Studies on the sandfly fauna (Diptera: Psychodidae: Phlebotominae) from transmission areas of American Cutaneous Leishmaniasis in state of Acre, Brazil

Alfredo CR Azevedo/+, Simone M Costa, Maria CG Pinto[1], Janis L Souza[1], Henrique C Cruz[1], Joaquim Vidal[1], Elizabeth F Rangel

Laboratório de Transmissores de Leishmanioses, Instituto Oswaldo Cruz-Fiocruz, Av. Brasil 4365, 21040-900 Rio de Janeiro, RJ, Brasil
[1]Secretaria de Saúde do Estado do Acre, Rio Branco, AC, Brasil

Studies were undertaken on the phlebotomines in the municipalities of Bujari, Xapuri and Rio Branco in the state of Acre. The abundance of species on the ground and in the tree canopy was estimated by Standardized Index of Species Abundance. Of the 52 species identified, Lutzomyia (N.) antunesi, Lutzomyia (N.) whitmani, Lutzomyia (P.) davisi, Lutzomyia migonei, Lutzomyia (N.) umbratilis, Lutzomyia (N.) flaviscutellata, Lutzomyia (T.) ubiquitalis, Lutzomyia (P.) hirsuta hirsuta, Lutzomyia (P.) paraensis *and* Lutzomyia (P.) ayrozai *are known to be vectors of* Leishmania*, the causative agent of American cutaneous leishmaniasis.* Lutzomyia (T.) auraensis, Lu. (N.) antunesi, Lu. (N.) whitmani *and* Lu. (P.) davisi *accounted for 66.95% of the specimens collected.* Lu. (N.) whitmani *was the most abundant species, followed by* Lu. (N.) antunesi *and* Lu. (P.) davisi. Lu. (N.) antunesi *was the most abundant species in the soil as well as in the canopy.* Lu. (N.) umbratilis *occurred in all three municipalities and was the fifth most abundant species in the Chico Mendes Municipal Park in Rio Branco. It was collected on both the ground level as well as in the canopy; however, it was more frequently collected in the tree canopy. The present study suggests the existence of three transmission cycles of* Leishmania *in Acre, including the transmission of* Leishmania (V.) guyanensis *by* Lu. (N.) umbratilis *south of the Amazon River.*

Key words: Phlebotomine fauna - *Lutzomyia* - vector ecology - American Cutaneous Leishmaniasis - state of Acre - Brazil

American cutaneous leishmaniasis (ACL) is widely distributed in Latin America. In Brazil, the disease is regarded as an emerging infection and the prevalence of ACL is increasing as a result of the deforestation that occurs for the exploitation of natural resources, the expansion of agriculture and the construction of dams and hydroelectric projects. Within Brazil, ACL may present in a classic epidemiological pattern or as an epidemic surge associated with these activities (Lainson 1988, Rangel 1995, Rangel & Lainson 2003, MS 2006). In an attempt to analyze the spatial distribution of the disease within Brazil, the National Leishmaniases Program of the Health Ministry has been analyzing disease circuit of ACL by associating the occurrence of human cases with specific socio-environmental variables (MS 2006).

Within this context, Amazonia has been identified as having an epidemiological circuit of rural and occupational variables that are associated with the destruction of forest. Within this region, the state of Acre has reported a large number of mucosal leishmaniasis cases. As reported by the Health Secretary of the state of Acre, the majority of patients were male (72%) and over 10 years of age (89%). Moreover, from 1987-2007, the number of cases reported each year has increased and in this last year 1,100 cases were reported.

Recently, Tojal et al. (2003, 2006) characterized the *Leishmania* species isolated from the cutaneous lesions of patients in Rio Branco city, *Leishmania (Viannia) braziliensis*, *Leishmania* (*V.*) *guyanensis*, *Leishmania* (*V.*) *lainsoni* and a hybrid of *Leishmania* (*V.*) *naiffi* and *L.* (*V.*) *lainsoni*.

Studies on sandfly fauna in the state of Acre are still limited (Martins & Silva 1964, Arias et al. 1984, Silva-Nunes et al. 2008). This study aims to broaden the knowledge of phlebo-tomine fauna, detailing the species considered to be potential vectors in Acre and study aspects of their ecology.

MATERIALS AND METHODS

Study area - The state of Acre has a tropical wet and dry climate (Köppen classification) with yearly temperatures ranging from 24.5-32°C. There are two very distinct seasons: a dry season and a wet season. During the dry season, which starts in March and extends through October, rain is absent, although the *friagens* (a local term for the cold fronts of polar origin that invade the Amazon) are particularly common. The rainy season, which is characterized by constant (daily) and abundant rain, is from November until April. The atmospheric humidity reaches 90% with pluviometric indexes ranging from 1,600-2,750 mm a year.

