Molecular Typing of *Trypanosoma cruzi* Isolates, United States

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Studies have characterized *Trypanosoma cruzi* from parasite-endemic regions. With new human cases, increasing numbers of veterinary cases, and influx of potentially infected immigrants, understanding the ecology of this organism in the United States is imperative. We used a classic typing scheme to determine the lineage of 107 isolates from various hosts.

In Latin America, an estimated 10–12 million persons are infected with *Trypanosoma cruzi*, the etiologic agent of Chagas disease and a major contributor to heart disease within the region. Autochthonous human infections in the United States have been reported in 6 persons, with the most recent case reported from Louisiana (*1*). In addition, the parasite is euryxenous; it is able to infect a broad range of hosts, including domestic dogs, woodrats, raccoons, opossums, armadillos, and nonhuman primates.

Associations between host species and parasite genotype have been suggested and are important in understanding the domestic and sylvatic cycles of *T. cruzi* (*2*–*4*). Although studies conducted on US isolates suggest an association between *T. cruzi* genotype and host, these studies were limited because of low sample numbers, low host diversity, and narrow geographic distribution (*2*,*4*–*7*). In the current investigation, we used the molecular typing scheme proposed by Brisse et al. (*8*), in which isolates are delineated into 1 of the 6 lineages (types I and IIa–IIe) on the basis of size polymorphisms of several PCR markers. We then expanded characterization of US isolates and show additional evidence for correlations between host specificity and genotype of *T. cruzi*.

The Study

We analyzed 107 isolates of *T. cruzi* from multiple species of free-ranging and captive wildlife, domestic animals, triatomine bug vectors, and humans who were autochthonously infected in the United States. Some isolates were obtained as liquid nitrogen–stored parasites from the Centers for Disease Control and Prevention (Atlanta, GA, USA), the Institut Pasteur (Paris, France), and the Southeastern Cooperative Wildlife Disease Study (Athens, GA, USA) and were established in axenic liver infusion tryptose medium as described (*9*). Additional isolates were obtained from wild-trapped animals in axenic liver infusion tryptose medium or canine macrophage cell culture as described (*10*). Isolated DNA was used as template for PCR amplification of 3 gene targets, mini-exon, D7 divergent domain of 24S α rRNA, and 18S rRNA, according to published methods (*8*). Locality data and results of molecular typing of each isolate are shown in the online Appendix Table (available from www.cdc.gov/EID/content/14/7/1123-appT.htm). All animals used in this study were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee and under animal use protocol approved by the Institutional Animal Care and Use Committee at the University of Georgia.

Only 2 genotypes, *T. cruzi* I and *T. cruzi* IIa, were detected. Typical amplicon sizes of *T. cruzi* I and *T. cruzi* IIa isolates from the United States are shown in the Table. Atypical banding patterns and isolates that differ from the standard genotype from a particular host are also represented. With the exception of human isolates, 1 primate isolate, and a few raccoon isolates, placental mammalian isolates, including those from raccoons, domestic dogs, ring-tailed lemurs, and skunks, were characterized as type IIa (online Appendix Table). All remaining isolates, including those from Virginia opossums (*Didelphis virginiana*), triatomine vectors, humans, and rhesus macaques from the United States, were identified as type I (online Appendix Table).

Conclusions

In contrast to studies conducted on South American isolates, for which 6 genotypes of *T. cruzi* have been identified, only 2 genotypes (I and IIa) were identified in the current study. These data support results of investigations in Central America and Mexico in which a paucity of genotypes was found (*14*,*15*). Many investigations on *T. cruzi* evolutionary ecology have shown strict host–parasite specificity in regard to host species and parasite genotype (*2*–*4*), although exceptions have been observed. The presence of only 2 genotypes in the United States could be caused by a lack of introduction of other genotypes or a lower diversity of natural reservoir hosts for *T. cruzi* than in South America. A recent analysis of *T. cruzi* hosts in North and South America indicated that ≥48 host species representing 17 families were infected with ≥1 of the 6 strains (*4*). Only 6 of these hosts have established populations in the United States, and US isolates from these species were only characterized as types I or IIa (*4*).

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Our data for US isolates correspond with those of previous studies in which *Didelphis* spp. are reservoirs for type I *T. cruzi* (*4*); no infections with type II parasites were observed. The Virginia opossum (and its ancestors), which is the only marsupial present in the United States (it migrated from South America ≈4.5 million years ago), is a possible host for *T. cruzi* I. This evidence suggests that *T. cruzi* was not recently introduced into North America or the United States (*5*). Additionally, sufficient time may have passed for random and rare genetic exchange events to occur independent of those found in South American isolates (*13*), enabling the lineage to infect atypical reservoirs (i.e., raccoons) in North America.

