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VERSITA

Trypanosoma cruzi-triatomine associations and the presence of mixed infections in single triatomine bugs in Paraná state, Brazil

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Abstract

Eighteen strains of *Trypanosoma cruzi* isolated from two species of triatomines in the state of Paraná, Brazil, were characterized molecularly using three strategies: RAPD (randomly amplified polymorphic DNA) with four primers, analysis of the D7 polymorphic region of the 24Sα rDNA, and RFLP (restriction fragment length polymorphism) of region 5' of the mitochondrial gene COII (cytochrome oxidase subunit 2). The phenogram constructed with the RAPD data showed that only three strains isolated from *Panstrongylus megistus* collected in the Municipality of Arapongas were grouped together in a sub-branch. None of the other 15 strains could be clustered according to triatomine species or geographical origin. The strains were grouped with the *T. cruzi* I reference sample, indicating closer association with the sylvatic transmission cycle of *T. cruzi* in the state of Paraná. However, analyses of the rDNA and COII gene polymorphisms revealed the presence of populations from both *T. cruzi* I and II major lineages. In half of the analyzed triatomines, we found parasites from both lineages coinfecting the same bugs. Of these, most (6/9) were isolated from *Triatoma sordida*, and 3/9 from *Panstrongylus megistus*. These results contribute to a better comprehension of the ecoepidemiology of Chagas' disease in Paraná, and raise questions about the role of studies of polyclonal population dynamics for controlling the transmission of *T. cruzi* to humans in this region.

Key words

Trypanosoma cruzi, triatomines, RAPD, rDNA, mitochondrial DNA

Introduction

Trypanosoma cruzi, the etiological agent of Chagas' disease, has a complex life cycle, circulating among humans, triatomines, and sylvatic and domestic reservoirs. This disease has a strong social and economic impact, because approximately 15 million people may be infected with the parasite in Latin America (WHO 2002), including 3.5 million in Brazil (Dias 1997). In the state of Paraná, the prevalence of Chagas' disease is 4% (Camargo *et al*. 1984, Silveira and Resende 1994).

Studies have shown that *T. cruzi* is a heterogeneous species, consisting of several subpopulations with different biological, biochemical, and genetic characteristics (Morel *et al*. 1980, Brener 1992, Steindel *et al*. 1993, Oliveira *et al*. 1997, Gomes *et al*. 1998, Macedo *et al*. 2004). Two main evolutionary lineages, *T. cruzi* I and II, have been identified by different methods (Anonymous 1999). These lineages diverge widely from each other, as shown by different markers including isoenzymes, randomly amplified polymorphic DNA (RAPD), and polymorphisms of nuclear genes (24Sα rRNA and mini-exon; Souto *et al*. 1996, Fernandes *et al*. 1999) and mitochondrial genes (COII; Machado and Ayala 2001, 2002; Freitas *et al*. 2006). Strains belonging to the *T. cruzi* I lineage possess zymodeme Z1, 24Sα rDNA group 2, mini-exon group 2, and mitochondrial type A. Strains belonging to the lineage *T. cruzi* II possess zymodeme Z2, 24Sα rDNA group 1, mini-exon group 1, and mitochondrial type C.

Zingales *et al*. (1999) reported that *T. cruzi* I and II circulate in two different ecotopes, which represent, respectively, the sylvatic and domestic transmission cycles of Chagas' disease. Recently, the data above confirm Yeo *et al*. (2005). At least in Brazil, the strains of *T. cruzi* II appear to be exclusively responsible for tissue lesions in chronic Chagas' disease

