Sandflies (Diptera: Psychodidae) in rural and urban environments in an endemic area of cutaneous leishmaniasis in southern Brazil

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The high proportion of cases of cutaneous leishmaniasis reported amongst residents in the city of Bandeirantes, in the state of Paraná, Brazil, led the authors to investigate the phlebotomine fauna in both urban and rural environments. The sandflies were captured with automatic light traps from 07:00 pm-07:00 am fortnightly in 11 urban peridomiciles from April 2008-March 2009 and monthly in three ecotopes within four rural localities from April 2009-March 2010. In one of these latter localities, sandfly capture was conducted with white/black Shannon traps during each of three seasons: spring, summer and fall. A total of 5,729 sandflies of 17 species were captured. Nyssomyia neivai *(46.7%) and* Nyssomyia whitmani *(35.3%) were the predominant species. In this study, 3,865 specimens were captured with automatic light traps: 22 (0.083 sandflies/trap) in the urban areas and 3,843 (26.69 sandflies/trap) in the rural areas.* Ny. neivai *was predominant in urban (68.2%) and rural (42.8%) areas. A total of 1,864 specimens were captured with the white/black Shannon traps and* Ny. neivai *(54.5%) and* Ny. whitmani *(31.4%) were the predominant species captured. The small numbers of sandflies captured in the urban areas suggest that the transmission of* Leishmania *has occurred in the rural area due to* Ny. neivai *and* Ny. whitmani *as the probable vectors.*

Key words: cutaneous leishmaniasis - *Leishmania (Viannia) braziliensis* - sandflies - vectors

Leishmaniasis ranks second among the six most common infectious and parasitic diseases in the world and approximately 350 million people are at risk for infection. As many as 12 million people are believed to be currently infected, with approximately one-two million estimated new cases occurring every year (WHO 2010).

With the exception of Oceania and Antarctica, all continents have reported human cases of leishmaniasis. Cutaneous leishmaniasis constitutes a public health problem in 88 countries on four continents (the Americas, Europe, Africa and Asia) (WHO 2010). In Brazil, American cutaneous leishmaniasis (ACL) is a dermatological disease that calls for special attention due to the consequent risk of deformities and psychological effects. ACL affects both the social and economic lives of patients. ACL is widespread in Brazil and cases have been reported in all regions of the country. It affects both sexes and all age groups; however, 90% of ACL cases occur in patients over 10 years old and 74% in males. ACL is considered an occupational disease (MS 2007).

A total of 260,486 ACL cases were reported in Brazil between 2000-2009: 6,625 (2.6%) occurred in the southern region and 6,010 (90.7%) of those occurred in the state of Paraná (PR) [Health Informatics Department of the Brazilian Ministry of Health (datasus.gov.br/DATASUS/index.php)] (MS 2010). In this state, the majority of the cases have been concentrated in the northern areas since 1940 when an extensive clearing of native vegetation (dense and transitional tropical/subtropical forest) began (Teodoro et al. 1991). More recently, the disease has been associated with areas of intense soybean, corn and sugar cane cultivation and pastures near vestigial forests (Lonardoni et al. 2006).

Studies of the sandfly fauna in PR have been undertaken more extensively in the northern region, where the greatest numbers of ACL cases have been registered (Gomes & Galati 1977, Teodoro et al. 1991, 1993a, b, 2001, 2006, Teodoro & Kuhl 1997, Oliveira et al. 2000, Massafera et al. 2005). *Nyssomyia neivai* (Pinto, 1926), *Nyssomyia whitmani* (Antunes & Coutinho, 1939), *Pintomyia pessoai* (Coutinho & Barretto, 1940), *Pintomyia fischeri* (Pinto, 1926) and *Migonemyia migonei* (França, 1920) are the most commonly suspected vectors of *Leishmania* sp. found in PR (Zanzarine et al. 2005). *Ny. neivai* (denoted as *Lutzomyia intermedia, s.lat.* in many studies) and *Ny. whitmani* are consistently present in high numbers in peridomestic and domestic areas in most parts of northern PR (Teodoro et al. 1993a, b, Teodoro & Kuhl 1997, Oliveira et al. 2000, Massafera et al. 2005).

The high urban prevalence of ACL cases (81/123; 66%) between 2000-2009 in the municipality of Bandeirantes (Cruz 2010) created the need to investigate the behaviours of these insects in both urban and rural environments to better understand the epidemiology of the disease.

