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Infestation of *Mauritia flexuosa* palms by triatomines (Hemiptera: Reduviidae), vectors of *Trypanosoma cruzi* and *Trypanosoma rangeli* in the Brazilian savanna

Rodrigo Gurgel-Gonçalves[a,*], Carolina Cura[b], Alejandro G. Schijman[b], César A. Cuba Cuba[a]

[a] Laboratório de Parasitologia Médica e Biologia de Vetores, Faculdade de Medicina, Universidade de Brasília, Brasília, Distrito Federal, Brazil
[b] Laboratorio de Biología Molecular de la Enfermedad de Chagas, INGEBI-CONICET, Buenos Aires, Argentina

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ABSTRACT

To determine the infestation and trypanosome infection of triatomines captured in *Mauritia flexuosa* palm trees across its geographic distribution in the Brazilian savanna (*Cerrado*), we sampled 42 localities in eight states and in the Federal District, Brazil, between July 2005 and January 2010. Overall, 2154 specimens of the species *Rhodnius neglectus*, *Psammolestes tertius*, *Triatoma sordida*, and *Microtriatoma borbai*, were collected. Among the 341 palms sampled, 182 (53.3%) were infested with *R. neglectus*, which resulted in the capture of 1639 specimens (9.0 insects per infested palm). *P. tertius* occurred in 26 palms (8%), which resulted in the capture of 484 specimens (19 insects per infested palm). *T. sordida* ($n = 30$) and *M. borbai* ($n = 1$) occurred in only one location. From 537 *R. neglectus* examined, 44 were infected (8%) with *Trypanosoma rangeli* and/or *Trypanosoma cruzi* (Tc Id). *M. flexuosa* was previously recognized as a suitable breeding ecotope for *R. neglectus* in the Brazilian states of Minas Gerais, Goiás, Tocantins and the Federal District. Our results expand this distribution to other states (São Paulo, Bahia, Mato Grosso, Maranhão and Piauí), and also show that this particular palm tree harbors other triatomine species. Finally, we show that *R. neglectus* plays an important role in maintaining the enzootic circulation of *T. cruzi* and *T. rangeli* in the Brazilian savanna.

1. Introduction

The widespread occurrence of sylvatic triatomine species that sporadically invade or reinvade human dwellings is a major difficulty for the consolidation of Chagas disease vector control (Miles et al., 2003; Fitzpatrick et al., 2008; Guhl et al., 2009). The ecology and behavior of such species must be studied in their natural setting so that the domiciliation process may be better understood, enabling the development of new strategies for their surveillance.

Palm trees play a major role as breeding and foraging habitats for sylvatic triatomines, especially for the arboricolous (tree-living) *Rhodnius* species. Studies in different areas of Brazil have shown triatomine infestation rates in palm trees (Barretto et al., 1969; Miles et al., 1983a; Diotaiuti and Dias, 1984; Pinto and Bento, 1986; Teixeira et al., 2001; Gurgel-Gonçalves et al., 2004a; Sarquis et al., 2004; Dias et al., 2008; Abad-Franch et al., 2009, 2010). The wide distribution of palms trees, their morphological variability, which favors the maintenance of vertebrates and nesting bugs, and their various uses by humans, contribute to value the importance of this group in the context of American Trypanosomiasis. In this sense, the palm trees have been considered as ecological indicators of risk areas, because they are useful in identifying the presence of vectors and hosts of *Trypanosoma cruzi* and *Trypanosoma rangeli* (Miles et al., 1983b; Romaña et al., 1999; Gurgel-Gonçalves et al., 2004b; Dias et al., 2008; Lima et al., 2008; Abad-Franch et al., 2010; Justi et al., 2010).

Mauritia flexuosa is widespread in Brazil, where it is used in both traditional crafts and industry (Lorenzi et al., 2004). Although it is known that *Rhodnius neglectus* often infests *M. flexuosa* in *Cerrado*, the Brazilian savanna (Barretto et al., 1969; Mello, 1982; Gurgel-Gonçalves et al., 2003, 2004a; Gurgel-Gonçalves and Cuba, 2007, 2009; Abad-Franch et al., 2009), previous studies have not covered the whole distribution area of *M. flexuosa* in that biome. The present study aims to determine the infestation and trypanosome infection of triatomines captured in *M. flexuosa* palm trees across its geographic distribution in the Brazilian savanna. This will help expand the knowledge of triatomine bugs from *Cerrado*, thus supporting strategies for surveillance and control of Chagas disease vectors in Brazil.

