

Emerging Chagas Disease: Trophic Network and Cycle of Transmission of *Trypanosoma cruzi* from Palm Trees in the Amazon

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A trophic network involving molds, invertebrates, and vertebrates, ancestrally adapted to the palm tree (*Attalaea phalerata*) microhabitat, maintains enzootic *Trypanosoma cruzi* infections in the Amazonian county Paço do Lumiar, state of Maranhão, Brazil. We assessed seropositivity for *T. cruzi* infections in the human population of the county, searched in palm trees for the triatomines that harbor these infections, and gathered demographic, environmental, and socioeconomic data. *Rhodnius pictipes* and *R. neglectus* in palm-tree frond clefts or in houses were infected with *T. cruzi* (57% and 41%, respectively). Human blood was found in 6.8% of *R. pictipes* in houses, and 9 of 10 wild *Didelphis marsupialis* had virulent *T. cruzi* infections. Increasing human population density, rain forest deforestation, and human predation of local fauna are risk factors for human *T. cruzi* infections.

The tropical moist broadleaf forests of Latin America are an important region for conservation of biodiversity (1). In the Amazon Basin (area 8,214,284 km²), blocks of original habitat are still intact, while some ecoregions¹ are almost completely converted or degraded, allowing major components of biodiversity to steadily erode (1,2). Populations of several endangered wildlife species have declined, and human habitat and land use are considered a threat to most native species and communities.

Palm trees propagate in ecoregions of the Amazon Basin ecosystem amid other vegetation or in enormous palm forests. Of approximately 2,800 palm species worldwide, 387 (13.8%) are native to the basin (3,4). Palm trees have been

used to study the evolution of biological diversity and are excellent markers of ecologic fitness in the Amazon Basin (2,4). In addition, these trees may play an important role in the forest ecosystem. Palm trees produce 15 tons of dry organic material per hectare per year (threefold more than other species of trees) and recuperate more rapidly after fire than other forest species. A single native palm tree may serve as shelter and food for diverse fauna (wild mammals, snakes, scorpions, spiders, amphibians, and many species of insects). Palm trees are also an important economic resource for residents of the Amazon region, who collect and sell palm roots, stipe, leaves, fruits, seeds, heart of palm, and inflorescences (4,5). The babassu palm (*Attalaea phalerata*) reaches an average density of 200 trees per hectare in the state of Maranhão, but lower densities were reported in the states of Piauí, Goiás-Tocantins, and Mato Grosso (5), where babassu trees number an estimated 11×10^8 .

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¹In this study, a major ecosystem is defined as a set of ecoregions of comparable dynamics, response characteristics to disturbance, species diversity, and conservation needs. An ecoregion is a geographically distinct set of natural communities with similar species, ecologic dynamics, environmental conditions, and ecologic interactions critical for long-term persistence (1).

Not much attention has been given to human health conditions in this ecosystem (6), probably because pathologic conditions in tropical broad-leaf forests are difficult to quantify in these isolated, often impoverished communities. Nineteen sylvatic species of triatomines were identified in the Amazon Basin (7-10), six in association with palm tree microhabitats (Table 1). Eleven of

Table 1. Reservoir hosts, triatomines and palm trees participating in the life cycle of transmission of *Trypanosoma cruzi* in the Amazon Basin

Mammal hosts of *T. cruzi*^a

- Primata
- Marsupialia
- Edentata
- Rodentia
- Carnivora
- Artiodactyla
- Chiroptera

Triatomines

- Belminus herreri*
- Cavernicola lenti*
- C. pilosa*^b
- Eratyrus mucronatus*^b
- Microtriatoma trinidadensis*^b
- Panstrongylus geniculatus*^b
- P. rufotuberculatus*^b
- P. lignarius*^b
- P. arthruri*
- Rhodnius brethesi*
- R. nasutus*
- R. neglectus*^b
- R. paraensis*^b
- R. pictipes*^b
- R. prolixus*^b
- R. robustus*^b
- Triatoma maculata*
- T. rubrofasciata*
- T. rubrovaria*

Palm trees

- Acrocomia aculeata*
- A. sclerocarpa*^c
- Astrocaryum aculeatum*
- Attalaea phalerata*^c
- A. vulgare*
- Bactris gasipaes*
- Euterpe oleracea*^c
- E. precatória*
- Leopoldina piassaba*
- Mauritia flexuosa*^c
- Maximiliana elegans*^c
- M. regia*
- Oenocarpus bacaba*
- O. bataua*
- O. mapora*
- Phytelephas macrocarpa*
- Scheelea martiana*
- Sheelea sp.*^c

^a>100 mammal wildlife species are reservoirs of *T. cruzi* (11).
^bTriatomine species found with *T. cruzi* infection (12-14).
^cPalm species with triatomines infected with *T. cruzi* (13,15,16).

these species were infected with *Trypanosoma cruzi* or *T. cruzi*-like flagellates (17,18). None of these triatomine species, with the possible exception of *T. rubrofasciata*, have adapted to human habitats in the Amazon Basin (19). Since 1924, when *T. cruzi* infection in wild squirrel monkeys (*Crisotrix sciureus*) was described (20), sporadic human *T. cruzi* infections have been reported in the basin (21). However, this enzootic protozoan infection received attention only after 1969, when acute cases of human Chagas disease were described in Belém, State of Pará, Brazil (22,23). Further evidence shows that *T. cruzi* infections are endemic in the Amazon Basin (12-15,21-28) (Figures 1 and 2).

