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

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We report the characterization of *Trypanosoma cruzi* of southern South American origin among humans, domestic vectors, and peridomestic hosts in Colombia using high-resolution nuclear and mitochondrial genotyping. Expanding our understanding of the geographic range of lineage TcVI, which is associated with severe Chagas disease, will help clarify risk of human infection for improved disease control.

Chagas disease is the most common parasitic infection in Latin America, annually affecting ≈5–6 million persons and putting another 70 million at risk (*1*). The etiologic agent, *Trypanosoma cruzi*, displays remarkable genetic diversity, which is widely thought to contribute to the considerable biologic, epidemiologic, and clinical variation observed in regions where the disease is endemic (*2*). Seven discrete typing units (DTUs) are currently recognized (TcI–TcVI and TcBat) (*2*); TcV and TcVI are natural interlineage hybrids of TcII and TcIII (*3*). It is unknown whether these hybrids arose from multiple independent recombination events (*3*) or a single incidence of hybridization followed by clonal divergence (*4*). Molecular dating indicates these lineages evolved recently (<1 million years ago) (*3*,*4*), suggesting that genetic exchange may still be driving the emergence of novel recombinants (*3*,*4*).

Historically, most *T. cruzi* DTUs have had broadly distinct, but often overlapping, geographic and ecologic distributions (*2*). TcV and TcVI are largely confined to domestic transmission cycles and are sympatric with severe chronic and congenital human disease in southern South America (*2*). Increased sampling indicates that the geographic ranges of TcV and TcVI are more extensive than previously suggested. Putative domestic hybrid strains were identified recently as far north as Colombia (*5*); it is unclear whether these are bona fide TcV and TcVI isolates (suggesting long-range introduction) or progeny of a novel, independent, and local recombination event(s). Elucidation of the molecular epidemiology of TcV and TcVI has been complicated by limited sample collections and difficulties distinguishing these genotypes from their parental DTUs (*6*) and each other (*7*). We undertook high-resolution nuclear and mitochondrial genotyping of hybrid clones from Colombia to resolve their putative status as novel recombinants and provide further insights into the evolutionary origin(s) of TcV and TcVI.

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The Study

For analysis, we assembled a panel of 57 *T. cruzi* biologic clones from a range of representative hosts/vectors across South America: 24 uncharacterized clones from Colombia and 33 reference clones (Figure 1; online Technical Appendix 1 Table 1, http://wwwnc.cdc.gov/EID/article/22/8/15-0786-Techapp1.pdf). From 2002–2010, we isolated the uncharacterized clones from humans; triatomine vectors (*Panstrongylus geniculatus, Rhodnius prolixus*, and *Triatoma venosa* insects); and sylvatic mammalian hosts (*Dasypus* spp. armadillos) in 3 *T. cruzi*–endemic departments in northern Colombia.

We genotyped all isolates using nuclear housekeeping genes *GPX*, *GTP*, *Met-II*, *TcAPX,* and *TcMPX* (*6*,*8*) (online Technical Appendix 1 Table 2); 25 microsatellite loci (online Technical Appendix 1 Table 3) (*9*); and 10 mitochondrial gene fragments (*10*). Diploid multilocus sequence typing (MLST) data were analyzed by locus and concatenated according to their relative chromosomal positions in MLSTest (*11*); heterozygous variable sites were handled as average states. Gene haplotypes were inferred using PHASE version 2.1 (*12*). PCR products were cloned and sequenced to confirm ambiguous gene phases. We constructed maximum-likelihood and Bayesian phylogenies for nuclear haplotypic and concatenated mitochondrial data (*13*).

For microsatellite loci, we defined sample clustering using a neighbor-joining tree based on pairwise distances between multilocus genotypes (Figure 2) (*13*). We calculated DTU-level heterozygosity (Bonferroni-corrected) and evaluated genetic diversity using sample size–corrected allelic richness and private allele frequency per locus (Table). To examine TcV/TcVI allele inheritance, we classified genotypes at each locus as hybrid (TcII/TcIII) or nonhybrid (TcII/TcII or TcIII/TcIII) based on the presence or absence of specific parental alleles (online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/22/8/15-0786-Techapp2.xlsx).

