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Importation of Hybrid Human-Associated *Trypanosoma cruzi* Strains of Southern South American Origin, Colombia

Technical Appendix 1

Technical Appendix 1 Table 1. Panel of Colombian biologic clones and reference clones assembled for analysis.

Strain code	Host/vector	Department	Country*	Discrete typing unit
Colombian Clones†				
EB cl4‡	*Homo sapiens* neonate (suspected congenital infection)	Boyaca	Colombia	TcII
EB cl6	*Homo sapiens* neonate (suspected congenital infection)	Boyaca	Colombia	TcII
EB cl20	*Homo sapiens* neonate (suspected congenital infection)	Boyaca	Colombia	TcII
PGPA2 cl6	*Panstrongylus geniculatus*	Casanare	Colombia	TcII
PGPA2 cl7	*Panstrongylus geniculatus*	Casanare	Colombia	TcII
PGPA2 cl10	*Panstrongylus geniculatus*	Casanare	Colombia	TcII
CM17	*Dasypus sp.*	Carimagua	Colombia	TcIII
CM25 cl2	*Dasypus novemcinctus*	Carimagua	Colombia	TcIII
SLDN1 cl6	*Dasypus novemcinctus*	Casanare	Colombia	TcIII
TV cl9	*Triatoma venosa*	Boyaca	Colombia	TcIII
AACf2 cl11	*Canis familiaris*	Casanare	Colombia	TcVI
DA cl1	*Homo sapiens* adult (suspected congenital transmitter)	Boyaca	Colombia	TcVI
DA cl2	*Homo sapiens* adult (suspected congenital transmitter)	Boyaca	Colombia	TcVI
PG98 cl1	*Panstrongylus geniculatus*	Antioquia	Colombia	TcVI
PG98 cl7	*Panstrongylus geniculatus*	Antioquia	Colombia	TcVI
Rp540 cl4	*Rhodnius prolixus*	Casanare	Colombia	TcVI
Rp540 cl6	*Rhodnius prolixus*	Casanare	Colombia	TcVI
Rp540 cl7	*Rhodnius prolixus*	Casanare	Colombia	TcVI
Rp540 cl8	*Rhodnius prolixus*	Casanare	Colombia	TcVI
Rp540 cl9	*Rhodnius prolixus*	Casanare	Colombia	TcVI
VS cl6	*Homo sapiens* neonate (suspected congenital infection)	Boyaca	Colombia	TcVI
VS cl7	*Homo sapiens* neonate (suspected congenital infection)	Boyaca	Colombia	TcVI
VS cl8	*Homo sapiens* neonate (suspected congenital infection)	Boyaca	Colombia	TcVI
VS cl10	*Homo sapiens* neonate (suspected congenital infection)	Boyaca	Colombia	TcVI
Reference Clones§				
CBB cl2	*Homo sapiens*	Tulahuén	Chile	TcII
Chaco23 col4	*Triatoma infestans*	Pr. Hayes	Paraguay	TcII
Esm cl3	*Homo sapiens*	*São Felipe*	Brazil	TcII
IVV cl4	*Homo sapiens*	Cuncumen	Chile	TcII
Pot7a cl1	*Triatoma infestans*	San Martin	Paraguay	TcII
Pot7b cl5	*Triatoma infestans*	San Martin	Paraguay	TcII

Strain code	Host/vector	Department	Country*	Discrete typing unit
Rita cl5	*Homo sapiens*	*São Felipe*	Brazil	TcII
T665 cl1	*Triatoma infestans*	Pr. Hayes	Paraguay	TcII
Tu18 cl2	*Triatoma infestans*	Tupiza	Bolivia	TcII
85/847 cl2	*Dasypus novemcinctus*	Alto Beni	Bolivia	TcIII
ARMA13 cl1	*Dasypus novemcinctus*	Campo Lorro	Paraguay	TcIII
ARMA18 cl3	*Dasypus novemcinctus*	Campo Lorro	Paraguay	TcIII
JA2 cl2	*Monodelphis sp.*	Amazonas	Brazil	TcIII
M5631 cl5	*Dasypus novemcinctus*	Marajo	Brazil	TcIII
M6421 cl6	*Homo sapiens*	Belém	Brazil	TcIII
SABP19 cl1	*Triatoma infestans*	Vitor	Peru	TcIII
X109/2	*Canis familiaris*	Makthlawaiya	Paraguay	TcIII
X9/3	*Canis familiaris*	Makthlawaiya	Paraguay	TcIII
92.80 cl2	*Homo sapiens*	Santa Cruz	Bolivia	TcV
Bug 2148 cl1	*Triatoma infestans*	Rio Grande do Sul	Brazil	TcV
Chaco2 cl3	*Triatoma infestans*	Chaco	Paraguay	TcV
PAH179 cl5	*Homo sapiens*	Chaco	Argentina	TcV
Para4 cl3	*Triatoma infestans*	Paraguari	Paraguay	TcV
Para6 cl4	*Triatoma infestans*	Paraguari	Paraguay	TcV
Sc43 cl1	*Triatoma infestans*	Santa Cruz	Bolivia	TcV
Vinch101 cl1	*Triatoma infestans*	Limari	Chile	TcV
Chaco17 col1	*Triatoma infestans*	Chaco	Paraguay	TcVI
CL Brener	*Triatoma infestans*	Rio Grande do Sul	Brazil	TcVI
EPV20–1 cl1	*Triatoma infestans*	Chaco	Argentina	TcVI
LHVA cl4	*Triatoma infestans*	Chaco	Argentina	TcVI
P251 cl7	*Homo sapiens*	Cochabamba	Bolivia	TcVI
Tula cl2	*Homo sapiens*	Tulahuén	Chile	TcVI
VFRA1 cl1	*Triatoma infestans*	Francia	Chile	TcVI

