Infection, Genetics and Evolution 58 (2018) 171–180

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Analysis of over 1500 triatomine vectors from across the US, predominantly Texas, for *Trypanosoma cruzi* infection and discrete typing units

Rachel Curtis-Robles[a], Lisa D. Auckland[a], Karen F. Snowden[b], Gabriel L. Hamer[c], Sarah A. Hamer[a,*]

[a] Department of Veterinary Integrative Biosciences, 4458 TAMU, Texas A&M University, College Station, TX 77843, USA
[b] Department of Veterinary Pathobiology, 4467 TAMU, Texas A&M University, College Station, TX 77843, USA
[c] Department of Entomology, 2475 TAMU, Texas A&M University, College Station, TX 77843, USA

ARTICLE INFO

Keywords:
Discrete typing unit
Texas
Triatomine
Trypanosoma cruzi
United States
Vector

ABSTRACT

Across the Americas, triatomine insects harbor diverse strains of *Trypanosoma cruzi* (*T. cruzi*), agent of Chagas disease. Geographic patterns of vector infection and parasite strain associations, especially in vectors encountered by the public, may be useful in assessing entomological risk, but are largely unknown across the US. We collected *Triatoma* spp. from across the US (mainly Texas), in part using a citizen science initiative, and amplified *T. cruzi* DNA to determine infection prevalence and parasite discrete typing units (DTUs). We found 54.4% infection prevalence in 1510 triatomines of 6 species; prevalence in adult *T. gerstaeckeri* (63.3%; $n = 897$) and *T. lecticularia* (66.7%; $n = 66$) was greater than in *T. sanguisuga* (47.6%; $n = 315$), *T. indictiva* (47.8% $n = 67$), *T. rubida* (14.1%; $n = 64$), and *T. protracta* (10.5%; $n = 19$). The odds of infection in adults were 9.73 times higher than in nymphs (95% CI 4.46–25.83). PCR of the spliced leader intergenic region (SL-IR) and/or the putative lathosterol/episterol oxidase TcSC5D gene revealed exclusively *T. cruzi* DTUs TcI and TcIV; 5.5% of *T. cruzi*-positive samples were not successfully typed. *T. gerstaeckeri* ($n = 548$) were more frequently infected with TcI (53.9%) than TcIV (34.4%), and 11.9% showed mixed TcI/TcIV infections. In contrast, *T. sanguisuga* ($n = 135$) were more frequently infected with TcIV (79.3%) than TcI (15.6%), and 5.2% showed mixed infections. Relative abundance of parasite DTUs varied spatially, with both TcI and TcIV co-circulating in vectors in central Texas, while TcIV predominated in northern Texas. Given prior findings implicating TcI in human disease and TcI and TcIV in animal disease in the US, knowledge of spatial distribution of *T. cruzi* infection and DTUs in vectors is important to understanding public and veterinary health risk of *T. cruzi* infection.

1. Introduction

Throughout the Americas, the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) is responsible for an estimated burden of Chagas disease exceeding 5.7 million people (World Health Organization, 2015). Transmission is primarily through the infective feces of triatomine insects, although transmission can also occur congenitally, through organ transplant and blood transfusion, and through consumption of contaminated food and drink (Bern et al., 2011). Infection with *T. cruzi* ranges from asymptomatic to clinical presentation of acute or chronic cardiomyopathy and, less commonly, gastrointestinal complications (Bern et al., 2011).

T. cruzi exhibits remarkable genetic variation (Miles et al., 2009; Zingales et al., 2009, 2012), and current classification delineates six major discrete typing units (DTUs), TcI - TcVI, and a seventh, TcBat lineage, each with particular ecological and epidemiological associations (Zingales et al., 2012). In the Southern Cone region of South America, for example, TcII, TcV, and TcVI have been mainly documented in domestic transmission cycles; in contrast, TcIII and TcIV are more typically associated with sylvatic transmission cycles in Brazil and northern South America (Miles et al., 2009; Zingales et al., 2012). TcI is the most genetically diverse DTU and is found throughout the Americas with variable domestic and sylvatic associations (Zingales et al., 2012), which has resulted in additional proposed subdivisions (Zumaya-Estrada et al., 2012). Understanding the effect of parasite strain on disease progression is complicated by co-infections, transmission mode, and individual host immune function (Messenger et al., 2015a; Zingales et al., 2012).

In the southern US, *T. cruzi* actively circulates through vector, wildlife, and domestic dog populations (Brown et al., 2010; Burkholder

* Corresponding author.
E-mail addresses: RCurtis@cvm.tamu.edu (R. Curtis-Robles), LAuckland@cvm.tamu.edu (L.D. Auckland), KSnowden@cvm.tamu.edu (K.F. Snowden), GHamer@tamu.edu (G.L. Hamer), SHamer@cvm.tamu.edu (S.A. Hamer).

https://doi.org/10.1016/j.meegid.2017.12.016
Received 10 August 2017; Received in revised form 4 December 2017; Accepted 15 December 2017
Available online 18 December 2017

et al., 1980; Curtis-Robles et al., 2015, 2016; Kjos et al., 2008, 2009), and autochthonous human Chagas disease is also documented in the US (Cantey et al., 2012; Dorn et al., 2007; Garcia et al., 2015). Standard diagnostic approaches are typically based on antibody detection and do not determine parasite strain, so the increasing diagnoses of human and animal *T. cruzi* infections in the US are not generally accompanied by a concomitant increase in the knowledge of which strains are implicated in disease. Strain-typing approaches that have been applied to samples from the US include methods based on enzyme profiles (Barnabé et al., 2001; Beard et al., 1988), PCR product sizes of several genetic targets (Garcia et al., 2017; Herrera et al., 2015; Roellig et al., 2008), and DNA sequence analysis (Buhaya et al., 2015; Curtis-Robles et al., 2016; Garcia et al., 2017; Herrera et al., 2015; Shender et al., 2016). There have been seven definitively typed human infections in the US, all of which were TcI (Garcia et al., 2017; Roellig et al., 2008). Limited studies of dog and wildlife populations have revealed an association of TcI with opossums and TcIV with dogs and raccoons (Curtis-Robles et al., 2016; Roellig et al., 2008). Recently, TcII was found in small rodents from Louisiana (Herrera et al., 2015), and insects in California were found to harbor isolates closely related to the TcII and TcVI group members (Hwang et al., 2010). Triatomine vectors have been found infected with TcI in a limited number of samples from California, Florida, Georgia, Louisiana, and Texas (Barnabé et al., 2001; Beard et al., 1988; Buhaya et al., 2015; Herrera et al., 2015; Roellig et al., 2008; Shender et al., 2016); TcIV in triatomines has been documented rarely in California, Georgia, and Texas (Barnabé et al., 2001; Roellig et al., 2008; Shender et al., 2016).

Despite the recognized ecological and potential epidemiological importance of *T. cruzi* genetic variation, the majority of data are from South America (Brenière et al., 2016). Few studies have determined the DTUs of parasite isolates from various hosts in the US, and none have examined relative spatial distribution across a broad area. Knowledge of the prevalence and distribution of *T. cruzi* in US triatomine vectors—particularly those encountered by humans in peridomestic settings—would allow a greater understanding of disease risk and potential consequences. In this study, we determined *T. cruzi* infection prevalence in six triatomine species from Texas and other states. We analyzed samples using multiple PCR assays and assessed risk factors for infection through logistic regression. Given interests in vector associations and spatial distributions of *T. cruzi* DTUs in the US, we compared DTUs found in the two most frequently collected species and mapped occurrences of *T. cruzi* DTUs. We discuss the results with particular attention to potential drivers of differential DTU occurrence in species of triatomines found across the US.

2. Materials and methods

2.1. Sample collection and preparation

Triatomine specimens were collected from April 2013–November 2016 via citizens through a citizen science program (Curtis-Robles et al., 2015) and via our laboratory members using traditional entomological techniques (black light, mercury vapor light, and active searching of environments). Triatomines were morphologically identified to species (Lent and Wygodzinsky, 1979), sexed, and dissected using sterile instruments after specimens were soaked with 10% bleach solution and rinsed with distilled water to reduce risk of contamination with exogenous DNA. Dissections were conducted by carefully cutting off the connexivum, snipping 1–2 terminal abdominal segments, and removing the dorsal portion of the abdominal wall to reveal and remove the hindguts. In cases where hindguts were desiccated, the snipped terminal segments were also included in the sample to increase the sample amount. DNA from triatomine hindguts was extracted using commercially-available extraction kits (Omega E.Z.N.A. Tissue DNA Kit (a spin column-based kit), Omega Bio-tek, Norcross, GA for almost all (93.0%; Table S1) samples collected in 2013–2014; KingFisher Cell and Tissue kit (a magnetic-particle-based kit), Thermo Fisher Scientific, Waltham, MA for almost all (96.1%; Table S1) samples collected in 2015–2016).

