

Seasonality of *Lutzomyia intermedia* and *Lutzomyia whitmani* (Diptera: Psychodidae: Phlebotominae), Occurring Sympatrically in Area of Cutaneous Leishmaniasis in the State of Rio de Janeiro, Brazil

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Lutzomyia intermedia and *Lutzomyia whitmani* occur sympatrically in the district of Posse, municipality of Petrópolis, in the State of Rio de Janeiro (114 km from the city of Rio de Janeiro), where sand flies were captured weekly for three consecutive years in both the peridomicile and forest, in two first years from 18:00 to 22:00 h, using a Shannon trap and CDC light traps. The anthropophilic habit was assessed by recording the specimens that bit the human captors. Considering the different capture types and sites, the following species were identified: *Brumptomyia brumpti*, *B. cardosoi*, *B. guimaraesi*, *Lutzomyia amarali*, *L. longipalpis*, *L. microps*, *L. costalimai*, *L. edwardsi*, *L. firmatoi*, *L. migonei*, *L. termitophila*, *L. tupynambai*, *L. fischeri*, *L. pessoai*, *L. lutziana*, *L. shannoni*, *L. barretto*, *L. intermedia*, *L. whitmani*, *L. lloydi*, and *L. minasensis*. *L. intermedia* (38.3%) and *L. whitmani* (38.6%) predominated. *L. intermedia* predominated in the peridomicile, where it displayed more intense anthropophily, while *L. whitmani* displayed greater density in the banana plantations and forest, preferential sites for biting humans. Studies on seasonality showed that while *L. intermedia* displayed greater density in the hottest months of the year (December, January, and February), *L. whitmani* was abundant in the coolest months (June, July, and August), although both occurred throughout the year.

Key words: *Lutzomyia intermedia* - *Lutzomyia whitmani* - sand fly fauna - seasonality - sympatric species - Rio de Janeiro - Brazil

Foci of American cutaneous leishmaniasis (ACL) in the State of Rio de Janeiro have been described since the beginning of the last century. In the last 25 years, several outbreaks have been reported in different places, related to new human settlements in recently deforested areas. This epidemiological profile has been observed mainly in the Southeast of Brazil, and peridomiciliary transmission should be considered in these cases (Rangel 1995). Currently it is possible to observe the disease around metropolitan areas, where the Atlantic Forest has been devastated over the course of the years.

Lutzomyia intermedia (Lutz & Neiva, 1912) is probably the main vector of *Leishmania (Viannia) braziliensis* in the State of Rio de Janeiro (Aragão 1922, Nery-Guimarães 1955, Menezes et al. 1974, Araújo-Filho 1978, Sabroza 1981, Coutinho et al. 1981, Rangel et al. 1984, 1986, 1990), with a domiciliary and peridomiciliary transmission cycle.

In 1996, we were informed by the Center for Zoonoses of the Petrópolis Municipal Health Department about the occurrence of human cases [due to *L.(V.) braziliensis*], dogs with high serum titers, and dogs and horses with

suspicious lesions in the district (township) of Posse, in the northern region of the municipality (county) of Petrópolis, in the State of Rio de Janeiro.

Preliminary studies on the sand fly fauna showed the simultaneous occurrence of *L. intermedia* and *L. whitmani* (Antunes & Coutinho 1939), apparently for the first time, with a similar density and being a more aggressive man-biter than *L. intermedia* in the same transmission area for cutaneous leishmaniasis in the State of Rio de Janeiro.

Therefore, the aim of this study, besides the knowledge of local sand fly species, was to analyze aspects related to the seasonality and anthropophilic behaviour of *L. intermedia* and *L. whitmani*, evaluating the possibility of these vector species to be sharing the transmission mechanism for *L. (V.) braziliensis* in the region.

MATERIALS AND METHODS

Study area - The district of Posse is a rural area in the northern part of the municipality of Petrópolis, 112 km from the city of Rio de Janeiro (Fig. 1). In the past, this area was covered by primary forest, while currently, due to heavy anthropic activity, a major portion of the native forest has been replaced by farm crops and especially pasture.

Due to its geographic location and relief, the area is classified as having a super-humid and humid mild mesothermic tropical climate. This type of climate occurs in the Serra do Mar mountain range, where the altitude is 700 m above sea level, which is the case for the area under study. Annual mean temperatures vary between 18° and

This work was supported by Fiocruz and CNPq with additional support from The Wellcome Trust.