Sandfly collections - Collections were undertaken in the municipalities of Bujari (Area I, highway BR-364 km

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+ Corresponding author: alcaraze@ioc.fiocruz.br
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98), Xapuri (Area II, Barro Alto, Barra, Sibéria, Nazaré and Floresta) and Rio Branco (Area III, Chico Mendes Municipal Park, Area IV, highway BR-317 km 38, and Area V, highway AC-090 km 75) from 2002 through 2006. As suggested by the Health Secretary of the state of Acre, two of the locations (Bujari, Area I, and Rio Branco, Area III) were considered strategic areas for regular collections since these municipalities have been reported high incidences of human ACL cases.

Area I is in the Bujari Municipality (09°49'50"S, 67°57'08"W), nearly 28 km from Rio Branco. The economy of Bujari is based on subsistence agriculture, cattle breeding, fish farming [e.g. tambaqui (*Colossoma macropomum)*, curimatã (*Prochilodus* spp.) and pirarucu (*Arapaima* spp.)], rubber extraction (*Hevea brasiliensis)*, chestnut production (*Bertholletia excelsa*), copaiba oil (*Copaifera* spp.) and the collection of wild seeds. Sandfly collections were conducted in the Antimari state forest with an area of 3,397.9 km^2 ramal Espinhara [(highway BR-364 km 98)], where there is wood extraction.

Rio Branco Municipality (09°58'29"S, 67°48'36"W), Area III: Chico Mendes Municipal Park is located on highway AC-040 10 km from the city of Rio Branco. The park occupies an area of 52 ha and is considered one of the last remaining areas of primary forest where very important representative species of fauna and flora live: chestnut trees, rubber trees, the squirrel-monkey (*Saimiri boliviensis*), the saki (*Pithecia irrorata*), the owl-monkey (*Aotus nigriceps*), the tamarin (*Saguinus labiatus*), the paca (*Agouti paca*), the squirrel (*Sciurus* spp.), sloths (*Bradypus variegatus*, *Choloepus didactylus* and *Choloepus hoffmanni*), the boa (*Boa constrictor*), the liana (*Chironius bicarinatus*) and several species of lizards and birds.

Sandfly capture and identification - The phlebotomines were collected in forested areas impacted by man (Area I) and in a park (Area III), using CDC light traps. Ground level captures (50 cm high) were made in Areas I and III, as well as in the tree canopy (15-18 m) in Area III. Eight CDC light traps were mounted (ground and/or canopy) at different times between 17:00h and 21:00h providing a total of 17 h capture per trap. Forty sandfly collections were carried out during the months of February/April and August/October from 2003-2006. The methods of collection used in areas II, IV and V; however collections at these areas were performed only eventually to describe local sandfly fauna in comparison to areas I and III (during the period of 2002 and 2003) were identical to those applied in areas I and III. The sandfly specimens were mounted individually on slides and the identification of species followed the classification proposed by Young and Duncan (1994). The specimens of the *Oswaldoi* group were identified according to Galati (2003).

Statistical analysis - The Index of Species Abundance (ISA), Standardized Index of Species Abundance (SISA) (Roberts & Hsi 1979) and Shannon-Wiener Diversity Index (Shannon 1948) were used to analysis the data obtained in Areas I and III. The ISA values were determined using Excel 2002 (Microsoft) and were converted in SISA, to values between 0-1. In order to obtain the Shannon-Wiener Diversity Index, the DivEs software (WC Rodrigues) was used.

Natural infection - Some sandflies collect in Areas I and III were analyzed for natural infection by flagellates. The female sandfly guts were dissected in sterile PBS. If flagellates were observed in the gut, they were inoculated into the hind paws of Syrian hamsters (*Mesocriscetus auratus*; CEUA number P0097-01). Simultaneously, the sandfly species was identified by analysis of the spermathecae.

RESULTS

Sandfly fauna - A total of 7,302 specimens belonging to 52 species were collected in the five areas. Of these 16 are here registered for the state of Acre for the first time. Overall, 66.9% of the specimens collected were identified as *Lutzomyia* (*Trichophoromyia*) *auraensis*, *Lutzomyia (N.) antunesi*, *Lutzomyia (N.) whitmani* and *Lutzomyia (P.) davisi*. Some of the identified sandfly species are vectors of *Leishmania* species: *Lu. (N.) whitmani*, *Lu. (N.) antunesi, Lu. (P.) davisi, Lutzomyia (N.) umbratilis*, *Lutzomyia (N.) flaviscutellata*, *Lutzomyia (T.) ubiquitalis* (in Area II only), *Lutzomyia (P.) hirsuta hirsuta*, *Lutzomyia migonei* (Group *Migonei*), *Lutzomyia (P.) paraensis* and *Lutzomyia (P.) ayrozai* (Table I). A total of 915 h (Area I, 255 h; Area II, 210 h; Area III, 360 h; Area IV, 30 h and Area V, 60 h) were spent in captures. The best results were obtained in Area II, corresponding to 9.1 specimens per hour. In Areas I and III, 7.8 specimens per hour were observed.

