Genetic characterization of Trypanosoma cruzi DTUs in wild Triatoma infestans from Bolivia: predominance of TcI
Simone Brenière, Claudia Aliaga, Etienne Waleckx, Rocio Buitrago, R. Salas, Christian Barnabé, Michel Tibayrenc, François Noireau

To cite this version:
Simone Brenière, Claudia Aliaga, Etienne Waleckx, Rocio Buitrago, R. Salas, et al.. Genetic characterization of Trypanosoma cruzi DTUs in wild Triatoma infestans from Bolivia: predominance of TcI. PLoS Neglected Tropical Diseases, Public Library of Science, 2012, 6 (5), pp.e1650. 10.1371/journal.pntd.0001650. hal-01256078

HAL Id: hal-01256078
https://hal.archives-ouvertes.fr/hal-01256078
Submitted on 14 Jan 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Genetic Characterization of *Trypanosoma cruzi* DTUs in Wild *Triatoma infestans* from Bolivia: Predominance of TcI

Simone Frédérique Brenière¹,², Claudia Aliaga¹,², Etienne Waleckx¹,², Rosio Buitrago¹,², Renata Salas¹,², Christian Barnabé¹,², Michel Tibayrenc¹, François Noireau¹,³

¹ MIVEGEC (Université de Montpellier 1 et 2, CNRS 5290, IRD 224), Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle, Institut de Recherche pour le développement (IRD), Representation en Bolivie, La Paz, Bolivia
² Instituto Nacional de Laboratorios de Salud (INLASA), Laboratorio de Entomología Médica, La Paz, Bolivia
³ IIBISMED, Facultad de Medicina, Universidad Mayor de San Simón, Cochabamba, Bolivia

Abstract

**Background:** The current persistence of *Triatoma infestans* (one of the main vectors of Chagas disease) in some domestic areas could be related to re-colonization by wild populations which are increasingly reported. However, the infection rate and the genetic characterization of the *Trypanosoma cruzi* strains infecting these populations are very limited.

**Methodology/Principal Findings:** Of 333 wild *Triatoma infestans* specimens collected from north to south of a Chagas disease endemic area in Bolivia, we characterized 234 stocks of *Trypanosoma cruzi* using mini-exon multiplex PCR (MMPCR) and sequencing the glucose phosphate isomerase (*Gpi*) gene. Of the six genetic lineages ("discrete typing units"; DTU) (TcI-VI) presently recognized in *T. cruzi*, TcI (99.1%) was overdominant on TcIII (0.9%) in wild Andean *T. infestans*, which presented a 71.7% infection rate as evaluated by microscopy. In the lowlands (Bolivian Chaco), 17 "dark morph" *T. infestans* were analyzed. None of them were positive for parasites after microscopic examination, although one TcI stock and one TcII stock were identified using MMPCR and sequencing.

**Conclusions/Significance:** By exploring large-scale DTUs that infect the wild populations of *T. infestans*, this study opens the discussion on the origin of TcI and TcV DTUs that are predominant in domestic Bolivian cycles.

Introduction

*Trypanosoma cruzi*, the agent of Chagas disease, is a serious threat to health in the Americas, accounting for the highest disease burden in Latin America, with eight to nine million people infected and 25-90 million at risk [1–3]. This parasite, which belongs to the order Kinetoplastida, is mainly transmitted by blood-sucking bug vectors (Hemiptera, Reduviidae, Triatominae) but also by blood transfusion and oral transmission. Moreover, newborns can be infected through vertical transmission. There are currently 141 recognized species of triatomines, but only five of them, belonging to three genera (*Triatoma, Rhodinus, and Panstrongylus*) can be considered important vectors of Chagas disease [4]. With the exception of one species (*T. rubrofasciata*), all Triatominae have populations living in natural habitats in contact with wild mammals, birds, or reptiles [3–8]. *T. cruzi* is found in three overlapping ecosystems. One is related to the wild environment and involves wild populations of triatomines and mammals (sylvatic cycle); the second one depends on artificial structures surrounding human dwellings where vector populations associated to domestic and synanthropic animals live (peridomestic cycle); the third one occurs in dwellings and involves triatomines living indoors, humans, and domestic animals (domestic cycle).