Spellerberg and Hardes (29) describe the major threats to rain forest conservation as shifting agriculture, cattle ranching, logging, and industrialization (mining, hydroelectric dams), to which we add land colonization. We

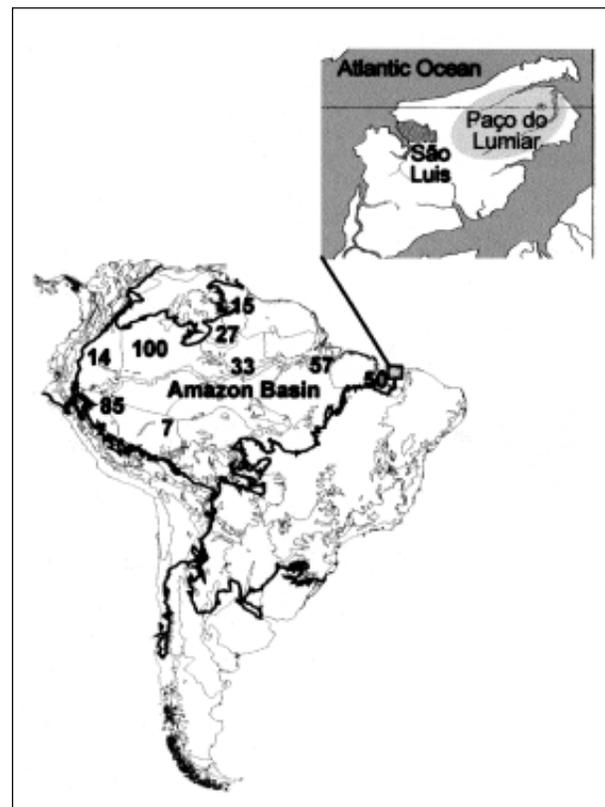


Figure 1. Human cases of acute human *Trypanosoma cruzi* infections in the Amazon Basin (19-28). French Guiana, 15; Colombia, 100; Ecuador, 14; Peru, 85; and Brazilian States: Amapá, 27; Acre, 7; Amazonas, 33; Pará, 57; and Maranhão, 50 cases. Insert shows Paço do Lumiar county in the island São Luís, State of Maranhão, an ecoregion vulnerable to human predation, where acute *T. cruzi* infections have been identified.

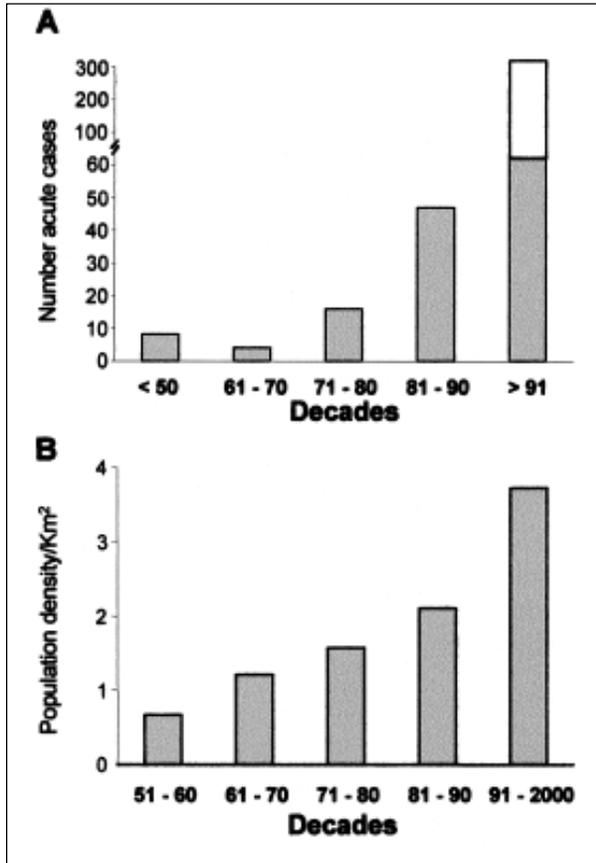


Figure 2. Human population growth and acute *T. cruzi* infections in the Brazilian Amazon Basin (19-28). In A, population density increased 2.5-fold in the last three decades; in B, acute *T. cruzi* infections increased several-fold in the same timespan. □ = break in scale.

hypothesized that certain effects of human economic activity (population growth, land use, deforestation, lack of environmental education programs, and human predation on mammals and birds) in a defined ecoregion of this major ecosystem may therefore pose a risk for outbreaks of acute Chagas disease. We evaluated these risk factors in the Tocantins River moist forest ecoregion (1), which is considered vulnerable to human intervention. We conducted a pilot study to assess seropositivity for *T. cruzi* infections in the human population of Paço do Lumiar County, state of Maranhão, Brazil, and to search for triatomines that harbor these infections in palm trees. We also gathered and analyzed demographic, environmental, and socioeconomic data.

Methods

The field study was carried out in Paço do Lumiar county (population 55,000), 20 km from São Luiz, capital of the state of Maranhão. The

county is part of the Tocantins moist forest ecoregion (1). We worked in 15 villages, separated by partly deforested argillaceous pathways with scattered houses, where mud-walled, thatch-roofed houses are usually located beneath or beside large palm trees. The surroundings consist of shady, partly deforested areas, where dogs, cats, chickens, pigs, cows, and horses live; no clear delineation separates peridomestic areas from the dense rain forest habitat of wild animals. The county's economy depends on subsistence agriculture and fishing. Raising domestic animals, producing manioc root flour and grains, and harvesting greens and fruits necessitate clearing areas of forest.