Statistical tests were applied to 4,805 collected specimens, obtained during the 615 h of capture in Areas I and III. *Lu. (N.) whitmani* was the most abundant species with a SISA of 0.718. The SISA for other *Leishmania* sandfly vectors were: *Lu. (N.) antunesi*, 0.670; *Lu. (P.) davisi*, 0.664; *Lu. (N.) umbratilis*, 0.374; *Lu. (P.) h. hirsuta*, 0.136; *Lu. (N.) flaviscutellata*, 0.118; *Lu. migonei*, 0.074; *Lu. (P.) paraensis*, 0.030 and *Lu. (P.) ayrozai*, 0.025 (Table II). Overall, these sandfly species accounted for 75.5% of the specimens collected.

Subgenera *Nyssomyia* (54.5%) and *Psychodopygus* (30.4%) species accounted for 84.9% of sandflies collected in Areas I and III (Table II). *Lu. davisi, Lutzomyia carrerai carrerai*, *Lutzomyia amazonensis*, *Lutzomyia chagasi* and *Lu. h. hirsuta* (subgenus *Psychodopygus*) accounted for 67% of the sandfly fauna in Area I, while *Lu. whitmani*, *Lu. antunesi*, *Lu. umbratilis*, *Lutzomyia richardwardi* and *Lu. flaviscutellata* (subgenus *Nyssomyia*) accounted for 86.4% in Area III (Fig. 1).

The highest number of sandfly species (34) was collected in Area I and as a result, Area I had the highest value for the Shannon-Wiener Diversity Index (0.90).

The species with the highest SISA values that were common to both areas were *Lu. (P.) davisi* (Area I SISA 0.792, Area III SISA 0.542), *Lu. (N.) antunesi* (Area I SISA 0.475, Area III SISA 0.801), *Lu. (N.) whitmani* (Area I SISA 0.686, Area III SISA 0.729), *Lu. (Lutzomyia) gomezi* (Area I SISA 0.372, Area III SISA 0.280).

TABLE I

Total and percentage of sandfly species collected in the Area I (Bujari municipality), Area II (Xapuri municipality), Areas III, IV, V (Rio Branco municipality), state of Acre, Brazil, 2002-2006

Species	Males	Females	Total	%
Lutzomyia (Trichophoromyia) auraensis	695	724	1,419	19.44
Lutzomyia (Nyssomyia) antunesi[c]	693	632	1,325	18.16
Lutzomyia (N.) whitmani[c]	821	347	1,168	16.00
Lutzomyia (Psychodopygus) davisi[c]	470	505	975	13.35
Lutzomyia (P.) carrerai carrerai	165	312	477	6.53
*Lutzomyia nevesi (*Group *Verrucarum)*	112	119	231	3.16
Lutzomyia (N.) umbratilis[c]	123	97	220	3.01
Lutzomyia (P.) hirsuta hirsuta[c]	110	106	216	2.96
Brumptomyia spp.[a]	100	60	160	2.19
Lutzomyia saulensis (Group *Saulensis*)	22	121	143	1.96
Lutzomyia (N.) richardwardi	25	101	126	1.73
Lutzomyia (Pressatia) choti	83	32	115	1.57
Lutzomyia (Lutzomyia) sherlocki	27	40	67	0.92
Lutzomyia (N.) shawi[b]	21	41	62	0.85
Lutzomyia (Lutzomyia) gomezi	9	51	60	0.82
Lutzomyia (Sciopemyia) sordellii	26	33	59	0.81
Lutzomyia (N.) flaviscutellata[c]	13	35	48	0.66
Lutzomyia (P.) amazonensis	29	12	41	0.56
Lutzomyia (Trichophoromyia) ubiquitalis[c]	27	14	41	0.56
Lutzomyia (P.) lainsoni[b]	5	33	38	0.52
Lutzomyia (Evandromyia) infraspinosa[b]	4	31	35	0.48
Lutzomyia (Psathyromyia) shannoni	2	27	29	0.40
Lutzomyia (S.) preclara[b]	2	25	27	0.37
Lutzomyia walkeri (Group *Migonei*)	15	9	24	0.33
Lutzomyia baculus (Group *Migonei*)	8	15	23	0.31
Lutzomyia serrana (Group *Verrucarum*)	5	14	19	0.26
Lutzomyia (P.) chagasi[b]	18	-	24	0.25
Lutzomyia migonei[c] (Group *Migonei*)	8	9	17	0.23
Lutzomyia (P.) dendrophyla	13	3	16	0.22
Lutzomyia peresi[b] (Group *Oswaldoi*)	-	15	15	0.21
Lutzomyia (Trichopygomyia) dasypodogeton[b]	13	1	14	0.19
Lutzomyia (Vinnamyia) furcata	8	5	13	0.18
Lutzomyia (P.) paraensis[b]	1	8	9	0.12
Lutzomyia (P.) damascenoi	-	7	7	0.10
Lutzomyia (P.) yulli yulli[b]	2	5	7	0.10
Lutzomyia (P.) ayrozai[b]	3	3	6	0.08
Lutzomyia sericea (Group *Migonei*)	2	4	6	0.08
Lutzomyia aragaoi (Group *Aragaoi*)	2	2	4	0.05
Lutzomyia (N.) olmeca bicolor	-	4	4	0.05
Lutzomyia (P.) lutziana	2	1	3	0.04
B. avellari	1	1	2	0.03
Lutzomyia (P.) corossoniensis[b]	2	-	2	0.03
Lutzomyia (P.) llanosmartinsi[b]	-	2	2	0.03
Lutzomyia (Micropygomyia) micropyga	1	1	2	0.03
Lutzomyia (E.) bourrouli	1	-	1	0.01
Lutzomyia (T.) elegans	1	-	1	0.01
Lutzomyia (T.) ininii[b]	1	-	1	0.01
Lutzomyia (E.) monstruosa	-	1	1	0.01
Lutzomyia pilosa[b] (Group *Pilosa*)	1	-	1	0.01
Lutzomyia (P.) punctigeniculata	-	1	1	0.01
Lutzomyia villelai[b] (Group *Oswaldoi*)	-	1	1	0.01
Total	3,692	3,610	7,302	100.00