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(Freitas *et al*. 2005). Furthermore, the two main lineages show different behaviors in regard to the host, for example: virulence, capacity of cellular invasion, and infectivity (reviewed by Mortara *et al*. 2005); drug susceptibility (Toledo *et al*. 2003); and different capacities for vector transmission (Lana *et al*. 1998). Additionally, there are some strains that could not be adequately grouped with either of these two principal lineages. Among these strains are those which possess mitochondria type B, such as those belonging to zymodeme Z3, and other hybrid strains such as those characterized as 24Sα rDNA group 1/2 (reviewed by Macedo *et al*. 2004). Through the work of Brisse *et al*. (2001), these unclassifiable strains were identified as belonging to different sublineages, the so-called TCIIa, TCIIc, TCIId, TCIIe (Brisse *et al*. 2001). Recently, however, Freitas *et al*. (2006) demonstrated that the TCIIc in fact corresponds to a third major lineage, distinct from *T. cruzi* I or *T. cruzi* II, which was termed *T. cruzi* III.

Several species of triatomines are vectors for *T. cruzi*, including *Triatoma infestans*, *Panstrongylus megistus*, *Triatoma sordida*, and *Rhodnius prolixus*. Of these, *T. sordida* and *P. megistus* are the predominant species found in north/northwest Parana (Toledo *et al*. 1997, Guilherme *et al*. 2001). In southern Brazil, *P. megistus* is present in wooded ecotopes, only sporadically invades houses, and very rarely establishes colonies in a domicile. In contrast, *T. sordida* is a species of the "cerrado" (savanna), where it is most frequently present in dead trees and bird nests. This species is markedly ornithophilic, but is able to colonize houses when it reaches a critical density at which the available food is exhausted (Silveira and Rezende 1994). It has been suggested that different tribes of triatomines are preferentially associated with distinct *T. cruzi* genetic groups, hosts, and environments. For example, strains of the *T. cruzi* I lineage are more often associated with vectors of the tribe Rhodniini, the marsupial *Didelphis*, and palm trees; whereas strains of the *T. cruzi* II lineage and hybrid strains are more associated with vectors of the tribe Triatomini and with terrestrial mammals (Gaunt and Miles 2000, Brisse *et al*. 2001, Gaunt *et al*. 2003, Yeo *et al*. 2005, Westenberg *et al*. 2006).

Published data show that natural populations of *T. cruzi* are polyclonal and that subpopulations can be under selective pressure depending on the genetic constitution of the vertebrate hosts (Mangia *et al*. 2001, Macedo *et al*. 2004), on the different species of triatomines in which the parasite develops (Nehme *et al*. 2001, Kinoshita *et al*. 2003), and on the genetic constitution of these parasites (Lana *et al*. 1998). These questions can be better explored using random molecular markers, such as RAPD (Steindel *et al*. 1993, Tibayrenc *et al*. 1993, Oliveira *et al*. 1997, Gomes *et al*. 1998), or specific markers designed for analysis of nuclear genes, for example rDNA (Souto and Zingales 1993, Souto *et al*. 1996) or mitochondrial genes such as COII (Freitas *et al*. 2006). Taken together, these markers allow each strain to be differentiated, due to the strong discriminatory power of RAPD and at the same time to identify the principal lineages to which each isolate belongs, through analyses of rDNA and COII.

Considering the distinct ecotopes and feeding habits of *P. megistus* and *T. sordida* in north/northwest Paraná, the purpose of this study was to verify if these two species of triatomines are preferentially associated with distinct populations of *T. cruzi*, through RAPD and analyses of the polymorphism of the rRNA and COII genes.

Materials and methods

Parasites

We analyzed 18 strains of *T. cruzi* isolated recently from peridomestic environments in different localities in the north and northwest part of the state of Paraná, southern Brazil. Table I lists the strains and their respective hosts, the isolation method, and the geographical origin.The strains were cultured in LIT medium at 28°C until they reached 1×10^9 cells/ml. These cells were successively washed in KRT (Krebs-Ringer-Tris) buffer, and the parasite mass obtained was stored at –20°C. For the RAPD analysis, reference samples of *T. cruzi* I (Sylvio) and *T. cruzi* II (Esmeraldo) were used. For the rDNA and COII analyses, the standard samples used were *T. cruzi* I (Col1.7G2), *T. cruzi* II (JG), *T. cruzi* III (4166), and hybrid *T. cruzi* (SO3cl5). Samples of *Trypanosoma rangeli* isolated in Uberaba (state of Minas Gerais) and in the state of Santa Catarina were also included in this study, in order to test whether this species was present among the 18 strains.

