Original article

New Ecoregional and Altitudinal Reports of Leishmaniosis Vectors (Psychodidae: Phlebotominae) in Southern Bolivia

Nuevos registros ecorregionales y altitudinales de vectores de leishmaniosis (Psychodidae: Phlebotominae) en el sur de Bolivia

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ABSTRACT

Introduction: The present study allows to estimate the potential role of the diversity of Phlebotominae (Diptera: Psychodidae) species, and their eco-epidemiological

aspects as vectors of leishmaniosis, mainly in regions where there is a suspected emergence or re-emergence of cases.

Objective: Characterizing the diversity of Phlebotominae (Diptera: Psychodidae) species and their eco-epidemiological aspects.

Methods: This study was conducted in the town of Caraparí, approximately 800 masl, within an inter-andean region in Tarija department from southern Bolivia. The captures were realized between October 2023 and June 2024 in households located on the outskirts of the urban area, using REDILA-BL light traps, and then, standardized microscopy techniques were applied to identify sandflies.

Results: 443 individuals were captured, of which 435 were classified in five species: *Lutzomyia cruzi, Evandromyia cortelezzii, Migonemyia migonei, Nyssomyia neivai, and Micropygomyia peresi.* Considerably, *Lu. cruzi* was the dominant species, representing 52.6% of total captures, followed by *Ev. cortelezzii and Mg. migonei*. The highest phlebotomine sandfly presence was observed between the moistest months (March and April), at dwellings with several inhabitants and different types of domestic animals.

Conclusions: The presence of medically important species, such as *Ny. neivai* (vector of cutaneous and mucocutaneous leishmaniosis) and *Lu. cruzi* (vector of visceral leishmaniosis) at a new ecoregion and altitudinal level, suggests an entomological risk for transmission in this region. This study provides new data of distribution of these vectors in southern Bolivia and underscores the need for continuous surveillance and control measures.

Keywords: Sandflies; *Lutzomyia cruzi*; *Nyssomyia neivai*; medical entomology; entomological risk.

RESUMEN

Introducción: Este estudio permite estimar los potenciales roles de la diversidad de especies de *Phlebotominae* (*Diptera: Psychodidae*) y sus aspectos eco-epidemiológicos, como vectores de leishmaniosis, principalmente en regiones donde existe una posible emergencia o re-emergencia de casos.

Objetivo: Caracterizar la diversidad de especies de *Phlebotominae* (*Diptera: Psychodidae*) y sus aspectos eco-epidemiológicos.

Métodos: Este estudio fue conducido en el poblado de Carapari, a aproximadamente 800 msnm dentro de una región inter-andina del departamento de Tarija al sur de Bolivia. Las colectas fueron realizadas entre octubre del 2023 y junio del 2024 en viviendas localizadas en la periferia del área urbana, esto usando trampas de luz REDILA-BL, para luego aplicar técnicas estandarizadas de microscopia para identificar flebótomos.

Resultados: 443 individuos fueron capturados, de los cuales 435 fueron clasificados dentro de cinco especies: *Lutzomyia cruzi*, *Evandromyia cortelezzii*, *Migonemyia migonei*, *Nyssomyia neivai* y *Micropygomyia peresi.* Considerablemente, *Lu. cruzi* fue la especie dominante, representando el 52.6% del total de capturas, seguido por *Ev. cortelezzii* y *Mg. migonei*. Además, la presencia de estos insectos fue más alta fue durante los meses húmedos (marzo y abril), en viviendas con varios habitantes y diferentes tipos de animales domésticos.

Conclusiones: La presencia de especies con importancia médica, como *Ny. neivai* (vector de la leishmaniosis cutánea y mucocutanea) y *Lu. cruzi* (vector de la leishmaniosis visceral) en una nueva ecorregión y nivel altitudinal, sugiere un riesgo entomológico de transmisión en esta región. Finalmente, este trabajo aporta nuevos datos de la distribución de dichos vectores en el sureste de Bolivia y resalta la necesidad por medidas de vigilancia y control continuo.

Palabras clave: moscas de la arena; *Lutzomyia cruzi*; *Nyssomyia neivai*; entomología médica; riesgo entomológico.