* Corresponding author at: Laboratório de Parasitologia Médica e Biologia de Vetores, Área de Patologia, Faculdade de Medicina, Universidade de Brasília, Campus Universitário Darcy Ribeiro, Asa Norte, CEP: 70904-970, Caixa Postal: 4569, Brasília, Distrito Federal, Brazil. Fax: +55 61 3273 3907.
E-mail addresses: rgurgel@unb.br, gurgelrg@hotmail.com (R. Gurgel-Gonçalves).

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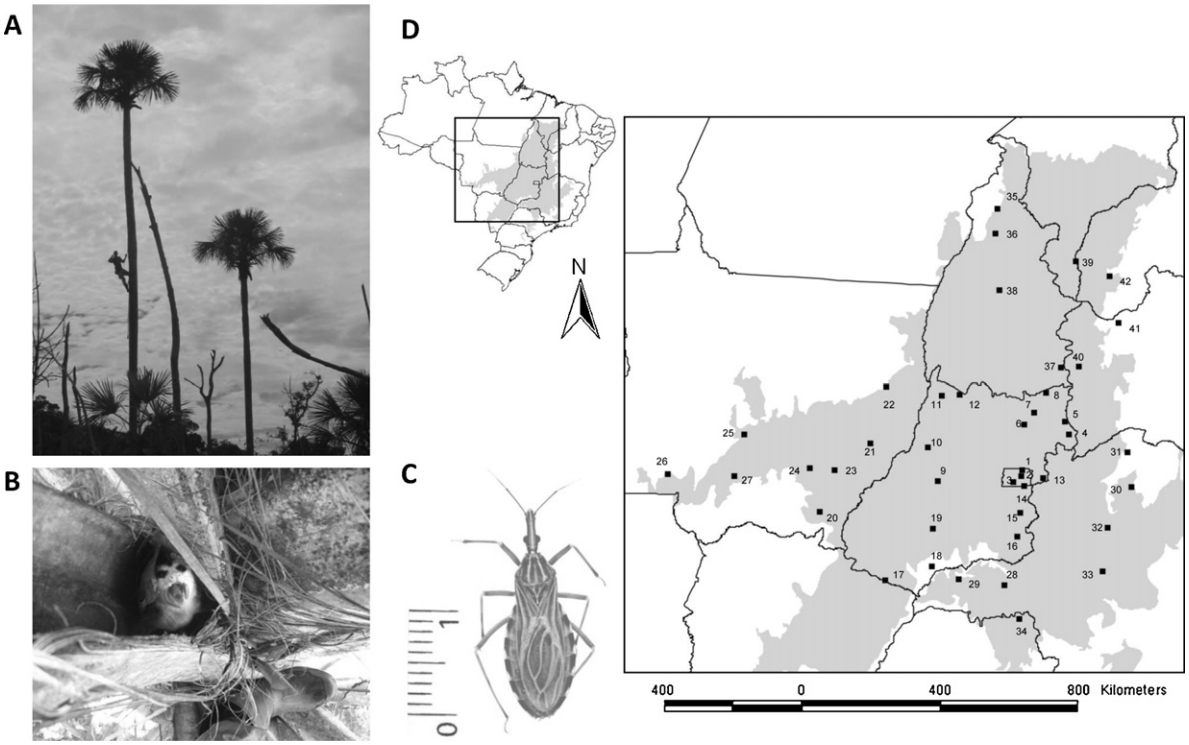

Fig. 1. (A) Capture of triatomine bugs in the *Mauritia flexuosa* palm tree. (B) *Didelphis albiventris*, marsupial frequently found in the palm tree crowns, and probable source of infection of *Trypanosoma cruzi* and *T. rangeli* for *Rhodnius neglectus* (C). (D) Study areas. The lines represent the limits of Brazilian states. The grey area represents the Brazilian savanna (*Cerrado*). The numbers represent the localities described in Table 1. Localities 1–3 and 14 were sampled in July 2005; 4–8, 13, 15, 16, 28–38, 40 and 41 in January 2006; 9–12, 17–27 in January 2007; 39 and 42 in January 2010.