Extraction and quantification of genomic DNA of T. cruzi

The DNA was extracted as described by Macedo *et al*. (1992). The cell mass was resuspended in 80 mM NaCl/45 mM

Table I. Strains of *Trypanosoma cruzi* isolated from *Triatoma sordida* and *Panstrongylus megistus* captured in peridomestic environments, northwest Paraná, Brazil

Strain	Host	Isolation method	Origin (PR/Brazil)
A3.16A	*T. sordida*	inoculation in mice	Sarandi
N2.14A	*T. sordida*	inoculation in mice	Paiçandu
N5.16A	*T. sordida*	xenoculture	Sarandi
N9.14A	*T. sordida*	xenoculture	Floresta
A1.14A	*T. sordida*	xenoculture	Paiçandu
N10.19	*T. sordida*	xenoculture	Paiçandu
N2.1A	*T. sordida*	inoculation in mice	Paiçandu
A2.1A	*T. sordida*	inoculation in mice	Paiçandu
A3.1A	*T. sordida*	inoculation in mice	Paiçandu
N120B	*P. megistus*	inoculation in mice	Doutor Camargo
PMARA31	*P. megistus*	inoculation in mice	Arapongas
PMARA38	*P. megistus*	inoculation in mice	Arapongas
PMARA40	*P. megistus*	inoculation in mice	Arapongas
PMARA41	*P. megistus*	inoculation in mice	Arapongas
PMARA60	*P. megistus*	inoculation in mice	Arapongas
PMARA61	*P. megistus*	inoculation in mice	Arapongas
PMARA67	*P. megistus*	xenoculture	Arapongas
PMARA68	*P. megistus*	xenoculture	Arapongas

EDTA, pH 8.0/1% SDS. Proteinase K (Sigma Company Ltd) was added at 0.1 mg/ml and the product was incubated at 37°C overnight. The extraction was done using phenol and phenol-chloroform, followed by precipitation with absolute ethanol.

The total DNA was resuspended in Low-TE buffer (Tris-HCl, pH 8.0/1 mM EDTA, pH 8.0) in a proportion of 100 µl/10^9 cells. The DNA solution was quantified by visual comparison, using known concentrations of DNA in 1% agarose gel electrophoresis followed by ethidium bromide staining. Following the DNA dosage was diluted to 1 ng/µl.

RAPD

Amplification was done in a MJ Research PTC-100 thermocycler in a final volume of 10 µl. In each reaction we used 1.0 U of Taq – DNA polymerase (Cenbiot, Rio Grande do Sul, Brazil), 125 µM of each dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, Tris-HCl (pH 8.5), 6.4 pmoles of the primer, and 1 ng DNA. The primers used were the M13F-40 "forward" (5'GTTTTCCCAGTCACGAC3'), L15996 (5'CTCCACCATTAGCACCCAAAGC3'), λGT11–F (5'GACTCCTGGAGCCCG3'), and A7 (5'GAAACGGGTG3'). The RAPD program consisted of an initial denaturation at 95°C for 5 min, 2 cycles of 30°C for 2 min for annealing, 1 min at 72°C for extension, and 30 s at 95°C for denaturing. This was followed by 33 cycles in which the annealing temperature was increased to 40°C. The final extension took place for 5 min at 72°C. After amplification, 5 µl of the product was submitted to electrophoresis in polyacrylamide gel at 4.5% (29:1), and visualized by silver staining as described by Oliveira *et al*. (1997). The gels were then photographed and dried between cellophane sheets.

