LSHTM Research Online

Entomological surveys of *Lutzomyia flaviscutellata* and other vectors of cutaneous leishmaniasis in municipalities with records of *Leishmania amazonensis* within the Bragança region of Pará State, Brazil

Bruno M. Carvalho[1/+], Thiago Vasconcelos dos Santos[2], Iorlando da R. Barata[2], José Aprígio N. Lima[2], Fernando T. Silveira[2], Mariana M. Vale[3], Paul D. Ready[4], Elizabeth F. Rangel[1]

[1] Laboratório Interdisciplinar de Vigilância Entomológica em Diptera e Hemiptera, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Avenida Brasil 4365, 21040-900, Manguinhos, Rio de Janeiro, RJ, Brazil

[2] Laboratório de Leishmanioses Prof. Dr Ralph Lainson, Instituto Evandro Chagas, Secretaria de Vigilância em Saúde, Ministério da Saúde, Rod. BR 316, Km 07 s/n, 67030-000, Levilândia, Ananindeua, PA, Brazil

[3] Laboratório de Vertebrados, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho, 373, 21941-902, Cidade Universitária, Rio de Janeiro, RJ, Brazil

[4] Department of Disease Control, Faculty of Infectious Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, U.K.

[+] Corresponding author: brunomc.eco@gmail.com

ABSTRACT

In southeast Amazon, *Lutzomyia (Nyssomyia) flaviscutellata* is the incriminated vector of *Leishmania (Leishmania) amazonensis*, a causative agent of zoonotic cutaneous leishmaniasis (CL). The optimal methods for surveying *Lu. flaviscutellata* were investigated in the Bragança region, northeast Pará State, Brazil, selected for the presence of *Le. amazonensis*. The performances of modified Disney traps and CDC light traps were compared in four ecotopes within and around four village transects during the wet and dry seasons. The physiological age of female sand flies was estimated and natural infection by flagellates was evaluated by dissection. Disney traps were better for detecting the presence of *Lu. flaviscutellata*, while CDC traps performed well for detecting *Lutzomyia (Nyssomyia) antunesi*, suspected vector of *Leishmania lindenbergi*. The former was more abundant during the wet season, when female flies were naturally infected with *Le. amazonensis*. These findings identified the environments of local transmission. In order to improve surveys of *Lu. flaviscutellata* as part of integrated epidemiological surveillance of CL, our recommendations include focusing vector surveys with Disney traps on forest fragments where people work, during the seasonal peak of the vector. Further field studies are required to make model-based predictions of seasonal variations in the vectorial capacity of vector populations.

Key words: *Lutzomyia flaviscutellata – Lutzomyia antunesi – Leishmania amazonensis* – cutaneous leishmaniasis – entomological surveys – Brazil

---

INTRODUCTION

A wide diversity of species of *Leishmania* parasites (Kinetoplastida, Trypanosomatidae), their mammalian hosts and sand fly vectors (Diptera: Phlebotominae) are involved in the transmission of human cutaneous leishmaniasis (CL) in the Americas. Recent investigations of CL foci in Brazil show the emergence of various transmission cycles associated with environmental change (Rangel & Lainson 2009, Ready 2013, Rangel et al. 2014). Seven species of *Leishmania* are responsible for CL in Brazil, namely *Leishmania (Leishmania) amazonensis*, *Le. (Viannia) braziliensis, Le. (Viannia) guyanensis, Le. (Viannia) naiffi, Le. (Viannia) shawi, Le. (Viannia) lainsoni* and *Le. (Viannia) lindenbergi* (Brasil 2007). Pará is the only Brazilian state that has recorded all seven parasites, with most CL caused by *Leishmania (Viannia)* species. Most cases of CL by *Le. amazonensis* diagnosed in the Leishmaniasis Laboratory Prof. Dr Ralph Lainson at Instituto Evandro Chagas come from the northeast of the state (FT Silveira, unpublished observations), and in part this prompted the current investigation in the Bragança region (Figure 1).

The sand fly incriminated as the main vector of *Le. amazonensis* in Brazil is *Lutzomyia (Nyssomyia) flaviscutellata* (Lainson & Shaw 1968, Rangel & Lainson 2009), a species widely distributed in South America, with occurrence records in three Brazilian biomes, namely Amazon, Cerrado and Atlantic Forest (Young & Duncan 1994, Carvalho et al. 2015). Its distribution and seasonality are influenced by precipitation (Shaw & Lainson 1972), and its current area of climatic suitability is predicted to expand slightly following the forecasted climate change (Carvalho et al. 2015). The vectorial role of a sand fly depends not only on vector competence, which is governed by its ability to facilitate the development of infective metacyclic forms of *Leishmania* species, but also on vectorial capacity, which depends partly on ecological associations (Ready 2013). The need to improve ecological surveys of *Lu. flaviscutellata* was brought to our attention during literature searches for location records required to construct ecological niche models and predictive distribution maps (Carvalho et al. 2015).

In the eastern Amazon region, four main types of forest can be recognized (Shaw & Lainson 1972, Ready et al. 1983). Multi-story "Terra firme" forests are rarely flooded seasonally by rivers or heavy rainfall; multi-story “Várzea” forests are riparian formations seasonally flooded by rivers; less vertically structured “Igapó” forests are flooded by heavy rainfall in the wet season; and, “Capoeira” woodlands are usually small forests or woods and copses of secondary growth that appear after deforestation. Intensive ecological research near Belém, the state capital of Pará, demonstrated that *Lu. flaviscutellata* is more abundant in “Igapó” and “Capoeira” forests in the dry season and wet season, respectively (Shaw & Lainson 1972, Shaw et al. 1972, Ready et al. 1983). However, the ecology of *Lu. flaviscutellata* has rarely been investigated elsewhere and, based on the experience in Belém, surveys of this vector might depend on using small mammal-baited traps (Shaw & Lainson 1968) rather than the CDC miniature light traps routinely favoured for sampling other sand fly species (Dorval et al. 2010, Ready 2013) as part of epidemiological surveillance (Brasil 2017). *Lutzomyia flaviscutellata* has not been recorded biting humans in large numbers in Pará State or elsewhere (Shaw & Lainson 1972, Ward et al. 1973, Ready et al. 1983), and so it has not been surveyed by using human-baited Shannon traps or by aspirating adult sand flies from the interior or exterior surfaces of human habitations and sheds.

