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Article *in* Pathogens and Global Health · October 2000

DOI: 10.1080/00034980050152058 · Source: PubMed

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Annals of Tropical Medicine & Parasitology, Vol. 94, No. 6, 623–631 (2000)

Lutzomyia whitmani (Diptera: Psychodidae) as vector of *Leishmania* (*V.*) *braziliensis* in Paraná state, southern Brazil

By E. LUZ*, N. MEMBRIVE†, E. A. CASTRO*, J. DEREURE‡, F. PRATLONG‡, J. A. DEDET‡, A. PANDEY§ and V. THOMAZ-SOCCOL*¶

**Laboratório de Parasitologia Molecular, Departamento de Patologia Básica, Universidade Federal do Paraná, Centro Politécnico Jardim das Américas, CEP 81531–990, Curitiba, Paraná, Brazil*

†*Laboratório de Entomologia médica da Fundação Nacional de Saúde MS, Rua das Pombas 1294, Arapongas, Paraná, Brazil*

‡*Laboratoire d'Ecologie Medicale, 163 Rue Auguste Broussonet, 34090 Montpellier, France*

§*Laboratório de Biotecnologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil*

Received 16 March 2000, Revised 14 June 2000, Accepted 16 June 2000

The phlebotomine sandflies in the northern areas of the state of Paraná, Brazil, particularly those in the '16a' health region, were investigated over a 3-year period. Using CDC light traps (with and without hamster bait) and Shannon traps (with lights and horse or human bait), 16 species were collected from seven municipal districts which were known foci for cutaneous leishmaniasis: Arapongas; Apucarana; Cambira; Marumbi; Faxinal; Florestópolis; and Sabáudia. Although the frequency at which each species was collected varied with the collection site, *Lutzomyia whitmani* predominated (62.0% of all the sandflies collected), followed by *Lu. fischeri* (13.3%), *Lu. pessoai* (10.8%), *Lu. migonei* (8.2%) and *Lu. intermedia* (2.8%). *Lutzomyia monticola*, *Lu. shanonni*, *Lu. firmatoi*, *Lu. lanei*, *Lu. alphabetica*, *Lu. misionensis*, *Lu. correalimai*, *Lu. cortellezzii*, *Lu. longipenis*, *Brumptomyia brumpti* and *B. nitzulescui* together represented the remaining 3.0% of the collected sandflies.

Three of the 1961 female sandflies collected and dissected in the municipal district of Cambira, where a recent case of cutaneous leishmaniasis had been registered, were found to have flagellates in their guts. All three were *Lu. whitmani*. The parasites from each of these infections were successfully isolated in NNN and 'Tobie and Evans' media and/or by inoculation into a hind foot of a golden hamster. The results of isoenzyme electrophoresis indicated that all three isolates were of *Leishmania* (*Viannia*) *braziliensis*.

At various times, human cases of cutaneous leishmaniasis have been widely reported in two areas of Paraná, in southern Brazil: the north of the state; and Vale da Ribeira, in the south–east. In the northern areas, the disease reached epidemic proportions between 1930 and 1950, as the state was colonised. Its incidence fell dramatically during the 1950s, as an indirect benefit of a spirited campaign to eradicate malaria by use of insecticides. Since the 1980s, however, leishmaniasis has again been endemic in the north of Paraná state (with annual incidence gradually increasing until it now stands at about 500 new cases/year), and epidemics occurred in 1992 and 1995 (Silveira *et al.*, 1990, 1996, 1999). The problem appears to have been exacerbated by the replacement of forest with various agricultural crops (coffee, soybean, maize and cotton) and pasture.

Although most of the human infections are known to be caused by *Leishmania* (*Viannia*) *braziliensis* (Silveira *et al.*, 1990, 1999; E. A.

¶ Author to whom correspondence should be addressed. E-mail: vasoccol@bio.ufpr.br; fax: + 55 41 2662042.

