Infection, Genetics and Evolution 22 (2014) 12–22

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

The role of sigmodontine rodents as sylvatic hosts of *Trypanosoma cruzi* in the Argentinean Chaco

M. Marcela Orozco [a,b], Romina V. Piccinali [a,b], Matías S. Mora [c], Gustavo F. Enriquez [a,b], M. Victoria Cardinal [a,b], Ricardo E. Gürtler [a,b,*]

[a] Laboratorio de Eco-Epidemiología, Departamento de Ecología, Genética y Evolución, Universidad de Buenos Aires, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina
[b] Instituto de Ecología, Genética y Evolución (IEGEBA), CONICET, Argentina
[c] Instituto de Investigaciones Marinas y Costeras (IIMyC, CONICET), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, 7600, Mar del Plata, Argentina

ARTICLE INFO

Article history:
Received 21 August 2013
Received in revised form 18 December 2013
Accepted 21 December 2013
Available online 3 January 2014

Keywords:
Trypanosoma cruzi
Rodents
Sigmodontinae
Molecular identification
Molecular diagnosis
Reservoir competence

ABSTRACT

The role of rodents in the sylvatic transmission of *Trypanosoma cruzi* has seldom been investigated using parasitological and molecular methods. We assessed the occurrence of *T. cruzi* in wild small rodents from Pampa del Indio, in the Argentinean Chaco, and identified the taxonomic status of positive rodents by sequencing a fragment of cytochrome b gene (cytb) and performing BLAST searches and phylogenetic analyses. A total of 176 Sigmodontinae rodents was captured in six surveys using 5425 trap-nights in a wide range of sylvatic habitats between 2009 and 2011. Host infection was determined by xenodiagnosis and by polymerase chain reaction amplification of the hyper-variable region of kinetoplast DNA minicircles of *T. cruzi* (kDNA-PCR) from blood samples. None of the 176 rodents examined was xenodiagnosis-positive. The prevalence of infection determined by kDNA-PCR from blood samples was 16.2% (95% confidence interval, 10.1–21.9%). Half of the infections detected by kDNA-PCR were confirmed by nuclear satellite DNA-PCR or by kDNA-PCR of the rectal contents of xenodiagnostic bugs. The 24 positive specimens were assigned to eight species, providing the first records of *T. cruzi* in *Akodon montensis*, *Akodon toba*, *Graomys chacoensis*, and *Oligoryzomys chacoensis*. The occurrence of *T. cruzi* infection in *Oligoryzomys nigripes*, *Calomys callosus*, *Necromys lasiurus* and *Oecomys* sp. (most probably *Oecomys mamorae*) from the Gran Chaco is also reported for the first time. Although sigmodontine rodents were frequently infected, the intensity of bug rectal infection with *T. cruzi* was below the detection limit of xenodiagnosis (subpatent infectiousness to bugs), indicating they had a low reservoir host competence.

1. Introduction

Chagas disease is a zoonosis caused by *Trypanosoma cruzi*, a kinetoplastid protozoan with a broad geographic range extending from Southern USA to Southern South America (Gaunt and Miles, 2000). The transmission of *T. cruzi* occurs in a great variety of domestic and sylvatic habitats. The sylvatic cycle includes numerous species of triatomine bugs and at least 180 species of wild mammals, some of which can act as reservoir hosts in different ecological regions whereas other host species may play secondary or dead-end roles (World Health Organization, 2002; Chaves et al., 2007). Wild and synanthropic rodent species were implicated in the transmission of *T. cruzi* in several regions (Noireau et al., 2009; Rozas et al., 2007). However, the role of sylvatic rodents in the transmission cycles of *T. cruzi* has rarely been investigated (Ramsey et al., 2012; Rojas Cortez et al., 2006).

Rodentia includes almost half of all mammalian extant species (Wilson and Reeder, 2005). Rodents usually live in close association with humans and have an important role in the transmission of infectious diseases, acting as sources, hosts or reservoirs of multiple pathogens (Kruse et al., 2004). In the Gran Chaco ecoregion (The Nature Conservancy, 2005), there is a large diversity and abundance of sigmodontine (Cricetidae: Sigmodontinae) rodents, one of the most diversified and complex groups of New World mammals, which includes 84 endemic genera (D'Elía et al., 2007). Sigmodontine rodents are frequently found around or inside human dwellings in close proximity to domestic animals, a characteristic that makes them potential hosts of *T. cruzi* (Rademaker et al., 2009). Additionally, small rodents may also be involved in the transmission of trypanosomes by the oral route because they are prey of many carnivorous species (Herrera et al., 2007).

The occurrence of *T. cruzi* infection in synanthropic rodents has received much more attention than in wild rodents (Noireau et al., 2009; Jansen and Roque, 2010). *T. cruzi* has occasionally been

* Corresponding author at: Laboratorio de Eco-Epidemiología, Departamento de Ecología, Genética y Evolución, Universidad de Buenos Aires, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina. Tel./fax: +54 11 4576 3318.
E-mail address: gurtler@ege.fcen.uba.ar (R.E. Gürtler).

1567-1348/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2013.12.020

identified in various species of Sigmodontinae (*Calomys musculinus*, *Calomys laucha* and *Akodon dolores*) from the Chaco and Espinal ecoregions in Argentina (Basso et al., 1977, 1982; Moretti et al., 1980). More recently, the use of the polymerase chain reaction amplification of the hyper-variable region of kinetoplast DNA minicircles (kDNA-PCR) detected *T. cruzi* in *C. musculinus*, *Graomys griseoflavus*, *Phyllotis darwini* and *Akodon molinae* in mid-western Argentina (Brigada et al., 2010). None of the 151 rodents captured in the Dry Chaco of Santiago del Estero was xenodiagnosis-positive (Ceballos et al., 2006), but one *Graomys centralis* and five unidentified small rodents were *T. cruzi*-seropositive by two methods (Leonardo A. Ceballos et al., unpublished data). In the Humid Chaco, all sigmodontine specimens from various genera were negative by xenodiagnosis in Tres Estacas (Diosque et al., 2004), and by xenodiagnosis and kDNA-PCR from blood samples in Pampa del Indio (Alvarado-Otegui et al., 2012). Subsequent surveys conducted in Pampa del Indio found various species of armadillos and marsupials infected with *T. cruzi*, and two rodent specimens positive by two different kDNA-PCR assays (Orozco et al., 2013). Unfortunately, the taxonomic status of the two infected rodents was unknown (see below).

Here we pursued two complementary goals: (1) to assess the occurrence of *T. cruzi* infection and infectiousness to the vector in a larger sample of wild rodents captured in a wider range of environments (i.e., protected and disturbed areas, at ground level and on tree branches) in Pampa del Indio, and (2) to identify the species of *T. cruzi*-positive rodents using the mitochondrial cytochrome b (cytb). We choose to use DNA sequences for species identification because they provide a large amount of data for phylogenetic analysis at the expense of relatively little effort, and a small amount of tissue may provide a high yield of good-quality DNA. This methodology has high reproducibility, and offers the advantage of not needing to kill or remove specimens from the study area. This is especially important when species identification is uncertain and fieldwork is conducted in protected areas from regions where the rodent fauna is rich, as in the present study.

2. Materials and methods

2.1. Study area

Field studies were conducted in the municipality of Pampa del Indio (25° 55′S 56° 58′W), Chaco Province, Argentina, which has been described elsewhere (Gurevitz et al., 2011; Orozco et al., 2013) (Fig. 1). Samples for this study were taken from six wild mammal surveys conducted in a well-defined rural area (45000 ha) and in a neighboring protected area (Pampa del Indio Provincial Park, 8633 ha) in July, August and November 2009, July and August 2010, and March 2011 (i.e., includes winter, spring and late summer).

2.2. Rodent sampling and handling

Rodents were live-captured in forest and grassland habitats using pitfall and Sherman traps whose individual location was georeferenced (Garmin Legend C) as described in Orozco et al. (2013). Sherman traps were located both at ground level and on tree branches (1–2.5 m high), and were baited with seeds, fresh and dry fruits, pellets of peanut butter, oatmeal and vanilla essence. Pitfall traps were set up along transect lines in stations deployed every 10 m. All traps were checked and re-baited every morning.

Biosafety and animal processing procedures were performed according to protocols approved by the Argentinean "Dr. Carlos Barclay" Ethical Committee. Transit permits were obtained from the provincial government. The captured animals were anesthetized using Isoflurane® delivered with a vaporizer (IsoTec®, Datex-Ohmeda GE Healthcare), and achieved by using 2–3% isoflurane in medicinal oxygen (0.25–3 lt/min), administered in an anesthetic chamber and then through small home-made masks. Medicinal O_2 (0.25–3 lt/min) was used during induction and maintenance of inhalatory anesthesia; our experience indicates that this procedure does not affect the survival or normal performance of individuals. All animals were sexed and weighed with a spring scale (Pesola®, Switzerland). Tissue samples were taken by ear punch and were stored in 1.5 ml tubes with 0.5 ml of 1% phosphate buffered saline at −20 °C. Blood samples were drawn by venipuncture (0.03–0.4 ml, depending on the rodent's weight), and diluted 1:1 in guanidine buffer for PCR. All rodents were examined by xenodiagnosis using five laboratory-reared, uninfected fourth-instar nymphs of *Triatoma infestans* contained in wooden boxes applied on the host's belly during 25 min and checked visually whether they had engorged to a large extent (Ceballos et al., 2006). All animals were released at the capture site after full recovery.

