Composition and distribution of medically important phlebotomines (Diptera: Psychodidae) in the municipalities of Tierralta and Valencia (Córdoba, Colombia)

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ABSTRACT

Background & objectives: Ecoepidemiological studies of cutaneous leishmaniasis and regular monitoring of *Lutzomyia* species have generated a knowledge base that can be used for control and prevention strategies targeted at the disease transmission dynamics in focal areas of Colombia. In this study, the presence and spatial distribution of phlebotomines of medical importance in the municipalities of Tierralta (El Loro and Tuis Tuis villages) and Valencia (Guadua and Mieles villages) were determined.

Methods: Entomological surveys were performed in 2015 (months of June, September and November) and samples were collected via CDC-traps located in intradomicilary and peridomiciliary areas in the municipalities of Tierralta and Valencia (Department of Córdoba, Colombia). Active searches were also carried out with a mouth aspirator to collect adult phlebotomines from resting sites. ANOVA and Kruskal-Wallis tests were performed to assess if the differences between the communities of phlebotomines. Spatial distribution maps of the Lutzomyia species were generated.

Results: A high species diversity of *Lutzomyia* was observed with a total of 1677 *Lutzomyia* individuals belonging to 12 species. Among these species, *Lu. panamensis* was the most abundant (80.18%). The composition of the intradomicilary and peridomiciliary phlebotomines varied significantly (F = 0.9962; df = 1; p = 0.02895). Species like *Lu. carpenteri, Lu. camposi, Lu. dysponeta, Lu. atroclavata* and *Lu. yuilli yuilli* were recorded for the first time in the Department of Córdoba, Colombia.

Interpretation & conclusion: The spatial distribution shows that Lu. panamensis and Lu. gomezi are predominant and present in areas with high concentration of houses. This study provides basic information on new records of phlebotomines in the Department of Córdoba. The results suggest that greater vector-human contact occurs in the peridomiciliary environment and that a high number of Lutzomyia species associated with the transmission of leishmaniasis are present in Colombia.

Key words Colombia; Córdoba; cutaneous leishmaniasis; Lutzomyia panamensis; spatial distribution

INTRODUCTION

Leishmaniasis in the Americas represents a serious public health problem because of its morbidity and wide geographical distribution¹. This tropical disease is continually introduced into urban environments because of environmental changes and the adaptive nature of the *Lutzomyia* insect vectors²⁻³.

The females of some *Lutzomyia* species are the main risk factor in the transmission cycle of leishmaniasis due to their vector competence and haematophagous habit, which allows them to transmit one or several species of *Leishmania* to humans^{1,4}. Therefore, ecoepidemiological studies including the identification of phlebotomines are important. In Colombia, several studies have been carried out to monitor the distribution and infection rates of *Leishmania* in phlebotomine sandflies and mammals (canines and *Didelphis*). These studies were performed in the departments of Magdalena, Cesar, Córdoba, Bolivar and Sucre⁵⁻¹² from the Caribbean coast region. Nevertheless, in the department of Córdoba, the dynamics of disease transmission and the spatial distribution of insect vectors over the last decade are not well understood.

Previous studies on leishmaniasis in Córdoba have been carried out in the municipalities of San Andrés de Sotavento, Cereté, Chinú, Sahagún and Tierralta¹³⁻¹⁶. These studies identified 12 *Lutzomyia* species in the Department of Córdoba^{12, 17}. Among these species, *Lu. gomezi*, *Lu. longipalpis, Lu. cayennensisc, Lu. panamensis and* *Lu. evansi* are of importance because they have been found naturally infected with different *Leishmania* species^{1,5}.

In the Department of Córdoba, ecoepidemiological studies of the leishmaniasis are essential because of the high number (2155) of cutaneous leishmaniasis (CL) cases reported from 2010–2015 (National Institute of Health, Colombia, 2010–2015), lack of updated taxonomic inventories of phlebotomine sandflies, and their bioecology. In this department, the municipalities of Tierralta and Valencia have reported highest number of CL cases (Valencia = 65; Tierralta = 790) (Departmental Secretary of Health, Cordoba department, 2010–2015). Nevertheless, information on disease transmission dynamics and the phlebotomine species composition is lacking.