2. Materials and methods

2.1. Study areas

Between July 2005 and January 2010, 341 palm trees were sampled in 42 localities from eight Brazilian states (Goiás, Mato Grosso, Minas Gerais, Tocantins, Bahia, Piauí, Maranhão and São Paulo) and in the Federal District (Fig. 1). The sampling covered almost the entire distribution of the Brazilian savanna (*Cerrado*), the second largest biome in Brazil, occupying approximately 25% of the national territory (Klink and Machado, 2005). The climate is tropical savanna, with two definite seasons: the rainy season (October–April) and the dry season (May–September), when the relative humidity may drop to less than 20%. Forests, savanna and grassland are found in the biome, which presents eleven major vegetation types (Ribeiro and Walter, 1998). Insect sampling was carried out in permanent wet grasslands with populations of *M. flexuosa* palm trees, known as "buriti" (Ribeiro and Walter, 1998). This vegetation type has an important role in the maintenance of water resources and is currently regarded as a permanent protection area by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA).

2.2. Palm tree sampling and triatomine collection

Triatomines were manually collected following standard methodology (Gurgel-Gonçalves et al., 2003). In each area the search was made in those palm trees that showed evidence of vertebrate colonization, especially birds, which would provide a feeding source for the insects. Ladders and ropes were used to reach the palm tree crowns, from where organic matter, vegetation and abandoned nests were collected and placed into plastic bags. This material was then lowered to the ground and placed over a white cloth to facilitate triatomine detection. Additionally, we performed an active search of bugs between the bases of the leaves with a tweezer. The collection of insects was done with prior licensing from IBAMA. The insects were separated by sex and nymphal stage, identified using keys of Lent and Wygodzinsky (1979) and examined for infection with trypanosomes.

2.3. Identification of T. cruzi and T. rangeli

The phenotypic identification of the parasites was made by observation under a microscope of Giemsa-stained insect feces. Parasite DNA was extracted from abdomen samples and feces/urine from infected bugs, and from parasite culture stocks (blood agar). Feces and culture samples impregnated on filter paper were processed as reported in Machado et al. (2000), with modifications. Briefly, 200 μl bi-distilled water was added to round filter paper pieces and boiled in eppendorf microtubes for 15 min. After cooling at room temperature, they were centrifuged at 13,000 rpm for 5 min, and the appropriate volume of the supernatant was directly applied to the PCR reaction. Abdomen samples were processed using the High Pure PCR Template Preparation Kit (Roche, Germany) following the protocol recommended for mammalian tissue with 24-h lysis (Pizarro et al., 2007).

Subsequently, PCR was performed for molecular identification of kinetoplastid DNA (kDNA) of *T. cruzi* as described in Burgos et al. (2007). A panel of PCR positive samples was further characterized to identify the discrete typing units (DTUs, Zingales et al., 2009) by means of PCR strategies targeted to the intergenic region of mini-exon genes (SL-IR) as reported (Marcet et al., 2006). When DTU I was detected, the SL-IR 475 bp amplicons were purified and sequenced, in order to identify Tc I SL-IR genotypes as described in Cura et al. (2010). Differential detection of *T. rangeli* and *T. cruzi* was performed by a PCR strategy targeted to the D7 domain of 24sα ribosomal DNA (rDNA) with primers D75 and D76, and confirmed by sequencing, according to Schijman et al. (2006).

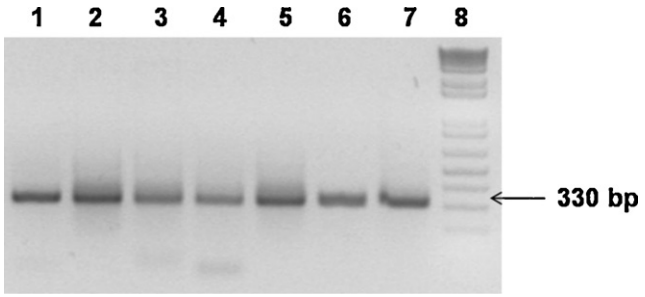

Fig. 2. kDNA PCR results. Lanes 1–5: fecal samples from *Rhodnius neglectus* taken on filter paper (1–5: Monte Alegre do Piauí-PI). Lanes 6 and 7: amplification products of 5 and 50 fg, respectively, of *T. cruzi* strain CL-Brener DNA. Lane 8: 1 kb DNA ladder.

2.4. Entomological indices

For each locality we calculated the following entomological indices: (i) number of palm trees infested/sampled, (ii) number of bugs per infested palm, (iii) number of adults/nymphs, (iv) number of males/females and (v) number of insects infected/examined.

3. Results

A total of 2154 specimens of *R. neglectus*, *Psammolestes tertius*, *Triatoma sordida* and *Microtriatoma borbai*, were collected. Among the 341 palm trees sampled, 182 were infested with *R. neglectus* (53.3%). We collected 1639 specimens of *R. neglectus*, with an average of 9.0 insects per infested palm (ranging from 1 to 124 insects). We detected a high percentage of nymphs' specimens of *R. neglectus* (70% of all specimens captured). We captured more nymphs than adults (ratio adult/nymph <1) in 37 localities. Among adults, a higher proportion of males were observed in most populations (ratio male/female >1, Table 1).

