

First report of *Rhodnius montenegrensis* (Hemiptera: Reduviidae: Triatominae) infection by *Trypanosoma rangeli*

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ABSTRACT

Introduction: This study reports for the first time the infection of *Rhodnius montenegrensis* by *Trypanosoma rangeli*. **Methods:** The triatomines were manually collected in *Attalea speciosa* in the municipality of Buritis, Rondônia. The identification of the trypanosomatid species was confirmed by multiplex PCR. **Results:** All of the collected triatomines were *R. montenegrensis*. The analysis confirmed that all of the adults were infected with the epimastigote form of *T. rangeli*. **Conclusions:** This report of a new vector of *T. rangeli* raises a warning for the State of Rondônia because the simultaneous presence of *T. rangeli* with *T. cruzi* in the same geographic region enables the occurrence of mixed infections in hosts and vectors, which complicates the differential diagnosis.

Keywords: Triatominae. *Trypanosoma rangeli*. *Rhodnius montenegrensis*. Western Amazon.

Trypanosoma rangeli is a heterogeneous hemoflagellate protozoan discovered in Venezuela in the intestinal contents of *Rhodnius prolixus* and, in addition to *Trypanosoma cruzi*, is the only trypanosome parasite of man found in Central and South America¹.

Trypanosoma rangeli has been reported in Colombia, Mexico, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Panama, Guyana, Fr Guyana, Uruguay, Paraguay, Peru, Chile, Venezuela, Trinity, Argentina, Ecuador, Bolivia and Brazil^{2,3}. In Brazil, it was found in wild animals and triatomines in the south (Santa Catarina), southeast (Minas Gerais), northeast (Ceará, Alagoas and Bahia), central west (Federal District, Goiás, Mato Grosso and Mato Grosso do Sul) and north (Amazonas, Pará, Rondônia, Acre and Tocantins) regions^{2,4,5}.

From the earliest reports of human infection by *T. rangeli* in the Americas, more than 2,700 cases of human rangeliellosis have been confirmed⁶. The only cases of human infection reported in Brazil have been in the States of Amazonas and Bahia^{7,8}, but in contrast to *T. cruzi*, *T. rangeli* is not considered pathogenic to its vertebrate hosts³.

The transmission of *T. rangeli* to vertebrates is mainly related to species of triatomines of the genus *Rhodnius*, with 12 species reported as their natural vectors; of these 12 species, 6 are found

in Brazil (*Rhodnius neglectus*, *Rhodnius nasutus*, *Rhodnius neivai*, *Rhodnius domesticus*, *Rhodnius pictipes* and *Rhodnius robustus*)^{2,9,10}. The latter species may have been erroneously described in the State of Rondônia by some researchers^{11,12} because of its similarity to *R. montenegrensis*, which had not been described at that time.

Rhodnius montenegrensis was first described in 2012 from specimens collected in the municipality of Monte Negro, Rondônia, Brazil. Initially, it was identified as *R. robustus*, but subsequent studies showed that it was a new species¹³.

The present study reports the first documented infection of *Rhodnius montenegrensis* (Hemiptera: Reduviidae: Triatominae) by *Trypanosoma rangeli*.

Monthly collections were performed with permission from the Brazilian Institute of Environment and Renewable Natural Resources (*Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* - IBAMA), permanent license No. 14934-1 from June to December of 2012 in the rural pasture area in the municipality of Buritis, Rondônia (Latitude 9°57'32.3"S - Longitude 64°08'05.2"W) (**Figure 1**).

Triatomines were collected from 14 specimens of *Attalea speciosa* (babassu) twice a month in the morning after downing a tree with a chainsaw and removing the bracts. Triatomines were manually collected through active searching. The *A. speciosa* were randomly selected, drawing any 1 from a group of 20.

