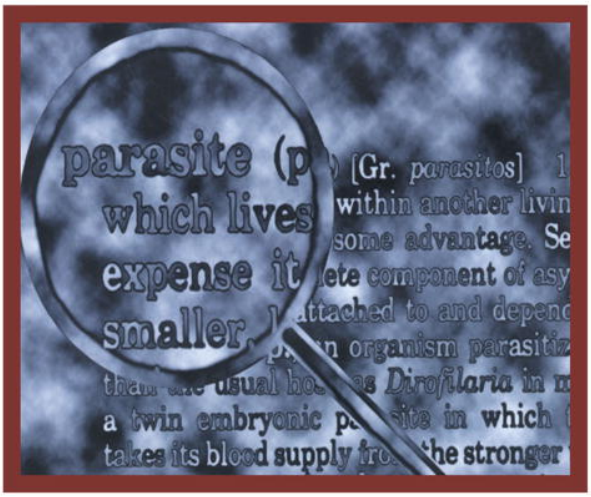
Volume 56, issue 2 June 2007 ISSN 1383-5769

PARASITOLOGY INTERNATIONAL



www.elsevier.com/locate/parint

Available online at www.sciencedirect.com
ScienceDirect
Parasitology International 56 (2007) 119–128
www.elsevier.com/locate/parint

Mapping of the distribution of *Trypanosoma cruzi* infection among small wild mammals in a conservation unit and its surroundings (Northeast-Brazil) ☆

S.C.C. Xavier [a], V.C. Vaz [a,c], P.S. D'Andrea [c], L. Herrera [a], L. Emperaire [e], J.R. Alves [e], O. Fernandes [d], L.F. Ferreira [b], A.M. Jansen [a,*]

[a] *Laboratory of Biology of Tripanosomatid, Department of Protozoology, Oswaldo Cruz Institute — IOC/FIOCRUZ, Av. Brasil 4365, Zip Code 21045-900, Rio de Janeiro, Brazil*
[b] *Laboratory of Cartography, Cartographic Engineering Department — IME, Praça General Tiburcio 80/DE-6, Zip Code 22290-270, Rio de Janeiro, Brazil*
[c] *Laboratory of Biology and Parasitology of Wild Mammals Reservoir, Department of Tropical Medicine, Oswaldo Cruz Institute — IOC/FIOCRUZ, Av. Brasil 4365, Zip Code 21040-900, Rio de Janeiro, Brazil*
[d] *Laboratory of Molecular Epidemiology and Infectious Diseases, Department of Tropical Medicine, Oswaldo Cruz Institute — IOC/FIOCRUZ, Av. Brasil 4365, Zip Code 21040-900, Rio de Janeiro, Brazil*
[e] *Institut de Recherche pour le Développement, UR no. 16, C.P. 7091, Lago Sul, Brasília, DF 71619-970, Brazil*

Received 21 July 2006; received in revised form 4 January 2007; accepted 5 January 2007
Available online 18 January 2007

Abstract

Maps are a useful tool that permits correlation of landscapes with hotspots of parasite transmission. Here, they were used as a tool for geovisualization to evaluate variables involved in the transmission of *Trypanosoma cruzi* among small wild mammals in an area endemic for Chagas disease, the "Serra da Capivara" National Park (PARNA) and its surroundings in Piauí State, Northeast Brazil. The implementation of a Geographical Information System (GIS) allowed the observation that a previously noted aggregated distribution of *Triatoma sordida* and *Triatoma brasiliensis*, *T. cruzi* prevalence and infection pattern of small wild mammals was directly or indirectly influenced by the local relief and human action. Small mammalian species diversity was higher in mesic refugia inside the park and in its buffer zone and lower in the disturbed area by anthropic activities. *Didelphis albiventris* was more abundant in the areas affected by human action. *Thrichomys laurentius* demonstrated to be an eclectic species and a competent reservoir of *T. cruzi*, being infected in all study areas. Small wild mammals infected with the TCII genotype of *T. cruzi* were localized only in the buffer zone of PARNA while TCI infected specimens were found in both areas, inside the PARNA and its buffer zone. The impact of biodiversity loss on the transmission cycle of *T. cruzi* in the wild environment was discussed.

Keywords: Geographical Information System — GIS; *Trypanosoma cruzi*; Triatomines; Wild reservoir; Ecoepidemiology; Semi-arid ecosystems

1. Introduction

Trypanosoma cruzi, the etiological agent of Chagas disease, is a digenetic trypanosomatid that is characterized by a significant genetic variability, mainly attributed to a long clonal evolution process [1,2]. The distribution of the two main genotypes of the parasite, respectively TCI and TCII, in the wild environment, as well as the relatedness of the subpopulation termed Z3 to these genotypes is still under debate [3,4]. Notwithstanding the several cases of Chagas disease that still may be found in South American countries, Brazil is currently considered free from vectorial transmission of *T. cruzi* [5,6]. This is due to the massive campaigns launched by the South Cone Initiative that achieved the control of the main vector, *Triatoma infestans*, a species introduced in Brazil that used to be restricted to the domiciliary environment.

Chagas disease epidemiology still remains a challenge given that the sylvatic transmission cycle of the parasite occurs in a

☆ The present work has the endorsement of the Ethical Commission for Experimentation with Animal Models (CEUA) from Fundacão Oswaldo Cruz — FIOCRUZ, RJ, Brazil, registration number: P0007.

* Corresponding author. Tel.: +55 21 2598 4324; fax: +55 21 2560 6572.
E-mail address: jansen@ioc.fiocruz.br (A.M. Jansen).

1383-5769/$ - see front matter © 2007 Published by Elsevier Ireland Ltd.
doi:10.1016/j.parint.2007.01.003

complex trophic network that includes several mammalian species [3]. The complexity of the epidemiology of Chagas disease is exemplified by the recently described new epidemiological features expressed by outbreaks of human disease probably due the oral route described in Amazonia [7], and, more recently, in 2005, in the Santa Catarina, a southern area of the Atlantic Coastal Rain Forest, where no domiciliation of triatomines had been reported up to now. In both biomes the disease resulted in severe symptoms and death of a number of infected people already during the acute phase [8]. These outbreaks show the importance of maintaining surveillance programmes in the light of biogeography of the transmission cycles in the wild mainly if it is considered that distinct scenarios of the enzootia may occur in a same biome and even in a same forest fragment [9,10].

Remote Sensing (RS), image processing and Geographic Information Systems (GIS) are frequently used in public health studies to analyze the spatial distribution of the elements that are linked to the transmission of vector borne diseases and its relationships with environmental variables that may or not influence the distribution and abundance of these elements. Moreover, the follow-up by GIS allows the evaluation of temporal and spatial evolution of parasitic infections and their risk factors for policy of prevention and control [11,12]. Such studies have been described in literature [13], although spatial analyses of *T. cruzi* transmission in the wild by GIS are scarce up to now [14].

