Biota Neotropica 22(2): e20211263, 2022
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Entomological survey of phlebotominae sand flies (diptera: psychodidae) and vector species in the tegumentary leishmaniasis endemic area in eastern brazilian Amazon, Amapá state

Tiago Silva da Costa[1,2*], ***Ricardo Marcelo dos Anjos Ferreira***[1], ***Gabriel Silva Santos***[3],

Manoel Daltro Garcia Júnior[1,2], ***Camila Barbosa Pinto***[1,4] **&** ***Raimundo Nonato Picanço Souto***[1,2]

[1]*Universidade Federal do Amapá, Laboratório de Arthropoda, Campus Universitário Marco Zero do Equador, Rodovia Juscelino Kubitschek de Oliveira, KM 02, Bairro Zerão, 68902-280, Macapá, AP, Brasil.*
[2]*Universidade Federal do Amapá (UNIFAP), Programa de Pós-Graduação em Biodiversidade Tropical (PPGBIO), Macapá, AP, Brasil.*
[3]*Universidade do Estado do Rio de Janeiro, Programa de Pós-Graduação em Ecologia e Evolução, Rua São Francisco Xavier, 524, PHLC 220, Maracanã, Rio de Janeiro, RJ, Brasil.*
[4]*Universidade Federal do Amapá, Curso de Ciências Biológicas - Campus Universitário Marco Zero do Equador – Rodovia Juscelino Kubitschek de Oliveira – KM – 02 - Bairro Zerão – CEP: 68902-280, Macapá, AP, Brasil.*
[*]*Corresponding author: tiago_sc@hotmail.com*

DA COSTA, T.S., FERREIRA, R.M.A, SANTOS, G.S., GARCIA JÚNIOR, M.D., PINTO, C.B., SOUTO, R.N.P. **Entomological survey of phlebotominae sand flies (diptera: psychodidae) and vector species in the tegumentary leishmaniasis endemic area in eastern brazilian Amazon, Amapá state.** Biota Neotropica 22(2): e20211263. https://doi.org/10.1590/1676-0611-BN-2021-1263

Abstract: American tegumentary leishmaniasis is an endemic that has increased considerably in recent decades in the Amazon region, sand flies are the vectors of the transmission of the protozoan that causes leishmaniasis, so the objective of this study was to carry out a survey of the diversity of species and the presence of *Leishmania* DNA in vectors circulating in three endemic counties for tegumentary leishmaniasis in the eastern Brazilian Amazon (Amapá state, Brazil). Using CDC light traps, a total of 10,773 specimens were collected between February 2019 and February 2020, representing 64 species in 15 genera. The vector specie *Nyssomyia umbratilis* Ward and Frahia, 1977 was the predominant species (13.20% of the total), being collected in all three counties, followed by *Trichopygomyia trichopyga* Floch & Abonnenc, 1945 (11.41%), *Trichophoromyia ubiquitalis* Mangabeira,1942 (9.47%) and *Nyssomyia anduzei* Rozeboom, 1942 (7.61%). For the identification of *Leishmania* DNA, 775 pools of unengorged females were used, of which 5 tested positive, 2 of *Nyssomya umbratilis* Ward & Fraiha,1977, 1 of *Nyssomyia anduzei* and 2 of *Psychodopygus davisi* Root,1934, demonstrating a natural total infection rate of 0.64%. This study increases the knowledge of vector diversity, as well as identifying *Leishmania* spp. in circulation in the eastern region of the Amazon.

Keywords: *Entomological surveillance; PCR; Molecular detection; Sand flies Diversity.*

Levantamento entomológico de flebotomíneos (Diptera: Psychodidae) e espécies vetoras na área endêmica de leishmaniose tegumentar na Amazônia oriental brasileira, Estado do Amapá

Resumo*:* A leishmaniose tegumentar americana é uma endemia que aumentou consideravelmente nas últimas décadas na região amazônica, os flebotomíneos são os vetores da transmissão do protozoário causador da leishmaniose, portanto o objetivo deste estudo foi realizar um levantamento da diversidade de espécies e a presença de DNA de *Leishmania* em vetores que circulam em três municípios endêmicos de leishmaniose tegumentar na Amazônia oriental brasileira (Amapá, Brasil). Usando armadilhas luminosas do tipo CDC, um total de 10.773 espécimes foram coletados entre fevereiro de 2019 e fevereiro de 2020, representando 64 espécies em 15 gêneros. As espécie vetoras - singular *Nyssomyia umbratilis* Ward e Frahia 1977 foram as espécies predominantes (13,20% do total), sendo coletadas nos três municípios, seguido por *Trichopygomyia trichopyga* Floch & Abonnenc, 1945 (11,41%), *Trichophoromyia ubiquitalis* Mangabeira, 1942 (9,47%) e *Nyssomyia anduzei* Rozeboom, 1942 (7,61%). Para a identificação do DNA de *Leishmania*, foram utilizados 775 pools de fêmeas não ingurgitadas, dos quais 5 foram positivos, 2 de *Nyssomya umbratilis* Ward & Fraiha, 1977, 1 de *Nyssomyia anduzei* e 2 de *Psychodopygus davisi* Root, 1934, demonstrando uma taxa de infecção total de 0,64%. Este estudo aumenta o conhecimento da diversidade de vetores, bem como a identificação das espécies de *Leishmania* spp. em circulação na região oriental da Amazônia.

Palavras-chave: *Vigilância Entomológica; PCR; Detecção Molecular; Diversidade de Flebotomíneos.*

Introduction

Sand flies (Diptera: Psychodidae) are small insects that play a fundamental role in the transmission of protozoa of the genus *Leishmania* Ross (Kinetoplastida: Trypanosomatidae), which are the etiological agents of American Tegumentary Leishmaniasis (ATL) and Visceral Leishmaniasis (VL) in humans (Ready 2013). Leishmaniasis infects approximately 12 million people around the world, with approximately 600,000 new cases being reported each year. It is estimated that 90% of ATL cases occur in Latin America (Bolivia, Peru, and Brazil) and Middle East (Iran, Saudi Arabia, Syria, Afghanistan) (WHO 2021).

