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Communication/Comunicação

Natural infection of triatomines (Hemiptera: Reduviidae) by trypanosomatids in two different environments in the municipality of Ouro Preto do Oeste, State of Rondônia, Brazil

Infecção natural de triatomíneos (Hemiptera: Reduviidae) por tripanosomatídeos em dois ambientes distintos no município de Ouro Preto do Oeste, Estado de Rondônia, Brasil

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ABSTRACT

Introduction: This study analyzed the occurrence and the contamination of triatomines by trypanosomatids in *Orbignya speciosa* (babassu) specimens in the State of Rondônia, Brazil, in two different environments (pasture and woods). **Methods:** Capture of triatomines on babassu and microscopic search for trypanosomatids in their digestive tube were carried out. **Results:** Four hundred ninety-four (494) specimens were captured (*Rhodnius prolixus* and *R. robustus*), of which 35.6% of the triatomines were positive for trypanosomatids. **Conclusions:** The high index of natural infection along with the abundance of triatomines points out to the necessity to create an epidemiological surveillance system to monitor vector-borne transmission and deepen the studies on the ecology of such vectors in the Amazon.

Keywords: Triatomines. Chagas' disease. Amazon.

RESUMO

Introdução: O estudo analisou a ocorrência e o índice de contaminação por tripanosomatídeos de triatomíneos em *Orbignya speciosa* no Estado de Rondônia, Brasil, em 2 ambientes (pastagem e floresta). **Métodos:** Captura em palmeiras e esfregaços do conteúdo do tubo digestivo dos triatomíneos analisados por microscopia óptica. **Resultados:** Capturaram-se 494 espécimes (*Rhodnius prolixus* e *R. robustus*), sendo que 35,6% dos triatomíneos estavam positivos para tripanosomatídeos. **Conclusões:** O elevado índice de infecção natural, aliados à abundância de triatomíneos, mostram a necessidade de se criar um sistema de vigilância epidemiológica para monitorar a transmissão vetorial e aprofundar estudos sobre a ecologia destes vetores na Amazônia.

Palavras-chaves: Triatomíneos. Doença de Chagas. Amazônia.

Triatomines, also known as conenose bugs, kissing bugs, or assassin bugs, are insects largely spread throughout the Americas and are found from the South of the United States through the South of Argentina. They are of great importance, as they may convey the South American Trypanosomiasis, also called Chagas' disease¹.

In the State of Rondônia, the occurrence of seven species of triatomines has been reported in four genera, namely: *Rhodnius robustus*, *Rhodnius prolixus*, *Rhodnius pictipes*, *Rhodnius milesi*, *Panstrongylus geniculatus*, *Eratyrus mucronatus*, and *Triatoma rubrovaria*. However, *T. rubrovaria* is probably a taxonomic mistake².

Many studies have pointed out the importance of babassu as a natural biotope, as they shelter a diversity of mammalian fauna such as bats, rats, opossums, and monkeys, besides snakes, birds, scorpions, amphibians, and a great variety of arthropods³. Such environment embodies a combination of shelter and food resources for the growth and procreation of triatomines, specially the species of the genus *Rhodnius*³, whose distribution on the sylvatic environment is strongly related to the distribution of palm trees⁴. On the other hand, the species of the *Triatoma* and *Panstrongylus* genus live rather in association with terrestrial hosts⁵. Over their evolutionary processes, only few species have adapted themselves to households and peridomestic animals⁶.

This study aimed to analyze the occurrence and the contamination of triatomines found in palm trees, such as *Orbignya speciosa* (babassu), by trypanosomatids in two different environments (primary forest and pasture) in the State of Rondônia, Brazil.

The study was carried out in the municipality of Ouro Preto do Oeste (S10°44'53," W62°12'57"), located in the State of Rondônia, whose population is estimated to be at 36,725 inhabitants settled in a 1,970-km² area (population density of 18.6 inhabitant/km²), located at 325km from the state capital, the City of Porto Velho (Figure 1).

The research was carried out in two different environments one in a primary forest and the other in a pasture area. The collection of insects was done twice a month one in each environment within the period from February 2009 to January 2010, totaling 24 collections.

