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[1]Universidade Federal do Acre, Programa de Pós-Graduação Stricto Sensu em Ciências da Saúde na Amazônia Ocidental, Rio Branco, Acre, Brazil.

[2]Universidade Federal do Acre, Campus Floresta, Centro Multidisciplinar, Cruzeiro do Sul, Acre, Brazil

[3]Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância das Doenças Transmissíveis, Brasília, Distrito Federal, Brazil

[4]Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Ciências Farmacêuticas, Departamento de Ciências Biológicas, Araraquara, São Paulo, Brazil

[5]Universidade Estadual Paulista Júlio de Mesquita Filho, Programa de Pós-Graduação Stricto Sensu em Biociências e Biotecnologia, Araraquara, São Paulo, Brazil

[6]Universidade de São Paulo, Instituto de Ciências Biomédicas 5, Monte Negro, Rondônia, Brazil

[7]Centro Universitário São Lucas/Afya, Departamento de Medicina, Porto Velho, Rondônia, Brazil

[8]Centro de Pesquisa em Medicina Tropical de Rondônia, Porto Velho, Rondônia, Brazil.

[9]Instituto Nacional de Epidemiologia da Amazônia Ocidental, Porto Velho, Rondônia, Brazil

[10]Universidade Federal do Acre, Programa de Pós-Graduação Stricto Sensu em Ciência, Inovação e Tecnologia para Amazônia, Rio Branco, Acre, Brazil

[11]Universidade Federal do Acre, Colégio de Aplicação, Rio Branco, Acre, Brazil

Correspondence to: Dionatas Ulises de Oliveira Meneguetti
Universidade Federal do Acre, Colégio de Aplicação, Rodovia BR 364, Km 04, Distrito Industrial, CEP 69920-900, Rio Branco, AC, Brazil
Tel: +55 68 99217-9229.

E-mail: dionatas@icbusp.org

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BRIEF COMMUNICATION

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Trypanosoma cruzi vectors in a periurban area of the Western Brazilian Amazon

Madson Huilber da Silva Moraes[1], Adila Costa de Jesus[1,2], Fernanda Portela Madeira[1,2], Gilberto Gilmar Moresco[3], Jader de Oliveira[4,5], João Aristeu da Rosa[4,5], Luís Marcelo Aranha Camargo[1,6,7,8,9], Paulo Sérgio Bernarde[1,2], Dionatas Ulises de Oliveira Meneguetti[1,10,11]

ABSTRACT

The etiological agent of American trypanosomiasis is the protozoan *Trypanosoma cruzi*, typically transmitted by triatomines. The aim of this study was to investigate the triatomine fauna and trypanosomiasis infections in Acre State, Western Brazilian Amazon. Insect collection was performed by dissecting palm trees and installing traps. We found that *T. cruzi* infection rate was 24.5% and *Rhodnius pictipes* (57.1%) was the most abundant triatomine species. Health education as well as epidemiological and entomological surveillance are necessary to diagnose and prevent new cases of Chagas disease in the region.

KEYWORDS: Triatominae. Trypanosomiasis. *Trypanosoma cruzi*. Western Amazon. *Rhodnius pictipes.*

INTRODUCTION

American trypanosomiasis, also known as Chagas disease, is a neglected tropical disease with approximately 6 million people infected worldwide[1]. It is caused by the protozoan flagellate *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae)[2] and is transmitted by insects of the Reduviidae family and the Triatominae subfamily[3].

Most triatomine species are wild although they exhibit synanthropic behavior and can be found in various habitats, such as bird nests, under tree barks or hollow trees, in palm trees, bromeliads, vertebrateburrows , among others, as long as they provide shelter and food source[4]. There are 11 known triatomine species in Acre State, Brazil (Rhodnius robustus, first described by Stal, 1872; R. montenegrensisby Rosa *et al*., 2012; R. pictipesby Stal, 1872; R. neglectusby Lent, 1954; R. staliby Lent, Jurberg and Galvão, 1993; Panstrongylus geniculatusby Latreille, 1811; P. megistusby Burmeister, 1835; P. lignariusby Walker, 1873; *Panstrongylus rufotuberculatus* by Champion, 1899; Eratyrus mucronatusby Stal, 1859; and Triatoma sordidaby Stål, 1859)[5].

However, most of the species described in the previous paragraph, have not yet been recorded in Jurua Valley, a region that borders Peru, and has Cruzeiro do Sul as its main city, a region in which Chagas disease outbreaks have already occurred, but only a few studies have been carried out in this area[6]. This is the reason why additional studies are welcome in this region in order to understand the diversity of Chagas disease vectors. Therefore, the present study aimed to investigate the triatomine species occurring in Cruzeiro do Sul, and the presence of trypasonomiasis infections.

