Acta Tropica 151 (2015) 178–181

Contents lists available at ScienceDirect

Acta Tropica

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

Temporal variation in *Trypanosoma cruzi* lineages from the native rodent *Octodon degus* in semiarid Chile

Carezza Botto-Mahan[a], Gemma Rojo[b], Alejandra Sandoval-Rodríguez[a], Fabiola Peña[c], Sylvia Ortiz[b], Aldo Solari[b,*]

[a] Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
[b] Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile
[c] Life Science Faculty, Utrecht University, Utrecht, Netherlands

ARTICLE INFO

Article history:
Received 1 March 2015
Received in revised form 9 June 2015
Accepted 11 June 2015
Available online 15 June 2015

Keywords:
Chagas disease
Wild reservoir
Trypanosoma cruzi
DTU
Chile

ABSTRACT

Chagas disease is a zoonosis caused by the protozoan parasite *Trypanosoma cruzi* and transmitted by triatomine insects to several mammalian species acting as reservoir hosts. In the present study, we assess *T. cruzi*-prevalence and DTU composition of the endemic rodent *Octodon degus* from a hyper-endemic area of Chagas disease in Chile. Parasite detection is performed by PCR assays on blood samples of individuals captured in the austral summers of 2010–2013. The infection level in rodents differed in the summers of these four years between 18% and 70%. Overall, infected *O. degus* showed similar *T. cruzi*-DTU composition (TcI, TcII, TcV and TcVI lineages) among years, corresponding to single and mixed infection, but the relative importance of each DTU changed among years. In 2013, we detected that only three out of the four *T. cruzi*-DTU found in *O. degus* were present in the endemic triatomine *Mepria spinolai*. We suggest that *O. degus*, an abundant long-lived rodent, is an important native reservoir of *T. cruzi* in the wild transmission cycle of Chagas disease and it is able to maintain all the *T. cruzi*-DTUs described in semiarid Chile.

1. Introduction

Chagas disease, considered one of many neglected tropical diseases, is a zoonosis caused by the parasite *Trypanosoma cruzi* that is transmitted by triatomine insects to several mammalian species (Leslie, 2011). The protozoan *T. cruzi* multiplies and differentiates in the digestive tract of the insect vector, and infection of mammalian hosts occurs by contamination of mucous membranes with insect-infected faeces containing the metacyclic trypomastigote stage of the flagellate (Kollien and Schaub, 2000). The taxon *T. cruzi* is divided into six discrete typing units (DTUs): TcI-TcVI, including Tcbat (Zingales et al., 2012).

In hyper-endemic areas of Chagas disease in Chile, infection levels can reach up to 46% in the main native vector *Mepraia spinolai* and 61% in native mammals (Botto-Mahan et al., 2005; Rozas et al., 2007). Several of these mammalian species may act as reservoir hosts because the infectious agent seems to be nonpathogenic (Campos et al., 2010; Botto-Mahan et al., 2012). Four *T. cruzi*-DTUs circulate in wild mammals of the endemic area (TcI, TcII, TcV, and TcVI); however, other unknown DTUs are also present, but at very low rates (0–14%) (Rozas et al., 2007). Preliminary information on *T. cruzi*-DTUs circulating in *M. spinolai* of the same endemic area indicates a strong similarity [TcI, TcII, TcV and TcVI] (Coronado et al., 2009).

The endemic Chilean caviomorph rodent *Octodon degus* (Octodontidae) is a slow-reproducing species with diurnal activity that lives in large colonies of up to 100 individuals (Muñoz and Yáñez, 2000). *O. degus* individuals exhibit gregarious behavior, strong fidelity to their burrows and their colonies share the same habitats with *M. spinolai* colonies (Oda et al., 2014). This abundant rodent is mainly found in semiarid and Mediterranean ecosystems, where it feeds on grasses, shrub leaves, and seeds (Previtali et al., 2009). Under field conditions, it shows an average lifespan of approximately 3 years, but in captivity it can live 6–8 years (Previtali et al., 2009). In an early study, microscopic blood examination and xenodiagnosis with Triatoma infestans performed on *O. degus* reported a very low infection rate (8.9%) (Whiting, 1946). However, in recent years molecular evidence indicated that *T. cruzi*-prevalence in wild populations of this rodent ranges from 18.0–70.4%, probably depending on climatic conditions (Botto-Mahan et al., 2010; Jiménez et al., 2015). In addition, under field conditions inter-annual survival does not differ between infected and non-infected *O. degus*, which turns this rodent species into a potentially important native asymptomatic reservoir of *T. cruzi*

* Corresponding author at: Programa Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Universidad de Chile, Independencia 1027, Santiago, Chile. Fax: +56 2 2735 5580.
E-mail address: asolari@med.uchile.cl (A. Solari).

http://dx.doi.org/10.1016/j.actatropica.2015.06.008
0001-706X/

(Botto-Mahan et al., 2012). This finding is also supported by a laboratory study in which wild caught *T. cruzi*-infected *O. degus* survived for up to two years after field capture (Campos et al., 2010). Since triatomines can be infected sucking on naturally infected *O. degus* captured at the field, this rodent is a competent reservoir. According to xenodiagnoses using *T. infestans* and *M. spinolai*, the latter is the better vector (Campos et al., 2007).

