Molecular Detection of *Leishmania* in Sand Flies (Diptera: Psychodidae: Phlebotominae) Collected in the Caititu Indigenous Reserve of the Municipality of Lábrea, State of Amazonas, Brazil

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J. Med. Entomol. 51(6): 1276–1282 (2014); DOI: http://dx.doi.org/10.1603/ME14025

ABSTRACT Phlebotominae sand flies are of medical importance because they are vectors of human pathogens, such as protozoa of the genus *Leishmania* Ross, etiological agent of American cutaneous leishmaniasis (ACL). In Lábrea, a municipality in the state of Amazonas, Brazil, ACL is primarily associated with subsistence activities, such as collection and extraction of forest products, undertaken by both indigenous and nonindigenous people. Data on ACL in indigenous populations are scarce, such that there is little information on the identity of the etiologic agent(s), reservoir host(s) and insect vector(s). The aim of this work was to study the sand fly fauna collected during an 8-d surveillance of different habitats in the Indigenous Reserve Caititu, Lábrea. In total, 1,267 sand flies were collected in different habitats for eight consecutive days, of which 819 (64.6%) were females and 448 (35.4%) males, from 10 genera and 32 species. The most abundant genera were *Psychodopygus* (34.3%), *Trichophoromyia* (22.9%), and *Nyssomyia* (15.3%). The most abundant species were *Trichophoromyia ubiquitalis* (Mangabeira) ($n = 235$, 18.5%), *Psychodopygus davisi* (Root) ($n = 228$, 18.0%) and *Nyssomyia antunesi* (Coutinho) ($n = 135$, 10.7%). Direct sequencing of polymerase chain reaction products demonstrated the presence of *Leishmania* (*Leishmania*) *amazonensis* and *Leishmania* (*Viannia*) *braziliensis* in the following species of sand flies: *Evandromyia apurinan* (Shimabukuro, Silveira, & Silva), *Nyssomyia umbratilis* (Ward & Fraiha), *Nyssomyia yuilli yuilli* (Young & Porter), *Ps. davisi*, *Sciopemyia servulolimai* (Damasceno & Causey), and *Th. ubiquitalis*. The presence of natural infection by *Leishmania* detected in the sand fly species investigated in this study suggests their possible role in the transmission cycle of ACL in the studied area.

KEY WORDS Amazon, cutaneous leishmaniasis, indigenous people, sand fly

Sand flies are insects belonging to the order Diptera, family Psychodidae, and subfamily Phlebotominae. There are ≈900 known species in the world, of which 521 are valid species described for the Americas, with 133 species recorded only in the state of Amazonas (Galati 2003, Shimabukuro and Galati 2010, Figueira et al. 2013).

American cutaneous leishmaniasis (ACL) is a disease caused by 29 different *Leishmania* species, which infect the skin and mucous membranes. Transmission between 30 vertebrate hosts occurs with the bite of various species of sand flies (Lainson et al. 1994). The main vector species of ACL known in Brazil are *Bichromomyia flaviscutellata* (Mangabeira), *Nyssomyia whitmani* (Antunes & Coutinho), *Nyssomyia umbratilis* (Ward & Fraiha), *Nyssomyia intermedia* (Lutz & Neiva), *Psychodopygus wellcomei* Fraiha, Shaw & Lainson, and *Migonemyia migonei* (França) (Brasil 2007). However, there are also studies throughout Brazil, providing information on the fauna and biology of the insect vector involved in the transmission of ACL, particularly in the state of Amazonas (Castellón et al. 2000; Alencar 2007; Barbosa et al. 2008; Alves et al. 2011, 2012; Guerra et al. 2011; Figueira et al. 2013).

ACL is primarily zoonotic, but can infect humans, which are involved secondarily (Lainson and Shaw 1972, Lainson 1988; Brasil 2007). Several animals have been found infected, including wild, synanthropic, and domestic animals, such as sloths, anteaters, opossums, raccoons, rodents, dogs, and domestic cats (Brasil 2007, World Health Organization [WHO] 2010).