MATERIALS AND METHODS

Area of study - The municipality of Bandeirantes is located in northern PR ("Old North"). The town of Bandeirantes is located at 23º06'36"S 50º27'28"W and 420 m above sea level (asl). In 2010, the municipality had 32,182 inhabitants and the urban population was 28,382

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[Brazilian Institute of Geography and Statistics (IBGE) (ibge.gov.br/cidadesat/topwindow.htm?1)].

The municipality has an area of 446,301 km², including the urban area of 12 km². The soil is a ferruginous clay composed of basalt, also known as red soil (Panchoni et al. 2008).

The annual rainfall ranges from 1,300-1,400 mm, with approximately 150-200 mm in January and approximately 50-75 mm in June and July. The prevailing winds vary from southwesterly to northwesterly with an average speed of 2.5 m/s. The climate is subtropical with hot summers and infrequent frosts during the winter in the lowlands. High levels of rainfall occur in the summer months, but there is no clearly defined dry season. The average annual temperature is 21ºC, the monthly average in the summer is between 22-28ºC and the monthly average during the winter is between 14-18ºC (Panchoni et al. 2008).

The residual native vegetation of Bandeirantes consists of Atlantic semideciduous forest [Secretary of Environment (sema.pr.gov.br/)]. The basis of the municipal economy is sugar cane cultivation [IBGE (ibge.gov.br/cidadesat/topwindow.htm?1)].

Capture sites – Cases of cutaneous leishmaniasis were reported between 2000-2009 in all of the sampling sites. Eleven urban sites were sampled: (1) Itapeva smallholding (near the old piggery), (2) Souto smallholding (henhouse), (3) União suburb (a tree in the backyard and several dogs), (4) Lordani neighbourhood (deposit of recyclable materials), (5) Humberto Teixeira neighbourhood (jaboticaba tree close to dwelling), (6) house in front of the cemetery, (7) Clubhouse of the Athletic Association of the Bank of Brazil (avocado tree near the house of the caretaker), (8) Bela Vista neighbourhood (guava tree in the peridomicile near a sugarcane plantation), (9) Faculties Luis Meneghel (FALM) (tree next to the Nursing department), (10) rural village one (avocado tree) and (11) rural village two (pigsty). Although points 10 and 11 are called "rural villages", both are included in the urban area of Bandeirantes and are located approximately 3 km from the city centre. In the rural area, three ecotopes in each of the following four neighbourhoods were investigated. (1) Jacutinga locality - the ecotopes sampled are situated in a fishing are (a large leisure area open to visitors from Tuesday-Sunday; on weekends the number of visitors is higher and they usually remain until late at night): (1.1) pigsty (10 m from the house and 130 m from the forest edge), (1.2) forest edge (under a guava tree, 120 m from the dwelling and 200 m from the lake) and (1.3) peridomicile. (2) Tabuleta - this locality consists of a small social centre with a church, soccer field and several plots of land where sugar cane, coffee, alfalfa, soybeans and corn, among other crops, are cultivated; the residence of an ACL case is also located there. The ecotopes sampled were: (2.1) a tree close to the veranda of a dwelling, (2.2) peridomicile, an avocado tree used as a perch by chickens and (2.3) peridomicile, a tree close to the pigsty. (3) Perobinha - in this locality there is a village with approximately 40 plots of land (5,000 m²) where families grow fruits and vegetables and breed pigs, poultry and cattle. This locality also has a social centre with a primary school, church, meeting hall and soccer field and the dwelling of an ACL case is also found there. The ecotopes sampled were: (3.1) peridomicile, under a jaboticaba tree close to the house, (3.2) under an orange tree close to a pigsty and a dwelling and (3.3) a bamboo grove 10 m from the dwelling and next to a pasture, a pond and a small forest. Lastly, (4) Cabiúna - where there are settlements and a small social centre with a bar, school, church and soccer field. The ecotopes sampled were: (4.1) a pigsty close to a dwelling, (4.2) a pigsty situated at the forest edge (50 m from the same dwelling) and (4.3) the forest edge (under a tree and 50 m away from point 4.2).

Collection and identification of sandflies - Sandflies were captured in the urban areas using automatic light traps (Galati et al. 2010) installed fortnightly between 06:00 pm-07:00 am from April 2008-March 2009 in 11 peridomiciles. In the rural areas, two techniques were used: automatic light traps installed one day per month at the 12 points sampled from 06:00 pm-07:00 am from April 2009-March 2010 and at one of these sites (Jacutinga neighbourhood - forest edge of the fishing area), a modified black and white Shannon trap was also used (Galati et al. 2001). This trap was used once during each of the following seasons: spring 2009 (06:00 pm-03:00 am), summer 2010 (06:00 pm-04:00 am) and fall 2010 (06:00 pm-06:00 am). The captures in the spring and summer were interrupted before 06.00 am due to heavy rains with winds.