2.2. Detection of T. cruzi infection

T. cruzi infection status was determined by amplification of a 166-bp region of repetitive nuclear satellite DNA using a TaqMan qPCR reaction with Cruzi 1/Cruzi 2 primers and Cruzi 3 probe (Duffy et al., 2013; Piron et al., 2007). This approach has previously been shown as both sensitive and specific for *T. cruzi* (Schijman et al., 2011). Reactions consisted of 5 μL of template DNA, primers at a final concentration of 0.75 μM each, 0.25 μM of probe, and iTaq Universal Probes Supermix (Bio-Rad Laboratories, Hercules, CA), in a total volume of 20 μL run on a Stratagene MxPro3000 instrument (Agilent Technologies, Santa Clara, CA), following previously described thermocycling parameters (Duffy et al., 2013), except with a reduced, 3-min, initial denaturation. Our internal laboratory validations—experiments using serial dilutions of *T. cruzi* TcIV DNA from a *T. gerstaeckeri* specimen collected in Texas to calculate limit of detection and PCR efficiency for this assay—defined samples with cycle threshold (Ct) values of < 33 as positive, and samples with Ct values of > 35 as negative. Samples with Ct values of 33–35 were considered equivocal and final infection status was based on the result on the SL-IR qPCR assay (see below). No-template controls were included in each set of DNA extractions, and molecular grade water was included as negative controls in all PCRs. All PCRs were run with a *T. cruzi* positive control, including DNA extracted from Sylvio-X10 CL4 (ATCC 50800, American Type Culture Collection) or *T. cruzi*-positive field-collected samples from triatomines, dogs, and wildlife (infections with either TcI or TcIV).

2.3. T. cruzi DTU determination

All *T. cruzi* positive and equivocal samples were subjected to additional reactions to amplify the putative lathosterol/episterol oxidase gene (TcSC5D) and/or nuclear spliced leader intergenic region (SL-IR) for DTU typing of *T. cruzi*. Originally, we attempted amplification of the TcSC5D region and sequenced the product to determine the DTU of *T. cruzi* in the vector hindgut DNA extracts (Cosentino and Agüero, 2012). During the course of this research, a probe-based multiplex qPCR based on SL-IR amplification was published (Cura et al., 2015). Subsequent to establishing this DTU-typing qPCR in our lab, we ran all previously untyped *T. cruzi* positive and equivocal samples, as well as a subset of samples successfully typed using the TcSC5D PCR, using this method.

2.3.1. TcSC5D gene amplification for DTU determination

Initially, we attempted amplification of the TcSC5D gene (Cosentino and Agüero, 2012) of any sample generating a positive *T. cruzi* result on the Cruzi 1/2/3 qPCR. However, *T. cruzi*-positive samples with relatively high Ct value (indicative of low parasite burdens) were not successfully amplified with the TcSD5D assay, perhaps reflecting the fact that the TcSC5D PCR was developed using *T. cruzi* pure cultures (Cosentino and Agüero, 2012) and seems to be less successful in analyzing field-collected samples with mixed populations of host and parasite DNA (Cominetti et al., 2014; Curtis-Robles et al., 2016; although see Buhaya et al., 2015). Thereafter, we focused on TcSC5D gene amplification from samples with greater concentrations of *T. cruzi* DNA, using a cut-off of Ct values of < 17 on the TaqMan qPCR. Reactions consisted of 1 μL extracted DNA, 0.75 μM of each primer, and FailSafe PCR Enzyme Mix with PreMix E (Epicentre, Madison, WI) in a total volume of 15 μL. The 832-bp amplicons were visualized on 1.5% agarose gels stained with ethidium bromide. In some cases, samples that did not amplify were tested again on this assay using the same conditions or a 1:10 dilution of the DNA template. Amplicons were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) and bi-directionally sequenced using Sanger sequencing (Eton Bioscience, Inc., San

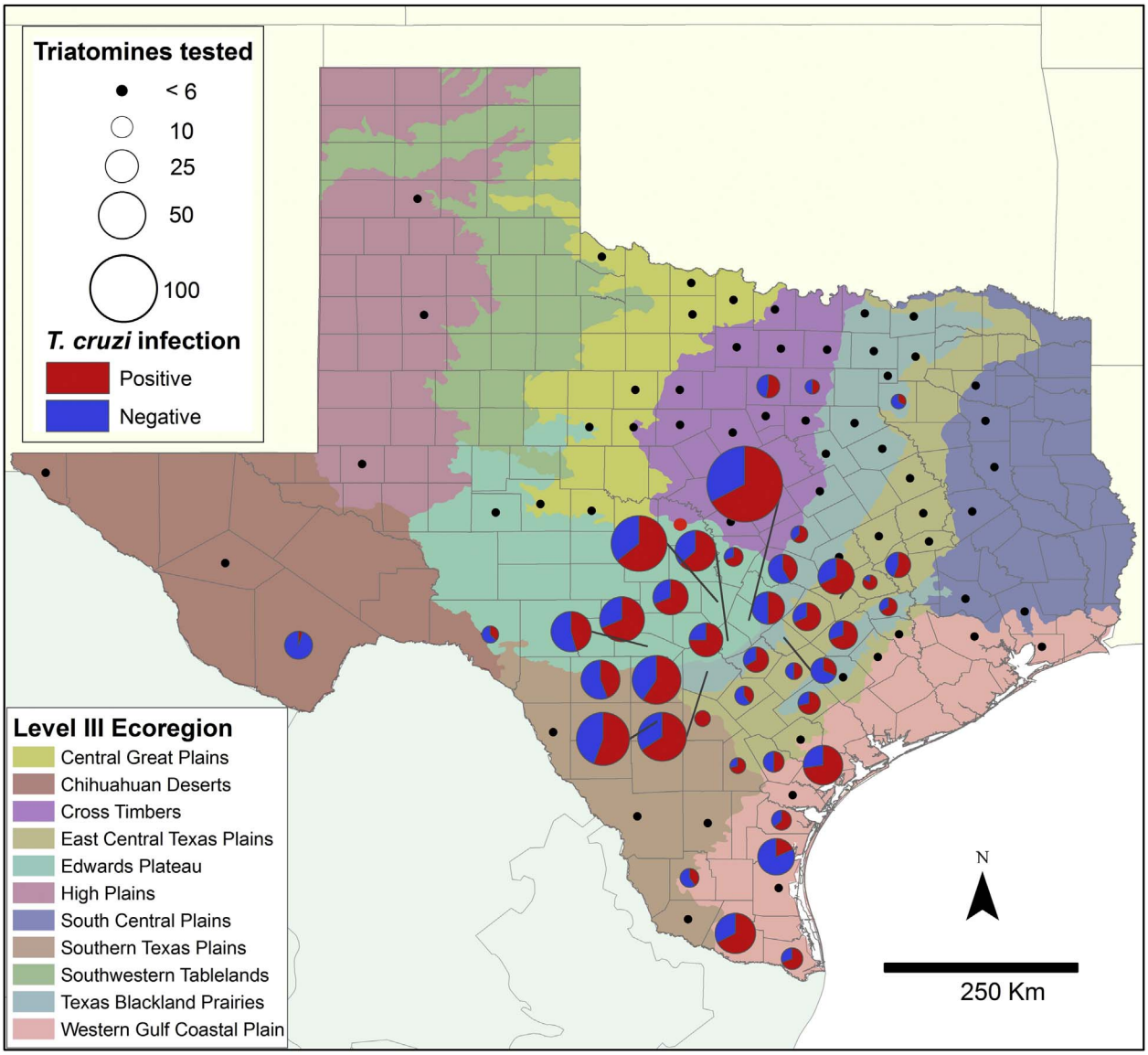

Fig. 1. County distribution of *T. cruzi*-infected triatomines in Texas. Pie charts showing infection prevalence are displayed for counties from which at least six triatomines were tested. Circle size is proportional to the number of triatomines tested from each county. Any county with at least 4 specimens tested had at least one positive. Vector infection prevalence exceeded 30% across 37 counties with at least 6 specimens tested, with the exception of two counties: Kleberg County (n = 38) with an infection of prevalence of 18.4% and Brewster County (n = 22) with an infection prevalence of 4.5%. All samples in Kleberg County originated from a single canine kennel, and all samples in Brewster County were *T. rubida*.

Diego, SA). Sequences and chromatographs were viewed with Geneious version R7 software (http://www.geneious.com) (Kearse et al., 2012) for quality, forward and reverse sequence alignment, and examination of eight key discriminant SNPs to designate DTU and discern double nucleotide peaks at SNPs (see Fig. 1A of Cosentino and Agüero, 2012). This gene target has 81 fixed differences, 70 or which were synonymous and 11 of which were non-synonymous (Cosentino and Agüero, 2012). All *T. cruzi* TcI and TcIV sequences were deposited to GenBank [a total of 222 sequences; MF190169-MF190186; MG213863-MG214066]; sequences representing mixed infections were not submitted.

2.3.2. SL-IR qPCR for DTU determination

A probe-based multiplex qPCR based on amplification of the nuclear spliced leader intergenic region (SL-IR) (Cura et al., 2015) was used to determine discrete typing units (DTUs) of a subset of samples successfully typed on TcSC5D, as well as all other samples that were positive and equivocal for detection of *T. cruzi* on the Cruzi 1/2/3 qPCR assay. Reactions were 20 µL total volume using a QIAGEN Multiplex PCR Kit (QIAGEN, Valencia, CA) using modifications we have previously described (Curtis-Robles et al., 2017a). Samples that were negative in the initial attempt on this assay, including those with non-exponential amplification curves, were diluted 1:10 and SL-IR amplification was attempted a second time. Samples that did not amplify at a 1:10 dilution were attempted a third time, using twice the original volume of extracted DNA. Samples that did not produce results after three attempts were considered untypeable. Samples that were untypeable after being positive on Cruzi 1/2/3 qPCR were still considered infected, while samples that were untypeable after being equivocal on the Cruzi 1/2/3 qPCR were considered uninfected with *T. cruzi*.

