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**Title:** Predominance of *Trypanosoma cruzi* I among Panamanian sylvatic isolates

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**Abstract**

*Trypanosoma cruzi* is throughout Panama, which is in agreement with the widespread of the sylvatic vectors implicated in the transmission. Eco-epidemiological changes in some regions of the country have led to a successful dissemination of the palm-tree *Attalea butyracea* and a possible adaptation of the primary vector of Chagas' disease to human settlements. These facts might increase both vector-human contact and human infection with different potentials *T. cruzi* genotypes and make therefore necessary a study to disclose Panamanian *T. cruzi* make-up. In this study, 71 *T. cruzi* isolates from *Rhodnius pallescens* were analyzed using mini-exon gene and sequence-characterized amplified region markers. The analyzed strains were *T. cruzi* lineage I. This finding along with prior results indicates that *T. cruzi* I is the principal genotype circulating in both sylvatic and domestic/peridomestic cycles and consequently responsible for the disease in the country.

**Key words:** Chagas' disease; *Trypanosoma cruzi* I; *Rhodnius pallescens*; Panama

There is a high morbidity and mortality caused by Chagas' disease from *Trypanosoma cruzi* infections. This disease has a broad spectrum of clinical presentations which vary in different geographical regions, possibly reflecting both parasite and host genetic factors (Vargas et al., 2004). Although *T. cruzi* undergoes predominantly clonal evolution, events of genetic exchange between sub-divisions of this parasite have been confirmed (Tibayrenc and Ayala 1988; Westenberger et al., 2006). However, these rare events of genetic exchange do not explain the overall genetic diversity found in *T. cruzi* (Tibayrenc and Ayala, 2002). Even though the genetic diversity of *T. cruzi* is still under study, researchers in the area have grouped this parasite into two major phylogenetic lineages, *T. cruzi* I and *T. cruzi* II (Satellite Meeting, Rio de Janeiro, Anon, 1999). Since then, *T. cruzi* II has been further subdivided into five well defined subgroups (Brisse et al., 2000).

Both lineages circulate in two transmission cycles; a sylvatic cycle involving different species of triatomine vectors and wild animals, and a domestic/peridomestic cycle in which household animals and humans act as reservoirs (Brisse et al., 2000a). In Panama, the vectors which transmit Chagas' disease are found in both sylvatic and peridomestic environments (palm-tree) in balance with the animals they feed on. *Rhodnius pallescens*, the main vector of *T. cruzi* in Panama, has regularly been found in sylvatic and domestic/peridomestic settings associated with palm-trees (*A. butyracea*) and opossum (Vasquez et al., 2004). Eco-environmental changes caused by human activities have led to a successful dissemination of *A. butyracea* from the forest to domestic/peridomestic settings (Romaña et al., 2000). This fact makes difficult the distinction between sylvatic and domestic transmission cycles of Chagas' disease in this country. Moreover, *R. pallescens* in the process of adaptation to human domestic

environments has been found in Panama (Calzada et al., 2006). This fact is important since a possible domiciliation of *R. pallescens* could increase human-vector contact and thus human infection by different *T. cruzi* genotypes from this sylvatic vector. Panamanian patients present the cardiac form of Chagas' disease rather than the digestive form. This cardiac tropism may be the result of a different histotrophic characteristic of the *T. cruzi* clonal lines circulating in the country. Likewise, the digestive form has not been found in other North and Central America countries (Ruíz et al., 2005) and northern South America countries where a specific *T. cruzi* genotype prevail (Añez et al., 2004).

These data support the idea that in Panama as in some other countries, there might be a different epidemiological picture of Chagas disease. This fact indicates we need a better understanding of the population structure of *T. cruzi*, especially the parasite circulating in the sylvatic cycle which represents a source of parasites that might be a threat for humans. This is of paramount importance because genotyping of sylvatic *T. cruzi* isolates will permit us to determine the genetic make-up of *T. cruzi* parasites and compare their genetic constitution with the different clinical profiles found in the country.