In Brazil, ATL is a disease that requires attention due to the growing number of cases and the characteristics of the country that are conducive to the development of the disease, such as the great diversity of phlebotomine vectors of several *Leishmania* species that occur in the country (Brazil et al. 2014). Currently there are approximately 1,000 species of sand flies described in the world, 530 in the Americas and approximately 280 species have already been found in Brazil (Shimabukuro et al. 2017, Galati 2018), of these, at least 19 species are identified as proven or probable vectors of medical-veterinary importance (Aguiar & Medeiros 2003). According to the Notifiable Diseases Information System (SINAN), from the Ministry of Health, between 2003 and 2018, more than 300,000 cases of ATL were reported, with an average of 21,158 cases per year. ATL is largely neglected, and Brazil is responsible for the majority of human cases in the West (Alvar et al. 2012, Da Silva et al. 2020). The northern region of the country, where the state of Amapá is located, it is responsible for the largest number of cases in the period (Brasil 2019).

The state of Amapá, located in the Eastern Amazon, has environmental conditions that support one of the greatest biodiversity in the world, which provide opportunities for the development of vector-host relations and, consequently, of the leishmaniasis cycle (Ellwanger et al. 2020). To date there has been a record of five species of *Leishmania* coexisting in the region: *Leishmania (Viannia) guyanensis* Floch, 1954, *L. (V.) braziliensis* Vianna, 1911, *L. (V.) lainsoni* Silveira, Shaw, Braga & Ishikawa, 1987, *L. (V.) naiffi* Laison and Shaw, 1989, *L. (Leishmania) amazonensis* Lainson and Shaw, 1972. *L. (V.) guyanensis* being responsible for 80% of ATL cases in this area (Brasil 2017, de Souza et al. 2017). Despite the high incidence of ATL in the region, there are few studies that study the diversity of the sandfly and *Leishmania* fauna (Costa et al. 2021). The aim of this study is to characterize the sandfly fauna, as well as their natural infection by *Leishmania* spp. in an endemic region for ATL in the state of Amapá (AP), eastern Amazon.

Material and Methods

1. Study area

This study was conducted in the rural area of three counties in the state of Amapá (AP): **Mazagão – P1** (0° 6' 54" S, 51° 17' 20" W)**, Porto Grande – P2** (0° 53' 45" N, 52° 0' 7" W) and **Serra do Navio – P3** (0° 53' 45" N, 52° 0' 7" W) (Figure 1), located in the eastern Amazon, northern region of Brazil. According to Köeppen's classification criteria, the region's climate is in the humid tropical category, predominantly in the Am category, with the mildest month temperature above 18°C and monthly average rainfall below 60mm (Tavares 2014, Garcia et al. 2020). The collections were carried out in areas characterized as dry land with predominant vegetation of dense rainforest. The research was carried out in areas of proven ATL transmission in the three counties, in which an increase in cases was detected in recent years by the state agency for Health Surveillance (SVS 2019). Records have shown that human ATL cases have increased in rural areas in villages that are close to forested areas, and where villagers engage in agriculture and extractive activities of forest products.

2. Sandfly collections and morphological identification

The sand flies were captured in villages in rural areas of the three counties with Center for Disease Control (CDC) light traps placed in the peridomicile of homes that were selected for their proximity to the forest and the presence of chicken coops and pig breeding, characteristics that favor the presence of sand flies. The traps worked for three consecutive nights from 6:00 pm to 7:00 am, monthly for one year (February 2019 to February 2020), totalizing 468 hours of capture at each collection point. The 30 traps were distributed in locations with ATL human case records, in each of the three selected counties.

The collected insects were taken to the Arthropoda Laboratory (ArtroLab) at the Federal University of Amapá (UNIFAP) for screening by sex and dissection process. The final three segments of the abdomen and the head were removed for mounting on Berlese liquid glass slides. The rest of the phlebotomine bodies were conditioned in 94% ethanol at -20ºC for the subsequent extraction of genomic DNA. Species identification was based on the morphology of male genitalia and the spermatheca and by the characters present in female's head, , using the updated classification key developed by Galati in 2003 (Galati 2019), and following the abbreviation of the genera proposed by Marcondes (2007).

3. Molecular detection of Leishmania

The thorax of unengorged female sand flies were grouped according to date, species, and county of collection to form pools with 2 to 10 specimens of the 65 species collected. DNA extraction and Polymerase Chain Reaction (PCR) were performed to amplify the molecular targets of the region of kinetoplast DNA minicircles (*k*DNA) and *hsp*70, as described elsewhere (Pereira Júnior et al. 2015, Resadore et al. 2017). For the negative control, purified water was used and for the positive controls, DNA from *Le. (L.) amazonensis* Lainson & Shawn, 1972 and *Le. (V.) braziliensis* Vianna, 1911 strains.

4. Data analysis

The collection effort and the number of species in the study were measured using the non-parametric Jackknife1 estimator, generating a rarefaction curve for each of the collection points using the R software (R Core Team 2021) using the vegan package (Oksanen et al. 2019).

The sequences of the *hsp*70 molecular target were analyzed using Phred, Phrap and Consend software (Ewing & Green 1998), with the minimum value defined as Q=30. The identification of *Leishmania* species was performed by comparing the consensus sequences obtained in the study with reference sequences deposited in the GenBank database (http: www.ncbi.nlm.nih.gov/genbank), using the BLAST tool (Basic Local Alignment Search Tool) (Altschul et al. 1990).

