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HEALTH SCIENCES – SHORT COMMUNICATION

Detection of *Leishmania* DNA and identification of blood meal sources in sand flies (Psychodidae: Phlebotominae) from an Amazonian endemic area of cutaneous leishmaniasis

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ABSTRACT

Cutaneous leishmaniasis is endemic in the state of Acre, western Amazonia, but natural vectors and reservoirs are poorly known. This study investigated *Leishmania* infection and blood meal sources in sand flies in the town of Sena Madureira. Sand flies were captured using CDC light and Shannon traps. *Leishmania* DNA was detected using PCR targeting *hsp70*; using PCR against *cytb* from engorged females' blood samples. A total of 560 sand flies from 12 genera were collected, with *Psychodopygus* representing 56.6% of specimens. *Leishmania* DNA was detected in 12 samples, sequencing confirmed *L. (Viannia) braziliensis* infection in *Nyssomyia fraihai* and *Psychodopygus hirsutus*. Blood-meal testing identified *cytb* sequences matching human (5 flies), *Tamandua tetradactyla* (2 flies), and *Cebus unicolor* DNA (1 fly). This study provides new evidence of *Leishmania* circulation in anthropic environments, highlighting potential risk for human infection and provides new methods for understanding leishmaniasis transmission.

KEYWORDS: cutaneous leishmaniasis, vectors, *Leishmania* sp., wild animals

Detecção de DNA de *Leishmania* e identificação da fonte alimentar sanguínea em flebotomíneos (Psychodidae: Phlebotominae) em área endêmica de leishmaniose cutânea na Amazônia

RESUMO

A leishmaniose cutânea é endêmica no estado do Acre, na Amazônia Ocidental, porém os vetores e reservatórios naturais ainda são pouco conhecidos. Este estudo investigou a infecção por *Leishmania* e as fontes de repasto sanguíneo em flebotomíneos no município de Sena Madureira. Os flebotomíneos foram capturados utilizando armadilhas luminosas CDC e armadilhas de Shannon. O DNA de *Leishmania* foi detectado por PCR direcionada ao gene *hsp70*; as fontes de sangue foram identificadas por PCR para o gene *cytb* a partir de amostras de fêmeas ingurgitadas. Ao todo, foram coletados 560 flebotomíneos pertencentes a 12 gêneros, sendo *Psychodopygus* responsável por 56,6% dos espécimes. O DNA de *Leishmania* foi detectado em 12 amostras, identificando *L. (V.) braziliensis* em *Nyssomyia fraihai* e *Psychodopygus hirsutus*. A análise de DNA mitocondrial em 12 flebotomíneos alimentados identificou sangue de *Homo sapiens* (n = 5), *Tamandua tetradactyla* (n = 2) e *Cebus unicolor* (n = 1). Este estudo fornece novas evidências da circulação de *Leishmania* em ambientes antrópicos, destacando o potencial risco de infecção humana, além de apresentar novas abordagens para a compreensão da transmissão da leishmaniose.

PALAVRAS-CHAVE: leishmaniose cutânea, vetores, *Leishmania* sp., animais silvestres

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Cutaneous leishmaniasis (CL) is a neglected enzootic and zoonotic disease caused by protozoan parasites of the genus *Leishmania* and transmitted by sand flies. In Brazil, CL is a major public health concern in both rural and urban areas, where a variety of *Leishmania* species circulate among multiple vector species and vertebrate hosts (Brazil *et al.* 2015; Shaw 2025). In 2023, more than 50% of reported cases occurred in the Northern region of Brazil, with the state of Acre presenting the highest detection rate (691 cases per 100,000 inhabitants) and accounting for 76.9% of the cases reported in the region (Brasil 2024).

