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Insights into the transmission cycle of cutaneous leishmaniasis from an endemic community in rural Guatemala

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ABSTRACT

Cutaneous leishmaniasis (CL) is a parasitic vector-borne disease prevalent in 90 countries. Despite its endemicity in Guatemala, key transmission factors are still unknown. To address this, we characterized sand fly populations and *Leishmania* parasites in a Guatemalan community in 2022. We visited the households of 23 patients with lesions compatible with CL, sampled for sand flies and analysed *Leishmania* spp. presence in patients' skin scrapings and in collected sand flies. We collected 93 sand flies, predominantly females including the vector species *Nyssomyia ylephiletor*, *Bichromomyia olmeca* and *Lutzomyia cruciata*. *Nyssomyia ylephiletor* was the most abundant species indoors. Four *Leishmania* spp. were identified including *L. panamensis*, *L. guyanensis*, *L. braziliensis* and *L. infantum* in CL lesions, *L. guyanensis* complex species (*L. guyanensis* or *L. panamensis*) and *Leishmania* sp. in sand flies. Sand fly species positive for *Leishmania* spp. were *Ny. ylephiletor*, *Dampfomyia deleoni*, *Dampfomyia* sp. and *Brumptomyia* sp. Blood-meal analysis revealed human and pig blood in engorged *Ny. ylephiletor* collected inside and in the proximity of the households. This is the first report of *L. guyanensis* in Guatemalan patients and provides insights into CL transmission dynamics, suggesting potential indoor transmission, pending more studies.

1. Introduction

Cutaneous leishmaniasis (CL) is a neglected tropical disease prevalent in 90 countries (Ruiz-Postigo et al., 2023) particularly in low-middle-income territories. CL is caused by *Leishmania* parasites transmitted by female sand flies during blood-feeding. In 2023, 272,098 new CL cases were reported worldwide; of these 35,135 were reported in the Americas (Jain et al., 2024).

Guatemala, a Central American country, is endemic for two clinical forms of leishmaniasis: visceral and CL. The incidence of CL increased in endemic areas of Guatemala in the last decade, rising from 28.9/100,000 people in 2012 to 72.26/100,000 people in 2019 (Ruiz-Postigo et al., 2020); 1134 cases were reported across the country in 2022 (PAHO/WHO, 2023). Evidence from studies carried out in the 80's and 90's in northern Guatemala showed that CL was caused almost exclusively by *L. braziliensis* (~75% of cases) and *L. mexicana* (~25% of cases) (Navin et al., 1988), but recent studies suggest the *L. guyanensis* species complex to be involved in other endemic regions of the country (Montalvo et al., 2017). Nevertheless, knowledge about transmission drivers and vector species involved remains limited. Our recent literature review highlighted the pressing need to obtain updated data to better understand the CL transmission cycle (Lopez et al., 2023). Entomological and ecological studies are key as the vector and transmission dynamics in Guatemala are still unknown. Of the 35 sand fly species previously reported in the country, at least 11 have been associated with leishmaniasis transmission in other endemic countries in Latin America including *Lutzomyia cruciata*, *Nyssomyia ylephiletor*, *Pintomyia ovallesi* and *Bichromomyia olmeca* (Lopez et al., 2023) but none has been

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incriminated as true vector in the country. Also, no reservoir has been found for the species of *Leishmania* present in Guatemala. The reservoirs for *L. braziliensis* and *L. mexicana* are known from other endemic countries and include opossums, rodents, skunks and monkeys for *L. braziliensis* and rodents for *L. mexicana* (Roque and Jansen, 2014). In Guatemala, CL has been predominantly detected in patients with outdoor occupational exposure (Copeland et al., 1990).

Despite the increase in CL cases over the last ten years in Guatemala (MSPAS, 2019; Ruiz-Postigo et al., 2020), comprehensive information on the *Leishmania* species and vectors is lacking, hampering the development of appropriate control and preventive measures (PAHO/WHO, 2022). Research on the main components of the transmission cycle of CL in Guatemala have been lacking since the early 2000's, creating a need to update the information. To address this gap, an observational descriptive study was conducted in an endemic community in Guatemala from March to August 2022 to assess possible vectors and parasite species in sand flies and patients.

