

# ***Trypanosoma cruzi* I genotype among isolates from patients with chronic Chagas disease followed at the Evandro Chagas National Institute of Infectious Diseases (FIOCRUZ, Brazil)**

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## **Abstract**

**Introduction:** *Trypanosoma cruzi* is the etiologic agent of Chagas disease in humans, mainly in Latin America. Trypanosome stocks were isolated by hemoculture from patients followed at Evandro Chagas National Institute of Infectious Diseases (FIOCRUZ) and studied using different approaches. **Methods:** For species and genotype identification, the stocks were analyzed by parasitological techniques, polymerase chain reaction assays targeted to specific DNA sequences, isoenzyme patterns, besides sequencing of a polymorphic locus of *TcSC5D* gene (one stock). **Results:** The isolates presented typical *T. cruzi* morphology and usually grew well in routine culture media. Metacyclic trypomastigotes were found in cultures or experimentally infected *Triatoma infestans*. All isolates were pure *T. cruzi* cultures, presenting typical 330-bp products from kinetoplast DNA minicircles, and 250 or 200-bp amplicons from the mini-exon non-transcribed spacer. Their genetic type assignment was resolved by their isoenzyme profiles. The finding of TcI in one asymptomatic patient from Paraíba was confirmed by the sequencing assay. TcVI was found in two asymptomatic individuals from Bahia and Rio Grande do Sul. TcII was identified in six patients from Pernambuco, Bahia and Minas Gerais, who presented different clinical forms: cardiac (2), digestive with megaesophagus (1), and indeterminate (3). **Conclusions:** The main *T. cruzi* genotypes found in Brazilian chronic patients were identified in this work, including TcI, which is less frequent and usually causes asymptomatic disease, unlike that in other American countries. This study emphasizes the importance of *T. cruzi* genotyping for possible correlations between the parasite and patient' responses to therapeutic treatment or disease clinical manifestations.

**Keywords:** *Trypanosoma cruzi* genotypes. Chronic Chagas disease. TcI. Paraíba State. Brazil.

## **INTRODUCTION**

*Trypanosoma cruzi* Chagas, 1909 is the etiologic agent of the American trypanosomiasis or Chagas disease, one of the most important neglected human infections. About 6-7 million people worldwide are infected with *T. cruzi*, mainly in endemic areas of 21 countries of Latin America<sup>1</sup>. Since the 1990s, several successful initiatives for controlling the parasite vectors, and preventing transmission by blood transfusion or organ transplantation have drastically reduced the number of new cases of Chagas disease<sup>2</sup>. However, the international migration, generally of asymptomatic patients, from Latin American to non-endemic countries of North America (United States and

Canada), Western Pacific region (mainly Japan and Australia), and Europe (Spain, Portugal, France, and other countries), has spread the Chagas disease by non-vectorial routes. Nowadays, this disease has become an emerging global health problem<sup>3-5</sup>.

*Trypanosoma cruzi* is composed of heterogeneous populations of the parasite that circulate among humans, vectors, domestic animals and wild reservoirs<sup>6-8</sup>. The high genetic variability of *T. cruzi* has been confirmed by different approaches, and this species was classified in different sub-groups, as zymodemes<sup>6,7,9</sup>, major groups or lineages<sup>10,11</sup>, and thereafter in six discrete typing units (DTUs)<sup>12</sup>, which correspond to the six clusters of isozyme genotypes found by Tibayrenc and Ayala<sup>13</sup>. According to consensus among specialists, the main *T. cruzi* genetic types were correlated to those six DTUs, and they were renamed *Trypanosoma cruzi* I-VI (TcI-TcVI)<sup>7,14,15</sup>. Subsequently, a new genotype closely related to TcI was described in Brazilian bats<sup>16</sup> and named Tcbat or TcVII, although without consensus regarding its DTU assignment<sup>8,17,18</sup>.

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In Brazil, TcI (formerly Z1) is widely distributed among mammalian hosts within the wild cycle, mainly the common opossum, *Didelphis marsupialis*, but it can also be found in human acute infections and more rarely in chronic patients<sup>6,7,19-23</sup>. Otherwise, TcII (formerly Z2) and TcVI (formerly ZB or Paraguayan Z2) are mainly restricted to hosts within the domiciliary habitats, including humans<sup>7,8</sup>. All *T. cruzi* genotypes can be found in asymptomatic chronic patients. However, TcII is the most important agent of severe heart disease, including megacardia, and digestive syndromes, such as megaesophagus and megacolon, mainly in central and eastern regions of Brazil<sup>6-8,19,20</sup>. The TcVI genotype is prevalent in Southern Brazil (Rio Grande do Sul), and it has been also associated with cardiac and digestive forms<sup>7,8,24</sup>.

