

## Article/Artigo

# Ecological aspects of the sandfly fauna (Diptera, Psychodidae) in an American cutaneous leishmaniasis endemic area under the influence of hydroelectric plants in Paranapanema River, State of Paraná, Brazil

Aspectos ecológicos da fauna flebotomínea (Diptera, Psychodidae) em área endêmica de leishmaniose tegumentar americana, sob influência de complexo hidrelétrico no rio Paranapanema, Estado do Paraná, Brasil

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#### **ABSTRACT**

Introduction: An epidemiological study was undertaken to identify determinant factors in the occurrence of American cutaneous leishmaniasis in areas under the influence of hydroelectric plants in Paranapanema River, State of Paraná, Brazil. The ecological aspects of the phlebotomine fauna were investigated. Methods: Sandflies were sampled with automatic light traps from February 2004 to June 2006 at 25 sites in the urban and rural areas of Itambaracá, and in Porto Almeida and São Joaquim do Pontal. Results: A total of 3,187 sandflies of 15 species were captured. Nyssomyia neivai predominated (34.4%), followed by Pintomyia pessoai (32.6%), Migonemyia migonei (11.6%), Nyssomyia whitmani (8.8%), and Pintomyia fischeri (2.7%), all implicated in the transmission of Leishmania. Males predominated for Ny. neivai, and females for the other vector species, with significant statistical differences (p < 0.001). Nyssomyia neivai, Pi. pessoai, Ny. whitmani, Brumptomyia brumpti, Mg. migonei, and Pi. fischeri presented the highest values for the Standardized Species Abundance Index (SSAI). The highest frequencies and diversities were found in the preserved forest in Porto Almeida, followed by forests with degradation in São Joaquim do Pontal and Vila Rural. Conclusions: Sandflies were captured in all localities, with the five vectors predominating. Ny. neivai had its highest frequencies in nearby peridomestic environments and Pi. pessoai in areas of preserved forests. The highest SSAI values of Ny. neivai and Pi. pessoai reflect their wider dispersion and higher frequencies compared with other species, which seems to indicate that these two species may be transmitting leishmaniasis in the area.

Keywords: American cutaneous leishmaniasis. Hydroelectric plants. Phlebotomine. Ecological aspects. Vectors.

#### **RESUMO**

Introdução: Em estudo epidemiológico desenvolvido para identificar fatores determinantes na ocorrência da leishmaniose tegumentar americana em áreas impactadas por complexo hidrelétrico no rio Paranapanema, Estado do Paraná, Brasil, foram investigados aspectos ecológicos da fauna flebotomínea. Métodos: Os flebotomíneos foram amostrados com armadilhas automáticas luminosas de fevereiro/2004 a junho/2006, em 25 pontos, distribuídos na área urbana, periurbana e rural da Cidade de Itambaracá e Vila Rural, além das localidades de Porto Almeida e São Joaquim do Pontal. Resultados: No total capturouse 3.187 flebotomíneos de 15 espécies. Predominaram Nyssomyia neivai (34,4%), Pintomyia pessoai (32,6%), Migonemya migonei (11,6%), Nyssomyia whitmani (8,8%) e Pintomyia fischeri (2,7%), todas com capacidade de transmissão de Leishmania. Para Ny. neivai, predominaram os machos e para as outras vetoras, fêmeas, com diferença estatística significante (p < 0.001). Segundo o índice de abundância das espécies padronizado, as mais abundantes foram: Ny neivai, Pi. pessoai, Ny. whitmani, Brumptomyia brumpti, Mg. migonei e Pi. fischeri. As maiores frequências e diversidade das espécies foram encontradas em Porto Almeida em áreas com matas mais preservadas, seguida por São Joaquim do Pontal e Vila Rural, com matas mais degradadas. Conclusões: Os flebotomíneos foram capturados em todas as localidades amostradas, com predominância de cinco espécies vetoras, destacando-se Ny. neivai, com suas frequências mais elevadas nas proximidades de peridomicílios e *Pi. pessoai* nas áreas de matas mais preservadas. Os altos índices de abundância das espécies padronizados para Ny. neivai e Pi. pessoai indicam que estas duas espécies podem transmitir a leishmaniose tegumentar na área investigada.

Palavras-chaves: Leishmaniose tegumentar americana. Complexo hidrelétrico. Flebotomíneo. Aspectos ecológicos. Vetores.

