
**EPIDEMIOLOGY AND DISTRIBUTION OF PHLEBOTOMINE
SAND FLIES (DIPTERA: PHLEBOTOMINAE) IN
BLUMENAU, SC, BRAZIL, AN AREA OF TRANSMISSION OF
AMERICAN CUTANEOUS LEISHMANIASIS**

Suelen Cristina Grott¹, Juliane Araújo Greinert-Goulart², Camila Mafalda Rodrigues², Mário Steinde³, Marcelo Schaefer⁴ and Carlos Brisola Marcondes³.

ABSTRACT

Since 259 cases of cutaneous leishmaniasis were recorded in the year 2005 in the city of Blumenau, in the southern state of Santa Catarina, Brazil. In order to obtain more information on the transmission cycle of the disease in this city, this study aimed to conduct a survey of patients affected by American Cutaneous Leishmaniasis (ACL) and to evaluate and characterize the sand fly fauna in the city. A questionnaire was completed by the patient or guardian, containing eleven questions about their health, housing and its surroundings, proximity of the forest and presence of domestic and wild animals when confirmed cases of ACL were found. Collections of sand flies were performed in 14 residences divided between four districts (Badenfurt, Central Itoupava, Old and Progress). These districts were chosen because they showed the highest number of cases. The insects were collected for three consecutive nights per month, from April to October 2008, using CDC traps. From the households selected for the survey, fifteen patients were identified, 53.3% female and 46.6% male. All residences were within 200 m of the forest, 85.7% reported the presence of wildlife near homes and 72.2% reported having a domestic animal. Two hundred and seventy-five specimens from eight species were obtained. *Nyssomyia neivai* (42.9%) and *Pintomyia fischeri* (38.2%) were the most prevalent species.

KEY WORDS: Diptera; psychodidae; *Leishmania*; Blumenau; vectors.

-
- 1 Mestranda Programa de Pós Graduação em Engenharia Ambiental da Universidade Regional de Blumenau (FURB), Blumenau, SC, Brazil.
 - 2 Departamento de Ciências Naturais of FURB, Blumenau, SC, Brazil.
 - 3 Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil.
 - 4 Secretaria Municipal de Saúde, Blumenau, SC, Brazil.

Corresponding author: Suelen Cristina Grott, Universidade Regional de Blumenau, Rua Antônio da Veiga, 140. CEP 89012-900, Blumenau, Santa Catarina, Brazil. E-mail: suelengt@bol.com.br

Received for publication: 13/11/2014. Reviewed: 11/12/2014. Accepted: 13/12/2014.

RESUMO

Epidemiologia e distribuição de flebotomíneos (Diptera: Phlebotominae) em áreas de transmissão da leishmaniose tegumentar americana – Blumenau-SC, Brasil.

Em 2005, 259 casos de leishmaniose tegumentar americana (LTA) foram registrados no município de Blumenau, sul do estado de Santa Catarina. Visando à obtenção de maiores informações sobre o ciclo de transmissão da doença no município, o objetivo deste estudo foi realizar um levantamento dos pacientes afetados por LTA, avaliar e caracterizar a fauna de flebotomíneos na cidade. Nas residências com casos confirmados de LTA, aplicou-se um questionário ao paciente ou responsável contendo 11 perguntas sobre as condições sanitárias da residência, seu entorno, proximidade da floresta e presença de animais domésticos e silvestres. As coletas dos flebotomíneos foram realizadas em 14 residências distribuídas em quatro bairros (Badenfurt, Itoupava Central, Velha e Progresso). Os bairros foram escolhidos por apresentarem o maior número de casos da doença. Os insetos foram coletados durante três noites consecutivas por mês, de abril a outubro de 2008, usando-se armadilhas CDC. Dos domicílios selecionados para a pesquisa, 15 pacientes foram identificados, sendo 53,3% do sexo feminino e 46,6% do sexo masculino. Todas as residências estavam a menos de 200 m da floresta, 85,7% relataram a presença de animais selvagens perto das casas e 72,2% relataram ter algum animal doméstico. Foram obtidos 175 exemplares de 8 espécies, sendo *Nyssomyia neivai* (42,9%) e *Pintomyia fischeri* (38,2%) as prevalentes.

