

## Accepted Manuscript

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PII: S0001-706X(15)30128-5  
DOI: <http://dx.doi.org/doi:10.1016/j.actatropica.2015.10.005>  
Reference: ACTROP 3749

To appear in: *Acta Tropica*

Received date: 14-2-2015  
Revised date: 5-10-2015  
Accepted date: 6-10-2015

Please cite this article as: Paternina, Luís E., Verbel-Vergara, Daniel, Romero-Ricardo, Luís, Pérez-Doria, Alveiro, Paternina-Gómez, Margaret, Martínez, Lily, Bejarano, Eduar E., Evidence for anthropophily in five species of phlebotomine sand flies (Diptera: Psychodidae) from northern Colombia, revealed by molecular identification of bloodmeals. *Acta Tropica* <http://dx.doi.org/10.1016/j.actatropica.2015.10.005>

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Evidence for anthropophily in five species of phlebotomine sand flies (Diptera: Psychodidae) from northern Colombia, revealed by molecular identification of bloodmeals

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## Graphical Abstract



## Highlights

- Ten species of vertebrates provided sources of blood for eight species of phlebotomine sand flies.
- Anthropophily was determined for *Lu. evansi*, *Lu. panamensis*, *Lu. micropyga*, *Lu. shannoni* and *Lu. atroclavata*.
- Cattle, donkeys, humans and pigs were the principal sources of blood for *Lu. evansi*.

## ABSTRACT

Identification of the bloodmeal sources of phlebotomine sand flies is fundamental to determining which species are anthropophilic and understanding the transmission of *Leishmania* parasites in natural epidemiological settings. The objective of this study was to identify sand fly bloodmeals in the mixed leishmaniasis focus of the department of Sucre, northern Colombia. In all 141 engorged female sand flies were analyzed, after being captured in intradomiciliary, peridomiciliary and extradomiciliary habitats with Shannon and CDC traps and by active searching in diurnal resting sites. Bloodmeals were identified by sequencing and analysis of a 358 bp fragment of the mitochondrial gene Cytochrome b (CYB) and a 330 bp fragment of the nuclear gene prepronociceptin (PNOC). Using both genes 105 vertebrate bloodmeals were identified, with an efficiency of 72% for CYB but only 7% for PNOC. Ten species of vertebrates were identified as providing bloodmeal sources for eight sand fly species: *Homo sapiens* (*Lutzomyia evansi*, *Lu. panamensis*, *Lu. micropyga*, *Lu. shannoni* and *Lu. atroclavata*), *Equus caballus* (*Lu. evansi*, *Lu. panamensis* and *Lu. cayennensis cayennensis*), *Eq. asinus* (*Lu. evansi* and *Lu. panamensis*), *Bos taurus*

(*Lu. evansi*, *Lu. panamensis* and *Lu. c. cayennensis*), *Tamandua mexicana* (*Lu. shannoni* and *Lu. trinidadensis*), *Proechimys guyanensis* (*Lu. evansi*, *Lu. panamensis* and *Lu. c. cayennensis*), *Mabuya* sp. (*Lu. micropyga*), *Sus scrofa* (*Lu. evansi* and *Lu. gomezi*) and *Gallus gallus* (*Lu. evansi*). Cattle, donkeys, humans and pigs were significantly more important than other animals ( $P = 0.0001$ ) as hosts of *Lu. evansi*, this being the most abundant sand fly species. The five *Lutzomyia* species in which blood samples of human origin were detected included *Lu. micropyga* and *Lu. atroclavata*, constituting the first evidence of anthropophily in both species.

Keywords: Lutzomyia; Sandflies; Bloodmeals; Molecular analysis; Leishmaniasis; Colombia

## 1. Introduction

Leishmaniasis is the second most important protozoan disease of man worldwide, exceeded only by malaria. It is however categorized by the World Health Organization as one of the neglected tropical diseases (WHO, 2010a). Leishmaniasis is present in most of Colombia, cutaneous leishmaniasis (CL) being the most frequent form, accounting for 98% of all cases (Zambrano & Gutiérrez, 2011). More than 10 million people in Colombia are at risk of contracting the disease, with 17,420 new cases per year recorded between 2005 and 2009 although the true annual incidence is estimated at between 48,800 and 80,100 cases (Alvar *et al.*, 2012).

One of the most important macrofoci of leishmaniasis in Colombia encompasses the departments of Bolívar, Córdoba and Sucre, accounting for 83% of CL cases from the country's Caribbean region. This macrofocus is also responsible for 71% of Colombian visceral leishmaniasis (VL) cases as well as sporadic reports of mucocutaneous leishmaniasis (MCL) (INS, 2005–2014). The dominant sand fly species is *Lu. evansi* (Pérez-Doria *et al.*, 2008b), already recognised as a vector of *Le. infantum* (Travi *et al.*, 1996; Cocheró 2002), although at least four different *Leishmania* species circulate in the area (Martínez *et al.*, 2010) and its phlebotomine fauna includes 29 species of *Lutzomyia* (Bejarano & Estrada, 2015). Recently, *Lu. evansi* was found naturally infected with *Le. braziliensis* (Bejarano *et al.*, 2012), suggesting it could be associated with transmission of this parasite in the region.

Although *Lu. evansi* bites humans, pigs, donkeys, chickens, opossums and dogs in the region (Vélez *et al.*, 1995; Montoya-Lerma & Lane, 1996; Cocheró, 2002), it is not known

what other species of animals act as sources of bloodmeals for this vector. Furthermore, the hosts of the less abundant *Lutzomyia* species in the region are unknown. The amplification and sequencing of vertebrate genes from engorged phlebotomine sand flies offers a sensitive and reproducible method of identifying the animals from which these insects obtained their bloodmeals. The principal advantage of sequencing relates to its high specificity, which allows unsuspected relationships between sand flies and vertebrates to be discovered. These would not be detected by serological or molecular methods that do not involve sequencing (Jaouadi *et al.*, 2013).

