

Phlebotomine sand flies (Diptera, Psychodidae) in an American tegumentary leishmaniasis transmission area in northern Espírito Santo State, Brazil

Fauna de flebotomíneos (Diptera, Psychodidae) em área de transmissão de leishmaniose tegumentar americana no norte do Estado do Espírito Santo, Brasil

Thieres Marassati das Virgens ¹
 Claudiney Biral dos Santos ²
 Israel de Souza Pinto ¹
 Kleber Silveira da Silva ¹
 Fernanda Cristina Leal ¹
 Aloísio Falqueto ¹

¹ Unidade de Medicina Tropical, Universidade Federal do Espírito Santo, Vitória, Brasil.

² Núcleo de Entomologia, Secretaria de Saúde do Estado do Espírito Santo, Vitória, Brasil.

Correspondência

T. M. Virgens
 Unidade de Medicina Tropical, Universidade Federal do Espírito Santo, Av. Marechal Campos 1468, Vitória, ES 29040-090, Brasil.
 thieres@faceli.edu.br
 deagrizzi@hotmail.com

Abstract

American tegumentary leishmaniasis is endemic to the Espírito Santo State, Brazil, where it is widely distributed. The composition of the phlebotomine sand fly fauna in an American tegumentary leishmaniasis focus was determined by monthly sampling, using Shannon light traps in an Atlantic Forest reserve and adjacent habitat that had been modified by human activity. Seasonal fluctuations in numbers of the most abundant species were also monitored from June 2004 to May 2006. Of the 6,176 specimens collected, 47.4% were captured in the forest and 52.6% in the disturbed habitat. Although *Lutzomyia davisi* (60.8%) predominated in specimens from the forest, those captured near human dwellings consisted almost entirely of *Lu. choti* (72%) and *Lu. intermedia* (24.3%). All three species occurred throughout the year. Based on our findings, *Lu. intermedia* probably acts as the principal domestic *Leishmania vector* in the study area.

Leishmaniasis; Psychodidae; Vector Ecology

Introduction

American tegumentary leishmaniasis is considered one of the six most important parasitic infectious diseases worldwide (World Health Organization. <http://www.who.int>, accessed on 10/Oct/2006). The disease constitutes a major public health problem in Brazil, where it occurs in all 26 States ¹. American tegumentary leishmaniasis is endemic to the State of Espírito Santo, where it is widely distributed, threatening a significant proportion of the rural population ^{2,3,4}.

Northern Espírito Santo was covered by Atlantic Forest until the mid-20th century, and American tegumentary leishmaniasis does not appear to have constituted a serious public health problem for the original settlers. Furthermore, there are no records of cases of mucocutaneous leishmaniasis resulting from old infections with *Leishmania braziliensis* in the region.

Beginning in the 1980s, the suspension of insecticide use for malaria control may have favored invasion of houses by sand flies and coincided with the appearance of the first American tegumentary leishmaniasis cases in northern Espírito Santo. The study area was selected in order to obtain information to help elucidate the American tegumentary leishmaniasis epidemiological profile in the region, where human cases have been observed for about 20 years. A total of 2,132 cases were reported from 1986 to 2003 ⁵.

The phlebotomine sand fly *Lutzomyia intermedia* (Lutz & Neiva, 1912) predominates in peridomiciliary captures from old established rural communities. This species has been incriminated as the main vector of *L. braziliensis* in Southeast Brazil^{6,7,8}, although *Lu. whitmani* (Antunes & Coutinho, 1939) and *Lu. migonei* (França, 1920) are also frequently found biting humans in the peridomicile⁹.

The lack of studies on the phlebotomine sand fly fauna and potential *Leishmania* vectors in northern Espírito Santo motivated this study, the objective of which was to determine the composition of the phlebotomine sand fly fauna in an Atlantic Forest reserve and adjacent habitat disturbed by human activity. Seasonal population fluctuations of the main species in both environments were also monitored, providing information to help clarify the sylvatic and domiciliary American tegumentary leishmaniasis transmission cycles of *Leishmania* in the study area.

Material and methods

Study area

Two distinct habitats were chosen for sampling:

- Environment I: forest in the Sooretama Biological Reserve (18°33'-19°05'S; 39°55'-40°15'W), comprising an area of approximately 24,000ha, bordered by the municipalities (counties) of Linhares, Sooretama, Jaguaré, and Vila Valério. The principal vegetation type is Atlantic Rainforest, harboring a fauna that is similar in many ways to that of the Amazon region, as well as large numbers of endemic species¹⁰. The climate is hot, humid, and tropical, with a mean annual temperature of 23°C. Together with the Vale do Rio Doce Reserve, this area constitutes the largest remnant of the Atlantic Forest in the State of Espírito Santo¹⁰.
- Environment II: the disturbed habitat surrounding human dwellings in Jurama (18°59'S; 40°15'W), a rural area in the municipality of Vila Valério (Figure 1). This locality is situated at least 800m from the reserve and has steep hillsides largely occupied by coffee plantations and pasture. Most of the houses are rustic and surrounded by pigsties, henhouses, and cowsheds.

Phlebotomine sand fly captures

Modified Shannon light traps¹¹ were installed within the forest and peridomicile. Monthly sampling was performed simultaneously in the two habitats during the first 3 hours after sunset,

from June 2004 to May 2006. In total, 72 hours of sampling per trap were performed in each habitat.

Mounting and identification of phlebotomine sand flies

Specimens were mounted using the Barreto & Coutinho technique¹² and identified as to species according to the Young & Duncan taxonomic criteria¹³. Specimens whose taxonomic characters were damaged or obscured were identified to the generic level. Sample material was deposited in the entomology collection of the Department of Tropical Medicine at the Federal University in Espírito Santo.

