Molecular Typing of *Trypanosoma cruzi* Isolates, United States

Dawn M. Roellig,* Emily L. Brown,* Christian Barnabé,† Michel Tibayrenc,† Frank J. Steurer,‡ and Michael J. Yabsley*

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–Ie) 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, triatome 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*), triatome 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).
Our data for US isolates correspond with those of previous studies in which Didelphis spp. are reservoirs for type I *Trypanosoma 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*. 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 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.

Acknowledgments

We thank B. Wilcox, B. Hanson, and D. Kavanaugh for field assistance; C. Paddock for providing 1 isolate used in the study; and P. Dorn for providing blood for isolation of 1 isolate.

This study was supported by grant R15 AI067304 from the National Institutes of Health, National Institute of Allergy and Infectious Diseases.

Ms Roellig is a doctoral student in infectious diseases at the University of Georgia. Her research interests are vector-borne zoonotic diseases, including Chagas disease in wildlife and tick-borne rickettsial pathogens.

References

1. Dorn PL, Perniciaro L, Yabsley MJ, Roellig DM, Balsamo G, Diaz J, et al. Autochthonous transmission of *Trypanosoma cruzi*, Louisiana. Emerg Infect Dis. 2007;13:605–7.
2. Clark CG, Pung OJ. Host specificity of ribosomal DNA variation in sylvatic *Trypanosoma cruzi* from North America. Mol Biochem Parasitol. 1994;66:175–9. DOI: 10.1016/0166-6851(94)90052-3

| Table. Approximate amplicon sizes of gene targets and lineage determination in *Trypanosoma cruzi* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Strain                          | Mini-exon, bp   | 24S 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 c3                      | 350             | 110             | 175             | I               |
| FL Rac 13                        | 350             | 110, 120        | 155, 175        | IIa†            |
| FL Rac 46                        | 400             | 110, 120        | 155             | IIa†            |
| Griffin Dog                      | 350             | 110, 120        | 155             | IIa†            |
| Monk RH89–40                     | None            | 110             | 155             | 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.
3. Briones MR, Souto RP, Stolf BS, Zingales B. The evolution of two Trypanosoma cruzi subgroups inferred from rRNA genes can be correlated with the interchange of American mammalian faunas in the Cenozoic and has implications to pathogenicity and host specificity. Mol Biochem Parasitol. 1999;104:219–32. DOI: 10.1016/S0166-6851(99)00155-3

4. Yeo M, Acost N, Llewellyn M, Sánchez H, Adamson S, Miles GA, et al. Origins of Chagas disease: Didelphis species are natural hosts of Trypanosoma cruzi I and armadillos hosts of Trypanosoma cruzi II, including hybrids. Int J Parasitol. 2005;35:225–33. DOI: 10.1016/j.ijpara.2004.10.024

5. Barnabé C, Yaeger R, Pung O, Tibayrenc M. Trypanosoma cruzi: a considerable phylogenetic divergence indicates that the agent of Chagas disease is indigenous to the native fauna of the United States. Exp Parasitol. 2001;99:73–9. DOI: 10.1006/expr.2001.4651

6. Miles MA, Souza A, Povoa M, Shaw JJ, Lainson E, Toye PJ. Isozymic heterogeneity of Trypanosoma cruzi in the first autochthonous patients with Chagas’ disease in Amazonian Brazil. Nature. 1978;272:819–21. DOI: 10.1038/272819a0

7. Yabsley MJ, Noblet GP. Biological and molecular characterization of a raccoon isolate of Trypanosoma cruzi from South Carolina. J Parasitol. 2002;88:1273–6.

8. Brisse S, Verhoef J, Tibayrenc M. Characterisation of large and small subunit rRNA and mini-exon genes further support the distinction of six Trypanosoma cruzi lineages. Int J Parasitol. 2001;31:1218–26. DOI: 10.1016/S0020-7519(01)00238-7

9. Castellani O, Ribeiro LV, Fernandes JF. Differentiation of Trypanosoma cruzi in culture. J Protozool. 1967;14:447–51.

10. Yabsley MJ, Norton TM, Powell MR, Davidson WR. Molecular and serologic evidence of tick-borne ehrlichiae in three species of lemans from St. Catherine’s Island, Georgia, USA. J Zoo Wildl Med. 2004;35:503–9.

11. de Freitas JM, Augusto-Pinto L, Pimenta JR, Bastos-Rodrigues L, Gonçalves VF, Teixeira SM, et al. Ancestral genomes, sex and the population structure of Trypanosoma cruzi. PLoS Pathog. 2006;2:e24. DOI: 10.1371/journal.ppat.0020024

12. Brisse S, Barnabé C, Tibayrenc M. Trypanosoma cruzi clonal diversity: identification of discrete phylogenetic lineages by random amplified polymorphic DNA and multilocus enzyme electrophoresis analysis. Int J Parasitol. 2000;30:35–44. DOI: 10.1016/S0020-7519(99)00168-X

13. Machado CA, Ayala FJ. Nucleotide sequences provide evidence of genetic exchange among distantly related lineages of Trypanosoma cruzi. Proc Natl Acad Sci U S A. 2001;98:7396–401. DOI: 10.1073/pnas.121187198