a: *B. brumpti* and *B. pentacantha*; *b*: species cited by the 1st time in the state of Acre; *c*: sandfly vectors of American cutaneous leishmaniasis.

TABLE II

Total, percentage, specimens dissected and SISA of the sand fly species collected at Areas I (Bujari municipality) and III (Rio Branco municipality), state of Acre, Brazil, 2003-2006

Species	Males	Females (Dissected)	Total	%	SISA	Rank
Lutzomyia (Nyssomyia) whitmani[b]	810	310 (192)	1,120	23.32	0.718	1st
Lutzomyia (N.) antunesi[b]	658	577 (286)	1,235	25.70	0.670	2nd
Lutzomyia (Psychodopygus) davisi[b]	376	436 (56)	812	16.90	0.664	3rd
Brumptomyia spp.[a]	86	59 (32)	145	3.02	0.436	4th
Lutzomyia (Nyssomyia) umbratilis[b]	122	94 (32)	216	4.50	0.374	5th
Lutzomyia (Lutzomyia) gomezi	9	46 (23)	55	1.14	0.341	6th
Lutzomyia nevesi (Group *Verrucarum*)	95	73 (54)	168	3.50	0.324	7th
Lutzomyia (Lutzomyia) sherlocki	23	34 (11)	57	1.19	0.245	8th
Lutzomyia (P.) carrerai carrerai	152	264 (43)	416	8.66	0.245	
Lutzomyia saulensis (Group *Saulensis*)	12	53 (8)	65	1.35	0.194	10th
Lutzomyia (Sciopemyia) preclara	2	25	27	0.56	0.145	11th
Lutzomyia (P.) hirsuta hirsuta[b]	105	95 (12)	200	4.16	0.136	12th
Lutzomyia (Trichophoromyia) auraensis	13	23 (13)	36	0.75	0.127	13th
Lutzomyia (Trichopygomyia) dasypodogeton	11	1	12	0.25	0.122	14th
Lutzomyia (N.) flaviscutellata	1	22 (2)	23	0.48	0.118	15th
Lutzomyia (Evandromyia) infraspinosa	4	16 (10)	20	0.42	0.116	16th
Lutzomyia (Vinnamyia) furcata[b]	8	5 (2)	13	0.27	0.114	17th
Lutzomyia (Psathyromyia) shannoni	1	12 (11)	13	0.27	0.106	18th
Lutzomyia (N.) shawi	4	9 (2)	13	0.27	0.093	19th
Lutzomyia (P.) dendrophyla	10	2	12	0.25	0.082	20th
Lutzomyia baculus (Group *Migonei*)	2	5 (4)	7	0.15	0.076	21th
Lutzomyia migonei (Group *Migonei*)[b]	7	2 (1)	9	0.19	0.074	22th
Lutzomyia (S.) sordellii	7	29 (2)	36	0.75	0.066	23th
Lutzomyia walkeri (Group *Migonei*)	12	6	18	0.37	0.061	24th
Lutzomyia peresi (Group *Oswaldoi*)	-	10 (10)	10	0.21	0.058	25th
Lutzomyia (N.) richardwardi	1	8 (5)	9	0.19	0.055	26th
Lutzomyia (Pressatia) choti	3	3	6	0.12	0.053	27th
Lutzomyia serrana (Group *Verrucarum*)	1	4	5	0.10	0.037	28th
Lutzomyia aragaoi (Group *Aragaoi*)	2	1	3	0.06	0.031	29th
Lutzomyia (N.) olmeca bicolor	-	4 (2)	4	0.08	0.031	30th
Lutzomyia (P.) paraensis[b]	1	8	9	0.19	0.030	31th
Lutzomyia (P.) ayrozai[b]	2	-	2	0.04	0.025	32th
Lutzomyia sericea (Group *Migonei*)	2	-	2	0.04	0.024	33th
Lutzomyia (P.) chagasi	-	17	17	0.35	0.022	34th
Lutzomyia (P.) amazonensis	2	-	2	0.04	0.020	35th
Lutzomyia (P.) lutziana	2	-	2	0.04	0.017	36th
Lutzomyia (P.) corossoniensis	2	-	2	0.04	0.016	37th
Lutzomyia (E.) monstruosa	-	1	1	0.02	0.015	38th
Lutzomyia villelai (Group *Oswaldoi*)	-	1	1	0.02	0.014	40th
Lutzomyia (T.) ininii	1	-	1	0.02	0.013	41th
Lutzomyia (E.) bourrouli	1	-	1	0.02	0.011	42th
Total	2,550	2,255 (813)	4,805	100.00		

