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**SHORT REPORT**

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# North American import? Charting the origins of an enigmatic *Trypanosoma cruzi* domestic genotype

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## Abstract

**Background:** *Trypanosoma cruzi*, the agent of Chagas disease, is currently recognized as a complex of six lineages or Discrete Typing Units (DTU): TcI-TcVI. Recent studies have identified a divergent group within TcI - TcI<sub>DOM</sub>. TcI<sub>DOM</sub> is associated with a significant proportion of human TcI infections in South America, largely absent from local wild mammals and vectors, yet closely related to sylvatic strains in North/Central America. Our aim was to examine hypotheses describing the origin of the TcI<sub>DOM</sub> genotype. We propose two possible scenarios: an emergence of TcI<sub>DOM</sub> in northern South America as a sister group of North American strain progenitors and dispersal among domestic transmission cycles, or an origin in North America, prior to dispersal back into South American domestic cycles. To provide further insight we undertook high resolution nuclear and mitochondrial genotyping of multiple Central American strains (from areas of México and Guatemala) and included them in an analysis with other published data.

**Findings:** Mitochondrial sequence and nuclear microsatellite data revealed a cline in genetic diversity across isolates grouped into three populations: South America, North/Central America and TcI<sub>DOM</sub>. As such, greatest diversity was observed in South America ( $A_r = 4.851$ ,  $\pi = 0.00712$ ) and lowest in TcI<sub>DOM</sub> ( $A_r = 1.813$ ,  $\pi = 0.00071$ ). Nuclear genetic clustering (genetic distance based) analyses suggest that TcI<sub>DOM</sub> is nested within the North/Central American clade.

**Conclusions:** Declining genetic diversity across the populations, and corresponding hierarchical clustering suggest that emergence of this important human genotype most likely occurred in North/Central America before moving southwards. These data are consistent with early patterns of human dispersal into South America.

**Keywords:** *Trypanosoma cruzi*, Maxicircle, Microsatellite, Chagas Disease, Phylogeography, Population genetics, TcI

## Findings

*Trypanosoma cruzi*, the aetiological agent of Chagas disease, infects 6-8 million people in Latin America, while some 25 million more are at risk of acquiring the disease [1]. Parasite transmission to mammal hosts, including humans, can occur through contact with the faeces of hematophagous triatomine bugs. However, non-vectorial routes are also recognized, including blood transfusion, organ transplantation, congenital transmission, and oral

transmission via ingestion of meals contaminated with infected triatomine feces [2,3].

*T. cruzi* (family Trypanosomatidae; Euglenozoa: Kinetoplastida) is most closely related to several widely dispersed species of bat trypanosomes [4]. Salivarian trypanosomes including medically important *Trypanosoma brucei* subspecies, represent a more divergent group [5]. The age of the split between the *T. cruzi*-containing and *T. brucei*-containing trypanosome lineages is thought to have been concurrent with the separation of Africa and South America/Antarctica/Australasia 100MYA [6], implying that *T. cruzi* and the other Schizotrypanum

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**Table 1 *Trypanosoma cruzi* I samples included in this study**