The collections done in a primary forest were carried out in the reserve of *Comissão Executiva do Plano da Lavoura Cacaueira* (CEPLAC) (S10°42'49," W62°13'31"), while the ones carried out in pasture areas were done in 12 farms: C1 — (S10°43'43," W62°14'29"); C2 — (S10°44'10," W62°16'00"); C3 — (S10°43'26," W62°16'51"); C4 — (S10°42'21," W62°17'90"); C5 — (S10°41'43," W62°16'29"); C6 — (S10°41'16," W62°13'34"); C7 — (S10°44'16," W62°12'37"); C8 — (S10°45'33," W62°13'32"); C9 — (S62°11'12," W62°11'12"); C10 — (S62°12'28," W62°12'28"); C11 — (S10°39'38," W62°15'34"); and C12 — (S10°45'26," W62°90'58"), all of them located in the vicinities of the municipality of Ouro Preto do Oeste.

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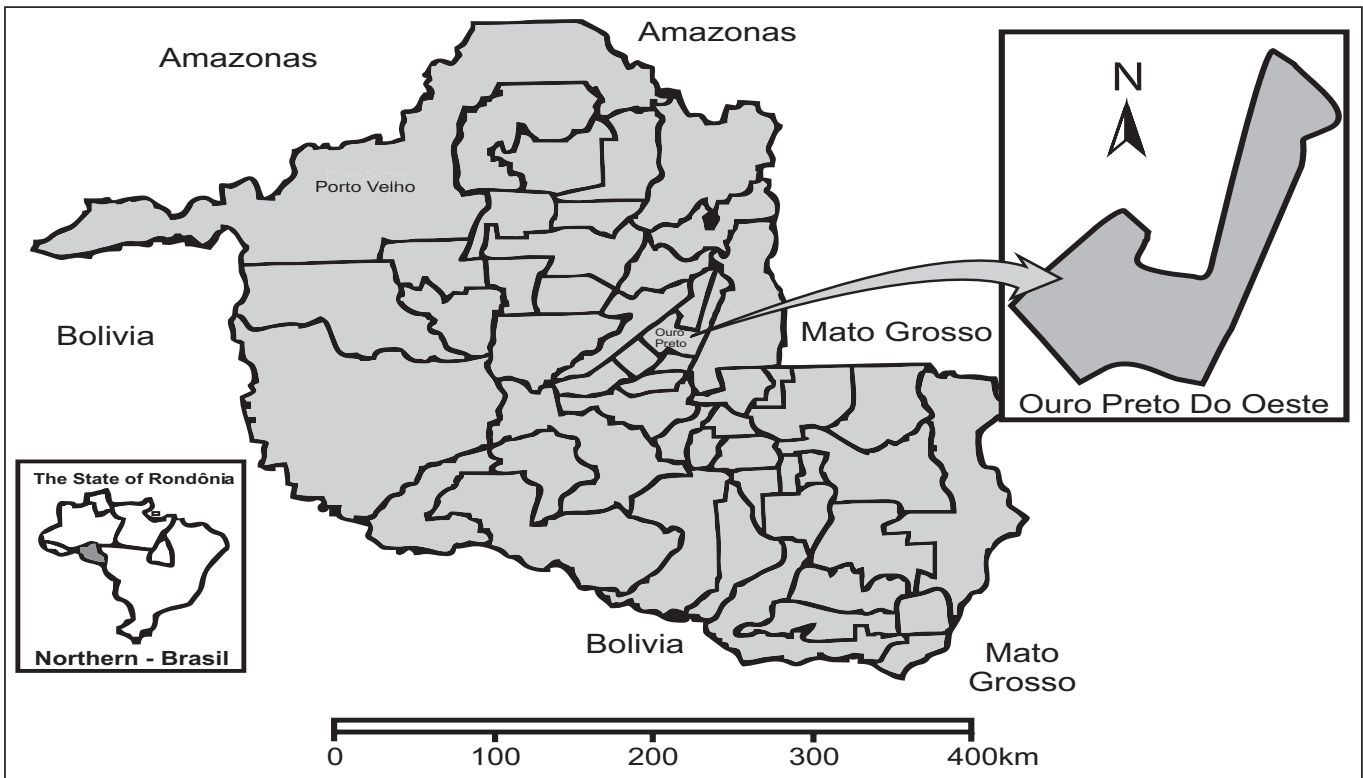


FIGURE 1 - Geographical location of the municipality of Ouro Preto do Oeste, State of Rondônia, Brazil.

In each collection, one babassu was examined (*Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* (IBAMA) permanent license nr. 14934-1). These trees were randomly picked out after sorting 20 palm trees in each area.

The babassu were cut off with a chain saw. Then, the bracts (where a large quantity of invertebrates and small vertebrates can inhabit) were taken off one-by-one in order to facilitate the capture of triatomines (Figure 2).



