, *Ps. ayrozai* and *Ps. geniculatus* respectively, are among those of low abundance in the PEI. Coincidentally, *Pa. lanei* had the second greatest frequency in both parks; iv) in the dark zone of the Morro Preto cave (PETAR) twice as many insects were captured as in the Colorida cave (PEI), 34 and 17, respectively; in the mouth of Morro Preto 13 times more insects were captured than in the Minotauro cave (PEI), 182 and 14, respectively; v) in the captures with Shannon traps and on the researchers females of *Ps. ayrozai* and *Ps. geniculatus* predominated in PETAR, while in the PEI the predominance was *Pi. monticola*'s. A series of other differences may be cited, but noteworthy in the PETAR was the capture of *Lutzomyia longipalpis,* the main vector of visceral leishmaniasis in the Americas, and of *Ny. intermedia*, and *Ny. neivai*, important vectors of cutaneous leishmaniasis in the Brazilian Southeast region, and the most frequent on the open camping site.

Lu. longipalpis, although widely distributed from Mexico to Argentina, mainly in savannah/cerrado, caatinga and semi-arid vegetation, and despite its being common in coastal areas of the Brazilian Northeast region and some others of the Southeast, such as the state of Rio de Janeiro, had not been found in the moist Atlantic forest of the Serra do Mar/ Paranapiacaba in São Paulo state, before this project. The finding of a female in the dark zone of the Morro Preto cave and one male in the Shannon trap installed close to the Santana cave suggests its association with this ecotope. Thus it is possible that during the cold, dry Würm-Wisconsin glacial period of the Pleistocene (13,000 and 20,000 years B.P.), populations of this species had dispersed throughout the area covered by semi-arid vegetation on the southeastern coast of Brazil, one of the three broad routes of expansion provided by this open vegetation formation (Brown & Ab' Saber, 1979). When this period finished and there was dispersion of dense and humid vegetation throughout the speleological province of the Ribeira Valley, populations of this sand fly were preserved in caves. Thus, probably the gene flux among other South American populations has been interrupted since then. As *Lu. longipalpis,* has, throughout the Americas, been considered a cryptic species-complex, consisting of at least four clades, the majority of Brazilian populations constituting one of them (Arrivillaga *et al.* 2003), the analysis of specimens of this speleological province will perhaps help us better to understand the relationship among the various South American populations.

The sympatric occurrence of *Ny. intermedia* and *Ny. neivai* in the Ribeira Valley had already been reported (Marcondes *et al.* 1998) after the latter species, previously considered a junior synonym of the former, had been validated by Marcondes (1996). The predominance of these two species at the camping site (a narrow open grassy strip) was not a surprise, since *Ny. intermedia s. lat,* had been at least 8 times more frequently captured on the edge of forest than within it in Pariquera Açu (Gomes & Galati 1987), and 3.9 times more frequently in the same circumstances in Pedro de Toledo (Domingos *et al.* 1998), both municipalities in the Ribeira Valley region. In the southwestern region of São Paulo state this same value was 5.1 times (Condino *et al.* 1998). In this latter area this taxon has been identified as *Ny. neivai* (Marcondes *et al.* 1998). Thus the presence of these two species at the camping site of Petar where people spend the night may expose the latter to the risk of infection by *Leishmania* since these sandflies are anthropophilic, as demonstrated for *Ny. neivai* by Gomes *et al.* (1989) and Pinto *et al.* (2001) and for *Ny. intermedia* by Aguiar *et al.* (1996) and Souza *et al.* (2001, 2005); they have also been found to be naturally infected by trypanosomatids: *Ny. neivai* by Forattini & Santos (1952), Forattini *et al.* (1972), Casanova *et al.* (1995) and Córdoba-Lanus *et al.* (2006) and *Ny. intermedia* by Aragão (1922), Rangel *et al.* (1984) and Pita-Pereira *et al*. (2005), and each species occurs with the highest frequencies in allopatric areas in the state of São Paulo (Marcondes *et al.* 1998) where the transmission of ACL occurs (Camargo-Neves *et al.* 2002).

The greatest frequencies of *Pa. pascalei* at the sites situated on the track to Morro Preto cave, including the Morro Preto cave mouth, and the domicile, all these in hilly areas, suggest a higher concentration of armadillo borrows in this terrain, since this sand fly is clearly associated with these ecotopes (Galati *et al.* 2010). Similar results were

Table III. Standardized Index Species Abundance obtained in the captures with automatic light traps in eleven ecotopes of the PETAR, between January 2001 to December 2002.

