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Trypanosoma cruzi III causing the indeterminate form of Chagas disease in a semi-arid region of Brazil

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SUMMARY

Objective: *Trypanosoma cruzi* is subdivided into six discrete typing units (DTUs), TcI–TcVI. The precise identification of each can contribute to tracking wild DTUs that invade the domiciliary environment.
Methods: Twenty *T. cruzi* stocks isolated from 16 chagasic patients, two *Panstrongylus lutzi*, one *Galea spixii*, and one *Euphractus sexcinctus*, from different localities in the State of Rio Grande do Norte, Brazil, were characterized by genotyping the 3′ region of the 24Sα rRNA gene, the mitochondrial cytochrome oxidase subunit 2 gene, and the spliced leader intergenic region.
Results: TcIII was identified in 18.7% (3/16) of patients from different municipalities, as well as in *P. lutzi*, *G. spixii*, and *E. sexcinctus*, indicating the connection between the sylvatic and domestic cycles in this Brazilian semi-arid region. TcI and TcII were also detected, in 37.5% (6/16) and 43.8% (7/16) of patients, respectively. These DTUs were associated with cardiac, digestive, and indeterminate clinical forms, while TcIII was identified only in patients with the indeterminate form.
Conclusions: The occurrence of these DTUs reveals important phylogenetic diversity in *T. cruzi* isolates from humans. TcIII is reported for the first time in northeastern Brazil. These findings appear to indicate an overlap between the sylvatic and domestic transmission cycles of the parasite in this region.
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1. Introduction

Chagas disease is a sylvatic enzootic disease distributed widely in Latin America. It is transmitted in nature between triatomines and mammals and has become an anthropozoonosis due to anthropic changes in the natural environment.[1,2] Increasing human occupation of the semi-arid environment has unleashed triatomine dispersion, expanding the transmission cycle of the parasite to human dwellings.[3] The northeast of Brazil encompasses extensive areas of territory with a semi-arid climate, constituted by the Caatinga biome, and has a large quantity of low quality housing in rural areas. These factors promote the colonization by insects originating from the wild environment.[3,4] This region is the center of dispersion and has high concentrations of *Triatoma brasiliensis* and *Triatoma pseudomaculata*.[3,5] Similarly, high rates of natural infection by *Trypanosoma cruzi* in *Panstrongylus lutzi* may play an important role,[6] together with wild mammals found in this region, which have been indicated as hosts in different areas.[7–9]

T. cruzi populations show a high degree of intraspecific variability, as detected by biological, biochemical, immunological, and genetic markers.[10] Recently, consensus was reached among researchers to rename isolates of *T. cruzi* by assigning them one of six discrete typing units (DTUs; TcI–TcVI) based on different genetic markers.[11] Of note, DTUs II and III were formerly known as TcIIb and TcIIc.[12]

Geographically, TcI is dispersed widely in the Americas, maintains more affinity with marsupials than other mammals,[13] and is found throughout the range of triatomine distribution, such that this DTU is associated with sylvatic and domestic cycles. Recent studies have confirmed the presence of the TcI genotypes in

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Argentina, Brazil, Bolivia, Chile, Colombia, Mexico, Panama, Paraguay, French Guiana, Venezuela, and the USA.[14,15] Human infection with TcI in Brazil is commonly concentrated in the Amazon basin, in the northern part of South America and Central America.[16] In some countries, like Colombia, TcI is associated with chagasic cardiomyopathy,[17] while in others it shows low pathogenicity.[18] This DTU has been found from Argentina to the USA, while TcII–VI are distributed from the Amazon basin to southern Argentina. However, it is possible to find regions where all six DTUs are present, such as in Colombia, where TcI is reported predominantly, together with low proportions of DTUs II, III, IV, and VI.[19–23] The wide genetic diversity of TcI in the Americas has been established, as has the geographical distribution of TcI genotypes throughout the Americas, confirming that it is most prevalent in Colombia.[24]

TcII is predominant in the southern and central regions of South America, but its true extent remains unclear. It has mostly been isolated in domestic transmission cycles from vectors, primates, and sporadically from other mammals.[25,26] This DTU is found in different clinical forms and can be associated with distinct symptoms and varying degrees of pathological processes in all the clinical forms of the disease.[27]