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Received 1 October 2001

Accepted 29 April 2002



Fig. 1: district of Posse, Petrópolis municipality, State of Rio de Janeiro, Brazil.

19°C, and even during the summer the mean temperatures are rarely over 20°C. During the winter the mean minimum temperature falls to 10°C (National Department of Water and Electric Power, National Institute of Meteorology).

The district of Posse is located in an area of the Serra do Mar where rocky outcroppings indicate abundant presence of granite and gneiss, in various stretches of the mountain range.

Our capture station was set up on the “Sítio Catavento” (Windmill Farm), where a human case of leishmaniasis due to *L. (V.) braziliensis* had been diagnosed.

Capture sites - To conduct the survey of sand fly fauna, local distribution of species by type of capture, activity, seasonality, and anthropophilic habit, we used two capture stations: Station 1 (peridomicile): anthropic areas, recent deforestation, with human dwellings, shelters for domestic animals (a chicken coop and kennel), and fruit trees, where bananas were the most abundant fruit crop. Station 2 (forest): part of the farm is covered by mountainous semideciduous seasonal forest (primary and secondary vegetation in the early, medium and advanced stages of regeneration of the Atlantic Rain Forest, in the State of Rio de Janeiro by report of the Municipal Government of Petrópolis). This type of vegetation is conditioned by a double climatic seasonality, including one tropical season with heavy summer rains followed by an intense drought, and the other subtropical, without a proper dry period, but with a physiological drought caused by the cold winter temperature. The climates establish a foliar seasonality in the dominant arboreal species, which adapt

to the alternating lack of water and drop in temperature in the cold months. The predominant smaller plant and shrub species are the following: “romerillo” (*Baccharis dracunculifolia*), “assa-peixe” (*Vernonia polyanthes*); “lantana” (*Lantana camara*); rattlesnake fern (*Crotalaria mucronata*); “erva-colégio” (*Elephantopus mollis*); nightshade (*Solanum aculeaisum*); “jurubeba” (*Solanum paniculatum*); “pindova” (*Atalea humilis*); “pixirica” (*Clidemia hirta*); and “sape” grass (*Imperata brasiliensis*). The most frequent ligneous species indicative of this stage in the area are: “angico” (*Anadenanthera colubrina*); “crindiúva” (*Trema micrantha*); trumpetwood (*Cecropia* spp.); “marica” (*Mimosa bimucromata*); and “sangue-de-dragão” (*Croton urucuana*). The vines are predominantly ligneous. The following species are also present: “açoita-cavalo” (*Luheta grandiflora*); “tecoma” (*Tabebuia chrisotricha*); and “senna” (*Senna multijuga*) (Municipal Government of Petrópolis).

A comparative entomological survey of the two areas (located 200 m apart) was conducted weekly for two consecutive years, from 18:00 to 22:00 h. The data presented in the table and figures represents the sum of the four captures performed each month.

Captures in the domicile and peridomicile - Station 1 employed three CDC light traps (Sudia & Chamberlain 1962): one in the chicken coop, another on the outside wall of the house (10 m apart), and a third one in the banana grove (80 m from the house). Active captures on the outside wall of the house were conducted on the opposite side from where the light trap was placed. Attraction to human bait was evaluated together with the captures on the walls of the house. Species found biting human captors were separated from those captured on the walls.

Captures in the forest - Station 2 included CDC light traps at different points: one on the slope close to crevices in the rocks; another in a small cave; and a third one above the tabular roots of mature trees (located 15 m apart from each other). A Shannon trap (Shannon 1939, Alexander 2000) was also used to capture sand flies 245 m from the house.

Sand fly identification - The specimens captured and fixed in alcohol at 70% underwent a process and were mounted on microscope slides with slide covers and fixed in Berlese liquid. Identification of specimens was performed using the classification proposed by Young and Duncan (1994).

Data analysis - Analysis of variance, investigating the effect of seasonal and microgeographic variation and the different capture types on the relative frequencies of *L. intermedia* and *L. whitmani* was conducted after angular transformation. Captures performed in the same months in different years were treated as replicates. Analysis of the association between the number of sand flies captured in the two years and environmental variables (precipitation and high and low temperatures) used Spearman’s rank correlation (Sokal & Rohlf 1995).