The present paper describes the characterization and DTU identification of nine *T. cruzi* isolates obtained from patients with chronic Chagas disease who were under ambulatory care at the Evandro Chagas National Institute of Infectious Diseases (INI, FIOCRUZ, Brazil). The finding of TcI among these isolates is emphasized, as well as the importance of the routine genotyping of samples from patients with Chagas disease to better understand possible correlations between the parasite and the human responses to drug treatment, or even disease clinical outcomes.

## METHODS

### Patients and trypanosome cultures

Patients with chronic Chagas disease who donated blood samples for the trypanosome isolation were under clinical care at INI and had not yet begun chemotherapeutic treatment. They presented different clinical forms and proceeded from the States of Pernambuco, Paraíba, Bahia, Minas Gerais, and Rio Grande do Sul. These patients were of both sexes and aged 44-66 years, as shown in **Table 1**.

For trypanosome isolation in cultures, blood samples (5mL) from each patient were collected in Vacutainer® tubes [ethylenediaminetetraacetic acid (EDTA), as an anticoagulant], divided in aliquots (~1.2 mL), and thereafter seeded into 16×150-mm screw-cap tubes containing blood-agar slants (NNN) overlaid with liver infusion tryptose broth (LIT) supplemented with 10% or 20% fetal calf serum, as described elsewhere<sup>25</sup>. Positive hemocultures were subsequently maintained in LIT and/or NNN+LIT at 27.3±0.4°C. When rich cultures from each isolate were obtained, samples of them were cryopreserved in liquid nitrogen (stabilates), which previously received a name and a code number for deposit in the Trypanosomatid Collection at the Oswaldo Cruz Institute (CT-IOC). For characterization studies, the stocks usually proceeded from stabilates, being grown in axenic cultures as aforementioned. After several passages, the subcultures only returned to the cryobank with another code number.

The following *T. cruzi* stocks were used as references: Y (CT-IOC 106), CL Brener (CT-IOC 005), Dm28c (CT-IOC 010), F (CT-IOC 003), and Colombian (CT-IOC 004). The references

TABLE 1

Data on the patients with chronic Chagas disease who donated blood for parasite isolation, and data on the isolates of *Trypanosoma cruzi* obtained from them, including their code number in the Trypanosomatid Collection (CT-IOC), molecular and biochemical characterization, and genotype assignment.

Patients	Data of the patients			<i>Trypanosoma cruzi</i> isolates					Molecular and biochemical analyses			Genotype
	Age (years)	Sex	Origin (Brazilian states)	Clinical form	Stock	CT-IOC	kDNA*	Mini-exon*	Zymodeme	DTUs		
L.F.S.	49	M	PE	Indeterm.	LFS49	537/542**	330	250	Z2	TcII		
J.M.C.	55	M	MG	Digestive (1)	JMC55	538	330	250	Z2	TcII		
M.C.J.B.	51	F	BA	Indeterm.	MCJB51	539	330	250	ZB	TcVI		
L.T.A.	47	F	RS	Indeterm.	LTA47	540	330	250	ZB	TcVI		
J.N.S.	65	M	PB	Indeterm.	JNS65	541	330	200	Z1	TcI***		
J.M.M.	51	M	BA	Indeterm.	JMM51	543	330	250	Z2	TcII		
J.J.C.	66	M	PE	Cardiac (2)	JJC66	544	330	250	Z2	TcII		
M.N.A.G.	44	F	MG	Cardiac (2)	MNAG44	545	330	250	Z2	TcII		
C.S.	53	M	MG	Indeterm.	CS53	553	330	250	Z2	TcII		

**M:** male; **F:** female; **PE:** Pernambuco; **MG:** Minas Gerais; **BA:** Bahia; **RS:** Rio Grande do Sul; **PB:** Paraíba; **Indeterm.:** indeterminate; (1) megaesophagus; (2) cardiac form, stage A<sup>33</sup>; **CT-IOC IOC:** Trypanosomatid Collection at the Oswaldo Cruz Institute; **kDNA:** kinetoplast DNA; **DTUs:** discrete typing units (TcI, TcII, TcVI). \*Data in base pairs (bp). \*\*CT-IOC 542 is a subculture of the isolate CT-IOC 537. \*\*\*Genotype confirmed by DNA sequencing and BLAST analysis.

of *T. rangeli* were H14 (CT-IOC 038; KP1+), SC-61 (CT-IOC 272; KP1-), and two isolates from Brazilian patients with Chagas disease<sup>25</sup>: US42 (CT-IOC 535) and APS56 (CT-IOC 536 and its subculture CT-IOC 546).

### Parasitological characterization

For trypanosome species identification, each isolate was first analyzed with regard to its morphological peculiarities in Giemsa-stained smears, as seen under optical microscopy ( $\times 1,000$ ), by comparing them with typical *T. cruzi* and *T. rangeli* forms<sup>25,26</sup>. Two biometrical parameters for distinguishing these species were also examined: the total length (TL, flagellum included) of the trypomastigotes and the length of rod-like kinetoplasts at their major axis (KL) of the epimastigotes<sup>25</sup>.