rDNA

A divergent domain of the 24Sα ribosomal RNA gene was amplified using the primers D71 (5'AAGGTGCGTCGACAGTGTGG) and D72 (5'TTTTCAGAATGGCCGAACAGT), as described by Souto *et al*. (1996). After denaturing at 94°C for 4 min, the samples were submitted to 30 cycles at three temperatures (94°C/1 min, 60°C/1 min and 72°C/1 min). The reaction products were observed by silver staining in 7.5% polyacrylamide. Typically, products with 125 bp characterized strains of rDNA group 1 (*T. cruzi* II), products with 110 bp identified strains of rDNA group 2 (*T. cruzi* I), and products of both sizes characterized strains of rDNA group 1/2 (Souto *et al*. 1996, reviewed by Macedo *et al*. 2004).

COII

Amplification of subunit 2 of the cytochrome oxidase mitochondrial gene (COII) was carried out as recently described by Freitas *et al*. (2006), using the primers Tcmit-10 (5'-CCATATATTGTTGCATTATT-3') and Tcmit-21 (5'-TTGTAATAGGAGTCATGTTT-3'), designed to amplify a 375 bp DNA fragment of the *T. cruzi* maxicircle. Briefly, 1 ng of DNA was amplified in a total of 30 cycles in the following conditions: 30 s for denaturing at 94°C, annealing of the primer for 2 min at 48°C, and extension of the primer for 2 min at 72°C. In the amplified fragment of approximately 375 bp there are two polymorphic *Alu* sites at positions 128 and 313. The former is present only in mitochondrial type A (*T. cruzi* I), and the second only in mitochondrial type C (*T. cruzi* II); both are lacking in mitochondrial type B (*T. cruzi* III). Thus, for the RFLP analysis, the PCR products were digested with the *Alu*I restriction enzyme for 16 h according to instructions from the manufacturer (Promega) (Freitas *et al*. 2006). The digested products were analyzed in polyacrylamide gels visualized by silver staining.

Data analysis

The multiple band profiles on polyacrylamide gel of *T. cruzi* strains obtained by RAPD were visually scored and analyzed for polymorphism based on the presence and absence of bands. Based on RAPD data, distance matrixes between strains, taken two by two, were obtained by the arithmetic complement of Jaccard's similarity coefficient, using the FreeTree program (Pavlicek *et al*. 1999). Clustering was analyzed with the algorithm UPGMA (Unweighted Pair Group Methods of Arithmetic Means), according to Sneath and Sokal (1973), using the FreeTree program. Bootstrap analyses were based on 10,000 resamplings. Better presentation of the tree topology was obtained using MEGA 2.1 software (Kumar *et al*. 2001).

Results

RAPD

Figure 1A shows a representative RAPD gel obtained with the M13F-40 primer. Patterns of multiple bands, varying between 200 and 2,000 base pairs, are visible. No identical RAPD profile was observed among any of the strains studied. In general, the strains showed very similar band patterns. An average of 56% of the bands was shared between any two strains analyzed. Similar results were obtained for the other primers used. The phylogenetic relationship among strains was first obtained with 72 RAPD markers from the M13F-40 primer. Data were entered into a distance matrix of the complement of Jaccard's similarity coefficient and used to construct a phenogram (Fig. 1B). This showed that the strains could not be grouped according to triatomine species or geographical origin.