The current report investigates the eco-epidemiology of CL in municipalities within the Bragança region of Pará State where human infections of *Le. amazonensis* have been recorded recently. These locations were selected with the aim of assessing how the sampling of *Lu. flaviscutellata* can be used to optimize eco-epidemiological surveys of CL in transmission areas of *Le. amazonensis*.

MATERIALS AND METHODS

Study area - Sand fly populations were sampled along four transects surrounding three small villages in northeast Pará State in the municipalities of Bragança (Alto Alegre and Vila do Almoço with one transect each) and Tracuateua (Vila Martins with two transects) (Figure 1). These rural communities are composed mostly of farmers. Their daily activities include long periods of work near forest fragments, which frequently last until dusk when many sand fly species start to search for blood meals and to transmit *Leishmania* species. The Brazilian Ministry of Health has records of 271 autochthonous CL cases in Bragança and 32 in Tracuateua for the period 2001-2014

(SINAN 2017, Brasil 2017). We found no records of human visceral leishmaniasis (VL) cases in the two municipalities.

The soils along the sand fly sampling transects were not "white sands", although such soil types do occur in other parts of the region (IBGE 2017). The climate in the Bragança region is typical of southeast Amazonia: two seasons with marked differences in precipitation. The rainy season starts in December and peaks between February and March. In the following months, precipitation gets progressively lower until the peak of the dry season from October to November (INMET 2015).

Sand fly sampling and species identification - Trapping was carried out in July 2014, December 2014 and March 2015, in order to sample sand flies in seasons with different precipitation levels. Four transects were established that included "Igapó" forest fragments and/or "Capoeira" woodland and/or peridomestic areas (Figure 2). Each transect had six sand fly capture sites situated 100 m apart, in each of which two traps were suspended from low tree branches or from man-made supports in peridomestic areas (Figure 3). One was a modified Disney trap (Disney 1966, Shaw & Lainson 1968), with its animal bait and sticky collection tray placed 20 cm above the ground and protected from rain by a plastic sheet. The other was a CDC miniature light trap (Sudia & Chamberlain 1962), with the trap entrance approximately 1 m above the ground and the collection net protected from rain by a plastic bag. The two traps at each capture site were set approximately 10 m apart and separated by trees. Traps were set from sunset to the next morning (12 h) for two consecutive nights in each capture station. The total sampling effort was 144 trap-nights (or 1728 hours) for each trap type.

These sand fly captures were authorized by the "System of Authorization and Information on Biodiversity" of the Brazilian Ministry of Environment (SISBIO, protocol number 43672-1). Modified Disney traps were baited with laboratory-bred hamsters (*Mesocricetus auratus*) that were provided with food and water supplies. The trapping protocol was authorized by the Ethics Committee on Animal Use of the Instituto Evandro Chagas (CEUA/IEC, protocol number 0010/2014).

Using a binocular microscope with up to x 50 magnification, captured sand flies were separated by gender. Each female was then dissected, in order to assess physiological age and in preparation for mounting in Berlese fluid on slides for identification according to the taxonomic keys of Young & Duncan (1994).

Physiological age of sand flies and natural infection of females by Leishmania - Males where examined for rotation of the external genitalia, which is usually completed within 24 h of emergence from the pupa (Ready 2013). Captured females were washed in sterile water and then dissected in sterile physiological saline (Ryan et al. 1987). Internal organs were then examined to determine parity, developmental stage of eggs, the stage of blood meal digestion and the presence of any flagellates (Ready et al. 1983, 1984) (Table 1).

If flagellates were present, their position in the midgut was recorded (Ryan et al., 1987), and the dissected female was promptly macerated in a sterile 1.5 ml microtube with 0.1 ml of sterile physiological saline prior to trying to culture and identify the infection. Each homogenate was inoculated into Difco B45 culture medium and into a hind leg of two hamsters for *in vitro* and *in vivo* isolation trials, respectively (Ryan et al., 1987).

Following inoculation, *in vitro* culture tubes were maintained at 25°C and any parasites microscopically detected after 10, 15 and 20 days. Flagellates in positive cultures were characterized by Polymerase Chain Reaction (PCR) amplification of the Mini-exon gene of *Leishmania* (Fernandes et al. 1994). This PCR specifically amplifies a 400 bp DNA fragment from viscerotropic *Leishmania (Leishmania) infantum chagasi*, a 250 bp DNA fragment from dermotropic *Leishmania (Viannia)* species and a 350 bp DNA fragment from *Le. amazonensis*. DNA fragments were sized by electrophoretic fractionation in 1% agarose gels using the 1 kb reference DNA ladder (Fernandes et al., 1994). Each batch of PCRs always included negative controls and positive controls using samples of the *Le. amazonensis* reference strain IFLA/BR/1967/PH8.

Inoculated hamsters were kept in cages with good air circulation, provided with food rations and water, and not sedated. The animals were periodically examined for cutaneous lesions. If the animals did not produce any clinical signs after two months of quarantine, they were euthanized as negative (CEUA/IEC, protocol number 0010/2014).

Data analysis - Relative frequencies of sand fly species were expressed as number of captured specimens per trap-night. Precipitation data from weather station 82145 in Tracuateua municipality were obtained from the National Institute of Meteorology (INMET 2015).

Statistical analysis used R software (R Core Team, 2017) to compare: sand fly frequencies in different ecotopes by Analysis of Variance (ANOVA), captured sand flies in different seasons by the Student's t-test, female parous rates in different ecotopes by the Chi-square test, and probability of detecting sand fly presence also by the Chi-square test. The threshold for considering statistical significance was $p > 0.05$.

RESULTS

Sand fly fauna and seasonality - The sand fly surveys resulted in 1,394 captured specimens belonging to 11 species of genus *Lutzomyia* and one undetermined species of genus *Brumptomyia* (Table 2). Three of the detected species have been incriminated as vectors elsewhere in Brazil: *Lu. flaviscutellata* and *Lutzomyia (Nyssomyia) antunesi* are incriminated vectors of CL and *Lutzomyia (Lutzomyia) longipalpis* is the main vector of VL. The most abundant species were *Lu. flaviscutellata* (N = 1,011) and *Lu. antunesi* (N = 356), with their females predominating over males in Disney traps and CDC traps, respectively. Each of the other species was represented by less than 10 captured individuals (Table 2).