The biological behavior of these stocks was analyzed according to their ability to grow in routine culture media (LIT and NNN+LIT) and their cellular differentiation to typical *T. cruzi* metacyclic trypomastigotes. The percent of these stages was evaluated by counting 100-200 randomly chosen forms in Giemsa-stained slides. If necessary, the occurrence of metacyclics was searched in the gut of *Triatoma infestans* (third instar nymphs) experimentally infected through an artificial system (MM Lima: Personal Communication).

### Molecular and biochemical characterization

For molecular and biochemical analyses, parasite cells from axenic ecultures of each isolate were harvested by centrifugation (1,500 $\times$ g, 15 minutes, 4°C), washed twice in saline plus EDTA (0.1M, pH 8.0), and the pellets stored in liquid nitrogen until use. Genomic DNA from each isolate was extracted using DNAzol (Invitrogen), according to the manufacturer's instructions.

All isolates were first analyzed by polymerase chain reaction (PCR) targeted to sequences of their kDNA minicircles using the primers Tc121/Tc122, which reveal a single 300-bp amplicon (*T. cruzi*) or 760-bp and ~330-bp products (*T. rangeli*)<sup>25,27</sup>. Thereafter, a PCR multiplex assay based on the non-transcribed spacer of the mini-exon gene was performed using the primers Tc1/Tc2/Tc3/Tr/ME<sup>28,29</sup>, which can yield products with 200bp (TcI), 250bp (TcII and TcVI), 150bp (TcIII and TcIV), and 100bp (*T. rangeli*). The amplicons were electrophoresed on 1.6% agarose gels, stained with ethidium bromide, and visualized and photographed under ultraviolet light.

All isolates were also analyzed by multilocus enzyme electrophoresis (MLEE) at four selected *loci*, which enable the identification of *T. cruzi* DTUs, their mixtures, *T. rangeli*, and other trypanosome species<sup>13,24,25,28,30-33</sup>, as follows: malate dehydrogenase (MDH, E.C.1.1.1.37), glucose phosphate isomerase (GPI, E.C.5.3.1.9), phosphoglucomutase (PGM, E.C.2.7.5.1), and malic enzyme (ME, E.C.1.1.1.40).

For *T. cruzi* genotype confirmation, the isolate JNS65 (CT-IOC 541) was also analyzed using a PCR assay based on a 832-bp fragment of the *TcSC5D* gene from the *T. cruzi* CL-Brener genome (TcCLB.473111.10 and TcCLB.507853.10 *loci*) with the primers *TcSC5D*-fwd (5'-GGACGTGGCGTTTGATTAT-3') and *TcSC5D*-rev (5'-TCCCATCTTCTCGTTGACT-3')<sup>17</sup>. The amplified products were monitored on agarose gel electrophoresis

stained with 3% GelRed (Biotium). For amplicon sequencing, we used a terminator kit (BigDye, Applied Biosystems), and the sequencing was performed using a DNA analyzer (ABI PRISM® 3730, Applied Biosystems) at the Fiocruz Genomics Technological Platform. The acquired sequences were compared with others from the GenBank through the Basic Local Alignment Search Tools (BLAST).

### Ethical considerations

The inclusion of patients in this study and blood collections were performed after receiving approval from the Fiocruz Ethical Committee for Research in Humans (approval no.: 0050.0.009.000-05).