The insects were sacrificed with chloroform and packed into entomological boxes. At the Public Health Entomology Laboratory/Phlebotominae of the Department of Epidemiology, School of Public Health, University of São Paulo, the sandflies were separated from the other insects captured. After clarification, they were identified in accordance with Galati's keys (Galati 2003).

Statistical analysis - The numerical abundance according to the spatial distribution of sandfly species was calculated based on the Standardized Index of Species Abundance (SISA) (Roberts & Hsi 1979) and the values that are closest to 1.0 represent the most abundant species. The index was calculated for all the species collected using the CDC traps in rural localities. The Williams' geometric average (Haddow 1960), which reflects the frequency and regularity of the species in the collections, was used to evaluate the activity of the most abundant sandfly species. The Shannon diversity index, which indicates the diversity and frequency of the sandfly species at particular capture sites, and Pielou's evenness index were obtained in accordance with the formulas presented by Service (1993).

The pluviometric data and temperature were obtained from the meteorological station closest to Bandeirantes (50 km away), which was situated in the municipality of Ourinhos, in the state of São Paulo, from the Agrometeorology Information Center (ciiagro.sp.gov.br/dados/entrada.htm).

RESULTS

Using the two techniques (automatic light and Shannon traps) in the urban (11 sites) and rural environments (13 sites), 5,729 sandflies belonging to 17 species of four subtribes were captured: Brumptomyiina - *Brumptomyia brumpti* (Larrousse, 1920), *Brumptomyia cunhai* (Mangabeira, 1942) and *Brumptomyia nitzulescui* (Costa Lima, 1932), Lutzomyiina - *Evandromyia correalimai* (Martins, Coutinho & Luz, 1970), *Evandromyia cortelezzii* (Brèthes, 1923), *Expapillata firmatoi* (Barretto, Martins & Pellegrino, 1956), *Mg. migonei*, *Pintomyia christenseni* (Young & Duncan, 1994), *Pi. fischeri*, *Pi. pessoai* and *Sciopemyia sordellii* (Shannon & Del Ponte, 1927), Psychodopygina - *Ny. neivai, Ny.whitmani* and *Psathyromyia* sp. (Floch & Chassignet, 1947) and Sergentomyiina - *Micropygomyia ferreirana* (Barretto, Martins & Pellegrino, 1956) and *Micropygomyia longipennis* (Barretto, 1946) (Table I).

In the urban area, 264 collections with automatic light traps were performed, but only 22 specimens (0.083 sandflies/trap) belonging to six species were captured: *Br. brumpti*, *Ev. cortelezzii*, *Ny. neivai*, *Ny. whitmani*, *Pi. pessoai* and *Sc. sordellii*. *Ny. neivai* predominated (65%) (Table I). Of the 11 sites sampled, no sandflies were obtained at two locations (the henhouse on the Souto smallholding and jaboticaba tree in the Humberto Teixeira neighbourhood). The sites with the largest number of sandflies (three specimens each) were the "rural village" suburb, the Itapeva smallholding, the house in front of the cemetery and FALM.

A total of 3,843 specimens were captured with automatic light traps (144 traps installed) in the rural area, yielding an average of 26.69 sandflies/trap. The most prevalent species were: *Ny. neivai* (42.83%), *Ny. whitmani* (37.34%), *Br. brumpti* (8.07%) and *Pi. pessoai* (6.12%). *Ny neivai* was the most abundant (SISA = 0.897), followed by *Ny. whitmani* (0.862), *Pi. pessoai* (0.710), *Pi. fischeri* (0.399) and *Mg. migonei* (0.339). Of the total of sandflies captured in the rural areas, 65.31% were collected in Jacutinga (96.2% of which were collected at the

TABLE I

Numbers and percentages of sandflies captured with automatic light and Shannon traps by species, sex and period, rural and urban areas of the municipality of Bandeirantes, state of Paraná, April 2008-April 2010