Climate data

Climate data (mean temperature, rainfall, and relative humidity) were obtained from the weather station of the National Institute of Meteorology located in the Espírito Santo State Institute for Research, Technical Assistance, and Rural Extension (INCAPER) in Linhares municipality, approximately 30km from the study area.

Statistical analysis

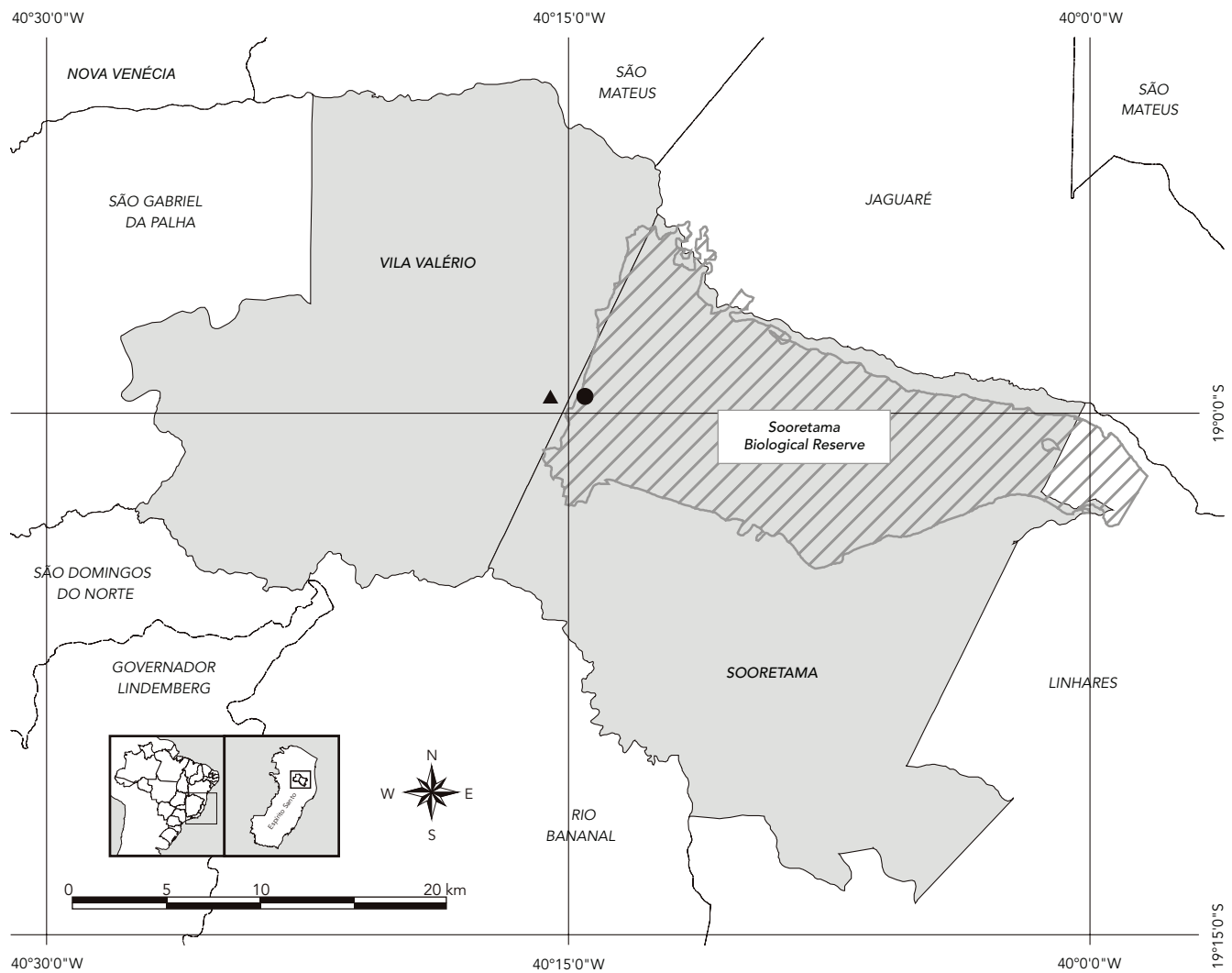
Spearman's non-parametric test was used to determine the correlation between the climate data and the most abundant species in the different habitats. The Mann-Whitney test was used to verify differences between numbers of specimens in the most abundant species collected in the two habitats. Differences were considered significant at $p < 0.05$.

Results

A total of 6,176 specimens from genera *Brumptomyia* and *Lutzomyia* were captured during the study. The species captured were: *B. figueiredoi* Mangabeira & Sherlock, 1961; *Lu. ayrozai* (Barretto & Coutinho, 1940); *Lu. callipyga* Martins & Silva, 1965; *Lu. choti* (Floch & Abonnenc, 1941); *Lu. davisii* (Root, 1934); *Lu. ferreirana* (Barreto, Martins & Pellegrino, 1956); *Lu. fischeri* (Pinto, 1926); *Lu. intermedia* (Lutz & Neiva, 1912); *Lu. lanei* (Barreto & Coutinho, 1941); *Lu. lenti* (Mangabeira, 1938); *Lu. lutziana* (Costa Lima, 1932); *Lu. matosi* (Barretto & Zago, 1956); *Lu. migonei* (França, 1920); *Lu. pascalei* (Coutinho & Barretto, 1940); *Lu. pellowi* (Sherlock & Alencar, 1959); *Lu. pestanaei* (Barretto & Coutinho, 1941); *Lu. quinquefer* (Dyar, 1929); *Lu. schreiberi* (Martins, Falção & Silva, 1975); *Lu. shannoni* (Dyar, 1929), and

Figure 1

Geographic locations of study area and sand fly capture points in the municipalities of Sooretama (●: forest habitat) and Vila Valério (▲: disturbed habitat), Espírito Santo State, Brazil.