From 537 *R. neglectus* examined, 44 were infected (8%) with *T. cruzi* and/or *T. rangeli* (Table 1). Localities that had the highest rates of trypanosome infection were Monte Alegre do Piauí (state of Piauí), Alto Paraíso (state of Goiás), Araguaina (state of Tocantins), Ribeirão Cascalheira (state of Mato Grosso) and Ituiutaba (state of Minas Gerais). kDNA-PCR positive results were obtained in abdomen and filter paper samples from bugs captured in nine localities. As an example, kDNA-PCR results from bugs captured in Monte Alegre do Piauí were shown in Fig. 2. Triatomine infection by *T. rangeli* was confirmed in samples with positive kDNA results by means of 24sα rDNA PCR (Fig. 3). Fifteen of the 240 bp amplicons were purified, sequenced and aligned with reference *T. rangeli* sequences, being 100% homologous. These sequence data are available in GenBank database under the accession numbers JN016743–JN016745 and JN673224–JN673235. In seven localities, such as Monte Alegre do Piauí, mixed infections by *T. rangeli* and *T. cruzi* were detected in triatomines (Table 1). Overall, we identified mixed infections by Tc I and *T. rangeli* in nine samples, inferred by the amplification of two 24sα rDNA PCR products (Fig. 3), and also by mixed results obtained in the 24sα rDNA and SL-IR PCR assays. As an example, the sample of Taguatinga-TO amplified both the band of 240 bp in the 24sα rDNA PCR (*T. rangeli*), and the 475 bp product in the SL-IR PCR (*T. cruzi* I) (Figs. 3 and 4). Sequencing the Tc I SL-IR products (Fig. 4) allowed identification of Tc Id SL-IR genotype (GenBank accession numbers GQ398813 and JN673236–JN673244).

The second most frequent triatomine species in *M. flexuosa* was *P. tertius*, occurring in 26 of 341 palms trees (8%), which resulted in the capture of 484 specimens (19 insects per infested palm) (Table 2). Overall, 60% of *P. tertius* specimens captured were nymphs but in some palms a higher proportion of adults were observed (Table 2). A higher proportion of males were observed in most populations (ratio male/female > 1, Table 2). No specimen of *P. tertius* was examined due to the strictly bird-feeding habit in natural conditions. *T. sordida* occurred in only one locality (Buritizeiro, state of Minas Gerais), where four of the 13 palm trees sampled were infested (31%) resulting in 30 specimens caught (nymphs) with 7.5 bugs per infested palm. None of the 10 *T. sordida* examined bugs were infected by trypanosomes. A female specimen of *M. borbai* was captured in Alto Garça (state of Mato Grosso). A co-occurrence of *R. neglectus* and *P. tertius* was detected in 14 locations (Tables 1 and 2). *R. neglectus* also occurred in palm trees where specimens of *M. borbai* and *T. sordida* were captured. Overall, the percentage of infested palm trees by more than one triatomine species was 15%.

4. Discussion

M. flexuosa was previously recognized as an important breeding site for *R. neglectus* in the Brazilian states of Minas Gerais, Goiás, Tocantins and the Federal District (Barretto et al., 1969; Mello, 1982; Gurgel-Gonçalves et al., 2003, 2004a; Gurgel-Gonçalves and Cuba, 2007, 2009; Abad-Franch et al., 2009). The present study expands the occurrence to other states (São Paulo, Bahia, Mato Grosso, Maranhão and Piauí) throughout the *Cerrado* biome. In the Amazon basin, other *Rhodnius* species are found (e.g. *R. robustus*, *R. pictipes*) mainly in *Attalea speciosa* palm trees (Miles et al., 1983a; Abad-Franch et al., 2010). There is little evidence available of *R. neglectus* occurrence in this hydrographic basin (Abad-Franch and Monteiro, 2007). Moreover, ecological niche modeling of *R. neglectus* did not show potential areas of the species occurrence in the Amazon basin. Thus, *M. flexuosa* would be an indicator of the presence of *R. neglectus* within the *Cerrado* and transition areas with other biomes (Batista and Gurgel-Gonçalves, 2009; Gurgel-Gonçalves and Cuba, 2009). Future studies sampling *Rhodnius* populations in *M. flexuosa* across the major rivers, savannas and wetlands of Amazonia could confirm this hypothesis.

