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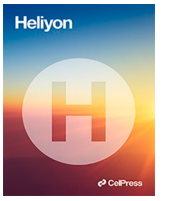

First report of *Rhodnius stali* Lent, Jurberg & Galvão, 1993 (Hemiptera: Reduviidae: Triatominae), vector of *Trypanosoma cruzi* (TcI) and *Trypanosoma rangeli* (TrA), in Rondônia, Southwestern Brazilian Amazonia

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ABSTRACT

Triatomines are hematophagous insects of epidemiological importance as they are vectors of Chagas disease. The first report of *Rhodnius stali* Lent, Jurberg & Galvão, 1993 in Rondônia, Brazil, is described. The insects were captured on palm trees of the genus *Oenocarpus* sp. Two adult male specimens of *R. stali* were identified and were found to be infected with *Trypanosoma cruzi* and *Trypanosoma rangeli*. The confirmation of this *Rhodnius* species in Rondônia increases the number of triatomines from nine to ten species in this state.

Introduction

In the Amazon region, currently eleven species of the genus *Rhodnius* are known, a genus that is distributed from Central to South America [1]. *Rhodnius* comprises twenty-one species (Zhao; Galvão; Cai, 2021), of which ten are found in Northern Brazil [2]. *Rhodnius* exhibits its species diversity strictly associated with palm trees in both the wild and peridomestic cycles [3]. This association is important in terms of public health because the insects that live in these palm trees can invade nearby homes, overall attracted by light [4]. The migration of these vectors from palm trees to houses can undermine vector control programs, as it can lead to reinfestation of homes treated with insecticides [5], and represent a risk of emerging *T. cruzi* transmission in peridomestic and domestic environment in Amazonia (Depickère et al., 2023; [6].

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Transmission of human trypanosomiasis in the Amazon region has been increasing, possibly due to the contact of humans with the rich fauna of vectors and animal reservoirs, with growing human population and migratory movements of people causing deforestation and invading the natural habitat of these insects. Transmission of Chagas disease in Brazilian Amazon region relied mostly on *Rhodnius* species and has spreading due to limited vector surveillance campaigns, and public health education [7–9]; Coura; Junqueira, 2012; Vergara-Mesa et al., 2022).

In the Amazon context Chagas disease is an emerging disease, most confirmed outbreaks of acute disease through epidemiological investigations indicate non-vectorial transmission involving palm fruit juices [10,11]; Vergara-Mesa et al., 2022). From 2001 to 2018, acute cases of Chagas disease were concentrated in the North region, representing more than 50 % in relation to the other regions (South, Southeast, Central-West and Northeast) and were predominantly oral [12]. According to a systematic review and meta-analysis, oral Chagas disease has been increasing in the last two decades, but lethality has reduced by improvement on diagnosis and treatment, and all cases were from Latin America, particularly Brazil, and caused mostly by the ingestion of acai fruit [13].

Palms are considered an important ecotope for triatomines, providing ideal conditions for their development [3,14,15], particularly for species of the genus *Rhodnius*. Palm-vector association enhances the occurrence of domiciliary and peridomiciliary invasion in the Brazilian Amazon region [3,16], this makes the genus *Rhodnius* the most important for the region due to its abundance in peridomestic palms and its close association in the Amazon region, as well as its involvement in reported outbreaks of oral transmission, during which they are found invading homes and spaces. Therefore, this study aims to describe the first report of the occurrence of *Rhodnius stali* in the state of Rondônia, Brazil, and the identification of trypanosomes they can harbor and transmit.

The collections were conducted in 2020 within the Rio Cautário Extractive Reserve, located in the municipality of Guajará-Mirim, Rondônia, bordering the city of Costa Marques in Rondônia and the country of Bolivia (Fig. 1). Triatomine bugs were captured through active search methods on palm trees of the *Oenocarpus* sp. genus. Nine palm trees were sampled, and two tested positive for *Rhodnius stali*. It is worth noting that the insects were found in the canopy of the palms, in areas with accumulations of materials and bracts. Collection tools included cutting equipment and tweezers. The bracts were individually removed, and a meticulous analysis of the material present between them was conducted.