2.3. Diagnosis of T. cruzi

As a first step when examining the xenodiagnostic bugs of an individual host, the rectal contents from two bugs were diluted with physiological saline solution and examined individually at 400× magnification (Zeiss) 30 days after exposure to the host. If negative, the rectal contents from the rest of the insects were pooled and examined. All negative bugs were re-examined 60 days after initial exposure (i.e., classic xenodiagnosis).

Parasite DNA was extracted from blood samples mixed with Guanidine hydrochloride–EDTA buffer (GEB) and boiled for 15 min using the DNeasy Blood & Tissue Kit and manufacturer's instructions (QIAGEN Sciences, Germantown, MD, USA). Samples were examined initially by polymerase chain reaction amplification of the 330-basepair (bp) fragment from the kinetoplast DNA minicircles of *T. cruzi* (kDNA-PCR) using primers and cycling conditions described (Burgos et al., 2007). Positive samples were examined by nuclear satellite DNA-PCR (Sat-DNA-PCR). Satellite hot-start PCR was performed using a mix composed of 1× Taq Platinum amplification buffer, 0.25 mM deoxynucleotide triphosphate solution (dNTPs), 3 mM $MgCl_2$ solution, 0.6 U Taq Platinum (Invitrogen, Brazil), 0.5 mM Sat-DNA specific primers cruzi 1 (5′-ASTCGGCTGA TCGTTTTCGA-3′) and cruzi 2 (5′-AATTCCTCCAAGCAGCGGATA-3′) (Piron et al., 2007), 5 μl of DNA sample and a quantity of water sufficient to give a final volume of 30 μl. Cycling parameters were one step of 5 min at 94 °C; 40 cycles of 1 min at 94 °C, 30 s at 68 °C and 1 min at 72 °C, and one final extension step of 10 min at 72 °C. 166 bp Sat-DNA-PCR products were analyzed in 3% agarose gels (Invitrogen, Carlsbad, CA, USA) stained with Gel Red (Biotium, Hayward, CA, USA).

In samples positive by kDNA-PCR and negative by Sat-DNA-PCR, DNA from the rectal contents of all xenodiagnostic bugs was extracted with DNAzol (Invitrogen, Carlsbad, CA, USA) and subsequently tested by kDNA-PCR as previously described (Maffey et al., 2012; Orozco et al., 2013). All xenodiagnostic bugs had previously been negative by microscopical examination at 400×. Subpatent infectiousness to bugs stratified by rodent species was calculated as the percentage of kDNA-PCR positive *T. infestans* divided by the total number of insects examined for infection by kDNA-PCR.

2.4. Identification of rodent species

DNA from the ear tissues of *T. cruzi*-positive rodents was extracted using a protocol that included sodium-dodecyl-sulfate

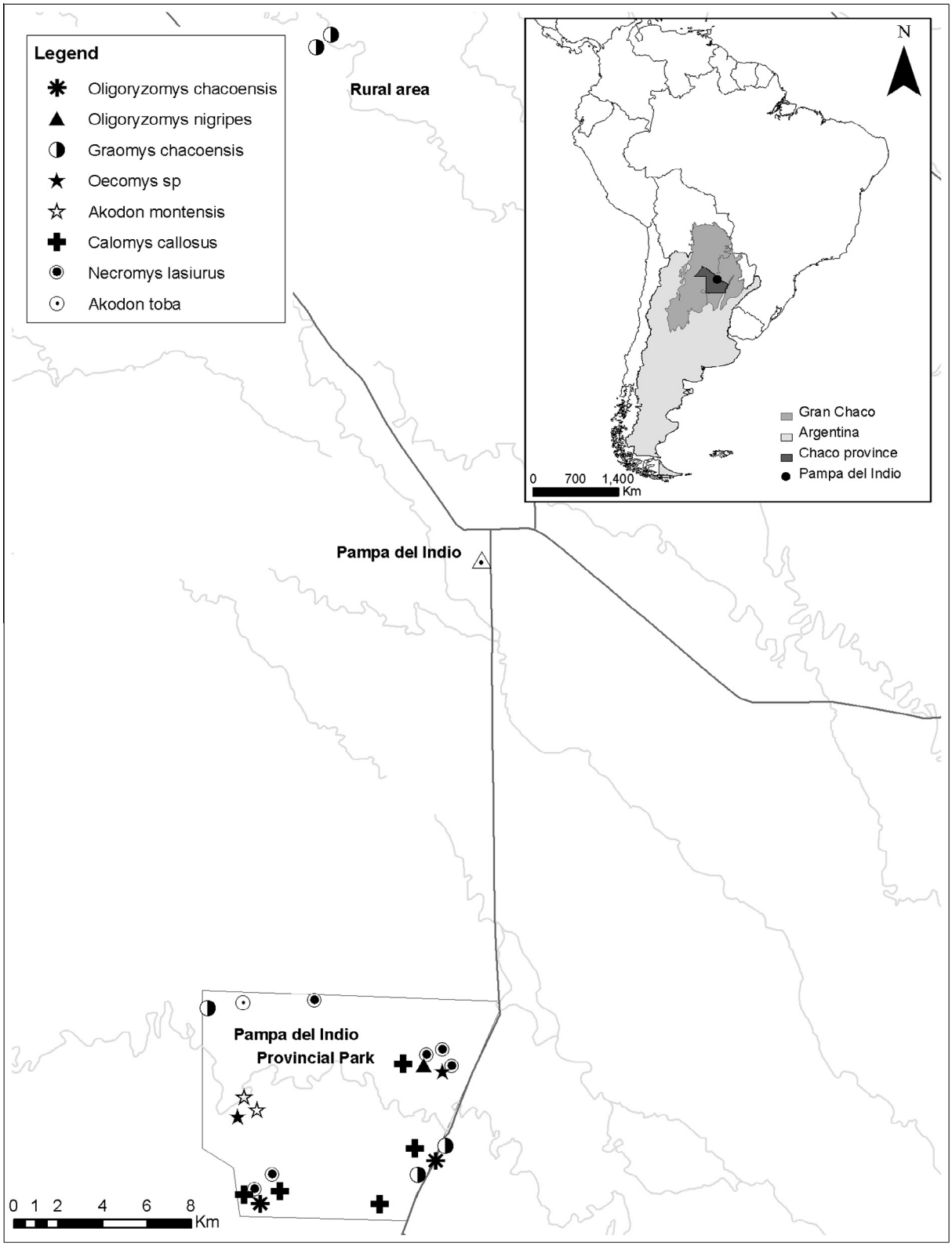

Fig. 1. Geographic location of *T. cruzi*-infected rodents in Pampa del Indio, Argentina. Infected species are represented with different symbols.

(SDS), proteinase K, NaCl and isopropanol (modified from Miller et al., 1988). An 801-bp fragment of the mitochondrial cytochrome b gene (cytb) was amplified by PCR using the primers MVZ05 (5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′) and MVZ16 (5′-AAA TAGGAARTATCAYTCTGGTTTRAT-3′; Smith and Patton, 1993). This widely used gene was useful to resolve phylogenetic relationships in Sigmodontinae (D'Elía, 2003; Steppan et al., 2007).

Reactions were performed in a final volume of 50 μl containing 8 μl of dNTPs (1.25 mM each), 10 μl of 5× reaction buffer, 3 μl of $MgCl_2$ (25 mM), 0.2 μl of GoTaq DNA polymerase (Promega,

Madison, WI, USA) 5 U/μl, 100 ng of sense and antisense primers, and 50–100 ng of DNA. Amplifications were carried out in a BIO-RAD thermal cycler (BIO-RAD, Hercules, CA, USA) with the protocol described by González-Ittig et al. (2007) at an annealing temperature of 41 °C. The amplified samples were run on a 1% agarose gel and purified with Qiaquick Gel Extraction kit (Qiagen, Germantown, MD, USA). The purified PCR products were used as a template for direct sequencing of both strands using the same primers as in the amplification. Sequences were obtained in Macrogen (Inc., Seoul, Korea) in an ABI PRISM 3730 DNA Analyzer automated sequencer (Applied Biosystems, Foster City, CA), and aligned manually or using MEGA 5.1 software (Tamura et al., 2011). Newly reported sequences are available at GenBank under accession numbers KF207841–KF207864.