The distribution of sandflies is an ecoepidemiological component that also needs to be included in poorly explored areas due to high habitat diversity, different physical reliefs, and the temperature gradient prevailing in the study area. Furthermore, systematic surveys are required considering that the distribution of sandflies could be affected by climate change and by variations in ecological conditions due to changes in landuse patterns and settlements.

The aim of this study was to investigate the composition and current spatial distribution of *Lutzomyia* species in four villages located in the municipalities of Tierralta and Valencia in the Department of Córdoba.

MATERIAL & METHODS

Study area

Entomological surveys were performed in 2015 (months of June, September and November) in the municipalities of Tierralta and Valencia (Department of Córdoba, Colombia). In each of these municipalities, two villages were selected based on the number of leishmaniasis cases reported by the local health department of Córdoba. In Tierralta, the selected villages were El Loro and Tuis Tuis; and in Valencia, Mieles and Guadual were taken as study villages (Fig. 1)¹⁸. In these villages, the number of houses ranged between 70 and 100.

Valencia is located in south of the Department of Córdoba, 60 m above sea level. The average temperature is 28°C with a warm semi-humid climate influenced by the northern trade winds. The city is located between the Abibe Mountains and the Sinú Valley and exhibits varied topography¹⁸⁻¹⁹. Tierralta is located 5 m above sea level and has an average temperature of 27 °C. This municipality is bordered in the north withValencia and covers much of the Nudo de Paramillo natural National Park, which explains its considerable biological diversity¹⁸⁻¹⁹.

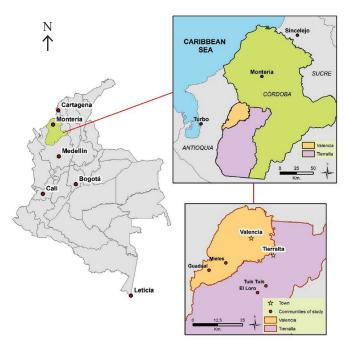


Fig. 1: Geographical locations of the phlebotomine collection areas in the municipalities of Tierralta (Tuis Tuis and El Loro) and Valencia (Mieles and Guadual) in the Department of Córdoba, Colombia.

In general, the studied areas harbour several habitats, such as gallery forests, stubbles, pastures, intervened natural forests, secondary forests, grass and crops (mangoes, bananas, yuccas, guaduales, and papayas)¹⁸⁻¹⁹. In addition, domestic animal breeding including cattle, sheep and poultry; and timber production including oak, cedar, campano, teak and a wide variety of native trees (abarcos, manzabalos, and brazilete), are prominent in these areas¹⁹.

Collection of adult phlebotomines

Entomological surveys were conducted consecutively over a period of six days in the months of June, September and October, 2015 in the study area. The methodology used to collect adult phlebotomines involved the installation of 20 CDC-type traps in the peridomiciles and intradomiciles of 10 houses in each village. The traps were activated between 1700 and 0600 hrs²⁰⁻²¹. Active searches (at 0500 and 1000 hrs) were also carried out to collect adult phlebotomines from resting sites with a mouth aspirator²¹.

Processing and identification of adult phlebotomines

The collected phlebotomines were kept in 1.5 ml vials with isopropanol at -20° C, properly labeled with information about the house, number of specimens and coordinates. The taxonomic identifications followed clearing and mounting protocols²¹⁻²², exposing the collected speci-

mens to a lactophenol solution (1:1, lactic acid-phenol) for 24 h. Permanent mounts were used to establish a reference collection of the species (males and females) using Canada balsam as the mounting medium.