Only four genes have been used to date for studies of bloodmeal ingestion in sand flies, *i.e.*, the mitochondrial gene Cytochrome b (CYB), the nuclear gene coding for prepronociceptin (PNOC) and, more recently, the 12S and 16S mitochondrial rRNA genes (Valinsky *et al.*, 2014). CYB is the gene that has been more widely used in phlebotomines (Quaresma *et al.*, 2012; Maia *et al.*, 2015; Sales *et al.*, 2015), since the identification of ingested blood is facilitated by the large set of vertebrate species sequences available on GeneBank (Johns & Avise, 1998). PNOC, which has been proposed as an alternative marker has only been used sporadically in sand flies (Haouas *et al.*, 2007; Jaouadi *et al.*, 2013; Baum *et al.*, 2015). Its amplification is only viable in mammals, which have the greatest epidemiological importance as reservoirs of *Leishmania*. However before the present study the two markers had not been used together in phlebotomines.

The objective of the present study was to identify sand fly bloodmeal sources from the mixed focus of leishmaniasis in the department of Sucre, Colombia, using CYB and PNOC as markers.

## 2. Materials and methods

### 2.1. Study area

The study was carried out in the department of Sucre, northern Colombia, a focus of active transmission of both cutaneous and visceral leishmaniasis in humans. Sucre represents an epidemiological corridor between the departments of Bolívar and Córdoba, ecologically classified as being within the “Tropical Dry Forest” life zone (TdF), with a mean temperature of 27.5°C, relative humidity of 77–80% and mean annual precipitation of 1000–1300 mm (Holdridge, 1967).

Four municipalities in the department of Sucre were selected for the study: Ovejas, Los Palmitos, Sincelejo and Colosó (Figure 1). Two houses in the peri-urban area of Ovejas were selected, located in the La Paz neighbourhood (9° 31' 36.25" N, 75° 13' 38.65" W) and La Troncal sector (9° 31' 28.64" N, 75° 13' 37.25" W), inhabited by people who had clinical histories of CL. In Los Palmitos a dwelling in the El Piñal settlement was chosen (9° 27' 42.57" N, 75° 13' 12.53" W), also inhabited by people with a history of CL. In Sincelejo the study was carried out in the urban area, in two parks: “Las Iguanas” and “Scouts” of the neighbourhoods Venecia (9° 18' 17.15" N, 75° 22' 36.62" W) and Los Libertadores (9° 18' 11.55" N, 75° 23' 56.51" W) respectively, as well as in a house of the Sabanas del Potrero district (9° 15' 12.36" N, 75° 26' 15.73" W) on the outskirts of the city. The study in Colosó was carried out in the rural area, specifically the Reserva Forestal Protectora Serranía de Coraza y Montes de María (9° 31' 49.59" N, 75° 21' 04.08" W). Although no catalogue of the species of domestic and wild animals in the study areas was available, dogs, poultry and pigs were observed in the peridomiciliary area of dwellings in

Ovejas, Los Palmitos and Sabanas del Potrero. Cattle were seen in pastures in the surrounding countryside of the four municipalities sampled, together with donkeys and smaller numbers of horses, as expected given that the main economic activity of Sucre is ranching. A checklist was available for the municipality of Colosó, where 58 species of birds and 28 of wild mammals have been recorded (Galván-Guevara, 2010).

## *2.2. Sampling and taxonomic determination of phlebotomine sand flies*

In the municipalities of Ovejas and Los Palmitos phlebotomines were collected by systematic sampling between April 2010 and April 2011. Sampling was carried out using a Shannon trap which was installed one per month in the peridomicile and manned by two or three people, between 18:00 and 21:00 h (Alexander, 2000). One CDC trap was distributed indoors and two in the peridomicile of each dwelling chosen for the study and activated from 18:00 to 06:00 h, on two consecutive nights each month. Active searching for phlebotomines was done using electric and mouth aspirators between 06:00 and 12:00 h in trees used as diurnal resting sites by the insect around each dwelling. In Sincelejo and Colosó, phlebotomines were collected over shorter periods of a single day or night per sampling site. In Sincelejo an active search was carried out between 06:00 and 12:00 h, in trees used as diurnal resting sites while in Colosó a CDC trap was used, hung in the forest between 18:00 and 06:00 h.

Either on the same day or the morning after being collected, sand flies were transported dry back to the biomedical laboratory of the University of Sucre, where bloodfed specimens were separated and preserved at -20°C until they could be processed by molecular biology.

Unlike the methodology traditionally used in molecular studies of insect vectors, taxonomic determination of the species was carried out following DNA extraction. The entire body of each phlebotomine, recovered after after DNA extraction, was immersed for 24 h in lactophenol, a 1:1 (v/v) mixture of lactic acid and phenol, in order to remove the hairs and clarify the exoskeleton, allowing internal structures to be observed. Species were determined using the head and its appendages, the thorax with wings and legs, and the abdomen with the internal genital structures. The key of Galati (2011) was used but following the taxonomic classification scheme of Young & Duncan (1994) which recognises only three New World genera. Given the predominance of *Lu. evansi* in the study area, only blood-fed examples of this species from the municipalities of Ovejas and Los Palmitos were selected for the molecular analysis. For the other sand fly species all blood-fed specimens collected in the four municipalities were used.

### 2.3. *Extraction and amplification of DNA from bloodmeal sources*

Female sand flies with complete or partial bloodmeals were used indiscriminately in this study. Nucleic acid was extracted from each engorged female using the protocol described by Pérez-Doria *et al.* (2008a), with three modifications: (1) the specimen was not macerated, to allow the entire exoskeleton to be recovered for taxonomic determination, (2) proteinase K was added and (3) the preparation was incubated for 2 h at 65°C. The DNA extracted was resuspended in 60uL of ultrapure water. Bloodmeal sources were identified using the mitochondrial gene CYB and the nuclear gene which codes for PNOC. Amplification of CYB was performed with the primers L14841 (5'-CCA TCC AAC ATY

TCA DCA TGA TGA AA-3') and H15149 (5'-GCH CCT CAG AAT GAT ATT TGK CCT CA-3') which amplify a 358bp fragment in vertebrates (Kocher *et al.*, 1989), while amplification of the PNOC gene in mammals was carried out using the primers PNOCF (5'-GCA TCC TTG AGT GTG AAG AGA A-3') and PNOCR (5'-TGC CTC ATA AAC TCA CTG AAC C-3') which generate a fragment of approximately 330bp (Haouas *et al.*, 2007). During the molecular assays dog DNA was used as a positive control to evaluate whether inhibition of the PCR had occurred and ultrapure water as a negative control to determine whether there had been contamination.