The transect in the village of Alto Alegre (Bragança municipality) contained mostly peridomestic trap sites, but the other three transects contained mainly forest ecotopes. "Igapó" forest had higher species richness and frequency (10 species; 8.64 specimens per trap-night) when compared with captures performed in "Capoeira" woodland (four species, 5.95 specimens per trap-night) and in peridomestic areas (five species, 1.43 specimen per trap-night) (Table 3). The epidemiologically relevant species *Lu. flaviscutellata* and *Lu. antunesi* were detected in all three ecotopes, while the three captured females of *Lu. longipalpis* were found exclusively in "Igapó" forest fragments (Table 3).

During the rainy season, the frequency of *Lu. flaviscutellata* was higher (18.10 specimens per trap-night) than in captures performed in the dry season, in July 2014 (0.61 specimens per trap-night) and December 2014 (2.21 specimens per trap-night) (Figure 4A), and these differences were statistically significant ($t = -3.702$, $p = 0.0003$). These seasonal differences were not as marked for *Lu. antunesi* (Figure 4B), and the differences were not statistically significant (t = -1.7364, p = 0.0844). *Lutzomyia longipalpis* was captured only in the rainy season, in March 2015.

Physiological age of sand flies - Every captured male had its external genitalia rotated through 180º, indicating that none of the collections was made near a site when flies were emerging from pupae.

The evaluation was made for 310 females of all captured species. However, some features were not visible in all specimens because of loss of material during dissection. In total, the nulliparous females predominated over parous females. *Lutzomyia flaviscutellata* had a statistically higher parous rate in "Capoeira" woodland (52%) when compared with rates in "Igapó" forest (26%) and peridomestic areas (46%) (Table 4). Parous rates of *Lu. antunesi* were approximately 25% in both forest ecotopes, but only one female (nulliparous) was captured peridomestically (Table 4).

Of the 12 gravid females captured, most were from forest fragments (Table 5). Only three out of 235 *Lu. flaviscutellata* (all from "Igapó" forest in the dry season) and five out of 65 *Lu. antunesi* (from both forest types in the dry season) were gravid, and the single gravid female captured peridomestically was identified as *Lu. gomezi*.

Natural infections of sand flies by Leishmania - In total 1,087 female sand flies were dissected and examined for natural infections (Table S1). Four *Lu. flaviscutellata* females were positive for flagellate infections, three in December 2014 and one in March 2015, all in "Igapó" forest fragments of Vila Martins, Tracuateua municipality. In one of the dissected females captured in December 2014, it was possible to determine a suprapylarian position of the flagellates suggestive of *Leishmania (Leishmania)* species and the gut contained no blood meal. Of the remaining infected females, flagellates were dispersed in the midgut. Of the 905 uninfected females, five had well-digested ("late") blood meals and 51 had recent ones, and most of the latter were captured in the hamster-baited traps.

No infections were found in other sand fly species, but blood meals were found in six out of 156 *Lu. antunesi* (Table S1).

One of the four *Leishmania* cultures from *Lu. flaviscutellata* was positive, and this was identified as *Le. amazonensis* by the amplification of a c. 350 bp Mini-exon DNA fragment by PCR. The gut contained vestiges of a late blood meal, and the fly was captured in December 2014, making at least one out of four infections suprapylarian.

The *Le. amazonensis* strain was deposited in the *Leishmania* collection of the Leishmaniasis Laboratory Prof. Dr Ralph Lainson (Instituto Evandro Chagas) under the reference code IFLA/BR/2014/M31165.

The *in vivo* isolation trials were negative, as no hamster developed clinical symptoms during quarantine.

Efficiency of sand fly surveys - For each of the two abundant species, *Lu. flaviscutellata* (Table S2) and *Lu. antunesi* (Table S3), the presences or absences in trap sites were tabulated for each of the four transects and the results were partitioned by month of visit, ecotope, trap type and trap site. There were significant differences in the probability of detecting the presence of *Lu. flaviscutellata* among different months of visit (X^2=22.163; df=2; p < 0.001), different trap types (X^2=25.025; df=1; p < 0.001) and different trap sites (X^2=18.209; df=3; p < 0.001), but not between ecotopes (X^2=3.785; df=2; p = 0.151). Similarly, there were significant differences in the probability of detecting the presence of *Lu. antunesi* among different months of visit (X^2=22.163; df=2; p < 0.001), different trap types (X^2=22.188; df=1; p < 0.001) and different trap sites (X^2=7.587; df=3; p < 0.055), but not between ecotopes (X^2=4.1697; df=2; p = 0.124).

DISCUSSION

Likelihood of Lu. flaviscutellata *transmitting* Le. amazonensis i*n different ecotopes and seasons in the Bragança region* - This vector is widely distributed across Amazonia including Pará State, where there are records of its occurrence in 43 municipalities (Carvalho et al. 2015). Results shown here agree with previous observations of its sylvatic habits and the higher attractiveness of animal-baited traps compared to light traps (Shaw & Lainson 1968, Dorval et al. 2007, 2010). Captures in “Igapó” forest fragments showed 5.7 sand flies per trap-night when compared to 5.3 in “Capoeira” woodlands. Previous studies have shown higher species richness and abundance in “Terra firme” forests in Tefé, Amazonas State (Pereira Junior et al. 2015) and in Paragominas, a municipality in northeast Pará State, where secondary "Terra firme" forest fragments had higher sand fly richness and abundance when compared to “Várzea” forests (Rebêlo & Oliveira-Pereira 2001). In areas close to the Jari River, which divides Pará and Amapá states, *Lu. flaviscutellata* was abundant in lowland forests during the dry season, while in the rainy season it prevailed in upland forests (Ready et al. 1983).

Shaw & Lainson (1972) suggested that the population dynamics of *Lu. flaviscutellata* near Belém, the state capital of Pará, was influenced by the effect of rainfall on the local water table, with monthly rainfall above 250 mm being harmful to the species, leading to population decline during the rainy season and growth during the dry season in flooded forests. Our observations were different, with the frequency of the vector being higher at the peak of the rainy season, in March 2015, when monthly precipitation was above 600 mm. This would be consistent with the earlier findings if the forests surveyed in Bragança had lower water tables than those close to the River Guamá near Belém. There is evidence that the species might maintain viable populations in seasonally flooded areas because its eggs are resistant to long periods of immersion (Ward & Ready 1975, Ready et al. 1983). Despite this, our data is insufficient to support a conclusive hypothesis on the seasonality of *Lu. flaviscutellata* in relation to precipitation, because sampling took place only in three isolated periods of the year. To explore effectively the effect of seasonality on the vector's populations, long-term studies must be done, such as the ones conducted in forest fragments near Belém (Shaw & Lainson 1972).

