

A STUDY OF SANDFLY SPECIES (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE) IN A FOCUS OF CUTANEOUS LEISHMANIASIS IN THE MUNICIPALITY OF BATURITÉ, CEARÁ, BRAZIL

ALFREDO C. R. AZEVEDO & ELIZABETH F. RANGEL

Instituto Oswaldo Cruz, Departamento de Entomologia, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil

*In a study on putative vectors of cutaneous leishmaniasis in Baturité, Ceará State, Brazil, six sandfly species were identified: Lutzomyia longipalpis, L. wellcomei, L. peresi, L. whitmani, L. shannoni and L. migonei.*

*In general L. whitmani and L. migonei were the predominant species. They were collected in peridomiliary areas and were attracted by man and equines. L. whitmani was the most anthropophilic species. Studies using animals as bait showed that blood-feeding occurs throughout the night.*

*Previously L. whitmani had been found infected with a Leishmania species of the braziliensis complex and L. migonei was infected with peripylarian flagellates. In combination these findings suggest that they may be responsible for peridomestic transmission of cutaneous leishmaniasis.*

Key words: sandfly species – cutaneous leishmaniasis – Ceará State

To gain information on sandfly species and their relation to cutaneous leishmaniasis in Ceará State, we started surveying an endemic area in Aquiráz municipality in 1987 (Rangel et al., 1989), but discontinued the study when the place was sprayed with DDT, as a control measure by SUCAM. We consequently moved to another municipality, Baturité, from where cases of the disease were being reported.

The purpose of our survey was to identify the local sandfly fauna, study some aspects of the biology of the species and try to discover the vector.

#### MATERIALS AND METHODS

*Study area* – Baturité municipality is located at about 106 km from Fortaleza City, at 4° 26' latitude South and 39° 3' longitude West. In the past, it was covered by tropical forest, which has been replaced by plantations

of bananas and sugar-cane. We worked in three localities: Mondego (540 m. alt.), Tijuca (550 m. alt.) and Pé-do-Candeia (140 m. alt.).

*Meteorological data* – In the last 10 years the average temperature ranged between 20-21 °C; the relative humidity was 80-83%, during August and September, and annual rainfall 1.600-1.800 mm, according to 6<sup>o</sup> Distrito de Meteorologia do Rio de Janeiro.

*Sandfly captures* – These were carried out daily, between 6 p.m. and 8 p.m., during August and September 1989, and consisted of collections in peri-domestic sites (up to 10 m distance from houses) using human and animal baits (equines), and CDC and Falcão light traps in domestic animal shelters. Each method of capture was used in all sites. For the study of hourly biting-activity, separate, hourly captures were made, from 6.0 p.m. to 6.0 a.m., using a equine (mule) bait.

#### RESULTS

*Sandfly fauna* – During a total of 108 h we captured 11,431 sandflies belonging to the following species, named according to the classification given by Martins et al. (1978):

Work supported by NIH ICIDR grant A116305 and FAPERJ grant E-29/170.227/90.

Received 9 April 1991.

Accepted 12 August 1991.

TABLE I

Sandflies collected by all methods of capture in endemic area of cutaneous leishmaniasis surveyed in Baturité municipality, Ceará, Brazil, August-September, 1989

Sandfly species	Number			%		
	Females	Males	Both Sexes	Females	Males	Both Sexes
<i>L. migonei</i>	1177	4989	6166	21.6	83.6	53.9
<i>L. whitmani</i>	3452	876	4328	63.2	14.7	37.9
<i>L. (Psychodopygus) spp<sup>a</sup></i>	745	7	752	13.6	0.1	6.6
<i>L. shannoni</i>	89	69	158	1.6	1.2	1.4
<i>L. (Psathyromyia) spp</i>	1	17	18	0	0.3	0.2
<i>L. longipalpis</i>	2	6	8	0	0.1	0
<i>L. peresi</i>	—	1	1	—	0	0
Total	5466	5965	11431	100	100	100

a: males *L. wellcomei*, females series *squamiventris*, which includes *L. wellcomei* and other morphologically indistinguishable species.