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Introduction

Phlebotominae is a group of hematophagous insects of the family Psychodidae (Diptera) known as sandflies, the main vectors of protozoan parasites of genus *Leishmania*, the etiologic agents of leishmaniosis.(1) Those vectors are endemic in forested, rural, and urban environments in almost all continents, principally in tropical and subtropical areas.(2) Their global diversity spans 994 described species,

at least 530 in the Americas, and 10% are considered of medical-veterinary importance.[3,4] Thereby, understanding the cycles of transmission, the vectors, and the reservoirs could help to assess the epidemiology and ecology of this vector-borne disease.[3]

Bolivia reports all clinical manifestations of leishmaniosis (cutaneous, mucous, mucocutaneous, and visceral) in seven of its nine departments. Bolivia, along with Brazil, Ethiopia, and Peru, are considered as the territories where most cases of mucocutaneous leishmaniosis (MCL) occur in the world.[5] In 2022, more than two thousand cases were reported in this country; 85.5% cutaneous, 10.7% mucous, 2.7% mucocutaneous, and 1 % visceral, with an incidence fluctuating over the years with a tendency to increase.[6]

In Bolivia, 121 species of sandflies were reported and ten are incriminated as vectors of *Leishmania* spp.[6,7] Such species are; *Lutzomyia longipalpis* (*Lutz & Neiva* 1912) and *Lu. cruzi* (*Mangabeira* 1938) vectors of the parasite that produce Visceral Leishmaniosis (VL), and *Lutzomyia nuneztovari* (*Ortiz* 1954), *Lutzomyia carrerai* (*Barretto* 1946), *Lutzomyia yucumensis* (*Le Pont*, *Caillard*, *Tibayrenc* & Desjeux 1986), *Ny. neivai* (Pinto 1926), *Nyssomyia shawi* (Ward & Ready 1981), *Nyssomyia flaviscutellata* (*Mangabeira* 1942), *Psychodopygus ayrozai* (*Barretto & Coutinho* 1940) and *Psychodopygus llanosmartinsi* (*Fraiha & Ward* 1980) who transmit cutaneous leishmaniosis (CL) and MCL.[6,7]

In Southern Bolivia in 2022, two children died due to VL in the department of Tarija in Villa Montes city, and in the first 20 epidemiological weeks of 2024 there were fourteen confirmed CL cases in other nearby municipalities.[6,8] Moreover, so far, three species of vectors have been reported in this region to date: *Lu. cruzi*, *Lu. longipalpis* and *Ny. neivai*.[6] Based on this health issue in the south of Bolivia and the few entomological studies, it was sought to determine the diversity of *Phlebotominae* species in a locality from this region and propose the species with a potential involvement in leishmaniosis transmission, and thus, take actions to prevent the emergence of this disease.

Methods

Study area

This study was carried out in the locality of Carapari within the department of Tarija - Bolivia (fig. 1), which border at north with Villamontes and Entre Rios municipalities,

at the west with the municipality of Padcaya, at the east with the municipality of Yacuiba and at the south with the Republic of Argentina. This town is located within an inter-andean valley between ecoregions of Chaco Serrano and Tucumano-Boliviano forests also called Southern Andean Yungas, which is characterized by a subtropical humid climate with rainy summers and dry winters.[9] Its urban area has a mean height of 830 meters above sea level (masl), surrounded for wooded mountain ranges up to 1300 masl and have a mean population of 16000 inhabitants dedicated to manual agriculture of corn, peanuts, soy and small cattle, also the oil exploitation has an important contribution to municipality economy.