In Acre, several sand fly species are vectors of *Leishmania (Viannia) braziliensis* Vianna, 1911, *L. (V.) guyanensis* Floch, 1954, *L. (V.) lainsoni* Silveira *et al*., *1987,* and *L. (V.) shawi* Lainson *et al.,* 1989 (Ávila *et al.* 2018; Araujo-Pereira *et al.* 2020; Brilhante *et al.* 2022; Barroso *et al.* 2025). Continued study of phlebotomine sand flies, combined with *Leishmania* infection and blood meal analysis, is essential for guiding surveillance efforts in endemic areas (Shaw 2025). Thus, we investigated *Leishmania* infection and identified blood meal sources in sand flies collected from rural communities with confirmed human cases of CL in the municipality of Sena Madureira, Acre State, Brazil.

We collected sandflies in four rural locations, chosen based on accessibility, logistics, and reports of human infections: Cazumbá-Iracema - CI (09°08'30.8"S; 68°55'40.4"W; August 2022), Novo Amparo - NA (09°04'16.0"S; 68°27'02.0"W; March 2023), Joaquim José de Matos - JJM (09°23'35.6"S; 68°35'37.8"W; July 2023), and São José - SJ (09°22'28.6"S; 68°43'29.0"W; November 2023) (figure 1).

Sand flies were collected for two consecutive nights using CDC light traps (18:00–07:00) and a Shannon trap (18:00–20:00). On the first night, six CDC traps were installed in two houses with confirmed human CL cases (indoors, areas near animal shelters, and at the forest edge close to the households), along with a Shannon trap at the forest edge (figure 2). On the second night, five CDC traps and one Shannon trap were placed along trails in primary forest fragments.

At each locality, CDC sampling included 2 trap-nights (26 h) in each intradomicile, peridomicile, and forest-edge setting, and 5 trap-nights (65 h) in primary forest fragments. Shannon traps covered 2 trap-nights (4 h), one at the forest edge and one in primary forest fragments. Specimens were preserved in 70% ethanol, according to trapping localities and collection ecotype, and identified morphologically following Galati (2024).

Non-engorged females were tested individually when unique for a given species and collection site or grouped into pools of up to five specimens of the same species and locality when more than one individual was available. Engorged females were analyzed individually for blood meal identification and *Leishmania* infection detection.