To identify the remaining collected specimens, semipermanent mounts were performed using concave blades with lactophenol solution that were subsequently preserved in 70% ethyl alcohol at 4°C in labeled cryovials. Preserved sandflies were identified using the taxonomic keys developed by Young & Duncan²¹; and Galati²². The adult specimen collection was registered in the Unit of Medical Entomology of the Control and Study Program of Tropical Diseases (PECET, Universidad de Antioquia), and the Laboratory of Biomedical Investigations of the Universidad delSinú (Elias Bechara Zainúm).

Spatial distribution and relative species richness of adult phlebotomines

Coordinates of georeferenced CDC-traps in decimal degrees were obtained using a Garmin eTrex GPS. The data were processed in MapSource/Base Camp and subsequently downloaded as a CSV file into Excel (Comma delimited). The entomological information and shapefile-based layers (contour lines, rivers, road networks, *etc.*) were entirely managed in ArcGIS 10.3.1. However, >200 houses in the villages of El Loro, Tuis Tuis, Mieles and Guadual were mapped using a Google Earth Landsat image of June 2015.

Subsequently, contour lines were converted from topo to raster using the Spatial Analyst, Interpolation and Topo to Raster tools. The reference system was standardized using the Universal Transverse Mercator (UTM, Zone 18N) system for the projected coordinates and the World Geographic System (WGS, 1984) system for the geographic coordinates. For all cases, MAGNA was the reference datum. The entomological database was introduced into the "CDC traps" layer, which provides the junction field code of the trap, using the Join and Relates tool. Finally, all of the editing (symbols, labels and source) was performed in Layer Properties, which allowed for a clear visualization of the information of interest.

Data analysis

The relative abundance of the adult phlebotomines collected in each study area was tabulated and described by relating their presence in the peridomiciles and intradomiciles. ANOVA and Kruskal-Wallis tests were performed to assess if the differences between the communities of phlebotomines present in the four villages and their compositions with respect to the houses were significant. A hierarchical cluster dendrogram was calculated and generated by the Jaccard similarity index using the UP-GMA algorithm in the PAST 3 program.

RESULTS

Taxonomic list and relative abundance of phlebotomines

A total of 1635 specimens were collected using CDC traps in the municipalities of Valencia (656) and Tierralta (979) (Table 1). Twelve *Lutzomyia* species were identified morphologically (Table 1), of which *Lu. panamensis* was the most abundant in Valencia (475; 72.21%) and Tierralta (836; 85.3%) followed by *Lu. dubitans* (138; 8.48%) and *Lu. gomezi* (128; 7.83%), with especially high numbers in the Tuis Tuis and Mieles villages (Table 1). The active searches of the extradomiciliar environments collected 42 specimens corresponding to *Lu. panamensis* (El Loro = 20, Guadual = 5); *Lu. gomezi* (El Loro = 2; Guadual = 1); *Lu. shannoni* (El Loro = 1) and *Lu. trinidadensis* (El Loro = 11, Guadual = 2).

Presence of medically important phlebotomines with respect to location and intradomicile area

In most villages, the number of phlebotomines was found to be higher in the peridomiciliary environment (El Loro = 276; Tuis Tuis = 561; Mieles = 496) (Table 1) except in Guadual, where relative abundances were lower (Peridomicile = 56; 8.53%). In general, several species associated with the transmission of leishmaniasis were identified in the intradomicile area, such as *Lu. panamensis, Lu. gomezi* and *Lu. cayennensis c.* (Table 1).

In the municipality of Tierralta, specifically in El Loro village, seven medically important species were found, viz. Lu. panamensis, Lu. gomezi, Lu. cayennensis c., Lu. micropyga, Lu. shannoni, Lu. trinidadensis and Lu. yuilli yuilli, and predominance of female sandflies was observed in the peridomicile area (Table 1). In Tuis Tuis village, fewer medically important species were collected in the peridomicile (Lu. panamensis, Lu. gomezi and Lu. yuilli yuilli), although a considerable number of females of these species were collected in the intradomicile, which represents a risk factor for the transmission of the disease (Table 1). In Guadual village (Valencia), only two species considered to be vectors were found (Lu. panamensis and Lu. gomezi), with low proportion of females in both the environments (Table 2). The Mieles village presented high species diversity and high numbers of the vector species were identified, including Lu. panamensis, Lu. gomezi, Lu. cayennensis c., and Lu. trinidadensis, as well as Lu. atroclavata that was only collected in this location (Table 2).