After collection, the insects were taken to the laboratory of the Federal Institute of Rondônia (IFRO) campus in Guajará-Mirim, where they were sorted and sent to the Parasitology Laboratory of the Faculty of Pharmaceutical Sciences - Unesp/Araraquara for specific confirmation through male genitalia examination. The diaphanization and examination of the morphological structures of the

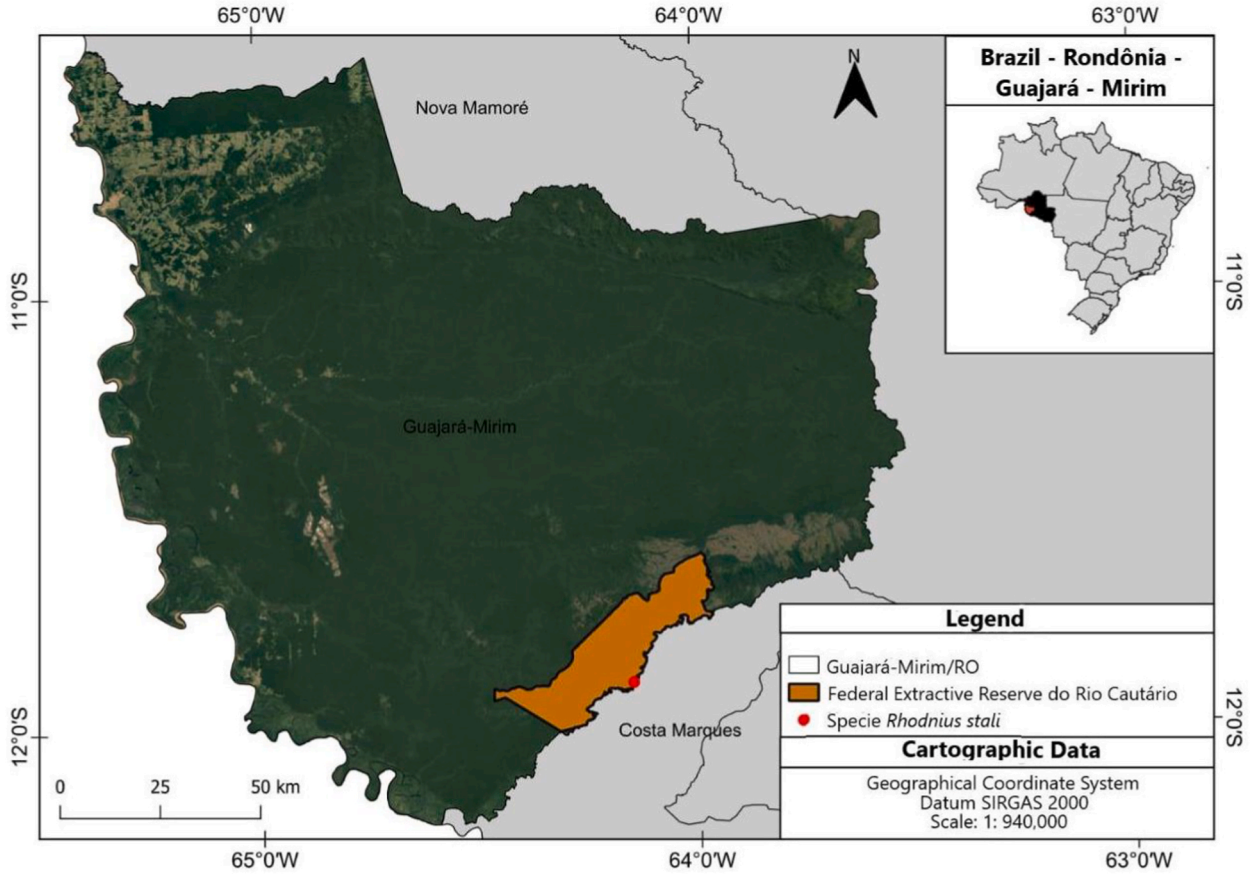

Fig. 1. Location of *Rhodnius stali* collection in the Rio Cautário Extractive Reserve, municipality of Guajará-Mirim, Rondônia, Brazil.

collected specimens were performed as described by Refs. [17,18].