#### 2.4. DNA sequencing and determination of species of vertebrates

The amplicons of the CYB and PNOC genes were submitted to DNA sequencing in both senses of the nucleic acid chain with a 3730XL capillary electrophoresis apparatus. The electrophoregrams of the nucleotide sequences were verified and edited with the MEGA 5.2 program to obtain sequences for each DNA strand and consensus sequences for each sample. Both DNA strand sequences of each bloodmeal source were analyzed independently to guarantee reproducibility of the results. The CYB and PNOC sequences were compared with those available on GeneBank (<http://www.ncbi.nlm.nih.gov/>) by means of Linnaeus BlastN with the Geneious 5.4 software. The animal species to which the blood in the sand fly guts belonged were determined by hierarchical taxonomy; by ranges of identity; and using the criteria of maximum identity, coverage and total punctuation in the multiple alignment.

## 2.5. Data analysis

A descriptive analysis of the findings was carried out, followed by a comparative analysis of the results obtained with the CYB and PNOC genes. A correspondence analysis was then carried out for which a species/species data matrix between sand flies and bloodmeal sources was constructed to represent the interrelationships detected in our study, using the programs InfoStat 2011e and PAST 2.05. The possible associations found by correspondence analysis were assigned a statistical value with the InfoStat 2011e program.

For those sand fly species that were most abundant and thus able to provide sufficient data, a Chi-Squared test ( $\chi^2$ ) was computed to establish whether there were significant differences in host utilization. In this study we preferred to use the term “host utilization” rather than “preference”, since there are multiple factors besides preference that together determine whether a sand fly exploits a particular vertebrate species as a source of blood (Kent, 2009). The human blood index (HBI) was also calculated. This compares the number of bloodmeals of human origin against the total number of bloodmeals identified.

## 3. Results

### 3.1. Identification of phlebotomine sand flies

In the four municipalities selected for the study 12,077 phlebotomines were collected, belonging to 11 species of the genus *Lutzomyia* and one of *Brumptomyia*. The most abundant species was *Lu. evansi* (90%), followed by *Lu. panamensis* (4%), *Lu. gomezi* (1.4%), *Lu. cayennensis cayennensis* (1.4%), *Lu. micropyga* (1.0%), *Lu. dubitans* (0.5%), *Lu. trinidadensis* (0.4%), *Lu. atroclavata* (0.3%), *Lu. rangelliana* (0.1), *Lu.*

*venezuelensis* (0.04%), *Lu. shannoni* (0.01), unidentified *Lutzomyia* spp (0.84%) and *Brumptomyia* sp (0.01%). A total of 141 engorged female sand flies was analyzed by molecular techniques to identify their bloodmeal sources (Table 1).

### 3.2. Efficiency of the PNOC and CYB genes

Using PNOC-PCR a fragment of approximately 330bp was amplified in 30 of the 141 specimens processed (21%). However, only 10 of these samples were appropriated for direct sequencing since the others presented non-specific bands that could not be removed, even after modification of the PCR cocktail and amplification profile. The limited amplification success of the PNOC gene contrasted with the 141 amplicons obtained using CYB-PCR (141/141, 100%) but sequencing of the latter subsequently revealed that 37 of them in fact corresponded to DNA of the insects analyzed. The 151 amplicons (10 PNOC and 141 CYB) derived from the 141 sand flies analyzed were used to determine the identities of 105 vertebrates (Table 1), 37 of the other sequences being discarded for being of phlebotomine origin and four for providing illegible electropherograms. Individually, the vertebrate identification efficiencies were 7% and 72% for the PNOC and CYB genes respectively.

### 3.3. Identification of bloodmeals

Ten species of *Lutzomyia* were identified among the 141 engorged female sand flies examined in this study (Table 1). In turn, the 105 bloodmeals identified as being from vertebrates belonged to 10 species distributed among six families of mammals, two of reptiles and one of birds (Table 1). All of the vertebrate bloodmeal sequences showed 99-

100% similarity to reference sequences from Genbank and were successfully identified to species level, with the exception of three bloodmeals of *Lu. micropyga*, which showed 92% similarity and were only identified to genus level.

#### 3.4. Detection of anthropophily and mixed bloodmeals

Using both genes anthropophily was detected in five of the eight sand fly species *i.e.*, *Lu. evansi*, *Lu. panamensis*, *Lu. micropyga*, *Lu. shannoni* and *Lu. atroclavata*, with a HBI of 14/81 (17%) for the first of these. The PNOC gene concurred with CYB in the identification of six bloodmeal samples and there were discrepancies in the identification of four, indicating that these represented mixed bloodmeals. It was thus detected that two females of *Lu. panamensis* had fed on both *Eq. asinus* (CYB) and *Ho. sapiens* (PNOC), another female of the same species on *Bos taurus* (CYB) and *Ho. sapiens* (PNOC), and one female *Lu. micropyga* on *Mabuya* sp. (CYB) and *Ho. sapiens* (PNOC).

#### 3.5. Associations between phlebotomine sand flies and vertebrates

The associations between sand flies and their bloodmeal sources, each assigned a value by means of a multiple correspondence analysis, were able to explain 89% of the observations (Axis 1 = 39.30% + Axis 2 = 32.55%+ Axis3 = 17.08%) allowing three important associations to be noted: I) *Lu. micropyga* with *Mabuya* sp. + *Anolis* sp., II) *Lu. c. cayennensis* with *Pr. guyanensis* + *Eq. caballus*, and III) *Lu. evansi* with *Bos taurus* + *Eq. asinus* (Figure 2).

### 3.6. Host utilization by *Lu. evansi*

Significant differences were found between the frequencies of the vertebrate species that provided sources of blood for *Lu. evansi* ( $P = 0.0001$ ), with *Bos taurus*, *Eq. asinus*, *Ho. sapiens* and *Sus scrofa* predominating.