ISSN 0003-4983 (print) ISSN 1364-8594 (online)/00/060623-09
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Castro, V. Thomaz-Soccol, N. Membrive and E. Luz, unpubl. obs.), control is made difficult by a lack of information on the vectors of this parasite. The local phlebotomine fauna was studied by Teodoro *et al.* (1991, 1993*a*, *b*) and various species of sandfly have been incriminated as the vector. However, the parasite has never been isolated from any local sandfly and characterised. The main aim of the present study was to find and identify naturally infected vectors, by dissecting sandflies caught in known foci of cutaneous leishmaniasis and typing any flagellates observed.

MATERIALS AND METHODS

Study Area

The Brazilian state of Paraná covers an area of approximately 201 000 km^2, at about 22°29′ S and 26°42′ W. In terms of climate and hydrology, the state occupies a transition zone between tropical and subtropical areas. The general area of the present study, in north–western Paraná state, has a subtropical climate and was covered in forest until the 1930s. People began to move into the region in the 1940s and this immigration intensified in the 1950s and 1960s. All but a few remnants of the original forest have now been cleared, largely to make way for coffee plantations. Sandflies were collected in 10 rural areas in seven municipalities where cutaneous leishmaniasis is endemic: Arapongas; Apucarana; Cambira; Marumbi; Faxinal; Florestópolis; and Sabáudia (Fig. 1). The collections were made in traps set near houses which were 50–400 m from patches of residual forest (the creeping flora originally present below the trees had been cleared), in the residual forest itself, or on its edge. All the houses investigated had been built to face south–west and traps were installed behind them.

Sandfly Collection

Sandflies were collected in CDC light traps with or without hamster bait or, using mouth aspirators, from illuminated Shannon traps baited with a man or horse. The traps were operated between 20.00 and 24.00 hours, on 54 nights at various times of year between May 1996 and June 1999. Towards the end of the study (December 1998–June 1999), particular attention was paid to areas, in six of the municipal districts investigated, where new cases of cutaneous leishmaniasis had been reported. Female flies collected during this period were dissected (see below).

All the sandflies caught were put in plastic tubes (3 cm × 2 cm) and stored at 4°C until they reached a laboratory. All the sandflies were identified to species from the morphology of a spermatheca and the cibarium (females) or external genitalia (males). [Some of the females (10%) were preserved in 95% ethanol for later identification.]

The females collected towards the end of the study (December 1998–May 1999), in all the study municipalities except Faxinal, were dissected on arrival at the laboratory. Each gut was examined on a sterile slide. Samples from any flagellate-positive gut were taken up in sterile Pasteur pipettes and inoculated into NNN and 'Tobie and Evans' culture media. Simultaneously, another sample of the flagellates from each positive fly was inoculated into a hind foot of a golden hamster (*Mesocricetus auratus*).

Identification of Flagellates

Any successful isolate of parasites from a sandfly was characterised by isoenzyme electrophoresis, using starch gel (Rioux *et al.*, 1990; Thomaz-Soccol, 1993) and staining for 11 enzymes: malic dehydrogenase (ME; EC 1.1.1.40); glucose-6-phosphate dehydrogenase (G-6-PD; EC 1.1.1.49); NADH diaphorase (DIA; EC 1.6.2.2); purine nucleoside phosphorylase (NP_1; EC 2.4.2.1); purine nucleoside phosphorylase (NP_2; EC 2.4.2.1*); glutamate oxalacetate transaminase 1 and 2 (GOT_1 and GOT_2; EC 2.6.1.1); phosphoglucomutase (PGM; EC 5.4.2.20); fumarate hydratase (FH; EC 4.2.1.2); mannose phosphate isomerase (MPI; EC 5.3.1.8); and glucose phosphate isomerase (GPI; EC 5.3.1.9).