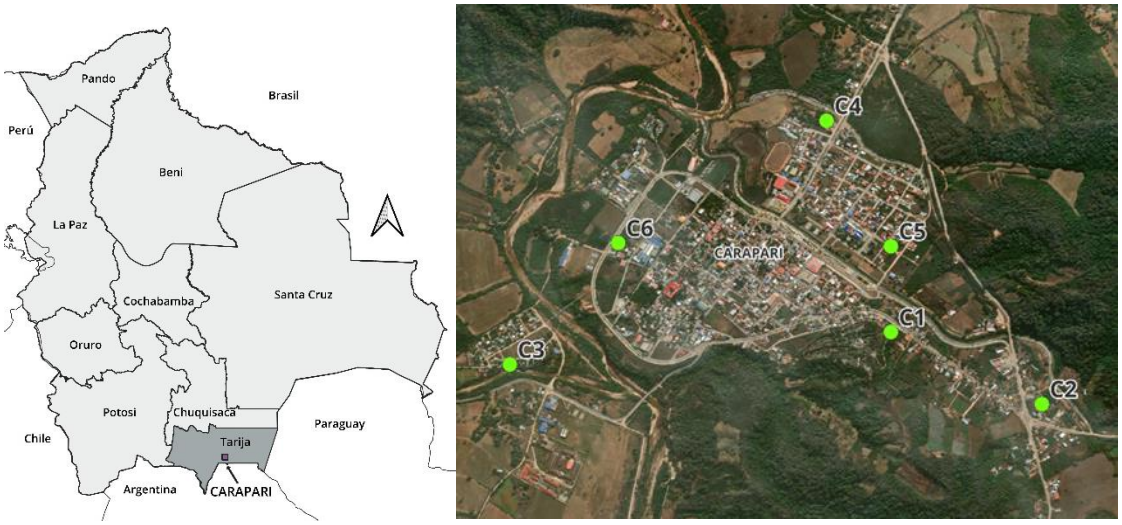

Fig. 1 - Study area; Left, location of Carapari town from Tarija department at southern Bolivia; Right, distribution of studied households (yellow points C1-C6) in the urban area.

Sampling methodology

The samplings were carried out during nine months, from October 2023 to June 2024, encompassing before, during and after rainy season in the region. REDILA-BL light traps[10] were used to capture alive nocturnal insects, built by the medical entomology laboratory team of the Centro Nacional de Enfermedades Tropicales (CENETROP), following the instructions of the Red de Investigación de la Leishmaniasis en la Argentina (REDILA) guide.[11] The traps were placed in six inhabited dwellings in outskirts of the urban area of Carapari (C1-C6) (fig. 1), near

dense vegetation, watercourses, crops and animal breeding areas to increase chances of capture, this according to the criteria of OPS to entomological surveillance of phlebotomine sandflies.[12]

In each selected household, the occupants were informed that the traps posed no risk to people or domestic animals, prior to obtaining their verbal consent. Then, georeferenced data and environmental conditions were recorded, as temperature and relative humidity, but precipitation was not recorded during samplings due to logistical limitations, so data of monthly reports in a nearest weather station from Yacuiba (36.5 km from Carapari) of the National Meteorological and Hydrological Service of Bolivia were consulted.[13] Additionally, other characteristics such as the number of inhabitants and the type of domestic animals in each house were recorded, all that, using the sampling sheets to phlebotomine sandflies studies of OPS.[12]

Afterward, the samplings consisted in installing two light-traps per house, one intra-domicile (indoors, inside the household) and one peri-domicile (outdoors, outside the household but within the owner's property), activated between 6:00 p.m. until 6:00 a.m., that is, all night long for three consecutive nights per month.[11] Hence, 12 light-traps were installed, totaling 27 capture nights (324 hours), then after each sampling night, the captured insects were transported to the Vectors Laboratory of the aforementioned town, to separate the sandflies from other insects and by gender, utilizing magnifying glasses and preserving them in 70% alcohol.[12]

Specimen identification

Subsequently, the preserved individuals were clarified (diaphanization) through a chemical process and mounted to microscopy for the Vector Control team of Carapari municipality, trained by the CENETROP's team of entomologists, following standardized methodology for this group of dipterans.[11] Later, the specimens were sent to the medical entomology laboratory of CENETROP in Santa Cruz de la Sierra city, to identify individuals taxonomically through internal and external morphology utilizing optical microscopy Olympus (CX31RBSFA-Tokyo, Japan) and dichotomous keys from *Young & Duncan*,[2] *Galati*[4] and *Forattini papers*.[14]

Data analysis

Finally, all data were entered into a spreadsheet database in Microsoft Excel to summarize sandflies collections for descriptive results and graphics. Statistical

tests for numerical non-parametric data were carried out in R program 4.3.2, such as the Kruskal-Wallis test to points of sampling, because they are unrelated groups, and the Friedman test to months of study, because they are related groups.[15]

Results

A total of 443 phlebotomine sand flies were captured in Carapari during the 9 months of study in all the sampled households, that is, resulting in 100% infestation positivity. Hence, 435 specimens of the total were identified taxonomically, exhibiting a diversity of five species: *Lu. cruzi*, *Ev. cortelezzii* (*Brethes* 1923), *Mg. migonei* (*Franca* 1920), *Ny. neivai* and *Mi. peresi* (*Mangabeira* 1942) (fig. 2). Nonetheless, 1.8% of individuals were not identified due to reproductive organs being damaged, precluding their recognition, likely during field capture or laboratory processing.