TABLE II

Sandflies collected by all methods of capture in each of the endemic localities of cutaneous leishmaniasis — Pé-do-Candeia, Tijuca and Mondego surveyed in Baturité municipality, Ceará, Brazil, August-September, 1989

Sandfly species	Number		
	Pé-do-Candeia (140 m. alt.) 48 h	Tijuca (550 m. alt.) 44 h	Mondego (540 m. alt.) 16 h
<i>L. migonei</i>	635	5466	65
<i>L. whitmani</i>	1356	2626	346
<i>L. (Psychodopygus) spp</i>	113	637	2
<i>L. shannoni</i>	20	130	6
<i>L. (Psathyromyia) spp</i>	5	14	1
<i>L. longipalpis</i>	3	1	4
<i>L. peresi</i>	—	1	—
Total	2132	8875	424

*Lutzomyia (Lutzomyia) longipalpis* (Lutz & Neiva, 1912)

*Lutzomyia (Psychodopygus) wellcomei* (Fraiha, Shaw & Lainson, 1971)

*Lutzomyia (Psychodopygus) spp.*

*Lutzomyia (Helcocyrtomyia) peresi* (Mangabeira, 1942)

*Lutzomyia (Nyssomyia) whitmani* (Antunes & Coutinho, 1939)

*Lutzomyia (Psathyromyia) shannoni* (Dyar, 1929)

*Lutzomyia (Psathyromyia) spp.*

*Lutzomyia migonei* (França, 1920).

The name *Lutzomyia (Psychodopygus) spp.* refers to sandflies of the *squamiventris* series: the males are *L. wellcomei*; the females, however could belong to the same species or to other morphologically indistinguishable species, such as *L. squamiventris*, *L. chagasi*, *L. complexus* or even *L. maripaensis*.

Table I shows that in this focus of cutaneous leishmaniasis the more numerous species of sandflies were *L. migonei* and *L. whitmani*, corresponding to 91.8% of the local fauna. In

TABLE III

Sandflies collected on human bait, on animal bait and in light traps in the three endemic localities of cutaneous leishmaniasis surveyed in Baturité municipality, Ceará, Brazil, August-September, 1989

Sandfly species	On human bait 20 h		On animal bait 34 h		In light-traps 54 h	
	Females	Males	Females	Males	Females	Males
<i>L. migonei</i>	14	6	1155	4935	8	48
<i>L. whitmani</i>	693	11	2371	603	388	262
<i>L. (Psychodopygus) spp</i>	92	—	646	6	7	1
<i>L. shannoni</i>	27	—	61	67	1	2
<i>L. (Psathyromyia) spp</i>	—	—	—	9	1	8
<i>L. longipalpis</i>	1	—	1	—	—	6
<i>L. peresi</i>	—	—	—	1	—	—
Total	827	17	4234	5621	405	327
	844		9855		732	

far fewer numbers were specimens of the *squamiventris* series of the subgenus *Psychodopygus* — 6.6%; the remaining species were even more scarce.

Table II shows that while in Tijuca *L. migonei* was much more numerous than *L. whitmani*, the opposite occurred in Pé-do-Candeia and Mondengo, suggesting that altitude was not the factor responsible for this variable distribution.

**Frequency of species caught with different baits** — In Table III it can be seen that, on human bait, *L. whitmani* was by far the dominant sandfly species, comprising 83.4% of all the total catch. After this the order of frequency was *L. (Psychodopygus) spp.* (10.9%), *L. shannoni* (3.2%) and *L. migonei* (2.4%).