Physiological age, vector dispersal and transmission - Males with unrotated genitalia suggest the proximity of larval sites (Ready 2013). In the current study, all males of of *Lu. flaviscutellata* had rotated external genitalia, which suggests that the traps were placed away from active sites of adult emergence from pupae. The observed parous rates of females (26-52%) are consistent with previous studies in Belém, where the range was 36-63% (Lewis et al. 1970). Females of this species were gonotrophically concordant when reared in the laboratory (Ready et al. 1984), meaning they need another blood meal after egg-lay in order to transmit *Leishmania* to a new host (Forattini 1973, Ready 2013). The detection of 46% parous females in peridomestic areas indicates that they obtained a blood meal (when they could have become infected), survived oviposition and might have been captured when actively searching for a new blood source, as evidenced by their capture in rodent-baited Disney traps. Like the single natural infection with *Le. amazonensis* detected by us, all previous infections were found in forest or forest fragments near Belém (Shaw & Lainson, 1968) or elsewhere in Pará State (Ward et al. 1973, Arias et al. 1985, Ryan et al. 1987), and Mato

Grosso do Sul State (Brilhante et al. 2015). This indicates a higher risk of transmission to humans when they enter the forest, and not close to their residences. The absence of blood meals in the midgut of three of our four infected females is additional evidence of the vector competence of *Lu. flaviscutellata*, because it indicates parasites were acquired in a previous blood meal, successfully escaped the peritrophic membrane and colonized the midgut, ready for their transmission to the next mammalian host. There is also evidence that this sand fly transmits Pacui virus in the forests near Belém (Aitken et al. 1975).

Lainson et al. (1994) suggested the hypothesis that *Lu. flaviscutellata* would become progressively better adapted to environments modified by humans, based on its presence in a range of primary and secondary forest types (Shaw & Lainson 1972). Near the River Jari in north Pará, this vector was abundant in plantations of small exotic trees (African *Gmelina* species and Caribbean Pine), which might be ecologically similar to clearings in primary forests (Ready et al. 1983). More recently, the vector was detected in peridomestic areas in several Brazilian localities outside the Amazon (Andrade Filho et al. 2001, Oliveira et al. 2003, 2006, Missawa & Dias 2007, Nunes et al. 2008, Andrade et al. 2009, Rebêlo et al. 2010, Vilela et al. 2011, 2013, Queiroz et al. 2012, Carvalho et al. 2013, Brito et al. 2014). The current results indicate that the vector is more abundant in sylvatic environments. Other comparative surveys found higher abundances in sylvatic compared with peridomestic ecotopes (Andrade et al. 2009, Almeida et al. 2013, Campos et al. 2013, Carvalho et al. 2013, Ramos et al. 2014). To date, there is no concrete evidence that this species might establish self-perpetuating colonies in peridomestic areas.

These observations are consistent with the source-sink metapopulation model of Pulliam (1988). This assumes the existence of habitat spots where population growth rate is positive (sources), from where individuals emigrate to other habitat spots, with negative growth rates (sinks). Populations of *Lu. flaviscutellata* in forest fragments would be sources, where natural resources are high, while peridomestic areas would be sink populations. Environmental impacts, such as deforestation, habitat fragmentation, and introduction of domestic animals would be the controlling agents of the dynamics between source and sink populations, as suggested for *Lutzomyia (Nyssomyia) neivai* (Quintana et al. 2012). Our observations of higher parous rates in the forest and absence of gravid females in peridomestic areas supports this interpretation. It also matches a sylvatic transmission cycle of *Le. amazonensis* involving rodent and marsupial hosts

(Lainson & Shaw 1968, 1969, 2005). The main determinant of the relative rarity of human cases caused by this parasite, compared with those caused by *Le. braziliensis*, is likely to be the behaviour of *Lu. flaviscutellata*, which exhibits low anthropophily (Lainson & Shaw 1968, Lainson et al. 1994, Silveira 2009, Dorval et al. 2007, 2010).

Likelihood of Lu. antunesi *transmitting* Leishmania *species in different ecotopes and seasons in the Bragança region* - The second-most abundant species, *Lu. antunesi*, has been suspected of transmitting *Le. lindenbergi* to humans (Silveira et al. 2002, Lainson & Shaw 2005). This parasite was described after discovery in a military training area in the outskirts of Belém, during a small CL outbreak in 1996 (Silveira et al. 2002). It predominated in forest fragments where infected soldiers used to train, and the vector demonstrated high anthropophily, but natural infections of *Le. lindenbergi* were not detected at the time (Silveira et al. 2002). Since then, there have been sporadic records of human CL caused by *Le. lindenbergi* in municipalities surrounding Belém, such as Benevides and Santa Bárbara do Pará, but there is no evidence of this parasite occurring in the Bragança region. Despite the dissection of 156 females of *Lu. antunesi*, no natural infections by *Leishmania* were found. Nevertheless, it is important that future studies evaluate the importance of this sand fly because of its high abundance in the area.

Presence of the vector of VL in the Bragança region - The detection of three females of *Lu. longipalpis* in "Igapó" forest in Tracuateua municipality was a novel record, but not unexpected given its widespread presence in sylvatic and peridomestic ecotopes in the state (Lainson & Rangel 2005). The detection of this VL vector characterizes the municipality as receptive for the disease, according to the stratification criteria of Brazilian Ministry of Health (Brasil 2014). The municipality should be surveyed not only for the presence of *Lu. longipalpis* but also for the occurrence of human and canine cases of VL. This VL vector, unlike *Lu. flaviscutellata*, is likely to establish self-perpetuating colonies in peridomestic areas (Feliciangeli 2004, Lainson & Rangel 2005, Salomón et al. 2015).

Optimizing ecological surveys and epidemiological surveillance of Lu. flaviscutellata *and other sand fly vectors in northeast Pará State* - All four infected females were captured in forest fragments where residents work on manioc flour production. The current results lead to the conclusion that the residents of Tracuateua are at risk of infection by *Le. amazonensis* by the bite of *Lu. flaviscutellata* in a sylvatic and occupational transmission pattern. The Brazilian Ministry of Health recommends that health education activities are performed in such areas, to raise awareness of local residents and to supply them with information on adoptable measures to reduce human/vector contact. The CL surveillance manual recommends Disney traps in transmission areas of *Le. amazonensis* as a preferred method for monitoring the vector populations (Brasil 2017). However, the causative agent of CL is not usually typed to species. Epidemiological surveys by Health Departments must be cost-effective, and maybe Disney traps are not routinely used because of the need to train staff to maintain hamsters in laboratory colonies and in the field. Our survey showed that using the right trap is more important than surveying different ecotopes. With Disney traps, the probability of detection of *Lu. flaviscutellata* was three times higher compared with CDC miniature light traps (46% presence *versus* 15%), and this was highly significant ($p < 0.001$).