## 4. Discussion

### 4.1. Efficiency of the *PNOC* and *CYB* genes

Although the markers *CYB* and *PNOC* have been used previously to identify sand fly bloodmeals (Haouas *et al.*, 2007; Quaresma *et al.*, 2012), this is the first time they have been employed together, illustrating not only their different efficiencies but also the advantages of using both. It is noteworthy that the efficiency of *CYB* was 72%, while *PNOC* only reached 7%, a surprisingly low percentage given that at least 94% of the bloodmeals evaluated were from mammals, the group which constitutes the target for this marker. This finding contradicts the efficiency values of 29%, 65% and 79% reported by the only three previous studies of this gene in sand fly bloodmeals (Haouas *et al.*, 2007; Jaouadi *et al.*, 2013; Baum *et al.*, 2015). The differences observed between the *CYB* and *PNOC* genes could be due principally to enzymatic degradation of the DNA template in the sand fly gut. This would have a greater impact on *PNOC* since this only presents a single copy per cell, unlike *CYB* which as part of the mitochondrial genome presents hundreds or even thousands of copies per cell.

Comparative analysis of both genetic markers indicates that the nuclear gene *PNOC* should not be used alone for the molecular identification of bloodmeal sources in New

World sand flies. This is based on the difficulties encountered in its amplification for most of the samples analyzed, as well as the fact that it cannot detect bloodmeals from birds and reptiles. Although in the Americas these are not considered to be receptive to the development of *Leishmania*, they do have epidemiological relevance (Alexander *et al.*, 2002). Despite these limitations, when used in conjunction with CYB the PNOC gene is useful for the identification of complex biological relationships, particularly of mixed bloodmeals.

#### 4.2. Species of phlebotomine sand flies and vertebrates detected in bloodmeals

Among the different links within the epidemiological cycle of leishmaniasis, the vertebrate reservoirs that provide blood for sand flies are probably the least studied, because of the difficulties of sampling and analyzing these animals (Travi *et al.*, 1994, 1998; Alexander *et al.*, 1998). In the present study 10 vertebrates were identified that provide bloodmeal sources for eight sand fly species in the department of Sucre, providing evidence for the existence of biological relationships between these vectors and the local vertebrate fauna. Three of the five phlebotomine species from which human bloodmeals were detected are known to be *Leishmania* vectors, *i.e.*, *Lu. evansi*, *Lu. panamensis* and *Lu. shannoni* (Maroli *et al.*, 2013), notably the first of these based on previous reports of its natural infection with *Le. infantum* and *Le. braziliensis* in the region (Vélez *et al.*, 1995; Travi *et al.*, 1996; Bejarano *et al.*, 2012).

Bloodmeal analyses show that *Lu. evansi* feeds on at least seven different hosts, among which cattle, donkeys, humans and pigs are particularly important. This does not

necessarily infer an innate preference of the vector for these mammals, given that no survey of the relative abundance and availability of each vertebrate species in the study area was carried out. Nevertheless, this diversity of bloodmeal sources could partly explain the appearance of leishmaniasis cases in the outskirts of urban centres (Bejarano *et al.*, 2002; Cortés & Fernández 2008), where man becomes a new source of blood for the insect without it abandoning other, non-human sources including potential reservoirs of the parasite, and without the need for any extreme ecological adaptation. Cochero (2002) reported anthropophily of 34% for *Lu. evansi*, while Montoya & Lane (1996) observed that although females of this species are significantly more attracted to man, when humans are present it also feeds on dogs and opossums. The diversity of bloodmeal origins detected in the present study should be supported by observations of a *Lu. evansi* behavioural characteristic that satisfies one of the vector incrimination criteria, *i.e.*, that the species concerned should bite man and other vertebrates (WHO 2010b).

It is important to emphasize that several of the bloodmeals identified came from sand flies collected without attractants on diurnal resting sites (Table 1), yielding a more representative sample of the region's fauna, the predominance of *Lu. evansi* notwithstanding. The findings of bloodmeals from spiny rats, horses and cattle in *Lu. c. cayennensis* are noteworthy because they confirms the capacity of this species to feed on both large and small mammals even at low population densities (Cochero *et al.*, 2007; Cortés *et al.*, 2009) and challenge the classic premise that it feeds exclusively on lizards (Young & Arias, 1992).

Associations such as those of *Lu. micropyga* with reptiles of the genera *Mabuya* and *Anolis* are of particular interest although it was not possible to determine the exact species involved due to the taxonomic complexity of these genera, especially in Colombia and Venezuela. The similarity values of under 92% which BlastN revealed in this case indicate that the nucleotide sequences available on GeneBank do not correspond to the species present in the region studied (Whiting *et al.*, 2006). These records constitute the first reported identifications of bloodmeal sources for *Lu. micropyga* and build on the preliminary findings of Tesh *et al.* (1971), who reported bloodmeal sources of reptilian/amphibian origin for this sand fly, although it was not possible to establish the identity of the vertebrates involved due to the limitations of serological techniques at that time. Even more interesting is the finding of bloodmeals of human origin in *Lu. micropyga* and *Lu. atroclavata*, species of the subgenus *Micropygomyia* that are not attracted to light. According to Galati (2011) both species belong to the subtribe *Sergentomyiina*, which also includes *Sergentomyia minuta*, an Old World sand fly recently encountered engorged on blood of the mouse *Mus musculus* (Jaouadi *et al.*, 2013). This provides a further indication that these *Lutzomyia* species feed on mammals as well as poikilotherms.

Spiny rats of the genus *Proechimys*, which provided a source of bloodmeals for three sand fly species (Table 1), are a known reservoir of *Leishmania* spp. in other parts of the Neotropics (Lainson *et al.*, 1981; Dedet *et al.*, 1989) and thus may play the same role in the study area. However further studies of natural infections in this rodent are needed to establish whether it is involved in the epidemiology of leishmaniasis in the Caribbean region of Colombia.

The absence of blood from dogs in the samples identified is puzzling, since these animals were present in the peridomiciliary habitats studied. Furthermore the overall prevalence of *Leishmania* infection in the canine population of Sucre reaches 33.6% (Paternina-Gómez *et al.*, 2013), which implies contact with vectors of the parasite *per se*. Nevertheless, the present result concurs with those of previous studies which suggested that dogs are less attractive to some sand fly species than other potential sources of blood (Morrison *et al.*, 1993; Montoya & Lane, 1996; Oliveira *et al.*, 2008; Marassa *et al.*, 2013; Maia *et al.*, 2015).