| Strain code | Strain     | Host/vector                  | Country   | State      | Latitude | Longitude | Date | Population | Reference   |
|-------------|------------|------------------------------|-----------|------------|----------|-----------|------|------------|---|
| PALDA4      | PALDA4     | <i>Didelphis albiventris</i> | Argentina | Chaco      | -27.133  | -61.460   | 2001 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| PALDA21     | PALDA21    | <i>Didelphis albiventris</i> | Argentina | Chaco      | -27.133  | -61.460   | 2001 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| PALDA5      | PALDA5     | <i>Didelphis albiventris</i> | Argentina | Chaco      | -27.133  | -61.460   | 2001 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| PALDAV2     | PALDAV2^3  | <i>Triatoma infestans</i>    | Argentina | Chaco      | -27.133  | -61.460   | 2001 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| PALDA20     | PALDA20    | <i>Didelphis albiventris</i> | Argentina | Chaco      | -27.133  | -61.460   | 2001 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| COTMA38     | COTMA38    | <i>Akodon boliviensis</i>    | Bolivia   | Cotopachi  | -17.430  | -66.270   | 2004 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| P234        | P234       | <i>Homo sapiens</i>          | Bolivia   | Cochabamba | -17.380  | -66.160   | 1985 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| P238        | P238       | <i>Homo sapiens</i>          | Bolivia   | Cochabamba | -17.380  | -66.160   | 1985 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| P268        | P268       | <i>Homo sapiens</i>          | Bolivia   | Cochabamba | -17.380  | -66.160   | 1987 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| SJM22       | SJM22 cl1  | <i>Didelphis marsupialis</i> | Bolivia   | Beni       | -14.810  | -64.600   | 2004 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| SJM34       | SJM34      | <i>Didelphis marsupialis</i> | Bolivia   | Beni       | -14.810  | -64.600   | 2004 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| SJM37       | SJM37      | <i>Didelphis marsupialis</i> | Bolivia   | Beni       | -14.810  | -64.600   | 2004 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| SJM39       | SJM39 cl3  | <i>Didelphis marsupialis</i> | Bolivia   | Beni       | -14.810  | -64.600   | 2004 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| SJM41       | SJM41      | <i>Philander opossum</i>     | Bolivia   | Beni       | -14.810  | -64.600   | 2004 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| SJMC12      | SJMC12     | <i>Philander opossum</i>     | Bolivia   | Beni       | -14.810  | -64.600   | 2004 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| XE5167      | XE5167 cl1 | <i>Didelphis marsupialis</i> | Brasil    | Para       | -1.710   | -48.880   | 1999 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| IM4810      | IM4810     | <i>Didelphis marsupialis</i> | Brasil    | Manaus     | -3.070   | -60.160   | 2002 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| B2085       | B2085      | <i>Didelphis marsupialis</i> | Brasil    | Belem      | -1.360   | -48.360   | 1991 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| XE2929      | XE2929     | <i>Didelphis marsupialis</i> | Brasil    | Pará       | -5.830   | -48.030   | 1988 | SOUTH      | Messenger <i>et al.</i> , [12]                            |
| AAA1cl5     | AAA1cl5    | <i>Rhodnius prolixus</i>     | Colombia  | Casanare   | 4.150    | -71.200   | 2010 | SOUTH      | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| AAA7cl2     | AAA7cl2    | <i>Rhodnius prolixus</i>     | Colombia  | Casanare   | 5.100    | -71.600   | 2010 | SOUTH      | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| AAB3cl3     | AAB3cl3    | <i>Rhodnius prolixus</i>     | Colombia  | Casanare   | 4.150    | -71.200   | 2010 | SOUTH      | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| AAC1cl3     | AAC1cl3    | <i>Rhodnius prolixus</i>     | Colombia  | Casanare   | 5.100    | -71.600   | 2010 | SOUTH      | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| AACf1cl4    | AACf1cl4   | <i>Canis familiaris</i>      | Colombia  | Casanare   | 5.100    | -71.600   | 2010 | SOUTH      | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| AAD6cl6     | AAD6cl6    | <i>Rhodnius prolixus</i>     | Colombia  | Casanare   | 5.100    | -71.600   | 2010 | SOUTH      | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| CACQcl7     | CACQcl7    | <i>Homo sapiens</i>          | Colombia  | Santander  | 6.963    | -73.420   | 2009 | TclDOM     | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| CACQcl8     | CACQcl8    | <i>Homo sapiens</i>          | Colombia  | Santander  | 6.644    | -73.654   | 2009 | TclDOM     | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| DYRcl16     | DYRcl16    | <i>Homo sapiens</i>          | Colombia  | Boyacá     | 5.640    | -72.899   | 2007 | TclDOM     | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |

**Table 1 *Trypanosoma cruzi* I samples included in this study (Continued)**

|            |                |                               |           |                     |        |          |      |            |   |
|------------|----------------|-------------------------------|-----------|---------------------|--------|----------|------|------------|---|
| EBcl11     | EBcl11         | <i>Homo sapiens</i>           | Colombia  | Boyacá              | 5.130  | -73.119  | 2007 | TcIDOM     | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| FECcl10    | FECcl10        | <i>Homo sapiens</i>           | Colombia  | Boyacá              | 5.920  | -73.500  | 2001 | TcIDOM     | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| Td3cl11    | Td3cl11        | <i>Triatoma dimidiata</i>     | Colombia  | Boyacá              | 6.270  | -71.200  | 2000 | TcIDOM     | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| X-1084cl10 | X-1084cl10     | <i>Rhodnius prolixus</i>      | Colombia  | Boyacá              | 4.960  | -73.630  | 2010 | SOUTH      | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| X-236cl9   | X-236cl9       | <i>Rhodnius prolixus</i>      | Colombia  | Boyacá              | 4.960  | -73.630  | 2010 | SOUTH      | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| YAS1cl3    | YAS1cl3        | <i>Alouatta spp</i>           | Colombia  | Casanare            | 5.300  | -72.400  | 2010 | SOUTH      | Ramirez <i>et al.</i> , Molecular Ecology <i>In press</i> |
| 38         | 38             | <i>Triatoma dimidiata</i>     | Guatemala | Jutiapa             | 14.287 | -89.844  | 2000 | NORTH-CENT | This study  |
| 46         | 46             | <i>Triatoma dimidiata</i>     | Guatemala | Santa Rosa          | 14.177 | -90.303  | 2001 | NORTH-CENT | This study  |
| 66         | 66             | <i>Triatoma dimidiata</i>     | Guatemala | Jalapa              | 14.633 | -89.989  | 2001 | NORTH-CENT | This study  |
| 67         | 67             | <i>Triatoma dimidiata</i>     | Guatemala | Jutiapa             | 14.287 | -89.844  | 2001 | NORTH-CENT | This study  |
| 70         | 70             | <i>Triatoma dimidiata</i>     | Guatemala | Jutiapa             | 14.287 | -89.844  | 2001 | NORTH-CENT | This study  |
| 71         | 71             | <i>Triatoma dimidiata</i>     | Guatemala | Jalapa              | 14.633 | -89.989  | 2001 | NORTH-CENT | This study  |
| 83         | 83             | <i>Triatoma dimidiata</i>     | Guatemala | Chiquimula          | 14.768 | -89.458  | 2002 | NORTH-CENT | This study  |
| 95         | 95             | <i>Triatoma dimidiata</i>     | Guatemala | Chiquimula          | 14.768 | -89.458  | 2002 | NORTH-CENT | This study  |
| 100        | 100            | <i>Triatoma dimidiata</i>     | Guatemala | Santa Rosa          | 14.177 | -90.303  | 2002 | NORTH-CENT | This study  |
| 113        | 113            | <i>Triatoma dimidiata</i>     | Guatemala | Chiquimula          | 14.768 | -89.458  | 2002 | NORTH-CENT | This study  |
| 116        | 116            | <i>Triatoma dimidiata</i>     | Guatemala | Baja Verapaz        | 15.079 | -90.413  | 2002 | NORTH-CENT | This study  |
| 154        | 154            | <i>Triatoma dimidiata</i>     | Guatemala | Alta Verapaz        | 15.594 | -90.149  | 2002 | NORTH-CENT | This study  |
| DAVIScl1   | DAVIS 9.90 cl1 | <i>Triatoma dimidiata</i>     | Honduras  | Tegucigalpa         | 14.080 | -87.200  | 1983 | NORTH-CENT | Messenger <i>et al.</i> , 2012                            |
| ANITA II   | ANITA          | <i>Triatoma dimidiata</i>     | Mexico    | Campeche            | 19.188 | -90.300  | 2011 | NORTH-CENT | This study  |
| CAM6       | CAM6           | <i>Triatoma dimidiata</i>     | Mexico    | Campeche            | 19.188 | -90.300  | 2011 | NORTH-CENT | This study  |
| CRISTY     | CRISTY         | <i>Homo sapiens</i>           | Mexico    | San Luis Potosí     | 22.159 | -100.990 | 2007 | NORTH-CENT | This study  |
| MICH1      | MICH           | <i>Triatoma dimidiata</i>     | Mexico    | Michoacan           | 19.567 | -101.707 | 2011 | NORTH-CENT | This study  |
| NINOA      | NINOA          | <i>Homo sapiens</i>           | Mexico    | Oaxaca              | 17.054 | -96.714  | 1994 | NORTH-CENT | This study  |
| PLI        | PL             | <i>Dipetalogaster maxima</i>  | Mexico    | Baja California Sur | 26.044 | -111.666 | 2001 | NORTH-CENT | This study  |
| QROI       | QRO            | <i>Triatoma barberi</i>       | Mexico    | Queretaro           | 20.594 | -100.393 | 1986 | NORTH-CENT | This study  |
| TQI        | TQ             | <i>Triatoma pallidipennis</i> | Mexico    | Morelos             | 18.953 | -99.223  | 1991 | NORTH-CENT | This study  |

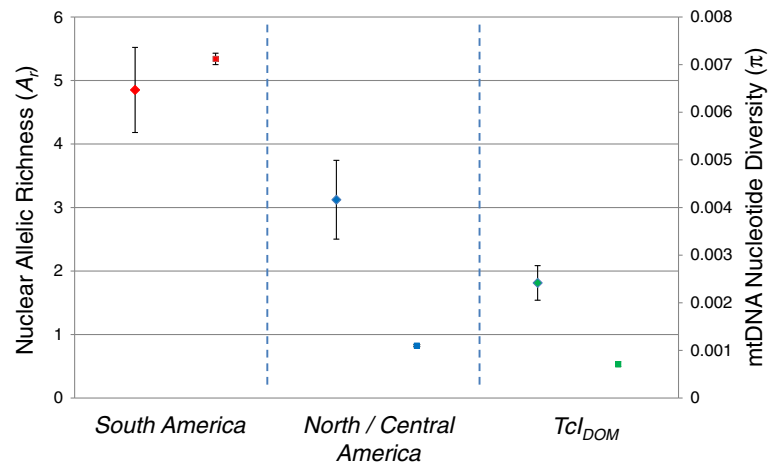
**Table 1 *Trypanosoma cruzi* I samples included in this study (Continued)**