All putative hybrids from Colombia were highly heterozygous and minimally diverse. They possessed TcII and TcIII alleles at an approximate 1:1 ratio and, compared with parental DTUs, they displayed fewer private alleles or single-nucleotide polymorphisms; these strains fulfilled all the expectations of progeny from a recent Mendelian hybridization event(s) (Table). Based on nuclear MLST and microsatellite data, all hybrids from Colombia were classified as TcVI, not novel recombinants.

Examination of TcII and TcIII alleles across 5 nuclear loci showed that hybrid haplotypes from Colombia were shared among other TcVI strains from the Southern Cone region of South America and showed negligible affinities to parental alleles from Colombia (online Technical Appendix 1 Figures 1, 2). Microsatellite profiles also supported this allopatric inheritance: only a minority of private parental alleles from Colombia were common to local TcVI hybrids. At mitochondrial loci, TcVI clones from Colombia were noticeably divergent from local TcIII maxicircle haplotypes and those observed in reference TcVI strains (Figure 2). Of note, 1 hybrid from Colombia (AACf2 cl11), which was unequivocally classified as TcVI by both types of nuclear loci, possessed a TcV-type mitochondria. All isolates in this study were biologic clones, ruling out mixed infections as a potential confounder.

Overall, our data support the hypothesis that 2 separate recombination events led to the formation of TcV and TcVI. These interlineage hybrids have distinct nuclear and mitochondrial MLST genotypes and related but independent microsatellite profiles, and most alleles that distinguish between hybrid DTUs (70.4% [38/54 alleles]) were also present in their corresponding parental strains. Interlineage differences (fixed at 84% [21/25 of loci]) between TcV and TcVI are not consistent with allelic sequence divergence (Meselson effect); for such divergence, a much higher frequency of private alleles, compared with parental genotypes, would be expected at rapidly evolving microsatellite loci.

TcVI clones from Colombia had more private microsatellite alleles per locus (0.86) than their southern counterparts (0.43), despite their unequivocal origin in the Southern Cone. This phenomenon could be attributable to de novo mutations or a founder effect with respect to the northerly introduction of TcVI. Support for the latter cause is evidenced by an overall reduction in genetic diversity among hybrids from Colombia compared with TcVI strains from the Southern Cone (allelic richness 1.87 vs. 2.46, respectively). However, we cannot discount some sampling bias because reference Southern Cone strains represented a much wider geographic range.

A novel observation among TcVI strains from Colombia was the presence of an anomalous TcV maxicircle.