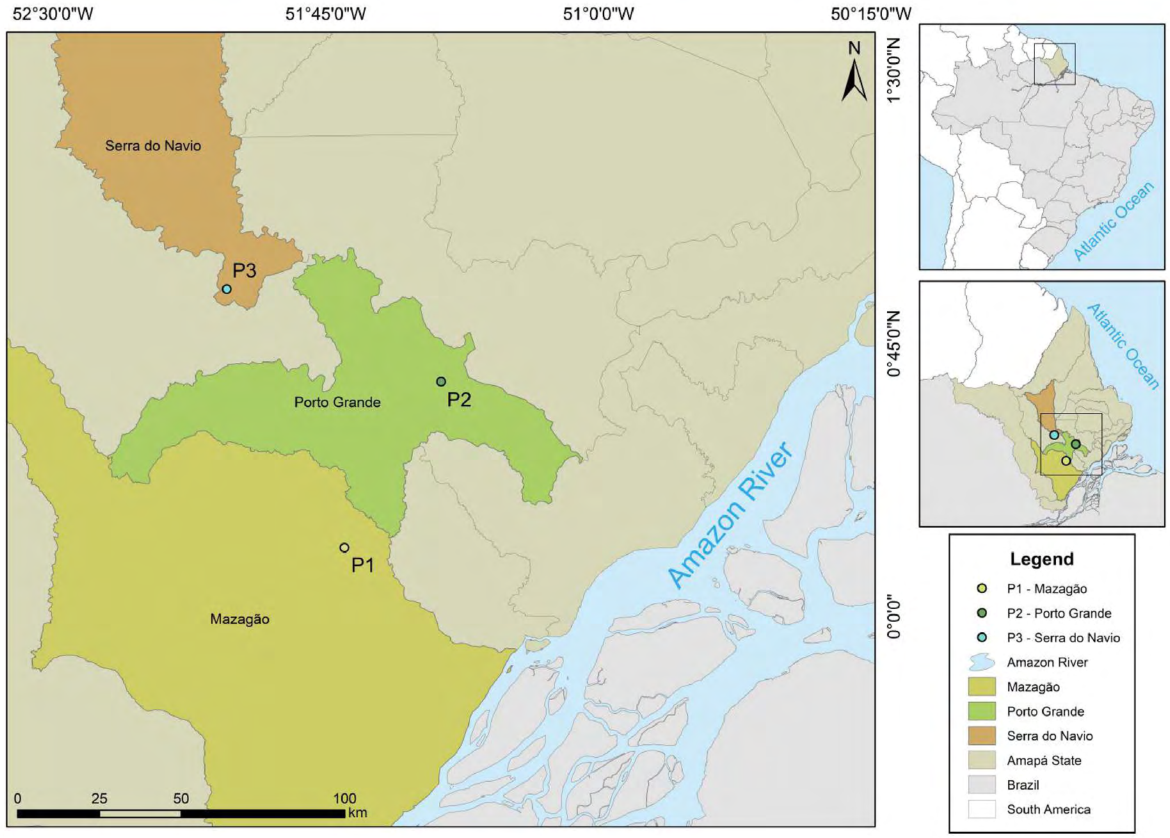

Figure 1. Map of the study area, location counties of Mazagão (P1), Porto Grande (P2) and Serra do Navio (P3), Amapá State, Brazil, where the Sand flies collections with the CDC light traps were carried out.

Results

The total number of sand flies collected in the three locations (P1, P2, P3) was 10,773 specimens, being: 4,512 males (41.89%) and 6,261 females (58.11%), totalizing 15 genera and 64 species (Table 1). The most abundant genera were *Nyssomyia* Barretto (3,559 individuals, 33.04%), *Trichophoromyia* Barreto (1,619, 15.02%), *Trichopygomyia* Barretto (1,278, 11.87%) and *Psychodopygus* Mangabeira (1,176, 10.92%). The genera collected in lesser abundance were *Pintomyia* Costa Lima (72 individuals, 0.67%), *Vianamyia* Mangabeira (65, 0.60%) and *Pressatia* Mangabeira (35, 0.32%). The most abundant species were *Ny. umbratilis* (n=1.422, 13.20%), *Ty. trichopyga* (n=1.229, 11.41%), *Th. ubiquitalis* (n=1020, 9.47%) and *Ny. anduzei* (n=820, 7.61%); these species accounted for 40.69% of the sand flies collections. The least abundant species were *Pa. bigeniculata* Floch & Abonnenc, 1941 (n=11,0.10%), *Br. beaupertuiy* Ortiz, 1954 (n=11,0.10%), *Pa. lutziana* Costa Lima, 1932 (n=8, 0.07%), *Lu. spatotrichia* Martins, Falcão & Silva, 1963 (n=6, 0.06%); among the less collected species is the vector *Ps. ayrozai* Barretto & Coutinho, 1940 (n=45, 0.42%).

Based on the data from the study, the rarefaction curves demonstrated a good result of the sampling efforts, with collection efficiency approaching 100% at the three collection points. The Jackknife 1 estimator showed that species richness corresponded to 96% in Mazagão (P1) and Porto Grande (P2), and 100% in Serra do Navio (P3) (Figure 2), demonstrating a tendency to stabilization in the three sampling points of the curve in an asymptote.

A total of 775 pools of unengorged females were formed for the detection of *Leishmania* DNA, of which PCR for molecular targets *k*DNA and *hsp*70 identified that a total of five pools were positive: two pools of *Ny. umbratilis* infected with *L. (V.) guyanensis* (query cover=100%, identity=100%, Genbank accession MW094227.1) collected in Porto Grande (P1) and Serra do Navio (P3), a pool of *Ny. anduzei* infected with *L. (V.) naiffi* (query cover=100%, identity=100%, Genbank accession MT469994.1) in Serra do Navio (P3), and two pools of *Ps. davisi* infected with *L. (V.) braziliensis* (query cover = 98%, identity = 98%, Genbank accession MT543301.1) collected in Mazagão (P2) and Serra do Navio (P3). The minimum infection rate (number of positive samples / total samples tested x 100) was calculated for each of the species that tested positive for *Leishmania* DNA: *Ny. umbratilis* (1.0%), *Ny. anduzei* (1,5%) and *Ps. davisi* (5.7%).

Discussion

Our sampling included 64 species of sand flies among the 77 recorded in the state of Amapá (Galati 2019), 50 in Mazagão (P1),49 in Porto Grande (P2) and 56 in Serra do Navio (P3). The highest number of species was found in Serra do Navio, which corroborates the 55 species already found in the county in a previous study (de Souza et al. 2017),

Table 1. Species of Sand flies from Amapá State, Eastern Amazon, Brazil, collected with CDC light traps from February 2019 to February 2020.