Species	ISA	SISA	Position
Br. cunhai	11.04545	0.442	9th
Br. nitzulescui	11.13636	0.437	10th
Br. carvalheiroi	15.36364	0.202	16th
Br. troglodytes	15.13636	0.215	15th
Ev. edwardsi	8.954545	0.558	6th
Ex. firmatoi	14.90909	0.227	14th
Lu. amarali	16.59091	0.134	18th
Lu. longipalpis	17.81818	0.066	20th
Mi. petari	12.86364	0.341	11th
Mg. migonei	13.86364	0.285	12th
Ny. intermedia	10.19091	0.489	8th
Ny. neivai	14.72727	0.237	13th
Pa. lanei	4.681818	0.795	3rd
Pa. pascalei	2.409091	0.922	1st
Pa. sp.	16.13636	0.159	17th
Pi. fischeri	6.772727	0.679	5th
Pi. monticola	9.409091	0.533	7th
Ps. ayrozai	3.727273	0.848	2nd
Ps. geniculatus	5.909091	0.727	4th
Sc. microps	17.45455	0.086	19th

observed by Aguiar *et al.* (1985) who captured *Psathyromyia barrettoi* (Mangabeira, 1942), also in light traps, a species that presents a close affinity with *Pa. pascalei*, presenting its highest frequency (46.0%) on the Serra dos Órgãos (380 m a.s.l.), state of Rio de Janeiro. On the other hand, Marcondes *et al.* (2001) captured *Pa. pascalei* with CDC traps in low frequencies (1.68% in canopy and 1.85% on the ground) in an Atlantic forest fragment in the low-lying eastern part of the Paraná state (10 m a.s.l).

The soil among the rocks close to the principal Santana cave mouth, among ficus tree roots and on the Morro Preto hill, seems to offer adequate breeding or resting sites for the anthropophilic species *Mg. migonei*, *Pi. fischeri*, *Pi. monticola*, *Pa. lanei*, *Ps. ayrozai* and *Ps. geniculatus* and also for *Mi. petari* which probably bites cold-blooded animals.

Of the two specimens of *Lu. longipalpis* captured in PETAR, one was caught in the dark zone of the Morro Preto cave and the other on a Shannon trap in front of the Santana cave mouth. *Ev. edwardsi*, having a frequency of 70.5% in the dark zone of the Morro Preto cave, also seems to be a species adapted to living in caves. But as this latter species was also captured in the external environment, they may both be considered troglophiles.

As the Morro Preto cave has a large entrance, many species may use the area as a resting site or be attracted by the light of the trap. As regards the predominance of *Pa. pascalei* (61.0%), the possibility of armadillo borrows in the soil in its interior, or very close to it, cannot be ignored since both sexes were captured in practically equal numbers; while at the other sites generally the males predominated.

As regards the association with the rains during the 30 days before the capture, all the four most abundant species showed positive correlations, but only *Pa. lanei* and *Ps. ayrozai* presented statistical significance ($p < 0.05$). In relation to the temperatures of the months, as distinct from the other three species which presented positive correlation, that for *Pa. pascalei* was negative, although without statistical significance. This probably occurred due to the increase of its population in the armadillo borrows during the driest and coldest period, as observed in the PEI (Galati *et al.* 2010). The highest frequencies observed in the Serra dos Órgãos, Rio de Janeiro state, from June to August, mainly in July, by Aguiar *et al*. (1985) for *Pa. barrettoi*, a species also belonging to the same subgenus (*Forattiniella*) of *Pa. pascalei,* seems to confirm that the coldest period is the most favorable for the capture of adults of this group of insects.