We have characterized the genetic profile of 71 *T. cruzi* isolates from *R. pallescens* 67 of which were collected on palm tree (n = 50) and four were found inside houses. The number of *T. cruzi* isolates from *R. pallescens* collected in various districts is listed in the Table. Faeces samples from *R. pallescens* were examined by light microscopy for parasitic protozoa and positive samples were subjected to passage in 3-weeks-old mice. To assess the infection in mice, blood samples were collected after two

weeks and daily evaluated by light microscopy for evidence of flagellates for a month thereafter. To produce parasite cultures, 100µl of blood was taken from positive infected mice and cultured in Seneckjies biphasic medium. Parasite cultures grown for 15 days were pelleted and DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega, USA) according to manufacturer instructions. We used a multiplex-PCR assay that can amplified subtelomeric sequences of *T. cruzi* and *T. rangeli* to determine the species following the procedure described elsewhere (Chiurillo et al., 2003). All the samples were found to be *T. cruzi* positive and there was no evidence of *T. rangeli* presence.

*Trypanosoma cruzi* genotyping was accomplished using a multiplex-PCR assay based on the amplification of the mini-exon gene (Souto et al., 1996). We also characterized *T. cruzi* isolates with sequence-characterized amplified region markers (SCAR) primers (Brisse et al., 2000) since lineage IIa and IIc can not be detected by the Mini-exon based PCR alone. PCR-amplifications on Panamanian *T. cruzi* isolates were performed using 25ng of DNA and the PCR-conditions previously determined for both techniques. All *T. cruzi* isolates showed characteristic products of *T. cruzi* I using SCARS primers and amplified a 350pb product consistent with *T. cruzi* I using the mini exon gene based approach.

As was expected, all *T. cruzi* isolates were found to be *T. cruzi* I by both techniques. This finding is in agreement with a previous study that determined *T. cruzi* I to be the primary genotype responsible for disease in humans from endemic areas in Panama who presented different clinical profiles (Sousa et al., 2006). Additionally, our results are supported by the isolation of *T. cruzi* I from two acute fatal cases of dogs and

one opossum which came from recognized endemic areas (data not shown). Altogether, these results suggest that the only genotype circulating in endemic areas of Panama is *T. cruzi* I.

In Panama, it is difficult to define the limits between sylvatic and domestic/peridomestic transmission cycles. There have been many eco-environmental changes caused by human intervention which has led to the successful spreading of the royal palm tree (*A. butyracea*) from sylvatic to peridomestic settings (Romaña et al., 2000). One important characteristic of most endemic areas, no matter which cycle is involved, is the constant presence of *Didelphis marsupialis*, primary blood source for *R. pallescens* (Vasquez et al., 2004). In addition, it is thought that *T. cruzi* I has an evolutionary history associated to both *Didelphis* living in arboreal habitats and *Rhodnius* species as primary vectors (Matthew et al., 2005). This fact could explain the high frequency of *T. cruzi* lineage I in humans and animals from sylvatic cycle as well. Nonetheless, we can not rule out the presence of other genotypes different than *T. cruzi* I as *D. marsupialis* along with other host reservoirs might be acting as natural filters selecting parasites sub-populations (*T. cruzi* I). Consequently, a more extensive study encompassing more animals, other species of vectors and vertebrate hosts including humans will help determine the overall genetic diversity of *T. cruzi* isolates coming from Panamanian sylvatic cycle.

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**TABLE**

*Trypanosoma cruzi* isolates from *Rhodnius pallescens* by locality

<i>R. pallescens</i> Habitat	Geographic Origin		No. of <i>T. cruzi</i> isolates
	Community	District	
Palm-trees	Burunga	Arraijan	1
	Loma Cova	Arraijan	24
	Mendoza	La Chorrera	20
	Caño Quebrado	La Chorrera	1
	Chilibre	Panama	21
Houses	Villa Grecia	Panama	1
	Lago Alajucla	Panama	1
	Buena Vista	Colon	2
	<b>Total</b>		<b>71</b>

# Panamá, República de Panamá



Instituto Conmemorativo Gorgas de Estudios de la Salud

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Editor-In-Chief  
Acta Tropica

Dear sir or madam:

We are pleased to submit you the paper entitled: "**Predominance of *Trypanosoma cruzi* I among Panamanian sylvatic isolates**" to be published in your journal if you consider it suitable.

This paper is an original research that reports the genetic characterization of *Trypanosoma cruzi* isolates from *Rhodnius pallescens*, the most important Chagas' disease vector in Panama. The results showed on this paper point at one genotype as the probably responsible of the disease in most endemic areas of Panama.

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Warm regards,

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