Period	Dec 2009-Feb, Apr 2010		April 2008-March 2009		April 2009-March 2010		
Method of capture	Shannon trap		Automatic light trap				
Environment	Rural		Urban		Rural		
Sex	M	F	M	F	M	F	Total
Species	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Brumptomyia brumpti	-	1 (0.1)	2 (15.4)	-	167 (8.6)	143 (7.5)	313 (5.5)
Brumptomyia cunhai	-	-	-	-	18 (0.9)	14 (0.7)	32 (0.5
Brumptomyia nitzulescui	-	-	-	-	4 (0.2)	1 (0.1)	5 (0.1)
Brumptomyia sp.[a]	-	-	-	-	0	3 (0.2)	3 (0.1)
Evandromyia correalimai	-	-	-	-	-	1 (0.1)	1 (0)
Evandromyia cortelezzii	-	-	1 (7.7)	1 (11.1)	1 (0.1)	10 (0.5)	13 (0.2)
Expapillata firmatoi	7 (0.7)	25 (3)	-	-	20 (1)	78 (4)	130 (2.3)
Migonemyia migonei	3 (0.3)	8 (1)	-	-	5 (0.3)	22 (1.1)	38 (0.7)
Pintomyia christenseni	-	1 (0.1)	-	-	-		1 (0)
Pintomyia fischeri	1 (0.1)	3 (0.4)	-	-	15 (0.7)	12 (0.6)	31 (0.5)
Pintomyia mamedei	-	1 (0.1)	-	-	-		1 (0)
Pintomyia pessoai	149 (14.3)	59 (7.1)	1 (7.7)	-	164 (8.5)	71 (3.7)	444 (7.7)
Sciopemyia sordellii	-	-	-	1 (11.1)	1 (0.1)	2 (0.1)	4 (0.1)
Micropygomyia ferreirana	-	-	-	-	-	4 (0.2)	4 (0.1)
Micropygomyia longipennis	-	2 (0.3)	-	-	1 (0.1)	3 (0.2)	6 (0.1)
Nyssomyia neivai	565 (54.5)	451 (54.5)	9 (69.2)	6 (66.7)	826 (42.8)	820 (42.9)	2,677 (46.7)
Nyssomyia sp.[a]	-	-	-	-	-	2 (0.1)	2 (0)
Nyssomyia whitmani	312 (30.1)	274 (33.1)	-	1 (11.1)	708 (36.7)	727 (38)	2,022 (35.3)
Psathyromyia sp.	-	2 (0.3)	-	-	-	-	2 (0)
Total	1,037 (100)	827 (100)	13 (100)	9 (100)	1,930 (100)	1,913 (100)	5,729 (100)

a: damaged, probably belonging to one of two species of the genus indicated; F: female; M: male.

forest edge), 24% in Perobinha, 10.3% in Cabiúna and 0.4% in Tabuleta. Notably, 62.8% of the specimens were collected at the Jacutinga forest edge and the most predominant species were *Ny. neivai* (28.2%) and *Ny. whitmani* (17.5%). The highest level of species richness was obtained in Jacutinga (14), followed by Perobinha (9), Cabiúna (7) and Tabuleta (4). The highest diversity index values were observed in Jacutinga at the forest edge under a guava tree (1.45) and in the pigsty (1.42). This index was zero in a tree close to the pigsty in the Tabuleta neighbourhood and this index was very low (0.48) in the jaboticaba tree close to the home at the Perobinha. In the ecotopes of the Tabuleta neighbourhood, the smallest numbers of insects were captured, but these specimens had the highest diversity indices (0.91-0.96). Two ecotopes in Perobinha (an orange tree close the pigsty and a bamboo grove) yielded the second and third greatest frequencies of sandflies captured (13.2% and 10.4%, respectively); however their diversity indices were the lowest of all the samples sites (0.45 and 0.43) and had a predominance of the two *Nyssomyia* species (Table II).

Amongst the most abundant species, *Ny. neivai* and *Ny. whitmani* predominated throughout the study period. *Ny. whitmani* showed higher monthly averages than *Ny. neivai* from January-May and in August. *Pi. pessoai* was the third most frequent species, with the highest averages captured in February and March. The highest average captures of *Ny. neivai* ranged from 4.28-2.31 insects/month and the highest average captures of *Ny. whitmani* ranged from 4.34-2.67 insects/month. The lowest average captures for both species occurred in July (Fig. 1).