2.4. Statistical methods

Project data are available from the Oak Trust digital repository through Texas A&M University at: http://hdl.handle.net/1969.1/165517. Data were analyzed using R software version 3.3.1 (R Development Core Team, 2008). We calculated the odds ratio of infection in nymphs vs. adults using package 'epitools'. To examine factors influencing the likelihood of an adult triatomine being infected, we

first evaluated putative predictor variables. Assessed variables were submission source (citizen science program or collected by our research team), sex (female, male, or unknown), status of vector when collected (alive, dead, or unknown), species, year of collection, season of collection (in 3-month quarters), and level III ecoregion (U.S. EPA (Environmental Protection Agency), 2012). DNA extraction kit type, which had changed over the course of the study, was also included as a potential predictor, since some previous studies have suggested that extraction method can affect detection of *T. cruzi* DNA in samples (Moreira et al., 2013; Schijman et al., 2011). Due to small sample sizes, some ecoregions were merged based on geographic proximity for the analysis including Chihuahuan Desert ($n = 24$) with High Plains ($n = 3$) and Central Great Plains ($n = 22$); as well as South Central Plains ($n = 5$) with Western Gulf Coastal Plains ($n = 164$). Triatomines from outside of Texas were grouped together in a "non-Texas" category. We evaluated the relationship between candidate variables and the infection status of adult triatomines through bivariable analyses using Chi-square tests. Factors with a $p < 0.25$ from the initial bivariable screening were subjected to tests of pairwise association using Chi-square tests followed by calculation of Cramer's V, using package 'vcd' in R. A logistic regression model was generated to investigate the effects of predictors identified by the previous methods on the infection status of a triatomine. Alternate models including pairwise interactions were constructed and assessed through comparison of corrected Akaike's Information Criterion (AIC) values (using package 'sme' in R) and with consideration of biological meaning. Referent categories for sex, species, season, and ecoregion were chosen according to those categories with the greatest numbers of specimens. Factors with values of $p < 0.05$ in the model output were considered significant. Odds ratios and 95% confidence intervals were calculated for each predictor.

To further investigate the effects of 'year' and 'extraction kit' on *T. cruzi* infection prevalence in adult triatomines with known year of collection (Table S1), we used a one-tailed 2-sample test for equality of proportions without continuity correction to compare the infection prevalence of samples collected 2013 to those collected in 2014 for only the Omega-extracted samples; we conducted the same analysis to compare the infection prevalence of samples collected in 2015 to those collected in 2016 for only KingFisher-extracted samples.

To evaluate patterns in the proportions of parasite DTUs within infected triatomine species, we calculated proportions infected with TcI, TcIV, and mixed infections. For those with values of 5 or more in each category, we used package 'DescTools' to calculate 95% confidence intervals using the Goodman method for simultaneous calculation of multinomial confidence intervals (Goodman, 1965). We calculated additional metrics for two main species of interest: *T. gerstaeckeri*, which was the most frequently tested species in the study area (Texas-focused), and *T. sanguisuga*, which was the second most frequently tested species in the study and which has an expansive range covering the eastern US. A Chi-square test was used to compare proportions of *T. gerstaeckeri* and *T. sanguisuga* carrying mixed infections vs. single DTU infections; in addition, the odds ratio and 95% confidence interval were calculated using package 'epitools'. A Chi-square test was used to compare proportions of *T. gerstaeckeri* and *T. sanguisuga* carrying TcI vs. TcIV infections; in cases of mixed infections, the number of mixed infections for each species was added to each of the single DTU cells for that species for the calculation. The odds ratio and 95% confidence interval were calculated using package 'epitools'.

All triatomine location data were geo-coded and mapped using ArcMap 10.1 (ESRI, Redlands, CA).

3. Results

3.1. T. cruzi infection

A total of 1510 triatomine insects collected from 17 states via citizens through the citizen science program (1365 adults, 51 nymphs) and via our laboratory members using standard entomological collecting techniques (92 adults, 2 nymphs) were tested for the presence of *T. cruzi* parasites using PCR, of which 822 (54.4%) were positive. There were 35 samples that gave equivocal results on the Cruzi 1/2/3 qPCR, of which 15 samples were successfully typed using the SL-IR qPCR (Table S2) and included in the total number of infected vectors and DTU typed samples; the 20 samples that were untypeable using the SL-IR qPCR were considered uninfected.

Triatomines were documented in 16 states other than Texas, with infection found in *T. protracta*, *T. rubida*, and *T. sanguisuga* (Table S3). Triatomines from Texas composed the majority of the samples ($n = 1396$, 57.2% infection prevalence). Triatomines were collected from 103 of 254 Texas counties. Infected triatomines were found in 70 counties (Fig. 1); any county with at least 4 specimens tested had at least one positive. Vector infection prevalence exceeded 30% across 37 counties with at least 6 specimens tested with the exception of two counties (Fig. 1): Kleberg County ($n = 38$) with an infection prevalence of 18.4%, and Brewster County ($n = 22$) with an infection prevalence of 4.5%. All samples in Kleberg County originated from a single canine kennel, and all samples in Brewster County were *T. rubida*.

Adults were more frequently (56.0%; $n = 1457$) infected than nymphs (11.3%; $n = 53$). The odds of infection in adults were 9.73 times (95% CI 4.46–25.83) higher than the odds in nymphs. Results of bivariable analysis of adult specimens ($n = 1457$; Table 1) indicated several potential predictors with $p < 0.25$, which were sex, species, year, season, ecoregion, and extraction kit type. Bivariable analysis also revealed extraction kit type was significantly associated with likelihood of being infected ($p < 0.001$); a greater percentage of samples that had been extracted using the Omega kit (64.3%; which had been used in 2013–2014) were found to be infected with *T. cruzi* compared to those extracted using the Kingfisher method (42.2%; which had been used in 2015–2016). Based on pairwise correlations (Table S4), year and extraction kit type were associated ($p < 0.001$, Cramer's V = 0.860). We chose to retain year as a potential predictor in the model and interpret results in light of the limitations introduced by changing the extraction kit during the study. Although most pairwise comparisons generated *p*-values < 0.001, all other pairs of potential predictors had Cramer's V values of 0.371 or less and were retained (Table S4).

A logistic regression model of infection in adult specimens included sex, species, year, season, and ecoregion (Table 2). When comparing alternate models including pairwise interaction terms, addition of an interaction term containing year and ecoregion resulted in a lower AIC_C than the model without any interaction terms included (AIC_C of 1803.8 vs. 1813.9). This interaction term was nonetheless removed, in consideration of potential sample bias introduced by differential effort by citizen scientists in different ecoregions by year, as the citizen science program expanded over the study period. Results showed that males had a greater odds of infection than females (OR = 1.41, 95% CI 1.11–1.78, $p = 0.005$; Table 2). Infection prevalence varied significantly across different triatomine species, ranging from 10.5% in *T. protracta* to 66.7% in *T. lecticularia* (Table 2). Specimens collected from 2014, 2015, and 2016 had lower odds of infection (OR = 0.34, OR = 0.29, OR = 0.21, respectively) than specimens collected in 2013 (Table 2), and odds of infection were lower (OR = 0.62) for specimens collected April–June than for those collected July–September. Compared to the referent ecoregion (Edwards Plateau), there were significantly lower odds of infection in the Southern Texas Plains (OR = 0.54, 95% CI 0.35–0.83, $p = 0.005$), the group that included Central Great Plains, Chihuahuan Desert, and High Plains (OR = 0.23, 95% CI 0.09–0.54, $p = 0.001$), and the group that included Western Gulf Coastal Plains and South Central Plains (OR = 0.65, 95% CI 0.45–0.96, $p = 0.031$).

When comparing the infection prevalence (Table S1) of only the Omega-extracted samples, we found the infection prevalence was lower in 2014 (59.0%; 95% CI 54.9–63.0%) than 2013 (79.1%; 95% CI 73.8–83.6%; $\chi^2 = 31.86$, $p < 0.01$). When comparing the infection

Table 1
Bivariable analysis of predictor variables of adult triatomine vector T. cruzi infection status (n = 1457 adult specimens).

Bivariable analysis using Chi-square or Fisher's exact tests was used to identify putative predictor variables.

Risk factor	No. tested (%)	No. *T. cruzi* positive (%)	*p*-value
Submission source			0.824
Collected by our research team	92 (6.3)	50 (54.3)	
Citizen science program	1365 (93.7)	766 (56.1)	
Sex			0.211
Female	821 (56.3)	444 (54.1)	
Male	608 (41.7)	357 (58.7)	
Unknown	28 (1.9)	15 (53.6)	
Collection status[a]			0.712
Alive	776 (53.3)	427 (55.0)	
Dead	189 (13.0)	109 (57.7)	
Unknown	492 (33.7)	280 (56.9)	
Species of adults			< 0.001
T. gerstaeckeri	897 (61.6)	568 (63.3)	
T. indictiva	67 (4.6)	32 (47.8)	
T. lecticularia	66 (4.5)	44 (66.7)	
T. protracta	19 (1.3)	2 (10.5)	
T. rubida	64 (4.4)	9 (14.1)	
T. sanguisuga	315 (21.7)	150 (47.6)	
Unknown species	29 (2.0)	11 (37.9)	
Year			< 0.001
2013	276 (18.9)	216 (77.9)	
2014	613 (42.1)	355 (57.9)	
2015	226 (15.5)	105 (46.5)	
2016	263 (18.1)	96 (36.5)	
Unknown	79 (5.4)	44 (55.7)	
Season[b]			< 0.001
January–March	9 (0.6)	3 (33.3)	
April–June	533 (36.6)	255 (47.8)	
July–September	724 (49.7)	448 (61.9)	
October–December	30 (2.1)	16 (53.3)	
Unknown	161 (11.1)	94 (58.4)	
Ecoregion			< 0.001
Edwards Plateau	562 (38.6)	365 (65.0)	
Central Great Plains, Chihuahuan Desert, and High Plains	49 (3.4)	7 (14.3)	
Cross Timbers	54 (3.7)	25 (46.3)	
East Central Texas Plains	182 (12.5)	117 (64.3)	
Southern Texas Plains	135 (9.3)	66 (48.9)	
Texas Blackland Prairies	148 (10.2)	91 (61.5)	
Western Gulf Coast Plain and South Central Plains	169 (11.6)	95 (56.2)	
Non-Texas	106 (7.3)	24 (22.6)	
Unknown	52 (3.6)	26 (50.0)	
Extraction kit			< 0.001
KingFisher	550 (37.7)	233 (42.4)	
Omega	907 (62.3)	583 (64.3)	

[a] Chi-square test was also conducted for only those specimens for which collection status was known; the *p*-value for the test was 0.565.