We were able to select an area for surveying *Lu. flaviscutellata*, based on the expert knowledge of the Evandro Chagas Institute staff concerning the landscape epidemiology of the transmission cycle near Belém and the known distribution of the parasite in Pará (Brasil 2017). The challenge is to make available this expertise to regional public health teams, together with recommendations for improving surveys of *Lu. flaviscutellata* as part of integrated epidemiological surveillance of CL. Our recommendations include the need to focus vector surveys with Disney traps on forest fragments where people are working, because *Lu. flaviscutellata* rarely visits peridomestic areas in the region. Such surveys should be performed preferably during the seasonal peak of the vector, in order to identify high-risk villagers and local communities that should receive recommendations for individual protection against sand fly bites, as determined by the CL surveillance manual (Brasil 2017). Further field studies are required to make model-based predictions of seasonal variations in the vectorial capacity of sylvatic populations of the vector.

ACKNOWLEDGEMENTS

To the following in the Leishmaniasis Laboratory Prof. Dr Ralph Lainson, Instituto Evandro Chagas, Belém: Fábio Medeiros for assistance with field work; and, Patricia Karla Ramos and Ana Camila Alves for the characterization of *Leishmania* strains.

AUTHORS' CONTRIBUTIONS

BMC, EFR, PDR and MMV conceived the study; BMC, TVS, IRB, JANL and FTS collected the data; BMC and PDR analysed the data and drafted the manuscript; and all authors read and approved the final manuscript.

REFERENCES

Aitken, T.H.G, J.P. Woodall, A.H.P. De Andrade, G. Bensabath, and R.E. Shope. 1975. Pacui virus, phlebotomine flies and small mammals in Brazil: an epidemiological study. Am. J. Trop. Med. Hyg. 24(2): 358-368.

Almeida, P.S., J.A. Leite, A.D. Araújo, P.M. Batista, R.B.S. Touro, V.S. Araújo, et al. 2013. Fauna of phlebotomine sand flies (Diptera, Psychodidae) in areas with endemic American cutaneous leishmaniasis in the State of Mato Grosso do Sul, Brazil. Rev. Bras. Entomol. 57(1): 105-112.

Andrade, A.R.O., V.L.B. Nunes, E.A.B. Galati, C.C.P. Arruda, M.F.C. Santos, M.E.G. Rocca, et al. 2009. Epidemiological study on leishmaniasis in an area of environmental tourism and ecotourism, State of Mato Grosso do Sul, 2006-2007. Rev. Soc. Bras. Med. Trop. 42(5): 488-493.

Andrade Filho, J.D., M.B. Valente, W.A. Andrade, R.P. Brazil, and A.L. Falcão. 2001. Flebotomíneos do Estado de Tocantins, Brasil (Diptera: Psychodidae). Rev. Soc. Bras. Med. Trop. 34(4): 323-329.

Arias, J.J., M.A. Miles, R.D. Naiff, M.M. Povoa, R.A. De Freitas, C.B. Biancardi, et al. 1985. Flagellate infections of Brazilian sand flies (Diptera: Psychodidae): Isolation in vitro and biochemical identification of Endotrypanum and Leishmania. Am. J. Trop. Med. Hyg. 34(6): 1098-1108.

Brasil. Ministério Da Saúde. 2017. *Manual de Vigilância da Leishmaniose Tegumentar Americana*. 2nd ed. Editora do Ministério da Saúde, Brasília.

Brasil. Ministério Da Saúde. 2014. *Manual de Vigilância e Controle da Leishmaniose Visceral*. 1st ed. Editora do Ministério da Saúde, Brasília.

Brilhante, A.F., V.L.B. Nunes, K.A. Kohatsu, E.A.B. Galati, M.E.G. Rocca, and E.A.Y. Ishikawa. 2015. Natural infection of phlebotomines (Diptera: Psychodidae) by

Leishmania (Leishmania) amazonensis in an area of ecotourism in Central-Western Brazil. J. Venom. Anim. Toxins Incl. Trop. Dis. 21: 39.

Brito, V.N., A.B.P.F. De Almeida, L. Nakazato, R. Duarte, C.O. Souza, and V.R.F. Sousa. 2014. Phlebotomine fauna, natural infection rate and feeding habits of *Lutzomyia cruzi* in Jaciara, state of Mato Grosso, Brazil. Mem. Inst. Oswaldo Cruz. 109(7): 899-904.

Campos, A.M., R. Matavelli, C.L.C. Dos Santos, L.S. Moraes, and J.M.M. Rebêlo. 2013 Ecology of Phlebotomines (Diptera: Psychodidae) in a Transitional Area between the Amazon and the Cerrado in the State of Maranhão, Brazil. J. Med. Entomol. 50(1): 52-58.

Carvalho, B.M., M. Maximo, W.A. Costa, A.L.F. Santana, S.M. Costa, T.A.N.C. Rego, et al. 2013. Leishmaniasis transmission in an ecotourism area: potential vectors in Ilha Grande, Rio de Janeiro State, Brazil. Parasit. Vectors. 6: 325.

Carvalho, B.M., E.F. Rangel, P.D. Ready, and M.M. Vale. 2015. Ecological niche modelling predicts southward expansion of *Lutzomyia (Nyssomyia) flaviscutellata* (Diptera: Psychodidae: Phlebotominae), vector of *Leishmania (Leishmania) amazonensis* in South America, under climate change. PLoS ONE. 10(11): e0143282.

Disney, R.H.L. 1966. A trap for phlebotomine sandflies attracted to rats. Bull. Entomol. Res. 56: 445-451.

Dorval, M.E.C., T.P. Alves, G. Cristaldo, H.C. Da Rocha, M.A. Alves, E.T. Oshiro, et al. 2010. Sand fly captures with Disney traps in area of occurrence of *Leishmania (Leishmania) amazonensis* in the State of Mato Grosso do Sul, mid-western Brazil. Rev. Soc. Bras. Med. Trop. 43(5): 491-495.