|           |               |                              |           |            |            |         |         |            |                        |
|-----------|---------------|------------------------------|-----------|------------|------------|---------|---------|------------|------------------------|
| XAL1      | XAL           | <i>Triatoma dimidiata</i>    | Mexico    | Veracruz   | 19.173     | -96.133 | 2003    | NORTH-CENT | This study             |
| 9209802P  | 9209802P c1   | <i>Didelphis marsupialis</i> | USA       | Georgia    | 32.430     | -83.310 | 1992    | NORTH-CENT | Messenger et al., [12] |
| 9307      | 93070103P c1  | <i>Didelphis marsupialis</i> | USA       | Georgia    | 32.430     | -83.310 | 1993    | NORTH-CENT | Messenger et al., [12] |
| ARMA      | USAARMA c13   | <i>Dasypus novemcinctus</i>  | USA       | Lousiana   | 30.500     | -91.000 | Unknown | NORTH-CENT | Messenger et al., [12] |
| USA       | USAPOSSUM c12 | <i>Didelphis marsupialis</i> | USA       | Lousiana   | 30.500     | -91.000 | Unknown | NORTH-CENT | Messenger et al., [12] |
| 9354      | 9354          | <i>Homo sapiens</i>          | Venezuela | Sucre      | 10.460     | -63.610 | 1999    | TcIDOM     | Messenger et al., [12] |
| 11541     | 11541         | <i>Homo sapiens</i>          | Venezuela | Merida     | 8.590      | -71.230 | 2003    | TcIDOM     | Messenger et al., [12] |
| 11713     | 11713         | <i>Homo sapiens</i>          | Venezuela | Lara       | 10.233     | -69.866 | 2003    | TcIDOM     | Messenger et al., [12] |
| 11804     | 11804         | <i>Homo sapiens</i>          | Venezuela | Portuguesa | 9.084      | -69.103 | 2003    | TcIDOM     | Messenger et al., [12] |
| 10462P2C3 | 10462P2C3     | <i>Homo sapiens</i>          | Venezuela | Miranda    | 10.266     | -66.485 | Unknown | TcIDOM     | This study             |
| 10462P2C7 | 10462P2C7     | <i>Homo sapiens</i>          | Venezuela | Miranda    | 10.080     | -66.449 | Unknown | TcIDOM     | This study             |
| 10968P1C1 | 10968P1C1     | <i>Homo sapiens</i>          | Venezuela | Sucre      | 10.406     | -63.298 | Unknown | TcIDOM     | This study             |
| ANT3P1C6  | ANT3P1C6      | <i>Homo sapiens (oral)</i>   | Venezuela | DC         | 10.500     | -66.951 | Unknown | SOUTH      | This study             |
| M13       | M13           | <i>Didelphis marsupialis</i> | Venezuela | Barinas    | 7.500      | -71.230 | 2004    | SOUTH      | Messenger et al., [12] |
| M16       | M16 c14       | <i>Didelphis marsupialis</i> | Venezuela | Barinas    | 7.500      | -71.230 | 2004    | SOUTH      | Messenger et al., [12] |
| M18       | M18           | <i>Didelphis marsupialis</i> | Venezuela | Barinas    | 7.500      | -71.230 | 2004    | SOUTH      | Messenger et al., [12] |
| M7        | M7            | <i>Didelphis marsupialis</i> | Venezuela | Barinas    | 7.500      | -71.230 | 2004    | SOUTH      | Messenger et al., [12] |
| 92122     | 92122102R     | <i>Procyon lotor</i>         | TcIV      | USA        | Georgia    |         |         | OUTGROUPS  | Messenger et al., [12] |
| CANIII    | CANII c1      | <i>Homo sapiens</i>          | TcIV      | Brazil     | Belem      |         |         | OUTGROUPS  | Messenger et al., [12] |
| CM17      | CM17          | <i>Dasypus spp.</i>          | TcIII     | Colombia   | Carimaga   |         |         | OUTGROUPS  | Messenger et al., [12] |
| X1060     | X10610 c15    | <i>Homo sapiens</i>          | TcIV      | Venezuela  | Guárico    |         |         | OUTGROUPS  | Messenger et al., [12] |
| ERA       | ERA c12       | <i>Homo sapiens</i>          | TcIV      | Venezuela  | Anzoátegui |         |         | OUTGROUPS  | Messenger et al., [12] |
| 10R26     | 10R26         | <i>Aotus spp.</i>            | TcIV      | Bolivia    | Santa Cruz |         |         | OUTGROUPS  | Messenger et al., [12] |
| SAIRI3    | Saimiri3 c1   | <i>Saimiri sciureus</i>      | TcIV      | Venezuela  | Venezuela  |         |         | OUTGROUPS  | Messenger et al., [12] |