2. Materials and methods

2.1. Study site

The study was conducted in a rural community of the municipality of Cobán, Alta Verapaz (AV), northern Guatemala. According to the last national census of 2018, approximately 8% (1,215,038) of the total population of the country lives in Alta Verapaz (INE, 2018), relying mainly on agriculture (SEGEPLAN, 2011). Cobán is classified as a subtropical wet forest according to the Holdridge life zones system (SEGEPLAN, 2011) and is predominantly inhabited by Mayan descendants. Cobán is located at 1316 m above sea level, with temperatures as low as 12 °C and as high as 27 °C and an average annual precipitation of 2000 mm (Bernal et al., 2023). The main economic activity in AV is agriculture, either for subsistence (maize and beans) or exportation (coffee and cardamom) (SEGEPLAN, 2011). In AV, 84% of the population is living in poverty (Owen et al., 2010). Around 77% of AV residents live in rural areas, with the city of Cobán being the main urban centre (INE, 2013). AV is endemic for CL and has reported an increase in the number of CL cases yearly (MSPAS, 2019). The increase of cases could be the result of improved surveillance or a change in the epidemiological scenario.

2.2. Study design

From March to August 2022, a descriptive cross-sectional study was conducted in an endemic rural community from Cobán, selecting households where CL cases were detected by the Ministry of Health (MoH). The study period covered both the dry and rainy season in the area. Demographic and lesion data, along with lesions' smears collected by the MoH for diagnosis were obtained from every patient in each household. Additionally, sand flies were collected monthly in patient's household with light traps, as described below. At the end of the study period, 21 households had been visited with 23 patients recruited. Most of the households were visited once, as only one patient was detected but one household was sampled three times as new cases emerged during the visits to the community. Diagnosed patients received free treatment from the MoH. The visited households were simple huts with tied wood planks, minimal room separation and palm leaf roofs. The location of the community and distribution of the households is presented in Fig. 1.

For sand fly collections, four households were selected each month, sampling all the houses at the end of the study period. CDC light traps (provided by the Centre for Disease Control and Prevention (CDC) to the Centre for Health Studies-Universidad del Valle de Guatemala (CHS-UVG) in previous studies) were placed in the selected households, four days a week, and three light traps per house in these environments: indoors, peri-domicile (i.e. next to animal sheds), and extra-domicile (i.e. forest edge). Traps operated from 18:00 to 06:00 h, for a total trapping effort of 288 trap-nights. The trapping bags were kept in ice and the sand flies were separated from the other arthropods, photographed and dissected into three segments: head, genital terminalia, and abdomen/

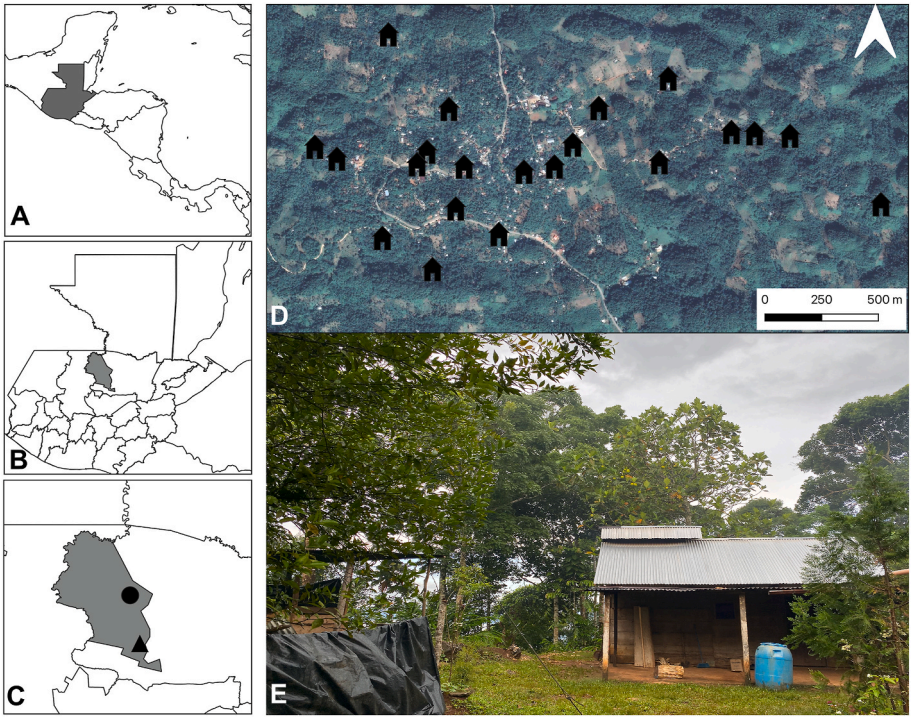

Fig. 1. Geographical location and description of the study area. **A** Guatemala in Central America. **B** Municipality of Cobán in Alta Verapaz. **C** Location of the rural community (*circle*) and the urban centre of Cobán (*triangle*). **D** Distribution of patients' households and sand fly trapping sites. **E** Typical house structure and environment of the study site.