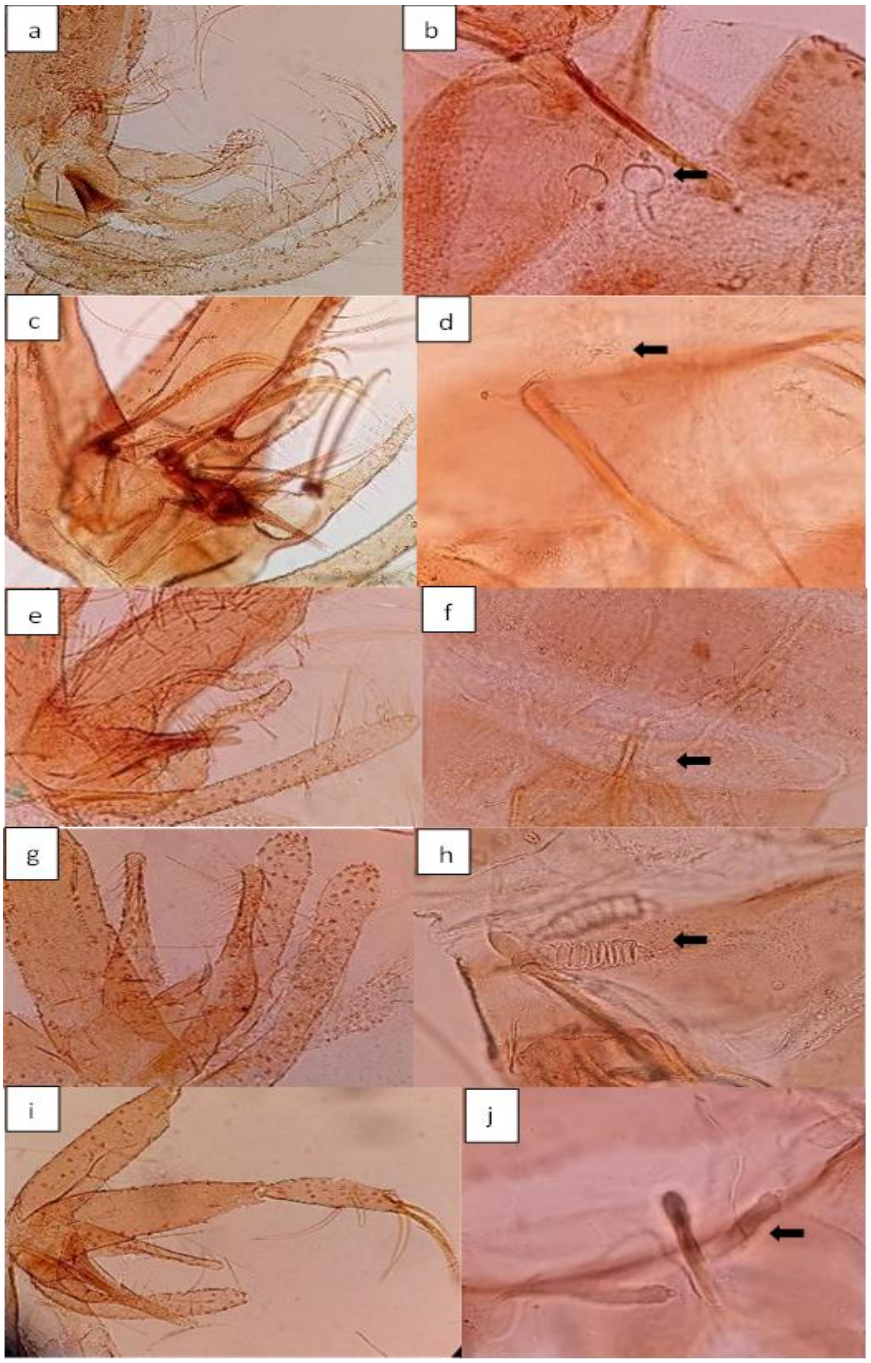

Fig. 2 - *Male terminalia* (left) and *Female genitalia* (right) of *Phlebotomine* species captured in Carapari: (a-b) *Ev. cortelezzii*; (c-d) *Lu. cruzi;* (e-f) *Mg. migonei*; (g-h) *Ny. neivai*; (i-j) *Mi peresi*. Arrows indicate female spermathecae.