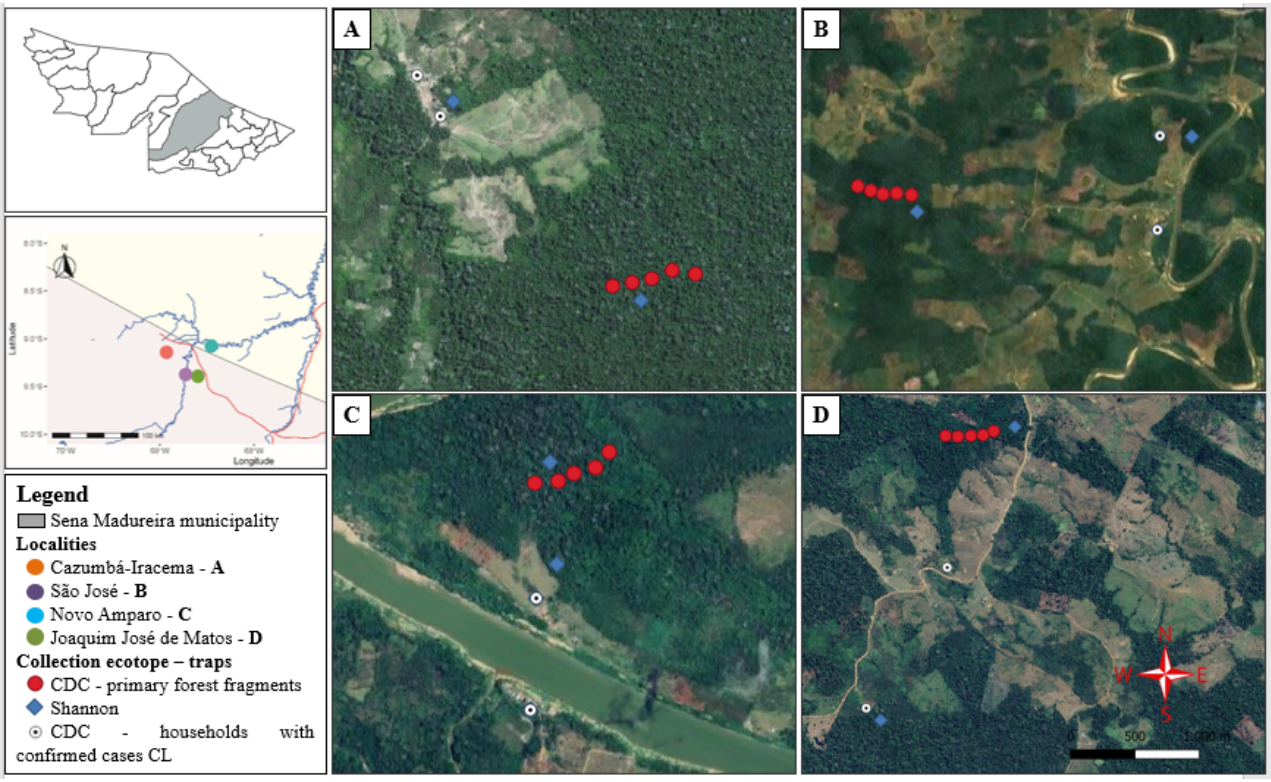

Figure 1. Geographic location with vegetation cover, of the sampling points in rural areas of the municipality of Sena Madureira, Acre State, Brazil.

Figure 2. Typical rural landscape in Acre State: (**A**) rural house showing the three ecotypes selected for trap placement; (**B**) intradomicile; (**C**) peridomicile area near domestic animal shelters; and (**D**) edge of forest fragment near residences.

DNA was extracted using the EXTRACTA*[32] system with the Extracta® DNA/RNA Kit for Pathogens (MPTA-B01K). *Leishmania* DNA was detected by PCR targeting *hsp70* (≈ 230 bp) (Graça *et al.* 2012), using *L. (V.) guyanensis* (MHOM/BR/1975/M4147; IOC/L0565) as positive control and *Psychodopygus davisi* (Root 1934) male sand flies and water as negative control. Blood meal sources were identified by PCR amplification and sequencing of a ~358-bp fragment of the mitochondrial cytb gene (Boakye *et al.* 1999), with *Homo sapiens* DNA as positive control and water as negative control. The IVS6 cacophony gene (≈220 bp) served as endogenous control in all reactions (Lins *et al.* 2006).

PCR reactions for *hsp70* and *cytb* were performed in 50 µL containing 35 µL ultrapure water, 5.0 µL 10× buffer, 1.5 µL $MgCl_2$ (50 mM), 1 µL dNTPs (2 mM), 1 µL of each primer (10 pmol), 0.5 µL Taq DNA polymerase (5 U μL^{-1}), and 5 µL extracted DNA. The IVS6 reaction was carried out in a final volume of 25 µL containing 17.5 µL ultrapure water, 2.5 µL 10× buffer, 0.75 µL $MgCl_2$ (50 mM), 0.5 µL dNTPs (2 mM), 0.5 µL of each primer (10 pmol), 0.25 µL Taq DNA polymerase (5 U μL^{-1}), and 2.5 µL extracted DNA.

Amplifications were carried out in a CFX96 Touch thermocycler with initial denaturation at 94 °C (2 min), 35 cycles of 94 °C (30 s), 56 °C (45 s), 72 °C (45 s), and final extension at 72 °C (5 min). PCR products were visualized on 2% agarose gels, positive amplicons were purified (PureLink PCR Purification Kit) and Sanger sequenced (Fiocruz platform RTP01A). Data was analyzed in Geneious 2023.0.3, compared by BLAST, and deposited in GenBank (PV587120–PV587121). The study was authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (permit n. 68547-1) and approved by the IOC/Fiocruz Research Ethics Committee (CAAE: 57573822.2.0000.5248).