We conducted a serologic survey to assess the prevalence of *T. cruzi* infection in 25,451 county residents >1 and <75 years of age (72% were ≤30 years of age) in these 15 villages with <1,200 houses. Fingerprick blood samples from study participants were collected onto Whatman (Clifton, NJ) 1-mm filter paper for seropositivity assessment. After air-drying at room temperature, each set of 10 blood samples was sealed in clean plastic wrap and kept dry in an ice box during the day of collection. The blood samples were then stored frozen until analysis. At the laboratory, filter paper blood samples were punched out and eluted in 100 µL of phosphate-buffered saline (PBS), pH 7.4, as described (30). The test was standardized for obtaining 5 µL of blood in 1 cm² of the filter paper, and serum proteins were eluted in 100 µL of PBS, pH 7.4, yielded a 1:20 final dilution for screening seropositivity. For quality control, 10% of the samples were analyzed by a second examiner.

Trapping Triatomines

The strategy for trapping sylvatic triatomine bugs derived from published work (12,17), as well as observations by local residents that triatomines attracted by light fly from palm trees to houses at night. A night visit to one house resulted in capturing two triatomines on the wall near a light bulb; both these specimens had protozoan flagellates in the intestinal contents.

Researchers and field workers spoke to residents at clubs or social organizations. Dried triatomines were displayed, and community leaders requested that triatomines in houses be captured and stored (in a 5x3-cm translucent plastic container with holes in the cap). This strategy proved efficient for collecting triatomines in the rainy season, when they invaded the houses.

We systematically dissected 23 palm trees (*A. phalerata*) in backyards in five villages of the county. Each tree was cut into segments: stipe, crown shaft, fronds, petiole, and leaves. Each segment was carefully searched for insects, mammals, bird nests, and animal vestiges.

During microhabitat dissection, we captured 67 nymphs and 95 adults of three species of triatomines. The precipitin test was used to type blood in the intestinal contents of 44 adult male and female triatomines. The test consisted of two-dimensional immunodiffusion of blood in the insects' intestinal fluid against taxon-specific antisera (31,32).

Trapping and Identifying Wild Animals

The rich bird fauna in the research area included *Aratinga jandaia*, *Buzeo magnirostris*, *Colombina passerine*, *Coragyps atratus*, *Crotophaga ani*, *Guira guira*, *Otus choliba*, *Pitangus sulphuratus*, *Turdus fumigatus*, and *Tyrannus muscivora*. We did not capture birds because they are refractory to *T. cruzi* infections. However, we captured sylvatic mammals (*Didelphis marsupialis*) near houses in the study area. These ancient marsupials eat palm-tree fruits and rest and nest in the clefts between the stipe and their fronds. They leave these hiding places at night to search for fruits, chicken eggs, baby chicks, and food scraps. Using nylon net or wooden box-traps baited with mango and banana, we captured 12 adult *D. marsupialis* but were unable to trap *Caluromys* sp. seen in the palm trees around the study area.

Biologic Characterization of Kinetoplastid Flagellates

Parasitic protozoa in the feces of insect vectors of Chagas disease infections or in blood agar broth were demonstrated directly by light microscopy. Flagellate protozoan infections in *D. marsupialis* were detected by xenodiagnosis or hemoculture (33). For xenodiagnosis, 20 first-instar uninfected nymphs of *Dipetalogaster maximus* took a blood meal from each adult *D. marsupialis* captured in the field. Thirty days later, the feces of the triatomines were examined by microscopy for flagellates. Any parasitic flagellates in the feces of triatomines or in hemocultures were subjected to passage in weanling mice. This procedure consisted of intraperitoneal injection of a saline dilution of the metacyclic flagellates into mice. Two weeks later,

trypomastigote forms of the parasite were identified in the blood of the mice, then 100 μ L of infected blood was seeded in blood-agar slants, the supernatant of which yielded parasitic forms, which were used for mass production in nutrient-rich liver infusion tryptose medium. One isolate from *R. pictipes* (*Rp1*) and three isolates from *D. marsupialis* (*Dm1*, *Dm2*, and *Dm3*) were characterized.

Phenotypic and Genotypic Characterization of *T. cruzi*-like Isolates

Specific antibodies in sera from Chagas disease patients with parasitologically confirmed *T. cruzi* infection were used as phenotypic markers for the counterpart herein called *T. cruzi*-like parasitic infection. Binding of antibodies to epimastigote forms grown in liver infusion tryptose (LIT) medium and to amastigote forms in sections of murine tissues was detected by indirect immunofluorescence assay (30,34). Epimastigote and amastigote forms of the archetype Berenice stock of *T. cruzi* were used as positive controls. For negative controls, parasitic forms from both sources were treated with sera of *T. cruzi* antibody-negative persons.