14. Bosseno M-F, Bernabé C, Gastélum EM, Kasten FL, Ramsey J, Espinoza B, et al. Predominance of Trypanosoma cruzi lineage I in Mexico. J Clin Microbiol. 2002;40:627–32. DOI: 10.1128/JCM.40.2.627-632.2002

15. Iwagami M, Higo H, Miura S, Yanagi T, Tada I, Kano S, et al. Molecular phylogeny of Trypanosoma cruzi from Central America (Guatemala) and a comparison with South American strains. Parasitol Res. 2007;102:129–34. DOI: 10.1007/s00436-007-0739-9

Address for correspondence: Dawn M. Roellig, Southeastern Cooperative Wildlife Disease Study, Department of Population Health, 589 DW Brooks Dr, Wildlife Health Bldg, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA; email: droellig@uga.edu

All material published in Emerging Infectious Diseases is in the public domain and may be used and reprinted without special permission; proper citation, however, is required.
### 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 Dog  | 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       | 9212102R†   | 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  | I       |
|               | FL Rac 15   | Wakulla Springs, FL  | I       |
|               | FL Rac 26   | Wakulla Springs, FL  | I       |
|               | FL Rac 30   | Wakulla Springs, FL  | I       |
|               | FL Rac 38   | Maclay State Park, FL| I/IIa   |
|               | FL Rac 4 PAD | Tallahassee, FL      | IIa     |
|               | FL Rac 40   | Wakulla Springs, FL  | I       |
|               | FL Rac 42   | Wakulla Springs, FL  | I       |
|               | FL Rac 46   | Tall Timbers, FL     | I       |
|               | FL Rac 48   | Maclay State Park, FL| I       |
|               | FL Rac 5    | Torreya State Park, FL| I       |
|               | FL Rac 50   | Wakulla Springs, FL  | I       |
|               | FL Rac 51   | Wakulla Springs, FL  | I       |
|               | FL Rac 7    | Lake Talquin, FL     | I       |
|               | FL Rac 9    | Torreya State Park, FL| I       |
|               | FR36#       | Pickens County, SC   | I       |
| GA Rac 103    | Ossabaw Island, GA | Ossabaw Island, GA | I       |
| GA Rac 104    | Ossabaw Island, GA | Ossabaw Island, GA | I       |
| GA Rac 107    | Ossabaw Island, GA | Ossabaw Island, GA | I       |
| GA Rac 108    | Ossabaw Island, GA | Ossabaw Island, GA | I       |
| GA Rac 111    | Ossabaw Island, GA | Ossabaw Island, GA | I       |
| GA Rac 121    | Ossabaw Island, GA | Ossabaw Island, GA | I       |
| GA Rac 124    | Ossabaw Island, GA | Ossabaw Island, GA | I       |
| GA Rac 134    | Whitehall Forest, GA | Whitehall Forest, GA | IIa     |
| GA Rac 135    | Whitehall Forest, GA | Whitehall Forest, GA | IIa     |
| GA Rac 137    | Whitehall Forest, GA | Whitehall Forest, GA | IIa     |
| GA Rac 141    | Whitehall Forest, GA | Whitehall Forest, GA | IIa     |
| GA Rac 142    | Whitehall Forest, GA | Whitehall Forest, GA | IIa     |
| GA Rac 143    | Athens, GA   | Athens, GA           | I       |
| GA Rac 144    | Athens, GA   | Athens, GA           | I       |
| GA Rac 147    | Woodbine, GA | Woodbine, GA         | I       |
| GA Rac 148    | Woodbine, GA | Woodbine, GA         | I       |
| GA Rac 186    | White Hall, GA | White Hall, GA       | I       |
| Code    | Location                         | Status |
|---------|----------------------------------|--------|
| GA Rac 2 | Ludwici, 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            | I      |

**Triatoma sanguisuga**

| Code    | Location               | Status |
|---------|------------------------|--------|
| Florida† | Gainesville, FL        | I      |
| Florida C16¶ | Gainesville, FL     | I      |
| Florida C1F8 | Gainesville, FL    | I      |
| T. sang 5 cl1† | Bulloch County, GA | I      |

**Triatoma gerstackeri**

| Code    | Location               | Status |
|---------|------------------------|--------|
| Triatoma 2 | Texas                 | I/IIa  |
| Triatoma 3 | Texas                 | I      |
| T.xtg2   | Texas                  | I      |

**Ring-tailed lemur**

| Code    | Location                  | Status |
|---------|---------------------------|--------|
| Nilda   | St. Catherine’s Island, GA| IIa    |
| Clarence | St. Catherine’s Island, GA| IIa    |
| Meg     | St. Catherine’s Island, GA| IIa    |

**Rhesus macaque**

| Code    | Location                  | Status |
|---------|---------------------------|--------|
| Monk RH89–40 | Atlanta, GA (CDC)  | I/IIa  |
| Texas Theis† | Not known                | I      |

**Nine-banded armadillo**

| Code    | Location                  | Status |
|---------|---------------------------|--------|
| Armadillo 1973† | New Orleans, LA   | I      |
| GA Arm 20 | Ossabaw Island, GA         | IIa    |
| USA Armadillo† | Southern Louisiana | I      |

**Striped skunk**

| Code    | Location                  | Status |
|---------|---------------------------|--------|
| GA Sk 1  | Ludwici, 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 an unspecified method (13).

#Characterized by using RAPD and mini-exon amplification (7).