The isolates were identified by comparison with standard reference strains (WHO, 1990) for *L. braziliensis* (MHOM/BR/84/LTB300, MHOM/BR/75/M2903 and MHOM/BR/

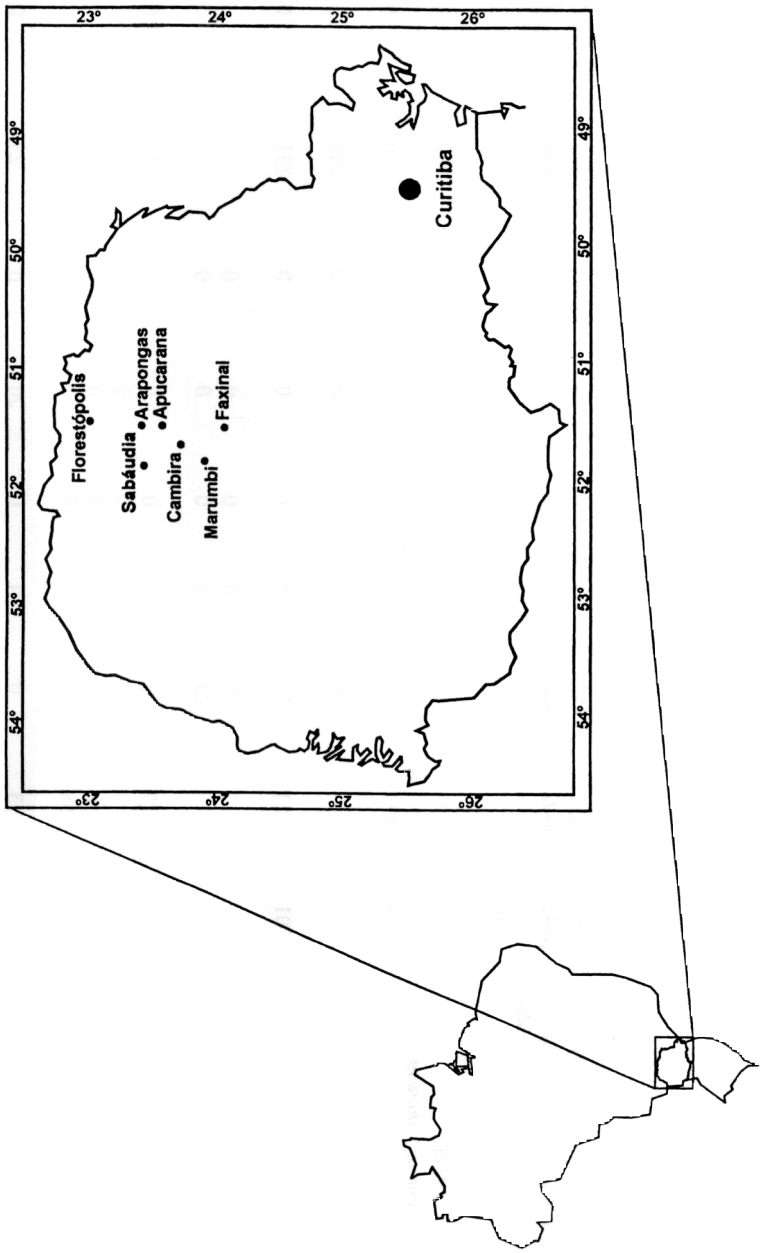

Fig. 1. Locations of the seven study areas in Paraná state, southern Brazil.

TABLE 1

The species and numbers of phlebotomine sandflies caught in seven municipalities in the north of Paraná state, Brazil, between May 1996 and June 1999