Dorval, M.E.C., T.P. Alves, A.G. Oliveira, R.P. Brazil, E.A.B. Galati, and R.V. Cunha. 2007. Modification of Disney trap for capture of sand flies (Diptera: Psychodidae: Phlebotominae). Mem. Inst. Oswaldo Cruz. 102(7): 877-878.

Feliciangeli, M.D. 2004. Natural breeding places of phlebotomine sandflies. Med. Vet. Entomol., 18: 71-80.

Fernandes, O., V.K. Murthy, U. Kurath, W.M. Degrave, and D.A. Campbell. 1994. Mini-exon gene variation in human pathogenic *Leishmania* species. Mol. Biochem. Parasitol. 66(2): 261-271.

Forattini, O.P. 1973. *Entomologia médica 4º Volume: Psychodidae. Phlebotominae. Leishmanioses. Bartonelose*. Edgard Blücher, São Paulo.

IBGE. Instituto Brasileiro de Geografia e Estatística. 2017. *Mapa de solos do Brasil.* Available: <https://mapas.ibge.gov.br/tematicos/solos>. Accessed: 20 Jul. 2017.

INMET. Instituto Nacional De Meteorologia. Banco de Dados Meteorológicos para Ensino e Pesquisa. 2015. Available: <http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep>. Accessed: 12 Dec. 2015.

Lainson, R., and E.F. Rangel. 2005. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. Mem. Inst. Oswaldo Cruz. 100(8): 811-827.

Lainson, R., and J.J. Shaw. 1968. Leishmaniasis in Brazil I. Observations on enzootic rodent leishmaniasis - Incrimination of *Lutzomyia flaviscutellata* (Mangabeira) as the vector in the lower Amazonian Basin. Trans. R. Soc. Trop. Med. Hyg. 62: 385-395.

Lainson, R. and J.J. Shaw. 1969. Leishmaniasis in Brazil III. Cutaneous leishmaniasis in an opossum, *Marmosa murina* (Marsupialia, Didelphidae) from the lower Amazon region. Trans. R. Soc. Trop. Med. Hyg. 63(6): 738-740.

Lainson, R. and J.J. Shaw. 2005. New World Leishmaniasis. In: L. Collier, A. Balows, and M. Sussman (eds.) *Topley & Wilson's Microbiology and Microbial Infections, 10th ed., Vol 5: Parasitology*. Hodder Arnold, London.

Lainson, R., J.J. Shaw, F.T. Silveira, A.A. De Souza, R.R. Braga, and E.A. Ishikawa. 1994. The dermal leishmaniases of Brazil, with special reference to the eco-epidemiology of the disease in Amazonia. Mem. Inst. Oswaldo Cruz. 89(3): 435-443.

Lewis, D.J., R. Lainson, J.J. Shaw. 1970. Determination of parous rates in Phlebotomine sandflies with special reference to Amazonian species. Bull. Entomol. Res. 60: 209-219.

Missawa, N.A., and E.S. Dias. 2007. Phlebotomine sand flies (Diptera: Psychodidae) in the municipality of Várzea Grande: an area of transmission of visceral leishmaniasis in the state of Mato Grosso, Brazil. Mem. Inst. Oswaldo Cruz. 102(8): 913-918.

Nunes, V.L.B., E.A.B. Galati, C. Cardozo, M.E.G. Rocca, A.R.O. De Andrade, M.F.C. Santos, et al. 2008. Estudo de flebotomíneos (Diptera, Psychodidae) em área urbana do município de Bonito, Mato Grosso do Sul, Brasil. Rev. Bras. Entomol. 52(3): 446-451.

Oliveira, A.G., J.D. Andrade Filho, A.L. Falcão, R.P. Brazil. 2003. Estudo de flebotomíneos (Diptera, Psychodidae, Phlebotominae) na zona urbana da Cidade de Campo Grande, Mato Grosso do Sul, Brasil, 1999-2000. Cad. Saude Publica. 19(4): 933-944.

Oliveira, A.G., E.A.B. Galati, O. Oliveira, G.R. Oliveira, I.A.C. Espindola, M.E.C. Dorval, et al. 2006. Abundance of *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae) and urban transmission of visceral leishmaniasis in Campo Grande, state of Mato Grosso do Sul, Brazil. Mem. Inst. Oswaldo Cruz. 101(8): 869-874.

Pereira Junior, A.M., C.B.G. Teles, A.P.A. Dos Santos, M.S. Rodrigues, E.F. Marialva, F.A.C. Pessoa, et al. 2015. Ecological aspects and molecular detection of *Leishmania* DNA Ross (Kinetoplastida: Trypanosomatidae) in phlebotomine sandflies (Diptera: Psychodidae) in terra firme and várzea environments in the Middle Solimões Region, Amazonas State, Brazil. Parasit. Vectors. 8: 180.

Pulliam, H.R. 1988. Sources, Sinks, and Population Regulation. Am. Nat. 132: 652-661.

Queiroz, M.F.M., J.R. Varjão, S.C. De Moraes, G.E. Salcedo. 2012. Analysis of sandflies (Diptera: Psychodidae) in Barra do Garças, State of Mato Grosso, Brazil, and the influence of environmental variables on the vector density of *Lutzomyia longipalpis* (Lutz & Neiva, 1912). Rev. Soc. Bras. Med. Trop. 45(3):313-317.

Quintana, M.G., M.S. Fernández, and O.D. Salomón. 2012. Distribution and abundance of Phlebotominae, vectors of leishmaniasis, in Argentina: spatial and temporal analysis at different scales. J. Trop. Med. 2012: 652803.

Ramos, W.R., J.F. Medeiroe, G.R. Julião, C.M. Ríos-Velásquez, E.F. Marialva, S.J.M. Desmouliére, et al. 2014. Anthropic effects on sand fly (Diptera: Psychodidae) abundance and diversity in an Amazonian rural settlement, Brazil. Acta Trop. 139: 44-52.

Rangel, E.F., and R. Lainson. 2009. Proven and putative vectors of American cutaneous leishmaniasis in Brazil: aspects of their biology and vectorial competence. Mem. Inst. Oswaldo Cruz. 104(7): 937-954.

Rangel, E.F., S.M. Costa, and B.M. Carvalho. 2014. Environmental changes and the geographic spreading of American cutaneous leishmaniasis in Brazil. In: D. Claborn (ed.) *Leishmaniasis – Trends in Epidemiology, Diagnosis and Treatment*. InTech, Rijeka.