In a previous study, results were reported of the prevalence of *T cruzi* infection in small wild mammals in three sites inside a biological reserve called "Serra da Capivara" National Park — PARNA, (localities — Pedra Solta, Zabelê and Sítio dos Oitenta), and two sites in its buffer zone (municipalities — Coronel José Dias and João Costa) in the Piauí State, Brazil [15]. This region displays the most ancient remnants of South American human occupation and was formerly highly endemic for Chagas disease. During the last ten years, no new case of the disease has been recorded, but several infected people, with both forms (cardiac and digestive) of the disease, still remain in 7that area [16]. Both main genotypes of the parasite as well as the Z3 subpopulation were found infecting rodents and marsupials. A focal distribution of the prevalence and profile of the infection of the examined mammals was also observed. Likewise, a grouped distribution of the two main triatomine species, *Triatoma sordida* and *Triatoma brasiliensis*, was noticed [15,17]. This led to reappraisal of the issue concerning the spatial distribution of the collection sites. In this sense a return to the study area to georeference the localities where triatomine and small wild mammals had been collected for application of a Parasitary GIS model that had been implemented in the Cartography Laboratory of the Institute Military of Engineering — IME. The study was also extended to the examination of small wild mammals for *T. cruzi* infection at two other sites, respectively inside PARNA (Guarita) and 10 km outside its buffer zone (Lagoa Dantas). The main purpose was to perform a spatial analysis considering environmental peculiarities in order to evaluate their influence on the mammalian and vectorial fauna composition, as well as on the distribution of *T. cruzi* infection and on the distribution of the main genotypes of the parasite.

In the present study the spatial exploratory analysis was used, in which the variables were studied using maps. Cartographic visualization based on the cartographic knowledge, graphic computation and semiology, provides the way to analyze patterns in spatial data [18]. Thematic maps were produced to represent the variables studied as well as the impact of biodiversity of small wild mammal species on the transmission cycle of the parasite.

2. Materials and methods

2.1. Regional characteristics

The study region is located between 8° and 9° 30′ S and 42° and 43° W. The PARNA — "Serra da Capivara" is a federal protected area and constitutes an important set of the Caatinga's biota characteristic of the semi-arid Northeast of Brazil. It is also one of the most important archeological sites in South America [19] (Fig. 1A).

South-east Piauí is registered as a megathermic semi-arid county [20]. The annual mean temperatures reach 28 °C. The rainy season extends from November to April with an average rainfall of 675 mm±247 mm and relative humidity of 80–90%. In the dry season, the relative humidity lies between 35 and 70%. Vegetation display the typical Caatinga features and relictual semi-deciduous forestal patches.

A plateau named Chapada Serra da Capivara, at a maximum height of 660 m, cuts PARNA horizontally.

The rural population has as a main activity, subsistence agriculture during the rainy season (beans, corn and cassava) associated with raising of goats and cattle.

2.2. Collection sites

The following sites originally cited in a previous study [15] were revisited in this study and geographically positioned with a Garmim III GPS receiver: (i) inside PARNA: Zabelê (Z), an area that displays the profile of high shrub land Caatinga; Pedra Solta (PS), a collection site localized on a plateau named Serra Branca that displays a vegetation of dense arboreal shrub land Caatinga and where a very active wild transmission cycle of *T. cruzi* among *T. laurentius* has been reported; Sitio dos Oitenta (S80), that also displays a low shrub land Caatinga; and (ii) in the buffer zone of PARNA: João Costa (JC) and Coronel José Dias (CJD) small towns surrounding (Fig. 1B).

2.3. New collection sites in the same study area

Small wild mammals were examined for *T. cruzi* infection and geographically positioned in (i) Guarita (G): this site is inside PARNA, 20 km distant from PS, on the same plateau. Similar to PS, the Caatinga of this site is extremely dense, difficult to penetrate, due to an abundance of liana and brushwood. (ii) Lagoa Dantas (LD): a small rural settlement, localized outside PARNA's buffer zone and nowadays under severe