DESCRITORES: Diptera; psychodidae; *Leishmania*; Blumenau; Vetores.

INTRODUCTION

American Cutaneous Leishmaniasis (ACL) is a parasitic infectious disease that affects humans and is caused by several species of protozoa of the genus *Leishmania* (20). It is an anthroponosis with natural foci in which the parasite is maintained in wild ecotopes between vectors and their vertebrate hosts (21). Cutaneous leishmaniasis constitutes a public health problem in 88 countries spread across four continents (Americas, Europe, Africa and Asia), with annual incidence from 1 to 1.5 million cases. It is considered by the World Health Organization (WHO) to be one of the six most important infectious diseases, given its high detection rate and ability to produce deformities. It is estimated that 350 million people are at risk with an estimated two million new cases of different clinical forms per year (15).

Until the 1980s, nineteen states in Brazil had reported cases of ACL, whereas by 2003, all twenty-seven Brazilian states presented autochthonous ACL transmission, demonstrating continued geographical expansion (16). Brazil is one of seven countries recording 90% of cutaneous leishmaniasis worldwide and Brazil reports an average of 28,000 cases per year (16).

In the context of public health, ACL is significantly increasing its importance due to increasing urbanization (3). Initially considered a zoonosis of wild animals which occasionally affected people in close proximity to forests, ACL began to occur in deforested rural and peri-urban regions (24). The transformations in the environment, caused by intense migration, the process of increasing urbanization and

socioeconomic pressures lead to the expansion of endemic areas and the emergence of new foci (5). Therefore, sand fly species, which somehow resist adverse conditions, are able to explore new environments, approaching the peridomestic environment more and more, and thus facilitating disease transmission (14).

The sand fly fauna in Santa Catarina is still poorly understood and the first human cases of ACL were recorded in the counties of Quilombo and Coronel Freitas, in the West region in 1987 (23). From 1990 to 1996, 62 imported cases were reported in the State of Santa Catarina (8). From 1997 several cases were being registered every year in the municipality of Piçarras, and in the Vale do Itajaí, and since then the area has been considered at risk for ACL transmission (13).

Since 2001, ACL has been considered an emerging disease in the state of Santa Catarina with 401 autochthonous cases reported between 2001 and 2009 (12). The first case of ACL in the city of Blumenau was reported in September 2005 and since then an increasing number of new cases has arisen in the municipality reaching 259 patients in 2008 according the Secretaria Municipal de Saúde. From 2009 onwards the annual incidence began to decline in the municipality, and in 2014 no cases were reported (2).

The aim of this study was to evaluate and characterize the specific sand fly fauna potentially involved in the transmission of ACL in the city of Blumenau, Santa Catarina. Except for studies on sand flies in a municipality in the East of the state (9, 10), no study in this part of the state has yet been conducted.

MATERIAL AND METHODS

Municipality description

The city of Blumenau, occupies 520 km² of the coastal plain of southern Brazil, northeastern state of Santa Catarina, with an average altitude of 21 m. The urban area occupies 36.5% of the city, compared to 63.5% considered rural, with irregular topography (19). The town is embedded in the Itajaí-Açú river basin, along with 53 other counties (Figure 1).

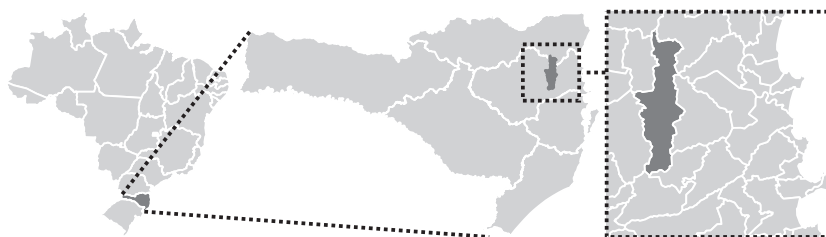


Figure 1. Map of the State of Santa Catarina and the location of the study area, the city of Blumenau (Source: adapted: <http://www.mapainterativo.ciasc.gov.br>).

The climate is classified as moist subtropical, for all seasons, characterized by hot and rainy summers with high humidity. The seasons are well defined, with drier winters. The remaining original vegetation is classified as Tropical Rain Forest or Tropical Atlantic Rainforest.