On the other hand, C4 point had the greatest abundance with the five species of sand flies, while C5 presented fewer individuals of only two species (table 1), however, there was no significant difference between the abundance of sampling points

(H=6.4945, df=5, p-value=0.261). Interestingly, dwellings with greater phlebotominae abundance and diversity (C1, C2, C4) had more than five human inhabitants with the presence of several types of domestic animals, between dogs, cats, pigs, and poultry, and were located at a height between 824 to 858 masl (table 1).

Table 1 - Data of phlebotomine sandflies captured per sampling sites in Carapari city from October 2023 to June 2024

Point	Latitude	Longitude	Masl	N	%	D	I	Domestic animals
C1	-21.8313471	-63.7363696	858	48	10,8	4	9	Dogs, cats, pigs, hens, ducks
C2	-21.8341640	-63.7304211	841	109	24,6	4	8	Dogs, cats
C3	-21.8326147	-63.7513846	799	33	7,4	3	5	Dogs, cats, hens
C4	-21.8230958	-63.7389059	824	21	47,4	5	5	Dogs, cats, pigs, hens, ducks
C5	-21.8279985	-63.7363699	826	11	2,5	2	3	Dogs
C6	-21.8278687	-63.7471162	814	32	7,2	5	3	Dogs, cats, hens

Legend: masl: meters above sea level, N: Number of individuals, D: Number of species, I: Number of Inhabitants per household.

With respect to species distribution between sampling points, *Ev. cortelezzii* and *Mg. migonei* were the most common species in all sampled dwellings and, although *Lu. cruzi* was reported in four points, it was the dominant species in them (table 2). Regarding the sex ratio, it was in general two males (M) by female (F) approximately (1.9 M: 1F), however, relative abundance by sex was different for each species, having a greater quantity of males of *Lu. cruzi* (6.4M: 1F) and *Ny. neivai* (4.7 M: 1 F), whereas females had a greater amount to *Ev. cortelezzii* (1.5 F: 1 M) and *Mg. migonei* (1.3 F: 1 M) (table 2). In addition, domiciliary location of *Lu. cruzi, Mg. migonei* and *Ev. cortelezzii*, both males and females, were frequently found in outdoors locations than indoors one. The other species were captured only in one type of location, *Ny. neivai* outdoors and *Mi. peresi* indoors (table 2).

Table 2 - Phlebotominae species found in Carapari during the 9 months of samplings.

Species	Total	%	Females	Males	Peri	Intra	Points
Ev. cortelezzii	126	29.0	75	51	68	58	C1;C2;C3;C4;C5;C6
Lu. Cruzi	229	52,6	31	198	221	8	C1;C2;C4;C6
Mg. migonei	61	14,1	34	27	37	24	C1;C2;C3;C4;C5;C6
Ny. neivai	17	3,8	3	14	17	0	C1;C2;C3;C4;C6
Mi. peresi	2	0,5	1	1	0	2	C4;C6
Total	435	100	144	291	343	92	

Legend: Peri: Peri-domicile or outdoors, Intra: Intra-domicile or indoors, %: relative abundance.

Regarding temporal variations, sandflies individuals were found in all sampling months in Carapari, being April when more captures were obtained (N=288) compared to the other months with significant difference between them (Fr=22.797, gf=8, *p*-value=0.003635). On the other hand, *Ev. cortelezzii* and *Mg. migonei* were the species most frequently caught from October to May, and *Lu. cruzi* was only recorded between March and June, but it was the most abundant (fig. 3a). Finally, between February and May, humidity exhibits an increase alongside the outbreak of sand flies, but temperature and regional precipitation exhibited a decrease (fig. 3b).