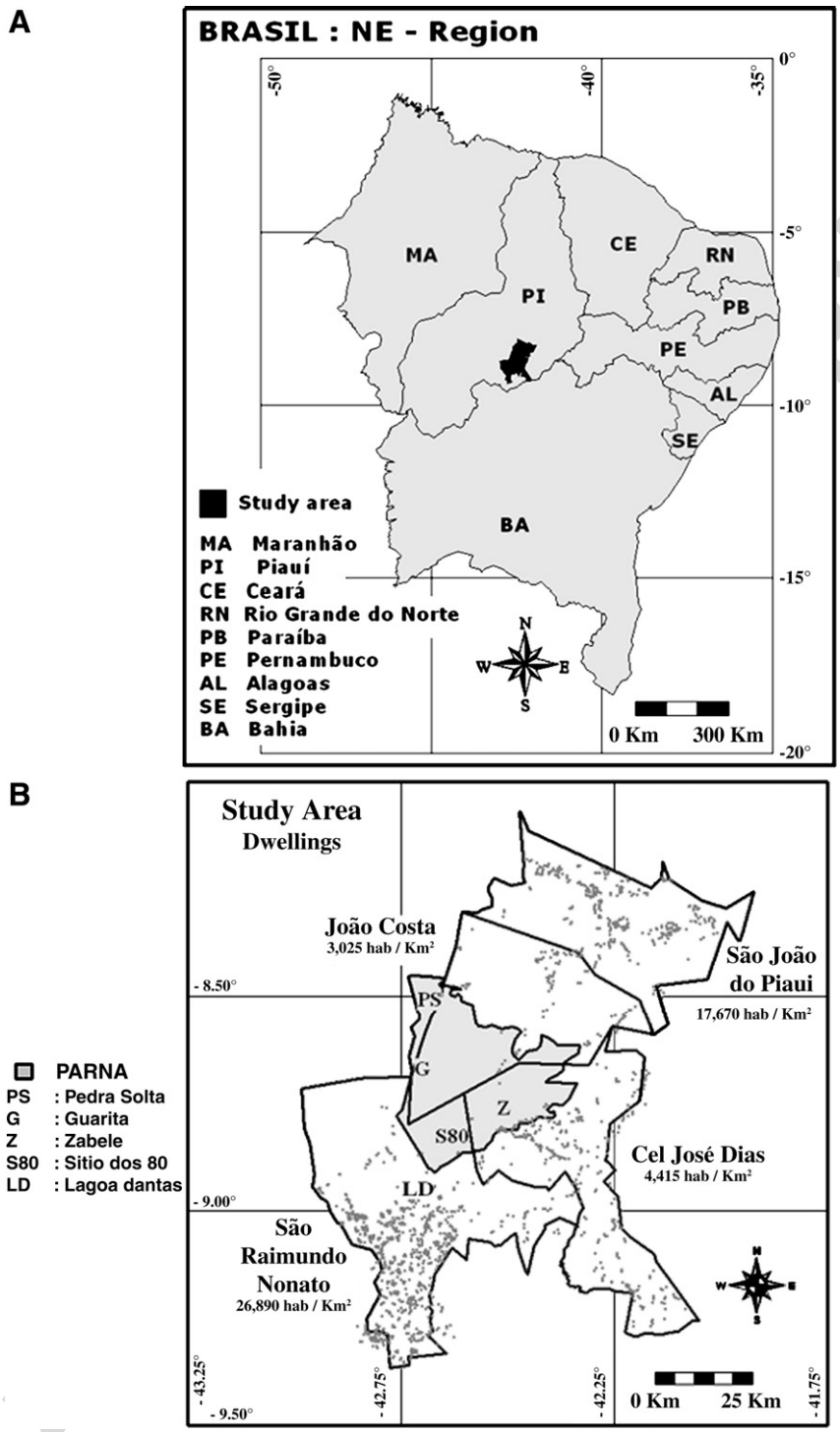


Fig. 1. A. Mapping of distribution of *Trypanosoma cruzi* in PARNA and it surroundings: study area is localized in the southeast region of Piauí, northeast region of Brazil. B. Mapping of distribution *of Trypanosoma cruzi* in PARNA and it surroundings: collection sites inside PARNA and it surroundings.

influence of human activities such as deforestation, burning and intense agriculture with corn and carob bean crops (Fig. 1B).

2.4. Positioning of the triatomine collection site

Besides all previous study sites, the triatomine collection site São João do Piauí (SJP) was revisited and geographically positioned. This site is localized beyond the mountain plateau. A total of 53 dwellings were considered. Inhabitants reported only occasional public fumigation.

The different abundance in the distribution of triatomine species in the northern and southern parts of the Chapada Serra da Capivara was compared using the Chi-Square test ($p \leq 0.05$).

2.5. Trapping and examination of small wild mammals

Small wild mammals of Guarita and Lagoa Dantas (in the São Raimundo Nonato – SRN – region) were trapped using live traps of Tomahawk and Sherman models (Tomahawk Live Traps Co. and H.B. Sherman Traps Inc.) baited with a mixture of peanut butter, banana, oat and bacon in the dry season, October of 2003 and May of 2004 respectively. The traps were settled at 20 m intervals in linear transects, during 5 nights, in all kinds of vegetational formations and habitats such as rocky, near to water sources and canyons in each site. The total capture effort was 900 and 850 trap-nights per locality, respectively. Blood for hemoculture and sera samples of the mammals was collected under anesthesia (Ketamine™, 50 mg/kg) by vein puncture.

The richness was calculated as being the number of species captured, and the Alpha diversity (Shannon index, H') as a local index of diversity for each area. These measures were computed using the statistical program STATECOL. The abundance per species (N) for each site was calculated as follows:

$$N = n/t$$

where n=total number of individuals captured per species and t=total sampling effort; the result was multiplied by 1000.

The total capture effort inside PARNA was 4187 trap-nights and outside PARNA was 3929 trap-nights.

The differences between the Alpha diversities inside and outside the PARNA were tested by one-way analysis of variance, and this analysis was run using the statistical program SPSS 10.01 ($p \leq 0.05$).

The natural *T. cruzi* infection of mammals was determined by hemocultures in axenic medium NNN (Novy, McNeal and Nicolle medium) with an Liver Infusion Triptose (LIT) overlay. Fortnightly examinations of the tubes were performed during 5 months. Additionally, the presence of specific antibodies was evaluated by an Indirect Imunofluorescence Assay (IFA) [21]. Priority was given for hemocultures when the amounts of collected blood were insufficient for both tests.

Only the species tested by IFA were considered to evaluate the prevalence of infection by *T. cruzi*. The independence between the prevalence observed inside and outside PARNA was tested using the Chi-Square test ($p \leq 0.05$).

2.6. Molecular characterization of T. cruzi isolates

Parasites obtained from the positive hemocultures were amplified in LIT medium up to a maximum of five passages and subsequently cryo-preserved for molecular characterization. The cryopreserved isolates were incubated with 0.5% Sodium Dodecyl Sulfate and genomic DNA was extracted from parasite lysates using phenol–chloroform 1:1 and precipitated with sodium acetate and ethanol. A miniexon Multiplex PCR assay was carried out in order to type *T. cruzi* isolates with TCI, TCII, Z3 or *Trypanosoma rangeli* specific primers [22]. The amplified PCR products were analyzed in Ethidium Bromide stained Agarose gel (3%) and visualized under Ultra Violet light.

In an additional experience, the obtained Z3 isolates were amplified in NNN culture and its nuclear DNA extracted for an first analysis of the 3′ untranslated region (3′ UTR) of the calmodulin gene [23].

Table 1
Total abundance (N), species richness and Shannon index (H') of small wild mammals captured inside and on the surroundings of the Serra da Capivara National Park (PARNA), Piauí, Brazil

	Inside PARNA				Outside PARNA		
	Z[1]	S80[1]	PS[1]	G[2]	LD[2]	JC[1]	CJD[1]
	N	*N*	*N*	*N*	*N*	*N*	*N*
Rodentia							
Bolomys lasiurus (Lund, 1841)	0.8						
Calomys expulsus (Lund, 1841)		0.9				1.34	1.3
Galea spixii (Wagler, 1831)	3.0		1.1	1.1			
Kerodon rupestris (Wied-Neuwied, 1820)						0.7	
Oligoryzomys stramineus (Bonvicino and Weksler, 1998)			1.1				
Rattus rattus (Linnaeus, 1758)	0.8	2.8					0,6
Rhiphidomys macrurus (Gervais, 1855)	0.8	8.5	1.1	4.4			5
Thrichomys laurentius (Thomas, 1904)	28.1	40.4	47.5	70	32.9	26.2	37.2
Marsupialia							
Didelphis albiventris (Lund, 1841)	2.3	1.9			10.6	6.7	0.6
Gracilinanus agilis (Burmeister, 1854)		3.8				4.0	
Monodelphis gr. *americana* (Müller, 1776)	0.8	1.9	2.2	1.1	9.4	2.0	2.5
Carnivora							
Conepatus simistriatus (Boddaert, 1785)	1.5						0.6
Richness (H')	8 (1.128)	7 (1.153)	5 (0.409)	4 (0.371)	3 (0.924)	6 (1.138)	7 (0.855)

Inside PARNA: Zabelê — Z; Sitio Oitenta — S80; Pedra Solta — PS; Guarita — G.
Outside buffer zone: Lagoa Dantas — LD; João Costa — JC and Coronel Jose Dias — CJD.
[1]Data published in Herrera et al. 2005.
[2]Our data.
(N, total number of individuals captured per species/total sampling effort; the result was multiplied by 1000).