## RESULTS

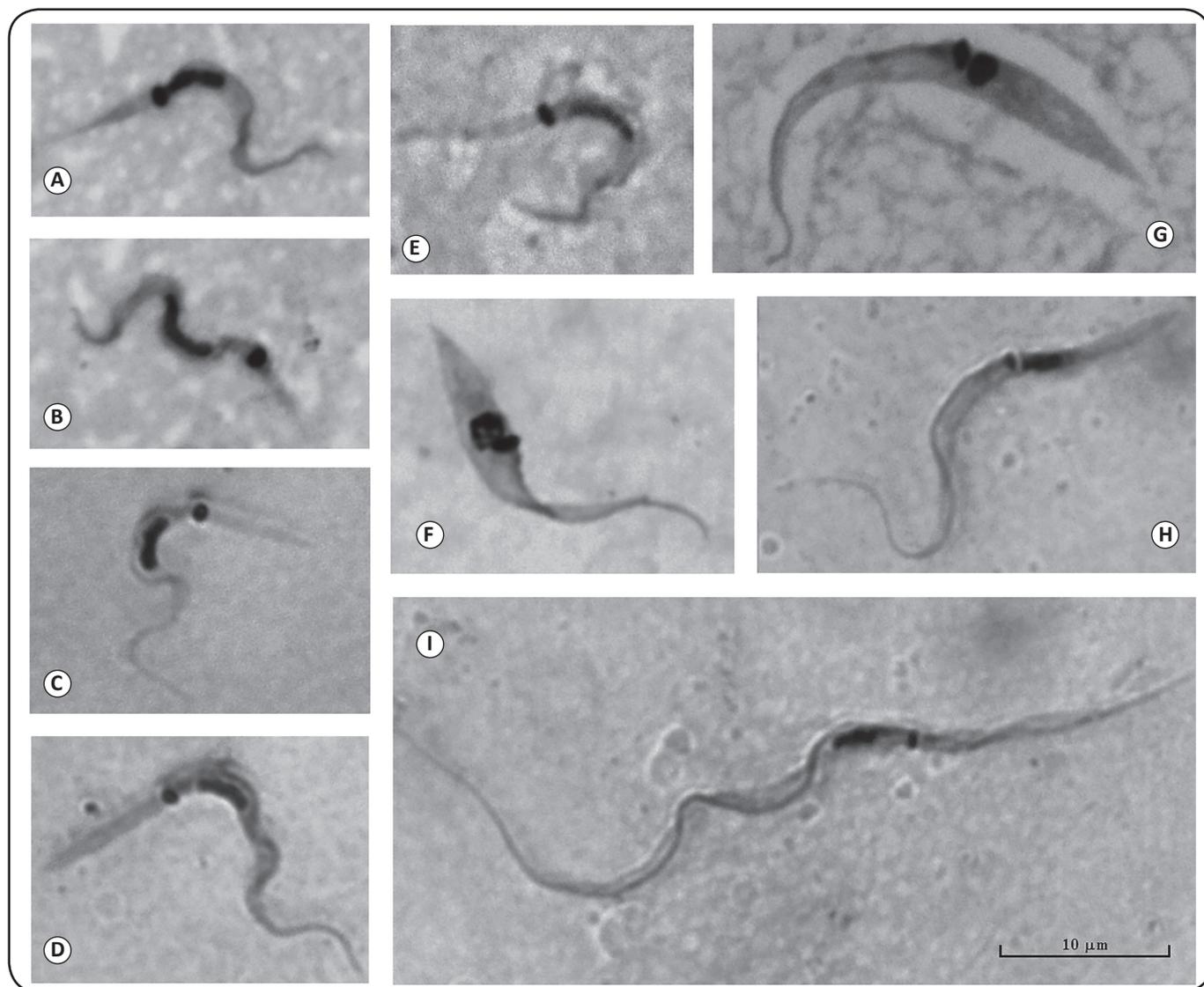
### Trypanosome cultures and parasitological characterization

The trypanosome cultures obtained from each patient and used throughout the present work were deposited in the Trypanosomatid Collection at the Oswaldo Cruz Institute, and identified with a name and a code number (CT-IOC), as shown in **Table 1**.

As seen in Giemsa-stained smears under light microscopy ( $\times 1,000$ ), all cultures only displayed typical *T. cruzi* stages regarding the general features of their epimastigotes and trypomastigotes with large kinetoplasts (**Figure 1A-G**), which were very distinct from those found in *T. rangeli* (**Figure 1H-I**). The TL of the trypomastigotes (metacyclics) averaged from 19.1 $\pm$ 2.5 $\mu$ m (CT-IOC 541) to 23.2 $\pm$ 0.5 $\mu$ m (CT-IOC 545), and the KL of the epimastigotes ranged from 1.6 $\pm$ 0.2 $\mu$ m (CT-IOC 544) to 1.8 $\pm$ 0.2 $\mu$ m (CT-IOC 553). Most of the isolates were able to grow both in LIT and NNN+LIT media, with the exception of the stock CT-IOC 538, which only grew in the latter condition. Typical *T. cruzi* metacyclic trypomastigotes (**Figure 1A-E**) were found in cultures of eight stocks at rates ranging from 2.9% (CT-IOC 538) to 19.8% (CT-IOC 543). The isolate CT-IOC 537/542 only presented metacyclics (16.7%) in the gut of experimentally infected *T. infestans*.

Results of the PCR using the primers Tc121/Tc122 showed that all isolates from the patients presented a single 300-bp amplicon derived from the kDNA minicircles, as the reference strain of *T. cruzi* (Y; CT-IOC 106) (**Figure 2A**). The multiplex PCR assay of the non-transcribed spacer of the mini-exon gene displayed 250-bp products in most isolates (CT-IOC 537-540, 543-545, and 553) and the Y reference strain (CT-IOC 106). Amplicons with 200bp were only found in the isolate JNS65 (CT-IOC 541) and TcI reference strains (CT-IOC 010 and 003) (**Figure 2B**).

