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ORIGINAL PAPER

Molecular characterization of *Trypanosoma cruzi* and infection rate of the vector *Triatoma dimidiata* in Costa Rica

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Abstract According to the genetic characterization by the analysis of the miniexon gene, strains of *Trypanosoma cruzi* can be classified into six discrete typing units (DTUs), and the DTU 1 into four distinct genotypes associated with different life cycles. While Chagas disease is endemic in Costa Rica, *T. cruzi* isolates from this region have never been genetically characterized. An analysis of 16 isolates from Costa Rica, based on miniexon gene analysis, showed the existence of two different haplotypes in the country, closely related to the Colombian haplotype group TcIa and to sequences from several Mexican isolates, with eight variable positions in the alignment and a variability of 2.6 % between the compared sequences. No relationship between the habitat, vector or host, and the haplotypes was found, suggesting an active flow of *T. cruzi* in the country. The present study also reports a very high infection rate (47.3 %, 26 out of 55 specimens) in a Costa Rican population of *Triatoma dimidiata*, the main vector of Chagas disease in this country. The distribution and abundance of the parasite and its main vector suggest a high risk of Chagas disease emergence in Costa Rica.

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Introduction

Trypanosoma cruzi, the etiologic agent of Chagas disease, shows a great genetic heterogeneity. In the last consensus nomenclature, *T. cruzi* isolates have been classified into six discrete typing units (DTUs), designated *T. cruzi* (Tc) I–VI (Zingales et al. 2009). The DTU TcI, previously known as TCI lineage, is prevalent in Central America and North America (Bosseno et al. 2002; Ruíz-Sánchez et al. 2005), although it is not the only DTU circulating in these regions (Roellig et al. 2008; Pennington et al. 2009).

The molecular characterization of this parasite can be approached with a wide range of techniques. Due to the large amount of information they provide, the most frequently used techniques are based on DNA hybridization and amplification, such as low-stringency single-primer polymerase chain reaction (Brito et al. 2008; Mejía-Jaramillo et al. 2009), single-stranded conformation DNA polymorphism (Higo et al. 2007), restriction fragment length polymorphism (Luna-Marín et al. 2009), random amplification of polymorphic DNA (Higo et al. 2007; Luna-Marín et al. 2009), and the sequencing of DNA molecular markers (Santos-Mallet et al. 2008; Venegas et al. 2010). In particular, the miniexon gene has been widely used as a taxonomic marker in *T. cruzi* and other kinetoplastids due to its high heterogeneity and the occurrence of large multicopy genomic arrays in the genome of these parasites (Thomas et al. 2005), and it is considered a

useful molecular marker for the assessment of TcI genetic variability (Fernandes et al. 2001; O'Connor et al. 2007; Santos-Mallet et al. 2008). Despite TcI was initially considered a relatively homogeneous clade, recent studies in isolates from Colombia have revealed four distinct haplotype groups for the miniexon gene, showing different habitat associations (Herrera et al. 2007, 2010).

Chagas disease is endemic in Costa Rica, and the prevalence of *T. cruzi* is mainly linked to zoonotic cycles (Zeledón et al. 1975; Calderón-Arguedas et al. 2002). Molecular characterization of *T. cruzi* has never been performed in Costa Rican isolates. Therefore, the aim of this work was to determine the DTU of *T. cruzi* samples from different hosts or vectors and different geographic locations of the country. In parallel, the infection rate of *T. cruzi* was analyzed in a Costa Rican population of *Triatoma dimidiata*, the main vector of *T. cruzi* in this country. This species is characterized by its capacity of occupying all kinds of habitats, complicating the control strategies against it. A high degree of recolonization of treated houses by peridomestic and wild adults has been reported in Costa Rica (Zeledón and Rojas 2006). Consistently, there is evidence of genetic flow among domestic, peridomestic, and sylvatic populations in the country (Blandón-Naranjo et al. 2010). In this context, elucidating the infection rate of this vector is highly relevant for the epidemiology of Chagas disease.

Material and methods

T. cruzi genetic characterization

A total number of 16 *T. cruzi* samples from five different Costa Rican provinces was analyzed (Fig. 1). These isolates were obtained from different hosts and vectors: three from humans, one from dog, one from opossum, nine from *T. dimidiata*, one from *Panstrongylus rufotuberculatus*, and one from *Rhodnius pallescens*. In addition, the samples were collected from different types of cycles, including domestic, peridomestic, and sylvatic (Table 1).