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### INTRODUCTION

The State of Paraná has been presenting a gradual increase in the number of municipalities with cases of American cutaneous leishmaniasis (ACL), accounting for 98% of the cases in Southern Brazil in 2000. Part of this increase, however, may be due to the improvement in the process of notification of the disease<sup>1</sup>.

American cutaneous leishmaniasis is endemic in a great part of the State of Paraná, occurring in 276 of its 399 municipalities<sup>2</sup>. In some localities in the north of the state the number of cases has increased, reaching epidemic proportions during particular periods and becoming a public health problem<sup>3,4</sup>.

The increase in ACL cases has been related to the deforestation of primary forests, leading to environmental changes and imbalances, or to haphazard urbanization and the construction of dams and hydroelectric plants. Under such circumstances the exposure of humans to the bite of sandfly vectors of Leishmania spp., whose natural habitats are still closely dependent on forests, has certainly increased<sup>5</sup>.

Nyssomyia whitmani, Nyssomyia intermedia s.lat., Migonemyia migonei, Pintomyia fischeri, and Pintomyia pessoai, which are the potential vectors of Leishmania, have been found in ACL endemic areas in the State of Paraná<sup>6-9</sup>. In Northern Paraná, where the highest incidences of the disease have occurred, studies undertaken during the 1990s detected a high prevalence of Ny. whitmani (67.8%), *Ny. intermedia s. lat.* (17.6%), and *Mg. migonei* (9%). Ny. whitmani and Ny. intermedia s. lat. have adapted to anthropic environments, playing a significant role in the transmission of the leishmaniasis agent9. Ny. whitmani has been found to be naturally infected by Leishmania braziliensis8, the agent of ACL with the widest distribution in endemic areas in Brazil.

The present study sought to investigate aspects of the behavior of the sandfly fauna with regard to the abundance, diversity, and evenness of the species in environments subject to varying degrees of anthropic action, and to identify the possible vectors of the agent of leishmaniasis in an area considered endemic for ACL, close to the hydroelectric plants Canoas I and II on the Paranapanema River in Northern Paraná, Brazil.

#### **METHODS**

#### Study area

The study was undertaken in two rural localities, São Joaquim do Pontal and Porto Almeida, and in the periurban and urban environments of Itambaracá municipality, State of Paraná, Brazil. The former two localities are situated near the hydroelectric plants Canoas I and II on the Paranapanema River and are, therefore, considered areas subject to the influence of that hydroelectric complex (Figure 1).

The municipality of Itambaracá, with an area of  $197,301 \, \mathrm{km}^2$ , is situated 402m above sea level and at  $23^{\circ}02'00''$  S,  $50^{\circ}22'00''$  W. It has 7,089 inhabitants, 5,302 of whom live in the urban area and 1,787 in the rural area.

The climate is mesothermic subtropical humid, with warm summers having more intense rains and an average temperature over 22°C, and winters with infrequent frosts, an average temperature below 18°C, and no clearly defined dry season.

Latosol Eutrophic Purple is the most prevalent type of soil. The remaining Atlantic forest areas are semi-seasonal deciduous. Agricultural production is the basis of the economy of the municipality.

#### Sandfly collections

For the sandfly captures, 25 automatic light traps of Center for Disease Control (CDC) type were installed once a month from 18:00 to 07:00h, one at each of the 25 sites, from February 2004 to June 2006.

These 25 sites were located in two rural localities near Canoas I, namely, São Joaquim do Pontal (eight traps) and Porto Almeida (nine traps), both in areas affected by the construction of the dam, and in the town of Itambaracá (eight traps), particularly in periurban areas and two peripheral areas, Vila Rural and *Pesque-e-Pague* (Fish-and-Pay), situated approximately 10km from the area affected by construction of the dam. In each of the areas sampled there was at least one autochthonous case of ACL recorded by the municipal health service. Some cases had wound scars and others active wounds.