We extracted DNA of parasitic forms from each of three flagellate protozoa derived from *D. marsupialis* and one isolate of *R. pictipes*. The epimastigote forms grown in LIT were used for extraction of nuclear and kinetoplast DNA, essentially as described (35). DNA samples were analyzed by polymerase chain reaction (PCR) with specific primers for the constant regions of minicircles of kDNA and for highly repetitive sequences of nuclear DNA of *T. cruzi*, as described (35-37). In addition, we used the rDNA nested set of primers D71/72A, which can amplify sequences of 125 and 110 base pairs (bp), respectively, from type II or I parasites (38-41). The reactions were run in parallel with 100 pg of protozoan flagellates *Rp1*, *Dm1*, *Dm2*, and *Dm3*. As positive controls, we used 100 pg of DNA from *T. cruzi* Berenice (Type 1) and *Dm28* (Type 2). Negative controls were 100 pg of DNA from *Leishmania braziliensis* (42) and *T. rangeli* (43,44).

Formalin-killed epimastigote forms of *T. cruzi*-like flagellates from *Rp1*, *Dm1*, *Dm2*, *Dm3*, and *Dm4* and the Berenice stock of *T. cruzi* were used. The probes consisted of a nuclear DNA sequence PCR amplified with primer sets TcZ1/2 (37). The probe was labeled with biotin

according to the manufacturer's protocol. Cells fixed in glass slides were hybridized with selected DNA probes and stained with fluorescein-labeled streptavidin (Sigma Chemical Co., St. Louis, MO).

Each of 10 *D. marsupialis* trapped in the wild and BALB/c mice receiving *T. cruzi*-like parasites isolated from triatomines were subjected to histopathologic study. The animals were euthanized, and tissue samples from organs and tissues were fixed in 10% formalin. Three representative sections of skeletal muscles, heart, esophagus, small and large intestine, liver, kidney, spleen, and lung were stained with hematoxylin and eosin for examination by microscopy. An average of six sections from the scent glands of the marsupials was taken for histopathologic study.

Results

By indirect immunofluorescence test for anti-*T. cruzi* antibodies in human blood collected on filter paper (30), 212 (0.83%) of persons tested had specific antibodies for *T. cruzi* infections (Figure 3). Positive serologic results in young populations indicate recent transmission and acute infection. Forty-six children ≤ 10 years of age (0.18% of the total study population) were antibody-positive for *T. cruzi* and were considered acutely infected.

Our results, which show seroprevalence of *T. cruzi* infections in the absence of hematophagous bugs or their vestiges (excreta and molted skins) in houses, prompted us to search for triatomines in the ecosystem where the population was infected or continues to be at risk. The strategy for capture of triatomines consisted in surveillance of houses by residents (household members captured bugs in the house and placed them in plastic containers) or in dissection of palm trees in backyards of houses (16,45-49). This householder-assisted surveillance and capture method yielded 52 triatomine bugs (36 *R. pictipes* and 16 *R. neglectus*). Triatomine excreta and molted skins in these houses were neither reported by inhabitants nor detected by field workers. Adult triatomines were captured in houses only during the rainy season. We also captured 133 triatomines in 23 palm trees cut down in backyards in five villages. Careful dissection of these trees allowed detection of different developmental stages of triatomines in clefts of palm frond-sheets (Figure 4, Tables 2-4).

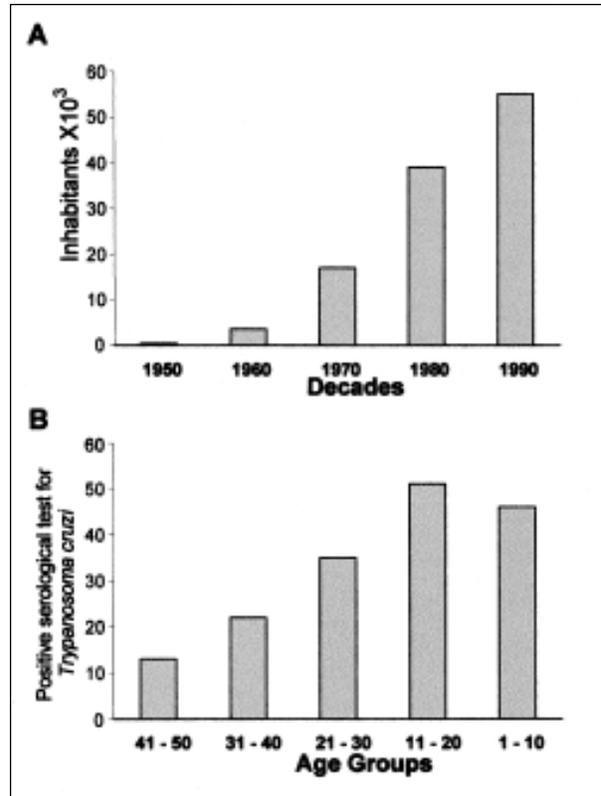


Figure 3. Human population growth and acute *Trypanosoma cruzi* infections in the Amazonian county Paço do Lumiar (19-28). In A, population density increased approximately fourfold in the last three decades. In B, autochthonous acute *T. cruzi* infections increased fourfold in the same timespan (comparing percentages of seropositivity in 41- to 50-year-olds vs. 11- to 20-year-olds), affecting younger age groups.



Figure 4. Palm tree (*Attalea phalerata*) frond-sheet microsystem and cycle of transmission of *Trypanosoma cruzi*. This natural dwelling of triatomine species and top predators (*Didelphis marsupialis*, circle) consists of clefts formed by the insertion of frond sheets into the stipe of babassu palm.