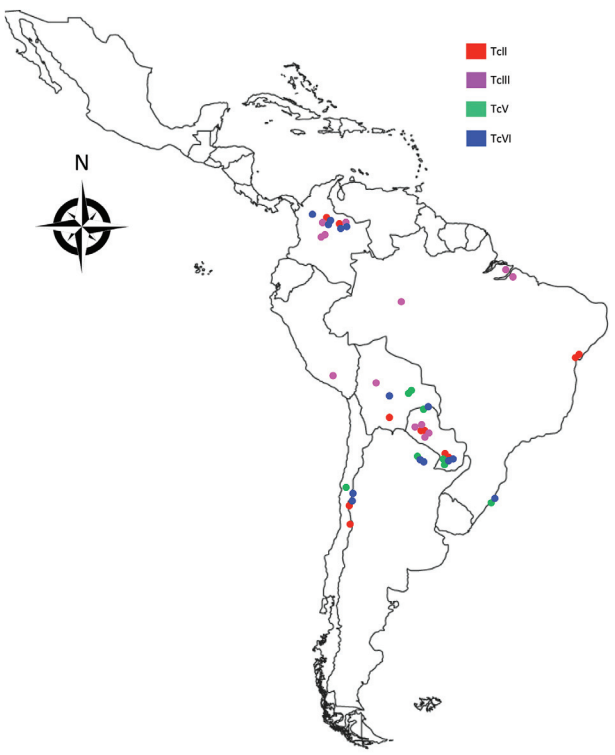

Figure 1. Geographic distribution of TcII, TcIII, TcV, and TcVI *Trypanosoma cruzi* clones, South America, 2002–2010. A total of 57 *T. cruzi* biologic clones were assembled for analysis. Of these, 24 were isolated from humans; triatomine vectors (*Panstrongylus geniculatus, Rhodnius prolixus*, and *Triatoma venosa* insects); and sylvatic mammalian hosts (*Dasypus* spp. armadillos) in Antioquia, Boyaca, and Casanare Departments in northern Colombia. The remaining 33 were reference clones derived from a range of representative hosts and vectors across South America (online Technical Appendix 1 Table 1, http://wwwnc.cdc.gov/EID/article/22/8/15-0786-Techapp1.pdf). Dots indicate geographic strain origin of biologic clones; colors denote isolate discrete typing units.

This pattern of inheritance could reflect 1) recent mitochondrial introgression from TcV into TcVI, leaving undetectable signatures of nuclear hybridization by our markers or, possibly, none at all (*10*,*14*), or 2) potential backcrossing of TcVI into TcIII. Genetic exchange has not been described among hybrid DTUs, but it might be expected to be more permissive between closely related strains (*14*). We also isolated hybrid AACf2 cl11 from a dog. *T. cruzi* hybridization has been proposed to arise within mammalian cells (*14*), and mixed infections in such hosts are common. Alternatively, TcV and TcVI may have evolved from the beneficiaries of different alleles during a single hybridization event between heterozygous parents with mixed TcIII-type mitochondrial complements; although, to date, reported levels of mitochondrial heteroplasmy in *T. cruzi* are low (*10*).

