

Triatominae (Hemiptera, Reduviidae) in the Pantanal region: association with *Trypanosoma cruzi*, different habitats and vertebrate hosts

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ABSTRACT

Introduction: The transmission cycle of *Trypanosoma cruzi* in the Brazilian Pantanal region has been studied during the last decade. Although considerable knowledge is available regarding the mammalian hosts infected by *T. cruzi* in this wetland, no studies have investigated its vectors in this region. This study aimed to investigate the presence of sylvatic triatomine species in different habitats of the Brazilian Pantanal region and to correlate their presence with the occurrences of vertebrate hosts and *T. cruzi* infection. **Methods:** The fieldwork involved passive search by using light traps and Noireau traps and active search by visual inspection. The light traps were placed at five selected points along forested areas for seven nights during each of the nine excursions. At each point where a light trap was set, eight Noireau traps were placed in palm trees and bromeliads. **Results:** In all, 88 triatomine bugs were collected: two and one individuals from light traps and Noireau traps, respectively; three from peridomestic areas; 23 in coati nests; and 59 in thornbird nests. In this study, active search in microhabitats showed higher efficiency than passive search, since 95% of the triatomine bugs were caught in nests. Further, triatomine bugs were only found to be infected by *T. cruzi* in coati nests. **Conclusions:** Coati nests might act as a point of convergence and dispersion for triatomine bugs and mammal hosts infected by *T. cruzi*, thereby playing an important role in the sylvatic cycle of *T. cruzi* in the Pantanal region.

Keywords: Host-parasite relationship. *Nasua nasua*. *Phacellodomus* sp. Triatominae. Wetlands.

INTRODUCTION

The Triatominae (Hemiptera, Reduviidae) currently include 148 species and 18 genera⁽¹⁾⁽²⁾. These species belonging to this subfamily can act as invertebrate hosts for *Trypanosoma cruzi* (Protozoa, Kinetoplastida, Trypanosomatidae), the important digenetic parasite of Chagas disease (CD). The life cycle of *T. cruzi* is enzootic to a large region extending from Patagonian Argentina to southern Texas; currently, 12 million people are estimated to be infected with this protozoon, and 60 million people live in areas at risk of CD worldwide⁽³⁾.

Triatomine bugs exclusively include blood-sucking insects. This behavior is exhibited at any stage of life and by both the sexes⁽¹⁾. In the absence of vertebrates, triatomines might

feed on the hemolymph of other invertebrates⁽¹⁾. The classical transmission of *T. cruzi* occurs through contamination of feces by epimastigote forms of *T. cruzi* at the time when triatomine bugs are feeding on blood of parasitic hosts⁽¹⁾. *T. cruzi* is also transmitted via the oral route into the sylvatic environment, and this mechanism of transmission has been considered responsible for the recent outbreaks of CD⁽⁴⁾⁽⁵⁾⁽⁶⁾. The remarkable diversity of wild triatomine bugs, along with constant anthropogenic changes in the natural environment, might favor contact between humans and free-roaming bugs, thus leading to *T. cruzi* infection⁽⁷⁾.

Several triatomine bugs maintain enzootic *T. cruzi* in the wild, and the spatial distribution of different triatomine bugs has been shown to be correlated with the availability of food resources and shelter in the natural environment⁽¹⁾. That is, *Cavernicola pilosa* are mainly found in caves that harbor bats, and *Panstrongylus geniculatus* are found in areas with armadillos and in their underground shelters. The southern tamandua (*Tamandua tetradactyla*) has been reported to be infected with *T. cruzi* owing to its close association with *Rhodnius robustus* in the Brazilian Amazon region⁽⁸⁾⁽⁹⁾. Moreover, several triatomine species such as *Rhodnius neglectus*, *Triatoma plantensis*, *T. sordida*, *Psammolestes arthuri*, *Psammolestes coreodes* and

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Psammolestes tertius are found in bird nests, although birds are refractory to infection by *T. cruzi*^{(1) (10) (11)}.