Several studies show that there is great variation in the infestation and density of *R. neglectus* in *M. flexuosa*. In Uberaba (Minas Gerais state), the infestation of *R. neglectus* in 32 palms sampled was 93.7% with a density of 15.6 bugs per palm (Barretto et al., 1969). In Mambaí (Goiás State) the infestation was 65.6% with a density of 4.2 individuals with the same sampling effort (Mello, 1982) and in Frutal (state of Minas Gerais) the infestation was 69.2% with a density of 9.5 individuals per palm tree, with a sampling effort of

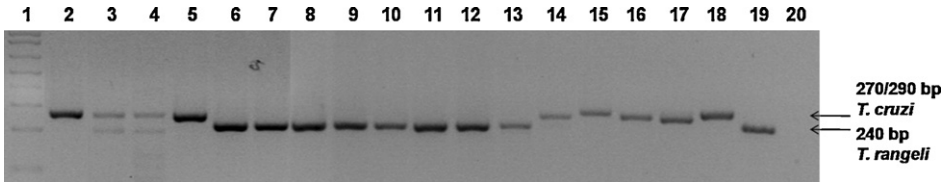

Fig. 3. Characterization of kDNA positive samples: 24sα rDNA PCR. Gel electrophoresis of 24sα rDNA amplification products from triatomine abdomen samples (*Rhodnius neglectus*) with primers D75 and D76. Lane 1: 1 kb DNA ladder; 2–5: samples from Monte Alegre do Piauí-PI; 6: Posse-GO; 7 and 8: Araguaína-TO; 9: Taguatinga-TO; 10: Palmas-TO; 11: Buritizal-SP; 12: Ituiutaba-MG; 13: São Desidério-BA; 14: Ribeirão Cascalheira-MT; reference *T. cruzi* and *T. rangeli* strains: Lane 15–18: CL-Brener (Tc VI); 16: G (Tc I); 17: PAH 265 (Tc V); 19: SC-58 (*T. rangeli*); 20: negative control.

Table 1
Entomological indices of *Rhodnius neglectus* in *Mauritia flexuosa* palm trees sampled in 42 localities in Brazil. Numbers on the second column indicate the geographic location shown in Fig. 1D.

Locality	n°	Palms sampled	Palms infested (%)	Bugs collected	Bugs/infested palm	Adult/nymph	Male/female	Examined	Infected (%)
Planaltina, DF	1	4	4 (100)	91	22.8	0.3	3.4	33	0
E.E. Águas Emendadas, DF	2	3	3 (100)	6	2.0	0.5	2	5	0
Reserva Ecológica do IBGE, DF	3	7	4 (57)	58	14.5	0.2	3.5	14	0
Mambaí, GO	4	14	7 (50)	50	7.1	0.3	1.8	7	0
Posse, GO	5	12	7 (58)	25	3.6	[a]	[a]	10	10[f]
Alto Paraíso, GO	6	12	8 (67)	94	11.8	0.4	2.9	7	28[g]
Teresina de Goiás, GO	7	13	7 (54)	22	3.1	0.1	[c]	5	0
Monte Alegre de Goiás, GO	8	1	1 (100)	4	4.0	[a]	[a]	4	0
Goiás Velho, GO	9	20	6 (30)	27	4.5	1.7	4.7	10	0
Mozarlândia	10	9	1 (11)	62	62.0	0.3	0.8	10	0
São Miguel do Araguaia, GO	11	17	6 (35)	16	2.7	0.6	1	3	0
Novo Planalto, GO	12	2	2 (100)	41	20.5	1.2	1.2	4	0
Cabeçeiras, GO	13	13	5 (38)	61	12.2	0.3	2.8	36	0
Cristalina, GO	14	9	9 (100)	48	5.3	0.5	2.2	28	0
Cristalina, GO	15	5	3 (60)	49	16.3	0.2	8	12	0
Campo Alegre, GO	16	10	7 (70)	37	5.3	0.3	1.3	12	8[g]
Aporé, GO	17	7	6 (86)	12	2.0	0.3	2	14	0
Quirinópolis, GO	18	1	1 (100)	4	4.0	[a]	[b]	0	0
Acreúna, GO	19	6	4 (67)	25	6.3	0.7	2.3	14	0
Alto Garça, MT	20	9	6 (67)	23	3.8	0.6	2	10	0
Ribeirão Cascalheira, MT	21	6	5 (83)	27	5.4	0.4	1	18	22[d,f]
Nova Xavantina, MT	22	4	4 (100)	87	21.8	2.3	0.7	30	0
Vila Paredão, MT	23	10	3 (30)	9	3.0	0.8	1	2	0
Primavera do Leste, MT	24	8	5 (63)	30	6.0	0.2	3	7	0
Diamantino, MT	25	4	4 (100)	85	21.3	0.5	1.5	20	0
Porto Esperidião, MT	26	4	1 (25)	3	3.0	[a]	[b]	0	0
Nossa Senhora do Livramento, MT	27	1	1 (100)	124	124.0	0.7	1.0	13	7[g]
Uberlândia, MG	28	5	4 (80)	53	13.3	0.3	0.7	13	15[g]
Ituiutaba, MG	29	8	7 (88)	53	7.6	1.4	1.8	24	17[e,f]
Mirabela, MG	30	10	6 (60)	35	5.8	0.6	3.3	10	0
P.N. Cavernas do Peruaçú, MG	31	4	3 (75)	27	9.0	0.3	0.8	13	0
Buritizeiro, MG	32	13	4 (31)	57	14.3	0.4	1.3	16	0
São João do Buriti, MG	33	7	1 (14)	4	4.0	[a]	[a]	2	0
Buritizal, SP	34	4	4 (100)	61	15.3	0.5	0.6	24	17[e,f]
Araguaína, TO	35	9	8 (89)	57	7.1	0.2	1.3	12	25[e]
Colinas, TO	36	14	3 (21)	5	1.7	0.2	[b]	4	0
Taguatinga, TO	37	9	3 (33)	56	18.7	0.2	1.7	37	8[d,e,f]
Palmas, TO	38	15	7 (47)	22	3.1	0.7	2	11	18[f]
Alto Parnaíba, MA	39	10	3 (30)	4	1.3	0.2	[b]	3	0
São Desidério, BA	40	4	3 (75)	15	5.0	1.1	0.6	9	11[e]
Santa Rita de Cássia, BA	41	11	4 (36)	16	4.0	0.3	3.0	2	0
Monte Alegre do Piauí, PI	42	7	2 (28)	54	27.0	0.3	1.5	29	55[d,f]