Source: Espírito Santo State Integrated System of Georeferenced Databases (GEOBASES).

Lu. yuilli yuilli Young & Porter, 1972. In all, 2,925 (47.4%) of the phlebotomines were captured in the forest and 3,251 (52.6%) from the adjacent disturbed habitat. More species ($n = 16$) were found in the forest.

When numbers from the two habitats were pooled, the most abundant species was *Lu. choti*, with 3,341 specimens (54.1% of the total), or 29.9% in the forest and 70.1% in the disturbed habitat. The second most abundant species was *Lu. davisii*, with 1,806 specimens (29.2%), almost

exclusively (98.5%) from the forest, followed by *Lu. intermedia*, with 804 specimens (13%) almost exclusively (98.1%) from the disturbed habitat.

There was no significant difference in the number of *Lu. choti* specimens captured in the forest and the disturbed habitats. However, significantly more *Lu. davisii* specimens were captured in the forest and more *Lu. intermedia* in the disturbed habitat ($p < 0.05$).

The predominant species in the forest were *Lu. davisii* (60.8%) and *Lu. choti* (34.1%). The

male/female ratios for these two species were approximately 1:4 and 1:1, respectively (Table 1).

Two species predominated in the disturbed habitat: *Lu. choti* and *Lu. intermedia* (72% and 24.3%, respectively). Males predominated in both species (approximately 2:1 for *Lu. choti* and 3:1 for *Lu. intermedia*) (Table 2).

In the forest, *Lu. davisi* was most abundant in the months of April, May, and June 2005, while *Lu. choti* reached its peak in October 2004 and February and May 2006. In the disturbed habitat, *Lu. choti* showed clear peaks in March and April 2005 and March and May 2006, while *Lu. intermedia* was most abundant in December 2004. Figures 2 and 3 show the monthly distribution of the three main species in the two habitats, together with mean monthly relative humidity and temperature. Statistical analysis did not show any significant correlation between monthly rainfall or temperature and seasonal patterns of abundance of any of these species in either habitat (Figures 4 and 5).

When mean monthly relative humidity was correlated with numbers of the most abundant

species in the different habitats, significant negative values were obtained for *Lu. choti* in the forest ($R = -0.50$, $P = 0.01$) and for *Lu. intermedia* in the disturbed habitat ($R = -0.52$, $P = 0.00$). A significant positive correlation was found for *Lu. davisi* in the forest ($R = 0.42$, $P = 0.03$), while there was no correlation in the disturbed habitat for *Lu. choti* ($R = 0.03$, $P = 0.88$).

Discussion

The high frequency of phlebotomine sand flies in the disturbed habitat confirms observations by other authors in different regions of Brazil^{2,14,15,16,17}. In Espírito Santo, Barros et al.² suggested that the construction of human dwellings in the middle of banana groves creates a favorable environment for these insects by providing both shelter and abundant blood meal sources. However, the current study showed a greater wealth of species in the forest, probably due to the greater variety of resting sites provided by plant cover.

Table 1

Relative numbers of males and females for sand fly species collected using Shannon traps in forest in the Municipality of Sooretama, Espírito Santo State, Brazil, June 2004 to May 2006.

Species	Male	Female	Total	%
<i>B. figueiredoi</i>	2	-	2	0.07
<i>Brumptomyia</i> sp. *	-	1	1	0.03
<i>Lu. ayrozai</i>	2	2	4	0.14
<i>Lu. callipyga</i>	5	-	5	0.17
<i>Lu. choti</i>	426	573	999	34.15
<i>Lu. davisi</i>	350	1,429	1,779	60.82
<i>Lu. ferreirana</i>	-	2	2	0.07
<i>Lu. fischeri</i>	6	33	39	1.33
<i>Lu. intermedia</i>	10	5	15	0.51
<i>Lu. lutziana</i>	13	-	13	0.45
<i>Lu. matosi</i>	1	-	1	0.03
<i>Lu. migonei</i>	2	4	6	0.21
<i>Lu. pascalei</i>	2	-	2	0.07
<i>Lu. quinquefer</i>	-	4	4	0.14
<i>Lu. schreiberi</i>	16	8	24	0.82
<i>Lu. shannoni</i>	3	2	5	0.17
<i>Lu. yuilli yuilli</i>	-	1	1	0.03
<i>Lutzomyia</i> spp. **	1	22	23	0.79
Total	839	2,086	2,925	100.00

* *Brumptomyia* sp. probably refers to *B. avellari* (Costa Lima, 1932), *B. cunhai* (Mangabeira, 1942), or *B. nitzulescui* (Costa Lima, 1932);

** *Lutzomyia* spp. refers to females or damaged males of *Lu. callipyga* Martins & Silva, 1965, *Lu. costalimai* (Mangabeira, 1942), or *Lu. tupynambai* Mangabeira, 1948.

Table 2

Relative numbers of males and females for sand fly species collected using Shannon traps in the disturbed habitat in the Municipality of Vila Valério, Espírito Santo State, Brazil, June 2004 to May 2006.