A total of 560 sand flies were collected, comprising 12 genera and 35 species (Table 1). Most were from JJM (23 species, 408 specimens) followed by CI (14 species, 68 specimens), SJ (13 species, 35 specimens) and NA (12 species, 49 specimens). Most specimens were in the genera *Psychodopygus* (56.6%), *Trichophoromyia* (14.3%) and *Nyssomyia* (12.0%). Two species were found indoors: *Nyssomyia antunesi* (Coutinho, 1939) and *Pressatia* sp. were found indoors in CI and SJ, respectively, and five species (*Br. Pentacantha, Pr. choti, Ps. claustrei, Ps. davisi* and *Th. octavioi*) were found in peridomiciliary areas. A total of 23 species were detected at the forest edge and 30 species in primary forest fragments.

Twelve of the 420 females analyzed were engorged, from which we had 193 samples (12 individuals and 181 pools) that we submitted to PCR. *Leishmania* DNA testing was positive for 12 out of 193 samples (5.6%), with positives at all four sites, including forest edges (CI, SJ, NA), primary forest fragments, and peridomestic areas (JJM). Sequencing confirmed *L. (V.) braziliensis* in one engorged *Ny. fraihai* (Martins, Falcão & Silva, 1979) and one non-engorged *Ps. hirsutus hirsutus* (Mangabeira, 1942) (Table 2). Mitochondrial DNA testing of 12 blood-fed sand flies was positive for

Table 1. Composition of the sand fly fauna in rural communities of the municipality of Sena Madureira, Acre, Brazil.

Species	Cazumbá-Iracema								Joaquim José de Matos								Novo Amparo								São José								Subtotal		Total	%
	ID		PD		FE		PF		ID		PD		FE		PF		ID		PD		FE		PF		ID		PD		FE		PF					
	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	M	F	M	F	M	F		
Bi. flaviscutellata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	1	**2**	**2**	**4**	**0.7**
Br. pentacantha	-	-	1	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	**5**	**0**	**5**	**0.9**
Brumptomyia sp.	-	-	-	-	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	**0**	**5**	**5**	**0.9**
Ev. saulensis	-	-	-	-	-	4	-	-	-	-	-	-	-	1	-	2	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	**0**	**15**	**15**	**2.7**
Lu. sherlocki	-	-	-	-	-	-	2	1	-	-	-	-	-	17	-	15	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	1	**4**	**34**	**38**	**6.8**
Ny. antunesi	1	-	-	-	-	-	-	1	-	-	-	-	-	1	-	2	-	-	-	-	-	1	-	1	-	-	-	-	1	-	2		**1**	**9**	**10**	**1.8**
Ny. anduzei	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**2**	**2**	**0.4**
Ny. fraihai	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	4	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-		**0**	**7**	**7**	**1.3**
Ny. shawi	-	-	-	-	2	-	4	18	-	-	-	-	-	3	-	11	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1		**6**	**34**	**40**	**7.1**
Ny. whitmani	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**5**	**5**	**0.9**
Ny. umbratilis	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**3**	**3**	**0.5**
Pi. nevesi	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	4	1	1	-	-	-	-	1	-	-		**2**	**7**	**9**	**1.6**
Pr. choti	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	3	1	-	-	**5**	**1**	**6**	**1.1**
Pressatia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	**0**	**3**	**3**	**0.5**
Pa. abonnenci	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**4**	**4**	**0.7**
Pa. aragaoi	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**4**	**4**	**0.7**
Pa. elizabethdorvalae	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**1**	**1**	**0.2**
Pa. pradobarrientosi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**	**0**	**1**	**0.2**
Ps. amazonensis	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**7**	**7**	**1.3**
Ps. ayrozai	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**5**	**5**	**0.9**
Ps. carrerai	-	-	-	-	-	-	-	1	-	-	-	-	-	-	34	42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**34**	**43**	**77**	**13.8**
Ps. chagasi	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**2**	**1**	**3**	**0.5**
Ps. claustrei	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**5**	**5**	**0.9**
Ps. davisi	-	-	-	-	1	-	5	4	-	-	1	-	-	5	7	25	-	-	-	-	-	-	-	1	-	-	-	-	5	11	2	10	**21**	**56**	**77**	**13.8**
Ps. francoisleponti	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**	**0**	**1**	**0.2**
Ps. Guyanensis series	-	-	-	-	-	-	-	-	-	-	-	-	-	27	-	30	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	**0**	**58**	**58**	**10.4**
Ps. hirsutus	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**21**	**21**	**3.8**
Ps. lainsoni	-	-	-	-	-	-	4	1	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**4**	**3**	**7**	**1.3**
Ps. llanosmartinsi	-	-	-	-	-	-	-	-	-	-	-	-	1	2	3	46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	**5**	**48**	**54**	**9.6**
Psychodopygus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0**	**2**	**2**	**0.4**
Th. auraensis	-	-	-	-	-	-	1	-	-	-	-	-	3	-	9	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	**20**	**0**	**20**	**3.6**
Th. octavioi	-	-	-	-	-	-	-	-	-	-	1	-	1	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**23**	**0**	**23**	**4.1**
Trichophoromyia sp.	-	-	-	-	-	-	-	1	-	-	-	-	-	8	2	23	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	**2**	**35**	**37**	**6.6**
Ty. dasypodogeton	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**	**0**	**1**	**0.2**
Vi. furcata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	**1**	**0**	**1**	**0.2**
Subtotal	**1**	**0**	**1**	**0**	**6**	**6**	**16**	**38**	**0**	**0**	**2**	**1**	**5**	**87**	**81**	**231**	**0**	**0**	**0**	**0**	**9**	**9**	**4**	**13**	**0**	**1**	**2**	**0**	**9**	**15**	**4**	**19**	**140**	**420**		
Total	**1**		**1**		**12**		**54**		**0**		**3**		**92**		**312**		**0**		**0**		**18**		**17**		**1**		**2**		**24**		**23**		**560**			
Richness	**1**		**1**		**5**		**13**		**0**		**3**		**20**		**21**		**0**		**0**		**6**		**7**		**1**		**2**		**6**		**9**		**35**			