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Table 1. Relative abundance of phlebotomine adults captured with CDC light-traps in the municipalities of Tierralta (El Loro & Tuis Tuis villages) and

13 (0.80) 6 (0.37) 3 (0.18) 4 (0.24) 3 (0.18) 1311 (80.18) 138 (8.44) 12 (0.73) 12 (0.73) 2 (0.12) 3 (0.18) 1635 (100) 1635 (100) 128 (7.83) Grand total 475 (72.21) 6 (0.91) 1 (0.15) 1 (0.15) 104 (15.85) 42 (6.40) 9 (1.37) 9 (1.37) 9 (1.37) 656 (100) 656 (100) Total 16 Ś Intradomi-56 (8.53) 60 I L 1 L 22 ciliary 34 0+ 2 \mathcal{C} 552 (84.14) Mieles 163127 496 (75.60) 60 Peridomiciliary 333 4 ∞ 258 0+ Valencia (Guadual & Mieles villages), Department of Córdoba, Colombia Valencia Intradomi-48 (7.31) 60 Ś \sim I ∞ ciliary 40 22 0+ 104 (15.85) Guadual Peridomi-23 56 (8.53) 60 20 \sim L ciliary 33 0+ S 3 34 (4.4) 86 (8.7) 4(0.4)3 (0.3) 3 (0.3) 2 (0.2) 2 (0.2) 4 (0.4) 2 (0.2) 3 (0.3) 836 (85.3) 979 (100) 979 (100) Total Intradomi-60 15 L 20 142 (14.50) ciliary 0+ 122 20 $\tilde{\mathbf{n}}$ 702 (71.70) Tuis Tuis 561 (57.30) Peridomi-Tierralta 87 60 74 \mathcal{C} ciliary 474 430 0+ 2 26 Peridomi-Total by Municipality 276 (28.19) 276 (28.19) El Loro 60 45 2 \sim 2 64 ciliary 212 <u>∞</u> 0+ 180 4 \sim \sim Total by collection Lu. cayennensis c. Lu. trinidadensis Lu. panamensis Lu. atroclavata Lu. yuilli yuilli Lu. carpenteri Lu. micropyga Lu. dysponeta Lu. shannoni Lu. dubitans Lu. camposi Total by sex Lu. gomezi Species location

 $\mathbb Q$ Females; $\mathcal S$ Males; Figures in parentheses indicate percentages.

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Table 2. *Lutzomyia* species with natural infections of *Leishmania* species according to historical reports of species associated with the transmission of cutaneous leishmaniasis and species that represent new records for the Department of Córdoba

