Research Article

Investigation of the triatomine (Hemiptera: Triatominae) fauna and its infection by *Trypanosoma cruzi* Chagas (Kinetoplastida: Trypanosomatidae), in an area with an outbreak of Chagas disease in the Brazilian South-Western Amazon

Investigación de la fauna triatomina (Hemiptera: Triatominae) y su infección por *Trypanosoma cruzi* Chagas (Kinetoplastida: Trypanosomatidae), en un área con un brote de enfermedad de Chagas en la Amazonía sudoccidental brasileña

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Abstract. The present study aims to describe the occurrence of triatomines and their infection rate by *Trypanosoma cruzi*, in an area of Chagas disease outbreak in Rodrigues Alves municipality, Acre state, Brazil. Collections were performed by passive search (capture of specimens by local people) and dissection of palm trees (*Attalea butyracea* and *Mauritia flexuosa*). Thirty two triatomines belonging to four species grouped into two genera (*Rhodnius* and *Eratyrus*) had *T. cruzi* infection rate of 15.6%. Only in *A. butyracea* triatomines were captured. The passive search was the most effective method for collection. The Nova Cintra triatomine fauna comprises four species and it is important to carry out future studies to understand the biology of these triatomines.

Key words: Chagas disease vectors, Trypanosomatids, medical entomology, Chagas disease and the Amazon.

Resumen. El presente estudio tiene como objetivo describir la aparición de triatominos y la infección causada por *Trypanosoma cruzi*, en un área de brote de la enfermedad de Chagas en el municipio de Rodrigues Alves, estado de Acre, Brasil. Las recolecciones se realizaron mediante búsqueda pasiva y disección en palmeras (*Attalea butyracea* y *Mauritia flexuosa*). Treinta y dos triatominos pertenecientes a cuatro especies agrupadas en dos géneros (*Rhodnius* y *Eratyrus*) tenían una tasa de infección por *T. cruzi* del 15,6%. Solo en *A. butyracea* se capturaron triatominos. La búsqueda pasiva fue el método

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más efectivo para la recolección. La fauna triatomina de Nova Cintra comprende cuatro especies, es importante realizar futuros estudios para comprender su biología.

Palabras clave: Vectores de la enfermedad de chagas, trypanosomatids, entomología médica, enfermedad de Chagas y la Amazonía.

Introduction

Chagas' disease (CD), also known as American trypanosomiasis, is an infectious disease whose etiologic agent is the protozoan *Trypanosoma cruzi* Chagas, 1909 (Kinetoplastida: Trypanosomatidae) (Chagas 1909; Castro *et al*. 2017) and the vectors are blood-sucking hemipteran insects, belonging to the Hemiptera order, Reduviidae family and Triatominae subfamily (Barbosa *et al*. 2015).

Although the Amazon region was excluded as a region of occurrence of CD until the mid-1970s, it has recently aroused the concern of the authorities in public health because of growing reports of this disease, which is still considered to be emerging in the region (Barbosa *et al*. 2015; Santana *et al.* 2019). Most of these cases are of transmission through contaminated foods, mainly regional drinks, such as the juice of "açaí" (*Euterpe precatoria* Mart.) (Barbosa *et al*. 2015). In addition to oral transmission, the possibility of vector transmission is another concern, given the existence of more than 30 species of triatomines described for the Amazon region (Castro *et al*. 2018).

In the Brazilian Amazon, Acre is considered one of the states with the greatest diversity of triatomines, with 11 species reported, all of them already found naturally infected with trypanosomatids (Oliveira *et al*. 2019). Although the first case of CD in the state of Acre was recorded in the 1980s (Barata *et al*. 1988), it was the outbreaks from 2016 onwards that raised the concern about the disease, given the increase in notifications and its intrinsic relationship with the consumption of contaminated food. An example is the outbreak that occurred in the municipality of Rodrigues Alves in 2016 (Oliveira *et al.* 2018). Since there are no studies on the vector species occurring in this region, studies are needed to increase the knowledge about this subject and thus raise the community's awareness of prophylactic measures, serving as a tool for guiding health surveillance in the establishment of strategies for combating new outbreaks. Thus, this study is aimed at investigating the occurrence of triatomines and their infection by *T. cruzi* in an area of CD outbreak in the municipality of Rodrigues Alves, Acre, Brazil, where an outbreak was reported with five cases confirmed during February 2016, all of them apparently caused by oral transmission, probably by consumption of "açaí" juice contaminated with feces from triatomines (Oliveira *et al.* 2018).

Material and Methods

The collections were made from August 2017 to December 2018, at the Nova Cintra Settlement Project (S 7°48,808' and W 72°40,480'), located in Rodrigues Alves, Acre, about 12 km away the urban area of the municipality (Fig. 1).