The seasonal distribution by site and ecotope of *Ny. neivai* and *Ny. whitmani* is presented in Table III. For the *Ny. neivai* captured in Jacutiga, the lowest Williams' averages were observed in all of the ecotopes during the fall and the highest Williams' averages were obtained in the spring. In Perobinha, the averages were remarkably higher in the summer on the edge of the forest and in the pigsty. In Cabiuna, the averages were generally low, with the exception of the pigsty and peridomicile during the summer. Concerning *Ny. whitmani* captured in Jacutinga, the lowest Williams' averages occurred in the fall for all the ecotopes and the highest occurred during the summer at the forest edge. In Perobinha, the averages were much higher in the summer for all of the ecotopes. In Cabiuna, the highest average occurred in the fall along the edge of the forest and in the summer the highest averages were found in the pigsty and the peridomicile.

Ten species were captured using the Shannon traps. The most frequently captured species were *Ny. neivai* (56.9%) and *Ny. whitmani* (32.8%) and the white trap captured significantly more specimens of these two taxa than the black trap (Table IV). In April 2010, the capture was conducted from 06:00 pm-06:00 am, but the other two captures were interrupted before 06:00 am due to heavy rain and rough winds. During the common capture period (06:00 pm-03:00 am), the highest average capture of *Ny. neivai* and *Ny. whitmani*, for both sexes, occurred using the white trap between 11:00 pm-12:00 am. Using the black trap, the females of these two species were most frequently captured between 09:00 pm-11:00 pm (Fig. 2).

DISCUSSION

The frequencies of the specimens of the subtribes Psychodopygina (83.2%), Lutzomyiina (10.5%), Brumptomyiina (6.1%) and Sergentomyiina (0.2%) found in this present study are in agreement with those found in this same municipality by Massafera et al. (2005). However, after capturing 38,662 sandfly specimens in 37 municipalities, da Silva et al. (2008) found that 86.1% of the sandflies belonged to Psychodopygina, 12.7% to Lutzomyiina, 0.8% to Sergentomyiina and 0.4% to Brumptomyiina; the two last subtribes inverted their positions in the ranking. However, within the subtribe Psychodopygina, the da Silva et al. (2008) study found that *Ny. neivai* (47.4%) predominated over *Ny. whitmani* (35.8%) and the frequency of *Pi. pessoai* (6.5%) was also close to that observed in the present study. These three species are considered vectors of ACL (Pessôa & Coutinho 1941, de Queiroz et al. 1994, Rangel & Lainson 2003, Pita-Pereira et al. 2005, Andrade Filho et al. 2007, Cruz et al. 2012). The predominance of *Ny. neivai* and its greater abundance have also been observed in the municipality of Itambaracá, which borders Bandeirantes (Cruz et al. 2012). In other studies, different results have been found for PR, where *Ny. whitmani* prevailed in the northwestern region (67.82%) (Teodoro et al. 1991), in the northern region (76.3%, 70%) (Teodoro et al. 1993a, Muniz et al. 2006) and even in Bandeirantes (58.9%) (Massafera et al. 2005). *Ny. neivai* and *Ny. whitmani* were also found to be the predominate species in 10 municipalities in the northwestern region of PR (Teodoro et al. 2006).

The sandflies captured in urban areas in Brazil have frequently demonstrated the ability of some species to adapt to anthropogenic environments (Teodoro et al. 1998). These authors also demonstrated the presence of nine sandfly species in the residual forests within the urban perimeter of Maringá and *Ny. whitmani* was overwhelmingly dominant. In the present study *Ny. neivai* was observed, although at a much lower frequency, to occupy urban environments. This species is also prevalent in the city of Piçarras in the state of Santa Catarina, near the homes of patients with ACL, where female sandflies naturally infected with *Leishmania* (*Viannia*) sp. have been found (Marcondes et al. 2009); this sandfly species has also been found in a periurban area of Porto Alegre in the state of Rio Grande do Sul (Pita-Pereira et al. 2009).

The high prevalence of *Ny. whitmani* in altered residual forests in urban environments in PR (Teodoro et al. 2001, Zanzarine et al. 2005) and in many rural regions of northern PR (da Silva et al. 2008) demonstrate that this species is adapting to the anthropic environment.

In Bandeirantes, the vector species *Ny. neivai*, *Ny. whitmani*, *Pi. pessoai, Pi. fischeri* and *Mg. migonei* have been frequently found in domestic animal shelters, woods and dwellings. *Pi. fischeri* naturally infected with *Leishmania braziliensis* has only recently been found in the Southeast Region of Brazil (Margonari et al. 2010, Rocha et al. 2010), but the other species have all been found naturally infected by *Leishmania* (*Viannia*) sp. in PR and in other areas of South America (de Queiroz et al. 1994, da Silva & Grunewald 1999, Luz et al. 2000, Pita-Pereira et al. 2005, 2009, Córdoba-Lanús et al. 2006, Marcondes et al. 2009).