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MATERIAL AND METHODS

The survey was conducted in the municipality of Cruzeiro do Sul, located in Acre State, Western Brazilian Amazon. It is located at latitude 07°39'54" South and longitude 72°39'1" West (Figure 1).

Collections were carried out in the periurban area, located on the side of the road leading to the BR 364 federal highway that connects most municipalities in Acre State.

Four quarterly collections were carried out from September 2017 to December 2018. The following capture methods were used: dissection of palm trees and passive search in the installed traps.

A total of eight palm trees were dissected, four specimens of *Mauritia flexuosa* (Buriti) and *Attalea butyracea* (Jaci), with one specimen per species per quarter. Before bracts dissection, the distancesbetween the selected palm trees (in meters) and the residence of the area owner was calculated by means of a 50-meter long measuring tape. Then, bracts were carefully examined.

For the triatomines capture, the following traps were used: Luiz de Queiroz-type light trap, Rafael and Gorayeb-type suspended trap and the traditional Malaise trap[7]. All traps remained installed for a period of approximately 12 h, from 6 pm to 6 am of the following day. This type of collection was performed every four months, for two consecutive nights. The collection team stayed close to the traps during the survey. The specimens were collected with authorization of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renovaveis - IBAMA (Brazilian Institute of the Environment and Renewable Natural Resources), license Nº 52260-1.

Collected triatomines were identified by means of their external morphological characteristics, based on Lent and Wygodzinsky's description[3], aside from other aspects clarified by Rosa[8] and Galvão[2]. Triatomines from the genus *Rhodnius* were bred until they reached the adult stage, and then the internal characteristics of the genitals were analyzed to identify the species[3,9].

Identification of trypanosomatids was performed by using the polymerase Chain Reaction (PCR). DNA was extracted from the rectal ampoule content of triatomines using the QIAGEN DNA mini kit (QIAGEN, Hilden, Germany). The PCR-multiplex was performed according to the method described by Fernandes[10] consisting of the amplification of fragment of the mini exon gene non-transcribed spacer which differs among the trypanosomatidae species (*T. cruzi* and *T. rangeli*) and *T. cruzi* strains (TCI and TCII). The fragments molecular weight ranged from 100 to 250 base

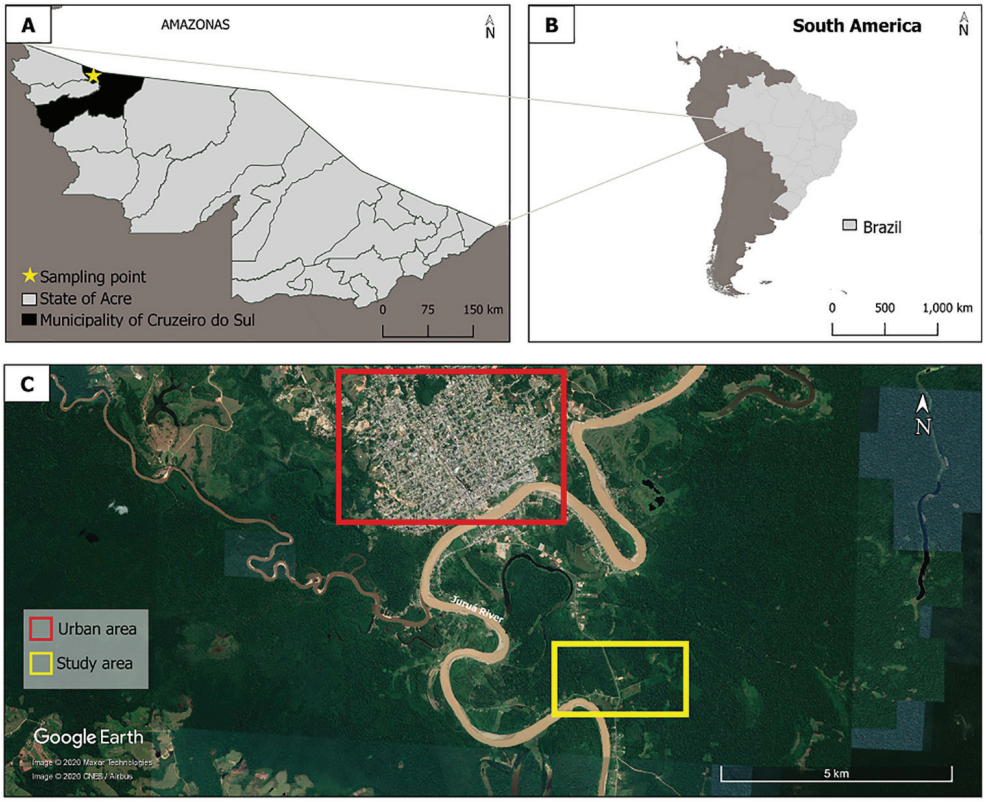

Figure 1 - Geographic location of Cruzeiro do Sul municipality and the collection area: A) Acre State; B) South America and Brazil; C) Urban area and the study area (Cruzeiro do Sul).