Pampa del Indio rodent sequences were used as queries to find highly similar cytb sequences deposited in GenBank with BLAST (Altschul et al., 1990), using the option nucleotide blast (blastn) and the megablast algorithm with the default options. Mean Kimura two-parameter (K2P) genetic distances within species obtained by BLAST were estimated using all the records available at GenBank and compared to the mean K2P distances between Pampa del Indio rodent sequences and all the sequences available at Genbank for the same species. All the calculations were made with MEGA 5.1.

We also performed maximum parsimony (MP), maximum likelihood (ML) and Bayesian (BA) phylogenetic analyses including our sequences, GenBank sequences with a high degree of similitude, and other reported sequences of Sigmodontinae from Northern Argentina in order to allow a complete biogeographic representation of sigmodontine species diversity (Ferro and Martinez, 2009; Jayat et al., 2006; Pardiñas and Teta, 2005; Teta et al., 2009). Cytb sequences from *Arvicola terrestris* (GenBank accession number DQ663669) and *Sigmodon hispidus* (GenBank accession number EU293755) were used as out-groups. The GenBank accession numbers of all the ingroup and outgroup sequences are in the Appendix (Table A).

All characters in the MP analysis were regarded as unordered and unweighted. The shortest phylogenetic trees were found with an heuristic search of 3000 replicates of random addition taxa plus the tree bisection-reconnection (TBR) branch swapping algorithm implemented in TNT (Goloboff et al., 2008). Most parsimonious trees were summarized in a strict consensus tree. Statistical support for clades was assessed by non-parametric bootstrap values based on 1000 replicate searches.

The BA phylogenetic tree was obtained with BEAST 1.5.4 (Drummond and Rambaut, 2007), which uses Bayesian Markov Chain Monte Carlo procedures. The program was run for 1×10^7 iterations and sampled every 1000 steps under a relaxed lognormal molecular clock with uniformly distributed priors. This analysis yielded 10000 final trees; 8000 from these trees were discarded as burn-into compute the 50% majority rule consensus tree; inferences were made with the last 2000 trees. To assess the robustness of parameter estimates, four independent chains were run with identical settings. Log-files were analyzed in Tracer 1.4.8 (Rambaut and Drummond, 2009) to evaluate Markov Chain Monte Carlo convergence within chains. The substitution model with more empirical support was estimated in jModelTest (Posada, 2008) using Akaike's Information Criterion. We used the GTR + I + G substitution model with four gamma categories, using a Yule branching rate prior, with rate variation across branches (Drummond and Rambaut, 2007).

The ML tree was found with MEGA 5.1 (Tamura et al., 2011). The program performs simultaneous nearest-neighbor interchanges to improve a reasonable topology of the starting tree. We ran MEGA considering the best substitution model inferred previously by jModelTest (Posada, 2008), where both the transitions-to-transversions ratio and gamma distribution parameters were empirically estimated. Consistency for internal branch and nodes was assessed using the standard bootstrapping method (sample with replacement and 1000 bootstrap replicates) implemented in MEGA. The topology of the phylogenetic tree was taken from BA analysis and was drawn with FigTree 1.4 (Rambaut, 2012).

3. Results

3.1. Infection

None of the 176 rodents examined by classic xenodiagnosis was positive. The overall prevalence of *T. cruzi* infection was 16.2% (95% confidence interval, 10.1–21.9%) among 148 rodents examined by molecular diagnostics. A total of 24 rodents was positive by kDNA-PCR from blood samples; 10 specimens were positive by kDNA-PCR of blood samples and bug rectal contents, and two were additionally positive by Sat-DNA-PCR (Table 1). Of the 24 positive rodents, 19 were captured in winter, and only 4 were caught in spring or summer. The overall prevalence of *T. cruzi* infection was 13.3% in spring-summer (November, March) and 16.9% in winter (July, August). Infection prevalence was fairly constant along the year and there were no statistically significant differences between seasons ($\chi^2 = 0.23$; df: 1; $P = 0.63$).

The overall subpatent infectiousness to bugs of the positive rodents was 16.7% among 72 insects with rectal contents examined by kDNA-PCR which had previously been negative by optical microscopy (Table 1).

3.2. Species identification

All *T. cruzi*-positive rodents were successfully sequenced for cytb. BLAST searches showed high degrees of similitude (100–99%) to DNA sequences of Sigmodontinae from Argentina, Paraguay and Brazil (Table 2). The eight species found positive by at least one of the PCR assays were *Oecomys* sp. (most probably *Oecomys mamorae*; mamore arboreal rice rat), *Oligoryzomys chacoensis* (Chaco pygmy rice rat), *Oligoryzomys nigripes* (black-footed pygmy rice rat), *Necromys lasiurus* (hairy-tailed bolo mouse), *Calomys callosus* (large vesper mouse), *Graomys chacoensis* (Chaco leaf-eared mouse), *Akodon montensis* (montane grass mouse) and *Akodon toba* (Chaco grass mouse).

The K2P genetic distances between the positive rodents and all the Genbank sequences available for the most similar species according to BLAST showed that mean values between samples and Genbank records were similar to mean values between Genbank sequences of the same species (Table 3). Divergence values were different among species and fluctuated between 0.4% and 2.3%.

Phylogenetic analyses were highly consistent among MP, AB and ML trees, recovering the same topology among sequences within each particular clade (Fig. 2). Bayesian inference showed high posterior probabilities for all major clades among different rodent genera. In general, ML reconstruction showed higher values of node support than the MP approach. All Sigmodontinae genera formed well-supported monophyletic groups with the exception of *Holochilus* (marsh rats). All Pampa del Indio rodent sequences were included in strongly supported clades and their identities were in agreement with the BLAST analysis: they clustered with sequences from *C. callosus* (5 individuals), *G. chacoensis* and *G. centralis* (5 individuals), *A. montensis* (2 individuals), *A. toba* (1 individual), *N. lasiurus* and *Necromys temchuki* (6 individuals), *Ol. nigripes* (1 individual), *Ol. chacoensis* (2 individuals), and *Oecomys* sp. (probably *Oe. mamorae*, 2 individuals).

Table 1
Distribution of rodent species infected with *T. cruzi* as determined by kDNA-PCR from blood samples, Sat-DNA-PCR from blood samples, and kDNA-PCR from rectal contents of xenodiagnostic bugs.

Species	No. of rodents positive by			No. of kDNA-positive xenodiagnostic bugs/No. examined (%)[b]
	kDNA-PCR from blood[a]	Sat-DNA-PCR from blood	kDNA-PCR from bug rectal contents	
Akodon montensis	2	0	0	0/9 (0.0)
Akodon toba	1	0	0	0/5 (0.0)
Necromys lasiurus	6	0	1	1/15 (6.7)
Calomys callosus	5	0	2	3/20 (15.0)
Graomys chacoensis	5	0	4	4/15 (26.7)
Oecomys sp.	2	1	1	2/2 (100.0)
Oligoryzomys chacoensis	2	1	1	1/2 (50.0)
Oligoryzomys nigripes	1	0	1	1/4 (25.0)
No. positive/No. examined (%)	24/148 (16.2)	2/24 (8.3)	10/22 (45.4)	12/72 (16.7)

[a] No rodent was positive for *T. cruzi* infection by xenodiagnosis.
[b] Subpatent infectiousness to bugs: calculated as the total number of kDNA-PCR positive *T. infestans* divided by the total number of insects examined for infection by kDNA-PCR. All xenodiagnostic bugs had previously been negative by microscopical examination.

4. Discussion

Our study describes a dramatically greater occurrence of *T. cruzi* in rodents than has previously been realized, albeit with subpatent infectiousness, and documents the first records of *T. cruzi* in *A. montensis*, *A. toba*, *G. chacoensis*, and *Ol. chacoensis*. Infections were also revealed in *Ol. nigripes*, *C. callosus*, *N. lasiurus* and *Oecomys* sp., apparently for the first time in the Gran Chaco. All of these host species are not restricted to a single closely related clade but belong to three different tribes (Phyllotini, Oryzomyini and Akodontini), showing the large capacity of *T. cruzi* to infect different rodent lineages. Compared with previous studies, the wider range of host species recorded may be related both to enhanced parasite detection by means of kDNA-PCR in blood samples and bug rectal contents, and the large catch effort invested in a wide diversity of habitats used by ground- and tree-dwelling rodent species. A major strength of our study is the sizable number of rodent species identified taxonomically using molecular methods and examined for *T. cruzi* infection with parasitological and molecular methods.