Studies reporting the incidence and prevalence of cases of human leishmaniasis in the state of Amazonas

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0022-2585/14/1276–1282$04.00/0

are scarce, and mostly concern the capital city of Manaus and its surrounding area north of the Amazon River, with few studies conducted south of the Amazon River. Detection of *Leishmania* was performed mainly in clinical samples in lesions from patients who received some form of medical care (Romero et al. 2002; Guerra et al. 2003, 2006, 2011; Teixeira et al. 2007, 2008; Figueira et al. 2008, Andrade et al. 2011, Benício et al. 2011; Chrusciak-Talhari et al. 2011; Coelho et al. 2011, Neves et al. 2011). However, there are some studies of *Leishmania* detection in sand flies from the state of Amazonas (Arias and Freitas 1978, Arias et al. 1987). In a study on vectors of ACL in the city of Manaus, the species *Ny. umbratilis* and *Nyssomyia anduzei* (Rozeboom) were found to be infected by *Leishmania* (*Viannia*) *guyanensis* (Arias and Freitas 1978). Arias et al. (1987) also conducted a study on vectors of ACL in Manaus and found *Leishmania* (*Leishmania*) *amazonensis* infecting *Bi. flaviscutellata* and *Bichromomyia olmeca nociva* (Young & Arias).

This work was conducted in Lábrea, a remote municipality in the State of Amazonas, south of the River Amazon, situated within the intact rain forest, where many ACL cases occur annually, with a significant number of cases probably being unreported. In this municipality, ACL is primarily associated with the extraction of forest products by indigenous and nonindigenous people. No published data on ACL in the indigenous population is available, but ≈12% of the population are from indigenous ethnic groups, and there is little information on the etiologic agent(s), nonhuman reservoir host(s), and insect vector(s) of *Leishmania* in this area (Guerra et al. 2011).

In this study, the sand flies collected in a small community, the Castanheira settlement of the Caititu Indigenous Reserve, Lábrea municipality, Amazonas state, are described, as are the findings of the presence of *Leishmania* DNA in the sand flies collected. The rate of natural infection of these insects is also estimated, we discuss possible vector species involved in the transmission cycle of ACL and *Leishmania* species circulating in the area.

Materials and Methods

Study Area. The municipality of Lábrea (07° 15′ S; 64° 47′ W) is located between the Madeira and Purus Rivers. The municipality has 41,600 inhabitants and an area of ≈68,234 km^2 (IBGE 2011). The municipality includes various conservation areas and indigenous reserves (areas of protected land occupied by indigenous people) that form a large continuum of preserved forest. The Caititu Indigenous Reserve is an area of 308,062 ha and a population of ≈1,022 inhabitants and inhabited by the Apurinã indigenous people. This Indigenous Reserve is located at ≈24 km from the urban area of Lábrea and has ≈70 inhabitants of the Apurinã ethnicity, according to the census of Subsistema de Atenção à Saúde Indígena/Secretaria Especial de Saúde Indígena.

Sand Fly Collection. Sand fly collection was conducted during 8 nights in February 2012. HP light traps (Pugedo et al. 2005), a modified version of a Centers for Disease Control and Prevention (CDC) light trap, were used, and installed at a height of up to 2 m in 10 randomly selected points along trails used for Brazil nut collection, and along the banks of the Poágua River stream, used by the inhabitants for their daily activities. Data collection was conducted under the Sistema de Autorização e Informação em Biodiversidade/Instituto Brasileiro de Meio Ambiente e dos Recursos Naturais Renováveis licenses (32971 and 15955-1). Sand flies were killed by freezing and placed individually into 1.5-mL microtubes containing 6% DMSO (dimethyl sulfoxide) and kept frozen at −20°C. In the laboratory, sand flies were kept under aseptic conditions and identified by dissection of the head and the genitalia. Identification to genus and species was performed according to the key by Galati (2003).