[b] Since there were small values in the January–March category, a Fisher's exact test was also conducted; the *p*-value using the Fisher's exact test was < 0.001.

prevalence of only the KingFisher-extracted samples, we found the infection prevalence was lower in 2016 (37.1%; 95% CI 31.3–43.4%) than in 2015 (46.7%; 95% CI 40.3–53.2%; $\chi^2 = 4.38$, $p = 0.02$).

3.2. *T. cruzi* DTUs

Across all infected triatomines subjected to the TcSC5D PCR, 66.2% were successfully typed (n = 349). Across all infected triatomines subjected to the SL-IR qPCR, 90.7% were successfully typed (n = 699; Table S2). Using the SL-IR assay, the majority of samples (71.7%) were successfully amplified from undiluted (1 ×) DNA. Those unsuccessfully amplified using undiluted DNA (n = 198) were attempted at a 1:10 dilution, of which 49.0% were successfully typed. Those unsuccessfully amplified undiluted or at a 1:10 dilution (n = 101) were attempted a final time with 2 × DNA, of which 35.6% were successfully typed. After these three failed attempts, a sample was considered untypeable (65 samples of 699; 9.3%).

Table 2
Regression models of *T. cruzi* infection in adult triatomines.

Associations between vector infection and predictor variables were quantified using a logistic regression model.

Variable	Infection prevalence (%)	Odds ratio	95% CI	*p*-value
Sex				
Female	54.1	Referent		
Male	58.7	1.41	1.11–1.78	0.005
Unknown	53.6	0.59	0.25–1.44	0.238
Species of adults				
T. gerstaeckeri	63.3	Referent		
T. indictiva	47.8	0.51	0.30–0.88	0.016
T. lecticularia	66.7	1.35	0.77–2.4	0.302
T. protracta	10.5	0.15	0.02–0.58	0.016
T. rubida	14.1	0.34	0.13–0.84	0.023
T. sanguisuga	47.6	0.54	0.38–0.77	0.001
Unknown adult	37.9	0.36	0.15–0.80	0.014
Year				
2013	77.9	Referent		
2014	57.9	0.34	0.24–0.49	< 0.001
2015	46.5	0.29	0.19–0.43	< 0.001
2016	36.5	0.21	0.14–0.32	< 0.001
Unknown	55.7	0.19	0.09–0.41	< 0.001
Season				
Jan–Mar	33.3	0.67	0.13–2.86	0.600
Apr–Jun	47.8	0.62	0.47–0.81	< 0.001
Jul–Sep	61.9	Referent		
Oct–Dec	53.3	0.73	0.33–1.63	0.435
Unknown	58.4	1.15	0.67–1.99	0.615
Ecoregion				
Edwards Plateau	65.0	Referent		
Central Great Plains, Chihuahuan Desert, and High Plains	14.3	0.23	0.09–0.54	0.001
Cross Timbers	46.3	0.80	0.42–1.53	0.505
East Central Texas Plains	64.3	1.28	0.87–1.90	0.206
Southern Texas Plains	48.9	0.54	0.35–0.83	0.005
Texas Blackland Prairies	61.5	1.24	0.83–1.87	0.302
Western Gulf Coast Plain and South Central Plains	56.2	0.65	0.44–0.96	0.031
Non-Texas	22.6	0.56	0.28–1.10	0.094
Unknown (Texas)	50.0	0.84	0.46–1.55	0.579

Of the 822 total *T. cruzi*-positive triatomines, *T. cruzi* DTU was successfully ascertained for a total of 777 samples (94.5%) using either or both of the typing methods (Table S5). Of the 231 samples successfully typed using the TcSC5D gene, inspection of eight key SNPs (noted in (Cosentino and Agüero, 2012) as able to discern all DTUs) revealed 81 were TcI and 141 were TcIV, and double nucleotide peaks indicating mixed TcI/TcIV infections occurred in an additional 9 samples. Average sequence length for the 222 single-DTU sequences (see Section 2.3.1. for GenBank accession numbers) was 708 base pairs, with a range of 481–747 base pairs. The total covered length captured 809 base pairs (of the 832 base pair gene), of which there were 75 variable sites. Of the 634 samples successfully typed using the SL-IR genetic region, 299 were TcI, 252 were TcIV, and 83 were classified as TcI/TcIV mixed infections. There were 88 samples for which both methods were successful, 74 (84.1%) had congruent results between methods (29 TcI, 41 TcI, 4 TcI/TcIV); there were 13 samples (14.8%) in which the SL-IR qPCR revealed TcI/TcIV mixed infections, but TcSC5D primers generated 1 TcI and 12 TcIV sequences; there was one sample (1.1%) categorized as TcI on SL-IR qPCR but TcI/TcIV mixed on TcSC5D. These 14 samples were all interpreted as mixed infections in our analyses.

Within individual triatomine species, parasite strain infection differed (Table 3). Significant differences in proportions of TcI, TcIV, and

Table 3
T. cruzi DTUs among infected adult triatomines.

Triatomines were collected from 2012 to 2016 through citizen science submission and standard entomological sampling. Not all samples were successfully typed, despite multiple attempts on the TcSC5D and/or SL-IR assay(s). Four typed nymphs (all TcI) are not included in this table (5 additional infected nymphs were not able to be typed). Confidence intervals (using Goodman's method for simultaneous calculation of multinomial confidence intervals) were calculated in cases where all three categories (TcI, TcIV, mixed) each had values of 5 or greater; confidence intervals were not calculated for overall totals because these proportions were influenced by varying sample sizes across species.

	Total typed	No. with only TcI	TcI %[a] (95% CI)	No. with only TcIV	TcIV %[a] (95% CI)	No. with mixed TcI/TcIV	Mixed %[a] (95% CI)
T. gerstaeckeri[b]	548	294	53.6 (48.4-58.8)	189	34.4 (29.7–39.6)	65	11.9 (8.9–15.7)
T. indictiva[c]	28	9	32.1	17	60.7	2	7.1
T. lecticularia[d]	42	9	21.4 (10.1-39.9)	25	59.5 (41.0–75.7)	8	19.0 (8.5–37.3)
T. protracta[e]	2	2	100.0	0	0.0	0	0.0
T. rubida[f]	7	6	85.7	1	14.3	0	0.0
T. sanguisuga[g]	135	21	15.6 (9.4-24.6)	107	79.3 (69.6–86.5)	7	5.2 (2.1–1.2)
Triatoma unknown sp. (adults)	11	4	36.4	7	63.6	0	0.0
Total	773	345	44.6	346	44.8	82	10.6

[a] Percentages are from the total number of successfully typed samples.
[b] 26 of 574 samples (4.5%) were not able to be successfully typed.
[c] 4 of 32 samples (12.5%) were not able to be successfully typed.
[d] 3 of 44 samples (6.8%) were not able to be successfully typed.
[e] 1 of 3 samples (33.3%) was not able to be successfully typed.
[f] 4 of 11 samples (36.4%) were not able to be successfully typed.
[g] 23 of 158 samples (14.6%) were not able to be successfully typed.

mixed infections were observed for each *T. gerstaeckeri* and *T. sanguisuga*, where the greatest proportion for *T. gerstaeckeri* was TcI infections (53.6%; 95% CI 48.4–58.8%) and the greatest proportion for *T. sanguisuga* was TcIV infections (79.3%; 95% CI 69.6–86.5%) (Table 3). In addition, *T. lecticularia* was observed to have a significant difference between proportion of TcI (21.4%; 95% CI 10.1–39.9%) and TcIV (59.5%; 95% CI 41.0–75.7%) infections, and between proportion of TcIV and mixed infections (19.0%; 95% CI 8.5–37.3%), but not between TcI and mixed infections (Table 3). Small sample sizes of the other species precluded any calculations regarding likelihood of being infected with TcI, TcIV, or mixed infections.

