



## Short communication

Phylogenetic reconstruction based on Cytochrome b (Cytb) gene sequences reveals distinct genotypes within Colombian *Trypanosoma cruzi* I populations<sup>☆</sup>

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## ABSTRACT

Chagas disease caused by *Trypanosoma cruzi* comprises an important problem of public health in the Americas. This parasite has been recently divided into six Discrete Typing Units (DTUs) due to its high genetic diversity. We sequenced the Cytochrome b (Cytb) gene of 70 *T. cruzi* I Colombian clones finding four genotypes related to transmission cycles of Chagas disease in Colombia and also to specific hosts of *T. cruzi*. The genotypes herein described based on Cytb gene sequences are in accordance with those found using the mini-exon gene and reveals once again the enormous genetic diversity at sub-DTU level evidenced in *T. cruzi* I.

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Chagas disease is a complex zoonosis caused by the parasite *Trypanosoma cruzi*. This pathology currently affects 15 million people, and 21 million are at risk of acquiring the infection. In America, there are approximately 41,200 new cases and 12,500 deaths annually (WHO, 2007). The nomenclature of *T. cruzi* has recently been modified to reflect its high genetic variability. Six Discrete Typing Units (DTUs) have been proposed in *T. cruzi* (Zingales et al., 2009). Several studies have focused on elucidating the genetic variability within *T. cruzi* (Tc) II–VI, revealing the presence of hybrid groups as supported by the evidence of genetic recombination *in vitro* (Gaunt et al., 2003). Accordingly, TcII is considered to be an homogeneous group, TcIII and TcIV are considered to be hybrid groups created by potential recombination events between TcI and TcII, and TcV and TcVI are considered to be hybrid groups created by potential recombination events between TcIII/TcIV and TcII, though this last statement remains controversial and requires further research (Gaunt et al., 2003; Sturm and Campbell, 2009; Westenberger et al., 2005). Despite the fact that the nomenclature proposed in 1999 determined TcI to be an homogeneous DTU (Anon, 1999), many studies have since reported the genetic variability of TcI using different molecular markers (Cura et al., 2010; Falla et al., 2009; Herrera et al., 2007, 2009; Llewellyn et al., 2009; Mejía-Jaramillo et al., 2009; Ocaña-Mayorga et al.,

2010; Salazar et al., 2006; Spotorno et al., 2008; Triana et al., 2006).

Studies using a large number of TcI isolates have shown the high degree of genetic diversity within this group. In 2007, four genotypes were proposed (Ia–Id) in relation to the transmission cycles of Chagas disease in Colombia (Herrera et al., 2007, 2009). In 2010, these four genotypes were confirmed within the American continent, showing a defined geographical distribution, and one new genotype (Ie) was related to the domestic cycle in Chile and the sylvatic cycle in Bolivia (Cura et al., 2010). The microsatellite motif of the intergenic region of the mini-exon gene has been demonstrated to be highly polymorphic (Fernandes et al., 1998; O'Connor et al., 2007; Tomasini et al., 2010). However, new studies, based on other molecular markers, are required to propose suitable subdivisions within TcI, due to the recent description of the importance of TcI in the development of cardiomyopathies in Argentina (Burgos et al., 2010) and cardiac alterations in Colombia (Ramírez et al., 2010). In 2007, subgroups were reported within TcI isolates based on genetic polymorphisms in the Cytochrome b (Cytb) gene sequence (Spotorno et al., 2008). The objective of this study was to evaluate the genetic variability within TcI clones using the sequences of Cytb genes in Colombian clones and to determine whether any kind of concordance could be observed with the established genotypes proposed using other molecular markers.

We used the Cytb gene sequences to examine the possible phylogenetic relationships between a well characterized set of TcI clones from different geographical regions of Colombia (Table 1). The strains were cloned and from five to ten clones were obtained

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**Table 1**  
Clones from the isolates analyzed using direct sequencing of Cytb gene that were genotyped as TcI based on the intergenic region of mini-exon gene and the divergent domain of the 24S $\alpha$  rDNA.