a: *B. brumpti* and *B. pentacantha; b*: sand fly vectors of American cutaneous leishmaniasis; SISA: Standardized Index of Species Abundance.

Overall, *Lu. (P.) davisi* (0.790) and *Lu. (N.) antunesi* (0.801) were the most abundant in Areas I and III, respectively (Fig. 2A, B).

The ranking of sandfly species that serve as vectors for *Leishmania* registered from areas I and III is shown in Fig. 3. *Lu. (N.) antunesi*, *Lu. (N.) whitmani, Lu. (N.) umbratilis*, *Lu. (P.) davisi*, *Lu. (N.) flaviscutellata* and *Lu. migonei* were collected in Areas I and III; *Lu. (P.) paraensis* and *Lu. (P.) ayrozai* were collected only in Area III and *Lu. (P.) h. hirsuta* was collected only in Area I. *Lu. (N.) antunesi* was the most abundant species in Area III (0.801), followed by *Lu. (N.) whitmani* (0.729) and *Lu. (N.) umbratilis* (0.573). In Area I, *Lu. (P.) davisi* was the most abundant (0.792). Fig. 4A shows the stratification of the most representative sandfly species. *Lu. (N.) whitmani* was the most numerous in the canopy

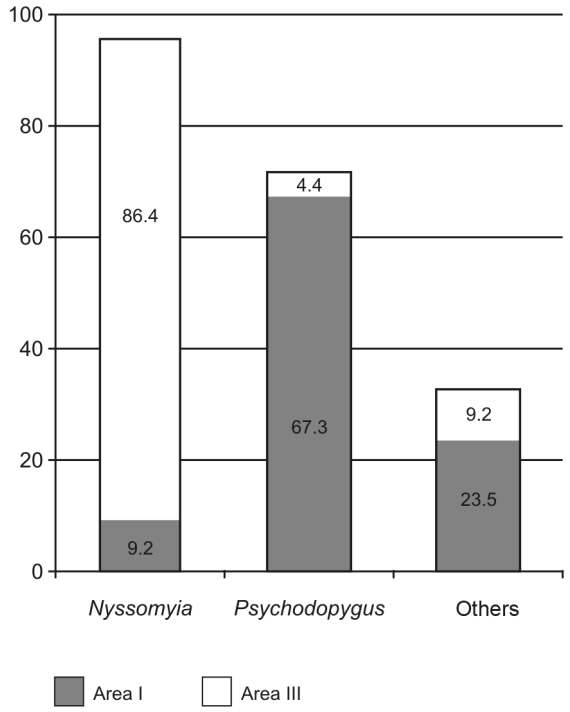

Fig. 1: species percentage of *Nyssomyia* and *Psychodopygus* subgenera in Areas I (Bujari municipality) and III (Rio Branco municipality), state of Acre, Brazil, 2003-2006.