species evolved exclusively in South America. Others propose an alternative origin of *T. cruzi* from an ancestral bat trypanosome potentially capable of long range dispersal [7]. Whilst the precise scenario for the arrival of ancestral *Schizotrypanum* lineages in South America is a matter for debate, the current continental distribution and genetic diversity of *T. cruzi* supports an origin within South America. Parasite transmission is maintained via hundreds of mammal and triatomine species in different biomes throughout South and Central America, as well as the southern states of the USA [8].

Biochemical and molecular markers support the existence of six lineages or Discrete Typing Units (DTU): TcI, -

TcVI agreed by international consensus ([9]. Each DTU can be loosely associated with a particular ecological and/or geographical framework [10]. TcI is ubiquitous among arboreal sylvatic foci throughout the geographic distribution of *T. cruzi* and is the major agent of human Chagas disease in northern South America. Several molecular tools now identify substantial genetic diversity within TcI [11-14]. Importantly these new approaches consistently reveal the presence of a genetically divergent and homogeneous TcI group (henceforth TcI<sub>DOM</sub> – previously TcIa/VEN<sub>DOM</sub>) associated with human infections from Venezuela to Northern Argentina, and largely absent from wild mammals and vectors sampled to date [14]. The origin of this clade is unclear, although recent work supports a



**Figure 1** Nucleotide diversity and allelic richness comparisons across North and South American *Trypanosoma cruzi* I populations. Left hand data points (diamond) indicate allelic richness  $\pm$  standard error over loci. Right hand data points (square) indicate nucleotide diversity ( $\pi$ )  $\pm$  standard error over pair-wise comparisons.

sister group relationship with TcI circulating in North America (e.g. [12,13]).

In this manuscript we have set out to evaluate the genetic diversity of TcI in North/Central America, undertaking a comparison with TcI diversity in South America, including TcI<sub>DOM</sub>. Our aim was to examine hypotheses describing the origin of the TcI<sub>DOM</sub> clade. We propose two possible scenarios: an emergence of TcI<sub>DOM</sub> in northern South America as a sister group of North American strains and dispersal among domestic transmission cycles, or an origin in North America, prior to dispersal back into South American domestic cycles, possibly anthropically. To provide further insight into this question we undertook high resolution nuclear and mitochondrial genotyping of multiple Central American strains (from areas of México and Guatemala) and included them in an analysis with other published data [11-13].

A panel of 72 TcI isolates and clones was assembled for analysis (Table 1) [11-16]. Of these, existing sequences and microsatellite data were available for 46 isolates [11,12]. Isolates were classified into three populations: TcI<sub>NORTH-CENT</sub>, TcI<sub>SOUTH</sub> and TcI<sub>DOM</sub>. TcI<sub>NORTH-CENT</sub> includes samples from the USA, México, Guatemala and Honduras; TcI<sub>SOUTH</sub> corresponds to South America (Argentina, Bolivia, Colombia, Venezuela and Brazil) and TcI<sub>DOM</sub> with exclusively domestic isolates from Colombia and Venezuela, already known to correspond to a genotype with restricted genetic diversity: TcIa, as previously described by Herrera *et al.*, (2007) [17] and VEN<sub>Dom</sub>, as described by Llewellyn *et al.*, (2009) [13]. Additional DTU isolates (TcIII-TcIV) were included as out-groups in the mitochondrial analysis.