DNA Extraction, Polymerase Chain Reaction (PCR), and Sequencing. Samples were organized in pools ranging from 2 to 10 individuals of the same species, and macerated with a sterile pestle. DNA extraction was performed using Gentra Puregene QIAGEN (EUD, Germantown, MD) extraction kit, following the manufacturer's specifications. PCR was performed using primers which amplify a 350-bp fragment of the *Leishmania* ribosomal internal transcribed spacer 1 (ITS1) region, following the protocol described by Schonian et al. (2003). The primers used in the reaction were LITSR 5′-CTG GAT CAT TTT CCG ATG-3′ and L5.8S 5′-TGA TAC CAC TTA TCG CAC TT-3′. The concentrations of the reagents used were: 1× PCR buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 1.5 mM MgCl2, 0.2 mM dNTPs, DMSO, 0.5 pmol LIT SR and L5.8S primers, 5U/μl Platinum*Taq*DNA Polymerase (Invitrogen, Carlsbad, CA), and H_2O. For each reaction, 3 μl of template DNA was used. PCR conditions were: initial denaturation at 95°C for 2 min, followed by 35 cycles of: denaturation at 95°C for 30 s, annealing at 53°C for 30 s and extension at 72°C for 60 s; with a final extension of 72°C for 10 min. The amplified products were visualized on 1.5% agarose gel stained with ethidium bromide, and analyzed using the photodocumentation L-PIX EX Biotechnology Loccus system. DNA extracted from reference strains of *Leishmania amazonensis* (IFLA/BR/67/PH8) *Leishmania braziliensis* (MHOM/BR/75/M2903), *Leishmania infantum* (MHOM/BR/74/PP75), and *L. guyanensis* (MHOM/BR/75/M4147) were used as positive controls and were kindly provided by the Grupo de Estudo em Leishmanioses of Centro de Pesquisas René Rachou – FIOCRUZ. Positive bands were excised from the agarose gel with the aid of sterile scalpel blades, then purification of DNA was performed with QIAquick gel extraction kit (QIAGEN), using the recommendations of the manufacturer. The genetic sequencing reaction was performed by the Sanger sequencing platform at CPqRR/FIOCRUZ to identify the *Leishmania* species detected by PCR. The raw sequencing files were analyzed using the program

Table 1. Sand fly species collected in the Indigenous Reserve Caititu, Lábrea, state of Amazonas, Brazil, February 2012