The finding of varying fractions of PCR-positive xenodiagnostic bugs gives further support to the fact that the study rodents were actually infected with *T. cruzi* and may eventually transmit it. We recorded three rodent specimens positive by kDNA-PCR that could not be further confirmed by kDNA-PCR of bug rectal contents or Sat-DNA-PCR of blood samples. In a parallel survey conducted in the same study area, xenodiagnosis and kDNA-PCR in domestic dogs and cats showed high levels of co-positivity and co-negativity (Enriquez et al., 2013). These apparently conflictive results can be explained by the existence of substantial differences in the number of copies of the molecular targets (Sat-DNA versus kDNA) per genome among *T. cruzi* genotypes. The Sat-DNA-PCR is expected to be more specific than kDNA-PCR because it amplifies nuclear DNA, whereas the much fewer number (4- to 10-fold less abundant) of satellite copies of the target nuclear gene reduces substantially the sensitivity of Sat-DNA-PCR in *T. cruzi* I (Duffy et al., 2009). Therefore, kDNA loci were recommended as a diagnostic tool for TcI (Schijman et al., 2011). The issue of lower detectability is further compounded by the small amount of blood (0.03–0.4 ml) obtained from the rodents for PCR. Taken together, the evidence suggests that the three rodent specimens that were kDNA-positive only were most likely infected with *T. cruzi*.

It is highly likely that the rodents were infected with *T. cruzi* I, as suggested by the generalized pattern shown in the Appendix (Table B) and findings in areas with shared ecological characteristics (Zingales et al., 2012). TcIV has not been found in the Gran Chaco yet, whereas the sylvatic reservoir hosts of TcII are little known (Zingales et al., 2012), with some scattered findings in Chile (Rozas et al., 2007) and Brazil (Herrera et al., 2007; Marcili et al., 2009). In contrast, TcI has repeatedly been found infecting sigmodontine and murine rodents from several locations in the Gran Chaco and elsewhere. In the Andean valleys of Cochabamba (Bolivia), mini-exon genotyping revealed only *T. cruzi* I in sigmodontine rodents including *Akodon boliviensis*, *Necromys (=Bolomys) lactens* and *Phyllotis osilae* (Rojas Cortez et al., 2006; Llewellyn et al., 2009). TcI was found in *Holochilus brasiliensis* from Paraguay and Brazil (Yeo et al., 2005; Marcili et al., 2009). In Muridae, TcI was also identified in *Rattus* sp. from Venezuela, Bolivia, Brazil, Ecuador and Argentina (Brenière et al., 1998; Yeo et al., 2005; Marcili et al., 2009; Añez et al., 2009; Llewellyn et al., 2009; Ocaña-Mayorga et al., 2010; Tomasini et al., 2011; Herrera et al., 2013). In other rodent families, other DTUs such as TcIII were found in *Thrichomys* sp., *Proechimys iheringi*, *Proechimys longicaudatus*, *Oxymycterus* sp., and *Oryzomys capito* from Brazil (Herrera et al., 2007; Marcili et al., 2009), whereas in Chile four DTUs including TcI were reported in *P. darwini*, *Octodon degus* and *Abrothrix olivaceus* (Rozas et al., 2007).

Sigmodontine rodents had very low reservoir host competence, as indicated by the simultaneous finding of kDNA-PCR positive rodents that had a negative classic xenodiagnosis in which the bugs were kDNA-PCR positive (i.e., subpatent infectiousness to bugs). This means that the intensity of bug rectal infections with *T. cruzi* was below the detection limit of xenodiagnostic tests in which *T. infestans* bugs had engorged substantially and were examined by optical microscopy at 400×. The study rodents most likely had very rare bloodstream trypomastigotes, and therefore the bugs feeding on them developed very low-density infections. A classic controversy about molecular or serological diagnosis arises from the fact that the presence of parasite DNA (or specific antibodies) does not equate to the presence of live parasites, and is not conclusive proof of whether a given species is a reservoir host (Chaves et al., 2007).

The extensive literature search of Sigmodontinae and *T. cruzi* infection in the Appendix (Table B) shows that very few species were found parasitologically positive by xenodiagnosis or hemoculture. Therefore, there are very few estimates of the infectiousness of sylvatic rodents and very few parasite isolates genotyped to DTU level. *C. callosus* showed a low prevalence of *T. cruzi* as detected by xenodiagnosis (1.2–15.4%) in Brazil and Bolivia (Mello, 1982; Noireau et al., 1997; Brenière et al., 1998), whereas *Oe. mamorae* displayed higher infection prevalence by microhematocrit (13%) and hemoculture (18–32%) (Herrera et al., 2007; Rademaker et al., 2009). In general, most sigmodontine species that have been considered infected with *T. cruzi* were found serologically positive and parasitologically negative, suggesting they have very low infectiousness (e.g., *H. brasiliensis*, *Cerradomys scotti*,

Table 2
Molecular identification of sigmodontine rodent species by BLAST searches. The individual samples of *T. cruzi*-positive rodents and the percentage of maximum similitude sequences (MS), accession number, species, locality, and collection vouchers of the most similar GenBank sequences are given.

Individual sample	MS	GenBank accession numbers	Species	Locality	Voucher
171, 252	99	EF531656	*Necromys lasiurus*	Berna, Santá Fé, Argentina	CNP 531
278, 306	99	EF531657	*Necromys lasiurus*	Berna, Santá Fé, Argentina	CNP 532
255	98	EF531655	*Necromys lasiurus*	Bahía Blanca, Buenos Aires, Argentina	CNP 520
300	99	EF531652	*Necromys lasiurus*	Cerro Ventana, Buenos Aires, Argentina	CNP 472
224, 308	99	EU579509	*Oecomys mamorae*	Rio Vermelho, Mato Grosso do Sul, Brazil	JLP 16961
68, 254, 377	99	DQ447282	*Calomys callosus*	Corumbá, Mato Grosso do Sul, Brazil	LBCE 5682
257, 279	100				
281, 282	99	EF101874	*Akodon montensis*	Mananciais da Serra, Paraná, Brazil	LMT 428
330	99	AY273910	*Akodon toba*	Paraguay	TK 66486
293	99	GU185910	*Oligoryzomys nigripes*	Camino Isla Cerrito, Chaco, Argentina	LIF 122
305, 386	99	EU258543	*Oligoryzomys chacoensis*	La Lomita, Boquerón, Paraguay	TK 62932
333, 359	99	FJ573154	*Graomys chacoensis*	Estancia Poguazú, Formosa, Argentina	CML 7540
363	100				
442	100	FJ573153	*Graomys chacoensis*	Route 95 near Riacho Pilaga, Formosa, Argentina	CML 7539
475	99				

Table 3
Pairwise K2P distances between Pampa del Indio rodents and all the Genbank records belonging to the most similar species according to BLAST. Intraspecific distances were calculated using all the records available at Genbank. In brackets: standard deviation.

Rodent ID	Mean K2P distance to the assigned species	Assigned species	Mean intraspecific K2P distance	Number of sequences available at Genbank
68	0.005 (±0.002)	*Callomys callosus*	0.006 (±0.001)	38
254	0.007 (±0.002)			
257	0.004 (±0.001)			
279	0.004 (±0.001)			
377	0.007 (±0.002)			
171	0.023 (±0.004)	*Necromys lasiurus*	0.025 (±0.003)	27
252	0.023 (±0.004)			
278	0.020 (±0.003)			
300	0.020 (±0.003)			
306	0.020 (±0.003)			
255	0.020 (±0.003)			
224	0.013 (±0.003)	*Oecomys mamorae*	0.019 (±0.004)	4
308	0.013 (±0.003)			
305	0.015 (±0.004)	*Oligoryzomys chacoensis*	0.014 (±0.003)	4
386	0.011 (±0.003)			
293	0.012 (±0.003)	*Oligoryzomys nigripes*	0.010 (±0.001)	36
281	0.011 (±0.003)	*Akodon montensis*	0.010 (±0.001)	84
282	0.010 (±0.003)			
330	0.004 (±0.002)	*Akodon toba*	0.005 (±0.002)	3
333	0.012 (±0.004)	*Graomys chacoensis*	0.012 (±0.004)	2
359	0.012 (±0.003)			
363	0.006 (±0.002)			
442	0.007 (0.002)			
475	0.007 (0.003)			

Oxymicterus gr. judex and *Delomys dorsalis*) (Herrera et al., 2007; Vaz et al., 2007).

One limitation of our assessment of rodent host competence is that trapping surveys were not equally balanced through the year, with four of them conducted in winter and only two in spring or late summer. Sigmodontine rodent populations in the region have two annual litters (in late spring and late summer) that lead to peak densities by the end of summer. In contrast, the study rodents were apparently more abundant (or more easily trapped) in the cold season than in warm season when more resources are available. Because most of the catches occurred in early winter (July), most of the specimens examined were adults from the late-summer cohort. If exposure to *T. cruzi* occurs early in life during a restricted time period (e.g., lactation or in the juvenile stage) and the natural course of infection progresses to a chronic phase with very low parasitemia, as in some laboratory mouse strains and experimentally-infected *C. callosus* (Borges et al., 1992; Mello et al., 1979), the infectiousness to the vector of adult rodents may be very low and subpatent. Future studies on the role of rodents as reservoir hosts should seek a more detailed representation of cohorts and age classes over the year.

