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Notes and Comments

Identification of sand flies (Diptera: Psychodidae) and blood meal sources in periurban areas of Ji-Paraná municipality, Western Brazilian Amazon

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(With 1 figure)

Leishmaniases is a disease caused by *Leishmania* protozoans which are transmitted to vertebrates by the bite of female sand flies. Epidemiological surveillance depends on the identification of vector species and their reservoirs, and identifying sand fly blood meal sources can be an effective means of identifying potential reservoirs in the Leishmaniasis transmission cycle (Roque and Jansen, 2014). In Rondônia State, approximately 1,000 new cases of cutaneous leishmaniasis (CL) occur annually (DATASUS, 2018). In Ji-Paraná municipality, which is located in the eastern mesoregion of Rondônia (see Figure 1), 418 cases of CL have been registered since 2007 (DATASUS, 2018); in spite of this, there is not much information about *Leishmania* infection, sand fly vectors and, their blood sources that occur there (Biancardi et al., 1982). The aims of our study was to verify these insects and possible blood meals in Ji-Paraná municipality.

Sand fly collections were made using light traps that were set for three consecutive nights in small forest fragments near eight periurban areas (see Figure 1C). Two collections were performed: one in November of 2017 and another in November of 2018. Males and females (head and last segments) were slide-mounted and morphologically identified (Galati, 2018).

In order to screen for the presence of *Leishmania* DNA, the thoraxes of non-engorged females were separated into pools and subjected to polymerase chain reaction assays using primers targeting *k*DNA (Pereira Júnior et al., 2019). The *Le. amazonensis* strain (IFLA/BR/1967/PH8) was used as the positive control and ultrapure water was used as the negative control. Blood meal sources were identified by submitting the thoraxes of engorged females to DNA extraction using the phenol/chloroform protocol; to avoid sample contamination, two samples containing a female with no blood present in the gut and one sample containing DNA-free water were used as negative controls. Extracted DNA was subjected to PCR assays targeting the *cyt*b mitochondrial region (Pereira Júnior et al., 2019).

A total of 1,331 sand flies comprising 28 species were identified (as shown in Table 1). The most abundant species were *Psychodopygus davisi* (491 individuals, 36.9%) and *Ps. hirsutus hirsutus* (391 individuals, 29.4%) (see Figure 1C). In Rondônia, *Ps. davisi* generally occurs in high abundance (Gil et al., 2003; Pereira Júnior et al., 2019). Specimens of both species have been found carrying *Leishmania naiffi* promastigotes (Gil et al., 2003); however, none of the 80 pools in our study exhibited the presence of *Leishmania* DNA.

Blood meal analysis revealed the DNA of *Homo sapiens* in one *Ps. davisi* female and four *Ps. hirsutus hirsutus* females, while DNA of the wild vertebrates *Alouatta seniculus* and *Dasypus sabanicola* was present in the gut of two *Ps. hirsutus hirsutus* females (as shown in Table 2). *Dasypus sabanicola* is not found in Brazil (Abba and Superina, 2010) and probably the DNA sequence could be another species of *Dasypus* genera with distribution in the Brazilian Amazon as *Dasypus novemcinctus* or *Dasypus kappleri*.

The finding that *Ps. hirsutus hirsutus* and *Ps. davisi* feed on wild vertebrates may help to improve our understanding of the hematophagous habits of these species as well as our understanding of the *Leishmania* transmission cycle in this region. The presence of *H. sapiens* DNA in engorged sand flies combined with the high abundance of these species indicates that humans have been exposed to bites from potential vectors in four of the eight localities in Ji-Paraná. Recently, Pereira Júnior et al. (2019) identified the DNA of humans and anteaters in sand flies from forest and peridomicile areas in Rondônia. Our results corroborate their findings and demonstrate that sand flies use a variety of blood meal sources, including humans, in Ji-Paraná; these findings increase our epidemiological knowledge of CL in the region.