Species	*No. of sandflies of both sexes caught in:*								
	Apucarana	*Arapongas*	*Marumbi*	*Cambira*	*Faxinal*	*Florestópolis*	*Sabáudia*	*Any site*	*% of collected sandflies*
Lutzomyia (Nyssomyia) whitmani (Antunes and Coutinho, 1939)	17 138	9894	11 206	2800	11	1615	72	42 736	61.95
Lutzomyia (Pintomyia) fischeri (Pinto, 1926)	3589	4028	1164	290		100	0	9172	13.3
Lutzomyia (Pintomyia) pessoai (Coutinho and Barreto, 1940)	5064	1227	995	64	0	67	0	7417	10.75
Lutzomyia migonei (França, 1920)	3143	1492	820	141	3	55	0	5654	8.2
Lutzomyia (Psychodopigus) intermedia (Lutz and Neiva, 1912)	239	737	8	448	487	0	0	1919	2.78
Lutzomyia (Psychodopygus) monticola (Costa Lima, 1932)	451	454	31	0	0	4	0	940	.36
Lutzomyia (Pintomyia) shanonni (Dyar, 1929)	139	132	33	16	0	10	0	330	0.48
Lutzomyia firmatoi (Barreto, Martins and Pellegrino, 1956)	50	167	26	0	0	0	0	243	0.35
Lutzomyia lanei (Barreto and Coutinho, 1941)	201	11	15	4	0	0	0	231	0.33
Lutzomyia alphabetica (Fonseca, 1936)	121	5	7	0	0	0	0	133	0.19
Lutzomyia misionensis (Castro, 1959)	33	6	23	1	0	0	0	63	0.09
Lutzomyia correalimai (Martius, Coutinho and Luz, 1970)	6	7	0	0	0	0	0	13	0.02
Lutzomyia cortellezzii (Brèthes, 1924)	0	0	2	2	0	5	0	9	0.01
Lutzomyia longipenis (Barreto, 1946)	2	0	0	0	0	0	0	2	0.003
Brumptomyia brumpti (Larrousse, 1920)	5	98	0	0	0	3	0	106	0.16
Brumptomyia nitzulescui (Costa Lima, 1932)	0	3	0	0	0	20	0	23	0.03
Any	30 181	18 261	14 330	3766	502	1879	72	68 991	100

TABLE 2
Sex ratios of the sandflies caught between May 1996 and June 1999

Species	No. caught			Sex ratio
	Male	Female	Any	
Lutzomyia whitmanii	18 187	24 549	42 736	0.74
Lu. pessoai	2398	5019	7417	0.48
Lu. fischeri	3466	5706	9172	0.60
Lu. migonei	3255	2399	5654	1.35
Lu. intermedia	874	1045	1919	0.83
Lu. monticola	105	835	940	0.12
Lu. shanoni	30	300	330	0.10
Lu. lanei	44	187	231	0.24
Lu. firmatoi	62	181	243	0.34
Lu. alphabetica	5	128	133	0.04
Lu. misionensis	4	59	63	0.06
Lu. correalimai	2	11	13	0.18
Lu. longipenis	1	1	2	
Lu. cortellezzii	3	6	9	0.5
Brumptomyia brumpti	47	58	106	0.82
B. nitzulescui	11	12	23	0.92
Any	28 495 (41.3%)	40 496 (58.7%)	68 991 (100.00%)	0.70

75/M2904) and *L. amazonensis* (MHOM/BR/73/M2269 and IFLA/BR/67/PH8).

RESULTS

Overall, over 216 h of trapping, 68 991 sandflies were caught. These belonged to 16 species: 14 of the genus *Lutzomyia* and two of *Brumptomyia* (Table 1). Just four species—*Lu. whitmani*, *Lu. fischeri*, *Lu. pessoai* and *Lu. migonei*—represented the bulk (97%) of the total collection. The ratio of males to females was 0.70 overall, but varied, from 0.04 (*Lu. alphabetica*) to 1.35 (*Lu. migonei*), with the species involved (Table 2).

In terms of species, the collections in the residual forest were slightly more diverse (16 species) than those made on the edges of the forests and in the peridomestic sites nearby (14 species). As demonstrated by the collection records for Apucarana (Table 3), most of the sandflies were caught either in an illuminated Shannon trap baited with a horse or in a CDC light trap modified by the addition of hamster bait. *Lutzomyia whitmani* was the predominant species in all months, all traps and all localities (Tables 1–4), peaking in abundance during summer (21 December–21 March) and autumn (22 March–20 June) (Fig. 2).