The proportions of infected *T. gerstaeckeri* vs. *T. sanguisuga* in which with TcI occurred (65.5% and 20.7%, respectively) vs. those in which TcIV occurred (46.3% and 84.4%, respectively) differed significantly ($p < 0.001$; Table 3). The odds of *T. gerstaeckeri* having a TcI infection were 5.72 (95% CI 3.72–9.07) times that of *T. sanguisuga*. Alternatively stated, the odds of *T. sanguisuga* having a TcIV infection were 5.72 times that of *T. gerstaeckeri*. The proportions (Table 3) of *T. gerstaeckeri* vs. *T. sanguisuga* with a single DTU infection (88.1% and 94.8%, respectively) vs. mixed infection (11.9% and 5.2%, respectively) differed significantly ($p = 0.035$). The odds of *T. gerstaeckeri* having a mixed infection were 2.41 (95% CI 1.15–5.94) times that of *T. sanguisuga*.

Both TcI and TcIV were found across the sampling area in Texas, although there were more TcIV isolates in north Texas (Fig. 2). In addition to the Texas triatomines, parasite typing from triatomines from other areas (Table S3) revealed DTU TcIV in *T. sanguisuga* from Alabama, Florida, Indiana, Kansas, Louisiana, Oklahoma, Tennessee, and Virginia; DTU TcI was found in *T. sanguisuga* from Louisiana and Florida, *T. rubida* from Arizona and New Mexico, and *T. protracta* from Arizona. Although 3 additional triatomines from other states were positive for *T. cruzi* DNA (Ct < 33 on Cruzi 1/2/3 qPCR), attempted strain-typing efforts were unsuccessful (Table S3).

4. Discussion

We tested triatomine insects collected from across the US for *T. cruzi* infection, and typed samples from infected triatomines to determine parasite DTUs. We found an overall infection prevalence of 54.4% ($n = 1510$) in triatomines from across a wide geographic distribution consisting of DTUs TcI and TcIV. These samples offer a perspective of landscape-level infection prevalence and DTU diversity. Further, as many of the insects we analyzed were encountered by members of the public as part of a citizen science program, these data afford a contemporary indication of the parasite DTUs to which humans are most likely to be exposed in the US.

The infection prevalence of 54% is in alignment with recent studies that found infection prevalence of 50.7 to 64.4% in Texas and Louisiana triatomines (Kjos et al., 2009; Waleckx et al., 2014; Wozniak et al., 2015). In all Texas counties from which at least 4 specimens were tested, *T. cruzi* DNA was detected in at least one triatomine, suggesting that uninfected populations of vectors are rare. We found no difference in infection prevalence in specimens collected alive vs. collected dead. This is consistent with previous findings (Smith et al., 2011), and suggests that parasite DNA degradation did not compromise our ability to screen dead specimens, which has practical implications for citizen science programs where keeping the vector alive is not advisable.

Adult specimens were nearly 10 times more likely to be infected with *T. cruzi* than nymphs; this is an expected finding considering vertical transmission of *T. cruzi* in the triatomine vector does not occur (Kirchhoff, 2011; Ryckman and Olsen, 1965). Additionally, triatomines take multiple blood meals throughout their lives, each potentially exposing them to *T. cruzi* infection. The two most frequently collected triatomine species were *T. gerstaeckeri* and *T. sanguisuga*, and infection was higher in *T. gerstaeckeri* than *T. sanguisuga*, consistent with previous Texas-focused reports (Kjos et al., 2009; Pippin, 1970). The odds of infection in *T. gerstaeckeri* were greater than for all other species, except *T. lecticularia*. There was also variation in infection prevalence across ecoregions, with lower odds of infection in vectors from ecoregions not in central Texas. Other ecoregions have markedly different climatic and biological conditions than central Texas, where the majority of specimens were collected. These significant differences in infection prevalence across species and ecoregions may be due to different vector-host associations, including differences in where eggs are laid and nymphs feed until molting to adults with potential to disperse (Lent and Wygodzinsky, 1979). Other drivers may include differing vector species, *T. cruzi* infection and potential transmission dynamics in different species of hosts.

We found that odds of infection in male triatomines were 1.41 times (95% CI 1.11–1.78) that of females, although infection between the sexes within each species was not examined. A previous study found

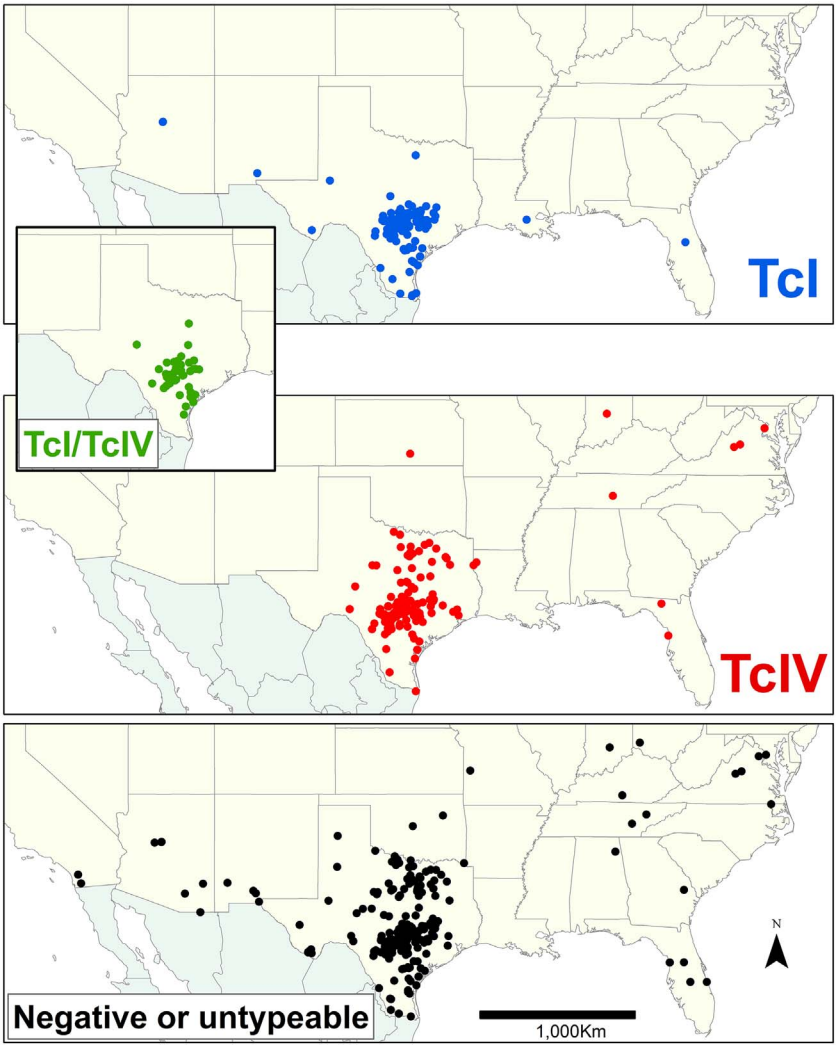

Fig. 2. *T. cruzi* from triatomine insects across Texas and the US. *T. cruzi* was typed to DTU and mapped by exact location of where each specimen was found. Not all typed triatomines were associated with an exact location, and therefore not all typed samples are shown here. For triatomine species distribution maps, please see (Curtis-Robles et al., in press).

variation in infection of *T. gerstaeckeri* by sex, site of capture, and year (Pippin, 1970), with greater infection prevalence in males in most cases. Other studies found no difference in infection prevalence in male and female *T. rubida* and *T. sanguisuga* (Reisenman et al., 2010; Waleckx et al., 2014). Although female triatomines have been reported to consume greater relative amounts of blood during a feeding (Klotz et al., 2009; Pippin, 1970; Reisenman et al., 2011), information regarding the frequency of feedings is lacking. More frequent meals, greater movement, and exposure to a greater variety of potentially infected hosts could potentially be drivers of differences in infection between males and females.

When analyzing seasonality, the greatest number of triatomines was collected in the July–September period. Although the odds of infection were similar in specimens collected from January–March and October–December in comparison to the referent group (July–September), the odds of infection in specimens collected April–June were 0.62 times that of specimens collected July–September (95% CI 0.47–0.81). This finding is in contrast to a prior study that did not find infection prevalence to increase in *T. gerstaeckeri* over the summer months (Pippin, 1970). Our finding is similar to a study that detected increasing infection prevalence in *T. gerstaeckeri* as summer progressed (Wozniak et al., 2015).

The model suggested that triatomines collected in 2013 were more likely to be infected than triatomines collected from 2014 to 2016. However, interpretation of this finding must consider that the extraction kit type was highly correlated with year, since extraction kit had changed during the study period. Extraction kit type was significantly associated with infection outcome in a bivariable analysis, as well as strongly associated with year of collection in pairwise correlation. Given this confounding relationship, it is difficult in the current study to clarify the contributions of extraction kit versus year to explaining variation in triatomine infection. Further investigation of possible differences in extraction kits is warranted. Nonetheless, infection prevalence significantly decreased from 2013 to 2014 when analyzing only samples extracted with the Omega kit; similarly, infection prevalence significantly decreased from 2015 to 2016 when analyzing only samples extracted with the KingFisher kit. Thus, these data suggest a decrease in prevalence over time that is not attributed to a change in extraction kit. Texas suffered a severe drought in 2011 (The National Drought Mitigation Center, 2011) and precipitation gradually increased over the next few years, until it returned to average conditions in 2016 (The National Drought Mitigation Center, 2016). Changes in vector or host densities related to climatic variability could influence vector infection prevalence.