Taking all RAPD data together, the proportion of shared bands, considering the mean of all four primers, was 70%, among the strains of *T. cruzi* isolated from *T. sordida*, as well as among those isolated from *P. megistus*. There was also a high percentage (68%) of shared bands between the isolated strains of *T. sordida* and *P. megistus*, indicating a strong genet-

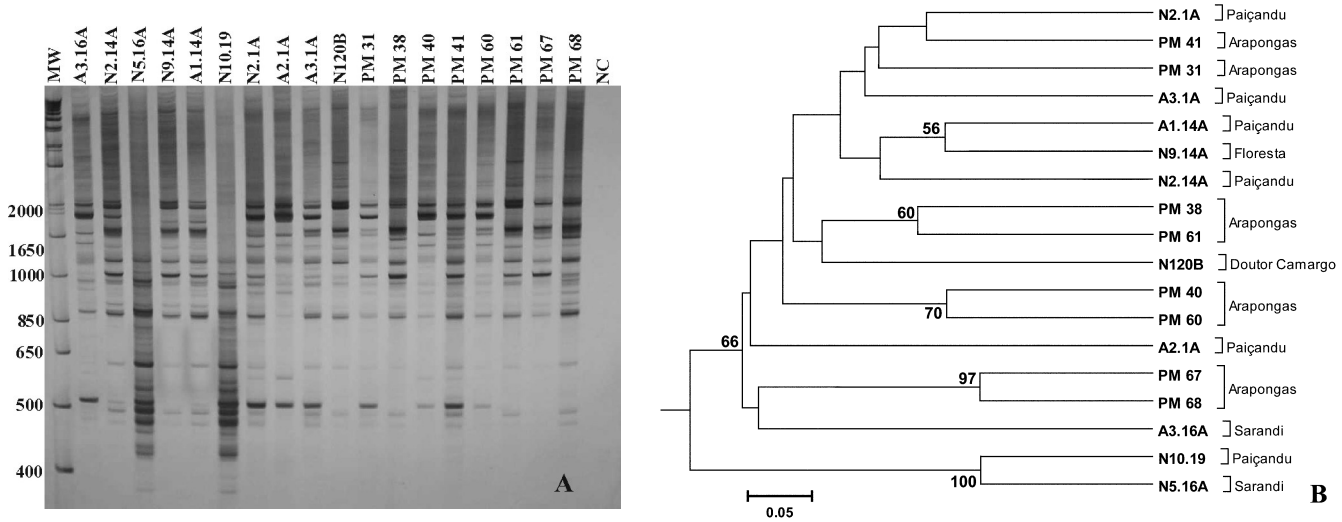

Fig. 1. A – RAPD profiles with the primer M13F-40 of 18 strains of *Trypanosoma cruzi* from northwest Paraná, in 4.5% polyacrylamide gel, silver-stained. Molecular weight size markers (MW) 1Kb Plus DNA Ladder (Invitrogen – Life Technologies) and (NC) negative control of the reaction which contains all the reagents except DNA; **B** – phenogram of 18 strains of *Trypanosoma cruzi*, constructed by the UPGMA method based on the complement of Jaccard's similarity coefficient from RAPD markers with primer M13F-40. The number on each node indicates bootstrap probability based on 10,000 resamplings; PM – PMARA

ic similarity between these strains. As seen in Figure 2, the phenogram constructed with the 210 RAPD markers from data with the four primers showed that only three strains isolated from *P. megistus* collected in the Municipality of Arapongas were grouped together in a sub-branch, and that most strains (15) could not be clustered according to triatomine species or geographical origin, as initially observed for the M13F-40 primer.