#### Localities and collection sites sampled

In São Joaquim do Pontal there is a district with several houses and small farms with areas close to degraded forests; this is the closest area subject to the influence of the Canoas I hydroelectric complex on the Paranapanema River. Four traps were installed in peridomiciles of the Patrimônio district. Four were installed in Monte Alto Farm: two in peridomiciles and two on the edge of riparian forest. I) Patrimônio district: a) house 1, peridomicile, at the right-hand entrance to the neighborhood, 1,500m away from the forest; b) house 2, peridomicile, at the left-hand entrance to the neighborhood, 1,400m from the forest; c) house 3, peridomicile, peripheral area, exit to the dam, 1,200m from the forest; d) house 4, peridomicile, peripheral area, exit to plantations, 1,500m from the forest. Around all the houses were yards and gardens with cats and

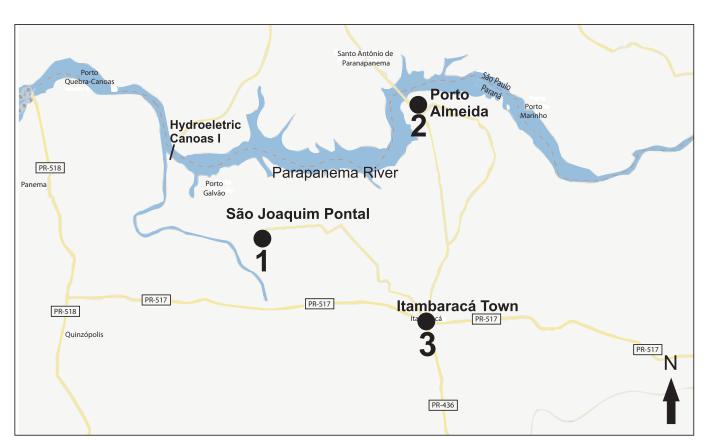


FIGURE 1 - Map of Paranapanema river and Itambaracá town, Brazil.

dogs, and at house 4 there were pigs and chickens. II) Monte Alto Farm: a) house 1 (colony 1), peridomicile, 217m from the forest; b) house 2 (colony 2), peridomicile, 230 m from the forest; c) electrical transformer, forest edge; d) shed, forest edge. At houses 1 and 2 in Monte Alto Farm there were cats, dogs, and chickens, and around the houses, a riparian forest, with wild animals.

In Porto Almeida are situated the ferryman's house, several small farms along the road, and the São José Farm. At all the sites there were cats, dogs, chickens, horses, cattle, sheep, and pigs. Near the sites where the traps were installed on São Jose Farm there was a large well-preserved riparian forest, with wild animals such as capybaras, peccaries, monkeys, rats, opossums, and others. I) Near the raft ferry to Cândido Mota, São Paulo State: a) the ferryman's house, peridomicile, 2.6km from the forest; b) house 2, Favoni's Farm, peridomicile, 1.95km from the forest; c) riparian forest; d) orchard (Strambecchi's small farm) 350m from the forest. II) São José Farm: a) chapel, forest edge (entrance to the farm); b) house (colony), peridomicile, 310m from the forest; c) road (crosses the farm), forest edge; d) riparian forest (near the Paranapanema River); e) gate (backyard of the farm), forest edge.

In Itambaracá City, the traps were installed at eight sites: four in *Vila Rural* (the houses presenting large backyards with orchards, gardens, pets, horses, chickens, and pigs); one in *Pesque-e-Pague*, near Vila Rural, having dogs, chickens, and horses; and three in urban and periurban areas, with pets and chickens. I) Vila Rural: a) house 1, peridomicile, near pigsty, 60m from the forest; b) edge of degraded forest; c) house 2, peridomicile, orchard, 250m from the forest; d) house 3, peridomicile, pigsty, 156m from the forest. II) *Pesque-e-Pague*: edge of degraded forest. III) Urban and periurban areas: a) municipal slaughterhouse, peridomicile, 240m from the forest; b) municipal supply house, peridomicile, 708m from the forest; c) Joel's small farm, peridomicile with domestic animals, 770m from the forest.

The sandflies collected were separated from the other insects at the *Laboratório de Entomologia em Saúde Pública/*Phlebotominae,

Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo (FSP/USP). The males and females were submitted to the technique of Maroli<sup>10</sup>, macerated using the technique of Forattini<sup>11</sup>, and identified according to Galati's identification key<sup>12</sup>.

#### Statistical analysis

The comparison of species abundance as between the different collection sites was calculated by the Standardized Species Abundance Index (SSAI)<sup>13</sup>, the species diversity by Shannon's index (H), and the evenness by the Pielou index (J)<sup>14</sup>. The  $\chi^2$  test was used to compare the numbers of males and females captured in each locality and the numbers of insects captured at the edge of forest and far from this.