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Remains of animal species (e.g., nests, hair, feathers) on which triatomine bugs prey were identified in palm trees in backyards in five villages. Twice when a tree was cut down, adult opossums (*Didelphis*, Figure 4) ran out of the fronds into the forest. Nests of marsupials and birds were easily detected on dissection of palm fronds and crowns. In addition to opossums and birds, we identified molds and captured and identified different species of various taxa of

invertebrate and vertebrate animals (50-60) in the 23 palm trees (Table 5, Figure 5). Molds were found in stipes, fronds, and crowns, and insects in roots, stipes, inflorescence, fruits, fronds, crowns, and leaves. The clefts formed by frond sheets were particularly rich in Amphibia, Arachnida, and Hemiptera. Triatomines were detected at the bottom of clefts where marsupials built their nests. Bird nests were found in the fronds and crowns where abundant species of insects were available for predation.

Table 2. Triatomine bugs infected with *Trypanosoma cruzi* in palm trees and houses, Paço do Lumiar, Maranhão, Brazil*

Triatomines	<i>Rhodnius</i>	<i>R.</i>	<i>Pan-</i>	Total
	<i>pictipes</i>	<i>neglectus</i>	<i>strongylus</i> <i>lignarius</i>	
Palm trees				
No. captured	89	33	11	133
% infected	68	39	27	57
Houses				
No. captured	36	16	-	52
% infected	28	31	-	41

*The triatomines nymphs and adults were captured either by careful dissection of palm trees or surveillance of houses (12). Flagellate forms in the feces of the Triatomines were detected by microscopy and further identified as *T. cruzi* (see text).

Table 3. Developmental stages of *Rhodnius pictipes*, *R. neglectus*, and *Panstrongylus lignarius* found in houses and palm trees *Attalea phalerata**

Reduviid species	<i>R. pictipes</i>	<i>R. neglectus</i>	<i>P. lignarius</i>
	Nymphs		
1st instar	4	3	1
2nd instar	14	-	-
3rd instar	9	5	1
4th instar	6	3	1
5th instar	14	4	2
Adults			
Male	27	10	3
Female	29	13	3
Total	103	38	11

*No nymphs were found in houses.

Table 4. Type of blood in triatomine bugs captured in palm trees and houses

Triatomine	Total	Bird/		Didelphis	Rodent	Didelphis/		Canine/
		Bird	Didelphis			Rodent	Human	
<i>Rhodnius pictipes</i>	28	26.6	26.6	26.6	13.4	6.8	6.8	-
<i>R. neglectus</i>	10	40	-	40	-	-	-	20
<i>Panstrongylus lignarius</i>	6	100	-	-	-	-	-	-
%		36.6	18.2	27.3	9.1	4.5	4.5	4.5

*The blood type in the gut of triatomine bugs was identified by the agarose gel precipitin test with taxon-specific antiserum (31,32).

Scarcity of blood flagellates in marsupials precluded detection by direct microscopy. However, the metacyclic flagellates recovered by xenodiagnosis were subinoculated in weanling mice. Two weeks after injection, trypomastigote forms of the parasitic protozoan morphologically indistinguishable from *T. cruzi* were detected in blood of the mice. To define and further characterize these isolates, we used phenotypic and genotypic molecular characterizations. In the first group, antibodies in sera of chronic Chagas disease patients reacted indistinctly with antigenic determinants in the surface of *T. cruzi* Berenice and with isolates *Dm1*, *Dm2*, and *Dm3* from *D. marsupialis* and with *Rp1* from *R. pictipes*.

Genotypic kDNA and nuclear DNA (nDNA) markers were used to genetically characterize these wild flagellate protozoan isolates. PCR amplification of template DNA from each of these *T. cruzi* isolates showed that kDNA primers S35/36 (35-37) amplified *T. cruzi*, a laboratory standard for virulent *T. cruzi*. In addition, when we used PCR with mini-exon intergenic spacer primers TC/TC1/TC2 (38) and rDNA primers D71/72 (39-41), amplification resulted in the same bands, using template DNA from wild *T. cruzi* *Dm 28c*, *Dm1*, *Dm2*, *Dm3*, and *Rp1* and from Berenice *T. cruzi* isolated from a patient with acute Chagas disease (Figure 6). These molecular features allowed classification of wild isolates of

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Table 5. Trophic network in randomly selected palm trees of an Amazonian county*