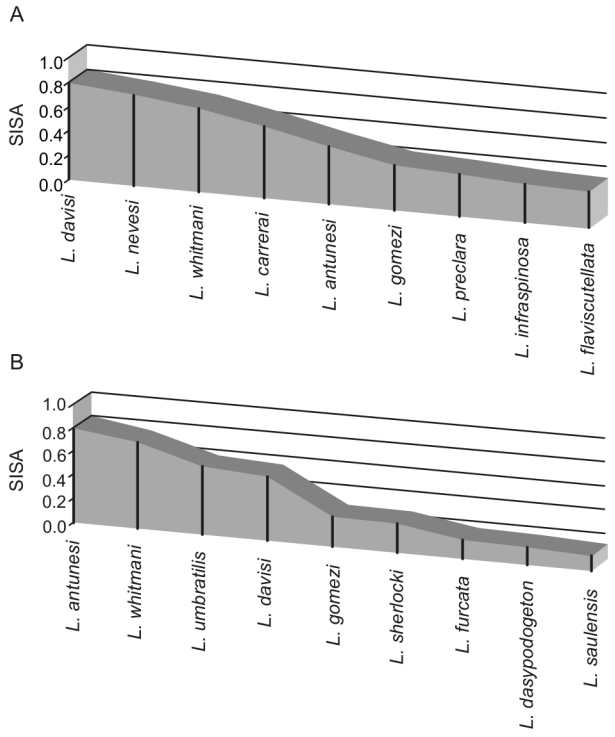

Fig. 2: Sandardized Index of Species Abundance (SISA) of the nine abundant species in Areas I (Bujari municipality) and III (Rio Branco municipality), state of Acre, Brazil,2003-2006. A: Area I; B: Area III.

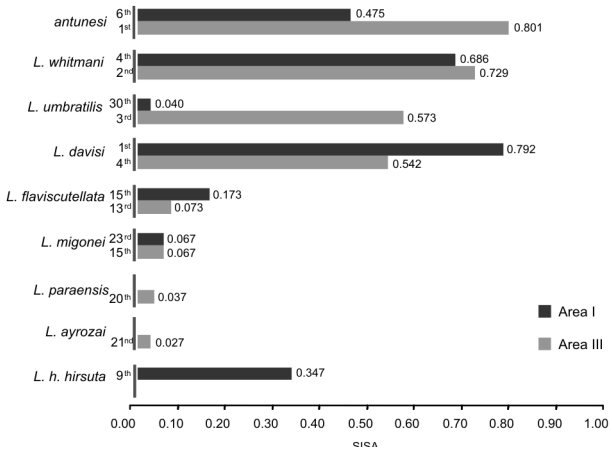

Fig. 3: Standardized Index of Species Abundance (SISA) and rank of medical important species in Areas I (Bujari municipality) and III (Rio Branco municipality), state of Acre, Brazil, 2003-2006.

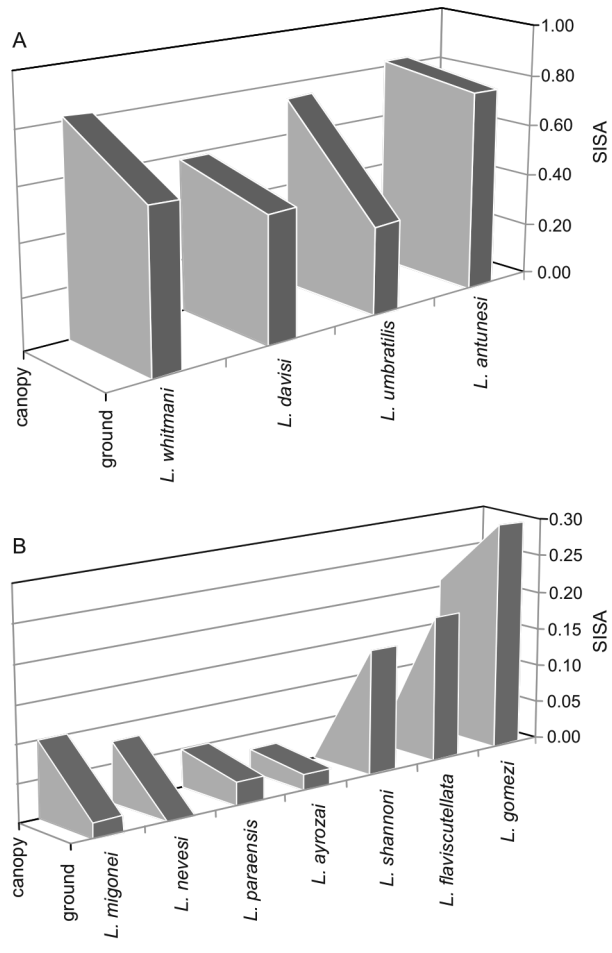

Fig. 4: Standardized Index of Species Abundance (SISA) on the ground and in the tree canopy, in Area III (Rio Branco municipality), state of Acre, Brazil, 2003-2006. A: species with highest values of SISA; B: other species.