Trypanosoma cruzi is a multi-host parasite and infects mammals belonging to different orders such Chiroptera, Primates, Rodentia, Artiodactyla, Marsupialia, Cingulata, Pilosa, and Carnivora^{(9) (11) (12)}. The enzootic cycle of *T. cruzi* in relation to mammalian hosts in the Pantanal region has been extensively investigated over the last decade. In fact, *T. cruzi* infection in this wetland has been reported in small mammals (rodents and marsupials), carnivores (coatis, crab-eating fox, crab-eating raccoon and ocelot), bats, armadillos, peccaries and feral pigs^{(12) (13) (14) (15) (16) (17)}.

Although described as a single species, *T. cruzi* population is known to be genetically diverse. At present, there is a consensus that *T. cruzi* strains encompass six discrete typing units (DTUs): *Trypanosoma cruzi* I (TcI) to *Trypanosoma cruzi* VI (TcVI). Furthermore, the new TcBat genotype was recently described, which is restricted to bats^{(18) (19)}. Moreover, Lima et al.⁽²⁰⁾ found wide genetic variation among TcI isolates in the different biomes of Brazil. Thus, the sylvatic cycles of *T. cruzi* can be considered to be unpredictable and multivariable complex systems that varyingly interact with different host species (mammals and triatomine bugs), leading to the genetic plasticity of *T. cruzi*. In the Pantanal region, coatis were found to be naturally infected with several subpopulations of *T. cruzi*⁽¹⁵⁾.

Although not many species are endemic to the Pantanal region, compared with the species abundance at Atlantic Forest and Brazilian Cerrado, the biodiversity of Pantanal region is considerably high⁽²¹⁾. In this biome, coatis inhabit various habitats, from the ground to treetops, connecting different *T. cruzi* cycles⁽¹⁶⁾. At present, knowledge regarding the diversity of triatomine bugs in the Pantanal wetland is lacking. Different bug species might be acting within different *T. cruzi* transmission cycles, which may or may not be connected.

This study aimed to investigate the presence of sylvatic triatomine bugs in different habitats and to correlate their presence with vertebrate hosts of *T. cruzi*. Further, we determined the parasitological prevalence of *T. cruzi* infection in invertebrate hosts in the Brazilian Pantanal region.

METHODS

Study area

The Pantanal is a large Neotropical wetland located in the center of South America. The climate is marked by two distinct seasons: a warm rainy period (October to March) and a cold dry period (April to September).

The fieldwork was conducted in the Southern Pantanal region, an area that has been described as having active *T. cruzi* transmission cycles in the last decade^{(12) (13) (14) (15) (16) (17)}. The investigation sites included (a) Nhumirim Ranch (NHUR), 18°59'17.07"S 56°37'8.65"W, which is a research station belonging to Embrapa Pantanal, along with neighboring farms with extensive cattle raising; and (b) sections of the MS-184 (67km) and MS-337

(30km) Park Roads (PRs). The phytophysiognomy of NHUR is characterized by (a) small elevations of continuous terrain covered by dense forested areas typical of the *Cerrado* biome, locally known as *cordilheiras*; (b) open savanna consisting of shrubs and scattered trees flooded seasonally; and (c) seasonally flooded grasslands. The PRs are dirt roads constructed on causeways 1-2m above the surrounding plains or at ground level in the high areas of floodplains. The terrain through which these highways pass is directly influenced by the rivers and tributaries that surround them: MS-184 by the Miranda and Abobral Rivers and MS-337 by the Negro River. The vegetation alongside these PRs comprises isolated trees and scrub vegetation.

Collection of triatomines

We collected data monthly between April and December 2013 through active and passive searches (light and Noireau traps). All field procedures and laboratory studies were conducted in accordance with a license granted by the Biodiversity Information and Authorization System of the Chico Mendes Institute for Biodiversity Conservation, under license number 37690-4. The present study was approved by the Ethics Committee for Animal Use of Dom Bosco Catholic University, Campo Grande, MS (under license number 12/2012).

Although small mammals have been described as hosts for *T. cruzi* in the studied area^{(13) (16)}, their nests are difficult to detect due to the lack of knowledge of their biology. Moreover, nests of small mammals are very small and found in burrow tunnels in the ground, natural cavities in trees, and high in trees out of leaves^{(22) (23)}. Thus, we used light traps to catch adult triatomines that use these habitats. The traps were divided into three compartments: (a) a light source consisting of a lamp with a light-emitting diode (LED) light bulb connected to a 12V battery; (b) a flap to intercept the flight of insects attracted by the light; and (c) a container for storing the insects. The light traps were set between 6:00pm and 6:00am on nights with a new moon. Two light traps were placed at each of the five selected sites along the *cordilheiras* in NHUR for seven nights, during each of the nine excursions.