thorax. The head and genital terminalia were stored in 70% (v/v) ethanol for morphological identification and the abdomen/thorax in RNA preserving solution (RNAlater, Invitrogen, Thermo Fisher Scientific, Waltham, USA) until transportation to the laboratory at the CHS-UVG for molecular analyses.

2.3. DNA extraction from sand flies

DNA was extracted for molecular identification of sand fly and *Leishmania* spp., and blood-meal analysis. For males, a non-destructive DNA extraction protocol was followed (Harrup, 2014). Briefly, each specimen was incubated overnight in 200 µl of an in-house tissue digestion solution (100 mM Tris-HCL, 200 mM NaCl, 0.2% Triton X-100, 5 mM EDTA, 200 µl/ml Proteinase K, and water) (Harrup, 2014) at 37 °C. After incubation, the specimens were removed from the solution and stored again at −20 °C for permanent mounting. DNA was precipitated in cold temperature with 3M sodium acetate and ethanol, and washed with 70% cold ethanol before resuspending with water (Harrup, 2014). For non-engorged females, the PureGenome™ Tissue DNA Extraction Kit (Novagen, Darmstadt, Germany) was used to extract DNA from the abdomen/thorax segments. For engorged abdomens of females, Qiagen DNeasy Blood & Tissue Kit (Qiagen, Baltimore, USA) was used to extract DNA for blood-meal analysis.

2.4. Sand fly identification

All sand flies were identified using two methods: morphologically using the key by Galati (2018), and *via* DNA barcoding (Hebert et al., 2003), targeting the 658-bp region of cytochrome *c* oxidase subunit 1 (*cox*1) gene. PCR was conducted using primers LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Adeniran et al., 2019), in a 20 µl reaction (3 µl of DNA extract, 1× Platinum II Hot Start Master Mix (Invitrogen, Thermo Fisher Scientific), 0.2 µM of each primer, and water). The cycling conditions were: initial denaturation at 94 °C for 1 min, 35 cycles at 94 °C for 30 s, 56 °C for 30 s, and 68 °C for 1 min, and a final extension step at 68 °C for 5 min, using DNA from *Aedes aegypti* Rockefeller as an internal positive control.

2.5. Blood-meal analysis of sand flies

Engorged females were screened by a 358-bp PCR of the vertebrate cytochrome *b* gene (*cytb*), using the primers L14841 (5′-CCA TCC AAC ATC TCA GCA TGA TGA AA-3′) and H15149 (5′-GCC CCT CAG AAT GAT ATT TGT CCT CA-3′) (Kocher et al., 1989). PCR reactions were performed in an Eppendorf Mastercycler Gradient 5331 with a 20 µl reaction (3 µl of DNA extract, 1× Platinum II Hot Start Master Mix (Invitrogen, Thermo Fisher Scientific), 0.2 µM of each primer, and water). Positive control was DNA from human-blood-fed *Aedes aegypti* Rockefeller. The cycling conditions were: initial denaturalization at 94 °C for 4 min, 35 cycles of 94 °C for 30 s, 58 °C for 1 min, 68 °C for 30 s and a final extension step at 68 °C for 5 min.

2.6. Clinical samples and demographic data

Tissue scrapings, routinely collected by the MoH for microscopic diagnosis were used to molecularly identify the *Leishmania* parasite responsible for the infection, with patient consent. Demographic data and lesion progression were gathered *via* questionnaires.

Genomic DNA from tissue smears for *Leishmania* identification was obtained by scraping the tissue into PBS, followed by extraction using the Qiagen DNeasy Blood & Tissue Kit (Qiagen) per the manufacturer's instructions.