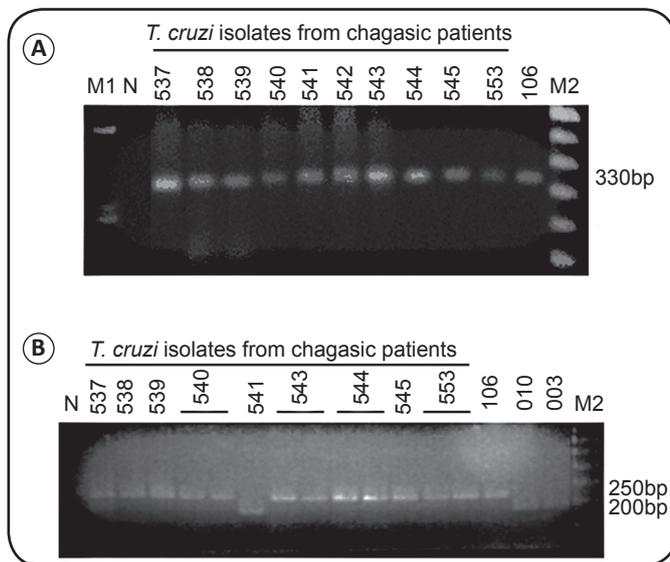
The isoenzyme analysis at MDH, PGM, and ME *loci* clearly distinguished *T. cruzi* from *T. rangeli* (**Figure 3** and **Figure 4**). The isoenzyme profiles of the *T. cruzi* stocks at GPI and PGM *loci* provided evidence of the zymodemes of the isolates from patients with Chagas disease, according to the reference strains (**Figure 4**). The Z2 pattern was found in six isolates (CT-IOC 537/542, 538, 543, 544, 545, and 553) and the Y reference strain (CT-IOC 106). The ZB pattern was found in the isolates CT-IOC 539, 540, and the CL Brener reference strain (CT-IOC 005). Only the isolate CT-IOC 541 (JNS65) presented the



**FIGURE 1 - Representative forms from axenic cultures of *Trypanosoma cruzi* and *Trypanosoma rangeli* isolates from patients with Chagas disease followed at the Evandro Chagas National Institute of Infectious Diseases (INI, FIOCRUZ).** *Trypanosoma cruzi* stages: metacyclic trypomastigotes (A-E), and epimastigotes (F, G). *Trypanosoma rangeli* stages: epimastigote (H), and trypomastigote (I) (CT-IOC 535). Compare the differences in size between the trypomastigotes of *T. cruzi* and *T. rangeli*, as well as their kinetoplasts. *Trypanosoma cruzi* genotypes are represented as follows. TcI: (A, B), CT-IOC 541. TcII: (C, D, F), CT-IOC 543 and 544. TcVI: (E, G), CT-IOC 539 and 540. Giemsa-stained smears under optical microscopy ( $\times 1,000$ ). All images have the same magnification.

ZI pattern, as Dm28c and Colombian stocks (CT-IOC 010 and 004, respectively). Otherwise, only discrete differences were found at the ME *loci* among the *T. cruzi* stocks, as follows (Figure 4). All Z2 strains presented identical profiles, and they could be distinguished from ZB isolates (CT-IOC 539, 540, and 005) at the ME-2 *locus*, and from Z1 stocks (CT-IOC 541, 010 and 004) at the ME-1 *locus*, being noteworthy that the isolate CT-IOC 541 and the Colombian strain (CT-IOC 004) presented the same pattern. Results and conclusions of the biochemical and molecular analyses are summarized in Table 1.

The acquired sequences from the amplification products of the isolate CT-IOC 541 were deposited in GenBank (accession number KX781993) and analyzed through BLAST. Its genetic similarity (99%) with several TcI strains and *Tcbat* (TCC1122 stock) was confirmed, as follows (GenBank accession numbers in parentheses): Sylvio X-10 (JN050585.1), PALV2 (JN050577.1), LL015 (JN050571.1), Dm28c (JN050567.1), JR cl4 (KC881183.1), Teda2 (JN050579.1), CAI72 (JN050565.1), and *Tcbat* (KC881185.1).

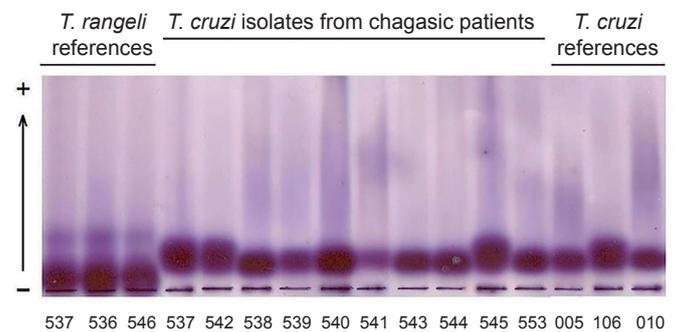


**FIGURE 2 -** Polymerase chain reaction products of *Trypanosoma cruzi* isolates from patients with Chagas disease followed at the Evandro Chagas National Institute of Infectious Diseases (INI, FIOCRUZ), and reference strains. **A:** Typical *T. cruzi* amplicons (330bp) derived from kDNA minicircles using the primers Tc121/Tc122. **B:** Amplicons from the mini-exon non-transcribed spacer using a polymerase chain reaction multiplex assay with the primers Tc1/Tc2/Tc3/Tr/ME; 200 and 250-bp bands were from TcI and TcII/TcVI genotypes, respectively. The numbers at the top of the gel indicate the code number of each stock in the Trypanosomatid Collection (CT-IOC). *Trypanosoma cruzi* reference strains: CT-IOC 106 (Y), 010 (Dm28c), and 003 (F). Negative control: (N). Molecular markers:  $\phi$ X174 DNA Hae digest (M1), and 100-bp ladder (M2).