R Core Team. 2017. *R: A language and environment for statistical computing*. R foundation for Statistical Computing, Vienna, Austria. Available: <http://www.R-project.org/.> Accessed: 13 Apr. 2017.

Ready, P.D. 2013 Biology of phlebotomine sand flies as vectors of disease agents. Annu. Rev. Entomol. 58: 227-250.

Ready, P.D. R. Lainson, and J.J. Shaw. 1983. Leishmaniasis in Brazil. XX: Prevalence of "enzootic rodent leishmaniasis" (*Leishmania mexicana amazonensis*) and apparent absence of pian-bois (*Le. braziliensis guyanensis*), in plantations of introduced tree species and in other non-climax forests in eastern Amazonia. Trans. R. Soc. Trop. Med. Hyg. 77: 775-785.

Ready, P.D., R. Lainson, T.J. Wilkes, and R. Killick-Kendrick. 1984. On the accuracy of age-grading neotropical phlebotomines by counting follicular dilatations: first laboratory experiments, using colonies of *Lutzomyia flaviscutellata* (Mangabeira) and *L. furcata* (Mangabeira) (Diptera: Psychodidae). Bull. Entomol. Res. 74: 641-646.

Rebêlo, J.M.M., A.N. Assunção Júnior, O. Silva, and J.L.P. Moraes. 2010. Ocorrência de flebotomíneos (Diptera, Psychodidae) em focos de leishmanioses, em área de ecoturismo do entorno do Parque Nacional dos Lençóis Maranhenses, Brasil. Cad. Saude Publica. 26(1): 195-198.

Rebêlo, J.M.M., and Y.N. Oliveira-Pereira. 2001. Flebotomíneos (Diptera, Psychodidae) de matas de terra firme e de várzea, do município de Paragominas, estado do Pará, Brasil. Acta Amaz. 31(1): 145-154.

Ryan, L., R. Lainson, and J.J. Shaw. 1987. Leishmaniasis in Brasil. XXIV. Natural flagellate infections of sandflies (Diptera: Psycodidae) in Pará State, with particular reference to the role of *Psychodopygus wellcomei* as the vector of *Leishmania braziliensis* in the Serra dos Carajás. Trans. R. Soc. Trop. Med. Hyg. 81: 353-359.

Salomón, O.D., M.D. Feliciangeli, M.G. Quintana, M.M.S. Afonso, and E.F. Rangel. 2015. *Lutzomyia longipalpis* urbanisation and control. Mem. Inst. Oswaldo Cruz. 110(7): 831-846.

Shaw, J.J., and R. Lainson. 1968. Leishmaniasis in Brazil: II. Observations on enzootic rodent leishmaniasis in the lower Amazon region – the feeding habits of the vector, *Lutzomyia flaviscutellata*, in reference to man, rodents and other animals. Trans. R. Soc. Trop. Med. Hyg. 62(3): 396-405.

Shaw, J.J., and R. Lainson. 1972. Leishmaniasis in Brazil: VI. Observations on the seasonal variations of *Lutzomyia flaviscutellata* in different types of forest and its relationship to enzootic rodent leishmaniasis (*Leishmania mexicana amazonensis*). Trans. R. Soc. Trop. Med. Hyg. 66(5): 709-717.

Shaw, J.J., R. Lainson, and R.D. Ward. 1972. Leishmaniasis in Brazil. VII. Further observations on the feeding habitats of *Lutzomyia flaviscutellata* (Mangabeira) with particular reference to its biting habits at different heights. Trans. R. Soc. Trop. Med. Hyg. 66(5): 718-723.

Silveira, F.T. 2009. Leishmaniose cutânea difusa (LCD) na Amazônia, Brasil: aspectos clínicos e epidemiológicos. Gaz. Med. Bahia 79(Supl.3): 25-29.

Silveira, F.T., E.A. Ishikawa, A.A. De Souza, and R. Lainson. 2002. An outbreak of cutaneous leishmaniasis among soldiers in Belém, Pará state, Brazil, caused by *Leishmania (Viannia) lindenbergi* n.sp. A new leishmanial parasite of man in the Amazon Region. Parasite. 9: 43-50.

SINAN. *Sistema de Informação de Agravos de Notificação*. 2017. Available: <http://www2.datasus.gov.br/DATASUS/index.php?area=0203&id=29878153>. Accessed: 20 Sep. 2017.

Sudia, W.D., and R.W. Chamberlain. 1962. Battery operated light trap, an improved model. Mosq. News. 22: 126-129.

Vilela, M.L., C.G. Azevedo, B.M. Carvalho, and E.F. Rangel. 2011. Phlebotomine fauna (Diptera: Psychodidae) and putative vectors of leishmaniases in impacted area by hydroelectric plant, state of Tocantins, Brazil. PLoS ONE. 6(12): e27721.

Vilela, M.L., D. Pita-Pereira, C.G. Azevedo, R.E. Godoy, C. Britto, and E.F. Rangel. 2013. The phlebotomine fauna (Diptera: Psychodidae) of Guaraí, state of Tocantins, with an emphasis on the putative vectors of American cutaneous leishmaniasis in rural settlement and periurban areas. Mem. Inst. Oswaldo Cruz. 108(5): 578-585.

Ward, R.D., R. Lainson, and J.J. Shaw. 1973. Further evidence of the rôle of *Lutzomyia flaviscutellata* (Mangabeira) as the vector of *Leishmania mexicana amazonensis* in Brazil. Trans. R. Soc. Trop. Med. Hyg. 67(4): 608-609.

Ward, R.D., and P. Ready. 1975. Chorionic sculpturing in some sandfly eggs (Diptera: Psychodidae). J. Entomol. Series A. 50: 127-134.

Young, D.G., and M.A. Duncan. 1994. Guide to the identification and geographic distribution of *Lutzomyia* sandflies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). Mem. Am. Entomol. Inst. 54: 1-881.

TABLES

Table 1: Character states of biological structures observed in dissected female sand flies.