Towards the end of the study collections were concentrated in and around the houses of recent cases of cutaneous leishmaniasis. These houses were all 15–100 m inside the forest. Overall, 5945 females from these targeted collections were dissected (Table 4), and three, all *Lu. whitmani* caught in Cambira in April–May 1999, were found to have flagellate infections (Table 5). Parasites from each of these infections were successfully isolated (to give IWHI/BR/99/CUR150, IWHI/BR/99/CUR151, and IWHI/BR/99/CUR 152), one straight from NNN and the other two (which initially produced contami-

TABLE 3

The numbers of female sandflies collected, in the municipality of Apucarana, in each of the trap–bait combinations used

	Shannon trap with light and:		*CDC light trap*		
Species	*Horse bait*	*Human bait*	*Without bait*	*Hamster bait*	*Total*
Lutzomyia whitmani	2480	8035	1257	5366	17 138
Lu. migonei	815	847	132	1349	3143
Lu. pessoai	455	2358	4	2247	5064
Lu. fischeri	331	1730	231	1297	3589
Lu. lanei	84	81	0	36	201
Lu. shanonni	77	44	0	18	139
Lu. alphabetica	35	71		14	121
Lu. monticola	32	247	3	169	451
Lu. intermedia	21	139	14	65	239
Lu. firmatoi	13	25	0	12	50
Lu. misionensis	2	25	0	6	33
Lu. correalimai		4	0	1	6
Lu. longipennis	2	0	0	0	2
Brumptomyia brumpti	0	5	0	0	5
Any	4348 (14.5%)	13 611 (45.1%)	1642 (5.5%)	10 580 (35.1%)	30 181 (100%)

nated cultures) by aspiration of material from the inoculation sites on the infected hamsters (only one of which developed a lesion). In the positive sandflies all the infections were peripylarian, with no blood in the guts, promastigotes and paramastigotes attached to the pylorus, and large numbers of promastigotes in the midgut. Isoenzymatic characterisation showed that all three isolates were identical to *Leishmania* (*V.*) *braziliensis* MHOM/BR/75/M2903.

DISCUSSION

In an endemic zone, successful control of leishmaniasis is easier if the causative agent, vector(s), reservoir hosts and transmission season are known. So far, in Paraná state, the causative agent of human cutaneous leishmaniasis has been identified [*Leishmania* (*V.*) *braziliensis*], but not the reservoirs or, until now, any vector. Since the prevalence of infection in the vector is usually low (see below), naturally infected sandflies are difficult to detect. In the absence of observed infection, the most abundant species of human-biting sandfly is often assumed to be the vector (Teodoro *et al.*, 1993*b*; Teodoro, 1995).

In the present studies, *Lu. whitmani* with natural infections of *Le. braziliensis* were observed. Although low, the infection rate (0.18% of the 1628 females dissected) is comparable with earlier observations. Galati *et al.* (1996), for example, found 0.16% of 613 female sandflies from Mato Grosso do Sul, in the Central–West region of Brazil, infected with promastigotes. Queiroz *et al.* (1994) reported 1.13% of the females they collected in Baturite, north–eastern Brazil, to be naturally infected.

According to the criteria of the World Health Organization (1990), *Lu. whitmani* can now be considered a vector of *Leishmania* (*V.*) *braziliensis* in Paraná state. This species is widely spread in all the areas where transmission occurs and adults are present in every month of the year. It is also strongly anthro-

TABLE 4

Numbers of female sandflies, of the five most common species, caught in Shannon traps and dissected during the summer months (representing 32 h of collection over eight nights) or the autumn months (28 h over seven nights)