[a] Only nymphs captured.
[b] Only females captured.
[c] Only males captured.
[d] *Trypanosoma cruzi* (TcId).
[e] *Trypanosoma rangeli*.
[f] Mixed infections TcI/*T. rangeli*.
[g] Parasites morphologically similar to *T. cruzi* after observation of Giemsa-stained insect feces.

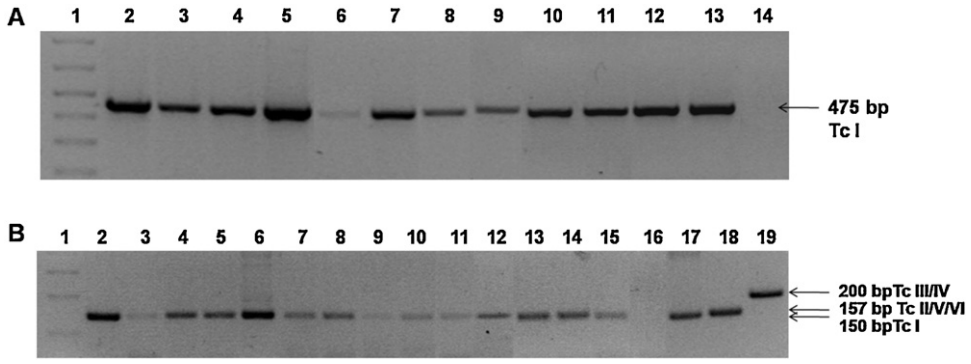

Fig. 4. Characterization of kDNA positive samples: SL-IR PCR. (A) Gel electrophoresis of SL-IR amplification products of fecal (f) and abdomen (a) samples from *Rhodnius neglectus* with primers UTCC-TC2. Lane 1: 1 kb DNA ladder; 2–5: Monte Alegre do Piauí-PI (a); 6 and 7: Taguatinga-TO (a); 8: Buritizal-SP (a); 9: Ribeirão Cascalheira-MT (a); 10 and 11: Monte Alegre do Piauí-PI (f); 13: Tc I reference strain G; 14: negative control. (B) Gel electrophoresis of SL-IR amplification products of fecal (f) and abdomen (a) samples with primers UTCC-Tcac: Lane 1: 1 kb DNA ladder; 2–6: Monte Alegre do Piauí-PI (a); 7 and 8: Taguatinga-TO (a); 9: Palmas-TO (a); 10: Buritizal-SP (a); 11: Ituiutaba-MG (a); 12: Ribeirão Cascalheira-MT (a); 13–15: Monte Alegre do Piauí-PI (f); 16: negative control; and *T. cruzi* reference strains: 17: G (Tc I); 18: PAH 265 (Tc V); and 19: Can III (Tc IV).