*References (*1*–*5*) describe the different geographic distributions, host/vector associations, and transmission cycles of *T. cruzi* DTUs in Colombia.

†Colombian clones were assigned to DTU-level by PCR amplification of the *SL-IR, 24α rDNA* and *18S rDNA* subunits according to (*6*). Putative hybrid strains were identified by either a double *24α rDNA* amplicon (125 and 140 bp) (TcV) or single *24α rDNA* amplicon (140 bp) and amplification of the A10 fragment of the *18S rDNA* subunit (TcVI) (525 or 630 bp), and confirmed by sequencing glucose-6-phosphate isomerase (*GPI*), as previously described (*6*).

‡Indicates multiple biologic clones derived from a single parasite strain.

§Reference clones were assigned to DTU-level using a triple-marker assay described by Lewis et al. (*7*).

Technical Appendix 1 Table 2. Intra-lineage diversity and properties of nuclear and mitochondrial MLST schemes*

T. cruzi DTU	Total no. isolates	Housekeeping gene																				nMLST†				mtMLST‡			
		GPX				*GTP*				*Met-II*				*TcAPX*				*TcMPX*											
		VS	ST	TE	DP	VS	ST	TE	DP	VS	ST	TE	DP	VS	ST	TE	DP	VS	ST	TE	DP	VS	ST	TE	DP	VS	ST	TE	DP
TcII	15 [6]	4 [0]	6 [1]	1.5 [0]	0.4 [0.17]	2 [0]	3 [1]	1.5 [0]	0.2 [0.17]	5 [0]	6 [1]	1.2 [0]	0.4 [0.17]	2 [0]	3 [1]	1.5 [0]	0.2 [0.17]	8 [0]	4 [1]	0.5 [0]	0.27 [0.17]	21 [0]	10 [1]	0.48 [0]	0.67 [0.17]	46 [25]	7 [3]	0.15 [0.12]	0.47 [0.5]
TcIII	13 [4]	10 [4]	8 [3]	0.8 [0.75]	0.62 [0.75]	2 [1]	3 [2]	1.5 [2.0]	0.23 [0.5]	10 [5]	7 [3]	0.7 [0.6]	0.54 [0.75]	4 [3]	5 [3]	1.25 [1.0]	0.38 [0.75]	1 [1]	3 [2]	3.0 [2.0]	0.23 [0.5]	27 [13]	13 [4]	0.48 [0.31]	1.0 [1.0]	107 [80]	10 [4]	0.093 [0.05]	0.77 [1.0]
TcV	8 [0]	0	1	0	0.125	0	1	0	0.125	17	2	0.12	0.25	9	4	0.44	0.5	5	2	0.4	0.25	31	5	0.16	0.63	6	8	1.33	1
TcVI	21 [14]	10 [2]	4 [3]	0.4 [1.5]	0.19 [0.21]	5 [5]	3 [3]	0.6 [0.6]	0.14 [0.21]	14 [0]	4 [1]	0.29 [0]	0.19 [0.07]	11 [11]	7 [4]	0.64 [0.36]	0.33 [0.29]	5 [0]	5 [1]	1.0 [0]	0.24 [0.07]	42 [12]	16 [9]	0.38 [0.75]	0.76 [0.86]	26 [26]	9 [7]	0.35 [0.27]	0.43 [0.5]

*Nos. in square brackets represent strains from Columbia. DP, no. of genotypes identified per total no. of isolates; DTU, discrete typing unit; MLST, multilocus sequence typing; mtMLST, mitochondrial MLST scheme, nMLST, nuclear MLST scheme, ST, no. of genotypes; TE, no. of genotypes identified per polymorphic site; VS, no. of variable sites.
†Based on 5 concatenated loci.
‡Based on 10 concatenated loci.