(0.816), while *Lu. (N.) antunesi* had the highest SISA value in the canopy (0.821). *Lu. (N.) umbratilis* was more abundant in canopy (0.740), compared to the ground level collections (0.343). Less representative species, namely *Lu. (N.) flaviscutellata* (0.193) and *Lutzomyia (Psathyromyia) shannoni* (0.164) were collected in ground level traps only, while *Lutzomyia nevesi* (Group *Verrucarum*) was only collected in the canopy (0.079) (Fig. 4B).

Natural infection - A total of 816 females belonging to 23 sandfly species were dissected. It was impossible to identify 12 *Brumptomyia* specimens, since *Brumptomyia brumpti* and *Brumptomyia avellari* females cannot be distinguished morphologically. *Lu. (N.) antunesi* and *Lu. (N.) whitmani* were the predominant species and in one specimen of each a natural infection of flagellates, probably Peripylarian promastigotes, in the pylorus and mid gut was observed. The flagellates were inoculated into hamsters but no lesion was observed and the characterization of the parasite was enabling.

DISCUSSION

The state of Acre is characterized by a great variety of ecosystems and habitats and it is rich in vegetation typologies, topographical gradients and different types of soils (IBGE 1996). Although 45.6% of its territory is occupied by protected areas, the state has been undergoing continuous deforestation, which has affected 19,200 km^2 (Souza Jr. et al. 2006). This deforestation occurs mostly for new agricultural settlements, constant slash and burn and extraction of timber for commercial purposes. Since 2002, a 34% increase in the annual deforestation rate has been observed for the whole state (878 km^2 a year). Among the municipalities that have lost their original forest cover, the most significant are Bujari (34%) and Rio Branco (25%) (Souza Jr. et al. 2006). Located in south-western Amazonia, the state of Acre sandfly fauna is typical to the Amazon region.

Among the total number of species present in the five areas, 16 species have not yet been registered in the state of Acre by Young and Duncan (1994), Aguiar and Medeiros (2003) or Galati (2003). Among the newly registered species were *L. (P.) paraensis* and *L. (P.) ayrozai,* both of which are of significant medical and epidemiological importance in the Amazon region. Although the females of *B. brumpti* (first occurrence) and *Brumptomyia pentacantha* are indistinguishable, and were therefore just classified as *Brumptomyia*, the identification of both species was possible due to the 92 male specimens of *B. brumpti* and the eight specimens of *B. pentacantha* found in the area. The identification of *Lu. peresi* and *Lutzomyia villelai* was possible by using the taxonomic key proposed by Galati (2003). Even though Young and Ducan (1994) described *Lu. villelai* as similar species to *Lutzomyia trinidadensis,* and previous studies have detected *Lu. trinidadensis* in the state of Acre, the species found in this study is in fact *Lu. villelai* as it lacks developed spines in the pharynges, an important morphological feature that distinguishes this species from *Lu. trinidadensis.*

The *Psychodopygus* and *Nyssomyia* subgenera are the most abundant species and also the most important vectors of *Leishmania* in the Amazon Region (Ward et al. 1973, Grimaldi et al. 1991, Rangel & Lainson 2003) and this was confirmed in the present study. The species of the *Nyssomyia* subgenus, in particular *Lu. antunesi* and *Lu. whitmani*, were predominant in Area III (Chico Mendes Municipal Park), which, despite being a protected park, is under significant human pressure from both the new dwelling areas and deforestation activities now surrounding the park, as well as the approximately 96,000 people per year who use area for recreation (JO Guimarães, park manager, personal communication). By contrast, Area I (ramal Espinhara) contained the highest frequency of species of the *Psychodopygus* subgenus. The predominance of the *Psychodopygus* subgenus, in addition to a higher Shannon-Wiener index of diversity value (0.90), are indications of a more preserved vegetation in Area I, which could be more structured in relation to Chico Mendes Municipal Park (Area III).

In confirmation of previous studies, this study suggests that changes to the forest have a profound impact on the composition and behaviour of the sandfly fauna (Arias & Freitas 1982, Ready et al. 1986, Azevedo et al. 2002). Azevedo et al. (2002) demonstrated that, based on the quantitative and qualitative analysis of the sandfly fauna in the Peixoto de Azevedo municipality (state of Mato Grosso), differences in the composition of the sandfly fauna between the primary forest (predominantly *Psychodopygus*) and the anthropic area (preimarily *Nyssomyia*) could be detected. In the areas studied in the state of Acre, nine potential *Leishmania* vectors were identified in Areas I and III. Among them, *Lu. (N.) whitmani*, *Lu. (N.) antunesi, Lu. (P.) davisi* and *Lu. (N.) umbratilis* are the most important due to their higher SISA values.