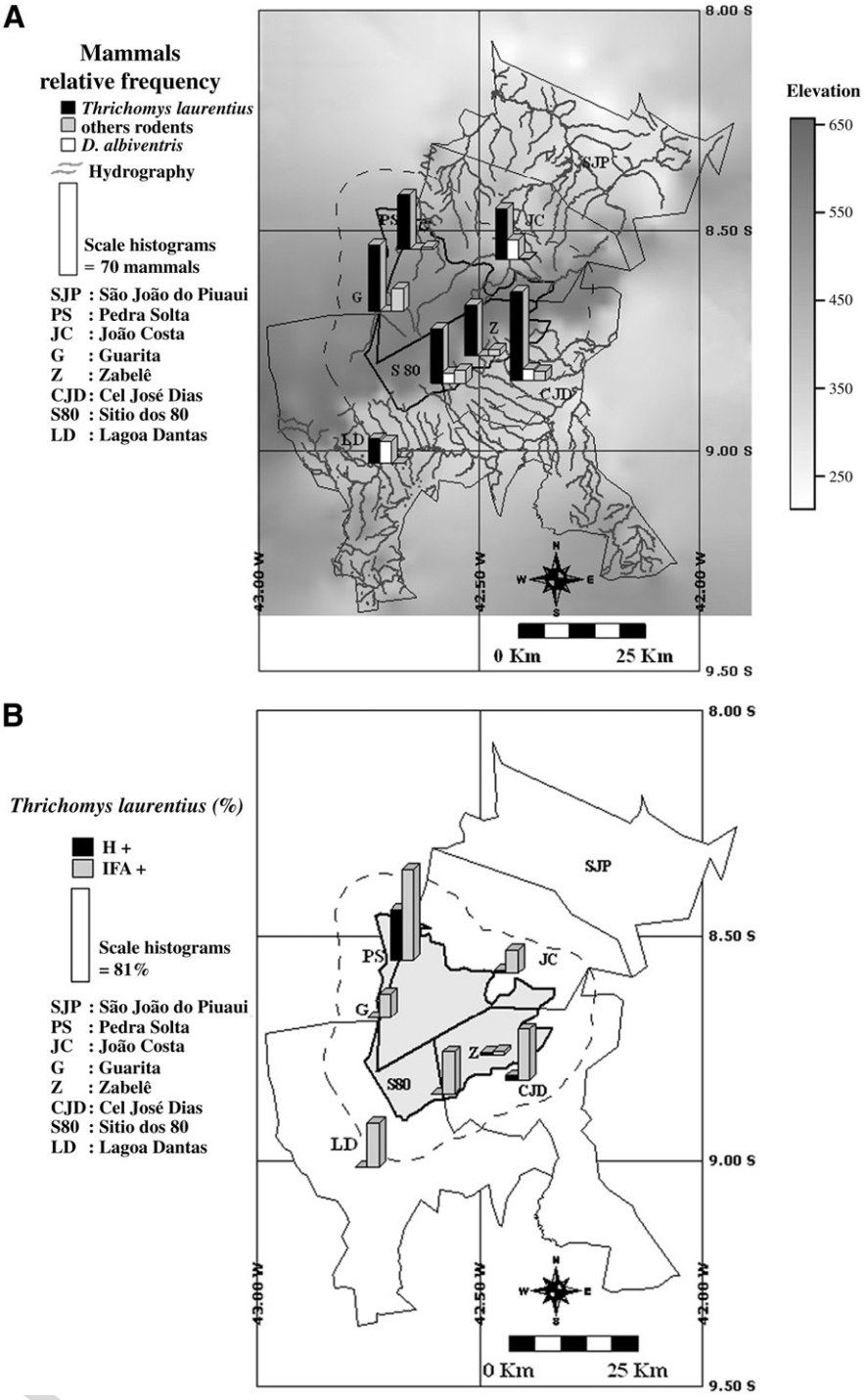


Fig. 2. A. Mapping of distribution of relative frequency of small wild mammals in PARNA and it surroundings: influence of local relief and hydrography on their occurrence. Black bars represent the frequency of *Thrichomys laurentius*; gray bars represent the frequency of other rodents; white bars represent the frequency of Marsupials. B. Mapping of distribution of infected *Thrichomys laurentius* in PARNA and it surroundings: black bars represent individuals with positive hemoculture and positive Indirect Immunofluorescense Assay (IFA); gray bars represent individuals with positive Immunofluorescense Assay (IFA) only. C. Mapping of distribution of *Trypanosoma cruzi* in PARNA and it surroundings: local relief and distribution of the two main triatomine species, *Triatoma sordida* and *Triatoma brasiliensis*. Distribution of infected *Thrichomys laurentius* (black rodent — infected *Thrichomys laurentius*; white rodent — not infected). D. Mapping of distribution of infected *Didelphis albiventris* in PARNA and its surroundings: black bars represent individuals with positive hemoculture and gray bars represent individuals with positive Indirect Immunofluorescense Assay (IFA). E. Mapping of distribution of *Trypanosoma cruzi* sub-populations in PARNA and it surroundings: black bars represent *T. cruzi* I, gray bars represent the *T. cruzi* II. White bars represent the animal infected wit *T. cruzi* Zimodeme 3.

The independence between the major *T. cruzi* sub-population distribution, according to conservation status of the study area, was tested using the Chi-Square test ($p \leq 0.05$).

2.7. Cartographic data

The original coordinates, referenced to WGS-84 (World Geodetic System 1984), were converted to SAD-69 (South American Datum 1969) according to the IBGE (Institute Brasilian of Geography and Statistics) methodology. In order to correlate the cartographic and environmental data in the study area, the focal scale 1/100,000 was used in agreement [24].

The data were filed in raster and vector formats. The analogical data for the base map was supplied by the Direction of Geographic Service (DSG — Brazilian Army Cartographic Service) in the Survey Division at Rio de Janeiro and by the Museum of the American Man Foundation in Piauí, Brazil, FUMDHAM.