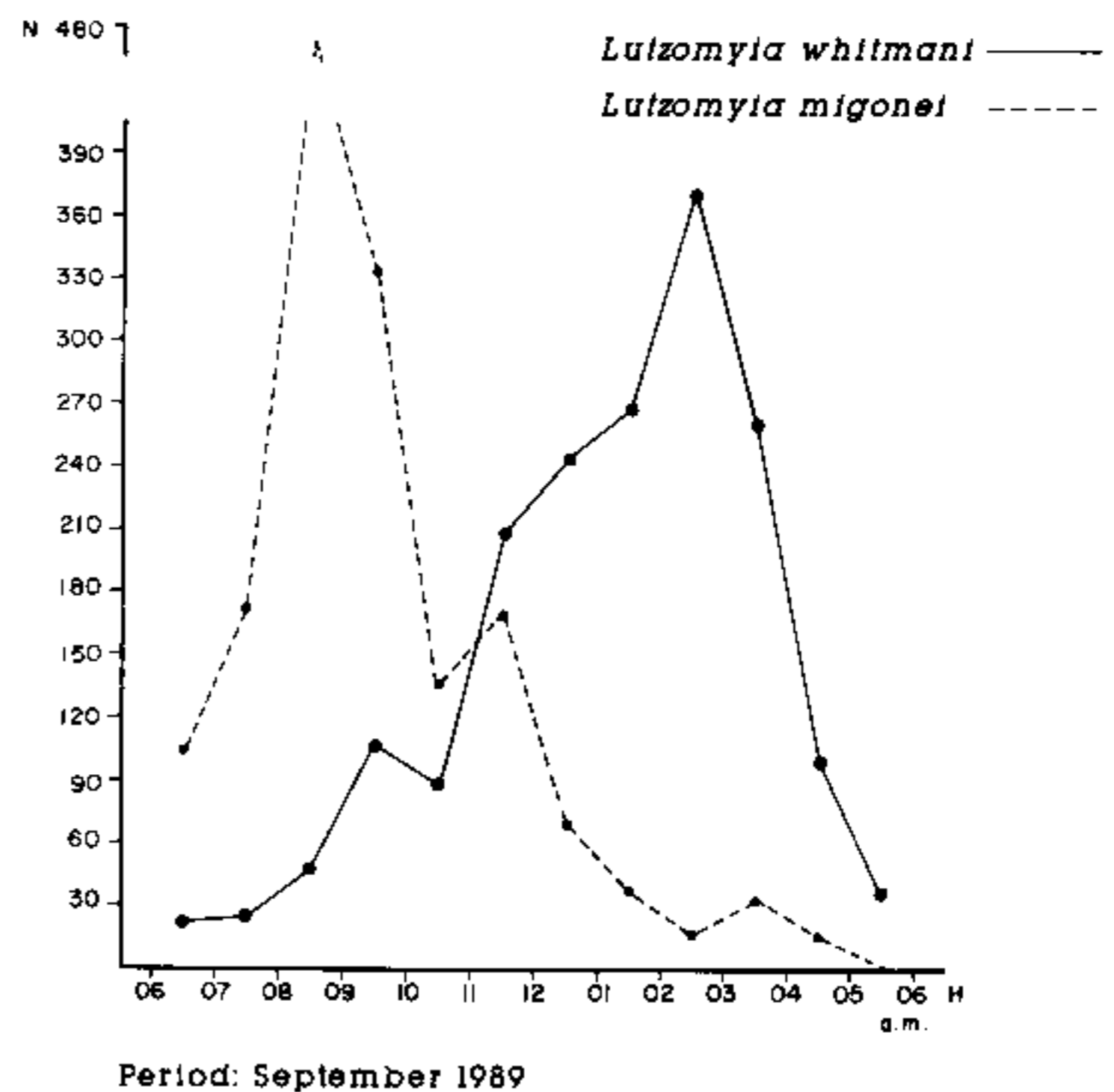
On animal bait, *L. migonei* was most abundant (61.8%) followed by *L. whitmani* (30.2%).

In light-traps *L. whitmani* was again predominant, with 88.8% of the total, while *L. migonei* formed only 7.7% of the catch.

If the sandfly sexes are compared, some other interesting results become apparent. On human bait, taking the sum of all species, female sandflies were much more abundant than males. When the species of sandflies are considered very few males of *L. whitmani* were attracted to human hosts, compared with *L. migonei*. No males of other species were caught on humans. On animals, however, the species most common were *L. migonei* and *L. shannoni*, and the number of male specimens

surpassed that of females. The predominance of females over males on human baits had been previously observed by Barretto (1943) in observations on sandflies of São Paulo State. Deane (1956) noted the same for *L. longipalpis* in Ceará State, and showed that males predominate on the hairy covering of animals (like donkeys, cows and dogs), while on humans males are mostly seen over the hair of the head.

**Hourly biting activity** — The captures performed during one whole night showed that for the two predominant species, the peak of hematophagic activity was earlier for *L. migonei* — from 7 to 9 p.m. — than for *L. whitmani* — between 1 and 3 a.m. (Fig.).



Sandfly fauna of Baturité, Ceará State: catching-rates of *Lutzomyia whitmani* and *L. migonei* during the night.

TABLE IV

Sandflies found naturally infected with peripylarian promastigotes in endemic localities of cutaneous leishmaniasis in Baturité municipality, Ceará, Brazil, August-September, 1989

Sandfly species	Caught on human bait			Caught on animal bait			Total		
	Exam.	Positive No.	%	Exam.	Positive No.	%	Exam.	Positive No.	%
<i>L. whitmani</i> <sup>a</sup>	458	4	0.9	435	3	0.7	893	7	0.8
<i>L. migonei</i> <sup>b</sup>	—	—	—	405	1	0.2	405	1	0.2
<i>L. (Psychodopygus) spp.</i>	30	—	—	111	—	—	141	—	—
<i>L. (Psathyromyia) spp.</i>	16	—	—	24	—	—	40	—	—
Total	504	4	0.8	975	4	0.4	1479	8	0.5

a: Pé-do-Candeia: 3 on human bait and 1 on animal bait.