The collections were made with a permanent license (license n° 52260-1) issued by the Brazilian Institute for the Environment and Renewable Natural Resources (Instituto Brasileiro de Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA). The technique used to collect triatomines consisted of passive search in households and active search through the dissection of two palm species: *Mauritia flexuosa* ("buriti") and *Attalea butyracea* ("jaci"). The bracts of two palm trees, one *M. flexuosa* and one *A. butyracea*, were dissected in each quarterly collection, totaling eight palm trees collected, 4 of each species.

The bracts were dissected using a chainsaw. They were gradually removed starting from the lower ones to the palm crown, with the specimens being manually collected, separated, and stored according to their stage of development.

The passive search performed in households consisted of distributing collection vials containing folded paper to community residents, along with the presentation of the morphological characteristics of the triatomines to the adults responsible for the households using printed images and specimens collected in their community.

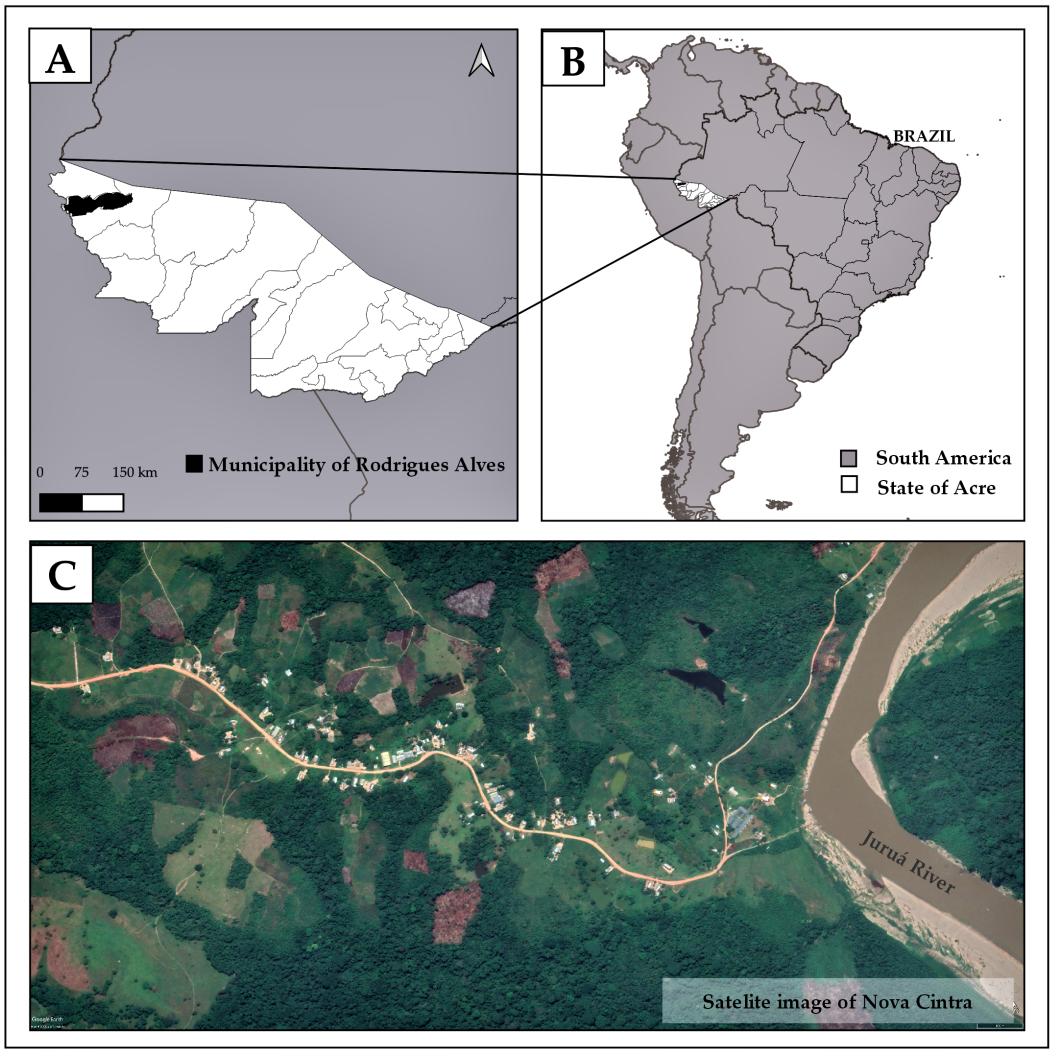

Figure 1. Geographic location of the study area. A) State of Acre; B) South America; C) Nova Cintra.