The analogical maps were digitalized in an OCÉ 4720 (600 dpi) B&W Scanner, geo-referenced using the I/IRAS B Image Raster Program (Micrograph Co.) and vectorized with a semi-automatic process (I/GEOVEC, Micrograph Co.). The data were unified (MicroStation 95, Bentley Co.) and validated (topologic structuration in MGE, Bentley Co.) to provide the GIS analysis.

The integration of the thematic and non-graphical (biological) data with the base map was done in data base file (DBF) and shape formats (SHP) used by ESRI ArcView 3.2a. This software was also used for the final implementation of the here named Parasitary GIS. The model was constructed using Geographical Information System (GIS) from individual data layers representing district boundaries, hydrography, elevation and dwellings.

2.8. Data analysis

In order to determine the distribution of TCI and TCII infected mammals, as well as the area of distribution of the triatomine species, data of collected insects, characterized isolates of the parasite, non infected or infected small wild mammal species considering positive hemocultures and IFA, were geo-referenced individually and/or paired and were registered in ArcView GIS 3.2a. Maps with the discriminated data/locality were prepared.

3. Results

3.1. Small wild animals studied

Diversity and abundance of small wild mammal species are displayed in Table 1, showing that diversity inside PARNA was the same to that outside PARNA ($F=0.003$; $p=0.960$). However, outside PARNA, where the areas are submitted to strong anthropic action, a higher number of *D. albiventris* was captured.

Concerning terrestrial relief, a variation in altitude in the range of ≅200 m and ≅660 m was verified in all studied sites. GIS analyses revealed that the richness of the small

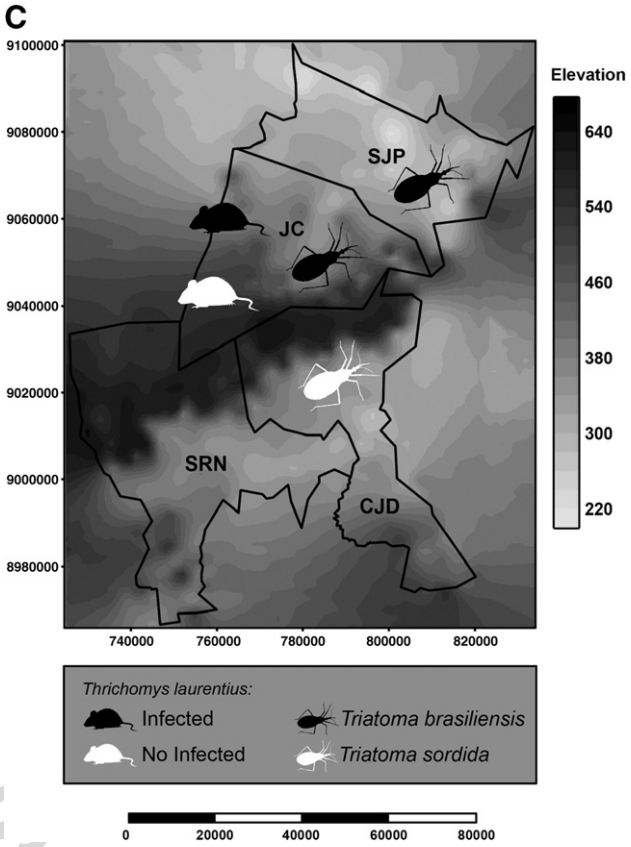


Fig. 2 (*continued*).

wild mammals was influenced by the local relief, with an additional more indirect effect due to water availability (Fig. 2A).

Indeed, the surrounding lowlands of PARNA, that are more xeric, displayed a lower richness of small wild mammals species captured. The most frequent species in the collection sites was *T. laurentius*, a caviomorph rodent that, in the semi-arid environment of Caatinga, lives mainly associated to rocky places (Fig. 2B).

Besides the animals collected by traps, 13 armadillos (12 *Dasypus novemcinctus* and 1 *Euphractus sexcinctus*) apprehended from illegal hunters by local authorities inside PARNA were examined for the presence of *T. cruzi* infection. Results from these analyses were passed onto these local authorities upon determination.

3.2. Collection of triatomines

In SJP a total of 702 triatomines (respectively 12% adults and 88% nymphs) were collected. Only *T. brasiliensis* could be observed. Infection was limited to two adults and one 5th instar nymph. The insects were not evenly distributed: there were heavily populated houses next to houses free of triatomines.

The present observation when analyzed through GIS, indicated that the mountain plateau acts as an ecological barrier for the *T. sordida* dispersion towards the northern part of the region (JC and SJP), explaining therefore the previously