Species	Male	Female	Total	%
<i>Lu. choti</i>	1,655	687	2,342	72.03
<i>Lu. davis</i>	12	15	27	0.83
<i>Lu. ferreirana</i>	1	-	1	0.03
<i>Lu. fischeri</i>	20	17	37	1.14
<i>Lu. intermedia</i>	607	182	789	24.30
<i>Lu. lanei</i>	-	1	1	0.03
<i>Lu. lenti</i>	-	1	1	0.03
<i>Lu. lutziana</i>	-	1	1	0.03
<i>Lu. migonei</i>	30	7	37	1.13
<i>Lu. pelsoni</i>	3	-	3	0.09
<i>Lu. pestanai</i>	-	2	2	0.06
<i>Lu. schreiberi</i>	3	3	6	0.18
<i>Lu. shannoni</i>	2	1	3	0.09
<i>Lu. yuilli yuilli</i>	-	1	1	0.03
Total	2,333	918	3,251	100.00

Figure 2

Association between relative humidity and mean temperature and sand fly population fluctuations in forest in the Municipality of Sooretama, Espírito Santo State, Brazil, June 2004 to May 2006.

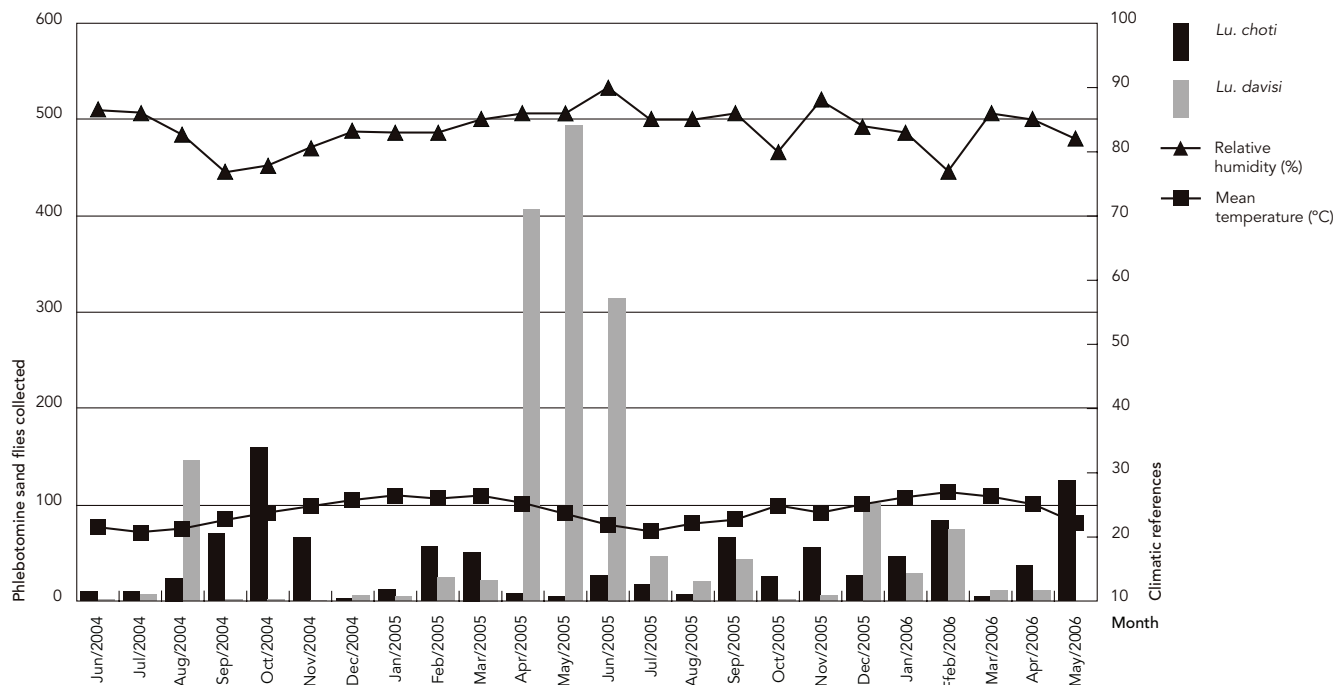
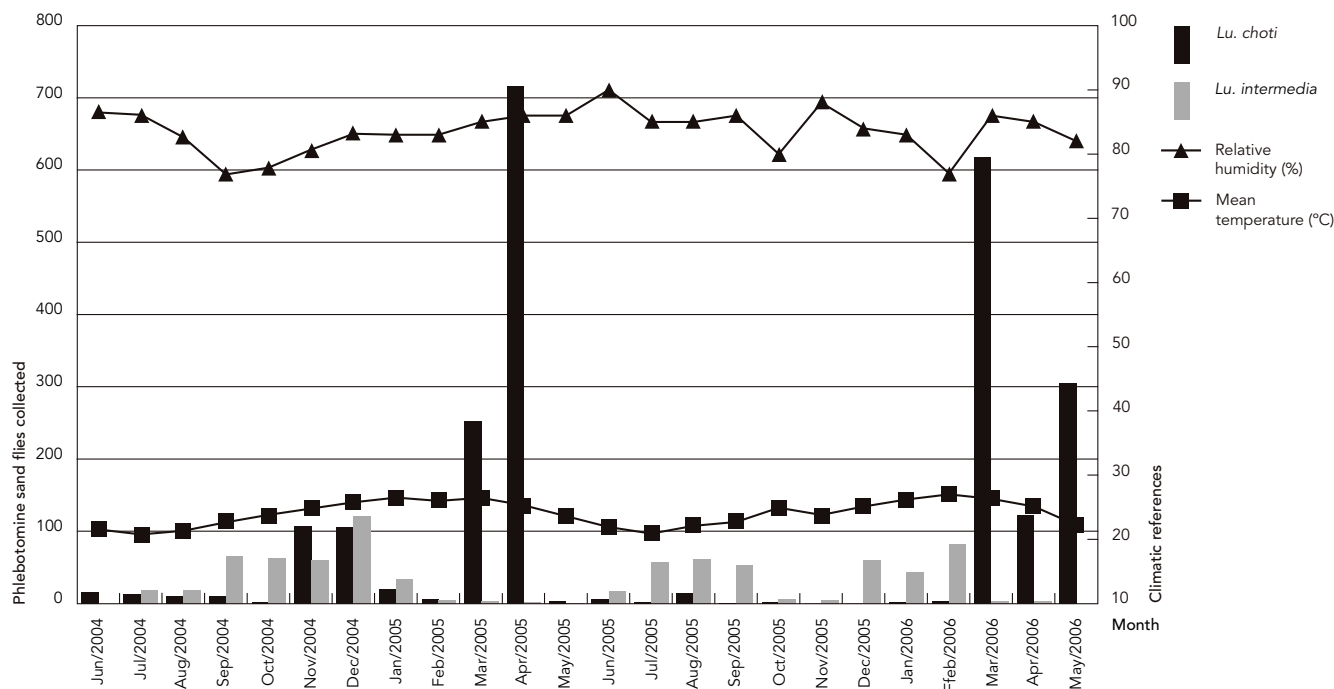


