

## PUBLIC HEALTH

## Sand Flies (Diptera: Psychodidae) in the Urban Area of the Municipality of Cianorte, Paraná State, Brazil

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## Flebotomíneos (Diptera: Psychodidae) na Área Urbana do Município de Cianorte, PR

RESUMO - A endemicidade da leishmaniose tegumentar americana (LTA) no município de Cianorte, PR, a ocorrência de casos humanos com provável local de infecção na área urbana, a presença de mata nativa modificada de preservação permanente no perímetro urbano e o desconhecimento da fauna de flebotomíneos no município motivaram a realização deste trabalho. Procurou-se conhecer a fauna, frequência e sazonalidade de flebotomíneos no peridomicílio e na floresta, na área urbana desse município. Os flebotomíneos foram coletados com armadilhas de Falcão instaladas no peridomicílio e na floresta, no período de julho de 2005 a junho de 2006. Foram coletados 755 flebotomíneos, com predomínio da espécie *Nyssomyia whitmani* (Antunes & Coutinho) (84,0%), seguida da espécie *Nyssomyia neivai* (Pinto) (12,7%). O número de flebotomíneos coletados foi maior no peridomicílio ( $P = 0.0068$ ). O pequeno número de flebotomíneos coletados, principalmente na floresta, pode estar relacionado às alterações ambientais realizadas no entorno do Parque Cinturão Verde que circunda a área urbana do município. Cinco espécies de flebotomíneos foram assinaladas na área urbana do município de Cianorte, com maior frequência no peridomicílio, especialmente nos meses de novembro a abril. Os resultados indicam a necessidade de se manterem as medidas que contribuem para evitar o contato do homem com os flebotomíneos, diminuindo o risco de transmissão de LTA.

PALAVRAS-CHAVE: Leishmaniose cutânea, mata nativa modificada

ABSTRACT - The endemicity of American cutaneous leishmaniasis (ACL) in the Cianorte municipality, Paraná State the occurrence of human cases with the probable infection locus in the urban area, the presence of a permanently preserved modified native forest in the urban perimeter, and the lack of knowledge of the fauna of sand flies in the municipality provided the impetus for this study. The objective of this study was to assess the fauna, frequency and seasonality of the sand flies in the peridomicile, forest and urban area of this municipality. Sand flies were collected using Falcão light traps installed in the peridomicile and forest, from July 2005 to June 2006. A total of 755 sand flies were collected; *Nyssomyia whitmani* (Antunes & Coutinho) (84.0%), followed by *Nyssomyia neivai* (Pinto) (12.7%) were the predominant species. The number of sand flies collected in the peridomicile was significantly higher than in the forest ( $P = 0.0068$ ). The small number of sand flies collected, especially in the forest, may be related to the modifications to the environment on the edge of the Parque Cinturão Verde, which surrounds the urban area of the municipality. Five species of sand flies were distinguished in the urban area of Cianorte, with greater frequencies found in the peridomicile, especially from November to April. Our data illustrate the necessity of maintaining the measures that contributed to reduce human contact with sand flies, thereby reducing the transmission risk of ACL.

KEY WORDS: Cutaneous leishmaniasis, urban area, native forest

According to World Health Organization (WHO) estimates, leishmaniasis occur in 88 countries, but notification is compulsory in only 32 of these countries. Of all cases of cutaneous leishmaniasis recorded, 90% occurred in only six countries: Iran, Saudi Arabia, Syria, Afghanistan, Brazil and Peru (WHO 2006). In Brazil, from 1980 to 2005, 610,205

cases were reported (Ministério da Saúde 2006a). The North region of Brazil has the highest rates of occurrence of American cutaneous leishmaniasis (ACL), followed by the Central-Western, Northeast, Southeast and South regions, which in 2005 had incidence rates per 100,000 inhabitants of 71.1; 33.3; 15.6; 3.5 and 2.0, respectively (Ministério da Saúde 2006b).

In the State of Paraná, ACL was confirmed early in the 20<sup>th</sup> century, and is officially reported since 1980. In the period from 1980 to 2005, 13,486 cases were reported in southern Brazil, with 13,316 (98.7%) cases occurring in the State of Paraná (Ministério da Saúde 2006b). Of the 399 municipalities in the state, 314 (78.7%) have reported cases of ACL (Ministério da Saúde 2006c).

The municipalities of the Northwest mesoregion of Paraná are included in the Paranapanema circuit of ACL outbreaks (FUNASA 2006). Among the municipalities covered by the 13<sup>th</sup> Health Region of Paraná, Cianorte and Jussara are noticeable due to their higher incidence rates than any other municipality in Brazil. In 2005, according to calculations based on data from the Ministério da Saúde (2006b, 2006c), the rates of incidence per 100,000 inhabitants in Cianorte and Jussara were 35.4 and 355.9, but only 14.1 and 4.2 for Brazil and Paraná, respectively.