#### **RESULTS**

At the 25 sites sampled in the three localities, i.e., São Joaquim do Pontal, Porto Almeida, and Itambaracá town (urban and periurban areas), 3,187 sandflies were captured, 46.2% of which males and 53.7% were females, belonging to 15 species, distributed in 4 subtribes: Brumptomyiina - Brumptomyia brumpti (Larrousse), Brumptomyia cunhai (Mangabeira), and Brumptomyia nitzulescui (Costa Lima); Lutzomyiina - Evandromyia bacula (Martins, Falcão & Silva), Evandromyia cortellezzii (Brèthes), Expapillata firmatoi (Barretto, Martins & Pellegrino), Migonemyia migonei (França), Pintomyia fischeri (Pinto), Pintomyia pessoai (Coutinho & Barretto), and Sciopemyia sordellii (Shannon & Del Ponte); Psychodopygina - Nyssomyia neivai (Pinto), Nyssomyia whitmani (Antunes & Coutinho), Psathyromyia aragaoi (Costa Lima), and Psathyromyia abonnenci (Floch & Chassignet); and Sergentomyiina -Micropygomyia ferreirana (Barretto, Martins & Pellegrino). Ny. neivai predominated (34.4%), followed by Pi. pessoai (32.6%), Mg. migonei (11.6%), Ny. whitmani (8.8%), and Pi. fischeri (2.7%) (Table 1).

TABLE 1 - Average of the number of specimens of both sexes captured per locality by species, number of specimens and frequency by species in the three localities: São Joaquim do Pontal, Porto Almeida, and Itambaracá City, February 2004 to June 2006.

Locality	São Joaquim do Pontal	Porto Almeida	Itambaracá		Total										
Sites of capture Sex Species	8	9	8		25		25	25							
		Both sexes	1	male	Fei	nale	Both sexes								
	n	n	n	n	%	n	%	n	%						
Brumptomyia brumpti	11	150	43	112	7.6	92	5.4	204	6.4						
Brumptomyia cunhai	-	61	1	35	2.4	26	1.5	61	1.9						
Brumptomyia nitzulescui	-	1	-	-	-	1	0.1	1	0.03						
Brumptomyia sp.	1	7	-	-	-	9	0.5	9	0.3						
Evandromyia bacula	-	2	1	1	0.07	2	0.1	3	0.1						
Evandromyia cortelezzii	2	5	8	6	0.4	8	0.5	14	0.4						
Expapillata firmatoi	-	13	-	6	0.4	7	0.4	13	0.4						
Micropygomyia ferreirana	-	4	-	-	-	4	0.2	4	0.1						
Migonemya migonei	1	337	32	114	7.7	256	14.9	370	11.6						
Nyssomyia neivai	47	351	697	643	43.6	452	26.4	1,095	34.4						
Nyssomyia whitmani	20	226	34	135	9.1	146	8.5	281	8.8						
Psathyromyia abonnenci	-	1	-	1	0.07	-	-	1	0.03						
Psathyromyia aragaoi	-	4	-	4	0.27	-	-	4	0.1						
Pintomyia fischeri	2	82	20	18	1.2	69	4.03	87	2.7						
Pintomyia pessoai	32	986	3	400	27.1	638	37.3	1,038	32.6						
Sciopemyia sordellii	-	1	1	-	-	2	0.1	2	0.06						
Total	116	2,231	840	1,475	100.0	1,712	100.0	3,187	100.0						

The number of sandflies captured by species captured in each of the three localities and the total numbers of specimens by sex and both sex as well as their frequencies in the three localities are presented in **Table 1**. In São Joaquim do Pontal, with 8 sites of capture, 116 insects were collected. In Porto Almeida (9 sites of captures), 2,231 specimens were captured and in Itambaracá town, also with 8 sites sampled, 840 sandflies. *Ny. neivai* (34.36%) and *Pi. pessoai* (32.57%) were the predominant species.

Of the total specimens captured in São Joaquim do Pontal, the closest area subject to the influence of the Canoas I hydroelectric complex, the greatest frequency occurred at the forest edge in Monte Alto Farm, at the following sites: electrical transformer (29.3%), shed (27.6%), house 2 (25.9%), and house 1 (12.1%).

At the same farm, the highest species richness occurred in the shed forested edge (6), and the highest diversity (1.31) and evenness (0.94) occurred at house 2. In Patrimônio, although sandflies were captured at all the sites sampled, the specimens totaled only 6. Of the 116 specimens captured in São Joaquim do Pontal, *Ny. neivai* predominated (40.5%); the females captured were significantly more numerous than the males. For the other two species with high frequencies, *Pi. pessoai* (27.6%) and *Ny. whitmani* (17.2%), the males predominated, but without any significant difference (Table 2).