Table 2
Entomological indices of *Psammolestes tertius* in *Mauritia flexuosa* palm trees sampled in 14 localities in Brazil. Numbers on the second column indicate the geographic location shown in Fig. 1D.

Locality	n°	Palms sampled	Palms infested (%)	Bugs collected	Bugs/infested palm	Adult/nymph	Male/female
Planaltina, DF	1	4	3 (75)	143	47.7	0.5	2.1
E.E. Águas Emendadas, DF	2	3	1 (33)	2	2.0	0.3	[a]
Reserva Ecológica do IBGE, DF	3	7	2 (28)	37	18.3	0.2	1.5
Mambaí, GO	4	14	3 (21)	71	23.1	1.1	0.9
Posse, GO	5	12	1 (8)	1	1.0	[b]	[b]
Cristalina, GO	14	9	3 (33)	60	20.0	0.4	2.6
Cristalina, GO	15	5	1 (20)	59	59.0	0.5	0.9
Acreúna, GO	19	6	1 (17)	33	33.0	3.1	1.1
Alto Garça, MT	20	9	2 (22)	20	10.0	1.5	2.0
Primavera do Leste, MT	24	8	2 (25)	5	2.5	0.3	[b]
Diamantino, MT	25	4	3 (75)	18	6.0	2.0	1.0
Uberlândia, MG	28	5	1 (20)	2	2.0	[d]	1.0
Buritizal, SP	34	4	1 (25)	1	1.0	[b]	[c]
São Desidério, BA	40	4	2 (50)	32	16.0	0.9	1.5

[a] Only nymphs captured.
[b] Only females captured.
[c] Only males captured.
[d] Only adults captured.

thirteen palm trees (Forattini et al., 1983). Gurgel-Gonçalves et al. (2004a) presented an infestation of 12–40% and density from 3.3 to 9.8 individuals per palm sampling 150 palm trees at five areas in the Federal District. Finally, rates of infestation of *M. flexuosa* by *R. neglectus* ranged from 70 to 95%, with average of seven insects per infested palm (Abad-Franch et al., 2009). In the present study, the infestation ranged from 11 to 100% with an average of 9 bugs per infested palm sampling 341 palm trees of 42 localities. The variation observed in infestation and density values must be the result of different sampling methods and other factors such as capture effort, characteristics of the geographic areas and period of capture (Gurgel-Gonçalves et al., 2003; Abad-Franch et al., 2010). However, these results clearly indicate that the palm *M. flexuosa* is a favorable breeding ecotope for the maintenance of *R. neglectus* populations in the *Cerrado*, as already suggested for other palm trees, such as *Attalea* spp. *Syagrus oleracea* and *Acrocomia aculeata* (Barretto et al., 1969; Diotaiuti and Dias, 1984; Abad-Franch et al., 2009).

The infestation values of *M. flexuosa* by *P. tertius* were lower than those observed for *R. neglectus*. This can be explained by the strong association between *P. tertius* and thornbirds (Silva and Lustosa, 1993; Gurgel-Gonçalves and Cuba, 2011). Thus, the development of colonies of *P. tertius* in *M. flexuosa* may be dependent on the presence of bird nests, mainly *Phacellodomus ruber* (Gurgel-Gonçalves et al., 2004a).

The predominance of nymphs and the higher proportion of males in *R. neglectus* populations from *M. flexuosa* were expected results considering the age structure generally observed in *Rhodnius* populations from palm trees (Barretto et al., 1969; Pizarro and Romaña, 1998; Gurgel-Gonçalves and Cuba, 2007). The percentage of *P. tertius* nymphs was lower than that of *R. neglectus*. In some areas we captured predominantly adults of *P. tertius*. The high proportion of adults in *P. tertius* populations from thornbird nests could be explained by a combination of high longevity and low dispersal of adult bugs from the nests. Also, the stable blood supply provided by birds would favor steady *P. tertius* infestation of thornbird nests across Brazilian *Cerrado* (Gurgel-Gonçalves and Cuba, 2011). Although *P. tertius* has been experimentally infected by *T. cruzi*, the bird-feeding behavior and strictly sylvatic habitats of this species do not contribute to vector transmission of this protozoan (Silva and Lustosa, 1993).