The presence of high numbers of sand flies has been reported in ACL endemic areas in northern Paraná (Teodoro *et al* 1993, 2001b, Luz *et al* 2000, Membrive *et al* 2004). Nowadays, the adaptation of sand flies to the available wild reservoirs in human environments, transmission of *Leishmania* in the peridomicile and domiciles of rural and urban zones has been favored, affecting humans and domestic animals, especially dogs (Aguilar *et al* 1986, 1989, Pirmez *et al* 1988, Lonardoní *et al* 1993, Teodoro *et al* 1993). In Paraná, earlier investigations showed a high incidence of sand flies in the domiciles and peridomicile of rural zones, where *Nyssomyia whitmani* (Antunes & Coutinho) and *Nyssomyia neivai* (Pinto) were the main species identified (Membrive *et al* 2004, Teodoro *et al* 2006a). The former species has been detected through natural *Leishmania* (*Viannia*) *braziliensis* (Vianna) infection in Paraná (Luz *et al* 2000). *Nyssomyia neivai* has also been found to predominate in rural areas where human cases have occurred (Brito *et al* 2002, Teodoro *et al* 2006a), and the vectorial potential of this species should

not be underestimated.

Many cases of ACL have been reported in the urban area of the municipality of Cianorte (Lima *et al* 2002), demonstrating the need for studies that help to clarify the epidemiology of ACL in the area. The objective of this study, therefore, was to study the fauna, frequency and seasonality of the sand flies in the peridomicile, forest and urban area of this municipality.

## Materials and Methods

**Area of study.** The municipality of Cianorte is situated in the Northwest mesoregion of the State of Paraná, southern Brazil (23°39'S, 52°38'W, 530 m altitude). It has a total area of 812 km<sup>2</sup> and an urban area of approximately 35 km<sup>2</sup> (Fig 1), with an urban population of 53,735 and a rural population of 8,401 (IBGE 2000). It has a mesothermal humid subtropical climate with hot summers with a tendency of heavy rains (mean temperature above 22°C), and winters with rare frosts (mean temperature below 18°C), without a defined dry season. It is located on the Third Paraná Plateau, in the sub-section of the large middle block of the Campo Mourão Plateau, in the region known as Arenito Caiuá, between the Ivaí and Piquiri rivers.

There is an area of modified native forest in the municipality known as the Parque Municipal Cinturão Verde, which is a remnant of a seasonal deciduous sub-montane forest. This forest is a permanent reserve, and has a border of approximately 7 km with the urban perimeter, with a pedestrian path running along it. The park has abundant fauna, composed of amphibians, arachnids, birds, insects, reptiles, and some mammals, in particular bush dogs, coatis, bats, armadillos, skunks, mouse-possums, capuchins, opossums, pacas, agoutis, wild mice, squirrels, hedgehogs and anteaters (Prefeitura do Município de Cianorte 2006).