FIGURE 2 - Active search for triatomines on *Orbignya speciosa* (babassu). A: Cutting off the bracts; B: Triatomine mating during the collection procedure; C: A triatomine being picked up.

The triatomines picked-up were sorted according to their nymphal stage and were then sent to the Centro Universitário Luterano de Ji-Paraná/Universidade Luterana do Brasil (CEULJI/ULBRA) — The Microscopy Laboratory of the Lutheran University Center of Ji-Parana (S10°51'48," W 61° 57'35") — in thermal boxes at room temperature where the digestive tube content was diluted in saline solution, prepared on microscope slides, and then examined with a microscope using a 16x and 40x objective lenses. The microscopic examination was rigorous, covered the whole slide, and was considered positive when flagellated protozoa were found.

Then, the smears were soaked in methanol, stained with Giemsa, and again observed by optical microscopy to confirm their positivity for the presence of trypanosomatids.

After the analysis, the triatomines were sent to the CEPLAC in the *estação experimental* (ESTEX) — Laboratory of Entomology of Ouro Preto do Oeste of the Rondônia (S10°43'33,"W62°14'20") for taxonomic identification according to their external morphological characteristics, by using the dichotomous key of Lent & Wygodzinsky, 19797.

In the 24 examined *babassus*, a total of 494 triatomines were found — 78 in the 12 *babassus* of the primary forest and 416 in the other 12 *babassus* of the pasture area. In both environments, 100% of the babassu picked-out were found to be positive for triatomines (Figure 3).

Overall, a density of 20.6 triatomines was found for each babassu — being 34.7 in the pasture area and 6.5 in the primary forest (Hartley's *t* test = 471.55, *p*<0.0000001).

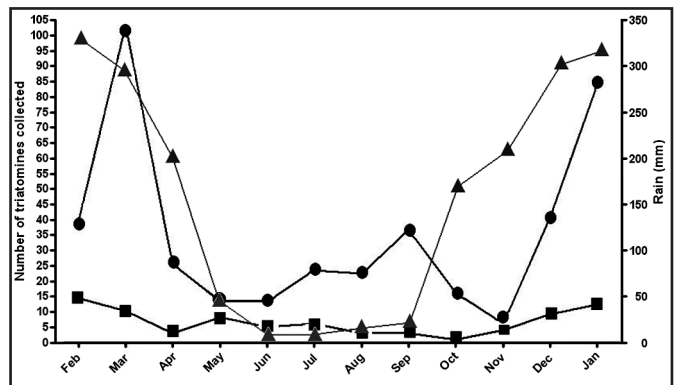


FIGURE 3 - Triatomines captured on *Orbignya speciosas* (babassu) located in a primary forest and pasture area, from February 2009 to January 2010, and the pluvial monthly average precipitation in the municipality of Ouro Preto do Oeste, State of Rondônia, Brazil.

●: pasture area, ■: primary forest, ▲: rain (mm).

In both environments, the period with greater triatomine capture index was from October to March (55.7 triatomines per month), the rainy season, when compared with the period from April to September (22.0 triatomines per month), the dry season (Hartley's f test = 22.0681, $p < 0.0000001$). The triatomines collected belong to the genus *Rhodnius* and are sorted into two species: *Rhodnius robustus* 459 (92.9%) and *Rhodnius prolixus* 35 (7.1%), with 390 (93.8%) *R. robustus* and 26 (6.2%) *R. prolixus* in the pasture area and 69 (88.5%) *R. robustus* and 9 (11.5%) *R. prolixus* in the primary forest, respectively.

The triatomines collected were sorted according to their stages and were analyzed for their positivity for trypanosomatids.

Out of the 494 specimens analyzed, 176 (35.6%) were found to be positive for trypanosomatids (not necessarily *Trypanosoma cruzi*). In the primary forest, the index, although higher (42.6%) when compared with the pasture area (34.1%), showed no statistical difference ($X^2 = 2.560$ and $p = 0.1096$), demonstrating no environmental influence on the results.

The numbers of captured triatomines contaminated by trypanosomatids morphologically resembling *T. cruzi* were: nymph-1 — 18 (0%); nymph-2 — 69 (2%); nymph-3 — 134 (19%); nymph-4 — 103 (44%); nymph-5 — 64 (40%); and adults — 106 (71.7%) ($X^2 = 122.066$ and $p \leq 0.0001$, 5 d.l.), suggesting that the higher the developmental stage of the triatomines, the higher the percentage of infection. In both environments, the highest contamination index was found in the adult stage: 72.2% in the primary forest and 71.6% in the pasture area.