T. cruzi isolates from human patients and animals were obtained from ELISA-positive blood samples, which were collected in heparin tubes and centrifuged to remove plasma. The pellet was washed in physiological saline and resuspended and finally cultured in Rugai medium (Rugai 1941) until parasites were observable. Isolates from Triatominae were obtained from insects with presence of *T. cruzi* in their feces. The feces were resuspended and inoculated intraperitoneally in 2-week-old mice, and blood from seropositive mice was cultured in LIT medium (Camargo 1964).

DNA extraction from cultures was performed with Wizard Genomic purification kit (Promega Corporation, Madison, WI, USA) following manufacturer's instructions. The miniexon gene was amplified by multiplex PCR using standard conditions and primers for this region, namely, TC, TCI, and TCII primers (Souto et al. 1996). The previously characterized strains Silvio and Y were used as positive controls for *T. cruzi* I and *T. cruzi* II, respectively. PCR products were purified with UltraClean™ PCR Clean-up DNA purification system (MoBio, Solana Beach, CA, USA) according to manufacturer's protocol. Amplification products were analyzed in 1 % agarose gel, and purified products were sequenced by the dideoxy chain-termination method, using the same amplification PCR primers. Sequences were assembled, aligned, and compared using the software MEGA 5 (Tamura et al. 2011). Sequences of *T. cruzi* miniexon from countries other than Costa Rica, located using the NCBI basic local alignment search tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi), were used for sequence analysis and comparison.

T. cruzi infection of *T. dimidiata*

A total of 55 *T. dimidiata* (Fig. 2) specimens from Monteverde (Province of Puntarenas) were examined for *T. cruzi* infection. Insects were classified according to the place of capture as sylvatic (6 specimens; 4 females and 2 males), peridomestic (18 specimens; 7 females and 11 males), and intradomestic (31 specimens; 13 females and 18 males). Infection was determined by observing the presence of parasites in a feces drop obtained by pressure to the abdomen, diluting it in saline solution, and observing in a light microscope.

Results

T. cruzi genetic characterization

Electrophoresis analysis of PCR amplicons of the miniexon gene showed a molecular weight of 350 bp for all the detected bands, indicating that all the samples belonged to the TCI lineage or DTU 1 (Souto et al. 1996). The sequences obtained from the amplicons exhibited a length of 312 bp and a 41.4 % AT content. The sequencing evidenced the existence of two different sequences with only one mutation between them—one transversion from T to A in the first position of the alignment. The sequence with a T in the first position of the alignment (samples group A, GenBank accession number JQ028863) was predominant, being present in 11 of the 16 samples (68.75 %), while the sequence with an A in the first position of the alignment (samples group B, GenBank accession number JQ028864) was present in the remaining 5 samples (31.25 %) (Table 1). No relationship between the presence of this mutation and the origin of the sample could be observed.

BLAST analysis showed that both sequences were very similar (99–100 % of query coverage and maximum identity)