## DISCUSSION

In a previous study, Sousa et al.<sup>25</sup> reported the finding of *T. rangeli* in two patients with Chagas disease who were under ambulatory care at the Evandro Chagas Clinical Research Institute (FIOCRUZ, Brazil), now known as INI. In the present paper, nine trypanosome isolates from patients with Chagas disease who were also followed at INI were characterized by different techniques for identifying the trypanosome species and its genetic type. Using classical parasitological approaches, such as the parasite morphological features (Figure 1), biometrical data, growth, and differentiation in routine culture media, all isolates under study were *T. cruzi*<sup>25,26,34</sup>. The confirmation that they were pure cultures was evidenced by their single 330-bp amplicon derived from the kDNA minicircles<sup>25,27</sup>, and their products from the mini-exon gene non-transcribed spacer<sup>28,29</sup> (Figure 2), which had 250bp in most isolates and 200bp only in one stock (CT-IOC 541) identified as TcI. The isoenzyme analyses also confirmed the presence of *T. cruzi* in all isolates (Figure 3), as well as resolved their DTU identification<sup>24,31,32,33</sup> (Figure 4 and Table 1).

The TcII genotype (formerly Z2) is the main agent of chronic Chagas disease in Brazil, and it can cause severe cardiomyopathy and digestive megasyndromes<sup>7,8,19,20</sup>. This genotype was found in isolates from six patients, two with the cardiac form in stage A<sup>35</sup> (CT-IOC 544, 545), one with megaesophagus (CT-IOC 538),