2.7. Leishmania identification in clinical samples and sand flies

Two fragments of the *Leishmania* heat-shock protein 70 gene (*hsp*70) were amplified from the DNA extracted from clinical samples and female sand flies: a 1286-bp fragment (PCR-F), using primers F25 (5′-GGA CGC CGG CAC GAT TKC T-3′) and R1310 (5′-CCT GGT TGT TGT TCA GCC ACT C-3′), and a 593-bp fragment (PCR-N), using primers F25 and R617 (5′-CGA AGA AGT CCG ATA CGA GGG A-3′) (Montalvo et al., 2012). All samples were initially tested with PCR-F and PCR-N was used only when amplification failed with the PCR-F, as sequencing of the PCR-N amplicons does not allow discrimination between species of the *L. guyanensis* complex, unlike sequencing of PCR-F amplicons (Montalvo et al., 2012). PCR was conducted in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems, Warrington, UK), with a 25 µl reaction (5 µl of DNA extract, 1× Hot Start Taq 2× Master Mix (New England Biolabs, Hitchin, UK), 0.5 µM of each primer, and water). Positive controls were DNA from *L. amazonensis* (MHOM/BR/73/M2269) or *L. guyanensis* (MHOM/BR/75/M4147). The cycling conditions were: initial denaturalization at 95 °C for 30 s, 35 cycles of 95 °C for 45 s, 61 °C for 1 min, and 68° for 90 s, and a final extension step at 68 °C for 5min.

2.8. Sequence analysis

PCR products were visualized in agarose gels, sequenced bidirectionally using the same PCR primer pair, and aligned to form consensus sequences in Geneious Prime® 2023.1.2. Consensus *cox*1 sequences were compared with those in the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert, 2007) to identify sand fly species. Additional confirmation involved barcode index number (BIN) separation and construction of a neighbour-joining dendrogram in MEGA X (Stecher et al., 2020). Sequences and chromatograms were uploaded to BOLD under "GTCL–Understanding cutaneous leishmaniasis in Guatemala". For *Leishmania hsp*70 and *cytb* PCR, consensus sequences were compared to GenBank entries, accepting those with > 98% similarity. *Leishmania* spp. DNA sequences from clinical samples were deposited in the GenBank database under the accession numbers OR551753-OR551763.

A copy of the article in Spanish is presented in Supplementary file S1.

3. Results

3.1. Sand fly diversity and distribution

Ninety-three sand flies were captured: 73 (78.5%) females and 20 (21.5%) males. Extra-domicile collections slightly surpassed intra-domicile ones (37.6% *vs* 35.4%), with peri-domicile yielding 27%. Seventy-six sand flies were identified at the species level (5 species), 15 at the genus level (Table 1), while two could not be identified at either level. *Nyssomyia ylephiletor*, *Lutzomyia cruciata* and *Brumptomyia mesai* were the most abundant species. Bioinformatic confirmation of species identification is provided in Supplementary file 2: Fig. S1 and Table S1.

Table 1
Abundance of the sand fly species collected during the study period.

Species	Females	Males	Total
Nyssomyia ylephiletor	26	–	26
Lutzomyia cruciata	20	4	24
Brumptomyia mesai	9	13	22
Dampfomyia sp.	9	2	11
Lutzomyia sp.	1	–	1
Brumptomyia sp.	2	1	3
Dampfomyia (*Coromyia*) *deleoni*	2	–	2
Bichromomyia olmeca	1	–	1
Pintomyia sp.	1	–	1
Total	71	20	91