Technical Appendix 1 Table 3. Panel of microsatellite loci and primers employed in this study*

Chromosome	Primer code	Repeat type	Forward/reverse primer (5′→3′)
6	6529$(CA)_a$	$(CA)_n$	TGTGAAATGATTTGACCCGA AGAGTCACGCCGCAAAGTAT
6	6529$(TA)_b$	$(TA)_n$	TGAAGGAGATTCTCTGCGGT CTCTCATCTTTTGTTGTGTCCG
6	mclf10	$(CA)_nA(CA)_n$	GCGTAGCGATTCATTTCC ATCCGCTACCACTATCCAC
10	6855(TA)(GA)	$(TA)_n(GA)_n$	TGTGATCAACGCGCATAAAT TTCCATTGCCTCGTTTTAGA
15	11863(CA)	$(CA)_n$	AGTTGACATCCCCAAGCAAG CCCTGATGCTGCAGACTCTT
19	10101(TA)	$(TA)_n$	AACCCGCGCAGATACATTAG TTCATTTGCAGCAACACACA
24	8741(TA)	$(TA)_n$	TGTAACGGTAGGTCTCAATTCG TTGCACTTGTGTATCTCGCC
27	10101(TC)	$(TC)_n$	CGTACGACGTGGACACAAAC ACAAGTGGGTGAGCCAAAAG
27	10101$(CA)_c$	$(CA)_n$	GTGTCGTTGCTCCCAAACTC AAACTTGCCAAATGTGAGGG
27	10101$(CA)_a$	$(CA)_n$	GTCGCCATCATGTACAAACG CTGTTGGCGAATGGTCATAA
34	6559(TC)	$(TC)_n$	CGCTCTCAAAGGCACCTTAC ATATGGACGCGTAGGAGTGC
37	10187(TTA)	$(TTA)_n$	GAGAGAGATTCGGAAACTAATAGC CATGTCCCTTCCTCCGTAAA
37	10187(CA)(TA)	$(CA)_n(TA)_n$	CATGTCATTAAGTGGCCACG GCACATGTTGGTTGTTGGAA
37	10187(TA)	$(TA)_n$	AGAAAAAGGTTTACAACGAGCG

Chromosome	Primer code	Repeat type	Forward/reverse primer (5′→3′)
			CGATGGAGAACGTGAAACAA
37	10187(GA)	$(GA)_n$	GTCACACCACTAGCGATGACA
			ACTGCACAATACCCCCTTTG
37	TcUn4	Unknown	ATGCTCCGCAACATATTACTCA
			GTCGAGCTTCTGTTGTTCCC
39	$6925(TG)_b$	$(TG)_n$	GAAACGCACTCACCCACAC
			GGTAGCAACGCCAAACTTTC
39	7093(TC)	$(TC)_n$	CCAACATTCAACAAGGGAAA
			GCATGAATATTGCCGGATCT
39	6925(CT)	$(CT)_n$	CATCAAGGAAAAACGGAGGA
			CGGTACCACCTCAAGGAAAG
39	$7093(TA)_c$	$(TA)_n$	CGTGTGCACAGGAGAGAAAA
			CGTTTGGAGGAGGATTGAGA
39	$6925(TG)_a$	$(TG)_n$	TCGTTCTCTTTACGCTTGCA
			TAGCAGCACCAAACAAAACG
39	7093(TCC)	$(TCC)_n$	AGACGTTCATATTCGCAGCC
			AGCCACATCCACATTTCCTC
40	11283(TCG)	$(TCG)_n$	ACCACCAGGAGGACATGAAG
			TGTACACGGAACAGCGAAG
40	$11283(TA)_b$	$(TA)_n$	AACATCCTCCACCTCACAGG
			TTTGAATGCGAGGTGGTACA
41	10359(CA)(GA)	$(CA)_n(GA)_n$	AGTCCTACTGCCTCCTTGCA
			CTGTTGGCGAATGGTCATAA

*A possible confounder that must be considered during data interpretation is that due to the high mutation rate of microsatellites, potentially as high as 1/1,000 cell divisions (*8*), and between different loci, some of the length variation observed may be de novo, arising during parasite isolation/culturing, not during natural strain evolution and transmission.