Porto Almeida was the locality that presented the highest number of insects (2,231) and of species (15) (**Table 3**), mainly at the São José Farm (2,067 specimens in 5 sites of collection). In the area near the ferry only 164 specimens were captured in the four sites sampled. In the two areas, although with greatly different numbers, the captures in the riparian forests presented the highest yield and species richness: 132 specimens and 9 species in the area close to the ferry, and 984 specimens and 11 species at the São José Farm. At the São José Farm more insects were captured at each of the sites situated on the forest edge than at that situated 300m from it, but the number of insects captured at each of the sites showed a significant difference ( $\chi^2 = 58.0$ ; p < 0.001; degrees of freedom = 3).

Regarding the species, in both areas, *Pi. pessoai* predominated with values almost equal in the area near the ferry (41.5%) and at

São José Farm (44.4%). However, *Ny. neivai* was the second predominant species in the former area (35.4%) and the third at São José Farm (14.2%) where it was outnumbered by *Mg. migonei* (16.2%). *Nyssomyia whitmani* represented about 10% of the specimens captured in both areas. Females of these species were captured in numbers significantly greater than the males.

The highest diversity (1.31) and evenness (0.95) in the area near the ferry were observed on the Strambecchi small farm; at São José Farm the highest diversity occurred in the riparian forest (1.74), but the greatest evenness in the colony was found 300m from the forest (0.86). (Table 4)

In the set of sites included within Itambaracá town, in the localities of Vila Rural and Pesque-e-Pague, very few sandflies (23 specimens) were captured at the four sites situated at distances ≥ 240m from the forest, while at those sites situated in the forest, on its edge or 60m from it, at least 50 insects were captured per site. In Vila Rural 87.8% of the specimens were captured at the sites associated with pigsty. In this locality, *Ny. neivai* predominated at all the sites except *Pesque-e-Pague*, representing 83% of all the specimens captured. Near the collection points at *Pesque-e-Pague* and *Vila Rural*, there were yards and gardens with dogs, cats, pigs, chickens, and horses.

The highest species richness occurred in the forest (8), and the highest diversity (1.20) and evenness (0.87) at the slaughterhouse. Unlike in the two other localities, males predominated significantly over females for the two most frequent species, *Ny. neivai* and *Ny. whitmani*.

Species abundance was evaluated by SSAI, according to which the closer the value is to one, the more abundant the species. The overall values obtained for all the localities together (São Joaquim do Pontal, Porto Almeida; Itambaracá town, Vila Rural and Pesque-e-Pague) were 0.87 (the most abundant) for Ny. neivai, 0.79 for Pi. pessoai, 0.50 for Ny. whitmani, 0.41 for Br. brumpti, 0.35 for Mg. migonei, 0.20 for Pi. fischeri, 0.20 for Br. sp., 0.14 for Ev. cortelezzii, 0.10 for Br. cunhai, 0.07 for Br. nitzulescui, 0.05 for Ex. firmatoi, 0.03 for Ev. bacula, 0.02 for Sc. sordellii, 0.01 for Mi. ferreirana, 0.01 for Pa. Aragaoi, and 0.01 for Pa. abonnenci.

TABLE 2 - Sandfly frequencies, by site sampled, male/female ratio, Shannon's diversity index (H), and Pielou's evenness index (J) in São Joaquim do Pontal and Itambaracá municipalities, February 2004 to June 2006.

								Loc	ality/s	ite									
		Monte Alto Farm										Patrin	nônio di	strict			_		
	5	hed					Electr	ical box	ĸ										
	Fore	est edge	Colo	ny 1	Colo	ny 2	forest edge		Hou	House 1		se 2	Hou	se 3	Hou	se 4			
Distance of forest	-		217m		230m		-		1,50	1,500m		1,400m		00m	1,500m		Tota		1
Species/sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	n	%	M/F
Brumptomyia brumpti	2	1	-	-	-	-	4	4	-	-	-	-	-	-	-	-	11	9.5	1.20
Brumptomyia sp.	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.9	-
Evandromyia cortelezzii	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	2	1.7	1.00
Migonemya migonei	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.9	-
Nyssomyia neivai*	3	19	-	4	8	4	2	3	-	1	-	1	1	-	-	1	47	40.5	0.42
Nyssomyia whitmani	-	1	1	6	10	1	-	-	-	1	-	-	-	-	-	-	20	17.2	1.22
Pintomyia fischeri	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	2	1.7	-
Pintomyia pessoai	1	3	1	1	5	-	11	9	-	-	-	-	-	1	-	-	32	27.6	1.29
Total	7	25	3	11	25	5	17	17	-	2	-	1	1	1	-	1	116	100.0	0.84
Both sexes (%)	27	7.6	12.1		25.9		29.3		1	.7	C	).9	1.	7	0.9				
Н	1.0	06	1.10		1.31		1.04		0.	.70		0	0.70		0			1.47	
J	0	59	0.	79	0	.94	0.7	75	1.	.00		0	1.0	00		0		0.71	