pairs. The primers used in the multiplex-PCR areTCI: (200 bp) (5'-ACACTTTCTGCGCTGATCG-3'); TCII: (250 bp) (5'-TTGCTCACTCGCTGCAT-3');Z3: (150 pb) (5'-CCGCGCACAACCCCTATAAAATG-3'); TR: (100 bp) (5'-CCTATTGTGATCCATCTTCG-3'); EXON: (5'-TACCAATATAGTACAGAACTG-3').

The master mix was composed of 100 pmol of each primer, 150 µM of dNTPs in a 10 mM buffer of Tris-HCl (pH 8.3), 1.5 mM of $MgCl_2$, 25 mM of KCl, 0.1 mg/mL of bovine albumin, and 2.5 U of TaqDNA Polymerase. Then, approximately 10 ng of genomic DNA were added, in a final volume of 50 µL. Amplifications consisted of an initial denaturation step of 5 min at 95 °C, followed by 34 cycles of 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C, and a final extension step at 72 °C for 10 min. In each PCR reaction, the following reference strains were used as controls: TC1 X10 Clone 1, TC2 Cepa Y, Z3 Emerald Clone 1, and *T. rangeli* R1625. Amplification products were analyzed in 2% ethidium bromide-stained agarose gels for 1 h at 100 volts. visualized under ultraviolet light.

RESULTS

During the study period, a total of 53 triatomines (all of them obtained by the species *A. butyracea* of palm trees dissection) belonging to three species: *Rhodnius montenegrensis* (Rosa *et al*., 2012), *Rhodnius pictipes* (Stål, 1872) and *Eratyrus mucronatus* (Stål, 1859) (all species previously reported in Acre State), They presented with an average infection rate of 24.5% by *T. cruzi* (Table 1), all of them, TCI strains. The species *R. pictipes* presented with the highest infection rate. In the present study, no *T. rangeli*-infected triatomes were found.

In the present study, the genus *Rhodnius* accounted for 96.2% of the total collected triatomines. The species *R. montenegrensis* was the one ranking second regarding the infection rate (28.5%).

Rhodnius sp1 and *Rhodnius* sp2 were the most captured in this study. They were classified in this way because they did not reach the adult stage, which is required to identify them using the internal genitalia analysis, and because they were very similar morphologically.

There was a predominance of the nymphal stages in the collections performed in relation to the development stage of the triatomines. In respect to the vectors natural infection, 13 triatomines tested positive for *T. cruzi,* indicating an infection rate of 24.5%. For *T. cruzi* infection, adults were found to be more contaminated than nymph stages. The percentage of infection of each development stage was: n1 (0.0%) n2 (11.1%), n3 (7.7%), n4 (38.5%), n5 (33.3%), and adult (50%), showing that the development stage has an influence on the percentage of infection ($p<0.05$ - chi-square test).

Table 1 - Species, number of collected triatomines, development stage and infection by *T. cruzi*.

Species	N/ Phase	Infected	Infection rate
R. montenegrensis	0/ Ni1	0	0%
	0/Ni2	0	0%
	1/Ni3	0	0%
	1/Ni4	0	0%
	1/Ni5	0	0%
	4/A	2	50%
	7 (Total)	**2**	**28.6%**
R. pictipes	0/Ni1	0	0%
	0/Ni2	0	0%
	1/Ni3	1	100%
	3/Ni4	2	66.7%
	1/Ni5	0	0%
	2/A	1	50%
	7 (Total)	**4**	**57.1%**
Rhodnius sp1* (pattern *R. montenegrensis*/ *R. robustus*)	0/Ni1	0	0%
	7/Ni2	1	14.3%
	10/Ni3	0	0%
	9/Ni4	3	33.3%
	6/Ni5	2	33.3%
	0/A	0	0%
	32 (Total)	**6**	**18.7%**
Rhodnius sp2* (pattern *R. pictipes*/ *R. stali*)	3/Ni1	0	0%
	2/Ni2	0	0%
	0/Ni3	0	0%
	0/Ni4	0	0%
	0/Ni5	0	0%
	0/A	0	0%
	5 (Total)	**0**	**0%**
E. mucronatus	0/Ni1	0	0%
	0/Ni2	0	0%
	1/Ni3	0	0%
	0/Ni4	0	0%
	1/Ni5	1	100%
	0/A	0	0
	2 (Total)	**1**	**50%**
Total	53	13	24.5

N = Sample number; Ni = Nymph; A = Adult; *Triatomines that did not reach the adult stage for the identification at the species level.