Species	P1	P2	P3	Total	% Total	MIR
Nyssomya umbratilis Ward & Fraiha, 1977	420	385	522	1327	13.20	1.0%
Trichopygomya trychopyga Floch & Abonnenc, 1945	450	255	524	1229	11.41	-
Trichophoromya ubiquitalis Mangabeira, 1942	357	204	459	1020	9.47	-
Nyssomya anduzei Rozeboom, 1942	227	280	313	820	7.61	1.5%
Nyssomya yuilli pajoti Abonnenc, Léger & Fauran, 1979	89	198	352	639	5.93	-
Nyssomya whitmani Antunes & Coutinho, 1939	159	200	256	615	5.71	-
Trichophoromya brachipyga Mangabeira, 1942	225	157	182	564	5.24	-
Psychodopygus squamiventris maripaensis Floch & Abonnenc, 1946	109	79	255	443	4.11	-
Evandromyia infraspinosa Mangabeira, 1941	78	117	180	375	3.48	-
Psychodopygus davisi Root, 1934	118	87	125	330	3.06	5.7%
Migonemyia migonei França, 1920	45	74	92	211	1.96	-
Evandromyia monstruosa Martins, Falcão & Silva, 1965	59	77	64	200	1.86	-
Migonemyia pilosa Damasceno & Causey, 1944	35	49	77	161	1.49	-
Migonemyia micropyga Mangabeira, 1942	21	40	78	139	1.29	-
Evandromyia brachyphalla Mangabeira, 1941	18	45	75	138	1.28	-
Bichromomyia flaviscutellata Mangabeira, 1942	45	33	57	135	1.25	-
Brumptomyia cunhai Mangabeira, 1942	54	-	77	131	1.22	-
Psychodopygus paraensis Costa Lima, 1941	42	12	74	128	1.19	-
Evandromyia bacula Martins, Falcão & Silva, 1965	15	46	65	126	1.17	-
Evandromyia sericea Floch & Abonnenc, 1944	-	78	72	120	1.11	-
Brumptomyia travassosi Mangabeira, 1942	7	-	94	101	0.94	-
Lutzomyia gomezi Nitzulescu, 1931	28	35	19	82	0.76	-
Psathyromyia inflata Floch & Abonnenc, 1944	14	22	45	81	0.75	-
Migonemyia oswaldoi Mangabeira, 1942	-	41	38	79	0.73	-
Evandromyia walkeri Newstead, 1914	78	-	-	78	0.72	-
Psychodopygus amazonensis Root, 1934	-	27	48	75	0.70	-
Sciopemyia fluviatilis Floch & Abonnenc, 1944	22	18	35	75	0.70	-
Evandromyia bourroli Barretto & Coutinho, 1941		25	49	74	0.69	-
Evandromyia pinottii Damasceno & Arouck, 1956	25	42	-	67	0.62	-
Psychodopygus claustrei Abonnenc, Léger & Fauran, 1979	22	18	27	67	0.62	-
Psathyromyia dreisbachi Causey & Damasceno, 1945	22	13	30	65	0.60	-
Migonemyia bursiformis Floch & Abonnenc, 1944	18	30	15	63	0.58	-
Micropygomyia longipennis Floch & Abonnenc, 1944	24	20	17	61	0.57	-
Micropygomyia rorotaensis Floch & Abonnenc, 1944	17	25	19	61	0.57	-
Nyssomya antunesi Coutinho, 1939	28	4	18	50	0.46	-
Trichopygomya depaquiti Floch & Abonnenc, 1944	25	-	24	49	0.45	-
Psychodopygus hirsutus Mangabeira, 1942	25	5	18	48	0.45	-
Evandromyia saulensis Floch & Abonnenc, 1943	45	-	-	45	0.42	-
Psychodopygus ayrozai Barretto &Coutinho, 1940	12	8	25	45	0.42	-
Pintomyia damascenoi Mangabeira, 1941		28	16	44	0.41	-
Micropygomyia chassigneti Floch & Abonnenc, 1944		15	27	42	0.39	-
Psychodopygus corossoniensis Le Pont & Pajot, 1978	18		22	40	0.37	-
Psathyromyia aragaoi Costa Lima, 1932		15	22	37	0.34	-
Trichophoromya ininii Floch & Abonnenc, 1943	12	5	18	35	0.32	-
Viannamyia tuberculata Mangabeira, 1941	15	19		34	0.32	-

Continua...

...Continuation

Psathyromyia runoides Fairchild & Hertig, 1953		12	20	32	0.30	-
Viannamyia furcata Mangabeira, 1941	3		28	31	0.29	-
Psathyromyia pradobarrientosi Le Pont, Matias, Martinez & Dujardin, 2004	25			25	0.23	-
Evandromyia evandroi Costa Lima & Antunes, 1936	-	24	-	24	0.22	-
Sciopemyia sordelli Shannon & Del Ponte, 1927	12	-	9	21	0.19	-
Lutzomyia evangelistai Martins & Fraiha, 1971	-	19	-	19	0.18	-
Pressatia choti Floch & Abonnenc, 1941	-	2	17	19	0.18	-
Evandromyia inpai Young & Arias, 1977	1	-	16	17	0.16	-
Evandromyia williamsi Damasceno, Causey & Arouck, 1945	5	12	-	17	0.16	-
Pressatia trispinosa Mangabeira, 1942	7	-	9	16	0.15	-
Lutzomyia carvalhoi Damasceno, Causey & Arouck, 1945	-	14	2	16	0.15	-
Pintomyia pacae Floch & Abonnenc, 1943	5	-	10	15	0.14	-
Brumptomyia pintoi Costa Lima, 1932	-	7	8	15	0.14	-
Nyssomyia richardwardi Ready & Fraiha, 1981	8	-	5	13	0.12	-
Pintomyia serrana Damasceno & Arouck, 1949	4	-	9	13	0.12	-
Brumptomyia beaupertuiy Ortiz, 1954	5	-	6	11	0.10	-
Psathyromyia bigeniculata Floch & Abonnenc, 1941	5	2	4	11	0.10	-
Psathyromyia lutziana Costa Lima, 1932	1	3	4	8	0.07	-
Lutzomyia spathotrichia Martins, Falcão & Silva, 1963		1	5	6	0.06	-
Total of individuals	3157	2899	4717	10773	100%	-
Total species	50	49	56	64		

Collection points: P1: Mazagão; **P2:** Porto Grande; **P3:** Serra do Navio; **MIR:** Minimum Infection Rate; The abbreviation of genera is in agreement with Marcondes (2007).

the present study found the highest number (83.1%) of species among all species with occurrence for the state. Our survey demonstrated a great range of sandfly species in the three collection sites, demonstrating the great diversity of the sandfly fauna in the Eastern Amazon. The diversity found in the study generally corresponded to the pattern of studies carried out in forest areas that demonstrate the phlebotomine fauna usually composed of a few dominant species and many species with specimens (Rosário et al. 2016)

The genus *Nyssomia* is of great importance for surveillance studies and understanding of the ecopidemiology of ATL, occurring from North America (Mexico) to South America (Argentina), with a total of seven species proven or suspected of transmission of ATL (Marcondes et al. 1998). Three species of the genus showed great abundance: *Nyssomyia umbratilis* (13.2%), *Nyssomyia anduzei* (7.61%) and *Nyssomyia whitmani* Antunes & Coutinho, 1939 (5.71%), these species identified as vectors in Brazil (Brasil 2017) and already associated with ATL transmission in the state of Amapá in previous studies (De Souza et al. 2017). *Ny. umbratilis* is a constant presence in endemic areas for ATL (Pinheiro et al. 2008), and is considered one of the main vectors for *L. (V.) guyanensis* in most of Latin America, in countries such as Brazil, Bolivia, Colombia, Peru, Venezuela, Suriname and French Guiana (Brazil et al. 2014), as well as in the Amazon region (Brasil 2019). *Nyssomyia anduzei* is considered a secondary vector of *L. (V.) guyanensis* and appears as the fourth species with the largest sampling in the present study, which corroborates studies carried out in the Western Amazon region (Barbosa et al. 2008, Grimaldi et al. 1991a), *Ny. whitmani* is considered one of the vectors of great medical importance in the Amazon region as it can transmit *L (V.) braziliensis, L. (V.) shawi* and *L. (V.) guyanensis* (Lainson et al. 1994), this species is considered a wild species and in the northern region of Brazil it has a lower anthropophilic habit (Silveira et al. 1991), having been identified previously infected with *Leishmania* in the region (Rangel & Lainson 2009).