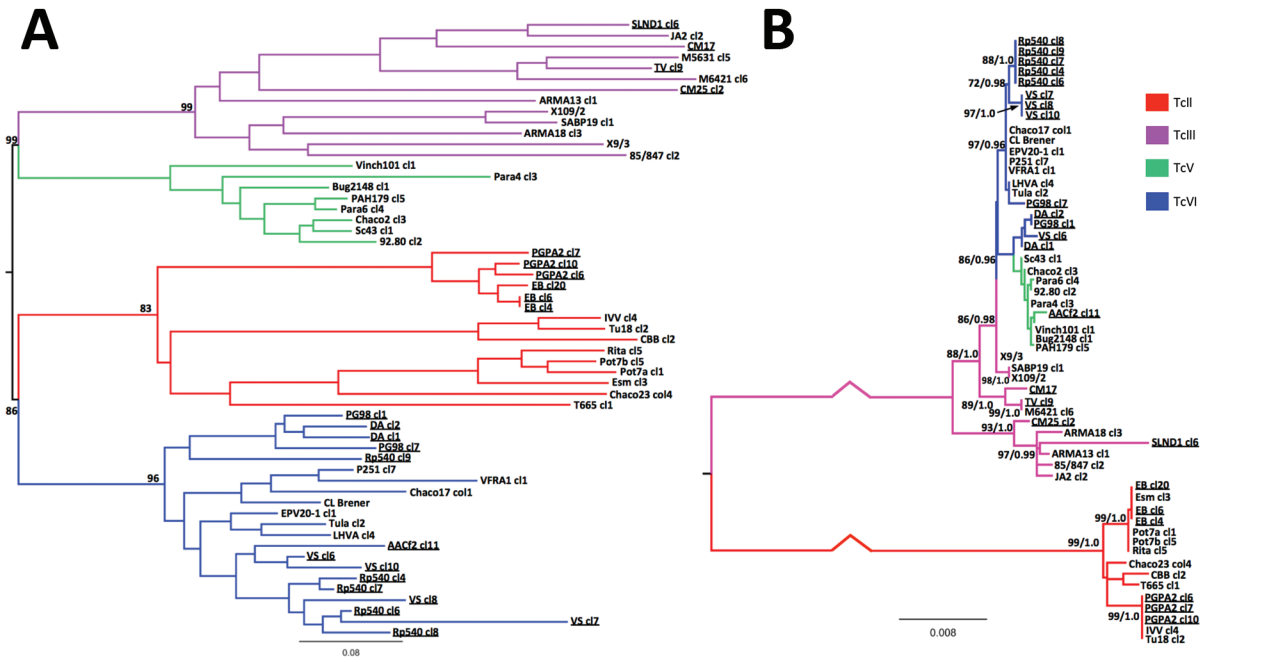

Figure 2. Phylogenetic trees showing relationships between *Trypanosoma cruzi* hybrids from Colombia and reference *T. cruzi* strains from across South America. A) Unrooted neighbor-joining tree based on pairwise distances between microsatellite loci. B) Maximum-likelihood tree from concatenated maxicircle sequences. Pairwise distance–based bootstrap values were calculated as the mean across 1,000 random diploid resamplings of the dataset; those >70% are shown for relevant nodes. A maximum-likelihood topology was constructed from concatenated maxicircle sequences for all clones. The most appropriate nucleotide substitution model was the general time reversible plus gamma distribution (9 substitution rate categories) based on the Akaike information criterion. 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 Hasegawa-Kishino-Yano plus gamma distribution model), respectively. Note that strain AACf2 cl11 is phylogenetically incongruent between nuclear and mitochondrial topologies. Branch colors indicate isolate discrete typing unit. Labels for clones from Colombia are underlined. Scale bars indicate genetic distance (A) and nucleotide substitutions per site (B).

Conclusions

Our understanding of the geographic and ecologic distribution of *T. cruzi* DTUs is changing because of parallel improvements in sampling strategies and genotyping techniques. Human Chagas disease in Colombia is currently associated with DTUs TcI, TcII (to a lesser extent), and oral outbreaks of TcIV (*5*). In this study, we isolated *T. cruzi* hybrids from 3 domestic triatomine vectors, a peridomestic dog, and congenital infections among local patients. Given that no reservoir hosts of TcV and TcVI have been described (*15*), the hybrids from Colombia are more likely the result of long-range anthropogenic introduction than local sylvatic invasion, especially considering the successful establishment of these DTUs among domestic infections in the Southern Cone. Further intensive sampling efforts in northern South America are warranted to elucidate the transmission cycle ecology of TcVI and to accurately assess the epidemiologic risk of

Table. Population genetic parameters for *Trypanosoma cruzi* discrete typing units, South America, 2002–2010*

Discrete typing unit	No. multilocus genotypes/no. isolates	Proportion shared alleles ± SD	No. polymorphic loci	Mean no. private alleles per locus ± SE	Mean A_r ± SE†	Mean expected/observed heterozygosity†	% Loci with deficit/excess heterozygosity‡
TcII	14/15 (5/6)	0.44 ± 0.23 (0.062 ± 0.053)	24 (15)	1.76 ± 0.20 (0.68 ± 0.14)	3.94 ± 0.29 (1.65 ± 0.12)	0.58/0.65 (0.91/0.58)	29.2/20.8 (40.0/0)
TcIII	13/13 (4/4)	0.48 ± 0.15 (0.30 ± 0.16)	22 (21)	2.35 ± 0.48 (1.76 ± 0.27)	4.26 ± 0.43 (2.35 ± 0.18)	0.45/0.70 (0.46/0.69)	4.5/27.3 (9.5/38.1)
TcV	8/8	0.15 ± 0.092	22	0.16 ± 0.07	2.38 ± 0.20	0.85/0.58	54.6/4.5
TcVI	21/21 (14/14)	0.24 ± 0.87 (0.22 ± 0.103)	21 (20)	0.43 ± 0.12 (0.86 ± 0.20)	2.46 ± 0.21 (1.87 ± 0.11)	0.60/0.49 (0.71/0.54)	41.7/16.7 (40.0/15.0)

*Values represent findings for reference clones derived from a range of representative hosts and vectors across South America and, in parentheses, clones isolated from humans, triatomine vectors, and sylvatic mammalian hosts in northern Colombia. Values were calculated from microsatellite data for 25 analyzed loci. Ar, allelic richness.
†Across all loci.
‡After sequential Bonferroni correction.

human Chagas disease associated with this low-diversity hybrid lineage.