Dissecting palm trees was the most efficient collection method for triatomines in this study based on the total number of captured specimens. The dissected palm trees were located 50 to 600 meters away from the home of the area owner, 50% of them were positive for triatomines and the distance with the largest number of collected specimens was 50 to 200 meters (Table 2).

By the method of palm dissection, an average density of 6.6 triatomines per palm tree was found. In the present study, no triatomines were collected from *M. flexuosa* palm tree species.

Table 2 - Distance between the residence and the palm tree, and average number of triatomines collected per palm tree.

DRP*(meters)	TTC**	TDP***	MTP****
50-200	35	3	11.7
200-400	9	2	4.5
400-600	9	3	3.0

DRP* = Distance between residence and palm tree (m); TTC** = Total triatomines collected; TDP*** = Total dissected palms; MTP**** = Average triatomines by palm tree.

DISCUSSION

The species *R. pictipes* and *R. montenegrensis* presented with the highest and second highest infection rates in this study, respectively. The former is one of the main triatomine species labelled as a vector, that can also transmit *T. cruzi* orally in Amazonia[11]. The second triatomine has epidemiological relevance in this region due to the confirmation of infections transmitted by this trypanosomatid[12].

Some species of the genus *Rhodnius* were not identified owing to their development stage but were classified according to their characteristics similarity. A recent phylogenetic study indicated that there is a close relationship between the species *R. montenegrensis* and *R. robustus*[12]; similar to *R. stali* and *R. pictipes*, which are considered morphologically similar and phylogenetically related[13].

The species *E. mucronatus,* also captured in this study, is considered wild but can be found in home environments, including reports of colonization in homes in Bolivia, a neighboring country of Acre, State confirming its potential for domicile trasmission[14].

The results associated with the predominance of nymph stages during this study are in agreement another survey conducted in the Rondonia State, where they have also collected a high number of nymphs in relation to the number of adults[15].

T. cruzi natural infection rate in the vectors was 24.5%. Compared to recent studies in the Amazon region, a lower percentage was observed than those observed by Meneguetti *et al.*[15], who described an infection rate of 35.6%, and Bilheiro *et al.*[12] (36.7%).

The identification of TCI strains of *T. cruzi*, followed nomenclature DTUs guidelines which use TC[16]. For the genotypes found using the mini-exon primer, TCI, TCII and Z3 were used, according to the 1999 consensus[17]. Another study carried out in Acre State used the same method of molecular analysis and the authors have also found only *T. cruzi* TCI strains[18].

The percentage of infection may be influenced by the development stage. In this study, adults were more contaminated than nymphs. Similar data were found in Rondonia State, where it was observed that the higher the development stage, the higher the trypanosomatid infection rate, probably because they performed more blood meals[12,15].

Also in Rondonia Stae, an association between the occurrence of triatomines and the proximity of examined palms to the homes was observed, suggesting that there is greater availability of food resources[19] near homes, a situation confirmed by this study.

Regarding the mean density index of dissected palm trees found in this research, previous reports that used a similar capture method in the genus *Attalea* obtained indexes of 3,414 and 8,310, and 20,613 in the genus *Orbignya*.

Triatomines infestation of the species *R. neglectus* Lent, 1954 and *Psammolestes tertius* Lent & Jurberg, 1965 was confirmed in a study carried out in the Federal District, in *M. flexuosa*[20], unlike this research as *M. flexuosa* triatomines were captured in palm trees of this species. The fact that there is no record on the occurrence of *R. neglectus species* in the region of Jurua Valley and no report of *P. tertius species* in Acre State might explain the negative results in the present study.

The absence of triatomines in *M. flexuosa* palm trees is actually a positive result, since this species has cultural, social and economic relevance; the fruit from this plant, popularly known as *buriti*, is the source of several products and food consumed *in natura* or after processing for wine preparation, which is a traditional local drink, marketed and consumed by a large part of the population. It was found that the triatomine fauna occurring in the study area was composed of *R. montenegrensis*, *R. pictipes* and *E. mucronatus.* All species collected were positive for *T. cruzi* infection.

The capture record of triatomine fauna with positive detection of *T. cruzi* in palm trees close to the houses reflects the possibility of human infection by the vector, so that activities related to insect control are required. In addition, it is essential to implement health education actions for residents of areas at risk, with emphasis on general aspects of Chagas disease and the identification of triatomines as a prophylactic measure to prevent the occurrence of *T. cruzi* transmission by both, vectors and the oral form, which is the main form of transmission in Acre State[6] due to cultural habits and lack of hygiene in the preparation of food, especially those from palm trees.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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