The collected specimens were sent in a cooler at room temperature to the microscopy laboratory at the School of Education and Environment (*Faculdade de Educação e Meio Ambiente* - FAEMA) in the municipality of Ariquemes, Rondônia, where wet mount analysis and smear analysis of

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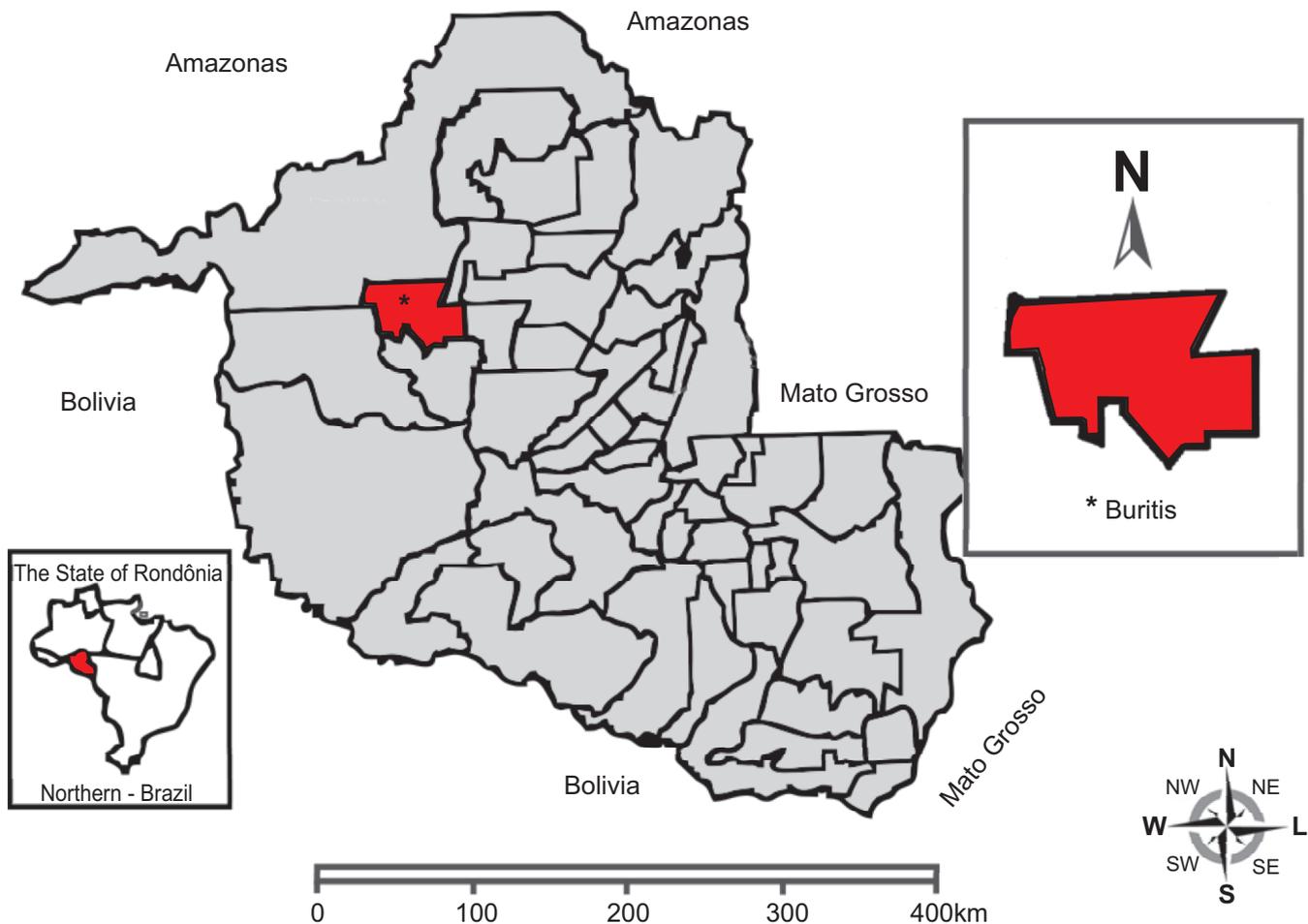


FIGURE 1 - Geographical location of the municipality of Buritis, State of Rondônia, Brazil.

the adult triatomines' rectal contents were performed with an optical microscope at 1600X magnification after staining with triarylmethane (0.1%), xanthene (0.1%) and thiazine (0.1%) (Figure 2).

The identification of the trypanosomatid species was confirmed by multiplex polymerase chain reaction (PCR) in collaboration with the Department of Parasitology at the Institute of Biomedical Sciences, São Paulo University (ICB-USP), São Paulo (SP), Brazil.