International code	Abbreviated code	Host/vector	Geographical origin	Cycle	Mini-exon genotype	Cytochrome b genotype
MHOM/CO/92/FCH/6	FcH cl6	<i>Homo sapiens</i>	Norte de Santander	Domestic	Ib	A
MHOM/CO/04/MG/15	MG cl 15	<i>Homo sapiens</i>	Arauca	Domestic	Ib	A
MHOM/CO/92/JL/5	JL cl 5	<i>Homo sapiens</i>	Arauca	Domestic	Ib	A
MHOM/CO/03/CG/6	CG cl 6	<i>Homo sapiens</i>	Caquetá	Domestic	Ia	A
MHOM/CO/05/JEM/2	JEM cl 2	<i>Homo sapiens</i>	Putumayo	Domestic	Ia	A
MHOM/CO/01/DA/14	DA cl 14	<i>Homo sapiens</i>	Boyacá	Domestic	Ib	A
MHOM/CO/09/EH/1	EH cl 1	<i>Homo sapiens</i>	Santander	Domestic	Ib	A
MHOM/CO/07/EB/10	EEBB cl 10	<i>Homo sapiens</i>	Boyacá	Domestic	Ia	A
MHOM/CO/07/EM/12	EM cl 12	<i>Homo sapiens</i>	Boyacá	Domestic	Ia	A
MHOM/CO/YLY/14	YLY cl 14	<i>Homo sapiens</i>	Arauca	Domestic	Ia	A
MHOM/CO/07/DYR/15	DYR c 15	<i>Homo sapiens</i>	Boyacá	Domestic	Ib	A
MHOM/CO/FEC/15	FEC cl 15	<i>Homo sapiens</i>	Boyacá	Domestic	Ib	A
MHOM/CO/SP/12	SP cl 12	<i>Homo sapiens</i>	Casanare	Domestic	Ib	A
MHOM/CO/09/XCh/14	Xch cl 14	<i>Homo sapiens</i>	Santander	Domestic	Ib	A
MHOM/CO/07/SEV/12	SEV cl12	<i>Homo sapiens</i>	Boyacá	Domestic	Ib	A
MHOM/CO/09/CACQ/14	CACQ cl 14	<i>Homo sapiens</i>	Santander	Domestic	Ib	A
MHOM/CO/09/LER/11	LER cl 11	<i>Homo sapiens</i>	Santander	Domestic	Ib	A
MHOM/CO/09/LJVP/7	LJVP cl 7	<i>Homo sapiens</i>	Santander	Domestic	Ib	A
MHOM/CO/10/SMA/8	SMA cl 8	<i>Homo sapiens</i>	Santander	Domestic	Ib	A
MHOM/CO/09/LCV/3	LCV cl3	<i>Homo sapiens</i>	Santander	Domestic	Ib	A
MCAN/CO/00/H10/10	H10 cl 10	<i>Canis familiaris</i>	Boyacá	Peridomestic	Ib	B
MCAN/CO/10/AACf1/2	AACf1 cl2	<i>Canis familiaris</i>	Casanare	Peridomestic	Ib	B
ITr dimidiata/CO/Td3/10	Td3 cl 10	<i>Triatoma dimidiata</i>	Boyacá	Peridomestic	Ib	B
ITr dimidiata/CO/Td11/7	Td11 cl 7	<i>Triatoma dimidiata</i>	Boyacá	Peridomestic	Ib	B
ITr dimidiata/CO/Td/9	Td cl 9	<i>Triatoma dimidiata</i>	Boyacá	Peridomestic	Ib	B
IRHO/CO/SN11/3	SN11 cl 3	<i>Triatoma dimidiata</i>	Magdalena	Peridomestic	Ib	B
MDID/CO/00/Dm11/17	Dm11 cl 17	<i>Didelphis marsupialis</i>	Tolima	Synanthropic	Id	C
MDID/CO/D1/14	D1 cl 14	<i>Didelphis marsupialis</i>	Tolima	Synanthropic	Id	C
MDID/CO/00/Dm38/16	Dm38 cl 16	<i>Didelphis marsupialis</i>	Tolima	Synanthropic	Id	C
MDID/CO/7/6	Dm7 cl 6	<i>Didelphis marsupialis</i>	Tolima	Synanthropic	Id	C
MDID/CO/10/YDm1M/2	YDm1M cl 2	<i>Didelphis marsupialis</i>	Casanare	Synanthropic	Id	C
MDID/CO/10/YDm1B/3	YDm1B cl 3	<i>Didelphis marsupialis</i>	Casanare	Synanthropic	Id	C
MDID/CO/D16/10	D16 cl 10	<i>Didelphis marsupialis</i>	Tolima	Synanthropic	Id	C
MDID/CO/D18/10	D18 cl 10	<i>Didelphis marsupialis</i>	Tolima	Synanthropic	Id	C
MDID/CO/10/AADm1/1	AADm1 cl 1	<i>Didelphis marsupialis</i>	Casanare	Synanthropic	Id	C