The second major natural reservoir of *T. cruzi* in the United States is the raccoon. In general, the nonprimate placental mammals in our study were infected with type IIa, a strain that is commonly found in sylvatic cycles in the Southern Cone of South America. Our data confirm previous typing of US isolates by multilocus enzyme electrophoresis or random amplified polymorphic DNA analysis (*5*), in which 11 raccoons from Georgia were characterized as zymodeme 3 (equivalent to IIa). Although raccoons are predominately infected with *T. cruzi* IIa, 4 known exceptions include 3 isolates from Georgia and Florida in the current study and 1 raccoon from Louisiana from a previous study (*5*).These data are in contrast to typing data for Virginia opossum isolates, which have all found *T. cruzi* I. This finding suggests that opossums primarily maintain persistent infections with *T. cruzi* I.

All characterized human isolates from autochthonous US cases of infection with *T. cruzi* are *T. cruzi* I. This genotype is predominantly responsible for Chagas disease north of the Amazon Basin and is part of the domiciliary cycle of the parasite. Our findings correspond with data from Mexico where *T. cruzi* I is the predominate strain detected in humans (*14*). It would be useful to differentiate biologic characteristics and polymorphisms by using additional gene targets in human type I isolates and compare them with those in opossum, triatomine vectors, and rhesus macaque isolates from the United States. Additionally, comparing these US isolates and Mexican reference strains with those from South America may indicate why type I typically infects humans in North America and multiple strains are found in humans in South America.

Our results provide additional evidence that *T. cruzi* has distinct genotypes that preferentially infect 1 host species or a group of hosts. Humans and marsupials are typically infected with type I *T. cruzi*, but raccoons, skunks, domestic dogs, and prosimians are typically infected with type IIa. Although we only detected *T. cruzi* I in triatomid bugs, other studies have detected *T. cruzi* IIa in triatomids from the United States (*5*). The mechanism is unknown by which persistent infections with a particular genotype of *T. cruzi* develop in certain hosts. Further analysis of isolates from an increased host diversity and geographic range should be pursued. Determining basic infection dynamics of reservoir hosts experimentally infected with various *T. cruzi* genotypes may provide additional insight into the host–parasite dichotomy.

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Table. Approximate amplicon sizes of gene targets and lineage determination in *Trypanosoma cruzi*

Strain	Mini-exon, bp	24S α rRNA, bp	18S rRNA, bp	Lineage
FL Opo 15*	350	110	175	I
GA Rac 103*	None	120	155	IIa
FL Rac 5*	400	120	155	IIa
93053103R cl3	350	110	175	I
FL Rac 13	350	110, 120	155, 175	I/IIa†
FL Rac 46	400	110, 120	155	I/IIa†
Griffin Dog	350	110, 120	155	I/IIa†
Monk RH89–40	None	110	155	I/IIa†

*Denotes isolates used as representative banding patterns seen for classic lineage typing.
†Because of atypical banding patterns, a clear definition of an isolate as type I vs. type IIa could not be obtained.

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Appendix Table. Origin and lineage identification of 107 US isolates of *Trypanosoma cruzi* used in the study*

Host	Isolate	Site of origin	Lineage
Human	CA R	California	I
	Corpus Christi†	Corpus Christi, TX	I
	LC *T cruzi*	New Orleans, LA	I
	TC California†	Lake Don Pedro, CA	I
	TX D	Alano, TX	I
Domestic dog	Caesar Dog	Not known	IIa
	Dog Theis†‡§	Not known	IIa
	Griffin Dog	Hillsboro, TN	I/IIa
	OK Dog	Bartlesville, OK	IIa
	Samantha Dog	South Carolina	IIa
	Smokey	South Carolina	IIa
	USA Dog Y†	California	IIa
Virginia opossum	92101601P†	Statesboro, GA	I
	93041401P cl1†	Statesboro, GA	I
	93070103P cl2†	Fort Stewart, GA	I
	FL Opo 15	Maclay State Park, FL	I
	FL Opo 17	Wakulla Springs, FL	I
	FL Opo 18	Wakulla Springs, FL	I
	FL Opo 2	Wakulla Springs, FL	I
	FL Opo 3	Wakulla Springs, FL	I
	FL Opo 717	Tampa, FL	I
	GA Opo 43	Chatham County, GA	I
	GA Opo 75	White Hall, GA	I
	Opossum 1970†	New Orleans, LA	I
	USA Opossum†	Southern Louisiana	I
	AU8	Auburn, AL	I
	FH4	Southern Georgia	I
Raccoon	92122102R†	Statesboro, GA	IIa
	93040701R cl1†	Statesboro, GA	IIa
	93053102R cl4†	Harrold Preserve, GA	IIa
	93053103R cl3	Harrold Preserve, GA	I
	93071502R cl2†	Fort Stewart, GA	IIa
	93072805R cl3†	Fort Stewart, GA	IIa
	FL Rac 13	Maclay State Park, FL	I/IIa
	FL Rac 14	Wakulla Springs, FL	IIa
	FL Rac 15	Wakulla Springs, FL	IIa
	FL Rac 26	Wakulla Springs, FL	IIa
	FL Rac 30	Wakulla Springs, FL	IIa
	FL Rac 38	Maclay State Park, FL	IIa
	FL Rac 4 PAD	Tallahassee, FL	IIa
	FL Rac 40	Wakulla Springs, FL	IIa
	FL Rac 42	Wakulla Springs, FL	IIa
	FL Rac 46	Tall Timbers, FL	IIa
	FL Rac 48	Maclay State Park, FL	IIa
	FL Rac 5	Torreya State Park, FL	IIa
	FL Rac 50	Wakulla Springs, FL	IIa
	FL Rac 51	Wakulla Springs, FL	IIa
	FL Rac 7	Lake Talquin, FL	IIa
	FL Rac 9	Torreya State Park, FL	IIa
	FR36#	Pickens County, SC	IIa
	GA Rac 103	Ossabaw Island, GA	IIa
	GA Rac 104	Ossabaw Island, GA	IIa
	GA Rac 107	Ossabaw Island, GA	IIa
	GA Rac 108	Ossabaw Island, GA	IIa
	GA Rac 111	Ossabaw Island, GA	IIa
	GA Rac 121	Ossabaw Island, GA	IIa
	GA Rac 124	Ossabaw Island, GA	IIa
	GA Rac 134	Whitehall Forest, GA	IIa
	GA Rac 135	Whitehall Forest, GA	IIa
	GA Rac 137	Whitehall Forest, GA	IIa
	GA Rac 141	Whitehall Forest, GA	IIa
	GA Rac 142	Whitehall Forest, GA	IIa
	GA Rac 143	Athens, GA	IIa
	GA Rac 144	Athens, GA	IIa
	GA Rac 147	Woodbine, GA	IIa
	GA Rac 148	Woodbine, GA	IIa
	GA Rac 186	White Hall, GA	IIa