RESULTS

Sand fly fauna - In 384 h, a total of 45,170 sand flies were captured: 19,365 females and 25,805 males, considering the different months, capture sites, and types of cap-

ture. Three species of *Brumptomyia* (França & Parrot 1921) and 20 species of *Lutzomyia* (França 1924) were listed. *L. intermedia* (38.3%) and *L. whitmani* (38.6%) predominated, with other species displaying low percentages (Table I).

Table II presents the capture data from Station 1, demonstrating that *L. intermedia* was the predominant species in the peridomicile (57.7%) where it was the most

anthropophilic species followed by *L. whitmani* (22.6%) (Fig.2), and it is important to highlight that both were captured on the walls of the house. *L. migonei*, *L. fisheri*, and *L. quinquefer* were also present in the peridomicile, although in smaller proportions. However, in the banana grove *L. whitmani* predominated over *L. intermedia*, and there was also a greater diversity of sand fly species.

In Station 2, located in the forest, there was a major

TABLE I
Sand fly fauna in Posse, State of Rio de Janeiro: number and percentage of collected sand fly species in different areas. July 1996 to June 1998

Species	Results			
	Number collected		Total	Percentage
	F	M		
<i>Brumptomyia brumpti</i> (Larrousse,1920)	43	65	108	0.2
<i>B. cardosoi</i> (Barretto & Coutinho,1941)	22	23	45	0.1
<i>B. guimaraesi</i> (Coutinho & Barretto,1941)	37	26	63	0.1
<i>Lutzomyia amarali</i> (Barretto & Coutinho, 1940)	31	37	68	0.2
<i>L. longipalpis</i> (Lutz & Neiva, 1912)	11	24	35	0.1
<i>L. microps</i> (Mangabeira,1942)	26	30	56	0.1
<i>L. costalimai</i> (Mangabeira,1942)	18	61	79	0.2
<i>L. edwardsi</i> (Mangabeira, 1942)	46	60	106	0.2
<i>L. firmatoi</i> (Barretto,Martins & Pellegrino,1956)	37	68	105	0.2
<i>L. migonei</i> (França, 1920)	697	1,059	1,756	3.9
<i>L. termitophila</i> Martins, Falcão & Silva,1964	10	22	32	0.1
<i>L. tupynambai</i> (Mangabeira, 1942)	90	88	178	0.4
<i>L. fisheri</i> (Pinto,1926)	1,764	2,275	4,039	8.9
<i>L. pessoai</i> (Coutinho & Barreto,1940)	24	22	46	0.1
<i>L. lutziana</i> (Costa Lima,1932)	12	20	32	0.1
<i>L. shannoni</i> (Dyar,1929)	22	46	68	0.2
<i>L. barrettoii</i> (Mangabeira, 1942)	123	131	254	0.6
<i>L. intermedia</i> (Lutz & Neiva,1912)	7,548	9,749	17,297	38.3
<i>L. whitmani</i> (Antunes & Coutinho, 1939)	7,507	9,930	17,437	38.6
<i>L. lloydi</i> (Antunes,1937)	32	41	73	0.2
<i>L. minasensis</i> (Mangabeira,1942)	35	39	74	0.2
<i>L. quinquefer</i> (Dyar, 1929)	531	1,286	1,817	4.0
<i>L. monticola</i> (Costa Lima, 1932)	699	703	1,402	3.1
Total	19,365	25,805	45,170	100