A. montensis and *A. toba* are here described as hosts of *T. cruzi* for the first time (Roque et al., 2008; Alvarado-Otegui et al., 2012; Ceballos et al., 2006). *A. montensis* (originally named as a subspecies of *Akodon arviculoides* by Cabrera, 1961) frequently occurs in the Atlantic forest and Cerrado. Typical of the Gran Chaco (Jayat et al., 2008), *A. toba* occupies agricultural habitats near domestic environments, pastures and woodlands. Although other species within the genus *Akodon* were found infected with *T. cruzi* in Argentina, Bolivia and Brazil, such as the two specimens of *A. boliviensis* from the Andean valleys of Cochabamba that were infected with TcI (Llewellyn et al., 2009), most surveys did not identify the species status of the rodents examined (see Appendix, Table B).

Our study shows molecular evidence of infection with *T. cruzi* in *G. chacoensis*. Recent analyses based on cytogenetics, molecular markers and geometric morphometry of the skull suggest the existence of only one *Graomys* species (*G. chacoensis*) in the Chaco eco-region, with *G. centralis* and *Graomys medium* taken as junior synonyms (Ferro and Martinez, 2009; Martínez and Di Cola, 2011). A longitudinal study in the Dry Argentinean Chaco reported the finding of one specimen of *G. centralis* (i.e., *G. chacoensis*) serologically

Fig. 2. Majority rule consensus phylogenetic tree resulting from Bayesian inference analysis of cytochrome-b gene sequences recovered from specimens of Sigmodontinae rodents. Numbers indicate support from posterior probability (from 0 to 1), maximum-likelihood bootstrap (%), and maximum parsimony bootstrap (%), respectively. Posterior probabilities lower than 0.5, or values of node supports lower than 50% for parsimony and likelihood analyses are not shown. Pampa del Indio rodents are given in bold. The Bayesian phylogram was modified with the option "equal" of FigTree 1.4 for a better visualization of the clades.

positive for *T. cruzi* by two methods and negative by xenodiagnosis (Leonardo A. Ceballos et al., unpublished data). In addition, *T. cruzi* was found in the bloodstream of *G. griseoflavus* in mid-western Argentina (Brigada et al., 2010), but not in Paraguay (Yeo et al., 2005). This species was described from specimens collected in the Monte and Patagonic steppe eco-regions (Lanzone et al., 2007). Our phylogenetic analyses support the hypothesis of the presence of a single *Graomys* species in the humid Chaco because the specimens studied formed a monophyletic group with *G. chacoensis* and *G. centralis* but not with *G. griseoflavus*.

The finding of *T. cruzi* in *Ol. chacoensis* was less surprising because several species of *Oligoryzomys* have been found infected in Brazil and Bolivia (Marcili et al., 2009; Noireau et al., 1997). *Ol. nigripes* was found seropositive for *T. cruzi* and negative by hemoculture and blood smears (Vaz et al., 2007). This species has very broad trophic spectrum and microhabitat requirements (Weksler and Bonvicino, 2005), occupying open areas, grasslands, and agricultural frontiers.

Necromys (=*Bolomys*) *lasiurus* (originally named as *Zygodontomys lasiurus*) was found infected by xenodiagnosis with a prevalence of 3% in Brazil (Mello, 1982). *N. temchuki* has been taken as a junior synonym of *N. lasiurus* (D'Elía et al., 2008). The related species *N. lactens* had high seroprevalence and positivity by hemoculture and microhematocrit in the Andean valleys of Bolivia where it was closely associated with *P. osilae*, *A. boliviensis* and *T. cruzi*-infected sylvatic *T. infestans* (Rojas Cortez et al., 2006).

Likewise in our study, *C. callosus* has been found infected with *T. cruzi* in peridomestic and cattle-ranching areas from Bolivia and Brazil (Herrera et al., 2007; Mello, 1982) and in domestic areas infested with *Triatoma sordida* (Brenière et al., 1998; Noireau et al., 1997). *C. callosus* is a terrestrial rodent usually found in disturbed environments and in open vegetation formations (Dunnum et al., 2008a). This species was highly susceptible to sylvatic strains of *T. cruzi* (Magalhães-Santos et al., 2004), and was able to control the intensity of parasitemia and survive (Borges et al., 1992; Mello et al., 1979). Other *Calomys* species were occasionally found infected with *T. cruzi* in the Monte ecoregion of Argentina (Basso et al., 1977; Brigada et al., 2010; Moretti et al., 1980) (Appendix, Table B).

The two positive specimens of *Oecomys* were most likely *Oe. mamorae* – an arboreal, solitary species with nocturnal activity (Dunnum et al., 2008b). Although the occurrence of the genus *Oecomys* in Argentina has not been fully confirmed (Dunnum et al., 2008b), it was reported in a few locations with gallery forests (Pardiñas and Teta, 2005; Teta et al., 2009). The two specimens most likely belonged to a single species closely related to *Oe. mamorae* or *Oe. superans* (Pardiñas and Ramírez-Llorens, 2005). *Oe. mamorae* has features that potentially make it a suitable host for the transmission of *T. cruzi* in both peridomestic and sylvatic habitats: they are abundant and occupy various habitats; use all forest levels; nest in tree holes, epiphytes and among palm leaves, and often invade houses with thatched roofs (Dunnum et al., 2008b). In the Brazilian Pantanal, *Oe. mamorae* apparently acts as a maintenance host of *T. cruzi* and *Trypanosoma evansi* (Herrera et al., 2005, 2007; Rademaker et al., 2009).

There are a few additional records of other species of Sigmodontinae infected with *T. cruzi* in South America. Several studies reported *T. cruzi* isolates from *A. boliviensis, H. brasiliensis, Nectomys squamipes* and *O. capito*, but unfortunately did not report the number of specimens examined or the data sources (Yeo et al., 2005; Marcili et al., 2009). Natural infections with *T. cruzi* were prevalent in rodents from the family Echimyidae (*Thrichomys apereoides* and *Echimys dasythryx*) as determined by xenodiagnosis (Mello, 1982; Steindel et al., 1995) and hemoculture (*Clyomys laticeps*, *Thrichomys pachyurus*, *T. apereoides*, and *Thrichomys laurentius*) (Herrera et al., 2005, 2007; Roque et al., 2005; Rademaker et al., 2009).

Proper taxonomic identification is crucial for host incrimination and to establish the potential role of a given host species in multihost transmission cycles. Our study provides an alternative to euthanasia of rodents for the identification of live specimens with high reproducibility. All BLAST-based comparisons of rodent DNA sequences gave a very high degree of similitude (99–100%) between specimens from Pampa del Indio and the Gran Chaco region. Most matching sequences came from rodents collected from Paraguay, Southern Brazil and Northeastern Argentina, and the results were confirmed using several phylogenetic analyses.

Molecular taxonomy offers the relative advantage of providing a universally applicable tool to any kind of organism and has a central role in species identification, but it is not free of problems such as taxonomic reliability and insufficient annotations at sequence databases (Nilsson et al., 2006). Specifically, the use of a single gene region for taxon identification could be problematic because identical mitochondrial DNA sequences can be present in closely related species due to introgression or incomplete lineage sorting (Valentini et al., 2008). This is particularly true for mitochondrial genes, which generally do not recombine and are maternally inherited as a whole. The idea of a barcoding approach requires an extensive database where gene regions are broadly represented and comprise the biggest possible number of genera and species. We consider that *cytb* is the most suitable gene for this purpose because it is widely represented in Sigmodontinae, particularly in those species reported in the Gran Chaco region. From the 8037 records of Sigmodontinae sequences at Genbank (last accessed 2013/14/11), 5829 (73%) are from mitochondrial genes, and within this group, records from *cytb* constitute 55% of all sequences. Another candidate marker for Sigmodontinae barcoding is *COI* (1704 records), which has been used recently for the identification of wildlife reservoirs of zoonoses (Müller et al., 2013). However, in our study, *COI* would not provide additional phylogenetic evidence of species identification because its evolutionary history is not independent of the one of *cytb*. In addition, there are no *COI* records for *G. chacoensis*, *Ol. chacoensis*, *Oe. mamorae*, *A. toba* and *C. callosus*.

Although the possibility of misclassification due to mitochondrial DNA introgression could not be completely ruled out, it does not seem to be common in Sigmodontinae. The accidental amplification of nuclear copies of mitochondrial DNA (mitochondrial pseudogenes) or heteroplasmy can also be excluded due to the lack of multiple bands on agarose gels of PCR products, double peaks, background noise, and ambiguity in sequence chromatograms and indels and/or stop codons in the reading framework of the sequences. An additional evidence that supports our species taxon hypotheses, although controversial (see Valentini et al., 2008 for references), is the fact that genetic distances between our sequences and Genbank records ($\leqslant$2.3%) are within the range of intraspecific values for Sigmodontinae (Rosa et al., 2012 and references therein).