References

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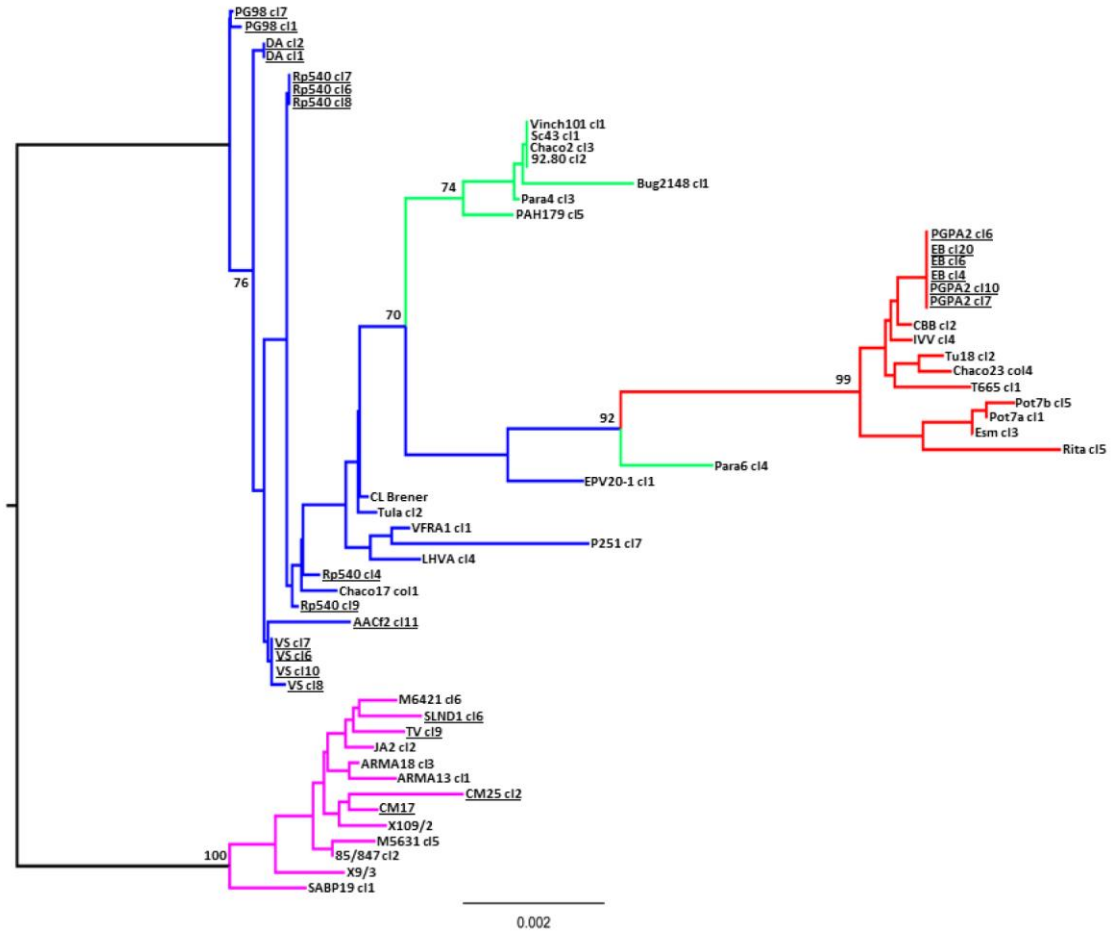
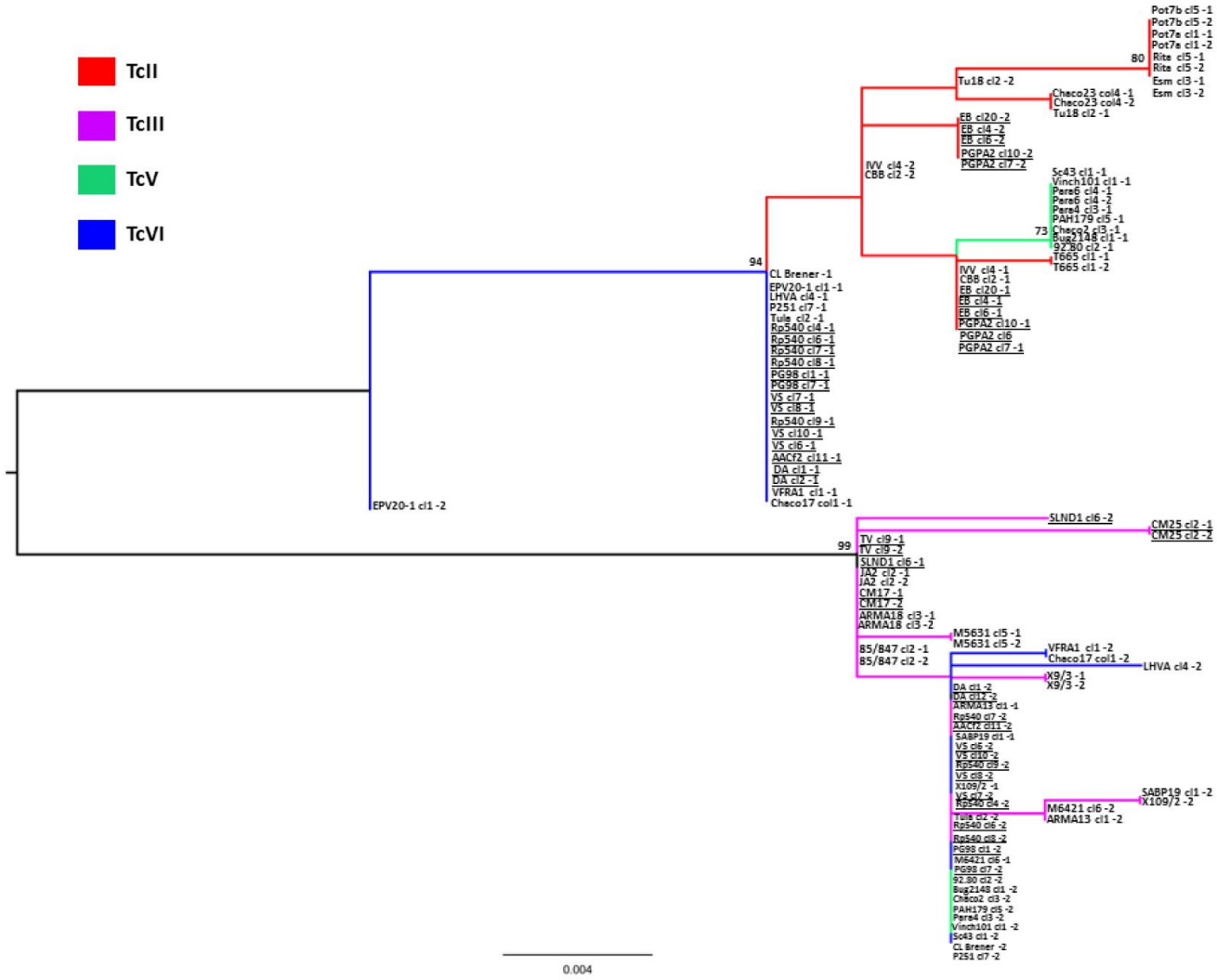