T. sordida has been reported in several genera of palms such as *Acrocomia*, *Attalea*, *Butya*, *Copernicia*, *Mauritia*, and *Syagrus* (Carcavallo et al., 1998; Bar and Wisnivesky-Colli, 2001). In Uberaba (Minas Gerais state), the infestation of *T. sordida* in 32 *M. flexuosa* palm trees sampled was 50% with a density of 5.2 bugs per palm and a *T. cruzi* infection rate of 12.7% (Barretto et al., 1969). These entomological indices are higher than those found in the present study.

To our knowledge, the occurrence of *M. borbai* in *M. flexuosa* in the state of Mato Grosso is described for the first time in this paper. The species is generally found in marsupial shelters (*Didelphis* sp.) and rodent nests in trees and bromeliads (Lent and Wygodzinsky, 1979; Carcavallo et al., 1998) and its occurrence has been described in the states of Goiás, Paraná and São Paulo (Rodrigues et al., 1992; Carcavallo et al., 1999). The finding of *M. borbai* in *M. flexuosa* should be associated with the presence of *Didelphis albiventris* in palm trees and our result extends the geographical distribution of this triatomine species in Brazil.

The data show that *R. neglectus* plays an important role in maintaining the enzootic circulation of *T. cruzi* and *T. rangeli* in the Brazilian savanna. In some areas mixed infections were detected in wild *R. neglectus* specimens as already observed in other areas and vector species (Ramirez et al., 2002; Pavia et al., 2007; Dias et al., 2008; Pineda et al., 2008; Thekisoe et al., 2010). The higher frequency of birds in *M. flexuosa* palm tree crowns could explain the low overall infection rate of our study (8%) when compared with other *Rhodnius* species in palms in the Amazon basin that have higher rates of infection (e.g. Miles et al., 1983a). However, *R. neglectus* also feeds in mammals and high rates of *T. cruzi* and *T. rangeli* infection of this species have been described in Brazil (Barretto et al., 1969; Ramirez et al., 2002; Gurgel-Gonçalves et al., 2004b; Abad-Franch et al., 2009). Our results extend these observations and confirm that these trypanosomes are widely distributed in Brazil. To our knowledge, the occurrence of *T. rangeli* in the Brazilian states of Mato Grosso, Goiás, São Paulo and Piauí is described for the first time. Moreover, we confirm the occurrence of *T. rangeli* in the state of Tocantins, as already suggested by Diotaiuti et al. (1992). Thus, our results indicate a widespread distribution of *T. rangeli* in Brazil, in agreement with other studies (Maia da Silva et al., 2007, 2009).

In some *M. flexuosa* palm trees where we found *R. neglectus* specimens highly infected with trypanosomes, rodents, opossums (*D. albiventris*) and anteaters (*Tamandua tetradactyla*) were observed. The role of *D. albiventris* as a reservoir of *T. cruzi* and *T. rangeli* is widely known and recently *T. tetradactyla* was found infected with *T. rangeli* in the Amazon (Dias et al., 2010). In Monte Alegre do Piauí where we detected a *T. cruzi* infection rate of 55%, the distance between the palm trees and houses was about 100 m and we could expect triatomine invasion in human dwellings which represents a risk for *T. cruzi* transmission to humans.

T. cruzi Id populations are widely distributed in the Americas. They have been recognized in the sylvatic transmission cycle, mainly in marsupials and sylvatic triatomines in Brazil. However, they are also associated with domestic and/or peridomestic vectors in Argentina, Paraguay and Colombia. Moreover, human infection with *T. cruzi* Id was described in Argentina, French Guiana, Panamá and Venezuela (Cura et al., 2010).

5. Conclusions

T. sordida and *R. neglectus* are secondary vectors potentially involved in human Chagas disease transmission throughout its wide distribution across the Brazilian savanna. Household infestation has been reported in the states of Tocantins (Gurgel-Gonçalves et al., 2008), Goiás (Oliveira and Silva, 2007), Mato Grosso do Sul (Almeida et al., 2008), Minas Gerais (Diotaiuti et al., 1998; Monteiro et al., 2009), and São Paulo (Silva et al., 1999). The presence of triatomines infected with trypanosomes in palm trees near residences shows the potential risk of human infection. Thus, extensive longitudinal surveillance systems capable of detecting and eliminating synanthropic *T. sordida* and *R. neglectus* populations are therefore needed across the range of these species. This surveillance system in localities near the palm trees should also include health education for residents to learn how to detect triatomine bugs strengthening passive surveillance.

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