The high (100%) frequency of positive babassus for the presence of triatomines and the high density of 20.6 individuals for each palm tree are above the frequency found in other similar studies: In the City of Uberlândia, State of Minas Gerais (Southwest), the findings were 93.7% of the palm trees, with a density of 15.5 specimens⁸. In the City of Monte Negro (North), Rondônia, the frequency was 85%, with a density of 2.9 specimens⁹. In the federal district, the frequency varied between 1% and 40% and had an average of 3.3 to 9.0 specimens for each palm tree¹⁰.

The highest occurrence of triatomines took place in the pasture area with 416 bugs captured and an average of 34.7 specimens for each babassu, which is different from the number found in the primary forest in which the average was 6.5. Such smaller occurrence of triatomines in the primary forest is probably due to a greater biodiversity of trees, especially other palm trees (*Euterpe oleracea*, *Iriarteia* spp., *Oenocarpus bacaba*, and *Mauritia flexuosa*). Therefore, the vectors have more sheltering options and more food availability on several trees, which did not occur in the pasture area where the sheltering options and food availability are provided only by the *babassu* perfectly adapted to the pasture area. Due to the scarcity of options in this area, they are concentrated in the existing *babassu*.

The months of October and March, the period in which a greater quantity of triatomines were captured, are in conformity with the highest indexes of pluvial precipitation that occur in the municipality of Ouro Preto do Oeste, Rondônia. This is similar to other studies in which the highest capture index took place during the rainy periods³. Some studies on the dynamics of the triatomine populations indicate that its dispersion to form new colonies takes place in the rainy periods in a tropical environment¹⁰.

The prevalence of the *Rhodnius* genus on palm trees is reported in other studies carried out in deforested areas of the municipality of Manaus, state of Amazonas¹¹, and Monte Negro, State of Rondônia⁹.

The species *R. robustus*, that had a higher incidence, has already been found in the peridomestic habitats and habitats infected by *T. cruzi*, which is considered a potential vector for the transmission of Chagas' disease¹². Its occurrence is described in the States of Maranhão, Goiás, Amazonas, Pará^{7,13}, and Rondônia^{2,9}.

Rhodnius prolixus has been reported as having domiciliary transmission, and its domiciliation is taken as a consequence of deforestation, which makes it an important vector for the occurrence of Chagas' disease in Central America¹⁴. Its occurrence is described in the States of Maranhão, Goiás, Pará, Rio de Janeiro^{7,10,13}, Minas Gerais, São Paulo, Pernambuco, Piauí¹¹, and Rondônia^{2,9}.

The two species are distinguished by the following characteristics. *R. robustus* is considered larger species — length of males is 20-23.5 mm and of females is 23-26mm; the anteocular region is about four times as long as the postocular; and the distance between the eyes is dorsally smaller than or equal to the width of the eyes in dorsal view. *R. prolixus* is considered a smaller species — length of males is 17.5-20.0mm and of females is 19.5-21.5mm; the anteocular region is slightly over three times as long as the postocular; and in most specimens, the distance between the eyes is dorsally larger than the width of the eyes in dorsal view⁷.

The total index of 35.6% of positivity for triatomines infected with trypanosomatids is higher than the 23.7% registered in a research carried out in Monte Negro, Rondônia⁹. Nevertheless, the previous study reports the contamination by *T. cruzi* detected by culture, and the present study reports contamination based on the observation of flagellated protozoa using the optical microscopy. It is not possible to determine the species principally because of the common occurrence of *Trypanosoma rangeli* in triatomines of the genus *Rhodnius*. With that, future studies to better characterize the species of trypanosomatids occurring in triatomines in the state of Rondônia are recommended.

The high contamination index in the adult stage is due to the fact that adults have eaten more often, therefore increasing the probability of catching the etiologic agent from the hosts, as in this stage, they have wings and can move from one tree to another or even to the houses, increasing the potential of these insects being vectors of Chagas' disease.

Such data is worrisome, as in the pasture areas, there are plenty of *babassu* near the houses, and they may allow these vectors to infest the habitats and, consequently, carry the etiologic agent, *T. cruzi*, that causes the American Trypanosomiasis.

High natural infection indexes along with the abundance of triatomines show the need to create an epidemiologic surveillance system to monitor the occurrence of cases of vector-borne transmission of Chagas' disease and carry out further studies on the ecology of these vectors in the Amazon.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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