Our study has some additional limitations. Although our molecular taxon identifications appear to be accurate, complementary approaches based on other sources of evidence are needed for the definitive identification of the specimens. Our study did not seek to estimate the prevalence of *T. cruzi* infection for each rodent species and any related attributes, which would require a sizable research effort to identify the taxonomic status of >120 kDNA-PCR negative specimens. Additional searches for parasite amastigotes in host tissues may provide additional clues (Ramsey et al., 2012). The potential effects of anesthesia with inhaled isoflurane on host infectiousness as determined by xenodiagnosis are unknown and perhaps minor, given that inhaled isoflurane produces minimal cardiovascular and respiratory effects in rodents (Fish et al., 2008). Assessing the host competence of sylvatic rodent species presumably infected with TcI by means of a domestic vector species such as *T. infestans* may paradoxically be as good as or better than using

other sylvatic triatomines to detect infectious hosts (Schweigmann et al., 1997; Loza-Murguía and Noireau, 2010).

5. Conclusions

This study provides essential information on sigmodontine rodent species and their reservoir host competence for *T. cruzi* in the Argentinean Chaco and southern cone countries. Although a large number of rodent species was found infected, they may have a secondary role in vector-borne transmission compared with *Dasypus novemcinctus* armadillos and *Didelphis* opossums which have much greater infection prevalence and infectiousness to the vector (Orozco et al., 2013). Rodents usually are abundant in the Gran Chaco and frequently serve as prey for domestic and wild carnivores, and therefore may be involved in oral transmission cycles. Most of the sigmodontine species here studied successfully adapt to disturbed environments in close proximity to domestic habitats, and therefore may eventually contribute to the domestic or peridomestic transmission of *T. cruzi*. Arboreal species such as *Oecomys* sp. (which displayed high reservoir host competence elsewhere) may play an important role in sylvatic cycles involving tree-dwelling triatomine bugs. Whether the large population density and seasonal fluctuations of small rodents (with multi-annual cycles affected by recurrent droughts) may allow them to play a more prominent role in the sylvatic transmission of *T. cruzi* at particular times deserves further investigation. Additional studies are needed to identify the vector species involved, and the potential links between sylvatic rodents and peridomestic and domestic transmission cycles.

Acknowledgements

We are grateful to Julián Alvarado-Otegui, Leonardo Ceballos, Flavia Netto, Marina Leporace, Juan Pablo Arrabal, Yael Mariana Provecho, Pablo Teta, Emiliano Muschetto, Alfonso, Lescano and Blanco brothers for field and laboratory assistance. This study was supported by awards from the International Development Research Center (EcoHealth Program), Tropical Disease Research (UNICEF/PNUD/WB/WHO), and University of Buenos Aires to REG, and Agencia Nacional de Promoción Científica y Tecnológica to MSM and RVP. REG, MSM, RVP and MVC are members of CONICET Researcher's Career.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2013.12.020.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.
Alvarado-Otegui, J.A., Ceballos, L.A., Orozco, M.M., Enriquez, G.F., Cardinal, M.V., Cura, C., Schijman, A.G., Kitron, U., Gürtler, R.E., 2012. The sylvatic transmission cycle of *Trypanosoma cruzi* in a rural area in the humid Chaco of Argentina. Acta Trop. 124, 79–86.
Añez, N., Crisante, G., Añez-Rojas, N., Rojas, A., Moreno, G., Maia da Silva, F., Teixeira, M.M.G., 2009. Genetic typing of *Trypanosoma cruzi* isolates from different hosts and geographical areas of western Venezuela. Bol. Mal. Salud Amb. 49, 251–258.
Basso, B., Eraso, A., Moretti, E., Albesa, I., Kravetz, F., 1977. Natural infection of *Calomys musculinus* (Rodentia, Cricetidae) by *Trypanosoma cruzi*. Rev. Asoc. Argent. Microbiol. 9, 11–16.
Basso, B., Moretti, E., Albesa, I., Eraso, A., Kravetz, F., D'Alessandro, A., 1982. Natural infection of *Akodon dolores*, Thomas, 1916 (Rodentia, Cricetidae) by *Trypanosoma cruzi*. Rev. Inst. Med. Trop. Sao Paulo 24, 21–26.
Borges, M.M., De Andrade, S.G., Pilatti, C.G., do Prado Junior, J.C., Kloetzel, J.K., 1992. Macrophage activation and histopathological findings in *Calomys callosus* and Swiss mice infected with several strains of *Trypanosoma cruzi*. Mem. Inst. Oswaldo Cruz 87, 493–502.
Brenière, S.F., Morochi, W., Bosseno, M.F., Ordoñez, J., Gutierrez, T., Vargas, F., Yaksic, N., Noireau, F., 1998. *Trypanosoma cruzi* genotypes associated with domestic *Triatoma sordida* in Bolivia. Acta Trop. 71, 269–283.
Brigada, A.M., Dona, R., Caviedes-Vidal, E., Moretti, E., Basso, B., 2010. American tripanosomiasis: a study on the prevalence of *Trypanosoma cruzi* and *Trypanosoma cruzi*-like organisms in wild rodents in San Luis province, Argentina. Rev. Soc. Bras. Med. Trop. 43, 249–253.
Burgos, J.M., Altcheh, J., Bisio, M., Duffy, T., Valadares, H.M., Seidenstein, M.E., Piccinali, R., Freitas, J.M., Levin, M.J., Macchi, L., Macedo, A.M., Freilij, H., Schijman, A.G., 2007. Direct molecular profiling of minicircle signatures and lineages of *Trypanosoma cruzi* bloodstream populations causing congenital Chagas disease. Int. J. Parasitol. 37, 1319–1327.
Cabrera, A., 1961. Catálogo de los mamíferos de América del Sur. Rev. Mus. Argent. Cienc. Nat. 4, 309–732.
Ceballos, L.A., Cardinal, M.V., Vazquez-Prokopec, G.M., Lauricella, M.A., Orozco, M.M., Cortinas, R., Schijman, A.G., Levin, M.J., Kitron, U., Gürtler, R.E., 2006. Long-term reduction of *Trypanosoma cruzi* infection in sylvatic mammals following deforestation and sustained vector surveillance in northwestern Argentina. Acta Trop. 98, 286–296.
Chaves, L.F., Hernandez, M.J., Dobson, A.P., Pascual, M., 2007. Sources and sinks: revisiting the criteria for identifying reservoirs for American Cutaneous Leishmaniasis. Trends Parasitol. 23, 311–316.
D'Elía, G., 2003. Phylogenetics of Sigmodontinae (Rodentia, Muroidea, Cricetidae), with special reference to the akodont group, and with additional comments on historical biogeography. Cladistics 19, 307–323.
D'Elía, G., Pardiñas, U.F.J., Teta, P., Patton, J.L., 2007. Definition and diagnosis of a new tribe of sigmodontine rodents (Cricetidae: Sigmodontinae), and a revised classification of the subfamily. Gayana 71, 187–194.
D'Elía, G., Pardiñas, U.F.J., Jayat, J.P., Salazar-Bravo, J., 2008. Systematics of *Necromys* (Rodentia, Cricetidae, Sigmodontinae): species limits and groups, with comments on historical biogeography. J. Mammal. 89, 778–790.
Diosque, P., Padilla, A.M., Cimino, R.O., Cardozo, R.M., Negrette, O.S., Marco, J.D., Zacca, R., Meza, C., Juarez, A., Rojo, H., Rey, R., Corrales, R.M., Nasser, J.R., Basombrio, M.A., 2004. Chagas disease in rural areas of Chaco Province, Argentina: epidemiologic survey in humans, reservoirs, and vectors. Am. J. Trop. Med. Hyg. 71, 590–593.
Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.
Duffy, T., Bisio, M., Altcheh, J., Burgos, J.M., Diez, M., Levin, M.J., Favaloro, R.R., Freilij, H., Schijman, A.G., 2009. Accurate real-time PCR strategy for monitoring bloodstream parasitic loads in Chagas disease patients. PLoS Negl. Trop. Dis. 3, e419.
Dunnum, J., Vargas, J., Bernal, N., D'Elia, G., Pardinas, U., Teta., P., 2008a. *Calomys callosus*, IUCN 2013. IUCN Red List of Threatened Species, Version 2013.1. <www.iucnredlist.org>.
Dunnum, J., Vargas, J., Bernal, N., Pardiñas, U., Patterson, B., Teta, P., 2008b. *Oecomys mamorae*, IUCN 2013. IUCN Red List of Threatened Species, Version 2013.1. <www.iucnredlist.org>.
Enriquez, G.F., Cardinal, M.V., Orozco, M.M., Lanati, L., Schijman, A.G., Gürtler, R.E., 2013. Discrete typing units of *Trypanosoma cruzi* identified in rural dogs and cats in the humid Argentinean Chaco. Parasitology 140, 303–308.
Ferro, L.I., Martinez, J.J., 2009. Molecular and morphometric evidence validates a Chacoan species of the grey leaf-eared mice genus *Graomys* (Rodentia: Cricetidae: Sigmodontinae). Mammalia 73, 265–271.
Fish, R.E., Danneman, P.J., Brown, M., Karas, A.Z., 2008. Domestic and wild mammalian reservoirs. Anesthesia and Analgesia in Laboratory Animals. American College of Laboratory Animal Medicine Series, second ed., Academic Press Inc.
Gaunt, M., Miles, M., 2000. The ecotopes and evolution of triatomine bugs (triatominae) and their associated trypanosomes. Mem. Inst. Oswaldo Cruz 95, 557–565.
Goloboff, P.A., Farris, J., Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. Cladistics 24, 1–13.
González-Ittig, R.E., Patton, J.L., Gardenal, C.N., 2007. Analysis of cytochrome-b nucleotide diversity confirms a recent range expansion in *Calomys musculinus* (Rodentia, Muridae). J. Mammal. 88, 777–783.
Gurevitz, J.M., Ceballos, L.A., Gaspe, M.S., Alvarado-Otegui, J.A., Enriquez, G.F., Kitron, U., Gurtler, R.E., 2011. Factors affecting infestation by *Triatoma infestans* in a rural area of the humid Chaco in Argentina: a multi-model inference approach. PLoS Negl. Trop. Dis. 5, e1349.
Herrera, C.P., Barnabe, C., Brenière, S.F., 2013. Complex evolutionary pathways of the intergenic region of the mini-exon gene in *Trypanosoma cruzi* TcI: a possible ancient origin in the Gran Chaco and lack of strict genetic structuration. Infect. Genet. Evol. 16, 27–37.
Herrera, H.M., Rademaker, V., Abreu, U.G., D'Andrea, P.S., Jansen, A.M., 2007. Variables that modulate the spatial distribution of *Trypanosoma cruzi* and *Trypanosoma evansi* in the Brazilian Pantanal. Acta Trop. 102, 55–62.
Herrera, L., D'Andrea, P., Xavier, S., Mangia, R., Fernandes, O., Jansen, A., 2005. *Trypanosoma cruzi* infection in wild mammals of the National Park "Serra da Capivara" and its surroundings (Piauí, Brazil), an area endemic for Chagasdisease. Trans. R. Soc. Trop. Med. Hyg. 99, 379–388.
Jansen, A.M., Roque, A.L.R., 2010. Domestic and wild mammalian reservoirs. In: Telleria, J., Tibayrenc, M. (Eds.), American Trypanosomiasis Chagas Disease. One Hundred Years of Research. Elsevier, pp. 249–276.
Jayat, J.P., Ortiz, P.E., Teta, P., Pardiñas, U.F.J., D'Elía, G., 2006. Nuevas localidades argentinas para algunos roedores sigmodontinos (Rodentia: Cricetidae). Mastozool. Neotrop. 13, 51–67.