Delineation of the study area

The choice of residences for trap placement was made in conjunction with the Blumenau Municipal Health Department. According the Secretaria Municipal de Saúde a total of 259 cases of ACL has been reported. After surveying these data, health workers and researchers conducted joint visits to all homes with reported cases of ACL. A questionnaire containing eleven closed questions was applied to the owner of the residence or the leishmaniasis patient to assess the conditions of the houses as well as their surroundings, such as the open sewer, garbage collection, presence of domestic animals (dogs, cats, horses etc.), proximity of the forest (0-400 m or more), problems and/or disturbance by mosquitoes biting and the characteristics of neighboring houses. Such information was necessary to identify the main characteristics of the local transmission of the disease in the city. The results obtained from the questionnaires were used to select the sampling sites.

Capture of Phlebotomines

Fourteen residences were used for sand fly collection: four in Badenfurt, four in Itoupava Central, two in Velha and four in Progresso. Samples were collected during the months of April, June, August and October 2008. Each week two residences in distinctive neighborhoods, were used as the collecting hub, totaling eight residences per month. Collections were intradomiciliary, peridomiciliary, from the forest edge, primary forest or secondary forest (about 40 meters from the forest edge). CDC traps were set up in buildings used as animal shelters: sties, kennels, chicken coops and pens. The traps were set up 1 meter above the ground, at 6:00 p.m. and always retrieved the following morning at 06:00 a.m. Traps were placed randomly without visible food source for insects at the forest edge and inside the forest. The traps were installed for three consecutive days at each collection point per month.

Insects captured were transported to the Parasitology Laboratory of the Regional University of Blumenau, stored in the freezer and subsequently screened under stereomicroscope for separation of sand flies. The screened specimens were mounted between slides and cover slips and identified according to the Galati classification(6); genera were abbreviated as proposed by Marcondes (9).

RESULTS

Analysis of the questionnaires

Thirty-eight questionnaires were applied by officials of the health department to patients or family members of people who had had ACL in the municipality of Blumenau/SC. In the 14 residences chosen for insect capture, 15 patients had been reported. Of these, eight (53.3%) were male and seven (46.6%) female. All were older than 15 years (Table 1).

Regarding the presence of pets in the chosen homes, all owners reported owning animals. Of these, 72.2% reported having a dog as a pet, followed by birds 14.8%, cats 11.1% and rabbits 1.8%. As for livestock, 28.5% of the respondents had reared animals, with chickens (40%) and ducks/geese (40%) the most frequent, followed by cattle, horses and pigs. The owners also reported the presence of wild animals in their homes or nearby, and the most cited were opossums (27.9%) and rats (18.6%). Armadillos, pacas, capybaras, agoutis, monkeys, bats, lizards and snakes were also cited. Of these, 85.7% had been sighted near the residences.

Table 1. Gender and age of ACL patients surveyed in Blumenau, SC.

Age (years)	Gender			Total	(%)
	Male	Female			
From 0 to 14	0	0		0	0
From 15 to 29	1	2		3	20.0
From 30 to 49	2	5		7	46.6
≥ 50	4	0		4	26.6
ND*	1	-		1	6.6
TOTAL	8	7		15	100

*ND: not determined. Source: Municipal Health Department (2008).

All residences were analyzed according to waste sorting and proximity to forest (less than 200 meters). Due to the proximity of the forest, 85.7% of residents reported being uncomfortable with “mosquitoes”, and the most common way of avoiding such nuisances was by using repellent (66.6%) and screening of windows (25%). No residence presented an open sewer.

Fruit trees, bamboo groves and other trees, and shrub vegetation were present at 71.4 % of the houses. These plantations contribute to the increase of organic matter in the area, which is suitable for the development of immature forms of sand flies (1).

Analysis of phlebotomines

From April to October 2008, the collection of sand flies resulted in the capture of 275 individuals from eight species, of which 113 (41.0%) were males and

162 (58.9%) females (Table 2). *Nyssomyia neivai* proved to be the most abundant species in different capture sites, followed by *Pi. fischeri*, but 96.2 % of the species were concentrated in Progresso (Table 3). At this point, samples were collected in the Private Reserve of Natural Patrimony - RPPN Bugerkopf (27° 00' S - 49° 04' W), which has 82.7 hectares and is located 12 km from Blumenau city center.