Population genetics analyses have shown that *T. cruzi* has a predominantly clonal mode of evolution and exhibits considerable phenotypic and genetic diversity [9]. This population genetics model refers to genetic clonality, i.e., limited or absent genetic recombination with persistence of durable multilocus associations, whatever the cytological mechanism of reproduction [10]. Six distinct genetic lineages or discrete typing units (DTUs) [11] have been described [12,13]. They have recently been validated by a committee of experts and labeled TcI to TcVI [14]. TcI is responsible for the large majority of human infections in the Amazon basin and more northern countries as well as part of the infections in South Cone countries of South America. It exhibits considerable genetic diversity [9,15,16] with possible subclustering [17,18]. TcII, V, and VI are mainly associated with domestic
cycles and prevalent in human infections in the Southern Cone countries; TcV and TcVI are hybrid genotypes, whose putative ancestors are TcII and TcIII [19,20]. Finally, TcIII and IV are more rarely sampled throughout the endemic area and seem to be specific to sylvatic cycles, with few reports of human infection.

In Bolivia, Triatoma infestans (Hemiptera: Reduviidae) remains the main domestic vector of Trypanosoma cruzi, the agent of the disease. This parasite presents a large genetic variability and it is important to know which T. cruzi genotypes are carried by the vectors. The authors found that in the wild T. infestans from the Bolivian Andean region, a principal group of genotype was circulating. In the lowlands (Bolivian Chaco), another additional genotype group was detected. Together with exploring at large scale which genotypes are infecting T. infestans wild populations, this study opens the discussion on the origin T. cruzi genotype groups. Also, this study completes our basic knowledge on T. cruzi subspecific genetic variability, and therefore brings new tools for molecular epidemiology of Chagas disease.

In this study, we applied the MMPCR and Gpi sequencing for the characterization of T. cruzi DTUs directly in the digestive tract of wild T. infestans collected in Bolivia.

Materials and Methods

Origin of T. infestans populations

The triatomines were sampled in sylvatic environments from April to November 2009 (Figure 1). Collections were carried out using mice-baited adhesive traps [40] in different ecotopes such as

Figure 1. Sampling sites of wild populations of Triatoma infestans in Bolivia. The sites were numbered from 1 to 36, Bolivian department names are indicated, for the DTU T. cruzi results see in Table 1.

doi:10.1371/journal.pntd.0001650.g001
under bush and bromeliads, rocks, burrows, hollow trees, and stone walls. The bugs were transported alive to the laboratory for species confirmation using morphological taxonomic keys [41].

Table 1 summarizes the geographical and ecotope origin of the collected T. infestans according to the ecoregions defined in [42]. Briefly, the majority of the bugs were collected in Andean valleys...
where sylvatic foci have been previously described [21,24] and the others were collected from new foci in the Bolivian Chaco where the "dark morph" type of *T. infestans* was discovered [22,26].

Before dissection, feces from each bug were examined for the presence of trypanosomatid by direct microscopic observation at 400 magnification (mo). Bugs were then dissected under a safety hood, and the digestive tracts stored at ~20°C.

Mini-exon multiplex PCR (MMPCR)

DNA was extracted from triatome digestive tracts with the QIAamp DNA mini kit (Quiagen, Courtaboeuf, France), according to the blood sample protocol. The multiplex primer set was as previously described: three oligonucleotides derived from the hypervariable region of *T. cruzi* mini-exon repeats, and a common downstream oligonucleotide, corresponding to sequences present in the best conserved region of the mini-exon gene used as opposing primer in the multiplex reaction. PCR conditions were according to Fernandes et al. [36], with slight modifications. DNA was amplified in a 25 µl reaction volume containing 1× reaction buffer, 1.5 mM MgCl$_{2}$, 50 µM of each nucleotide, 0.2 µM of each primer, 0.5 UI of Taq polymerase (Roche Applied Science, Penzberg, Germany). The amplifications were performed in a thermocycler (Eppendorf, Hamburg, Germany), in previously described conditions [36]. PCR products were separated on 3% agarose gel using the molecular weight marker Smart Ladder (Eurogentec, Angers, France) and visualized under UV with Ez-agarose gel using the molecular weight marker Smart Ladder (Eurogentec, Angers, France).