Jayat, J., D'elia, G., Teta, P., 2008. *Akodon toba*, IUCN 2013. IUCN Red List of Threatened Species, Version 2013.1. <www.iucnredlist.org>.

Kruse, H., Kirkemo, A., Handeland, K., 2004. Wild life as source of zoonotic infections. Emerg. Infect. Dis. 10, 2067–2072.

Lanzone, C., Novillo, A., Suárez, N.S., Ojeda, R.A., 2007. Cytogenetics and redescription of *Graomys* (Rodentia, Sigmodontinae) from Chumbicha, Catamarca, Argentina. Mastozool. Neotrop. 14, 249–255.

Loza-Murguía, M., Noireau, F., 2010. Vectorial capacity of *Triatoma guasayana* (Wygodzinsky & Abalos) (Hemiptera: Reduviidae) compared with two other species of epidemic importance. Neotrop. Entomol. 39, 799–809.

Llewellyn, M.S., Miles, M.A., Carrasco, H.J., Lewis, M.D., Yeo, M., Vargas, J., Torrico, F., Diosque, P., Valente, V., Valente, S.A., Gaunt, M.W., 2009. Genome-scale multilocus microsatellite typing of *Trypanosoma cruzi* discrete typing unit I reveals phylogeographic structure and specific genotypes linked to human infection. PLoS Pathog. 5, e1000410.

Maffey, L., Cardinal, M.V., Ordoñez-Krasnowski, P.C., Lanati, L.A., Lauricella, M.A., Schijman, A.G., 2012. Direct molecular identification of *Trypanosoma cruzi* discrete typing units in domestic and peridomestic *Triatoma infestans* and *Triatoma sordida* from the Argentine Chaco. Parasitology 139, 1570–1579.

Magalhães-Santos, I.F., Souza, M.M., Lima, C.S.C., Andrade, S.G., 2004. Infection of *Calomys callosus* (Rodentia Cricetidae) with strains of different *Trypanosoma cruzi* biodemes: pathogenicity, histotropism, and fibrosis induction. Mem. Inst. Oswaldo Cruz 99, 407–413.

Marcili, A., Lima, L., Valente, V.C., Valente, S.a., Batista, J.S., Junqueira, A.C.V., Souza, A.I., da Rosa, J.a., Campaner, M., Lewis, M.D., Llewellyn, M.S., Miles, M.a., Teixeira, M.M.G., 2009. Comparative phylogeography of *Trypanosoma cruzi* TCIIc: new hosts, association with terrestrial ecotopes, and spatial clustering. Infect. Genet. Evol. 9, 1265–1274.

Martínez, J.J., Di Cola, V., 2011. Geographic distribution and phenetic skull variation in two close species of *Graomys* (Rodentia, Cricetidae, Sigmodontinae). Zool. Anz. 250, 175–194.

Mello, D.A., 1982. Wild rodents, marsupials and triatominae captured in the municipality of Mambai-Goias. Natural infection by *Trypanosoma cruzi*. Rev. Saúde Públ. 16, 282–291.

Mello, D.A., Valin, E., Teixeira, M.L., 1979. Alguns aspectos do comportamento de cepas silvestres de *Trypanosoma cruzi* em camundongos e *Calomys callosus* (Rodentia). Rev. Saúde Públ. 13, 314–325.

Miller, S.A., Dikes, D.D., Polesky, H.H., 1988. A simple salting procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 215.

Moretti, E.R., Basso, B., Albesa, I., Eraso, A.J., Kravetz, F.O., 1980. Infección natural de *Calomys laucha* por *Trypanosoma cruzi*. Medicina (Buenos Aires) 40, 181–186.

Müller, L., Gonçalves, G., Cordeiro-Estela, P., Marinho, J.R., Althoff, S., et al., 2013. DNA barcoding of Sigmodontinae rodents: identifying wildlife reservoirs of zoonoses. PLoS ONE 8, e80282.

Nilsson, R.H., Ryberg, M., Kristiansson, E., Abarenkov, K., Larsson, K.-H., Kõljalg, U., 2006. Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PLoS ONE 1, e59.

Noireau, F., Brenière, S.F., Ordoñez, J., Cardozo, L., Morochi, W., Gurtierrez, T., Bosseno, M.F., Garcia, S., Vargas, F., Yacsic, N., Dujardin, J.C., Peredo, C., Wisniveski-colli, C., 1997. Low probability of transmission of *Trypanosoma cruzi* to humans by domiciliary *Triatoma sordida* in Bolivia. Trans. R. Soc. Trop. Med. Hyg. 91, 653–656.

Noireau, F., Diosque, P., Jansen, A.M., 2009. *Trypanosoma cruzi*: adaptation to its vectors and its hosts. Vet. Res. 40, 26.

Ocaña-Mayorga, S., Llewellyn, M.S., Costales, J.a., Miles, M.a., Grijalva, M.J., 2010. Sex, subdivision, and domestic dispersal of *Trypanosoma cruzi* lineage I in southern Ecuador. PLoS Negl. Trop. Dis. 4, e915.

Orozco, M.M., Enriquez, G.F., Alvarado-Otegui, J.A., Cardinal, M.V., Schijman, A.G., Kitron, U., Gürtler, R.E., 2013. New sylvatic hosts of *Trypanosoma cruzi* and their reservoir competence in the humid Chaco of Argentina: a longitudinal study. Am. J. Trop. Med. Hyg. 88, 872–882.

Pardiñas, U.F.J., Ramírez-Llorens, P., 2005. The genus *Oecomys* (Rodentia: Sigmodontinae) in Argentina. Mammalia 69, 103–107.

Pardiñas, U.F.J., Teta, P., 2005. Roedores Sigmodontinos del Chaco Húmedo de Formosa: aspectos taxonómicos y distribución geográfica. In: Di Giacomo, A.G., Krapovickas, S.F. (Eds.), Historia natural y paisaje de la Reserva El Bagual, Provincia de Formosa, Argentina. Inventario de la fauna de vertebrados y de la flora vascular de un área protegida del Chaco Húmedo. Temas de Naturaleza y Conservación 4. Aves Argentinas, Asociación Ornitológica del Plata, Buenos Aires, pp. 501–517.