TcIII is mostly associated with the sylvatic cycle in Brazil, with the terrestrial niche and *Dasypus novemcinctus* found over a vast range from western Venezuela to the Argentine Chaco.[28,29] Although rare in domestic transmission cycles, TcIII occurs at a relatively high frequency in the sylvatic environment and is associated almost exclusively with terrestrial transmission cycles and fossorial mammalian genera, including the *Cingulata* (armadillos) and terrestrial marsupials.[30–33] So far, few vector species have been incriminated in the sylvatic transmission of TcIII. *Panstrongylus geniculatus* and *Triatoma rubrovaria*, both mainly sylvatic vectors, are often, although not exclusively, associated with terrestrial ecotopes.[34] Although infrequent, the occurrence of TcIII in domestic transmission cycles implies its role as an agent of human disease.[28] Recent data have shown sporadic acute cases in humans within the Amazon basin.[29] In the study area, TcIII was identified in *T. brasiliensis* from the peri-domicile and *P. lutzi* captured in the wild environment. TcIII wild populations have always shown a high level of homozygosity, which is inconsistent with clonal propagation, although it is unclear whether this is explained by intra-lineage recombination or gene conversion.[34] TcIII is also occasionally isolated from domestic dogs[30,31] and other uncommon reservoirs and vectors.[6,7] This DTU therefore represents an important focus for study[28], as human populations are expanding into previously undisturbed cycles of natural transmission and secondary vector species are re-emerging from the sylvatic environment following the eradication of major domestic species.[35,36]

The diversity of natural *T. cruzi* populations involving TcI, TcII, and TcIII circulating among sylvatic and peri-domiciliary transmission cycles has been demonstrated in the west mesoregion of the State of Rio Grande do Norte.[6] Human infection with TcI and TcII was reported at the same time that TcI, TcII, and TcIII were isolated from *T. brasiliensis* and *P. lutzi*.[6,7,32] The presence of TcIII in triatomine vectors such as *T. brasiliensis* has prompted the search for these DTUs in humans to be extended, with the aim of understanding how the peri-domestic and domestic environments are connected to the wild in the semi-arid region.

2. Methods

2.1. Study area

The State of Rio Grande do Norte (RN) is located in northeastern Brazil between 4°49′53″S and 6°58′57″S, and 35°58′03″W and 38°36′12″W. It has a population of over three million inhabitants. The predominant biome is Brazilian savanna (Caatinga), described as a 'white forest' due to the lack of water, which means the plants are leafless over the dry months. This biome presents a large number of mammals, birds, reptiles, and shrubby plants, and undergoes long periods of drought over the years. A variety of triatomine endemic species have been reported over the last few decades, including *T. brasiliensis*, *T. pseudomaculata*, *Panstrongylus megistus*, *Rhodnius nasutus*, and *P. lutzi*, all found in the semi-arid region of the state.[4,5,32]

2.2. Origin of T. cruzi

A total of 266 chagasic patients were identified. These patients had positive ELISA (Chagatest recombinant ELISA v. 3.0 kit), positive hemagglutination inhibition (HAI) (Chagatest hemagglutination inhibition, screening A-V kit; Wiener Lab, Rosário, Argentina), and/or positive indirect immunofluorescence (IIF) test results. The HAI has a sensitivity of 100% and specificity of 98.7% according to the manufacturer[37]. The IIF, with a titer of 1:40 considered the cut-off point for this method, was performed using epimastigotes of *T. cruzi* Y (DTU II)[11] strain as antigen, maintained in acellular culture and fixed with 20% formaldehyde. After the exclusion of heart diseases (ischemic, valvular, and hypertensive), all patients aged 23 to 88 years were enrolled in the study. The same clinical–epidemiological protocol was applied throughout the physical examination, taking into account the patient's habits and concomitant diseases, including the presence of signs and symptoms general or specific to the cardiovascular and gastrointestinal systems. These patients were examined clinically and submitted to complementary tests, including an electrocardiogram, chest X-ray, and contrast radiography of the esophagus and colon. They were classified according to the clinical form of the disease as cardiac, digestive, or indeterminate, as recommended by the Brazilian Consensus on Chagas Disease.[38] The algorithm developed for patient classification is summarized in Figure 1.

This study was approved by the Research Ethics Committee of the State University of Rio Grande do Norte (COEP/UERN No. 027.2011) in Mossoró, Rio Grande do Norte, Brazil, and informed consent was obtained from the participants. The origin of *T. cruzi* and the patient's epidemiological and clinical characteristics are presented in Table 1.