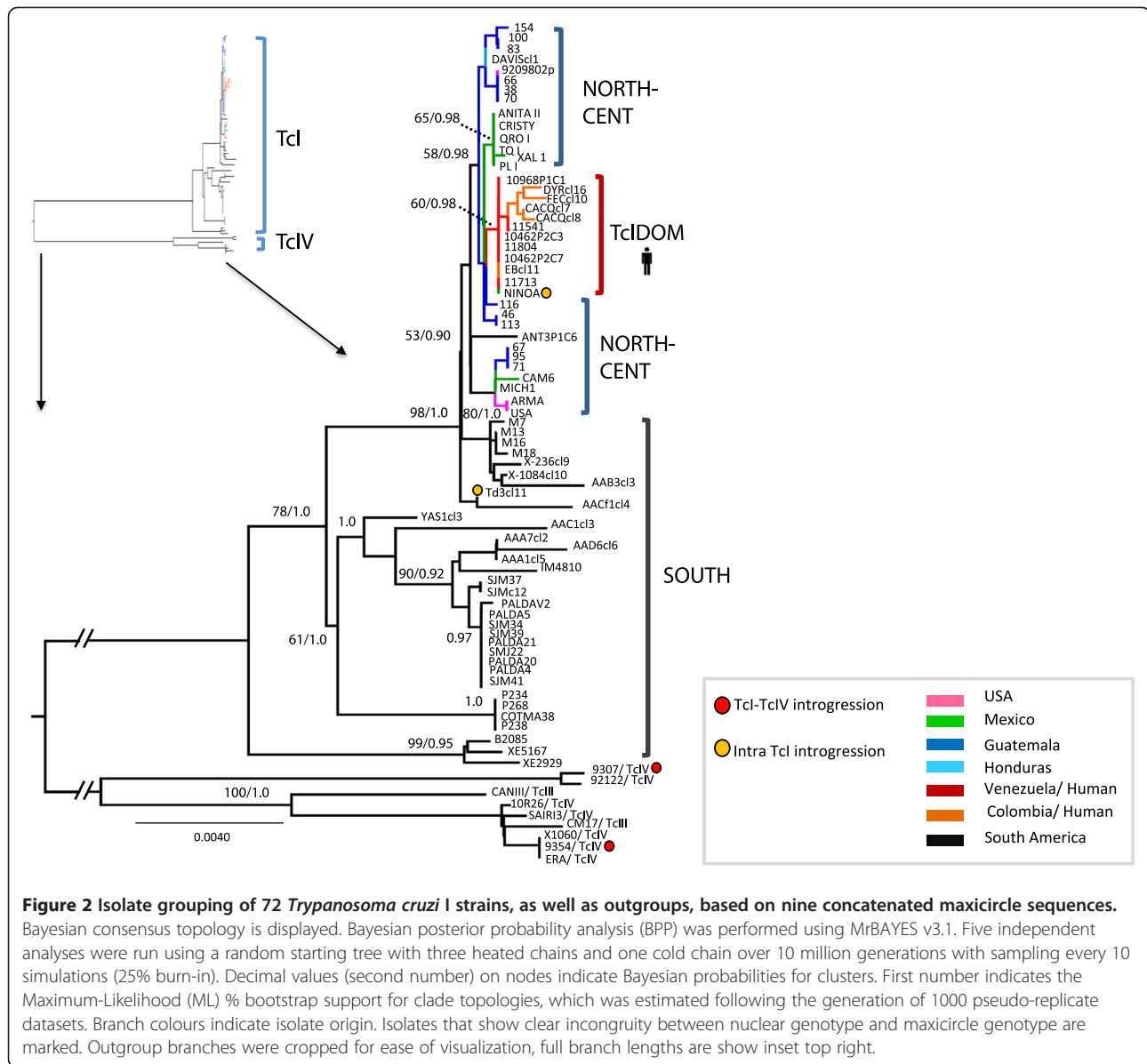
Isolates from México and Guatemala were characterized to DTU level via the amplification and sequencing

of glucose-6-phosphate isomerase (*GPI*) as previously described by Lauthier *et al.*, (2012) [18]. Subsequently, nine maxicircle gene fragments were amplified, sequenced and concatenated from the Mexican and Guatemalan strains according to Messenger *et al.*, 2012 (excluding *ND4*) [12]. Phylogenetic analysis was also conducted as in Messenger *et al.*, 2012 [12]. Nineteen nuclear microsatellite loci previously described by Llewellyn *et al.*, 2009 [13], were selected based on their level of TcI intra-lineage resolution. Microsatellite loci were amplified across 21 unpublished biological stocks from México and Guatemala. Reaction conditions were as described previously [13]. Dendrograms based on multilocus allele profiles were constructed also according to Llewellyn *et al.*, 2009 [13].

Maxicircle nucleotide diversity ( $\pi$ ) was calculated for TcI<sub>NORTH-CENT</sub>, TcI<sub>SOUTH</sub> and TcI<sub>DOM</sub> respectively in DNAsp v5 [19]. Nuclear allelic diversity was calculated for the same populations using allelic richness ( $A_r$ ) in FSTAT [20]. The resulting values are shown in Figure 1.