Piron, M., Fisa, R., Casamijtana, N., Lopez-Chejade, P., Puig, L., Verges, M., Gascón, J., Gomez 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.

Posada, D., 2008. JModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.

Rademaker, V., Herrera, H.M., Raffel, T.R., D'Andrea, P.S., Freitas, T.P.T., Abreu, U.G.P., Hudson, P.J., Jansen, A.M., 2009. What is the role of small rodents in the transmission cycle of *Trypanosoma cruzi* and *Trypanosoma evansi* (Kinetoplastida Trypanosomatidae)? A study case in the Brazilian Pantanal. Acta Trop. 111, 102–107.

Rambaut, A., Drummond, A.J., 2009. Tracer v1.5. Program Distributed by the Authors. <http://tree.bio.ed.ac.uk/software/tracer/> (last accessed 08.02.13).

Rambaut, A., 2012. FigTree v1.4. Program Distributed by the Authors. <http://tree.bio.ed.ac.uk/software/figtree/> (last accesed 08.02.13).

Ramsey, J.M., Gutierrez-Cabrera, A.E., Salgado-Ramirez, L., Peterson, A.T., Sanchez-Cordero, V., Ibarra-Cerdena, C.N., 2012. Ecological connectivity of *Trypanosoma cruzi* reservoirs and *Triatoma pallidipennis* hosts in an anthropogenic landscape with endemic Chagas disease. PLoS ONE 7, e46013.

Rojas Cortez, M., Pinho, A.P., Cuervo, P., Alfaro, F., Solano, M., Xavier, S.C.C., D'Andrea, P.S., Fernandes, O., Torrico, F., Noireau, F., Jansen, A.M., 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.

Roque, A.L., D'Andrea, P.S., de Andrade, G.B., Jansen, A.M., 2005. *Trypanosoma cruzi*: distinct patterns of infection in the sibling caviomorph rodent species *Thrichomys apereoides laurentius* and *Thrichomys pachyurus* (Rodentia, Echimyidae). Exp. Parasitol. 111, 37–46.

Roque, A.L., Xavier, S.C., da Rocha, M.G., Duarte, A.C., D'Andrea, P.S., Jansen, A.M., 2008. *Trypanosoma cruzi* transmission cycle among wild and domestic mammals in three areas of orally transmitted Chagas disease outbreaks. Am. J. Trop. Med. Hyg. 79, 742–749.

Rosa, C.C., Flores, T., Pieczarka, J.C., Rossi, R.V., Sampaio, M.I.C., Rissino, J.D., Amaral, P.J.S., Nagamachi, C., 2012. Genetic and morphological variability in South American rodent *Oecomys* (Sigmodontinae, Rodentia): evidence for a complex of species. J. Genet. 91, 265–277.

Rozas, M., Botto-Mahan, C., Coronado, X., Ortiz, S., Cattan, P.E., Solar, A., 2007. Coexistence of *Trypanosoma cruzi* genotypes in wild and periodomestic mammals in Chile. Am. J. Trop. Med. Hyg. 77, 647–653.

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.

Schweigmann, N.J., Pietrokovsky, S., Conti, O., Escosteguy, M., Bottazzi, V., Solarz, N., Wisnivesky-Colli, C., 1997. Infection of *Triatoma guasayana, Triatoma sordida* and *Triatoma infestans* by *Trypanosoma cruzi* from a naturally infected opossum. Mem. Inst. Oswaldo Cruz 92 (151), 152.

Smith, M.F., Patton, J.L., 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biological Journal of Linnean Society 50, 149–177.

Steindel, M., Toma, H., Ishida, M.M.I., Murta, S.M.F., de Carvalho Pinto, C.J., Grisard, E.C., Schlemper Jr., B.R., Riveiro-Rodriguez, R., Romanha, A.J., 1995. Biological and isoenzimatic characterization of *Trypanosoma cruzi* strains isolated from sylvatic reservoirs and vectors from the state of Santa Catarina, Southern Brazil. Acta Trop. 60, 167–177.

Steppan, S.J., Ramirez, O., Banbury, J., Huchon, D., Pacheco, V., Walker, L.I., Spotorno, A.E., 2007. A molecular reappraisal of the systema tics of the leaf-eared mice *Phyllotis* and their relatives. In: Kelt, D.A., Lessa, E., Salazar-Bravo, J.A., Patton, J.L. (Eds.), The Quintessential Naturalist: Honoring the Life and Legacy of Oliver P. Pearson. University of California Publications in Zoology, Berkeley and Los Angeles, pp. 799–826.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Teta, P., Pereira, J.A., Muschetto, E., Fracassi, N., 2009. Mammalia, Didelphimorphia, Chiroptera, and Rodentia, Parque Nacional Chaco and Capitán Solari, Chaco Province, Argentina. Check List 5, 144–150.

The Nature Conservancy, Fundación Vida Silvestre Argentina, Fundación para el Desarrollo Sustentable del Chaco, Wildife Conservation Society Bolivia, 2005. Evaluación Ecorregional del Gran Chaco Americano/Gran Chaco Americano Ecoregional Assessment. Buenos Aires. Fundación Vida Silvestre Argentina. <http://www.vidasilvestre.org.ar/descargables/bosques_selvas/chaco/dossier.pdf>.

Tomasini, N., Lauthier, J.J., Rumi, M.M.M., Ragone, P.G., D'Amato, A.A.A., Brandan, C.P., Cura, C.I., Schijman, A.G., Barnabé, C., Tibayrenc, M., Basombrío, M.A., Falla, A., Herrera, C., Guhl, F., Diosque, P., 2011. Interest and limitations of Spliced Leader Intergenic Region sequences for analyzing *Trypanosoma cruzi* I phylogenetic diversity in the Argentinean Chaco. Infect. Genet. Evol. 11, 300–307.

Valentini, A., Pompanon, F., Taberlet, P., 2008. DNA barconding for ecologists. Trends Ecol. Evol. 24, 110–117.

Vaz, V.C., D'Andrea, P.S., Jansen, A.M., 2007. Effects of habitat fragmentation on wild mammal infection by *Trypanosoma cruzi*. Parasitology 134, 1785–1793.

Weksler, M., Bonvicino, C.R., 2005. Taxonomy of pigmy rice rats genus *Ologoryzomys* Bangs, 1900 (Rodentia, Sigmodontinae) of the Brazilian Cerrado, with the description of two new species. Arq. Mus. Nac. 63, 113–130.

Wilson, D.E., Reeder, D.M., 2005. Mammal Species of the World. A Taxonomic and Geographic Reference, third ed. Johns Hopkins University Press, Baltimore.

World Health Organization, 2002. Control of Chagas Disease: Second Report of the WHO Expert Committee. World Health Organization, Geneva.

Yeo, M., Acosta, N., Llewellyn, M., Sánchez, H., Adamson, S., Miles, G., López, E., González, N., Patterson, J., Gaunt, M., Rojas de Arias, A., Miles, M., 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, 225–233.
Zingales, B., Miles, M.A., Campbell, D.A., Tibayrenc, M., Macedo, A.M., Teixeira, M.M., 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.

Further reading

Barretto, M.P., 1985. Reservorios del *Trypanosoma* (Schizotrypanum) *cruzi* (Chagas 1909). In: Carcavallo, R.U., Rabinovich, J.E., Tonn, R.J. (Eds.), Factores biológicos y ecológicos en la enfermedad de Chagas. Organización Panamericana de la Salud/Servicio Nacional de Chagas, Buenos Aires, Argentina, pp. 275–288.
Forattini, O.P., Juarez, E., Rabello, E.X., Pattoli, D., Corrêa, R.R., 1969. Infestação domiciliar por *Triatoma infestans* e alguns aspectos epidemiológicos da Tripanossomose Americana em área do Estado de São Paulo, Brasil. Rev. Saude Publ. 3, 159–172.
Gurgel-Gonçalves, R., Ramalho, E., Duarte, M., Palma, A., Abad-Franch, F., Carranza, J., Cuba, C., 2004. Enzootic transmission of *Trypanosoma cruzi* and *Trypanosoma rangeli* in the federal district of Brazil. Rev. Inst. Med. Trop. Sao Paulo 46, 323–330.
Mello, D.A., 1981. Aspectos do ciclo silvestre do *Trypanosoma cruzi* em regioes de cerrado (Município de Formosa, Estado e Goiás). Mem. Inst. Oswaldo Cruz 76, 227–246.
Xavier, S.C., Vaz, V.C., D'Andrea, P.S., Herrera, L., Emperaire, L., Alves, J.R., Fernandes, O., Ferreira, L.F., Jansen, A.M., 2007. Mapping of the distribution of *Trypanosoma cruzi* infection among small wild mammals in a conservation unit and its surroundings (Northeast-Brazil). Parasitol. Int. 56, 119–128.