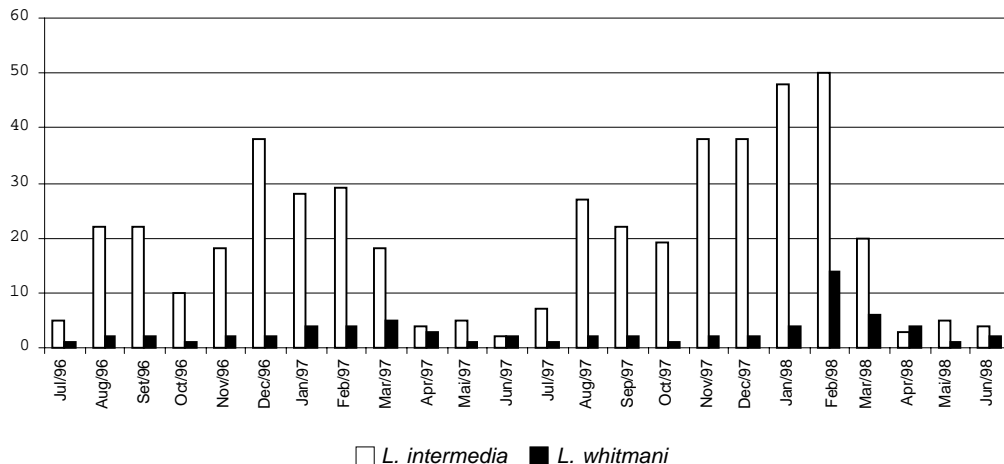


Fig. 2: collected *Lutzomyia intermedia* and *Lutzomyia whitmani* on human bait in peridomicile.

predominance of *L. whitmani* (63.5%) in all types of captures and it was also its preferential site for biting humans (Fig. 3). The second most frequently captured species was *L. fisheri* (14.6%), which occurred preferentially in this environment. *L. intermedia* was also present in the forest, but with a lower density (7.9%) (Table III).

Observations pertaining to sand fly seasonality showed that while *L. intermedia* predominated in the hottest months of the year (December, January, and February), *L. whitmani* was abundant in the coolest months (June, July, and August), although both were present throughout the year (Fig. 4). This seasonal fluctuation was confirmed by the highly significant correlations between the number of sand flies of the two species captured monthly during the two study years (*L. intermedia*, $R = 0.81$, $p < 0.002$; *L. whitmani*, $R = 0.98$, $p < 0.0001$). Temperature and precipitation data provided by the National Institute of Meteorology demonstrate this seasonality (Fig. 2), and significant correlations were present between the number of specimens from the two species and these environmental variables (Table IV).

Analysis of variance was conducted, comparing the relative frequency of *L. intermedia* in different months and types of capture. The results indicate highly significant effects for the month ($F_{[11, 108]} = 27.43$; $P < 0.0001$) and type of capture ($F_{[8, 108]} = 206.29$; $P < 0.0001$), as well as for interaction between the two ($F_{[88, 108]} = 6.34$; $P <$

0.0001). Similar results were obtained for *L. whitmani* (month: $F_{[11, 108]} = 73.02$; $P < 0.0001$; type of capture: $F_{[8, 108]} = 195.76$; $P < 0.0001$; and interaction: $F_{[88, 108]} = 8.97$; $P < 0.0001$). Likewise, significant effects for capture site and season of the year were observed when the months of the year were divided ("nested analysis") into two seasons (October-March, hot and rainy; April-September, cold and dry) and the captures were divided into peridomicile and forest. Thus, these results indicate the occurrence of microgeographic and seasonal variation in the relative frequencies of the two species.

Seasonal and microgeographic variations in the numbers of *L. intermedia* and *L. whitmani* have an important effect on the probability of humans being bitten by one of these two vectors, depending on the area and time of year, where the number of sand flies from the two species and captured with human bait over the two years is shown for both the peridomicile (Fig. 3) and the forest (Fig. 4).

DISCUSSION

Since the beginning of the last century in Rio de Janeiro *L. intermedia* has been considered as a vector of cutaneous leishmaniasis. Aragão (1922), based on the association between a high density of *L. intermedia* and the presence of the disease in Águas Férreas in the Laranjeiras neighborhood, incriminated this sand fly as the potential vector of *L. (V.) braziliensis* in the Southeast of Brazil.

TABLE II
Sand fly fauna in Posse, State of Rio de Janeiro: number and percentage of collected species in peridomiciliary area.
July 1996 to June 1998

