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Short communication

Tamandua tetradactyla Linnaeus, 1758 (Myrmecophagidae) and *Rhodnius robustus* Larrousse, 1927 (Triatominae) infection focus by *Trypanosoma rangeli* Tejera, 1920 (Trypanosomatidae) in *Attalea phalerata* Mart. ex Spreng (Arecaceae) palm tree in the Brazilian Amazon

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ABSTRACT

A sylvatic infection focus of *Trypanosoma rangeli*, whose cycle involves the anteater *Tamandua tetradactyla* and triatomine insect *Rhodnius robustus* was observed in a pasture-dominated landscape of the rural riparian community of São Tomé located along the Tapajós river in the municipal district of Aveiro (State of Pará, Brazil), the Brazilian Amazon region. During a field work campaign with the objective of Chagas disease diagnosis in the Tapajós region, an anteater and 31 triatomines were found inhabiting in the same *Attalea phalerata* palm tree crown. Collected triatomines were identified as *R. robustus* with morphological and molecular procedures. The analysis of infection by *T. rangeli* using the repetitive ARN nucleolar Cl1 (sno-RNA-Cl1) gene showed that 25 triatomines of all stages were infected by *T. rangeli* (total infection rate of 80.6%). Infection by *Trypanosoma cruzi* using mini-exon markers was not identified. Examination of the digestive content of the triatomines demonstrated that the only feeding source found was the anteater. These results demonstrate that *T. tetradactyla* can be an important reservoir for *T. rangeli* and a good vehicle of the parasite within the Brazilian Amazon region.

1. Introduction

The Triatominae (Heteroptera, Reduviidae), vectors of *Trypanosoma cruzi* Chagas, 1909, the ethiological agent of Chagas disease, are also vectors of the parasite *Trypanosoma rangeli* Tejera, 1920, both of the Trypanosomatidae family (Euglenozoa, Kinetoplastidae) and widely distributed throughout Central and South Americas.

T. cruzi and *T. rangeli* have a sympatric distribution in some areas producing mixed infections in vectors or in vertebrates. Moreover *T. rangeli* bears similarities with *T. cruzi* regarding surface antigens, impairing the diagnosis of Chagas disease when serological assays are carried out (D'Alessandro, 1976).

T. rangeli is mainly transmitted via saliva and occasionally by feces but is not pathogenic to vertebrates hosts where it is found in low density (D'Alessandro, 1976). However, in triatomines, *T. rangeli* is a good biological control because it can cause histopathological injuries, which can induce difficulties or even have lethal effects in mouting and feeding (Añez et al., 1987; Watkins, 1971). The species in the genus *Rhodnius* are considered the most important vectors of *T. rangeli*, being capable of transmitting the parasite both under natural and experimental conditions (Guhl and Vallejo, 2003). Unlike *T. cruzi* whose development in triatomines is entirely restricted to the gut, *T. rangeli* multiplies in the gut but completes its development in the insect salivary glands, where metacyclogenesis takes place (Añez and East, 1984; Guhl and Vallejo, 2003).

Like *T. cruzi*, *T. rangeli* also lacks mammalian host specificity and has been reported infecting a variety of mammals such Edentata, Rodentia, Carnivora, Primates and Marsupialia (D'Alessandro and Saraiva, 1992). The first report about the presence of *T. rangeli* in

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Brazil was done by Deane (1958) in *Didelphis marsupialis* in the state of Pará. In the Brazilian Amazon, Miles et al. (1983) found the mammals *D. marsupialis*, *Metachirus nudicaudatus*, *Tamandua tetradactyla* and *Nasua nasua* infected by *T. rangeli*. However, various studies documented the occurrence of *T. rangeli* in different regions of Brazil – including tropical humid and dry forest (Barrett and Oliveira, 1977; Steindel et al., 1991; Diotaiuti et al., 1992; Ramirez et al., 1998; Dias et al., 2007) – especially in on-human primates like tamarins and marmosets (Maia da Silva et al., 2008).

In the present study, we report on *T. rangeli* sylvatic infection involving the vector *Rhodnius robustus* collected in the palm tree *Attalea phalerata* and the Edentata *T. tetradactyla*, in the Tapajós region of the Brazilian Amazon where natural habitats have been modified by humans. On the sampled vectors, we investigated both the molecular identification of the host-feeding sources and trypanosome infection using respectively sequences of mtDNA *cytochrome b* gene and ARN nucleolar Cl1gene (sno-RNA-Cl1) for *T. rangeli* and non-transcribed intergenic region of mini-exon gene for *T. cruzi*.

2. Materials and methods

Triatominae were collected from a palm tree *A. phalerata*, named “uricuri” (S03.98715 W55.55081) in a cattle pasture along the Tapajós River in the community of São Tomé, municipal district of Aveiro, Pará, Brazil. The population of the community near the pasture is composed by 23 families native to the region. Generally, the population lives on self-subsistence, growing corn, rice, beans and manioc, practicing the cultivation after slash and burn. In the surrounding vegetation, *A. phalerata* is constantly associated with *Attalea maripa* (“inajá”) and *Attalea speciosa* (“babaçu”). Although a large part of the vegetation is composed by cleared land and pastures, it is still possible to find fragments of the Amazon forest.

The triatomines sampling and the observation of the anteater *T. tetradactyla* was realized on May 13th 2009, using palm tree dissection according to Diotaiuti and Dias (1984). The anteater was removed and walked to the closest palm tree, climbing to its crown.

The insects collected were transferred to the laboratory. Live insects were fed on hens and dead insects were placed in absolute alcohol. The identification of the triatomines was carried out morphologically according to Lent and Wygodzinsky (1979), and molecularly using *cytochrome b* mtDNA sequences according to Monteiro et al. (2003).