Other species with medical importance such as *Bichromomyia flaviscutellata* Mangabeira 1941*, Tricophoromyia ubiquitalis. Psychodopygus squamiventris maripaensis* Floch & Abonnenc, 1946 and *Migonemia migonei* França,1920 were found at the three collection points in peridomicile areas. This is a factor that should be carefully observed as finding females of these species in these environments may indicate that the species are looking for blood meal sources in the domestic environment or being attracted because of the lights in the houses; the presence of these vectors near the dwellings greatly increases the risk of contracting ATL in the home environment (Tanure et al. 2015).

For the incrimination of *Leishmania* vectors, one of the crucial points is to determine the occurrence of natural infection in sandfly populations, identifying possible vector species. The test considered that the gold standard for the natural detection of infection is the dissection of the digestive tract to indicate the presence of *Leishmania* promastigotes by light microscopy (Kato et al. 2005), but this method is extremely laborious and requires the dissection of a large number of specimens, isolation and culture of protozoa from dissected sand flies. Thus, molecular techniques such as PCR for the detection of *Leishmania* DNA have been increasingly used in studies with sand flies (Teles et al. 2016, Da Silva et al. 2020). The minimum infection rate of *Leishmania* DNA detected with molecular methods was 0.64%, a result compatible

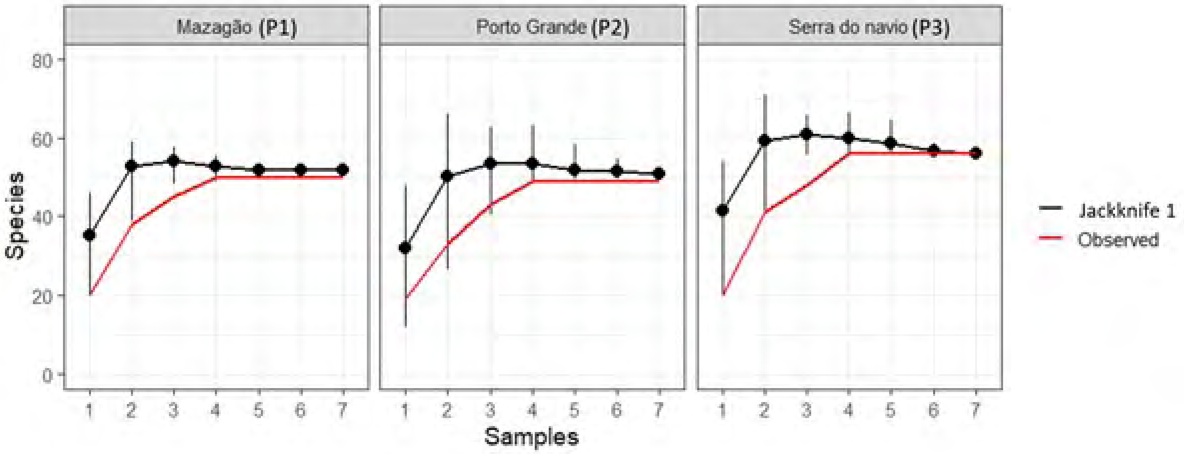

Figure 2. The rarefaction curves (observed and estimates by jackknife 1) of sand flies species collected in the Mazagão (P1), Porto Grande (P2) and Serra do Navio (P3), Amapá State, Brazil.

with a study conducted in the state of Amapá (0.78%) (Vasconcelos Dos Santos et al. 2019), as well as in other states in the Amazon region, such as Rondônia (0.28%) (Resadore et al. 2019), Amazonas (0.83%) and Acre (0.99%).

The DNA of the species *L. (V.) guyanensis* was detected in two samples of *Ny. umbratilis* in the collection locations (P1 and P3). This sandfly species is pointed out as the main vector of *L. (V.) guyanenis* in northern Brazil (Gil et al. 2009). Several other studies conducted in areas endemic to ATL found this sandfly species infected with *L. (V.) guyanensis* (Lainson et al. 1981, Pinheiro et al. 2008), including in studies previously conducted in the state of Serra do Navio (P3) (de Souza et al. 2017) and in Oiapoque, a region on the state border with French Guiana (Vasconcelos Dos Santos et al. 2019), where it is considered the main vector (Vasconcelos dos Santos et al. 2018). Infection by *L. (V.) guyanensis* represents a high risk of complications for human health, as the protozoan is resistant to the drug most used in clinical practice in Brazil, Glucantime (Brasil 2017).

Nyssomyia anduzei was found infected with the DNA of *L. (V.) naiffi* in P3 (Serra do Navio). This species is considered a secondary vector of *L. (V.) guyanensis* in the region (Rangel & Lainson 2009, Chagas et al. 2018), but has already been found infected with *L. (V.) naiffi* DNA in the state of Amapá (de Souza et al. 2017), demonstrating its likely participation in the cycle of leishmaniasis in the region. This species of *Leishmania* has been commonly associated with the *Ps. squamiventris maripaensis* vector in northern Brazil (Naiff et al. 1991), French Guiana (Fouque et al. 2007) and Suriname (Kent et al. 2013). In Brazil, *L.(V.) naiffi* has already been reported in other states of the Amazon region such as Pará (Lainson et al. 1981), Amazonas (Grimaldi et al. 1991a) and Acre (Tojal da Silva et al. 2006).

The *Psychodopygus davisi* species was found infected with *L. (V.) braziliensis*. The species is considered a potential vector for this species of *Leishmania*, having already been found in previous studies in forest environments infected by both *L. (V.) braziliensis* and *L. (V.) naiffi* (Grimaldi et al. 1991b, Gil et al. 2003). This species of sandfly is considered one of the main vectors of the etiological agent of Leishmaniasis, so it should be considered carefully in entomological surveys as it has relevant characteristics such as a high level of anthropophily. In Brazil, the transmission of *L. (V.) braziliensis* has as potential vectors 17 species of sand flies. In Amapá the DNA of *L. (V.) braziliensis* was found in other species of sand flies such as *Th. ininni* Floch & Abonnenc 1943, *Ny. umbratilis* and *Ev. infraspinosa* Mangabeira, 1941 (Vasconcelos Dos Santos et al. 2019).