At each point where a light trap was set, eight Noireau traps were placed in the following microhabitats: palm trees (*Attalea phalerata*) and bromeliads (*Bromelia balansae*). These traps followed the model proposed by Noireau et al.⁽²⁴⁾. For each trap, one mouse was used as live bait. Sawdust and food were placed together with the mouse on each bug-catching night. In all, 40 Noireau traps were used on each night, placed in the late afternoon, and left there between 6:00pm and 6:00am.

We also searched for triatomines by using active searches in the peridomestic areas (around human dwellings, especially animal enclosures) and in arboreal nests that have been described as habitats of triatomine bugs^{(1) (2)}: thornbird nests (*Phacellodomus* sp., Furnariidae, Passeriformes) along the PR (**Figure 1**) and nests of coatis (*Nasua nasua*, Procionidae, Carnivora) in the *cordilheiras* (**Figure 2**). Nests of both the species were carefully placed in plastic bags, identified, and transported to the laboratory for detecting triatomines.



FIGURE 1 - Nests of thornbirds (*Phacellodomus* sp., Furnariidae, Passeriformes) collected in the Southern Pantanal region of Brazil for searching for triatomines. Corumbá, State of Mato Grosso do Sul.

Identification of the triatomines collected

All triatomines collected were stored in 50-mL Falcon tubes that were identified with the capture location and date. The triatomines were classified according to their external morphology by Dr. José Jurberg of the National and International Reference Laboratory for Triatomine Taxonomy, Oswaldo Cruz Institute [Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, RJ], by referring to the dichotomous key developed by Lent and Wygodzinsky⁽¹⁾. Since the accurate morphological identification to species level is possible only with adult specimens, the immature forms collected were classified only until genus.

Identification of *Trypanosoma cruzi*

Parasitological tests for *T. cruzi* infection were performed at the Trypanosomatid Biology Laboratory, Oswaldo Cruz Institute, Rio de Janeiro. The intestines of live insects were examined to determine the presence of parasites. The intestinal contents were macerated with two drops of 0.85% saline solution containing antimycotics/antibiotics. A sample of the final product was then examined under an optical microscope to detect *T. cruzi* (fresh examination).



FIGURE 2 - Nests of coati (*Nasua nasua*) collected from the Southern Pantanal region of Brazil for searching for triatomines. Corumbá, State of Mato Grosso do Sul.

RESULTS

The capture methods used and the number of evolution stages among the triatomines collected (total, 88 specimens) are shown in **Table 1**. The active searches showed greater efficiency than passive searches: while only three triatomine specimens were collected using light traps ($n = 2$) and Noireau trap ($n = 1$), 85 triatomine bugs were collected by active searches.

We found that 10% (3/31) of coati nests inspected were colonized by triatomine bugs (one *T. sordida*, one *Rhodnius* sp., and 21 *Triatoma* sp.). The prevalence of *T. cruzi* in the triatomine bugs collected from a single coati nest was 70% (16/23).

Further, 46% (11/24) of thornbird nests were colonized by triatomines. All the triatomine bugs collected were identified as *Psammolestes coreodes* ($n = 59$) on the basis of morphological features. The immature forms found in the thornbird nests would have been *P. coreodes* since all these individuals were collected along with *P. coreodes* adults. No infection by *T. cruzi* was observed in any of the *P. coreodes* analyzed, although small mammals, host for *T. cruzi* in the region^{(13) (16)}, might inhabit bird nests⁽²⁵⁾. During the active searches around dwellings, we collected triatomines from a stable (one specimen of *T. sordida* and one of *Triatoma* sp.) and a henhouse (one of *T. sordida*).

DISCUSSION

Our results showed that active searching in microhabitats, such as arboreal nests of birds and coatis, has a high degree of capture success, since this method allowed capturing 95% of the triatomine bugs. Active searching is an efficient method for collecting wild triatomines and, when the local community is evolved (which was not noted in this study), this methodology becomes even more efficient⁽²⁶⁾.

TABLE 1 - Species of Triatomine bugs captured with reference to different methods, evolutive stages, infection by *Trypanosoma cruzi*, capture effort, and capture success in the Brazilian Pantanal.