Genus	Species	CDC1		CDC2		CDC3		CDC4		CDC5		CDC6		CDC7		CDC8		CDC9		CDC10		Aspiration		Total
		F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	
Bichromomyia	*flaviscutellata*	–	–	–	–	–	–	–	–	–	–	–	–	1	–	2	–	2	–	2	–	–	–	7
Evandromyia	*apurinan*	–	–	3	–	–	–	1	–	–	–	5	–	2	–	1	–	1	–	–	–	–	–	13
	bacula	–	–	3	–	–	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	5
	begonae	5	–	20	–	1	–	–	–	2	–	4	–	3	–	–	–	–	–	–	–	–	–	35
	infraspinosa	1	–	3	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	6
	saulensis	–	–	–	–	–	–	–	–	–	–	–	–	1	1	5	–	–	–	–	–	–	–	7
	walkeri	–	–	1	–	–	–	–	–	–	–	–	2	–	1	–	–	–	–	1	–	–	–	5
	sp.	–	–	1	1	–	–	–	–	–	1	–	–	1	–	–	–	–	1	–	–	–	–	5
Lutzomyia	*sherlocki*	–	–	5	–	3	–	2	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	11
	sp.	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Micropygomyia	*pilosa*	–	–	2	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	3
Nyssomyia	*anduzei*	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1	2
	antunesi	2	–	13	2	4	–	8	–	14	4	20	5	38	7	5	–	3	–	4	3	–	3	135
	umbratilis	2	–	5	–	1	–	5	–	1	–	5	–	7	–	–	–	–	–	–	–	–	–	26
	richardwardi	–	–	–	–	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	2
	yuilli yuilli	5	–	6	–	6	–	3	–	–	–	6	–	1	–	–	–	–	–	–	–	–	–	27
	sp.	–	–	–	1	1	–	2	–	3	–	–	–	2	–	–	–	–	–	–	–	–	–	9
Pressatia	sp.	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Psathyromyia	*abunaensis*	–	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	–	–	–	–	–	–	2
	aragaoi	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1
	barrettoi barrettoi	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	2
	coutinhoi	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1
	dendrophyla	–	–	–	2	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	2	5
	sp.	1	–	2	–	–	–	1	–	–	–	2	–	–	–	–	–	–	–	–	–	5	–	11
Psychodopygus	*ayrozai*	–	1	2	1	1	1	1	–	8	–	15	9	9	7	–	1	–	–	–	–	–	–	56
	carreirai	1	–	1	–	–	–	–	–	6	–	–	2	4	–	2	–	–	–	–	1	–	–	17
	chagasi	–	2	–	–	–	–	–	1	–	2	–	2	–	6	–	–	–	–	–	–	–	–	13
	claustrei	1	1	–	–	1	1	–	1	1	–	2	5	1	5	–	–	–	–	–	2	–	–	21
	davisi	7	6	3	4	2	3	7	1	97	4	19	2	46	9	7	1	6	1	–	2	–	1	228
	llanosmartinsi	–	–	–	–	–	–	–	–	15	–	1	–	3	3	1	–	1	–	–	–	–	–	24
	paraensis	2	1	4	–	1	1	2	2	1	–	23	6	5	2	–	–	–	–	–	–	–	–	50
	series *chagasi*	1	–	–	–	–	–	1	–	16	–	2	1	4	1	–	–	–	–	–	–	–	–	26
	sp.	4	2	3	–	1	1	–	1	8	–	6	5	2	4	–	–	1	–	–	–	–	–	38
Sciopemyia	*servulolimai*	–	–	18	4	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	23
	sordellii	9	1	25	–	–	–	5	1	3	–	15	5	3	1	–	1	–	–	–	2	–	–	71
	sp.	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Trichophoromyia	*flochi*	–	1	–	1	–	1	–	2	–	–	–	10	–	7	–	2	–	1	–	31	–	–	56
	ubiquitalis	–	8	2	1	2	2	4	12	4	7	16	48	15	15	6	8	1	–	19	61	–	4	235
	sp.	–	–	–	1	2	–	1	1	2	–	6	4	1	4	2	1	–	1	10	17	–	–	53
Viannamyia	*furcata*	–	–	–	–	1	–	1	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	3
	tuberculata	–	–	1	–	–	–	–	–	–	–	2	–	1	–	–	–	–	–	–	–	–	–	4
Without identification		–	1	–	3	–	–	–	3	–	–	3	6	1	5	–	1	–	–	1	–	–	2	26
		42	24	123	21	27	11	47	25	182	18	157	116	152	81	31	15	15	4	38	119	5	14	
Total		66		144		38		72		200		273		233		46		19		157		19		1,267

FinchTV 1.4 (Geospiza Inc., Seattle, WA). BLAST (www.ncbi.nlm.nih.gov/BLAST) was used to identify homologous sequences in GenBank and the resulting sequences were aligned in the program MEGA5 (Kumar et al. 2008).

Estimation of *Leishmania* Infection Rate. The infection rate was calculated using the formula described in Paiva et al. (2007).

Results

In total, 1,267 sand flies belonging to three subtribes, Lutzomyiina, Psychodopygina, and Sergentomyiina, were collected in the Castanheira settlement in a 98-h collection effort. Of these, 819 (65%) were females and 448 (35%) males. The insects were from 32 species in 10 genera: *Bichromomyia*, *Evandromyia*, *Lutzomyia*, *Micropygomyia*, *Nyssomyia*, *Psathyromyia*, *Psychodopygus*, *Sciopemyia*, *Trichophoromyia*, and *Viannamyia* (Table 1).

It was not possible to identify 26 (2%) specimens collected because of loss of morphological structures used for taxonomic identification. For the same reason, 119 (9%) specimens were only identified to genus level.

Overall, the most abundant species collected were, in order: *Trichophoromyia ubiquitalis* (Mangabeira) ($n = 235, 19\%$), *Psychodopygus davisi* (Root) ($n = 228$, 18%), and *Nyssomyia antunesi* (Coutinho) ($n = 135$, 11%). The remaining less abundant species comprised 524 (41%) specimens.