Medically important species	Tierralta	Valencia	<i>Leishmania</i> parasites (Natural infection)	Notes
Lu. panamensis	El Loro, Tuis Tuis	Guadual, Mieles	Le. panamensis, Le. braziliensis	Anthropophilic, wide distribution, relict preference for secondary forest, abundant in dry and rainy periods.
Lu. gomezi	El Loro, Tuis Tuis	Guadual, Mieles	Le. panamensis, Le. braziliensis, Le. colombiensis	Anthropophilic, ubiquitous in various habitats, preference for low-lying areas in forested areas.
Lu. shannoni	El Loro	_	Le. mexicana, Le. panamensis, Le. infantum	Autogenous, feeds on a variety of mammals, potential vector for visceral leishmaniasis, natural infection of vesicular stomatitis virus serotype New Jersey.
Lu. cayennensis c.	El Loro, Tuis Tuis	Mieles	Non-identified promastigotes, <i>Le. panamensis</i>	Ubiquitous in different habitats, feeds on cold- blooded vertebrates (lizards), rests on the walls inside homes.
Lu. trinidadensis	El Loro	Mieles	<i>Le. venezuelensis</i> Non-identified promastigotes	Feed on cold-blooded vertebrates.
Lu. micropyga	El Loro, Tuis Tuis	-	Non-identified promastigotes	Anthropophilic, ubiquitous in diverse habitats, often collected in tree roots.
Lu. yuilli yuilli	El Loro, Tuis Tuis	_	Le. panamensis	Anthropophilic, adaptation to human-modified environments and crops in the peridomiciliary and intradomicile areas.
New Records				
Lu. carpenteri	El Loro	Mieles	_	Belize, Colombia, Costa Rica, México and Panamá. Sucre, Antioquia, Caldas, Chocó, Cundinamarca, Guainía, Guaviare, Huila, Santander, Tolima and Magdalena. Not anthropophilic, and its role in the transmission of <i>Leishmania</i> in animals is uncertain.
Lu. camposi	El Loro, Tuis Tuis	Guadual, Mieles	_	Costa Rica, Panamá, Ecuador, Colombia, Antioquia, Boyacá, Caldas, Chocó, Nariño, Santander, Tolima and Valle del Cauca. Second report for the Caribbean coast. The female is virtually indistinguishable from other species of the subgenus <i>Pressatia</i> . zoophilic.
Lu. dysponeta	-	Mieles	_	Costa Rica, Panamá, Ecuador, Colombia, Venezuela. Boyacá, Chocó, Guaviare Meta, Nariño and Tolima. Second report for the Caribbean coast. Associated with rural and fragmented environments. zoophilic.
Lu. atroclavata	Tuis Tuis	Mieles		Costa Rica, Panamá, Ecuador, Colombia, Venezuela. Boyacá, Caldas, Cundinamarca, Guajira, Huila, Magdalena, north of Santander, Santander, Sucre and Tolima.
Lu. yuilli yuilli	El Loro, Tuis Tuis	-	_	Bolivia, Brazil, Ecuador, Colombia, Peru and Venezuela. Amazonas, Antioquia, Caquetá, Guaviare, Guanía, Meta, Putumayo and Santander. Presence from 250–1550 masl.

Spatial distribution of phlebotomines

An inter-village analysis of the *Lutzomyias* spatial distribution maps (Figs. 2–4), revealed predominance of *Lu. panamensis*, a medically important species, comprising 80.18% of the total number of captures. However, the proportion of *Lu. panamensis* in Guadual was significantly lower. The medically important species *Lu. gomezi*, and *Lu. dubitans* represented 7.8 and 8.44% of the total captures, respectively, and displayed homogeneous distribution in all villages, except for El Loro village.

The intra-village analysis showed a higher concentration of phlebotomines at El Loro village (Fig. 2), which is situated close to the foothills of the Nudo de Paramillo natural Reserve. Additionally, geographically proximate areas displayed high vector species richness with notable differences in their abundances (*Lu. yuilli yuilli, Lu. trinidadensis, Lu. shannoni* and *Lu. gomezi*). Tuis Tuis village (Fig. 3) had three clearly spaced areas where phlebotomines were strictly associated with urban settlements. Guadual village (Valencia), (Fig. 4) has low forest cover and the human population is concentrated, displaying a higher relative abundance and spatial distribution of the species *Lu. dubitans* and *Lu. gomezi* in the peripheral area

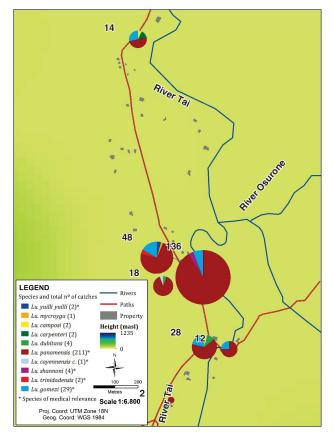


Fig. 2: Spatial distribution and relative abundance of adult phlebotomines collected in the intradomiciles and peridomiciles in the Village of El Loro, in the Municipality of Tierralta, Department of Córdoba.

as compared with the abundance and distribution of *Lu*. *panamensis* in the other villages.