## 5. Conclusions and perspectives

The mitochondrial gene CYB was found to be more efficient than the nuclear gene PNOG in identifying phlebotomine sand fly bloodmeals from the mixed leishmaniasis focus of the department of Sucre, Colombia. The results indicate that mammals constitute the principal source of blood for sand fly populations in the region, with birds and reptiles being much less important. The detection of bloodmeals of human origin in *Lu. evansi*, *Lu. panamensis*, *Lu. micropyga*, *Lu. shannoni* and *Lu. atroclavata* is particularly noteworthy. Although this alone does not mean that all of these species are vectors of *Leishmania* spp., it provides evidence that they are anthropophilic and may have as-yet undefined roles in the epidemiological cycle of leishmaniasis in the region.

**Funding**

This study was financed by the University of Sucre and the Administrative Department of Science, Technology and Innovation – Colciencias (grant no. 112945921432).

**Conflict of interest**

The authors have no conflict of interest to declare.

**Acknowledgments**

We thank Suljei Cochero of the Secretaría de Salud Departamental de Sucre for her collaboration in the sampling of entomological material. We thank Bruce Alexander of Xeroshield Ltd. (Roslin, Scotland) for critical review and English revision of the manuscript.

**Summary**

Chickens and pigs in peridomiciliary environment of the El Piñal rural settlement, municipality of Los Palmitos, Colombia.

## References

- Alexander, B., 2000. Sampling methods for phlebotomine sand flies (Diptera: Psychodidae). *Med. Vet. Entomol.* 14, 1–13.
- Alexander, B., de Carvalho, R.L., McCallum, H., Pereira, M.H., 2002. Role of the domestic chicken (*Gallus gallus*) in the epidemiology of urban visceral leishmaniasis in Brazil. *Emerg. Infect. Dis.* 8, 1480–1485.
- Alexander, B., Lozano, C., Barker, D.C., McCann, S.H., Adler, G.H., 1998. Detection of *Leishmania (Viannia) braziliensis* complex in wild mammals from Colombian coffee plantations by PCR and DNA hybridization. *Acta Trop.* 69, 41–50.
- Alvar, J., Vélez, I.D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J., den Boer, M., 2012. Leishmaniasis worldwide and global estimates of its incidence. *PLoS ONE* 7, e35671. doi:10.1371/journal.pone.0035671.
- Bejarano E.E., Estrada L.G., 2015. Family Psychodidae, in: Wolff, M., Nihei, S.S., Pérez, S.P., & de Carvalho, C.J.B. (Eds.), *Catalogue of Diptera of Colombia*. Zootaxa, In Press.
- Bejarano, E.E., Pérez-Doria, A., Paternina, L.E., Paternina-Gómez, M., Martínez, L., 2012. Natural infection of *Lutzomyia evansi* (Diptera: Psychodidae) with *Leishmania (Viannia)* spp. in northern Colombia. *Am. J. Trop. Med. Hyg.* 87(Suppl), 173.
- Bejarano, E.E., Uribe, S., Rojas, W., Velez, I.D., 2002. Phlebotomine sand flies (Diptera: Psychodidae) associated with the appearance of urban leishmaniasis in the city of Sincelejo, Colombia. *Mem. Inst. Oswaldo Cruz* 97, 645–647.

- Cochero, S., 2002. Papel de *Lutzomyia evansi* como vector de leishmaniasis visceral en Los Montes de María. Departamento de Biología y Química, Universidad de Sucre. Sincelejo, Colombia.
- Cochero, S., Anaya, Y., Díaz, Y., Paternina, M., Luna, A., Paternina, L., Bejarano, E.E., 2007. Infección natural de *Lutzomyia cayennensis cayennensis* con parásitos tripanosomatídeos (Kinetoplastida: Trypanosomatidae) en Los Montes de María, Colombia. Rev. Cubana Med. Trop. 59, 35–39.
- Cortés, L.A., Fernández, J.J., 2008. Species de *Lutzomyia* en un foco urbano de leishmaniasis visceral y cutánea en El Carmen de Bolívar, Bolívar, Colombia. Biomédica 28, 433–440.
- Cortés L., Pérez-Doria, A., Bejarano, E.E. 2009. Phlebotomines (Diptera: Psychodidae) antropofílicos de importancia en salud pública en Los Montes de María, Colombia. Rev. Cubana Med. Trop. 61, :220–5.
- de Oliveira, A.G., Marassá, A.M., Consales, C.A., Dorval, M.E., Fernandes, C.E., de Oliveira, G.R., Brazil, R.P., Galati, E.A.B., 2008. Observations on the feeding habits of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae) in Campo Grande, an endemic area of visceral leishmaniasis in Mato Grosso do Sul, Brazil. Acta Trop. 107, 238–241.
- Dedet, J.P., Gay, F., Chatenay, G., 1989. Isolation of *Leishmania* species from wild mammals in French Guiana. Trans. R. Soc. Trop. Med. Hyg. 83, 613–615.

- Galati, E.A.B., 2011. Phlebotominae (Diptera, Psychodidae). Classificação, morfologia, terminologia e identificação de Adultos. Vol. I. Apostila de Bioecologia e Identificação de Phlebotominae 2011. Universidade de São Paulo, Brasil.
- Galván-Guevara, S., 2010. Mamíferos y aves silvestres registrados en una zona de Los Montes de María, Colosó, Sucre, Colombia. Rev. colombiana Cienc. Anim. 2, 45–57.
- Haouas, N., Pesson, B., Boudabous, R., Dedet, J.P., Babba, H., Ravel, C., 2007. Development of a molecular tool for the identification of *Leishmania* reservoir hosts by blood meal analysis in the insect vectors. Am. J. Trop. Med. Hyg. 77, 1054–1059.
- Holdridge, L.R., 1967. Life zone ecology. Tropical Science Center, San José.
- INS, 2005–2014. Subdirección de Vigilancia y Control en Salud Pública. Sistema de Vigilancia en Salud Pública – SIVIGILA. Años 2005–2014. Instituto Nacional de Salud, Bogotá.
- Jaouadi, K., Haouas, N., Chaara, D., Boudabous, R., Gorgii, M., Kidar, A., Depaquit, J., Pratlong, F., Dedet, J.P., Babba, H., 2013. Phlebotomine (Diptera, Psychodidae) bloodmeal sources in Tunisian cutaneous leishmaniasis foci: could *Sergentomyia minuta*, which is not an exclusive herpetophilic species, be implicated in the transmission of pathogens?. Ann. Entomol. Soc. Am. 106, 79–85.
- Johns, G., Avise, J., 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial Cytochrome b gene. Mol. Biol. Evol. 15, 1481–1490.
- Kent, R.J., 2009. Molecular methods for arthropod bloodmeal identification and applications to ecological and vector-borne disease studies. Mol. Ecol. Resour. 9, 4–18.

- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. U.S.A.* 86, 6196–6200.
- Lainson, R., Shaw, J.J., Ready, P.D., Miles, M.A., Póvoa, M., 1981. Leishmaniasis in Brazil: XVI. Isolation and identification of *Leishmania* species from sandflies, wild mammals and man in north Para State, with particular reference to *L. braziliensis guyanensis* causative agent of "pian-bois". *Trans. R. Soc. Trop. Med. Hyg.* 75, 530–536.
- Maia, C., Parreira, R., Cristóvão, J. M., Freitas, F. B., Afonso, M. O., Campino, L., 2015. Molecular detection of *Leishmania* DNA and identification of blood meals in wild caught phlebotomine sand flies (Diptera: Psychodidae) from southern Portugal. *Parasit. Vectors* 8, 173.
- Marassá, A.M., Galati, E.A.B., Bergamaschi, D.P., Consales, C.A., 2013. Blood feeding patterns of *Nyssomyia intermedia* and *Nyssomyia neivai* (Diptera, Psychodidae) in a cutaneous leishmaniasis endemic area of the Ribeira Valley, State of São Paulo, Brazil. *Rev. Soc. Bras. Med. Trop.* 46, 547–554.
- Maroli, M., Feliciangeli, D., Bichaud, L., Charrel, R., Gradoni, L., 2013. Phlebotomine sandflies and the spreading of leishmaniasis and other diseases of public health concern. *Med. Vet. Entomol.* 27, 123–147.

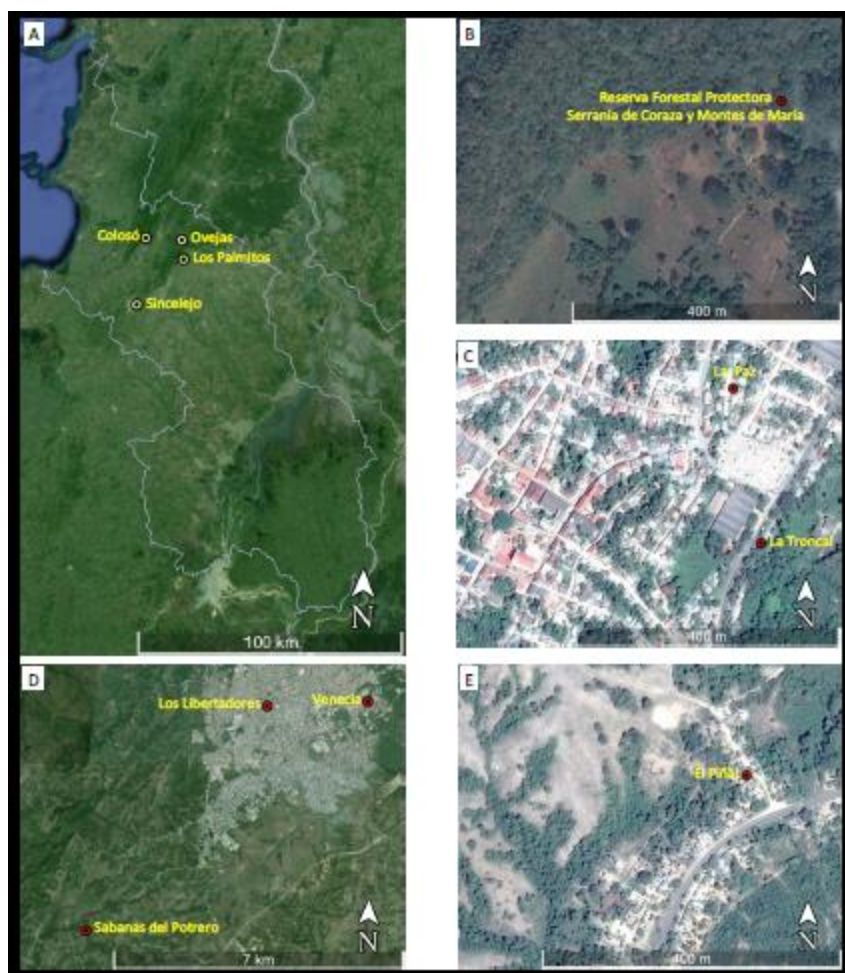
- Martínez, L., Rebollo, J., Luna, A., Cochero, S., Bejarano, E.E., 2010. Molecular identification of the parasites causing cutaneous leishmaniasis on the Caribbean coast of Colombia. *Parasitol. Res.* 106, 647–652.
- Montoya-Lerma, J., Lane, R.P., 1996. Factors affecting host preference of *Lutzomyia evansi* (Diptera: Psychodidae), a vector of visceral leishmaniasis in Colombia. *Bull. Entomol. Res.* 86, 43–50.
- Morrison, A.C., Ferro, C., Tesh, R.B., 1993. Host preferences of the sand fly *Lutzomyia longipalpis* at an endemic focus of American visceral leishmaniasis in Colombia. *Am. J. Trop. Med. Hyg.* 49, 68–75.
- Paternina-Gómez, M., Díaz-Olmos, Y., Paternina, L.E., Bejarano, E.E., 2013. Alta prevalencia de infección por *Leishmania* (Kinetoplastidae: Trypanosomatidae) en perros del norte de Colombia. *Biomédica* 33, 375–382.
- Pérez-Doria, A., Bejarano, E.E., Sierra, D., Vélez, I.D., 2008a. Molecular evidence confirms the taxonomic separation of *Lutzomyia tihuiensis* from *Lutzomyia pia* (Diptera: Psychodidae) and the usefulness of pleural pigmentation patterns in species identification. *J. Med. Entomol.* 45, 653–659.
- Pérez-Doria, A., Hernández-Oviedo, E., Bejarano, E.E., 2008b. *Lutzomyia* (Diptera: Psychodidae) from the reserva Serranía de Coraza y Montes de María, Colombia. *Rev. Colomb. Entomol.* 34, 98–101.
- Quaresma, P.F., Carvalho, G.M., Ramos, M.C., Andrade-Filho, J.D., 2012. Natural *Leishmania* sp. reservoirs and phlebotomine sandfly food source identification in Ibitipoca State Park, Minas Gerais, Brazil. *Mem. Inst. Oswaldo Cruz* 107, 480–485.