Figure 3

Association between relative humidity and mean temperature and sand fly population fluctuations in disturbed habitat in the Municipality of Vila Valério, Espírito Santo State, Brazil, June 2004 to May 2006.



The abundance of *Lu. choti* in the study area and its occurrence in both habitats suggests that the species has eclectic feeding habits. *Lu. choti* is the only one of the three most abundant sand fly species that is not a suspected *Leishmania* vector, since it has not been recorded in the principal ATL-endemic areas of Espírito Santo. Furthermore, there are no reports of this species' natural infection with the parasite¹⁸. However, Brandão-Filho et al.¹⁹ suggested that *Lu. choti* may be an ATL vector, based on its predominance in the Zona da Mata region of the Brazilian State of Pernambuco.

Although highly anthropophilic, *Lu. davisi* does not appear to be involved in ATL transmission in the study area, based on the absence of reported cases from the period when deforestation occurred. It is probably a sylvatic species and is common in humid regions of the Amazon²⁰. This species is involved in the transmission of *L. (Viannia) naiffi* Lainson & Shaw, 1989 in the Brazilian state of Rondônia²⁰. Grimaldi Jr. et al.²¹ isolated *L. (V.) braziliensis* from naturally infected *Lu. davisi* in the Amazon region. There is no clear evidence of sylvatic infection of humans by *Leish-*

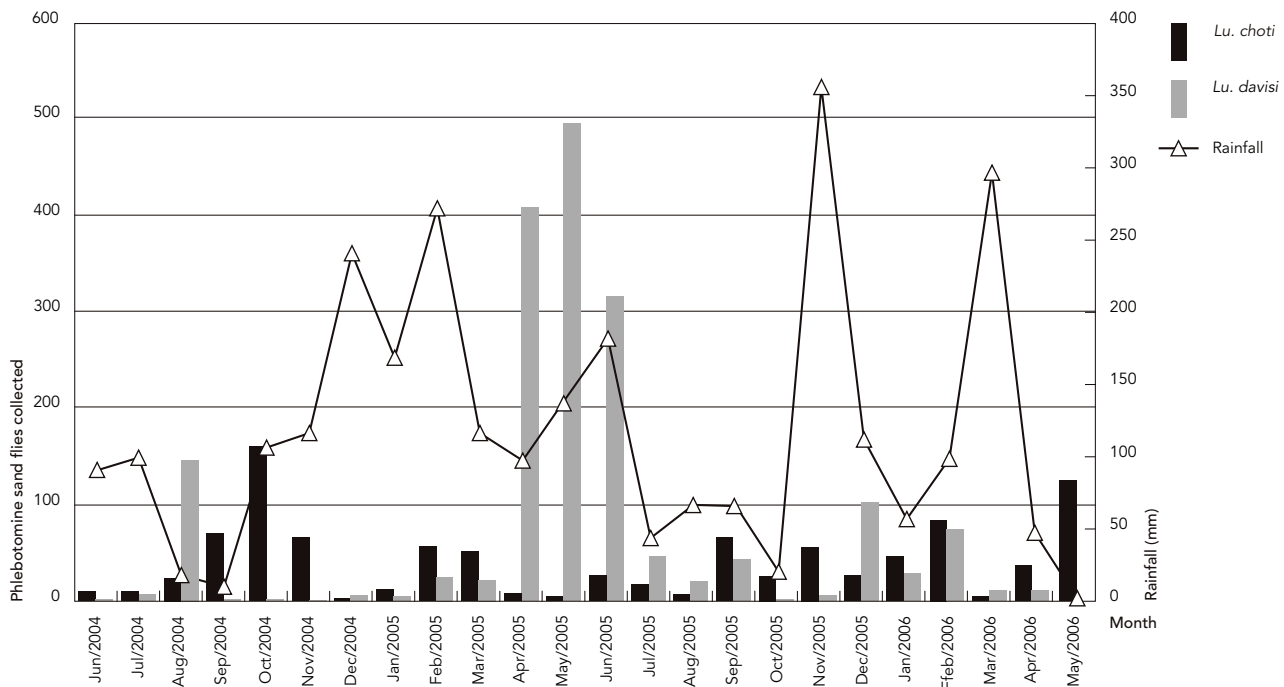
mania in Espírito Santo, since the disease attacks adults and children of both sexes, characteristic of an intradomestic transmission pattern². Since this species belongs to a group (*Psychodopygus*) including several species incriminated as vectors, it deserves close and thorough observation in the area.