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Dr. Messenger is postdoctoral researcher at the London School of Hygiene and Tropical Medicine. Her research interests include population genetics, molecular epidemiology, clinical parasitology, and disease control.

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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.

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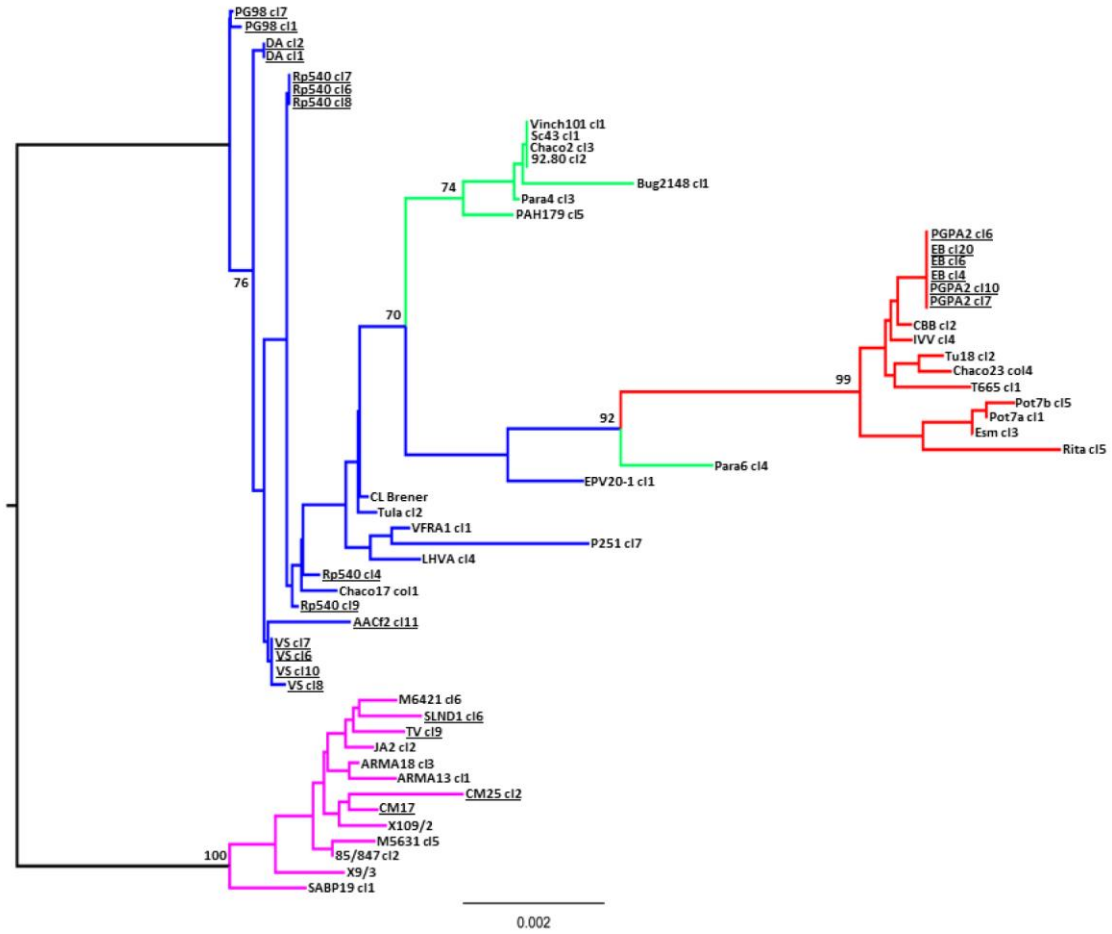