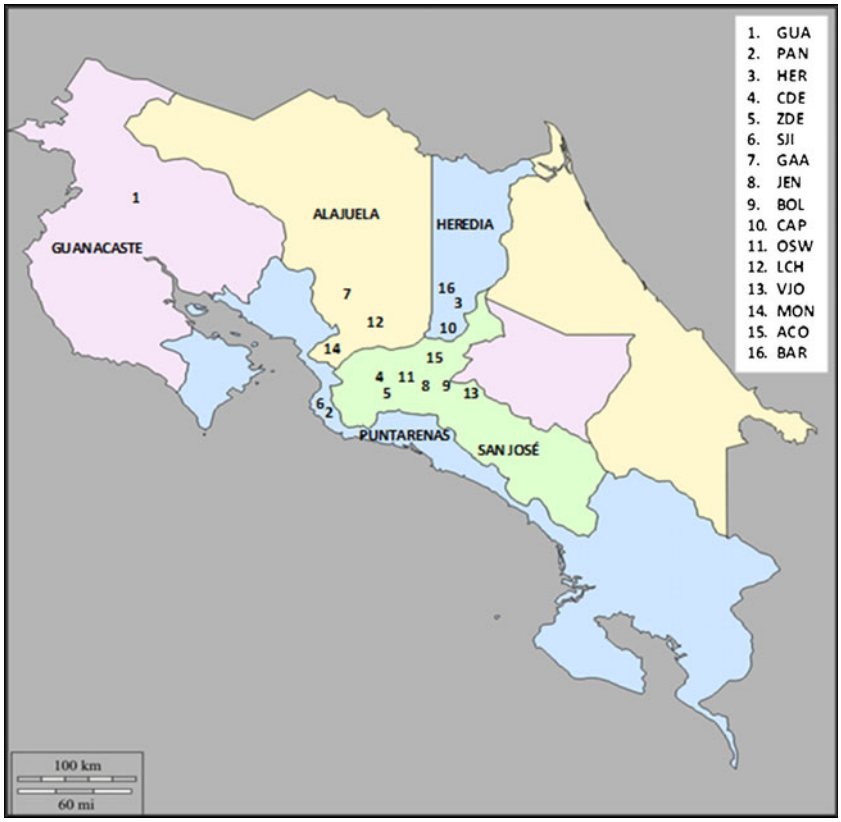

Fig. 1 Geographical location of the 16 *T. cruzi* samples

to several isolates with different geographical origins: the *T. cruzi* strains EBE (EU344771) and EMA (EU344772) from Colombia (Falla et al. 2009) and the strains Cari137 (EF576824), Gue536 (EF576827), H1 (EF576846), and Sba54 (EF576847) from Mexico (O'Connor et al. 2007). The variability between the compared sequences was 2.6 %, with eight variable positions (Table 2).

Other sequences found in GenBank with high similarity to the Costa Rican isolates analyzed herein were the strains USA115 (GU179075) and Flop2 (GU179077) from the

Table 1 Origin of the *T. cruzi* isolates and sequence group

Sample code	Host/vector	Geographical origin	Cycle type	Sequence group (GenBank accession number)
BOL (9)	Dog (*Canis familiaris*)	San José	Peridomestic	A (JQ028863)
JEN (8)	Human (*Homo sapiens*)	San José	Domestic	B (JQ028864)
SJI (6)	Human (*H. sapiens*)	Puntarenas	Domestic	A (JQ028863)
GAA (7)	Human (*H. sapiens*)	Alajuela	Domestic	A (JQ028863)
ZDE (5)	Opossum (*Didelphis marsupialis*)	San José	Sylvatic	A (JQ028863)
OSW (11)	Triatomine (*T. dimidiata*)	San José	Sylvatic	B (JQ028864)
CAP (10)	Triatomine (*T. dimidiata*)	Heredia	Domestic	A (JQ028863)
VJO (13)	Triatomine (*T. dimidiata*)	San José	Domestic	B (JQ028864)
MON (14)	Triatomine (*T. dimidiata*)	Puntarenas	Domestic	A (JQ028863)
ACO (15)	Triatomine (*T. dimidiata*)	San José	Domestic	A (JQ028863)
BAR (16)	Triatomine (*T. dimidiata*)	Heredia	Peridomestic	A (JQ028863)
GUA (1)	Triatomine (*T. dimidiata*)	Guanacaste	Peridomestic	B (JQ028864)
HER (3)	Triatomine (*T. dimidiata*)	Heredia	Peridomestic	A (JQ028863)
CDE (4)	Triatomine (*T. dimidiata*)	San José	Peridomestic	A (JQ028863)
PAN (2)	Triatomine (*P. rufotuberculatus)*	Puntarenas	Sylvatic	A (JQ028863)
LCH (12)	Triatomine (*R. pallescens*)	Alajuela	Sylvatic	B (JQ028864)

Figure 1 strain numbers are indicated in parenthesis

Fig. 2 Adult female of *T. dimidiata* from Costa Rica. Courtesy of Carlos Hernández, INBio

USA, the strains PASCh (GU179064), FRA (FJ713371), and Tev91 cl5 (FJ713402) from Argentina, and the strain TCC (FJ713401) from Chile (Cura et al. 2010). These sequences were not included in comparative analysis because of their shorter length and because they only overlap in a fragment of 265 bp with the sequences described in the present study.

T. cruzi infection rates in *T. dimidiata*

Twenty-six out of 55 *T. dimidiata* specimens (47.3 %) were infected with *T. cruzi*. Regarding the habitat where the insects were captured, the infection rates were 83.3 % for the sylvatic habitat (5 of 6 positive specimens), 44.4 % for the peridomestic habitat (8 of 18 positive specimens), and 41.9 % for the intradomestic habitat (13 of 31 positive specimens). Twelve out of 24 *T. dimidiata* female specimens were infected with *T. cruzi* (50.0 %), while 14 out of 31 male specimens were infected (45.2 %).