Given its high prevalence in the disturbed habitat, *Lu. intermedia* can be considered the main vector in the study area, confirming observations by other authors in Espírito Santo^{9,22,23}. This sand fly is abundant in American tegumentary leishmaniasis foci in Southeast Brazil^{8,9,15,17,24} and is the suspected vector of *L. (V.) braziliensis* in the area, specimens having been found naturally infected with the parasite^{6,25,26}.

Destruction of primary forest in northern Espírito Santo from the 1940s onwards appears to have favored increased density of *Lu. intermedia* in disturbed habitats, indicating that this species is able to adapt to deforested environments²⁷. Lima²⁸ suggested that *Lu. intermedia* was pre-adapted to open habitats, drawing on humans and domestic mammals as blood meal sources.

Figure 4

Association between rainfall and sand fly population fluctuations in forest in the Municipality of Sooretama, Espírito Santo State, Brazil, June 2004 to May 2006.



The adaptation of *Lu. intermedia* to the disturbed habitat would explain changes in the epidemiological profile of American tegumentary leishmaniasis in Espírito Santo, where intradomestic transmission of *Leishmania* is known to occur². The low frequency of this species in the forest supports this hypothesis.

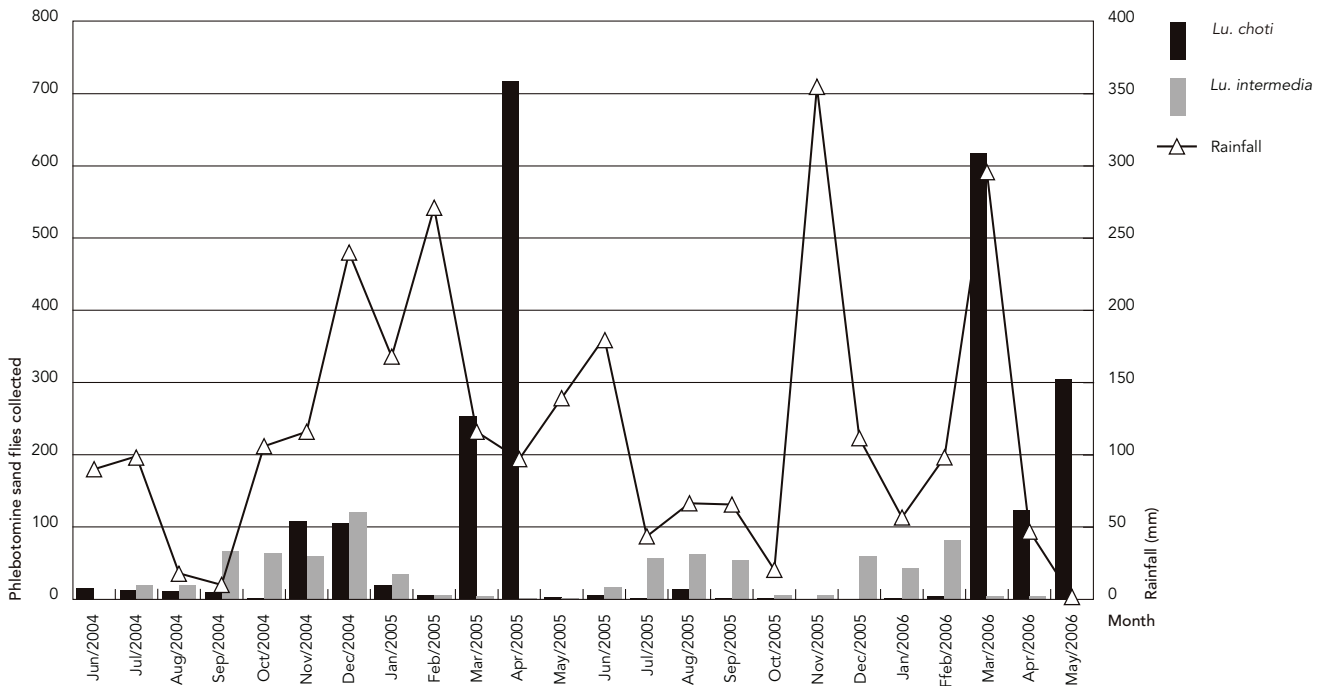
Although *Lu. intermedia* is highly anthropophilic, it is also strongly attracted to dogs and horses, which may act as secondary reservoirs of *L. (V.) braziliensis*^{22,29,30,31,32}. The species *Lu. choti*, *Lu. davisii*, and *Lu. intermedia* occurred in almost all months of the study period. *Lu. intermedia* frequently presents peaks in hotter, more humid months^{14,33}. In the present study, low numbers of this species were recorded dur-

ing and after periods of high rainfall, suggesting that intense rain is unfavorable to the development of immature forms, since breeding sites in disturbed habitats would suffer greater impact from extreme weather conditions. More detailed studies are needed to understand the seasonal population fluctuations of the main species. To meet all the requirements of Killick-Kendrick³⁴, studies on natural infection are needed to expand knowledge on the role of some vector species.

Finally, the higher frequency of *Lu. intermedia* in the disturbed habitat confirms its epidemiological importance, highlighting the risk of geographic expansion of American tegumentary leishmaniasis as this species occupies new areas from which native vegetation has been removed.

Figure 5

Association between rainfall and sand fly population fluctuations in disturbed habitat in the Municipality of Vila Valério, Espírito Santo State, Brazil, June 2004 to May 2006.