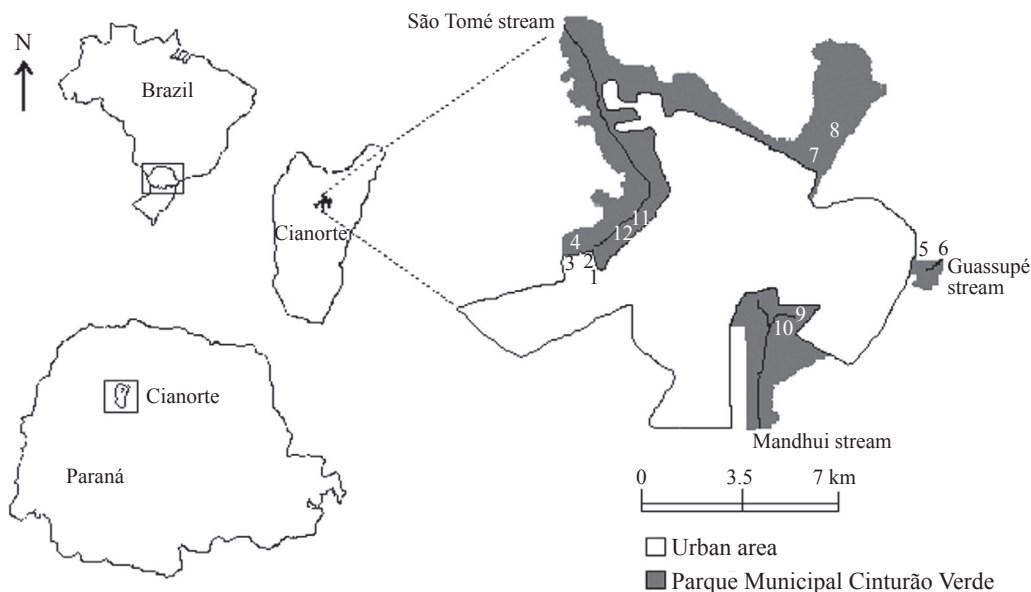


Fig 1 Location of the municipality of Cianorte, PR, Brazil, and trap distribution for collection of sand flies. Numbers 1 to 12 correspond to traps A1 to A12.

**Collection and identification of sand flies.** The collections of sand flies were carried out with Falcão light traps from July 2005 to June 2006, from 9 pm to 3 am, totaling 78h of collection for each trap. The collections were carried out every 14 days with six traps each time, alternating the collection sites as a function of the distance between them and resulting in 13 collections in each site.

The traps were distributed: i) in a plant nursery, considered as a peridomiciliary area, located next to the Parque Municipal Cinturão Verde; the traps were installed on the veranda of the domicile (trap A1), next to the nursery shed (A2), next to the greenhouse (A3) and in a tree reserve at the end of the shed (A4); ii) in a farm with rural characteristics, considered as a peridomiciliary area, located next to the park, which had a house, a barn, an orchard, a vegetable garden, a pigsty, a chicken coop and dogs; a trap was installed in a jaboticaba tree (*Myrciaria cauliflora*) next to the domicile (A5), and another in a breadfruit tree (*Artocarpus heterophyllus*), approximately 15 m from the domicile (A6); and iii) in different areas of the park, considered forest areas: on the Perobas Trail (A7 and A8), on Ambú Road (A9 and A10) and on Dom Pedro II Road (A11 and A12), all at 5 and 40 m from the edge of the forest, respectively.

The collected sand flies were kept in small cardboard boxes and later processed and identified according to Forattini (1973). The nomenclature of the sandfly species followed that of Galati (2003).

The environmental temperature and relative humidity of the air were measured at the beginning and end of each collection, using a digital thermo-hygrometer (Alla France, France).

**Statistical analysis.** The mean hourly rates of the collections were used to draw the sand fly monthly distribution graph. The Chi-square test ( $\chi^2$ ) was used to analyze the number of *N. whitmani* and *N. neivai* specimens and the proportion of male and female *N. whitmani* sand flies collected in the peridomicile and in the forest. The number and the mean number of sand flies collected in the different traps in the peridomicile and in the forest, as well as the mean hourly rates of sand flies collected in the different months were compared by Kruskal-Wallis test. The correlation of the number of sand flies collected with the environmental temperature and the air relative humidity was analyzed. The software Statistica 6.0 was used, with a 5% level of significance.

## Results

In the environments studied, 755 specimens of sand flies belonging to five species were collected. The predominant species was *N. whitmani*, representing 84.0% of the specimens collected, followed by *N. neivai* (12.7%), *Pintomyia monticola* (Costa Lima) (1.8%), *Pintomyia pessoai* (Coutinho & Barretto) (1.2%) and *Migonemyia migonei* (França) (0.3%). Four hundred and nineteen (55.5%) of the sand flies were females.

Of the 601 (79.6%) specimens collected at the nursery and farm (peridomicile), 322 (53.6%) were females. Within the Parque Municipal Cinturão Verde (Forest), 154 (20.4%)

specimens were collected, of which 97 (63.0%) were females. The proportion of females captured in relation to males was significantly higher in the forest than in the peridomicile ( $P = 0.0361$ ). The proportion of females was also higher in the forest ( $P = 0.0378$ ) when considering only the *N. whitmani* species. There was no significant difference in the proportions of *N. whitmani* to *N. neivai* collected between the peridomicile and the forest ( $P = 0.1518$ ).

Three-hundred and fifty-five specimens (47.0%) were collected in the plant nursery. The majority of them (236) were captured in the trap installed on the veranda of the domicile (trap A1). A total of 246 specimens (32.6%) were collected at the farm, with 158 specimens caught in trap A5, installed in the jaboticaba tree (*M. cauliflora*) next to the domicile, and 88 caught in trap A6, installed in the breadfruit tree (*A. heterophyllus*). There was no significant difference between the number of sand flies collected in the different traps installed in the peridomiciles of the nursery and the farm ( $P = 0.1111$ ).