In total, 559 (68.3%) female specimens belonging to 82 sand fly pools were used for molecular detection of *Leishmania* using the target ITS1. Seven pools were considered positive for the presence of *Leishmania* and belonged to six species: *Evandromyia apurinan* Shimabukuro, Silveira, & Silva, *Ny. umbratilis*, *Nyssomyia yuilli yuilli* (Young & Porter), *Ps. davisi*, *Sciopemyia servulolimai* (Damasceno & Causey), and *Th. ubiquitalis*. The following species of parasites were identified by genetic sequencing: *L. amazonensis*, *L. braziliensis*, and a sample identified only to the level of subgenus *Leishmania* (*Viannia*) sp. (Table 2).

The minimum estimated rate of infection was 0.84%, five pools had minimal infection rate equal to 0.12%, these species belong to the *Ev. apurinan*, *Ny. umbratilis*, *Ny. yuilli yuilli*, *Ps. davisi*, and *Sc. servulolimai*. The species *Th. ubiquitalis* presented the highest infection rate, with a value of 0.24%. This is the first

Table 2. Positive samples for *Leishmania* per species of sand flies collected in the Caititu Indigenous Reserve, Lábrea, state of Amazonas, Brazil, in February 2012, and number of pools used

Species	Positive samples			
	L. (L.) amazonensis	*L. (V.) braziliensis*	*Leishmania (Viannia)* sp.	No. of pools (specimens per pool)
Ev. apurinan	–	1	–	3 (3–4)
Ny. umbratilis	1	–	–	3 (4–8)
Ny. yuilli yuilli	1	–	–	2 (4–5)
Ps. davisi	–	1	–	18 (1–10)
Sc. servulolimai	1	–	–	2 (9–10)
Th. ubiquitalis	1	–	–	8 (2–10)
Th. ubiquitalis	–	–	1	8 (2–10)
Total	4	2	1	44 (311)

record of the presence of DNA of *Leishmania* (*V.*) *braziliensis* in *Ev. apurinan.*

Discussion

In this study, 32 species of sand flies from the municipality of Lábrea were collected representing ≈24% of the known diversity of sand fly species in the state of Amazonas (Figueira et al. 2013).

The most abundant genera were *Psychodopygus*, followed by *Trichophoromyia* and *Nyssomyia.*

In previous studies of sand flies from the state of Amazonas, *Trichophoromyia* was found to be the most abundant genus in the municipalities of Itacoatiara (Pessoa et al. 2007), Manacapuru (Silva et al. 2007), and Borba and Maués (Alves et al. 2012). In Manaus, *Nyssomyia* was the most abundant genus collected (Arias et al. 1987; Dias-Lima et al. 2002; Feitosa and Castellón 2004, 2006; Guerra et al. 2006; Barbosa et al. 2008). *Trichophoromyia* and *Nyssomyia* were both the most abundant in Tefé municipality according to Barrett et al. (1996). In the municipality of Coari, *Psychodopygus* was the most abundant (Castellón et al. 2000), and to the south of the state, *Nyssomyia* was reported as the most abundant (Figueira et al. 2013), in a previous study in the municipality of Lábrea.

The most abundant species *Th. ubiquitalis*, *Ps. davisi*, and *Ny. antunesi* have also been recorded in the state of Amazonas. *Th. ubiquitalis* was the most abundant in the municipalities of Borba and Maués, and *Ps. davisi* was the fourth most abundant species collected in the municipality of Nhamundá, while *Ny. antunesi* was among the species with lower abundance (Alves et al. 2012). The difference between the abundance of *Ny. antunesi* found between this work and that of Alves et al. (2012), which was carried out in undisturbed terra-firme forest, may be related to greater adaptability of this species to more open areas, as the dispersal of sand fly species may be related to factors such as the search for adequate resting sites and response to light stimuli (Galati et al. 2009); conditions that are found in the Castanheira settlement, which features small-scale pasture areas; gardens where manioc, banana, and sugar cane are planted by the Apurinã residents; presence of domestic animals; and the use of lamps and lanterns as light sources by indigenous people might account for high abundance of this *Ny. antunesi.*