2.3. Isolation and culture of T. cruzi

Parasite isolation from humans was performed by hemoculture[39] and from triatomine bugs and sylvatic mammals by xenoculture methods.[40] Hemoculture was performed for all chagasic patients and *T. cruzi* was isolated from 20 of them (20/266, 7.5%). In order to minimize parasite selection, *T. cruzi* was maintained in culture for a short period with three or four successive passages in LIT (Liver Infusion Tryptose) medium. Only 16 patients returned for the clinical examinations. Four additional isolates were obtained from the sylvatic environment, one from *Galea spixii* Wagler 1831, one from *Euphractus sexcinctus* Linnaeus 1758, and two from *P. lutzi*, and these were included in the study. Cultures of *T. cruzi* epimastigotes at 10^6/ml were washed three times in Krebs–Ringer–Tris pH 7.3, centrifuged at 2000 × *g* for 15 min at 4 °C, and were then stored at −20 °C until DNA extraction and genetic characterization. Genomic DNA from the cultured parasite was obtained by phenol–chloroform method and was used as the template for PCR assays.[41]

2.4. Genetic characterization of T. cruzi DTUs

The protocol used for genotyping was that proposed by D'Ávila et al.,[42] using a three-step assay (Figure 2). Polymorphism

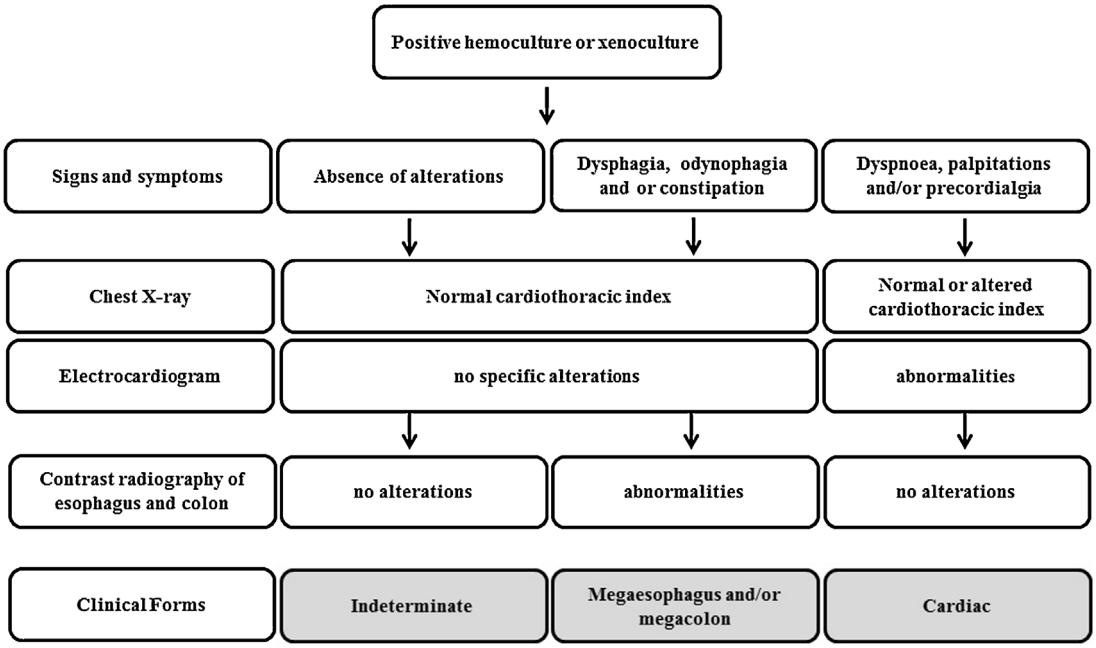

Figure 1. Flowchart for patient classification, as recommended by the Brazilian Consensus on Chagas Disease.[38]

analysis of the mitochondrial cytochrome oxidase subunit 2 (COII) gene was performed with Tcmit-10 (5′-CCATATATTGTTG-CATTATT-3′) and Tcmit-21 (5′-TTGTAATAGGAGTCATGTTT-3′) primers designed to amplify a fragment of 375 base pairs (bp) from *T. cruzi* maxicircle DNA.[42,43] On the basis of the restriction map of COII sequences, the AluI restriction endonuclease was chosen for use in restriction fragment length polymorphism (RFLP) analysis of the mitochondrial COII gene. This marker is able to distinguish haplotype A (TcI) and haplotype C (TcII) from other DTUs with haplotype B (TcIII–VI). Amplification of the D7 domain of the 24Sα rRNA gene was achieved by PCR with primers D71 (5′-AAGGTGCGTCGACAGTGTGG-3′) and D72 (5′-TTTTCA-GAATGGCCGAACAGT-3′), following previously described protocols.[44]

Table 1
Geographical origin, hosts, and genetic typing by three markers of *Trypanosoma cruzi* stocks from patients with different clinical forms, vectors, and reservoirs