At Mieles village (Fig. 5), three microfoci that presented greater spatial distributions were observed. A mi-

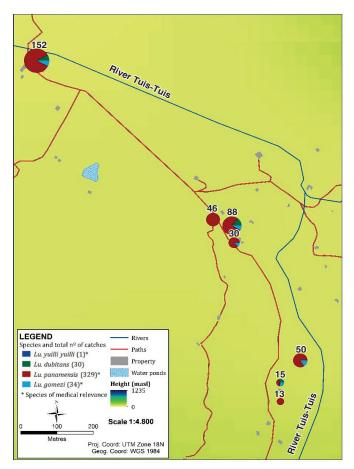


Fig. 3: Spatial distribution and relative abundance of adult phlebotomines collected in the intradomiciles and peridomiciles in the Village of Tuis Tuis, in the Municipality of Tierralta, Department of Córdoba.



Fig. 4: Spatial distribution and relative abundance of adult phlebotomines collected in the intradomiciles and peridomiciles in the Village of Guadual, in the Municipality of Valencia, Department of Córdoba.



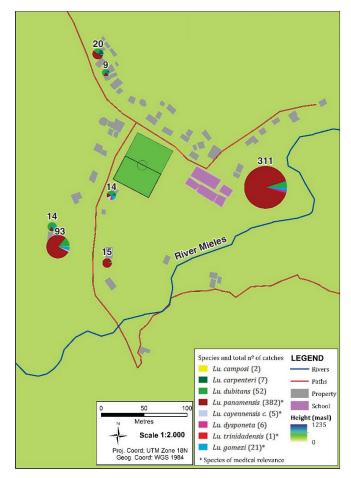


Fig. 5: Spatial distribution and relative abundance of adult phlebotomines collected in the intradomiciles and peridomiciles in the Village of Mieles, in the Municipality of Valencia, Department of Córdoba.

crofocus with high abundance of *Lu. panamensis* was identified next to a school located in a peridomiciliary environment. The other two microfoci were linked to areas with a higher population concentration, suggesting an intradomiciliary activity of *Lu. gomezi* and *Lu. dubitans*.

Analysis of richness and relative abundance of phlebotomines

Significant differences were observed in the composition of phlebotomines associated with house environments, *i.e.* intradomicilary vs peridomiciliary (F=0.9962; df=1; p=0.02895). The Jaccard similarity index revealed two distinct groups. The first group consisted of Mieles and Tuis Tuis villages with similarities in the composition of phlebotomines. This result might be related to the extent of forest cover near these villages, which promotes a greater richness of *Lutzomyia* species. The second group consisted of El Loro and Guadual villages presenting geographical and ecological conditions that are associated with altered or urbanized environments (Fig. 6).

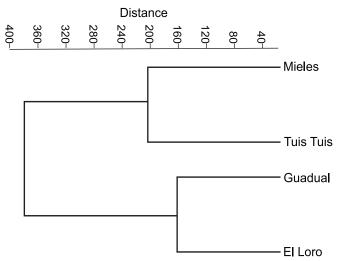


Fig. 6: Hierarchical cluster analysis using the UPGMA algorithm and Jaccard similarity index to compare the housing environment with respect to the communities of identified phlebotomines.

DISCUSSION

The description for the presence and spatial distribution of *Lutzomyia* species in the municipalities of Tierralta and Valencia in Córdoba can significantly contribute to the designing of methods and strategies for the control and prevention of leishmaniasis. Therefore, systematic studies of naturally infected phlebotomines as well as infected wild and domestic animals, which might have a role as potential vectors and reservoirs of *Leishmania*, are necessary. The large number of vector species and demographic conditions in the villages presented in the study (Table 2) indicate the risk factors for vector-human contact in the region.

The lists of phlebotomines identified in the municipalities of Tierralta and Valencia recognized as insect vectors of CL or naturally infected by *Leishmania*¹ include *Lu. panamensis, Lu. gomezi, Lu. shannoni, Lu. cayennensis c., Lu. trinidadensis, Lu. micropyga* and *Lu. yuilli yuilli* (Table 2). The presence of *Lu. panamensis* in Valencia and Tierralta as well as its wide spatial distribution are potentially interesting findings because *Lu. panamensis* is an abundant species that is primarily associated with the peridomicile transmission.