Resumo

A leishmaniose tegumentar americana é endêmica e amplamente distribuída no Estado do Espírito Santo, Brasil. Com o objetivo de avaliar a composição da fauna de flebotomíneos e verificar a flutuação sazonal, foram realizadas capturas mensais com armadilhas Shannon modificadas em reserva florestal de Mata Atlântica (Reserva Biológica de Sooretama) e ambiente antrópico adjacente, no período de junho de 2004 a maio de 2006. Foram capturados 6.176 espécimes, dos quais 47,4% ocorreram no ambiente florestal e 52,6%, no ambiente antrópico. Lutzomyia davisi (60,8%) predominou no ambiente florestal e Lu. choti (72%) seguida de Lu. intermedia (24,3%) predominaram no ambiente antrópico. As três espécies ocorreram ao longo de todo o ano. Os dados indicam que Lu. intermedia provavelmente atue como principal vetor domiciliar de Leishmania na área de estudo.

Leishmaniose; Psychodidae; Ecologia de Vetores

Contributors

T. M. Virgens participated in the study design, sand fly collection, identification of specimens, data analysis and interpretation, and writing of the manuscript. C. B. Santos participated in the sand fly collection, identification of specimens, and final editing of the manuscript. I. S. Pinto participated in the sand fly collection, identification of specimens, data analysis, and final editing of the manuscript. K. S. Silva and F. C. Leal participated in the sand fly collection, data acquisition, and final editing of the manuscript. A. Falqueto coordinated the study and the final editing of the manuscript.

Acknowledgments

The authors wish to thank the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) for permission to capture phlebotomines in the Sooretama Biological Reserve, INCAPER for providing meteorological data, the Espírito Santo State Health Department (SESA-ES) for support in field captures, and Bruce Alexander for assistance with the English translation.

References

- Basano AS, Camargo LMA. Leishmaniose tegumentar americana: histórico, epidemiologia e perspectivas de controle. *Rev Bras Epidemiol* 2004; 7:328-37.
- Barros GC, Sessa PA, Mattos EA, Carias VRD, Mayrink W, Alencar JTA, et al. Foco de leishmaniose tegumentar americana nos municípios de Viana e Cariacica, estado do Espírito Santo, Brasil. *Rev Saúde Pública* 1985; 19:146-53.
- Sessa PA, Barros GC, Mattos EA, Carias VRD, Alencar JTA, Delmaestro D, et al. Distribuição geográfica da leishmaniose tegumentar americana no estado do Espírito Santo, Brasil. *Rev Soc Bras Med Trop* 1985; 18:237-41.
- Falqueto A, Sessa PA, Ferreira AL, Vieira VP, Santos CB, Varejão JBM, et al. Epidemiological and clinical features of *Leishmania (Viannia) braziliensis* American cutaneous and mucocutaneous leishmaniasis in the State of Espírito Santo, Brazil. *Mem Inst Oswaldo Cruz* 2003; 98:1003-10.
- Secretaria de Saúde do Estado do Espírito Santo. Série histórica dos casos notificados período 1986-2004. Vitória: Programa de Controle das Leishmanioses, Secretaria de Saúde do Estado do Espírito Santo; 2004.
- Rangel EF, Souza NA, Wermelinger ED, Barbosa AF. Infecção natural de *Lutzomyia intermedia* Lutz & Neiva, 1912 em área endêmica de leishmaniose tegumentar no estado do Rio de Janeiro. *Mem Inst Oswaldo Cruz* 1984; 79:395-6.
- Marzochi MCA. Leishmanioses no Brasil. As leishmanioses tegumentares. *J Bras Med* 1992; 63: 82-104.
- Falqueto A. Especificidade alimentar de flebotomíneos em duas áreas endêmicas de leishmaniose tegumentar no estado do Espírito Santo, Brasil [Doctoral Dissertation]. Rio de Janeiro: Fundação Oswaldo Cruz; 1995.
- Ferreira AL, Sessa PA, Varejão JBM, Falqueto A. Distribution of sand flies (Diptera: Psychodidae) at different altitudes in an endemic region of American cutaneous leishmaniasis in the State of Espírito Santo, Brazil. *Mem Inst Oswaldo Cruz* 2001; 96:1061-7.
- Instituto de Pesquisas da Mata Atlântica. Conservação da Mata Atlântica no estado do Espírito Santo: cobertura florestal, unidades de conservação e fauna ameaçada. Vitória: Programa Centros para a Conservação da Biodiversidade/Conservação Internacional do Brasil/Instituto de Pesquisas da Mata Atlântica; 2004.
- Alexander B. Sampling methods for phlebotomine sand flies (Diptera: Psychodidae). *Med Vet Entomol* 2000; 14:1-13.
- Barreto MP, Coutinho JO. Processos de captura, transporte, dissecação e montagem de flebotomos. *Anais da Faculdade de Medicina da Universidade de São Paulo* 1940; 16:173-187.
- Young DG, Duncan MA. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). *Memoirs of the American Entomological Institute* 1994; 54: 1-881.
- Teodoro U, Salvia Filho VL, Lima ED, Spinosa RP, Barbosa OC, Ferreira MEMC, et al. Observações sobre o comportamento de flebotomíneos em ecótopos florestais e extraflorestais, em área endêmica de leishmaniose tegumentar americana, no norte do estado do Paraná, sul do Brasil. *Rev Saúde Pública* 1993; 27:242-9.
- Domingos MF, Carreri-Bruno GC, Ciaravolo RMC, Galati EAB, Wanderley DMV, Corrêa FMA. Leishmaniose tegumentar americana: flebotomíneos de área de transmissão, no município de Pedro de Toledo, região sul do estado de São Paulo, Brasil. *Rev Soc Bras Med Trop* 1998; 31:425-32.
- Mayo RC, Casanova C, Mascarini LM, Pignatti MG, Rangel O, Galati EAB, et al. Flebotomíneos (Diptera, Psychodidae) de área de transmissão de leishmaniose tegumentar americana, no município de Itupeva, região sudeste do estado de São Paulo, Brasil. *Rev Soc Bras Med Trop* 1998; 31:339-45.
- Brito M, Casanova C, Mascarini LM, Wanderley DMV, Corrêa FMA. Phlebotominae (Diptera: Psychodidae) em área de transmissão de leishmaniose tegumentar americana no litoral norte do estado de São Paulo, Brasil. *Rev Soc Bras Med Trop* 2002; 35:431-7.
- Oliveira-Pereira YN, Rebelo JMM, Moraes JLP, Pereira SRF. Diagnóstico molecular da taxa de infecção natural de flebotomíneos (Psychodidae, *Lutzomyia*) por *Leishmania* sp. na Amazônia maranhense. *Rev Soc Bras Med Trop* 2006; 39:540-3.
- Brandão-Filho SP, Brito MEF, Martins CAP, Sommer IB, Valença HF, Almeida FA, et al. Leishmaniose tegumentar americana em centro de treinamento militar localizado na Zona da Mata de Pernambuco, Brasil. *Rev Soc Bras Med Trop* 1998; 31:575-8.
- Gil LHS, Basano SA, Souza AA, Silva MGS, Barata I, Ishikawa EA, et al. Recent observations on the sand fly (Diptera: Psychodidae) fauna of the state of Rondônia, western Amazon, Brazil: the importance of *Psychodopygus davis* as a vector of zoonotic cutaneous leishmaniasis. *Mem Inst Oswaldo Cruz* 2003; 98:751-5.
- Grimaldi Jr. G, Momen H, Naiff RD, McMahon-Pratt D, Barrett TV. Characterization and classification of leishmanial parasites from humans, wild mammals, and sand flies in the Amazon region of Brazil. *Am J Trop Med Hyg* 1991; 44:645-61.
- Falqueto A, Sessa PA, Varejão JBM, Barros GC, Momen H, Grimaldi Jr. G. Leishmaniasis due to *Leishmania braziliensis* in Espírito Santo state, Brazil. Further evidence on the role of dogs as a reservoir of infection for humans. *Mem Inst Oswaldo Cruz* 1991; 86:499-500.
- Sessa PA, Falqueto A, Varejão JBM. Tentativa de controle da leishmaniose tegumentar americana por meio do tratamento dos cães doentes. *Cad Saúde Pública* 1994; 10:457-63.
- Saraiva L, Lopes JS, Oliveira GBM, Batista FA, Falcão AL, Andrade Filho JD. Estudo dos flebotomíneos (Diptera: Psychodidae) em área de leishmaniose tegumentar americana nos municípios de Alto Caparaó e Caparaó, estado de Minas Gerais. *Rev Soc Bras Med Trop* 2006; 39:56-63.