M: male; F: female; n: number; %: percentage; H: Shannon's diversity index; J: Pielou's evenness index. \*Difference statistically significant by the  $\chi^2$  test (p < 0.001; degree of freedom = 1) between the total of males and females.

TABLE 3 - Sandfly frequencies, by species, sex, site of capture, male/female ratio, Shannon's diversity index (H), and Pielou's evenness index (J) in Porto Almeida and Itambaracá municipalities, February 2004 to June 2006.

			Locali	ty/si	te		Locality/site															
	Near the ferry							São José Farm														
_	Fer	ry e	Fav	oni	Rip	arian	Stra	mbe	Cl	napel			R	oad	Ripa	arian	G	ate				
	ho	ous	Fa	rm	fo	rest	Fa	rm	fore	st edge	Co	lony	fores	t edge	for	rest	fores	t edge				
Distance of forest	2.6	km	1.95km		-		350m		-		300m				-		-		Total			
Species/sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	n	%	M/F	
Brumptomyia brumpti	-	-	-	-	6	2	2	1	13	12	6	4	29	13	22	21	5	14	150	6.7	1.24	
Brumptomyia cunhai	-	-	-	-	3	2	-	-	-	-	-	-	8	11	13	8	11	5	61	2.7	1.35	
Brumptomyia nitzulescui	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0.04	-	
Brumptomyia sp.	-	-	-	-	-	1	-	-	-	6	-	-	-	-	-	-	-	-	7	0.3	-	
Evandromyia bacula	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-		-	2	0.1	1.00	
Evandromyia cortelezzii	-	-	-	-	-	-	-	-	1	1	-	-	-	-	1	2	-	-	5	0.2	0.67	
Expapillata firmatoi	-	-	-	-	-	-	-	-	-	2	-	1	-	-	6	4	-	-	13	0.6	0.86	
Micropygomyia ferreirana	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	3	-	-	4	0.2	-	
Migonemya migonei	-	-	-	-	1	2	-	-	12	10	8	10	33	58	40	133	6	24	337	15.1	0.42	
Nyssomyia neivai	-	2	2	2	23	26	2	1	21	20	2	12	3	8	83	122	9	13	351	15.7	0.57	
Nyssomyia whitmani**	2	11	-	-	1	1	1	-	5	15	4	7	5	24	74	57	5	14	226	10.1	0.75	
Psathyromyia abonnenci	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	0.04	-	
Psathyromyia aragaoi	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	4	0.2	-	
Pintomyia fischeri*	-	-	-	-	-	1	-	-	1	5	-	-	1	8	9	47	4	6	82	3.7	0.22	
Pintomyia pessoai*	1	-	1	1	15	47	2	1	71	134	8	27	80	144	151	187	44	72	986	44.2	0.61	
Sciopemyia sordellii	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	0.04	-	
Total	3	13	3	3	49	83	7	3	125	207	28	61	163	266	400	584	84	149	2,231	100.0	0.61	
Both sexes (%)	0	.72	0.27	,	5.92		0.45		14.88		3.99		19.23		44.10		10.44			100.0		
Н	0	.60	0.65	,	1.	28	1.	31	1.	.36	1.	54	1.4	43	1.	.74	1	.58		1.67		
J	0	.55	0.93	,	0.	58	0.	95	0.	.57	0.	86	0.0	59	0.	.73	C	.76		0.60		

M: male; F: female; %: percentage; H: Shannon's diversity index; J: Pielou's evenness index \*Difference statistically significant (p < 0.001); \*\*(p < 0.05; degree of freedom = 2) between the total of males and females.