Network	Trophic level [†]	Palm tree localization	Local given names
Metaphyta:			
<i>Attalaea phalerata</i>	1	Rainforest	Babaçu
Mold: (50, 51)			
Meliolaceae	2	Stipe & crown shaft	Bolor
<i>Meliola acristae</i>			
Catabotrydaceae			
<i>Catobotrys decidium</i>			
Metazoa:			
Insecta (52, 53)			
Coleoptera			
Curculionidae			
<i>Homalinotus coriaceus</i>			
<i>Rhynchophorus palmarum</i>			
<i>R. barbrosstris</i>			
<i>Amerrhynus inca</i>	3	Stipe, fronds & crown	Besouro
Chrysomelidae	3	Crown	Barata do coqueiro
<i>Coralimela brunea</i>			
<i>Mecistomela marginata</i>			
Scarabaeidae	3	Roots	Rola bosta, broca raiz
<i>Strategus aloeus</i>			
Bruchidae	3	fronds	Broca, bicho do côco
<i>Pachimerus nucleorum</i>			
Homoptera			
Asphidae	3	Crown	Pulgão
<i>Cerataphis lataniae</i>			
Diaspididae	3	Crown	Cochonilha coqueiro
<i>Aspidiotus destructor</i>			
Lepidoptera			
Brassolidae	3	Crown	Borboleta
<i>Brassolis sophorae</i>			
<i>Brassopholis astyra astyra</i>			
Hymenoptera			
Formicidae			
<i>Acromyrmex histrix</i>			
<i>A. landolti</i>			
<i>A. laticeps migrosetosus</i>	2 & 3	Roots, stipe & fronds	Formiga
<i>A. nobilis</i>			
<i>A. lundii carli</i>			
Hemiptera			
Reduvidae (6, 7, 10, 17)	4	fronds & crown	Barbeiro
<i>Rhodnius pictipes</i>			
<i>R. neglectus</i>			
<i>Panstrongylus lignarus</i>			
Aracnidea, Araneae (38)	4		
Theraphosidae		fronds	Aranha
Amphibia	4	fronds	Perereca
<i>Scinax sp</i>			
Aves (56)	5	fronds & crown	
<i>Aratinga jandaia</i>			Jandaia
<i>Buzo magnirostris</i>			Gavião
<i>Colombina passerine</i>			Rolinha
<i>Coragyps atratus</i>			Urubú
<i>Crotophaga ani</i>			Anu
<i>Guira-guira</i>			Anu branco
<i>Otus choliba</i>			Caburé
<i>Pizangus sulphuratus</i>			Bem-te-ví
<i>Turdus fumigatus</i>			Sabiá
<i>Tyranus muscivora</i>			Tesourinha
Marsupialia (54-59)	5	fronds	
<i>Didelphis marsupialis</i>			Mucura
<i>Caluromys sp.</i>			Cuíca

*Palm trees were randomly selected from five different localities (backyards) in the Paço do Lumiar county.

[†]Trophic levels in microhabitat following the flux of energy: 1) Palm tree, predated by molds, insects, birds and mammals; 2) molds, predated by Formicinae and Ponarinae; 3) Insects, predated by arachnids, amphibians and birds; (4) Arachnids and amphibians, predated by birds; 5) Birds and mammals, predated by hematophagus insects and man (50-60).

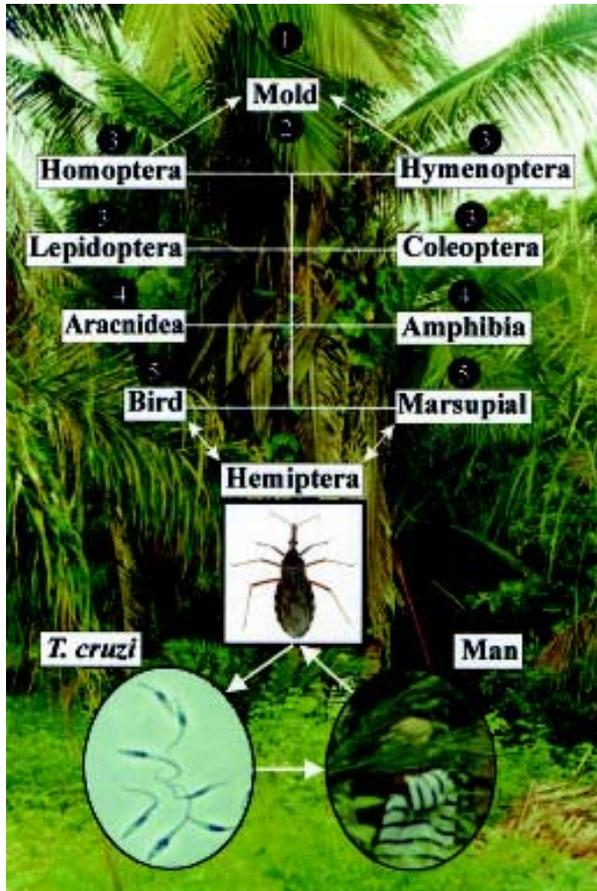


Figure 5. The informal economy: man carrying a babassu palm frond to be sold as soft thatch. A single palm tree enclosing five trophic levels associated with the *Rhodnius pictipes* (Hemiptera: Reduviidae) transmission of *T. cruzi* in the babassu microsystem.

T. cruzi as phylogenetic type I, while Berenice was *T. cruzi* II (11,38-41). These results were further confirmed by in situ hybridization of wild *T. cruzi* with a biotinilated 198-bp sequence derived from Berenice template DNA (Figure 7), which was amplified with specific nDNA primers Tcz1/2 (37).

Conclusions

Several authors (7,8,17-19,12) described *T. cruzi*-like flagellate protozoans (instead of *T. cruzi*) in the blood of various classes of mammals and in the feces of triatomine species from the Amazon Basin. We detected blood flagellates in 9 of 10 Didelphidae captured in backyards of houses in Paço do Lumiar county. In addition, we isolated flagellates from feces of *R. pictipes*, which showed the same morphologic features described for *T. cruzi*. In biological characterizations, these