The Shannon traps tend to capture more anthropophilic species. Among the 13 species captured on these traps, only *Ev. edwardsi* and *Mi. petari* are known as non-anthropophilic. Thus the higher frequencies of *Ps. ayrozai* and *Ps. geniculatus* suggest that they are the most bothersome species to humans in the PETAR, confirmed by their capture while biting humans. *Ps. ayrozai,* also found in the Atlantic forest of the Ribeira Valley, São Paulo state (Gomes & Galati 1989) and on the Serra dos Órgãos, Rio de Janeiro state (Aguiar *et al.* 1985) has presented a high degree of anthropophily, 69.3% and 57.5%, respectively, of the specimens being attracted by human bait at ground level; but though less anthropophilic in Amazonia, it is suspected of transmitting *Leishmania naiffi,* for which armadillos are the reservoir, to humans (Rangel & Lainson 2003). In forests of the Bahia states it was found to be predominant species (31.15%) (Vexenat *et a*l. 1986) and was highly susceptible to experimental infection by *Leishmania* (*L.*) *forattinii* Yoshida *et al.* 1993 from a wild rodent (Barretto *et al.* 1986; Lainson & Shaw, 2005). The positive correlation of *Ps. ayrozai* with the most humid period was also observed by Aguiar *et al.* (1985) and Marcondes *et al.* (2001).

Whereas in several other Atlantic and Amazonian areas *Ps. geniculatus* has been frequently captured, although with relatively low frequencies, in the Atlantic forest of Paraná state it has been found to be the second most prevalent species. Its role as vector of *Leishmania* has not yet, as far as we know, been demonstrated. The highest values for the capture of *Ps. geniculatus* occurred in April, as has also been recorded by Marcondes *et al.* (2001).

Pa. lanei, the third most abundant species captured with automatic light traps and the fourth on Shannon traps, also captured biting humans, though it is commonly found in other areas of the Ribeira Valley (Gomes & Galati 1987, 1989; Gomes *et al.* 1990), has had no role as a vector of *Leishmania* attributed to it.

Pi. fischeri was not captured biting humans in the PETAR, but was the fifth in abundance in the captures with automatic light traps and the third on the Shannon traps. Although its role as vector of *Leishmania* has not been conclusively established since it has not been found naturally infected by *Leishmania*, because of its high degree of anthropophily and its abundance in areas with transmission of cutaneous leishmaniasis it may be considered as a secondary vector (Rangel & Lainson 2003).

In brief, comparing the results obtained in the two years of captures in the two reserves, PETAR and PEI (the other sampled area of the ecological continuum of the Ribeira Valley), the mean number of insects collected with automatic traps in the former was significantly greater than that in the latter. Though the most abundant species captured in both areas present a close association with armadillos, in the PETAR, the known anthropophilic species, *Ps. ayrozai*, *Ps. geniculatus*, *Ny. intermedia, Ny. neivai, Pi. fischeri, Mg. migonei* and *Lu. longipalpis*, were more numerous than in the PEI, the opposite occurred with *Pi. monticola*, also anthropophilic. Thus while a greater number of species may bother visitors to the PETAR, all of them are known to present nocturnal habits, while this last species also bites in the diurnal period. Among the species captured in the PETAR, *Lu. longipalpis* and those of the genus *Nyssomyia* call for special attention due to their respective involvement in the American visceral and cutaneous leishmaniases. Though the frequencies of these species were not very high in the preserved environments sampled, it is also necessary to investigate neighboring deforested areas with established human and domestic animal populations, in which these vectors may find more food sources as well as more adequate conditions for their development, with a consequent increase in the risk of transmission of these parasite diseases.

Table IV. Number of phlebotomines, by species and sex, captured on the black or white Shannon traps in the Santana nucleus – PETAR, from April 2001 to December 2002.

Species / Trap, Sex	Black		White		Total			
	M	F	M	F	M	F	MF	%
Ev. edwardsi	-	1	-	-	-	1	1	0.94
Lu. longipalpis	1	-	-	-	1	-	1	0.94
Mi. petari	1	-	-	-	1	-	1	0.94
Mg. migonei	-	-	-	1	-	1	1	0.94
Ny. intermedia	4	2	-	-	4	2	6	5.60
Ny. neivai	1	-	-	-	1	-	1	0.94
Pa. lanei	-	1	-	12	-	13	13	12.15
Pa. sp.	-	1	-	-	-	1	1	0.94
Pi. fischeri	-	8	1	6	1	14	15	14.00
Pi. misionensis	-	1	-	-	-	1	1	0.94
Pi. monticola	-	7	-	2	-	9	9	8.41
Ps. ayrozai	4	6	-	12	4	18	22	20.55
Ps. geniculatus	8	21	2	4	10	25	35	32.71
Total	19	48	3	37	22	85	107	100.00

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