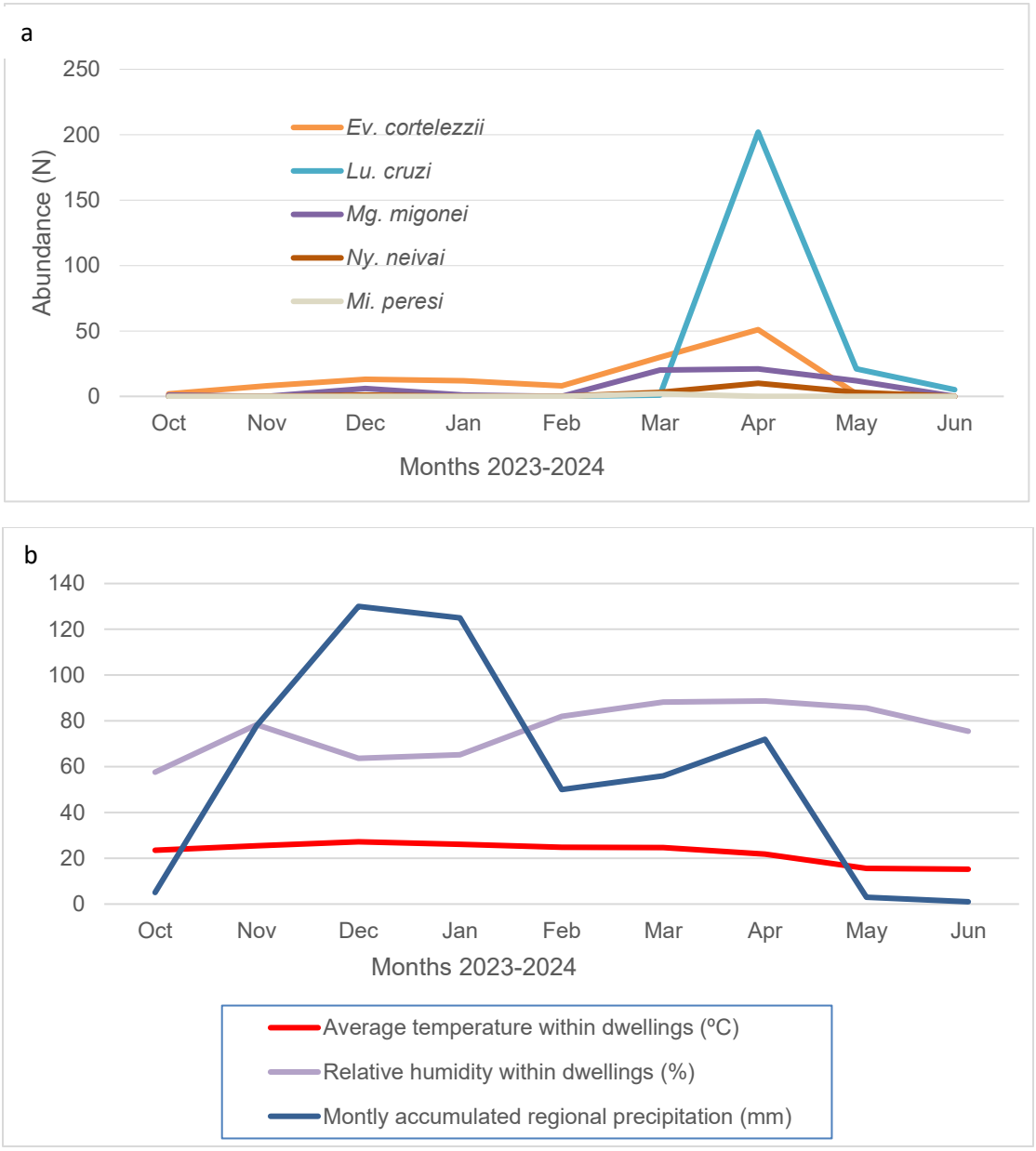

Fig. 3 - Temporal patterns of a) abundance of sandflies in Carapari during the nine months of sampling and b) environmental conditions at sampling sites during the study.