In total, 26 species have been recorded for the municipality of Lábrea by a recent study (Figueira et al. 2013). Of these, seven taxa were not found in the current study: *Bi. olmeca bicolor*, *Ev. sipani*, *Micropygomyia rorotaensis* (Floch & Abonnenc), *Pintomyia christenseni* (Young & Duncan), *Pressatia trispinosa* (Mangabeira), *Psathyromyia campbelli* (Damasceno, Causey & Arouck) and *Psathyromyia* (*Forattiniella*) sp. The study by Figueira et al. (2013) was conducted as part of entomological surveillance for leishmaniasis vectors and anthropic environments were sampled and may have influenced the fauna composition. Even though our sampling area here was smaller, we found a higher species richness consisting of 32 species compared with the 26 previously identified in the municipality by Figueira et al. (2013). The abundance for most species collected was also different and only *Ny. antunesi* was abundant in both studies. The species *Ev. walkeri* and *Mi. rorotaensis* were more abundant in the study of Figueira et al. (2013), while *Th. ubiquitalis* and *Ps. davisi* were more abundant in the Castanheira settlement.

The small number of individuals of *Ny. umbratilis* found in our study contrasts with the results found in the cities of Manaus and Itacoatiara, north of Lábrea, where the sand fly fauna was predominantly composed of *Ny. umbratilis* (Feitosa and Castellón 2004, 2006; Guerra et al. 2006; Pessoa et al. 2007; Barbosa et al. 2008). In these same studies in Itacoatiara and Manaus, *Th. ubiquitalis*, *Ps. davisi*, and *Ny. antunesi* were found in small numbers. The difference in environment, the degree of human disturbance and land use may be related to the difference between the sand fly fauna (Ready et al. 1986), as the work carried out north of the Amazon River were conducted in urban or peri-urban areas of the city of Manaus, as well as logging areas in Itacoatiara.

In the Amazon, the following species already incriminated as vectors were found in our study: *Bi. flaviscutellata* (Lainson and Shaw 1968), *Ny. umbratilis* (Arias and Freitas 1978), *Ny. anduzei* (Lainson et al. 1976) and *Ny. yuilli yuilli* (Santamaría et al. 2006), while the following sand fly species are suspected vectors: *Th. ubiquitalis* (Silveira et al. 1991), *Ps. ayrozai* (Rangel and Lainson 2003), *Ps. davisi* (Gil et al. 2003), *Ps. paraensis* (Silveira et al. 1991), *Ny. antunesi* (Silveira et al. 2002). In the current study, we observed the presence of *Leishmania* DNA in the sand fly species: *Evandromyia apurinan*, *Ny. umbratilis*, *Ny. yuilli yuilli*, *Ps. davisi*, *Sciopemyia servulolimai*, and *Th. ubiquitalis.*

In this work, we report the first record of infection based on the presence of DNA from *L. amazonensis* in the sand fly species *Sc. servulolimai.* This species has been found in the state of Roraima infected with parasites of the genus *Trypanosoma* by dissection of the midgut in a study that investigated the phylogenetic relationships among anuran trypanosomes and sand flies, and suggested that this species feeds on amphibians instead of warm-blooded animals, such as mammals (Ferreira et al. 2008). Our finding suggests

that either *L.* (*L.*) *amazonensis* might be circulating among amphibians or *Sc. servulolimai* might be able to feed in mammals, this latter assumption seems less likely because the mouthparts of species from the genus *Sciopemyia* are very short and delicate, with reduced number of teeth in the mandibles and maxillae. This is also the first record of the presence of DNA of *Leishmania* (*V.*) *braziliensis* in *Ev. apurinan.*