 $TABLE\ 4-Frequencies\ of\ sandflies\ captured\ with\ automatic\ light\ traps\ by\ sites\ of\ collection,\ Shannon's\ index\ (H),\ and\ Pielou's\ evenness\ index\ (J),\ in\ urban\ and\ periurban\ areas\ of\ Itambaracá\ municipality,\ and\ male/female\ ratio,\ February\ 2004\ to\ June\ 2006.$ 

			Locality	/site			Local	lity/si	te		Locali	ty/site							
			City	,			Pes	que			Vila l	Rural							
	Sup	ply	Joel's	small			e Pa	gue	Hou	ıse 1									
	hou	ise	fa	rm	Slaught	erhouse	for	est	n	ear	Hou	ise 2	Hou	ise 3					
	perido	micile	perido	micile	perido	micile	eć	lge	piş	gsty	orc	hard	Pig	gsty	fo	rest			
Distance of forest	708	3m	77	0m	24	0m		-	60	)m	25	0m	15	0m		0		Total	
Species/sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	n	%	M/F
Brumptomyia brumpti*	-	-	-	-	-	-	19	18	-	-	1	-	-	-	3	2	43	5.1	0.2
Brumptomyia cunhai	-	-	-	-	-	-	-	1	-	-	-	-	-	-		-	1	0.1	-
Evandromyia bacula	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0.1	-
Evandromyia cortelezzii	-	-	-	-	-	-	1	2	-	-	1	-	-	3	1	-	8	0.9	0.6
Migonemya migonei	-	-	-	-	1	1	1	-	-	-	-	-	1	1	10	17	32	3.8	0.7
Nyssomyia neivai*	2	2	3	2	4	2	3	5	269	104	-	-	149	46	54	52	697	83.0	2.27
Nyssomyia whitmani*	-	-	-	-	-	-	-	-	23	6	-	-	3	-	-	2	34	4.0	3.25
Pintomyia pessoai	-	-	-	-	1	4	-	-	2	-	-	-	5	-	1	7	20	2.4	0.8
Pintomyia fischeri	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	2	3	0.4	0.5
Sciopemyia sordellii	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	0.1	-
Total	2	2	3	2	7	7	24	26	294	110	2	-	158	51	69	83	840	100.0	1.92
Both sexes (%)	C	.5	C	0.6		1.7		6.0		48.0		0.2	25.0		18.0		100.0		
Н		0	0		1.20		0.84		0.	30	0.70		0.35		1.01		0.73		
J		0		0	0.	.87	0.	52	0.	27		1.0	0.	.20	0.49		0.32		

M: male; F: female; %: percentage; H: Shannon's diversity index; J: Pielou's evenness index \*Difference statistically significant (p < 0.001) between the total of males and females.

#### **DISCUSSION**

American cutaneous leishmaniasis persists in Paraná, especially in the north and northwest regions of the state, where it occurs in the majority of municipalities, including Itambaracá, where this study was undertaken and where the rich soils and flat lands associated with the intense agricultural activity of the north of the state favor contact between the vectors and the human population. Even the land that is inadequate for agriculture is used for cattle raising, which means that little residual forest remains. What remains, however, is sufficient to maintain the *Leishmania* vector population.

Prior to the construction of the hydroelectric plant, the area where the plant is situated had already presented degradation due to anthropic actions. The construction of the dam, however, led to the massive deforestation of riparian vegetation and promoted changes in the sandfly fauna that moved to the peridomicile in the neighborhoods near the affected area, as indicated by their presence in this environment. This increased the risk of transmission of the cutaneous leishmaniasis agent. In other localities, during the construction of other hydroelectric power stations, the presence of sandfly leishmania vectors has already been demonstrated <sup>15,16</sup>.

The adaptation of sandflies to the peridomiciliary environment was confirmed by the presence of several species in rural districts and periurban areas, even though in small numbers, during the research project. It is worth mentioning that some of them have been identified as ACL agent vectors<sup>17</sup>.

It deserves highlighting that in the region where the study was undertaken several agricultural and leisure activities, such as fishing and swimming, are practiced; there are soccer fields, intensively used at weekends, close to the sites sampled, where the activities continue until twilight, thus representing a risk factor for the transmission of the disease.

The vegetation, pets, and domestic and other animals in São Joaquim do Pontal, Porto Almeida, and the urban area of Itambaracá are attractive to sandflies, increasing the risk of ACL transmission in these locations.

Sandfly breeding and/or resting places are characterized by high humidity and low direct sunlight, which occur in forests or some particular peridomiciliary conditions<sup>17</sup>. The tendency of the adults is not to fly farther than about 180m from their shelters<sup>18-20</sup>, and the fact that sandflies with vectorial capacity were also observed in the periurban area of the City of Itambaracá may indicate that anthropic action has attracted these vectors to that environment.