Species	Walls of houses		CDC light trap		CDC light trap in chicken coop		Human bait	Banana plantation		Results			Percentage
	F	M	F	M	F	M		CDC light trap		Number collected		Total	
							F	M	F	M	Total		
<i>B. brumpti</i>	0	0	0	0	0	0	0	7	9	7	9	16	0.1
<i>B. cardosoi</i>	0	0	2	0	0	0	0	0	3	2	3	5	0.0
<i>B. guimaraesi</i>	0	0	3	0	0	0	0	1	4	4	4	8	0.0
<i>L. amarali</i>	0	0	0	2	0	0	0	6	1	6	3	9	0.0
<i>L. longipalpis</i>	2	2	1	0	5	17	0	0	1	8	20	28	0.1
<i>L. microps</i>	0	0	0	0	0	0	0	0	0	0	0	0	0.0
<i>L. costalimai</i>	0	8	0	0	0	0	0	4	16	4	24	28	0.1
<i>L. edwardsi</i>	0	0	0	0	0	0	0	23	25	23	25	48	0.2
<i>L. firmatoi</i>	3	3	0	5	1	0	0	4	18	8	26	34	0.1
<i>L. migonei</i>	42	139	35	54	297	527	26	86	108	486	828	1,314	4.8
<i>L. termitophila</i>	0	0	0	0	7	12	0	1	3	8	15	23	0.1
<i>L. tupynambai</i>	6	5	0	0	0	0	0	16	15	22	20	42	0.2
<i>L. fisheri</i>	44	264	26	44	146	203	21	300	421	537	932	1,469	5.3
<i>L. pessoai</i>	0	0	0	1	0	0	0	0	0	0	1	1	0.0
<i>L. lutziana</i>	0	0	0	0	0	0	0	0	0	0	0	0	0.0
<i>L. shannoni</i>	0	1	0	0	0	0	0	4	8	4	9	13	0.0
<i>L. barretto</i>	0	0	0	0	11	5	0	32	24	43	29	72	0.3
<i>L. intermedia</i>	621	1,872	939	1,652	3,908	4,220	482	986	1,230	6,936	8,974	15,910	57.8
<i>L. whitmani</i>	122	403	83	195	294	400	70	1,900	2,769	2,469	3,767	6,236	22.6
<i>L. lloydi</i>	0	2	0	0	0	0	0	11	12	11	14	25	0.1
<i>L. minasensis</i>	0	0	4	3	0	0	0	4	7	8	10	18	0.1
<i>L. quinquefer</i>	315	929	61	129	125	186	1	29	38	531	1,282	1,813	6.6
<i>L. monticola</i>	12	0	0	0	12	0	0	171	237	195	237	432	1.6
Total	1,167	3,628	1,154	2,085	4,806	5,570	600	3,585	4,949	11,312	16,232	27,544	100

B: *Brumptomyia*; L: *Lutzomyia*

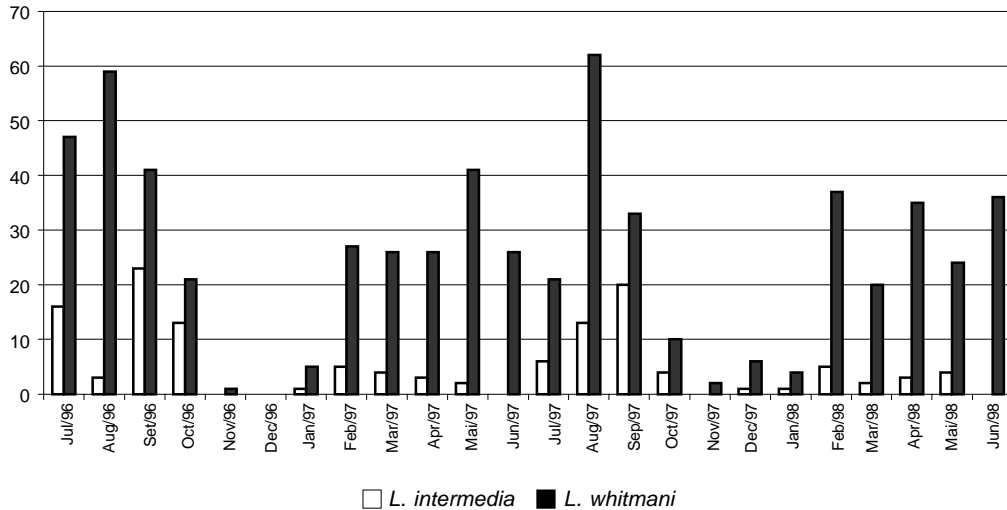


Fig. 3: collected *Lutzomyia intermedia* and *Lutzomyia whitmani* on human bait in the forest.