MDID/CO/10/SLDm2/9	SLDm2 cl 9	<i>Didelphis marsupialis</i>	Casanare	Synanthropic	Id	C
MDID/CO/10/SLDm1/9	SLDm1 cl 9	<i>Didelphis marsupialis</i>	Casanare	Synanthropic	Id	C
MDID/CO/10/NDm1/7	NDm1 cl 7	<i>Didelphis marsupialis</i>	Casanare	Synanthropic	Id	C
XXX/CO/00/Cepa2/6	Cepa 2 cl 6	Rodent	Casanare	Synanthropic	Id	C
XXXX/CO/91/Gal61/16	Gal61 cl 16	Rodent	Sucre	Synanthropic	Id	C
XXXX/CO/SR2/7	SR2 cl 7	Rodent	Casanare	Synanthropic	Id	C
MRAT/CO/10/NR1/10	NR1 cl 10	<i>Rattus rattus</i>	Casanare	Synanthropic	Id	C
MTAM/CO/10/YTT1/1	YTT1 cl 1	<i>Tamandua tetradactyla</i>	Casanare	Sylvatic	Id	C
MALO/CO/10/YAS1/2	YAS1 cl 2	<i>Alouatta spp.</i>	Casanare	Sylvatic	Id	C
IRHO/CO/SN5/7	SN5 cl 7	<i>Rhodnius prolixus</i>	Magdalena	Sylvatic	Id	D
IRHO/CO/10/AAD6/7	AAD6 cl 7	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/YB1/2	YB1 cl 2	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/YD1/5	YD1 cl 5	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/AAC1/3	AAC1 cl 3	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/AAB3/2	AAB3 cl 2	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/AAA7/5	AAA7 cl 5	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/SLF5/10	SLF5 cl 10	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/SLA9/7	SLA9 cl 7	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/NA3/4	NA3 cl 4	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/NB2/5	NB2 cl 5	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/SLB3/2	SLB3 cl 2	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/SLD2/1	SLD2 cl 1	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/10/NC2/9	NC2 cl 9	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/X380/11	X380 cl 11	<i>Rhodnius prolixus</i>	Boyacá	Domestic	Ic	D
IRHO/CO/X236/8	X236 cl 8	<i>Rhodnius prolixus</i>	Boyacá	Domestic	Ib	D
IRHO/CO/X1082/9	X1082 cl 9	<i>Rhodnius prolixus</i>	Boyacá	Domestic	Ib	D
IRHO/CO/X1544/10	X1544 cl 10	<i>Rhodnius prolixus</i>	Boyacá	Domestic	Ib	D
IRHO/CO/00/N5P14/7	N5P14 cl 7	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/00/Rp540/9	Rp540 cl 9	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IRHO/CO/00/PAL/7	Palmas cl 7	<i>Rhodnius prolixus</i>	Casanare	Sylvatic	Id	D
IEcuspidatus/CO/10/SLD1Ec/6	SLD1Ec cl 6	<i>Eratyrus cuspidatus</i>	Casanare	Sylvatic	Id	D
Itmaculata/CO/10/TmPA1/6	TmPA1 cl 6	<i>Triatoma maculata</i>	Casanare	Sylvatic	Id	D
XXX/CO/00/Coy11/12	Coy11 cl12	<i>Rhodnius colombiensis</i>	Tolima	Sylvatic	Id	C
IRPAL/CO/00/Necocli/6	Necocli cl 6	<i>Rhodnius pallescens</i>	Antioquia	Sylvatic	Id	D
ITr venosa/CO/04/TV/9	TV cl 2	<i>Triatoma venosa</i>	Boyacá	Sylvatic	Id	D

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