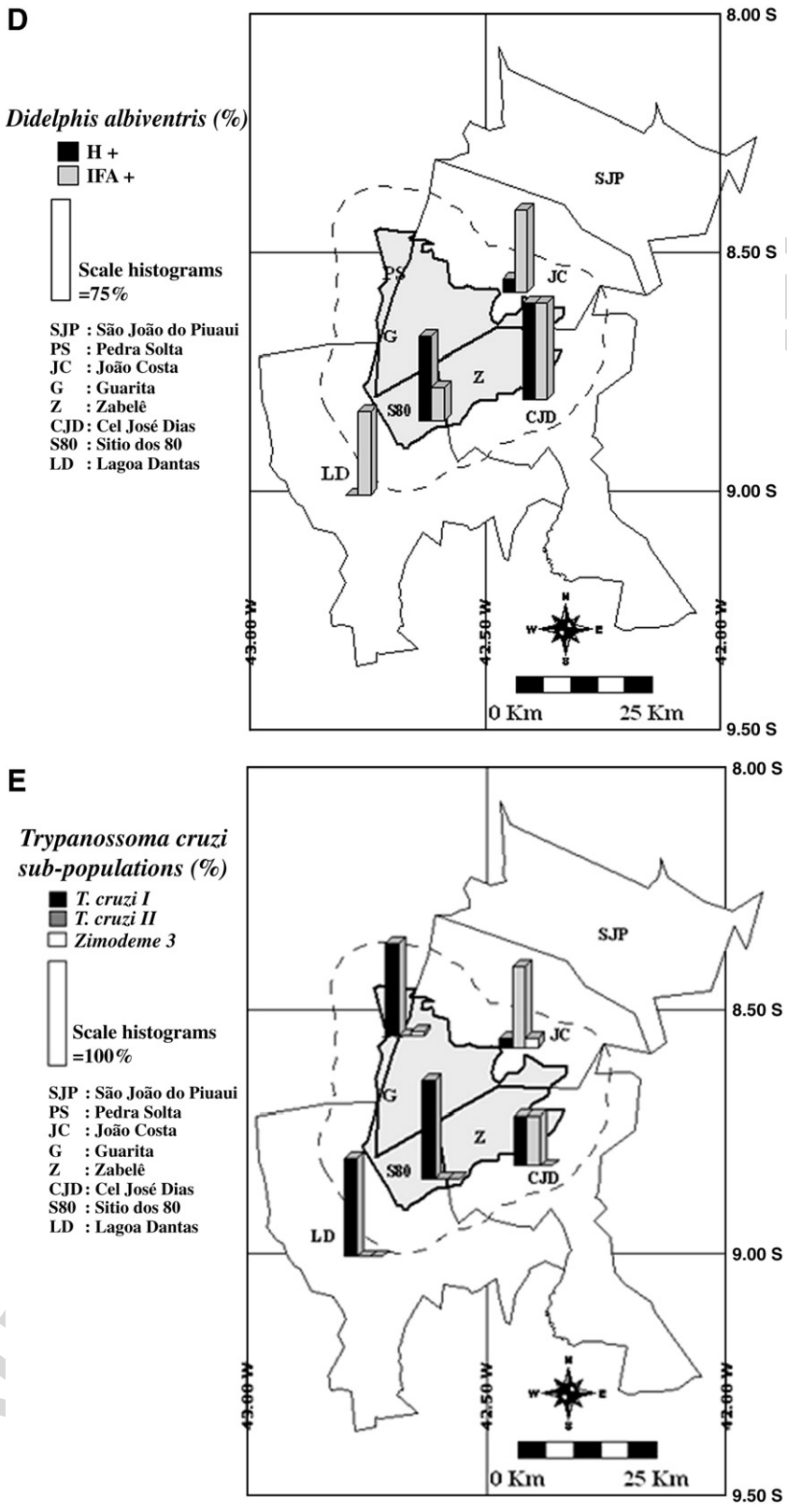


Fig. 2 (*continued*).

observed predominance of *T. sordida* in relation to *T. brasiliensis* in CJD, contrasting with the opposite scenario observed in JC ($\chi^2 = 1393$, $p < 0.001$; $df = 1$) (Table 2 and Fig. 2C).

3.3. T. cruzi infection pattern and small wild mammal distribution

Table 3 summarizes the results concerning prevalence of infection, richness of mammalian species and profile of infection

Table 2
Distribution of triatomine species and infection by *Trypanosoma cruzi* in the northern and southern parts of the "Chapada Serra da Capivara", analyzed by GIS

Herrera et al., [17]				Present study
Southern		Northern		
Sites	CJD	PS	JC	SJP
Triatomine species	+/total adults	+/total adults	+/total adults	+/total adults
Triatoma brasiliensis	1/84	8/10	81/1331	3/85
Triatoma sordida	6/371	–	0/14	–

Inside PARNA: Pedra Solta — PS; PARNA buffer zone: Coronel Jose Dias (CJD); João Costa (JC) and São João do Piauí (SJP).
– Species not captured.

Table 4
Distribution of *Trypanosoma cruzi* sub-populations in PARNA and it surroundings (*N* — isolated total number per collection sites)

Trypanosoma cruzi sub-populations	Inside PARNA		Outside PARNA		
	S80[1]	PS[1]	LD[2]	JC[1]	CJD[1]
	N	*N*	*N*	*N*	*N*
T. cruzi I	1	19	1	2	2
T. cruzi II				19	2
Zimodeme 3		1		2	

Inside PARNA: Sitio Oitenta — S80 and Pedra Solta — PS.
Outside buffer zone: Lagoa Dantas — LD; João Costa — JC and Coronel Jose Dias — CJD.
[1]Data published in Herrera et al. 2005.
[2]Our data.

of small wild mammals in G and LD. Briefly, in G a higher richness of mammalian species ($n=5$) was noticed in comparison to LD ($n=3$). Positive serological titers were observed in two rodent species, respectively, *T. laurentius* and *Rhiphidomys macrurus*. However, none of the 14 animals that tested positive by IFA displayed positive hemocultures. A different picture was observed in LD: in this heavily disturbed area, only 3 mammalian species could be trapped, and total number of seropositive animals observed by IFA was higher than in the G locality ($\chi^2=5143$, $p<0.05$; $df=1$). The only mammal species, *D. albiventris* with positive hemoculture ($n=1$) was also found in LD.

Parasitary GIS showed that *D. albiventris*, the species that displayed higher parasitemias as expressed by positive hemoculture, was more frequently found near human settlements (Fig. 2D). This species was also found at the border of PARNA and in its buffer zone, but not in the central part of PARNA (Fig. 2A). A distinct picture was noticed for *T. laurentius*, the more commonly found mammalian species, that demonstrated to be more habitat generalist in spite of being more frequent in rocky microenvironments (Fig. 2A). Specimens of *T. laurentius* with a higher potential of transmissibility, as expressed by positive hemocultures, were observed in the PS, Z, CJD and JC collection sites. None of armadillos analyzed was positive by *T. cruzi*.

3.4. Distribution of *T. cruzi* genotypes

Table 4 summarizes the results concerning the characterization of the *T. cruzi* isolates and their distribution. Parasitary GIS demonstrated that all animals infected with the genotype TCI were localized inside PARNA and in its buffer zone. The isolate obtained from the single animal that displayed positive hemoculture collected in LD, a locality that is heavily influenced by human activity, was also of the genotype TCI. Concerning distribution of the TCII genotype, Parasitary GIS demonstrated that the *T. cruzi* II infected hosts and vectors were localized only in the buffer zone of PARNA, corresponding to, respectively, JC and CJD, both counties where human cases of the infection were previously registered [16]. Z3 infected rodents were localized both inside PARNA and in its buffer zone isolated. This *T. cruzi* subpopulation was grouped with TCI [23] (Fig. 2E).

These data indicated that distribution of *T. cruzi* subpopulation is indirectly related with the conservation status of the study area. A higher number of TCI isolates was observed in the collections sites inside PARNA; TCII isolates were only detected in the collections sites localized in the buffer zone ($\chi^2=6421$ $p<0.05$; $df=2$) (Table 4 and Fig. 2E).