		No. of females caught		
Study area	*Species*	*Summer*	*Autumn*	*Any time*
Florestópolis	*Lu. whitmani*	953	423	1376
	Lu. fischeri	70	9	79
	Lu. pessoai	33	20	53
	Lu. migonei	20	2	22
	Lu. intermedia			2
	All five	1077	455	1532
Apucarana	*Lu. whitmani*	406	612	1018
	Lu. fischeri	70	47	117
	Lu. pessoai	30	44	74
	Lu. migonei	5	11	16
	Lu. intermedia	10	23	33
	All five	521	737	1258
Cambira	*Lu. whitmani*	120	1508	1628
	Lu. fischeri	15	44	59
	Lu. pessoai	10	22	32
	Lu. migonei	3	13	16
	Lu. intermedia	58	168	226
	All five	206	1755	1961
Marumbi	*Lu. whitmani*	218	120	338
	Lu. fischeri	15	7	22
	Lu. pessoai	4	1	5
	Lu. migonei	4		5
	Lu. intermedia	2	0	2
	All five	243	129	372
Arapongas	*Lu. whitmani*	290	430	720
	Lu. fischeri	11	2	13
	Lu. pessoai	10	2	12
	Lu. migonei	3		4
	Lu. intermedia		0	
	All five	315	435	750
Sabáudia	*Lu. whitmani*	38	34	72
	Lu. fischeri	0	0	0
	Lu. pessoai	0	0	0
	Lu. migonei	0	0	0
	Lu. intermedia	0	0	0
	All five	38	34	72

pophilic. The natural infections observed were peripylarian (a characteristic of the *Viannia* subgenus), and (compatible with vector capacity) they were also intense and included paramastigotes attached to the pylorus.

ACKNOWLEDGEMENTS. The authors thank L. Hennig, for her technical assistance. This investigation was partly supported by the Brazilian National Council of Scientific Development and Technology (CNPq).

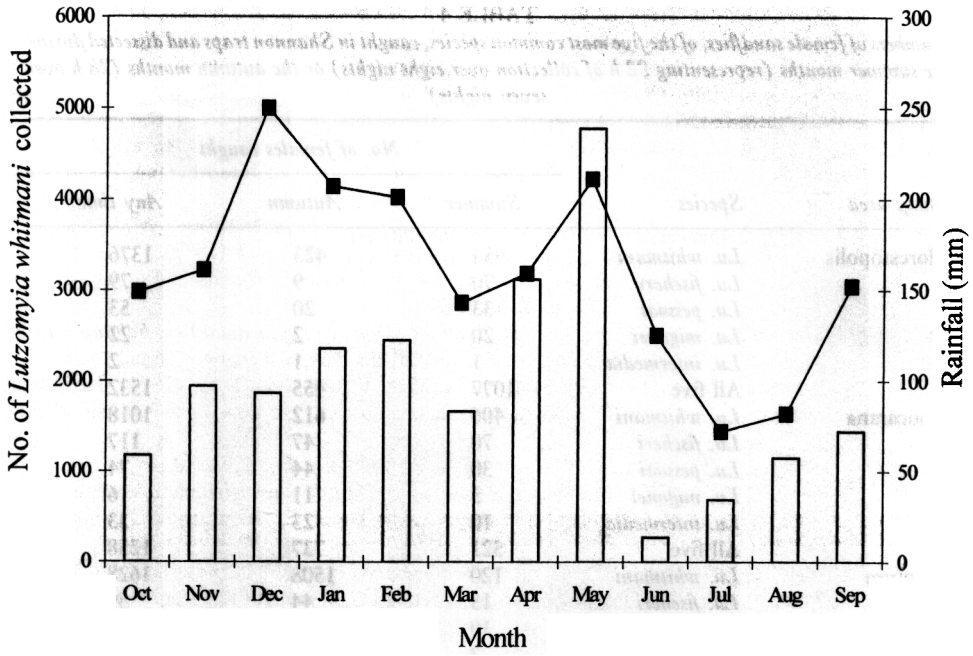

Fig. 2. Monthly variation in the numbers of *Lutzomyia whitmani* collected (□) and the rainfall (■), in the north of Paraná state, between October 1997 and September 1998.

TABLE 5

Prevalence of Leishmania *(*Viannia*)* braziliensis *in the female* Lutzomyia whitmani *collected in northern Paraná state, southern Brazil, between December 1998 and June 1999*

	Collected			
	December 1998	*April 1999*	*May 1999*	*Any time*
No. of females examined	93	685	850	1628
No. of females infected	0		2	3
% of females infected	0	0.14	0.23	0.18

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