Structure	Character state observed	Interpretation	Reference
Ovaries	Contracted	Nulliparous female	Lewis et al. 1970;
	Distended	Parous female	Ready et al. 1984.
Eggs	Light colour, relatively short	Immature eggs	Forattini et al. 1973
	Dark colour, relatively long	Mature eggs	
Blood meal	Enlarged midgut with red blood	Recent blood meal	Forattini et al. 1973
	Normal midgut with dark blood remains	Late blood meal	
Presence of flagellates	Located in the hindgut	Suggestive of *Leishmania (Viannia)* species	Lainson & Shaw 2005
	Located in the foregut and/or midgut only	Suggestive of *Leishmania (Leishmania)* species	

Table 2: Sand fly species and number of individuals by ecotope and gender captured in the Bragança region in July 2014, December 2014 and March 2015.

Species	**"Igapó" Forest**		**"Capoeira" Forest**		**Peridomestic areas**		**Total**		
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M+F**
*Lutzomyia flaviscutellata**	45	571	47	285	7	56	99	912	1,011
*Lutzomyia antunesi**	88	209	10	29	5	15	103	253	356
Lutzomyia gomezi	0	2	0	3	0	1	0	6	6
Lutzomyia sordellii	0	5	0	0	1	0	1	5	6
Lutzomyia furcata	0	4	0	0	0	0	0	4	4
*Lutzomyia longipalpis**	0	3	0	0	0	0	0	3	3
Lutzomyia carrerai carrerai	0	2	0	0	0	0	0	2	2
Lutzomyia infraspinosa	0	2	0	0	0	0	0	2	2
Lutzomyia bacula	0	1	0	0	0	0	0	1	1
Lutzomyia micropyga	0	1	0	0	0	0	0	1	1
Lutzomyia trinidadensis	0	0	0	0	1	0	1	0	1
Brumptomyia sp.	0	0	0	1	0	0	0	1	1
Total	**133**	**800**	**57**	**318**	**14**	**72**	**204**	**1,190**	**1,394**
	933		**375**		**86**				

M: males; F: females

* Species incriminated as vectors elsewhere in Brazil.

Table 3: Sand fly frequency (specimens per trap-night) by ecotope and trap type captured in the Bragança region in July 2014, December 2014 and March 2015.

Species	**"Igapó" Forest**		**"Capoeira" Forest**		**Peridomestic areas**		**Total**		***p*-value***
	CDC	**Disney**	**CDC**	**Disney**	**CDC**	**Disney**	**CDC**	**Disney**	
Lu. flaviscutellata	0.58	10.64	0.42	9.97	0.07	2.03	0.40	8.25	0.171
Lu. antunesi	5.60	0.00	1.23	0.03	0.63	0.03	3.11	0.02	0.255
Lu. gomezi	0.04	0.00	0.10	0.00	0.03	0.00	0.05	0.00	0.380
Lu. sordellii	0.09	0.00	0.00	0.00	0.03	0.00	0.05	0.00	0.706
Lu. furcata	0.08	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.099
Lu. longipalpis	0.06	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.568
Lu. carrerai carrerai	0.04	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.320
Lu. infraspinosa	0.04	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.568
Lu. bacula	0.02	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.568
Lu. micropyga	0.02	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.568
Lu. trinidadensis	0.00	0.00	0.00	0.00	0.03	0.00	0.01	0.00	0.241
Brumptomyia sp.	0.00	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.265
Total	**6.57**	**10.64**	**1.77**	**10**	**0.80**	**2.07**	**3.75**	**8.26**	
	8.64		**5.95**		**1.43**				

*Analysis of variance (ANOVA) of sand fly frequencies between ecotopes.

Table 4: Number of nulliparous and parous females and parous rates of sand flies captured in different ecotopes in the Bragança region in July and December 2014.

Species	**"Igapó" Forest**			**"Capoeira" Forest**			**Peridomestic areas**			***p*-value***
	NP	**P**	**%**	**NP**	**P**	**%**	**NP**	**P**	**%**	
Lu. flaviscutellata	97	34	26%	14	15	52%	7	6	46%	0.013**
Lu. antunesi	12	4	25%	10	3	23%	1	0	0	0.848
Lu. bacula	1	0	0	0	0	0	0	0	0	n.a.
Lu. carrerai carrerai	1	0	0	0	0	0	0	0	0	n.a.
Lu. infraspinosa	1	0	0	0	0	0	0	0	0	n.a.
Total	**112**	**38**	**25%**	**24**	**18**	**43%**	**8**	**6**	**43%**	

NP: nulliparous; P: parous; %: parous rate (P/NP+P); n.a.: not applicable.

*Chi-square test of parous rates between ecotopes.

**Number of parous/nulliparous females is statistically different between ecotopes.

Table 5: Number of female sand flies with eggs at different developmental stages collected in three ecotopes in the Bragança region in July and December 2014.

Species	**"Igapó" Forest**			**"Capoeira" Forest**			**Peridomestic areas**			**Total**		
	A	**I**	**M**	**A**	**I**	**M**	**A**	**I**	**M**	**A**	**I**	**M**
Lu. flaviscutellata	178	2	1	35	0	0	19	0	0	232	2	1
Lu. antunesi	32	1	3	26	0	1	2	0	0	60	1	4
Lu. carrerai carrerai	1	1	1	0	0	0	0	0	0	1	1	1
Lu. gomezi	1	1	0	0	0	0	0	0	1	1	1	1
Lu. infraspinosa	2	0	0	0	0	0	0	0	0	2	0	0
Lu. bacula	1	0	0	0	0	0	0	0	0	1	0	0
Lu. furcata	1	0	0	0	0	0	0	0	0	1	0	0
Total	**216**	**5**	**5**	**61**	**0**	**1**	**21**	**0**	**1**	**298**	**5**	**7**

A: eggs absent; I: immature eggs; M: mature eggs.

T1: Vila Martins, Tracuateua; T2: Alto Alegre, Bragança; T3: Almoço, Bragança; T4: Vila Martins, Tracuateua.

T1: Vila Martins, Tracuateua; T2: Alto Alegre, Bragança; T3: Almoço, Bragança; T4: Vila Martins, Tracuateua.

FIGURE LEGENDS

Figure 1: Location of study region, trapping sites and other named localities in Pará State.

Figure 2: Images of sites typical of the three ecotopes surveyed in the Bragança region. A) Igapó forest, B) Capoeira woodland, C) peridomestic area.

Figure 3: Images of the two trap types set in typical locations. A) Modified Disney trap, B) CDC miniature light trap.

Figure 4: Frequencies of *Lu. flaviscutellata* and *Lu. antunesi* in sites sampled during the three visits to the Bragança region, and the monthly precipitation recorded in Tracuateua municipality.