Tijuca: 1 on human bait and 1 on animal bait.

Mondego: 1 on animal bait.

b: Tijuca: 1 on animal bait.

#### Natural infection of sandflies by flagellates

— Table IV gives some results which have already been referred to in previous studies in this endemic area (Azevedo et al., 1990a, b).

#### DISCUSSION

In Baturité there are probably more species of sandflies than the six listed here. In the series *squamiventris* of subgenus *Psychodopygus*, we confirmed the presence of *L. wellcomei* on male morphology, but females of this species are morphologically similar to those of the other species, such as *L. squamiventris*, *L. chagasi*, *L. complexus* and *L. maripaensis* (Fraiha et al., 1986). In our material, we noted differences concerning the number of vertical teeth on the cibarium, length and morphology of individual duct of spermathecae and length of the third antennal segment. It is intended, therefore, to undertake morphometric study in species of the *squamiventris* series in order to better identify them.

*Lutzomyia (P.) wellcomei* was found to be the only species among 450 sandflies caught by Ready et al. (1983), using a man-baited Shannon-trap, in forest in the Serra de Baturité, Ceará, and these authors also suggested that collections of "*L. (P.) squamiventris*" made by Lucena (1953) from human bait in Pacoti, Ceará, were probably *L. (P.) wellcomei*.

The few specimens of the subgenus *Psathyromyia* caught in the present study are at present being examined in greater detail.

In Baturité, among the peridomestic sandflies, we found *L. whitmani* and *L. migonei* to be predominant. Both feed on man but *L. whitmani* does so in much higher numbers. During the night they bite at any hour, but the peak of feeding activity on a mule was found to be during the first half of the night for *L. migonei*, and during the second for *L. whitmani*.

Recently, in this same focus in Baturité, Azevedo et al. (1990a, b) reported the finding of *L. whitmani* naturally infected with a *Leishmania* of the *braziliensis* complex and *L. migonei* harbouring peripylarian flagellates which possibly belong to the same parasite.

Sandfly surveys have been carried out in the past in Ceará State, without incriminating any species as a vector of cutaneous leishmaniasis (Lucena, 1953; Alencar & Sherlock, 1956; Mangabeira, 1940 in Sherlock, 1969). However, previous studies have already reported the occurrence of *L. whitmani* and *L. migonei* in the mountainous regions in the west of Ceará State, indicating that their distribution is coincidental with that of cutaneous leishmaniasis (Deane & Deane, 1955).

The finding of *L. wellcomei* in the forest of the Serra de Baturité (Ready et al., 1983) suggested that this species might be a vector of cutaneous leishmaniasis in such areas of Ceará, as it is in the forests of the Serra dos Carajás, Pará State (Lainson et al., 1973).

The predominance of *L. whitmani* and *L. migonei* and the finding of both naturally infected with promastigotes suggests that these two species may act as periodomestic vectors of cutaneous leishmaniasis in Baturité, where only *L. (Viannia) braziliensis* has been isolated from patients. *L. whitmani* is the more important due to its attraction to humans: *L. migonei* may be a secondary vector.

These species have already been regarded as probable vectors of *L. (V.) braziliensis* in other endemic areas of Brazil: *L. whitmani* in the States of São Paulo, Minas Gerais and Bahia (Pessoa & Coutinho, 1941; Mayrink et al., 1979; Hock et al., 1986), and *L. migonei* in the States of São Paulo and Rio de Janeiro (Pessoa & Pestana, 1940; Araújo-Filho, 1979; Rangel et al., 1986).

As in Baturité, several studies on the ecology of *L. whitmani* in the States of São Paulo, Minas Gerais and Bahia have shown that this species feeds readily on humans and is adapted to peri-domestic areas (Pessoa & Coutinho, 1941; Mayrink et al., 1979; Vexenat et al., 1986). In Amazonian Brazil, however, *L. whitmani* is a silvatic, with little tendency to bite man and shows no inclination to invade human dwellings (Lainson, 1983; Shaw & Lainson, 1987). These ecological differences and habits suggest that either *L. whitmani* has adapted to the new environmental habitats resulting from deforestation or it is a complex of cryptic species (Lainson, 1988; Rangel et al., 1990).