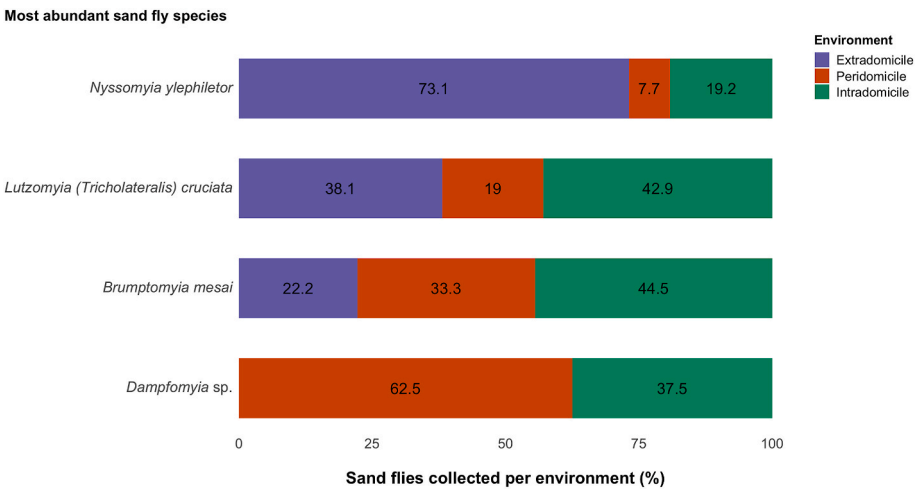

Fig. 2. Distribution per trapping environment of the most abundant sand fly species. Species are represented in order of relative abundance with *Ny. ylephiletor* being the most abundant with 26 individuals, followed by 24 of *Lu. cruciata*; 22 of *Brumptomyia mesai* and 11 of *Dampfomyia* sp. Numbers inside the bars represent the percentage of collected flies in a given environment.

Four species yielded more than 10 individuals, representing 91.2% of the total collected individuals. Five sand fly species were collected in multiple trapping environments. Eight taxa were captured in both the extra-domicile and peri-domicile, comprising 35.7% and 21.8% of the females collected, respectively; 42.5% of the females was collected indoors and belonged to four species. More than 70% of *Ny. ylephiletor* was found indoors, whereas *Lu. cruciata* was mostly found in the extra-domicile (45%). Fig. 2 shows the distribution per each trapping environment of the most abundant species collected.

3.2. Sand fly blood-meal sources

Three *Ny. ylephiletor* had blood visible in the abdomen. Two were captured in the intra-domicile, and one was captured in the peri-domicile. The blood sources were identified as human (2 samples, indoors and peri-domicile) and domestic pig (1 sample, indoors).

3.3. Leishmania spp. DNA in sand flies

All female sand flies were screened for the presence of *Leishmania* spp. DNA, including those lacking a visible blood meal. No *Leishmania* spp. DNA was detected in any of the sand flies with a visible blood meal analysed. Four of 73 females (5.5%) sand flies were *Leishmania* PCR-positive: three for *L. guyanensis* species complex and one for unidentified *Leishmania* species (Table 2).

Table 2
Leishmania species identified according to sand fly species and environment.

Sand fly species	Environment	*Leishmania* species	*n* (%)
Nyssomyia ylephiletor	Intra-domicile	*Leishmania* sp.	26 (3.8%)
Dampfomyia deleoni	Extra-domicile	*L. guyanensis/L. panamensis*	2 (50.0%)
Brumptomyia sp.	Extra-domicile	*L. guyanensis/L. panamensis*	2 (50.0%)
Dampfomyia sp.	Peri-domicile	*L. guyanensis/L. panamensis*	9 (11.1%)

Notes: The method used allows to confirm the presence of the *L. guyanensis* complex but does not allow discrimination among its species. *Leishmania guyanensis* and *L. panamensis* are both presented since the parasite can be either.
Abbreviations: *n*, number of sand flies screened; %, percentage by species of sand fly positive to *Leishmania* DNA.

Table 3
Demographic characteristics of patients diagnosed with cutaneous leishmaniasis from March to August 2022 at the study site.

Characteristic	*n* (%)
Age in years, mean (range)	34 (12–65)
Number of lesions per participant, mean (range)	1.2 (1–3)
Sex	
Male	13 (56.0)
Female	10 (44.0)
Maya Q'eqchi' ethnic group	23 (100)
Previous leishmaniasis	2 (8.0)
Location of lesions	
Lower legs	10 (34.5)
Hands	5 (17.2)
Neck	3 (10.3)
Feet	3 (10.3)
Forearms	2 (7.0)
Back	2 (7.0)
Upper legs	2 (7.0)
Ears	1 (3.4)
Arms	1 (3.4)

3.4. Characteristics of CL patients

CL was diagnosed by microscopic identification of lesion scrapings of 23 people, 13 males and 10 females, all of the Maya ethnic group (Table 3). The age-specific distribution of the cases was five 12–17 years-old, and 18 adults > 18 years-old. Lesions were localized in the limbs, neck and back, including 35% in the lower part of the leg and 17% on the hands.