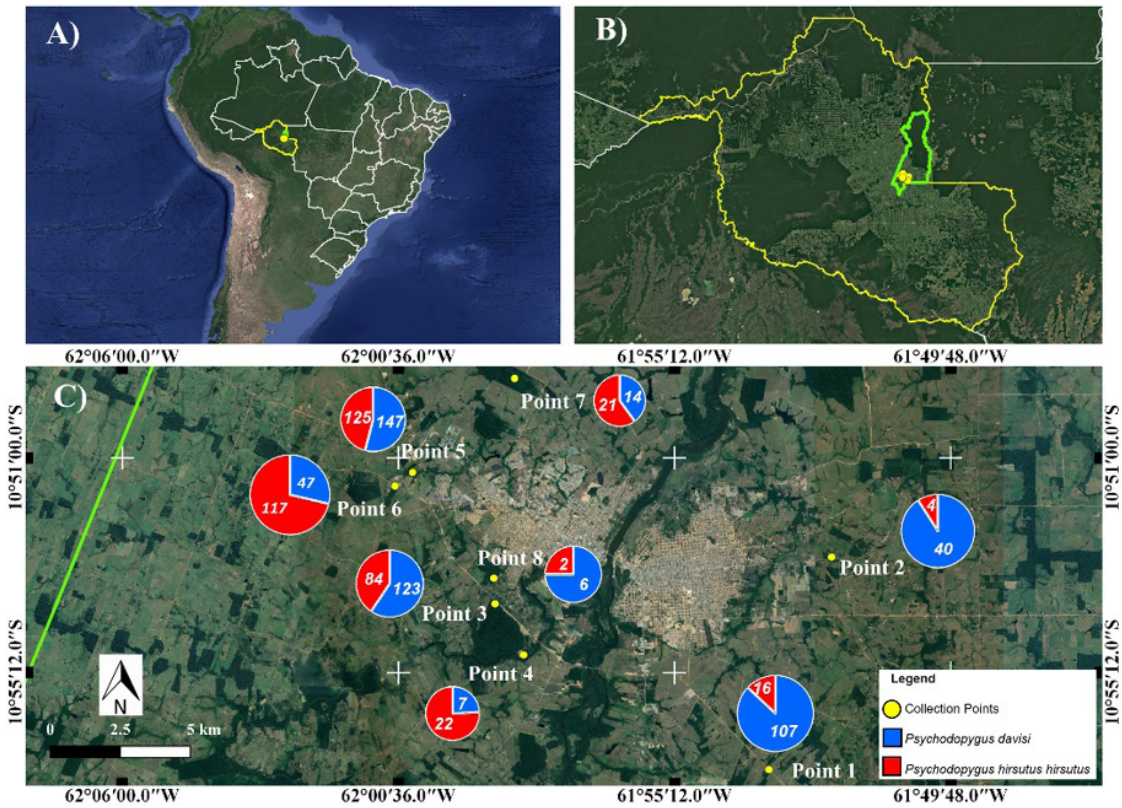

Figure 1. (A) South America map with Brazil Map delimited by white line indicating the states limits and in yellow the limits of the Rondônia State; (B) Map of Rondônia state in a large view (yellow line) and in green line indicating the limits of Ji-Paraná municipality; (C) Collection points distributed among periurban areas of Ji-Paraná with the number of *Psychodopygus davisi* (blue) and *Ps*. *hirsutus hirsutus* individuals (red) given for each location.

Table 1. Sand fly fauna collected at eight points in Ji-Paraná municipality in November of 2017 and 2018. Numbers in parentheses represent engorged females.