TABLE II

Numbers of phlebotomines by sex captured with automatic light traps in each site of the rural localities sampled and Shannon diversity and Pielou evenness indices and standardised index of species abundance (SISA), municipality of Bandeirantes, state of Paraná, April 2009-March 2010

Locality		1. Jacutinga						2. Tabuleta						3. Perobinha						4. Cabiúna						Total		SISA
Site		1.1		1.2		1.3		2.1		2.2		2.3		3.1		3.2		3.3		4.1		4.2		4.3				
Species	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	%	M/F
Brumptomyia brumpti		-	-	165	141	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	2	-	-	310	87	0.187
Brumptomyia cunhai		1	3	16	11	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	32	0.83	0.170
Brumptomyia nitzulescui		-	-	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0.13	043
Brumptomyia sp.		-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	08	020
Evandromyia correalimai		-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	03	006
Evandromyia cortelezzii		-	5	1	3	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	11	0.29	0.155
Expapillata firmatoi		1	-	18	77	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	98	2.55	0.167
Micropygomyia ferreirana		-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	0.10	032
Micropygomyia longipennis		-	-	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	0.10	032
Migonemyia migonei		-	1	3	13	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	4	1	1	-	1	27	0.70	0.339
Nyssomyia neivai		22	13	567	516	2	15	2	-	4	-	-	-	9	4	80	80	79	146	27	16	24	20	10	10	1,646	42.83	0.897
Nyssomyia sp.		-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	05	0.123
Nyssomyia whitmani		8	10	369	307	4	3	1	-	-	2	-	-	2	1	197	132	38	125	65	106	13	28	11	13	1,435	37.34	0.862
Pintomyia fischeri		-	-	-	2	-	-	-	-	-	1	-	-	-	-	-	5	-	3	1	-	13	1	1	-	27	0.70	0.399
Pintomyia pessoai		4	1	136	51	-	2	2	-	2	-	-	-	-	-	3	4	3	3	11	7	1	2	2	1	235	6.12	0.710
Sciopemyia sordellii		1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	3	08	0.109
Total		37	33	1,280	1,134	6	20	5	-	6	4	-	-	11	5	281	225	122	278	105	134	53	54	24	26	3,843	100	-
Shannon diversity		1.42		1.45		0.83		1.33		1.27		-	-	0.48		0.80		0.83		0.89		1.27		1.12		-	-	-
Pielou evenness		0.68		0.54		0.75		0.96		0.91		-	-	0.69		0.45		0.43		0.50		0.71		0.63		-	-	-

1.1: pigsty, 10 m away from the house and 130 m of edge forest; 1.2: forest edge, guava tree 120 m away from the residence and 200 m from the fishing ground; 1.3: peridomicile; 2: residence of an American cutaneous leishmaniasis (ACL) case; 2.1: tree close to the veranda of home; 2.2: peridomicile, avocado tree used as a perch for birds; 2.3: peridomicile, tree close to pigsty; 3: residence of an ACL case; 3.1: peridomicile in a jaboticaba tree close to the home; 3.2: orange tree close to the pigsty and residence; 3.3: bamboo, distant 10 m from the residence, next to a pasture, a pound and a small forest; 4.1: pigsty close to the residence; 4.2: pigsty situated at the forest edge, 50 m far from the residence; 4.3: forest edge tree (50 m far the point 4.2); F: female; M: male.

The greatest species richness and highest diversity index were observed in a rural district in Jacutinga, near the forest edge and a pigsty, possibly due to the following characteristics: the presence of dense forest near the domestic animal shelters and the presence of orchards and the accumulation of organic matter. These factors are considered favourable for sandfly breeding sites. The high species balance in Tabuleta can be explained by the similar frequencies amongst the species. The low frequency of specimens and low level of species richness at this site may be attributed to the absence of dense forest and the constant presence of strong winds. However, sandfly vectors are still present, which explains the occurrence of cutaneous leishmaniasis in a resident of this site.

The proximity of domestic animal shelters to dwellings, the accumulation of organic matter (leaves and fallen fruit, agricultural waste, animal droppings and household food scraps), wet soil due to the discharge of water from domestic use and the shade of trees are favourable for the formation of outdoor sandfly breeding sites (Massafera et al. 2005). These conditions are observed in rural areas of PR and were also present in the area studied, resulting in large numbers of sandflies collected around the dwellings and in domestic animal shelters.