3.5. Leishmania spp. in clinical samples

Leishmania spp. found in 11 clinical samples analysed by PCR (10 by PCR-F and 1 by PCR-N) included 5 *L. panamensis*, 3 *L. guyanensis*, 1 *L. guyanensis* complex (*L. guyanensis* or *L. panamensis*), 1 *L. braziliensis* and 1 *L. infantum* (Table 4). The remaining samples had a positive microscopic diagnosis by the MoH but were unavailable for molecular analysis.

Table 4
Species of *Leishmania* identified in clinical samples (lesion scraping) of cutaneous leishmaniasis patients from the study site.

Species	No. of clinical samples (%)
L. panamensis	5 (46)
L. guyanensis	3 (27)
L. braziliensis	1 (9)
L. infantum	1 (9)
L. guyanensis/L. panamensis	1 (9)
Total	11

4. Discussion

This study provides the first insights into sand fly abundance and *Leishmania* spp. diversity in Alta Verapaz, Guatemala, offering a preliminary understanding of CL transmission dynamics. Sand flies had been previously sampled in endemic areas of Petén, Guatemala, particularly in forested areas (Rowton et al., 1991; Perich et al., 1995) but their small-scale distribution and behaviour around human habitats was not known.

Rowton et al. (1991) collected 135,372 sand flies throughout a year in a primary forest of Petén using human-landing collections, with *Pintomyia ovallesi* (as its synonym *Lu. ovallesi*, see Galati and Rodrigues, 2023) being the most prevalent (87.5%). Similarly, sand flies were collected in forested habitats after insecticide spraying in Petén using CDC light traps. In the study by Perich et al. (1995), 2876 sand flies were collected in 13 trapping nights, with *Psychodopygus panamensis* (as its synonym *Lu. panamensis*, see Galati and Rodrigues, 2023) being the most prevalent. In our study, *Ny. ylephiletor*, *Lu. cruciata* and *Brumptomyia mesai* were the most prevalent sand fly species, the first two being confirmed vectors of *Leishmania* spp. causing CL in Central America and Mexico (Pech-May et al., 2010; Dutari and Loaiza, 2014). Previously in Guatemala, *L. braziliensis* (Rowton et al., 1991) and *L. mexicana* (Porter et al., 1987) were detected in *Ny. ylephiletor*, while an unidentified species was found in *Lu. cruciata* (Rowton et al., 1991), though further incrimination was achieved. In our study, *Ny. ylephiletor* emerged as an abundant species indoors, a behaviour uncommon in Central America (Fairchild and Hertig, 1952; Rowton et al., 1991). The presence of pig and human blood in *Ny. ylephiletor* abdomen highlights its opportunistic foraging habits (Dutari and Loaiza, 2014). The indoor presence *Ny. ylephiletor* increases the risk of parasite transmission to humans and domestic animals. Moreover, *Lu. cruciata* showed habitat generality (Castillo et al., 2015), as it was collected across the environments. Its competence for the parasite species in this study is unknown. Despite not detecting *Leishmania* DNA in any *Lu. cruciata* in our study, this species can play an important role in the transmission of *L. mexicana* (Pech-May et al., 2010) if the parasite is introduced in the area. Overall, the sampling effort of sand flies yielded relatively low abundance of sand flies. As mentioned before, in another endemic area of Guatemala, more than 2876 sand flies were collected in 13 trapping nights (Perich et al., 1995), while our effort of 288 trap/nights resulted in only 93 sand flies. Although no previous data are available for the region, it should be investigated if there is a seasonal trend in sand fly abundance and if it corresponds with CL cases, using a variety of sampling methods.

The presence of *Leishmania* spp. DNA across different sand fly species underscores complex interactions within the transmission cycle. Finding DNA of *Leishmania* spp. in a sand fly does not indicate vectorial competence, as it does not differentiate if the parasite has differentiated into infective form or will be expelled *via* excretion (Pruzinova et al., 2018). DNA of *Leishmania* spp. has previously been reported in *Brumptomyia messai* and *Dampfomyia beltrani*, without a role in the transmission of human parasites (Lozano-Sardaneta et al., 2020). In summary, while entomological studies have identified sand flies as mainly forest-associated in Guatemala (Rowton et al., 1991; Perich et al., 1995), our data show their presence near the households, suggesting potential indoor transmission. Additionally, CL cases among women and children supports the idea of domestic transmission, as seen in Colombia (Rodríguez-Villamizar et al., 2006) and Panama (Saldana et al., 2013).