Table 2 Variable positions found in the alignment between groups A and B sequences and highly similar DTU I strains found through BLAST in GenBank

		Variable positions		
Sequence	GenBank acc. number	1	33333 00000 23456	33 00 89
Group A	JQ028863	T	CGACT	CG
Group B	JQ028864	A	CGACT	CG
EBE	EU344771	T	GCACT	CG
EMA	EU344772	A	GCACT	CG
Cari137	EF576824	A	GCCGA	TT
Gue536	EF576827	A	GCCGA	TT
H1	EF576846	A	GCCGA	TT
Sba54	EF576847	A	GCCGA	TT

Discussion

T. cruzi I has been reported to be associated with domestic cycles in southern and northern South America and sylvatic cycles in Central and North America. It is the most prevalent DTU affecting the northern countries of America, with sporadic cases in the southern countries. Recent studies have confirmed the presence of the TcI genotype in Argentina, Brazil, Bolivia, Chile, Colombia, Mexico, Panama, Paraguay, French Guiana, Venezuela, and the USA (Luna-Marín et al. 2009; Cura et al. 2010; Venegas et al. 2010; Guhl and Ramírez 2011). The geographical dispersion of *T. cruzi* DTUs could respond to two evolutionary phenomena: (1) a specific association between *T. cruzi* strains and certain mammalian hosts or (2) a geographical structuring between the strains of North and South America. In the case of *T. cruzi* I, a strong association with the *Didelphis* genus has been observed, while other DTUs show a preferable association with Armadillos (Yeo et al. 2005; O'Connor et al. 2007). From a clinical view, TcI is specifically related to chagasic cardiopathies, and the TcIa genotype is commonly found in the bloodstream of chronic patients (Venegas et al. 2010; Guhl and Ramírez 2011).

The sequences obtained for the Costa Rican isolates analyzed in the present work correspond to the group of sequences included in the genotype described in Colombia as TcIa, which exhibits a specific pattern at positions 28, with an adenine and a motif TGTGTG at positions 35–40. This genotype is associated with human infection and domestic vectors in Colombia (Herrera et al. 2007, 2010). In consistence with our results, this genotype is circulating in Costa Rica in all kinds of habitats, hosts, and reservoirs, although it is probably mostly linked to zoonotic cycles, including *Didelphis* as mammal host, since the disease has a relatively low impact in humans in this country (Calderón-Arguedas et al. 2002).

The vector infection rate detected in the present analysis of Costa Rican specimens (47.2 %) is one of the highest ever detected in a *T. dimidiata* population, only comparable with studies conducted in Guayaquil, Ecuador, 50 % infected (Gómez-Lince 1968) and in Barro Colorado, Panamá, 43 % infected (Sousa et al. 1983). Lower infection rates have been reported in other countries: 16 % in Yucatan, Mexico (Guzmán-Marin et al. 1992), and between 9.7 and 29.1 % in different departments of Guatemala (Monroy et al.

2003a, b). In Costa Rica, there is only one previous study of the infection rate of this vector, conducted between 2001 and 2002 in the Province of Heredia. This study revealed infection rates between 22.5 and 31.4 % in domestic specimens of *T. dimidiata* (Zeledón et al. 2005). In contrast, our findings, herein, show a remarkably high infection rate in the domestic habitat (41.9 %). Moreover, the infection rate that we found in the sylvatic habitat (83.3 %) exceeds all previous infection studies conducted in this vector and suggests that wild animals acting as reservoirs of the disease in the area of Monteverde may also be highly infected with *T. cruzi*.

T. dimidiata is a greatly widespread species in all Central America, exhibiting genetic flow between the populations of different habitats, and a very fast reinfestation of the houses after the insecticide treatments (Blandón-Naranjo et al. 2010; Zeledón and Rojas 2006). Given that *T. dimidiata* is currently the main vector of Chagas disease in Costa Rica, its ubiquitous presence in different habitats (Zeledón et al. 2001), together with the high infection rates reported in the present study underline the high risk of human infection in some areas of the country. In addition to *T. dimidiata*, *T. cruzi* is also circulating among other vectors in Costa Rica, such as *P. rufotuberculatus* and *R. pallescens*; species are mainly sylvatic, but potential secondary vectors. Factors as environmental damage can increase the risk of *T. cruzi* transmission in the peridomestic areas by these vectors, as it has been reported to occur in Northeastern Brazil with species such as *Triatoma pseudomaculata* and *Rhodnius nasutus* (Brito et al. 2008).

In summary, the present study establishes the basis for future analysis, involving *T. cruzi* in Costa Rica. Further studies are needed to elucidate the life cycles of the parasite, its relation with the vectors present in the country, and the clinical forms of the disease. This information will significantly improve the management of the treatment and control strategies of Chagas disease.

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