The increased attractiveness of the Shannon traps (310 sandflies/trap/person) compared with the automatic light traps (26.6 sandflies/trap) may be an indication of the high anthropophily of the two predominant species. The significantly increased attractiveness of the white Shannon trap compared with that of the black trap was observed for both *Ny. neivai* and *Ny. whitmani*. A similar result was found in the Ribeira Valley in SP for *Ny. neivai* (Galati et al. 2010); however, the black trap attached significantly more *Ny. whitmani* than the white trap on the Bodoquena Range in the state of Mato Grosso do Sul (MS) (Galati et al. 2001). The monthly variation of *Ny. neivai* and *Ny. whitmani* show that they have great plasticity; although they predominated during the

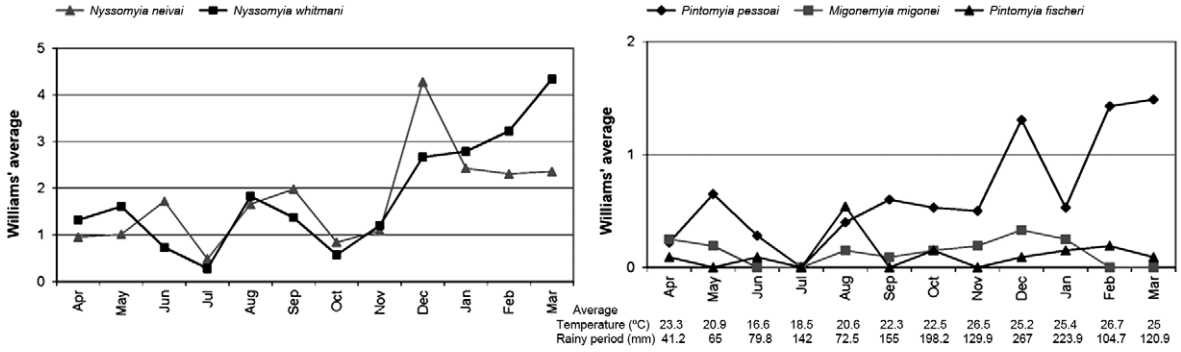

Fig. 1: the monthly Williams' averages of the sandfly vectors collected in the rural areas with CDC traps, municipality of Bandeirantes, state of Paraná, April 2009-March 2010.

TABLE III

Williams' average of *Nyssomyia neivai* and *Nyssomyia whitmani* captured with automatic light traps in rural area by site, season and ecotope, municipality of Bandeirantes, state of Paraná, April 2009-March 2010

Species		*Ny. neivai*			*Ny. whitmani*		
Site	Season	Forest edge	Pigsty	Peridomicile	Forest edge	Pigsty	Peridomicile
Jacutinga	Fall	14.8	0.6	0.26	14.1	0.26	0
	Winter	59.4	2.8	0.44	28.1	1.71	0
	Spring	121.8	3.1	18	46.9	1.47	0.82
	Summer	116.7	2.3	0.91	65.3	18	0.44
Perobinha	Fall	23	4.42	0.26	1	4.42	0
	Winter	1.41	1.41	1.15	0.44	1.41	0.26
	Spring	3.16	0.71	0	1.15	0.71	0
	Summer	35.31	35.25	0.81	41.38	35.25	0.44
Cabiuna	Fall	1.57	0.91	0.71	2.53	1.15	1.71
	Winter	0	0.91	0.82	0	1.22	2.68
	Spring	0.58	0.26	1.88	0.44	0	2.82
	Summer	0.44	2.96	2.53	0	3.76	10.2

rainy periods, they also peaked during the dry season. This trend was also observed for the *Ny. neivai* by Galati et al. (2010), but not for the either species by Massafera et al. (2005), whose peaks occurred only during the rainy season. On the other hand, *Ny. whitmani* only peaked during the dry periods in MS (Galati et al. 1996).

Using the white Shannon traps, both sexes of *Ny. neivai* were captured at the greatest frequencies from 10:00 pm-02:00 am. In the Ribeira Valley, SP (Galati et al. 2010) and in Bandeirantes (Massafera et al. 2005), the peak frequencies of *Ny. neivai* capture occurred from 07:00 pm-11:00 am. For *Ny. whitmani* captured using the white trap, there were two peaks for the females and the last peak was at a time close to that found in Maringá, PR (12:00 am-01:00 am) (Teodoro et al. 2003), but differed from that in Bandeirantes (08:00 pm-10:00 pm) (Massafera et al. 2005) and in the municipality of Corguinho, MS (06:00 pm-07:00 pm) (Galati et al. 1996).