Discussion

Similar studies with geographical proximity exhibit equal results, in Argentina within a transitional ecoregion between Southern Andean Yungas and Chaco Dry Forest near the border with Bolivia, an almost identical diversity were recorded,[16,17] among which *Ev. cortelezzii* and *Ny. neivai* were the most abundant species, followed by *Lu. longipalpis* a sibling species of *Lu. cruzi*. Likewise in Bolivia, the presence of three

species, *Ny. neivai*, *Mg. migonei* and *Ev. cortelezzii* were reported to the municipality of Bermejo in the same ecoregion at southwest of *Carapari*,[18] and the presence of *Lu. cruzi* were recorded in the Chaco ecoregion of Villa Montes municipality, at north of study area.[6] Elsewhere, *Galindo*[19] and *Brazil et al.*[20] found in Santa Cruz department, at eastward of Bolivia, that *Lu. cruzi* are the prevalent species in rural areas within a transition region between Cerrado and Chiquitania Dry forests, and in Brazil near the border with Bolivia in the ecoregions of Pantanal, high presence of the last mentioned species were recorded in urban, rural and forested areas.[21,22]

On the other hand, several studies as *Barrios et al.*[22] and *Bustamante et al.*[23] also found that male sand flies were more abundant with a noticeably presence in peri-domicile samplings. These scores are due to the method of capture employed, since males are more attracted to light traps than females and for the greater accessibility to food outdoors.[2] Moreover, in the study of Quintana *et al.*,[16] the presence of *Lu. longipalpis* was significantly related with the presence of dogs, hens, fruit tree cover and decomposing organic matter. Similarly, in Carapari, *Lu. cruzi* presents a high abundance in dwellings with several domestic animals (dogs, poultry, and pigs) and with more than five inhabitants. In fact, this high presence of sand flies related to people and domestic animals is due to their vertebrate blood diet.[1]

There are scarce studies of seasonality in phlebotominae populations, especially in Bolivia. *Forattini*[14] indicates that assemblages of sandflies vary among rainy and dry spells, decreasing during this latter, assertion corroborated by some studies that found population increase during rainy months.[22,23] Aversely to those results, in Carapari the diversity of sand flies was higher just after the rainy season and relative humidity seemed to have a positive relation with the increase of sand flies capture. Perhaps, these vectors are not influenced significantly by precipitation, whereas humidity and temperature are more important factors, due to these parameters vary a bit between seasons in a microhabitat level (households).[22] Such relations are so important to study and detect in advance, since these changes could imply an increase in likelihoods of emergence or re-emergence of leishmaniosis in humans and domestic animals.

Historically, there are no reports of *Lu. cruzi* in Andean environments and few records occurred in Chaco ecoregion shared between Argentina, Bolivia and Paraguay, probably due to the few studies in this region and not due to the low abundance of this VL vector.[16] Furthermore, *Oliveira et al.*[21] report a maximum elevation of 741 masl to the altitudinal range of this species in the geographical formation of the Central Brazilian Shield. Therefore, in the present study, *Lu. cruzi* is reported for the first time in a valley of the Southern Andean Yungas ecoregion of

Bolivia expanding its spatial distribution to the south of this country and towards a maximum altitude of 858 masl.

Recently, in nearby localities, Municipal Departments of Zoonosis revealed in recent years that up to 15% of domestic dogs are infected with leishmaniosis in Villa Montes[24] and Yacuiba (personal communication), highlighting that dogs are the main reservoir of VL in urban environments.[14] The species *Mg. migonei* frequently captured in southern Bolivia, is considered as a putative vector of VL in Brazil,[25] therefore it should be taken into account. On the other hand, in Bermejo municipality several cases of CL and MCL in humans were reported[19] and the potential vectors of these two last diseases are also present in Carapari (*Ev. cortelezzii, Ny. neivai* and *Mi. migonei*).[6,12]

In addition, the present research had some limitations, such as the sampling period that could not complete a year due to logistical circumstances. Besides, it was not possible to carry out detection of *Leishmania* parasites in collected specimens, hence we encourage future research to do so. In the same way, the sandflies diversity must be corroborated through molecular identification of species, to determine possible genetic divergences in this population.

Finally, in conclusion, all the nearby previous reports of leishmaniosis cases in humans and domestic animals, along with our results of potential vector species of sandflies and new data about their distribution, suggest a risk of Leishmaniosis transmission in the study area. Therefore, further research are recommended in this Bolivian region, along with continuous entomological surveillance and community prevention actions.

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Conflicts of interest

The authors declare no conflicts of interest.

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