Technical Appendix Figure 1. Unrooted Neighbor-Joining tree based on five concatenated diploid nuclear MLST sequences. For each isolate, nuclear diploid sequence data were concatenated in order of their relative chromosomal positions (Met-II, GTP, TcMPX, TcGPX and TcAPX, on chromosomes 6, 12, 22, 35 and 36, respectively). In MLSTest, phylogenetic incongruence between loci was assessed using the BIO-Neighbor Joining Incongruence Length Difference test (BIONJ-ILD) and evaluated by a permutation test with 1,000 replicates. A final Neighbor-Joining tree was constructed and statistical support was calculated as the mean across 1,000 randomizations and those >70% are shown for relevant nodes. Branch colors indicate isolate DTU (TcII, TcIII, TcV or TcVI). Colombian strain labels are underlined.



Technical Appendix Figure 2. Maximum-Likelihood tree constructed from Met-II haplotypes. Haplotypes for each nuclear gene were inferred using PHASE v2.1 software, which utilizes a modified Markov chain Monte Carlo (MCMC) algorithm to identify all unambiguous haplotypes within a population, i.e., those observed in strains which are homozygous at all variable sites or heterozygous at only a single polymorphic site.

Haplotypes in the remaining isolates, which are heterozygous at multiple sites (and therefore of ambiguous phase), are then estimated and a probability of uncertainty assigned to each phase call (latterly confirmed by PCR cloning if $p<0.95$). Maximum-Likelihood topologies were constructed using haplotypes for each individual nuclear locus. The phylogeny generated for Met-II, the most polymorphic target, is given as an example above. The most appropriate nucleotide substitution model was TrNef+G (three substitution rate categories) based on the AIC. Statistical support for major clades is given as equivalent bootstraps and posterior probabilities from consensus Maximum-Likelihood (1,000 pseudo-replicates) and Bayesian trees (based on the HKY+G model), respectively. Branch colors indicate isolate DTU (TcII, TcIII, TcV or TcVI). Colombian strain labels are underlined.