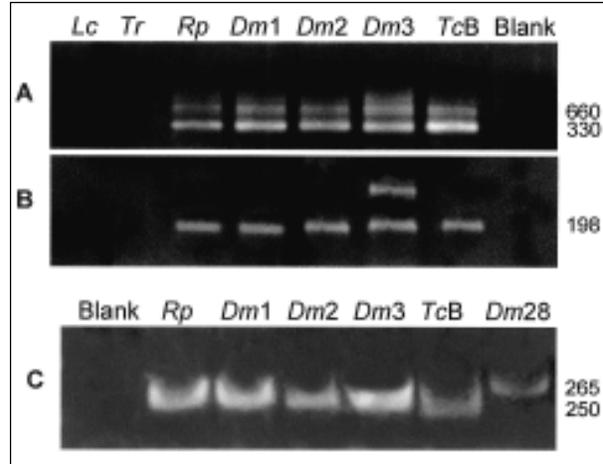


Figure 6. Genotypic characterization of wild-type flagellates by PCR amplification with rDNA and mini-exon specific primers derived from *Trypanosoma cruzi*. A, template DNAs amplified with mini-exon intergenic spacer primers (38): Blank, negative control; Tcb, archetypic type II *T. cruzi* Berenice; *Rp1*, *Dm1*, *Dm2*, and *Dm3*, flagellates isolated from *Rhodnius pictipes* and from *Didelphis marsupialis*; *Dm28*, standard type I, sylvatic *T. cruzi* isolate. B, same template DNAs amplified with rDNA primers (39-41). Tcb yielded typical 300-bp band of type II lineage, whereas *Rp1*, *Dm1*, *Dm2*, and *Dm3* and *Dm28* yielded a 350-bp band of type I *T. cruzi* lineage, with mini-exon spacer primers. In addition, Tcb yielded a typical 125-bp band of type II, whereas the sylvatic *T. cruzi* isolates yielded a 110-bp band of type I, with rDNA primers. These findings confirm sylvatic *Rp1*, *Dm1*, *Dm2*, and *Dm3* as *T. cruzi*.

flagellates induced low-level parasitemia in laboratory mice. However, histopathologic lesions in marsupials and laboratory mice were similar to those described in Chagas disease patients. Furthermore, nuclear DNA markers displayed all features of the *T. cruzi* standards Berenice and *Dm 28c T. cruzi*, which differed from those shown by *T. rangeli* and *L. braziliensis*. These data confirm the parasitic flagellates present in triatomines and mammals in this ecoregion as *T. cruzi*.

Birds are refractory to *T. cruzi* infections (33), but several authors (54-60) describe marsupials as important wildlife reservoirs of this parasitic protozoon. We used traps with fruit baits to capture 12 marsupials, which underwent karyotyping and parasitologic and pathologic examinations. The karyotyping confirmed these mammals as *D. marsupialis*. Nine of 10 trapped marsupials had protozoan blood flagellates isolated by xenodiagnosis or hemoculture. Histopathologic study of heart sections from

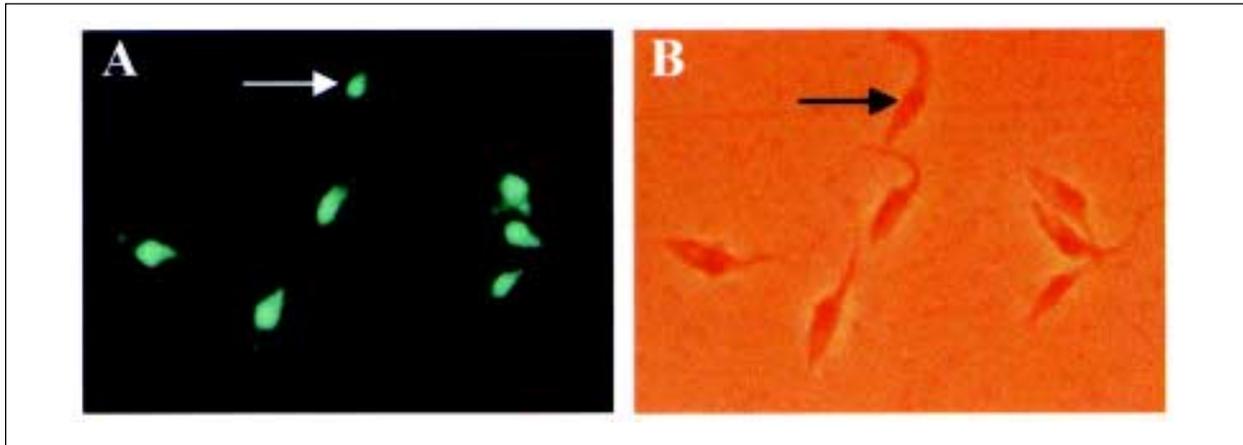


Figure 7. In situ hybridization of *Rhodnius pictipes*-derived flagellates with an archetypic Berenice *Trypanosoma cruzi*-specific probe. The byotynylated 198-bp sequence amplified with Tcz1/2 nuclear DNA probe (37) was revealed with fluorescein-conjugated streptavidin. This genotypic marker, representing 12.5% of total parasitic nuclear DNA, also confirms this sylvatic isolate as *T. cruzi*.

these infected animals showed typical myocarditis, characterized by mononuclear cell infiltrates and target cell lysis. Inflammatory infiltrates were seen in skeletal muscles, esophagus, and small and large intestines. Histopathologic study of representative tissue sections taken from the only opossum that did not have the parasite detected showed no histopathologic alterations (data not shown).