Species	P1	P2	P3	P4	P5	P6	P7	P8	Total	%
	♀/♂	♀/♂	♀/♂	♀/♂	♀/♂	♀/♂	♀/♂	♀/♂		
Bichromomyia flaviscutellata	5/2	1/0	1/5	-	0/2	0/2	2/4	-	**24**	**1.8**
Brumptomyia brumpti	-	-	4/7	1/3	-	-	-	-	**15**	**1.1**
Evandromyia saulensis	2/0	-	45/28	5/1	1/0	2/0	5/0	-	**89**	**6.7**
Evandromyia walkeri	2/1	1/0	8/7	-	-	-	-	-	**19**	**1.4**
Lutzomyia sherlocki	-	1/0	1/0	-	-	3/0	-	-	**5**	**0.4**
Microppygomyia rorotaensis	-	-	-	-	1/0	0/2	2/2	-	**7**	**0.5**
Micropygomyia villelai	0/3	-	-	-	-	2/0	2/0	-	**7**	**0.5**
Migonemyia migonei	-	1/0	1/0	-	-	-	-	-	**2**	**0.2**
Nyssomyia antunesi	8/0	1/3	24/5	2/0	4/0	7/0	12/3	-	**69**	**5.2**
Nyssomyia whitmani	1/0	0/1	2/2	-	1/2	4/1	10/3	0/1	**28**	**2.1**
Pintomyia nevesi	5/6	2/5	2/1	-	-	2/0	-	2/3	**28**	**2.1**
Pintomyia serrana	0/3	-	1/0	-	-	1/0	-	-	**5**	**0.4**
Psathyromyia campelli	-	-	-	-	-	-	1/0	-	**1**	**0.1**
Psathyromyia dendrophyla	-	-	2/0	-	-	-	-	-	**2**	**0.2**
Psathyromyia hermanlenti	-	-	-	0/1	1/0	0/3	-	-	**5**	**0.4**
Psathyromyia lutziana	1/0	-	1/0	-	-	-	0/1	-	**3**	**0.2**
Psathyromyia runoides	0/1	-	0/1	-	-	-	-	-	**2**	**0.2**
Psychodopygus ayrozai	-	-	1/0	-	-	-	-	-	**1**	**0.1**
Psychodopygus c. carrerai	-	4/4	5/1	2/1	5/0	9/18	5/3	-	**57**	**4.3**

Table 1. Continued...

Species	P1	P2	P3	P4	P5	P6	P7	P8	Total	%
	♀/♂	♀/♂	♀/♂	♀/♂	♀/♂	♀/♂	♀/♂	♀/♂		
Psychodopygus chagasi	-	1/0	3/0	3/0	1/0	-	8/0	-	**16**	**1.2**
Psychodopygus claustrei	0/4	0/1	1/6	0/1	0/4	0/3	-	0/1	**21**	**1.6**
Psychodopygus complexus	-	-	0/1	-	0/2	-	1/16	-	**20**	**1.5**
Psychodopygus davisi	25/82	14/26	38/85	4/3	94(1)/53	19/28	4/10	4/2	**491**	**36.9**
Psychodopygus geniculatus	-	-	-	-	0/1	2/3	-	-	**6**	**0.5**
Psychodopygus h. hirsutus	6/10	2/2	49/35	8(1)/14	103/22	65(3)/52	15/6	2/0	**391**	**29.4**
Sciopemyia servulolimai	-	-	-	-	-	-	1/1	-	**2**	**0.2**
Sciopemyia sordellii	-	-	-	-	-	0/1	8/2	-	**11**	**0.8**
Trichophoromyia clitella	-	0/1	0/2	-	-	0/1	-	-	**4**	**0.3**
Total	167	71	375	49	297	230	127	15	**1,331**	**100**

Table 2. Vertebrate species identified from sand fly females collected in Ji-Paraná municipality.

Sand fly species	Blood Meal	Accession	Location	Identity (%)	Total score	Query cover (%)	E-value
Ps. hirsutus	*Dasypus sabanicola*	KT818545.1	Point 3	95.74	448	79	1E-121
Ps. hirsutus	*Homo sapiens*	KX697544.1	Point 3	99.01	538	90	5E-149
Ps. hirsutus	*Homo sapiens*	LCO88150.1	Point 4	98.27	507	89	2E-139
Ps. davisi	*Homo sapiens*	KX697544.1	Point 5	96.58	477	85	1E+130
Ps. hirsutus	*Homo sapiens*	LCO88148.1	Point 6	97.82	473	84	2E-129
Ps. hirsutus	*Homo sapiens*	MH981639.1	Point 6	98.36	532	86	5E-147
Ps. hirsutus	*Alouatta seniculus*	KR902387.1	Point 6	96.45	507	99	1E-139

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