In the Parque Municipal Cinturão Verde, 154 (20.4%) specimens were caught in traps (A7, A8, A9, A10, A11, A12) installed in the interior of the modified native forest. There was no difference between the numbers of sand flies collected in these different traps ( $P = 0.7801$ ).

The mean number of sand flies caught per trap in the traps installed in the peridomicile (mean = 7.7) was significantly greater than that of the traps installed in the forest (mean = 2.0) ( $P = 0.0068$ ).

No correlation was found between the number of sand flies collected and the mean temperature ( $r = 0.2974$ ;  $P = 0.1400$ ) or air relative humidity ( $r = 0.0786$ ;  $P = 0.7030$ ) (Fig 2A). The largest mean hourly collection (MHC) of sand flies occurred in November, and the smallest in July. There were significant differences between the MHC obtained in the different months ( $P = 0.0198$ ) (Fig 2B).

## Discussion

All five sand fly species collected were previously reported in the municipality of Cianorte (Teodoro *et al* 2001b). However they represent a small number of species when compared with the number of species collected in ACL endemic areas of several municipalities in northern Paraná (Teodoro *et al* 1993, 2001b, 2006a, Membrive *et al* 2004). This can be explained by the high degree of modification in the vegetation that surrounds the urban area of Cianorte. Among them, *N. whitmani* and *N. migonei* were detected carrying a natural *L. braziliensis* infection in the State of Ceará (Azevedo *et al* 1990a, 1990b, Queiroz *et al* 1994), and infected specimens of *N. whitmani* were recently found in Paraná (Luz *et al* 2000).

In the present study, *N. whitmani* was the predominant species, both in the peridomicile and in the forest, followed by *N. neivai*. These species are dominant and very prevalent in the peridomicile of ACL-endemic areas in the State of Paraná (Membrive *et al* 2004, Teodoro *et al* 2006a). These last authors reported that *N. whitmani* prevails in areas of red and red-yellow latosols or nitosols, which retain higher humidity, whereas *N. neivai* predominates in more sandy soils, which are well drained and derive from Caiuá arenite.

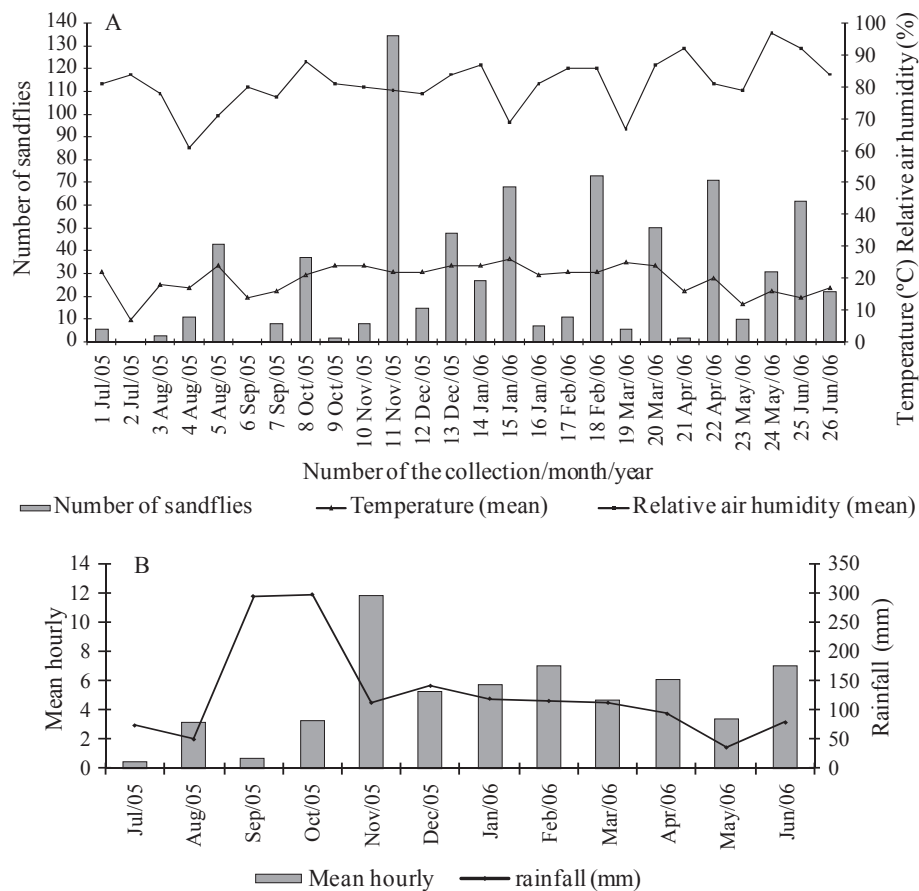


Fig 2 A) The mean temperature, air relative humidity and number of sand flies collected in the urban area of the municipality of Cianorte, State of Paraná, from July 2005 to June 2006; B) Monthly means of hourly collections of sand flies in the urban area of the municipality of Cianorte, and rainfall (Source: Cocamar – Cianorte/PR) during the period from July 2005 to June 2006.