PCR of the *T. cruzi* glucose-6-phosphate isomerase fragment

A 632 bp fragment was amplified with a set of primers, forward (Gpi-L) starting at position 591 of the gene (5' CGCCATGTGTGGAATATTGG-3') and reverse (Gpi-R) starting at position 1246 (5' TCCATGGTTTCCATGTCAG-3'), from a subsample of 15 DNAs which had given an intense MMPCR band. DNA was amplified in a 25 µl reaction volume containing 0.75 mM MgCl$_{2}$, 0.2 mM of each nucleotide, 0.4 µM of each primer, 2.5 UI of Taq DNA polymerase (Roche Applied Science, Penzberg, Germany), and 20 ng of DNA template. The amplification took place in a thermocycler (Eppendorf, Hamburg, Germany), with the following cycle conditions: 94°C for 3 min; 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min (35 cycles); 72°C for 5 min. Purification and direct sequencing of both strands of DNA amplicons were performed by the company MACROGEN (Seoul, South Korea).

Results

Mini-exon multiplex PCR (MMPCR) analysis

A total of 333 DNA samples from digestive tracts of wild *T. infestans* were processed in MMPCR for DTU identification. Among them 20.1% were adults of both sexes, 64.1% 4$^{th}$ and 5$^{th}$ instar nymphs, and 15.8% 2$^{nd}$ and 3$^{rd}$ instar nymphs. Before dissection, the bug feces were examined (85.0% of the total sample) using microscopy. The parasite infection rate was 71.7% in Andean specimens while no positive insect was found among the 17 specimens from Chaco (GC ecoregion). The accordance between microscopic observation and MMPCR was 82%, with 93.1% of positive MMPCR when mo was positive and 17.5% when mo was negative. The identification of the three DTUs was assessed by determining the molecular weight of the MMPCR products for each sample (Table 1, Figure 2). The results showed that the large majority (98.3%) of the 234 wild *T. infestans* specimens were infected by TcI (PCR products of 200 bp). Only one sample from an adult *T. infestans* ("dark morph" type) collected at site 32 (GC ecoregion) gave a 250 bp MMPCR product corresponding to TcII, TcV, or TcVI. Three other samples (sites 23, 25, and 29) gave a 150 bp MMPCR products corresponding to TcIII or TcIV. The MMPCR product of the specimen of the latter group captured at site 29 was sequenced and the DNA fragment (64 bp) matched the TcIII reference stock named M5631 (accession No AF050521.1 and AY367126.1, 98% identity).

Sequence variability of the *Gpi* gene from *T. cruzi* infecting wild *T. infestans*

A partial sequence of the *Gpi* obtained from 15 samples (13 from the set corresponding to TcI, one from the set corresponding to TcII, TcV, or TcVI, and one from the set corresponding to either TcIII or TcIV) were sequenced in order to explore the variability within TcI and to discriminate the DTUs within the other sets. The 458 bp partial sequences (starting at site 691 and ending at site 1148 of the entire CL Brener stock gene, accession no. XM815802.1) were aligned with the sequences corresponding to *T. cruzi* reference stocks belonging to the six DTUs previously deposited in GenBank (Table 2). With no ambiguity, each sequence under study had been attributed to a DTU. Within TcI, 3 sequences were observed: the most frequent (11 stocks) presented 100% identity with the two identical sequences from TcI reference stocks (OPS21 and P/209) deposited in GenBank;

See Table 2 for DTUs information.

![Figure 2. Illustrating electrophoresis of MMPCR products. Ez-vision stained 3% agarose gel containing MMPCR products obtained from DNA extracts of reference strains and current digestive tracts of *T. infestans*. Lane 1, sample Tor05; lane 2, PCR negative sample; lane 3, the molecular weight marker Smart Ladder (Eurogentec, Angers, France); lane 4–6, reference strains (M6241c6, P209ct1 and MNc12 respectively); lane 7–8, sample Char09 (two independent PCR); lane 9, sample Lur112. See Table 2 for DTUs information. doi:10.1371/journal.pntd.0001650.g002](image)
Table 2. Variable sites of glucose phosphate isomerase gene of *T. cruzi* identified in wild *T. infestans* compared with reference stocks.