Lutzomyia panamensis is a confirmed vector for CL and the results indicated that it might be the principal vector in these two municipalities. Bibliographic records show that *Lu. panamensis*, which is associated with urban environments and relicts of secondary forests close to peridomiciles, plays an important role in the transmission of CL²³. *Lutzomyia panamensis* has a preference for the forest floor and is also abundant during the dry and rainy seasons. Although, this species is considered anthropophilic, it also feeds on other hosts, particularly rodents^{1, 23}. In Colombia, *Lu. panamensis* is distributed in 18 of the 32 departments, and it was recently reported along with *Lu. gomezi* as a probable species responsible for the urban transmission of CL on the Caribbean coast of Colombia¹⁷.

The bioecology and epidemiological importance of *Lu. gomezi* in Colombia are related to its anthropophilic activity, high capacity for adaptation to urban environments and intradomiciliary behaviour, confirming it as a vector for CL transmission²³. *Lutzomia shannoni* was only collected in the village of El Loro. This species has wide range of feeding preferences²¹ and is considered a potential vector of visceral leishmaniasis and vesicular stomatitis virus of the New Jersey serotype²⁴⁻²⁵.

In a study carried out by Cochero and collaborators⁵ in the region of Montes de Maria, the natural populations of *Lutzomyia cayennensis c*. was found infected by unidentified species of flagellates. *Lutzomyia cayennensis c*. has a great capacity to adapt to different habitats and commonly bite reptiles. Several studies have suggested the need to determine the genus and the trypanosomatid species that naturally infects populations of *Lu. cayennensis c*. and whether this phlebotomine plays a role in maintaining the cycle of disease transmission^{5, 26}.

Female *Lu.trinidadensis* feeds on cold-blooded vertebrates, and populations have been found with natural infections of *Leishmania venezuelensis*²⁷ in Venezuela. *Lutzomyia micropyga* is anthropophilic, and in the town of Sincelejo (Department of Sucre), this species was recently reported to be naturally infected with trypanosomatid parasites. The *Lu. yuilli yuilli* was collected only in the Municipality of Tierralta and is considered a potential vector of CL. In the Department of Boyacá, this species was recently found to be naturally infected with *Le. panamensis* (Table 2).

Among the documented *Lutzomyia* species in the present study, five represent new records for the department of Córdoba, namely, *Lu. carpenteri* (El Loro and Mieles), *Lu. camposi* (Tierralta and Valencia), *Lu. dysponeta* (Mieles), *Lu. atroclavata* (Mieles) and *Lu. yuilli yuilli* (Tierralta). With this study the number of *Lutzomyia* species recorded in the department of Córdoba (Table 2) increased to 18 revealing a greater species diversity.

CONCLUSION

In conclusion, the municipalities of Valencia and Tierralta in the department of Córdoba, have been found to harbour a large number of species of *Lutzomyia*, which is an important genus in the transmission of CL in Colombia. *Lutzomyia panamensis* and *Lu. gomezi* were the most abundant taxa, with wider spatial distributions in the four villages explored. This study is the first record of the presence of *Lu. carpenteri*, *Lu. camposi*, *Lu. dysponeta*, *Lu. atroclavata* and *Lu. yuilli yuilli* in the department of Córdoba, Colombia.

Conflict of interest

All the authors declare that they have no conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the community members, community leaders and health centres in the municipalities of Tierralta and Valencia for allowing our group to perform field activities. The authors are also thankful to Mr Elkin Monterrosa and Andrés Velez Mira for their logistical and entomological support and Dr Richard Hoyos Onalbi for providing administrative and logistical management. The authors appriciate the support of the Governorship of Córdoba. Agreement 754–2013, Study Program Infection and Tropical Health, University of Sinú.

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Received: 28 September 2016

Accepted in revised form: 25 January 2017