ID - intradomicile; PD - peridomicile; FE - forest edge; PF - primary forest fragments; M - male; F - female.

humans (*Homo sapiens*, n = 5), southern tamandua (*Tamandua tetradactyla*, n = 2), and capuchin monkey (*Cebus unicolor*, n = 1) (Table 3). The *Ny. fraihai* positive for *L. braziliensis* had fed on *T. tetradactyla*. Remaining sequences (*hsp70* and *cytb*) had low quality or provided no database match.

Among the 35 species recorded, *Ps. carrerai* (Barretto, 1946) and *Ps. davisi* predominated (27.5% of specimens), consistent with previous reports from the state (Araujo-Pereira *et al.* 2020; Ávila *et al.* 2018; Brilhante *et al.* 2022). Most specimens and over 50% of *Leishmania* infections were detected in forested areas, suggesting a predominantly sylvatic transmission cycle. Nevertheless, the presence of anthropophilic species in peridomestic environments suggests peridomestic transmission.

Previous studies have identified *Ny. antunesi*, *Bi. flaviscutellata* (Mangabeira 1942) *Ps. hirsutus hirsutus*, and *Ps. ayrozai* (Barretto & Coutinho 1940) as frequent species at forest edges and in primary forest, incriminating them as *Leishmania* vectors in the region (Ávila *et al.* 2018; Araujo-Pereira *et al.* 2020; Barroso *et al.* 2025). In the present study, *Ps. hirsutus hirsutus* from primary forest fragments tested positive for *L. (V.) braziliensis*, consistent with previous reports of this species in CL transmission in Acre. *Ps. ayrozai* was recorded both at the forest edge near households and in

Table 2. Sand fly species that tested positive for *Leishmania* DNA in the municipality of Sena Madureira, Acre, Brazil.