| Name       | Accession no. | DTU | No. of current stock | Country | Area | Nucleotide position |
|------------|---------------|-----|----------------------|---------|------|---------------------|
| OPS21      | AY484472      | Tcl |                      | Venezuela|      | A A A T T T G T G A G C C A G T C G C T |
| P/209cl1   | AY484473      | Tcl |                      | Bolivia |      |                     |
| Aiq02C     | JN653335      | Tcl | 1                    | Bolivia |      |                     |
| Lur 112    | JN653324-34   | Tcl | 11                   | Bolivia |      |                     |
| Vis01c     | JN653336      | Tcl | 1                    | Bolivia |      |                     |
| Tu18c2     | AY484477      | Tcl |                      | Brazil  |      |                     |
| CB8c3      | AY484476      | Tcl |                      | Chile   |      |                     |
| Char09     | JN653338      | Tcl | 1                    | Bolivia | NA   |                     |
| M6241c6    | AY484478      | Tcl | III                  | Brazil  |      |                     |
| X110/8     | AY484479      | Tcl | III                  | Paraguay|      |                     |
| Tor05C     | JN653337      | Tcl | 1                    | Bolivia |      |                     |
| Canllc11   | AY484474      | Tcl | V                    | Brazil  |      |                     |
| EP272      | AY484475      | Tcl |                      | Colombia|      |                     |
| Mnc2       | AY484480      | Tcl |                      | Chile   |      |                     |
| Bug2148c11 | AY484481      | Tcl |                      | Brazil  |      |                     |
| CllBerner  | AY484482      | Tcl |                      | Brazil  |      |                     |
| TulaCl2    | AY484483      | Tcl |                      | Chile   |      |                     |

### Notes:
- **DTU**: Discrete Typing Unit;
- **A**: for Andean, **NA**: for Non-Andean (lowland);
- **Samples under study**;
- **Ten other samples had identical sequence, they were from Northern Andean area.**

**doi**: 10.1371/journal.pntd.0001650.t002
the two other sequences exhibited a single mutation and the Vis01 stock identified in a triatomine bug captured at site 27, presented a heterozygous pattern at nucleotide position 940. The sequence of the Char09 of the second set (corresponding to TcII, TcV, or TcVI), detected in a “dark morph” (site 32), presented 100% identity with two identical TcII reference stocks (Tu18d2 and CBBc13). For the sample of the last set corresponding to either TcIII or TcIV (Tor05 from site 25), the sequence presented 100% identity with two identical TcIII reference stocks (M6241d6 and X110/8).

Discussion

Recently, an active search for new foci of wild *T. infestans* in Bolivia enabled us to show that their distribution was broader than initially described [21,44]. Also, few data on the genetic characterization of *T. cruzi* stocks infecting these vector populations were available, apart from the work by Dujardin et al [35], conducted using multilocus enzyme electrophoresis, and the detection of the only TcI at Cotapachi 15 km west of Cochabamba city (Andean area) [25]. In the present context, where wild *T. infestans* highly infected can enter houses and recolonize them after domestic populations have been eliminated by insecticide spraying, it is important to know which *T. cruzi* DTUs are carried by the vectors. In this study, 234 *T. cruzi* stocks isolated from wild *T. infestans* were characterized by MMPCR. The vectors came from several areas mainly situated in two ecoregions in Bolivia, the Inter-Andean Dry Forest and the Gran Chaco where the “dark morph” was found. Regarding the detection of parasites in bugs, the correlation between detection of parasites in bugs, the correlation between detection of *T. infestans* and those infected by *T. cruzi* is very low in the domestic cycle than in sylvatic cycles. Selection of specific DTUs by hosts, considering that host diversity (human migration, triatomine transports) and by the selection of specific DTUs circulating in sylvatic cycles where the geographical distribution of the DTUs is skewed by passive transport of infective bugs and that the vectors came from several areas mainly situated in two ecoregions in Bolivia, the Inter-Andean Dry Forest and the Gran Chaco where the “dark morph” was found. Regarding the detection of parasites in bugs, the correlation between detection of infection by microscopy (mo) and by the method of MMPCR was high (82%). However, some infected bugs (mo positive) were MMPCR negative probably due to the presence of inhibitors factors of the polymerase in the DNA extracts. At the contrary, several samples mo negatives were MMPCR positive, which allowed us to detect and identify few strains in dark morph specimens. In the overall sample, the TcI DTU is widely dominant, but in the Andean and intermediate areas TcIII stocks were detected. In the lowlands, only TcI and TcII were characterized in the “dark morph” specimens. Interestingly, the DTU distribution in wild *T. infestans* is very different from that reported in domestic *T. infestans* collected before the vector control campaigns undertaken on a large scale in Bolivia since 2003; the frequencies of TcI only, TcV only, and mixed infections (TcI and TcV) were 38.6%, 16.8% and 32.7% respectively [31]. At the same time, TcV was mostly detected in patients during the chronic phase of the infection while both TcI and TcV were detected in younger patients with early infection [28,45]. As for the vectors, it was suggested that the domiciliation of *T. infestans* had taken place in high Andean valleys and that the dispersal of domestic *T. infestans* to other areas had occurred by human transport [46,47]. The current observations do not fit these hypotheses, since the only TcI (and to a lesser extent TcIII) would then have been introduced into domestic cycles but not TcV, unless it is assumed that TcV disappeared from the wild *T. infestans* cycle in the Andes valleys after its domiciliation.