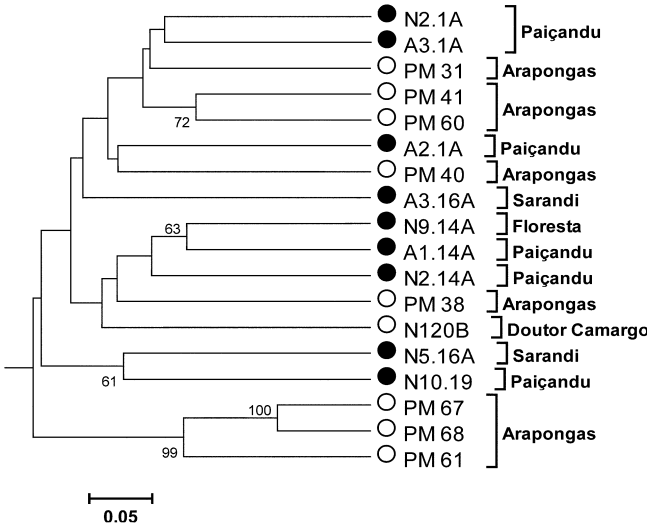

Fig. 2. Phenogram of 18 strains of *Trypanosoma cruzi*, constructed by the UPGMA method based on the complement of Jaccard's similarity coefficient from RAPD markers with four primers (M13F-40, L15996, lGT11 and A7). The number on each node indicates bootstrap probability based on 10,000 resamplings; PM – PMARA, white circle – *P. megistus*, black circle – *T. sordida*

The RAPD profile obtained with the λgt11 primer was initially used to estimate the genetic relationship between the strains studied and the reference samples *T. cruzi* I and II. All strains isolated from the two triatomine species in Paraná were grouped with the reference sample *T. cruzi* I (Sylvio). *T. rangeli* was not present among the strains studied (data not shown).

rDNA and COII

To confirm the lineages of the isolated populations of *T. cruzi*, the strains were analyzed for polymorphism of rDNA and COII; the results are summarized in Table II. In respect to rDNA, the majority (15/18) of the strains revealed amplified products of an unusual size of ~115 bp, and 9 strains showed amplification of more than one fragment (110/~115 + 125 bp). Of these, six (66.6%) were isolated from *T. sordida*, and three (33.4%) from *P. megistus*. RFLP analysis of the 5' region of the COII gene allowed us to confirm that the amplified product of ~115 bp was from strains of the lineage *T. cruzi* I (mitochondrial clade A), and that strains presenting more than one amplified rDNA fragment were mixtures of distinct populations, since they possessed two types of mitochondria (mitochondrial clades A and C) and not mitochondrial clade B, as would be expected for hybrid strains or from the *T. cruzi* III lineage.

Table II. Classification of the strains of *Trypanosoma cruzi* isolated from different triatomine species in northwest Paraná, Brazil, based on the 24Sα rRNA and COII gene polymorphisms

Strain or clone	Polymorphism of the D7 region of the 24Sα rRNA gene		Mitochondrial clade (RFLP of the 5' region of the COII gene)	Main lineages
	alleles (bp)	rDNA group		
A3.16A	~115; 125	1 + 2	A + C	[a]*T. cruzi* I* + [b]*T.cruzi* II
N2.14A	~115; 125	1 + 2	A + C	*T. cruzi* I* + *T.cruzi* II
N5.16A	~115	2	A	*T. cruzi* I*
N9.14A	~115	2	A	*T. cruzi* I*
A1.14A	~115	2	A	*T. cruzi* I*
N10.19	~115; 125	1 + 2	A + C	*T. cruzi* I* + *T.cruzi* II
N2.1A	~115; 125	1 + 2	A + C	*T. cruzi* I* + *T.cruzi* II
A2.1A	~115; 125	1 + 2	A + C	*T. cruzi* I* + *T.cruzi* II
A3.1A	~115; 125	1 + 2	A + C	*T. cruzi* I* + *T.cruzi* II
N120B	110	2	A	*T. cruzi* I
PMARA31	110; 125	1 + 2	A + C	*T. cruzi* I + *T.cruzi* II
PMARA38	110	2	A	*T. cruzi* I
PMARA40	~115; 125	1 + 2	A + C	*T. cruzi* I* + *T.cruzi* II
PMARA41	~115; 125	1 + 2	A + C	*T. cruzi* I* + *T.cruzi* II
PMARA60	~115	2	A	*T. cruzi* I*
PMARA61	~115	2	A	*T. cruzi* I*
PMARA67	~115	2	A	*T. cruzi* I*
PMARA68	~115	2	A	*T. cruzi* I*
Col1.7G2	110	2	A	*T. cruzi* I (Z1)
JG	125	1	C	*T. cruzi* II (Z2)
4166	119	Z3	B	[c]*T. cruzi* III (Z3)
SO3cl5	110; 125	1/2	B	*T. cruzi* hybrid (rDNA1/2)