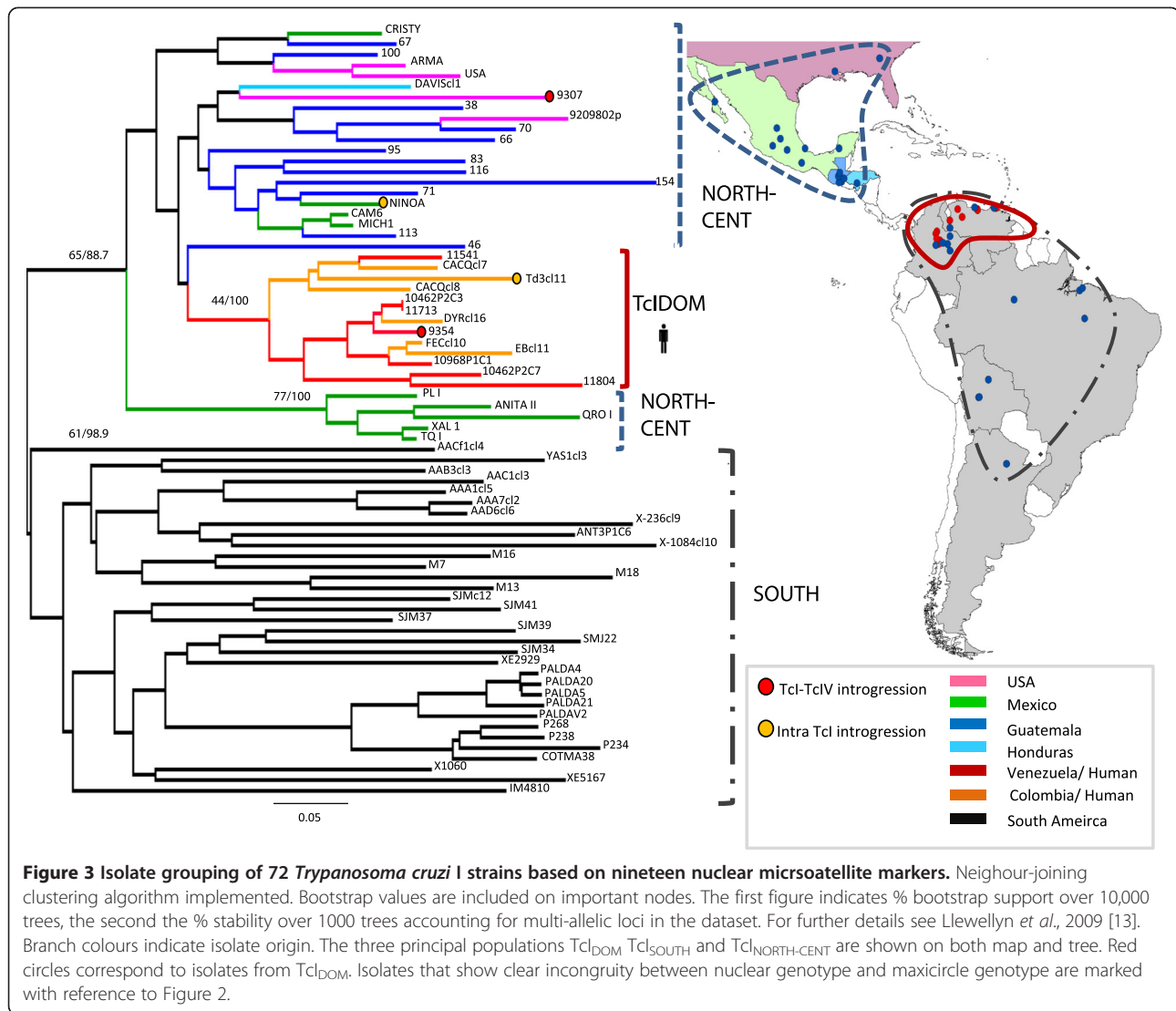
Nucleotide sequences per gene fragment are available from GenBank under the accession numbers *MURF1* (fragment a): JX431060 - JX431084; *MURF1* (fragment b): JX431085 - JX431109; *ND1*: JX431110 - JX431134; *ND5* (fragment a): JX431135 - JX431159; *ND5* (fragment b): JX431160 - JX431184; *9S rRNA*: JX431185 - JX431209; *12S rRNA*: JX431210 - JX431234; *COII*: JX431235 - JX431259 and *CYT b*: JX431260 - JX431284.

Across the 3,449 bp final concatenated alignment (including outgroups), a total of 374 variable sites were found. The mitochondrial phylogeny supported the presence of significant diversity among the isolates examined (Figure 2). TcI<sub>DOM</sub> strains formed a monophyletic clade [60% ML BS/0.98 BPP]. The principal division in the



phylogeny was between  $TcI_{SOUTH}$  and  $TcI_{DOM}/TcI_{NORTH-CENT}$  (98% ML BS/0.98 BPP). However, this division is incomplete, such that a subset of South American strains is also grouped with  $TcI_{DOM}$  and  $TcI_{NORTH-CENT}$ . Thus, it is not possible to conclude that  $TcI_{DOM}$  maxicircle sequences nest uniquely among those from  $TcI_{NORTH-CENT}$  strains. Conversely, a basal relationship of the  $TcI_{NORTH-CENT}$  to  $TcI_{DOM}$  is suggested at the level of nucleotide diversity by population (Figure 1), whereby  $TcI_{DOM} < TcI_{NORTH-CENT} < TcI_{SOUTH}$ . Low standard errors about the mean in all three populations, but especially in  $TcI_{DOM}$  and  $TcI_{NORTH-CENT}$ , suggest that sample size had little impact on the accuracy of estimation between populations.