Among the six *T. cruzi* DTUs, TcV and TcVI are composed of stocks that appear to be recent hybrids between TcII and TcIII [19]. Consequently, it is tempting to speculate that they might have arisen in an area where the putative parental DTUs coexist. Moreover, this hybridization event is still considered to have occurred much earlier than human colonization in South America [48]. Consequently, parental and hybrid DTUs are likely to coexist in the sylvatic cycle in a putative geographical area in South America. Lately, the Andean origin of *T. infestans* was challenged by the hypothesis of Chaquean origin [26,44,49,50]. If parental or hybrid DTUs are not found in the sylvatic cycle in the Andes, an alternative might be the Gran Chaco region. These is no information regarding the genetic characterization of *T. cruzi* in the sylvatic cycle at the Bolivian lowlands, except for a report of a TcVI stock isolated from a *Dileptus marcapacis* specimen captured on the Amazon slope [51]. In the Paraguayan Chaco, TcII, TcIII and TcV have been identified in different wild mammal species [52] and in the Argentinean Chaco TcI was identified in *Dileptus albiventris* and TcVI in one *Cunupia chinga* [53]. In spite of fairly scarce data, the hypothesis that hybrid DTUs may have originated in Chaco should be considered, especially considering the detection of all DTUs except for TcIV in the domestic cycle in the Bolivian Gran Chaco (unpublished data). The search for DTUs circulating in sylvatic cycles will provide more valid information on the evolution of *T. cruzi* than studies conducted in domestic cycles where the geographical distribution of the DTUs is skewed by passive transport of parasites (human migration, triatomine transports) and by the selection of specific DTUs by hosts, considering that host diversity is lower in the domestic cycle than in sylvatic cycles.

Acknowledgments

We are particularly grateful for the direction of the Inlasa (Instituto de Laboratorios de Salud, La Paz, Bolivia), and Dr. José Raphael Gutiérrez and Dr. Walter Agreda for hosting this work in the Department of Entomology, directed by Dr. Tamara Chavez.

Author Contributions

Conceived and designed the experiments: SFB CB FN. Performed the experiments: CA. Analyzed the data: SFB CA CB. Contributed reagents/materials/analysis tools: SFB CA EW RB RS FN. Wrote the paper: SFB CA CB MT FN.

References

1. Schmunis GA, Yadon ZE (2010) Chagas disease: a Latin American health problem becoming a world health problem. Acta Trop 113: 14–21.
2. Hotez PJ, Bottazzi ME, Franco-Paredes C, Ault SK, Perigo MR (2008) The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. Plos Negl Trop Dis 2: e300.
3. WHO (2007) World Health Organization Global health atlas. Available: http://www.who.int/globatlas/. Accessed 2 March 2008.
4. Noireau F, Dujardin JP (2010) Biology of Triatomiinae. In: Telleria J, Tibayrenc M, eds. American Trypanosomiasis Chagas disease One hundred years of Research. London: Elsevier; pp 149–164.
5. Rossono MF, Barnabe C, Sierra MJ, Kengue P, Guerrero S, et al. (2009) Wild ecotopes and food habits of Triatoma longipennis infected by *Trypanosoma cruzi* lineages I and II in Mexico. Am J Trop Med Hyg 80: 988–991.
6. Salvatella R, Calegari I, Puime A, Basamaja Y, Rosa R, et al. (1994) Feeding pattern of Triatoma rubrovaria (Blanchard, 1843) (Hemiptera, Triatominae) in peridomestic habitats, of a rural area of Uruguay. Rev Inst Med Trop Sao Paulo 36: 311–320.
7. Salvatella R, Rosa R, Basamaja Y, Puime A, Calegari I, et al. (1995) Ecology of *Trypanosoma cruzi* (Hemiptera, Triatominae) in wild and peridomestic environments of Uruguay. Mem Inst Oswaldo Cruz 90: 295–298.
8. Freitas SP, Loeser ES, Rodrigues DC, Freitas AL, Gonçalves TC (2005) Feeding patterns of *Triatoma paulistana* in the state of Ceará, Brazil. Rev Saude Publica 39: 27–32.
9. Tibayrenc M, Ward P, Moya A, Ayala FJ (1986) Natural populations of *Trypanosoma cruzi*, the agent of Chagas disease, have a complex multiconidial structure. Proc Natl Acad Sci USA 83: 115–119.
10. Tibayrenc M, Barnabe C, Telleria J (2010) Reticulate Evolution in *Trypanosoma cruzi*: Medical and Epidemiological Implications. In: Telleria J, Tibayrenc M,
eds. American trypanosomiasis Chagas disease One hundred years of research. Burlington: Elsevier. pp 475–488.