American trypanosomiasis has been considered an ancient zoonosis in which insect vectors and mammal hosts sympatrically occupy vast areas of South America (33). Wild *T. cruzi* infections and the bug vectors are syntopically adapted to mammalian host habitats under natural equilibrium. *T. cruzi* infections and human Chagas disease occur over a large geographic area, limited by parallels 42° N to 42° S (33). The Tocantins moist forest, much of which is subject to severe disturbance of the environmental equilibrium, provided conditions for disease outbreaks (61-64). The growing human populations encroaching on that natural ecoregion are fed upon by the triatomine vector *R. pictipes*, and cases of acute *T. cruzi* infections in humans have exponentially increased in the last three decades.

In this study, we describe a trophic network of five levels comprising different species dwelling in palm tree microhabitats. A single class of top predator mammal (Didelphidae) was found in the study area. The absence of other taxa of top wild predators upon which bugs feed may contribute to peridomiciliar and domiciliar

invasion during the wet season. This observation contrasts with earlier descriptions of seven families of mammals, belonging to Primates, Edentates, Marsupials, Carnivores, Rodents, and Chiroptera classes (10,55,57), which were hosts for triatomines in relatively undisturbed ecoregions. Elimination of a single class of invertebrate or vertebrate animals in a trophic network may be a major risk factor leading to more triatomine species entering houses and initiating a new cycle of transmission of *T. cruzi* infection.

In our study, a child ≤ 10 years of age with a positive immunofluorescence test (see methods) was considered a host of acute *T. cruzi* infection. Considering the age-specific prevalence of *T. cruzi* infections in adults (30) and the fact that for each acute case that is clinically identified an estimated 20 to 100 others are unrecognized (34), autochthonous human Chagas disease in the Amazon Basin may reach 7,860 to 39,300 cases. The latter figure is consistent with serologic evidence of *T. cruzi* infection in the Brazilian Amazon region presented in the national report on Chagas disease (65). The characteristics of transmission of infections described here do not indicate a need for insecticide spraying in the Amazon region, for the cycle of transmission of *T. cruzi* is deeply embedded in a natural trophic network comprising wild animals belonging to several classes and trophic levels.

Risk factors associated with the possibility of emergence of endemic Chagas disease in the

Amazon Basin have been described (10,12,17,19,25,54,55,62-64). First, the broadleaf moist rain forest ecosystem may be invaded by triatomine species (*T. infestans* and *T. rubrofasciata*), which are considered completely adapted to human domiciles, or by other triatomines (*P. megistus* and *T. brasiliensis*, *T. pseudomaculata*, and *T. sordida*), which can be found in different ecosystems but frequently enter and colonize houses (64). Second, several Amazon Basin triatomine species (*R. pictipes*, *R. prolixus*, *R. neglectus*, *R. nausutus*, *T. vitticeps*, *T. rubrovaria* and others) can adapt to human dwellings, where they could become important vectors of the *T. cruzi* infections (63-69). We found no vestiges of triatomine colonization in houses or their surroundings in our study area. We hypothesize that starving adult *R. pictipes* and *R. neglectus* may leave their natural shelters at night to feed on human hosts, probably attracted by light in the houses.

Factors associated with triatomines flying from palm tree to houses need to be clarified. The scarcity of birds and mammals during the wet season may be an important factor associated with anthropic predation and the presence of *T. cruzi*-infected insects in houses in the rainy season. Domiciliation of triatomines may not be required for an increasing endemicity of Chagas disease in the Amazon Basin (13,14,19). For example, sylvatic *Rhodnius brethesi* recently bit people harvesting palm fibers in Barcelos in the northwestern part of the State of Amazonas, 490 km upriver from Manaus, leading to an acute case of Chagas disease (13). Blood samples from residents of this locality tested by immunofluorescence assay had 12.5% positivity for anti-*T. cruzi* antibodies (70). These observations suggest that to prevent transmission of *T. cruzi* infections to humans in the Amazon Basin, new strategies are needed, which will not necessarily be similar to those used in controlling endemic Chagas disease in other ecosystems, such as the Cerrado and Caatinga ecosystems in Brazil (1,63,66,67,71).

The risk for endemic Chagas disease in the Amazon Basin appears related to deforestation and new population settlements, shifting cultivation, and rapid human colonization of the vectors' natural ecotope in the last three or more decades, as well as to lack of multiple blood sources (hosts) for the vectors in the rainy season. Amazon Basin periurban and urban areas where acute cases of

Chagas disease have been reported did not show signs of colonization of houses with triatomine bugs. Instead, transmission of sylvatic *T. cruzi* to humans has been associated with sylvatic species (*R. pictipes*, *R. neglectus*, *P. lignarius*), which fly from palm trees to houses. New prevention and control strategies should take into consideration risk factors leading to endemicity of the disease in the Amazon. An entomologic and epidemiologic system for surveillance of Chagas disease in Amazonia has been suggested (68,69).

Alternatively, the enormous task of controlling emerging Chagas disease in the Amazon Basin should rely initially on an information, education, and communication program, which encourages control measures by the householder (e.g., use of screens, bed nets, insecticide-treated fabrics, and vegetation management) (69-73). Such a program for prevention of contact with triatomines should be conducted directly in communities, elementary schools, and churches and social clubs, reinforced by social marketing and mass media communications. Further studies are also needed for identifying new and integrated (chemical and nonchemical) strategies required for controlling *T. cruzi* vectors in the Amazon Basin, which may not necessarily be similar to those already shown to be partially effective in controlling the domestic vectors of endemic Chagas disease in other ecosystems in the Americas.

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