Following observations in the Baturité focus of cutaneous leishmaniasis, Sallenave et al. (1990) reported unusually high infection-rates of *Leishmania (V.) braziliensis* in *L. longipalpis*, *L. whitmani* and *L. migonei*, as detected by "specific" DNA probes. Up till now, the only *Leishmania* recorded from *L. longipalpis* has been *L. (L.) chagasi* (of visceral leishmaniasis), and we feel that the records of these authors should be viewed with some caution. Regrettably we (A. C. R. Azevedo and E. F. Rangel) were included in the authorship of the paper without prior consultation.

#### ACKNOWLEDGEMENTS

To SUCAM (Superintendência de Campanhas de Saúde Pública, Brasil) for the facilities provided during the field work; to Raul G. Queiróz and Carlson P. Cabral for their collab-

oration in the field work; to Dr Antonio W. Vasconcelos for the help provided in the laboratory in the Ceará University; to Dr Leonidas M. Deane for his critical reading of the manuscript.

#### REFERENCES

- ALENCAR, J. E. & SHERLOCK, I., 1956. Fauna flebotômica do Ceará. Apresentado no 13<sup>o</sup> Congresso Brasileiro de Higiene, Fortaleza, mimeografado.
- ARAÚJO FILHO, N. A., 1979. *Epidemiologia da leishmaniose tegumentar na Ilha Grande*. Master Thesis, Universidade Federal do Rio de Janeiro, 144 p.
- AZEVEDO, A. C. R.; RANGEL, E. F.; COSTA, M. E.; DAVID, J.; VASCONCELOS, A. W. & LOPES, U. G., 1990a. Natural infection of *Lutzomyia (Nyssomyia) whitmani* (Antunes & Coutinho, 1939) by *Leishmania* of the *braziliensis* complex in Baturité, Ceará State, Northeast Brazil. *Mem. Inst. Oswaldo Cruz*, 85: 251.
- AZEVEDO, A. C. R.; RANGEL, E. F. & QUEIRÓZ, R. G., 1990b. *Lutzomyia migonei* (França, 1920) naturally infected with peripylarian flagellates in Baturité, a focus of cutaneous leishmaniasis in Ceará State, Brazil. *Mem. Inst. Oswaldo Cruz*, 85: 479.
- BARRETO, M. P., 1943. *Observações sobre a biologia, em condições naturais dos flebotomos do Estado de São Paulo (Diptera, Psychodidae)*. Thesis, Faculdade de Medicina, Universidade São Paulo, 162 p.
- DEANE, L. M., 1956. *Leishmaniose Visceral no Brasil. Estudos sobre reservatórios e transmissores realizados no Estado do Ceará*. Rio de Janeiro. Serviço Nacional de Educação Sanitária. Thesis de Livre-Docência, Fac. Medicina, Universidade São Paulo, 162 p.
- DEANE, L. M. & DEANE, M. P., 1955. Sobre a biologia do "*Phlebotomus longipalpis*" transmissor da leishmaniose visceral, em uma zona endêmica do Estado do Ceará. I. Distribuição, predominância e variação estacional. *Rev. Brasil. Biol.*, 15: 83-95.
- FRAIHA, H.; RYAN, L.; WARD, D. R.; LAINSON, R. & SHAW, J. J., 1986. *Psychodopygus leonidasdeanei* a new species of sandfly (Diptera: Psychodidae) from Pará State, Brazil. *Mem. Inst. Oswaldo Cruz*, 81: 333-339.
- HOCK, A.; RYAN, L.; VEXENAT, J. A.; ROSA, A. C. O. C. & BARRETO, A. C., 1986. Isolation of *Leishmania braziliensis braziliensis* and other trypanosomatids from Phlebotomine in mucocutaneous leishmaniasis endemic area, Bahia, Brazil. *Mem. Inst. Oswaldo Cruz*, 81 (Suppl.): 62.
- LAINSON, R., 1983. The American Leishmaniasis: some observations on their ecology and epidemiology. *Trans. R. Soc. Trop. Med. Hyg.*, 77: 569-596.
- LAINSON, R., 1988. Ecological interactions in the transmission of the leishmaniasis. *Phil. Trans. R. Soc. London B*, 321: 398-404.
- LAINSON, R.; SHAW, J. J.; WARD, R. D. & FRAIHA, H., 1973. Leishmaniasis in Brazil. IX. Considerations on the *Leishmania braziliensis* complex:

- importance of sandflies of the genus *Psychodopygus* (Mangabeira) in the transmission of *L. braziliensis* in north Brazil. *Trans. R. Soc. Trop. Med. Hyg.*, 67: 184-196.
- LUCENA, D. T., 1953. Flebótomos do Nordeste: Morfologia de algumas espécies e sua distribuição geográfica. *Papéis Avulsos*, II: 89-107.
- MANGABEIRA, O., 1940 in SHERLOCK, I., 1969. Sobre a sistemática e biologia dos *Phlebotomus* do Ceará. *Rev. Brasil. Malariol. Doenç. Trop.*, 21: 3-25.
- MARTINS, A. V.; WILLIAMS, P. & FALCÃO, A. L., 1978. *American sandflies (Diptera: Psychodidae, Phlebotominae)*. Rio de Janeiro. Academia Brasileira de Ciências, IV + 195 p.
- MAYRINK, W.; WILLIAMS, P.; COELHO, M. V.; DIAS, M. & MARTINS, A. V., 1979. Epidemiology of dermal leishmaniasis in the Rio Doce Valley, State of Minas Gerais, Brazil. *Ann. Trop. Med. Parasitol.*, 73: 123-137.
- PESSOA, S. B. & COUTINHO, J. O., 1941. Infecção natural e experimental dos flebótomos pela *Leishmania braziliensis* no Estado de São Paulo. *Hospital (R. J.)*, 20: 25-35.
- PESSOA, S. B. & PESTANA, B. R., 1940. Infecção natural do "*Flebotomus migonei*" por formas em leptomonas, provavelmente da *Leishmania braziliensis*". *Acta Médica*, 5: 106-111.
- RANGEL, E. F.; AZEVEDO, A. C. R.; VASCONCELOS, A. W.; ALENCAR, J. E.; LIMA, J. W. O. & DAVID, J., 1989. On the sandfly fauna of a focus of cutaneous leishmaniasis in Aquiráz Municipality, Ceará State, Brazil. *Mem. Inst. Oswaldo Cruz*, 84 (Suppl. II): 131.
- RANGEL, E. F.; LAINSON, R. & SOUZA, A. A., 1990. *Lutzomyia (Nyssomyia) whitmani* (Antunes & Coutinho, 1939) (Diptera, Psychodidae, Phlebotominae), a vector of cutaneous leishmaniasis in Brazil: is it a complex of cryptic species? *Mem. Inst. Oswaldo Cruz*, 85 (Suppl. I): 122.
- RANGEL, E. F.; SOUZA, N. A.; WERMELINGER, E. D.; AZEVEDO, A. C. R.; BARBOSA, A. F. & ANDRADE, C. A., 1986. Flebótomos de Vargem Grande, foco de leishmaniose tegumentar no Estado do Rio de Janeiro. *Mem. Inst. Oswaldo Cruz*, 81: 347-349.
- READY, P. D.; RIBEIRO, A. L.; LAINSON, R.; ALENCAR, J. E. & SHAW, J. J., 1983. Presence of *Psychodopygus wellcomei* (Diptera: Psychodidae), a proven vector of *Leishmania braziliensis braziliensis*, in Ceará State. *Mem. Inst. Oswaldo Cruz*, 78: 235-236.
- SALLENAVE, S. L.; VALIM, C.; DE QUEIRÓZ, R. G.; CABRAL, C. P.; DE SOUZA, R. N.; VASCONCELOS, I. A.; AZEVEDO, A. C. R.; RANGEL, E. & LOPES, U. G., 1990. Specific kinetoplast DNA probes in the detection of *Leishmania* in vertebrate and invertebrate hosts. *Mem. Inst. Oswaldo Cruz*, 85 (Suppl. I): 29.
- SHAW, J. J. & LAINSON, R., 1987. Ecology and Epidemiology: New World p. 292-363. In: W. Peters & R. Killich-Kendrick, (eds) *The Leishmaniasis in Biology and Medicine*, Academic Press, London.
- VEXENAT, J. A.; BARRETTO, A. C.; CUBA, C. C. & MARSDEN, P. D., 1986. Características epidemiológicas da leishmaniose tegumentar americana em uma região endêmica do Estado da Bahia. III. Fauna Flebotomínica. *Mem. Inst. Oswaldo Cruz*, 81: 293-302.