T. cruzi stocks	Host	Clinical form	Age, years	Sex	24Sα rDNA[a] (bp)	COII[b] (haplotype/bp)	SL-IR[c] (bp)	DTU	Municipality[d]
1150	Human	Indeterminate	44	Male	110	A/30, 81, 264	150	TcI	Caicó
1816	Human	Indeterminate	45	Female	110	A/30, 81, 264	150	TcI	Caicó
2137	Human	Cardiac	32	Female	110	A/30, 81, 264	150	TcI	S. Melo
2549	Human	Indeterminate	27	Female	110	A/30, 81, 264	150	TcI	Apodi
3188	Human	Digestive	62	Male	110	A/30, 81, 264	150	TcI	Assu
CBS 202	Human	Cardiac	88	Male	110	A/30, 81, 264	150	TcI	Caraúbas
240	Human	Cardiac	29	Male	125	C/81, 212	150	TcII	Caicó
1317	Human	Indeterminate	58	Male	125	C/81, 212	150	TcII	DSR
2934	Human	Indeterminate	44	Male	125	C/81, 212	150	TcII	Caicó
3973a	Human	Cardiac	72	Male	125	C/81, 212	150	TcII	Acari
RN26	Human	Digestive	56	Male	125	C/81, 212	150	TcII	SNN
RN79	Human	Cardiac	43	Female	125	C/81, 212	150	TcII	SNN
SM73	Human	Cardiac	64	Female	125	C/81, 212	150	TcII	S. Melo
105	Human	Indeterminate	39	Female	110	B/81, 294	200	TcIII	Caicó
CBS195	Human	Indeterminate	40	Female	110	B/81, 294	200	TcIII	Caraúbas
SM76	Human	Indeterminate	67	Female	110	B/81, 294	200	TcIII	S. Melo
Gs3	*G. spixii*	-	Adult	Male	110	B/81, 294	200	TcIII	Caraúbas
Es18	*E. sexcinctus*	-	Adult	Male	110	B/81, 294	200	TcIII	Caraúbas
Pl0213	*P. lutzi*	-	Adult	Male	110	B/81, 294	200	TcIII	SNN
Pl0812	*P. lutzi*	-	Adult	Female	110	B/81, 294	200	TcIII	SNN
Col1.7G2*[46]	Human	-	-	-	110	A/30, 81, 264	150	TcI	
JG*[47]	Human	-	-	-	125	C/81, 212	150	TcII	
RN19*[6]	Human	-	-	-	110	B/81, 294	200	TcIII	
AM64*[29]	Human	-	-	-	117/119	B/81, 294	200	TcIV	
3253*[e]	Human	-	-	-	110+125	B/81, 294	150	TcV	
CL-Brener*[48]	*T. infestans*	-	-	-	125	B/81, 294	150	TcVI	

bp, base pairs; Gs3, Galea spixii; Es18, Euphractus sexcinctus; Pl082 and Pl0213, Panstrongylus lutzi.
[a] Souto and Zingales.[44]
[b] Freitas et al.[43]; D'Àvila et al.[42]
[c] Burgos et al.[45]
[d] DSR, Dix Sept Rosado; S. Melo, Severiano Melo; SNN, Serra Negra do Norte.
[e] 3253, Lages-Silva et al. unpublished data.
* *T. cruzi* strains and clones used as controls.