Locality/Ecotype/ Sandfly Species	Total	BLAST Analysis (*hsp70*)				
		Parasite species	Accession number of the top hit	Coverage (%)	E-value	Identity (%)
Cazumbá-Iracema						
Forest Edge						
Brumptomyia sp.	2	*Leishmania* sp.	-	-	-	-
Joaquim J Matos						
Peridomicile						
Ps. claustrei	1	*Leishmania* sp.	-	-	-	-
Primary forest fragments						
Lu. sherlocki	1	*Leishmania* sp.	-	-	-	-
Ps. amazonensis	1	*Leishmania* sp.	-	-	-	-
Ps. hirsutus hirsutus	1	*L. (V.) braziliensis*	FR872763.1	100	5e-97	100%
Ps. carrerai	2	*Leishmania* sp.	-	-	-	-
Ps. Guyanensis series	1	*Leishmania* sp.	-	-	-	-
São José						
Forest Edge						
Ny. fraihai	1	*L. (V.) braziliensis*	MW507518.1	100	5e-44	100%
Novo Amparo						
Forest Edge						
Pi. nevesi	2	*Leishmania* sp.	-	-	-	-

Table 3. Identification of blood meals in sand flies collected in the municipality of Sena Madureira, Acre, Brazil.

Locality/Ecotype/ Sandfly Specie	Total	BLAST Analysis (cytb)				
		Blood source	Accession number of the top hit	Coverage (%)	E-value	Identity (%)
Cazumbá-Iracema						
Primary forest fragments						
Pi. nevesi	1	*C. unicolor*	PP454508.1	99	2e-148	99
Pa. aragaoi	1	*T. tetradactyla*	PQ181550.1	98	7e-97	88
	1	*H. sapiens*	AY509658.1	99	0.0	99
Joaquim J Matos						
Primary forest fragments						
Ps. amazonensis	1	*H. sapiens*	AY509658.1	99	0.0	100
São José						
Forest Edge						
Ny. fraihai	1	*T. tetradactyla*	PQ181550.1	100	3e-95	90
Pi. nevesi	1	*H. sapiens*	AY509658.1	99	0.0	100
Ps. davisi	2	*H. sapiens*	AY509658.1	99	0.0	100

primary forest fragments, supporting its known association with zoonotic and enzootic transmission cycles in the Amazon, given its ecological generalist habitat and anthropo-opportunistic feeding behavior.

Although *Ny. antunesi* was uncommon, it was captured indoors, in forest edges, and in primary forest fragments, demonstrating ecological plasticity. Despite testing negative for *Leishmania* DNA in this study, previous research in Acre found *L. (V.) braziliensis* and *L. (V.) guyanensis* in this fly, implicating it in CL transmission (Ávila *et al.* 2018; Araújo-Pereira *et al.* 2020; Carneiro *et al*. 2023). Its frequent occurrence in forest fragments close to dwellings, including in Rio Branco, suggests adaptation to human-modified environments (Brilhante *et al.* 2022; Carneiro *et al.* 2023).

The detection of *L. (V.) braziliensis* in *Ny. fraihai* and *Ps. hirsutus hirsutus*, captured in primary forest fragments and forest edges, is a descriptive record of parasite detection in the sampled area. This species has also been reported in Acre in sand flies (Teles *et al.* 2016; Araujo-Pereira *et al.* 2020; Brilhante *et al*. 2021), human (Teles *et al.* 2015; Araujo-Pereira *et al.* 2018) and domestic dogs (Brilhante *et al.* 2019).

Blood meal analysis identified human DNA and DNA from two sylvatic species in phlebotomine species captured in primary forest fragments and forest edge, indicating human exposure across different ecotopes. Previous studies in the state have also reported avian species, multiple mammalian hosts, and mixed feeding involving *H. sapiens* and *T. tetradactyla* (Ávila *et al.* 2018; Araújo-Pereira *et al.* 2020; Barroso *et al.* 2025).

Ten samples positive for the *hsp70* target could not be characterized due to low sequence quality, which may have led to underestimating *Leishmania* diversity. The use of pooled samples may also have influenced infection rate estimates, as it does not allow determination of individual infection status. The detection of *L. (V.) braziliensis* and the identification of blood meal sources demonstrate the efficacy of PCR-based methods for better characterizing parasite–host interactions, which is important for understanding disease dynamics.

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