Capture methods	Triatomine species	Evolutive stage	Triatomines infected by <i>T. cruzi</i>	Capture effort	Capture success
Light trap	1 <i>Rhodnius stali</i>	adult	0	7,560h	0.03 ^a
	1 <i>Triatoma sordida</i>	adult	0		
Noireau trap	1 <i>Triatoma</i> sp.	4 th stage nymph	0	26,400h	0.004 ^a
Peridomestic area	1 <i>Triatoma sordida</i>	adult	0	74 men	4.1 ^a
	1 <i>Triatoma sordida</i>	adult	0		
	1 <i>Triatoma</i> sp.	5 th stage nymph	0		
Coati nest	1 <i>Triatoma sordida</i>	adult	0	31 nests	0.74 ^b
	1 <i>Rhodnius</i> sp.	4 th stage nymph	0		
		one 3 rd stage nymph	16		
		one 4 th stage nymph			
	21 <i>Triatoma</i> sp.	nineteen 5 th stage nymphs			
Thornbird nest	59 <i>Psammolestes coreodes</i>	one 1 st stage nymph	0	24 nests	2.46 ^b
		eight 2 nd stage nymphs	0		
		ten 3 rd stage nymphs	0		
		three 4 th stage nymphs	0		
		three 5 th stage nymphs	0		
		34 adults	0		

T.: *Trypanosoma*; ^aTriatomines/100h; ^bTriatomines/nest.

The low capture success rate by using Noireau traps in this study might have been due to the wide availability of food sources for triatomine bugs in the area studied⁽²¹⁾. Studies using Noireau traps for catching triatomines in biomes such as the *Caatinga* and *Cerrado* have yielded better results⁽¹⁰⁾⁽²⁷⁾, probably because of the low abundance of food sources in these two biomes. In fact, as mentioned by Noireau et al.⁽²⁴⁾, the capture success rate of their traps was related to the quantity of food sources and number of triatomine specimens in the ecosystem sampled.

Although many species of insects (Coleoptera, Orthoptera and Hymenoptera) are known to be attracted to light traps (personal communications), the capture success for triatomines was low by using this method. We utilized LED light bulbs because they have a longer lifespan and better electrical efficiency than incandescent bulbs; however, they do not produce heat, which is one of the main ways to attract triatomine bugs⁽²⁸⁾. Similar low capture success rates were reported for the municipality of Diamantina, in southeastern Brazil, where only four specimens of triatomine bugs were caught using light traps with LED light bulbs over a one-year period⁽²⁹⁾.

The *Triatoma* spp. and *Rhodnius* spp. collected from coati nests have been described as ubiquitous sylvatic triatomine species. These triatomine bugs species are found in various natural ecotopes in the Neotropical region, from the leaves of palms, bromeliads, birds' nests, and the burrow of mammals⁽¹⁾⁽⁴⁾⁽⁹⁾, and feed on different hosts species⁽¹⁾. In contrast, *Psammolestes coreodes* found in thornbird nests has been described in close association with *Phacellodomus* sp., which is distributed throughout the

Southeastern region of Bolivia and Southwestern Brazil, Paraguay, and Argentina.

Nests of *Phacellodomus* are excellent habitats for *P. coreodes* in the southern Pantanal region, since almost half of the nests sampled were found to be colonized by triatomine species. Like other species of the genus *Psammolestes*, *P. coreodes* has strong ecological interactions with thornbird nests, especially *Phacellodomus* sp.⁽³⁰⁾⁽³¹⁾. This ecological association is directly linked to the biological characteristics of the birds' nesting habits: (a) large nests, with on average two to three incubation chambers for chicks; (b) both sexes incubate the eggs and rear the nestlings; (c) formation of territories with up to ten individuals that use the nests throughout the year for sleeping, incubating their eggs, and rearing their offspring; and (d) continuous presence of newborn young birds in the territory for up to 16 months, in order to help in feeding other chicks and to use the nests again for the subsequent breeding season⁽³²⁾.