[a]*T. cruzi* I* refers to strains which amplify the ~115 bp DNA locus (which is larger than that expected for the classic *T. cruzi* I of 110 bp). [b]*T. cruzi* II refers to the nomenclature as established in the Satellite Meeting (1999) and corresponds to TCIIb strains as denoted by Brisse *et al*. (2001). [c]*T. cruzi* III refers to a major line described by Freitas *et al*. (2006), and corresponds to the strains belonging to TCIIc as denoted by Brisse *et al*. (2001).

Discussion

In this study we sought to verify whether two species of triatomines were associated with distinct populations of *T. cruzi*. Strains of *T. cruzi* isolated from *T. sordida* and *P. megistus* were characterized, using three strategies of analysis of nuclear and mitochondrial DNA polymorphisms: RAPD, rDNA 24Sα, and RFLP of COII. Although a limited number of strains was analyzed, they were recently isolated from several localities in Paraná, and were representative of the north and northwest regions of the state.

The RAPD data obtained with the four primers indicated a close genetic similarity among the strains isolated from the two vector species. In general, the majority of the strains formed a closely related genetic group and could not be assigned to subgroups, either according to the hosts from which they were isolated, or their geographical origin. This genetic similarity detected by the RAPD is in accord with the results of Zalloum *et al*. (2005), who studied strains isolated from triatomines and sylvatic reservoirs in the same region. Limited genetic variability was also observed by Soccol *et al*. (2002), who analyzed, through isoenzymes, isolates of *T. cruzi* from two distinct regions of the state of Paraná, and by Carrasco *et al*. (1996), who observed homogeneous patterns of RAPD among strains of *T. cruzi* isolated from sylvatic reservoirs in different geographical areas of South and Central America. In this context, the genetic similarity observed among the samples studied here, appears to be a common finding for strains in the state of Paraná, and is probably not related to the method of isolation or to prolonged maintenance of the strains in the laboratory, because they were isolated in culture medium and immediately frozen at –196°C. This leads us to suppose that the triatomine species in northwest Paraná may be harboring populations of *T. cruzi* to which these species are more susceptible, and/or which are predominantly available in the region.

A preliminary estimate of the phylogenetic lineage of the studied strains was obtained by RAPD analysis using the primer λGT11. The phenogram constructed based on RAPD data with this primer indicated closer similarity of the populations analyzed with the *T. cruzi* I (Sylvio) reference sample, suggesting that the triatomines are predominantly associated with this lineage.

The same primer was also used to investigate the occurrence of *T. rangeli* in the populations isolated. Endemic and non-endemic regions of Chagas' disease in Brazil, such as Minas Gerais, Santa Catarina, and Amazonas, have *T. rangeli* as well as *T. cruzi* in the sylvatic environment (Steindel *et al*.

1991, Ramirez *et al*. 1998, Fernandes *et al*. 2001). Although up to now there are no reports of the presence of *T. rangeli* in the state of Paraná, this species was included in the study because the strains investigated were isolated from sylvatic hosts. Comparative analysis between the strains from northwest Paraná and samples of *T. rangeli* indicated that this species was not present among the strains studied here.

Confirmation of the principal lineages of the populations isolated was obtained by analysis of polymorphisms of the rDNA and COII genes. These analyses indicated the concomitant presence of populations of two principal lineages, *T. cruzi* I and II, with a predominance of *T. cruzi* I, as initially suggested by the RAPD data. These data are in agreement with recent findings that *T. cruzi* I and II circulate in north/northwest Paraná (Zalloum *et al*. 2005). These results also indicate that the strains analyzed here are more closely associated with the sylvatic transmission cycle of *T. cruzi* in this region, as reported for isolates from the sylvatic cycle in the most diverse endemic areas of Brazil and other countries of southern South America (Fernandes *et al*. 1997, Andrade 1999, Devera *et al*. 2003).