Progresso neighborhood also stood out with individuals of all eight species identified in the study. *Nyssomyia neivai* was the only species common to all neighborhoods, thereby indicating its possible role as a vector of ACL. The greatest quantity of sand flies were captured within the forest (29.8%), followed by the edge of the forest (22.1%) and animal shelters (19.2%).

Table 2. Phlebotomine sand flies collected in the municipality of Blumenau, SC, by species, sex and place of installation of the trap (April-October 2008).

Environment/ ecotopes	1*		2*		3*		4*		5*		Total	
Species / sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	Total	(%)
<i>Migonemyia migonei</i>	1	-	2	-	-	-	-	-	-	-	3	1.0
<i>Pintomyia fischeri</i>	6	8	1	8	5	16	4	23	6	28	105	38.1
<i>Evandromyia edwardsi</i>	-	2	-	-	1	-	-	-	-	6	9	3.2
<i>Lutzomyia sp.</i>	3	1	-	1	2	2	-	2	-	-	11	4.0
<i>Nyssomyia neivai</i>	18	10	9	6	17	12	12	6	20	8	118	42.9
<i>Psychodopygus ayrozai</i>	1	-	-	-	-	-	-	-	-	1	2	0.7
<i>Psathyromyia lanei</i>	-	-	-	-	2	1	2	2	8	1	16	5.8
<i>Psathyromyia pascalei</i>	-	-	-	-	-	1	-	-	2	-	3	1.0
<i>Psathyromyia shannoni</i>	1	1	-	-	1	1	-	2	1	1	8	2.9
No. individuals (%)	52 (18.9%)		27 (9.8%)		61 (22.1)		53 (19.2%)		82 (29.8%)			

*Ecotopes where the traps were installed: (1) Intradomicile; (2) Peridomicile; (3) Forest edge; (4) Animal shelter; (5) Inside the forest.

Table 3. Distribution of phlebotomine sand fly species listed by neighborhoods in the city of Blumenau, SC, from April to October 2008.

Districts	Progresso		Velha		Itoupava Central		Badenfurt		TOTAL	
Species	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
<i>Nyssomyia neivai</i>	39	36	14	4	10	6	5	4	68	50=118
<i>Pintomyia fischeri</i>	18	83	-	3	-	1	-	-	18	87=105
<i>Psychodopygus ayrozai</i>	-	2	-	-	-	-	-	-	-	2
<i>Evandromyia edwardsi</i>	1	7	-	1	-	-	-	-	1	8=9
<i>Psathyromyia lanei</i>	12	2	-	-	-	1	-	-	12	4=16
<i>Migonemyia migonei</i>	2	-	-	-	-	-	1	-	3	=3
<i>Psathyromyia shannoni</i>	3	5	-	-	-	-	-	-	3	5=8
<i>Psathyromyia pascalei</i>	2	1	-	-	-	-	-	-	2	1=3
<i>Lutzomyia sp.</i>	3	1	1	1	1	1	1	2	6	5=11
Total	217 (78.9%)		24 (8.7%)		20 (7.2%)		14 (5.0%)		275	

DISCUSSION

In Brazil ACL affects both sexes and all age groups, although predominantly among those >10 years old, representing 90% of the cases, and among males (74%) (16). These data corroborate the information obtained for all patients in Blumenau up to 2008, in which 89% of the reported patients were older than 15 years old, 10% aged under 14 years, 16.2% from 15 to 29 years old, 34.7% from 30 to 49, 23.9% 50 years or above and 15.0% of indeterminate age. Of the patients, 57.9% were male and 42.0% female (22). A more thorough study of population habits could provide more information on the site of transmission.

The presence of domestic and wild animals near the residences increases the risk of transmission of ACL to humans and domestic animals. In a study in the municipality of Doutor Camargo (PR), in which the influence of cleaning around houses was analyzed, the number of sand flies captured decreased by almost half after modifications to the environment, reducing shadow and vegetation and uncovering the soil (25). This emphasizes the importance of cleanliness of environments surrounding residences.