The sandfly fauna of the eastern Amazon region is still poorly known. In this study we demonstrate the high level of species diversity of sand flies in the state of Amapá, the three collection counties demonstrate a diversity of proven or putative vectors of *Leishmania* in the region, as well as the detection of three different species of the *Leishmania Viannia* complex in sandfly species that already have a history of vectors in the literature. Thus, our studies suggest that the sand flies found are acting as vectors in the ATL transmission cycle, as well as indicating a high risk of transmission in the three collection counties of the three main *Leishmania* species. Thus, it is necessary that measures against ATL transmission be planned for the state of Amapá, based on entomological inventories to monitor the vector arthropod species as well as the species infected by *Leishmania spp.* that cause ATL in Eastern Brazilian Amazon.

Acknowledgements

We would like to thank the residents of the Mazagão, Porto grande and Serra do Navio counties, who allowed sample collection on their property. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) [Finance Code 001]

Associate Editor

Gustavo Graciolli

Author Contributions

Tiago Silva da Costa: Substantial contribution in the concept and design of the study. Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

Ricardo Marcelo dos Anjos Ferreira: Contribution to data collection. Contribution to manuscript preparation.

Gabriel Silva Santos: Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

Manoel Daltro Nunes Garcia Junior: Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

Camila Barbosa Pinto: Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

Raimundo Nonato Picanço Souto: Substantial contribution in the concept and design of the study. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

AGUIAR, G. & MEDEIROS, W. 2003. Distribuição regional e habitats das espécies de flebotomíneos do Brasil. In Flebotomíneos do Brasil (E. F. Rangel & R. Lainson, eds) Fiocruz, Rio de Janeiro, p.207–255.

ALTSCHUL, S.F., GISH, W., MILLER, W., MYERS, E.W. & LIPMAN, D.J. 1990. Basic local alignment search tool. J. Mol. Biol. 215(3):403–410.

ALVAR, J., VÉLEZ, I.D., BERN, C., HERRERO, M., DESJEUX, P., CANO, J., JANNIN, J., DEN BOER, M. & WHO LEISHMANIASIS CONTROL TEAM. 2012. Leishmaniasis worldwide and global estimates of its incidence. PLoS One 7(5):e35671. https://doi.org/10.1371/journal.pone.0035671

BARBOSA, M.D.G.V., FÉ, N.F., MARCIÃO, A.H.R., SILVA, A.P.T., MONTEIRO, W.M. & GUERRA, J.A.D.O. 2008. Fauna de flebotomíneos (Diptera: Psychodidae) em um foco de leishmaniose tegumentar americana na área periurbana de Manaus, Estado do Amazonas. Rev. Soc. Bras. Med. Trop. 41(5):485–491. https://doi.org/10.1590/S0037-86822008000500010

BRASIL.2017. Manual De Vigilância Da Leishmaniose Tegumentar. Secretaria de Vigilância das Doenças Transmissíveis. Manual de vigilância da leishmaniose tegumentar.Brasília. http://bvsms.saude.gov.br/bvs/publicacoes/manual_vigilancia_leishmaniose_tegumentar.pdf (last acces 15/05/2021)

BRASIL. 2019. Boletim Epidemiológico: Vigilância em Saúde no Brasil 2003-2019. Ministério da Saúde. https://portalarquivos2.saude.gov.br/images/pdf/2019/setembro/25/boletim-especial-21ago19-web.pdf. (last acces 25/04/2021)

BRAZIL, R.P., RODRIGUES, A.A.F. & ANDRADE-FILHO, J.D. 2014. Sand Fly Vectors of *Leishmania* in the Americas - A Mini Review. Entomol. Ornithol. Herpetol. Curr. Res. 4: 144. https://doi.org/10.4172/2161-0983.1000144

CHAGAS, E.C.D.S., SILVA, A.S., FÉ, N.F., FERREIRA, L.S., SAMPAIO, V.D.S., TERRAZAS, W.C.M., GUERRA, J.A.O., SOUZA, R.A.F. De, SILVEIRA, H. & GUERRA, M.D.G.V.B. 2018. Composition of sand fly fauna (Diptera: Psychodidae) and detection of *Leishmania* DNA (Kinetoplastida: Trypanosomatidae) in different ecotopes from a rural settlement in the central Amazon, Brazil. Parasites Vectors 11:180. https://doi.org/10.1186/s13071-018-2743-6

COSTA, T.S. da, FERREIRA, R.M. dos A., SANTOS, G.S., GARCIA JÚNIOR, M.D., PINTO, C.B. & SOUTO, R.N.P. 2021. Ecological aspects and molecular detection of *Leishmania* DNA (Kinetoplastida: Trypanosomatidae) in phlebotomine sand flies (Diptera: Psychodidae) from a rural settlement in the Eastern Amazon, Brazil. Rev. Bras. Entomol. 65(3). https://doi.org/10.1590/1806-9665-rbent-2021-0065.

ELLWANGER, J.H., KULMANN-LEAL KULMANN-LEAL, BRUNA KAMINSKI, V.L., VALVERDE-VILLEGAS, J.M., VEIGA, A.B.G. DA, SPILKI, F.R., FEARNSIDE, P.M., CAESAR, L., GIATTI, L.L., WALLAU, G.L., ALMEIDA, S.E.M., BORBA, M.R., HORA, V.P. DA & CHIES, J.A.B. 2020. Beyond diversity loss and climate change: Impacts of Amazon deforestation on infectious diseases and public health. An. Acad. Bras. Cienc. 92(1):1–33.https://doi.org/10.1590/0001-3765202020191375

EWING, B. & GREEN, P. 1998. Base-Calling of Automated Sequencer Traces Using Phred. II. Error Probabilities. Genome Res. 8(3): 175-185. http://dx.doi.org/10.1101/gr.8.3.175

FOUQUE, F., GABORIT, P., ISSALY, J., CARINCI, R., GANTIER, J.-C., RAVEL, C. & DEDET, J.-P. 2007. Phlebotomine sand flies (Diptera: Psychodidae) associated with changing patterns in the transmission of the human cutaneous leishmaniasis in French Guiana. Mem. Inst. Oswaldo Cruz 102(1):35–40. https://doi.org/10.1590/S0074-02762007000100005

GALATI, E.A.B. 2018. Phlebotominae (Diptera, Psychodidae): Classification, Morphology and Terminology of Adults and Identification of American Taxa. In Brazilian Sand Flies Springer International Publishing, Cham, p.9–212.