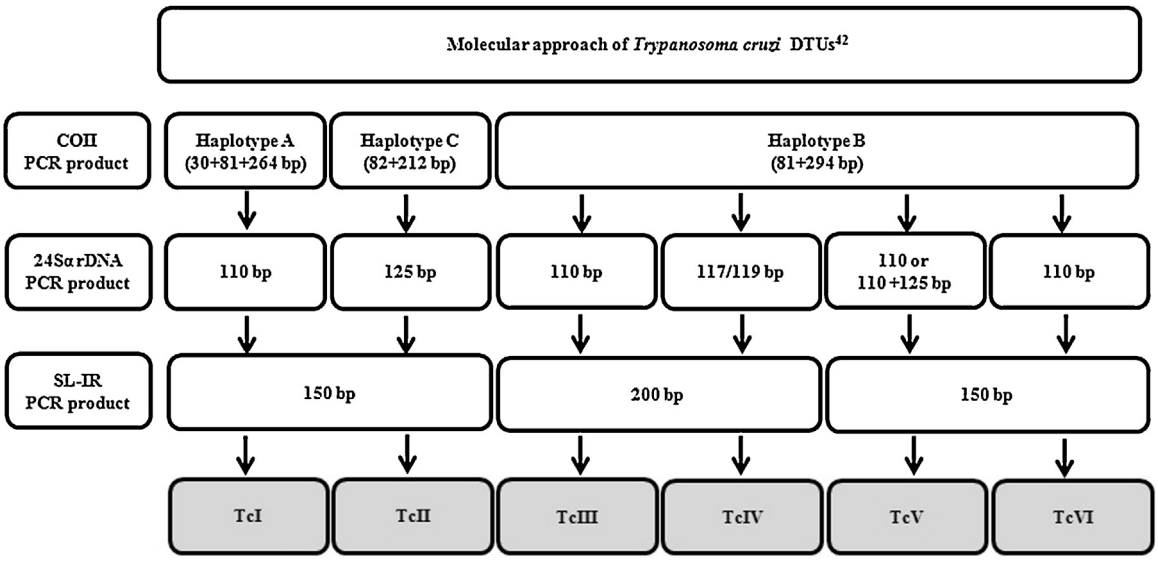

Figure 2. Protocol for genotyping proposed by D'Ávila et al.[42] using a three-step assay: polymorphism analysis of the mitochondrial cytochrome oxidase subunit 2 (COII) gene, amplification of the D7 divergent domain of the 24Sα rRNA gene, and amplification of the spliced leader intergenic region (SL-IRac).

The amplification of the spliced leader intergenic region (SL-IRac) genes was achieved with primers TcIII forward (5′-CTCCCCAGTGTGGCCTGGG-3′) and UTCC reverse (5′-CGTAC-CAATATAGTACAGAAACTG-3′). The PCR strategy with the SL-IRac gene was devised to distinguish populations belonging to *T. cruzi* III (amplicons of 200 bp) from *T. cruzi* I, *T. cruzi* II, and hybrid strains, which present fragments of approximately 150 to 157 bp.[45] The PCR products were analyzed by electrophoresis on 6.0% polyacrylamide gels and the DNA fragments were visualized by silver staining.

3. Results

Genetic profiles of the *T. cruzi* human stocks showed genetic diversity. Three human stocks amplified fragment 110 bp rDNA, COII haplotype B, and SL-IRac 200 bp, corresponding to TcIII. TcI was identified in six patients presenting 110 bp rDNA, COII haplotype A, and SL-IRac 150 bp. Seven TcII stocks were obtained from humans showing 125 bp rDNA, COII haplotype C, and SL-IRac 150 bp (Figure 3). All stocks obtained from *P. lutzi*, *G. spixii*, and *E. sexcinctus* also showed profiles corresponding to TcIII (Table 1).

The mean age of the 16 patients was 50.6 years. Patient 2549 (age 27 years) was the youngest and patient CBS202 (age 88 years) was the oldest. TcIII was identified in 18.7% (3/16) of human stocks, all of them thus far presenting the indeterminate clinical form. TcI was identified in 37.5% (6/16) of the human stocks: 50.0% (3/6) of those with the indeterminate clinical form, 33.3% (2/6) of those with the cardiac form, and 16.7% (1/6) of those with the digestive form. TcII was isolated from 43.8% (7/16) of humans stocks: 28.6% (2/7) of them with the indeterminate clinical form, 57.1% (4/7) with the cardiac form, and 14.3% (1/7) with the digestive form.

The geographical distribution of DTUs throughout the semi-arid area of Rio Grande do Norte showed a wide dispersion. TcIII was identified in different municipalities more than 100 km apart. TcI and TcII showed similar distribution, with no predominant area in the state. In Caraúbas, humans and sylvatic mammals (*G. spixii and E. sexcinctus*) were infected by TcIII. TcIII was also detected in *P. lutzi* obtained from a sylvatic reserve in the municipality of Serra Negra do Norte, indicating the presence of this DTU in sylvatic and peri-domestic environments and coexisting with TcI and TcII in the domestic cycle (Figure 4).

4. Discussion

This study identified DTU III in three chagasic patients of different ages with no parentage or evidence of oral transmission and living in municipalities approximately 100 km apart. TcIII is a sylvatic DTU found in Brazil and adjacent countries, rarely documented in human cases.[7,11,28] The triatomine species associated with TcIII are not well known, but may include terrestrial *Panstrongylus* and *Triatoma* genera. Genetic diversity and the spatial structure of populations within TcIII have been reported, with comparisons of samples from Brazil, Colombia, Venezuela, Bolivia, and Paraguay.[28]