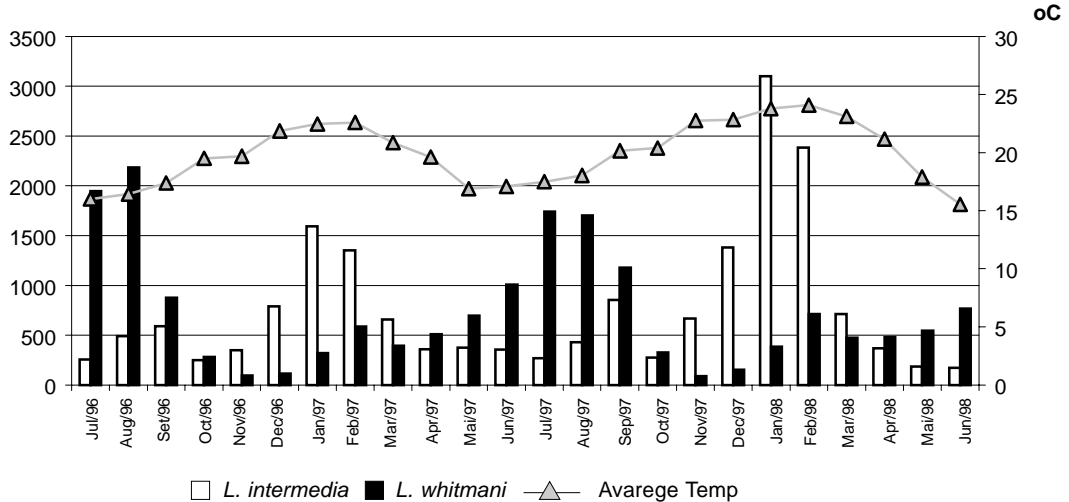


Fig. 4: seasonal variation of collected *Lutzomyia intermedia* and *Lutzomyia whitmani* in the peridomicile and in the forest.

Data from different studies have shown a significant percentage of this species in the domicile and peridomicile. Although being the more aggressive man-biter, *L. intermedia* appears to be strongly attracted by dogs and horses, suggested as secondary reservoirs of *L. (V.) braziliensis* (Menezes et al. 1974, Araújo Filho 1978, Sabroza 1981, Coutinho et al. 1981, Rangel et al. 1986, 1990, 1998).

L. intermedia appears to play an important role in the transmission mechanism of ACL in the states of Espírito Santo, São Paulo, and Rio de Janeiro. It has been found naturally infected with *Leishmania*, presumably *L. (V.) braziliensis*, in the states of Paraná (Forattini & Santos 1952), São Paulo (Forattini et al. 1972), and Rio de Janeiro (Rangel et al. 1984).

The first references to the role of *L. whitmani* as a vector of leishmaniasis date to the early 40s, based on studies in the Southeast region of Brazil, when Pessoa

and Coutinho (1940) found specimens of *L. whitmani* naturally infected with flagellates, presumably *Leishmania*, in São Paulo. In the Southeast of Brazil, the species has been found with a high density in endemic areas in the states of São Paulo, Minas Gerais, and Espírito Santo. Its presence has been recorded in animal shelters and the domicile and peridomicile.

In Rio de Janeiro, to date, the frequency of *L. whitmani* has always been recorded with low indices (Rangel et al. 1986, 1990, Carvalho 1993, Aguiar et al. 1993). More recent studies have identified *L. whitmani* in preserved areas, as well as in shelters for domestic animals, and it is also the second most frequent species to bite humans in the peridomicile (Souza et al. 2001). De Souza et al. (1995) in São José do Vale do Rio Preto, a neighbour municipality to our capture area (Fig. 1), showed *L. whitmani* as the second most frequently captured species, next to *L. intermedia*.

TABLE III

Sand fly fauna of Posse, Rio de Janeiro State: number and percentage of species caught in the forest. July 1996 to June 1998