The presence of a greater number of adults (58%) of *P. coreodes* found in our study is consistent with the findings of Bar et al.⁽³³⁾, who observed that 63% of the individuals present in the bird nests were adults. Likewise, Gurgel-Gonçalves and Cuba⁽³¹⁾ reported that 48% of the individuals in colonies of *P. tertius* were adults. In fact, colonies of *Psammolestes* sp. differ markedly from other triatomine genera because the immature stages are usually more prevalent in the colonies than adults, which generally account for less than 5%⁽³⁴⁾⁽³⁵⁾. Although little information is available on the ecological aspects of *P. coreodes*, the higher proportion of triatomine adults in thornbird nests can be explained by the greater longevity and lower dispersal of

adult bugs from the nests⁽³⁰⁾. In addition, the long-term continual presence of *Phacellodomus* sp. in the nests, together with the presence of a great variety of invertebrates, would provide food supply for adult triatomines throughout the year⁽³⁶⁾.

Besides birds, other taxa might inhabit or periodically use bird nests, and thus might serve as a food source for *Psammolestes* sp.^{(10) (37)}. We found one specimen of *Oecomys mamorae* in one of the thornbird nests collected. Since this rodent species is arboreal and has nocturnal habits, it might be using the bird nest as a dormitory or for reproduction. Rubio and Pinho⁽²⁵⁾ reported that *O. mamorae* use Furnariidae nests for shelter and nesting in northern Pantanal region, the municipality of Poconé.

Although natural *T. cruzi* infection in *Psammolestes* spp. has not been reported, experimental studies have shown that *P. coreodes* and *P. tertius* might become infected with *T. cruzi*^{(1) (38)}. Additionally, sylvatic *Triatoma platensis* inhabiting the nests of *Phacellodomus sibilatrix* were found to be naturally infected by *T. cruzi* in a typical area of the Chaco region of Argentina⁽³⁹⁾. Given that natural infection by *T. cruzi* in free-living small mammals is well documented in the Pantanal region^{(13) (16) (40)}, the presence of *O. mamorae* in thornbird nests might constitute a risk factor for *P. coreodes* infection by *T. cruzi*.

Our results showed that coati nests are colonized by triatomines of the genera *Triatoma* and *Rhodnius*. Coati nests serve as a shelter for various vertebrate and invertebrate animals⁽⁴¹⁾, which form sources of nourishment for triatomine bugs. Lima⁽⁴²⁾ reported that triatomine bugs were found in 30% (7/23) of coati nests sampled, which was higher than that found in our study. This suggests that nest infestation with triatomine bugs might differ even within the same landscape. In our case, the result might have been influenced by particular features of the areas sampled: Lima⁽⁴²⁾ collected samples only from the Nhumirim Ranch, which is an area with little livestock pressure⁽⁴³⁾, whereas our study was performed on traditional cattle-raising farms, with large densities of bovines⁽⁴⁴⁾. The aggregated distribution pattern of sylvatic triatomine populations has already been reported by Suarez-Davalos et al.⁽⁴⁵⁾.

Moreover, our data showed that 70% (16/23) of the triatomine bugs collected were infected by *T. cruzi*, similar to that reported by Alves⁽⁴⁶⁾ (66%; 16/23), who used the same triatomine bugs collected by Lima⁽⁴²⁾. In this regard, coati nests would act as an important link in the complex cycle of *T. cruzi* transmission in the Pantanal region, because coatis have high levels of *T. cruzi* prevalence and parasitemias⁽¹⁵⁾ and are found together with triatomine bugs infected by *T. cruzi* in their nests. When coatis are generalists regarding their diet⁽⁴⁷⁾, they become infected by *T. cruzi* by eating infected triatomine bugs, regardless of the classical vector transmission routes. Furthermore, since trans-ovarian transmission of *T. cruzi* has not been described for triatomines⁽¹⁾, the immature stages of triatomine bugs present in the coati nests would become infected because of feeding on coatis infected with *T. cruzi* per occasion of nocturnal rest in nests⁽⁴⁸⁾. Thus, after their development, adult triatomine bugs infected by *T. cruzi* might fly and colonize others ecological niches.

The life cycle of *T. cruzi* in the Pantanal wetland is complex and has a dynamic profile, because (a) *T. cruzi* infection has been described in a broad range of wild mammal species^{(12) (14) (15)}, (b) some wild triatomine bug species are found in different ecological niches, (c) different subpopulations of *T. cruzi* circulate in the study area⁽¹⁵⁾, and (d) constant environmental changes through deforestation occur to implement intensive cattle farming.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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