25. Forattini OP, Pattoli DBG, Rabello EX, Ferreira OA. Infecção natural de flebotomíneos em foco enzootico de leishmaniose tegumentar no estado de São Paulo, Brasil. *Rev Saúde Pública* 1972; 6:431-3.
26. Pita-Pereira D, Alves CR, Souza MB, Brazil RP, Bertho AL, Barbosa AF, et al. Identification of naturally infected *Lutzomyia intermedia* and *Lutzomyia migonei* with *Leishmania (Viannia) braziliensis* in Rio de Janeiro (Brazil) revealed by a PCR multiplex non-isotopic hybridisation assay. *Trans R Soc Trop Med Hyg* 2005; 99:905-13.
27. Gomes AC, Rabello EX, Santos JLF, Galati EAB. Aspectos ecológicos da leishmaniose tegumentar americana. 2. Estudo experimental da frequência de flebotomíneos a ecótopos artificiais com referência especial a *Psychodopygus intermedius*. *Rev Saúde Pública* 1980; 14:540-56.
28. Lima LC. Ruralização da *Lutzomyia intermedia*, um provável caso de pré-adaptação. *Rev Saúde Pública* 1986; 20:102-4.
29. Rangel EF, Souza NA, Wermelinger ED, Azevedo ACR, Barbosa AF, Andrade CA. Flebotomos de Vargem Grande, foco de leishmaniose tegumentar no estado do Rio de Janeiro. *Mem Inst Oswaldo Cruz* 1986; 81:347-9.
30. Rangel EF, Azevedo ACR, Andrade CA, Souza NA, Wermelinger ED. Studies on sandfly fauna (Diptera: Psychodidae) in a foci of cutaneous leishmaniasis in Mesquita, Rio de Janeiro State, Brazil. *Mem Inst Oswaldo Cruz* 1990; 85:39-45.
31. Falqueto A, Coura JR, Barros GC, Grimaldi Jr. G, Sessa PA, Carias VRD, et al. Participação do cão no ciclo de transmissão da leishmaniose tegumentar no município de Viana, estado do Espírito Santo, Brasil. *Mem Inst Oswaldo Cruz* 1986; 81:155-63.
32. Falqueto A, Varejão JBM, Sessa PA. Cutaneous leishmaniasis in a horse (*Equus caballus*) from endemic area in the state of Espírito Santo, Brazil. *Mem Inst Oswaldo Cruz* 1987; 82:443.
33. Condino MLE, Sampaio SMP, Henriques LF, Galati EAB, Wanderley DMV, Corrêa FMA. Leishmaniose tegumentar americana: flebotomíneos de área de transmissão no município de Teodoro Sampaio, região sudoeste do estado de São Paulo, Brasil. *Rev Soc Bras Med Trop* 1998; 31:355-60.
34. Killick-Kendrick R. Phlebotomine vectors of the leishmaniases: a review. *Med Vet Entomol* 1990; 4:1-24.

Submitted on 16/Jan/2008

Final version resubmitted on 08/Apr/2008

Approved on 20/May/2008