A previous study has shown a high frequency of TcIII in *T. brasiliensis* in the study area,[6] thus this vector species presents dual epidemiological behavior through its position in the sylvatic cycle and as a potential link to introducing these populations into the domiciliary cycle.[32] The geographical distribution of TcI and TcII in the same region indicates that TcIII is able to coexist with other DTUs in environments that support an overlap between sylvatic and peri-domestic transmission cycles of *T. cruzi*. The Caatinga is a biome that favors such an overlap,[32] where triatomines infected with TcIII, such as *P. lutzi*, may act in the sylvatic cycle, and *T. brasiliensis* infected with TcII and TcIII may act in both cycles. The additional data presented here corroborate the hypothesis that this biome sustains reservoirs and vectors of TcIII so close to human settlements that there are no barriers to this DTU reaching the peri-domicile. The destruction of the natural environment is forcing the proximity of wild triatomines to human dwellings.[16,49] However, in this biome, other factors contribute to strengthen the process, such as irregular vigilance against insects, which can contribute to the reinvasion of the peri-domicile and the transmission of TcI and TcIII throughout the environment.[6]

Studies have shown the presence of species of carnivores, marsupials, rodents, and domestic animals capable of hosting TcIII[7,8,28] that are sources of food for insects. Intense hunting of animals, such as *E. sexcinctus* and *G. spixii*, mammals already shown to be hosts of *T. cruzi*,[7–9] were also observed in the present study. It is common behavior in the semi-arid area to maintain animals captured while hunting in captivity for weeks, or even months, so that the animal can gain weight before slaughter. This could be one way by which sylvatic DTUs are transmitted to peri-domestic