- Sales, K.G., Costa, P.L., de Moraes, R.C., Otranto, D., Brandão-Filho, S.P., Cavalcanti, M.P., Dantas-Torres, F., 2015. Identification of phlebotomine sand fly blood meals by real-time PCR. *Parasit. Vectors* 8, 230.
- Svobodová, M., Alten, B., Zídková, L., Dvorák, V., Hlavacková, J., Mysková, J., Seblová, V., Kasap, O.E., Belen, A., Votýpka, J., Volf, P., 2009. Cutaneous leishmaniasis caused by *Leishmania infantum* transmitted by *Phlebotomus tobbi*. *Int. J. Parasitol.* 39, 251–256.
- Tesh, R.B., Chaniotis, B.N., Aronson, M.D., Johnson, K.M., 1971. Natural host preferences of Panamanian phlebotomine sandflies as determined by precipitin test. *Am. J. Trop. Med. Hyg.* 20, 150–156.
- Travi, B.L., Jaramillo, C., Montoya, J., Segura, I., Zea, A., Goncalves, A., Vélez, I.D., 1994. *Didelphis marsupialis*, an important reservoir of *Trypanosoma (Schizotrypanum) cruzi* and *Leishmania (Leishmania) chagasi* in Colombia. *Am. J. Trop. Med. Hyg.* 50, 557–565.
- Travi, B.L., Montoya, J., Gallego, J., Jaramillo, C., Llano, R., Vélez, I.D., 1996. Bionomics of *Lutzomyia evansi* (Diptera: Psychodidae) vector of visceral leishmaniasis in northern Colombia. *J. Med. Entomol.* 33, 278–285.
- Travi, B.L., Osorio, Y., Becerra, M.T., Adler, G.H., 1998. Dynamics of *Leishmania chagasi* infection in small mammals of the undisturbed and degraded tropical dry forests of northern Colombia. *Trans. R. Soc. Trop. Med. Hyg.* 92, 275–278.

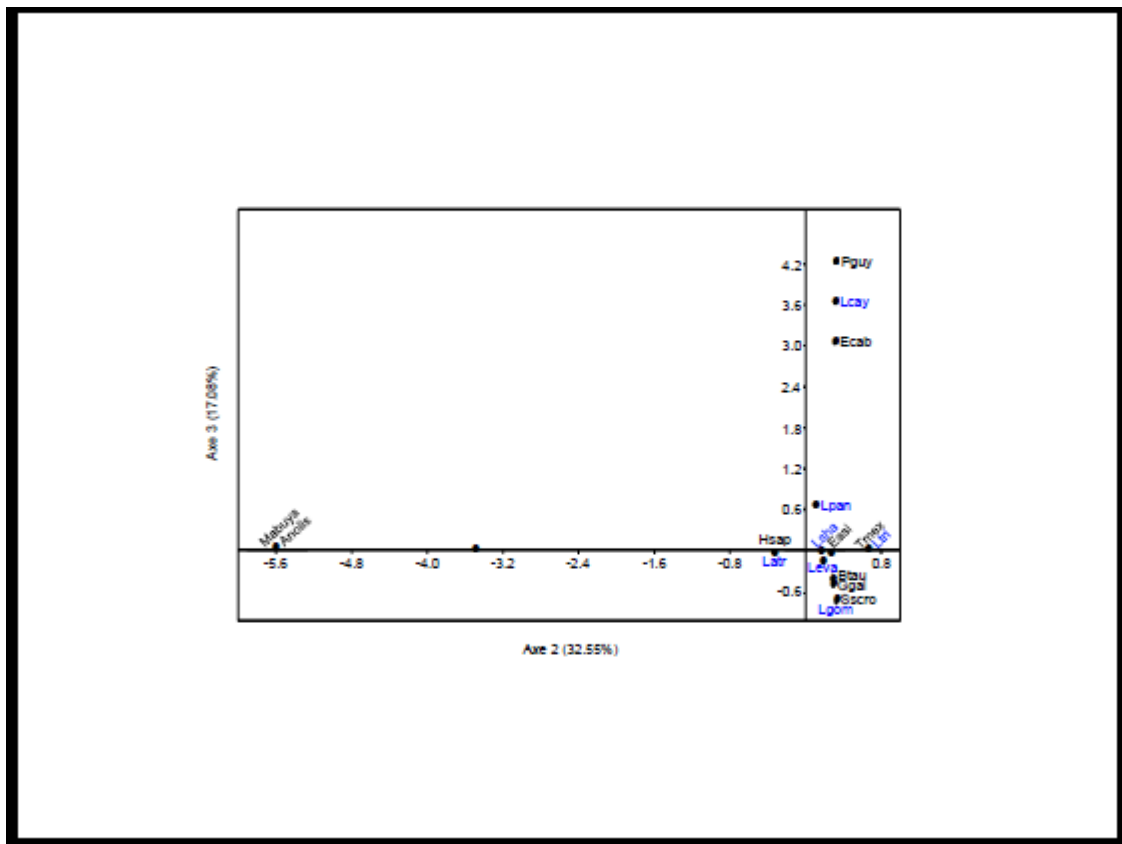
- Valinsky, L., Ettinger, G., Bar-Gal, G.K., Orshan, L., 2014. Molecular identification of bloodmeals from sand flies and mosquitoes collected in Israel. *J. Med. Entomol.* 51: 678–685.
- Vélez, I.D., Travi, B.L., Gallego, J., Palma, G.I., Agudelo, S.P., Montoya, J., Jaramillo, C., Llano, R., 1995. Evaluación ecoepidemiológica de la leishmaniasis visceral en la comunidad indígena Zenú de San Andrés de Sotavento, Córdoba: primer paso para su control. *Rev. Colomb. Entomol.* 21, 111–122.
- Whiting, A.S., Sites, J.W.S., Pellegrino, K.C.M., Rodrigues, M.T., 2006. Comparing alignment methods for inferring the history of the new world lizard genus *Mabuya* (Squamata: Scincidae). *Mol. Phylogenet. Evol.* 38, 719–730.
- WHO, 2010a. First WHO report on neglected tropical diseases 2010: working to overcome the global impact of neglected tropical disease. World Health Organization, Geneva.
- WHO, 2010b. Control of the leishmaniasis: report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis. Technical report series no. 949. World Health Organization, Geneva.
- Young, D.G., Arias, J.R., 1992. Flebótomos: vectores de leishmaniasis en las Américas. Cuaderno técnico no. 33. Organización Panamericana de la Salud (OPS), Washington.
- Young, D.G., Duncan, M.A., 1994. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). *Mem. Am. Entomol. Inst.* 54, 1–881.
- Zambrano, C.P., Gutiérrez, P., 2011. Situación de la leishmaniasis en Colombia. *Biomédica* 31(supl.3), 119.