For the analysis of the presence of trypanosomes, initially, optical microscopy (40x objective) was used to examine aliquots of feces diluted in saline solution (0.9 %). In case of a positive or negative result for trypanosomatids, total DNA extraction was performed. For this purpose, a conventional protocol described by Adams et al. (2008) was used, and for digestion of the feces/digestive tract, Digsol solution (50 mM Tris, 20 mM EDTA, 117 mM NaCl, and 1 % SDS) was used, along with proteinase K at 37 °C overnight. The solution was then precipitated with an ammonium acetate-based solution, centrifuged, and washed with 99.8 % and 70 % ethanol, respectively. After drying, the DNA was resuspended in 40 μl of TE buffer (Tris-EDTA) and stored at −20 °C [19]. After extraction, DNA concentration and quality were evaluated using the NanoDrop™ equipment (Thermo Scientific™ spectrophotometers).

For the identification of trypanosome species, the Fluorescent Fragment Length Barcoding (FFLB) method was used, which presents high sensitivity and precision in distinguishing species/genotypes of trypanosomes simultaneously (as in mixed infection) [20].

The DNA samples from triatomines was subjected to four PCR reactions with specific fluorescent primers, and the four PCR products analyzed in an ABI 3500 sequencer, and fluorescent peaks assessed using the GeneMapper Software v.4.0 (Applied Biosystems). Each trypanosome species/genotype has a unique a profile of four peaks of variable length, allowing for the identification of trypanosomes without the need of sequencing, as it is unlikely to find the same FFLB profile for different species [19,21,22]. The method of FFLB was developed for trypanosome surveys in tsetse flies, and recently adapted with success for identification and genotyping of both *T. cruzi* and *T. rangeli* directly in samples from digestive tract of triatomines[19,21,22]; Valença-Barbosa et al., 2021; Vergara-Mesa et al., 2022).

Two specimens of triatomine bugs were collected, identified as two adult males of *Rhodnius stali* (Fig. 2A–C). *Rhodnius stali* specimens have a yellowish-brown, dotted coloration, usually with small spots, measuring around 16.5–17 mm. They have an elongated head with an anteocular region. The pronotum is brown, covered with irregularly shaped black spots. The legs, coxae, trochanter, and femurs are yellowish and speckled with black as described in the diagnosis proposed by Ref. [17]. The identification of trypanosomes using FFLB revealed the presence of *Trypanosoma cruzi* of discrete typing units (DTU) TcI and *Trypanosoma rangeli* of lineage A (TrA) (Fig. 3).

New records. BRAZIL – Rondônia • Guajará-Mirim/Rio Cautário Extractive Reserve; Latitude: 11.897069, Longitude: 64.150294; 2020; leg. Menezes, ALR; CEJSMB, 2 ♂ (Fig. 2).

Rhodnius stali, according to Ref. [23]; inhabits the moist forests of southwestern Amazon and transitional ecoregions (mountainous Yungas, Chiquitano dry forests, and Beni-Cerrado-Pantanal savannas) [23], but in a community in Bolivia that suffered deforestation and construction of residences roofed by palm leaves, a process of domiciliation occurred [24], with this species representing the main vectors of Chagas disease in region of Bolivia [25]. Another study revealed *R. stali* in peridomicile areas and in palm trees (*Attalea phalerata*) in the Alto Beni region, Bolivia, being the likely vector responsible for the Chagas disease seropositivity in an indigenous population [26].

In 1993, the first record of *R. stali* species was reported, but it was only in 2009 that molecular studies confirmed the presence of *T. cruzi* (TcI) and *T. rangeli* (non-genotyped) in Mato Grosso do Sul, Brazil (Silva et al., 2009; Lent; [27]. In Brazilian Amazonia, first report of *R. stali* occurred in Acre, in 2015 [28], and *T. rangeli* was identified in this species [29]. In a community in the Juruá Valley, Acre, *R. stali* infected by *T. cruzi* was found in palm trees through passive search [30]. These studies highlighted the importance of