11. Tibayrenc M (1998) Integrated genetic epidemiology of infectious diseases: the Chagas model. Mem Inst Oswaldo Cruz 93: 577–580.

12. Barnabe C, Brisse S, Tibayrenc M (2000) Population structure and genetic typing of Trypanosoma cruzi, the agent of Chagas disease: a multilocus enzyme electrophoresis approach. Parasitolology 120: 513–526.

13. Brisse S, Barnabe C, Tibayrenc M (2000) Identification of six Trypanosoma cruzi phylogenetic lineages by random amplified polymorphic DNA and multilocus enzyme electrophoresis. Int J Parasitol 30: 35–44.

14. Zingales B, Andrade SG, Briones MR, Campbell DA, Chiari E, et al. (2009) A new consensus for Trypanosoma cruzi intraspecific nomenclature; second revision meeting recommending Tc1 to TcVI. Mem Inst Oswaldo Cruz 104: 1051–1054.

15. Llewellyn MS, Miles MA, Carrasco HJ, Lewis MD, Yeo M, et al. (2009) Genome-scale multilocus microsatellite typing of Trypanosoma cruzi discrete typing unit I reveals phylogenetic structure and specific genotypes linked to human infection. Plos Pathog 5: e1000410.

16. Lewicka K, Breniere SF, Barnabe C, Dedet JP, Tibayrenc M (1995) An isoenzyme survey of Trypanosoma cruzi genetic variability in sylvatic cycles from French Guiana. Exp Parasitol 81: 20–28.

17. Cura CI, Mejia-Jaramillo AM, Duffy T, Burgos JM, Rodrigues M, et al. (2010) Trypanosoma cruzi I genotypes in different geographical regions and transmission cycles based on a microsatellite motif of the intergenic spacer of spliced leader genes. Int J Parasitol 40: 1599–1607.

18. Herrera C, Guld F, Falla A, Fajardo A, Montilla M, et al. (2009) Genetic Variability and Phylogenetic Relationships within Trypanosoma cruzi I Isolated in Colombia Based on Miniexon Gene Sequences. J parasit Res pii 09736459.

19. Bourin T, Harrie F, Tibayrenc M, Oury B, Barnabe C (2006) Phylogenetic analysis of the glucose-6-phosphate isomerase gene in Trypanosoma cruzi. Exp Parasitol 113: 1–7.

20. Westenberger SJ, Barnabe C, Campbell DA, Sturm NR (2005) Two hybridization events define the population structure of Trypanosoma cruzi. Genetics 171: 527–543.

21. Buitrago R, Waleckx E, Bosseno MF, Zoveta F, Vidaurri P, et al. (2010) First report of widespread wild populations of Triatoma infestans (Reduviidae, Triatominae) in the valleys of La Paz, Bolivia. Am J Trop Med Hyg 82: 574–579.

22. Noireau F, Cortez MG, Monteiro FA, Jansen AM, Torrico F (2005) Can wild Triatoma infestans foci in Bolivia jeopardize Chagas disease control efforts? Trends Parasitol 21: 7–10.

23. Noireau F (2009) Wild Triatoma infestans, a potential threat that needs to be monitored. Mem Inst Oswaldo Cruz 104 Suppl 1: 69–64.