The occurrence of sand fly species in urban areas is due to the ability of these insects to adapt to profound changes in their natural habitats causing restriction of their ecological areas (26). The city of Blumenau, like many others, has a large number of residences in irregular areas, extending deforestation, and thus the invasion of houses by sand flies. In these places, domestic animal shelters are frequent and near both the residential and the forest areas. Raising animals for consumption, which is also common, changes the local vegetation, creating conditions for the sand fly species found in forested areas to reach the residences in search of food. The transmission of ACL depends on the exposure of humans and domestic animals to sand flies (7).

Given the predominance of *Ny. neivai* in all environments in this study, and its previous natural infection by *L. braziliensis* in the neighboring municipality of Piçarras (11), and in the states of Rio Grande do Sul (18) and Paraná (17), in addition to infection by *L. chagasi* in Florianópolis, Santa Catarina (4), the species is considered suspect and should be carefully analyzed. It is the only species found in all locations, enhancing its possible role in transmission.

Precarious housing hygiene, presence of moisture, soil and organic matter (leaves, fruit, pet feces, food and vegetable waste), besides animal shelters and proximity to the remaining forest were noticed in most houses where the sand fly collections occurred. These factors are crucial for influencing the quantity of sand flies that invade homes, increasing the risk of the inhabitants being infected with ACL.

CONCLUSION

Several sand fly species, some of them previously found infected by species of *Leishmania*, were found near and in houses of cutaneous leishmaniasis

patients in Blumenau. *N. neivai*, due to predominance and presence in all localities, seems to be the most suspect species, but other species must also be carefully analyzed.

ACKNOWLEDGEMENTS

To the Vigilância Sanitária and Epidemiológica of Blumenau/ SC, for their support in obtaining epidemiological data travel during the collections and the application of questionnaires in houses of the patients.

REFERENCES

1. Aguiar GM de, Medeiros WM de. Distribuição e habitats. In: Rangel EF; Lainson R. (org.). *Flebotomíneos do Brasil*. Fiocruz, Rio de Janeiro, 2003. p.207-255.
2. DATASUS. Informações epidemiológicas e morbidade. Disponível em: <http://www2.datasus.gov.br/DATASUS/index.php?area=0203>. Acesso em: 15 jan. 2014.
3. Dias ES, França-Silva JC, Silva EM, Monteiro MP, Kenia CM, Barata RA. Flebotomíneos (Diptera: Psychodidae) de um foco de leishmaniose tegumentar no Estado de Minas Gerais. *Rev Soc Bras Med Trop* 40: 49-52, 2007.
4. Dias SE. Detecção de infecção por *Leishmania* spp. em flebotomíneos coletados na cidade de Florianópolis, SC. Relatório Técnico-Científico, Centro de Referência em Competência Vetorial de Flebotomíneos, Laboratório de Leishmanioses/CPqRR/Fiocruz, Belo Horizonte, MG., 2010. 6 p.
5. Fundação Nacional de Saúde. *Manual de Controle da Leishmaniose Tegumentar Americana*. 5ª edição. Ministério da Saúde, Brasília, 2000.
6. Galati EAB. Morfologia e taxonomia. In: Rangel EF, Lainson R. *Flebotomíneos do Brasil*. Fiocruz, Rio de Janeiro, 2003.
7. Guerra JAO, Ribeiro JAS, Coelho LIARC, Barbosa MG, Paes GP. Epidemiologia da Leishmaniose Tegumentar na comunidade São João, Manaus, Amazonas, Brasil. *Cad Saúde Pública* 22: 2319-2327, 2006.
8. Lima Filho JHC, Steindel M. Aspectos clínicos e Epidemiológicos da Leishmaniose Cutânea no Estado de Santa Catarina. *Arq Catarinense de Medicina* 27: 25-31, 1998.
9. Marcondes CB. A proposal of generic and subgeneric abbreviations for phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae) of the world. *Entomol News* 118: 351-356, 2007.
10. Marcondes CB, Conceição MBE, Portes MGT, Simão BP. Phlebotomine sand flies in a focus of dermal leishmaniasis in the eastern region of the Brazilian State of Santa Catarina - preliminary results (Diptera: Psychodidae). *Rev Soc Bras Med Trop* 38: 353-355, 2005.
11. Marcondes CB, Bittencourt IA, Stoco PH, Eger I, Grisard EC, Steindel M. Natural infection of *Nyssomyia neivai* (Pinto, 1926) (Diptera: Psychodidae, Phlebotominae) by *Leishmania* (Viannia) spp. in Brazil. *Trans R Soc Trop Med Hyg* 103: 1093-1097, 2009.
12. Marlow MA, Da Silva Mattos M, Makowiecky ME, Eger I, Rossetto, Grisard CE; Steindel M. Divergent Profile of Emerging Cutaneous Leishmaniasis in Subtropical Brazil: New Endemic Areas in the Southern Frontier. *PLoS ONE* 8: e56177. doi:10.1371/journal.pone.0056177, 2013.
13. Makowiecki ME, Makowiecki YM. Aspectos clínicos e epidemiológicos dos casos de leishmaniose tegumentar americana diagnosticada em Santa Catarina. Anais do XLI Congresso da Sociedade Brasileira de medicina Tropical. Florianópolis. *Rev Soc Bras Med Trop* 38: 336, 2005.
14. Marzochi MCA, Marzochi KBF. Tegumentary and visceral leishmaniasis in Brazil. Emerging anthroozoonosis and possibilities for their control. *Cad Saúde Pública* 10: 359-375, 1994.
15. Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica. *Manual de vigilância da leishmaniose tegumentar americana*. 2. ed. Editora do Ministério da Saúde, Brasília, 2005.

16. Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica. *Manual de vigilância da leishmaniose tegumentar americana*. Editora do Ministério da Saúde, Brasília, 2007.
17. Oliveira DM, Reinhold-Castro KR, Bernal MVZ, Legriffon CM de O, Lonardon MVC, Teodoro U, Silveira TVG. Natural Infection of *Nyssomyia neivai* by *Leishmania* (*Viannia*) spp. in the State of Parana, Southern Brazil, Detected by Multiplex Polymerase Chain Reaction. *Vector Borne Zoonotic Dis II*: 137-143, 2011.
18. Pita-Pereira D, Alves CR, Souza MB, Brazil RP, Bertho AL, Barbosa AF, Britto CC. Identifications of naturally infected *Lutzomyia intermedia* and *Lutzomyia migonei* with *Leishmania* (*Viannia*) *braziliensis* in Rio de Janeiro (Brazil) revealed by a PCR multiplex non-isotopic hybridization assay. *Acta Trop* 99: 905-913, 2005.
19. Prefeitura Municipal de Blumenau. Disponível em: <http://www.blumenau.sc.gov.br>. Acesso em 05/01/2014.
20. Ross R. Further Notes on *Leishmania*'s bodies. *Br Med J II*: 1401, 1903.
21. Reis SR, Gomes LHM, Ferreira NM, Nery L da R, Pinheiro FG, Figueira L de P, Soares FV, Franco AMR. Ocorrência de flebotomíneos (Diptera: Psychodidae: Phlebotominae) no ambiente peridomiciliar em área de foco de transmissão de leishmaniose tegumentar no município de Manaus, Amazonas. *Acta Amaz* 43: 121-124, 2013.
22. Santa Catarina. Secretaria de Estado da Saúde. Diretoria de Vigilância Epidemiológica (SES/DIVE). Gerência de Controle de Zoonoses. Divisão de Vigilância de Vetores, Reservatórios e Outros. Programa de Vigilância da Leishmaniose Tegumentar Americana. Florianópolis, 2006/2008.
23. São Thiago PT, Guida U. Leishmaniose Tegumentar no oeste de Santa Catarina: (Brasil). *Rev Soc Bras Med Trop* 23: 201-203, 1990
24. Saúde Brasil – Uma análise da situação da saúde. Ministério da Saúde Brasília / DF, 2004.
25. Teodoro U, Silveira TG, Santos DR. Influência da reorganização, da limpeza do peridomicílio e da desinsetização de edificações na densidade populacional de flebotomíneos, no município de Doutor Camargo, Estado do Paraná, Brasil. *Cad Saúde Pública* 19: 1801-1813, 2003.
26. Ximenes MFFM, Silva VPM, Queiroz PVS, Rego MM, Cortez AM, Batista LMM. Flebotomíneos (Diptera: Psychodidae) e Leishmanioses no Rio Grande do Norte, Nordeste do Brasil - Reflexos do Ambiente Antrópico. *Neotrop Entomol* 36: 128-137, 2007.