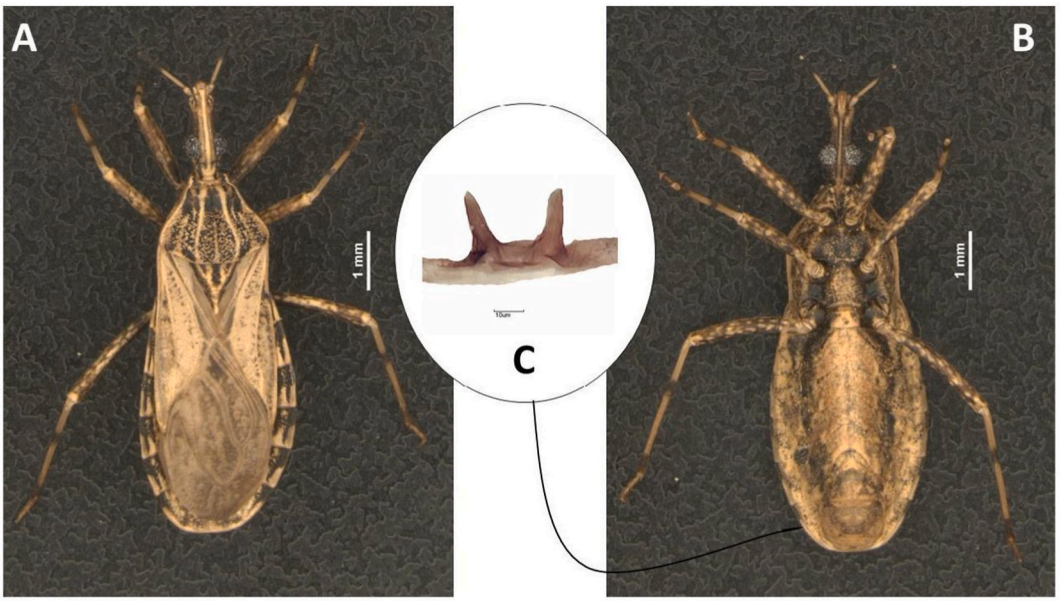

Fig. 2. Male *Rhodnius stali* collected in Guajará-Mirim in 2020, Rondônia, Brazil. A) Dorsal view. B) Ventral view. C) Median process of the pygophore.

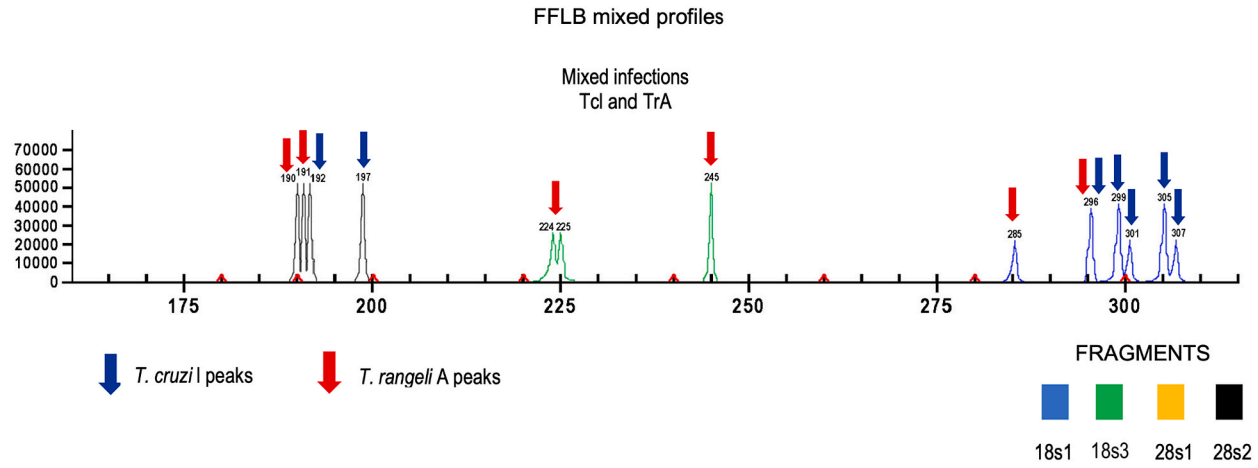

Fig. 3. FFLB profiles obtained with DNA samples from *R. stali*. The image shows different peaks that form the specific profile of each species/genotype. In this case, we have the occurrence of mixed infection of the protozoa *T. cruzi* (TcI) and *T. rangeli* (TrA).

describing the occurrence of *R. stali* and its potential role as vector for Chagas disease in Brazil. In addition, our findings exposed the possibility of trypanosome misidentification in vectors since mixed infections of *T. cruzi* with *T. rangeli* can occur in *R. stali*.