## Figure Captions

**Fig. 1.** Sand fly collection locations in northern Colombia. (A) Satellite image map of the department of Sucre, showing the four municipalities where phlebotomine sampling was carried out: (B) Colosó, (C) Ovejas, (D) Sincelejo, (E) Los Palmitos. Source: Google Earth Pro.



**Fig. 2.** Multiple correspondence analysis showing the relationships between species of phlebotomine sand flies (red) and the vertebrates that provide them with bloodmeals (black). The percentage of inertia explained by each axis is given. Sand fly species abbreviations are as follows; Leva, *Lu. evansi*; Lpan, *Lu. panamensis*; Lmic, *Lu. micropyga*; Lcay, *Lu. cayennensis cayennensis*; Lsha, *Lu. shannoni*; Lgom, *Lu. gomezi*; Latr, *Lu. atroclavata*; Ltri, *Lu. trinidadensis*. Vertebrate species abbreviations are as follows; Btau, *Bos taurus*; Easi, *Equus asinus*; Hsap, *Homo sapiens*; Sscr, *Sus scrofa*; Ecab, *Equus caballus*; Pguy, *Proechimys guyanensis*; Ggal, *Gallus gallus*; Tmex, *Tamandua mexicana*.



**Table**

**Table 1.** Sources of bloodmeals identified in female sand flies from the leishmaniasis focus of the department of Sucre, northern Colombia. CDC, CDC light trap; Sh, Shannon trap;

DRS, Diurnal Resting Sites; N, Number of insects; P, Peridomicile; I, Intradomicile; E: Extradomicile; NS, did not sequence well.

Sand fly species (N)	Blood source	Family	Ovejas						Los Palmitos			Sincelejo			Colosó	Total	
			La Troncal				La Paz		El Piñal			Iguanas	Scouts	Sabanas	Reserve		
			Sh	CDC	DRP		CDC		Sh	CDC	DRP	DRP	DRP	DRP	CDC		
			P	I	P	P	I	P	P	I	P	P	P	P	P		E
<i>Lu. evansi</i> (104)	<i>Bos taurus</i>	Bovidae	4	1	2	1	-	1	12	2	3	4	-	-	-	-	30
	<i>Equus asinus</i>	Equidae	-	-	2	2	-	-	2	1	-	12	-	-	-	-	19
	<i>Homo sapiens</i>	Hominidae	1	-	4	-	-	2	6	-	1	-	-	-	-	-	14
	<i>Sus scrofa</i>	Suidae	-	-	2	-	1	-	2	4	3	-	-	-	-	-	12
	<i>Gallus gallus</i>	Phasianidae	2	-	-	-	-	-	1	-	-	-	-	-	-	-	3
	<i>Equus caballus</i>	Equidae	-	-	1	1	-	-	-	-	-	-	-	-	-	-	2
	<i>Proechimys guyanensis</i>	Echimydae	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Insecta	-	2	-	1	4	3	1	-	3	3	3	-	-	-	-	20
	NS	-	-	-	1	-	-	1	-	1	-	-	-	-	-	-	3
<i>Lu. panamensis</i> (9)	<i>Equus asinus</i>	Equidae	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4
	<i>Homo sapiens</i>	Hominidae	1	-	-	-	-	-	-	-	-	-	-	-	-	2	3
	<i>Bos taurus</i>	Bovidae	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	<i>Equus caballus</i>	Equidae	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
	<i>Proechimys guyanensis</i>	Echimydae	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
	Insecta	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	2
<i>Lu. micropyga</i> (4)	<i>Mabuya</i> sp.	Scincidae	-	-	-	1	-	-	-	-	-	-	1	-	-	-	2
	<i>Anolis</i> sp.	Dactyloidea	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
	<i>Homo sapiens</i>	Hominidae	-	-	-	2	-	-	-	-	-	-	-	-	-	-	2
<i>Lu. c. cayennensis</i> (12)	<i>Equus caballus</i>	Equidae	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	<i>Bos taurus</i>	Bovidae	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
	<i>Proechimys guyanensis</i>	Echimydae	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	Insecta	-	5	-	2	-	-	-	-	-	-	-	-	2	-	-	9
<i>Lu. shannoni</i> (3)	<i>Homo sapiens</i>	Hominidae	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	<i>Tamandua mexicana</i>	Myrmecophagidae	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
	Insecta	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
<i>Lu. gomezi</i> (2)	<i>Sus scrofa</i>	Suidae	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2
<i>Lu. atroclavata</i> (1)	<i>Homo sapiens</i>	Hominidae	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
<i>Lu. trinidadensis</i> (1)	<i>Tamandua mexicana</i>	Myrmecophagidae	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
<i>Lu. dubitans</i> (3)	Insecta	-	-	-	1	-	-	-	-	1	1	-	-	-	-	-	3
<i>Lu. rangelifiana</i> (2)	Insecta	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	2
Total			20	1	18	12	5	6	24	13	11	19	1	5	3	7	145