24. Cortez MR, Empereur L, Piccalini RV, Gurtler RE, Torrico F, et al. (2007) Sylvatic Trypanosoma cruzi (Reduviidae, Triatominae) in the Andean valleys of Bolivia. Acta Trop 102: 47–54.

25. Cortez MR, Pihno AP, Cuervo P, Alfaro F, Solano M, et al. (2006) Trypanosoma cruzi (Kinetoplastida: Trypanosomatidae): ecology of the transmission cycle in the wild environment of the Andean valley of Cochabamba, Bolivia. Exp Parasitol 114: 305–313.

26. Noireau F, Flores R, Gurtler RE, Vargas F (1999) Trapping sylvatic Triatominae (Hemiptera: Reduviidae) in hollow trees. Trans R Soc Trop Med Hyg 93: 13–14.

27. Llewellyn MS, Lewis MD, Acosta N, Yeo M, Carrasco HJ, et al. (2009) Trypanosoma cruzi Ic: Phylogenetic and Phylogeographic Insights from Sequence and Microsatellite Analysis and Potential Impact on Emergent Chagas Disease. Plos Negl Trop Dis 3: e510.

28. Noireau F, Flores R, Vargas F (1999) Trapping sylvatic Triatominae (Reduviidae) in hollow trees. Trans R Soc Trop Med Hyg 93: 13–14.

29. Bent L, Wygodzinsky P (1979) Revision of the Triatominae (Hemiptera, Reduviidae), and significance as vectors of Chagas’ disease. Bull Am Museum Nat Hist 163: 125–520.

30. Busch PL, Beck SG, Gerckmann B, Carretero A (2008) La Diversidad biologica. In: Busch PL, M´trida G, eds. Biodiversidad: la riqueza de Bolivia Estado de conocimiento y conservacion. Santa Cruz de la Sierra: FAN Bolivia.

31. Hall TA (1999) BioEd: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

32. Waleckx E, Salas R, Huaman N, Buitrago R, Bosseno MF, et al. (2011) New insights on the Chagas disease main vector Triatoma infestans (Reduviidae, Triatominae) brought by the genetic analysis of Bolivian sylvatic populations. Infect Genet Evol 11: 1045–1057.

33. Breniere SF, Bosseno MF, Telleria J, Barnabe C, Yasuk N, et al. (1998) Different behavior of two Trypanosoma cruzi major clones: transmission and circulation in young Bolivian patients. Exp Parasitol 89: 285–295.

34. Schofield CJ (1988) Biosystematics of the Triatominae.: In: Service MW, ed. American trypanosomiasis Chagas disease One hundred years of research. Washington DC: American Association for the Advancement of Science. pp 294–312.

35. Cortez MR, Monteiro FA, Noireau F (2010) New insights on the spread of Triatoma infestans from Bolivia—implications for Chagas disease emergence in the southern cone. Infect Genet Evol 10: 350–353.

36. Machado GA, Ayala EF (2001) Nucleotide sequences provide evidence of genetic exchange among distantly related lineages of Trypanosoma cruzi. Proc Natl Acad Sci USA 98: 7396–7401.

37. Ceballos LA, Piccalini RV, Berkyun I, Kitzon U, Gurtler RE (2009) First insights of melanic sylvatic Triatoma infestans (Hemiptera: Reduviidae) colonies in the Argentine Chaco. J Med Entomol 46: 1195–1202.

38. Quisberth S, Waleckx E, Monje M, Chang B, Noireau F, et al. (2011) “Andean” and “non-Andean” TTS-2 and mstGB haplotypes of Triatoma infestans are observed in the Gran Chaco (Bolivia) population genetics and the origin of reinfection. Infect Genet Evol 11: 1006–1014.

39. Valette E, Breniere SF, Le Pont F, Desjeux P (1988) Zymodemes of Trypanosoma cruzi isolated from wild mammals in Bolivia. Mem Inst Oswaldo Cruz 83: 119–140.

40. Yeo M, Acosta N, Llewellyn S, Sanchez H, Adamson S, et al. (2005) 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 35: 223–233.

41. Cardenal MV, Lauricella MA, Ceballos LA, Lanati L, Marrellt PL, et al. (2008) Molecular epidemiology of domestic and sylvatic Trypanosoma cruzi infection in rural northwestern Argentina. Int J Parasitol 38: 1333–1343.