Both TcI and TcIV were documented across the state of Texas. The odds of *T. gerstaeckeri* having a TcI infection were 5.72 (95% CI 3.72–9.07) that of *T. sanguisuga*, and likewise, the odds of *T. sanguisuga* having a TcIV infection were 5.72 times that of *T. gerstaeckeri*. Samples submitted from the northern part of Texas were mainly TcIV; and this apparent geographic bias reflects that *T. sanguisuga* is more likely to be found in that part of the state (Curtis-Robles et al., in press; Kjos et al., 2009). We found TcI and TcIV in out-of-state samples as well. Previous

studies analyzing DTUs in small numbers of triatomine vectors have documented TcI in *T. sanguisuga* in Louisiana (Barnabé et al., 2001; Herrera et al., 2015), Florida (Barnabé et al., 2001; Beard et al., 1988; Roellig et al., 2008), Georgia (Roellig et al., 2008) and California (Shender et al., 2016), in *T. rubida* from west Texas (Buhaya et al., 2015), and in *T. gerstaeckeri* from Texas (Roellig et al., 2008). TcIV has been documented in *T. sanguisuga* from Georgia (Barnabé et al., 2001), and *T. protracta* from California (Shender et al., 2016). Mixed TcI/TcIV *T. cruzi* infections have been documented in one *T. protracta* from California, and one *T. gerstaeckeri* from Texas (Roellig et al., 2008). Additionally, a small number of *T. protracta* in California were found to be infected with isolates closely related to TcII and TcVI group members (Hwang et al., 2010).

The odds of *T. gerstaeckeri* having a mixed infection were 2.41 (95% CI 1.15–5.94) times that of *T. sanguisuga*. The presence of mixed DTU infections in vectors may reflect independent infections of triatomines acquired from different blood meals on different hosts, or, from a single blood meal on a co-infected host. Mammalian hosts co-infected with more than one DTU have been documented in multiple species the southern US (Curtis-Robles et al., 2016; Herrera et al., 2015; Roellig et al., 2008). A study in Venezuela revealed distinct patterns of DTU findings in human, vector, and wild hosts, suggesting that multi-host assessments of circulating DTUs are needed to better understand human and animal risk of infection with specific DTUs (Carrasco et al., 2012). Detected coinfections in vectors and hosts may underestimate true co-infection prevalences, given that both culture and PCR-based methodologies may be biased toward one strain over others. There is a need for concurrent investigations of vectors and hosts in the same geographic area to elucidate host sources of infection with different parasite DTUs relative to the DTUs in the vector community.

Sylvatic hosts are known to play an important role in triatomine infection, especially for US triatomine species that are less commonly associated with domestic colonization. Many wildlife species have been identified as infected with *T. cruzi* in Texas, including armadillos, opossums, raccoons, and woodrats (see review in Hodo and Hamer, 2017). Previous studies of US wildlife found predominantly TcI in opossums (Barnabé et al., 2001; Roellig et al., 2008) and TcIV in raccoons (Barnabé et al., 2001; Curtis-Robles et al., 2016; Roellig et al., 2008). One study in a focal area in west Texas found TcIV in a hispid cotton rat (*Sigmodon hispidus*) and rock squirrel (*Otospermophilus variegatus*), as well as TcI and TcIV in southern plains woodrats (*Neotoma micropus*) and striped skunks (*Mephitis mephitis*) (Charles et al., 2013). In addition to wildlife, canines have been shown to be infected with TcI and TcIV (Curtis-Robles et al., 2017a, 2017b; Patel et al., 2012; Roellig et al., 2008). The degree to which each host species plays a role in sustaining triatomine populations and serving as infection sources is unknown. Although there is a well-documented association between *T. protracta* and *Neotoma* spp. woodrats in the southwestern US (Charles et al., 2013; Packchanian, 1942; Ryckman et al., 1981), host utilization by other US triatomines species is not well known (Ibarra-Cerdeña et al., 2009).

Many *T. cruzi* strain-typing methods involve the propagation of the parasite in pure culture followed by multiple PCRs, with differentiation of strains based on amplicon presence/absence, amplicon size, and or multi-locus sequence typing (Messenger et al., 2015b). We originally sought an approach based on a single PCR and DNA sequencing to allow simple, rapid classification to DTU-level. We found the TcSC5D primer set an attractive option for DTU-level classification of *T. cruzi* from mixed DNA samples with high loads of parasite, as well as for its usefulness in determining potential mixed DTU infections based on examination of sequence chromatographs. However, this method was originally developed using pure parasite culture containing high concentrations of *T. cruzi* DNA (Cosentino and Agüero, 2012), and our current work as well as prior studies have had variable success amplifying the TcSC5D locus of DNA from field samples with low concentrations of *T. cruzi* DNA (Cominetti et al., 2014; Curtis-Robles et al., 2016). It is possible different DTUs of *T. cruzi* establish different parasite densities in triatomines, potentially leading to bias if our methods preferentially amplified DTUs that were more abundant. We had greater success in assigning DTU with a recently-published qPCR assay (Cura et al., 2015). In a small subset of samples typed using both methods (Table S5), the TcSC5D method revealed a single DTU in 13 of 17 cases (76.5%) in which the SL-IR method generated a mixed TcI/TcIV result; in contrast, the SL-IR method revealed a single DTU in 1 of 5 cases (20.0%) in which the TcSC5D generated a mixed TcI/TcIV result. Both typing methods have limitations: TcSC5D relies on success of both PCR and sequencing, while SL-IR qPCR may be affected by PCR artifact and is not confirmed by sequencing. Overall the SL-IR qPCR appears to be more useful for DTU typing in field-collected samples with variable concentrations of *T. cruzi* DNA. In addition, the multiplex probe design allows for detection of multiple co-infecting DTUs in one reaction.

This study represents the largest number of typed *T. cruzi* samples from US triatomines to date, and provides a base for future studies investigating the within-DTU genetic variations of *T. cruzi*. The small number of *T. cruzi* infections in humans in the US that have been definitively typed were all TcI (Garcia et al., 2017; Roellig et al., 2008), and our findings of TcI in vectors encountered by the public are therefore directly important for assessing human health risk. Although TcIV is known to infect dogs and wildlife in the US (Curtis-Robles et al., 2016; Curtis-Robles et al., 2017a; Patel et al., 2012; Roellig et al., 2008), whether triatomines infected with TcIV are a risk to human health is unknown. The growing datasets on the genetic variation of *T. cruzi* from natural transmission settings in the US may be valuable in understanding variation in disease progression and protecting veterinary and public health.

Acknowledgements

We thank Justin Bejcek, Bhagath Chirra, Adam Curtis, and Valery Roman-Cruz for laboratory and field assistance, as well as Alyssa Meyers and Jillian Wormington for discussion of statistical analyses. We are grateful to two anonymous reviewers for constructive comments. We thank hundreds of citizen submitters for their diligent collecting efforts.

Funding

This work was supported by the National Center for Veterinary Parasitology, the Texas A&M University-Consejo Nacional de Ciencia y Tecnologia Collaborative Research Grant Program, the Texas EcoLab Program, and a Texas A&M AgriLife Research Insect Vector Disease Seed Grant. Graduate student support was provided by the National Science Foundation Graduate Research Fellowship Program under Grant No. 1252521 (RC-R). We thank the family of Dr. Raymond Ryckman for supporting publication charges.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2017.12.016.

References

Barnabé, C., Yaeger, R., Pung, O., Tibayrenc, M., 2001. *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. 99, 73–79. http://dx.doi.org/10.1006/expr.2001.4651.

Beard, C.B., Young, D.G., Butler, J.F., Evans, D.A., 1988. First isolation of *Trypanosoma cruzi* from a wild-caught *Triatoma sanguisuga* (LeConte) (Hemiptera: Triatominae) in Florida, USA. J. Parasitol. 74, 343–344.

Bern, C., Kjos, S., Yabsley, M.J., Montgomery, S.P., 2011. *Trypanosoma cruzi* and Chagas' disease in the United States. Clin. Microbiol. Rev. 24, 655–681. http://dx.doi.org/10.1128/CMR.00005-11.

Brenière, S.F., Waleckx, E., Barnabé, C., 2016. Over six thousand *Trypanosoma cruzi* strains classified into discrete typing units (DTUs): attempt at an inventory. PLoS Negl. Trop. Dis. 10, e0004792. http://dx.doi.org/10.1371/journal.pntd.0004792.

Brown, E.L., Roellig, D.M., Gompper, M.E., Monello, R.J., Wenning, K.M., Gabriel, M.W., Yabsley, M.J., 2010. Seroprevalence of *Trypanosoma cruzi* among eleven potential reservoir species from six states across the southern United States. Vector-Borne Zoonotic Dis. 10, 757–763. http://dx.doi.org/10.1089/vbz.2009.0009.

Buhaya, M., Galvan, S., Maldonado, R., 2015. Incidence of *Trypanosoma cruzi* infection in triatomines collected at Indio Mountains Research Station. Acta Trop. 150, 97–99. http://dx.doi.org/10.1016/j.actatropica.2015.07.004.

Burkholder, J.E., Allison, T.C., Kelly, V.P., 1980. *Trypanosoma cruzi* (Chagas) (Protozoa: Kinetoplastida) in invertebrate, reservoir, and human hosts of the Lower Rio Grande Valley of Texas. J. Parasitol. 66, 305–311.

Cantey, P.T., Stramer, S.L., Townsend, R.L., Kamel, H., Ofafa, K., Todd, C.W., Currier, M., Hand, S., Varnado, W., Dotson, E., Hall, C., Jett, P.L., Montgomery, S.P., 2012. The United States *Trypanosoma cruzi* infection study: evidence for vector-borne transmission of the parasite that causes Chagas disease among United States blood donors. Transfusion 52, 1922–1930. http://dx.doi.org/10.1111/j.1537-2995.2012.03581.x.