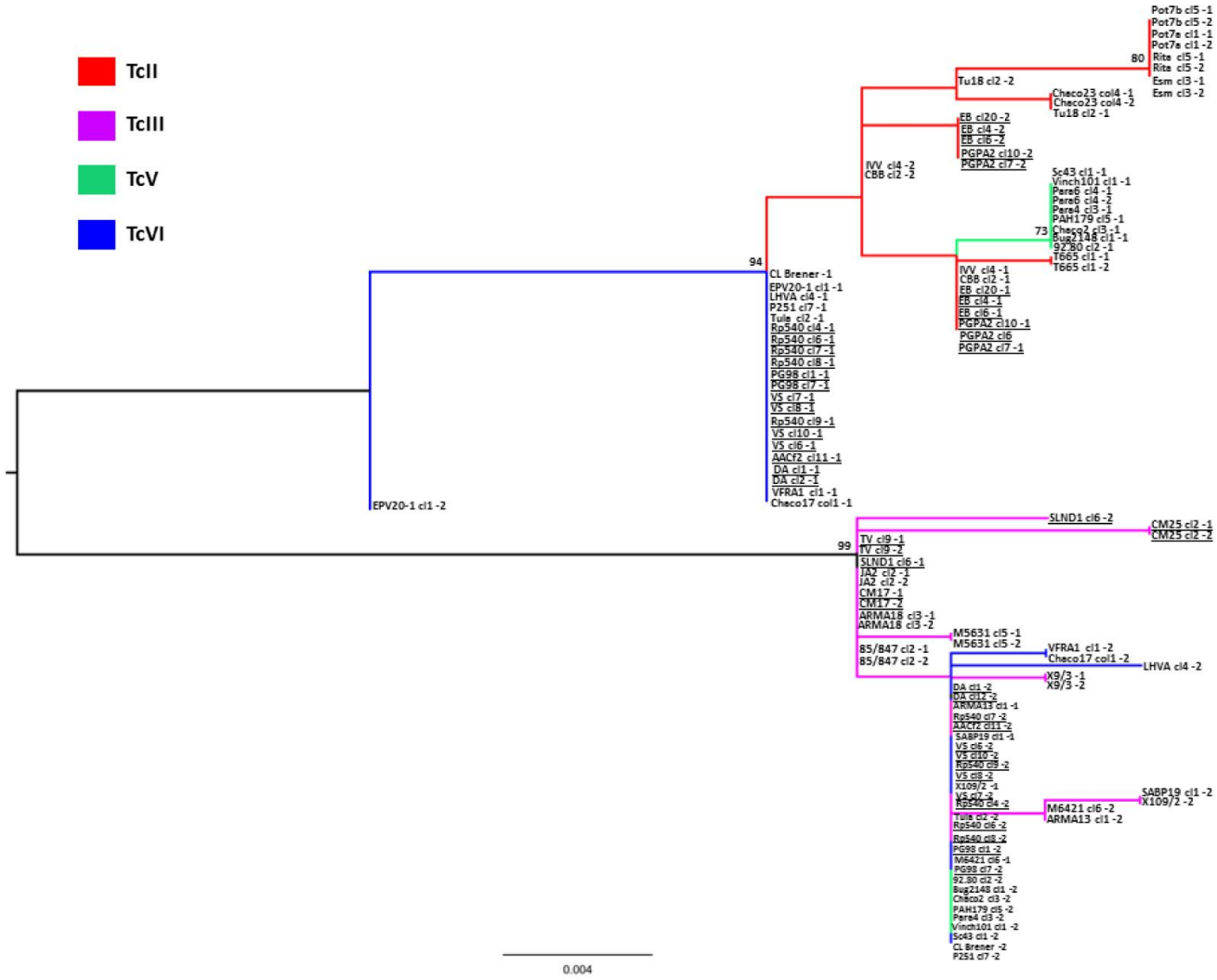


Technical Appendix Figure 1. Unrooted Neighbor-Joining tree based on five concatenated diploid nuclear MLST sequences. For each isolate, nuclear diploid sequence data were concatenated in order of their relative chromosomal positions (Met-II, GTP, TcMPX, TcGPX and TcAPX, on chromosomes 6, 12, 22, 35 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.