At the 25 sampled points in the three localities, i.e., São Joaquim do Pontal, Porto Almeida, and Itambaracá urban and periurban areas (ITC), 15 species were captured, all already reported for the State of Paraná<sup>21</sup>, with the techniques employed.

The predominance of *Ny. neivai*, calculated by SSAI and as observed in the present study, has not always been seen in other areas of Paraná. In some studies *Ny. whitmani* has predominated: in the north of Paraná, 76.3%<sup>22</sup>, 58.9%<sup>23</sup> and 70%<sup>24</sup>, and in the northwest, 67.8%<sup>25</sup>. In the northwest, *Ny. neivai* predominated in 10 of the towns investigated<sup>26</sup>.

It is important to remember that *Ny. neivai* has been considered a junior synonym of *Ny. intermedia*<sup>27</sup>. In the State of São Paulo, *Ny. intermedia* occurs in coastal and *Ny. neivai* in plateau areas<sup>26</sup>.

In the present study, males of *Ny. neivai* were less attracted to the light traps than were females in São Joaquin do Pontal and Porto Almeida, while in the ITC males predominated with a significant difference. This predominance of males may indicate the proximity of breeding places, since they have more limited dispersion capacity<sup>22,28,29</sup>.

*Ny. neivai* was found at almost every site sampled, including those in Porto Almeida and São Joaquim do Pontal affected by the construction of the hydroelectric plants, as well as at the sites in the anthropic areas of Itambaracá town, where the highest numbers of this insects were observed. On the other hand, *Pi. pessoai, Ny. whitmani*, and *Mg. migonei* were more numerous in the more preserved areas (Porto Almeida -São José Farm) (**Table 1**) but were observed in all the areas sampled.

The greatest abundance of *Ny. neivai* can be explained by its adaptation to residual degraded forests and to peridomiciles, as was observed by Aguiar et al.<sup>6</sup> in their study, in which they found a predominance of this species near the residences (48.2%) and relatively low frequency in the forest margin (17.7%).

The highest diversity and abundance indices in Porto Almeida can be explained by the existence of more extensive preserved forest areas than were to be found in the other two localities.

In brief, in the municipality of Itambaracá, Paraná, in the area of influence of the hydroelectric complex Canoas I, autochthonous cases of ACL were identified in all the localities where the study was carried out, and the possible risk factors for the transmission of the disease, i.e., adaptation of the sandfly vectors to the peridomiciliary environment and the predominance of the species that transmit the ACL agents in these environments, were analyzed.

The sandfly fauna, composed of 15 species, presented a predominance of the ACL vectors: *Ny. neivai, Pi. pessoai, Ny. whitmani, Mg. migonei*, and *Pi. fischeri*. These species were found in all the localities studied.

Deforestation resulting from anthropic action related to agriculture and cattle raising, environmental alterations due to the construction of the hydroelectric power stations in the region, and the existence of installations for the breeding of animals near the residences – in general less than 300 meters away – may be attracting these sandflies to peridomiciles.

The highest SSAI values of *Ny. neivai* and *Pi. pessoai* reflect their wider dispersion and constantly higher frequencies compared with other species. These parameters seem to indicate that these two species may be transmitting the leishmaniasis agent in the area investigated.

In Itambaracá town, the males were more attracted to light traps, which may indicate the proximity of breeding places. In São Joaquin do Pontal and Porto Almeida, females, especially of *Ny. neivai*, were more attracted to light traps, which may represent a risk factor for the transmission of the disease, considering that the larger part of the collection sites were near the houses.

The highest species abundance and diversity observed in *Porto Almeida*, at the *São José* Farm, may be due to the presence of preserved forests. In these ecosystems, *Pi. pessoai* predominated. However, in the localities within Itambaracá City, with *Vila Rural* and *Pesque-e-Pague* having less dense vegetation, *Ny. neivai* predominated near the peridomiciles, indicating a process of adaptation, mainly to this environment, which can be considered a risk factor for the transmission of the disease.

Other risk factors were identified, such as the presence of wild animals, including capybaras and opossums, and of domestic and other animals such as dogs, cattle, fowl, pigs, and equines. There was also the accumulation of organic matter, including animal manure, especially that of pigs, which attracts sandflies for two reasons: feeding (presence of animals) and breeding (environments rich in organic matter and shade). Such circumstances favor the contact of the human population with the vectors, thus constituting a risk factor for the acquisition of the disease.

#### **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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