Carrasco, H.J., Segovia, M., Llewellyn, M.S., Morocoima, A., Urdaneta-Morales, S., Martínez, C., Martínez, C.E., Garcia, C., Rodríguez, M., Espinosa, R., de Noya, B.A., Díaz-Bello, Z., Herrera, L., Fitzpatrick, S., Yeo, M., Miles, M.A., Feliciangeli, M.D., 2012. Geographical distribution of *Trypanosoma cruzi* genotypes in Venezuela. PLoS Negl. Trop. Dis. 6, e1707. http://dx.doi.org/10.1371/journal.pntd.0001707.

Charles, R.A., Kjos, S., Ellis, A.E., Barnes, J.C., Yabsley, M.J., 2013. Southern plains woodrats (*Neotoma micropus*) from southern Texas are important reservoirs of two genotypes of *Trypanosoma cruzi* and host of a putative novel *Trypanosoma* species. Vector-Borne Zoonotic Dis. 13, 22–30. http://dx.doi.org/10.1089/vbz.2011.0817.

Cominetti, M.C., Csordas, B.G., Cunha, R.C., Andreotti, R., 2014. Geographical distribution of *Trypanosoma cruzi* in triatomine vectors in the state of Mato Grosso do Sul, Brazil. Rev. Soc. Bras. Med. Trop. 47, 747–755. http://dx.doi.org/10.1590/0037-8682-0234-2014.

Cosentino, R.O., Agüero, F., 2012. A simple strain typing assay for *Trypanosoma cruzi*: discrimination of major evolutionary lineages from a single amplification product. PLoS Negl. Trop. Dis. 6, e1777. http://dx.doi.org/10.1371/journal.pntd.0001777.

Cura, C.I., Duffy, T., Lucero, R.H., Bisio, M., Péneau, J., Jimenez-Coello, M., Calabuig, E., Gimenez, M.J., Valencia Ayala, E., Kjos, S.A., Santalla, J., Mahaney, S.M., Cayo, N.M., Nagel, C., Barcán, L., Málaga Machaca, E.S., Acosta Viana, K.Y., Brutus, L., Ocampo, S.B., Aznar, C., Cuba Cuba, C.A., Gürtler, R.E., Ramsey, J.M., Ribeiro, I., VandeBerg, J.L., Yadon, Z.E., Osuna, A., Schijman, A.G., 2015. Multiplex real-time PCR assay using TaqMan probes for the identification of *Trypanosoma cruzi* DTUs in biological and clinical samples. PLoS Negl. Trop. Dis. 9, e0003765. http://dx.doi.org/10.1371/journal.pntd.0003765.

Curtis-Robles, R., Wozniak, E.J., Auckland, L.D., Hamer, G.L., Hamer, S.A., 2015. Combining public health education and disease ecology research: using citizen science to assess Chagas disease entomological risk in Texas. PLoS Negl. Trop. Dis. 9, e0004235. http://dx.doi.org/10.1371/journal.pntd.0004235.

Curtis-Robles, R., Lewis, B.C., Hamer, S.A., 2016. High *Trypanosoma cruzi* infection prevalence associated with minimal cardiac pathology among wild carnivores in central Texas. Int. J. Parasitol. Parasites Wildl. 5, 117–123. http://dx.doi.org/10.1016/j.ijppaw.2016.04.001.

Curtis-Robles, R., Snowden, K.F., Dominguez, B., Dinges, L., Rodgers, S., Mays, G., Hamer, S.A., 2017a. Epidemiology and molecular typing of *Trypanosoma cruzi* in naturally-infected hound dogs and associated triatomine vectors in Texas,USA. PLoS Negl. Trop. Dis. 11, e0005298. http://dx.doi.org/10.1371/journal.pntd.0005298.

Curtis-Robles, R., Zecca, I.B., Roman-Cruz, V., Carbajal, E.S., Auckland, L.D., Flores, I., Millard, A.V., Hamer, S.A., 2017b. *Trypanosoma cruzi* (agent of Chagas disease) in sympatric human and dog populations in “colonias” of the Lower Rio Grande Valley of Texas. Am. J. Trop. Med. Hyg. 96, 805–814. http://dx.doi.org/10.4269/ajtmh.16-0789.

Curtis-Robles, R., Lane, S., Levy, M., Hamer, S., Hamer, G., Bionomics and spatial distribution of Triatomine vectors of *Trypanosoma cruzi* in Texas and other southern states, USA. Am. J. Trop. Med. Hyg. Available online 6 Nov 2017. (In press) doi: https://doi.org/10.4269/ajtmh.17-0526.

Dorn, P.L., Perniciaro, L., Yabsley, M.J., Roellig, D.M., Balsamo, G., Diaz, J., Wesson, D., 2007. Autochthonous transmission of *Trypanosoma cruzi*, Louisiana. Emerg. Infect. Dis. 13, 13–15. http://dx.doi.org/10.3201/eid1304.061002.

Duffy, T., Cura, C.I., Ramirez, J.C., Abate, T., Cayo, N.M., Parrado, R., Bello, Z.D., Velazquez, E., Muñoz-Calderon, A., Juiz, N.A., Basile, J., Garcia, L., Riarte, A., Nasser, J.R., Ocampo, S.B., Yadon, Z.E., Torrico, F., de Noya, B.A., Ribeiro, I., Schijman, A.G., 2013. Analytical performance of a multiplex real-time PCR assay using TaqMan probes for quantification of *Trypanosoma cruzi* satellite DNA in blood samples. PLoS Negl. Trop. Dis. 7, e2000. http://dx.doi.org/10.1371/journal.pntd.0002000.

Garcia, M.N., Aguilar, D., Gorchakov, R., Rossmann, S.N., Montgomery, S.P., Rivera, H., Woc-Colburn, L., Hotez, P.J., Murray, K.O., 2015. Case report: evidence of autochthonous Chagas disease in southeastern Texas. Am. J. Trop. Med. Hyg. 92, 325–330. http://dx.doi.org/10.4269/ajtmh.14-0238.

Garcia, M.N., Burroughs, H., Gorchakov, R., Gunter, S.M., Dumonteil, E., Murray, K.O., Herrera, C.P., 2017. Molecular identification and genotyping of *Trypanosoma cruzi* DNA in autochthonous Chagas disease patients from Texas, USA. Infect. Genet. Evol. 49, 151–156. http://dx.doi.org/10.1016/j.meegid.2017.01.016.

Goodman, L.A., 1965. On simultaneous confidence intervals for multinomial proportions. Technometrics 7, 247–254.

Herrera, C.P., Licon, M.H., Nation, C.S., Jameson, S.B., Wesson, D.M., 2015. Genotype diversity of *Trypanosoma cruzi* in small rodents and *Triatoma sanguisuga* from a rural area in New Orleans, Louisiana. Parasit. Vectors 8, 1–9. http://dx.doi.org/10.1186/s13071-015-0730-8.

Hodo, C.L., Hamer, S.A., 2017. Toward an ecological framework for assessing reservoirs of vector-borne pathogens: wildlife reservoirs of *Trypanosoma cruzi* across the southern United States. ILAR J. 1–14. http://dx.doi.org/10.1093/ilar/ilx020.

Hwang, W.S., Zhang, G., Maslov, D., Weirauch, C., 2010. Short report: infection rats of Triatoma protracta (Ulher) with Trypanosoma cruzi in southern California and molecular identification of trypanosomes. Am. J. Trop. Med. Hyg. 83, 1020–1022.

Ibarra-Cerdeña, C.N., Sánchez-Cordero, V., Townsend Peterson, A., Ramsey, J.M., 2009. Ecology of north American Triatominae. Acta Trop. 110, 178–186. http://dx.doi.org/10.1016/j.actatropica.2008.11.012.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. http://dx.doi.org/10.1093/bioinformatics/bts199.

Kirchhoff, L.V., 2011. Epidemiology of American trypanosomiasis (Chagas disease). Adv. Parasitol. 75, 1–18. http://dx.doi.org/10.1016/B978-0-12-385863-4.00001-0.

Kjos, S., Snowden, K., Craig, T., Lewis, B., Ronald, N., Olson, J., 2008. Distribution and characterization of canine Chagas disease in Texas. Vet. Parasitol. 152, 249–256. http://dx.doi.org/10.1016/j.vetpar.2007.12.021.

Kjos, S.A., Snowden, K.F., Olson, J.K., 2009. Biogeography and *Trypanosoma cruzi* infection prevalence of Chagas disease vectors in Texas, USA. Vector-Borne Zoonotic Dis. 9, 41–50. http://dx.doi.org/10.1089/vbz.2008.0026.

Klotz, S.A., Dorn, P.L., Klotz, J.H., Pinnas, J.L., Weirauch, C., Kurtz, J.R., Schmidt, J., 2009. Feeding behavior of triatomines from the southwestern United States: an update on potential risk for transmission of Chagas disease. Acta Trop. 111, 114–118. http://dx.doi.org/10.1016/j.actatropica.2009.03.003.

Lent, H., Wygodzinsky, P., 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. Bull. Am. Mus. Nat. Hist. 163, 123–520.

Messenger, L.A., Miles, M.A., Bern, C., 2015a. Between a bug and a hard place: *Trypanosoma cruzi* genetic diversity and the clinical outcomes of Chagas disease. Expert Rev. Anti-Infect. Ther. 13, 995–1029. http://dx.doi.org/10.1586/14787210.2015.1056158.