The sites where *R. stali* was found includes coati burrows in the Pantanal of Mato Grosso do Sul, Brazil [31]. In Rio Branco, capital city of Acre, this species was captured using a light trap and by passive search surroundings *Attalea* palm trees [28]. In a region 648 km from the capital of Acre, an *R. stali* adult was collected in an *Attalea butyracea* palm [30]. In Bolivia, *R. stali* was found in *Attalea phalerata* located near infested residences, and also inside human houses and peridomiciliary environment (in a chicken coop), thus indicating a domiciliation process [24,26]. In 2022, an extensive collection was carried out in 6 cities in Bolivia, and *R. stali* was captured in two palm trees, *Copernicia alba* and *Attalea phaletara*[32]. Therefore, natural ecotopes of *R. stali* are possibly palm trees of different genera such as *Attalea*, *Copernicia* and, after this study, *Oenocarpus* sp.

T. cruzi is a complex taxon comprising seven DTUs. The DTUs TcI, TcIII, TcIV, and TcV genotypes of *T. cruzi* were related to oral outbreaks of acute Chagas disease (ACD) in Brazil, Colombia, Venezuela, Bolivia, and French Guiana [33]. In Brazil, there is a predominance of TcI and TcIV in oral transmission in the Amazon region [12,13,20,34,35]. *T. rangeli* is non-pathogenic to humans but is epidemiologically relevant as it can cause false-positive results in xenodiagnosis of triatomines, and microscopical and serological tests used to diagnose *T. cruzi* infection [36,37]. This species is widespread in Brazilian Amazonia, where three (TrA, TrB and TrE) of the five known genotypes are transmitted by *Rhodnius* spp. to a range of wild mammals, domestic dogs, and humans (Maia da Silva et al., 2007; Dario et al., 2021; [20].

In Rondônia, the following triatomines have already been reported: *Eratyrus mucronatus* Stal, 1859, *Panstrongylus geniculatus* (Latreille, 1811), *P. lignarius* (Walker, 1873), *P.megistus* (Burmeister, 1835), *P. rufotuberculatus* (Champion, 1899), *Rhodnius milesi* Carcavallo, Rocha, Galvão and Jurberg, 2001, *R. montenegrensis* [38]; *R. pictipes* Stål, 1872, *R. robustus* Larrousse, 1927 and *Rhodnius stali* Lent, Jurberg & Galvão, 1993 (Massaro; Rezende; Camargo 2008 [15,38]; Galvão; Justi 2015; [39–41]. This is the second report of *R. stali* in southwestern Amazon, after early reports in Acre [28,30]. First reports of *R. stali* in Brazilian territory were from Mato Grosso do Sul[15,42,43].

Our finding of *R. stali* in Rondônia indicates the need for meticulous entomological and epidemiological surveillance in the Brazilian Amazon. Additionally, accurate taxonomic identification, the study of the biology and geographical distribution of vectors and etiological agents of diseases are fundamental to design strategies for the prevention of Chagas disease in the Amazon region.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

André Luiz Rodrigues Menezes: Conceptualization, Formal analysis, Methodology, Validation, Writing – original draft, Writing –

review & editing. **Mariane Albuquerque Lima Ribeiro:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Lucas Nascimento da Cruz:** Data curation, Formal analysis. **Elaine Oliveira Costa de Carvalho:** Data curation, Formal analysis. **Jader de Oliveira:** Conceptualization, Data curation, Supervision, Validation, Writing – original draft, Writing – review & editing. **Marta Maria Geraldes Teixeira:** Data curation, Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing. **João Aristeu da Rosa:** Data curation, Funding acquisition, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

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