GALATI, E.A.B. 2019. Phlebotominae (Diptera, Psychodidae). Classificação, morfologia, terminologia e identificação de Adultos. Apostila da Disciplina HEP 5752—Bioecologia e Identificação de Phlebotominae 2019. I. https://edisciplinas.usp.br/mod/resource/view.php?id=2796254 (last acces 18/02/2021)

GARCIA, M.D.N., DAMASCENO, M.T.D.S., MARTINS, M.J.L., DA COSTA, T.S., FERREIRA, R.M.D.A. & SOUTO, R.N.P. 2020. New records of dragonflies and damselflies (Insecta: Odonata) from Amapá State, Brazil. Biota Neotrop. 21(1):1–7. https://doi.org/10.1590/1676-0611-BN-2020-1074 .

GIL, L.H.S., ARAÚJO, M. da S., VILLALOBOS, J.M., CAMARGO, L.M.A., OZAKI, L.S., FONTES, C.J.F., RIBOLLA, P.E.M., KATSURAGAWA, T.H., CRUZ, R.M., SILVA, A. de A. e & SILVA, L.H.P. da. 2009. Species structure of sand fly (Diptera: Psychodidae) fauna in the Brazilian western Amazon. Mem. Inst. Oswaldo Cruz 104(7):955–959. https://doi.org/10.1590/S0074-02762009000700002

GIL,L.H.S., BASANO, S.A., SOUZA, A.A., SILVA, M.G.S., BARATA, I., ISHIKAWA, E.A., CAMARGO, L.M.A. & SHAW, J.J. 2003. Recent observations on the sand fly (Diptera: Psychodidae) fauna of the State of Rondônia, Western Amazônia, Brazil: the importance of *Psychdopygus davisi* as a vector of zoonotic cutaneous leishmaniasis. Mem. Inst. Oswaldo Cruz 98(6):751–755. https://doi.org/10.1590/S0074-02762003000600007

GRIMALDI, G., MOMEN, H., NAIFF, R.D., MCMAHON-PRATT, D. & BARRETT, T. V. 1991a. Characterization and Classification of Leishmanial Parasites from Humans, Wild Mammals, and Sand Flies in the Amazon Region of Brazil. Am. J. Trop. Med. Hyg. 44(6):645–661. https://doi.org/10.4269/ajtmh.1991.44.645

GRIMALDI, G., MOMEN, H., NAIFF, R.D., MCMAHON-PRATT, D. & BARRETT, T. V. 1991b. Characterization and Classification of Leishmanial Parasites from Humans, Wild Mammals, and Sand Flies in the Amazon Region of Brazil. Am. J. Trop. Med. Hyg. 44(6):645–661.

KATO, H., UEZATO, H., KATAKURA, K., CALVOPIÑA, M., MARCO, J.D., BARROSO, P.A., GOMEZ, E.A., MIMORI, T., KORENAGA, M., IWATA, H., NONAKA, S. & HASHIGUCHI, Y. 2005. Detection and identification of Leishmania species within naturally infected sand flies in the andean areas of ecuador by a polymerase chain reaction. Am. J. Trop. Med. Hyg. 72(1):87–93.

KENT, A.D., DOS SANTOS, T. V., GANGADIN, A., SAMJHAWAN, A., MANS, D.R.A. & SCHALLIG, H.D.F.H. 2013. Studies on the sand fly fauna (Diptera: Psychodidae) in high-transmission areas of cutaneous leishmaniasis in the Republic of Suriname. Parasit. Vectors 6(1):318.

LAINSON, R., SHAW, J.J., READY, P.D., MILES, M.A. & POVOA, M. 1981. Leishmaniasis in Brazil: XVI. Isolation and identification of *Leishmania* species from sandflies, wild mammals and man in north Pará state, with particular reference to *L. braziliensis guyanensis* causative agent of "pian-bois. Trans. R. Soc. Trop. Med. Hyg. 75(4): 530-536. https://doi.org/10.1016/0035-9203(81)90192-9

LAINSON, R., SHAW, J.J., SILVEIRA, F.T., SOUZA, A.A.A. de, BRAGA, R.R. & ISHIKAWA, E.A.Y. 1994. The dermal leishmaniases of Brazil, with special reference to the eco-epidemiology of the disease in Amazonia. Mem. Inst. Oswaldo Cruz 89(3):435–443.

MARCONDES, C.B., LOZOVEI, A.L. & VILELA, J.H. 1998. Distribuição geográfica de flebotomíneos do complexo *Lutzomyia intermedia* (Lutz & Neiva, 1912) (Diptera, Psychodidae). Rev. Soc. Bras. Med. Trop. 31(1):51–58.

MARCONDES, C. B. 2007. A Proposal of Generic and Subgeneric Abbreviations for Phlebotomine Sandflies (Diptera: Psychodidae: Phlebotominae) of the World. Entomol. News 118:351–356.

NAIFF, R.D., FREITAS, R.A., NAIFF, M.F., ARIAS, J.R., BARRET, T. V., MOMEN, H. & GRIMALDI JÚNIOR, G. 1991. Epidemiological and nosological aspects of *Leishmania naiffi* Lainson & Shaw, 1989. Mem. Inst. Oswaldo Cruz 86(3):317–321.

OKSANEN, J., BLANCHET, F.G., FRIENDLY, M., KINDT, R., LEGENDRE, P., MCGLINN, D., MINCHIN, P.R., O'HARA, R.B., SIMPSON, B.L., SOLYMOS, P., STEVENS, M.H.H., SZOECS, E. & WAGNER, H. 2019. vegan: Community Ecology Package. R package version 2.5-5. https://CRAN.R-project.org/package=vegan

PEREIRA JÚNIOR, A.M., TELES, C.B.G., DE AZEVEDO DOS SANTOS, A.P., DE SOUZA RODRIGUES, M., MARIALVA, E.F., PESSOA, F.A.C. & MEDEIROS, J.F. 2015. Ecological aspects and molecular detection of *Leishmania* DNA Ross (Kinetoplastida: Trypanosomatidae) in phlebotomine sandflies (Diptera: Psychodidae) in terra firme and várzea environments in the Middle Solimões Region, Amazonas State, Brazil. Parasites Vectors 8(1):1–11.

PINHEIRO, F.G., LUZ, S.L.B. & FRANCO, A.M.R. 2008. Infecção natural por tripanosomatídeos (Kinetoplastida: Trypanosomatidae) em *Lutzomyia umbratilis* (Diptera: Psychodidae) em áreas de leishmaniose tegumentar americana no Amazonas, Brasil. Acta Amaz 38(1):165-172.