Lu. (N.) umbratilis is the most important vector of *L. (V.) guyanensis*, an etiological agent of ACL, which is characterized by multiple lesions (Ward & Fraiha 1977). Different studies have demonstrated the epidemiological importance of this species in the area north of the Amazon River, in the states of Pará (Lainson et al. 1976, 1979, 1981), and Amazonas, Brazil (Arias & Freitas 1977, 1978, Arias et al. 1981), in French Guiana (Gentile et al. 1981, Pajot et al. 1982) and in Ve-nezuela (Feliciangeli et al. 1985).

In all studies, the behavioural pattern of *Lu. (N.) umbratilis* is similar when compared to the areas evaluated here. This species is abundant and anthropophilic. Its preferred habitats are buttress roots, trunks and the tree canopy where it feeds on sloth. It is active during the twilight hours, although it may also bite during the first hours of the day (Rangel & Lainson 2003). The fact that *Lu. (N.) umbratilis* can be found infected with *L. (V.) guyanensis* exclusively in the area north of the Amazon River has raised doubts regarding the real taxonomy of this species. Arias and Freitas (1978) suggested that the Amazon River could function as a geographic barrier to the transmission of *L. (V.) guyanensis.* Furthermore, Lainson (1988) suggested that *Lu. (N.) umbratilis* could represent a complex of species, since it has not been observed transmitting *L. (V.) guyanensis* south of the Amazon River, perhaps due to the different behaviour between the northern

and southern populations of this species. While working in the municipality of Peixoto de Azevedo (an area with endemic cutaneous leishmaniasis), Azevedo et al. (2002) observed that *Lu. (N.) umbratilis* was one of the most abundant and anthropophilic sandfly species. The same study demonstrated the association of *Lu. (N.) umbratilis* with *L. (V.) braziliensis* as it was found in a naturally infected *Lu. (N.) umbratilis* specimen.

The studies carried out at Chico Mendes Municipal Park (Area III) revealed the presence of *Lu. (N) umbratilis* within two different strata, although *Lu. (N) umbratilis* was predominantly isolated in the tree canopy. In the tree canopy, *Lu. (N.) umbratilis* was the third most abundant species among all other collected sandflies. It is important to note that the vertebrate park fauna is composed of mammals belonging to the Xenarthra, Rodentia, Didelphimorphia and Primates orders. Among Xenarthra, the Conservation Unit inventory (JO Guimarães, personal communication) described three species of sloths including *C. didactylus,* a reservoir of *L. (V.) guyanensis*. In addition to the characterization of *Leishmania* isolated from patients in the municipality of Rio Branco (Tojal et al. 2006), the presence of *Lu. (N.) umbratilis* in the tree canopy and the presence of a natural reservoir of *L. (V.) guyanensis* within the areas studied provide strong evidence of the existence of a transmission cycle of *L. (V.) guyanensis* by *Lu. (N.) umbratilis*. Therefore, the evidence presented in this paper does not support the hypothesis of a possible *Lu. (N) umbratilis* complex in the region. The transmission of *L. (V.) guyanensis* by this sandfly species south of the Amazon River, even though the vector's behaviour is similar to that previously reported in literature, has been suggested for the first time.

According to the state of Acre's Health Secretary, and taking into consideration the findings of Tojal et al. (2006), most human cases of ACL in the region seem to be related to *L. (V.) braziliensis* infection. Given the frequency of *L. (V.) braziliensis* and its spatial distribution across the transmission areas, in addition to the fact that no known *L. (V.) braziliensis* vectors, such as *Lutzomyia (P.) wellcomei* and *Lutzomyia (P.) complexa,* were detected from Northern Brazil, *Lu. (N.) whitmani* could be a *Leishmania* vector for the municipalities investigated during the present study. Importantly, previous studies have connected *Lu. (N.) whitmani* with the transmission of *Leishmania (V.) shawi* in the Amazon region (Rangel et al. 1996, Lainson & Shaw 1998, Rangel & Lainson 2003, Costa et al. 2007).

In agreement with Tojal et al. (2006), who have isolated and characterized *L. (V.) lainsoni* from Acre's patients, and supported by the data presented in this study, the finding of *Lu.* (*T.*) *ubiquitalis* suggests that the transmission cycle in the state of Acre is similar to the transmission cycle reported for the state of Pará (Silveira et al. 1991, Lainson & Shaw 1998, Rangel & Lainson 2003). Furthermore, a previous report has already demonstrated the presence of *Lu.* (*T.*) *ubiquitalis* in the Acrelândia municipality and suggested that this sandfly species is a local vector of *Leishmania* (Silva-Nunes et al. 2008).

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