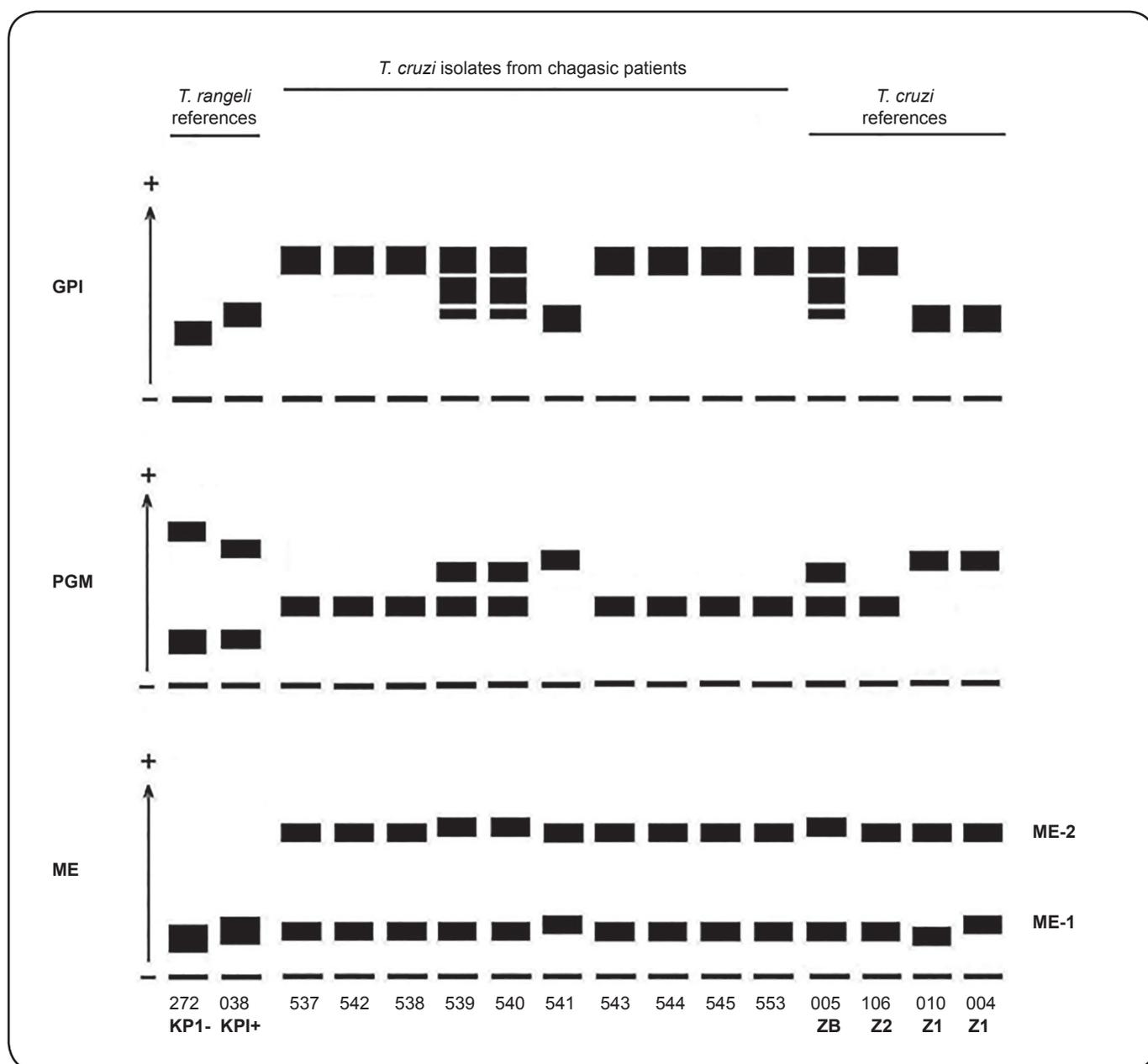


**FIGURE 3 -** Isoenzyme patterns at malate dehydrogenase locus displayed by *Trypanosoma cruzi* isolates from patients with Chagas disease followed at the Evandro Chagas National Institute of Infectious Diseases (INI, FIOCRUZ), and reference strains. The numbers at the bottom of the gel are the code numbers of each stock in the Trypanosomatid Collection (CT-IOC). *Trypanosoma rangeli* reference isolates from Chagas disease patients: CT-IOC 535 (US42), and 536/546 (AP56). *Trypanosoma cruzi* reference stocks: CT-IOC 005 (CL Brener), 106 (Y), and 010 (Dm28c). Note the clear distinction between *T. cruzi* and *T. rangeli* at this enzyme locus.

and the others with the indeterminate form (CT-IOC 537/542, 543, and 553). It is worth mentioning that the patient with megaesophagus proceeded from the Minas Gerais State, where this clinical form has been associated with TcII<sup>7,36</sup>. Although the present study did not aim to determine correlations between biological features of *T. cruzi* strains and Chagas disease clinical manifestations, it is interesting to note that the isolate from the patient with megaesophagus (CT-IOC 538) was the most fastidious in cultures, presenting the lowest rates of metacyclic trypomastigotes. The DTU TcVI (formerly ZB) was found in two isolates (CT-IOC 539 and 540), both from asymptomatic patients, one of them originating from the Rio Grande do Sul State, where this genotype is prevalent.

The finding of TcI (formerly Z1) in Brazilian patients with chronic Chagas disease is infrequent, and it usually produces mild disease, although its pathogenicity in acute infections is similar to that caused by TcII<sup>19,20</sup>. In the present study, TcI was identified in a single isolate (CT-IOC 541) from a chronic patient with the indeterminate form, who originated from the Paraíba State (Northeastern Brazil). A sequencing assay followed by BLAST analysis displayed its higher genetic similarity (99%) with several TcI strains, first Sylvio X10, a stock originally of a human case from the Pará State (Northern Brazil), besides a *Tcbat* stock<sup>16,17</sup>.

The genotype TcI had already been found in two patients from the Paraíba State by Barnabé et al.<sup>37</sup>, and in three individuals from another close State (Rio Grande do Norte) by Câmara et al.<sup>38</sup>. However, these authors did not mention their clinical forms. Barnabé et al.<sup>37</sup> also found TcI in *Triatoma pseudomaculata*, *T. brasiliensis*, and *Didelphis albiventris* from Paraíba, thus evidencing the existence of epidemiological conditions for circulation of this genotype there. Teixeira et al.<sup>39</sup> also isolated a TcI strain (José-IMT) from the myocardium of an end-stage chronic patient also from the Paraíba State. This is an uncommon disease outcome caused by this genotype



**FIGURE 4: Diagrammatic representation of the electrophoretic patterns of glucose phosphate isomerase (GPI), phosphoglucosmutase (PGM), and malic enzyme (ME) displayed by *Trypanosoma cruzi* isolates from patients with Chagas disease followed at the Evandro Chagas National Institute of Infectious Diseases (INI, FIOCRUZ), and reference strains.** The numbers at the bottom of the diagram are the code numbers of each stock in the Trypanosomatid Collection (CT-IOC). *Trypanosoma rangeli* KP1+ and KP1- references strains: CT-IOC 272 (SC-61) and 038 (H14), respectively. *Trypanosoma cruzi* reference stocks: CT-IOC 005 (CL Brener), 106 (Y), 010 (Dm28c), and 004 (Colombian). Zymodemes of the isolates from chagasic patients: Z1 (CT-IOC 541), Z2 (CT-IOC 537/542, 538, 543, 544, 545, and 553), and ZB (CT-IOC 539 and 540).

in Brazil, but it had been previously described by Barrett et al.<sup>19</sup> in a chronic patient from another Northeastern State (Bahia). More recently, Martins et al.<sup>23</sup> found TcI in chronic patients from the State of Rio Grande do Norte, three being asymptomatic, two with the cardiac form, and one with the digestive form. The finding of digestive form in an individual infected with TcI is surprising, since it is uncommon in Brazil and Latin American countries where this DTU is the main agent of Chagas disease<sup>7,8</sup>.