Species	In forest							Results			
	Shannon trap		CDC light trap		CDC light trap in the cave		Human bait	Number collected		Total	Percentage
	F	M	F	M	F	M	F	F	M		
<i>B. brumpti</i>	3	3	5	9	28	44	0	36	56	92	0.5
<i>B. cardosoi</i>	2	3	9	9	9	8	0	20	20	40	0.2
<i>B. guimaraesi</i>	2	2	6	6	25	14	0	33	22	55	0.3
<i>L. amarali</i>	12	14	1	6	12	14	0	25	34	59	0.3
<i>L. longipalpis</i>	0	2	3	2	0	0	0	3	4	7	0.0
<i>L. microps</i>	10	15	4	2	12	13	0	26	30	56	0.3
<i>L. costalimai</i>	2	6	0	11	12	20	0	14	37	51	0.3
<i>L. eduardsi</i>	13	14	8	20	2	1	0	23	35	58	0.3
<i>L. firmatoi</i>	20	20	0	0	9	22	0	29	42	71	0.4
<i>L. migonei</i>	149	152	50	79	0	0	12	211	231	442	2.5
<i>L. termitophila</i>	0	2	0	2	2	3	0	2	7	9	0.1
<i>L. tupynambai</i>	31	37	14	21	23	10	0	68	68	136	0.8
<i>L. fischeri</i>	730	935	215	241	102	167	180	1,227	1,343	2,570	14.6
<i>L. pessoai</i>	22	17	2	1	0	3	0	24	21	45	0.3
<i>L. lutziana</i>	5	9	4	7	3	4	0	12	20	32	0.2
<i>L. shannoni</i>	10	17	2	7	6	13	0	18	37	55	0.3
<i>L. barrettoi</i>	13	32	40	45	27	25	0	80	102	182	1.0
<i>L. intermedia</i>	242	358	163	290	77	127	130	612	775	1,387	7.9
<i>L. whitmani</i>	2,645	3,369	1,117	1,827	660	967	616	5,038	6,163	1,1201	63.5
<i>L. lloydi</i>	9	8	3	1	9	18	0	21	27	48	0.3
<i>L. minasensis</i>	5	7	2	5	20	17	0	27	29	56	0.3
<i>L. quinquefer</i>	0	0	0	4	0	0	0	0	4	4	0.0
<i>L. monticola</i>	221	239	50	47	122	180	111	504	466	970	5.5
Total	4,146	5,261	1,698	2,642	1,160	1,670	1,049	8,053	9,573	17,626	100

B: Brumptomyia; L: Lutzomyia

TABLE IV

Sperman Correlation (R) between the number of collected *Lutzomyia intermedia* (INTER) and *Lutzomyia whitmani* (WHITM) species and the environmental variations: monthly average of the highest temperature (TEMPMAX); monthly average of the lowest temperature (TEMPMIN) and monthly precipitation (PREC). July 1996 to June 1998

	R	P
INTER & TEMPMAX	.777198	.000008
INTER & TEMPMIN	.747444	.000027
WHITM & TEMPMAX	-.537424	.006763
WHITM & TEMPMIN	-.708723	.000106
WHITM & PREC	-.706087	.000116

In the district of Posse, *L. whitmani* was the second most frequently captured species, next to *L. intermedia*. Both species were present in the peridomicile. *L. whitmani* was the most frequent species in the banana grove, a transition area between the farm dwellings and the forest, and second the most frequently captured species on the walls of the house, showing the possibility of a certain degree of domiciliation, as occurs in other areas in the Northeast, Southeast, and South of Brazil. Considering both capture sites, it was the species that most frequently bit humans.

Barreto (1943) observed that *L. whitmani* was found more frequently in forest areas undergoing deforestation, although it invaded the domicile and was highly anthropophilic, in addition to avidly biting domestic animals. Its participation in the domicile was confirmed by Forattini (1960), who observed both *L. intermedia* and *L. whitmani* as predominant species during deforestation of primary forest. As the deforestation extended, there was a tendency for *L. whitmani* to disappear, suggesting that this species is more dependent on the primary forest than *L. intermedia*.

In the epidemiological model emerging from deforestation, the non-forest environment stands out, consisting mainly of the extra-domiciliary environment. In this new ecological situation, studies have demonstrated that the transmission of the parasite appears to depend on some species, highlighting *L. whitmani* and *L. intermedia* (Forattini et al. 1976, Taniguchi et al. 1991, Stolf et al. 1993).

L. intermedia and *L. whitmani* have been suggested as important vectors of cutaneous leishmaniasis in various regions of Brazil, demonstrating they are highly anthropophilic and abundant, in addition to natural infection with *L.(V.) braziliensis*. The data we obtained on seasonality, where *L. intermedia* predominates in the summer and *L. whitmani* in the winter, considering their seasonal habits as it was shown in this study, we suggest that the transmission may happen all over the year in this locality.

ACKNOWLEDGMENTS

To Ms Deise Aparecida Barrichelo, owner of the Sítio Catavento farm where the study was conducted, for her constant support and encouragement, and Mr Luís Henrique Costa for his technical support in the field and laboratory.

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