In contrast to studies performed in the 1980's and 1990's in northern Guatemala, where *L. braziliensis* and *L. mexicana* were the most abundant species (Navin et al., 1988), *L. guyanensis* complex was the most common in the present study, possibly suggesting a difference in parasite composition per endemic region. All the components of the transmission of the *L. guyanensis* species complex should be explored in Guatemala. The main reservoir, *Choloepus* spp. (sloths), of *L. panamensis* is not present in Guatemala. However, reported reservoirs for *L. guyanensis* such as rodents, armadillo (*Dasypus novemcinctus*), collared anteater (*Tamandua tetradactyla*) and kinkajou (*Potos flavus*) are all present in the region (Roque and Jansen, 2014). In addition, the finding of a *L. infantum* lesion on the foot of a patient was unexpected, as cutaneous lesions caused by this species have not yet been reported in Guatemala but are important in Brazil (Soares et al., 2024). In Honduras, Nicaragua and Costa Rica this species has been associated with atypical CL (Noyes et al., 1997). The patient in our study responded favourably to the standard CL treatment regime of 20 mg/kg of intramuscular antimonials for 20 days (MSPAS, 2021). Further studies are needed to describe the epidemiology and distribution of the cutaneous form of *L. infantum* in Guatemala.

It is worth noting that the descriptive nature and short duration of the study prevent drawing conclusive insights into the patterns of disease spread. The convenience sampling method adopted in this study allowed us to create a snapshot of sand fly and *Leishmania* spp. distribution, although limiting broader conclusions about year-round transmission dynamics. Regarding parasites, as DNA of *Leishmania* spp. was identified with different PCR conditions, a comparison between parasite strains found in sand flies and in humans was not possible thus preventing a confirmation of sand flies as vectors.

5. Conclusions

This study highlights the risk of CL indoor transmission in a rural community in Guatemala. *Nyssomyia ylephiletor* was collected mostly indoors and tested positive for *Leishmania* DNA and for human blood. Given its vector competence for *Le. panamensis* (Dutari and Loaiza, 2014), further research to understand its involvement in the transmission cycle is essential. Unlike the studies from the 1980's–1990's in Petén, in which a dominance of *L. braziliensis* and *L. mexicana* was observed in patient lesions (Herwaldt et al., 1992), our study shows a prevalence of *L. panamensis* and *L. guyanensis* in the samples and reports a cutaneous form of *L. infantum*. This diversity in species creates a need to (i) update the data from Petén, to confirm if there are changes in epidemiological scenarios, and (ii) describe other endemic areas where no data on species composition are available. This study contributes to regional knowledge of *Leishmania* spp. distribution. At a national level, the results should prompt the MoH to consider implementing indoor control and prevention measures for residents in endemic areas, raising awareness among the at-risk populations and including indoor prevention measures in their health education activities.

Ethical approval

Ethical approvals were granted by Research Ethics Committee of the Centre for Health Studies at Universidad del Valle de Guatemala (protocol 253-09-2021) and from the National Health Ethics Committee of the MoH (protocol 24–2021). Participants voluntarily consented to participate and signed consent forms. Minors under 18 provided informed assent, along with parental or legal guardian consent.

CRediT authorship contribution statement

Yaimie Lopez: Conceptualization, Data curation, Analysis, Funding

acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Aitor Casas-Sanchez**: Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. **Byron Arana:** Conceptualization, Supervision, Writing - review & editing. **Nidia Rizzo:** Conceptualization, Supervision, Writing – review & editing. **Erick Duran:** Conceptualization, Project administration, Resources, Writing - review & editing. **Norma Padilla:** Funding acquisition, Writing - review & editing. **Andrea de la Vega:** Investigation, Writing - review & editing. **Esteban Bustamante:** Investigation, Writing - review & editing. **Álvaro Acosta-Serrano:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Visualization, Writing – review & editing. **Renata Mendizabal-Cabrera:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2025.100244.

Data availability

The data supporting the conclusions of this article are included within the article and its supplementary files. Study datasets are available in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) under the accession numbers OR551753-OR551763 for *Leishmania* sequences and in BOLD (http://www.boldsystems.org) for sand fly species identification by DNA barcoding.

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