In Southeastern Brazil, the genotype TcI was reported in five chronic patients from the State of Minas Gerais<sup>40,41</sup>, three of them being asymptomatic and two others presenting clinical forms typically caused by TcII, which is prevalent in that state, thus suggesting mixed infections. More recently, Sangenis et al.<sup>42</sup> identified TcI mixed with TcVI in an asymptomatic patient from the Rio de Janeiro State. Otherwise, in the Amazon region (Northern Brazil), TcI is the prevailing DTU found in humans as the agent of acute infections<sup>6,7,21</sup>, but this genotype was also

identified in fourteen chronic patients with the indeterminate form of Chagas disease<sup>22,40</sup>.

Unlike that generally occurs in Brazil, TcI is the main agent of chronic Chagas disease in some American countries (e.g., Venezuela and Colombia), where the patients can develop severe and fatal cardiomyopathy (usually without digestive megasyndromes), as well as meningoencephalitis in immunocompromised individuals<sup>6-8,43-50</sup>. Such discrepancy deserves further investigations, but many factors should be considered for attempting to explain it, as the genetic diversity of both parasites and human beings, and the epidemiological conditions that favor the selection of *T. cruzi* genotypes by local vectors and hosts<sup>51-53</sup>.

TcI is the most abundant and widely dispersed of all *T. cruzi* genotypes, being found from the Southern North America to the Northern regions of Argentina and Chile<sup>54</sup>. The high genetic diversity within TcI was clearly shown by Tibayrenc and Ayala<sup>13</sup>, and thereafter mentioned by several authors<sup>7,8,55,56</sup>. Analysis of the spliced-leader intergenic region (SL-IR) of several TcI stocks evidenced five SL-IR groups (TcIa-TcIe) correlated with transmission cycles<sup>57-60</sup>. Other molecular studies displayed seven or three TcI subpopulations associated with their geographic distribution<sup>54,61</sup>. Llewellyn et al.<sup>54</sup> disclosed an exclusively domestic genotype (Ven<sub>DOM</sub>), subsequently named TcI<sub>DOM</sub>, which corresponds to the TcIa SL-IR group<sup>61</sup>. More recently, León et al.<sup>56</sup> suggested the subdivision of TcI only into two main groups (TcI<sub>DOM</sub> and sylvatic), proposing a PCR assay for identifying them. In the present study, the sequencing analysis of the TcI isolate (CT-IOC 541) showed that it is genetically closer to Sylvio X10, a stock identified as TcId<sup>60</sup>, a sub-group related to sylvatic cycles, which was also found by Câmara et al.<sup>38</sup> in Northeastern Brazil. It is worth mentioning that TcId (or TcI sylvatic) was identified in the heart and brain of patients with severe Chagas disease outside Brazil<sup>46,49</sup>. In the present study, the isolate CT-IOC 541 and the Colombian strain showed the same banding pattern at the ME-1 locus, which was distinct from that of the Dm28c stock (**Figure 4**), a finding that corroborates the variability within TcI.

Several authors have found correlations between the *T. cruzi* type and responses to chemotherapeutic treatments<sup>20,41,62-68</sup>. Together, these reports evidenced that the TcI strains were usually the most resistant to trypanocidal treatment, TcII stocks presented greatly variable responses, and TcVI isolates were the most susceptible. However, these associations have not been fully confirmed by other authors<sup>69-71</sup>. Accordingly, further long-term studies are necessary to investigate this issue, especially those monitoring the treatment responses of patients with Chagas disease whose parasite DTU has been identified. Routine *T. cruzi* genotyping can be feasible at medical centers with scientific research support, since presently, there are several available molecular techniques for its characterization<sup>8,14,17,72</sup>, and the classical MLEE analysis can reveal the main genetic groups, mixed stocks, and other trypanosome species<sup>14,25,30-33</sup>. Despite some controversies and exceptions, nowadays there are recommendations for treating all patients with chronic Chagas disease using new dosing strategies and drug combinations for preventing side

effects, thus increasing the chances of treatment completion and monitoring of patients for a longer time<sup>73-75</sup>. Identification of the *T. cruzi* genotype in samples from patients is also important to better understand the possible influence of the parasite type on the clinical manifestations of the human Chagas disease, as it seems to occur in individuals infected with TcI, who rarely present digestive forms.

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#### Conflict of interest

The authors declare that they have no conflict of interest.

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