	GA Rac 2	Ludiwici, GA	I
	GA Rac 206	Athens, GA	IIa
	GA Rac 208	White Hall, GA	IIa
	GA Rac 22	Victoria Bryant State Park, GA	IIa
	GA Rac 3	Athens, GA	IIa
	GA Rac 45	Skidaway Island, GA	IIa
	GA Rac 46	Skidaway Island, GA	IIa
	GA Rac 51	Skidaway Island, GA	IIa
	GA Rac 52	Skidaway Island, GA	IIa
	GA Rac 55	Skidaway Island, GA	IIa
	GA Rac 57	Skidaway Island, GA	IIa
	GA Rac 61	Skidaway Island, GA	IIa
	GA Rac 67	Athens, GA	IIa
	GA Rac 68	Athens, GA	IIa
	GA Rac 69	Athens, GA	IIa
	Maryland Rac	Laurel, MD	IIa
	STC 10R cl3†	St. Catherine's Island, GA	IIa
	STC 16R cl1†	St. Catherine's Island, GA	IIa
	STC 33R	St. Catherine's Island, GA	IIa
	STC 35R	St. Catherine's Island, GA	IIa
	STC 39R	St. Catherine's Island, GA	IIa
	STC 54R	St. Catherine's Island, GA	IIa
	STC 9R cl4†	St. Catherine's Island, GA	IIa
	TN Rac 18	Rutherford County, TN	IIa
Triatoma sanguisuga	Florida†	Gainesville, FL	I
	Florida C16¶	Gainesville, FL	I
	Florida C1F8	Gainesville, FL	I
	T. sang 5 cl1†	Bulloch County, GA	I
Triatoma gerstackeri	Triatoma 2	Texas	I/IIa
	Triatoma 3	Texas	I
	TxTg2	Texas	I
Ring-tailed lemur	Nilda	St. Catherine's Island, GA	IIa
	Clarence	St. Catherine's Island, GA	IIa
	Meg	St. Catherine's Island, GA	IIa
Rhesus macaque	Monk RH89–40	Atlanta, GA (CDC)	I/IIa
	Texas Theis†	Not known	I
Nine-banded armadillo	Armadillo 1973†	New Orleans, LA	I
	GA Arm 20	Ossabaw Island, GA	IIa
	USA Armadillo†	Southern Louisiana	I
Striped skunk	GA Sk 1	Ludiwici, GA	IIa

*GA, Georgia; TX, Texas; LA, Louisiana, CA, California; TN, Tennessee; OK, Oklahoma; FL, Florida; AL, Alabama; MD, Maryland; CDC, Centers for Disease Control and Prevention; SC, South Carolina.
†Characterized by using multilocus enzyme electrophoresis (MLEE) or random amplified polymorphic DNA (RAPD) analysis (*7*).
‡Characterized by using microsatellite, 24S α rRNA, and COII genetic analysis (*11*).
§Characterized by using RAPD and MLEE analysis (*12*).
¶Characterized by using an unspecified method (*13*).
#Characterized by RAPD and mini-exon amplification (*7*).