Table 3
Abundance of mammal species and prevalence of *Trypanosoma cruzi* infection by hemocultive (H) and immunofluoresence assay (IFA) in the National Park 'Serra da Capivara' (PARNA) and PARNA buffer zone, Piauí State, Brazil

Species diversity	Relative abundance	Guarita (G)		Lagoa Dantas (LD)		Total	
		Inside PARNA		Outside PARNA			
		H+/total	IFA+/total	H+/total	IFA+/total	H+/total	IFA+/total
Rodents							
Thrichomys laurentius	71 (73%)	0/52	11/50	0/19	7/18	0/71 (0%)	18/68 (26.5%)
Rhiphidomys macrurus	4 (4%)	0/4	3/4	–	–	0/4 (0%)	3/4 (75%)
Galea spixii	1 (1%)	0/1	0/1	–	–	0/1 (0%)	0/1 (0%)
Marsupials							
Didelphis albiventris	9 (8.4%)	–	–	1/9	6/9	1/9 (11%)	6/9 (66.7%)
Monodelphis domestica	9 (8.4%)	0/1	0/1	0/8	1/3	0/9 (0%)	1/4 (25%)
Total	106	0/70 (0%)	14/56 (25%)	1/36 (3%)	14/30 (46.7%)	1/106 (1%)	28/86 (32.5%)

*H=positive Hemoculture/total; **IFAs=positive Indirect Imunofluorescence Assay/total n.a.=not available.
– Species not captured.

4. Discussion

The sustainability of the success obtained by the Southern Cone Initiative in the control of Chagas disease, requires a more accurate knowledge of the factors that underlie the transmission cycle of this parasite in the wild, mainly, if it is considered that the epidemiology of this trypanosomiasis still displays unknown aspects. Indeed, the several outbreaks of Chagas disease ascribed to oral route in the in the Amazon region and in Santa Catarina are far from being clarified. Nevertheless, this is a highly complex study, since *T. cruzi* is a multihost parasite that displays a huge intraspecific heterogeneity and a complex transmission cycle that may exhibit local peculiarities even in a same biome.

Here, GIS showed the possible influence of the local relief in the transmission of *T. cruzi* in natural settings since it was observed that (i) the local relief hinders the northern dispersion of *T. sordida* explaining the distribution pattern of both major local triatomine species being therefore the reason for the higher abundance of *T. sordida* in relation to *T. brasiliensis* in CJD, (ii) an association exists between local hydrography and diversity of small wild mammals and (iii) that the local richness of small wild mammal species influences the overall *T. cruzi* prevalence.

The present results show that distribution of TCI and TCII in the study area was not directly determined by local relief. Moreover, the effect of local relief on the hydrography, and consequently on the mammalian species richness and on the vector fauna distribution, probably affect the transmission cycle of *T. cruzi.* Indeed, localities with higher mammalian richness, and consequently more complex host communities, offer a higher diversity of habitats to be exploited by *T. cruzi* subpopulations. These distinct habitats, represented by host species with distinct degrees of susceptibility, exert distinct selective pressures on the parasite subpopulations and consequently shape the local *T. cruzi* epidemiological scenario. In a similar way, the segregation of vector communities by relief can reduce the vectorial transmission of the parasite in the south part of the study area, because of the lesser competence of *T. sordida* in the transmission of *T. cruzi*, due to a lower rate of metacyclogenesis [17]. In addition, TCI display a broader dispersion in nature than TCII that is transmitted in more restricted cycles as is confirmed also by the herein presented data. Indeed, this genotype was detected only in JC and CJD.

The ability to transmit *T. cruzi* to its vectors depends, in the case of *T. cruzi*, on the parasitemia of the infected animal. In this sense, only *T. laurentius* of PS may actually be exerting the role of amplifier host [25], the higher prevalence of positive hemocultures observed in *T. laurentius* in this locality express a different profile of infection, in compared to other localities, that may be due to a recent epizootic outbreak. This situation contrasted with G locality, where infection by *T. cruzi* could be detected mainly by serological test (IFA), what suggested that *T. laurentius* in this locality appear to be acting as a maintenance host in an endemic cycle. This observation shows that the same mammal species may play distinct roles in the transmission cycle of a parasite even in a same biome. This point is reinforced by the absence of infection of armadillos that have previously been described together with opossums, as being the more important *T. cruzi* reservoirs [26].

That mammalian fauna diversity plays a role in the profile of the enzooty is shown by the high transmission focus described in a previous study, carried out in PS, a rocky environment where the more abundant species were the caviomorph rodents, *Kerodon rupestris* and *T. laurentius* [27]. Furthermore, here, a higher prevalence of infection was detected in LD that displays a lower richness of small wild mammalian species in comparison to G. All other sites studied showed a higher diversity index. Moreover, in LD where intense human action is exerted, a higher abundance of the synantropic marsupial species *D. albiventris* – considered to be an important reservoir of *T. cruzi* – was noticed. This increase of this marsupial species abundance is worth mentioning, especially as it has already been found infected with the TCII genotype that in Brazil is mainly associated with human infection [15,28].

The relief influences climatic conditions inside PARNA. The more mesic climate resultant of the presence of the plateau Serra da Capivara explains the richer mammalian fauna in the PARNA [29]. In addition to this, the infection of arboreal rodent *R. macrurus* shows that, inside PARNA, transmission of *T. cruzi* occurs in a complex way that involve both strata, ground and canopy.

Parasitary GIS revealed that the areas where triatomines have been collected in the peridomicile and inside domiciles, displayed homogeneous conditions concerning climate, relief and hydrography. It is highly probable that the observed differences concerning the presence of triatomines are explained by microclimatic conditions due to idiosyncrasies of humans managing their dwellings and yards [30,31].

Determination of the spatial distribution of the elements that compose the epidemiological chain of a parasitic disease is of pivotal importance for trend and risk evaluation. Moreover, it is worth mentioning that the control of a given multihost parasite based on the control of a single vector or host species may be insufficient given that parasite transmission very rarely relies in such a plain system. The simplification of mammalian host diversity, associated with an increase in the abundance of competent reservoir host species as described herein is certainly one of the risk factors involved in the reemergence of Chagas disease.

Acknowledgements

The authors thank lieutenant Fernandes for technical support, and Prof. Paulo Menezes, Laboratory of Cartography — UFRJ. We show gratitude to Dra. Vera Bongertz for her critical reading and revision of the manuscript. To Bruno Eschenazi for technical support treatment of the image. This study was supported by: IRD/CNPq No. 910157-00-6, FUMDHAM, FAPERJ; FIOCRUZ–Brazil, IME–Brazil.

References

[1] Tibayrenc M, Ward P, Moya A, Ayala FJ. Natural populations of *Trypanosoma cruzi*, the agent of Chagas disease, have a complex multiclonal structure. Proc Natl Acad Sci U S A 1986;83:115–9.