The collected specimens were identified based on the external morphological characteristics described by Lent & Wygodzinsky (1979), Rosa *et al*. (2012) and Galvão (2014). The species belonging to the genus *Rhodnius* Stål, 1859 had their genitalia analyzed for confirming the species (Lent and Wygodzinsky 1979; Rosa *et al*. 2012, 2014; Galvão 2014). The natural infection of triatomines by trypanosomatids was analyzed using feces obtained through compression of the insect's abdomen, followed by maceration and dilution in 0.9% saline solution. The resulting sample was mounted on a slide and examined using an optical microscope under 400-fold magnification Staining was performed with triarylmethane 0.1%, xanthene 0.1%, and thiazines 0.1%. Optical microscopy at 1000x magnification was used for examination.

The remaining portion of the material used for microscopy was inserted into microtubes containing absolute alcohol for biomolecular identification, which followed the protocol described by Fernandes *et al*. (2001), with the extraction of trypanosomatid DNA using the *Qiagen DNA extraction kit®*. Then, Polymerase Chain Reaction (PCR) technique was performed to amplify part of the non-transcribed spacer of the mini-exon gene that varied, according to the species *T. cruzi* (TCI and II) and *T. rangeli*. In the method, the generated fragments varied between 100 and 250 base pairs, and oligonucleotides primers with the following sequences were used: TCI: (200 bp) (5'ACACTTTCTGGCGCTGATCG); TCII: (250 bp) (5'TTGCTCGCACACTCG GCTGCAT); Z3: (150 bp) (5'CCGCGCACAACCCCTATAAAAATG); TR: (100 bp) (5'CCTATTGTGATCCCCATCTTCG); EXON: (5'TACCAATATAGTACAGAACTG).

Each reaction required 100 ppmol of each *primer*, 150 μM dNTPs, in 10 mM Tris-HCl buffer (pH 8.3), 1.5 mM MgCl2, 25 mM KCl, 0.1 mg/mL of bovine albumin, 2.5 U of TaqDNA Polymerase. Subsequently, 10 ng of the genomic DNA sample was added, resulting in a final volume of 50 μL with Type 1 water (Fernandes *et al.* 2001). The reaction started the cycle at 95°C for 5 minutes, followed by 34 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C, with a final extension of 10 minutes at 72°C. The reference strains used as control were: TC1 X10 Clone 1, TC2 Cepa Y, Z3 Esmeraldo Clone 1, and *T. rangeli* R1625. The amplified products were subjected to 2% agarose gel electrophoresis at 100 volts for 1 hour. After electrophoresis, DNA was revealed with ethidium bromide and visualized under ultraviolet light (Fernandes *et al*. 2001).

Results

Thirty-two triatomines belonging to four species grouped into two genera were collected throughout the study (Table 1). Infection of these triatomines by *T. cruzi* is also shown in table 1. There were no triatomines infected by *T. rangeli*.

Discussion

The low occurrence of triatomines collected from palm trees differs from other studies conducted in the Amazon region (Meneguetti *et al.* 2012) and may be related to the characteristics of the environment studied, with a lower density of palm trees of the genus *Attalea*. Data showing the absence of triatomines in *M. flexuosa* of the region differ from studies conducted with the same species of palm in another biome (Gurgel-Gonçalves *et al*. 2012), where species of triatominae that do not yet occur in the region of Juruá were collected.

The natural infection rate for *T. cruzi* was 15.6%, a percentage lower than that found in other studies conducted in the Amazon region, with rates of 23.7% (Massaro *et al.* 2008) and 35.6% (Meneguetti *et al*. 2012).

The species *R. montenegrensis* Rosa *et al.* (2012), represented 56% of the total of collected triatomines and 64% of those found within households. The higher occurrence of *R. montenegrensis* in the present study further strengthens the hypothesis of underreporting of the occurrence of triatomines of this species in the Amazon region, as it was only described in 2012 in the municipality of Monte Negro, Rondônia (Rosa *et al*. 2012), having been confirmed by karyotype and molecular analysis (Alevi *et al*. 2015; Carvalho *et al.* 2017). However, it is believed that its morphological similarity with *R. robustus* Larrousse, 1927, may have caused some errors in taxonomic study identities before 2012, as well as in subsequent identification using only external morphological characteristics.

The occurrence of *R. montenegrensis*, in this and other studies conducted in the state of Acre (Meneguetti *et al*. 2015; Ribeiro *et al*. 2019), added to the evidence of its infection by *T. cruzi* (Bilheiro *et al*. 2018) and *T. rangeli* (Meneguetti *et al*. 2015) and its occurrence in rural households (Meneguetti *et al.* 2015) and urban environment (Ribeiro *et al*. 2019), shows the

potential of this species for transmission of CD.