*Nyssomyia neivai*, which comprised 12.7% of the total sand flies collected, has not been found carrying natural *L. braziliensis* infection in Paraná, and *N. whitmani* may be the most important species in ACL epidemiology in Brazil (Costa et al 2007). However, the importance of *N. neivai* should not be underestimated because of its narrow distribution (Marcondes et al 1999, Casanova et al 2005). *Nyssomyia neivai* predominates in areas where human cases occur (Brito et al 2002), including the north of Paraná (Membrive et al 2004, Teodoro et al 2006a).

The proportion of female sand flies captured was higher in both environments studied (forest and peridomicile), and the higher proportion of female sand flies in the domicile than in the forest is already known (Domingos et al 1998, Mayo et al 1998, Teodoro et al 1998, Saraiva et al 2006). The fact that ACL infects humans in urban zones nearby forested areas is due to the persistence of the enzootic cycle of the parasite.

The highest sand fly MHCs occurred from November to April, representing 69.2% of the total MHCs. In these months, temperatures above 20°C were recorded at the time of collection, with rainfall between 100 and 150 mm. The highest rainfall was recorded in September and October, although the MHCs in these months were low. Although larger collections of sand flies were recorded in the warmer and humid months in Paraná (Massafera et al

2005, Reinhold-Castro et al 2008, Teodoro et al 2003a), we found no correlation between temperature or rainfall and the number of insects collected in our study. Generally, wind, low temperatures and rainfall affect the activity of sand flies, thereby reducing their population density (Aguiar et al 1985, Mayo et al 1998, Teodoro et al 2003a, Saraiva et al 2006).

In the peridomicile, 601 specimens were collected, representing 79.6% of the total number of sand flies collected. Although the differences in the numbers of sand flies collected in the traps installed in this environment were not significant, in the plant nursery, the number of specimens collected in the trap installed on the veranda of the domicile (A1) represented 31.3% of the total number of sand flies collected. The high number of specimens in this trap may be related to the presence of an electric light, inhabitants and/or animals that would serve as attraction factors (Teodoro & Kühl 1997, Teodoro et al 2006b). This could also explain the high number of insects (32.6%) collected by traps A5 and A6 installed at the farm. In addition to the presence of light and inhabitants, domestic animals, deposits of animal and plant organic matter, and humidity in the soil may all function as attractants to the flies (Teodoro et al 1993, 2001a, 2003b, Massafera et al 2005).

In the Parque Municipal Cinturão Verde, the six traps installed captured 154 specimens, 20.4% of the total number

of sand flies collected. Traps A7, A8, A9 and A10 were installed in a location where many ACL cases occurred in the urban area of the municipality from 1993 to 1998 (Lima *et al* 2002), and new human cases continue to be reported to the Illness Notification Information System, albeit in lower frequency. Although there is no previous information on the fauna of the sand flies in the municipality, the modifications carried out over the years (asphalt paving, periodic cleaning of litter and waste material from the borders and the interior of the forest, the construction of a walkway between the road and the edge of the forest, and the construction of a chain link fence to limit people access to the forest) may have contributed to the reduction of the occurrences of ACL in this area and explain the small number of sand flies collected. According to Teodoro *et al* (2003b), the reorganization and cleaning of the peridomicile significantly reduced the number of sand flies in a rural area (Recanto Marista) of the municipality of Doutor Camargo, Paraná, reducing the risk of ACL transmission to humans and domestic animals.

Even though no cases of ACL had been reported in the area where the traps A11 and A12 were installed, the same modifications had been carried out. In a study carried out in an area of modified primary forest reservation (Parque Ingá), on the urban perimeter of the city of Maringá, larger numbers of sand flies were collected in the interior of the forest and close to the shelters of captive wild animals (Teodoro *et al* 1998) in comparison to the results of this study. However, a larger number of traps were used, and some were placed inside the shelters of the wild animals. It is believed that the smaller number of sand flies collected in the present study, especially inside the Parque Municipal Cinturão Verde, is related to the modifications.

Our data demonstrated that only five species of sand flies occur in the urban area of the municipality of Cianorte, with the greatest frequency occurring in the peridomicile, especially from November to April, pointing to necessity of enforcing the control measures that contributed to the reduction of ACL transmission.

### Acknowledgments

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