[2] Buscaglia CA, Di Noia JM. *Trypanosoma cruzi* clonal diversity and the epidemiology of Chagas' disease. Microbes Infect 2003;5:419–27.
[3] Briones MRS, Souto RP, Stolf B, Zingales B. The evolution of two *Trypansoma cruzi* subgroups inferred from rRNA genes can be correlated with the interchange of American mammalian faunas in the Cenozoic and has implications to pathogenicity and host specificity. Mol Biochem Parasitol 1999;104:219–32.
[4] De Freitas JM, Augusto-Pinto L, Pimenta JR, Bastos-Rodrigues L, Gonçalves VF, Teixeira SMR, et al. Ancestral genomes, sex, and the population structure of *Trypanosoma cruzi*. PLoS Pathog 2006;2(3): 226–35.
[5] Schofield C, Dias J. The Southern Cone Initiative against Chagas disease. Adv Parasitol 1999;42:1–27.
[6] Aras R, Gomes I, Veiga M, Melo A. Vectorial transmission of Chagas' disease in Mulungu do Morro, Northeastern of Brazil. Rev Soc Bras Med Trop 2003;36:359–63.
[7] Coura JR, Junqueira AC, Fernandes O, Valente SA, Miles MA. Emerging Chagas disease in Amazonian Brazil. Trends Parasitol 2002;18:171–6.
[8] Geosentinel. The Global Surveillance Network of the ISTM and CDC. Chagas Disease Outbreak-Brazil, Santa Catarina; 2005. http://www.istm.org/geosentinel/advisory.html accessed 23.05.2005.
[9] Pinho AP, Cupolillo E, Mangia Rh, Fernandes O, Jansen AM. *Trypanosoma cruzi* in the sylvatic environment: distinct transmission cycles involving two sympatric marsupials. Trans R Soc Trop Med Hyg 2000;94:509–14.
[10] Lisboa CV, Mangia RH, Rubião E, Lima NRC, Xavier SCC, Picinatti A, et al. *Trypanosoma cruzi* transmission in a captive primate unit, Rio de Janeiro, Brazil. Acta Trop 2004;90:97–106.
[11] Thompson RA, Wellington DE, Oliveira-Lima J, Maguire JH, Braud DH, Scholl DT. Climatic and demographic determinants of American visceral leishmaniasis in northeastern Brazil using remote sensing technology for environmental categorization of rain and region influences on leishmaniasis. Am J Trop Med Hyg 2002;67:648–55.
[12] Cohen J, Gürtler R. Modeling household transmission of American trypanosomiasis. Science 2001;299:694–8.
[13] Dykes J, MacEachren AM, Kraak M. Exploring Geovisualization. 1st edition. Elsevier: International Cartographic Association0-08-044531-4; 2005. 710 pp.
[14] Durmonteil E, Gourbiere S. Predicting *Triatoma dimidiata* abundance and infection rate: a risk map for natural transmission of Chagas disease in the Yucatan Peninsula of Mexico. Am J Trop Med Hyg 2004;70:514–9.
[15] Herrera L, D'Andrea PS, Xavier SCC, Mangia RH, Fernandes O, Jansen AM. *Trypanosoma cruzi* infection in wild mammals of the National Park 'Serra da Capivara' and its surroundings (Piauí, Brazil), an area endemic for Chagas disease. Trans R Soc Trop Med Hyg 2005;99: 379–88.
[16] Borges-Pereira J, Fonseca de Castro J, Furtado Campos J. Study of the infection and morbidity of Chagas' disease in municipality of Joao Costa: National Park Serra da Capivara, Piaui, Brazil. Rev Soc Bras Med Trop 2002;35:315–22.
[17] Herrera L, Pinho AP, Lorosa E, Xavier SCC, Emperaire L, Mangia RH, et al. Studies of triatomine infection with *Trypanosoma cruzi* in João Costa, Piauí, Brazil. Acta Parasitol 2003;48:1230–2821.
[18] Sluter JR. New theoretical research trends in cartography. Rev Bras Cartogr 2001;53:29–37.
[19] Guidon N, Arnaud B. The chronology of the New World: two face of one reality. World Archaeol 1991;23:524–9.
[20] Thornthwaite CW. An approach toward a rational classification of climate. Geogr Rev 1948;38:55–94.
[21] Camargo ME. Fluorescent antibody test for the serodiagnosis of American trypanosomiais. Technical modification employing preserved culture forms of *Trypanosoma cruzi* in a slide test. Rev Inst Med Trop São Paulo 1966;8:227–34.
[22] Fernandes O, Santos S, Junqueira A, Jansen A, Cupolillo E, Campbell D, et al. Populational heterogeneity of Brazilian *Trypanosoma cruzi* isolates revealed by the mini-exon and ribosomal spacers. Mem Inst Oswaldo Cruz 1999;94:195–7.
[23] Brandão A, Fernandes O. *Trypanosoma cruzi*: mutations in the 3′ untranslated region of calmodulin gene are specific for lineages *T. cruzi* I, *T. cruzi* II, and the Zymodeme III isolates. Exp Parasitol 2006;112:247–52.
[24] Romaña C, Emperaire L, Jansen AM. Enfoques conceptuales propuestas metodológicas para el estudio de las interacciones entre el medio ambiente y la salud: aplicación a un programa de investigación sobre la tripanosomiasis americana. Cad Saúde Pública 2003;19:945–53.
[25] McMichael AJ. Environmental and social influences on emerging infectious diseases: past, present and future. Philos Trans R Soc Lond 2004;359:1049–58.
[26] Yeo M, Acosta N, Llewellyn M, Sánchez H, Adamson S, Miles GAJ, et al. Origins of Chagas disease: *Didelphis* species are natural hosts of *Trypanosoma cruzi* I and armadillos hosts of *Trypanosoma cruzi* II, including hybrids. Int J Parasitol 2005;35(2):225–33.
[27] Herrera L, Xavier SCC, Viegas C, Martinez C, Cotias PM, Carrasco H, et al. *Trypanosoma cruzi* in a caviomorph rodent: parasitological and pathological features of the experimental infection of *Thrichomys apereoides* (Rodentia, Echimyidae). Exp Parasitol 2004;107:78–88.
[28] Devera R, Fernandes O, Coura JR. Should *Trypanosoma cruzi* be called "*T. cruzi*" complex? A review of the parasite diversity and the potential of selecting population after in vitro culturing and mice infection. Mem Inst Oswaldo Cruz 2003;98:1–12.
[29] Mares MA, Willig MR, Lacher TE. The Brazilian Caatinga in South American zoogeography: tropical mammals in a dry region. J Biogeogr 1985;12:57–69.
[30] Lorenzo MG, Guarneri AA, Pires HHR, Diotaiuti L, Lazzari CR. Aspectos microclimáticos del hábitat de *Triatoma brasiliensis*. Cad Saúde Pública 2000;16:69–74.
[31] Walter A. Human activities and American trypanosomiasis. Rev Lit Parasite 2003;10:191–204.