The second highest most prevalent triatomine in this study was *R. pictipes* Stål, 1872, representing 25% of the total of triatomines collected, two of which were positive for *T. cruzi*. Both were collected from palm trees of the species *A. butyraceae,* which is considered a natural ecotope of this species in the wild (Galvão 2014). The specimens collected from palm trees were positive for *T. cruzi.* Similar data to this were found by other studies conducted in the state of Acre, where *R. pictipes* Stål, 1872 was collected in palm trees (Ramos *et al*. 2018) and households (Ribeiro *et al.* 2019), finding infection by *T. cruzi* in both environments. *R. pictipes* may also be associated with the first case of CD in the state of Amazonas (Fé *et al.* 2009), having a high prevalence both in wild and households in the city of Manaus, where 73.9% of the total specimens were found (Fé *et al.* 2009).

Rhodnius stali Lent, Jurberg & Galvão, 1993, represented 12.5% of the total of triatomines, with a record of infection by *T. cruzi.* There are confirmed reports of domiciliation in Bolivia, held as the most likely responsible for the transmission of *T. cruzi* in indigenous communities in Alto Beni (Matias *et al.* 2003). It occurs in the state of Acre (Meneguetti *et al.* 2016) and is also susceptible to infection by *T. rangeli*, which makes the differential diagnosis of trypanosomatids difficult (Castro *et al.* 2017), which indicate that mixed infections may be occurring in the state.

Table 1. Triatomine species collected and positivity for *T. cruzi* in the Nova Cintra, Acre, Brazil.

Species	Collection Method/ Amount	Positives for *T. cruzi*
Rhodnius montenegrensis Rosa *et al*., 2012	P (0)	-
	PD (0)	-
	I (18)	2 (11%)
	TR (0)	-
	T (18)	**2 (11%)**
Rhodnius pictipes Stål, 1872	P (2)	2 (100%
	PD (0)	-
	I (6)	-
	TR (0)	-
	T (8)	**2 (25%)**
Rhodnius stali Lent, Jurberg & Galvão, 1993	P (0)	0
	PD (0)	-
	I (4)	1 (25%)
	TR (0)	-
	T (4)	**1 (25%)**
Rhodnius sp.*	P (1)	0 (0%)
	PD (0)	-
	I (0)	-
	TR (0)	-
	T (1)	**0 (0%)**
Eratyrus mucronatus Stål, 1859	P (1)	0 (0%)
	PD (0)	-
	I (0)	-
	TR (0)	-
	T (1)	**-**
Total	**32**	**5 (15,6%)**

* Species whose identification was made impossible by the stage of development (nymph 1).
Legend: P: Palm tree. PD: Peridomicile, I: Intradomicile, TR: Trap and T: Total.

In addition to species belonging to the genus *Rhodnius*, a nymph of fifth stage, belonging to the genus *Eratyrus* Stål, 1859 was collected during dissection in *A. butyracea* and identified as *E. mucronatus* Stål, 1859. Although this species, which is a wild vector of CD, is considered to be of lesser epidemiological importance (Lent and Wygodzinsky 1979), it can quickly adapt to artificial ecotopes, when their natural habitats are destroyed by anthropic actions (Meneguetti *et al.* 2011) this species naturally infected by *T. cruzi*, also being reported in households in countries such as Bolivia, Peru, and Venezuela (Meneguetti *et al.* 2011). *E. mucronatus* was also observed in households in the state of Acre, where an *Attalea* palm tree was observed in the vicinity of the residence (Obara *et al.* 2013).

The passive search considered that the most effective method of sampling in the region was carried out by the research team and the inhabitants who identified the insect during the night. However, the invasion of triatomines in homes does not characterize domiciliation and can occur due to the chemical and luminous attraction within the households (Galvão 2014). The number of reports of triatomine intrusion is increasing among households in urban and peri-urban areas in the Amazon (Fé *et al.* 2009; Ribeiro *et al.* 2019), with only one report of domiciliation in the entire region (Ricardo-Silva *et al.* 2016). Therefore, the invasion of these vectors may also be due to the destruction of the nearby forest areas, causing wild species to be closer to residential areas.

The species of triatomines occurring in Nova Cintra are grouped into two genera (*Rhodnius* and *Eratyrus*), with a natural infection rate for *T. cruzi* of 15.6% and with low infestation rate in palm trees. The method for raising the awareness of the vector by the community residents was effective for the collection of adult triatomines that invade the residences at night and to guide measures to prevent the disease in the community.

It is important to conduct research to evaluate triatomines and their infection in the region to understand ecological aspects and possible changes in the current dynamics of CD transmission, thus preventing the occurrence of new outbreaks in the region.

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