Parasite deoxyribonucleic acid (DNA) was extracted from the triatomine rectal samples using a Qiagen DNA extraction kit. The multiplex PCR was performed according to Fernandes et al.¹⁴. This method amplifies a portion of the non-transcribed spacer of the mini-exon gene that varies between *T. cruzi* and *T. rangeli* species and between lines 1 and 2 of *T. cruzi*. The following primers were used: TC1, 5'-ACACTTCTGGCGCTGATCG-3'; TC2, 250bp, 5'-TTGCTCGCACACTCGGCTGCAT-3'; Z3, 150bp, 5'-CCGCGCACAACCCCTATAAAAATG-3'; TR, 100bp, 5'-CCTATTGTGATCCCCATCTTCG-3' and EXON, 5'-TACCAATATAGTACAGAACTG-3'. The reaction mixture consisted of 100pmol of each primer and 150µM dNTPs in a buffer composed of 10mM Tris-HCl (pH 8.3), 1.5mM MgCl₂,

25mM KCl, 0.1mg/ml bovine serum albumin, 2.5U of Taq DNA polymerase and 10ng of genomic DNA in a total volume of 50µL. The thermal cycling conditions were as follows: an initial step of 5min at 95°C, 34 cycles of 30sec at 94°C, 30sec at 55°C and 30sec at 72°C and a final extension of 10min at 72°C. The following reference strains were used as controls in each reaction: TC1, X10 Clone 1; TC2, Strain Y; Z3, Emerald Clone 1 and *T. rangeli* R1625. The amplified products were subjected to electrophoresis on a 2% agarose gel at 100V for 1h. After electrophoresis, the DNA was stained with ethidium bromide and visualized under ultraviolet light. A molecular marker of 50 base pairs was used as a size control for the amplified fragments.

The identification of the adult triatomine species was conducted based on the keys previously described by and Rosa et al.¹².

A total of 120 triatomines were collected, providing an average of 8.6 specimens per *Attalea speciosa*, which is below the average reported by other studies in the state^{9,11}. Of those triatomines, only 13 (10.8%) were adults, and all were *R. montenegrensis*.

The analysis confirmed that all of the adults were infected with trypanosomatids, with the *T. rangeli* in the epimastigote form.

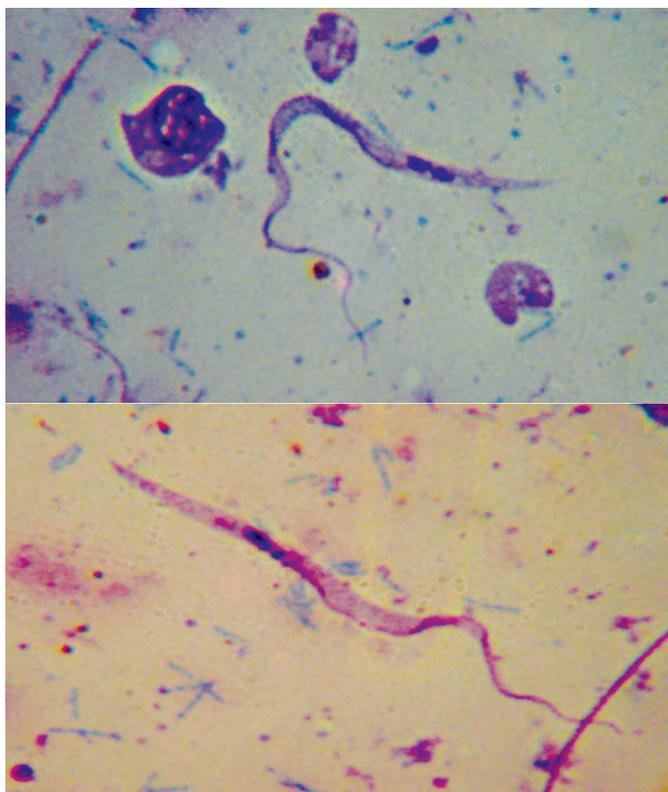


FIGURE 2 - Epimastigote form of *Trypanosoma rangeli* at 1600X magnification.

This work is the first report of the infection of *R. montenegrensis* by *T. rangeli*, which increases the total number of species of triatomines of the genus *Rhodnius* infected by this protozoan to 13, including 7 species found specifically in Brazil.

This report of a new vector of *T. rangeli* presents a warning to the State of Rondônia because the simultaneous presence of *T. rangeli* and *T. cruzi* in the same geographic region enables the occurrence of mixed infections in both vertebrate hosts and vectors, which complicates the differential diagnosis of an infection¹⁵. This possibility is concerning, especially in a state where there are reports of other vector species of *T. rangeli*, such as *R. pictipes* and *R. robustus*^{11,12}.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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