R CORE TEAM (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

RANGEL, E.F. & LAINSON, R. 2009. Proven and putative vectors of American cutaneous leishmaniasis in Brazil: Aspects of their biology and vectorial competence. Mem. Inst. Oswaldo Cruz 104(7):937–954.

READY, P.D. 2013. Biology of phlebotomine sand flies as vectors of disease agents. Annu. Rev. Entomol. 58:227–250. https://doi.org/10.1146/annurev-ento-120811-153557

RESADORE, F., JÚNIOR, A.M.P., DE PAULO, P.F.M., GIL, L.H.S., RODRIGUES, M.M.D.S., ARAÚJO, M.D.S., JULIÃO, G.R. & MEDEIROS, J.F. 2019. Composition and Vertical Stratification of Phlebotomine Sand Fly Fauna and the Molecular Detection of *Leishmania* in Forested Areas in Rondônia State Municipalities, Western Amazon, Brazil. Vector-Borne Zoonotic Dis. 19(5):347–357.

RESADORE, F., PEREIRA, A.M., CARVALHO, L.P.C., DE AZEVEDO DOS SANTOS, A.P., TELES, C.B.G. & MEDEIROS, J.F. 2017. Phlebotomine sand fly composition (Diptera: Psychodidae) and putative vectors of American cutaneous leishmaniasis in Porto Velho Municipality, Western Amazon, Brazil. J. Med. Entomol. 54(3):798–803.

ROSÁRIO, I.N.G., ANDRADE, A.J., LIGEIRO, R., ISHAK, R. & SILVA, I.M. 2016. Evaluating the Adaptation Process of Sandfly Fauna to Anthropized Environments in a Leishmaniasis Transmission Area in the Brazilian Amazon. J. Med. Entomol. 54(2): 450-452. doi: 10.1093/jme/tjw182

SHIMABUKURO, P.H.F., DE ANDRADE, A.J. & GALATI, E.A.B. 2017. Checklist of American sand flies (Diptera, psychodidae, phlebotominae): Genera, species, and their distribution. Zookeys 660:67–106.

DA SILVA, Y.Y., SALES, K.G.D.S., MIRANDA, D.E.D.O., FIGUEREDO, L.A., BRANDÃO-FILHO, S.P. & DANTAS-TORRES, F. 2020. Detection of *Leishmania* DNA in Sand Flies (Diptera: Psychodidae) from a Cutaneous Leishmaniasis Outbreak Area in Northeastern Brazil. J. Med. Entomol. 57(2):529–533.

SILVEIRA, F.T., SOUZA, A.A.A., LAINSON, R., SHAW, J.J., BRAGA, R.R. & ISHIKAWA, E.E.A. 1991. Cutaneous leishmaniasis in the Amazon region: natural infection of the sandfly *Lutzomyia ubiquitalis* (Psychodidae: Phlebotominae) by *Leishmania* (*Viannia) lainsoni* in Pará state, Brazil. Mem. Inst. Oswaldo Cruz 86(1):127–130.

DE SOUZA, A.A.A., DA ROCHA BARATA, I., DAS GRAÇAS SOARES SILVA, M., LIMA, J.A.N., JENNINGS, Y.L.L., ISHIKAWA, E.A.Y., PRÉVOT, G., GINOUVES, M., SILVEIRA, F.T., SHAW, J. & DOS SANTOS, T.V. 2017. Natural *Leishmania* (*Viannia*) infections of phlebotomines (Diptera: Psychodidae) indicate classical and alternative transmission cycles of American cutaneous leishmaniasis in the Guiana Shield, Brazil. Parasite 24:13. https://doi.org/10.1051/parasite/2017016

SVS (SISTEMA DE VIGILÂNCIA EM SAÚDE). 2019. Boletim Epidemiológico – Situação Epidemiológica da Leishmaniose Tegumentar no Estado do Amapá: Período de 2017 a junho de 2019.https://editor.amapa.gov.br/arquivos_portais/publicacoes/SVS_4d77b443923909a984f01e74bf38240f.pdf (last acces 14/01/2021)

TANURE, A., PEIXOTO, J.C., AFONSO, M.M. dos S., DUARTE, R., PINHEIRO, A. da C., COELHO, S.V.B. & BARATA, R.A. 2015. Identification of Sandflies (Diptera: Psychodidae: Phlebotominae) Blood Meals in an Endemic Leishmaniasis Area in Brazil.. Rev. Inst. Med. Trop. 57(4):321–324.

TAVARES, J.P.N. 2014. Características Da Climatologia De Macapá-Ap. CAMINHOS Geogr. - Rev. online 15(50):138–151.

TELES, C.B.G., SANTOS, A.P. de A. dos, FREITAS, R.A., OLIVEIRA, A.F.J. de, OGAWA, G.M., RODRIGUES, M.S., PESSOA, F.A.C., MEDEIROS, J.F. & CAMARGO, L.M.A. 2016. Phlebotomine sandfly (Diptera: Psychodidae) diversity and their *Leishmania* DNA in a hot spot of American Cutaneous Leishmaniasis human cases along the Brazilian border with Peru and Bolivia. Mem. Inst. Oswaldo Cruz 111(7):423–432.

TOJAL DA SILVA, A.C., CUPOLILLO, E., VOLPINI, A.C., ALMEIDA, R. & SIERRA ROMERO, G.A. 2006. Species diversity causing human cutaneous leishmaniasis in Rio Branco, state of Acre, Brazil. Trop. Med. Int. Heal. 11(9):1388–1398.

VASCONCELOS DOS SANTOS, T., DE PITA-PEREIRA, D., ARAÚJO-PEREIRA, T., BRITTO, C., SILVEIRA, F.T., PÓVOA, M.M. & RANGEL, E.F. 2019. *Leishmania* DNA detection and species characterization within phlebotomines (Diptera: Psychodidae) from a peridomicile-forest gradient in an Amazonian/Guianan bordering area.. PLoS One 14(7):e0219626.

VASCONCELOS DOS SANTOS, T., PRÉVOT, G., GINOUVÈS, M., DUARTE, R., SILVEIRA, F.T., PÓVOA, M.M. & RANGEL, E.F. 2018. Ecological aspects of Phlebotomines (Diptera: Psychodidae) and the transmission of American cutaneous leishmaniasis agents in an Amazonian/ Guianan bordering area. Parasit. Vectors 11(1):612.

WHO, 2021. Leishmaniasis factsheets. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis (last acces 28/04/2021).

Received: 05/08/2021
Accepted: 28/02/2022