The results of the natural infection rate found in this study are similar to findings of a recent study conducted at two sites in the city of Manaus using a molecular approach to detect the presence of *Leishmania* DNA in which infection rates were 0.4 and 1.6%, in *Nyssomyia umbratilis* (Pinheiro et al. 2010). In the states of Acre, Amazonas, Pará, and Rondônia, a study of natural infection in sand flies performing dissection of the midgut, reported natural infection rates of 13.4% for sand flies not identified to species level of the genus *Psathyromyia* sp.; 7.5% for the genus *Nyssomyia* sp.; 6.7% to *Lutzomyia* (*Tricholateralis*) sp.; and 0.5% for *Psychodopygus* sp. (Arias 1985). It is possible to observe a variation in the natural infection rates recorded for sand flies according to previous studies. This variation depends on the type of investigation used, specifically the method used for the detection of *Leishmania*, which may have a higher (DNA) or lower (dissection of midgut) sensitivity.

The application of ITS1 target for detection and identification of *Leishmania* has been reported in published studies, which successfully amplified this target in clinical samples (Schonian et al. 2003, Rotureau et al. 2006, Graça et al. 2012, Leelayoova et al. 2013). The same target was used in a study to detect DNA of *Leishmania* in sand flies collected in the state of Minas Gerais, where *Psychodopygus lloydi* (Antunes) found positive for DNA of *L.* (*V.*) *braziliensis* (Quaresma et al. 2012). For this work, the target ITS1 was sensitive for the detection of the parasite and a satisfactory tool when used in PCR-based sequencing approaches for detection and identification of *Leishmania.*

The species *L. amazonensis* has been found in the city of Manaus, isolated from *Bi. flaviscutellata* and *B. olmeca nociva* (Arias et al. 1987), and has been recorded from samples from lesions of patients who received medical care (Coelho et al. 2011), as well as mammals (*Didelphis marsupialis, Marmosa cinerea, Proechimys guiannensis, Dasyprocta aguti, Tupinambis nigropunctatus*) studied as reservoirs in the city of Manaus and also for the states of Amapá, Pará, and Rondônia (Arias and Naiff et al. 1981, Grimaldi et al. 1991).

The species *L.* (*V.*) *braziliensis* has also been found in the city of Manaus in the study of natural infection in sand flies (Arias and Freitas 1978) and lesions of patients (Romero et al. 2002, Guerra et al. 2011, Coelho et al. 2011, Chrusciak-Talhari et al. 2011, Neves et al. 2011, Benício et al. 2011), with records for the municipalities of Benjamim Constant, Coari, Eirunepé, Humaitá, Itacoatiara, Lábrea, Manacapuru, Nhamundá, Santa Isabel do Rio Negro, Tabatinga, Tapauá, and Tefé (Guerra et al. 2011). This parasite was also found infecting *Psychodopygus squamiventris* (Lutz & Neiva) and *Psychodopygus carrerai* (Barretto) in a study that included the states of Amapá, Amazonas, Pará, and Rondônia (Grimaldi et al. 1991).

For the subgenus *Viannia* species as *L.* (*V.*) *braziliensis*, *L.* (*V.*) *guyanensis*, and *L.* (*V.*) *naiffi* have been recorded in the state of Amazonas in various works ranging from studies of infection in sand flies, mammalian reservoirs, and lesions of patients who received any medical treatment (Arias and Freitas 1978, Arias and Naiff 1981, Grimaldi et al. 1991, Romero et al. 2002, Figueira et al. 2008, Guerra et al. 2011, Coelho et al. 2011, Chrusciak-Talhari et al. 2011, Neves et al. 2011, Benício et al. 2011).

Leishmania infection detected in vectors and suspected species found in this study suggests a role of these species in the transmission cycle of ACL in the Castanheira settlement, Lábrea, Amazonas, and the risk of infection in the study area. More studies should be performed in the area to contribute to the elucidation of the epidemiology of leishmaniasis in the state of Amazonas.

Acknowledgments

We thank the Programa de Apoio a Núcleos de Excelência – PRONEX/FAPEAM/CNPq for funding the project "Condições de vida e saúde de populações indígenas na Amazônia", of which this work was part, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for funding the project 474506/2012-6. We also thank Fundação Nacional do Índio/Lábrea for logistical support; and Luke Baton for revising and commenting on draft versions of this manuscript.

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Received 10 February 2014; accepted 5 September 2014.