A result that merits attention is the apparent concomitant occurrence of *T. cruzi* I and II in a higher number of isolated populations of *T. sordida* (6/9) in relation to *P. megistus* (3/9). This finding should be evaluated considering the data from this region. Under the epidemiological conditions of north/northwestern Paraná, *T. sordida* predominates in peridomestic environments, with high population densities, making possible diversification in feeding (Guilherme *et al*. 2001) this may have contributed to the higher percentage of vectors infected by both lineages of *T. cruzi*. Furthermore, the proportionally higher occurrence of *T. cruzi* II in *T. sordida* is in accord with the literature, which shows a greater association of this genetic lineage with species of the genus *Triatoma* (Lana *et al*. 1998, Pinto *et al*. 2000, Yeo *et al*. 2005). On the other hand, a larger number of strains isolated from *P. megistus* (6/9) were only from the *T. cruzi* I lineage, in relation to *T. sordida* (3/9). A possible explanation for this is that in southern Brazil, in contrast to the situation in other regions of the country, *P. megistus* is associated preferentially with the sylvatic environment and invades houses only sporadically (Silveira and Rezende 1994).

A curious result observed in the present study, and until now unreported in the literature, was the predominance of *T. cruzi* I strains that showed a band of approximately 115 bp, distinct from the usual rDNA products of 110 and/or 125 bp. Furthermore, in 50% of the samples, more than one fragment (110 or ~115 bp + 125 bp) was found. Fragments of different sizes (~117 and 119 bp) have been previously reported for hybrid strains or for strains of the lineages *T. cruzi* IIa, IIc, IId, and IIe (Brisse *et al*. 2001) or for the lineage *T. cruzi* III (Freitas *et al*. 2006). However, RFLP analysis of the COII gene indicated that those strains which showed the unusual 115 bp fragment actually belonged to the *T. cruzi* I lineage (mitochondrial type A), and that strains with more than one rDNA fragment corresponded to polyclonal strains, consisting of populations belonging to two different lineages, *T. cruzi* I and II (which possess type A and C mitochondria respectively), coexisting in the same vector. Hybrid strains or strains belonging to the recently described *T. cruzi* III lineage (which both possess type B mitochondria) were not isolated in this study.

Literature data show that certain subpopulations of *T. cruzi* can be selected, and that this depends on the genetic constitution of the vertebrate hosts (Mangia *et al*. 2001, Macedo *et al*. 2004) and of the different triatomine species in which the parasite developed (Nehme *et al*. 2001, Kinoshita *et al*. 2003). In the present study, the RAPD analysis with the four primers did not allow us to detect any clear correlation between the genetic characteristics of populations of *T. cruzi* in north/northwest Paraná with the vector species from which they were isolated. However, the rDNA/COII analysis indicated that *P. megistus* was preferentially infected with *T. cruzi* I, in relation to *T. sordida*. A possible explanation is that in this region *P. megistus* is more closely associated with the sylvatic environment where this lineage predominantly occurs.

The results of the present study allow us to conclude that the strains did not show a clear association with the vector species from which they were isolated, or with their geographical origin; however, there was a slight predominance of *T. cruzi* I in *P. megistus*, and a mixture of *T. cruzi* I and II in a larger number of populations isolated from *T. sordida*. The existence of coinfections (*T. cruzi* I plus *T. cruzi* II) in the same vector specimens certainly contributes to better comprehension of the Chagas' disease ecoepidemiology aspects in Paraná, and raises questions about the role of studies of polyclonal population dynamics for controlling the transmission of *T. cruzi* to humans in this region.

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