Distance-based clustering using the microsatellite dataset indicated the presence of several well defined clades (Figure 3). Importantly in this case the monophyly of North-Central American isolates was corroborated, and  $TcI_{DOM}$  clustered firmly within them (bootstrap 65%). By contrast, South American isolates fall into a divergent but diverse clade. Thus the nuclear data provide stronger support for divergence of  $TcI_{DOM}$  from within  $TcI_{NORTH-CENT}$  than the maxicircle phylogeny. Sample size-corrected allelic richness estimates are consistent with hierarchical patterns of clustering based on pair-wise genetic distances. As with the maxicircle dataset, there is a pronounced cline in diversity across the populations studied -  $A_r TcI_{DOM} < A_r TcI_{NORTH-CENT} < A_r TcI_{SOUTH}$  (Figure 1).



### TcI dispersion into Central and North America

Using a 100 MYA biogeographic calibration point [6], molecular clock analyses point to the origin of *T. cruzi* (*sensu stricto*) 5 – 1 MYA [21-23] and a most recent common ancestor for TcI at 1.3-0.2 MYA [22]. Reduced genetic diversity among North-Central American isolates by comparison to their southern counterparts is powerful evidence in support of others who suggest that TcI originated in South America [13,24]. The emergence of TcI in the South occurred prior to either migration across the Isthmus of Panama alongside didelphid marsupials during the Great American Interchange [25], or perhaps prior to northerly dispersal via volant mammals (e.g. bats).

### Origin of TcI<sub>DOM</sub>

Recent findings indicate a close resemblance between TcI<sub>DOM</sub> isolates from the northern region of South

America and parasite populations from Central and North America by the use of nuclear and mitochondrial markers [11-13]. Indeed SL-IR genotyping suggests a distribution for TcI<sub>DOM</sub> that now extends as far south as the Argentine Chaco, where multiple sequences have been identified from human and domestic vector sources [14]. Llewellyn *et al.*, (2009) originally hypothesised that a distinct human/domestic clade could be maintained despite the presence of nearby infective sylvatic strains due to the low parasite transmission efficiency by the vector [13]. In this case multiple feeds by domestic vector nymphs are required to infect individuals, as such human – human transmission is far more common than reservoir host - human transmission. Originally this hypothesis was developed to explain the epidemiology of Chagas disease in Venezuela. However, TcI<sub>DOM</sub> is clearly widespread and recent data propose a date for its emergence 23,000 ± 12,000 years ago [11].



This corresponds to the earliest human colonisation of the Americas [26]. Thus it seems that TcI<sub>DOM</sub> may be as ancient as humans in South America. Crucially, our data, which show that TcI<sub>DOM</sub> is nested among North and Central American strains, suggest that this widespread domestic *T. cruzi* genotype may actually have made first contact with man in North–Central America.

The expansion of limited diversity genotypes into domestic transmission cycles is a familiar story in *T. cruzi*. This phenomenon seems to have occurred almost simultaneously with TcI<sub>DOM</sub> (<60,000 YA) in the Southern Cone region but involving DTUs TcV and TcVI [22]. Nonetheless, static human population densities sufficient to support a sustained domestic cycle are presumably vital. For TcI<sub>DOM</sub>, patterns of genetic diversity suggest early colonizing Amerindians may have been responsible for its southerly migration and dispersal from North/Central America. However, such early settler populations were probably small, dynamic, and inherently unsuitable to sustain transmission of such a genotype. Many questions, therefore, remain unanswered regarding its emergence. Insight could perhaps be drawn from a better understanding of the current distribution and diversity of TcI<sub>DOM</sub> (including samples from the Southern Cone), patterns of vector population migrations, and even from analysis of ancient DNA (e.g. [27]). We hope this report serves to galvanize efforts towards this understanding, especially among researchers in Central and North America, where many of the answers lie.

#### Competing interests

The authors declare no competing financial interests. The funder played no role in the study design.

#### Authors' contributions

FZE wrote the article, performed the experiments and analysed the data. LAM analysed the data and wrote the article. MAM, TLO, PM, MDL contributed reagents and wrote the paper. CFL analysed the data. JMI, HC, MS contributed reagents. MSL conceived the experiments, analysed the data and wrote the article. All authors read and approved the final version of the manuscript.

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