For each triatomines two DNA extractions were performed, first using the legs and thorax of the insects for molecular identification of the triatomines and to confirm infection for *T. rangeli* (DNA A). A second extraction using the digestive tube containing blood meal was performed to identify the host-feeding sources and to confirm infection for *T. cruzi* (DNA B). This procedure was carried out for third instar nymphs to adults. For first and second instar nymphs, the DNA's extraction was performed with the whole insect (DNA AB). The DNA was extracted using DNease® Blood & Tissue Kit (Qiagen, USA).

For the Polymerase Chain Reaction (PCR), we used DNA A and AB for Triatominae identification with CYTB7432F and CYTB7433R primers (Monteiro et al., 2003) amplifying a 510 bp fragment of *cytochrome b* gene and for the molecular characterization of *T. rangeli* with the TrF/TrR2 primers (Pavia et al., 2007) amplifying a 620 bp fragment of the sno-RNA-Cl1 gene. We also tested other primers for the characterization of *T. rangeli*, like TrINT/TrINT-1/TrINT-2 primers for the amplification of the mini-exon gene (Grisard et al., 1999) and S35 and S36 primers that amplifying both kDNA of *T. rangeli* and *T. cruzi* (Sturm et al., 1989).

We used DNA B and AB for PCR targeted vertebrate hosts with the highly conserved primers L14841 (35mer) and H15149 (34mer) designated by Kocher et al. (1989) which amplified a 307 bp segment of *cytochrome b* gene (excluding primers) from a various panel of vertebrates and successfully used by Malmqvist et al. (2003) to identify the host species of blood feeding black flies. Bugs infection by *T. cruzi* was also checked in these DNA samples using a pool of three primers (TC/TC1/TC2) that amplify the non-transcribed intergenic region of *T. cruzi* mini-exon gene (Souto et al., 1996). DNA extracted from an individual of *Rhodnius prolixus* experimentally infected with *T. rangeli* (Choachi strain) and *T. cruzi* (CL strain) was used as positive controls.

For blood meal identification, the PCR reaction was realized in a total volume of 25 μL, including 4-6 μL (20–50 ng) of genomic DNA, 5 μL of 5× Green GoTaq® Flexi Buffer (Promega), 2 mM of $MgCl_2$, 0,2 mM of dNTP, 1 U GoTaq® Flexi DNA Polymerase (Promega) and 10 μM of each primer and were carried out on a thermal cycler Eppendorf Mastercycler EP Gradients. The others PCR reactions were carried out in a total volume of 10 μL, including 1 μL 5× Green GoTaq® Flexi Buffer (Promega), 1,5 mM of $MgCl_2$, 0,2 mM of dNTP, 0.5 U GoTaq® Flexi DNA Polymerase (Promega) 2 μM of each primer and it was carried out on a thermal cycler Biometra® T-1 Termoblock (Germany). The thermal profile for each PCR is found in Table 1. The amplified products were observed in 1% agarose gel, stained with ethidium bromid. PCR products were purified using the Kit Wizard SV Gel and PCR Clean-up System (Promega) and the DNA quantified on Nanodrop 2000c Spectrophotometer (Termo Fisher Scientific Inc.).

For cytochrome b amplicons, sequence reactions were performed using Bigdye® Terminator v3.1 Matrix Standard Kit (Applied Biosystem, UK) and sequence data were obtained using an automatic DNA sequencer (ABI PRISM 3130 Genetic Analyzer sequencer, Applied Biosystem, UK). For the identification of the

Table 1
The thermal profile for each Polymerase Chain Reaction, with the used primers.

Primers	Target	Thermal profile
L14841 and H15149[a]	cyt b mtDNA – blood meal sources	93 °C/1 min, 30 cycles of 93 °C/1 min, 50 °C/1 min, 72 °C/3 min and 72 °C/5 min
S35 and S36[b]	kDNA	95 °C/5 min, 30 cycles of 95 °C/1 min, 60 °C/1 min, 72 °C/1 min and 72 °C/5 min
TrINT/TrINT1/TrINT2[c]	Mini-exon gene *T. rangeli*	94 °C/10 min, 6 cycles of 94 °C/30 s, 45 °C/2 min, 72 °C/30 s, 26 cycles of 94 °C 30 s, 50 °C/1 min, 72 °C/30 s and 50 °C/1 min, 72 °C/5 min
TC/TC1/TC2[d]	Mini-exon gene *T. cruzi*	94 °C/5 min, 61 °C/30 s, 72 °C/30 s, 27 cycles of 94 °C 1 min, 61 °C/30 s, 72 °C/1 min and 94 °C/1 min, 61 °C/30 s, 72 °C/10 min
CYTB7432F and CYTB7433R[e]	Molecular characterization of triatomines	94 °C/3 min, 38 cycles of 94 °C/30 s, 47 °C/1 min, 72 °C/2 min and 72 °C/10 min
TrF and TrR2[f]	sno-RNA-Cl1 gene *T. rangeli*	95 °C/5 min, 15 cycles of 95 °C/30 s, 63 °C/1 min, 72 °C/30 s. 20 cycles of 95 °C/30 s, 61 °C/1 min, 72 °C/30 s and 72 °C 5 min

[a] Kocher et al. (1989).
[b] Sturm et al. (1989) and Vallejo et al. (2002).
[c] Grisard et al. (1999).
[d] Thermal profile modified of Souto et al. (1996).
[e] Monteiro et al. (2003).
[f] Thermal profile modified of Pavia et al. (2007).