TABLE IV

Numbers of sandfly specimens by species and sex captured with modified white and black Shannon trap and chi-square obtained between the numbers of insects on the two colour Shannon traps, Jacutinga neighbourhood, municipality of Bandeirantes, state of Paraná, 2009-2010

Traps		White		Black		White/black		χ^2	
Species	Sex	M	F	M	F	M	F	M	F
Brumptomyia brumpti		-	-	-	1	-	-	-	-
Expapillata firmatoi		-	5	7	20	-	0.25	-	9
Micropygomyia longipennis		-	1	-	1	-	1	-	-
Migonemyia migonei		1	7	2	1	0.5	7	-	-
Nyssomyia neivai		447	390	118	61	3.79	6.39	191.6	240
Nyssomyia whitmani		238	217	74	57	3.21	3.80	86.20	93.43
Psathyromyia sp.		-	1	-	1	-	1	-	-
Pintomyia christenseni		-	1	-	-	-	-	-	-
Pintomyia fischeri		-	1	1	2	-	0.5	-	-
Pintomyia pessoai		49	37	101	22	0.48	1.68	183	3.81
Total		735	660	303	166	2.42	3.97	179.8	295.4

F: female; M: male.

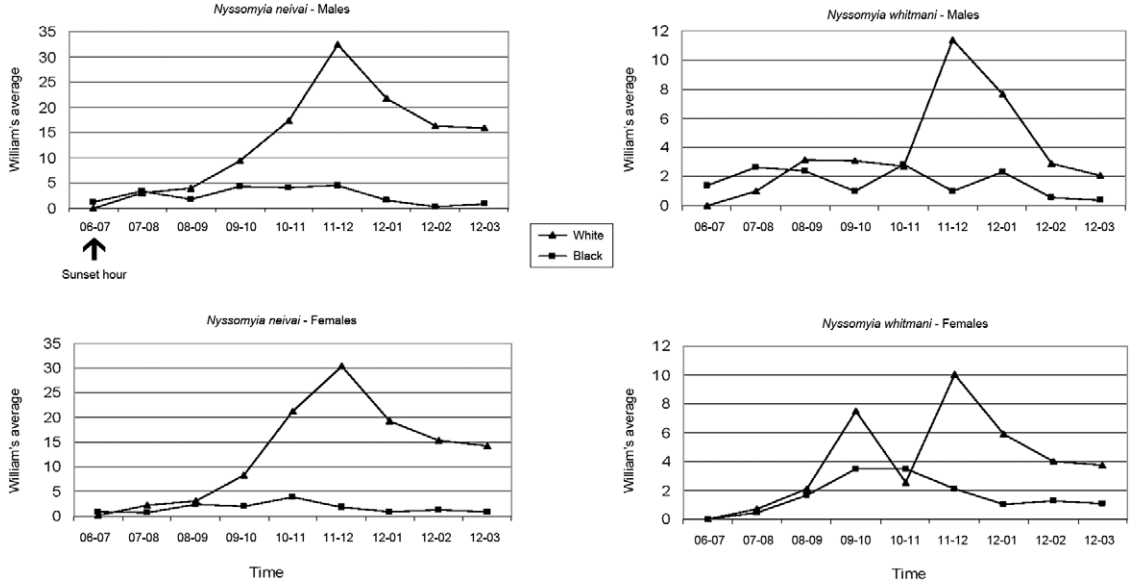

Fig. 2: hourly average of *Ny. neivai* and *Ny. whitmani* by time, sex and colour of the Shannon trap in the Jacutinga neighbourhood, municipality of Bandeirantes, state of Paraná, between December 2009-April 2010.

Although seasonality studies of sandflies are still needed, the results obtained in this present study suggest that the spring and summer are the seasons of greatest risk of human infection by *Leishmania*. A high frequency of sandflies was found in domiciliary areas and the observation that the highest frequencies occur late at night when people are resting suggests that the infection occurs at home. Some people may have been infected during leisure or occupational activities associated with the forest, especially in the Jacutinga neighbourhood; for example, people may have been infected at the fishing area, where the highest vector density occurred. Epidemiological research to investigate the probable sites where people acquire *Leishmania* infection is required to guide the entomological surveillance and control measures to diminish the sandfly population and educational activities to alert the public to the risks of infection should be implemented in Bandeirantes.

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