Messenger, L.A., Yeo, M., Lewis, M.D., Llewellyn, M.S., Miles, M.A., 2015b. Molecular genotyping of *Trypanosoma cruzi* for lineage assignment and population genetics. In: Peacock, C. (Ed.), Parasite Genomics Protocols, Methods in Molecular Biology. Springer New York, New York, NY, pp. 297–337. http://dx.doi.org/10.1007/978-1-4939-1438-8.

Miles, M., Llewellyn, M., Lewis, M., Yeo, M., Baleela, R., Fitzpatrick, S., Gaunt, M., Mauricio, I., 2009. The molecular epidemiology and phylogeography of *Trypanosoma cruzi* and parallel research on *Leishmania*: looking back and to the future. Parasitology 136, 1509–1528. http://dx.doi.org/10.1017/S0031182009990977.

Moreira, O.C., Ramírez, J.D., Velázquez, E., Dias Melo, M.F.A., Lima-Ferreira, C., Guhl, F., Sosa-Estani, S., Marin-Neto, J.A., Morillo, C.A., Britto, C., 2013. Toward the establishment of a consensus real-time qPCR to monitor *Trypanosoma cruzi* parasitemia in patients with chronic Chagas disease cardiomyopathy: a substudy from the BENEFIT trial. Acta Trop. 125, 23–31. http://dx.doi.org/10.1016/j.actatropica.2012.08.020.

Packchanian, A., 1942. Reservoir hosts of Chagas' disease in the state of Texas: natural infection of nine-banded armadillo (*Dasypus novemcinctus texanus*), house mice (*Mus musculus*), opossum (*Didelphis virginiana*), and wood rats (*Neotoma micropus micropus*), with *Trypanosoma cruzi* in the state of Texas. Am. J. Trop. Med. 22, 623–631.

Patel, J.M., Rosypal, A.C., Zimmerman, K.L., Monroe, W.E., Sriranganathan, N., Zajac, A.M., Yabsley, M.J., Lindsay, D.S., 2012. Isolation, mouse pathogenicity, and genotyping of *Trypanosoma cruzi* from an English Cocker Spaniel from Virginia, USA. Vet. Parasitol. 187, 394–398. http://dx.doi.org/10.1016/j.vetpar.2012.01.031.

Pippin, W.F., 1970. The biology and vector capability of *Triatoma sanguisuga texana* Usinger and *Triatoma gerstaeckeri* (Stål) compared with *Rhodnius prolixus* (Stål) (Hemiptera: Triatominae). J. Med. Entomol. 7, 30–45.

Piron, M., Fisa, R., Casamitjana, N., López-Chejade, P., Puig, L., Vergés, M., Gascón, J., Gómez i Prat, J., Portús, M., Sauleda, S., 2007. Development of a real-time PCR assay for *Trypanosoma cruzi* detection in blood samples. Acta Trop. 103, 195–200. http://dx.doi.org/10.1016/j.actatropica.2007.05.019.

R Development Core Team, 2008. R: A Language and Environment for Statistical Computing.

Reisenman, C.E., Lawrence, G., Guerenstein, P.G., Gregory, T., Dotson, E., Hildebrand, J.G., 2010. Infection of kissing bugs with *Trypanosoma cruzi*, Tucson, Arizona, USA. Emerg. Infect. Dis. 16, 400–405. http://dx.doi.org/10.3201/eid1603.090648.

Reisenman, C.E., Gregory, T., Guerenstein, P.G., Hildebrand, J.G., 2011. Feeding and defecation behavior of *Triatoma rubida* (Uhler, 1894) (Hemiptera: Reduviidae) under laboratory conditions, and its potential role as a vector of Chagas disease in Arizona, USA. Am. J. Trop. Med. Hyg. 85, 648–656. http://dx.doi.org/10.4269/ajtmh.2011.11-0137.

Roellig, D.M., Brown, E.L., Barnabé, C., Tibayrenc, M., Steurer, F.J., Yabsley, M.J., 2008. Molecular typing of *Trypanosoma cruzi* isolates, United States. Emerg. Infect. Dis. 14, 1123–1125. http://dx.doi.org/10.3201/eid1407.080175.

Ryckman, R.E., Olsen, L.E., 1965. Epizootiology of *Trypanosoma cruzi* in southwestern North America: part VI: insectivorous hosts of Triatominae-the epizootiological relationship to Trypanosoma cruzi (Kinetoplastida: Trypanosomidae) (Rodentia: Cricetidae). J. Med. Entomol. 2, 99–104.

Ryckman, R.E., Archbold, E.F., Bentley, D.G., 1981. The *Neotoma* group in north and central America: a checklist, literature review, and comprehensive bibliography (Rodentia: Cricetidae: Cricetinae). Bull. Soc. Vector Ecol. 6, 1–92.

Schijman, A.G., Bisio, M., Orellana, L., Sued, M., Duffy, T., Mejia Jaramillo, A.M., Cura, C., Auter, F., Veron, V., Qvarnstrom, Y., Deborggraeve, S., Hijar, G., Zulantay, I., Lucero, R.H., Velazquez, E., Tellez, T., Sanchez Leon, Z., Galvão, L., Nolder, D., Monje Rumi, M., Levi, J.E., Ramirez, J.D., Zorrilla, P., Flores, M., Jercic, M.I., Crisante, G.,

Añez, N., De Castro, A.M., Gonzalez, C.I., Acosta Viana, K., Yachelini, P., Torrico, F., Robello, C., Diosque, P., Triana Chavez, O., Aznar, C., Russomando, G., Büscher, P., Assal, A., Guhl, F., Sosa Estani, S., DaSilva, A., Britto, C., Luquetti, A., Ladzins, J., 2011. International study to evaluate PCR methods for detection of *Trypanosoma cruzi* DNA in blood samples from Chagas disease patients. PLoS Negl. Trop. Dis. 5, e931. http://dx.doi.org/10.1371/journal.pntd.0000931.

Shender, L.A., Lewis, M.D., Rejmanek, D., Mazet, J.A.K., 2016. Molecular diversity of *Trypanosoma cruzi* detected in the vector *Triatoma protracta* from California,USA. PLoS Negl. Trop. Dis. 10, e0004291. http://dx.doi.org/10.1371/journal.pntd.0004291.

Smith, B.A., Conlan, C., Hwang, W.S., Weirauch, C., 2011. Polymerase chain reaction detection of *Trypanosoma cruzi* in suboptimally preserved vectors and comparative infection rates 2007–2010 in Escondido, southern California. Vector-Borne Zoonotic Dis. 11, 2007–2008. http://dx.doi.org/10.1089/vbz.2011.0710.

The National Drought Mitigation Center, 2011. June 7, 2011 map. http://droughtmonitor.unl.edu/data/jpg/20110607/20110607_tx_trd.jpg (accessed 6.9.17).

The National Drought Mitigation Center, 2016. June 7, 2016 map. http://droughtmonitor.unl.edu/data/jpg/20160607/20160607_tx_trd.jpg (accessed 6.9.17).

U.S. EPA (Environmental Protection Agency), 2012. U.S. Level III and IV Ecoregions. https://www.epa.gov/eco-research/ecoregion-download-files-state-region-6#pane-41 (accessed 4.19.17).

Waleckx, E., Suarez, J., Richards, B., Dorn, P.L., 2014. *Triatoma sanguisuga* blood meals and potential for Chagas disease, Louisiana, USA. Emerg. Infect. Dis. 20, 2141–2143.

World Health Organization, 2015. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Wkly Epidemiol. Rec. 6, 33–44.

Wozniak, E.J., Lawrence, G., Gorchakov, R., Alamgir, H., Dotson, E., Sissel, B., Murray, K.O., 2015. The biology of the triatomine bugs native to south central Texas and assessment of the risk they pose for autochthonous Chagas disease exposure. J. Parasitol. 101, 520–528. http://dx.doi.org/10.1645/15-748.

Zingales, B., Andrade, S., Briones, M., Campbell, D., Chiari, E., Fernandes, O., Guhl, F., Lages-Silva, E., Macedo, A., Machado, C., Miles, M., Romanha, A., Sturm, N., Tibayrenc, M., Schijman, A., 2009. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem. Inst. Oswaldo Cruz 104, 1051–1054. http://dx.doi.org/10.1590/S0074-02762009000700021.

Zingales, B., Miles, M.A., Campbell, D.A., Tibayrenc, M., Macedo, A.M., Teixeira, M.M.G., Schijman, A.G., Llewellyn, M.S., Lages-Silva, E., Machado, C.R., Andrade, S.G., Sturm, N.R., 2012. The revised *Trypanosoma cruzi* subspecific nomenclature: rationale, epidemiological relevance and research applications. Infect. Genet. Evol. 12, 240–253. http://dx.doi.org/10.1016/j.meegid.2011.12.009.

Zumaya-Estrada, F.A., Messenger, L.A., Lopez-Ordonez, T., Lewis, M.D., Flores-Lopez, C.A., Martínez-Ibarra, A.J., Pennington, P.M., Cordon-Rosales, C., Carrasco, H.V., Segovia, M., Miles, M.A., Llewellyn, M.S., 2012. North American import? Charting the origins of an enigmatic *Trypanosoma cruzi* domestic genotype. Parasit. Vectors 5, 226. http://dx.doi.org/10.1186/1756-3305-5-226.