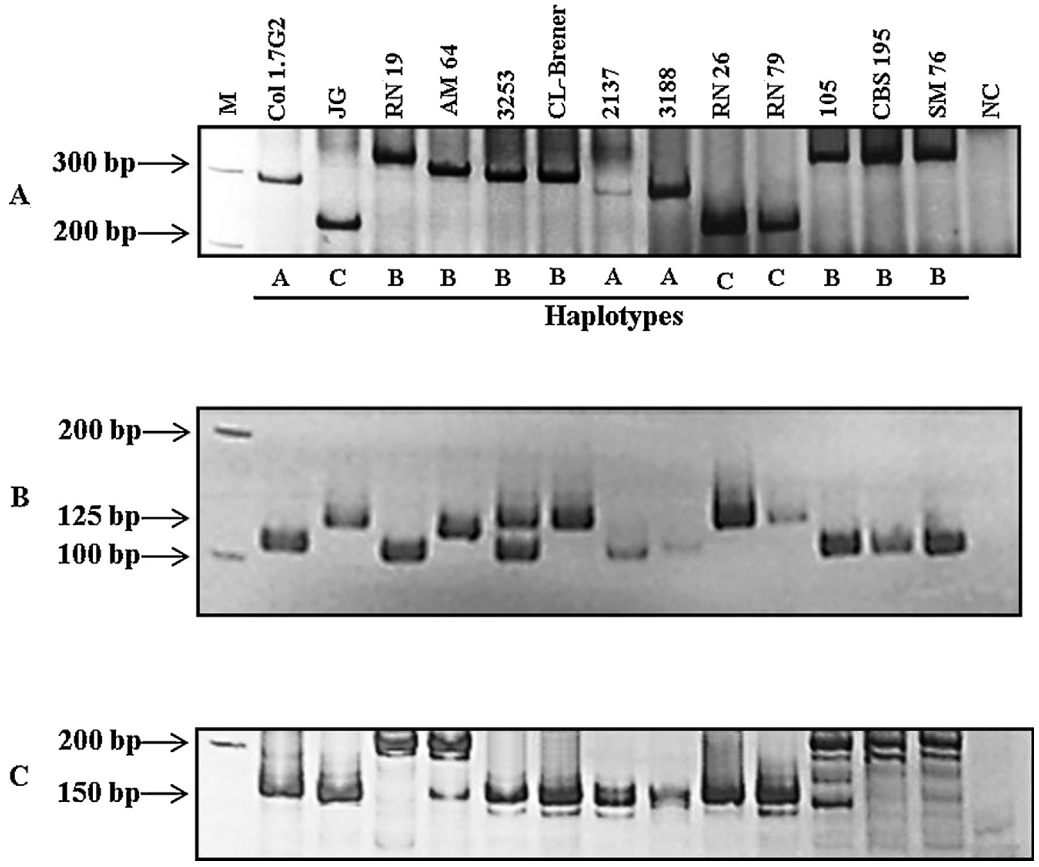

Figure 3. A RFLP analysis of the mitochondrial COII gene in the *Trypanosoma cruzi* isolates belonging to different haplotypes, obtained by polyacrylamide gel electrophoresis. Digestion of the DNA with AluI generates three RFLP patterns for the *T. cruzi* strains: restriction fragments of 264, 81, and 30 bp are classified as TcI (mitochondrial haplotype A; lane 1, Col1.7G2 clone), restriction fragments of 212 and 81 bp are classified as TcII (mitochondrial haplotype C; lane 2, strain JG), and restriction fragments of 294 and 81 bp are classified as TcIII–VI (mitochondrial haplotype B; lane 3, strain RN19; lane 4, strain AM64; lane 5, strain 3253; lane 6, CL Brener clone). Lanes 7–13 show *T. cruzi* samples of patients 2137, 3188, RN26, RN79, 105, CBS195, and SM76. (B) Analysis of 24Sα rRNA of *T. cruzi* isolates from chronic chagasic patients and controls. Lane 1, Col1.7G2 clone (~110 bp); lane 2, JG (~125 bp); lane 3, RN19 (~110 bp); lane 4, AM64 (~117/119 bp); lane 5, 3253 (~110 and 125 bp); lane 6, CL Brener clone (~125 bp); lanes 7–13, *T. cruzi* sample of patients 2137, 3188, RN26, RN79, 105, CBS195, and SM76. (C) The SL-IRac gene was used to separate isolates of TcIII and TcIV from other DTUs. Lanes 1, 2, 5 and 6: controls Col1.7G2 clone, JG, 3253, and CL Brener clone (fragments of 150–157 bp); lanes 4 and 5: controls RN19 and AM64 (fragments of 200 bp); lanes 7–13: *T. cruzi* sample of patients 2137, 3188, RN26, RN79, 105, CBS195, and SM76; lane M: molecular size marker; lane NC: negative PCR control.

triatomine bugs. Nevertheless, the most incisive factor appears to be the lack of any border between the rural and sylvatic zones, which allows the vectors and synanthropic mammals to remain in close contact with humans. It is likely that the absence of limits hinders epidemiological surveillance actions that should be permanent.

Patients in the present study infected with TcIII (105, CBS195, and SM76) only presented the indeterminate clinical form, which was also diagnosed in a patient from the State of Minas Gerais, Brazil.[42] These data suggest that TcIII has low pathogenic power, even in patients over the mean age (SM76, 67 years old), and since the three TcIII-infected patients lived in different municipalities, with no parentage or evidence of oral infection, it is probable that the infection was transmitted by the vector. A study in Colombia of chronic patients infected by TcIII, in whom mixed infection with TcI and TcII was detected,[50] demonstrated that it is hard to distinguish the role of each DTU in the heart diseases analyzed. Previous findings have shown that TcIII (formerly known as TcIIc) may be underreported from both domestic and sylvatic transmission cycles because certain genotyping methodologies fail to distinguish between TcIV (TcIIa) and TcIII.[51] TcIII has been associated with terrestrial ecotopes from different reservoirs and vectors.[7,33] In the Brazilian Amazon, Venezuela, and Colombia, TcI is the predominant DTU and the principal cause of both acute and cardiac Chagas disease, but not of the 'mega' syndromes, whilst TcIII causes sporadic acute cases of Chagas disease in the Brazilian Amazon basin.[49] Outbreaks of acute Chagas disease caused by both TcI and Z3 (TcIII or TcIV) have been reported.[52] However, data from Venezuelan localities have shown that TcI is responsible for human acute Chagas infection and for causing severe heart failure,[53] in contrast to an outbreak of acute cases caused uniquely by the genotype TcIII/Z3.[29] Three stocks from chronic chagasic patients (one with an asymptomatic form, two with a cardiac–digestive form) were found to be closely related to Z3/TcIII,[54] highlighting the need for the characterization of epidemiological and clinical traits associated with different genotypes of Chagas disease in the region under study.

In this study, TcI was associated with chronic chagasic patients presenting cardiac, digestive, and indeterminate clinical forms. Human infection with TcI has been reported in the State of Rio Grande do Norte, although with no association with clinical forms.[6] The recent description of the importance of TcI in the development of cardiomyopathies in Argentina[21] and cardiac alterations in Colombia[50,55] has drawn attention to the possibility that this DTU contributes to the pathogenicity of Chagas disease. In Colombia and Venezuela, TcI isolates have been associated not only with low parasitemia and very mild clinical symptoms, but also with severe acute symptomatic cases and death.[50,53] TcI haplotypes TcIa and TcId have been identified in different tissues from a heart-transplanted Chagas cardiomyopathy patient with reactivation, indicating histotropism.[14] A high percentage of isolates belonging to TcI haplotype TcId were identified in Venezuelan patients infected with oral outbreaks and there was evidence of multiclonal infections

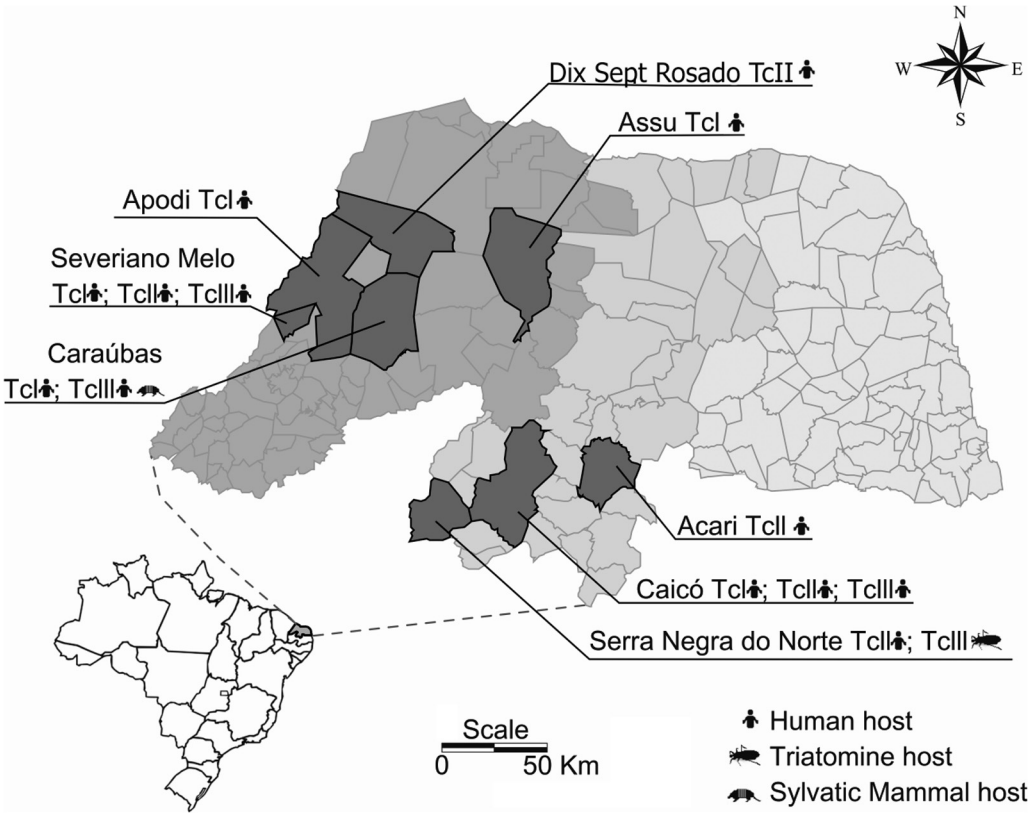

Figure 4. Distribution of discrete typing units (DTUs) of *Trypanosoma cruzi* in different municipalities of the State of Rio Grande do Norte, Brazil.

in patients, triatomine vectors, and reservoirs in the regions of these outbreaks, suggesting multiple infections in reservoirs from urban and rural areas.[56] Further studies are needed to determine whether the different clinical forms found here are relevant to some TcI haplotypes, immune responses of patients, or multiple infections by different DTUs not detected by the methodology used.

In this study, the distribution of TcI in patients from several municipalities, who did not share food sources capable of transmitting *T. cruzi*, suggests that transmission in the semi-arid area is vectorial and is not associated with oral outbreaks, as reported previously for this DTU.[57] Since TcI has been detected in humans, *T. pseudomaculata*, and *T. brasiliensis*[7,58], it is believed that the TcI present here could be associated with the *Triatoma* genus acting as vectors.

Chagasic patients with TcII presented cardiac, digestive, or indeterminate clinical forms, corroborating previous findings in which DTU II populations have been shown to play a major role in Brazilian chagasic patients.[18,27,42] This DTU is considered common in humans, with high homology among different hosts and locations in the semi-arid region.[32] *T. brasiliensis*, a vector capable of acting as a link between sylvatic and domestic cycles, has regularly been found to be infected.[6] TcII has also been observed in *Cingulata*, *Rodentia*, and *Primata*, which are distributed in many American regions, and these also occasionally carry TcIII.[26] These orders are common in the semi-arid region and are probably involved as reservoirs of this DTU.

The data presented here demonstrate that transmission cycles of *T. cruzi* in the semi-arid region can sustain epidemiological patterns of the parasite that are different to those of other regions of the country. Moreover, TcIII was identified for the first time in northeastern Brazil as a causative agent of chronic human disease; no similar reports from other states of the region have been verified. These findings alert us to the complexity of controlling the transmission of the parasite in such areas, where overlapping sylvatic and peri-domiciliary cycles occur.

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