In this study, we assess the temporal variability in *T. cruzi*-prevalence in the reservoir *O. degus* and evaluate the *T. cruzi*-DTU composition, along four consecutive years, from a highly endemic area in an attempt to answer the following questions: (a) Does *T. cruzi*-prevalence in *O. degus* vary in time? (b) Does *O. degus* transmit the same *T. cruzi*-DTUs over time? (c) Does *T. cruzi*-DTU composition in *O. degus* relate with *T. cruzi*-DTU composition found in *M. spinolai* in a particular year (2013)?

2. Materials and methods

2.1. Study site

Individuals of *O. degus* were collected from 2010 to 2013 at the Reserva Nacional Las Chinchillas (30° 30′S, 71°06′W; Coquimbo Region), a hyper-endemic zone of Chagas disease in Chile where the sylvatic and domestic transmission cycles overlap (Rozas et al., 2007). Several native (*Phyllotis darwini*, *Abrothrix* spp., *Abrocoma bennetti*, *Thylamys elegans, Oligoryzomys longicaudatus*) and introduced (*Oryctolagus cuniculus, Capra hircus, Canis lupus familiaris*) mammal species inhabit the study site as well as populations of the wild kissing bug *M. spinolai* (Botto-Mahan et al., 2005, 2010; Rozas et al., 2007).

2.2. Octodon degus *trapping and blood sample collection*

O. degus trapping was performed using 300 wire mesh live-animal-traps (a collapsible box of 24 × 8 × 9 cm), baited with oatmeal flakes and provided with cotton balls for bedding. Traps were arranged on three grids of 100 traps each. Each grid consisted of two lines of 50 traps separated 10 m one from each other set in ravines formed by two slopes. Small mammal collection was carried out for four to five nights from 19:00 to 09:00 h during the austral summer of 2010–2013 (first week of January), which coincides with rodent abundance peak. Capturing and handling procedures met the guidelines of the American Society of Mammalogists (Sikes and Gannon, 2011).

Each *O. degus* captured was sexed, weighed, and measured (total body length and tail length) under short-term isoflurane anesthesia (Lee, 2004; Jekl et al., 2011). On these individuals, 0.2 ml of blood was withdrawn by saphenous vein puncture with 21G needle (Johnson-Delaney, 2006; Morton et al., 1993) and mixed immediately with 6 M Guanidine-HCl – 0.2 M EDTA in a 1:1 proportion. Individuals were ear-tagged with a unique combination of numbers to exclude analyses of recaptures, and released at the capture point. DNA blood extraction was performed by using a DNA Miniprep Kit (Axy Prep Blood Genomic, CA, USA) according to manufacturer instructions and subsequently stored at –20 °C until molecular analyses. The Chilean Agriculture and Livestock Bureau permits N° 0048 and N° 7462, and National Forest Corporation permits N° 32/2009 and N° 61/2010 authorized this study.

2.3. Mepraia spinolai *trapping and rectal content collection*

During the first two weeks of January 2013, we geo-referenced 12 *M. spinolai* colonies (Precision: ±3 m) within the three sites where *O. degus* were collected (four colonies per site). The colonies inhabited rocky outcrops comprised of hundreds of small-to-medium size rocks exposed to hot and dry conditions. In each colony, one trained researcher manually collected kissing bugs during 1 h spans between 11 a.m. and 4 p.m., the time of day with maximum insect activity. The captured insects were classified according to their stage of development and individually stored to avoid potential cross-contamination with *T. cruzi*-infected faeces. At the laboratory, insects were killed and the abdomen compressed to obtain rectal contents. Each sample was mixed with 200 μL of bi-distilled water, boiled for 10 min and centrifuged at 10 000 g. Whole genomic DNA was isolated from faecal samples employing a Multisource Genomic DNA Miniprep Kit (Axygen, CA, USA) and stored at −20 °C until molecular analyses.

2.4. PCR detection of T. cruzi *in blood and rectal content samples*

The PCR method directed to kinetoplast DNA (kDNA) is highly specific (Junqueira et al., 2005) with an estimated sensitivity limit of 0.01 parasite equivalents/PCR assay (Schijman et al., 2003). The amplification reaction for rectal content and blood samples was performed in triplicate with oligonucleotides 121 (5′-AAA TAA TGT ACG G (T/G) GAG ATG CAT GA-3′) and 122 (5′ GGG TTC GATTGG GGT TGG TGT-3), which anneal to the four constant regions in minicircles of *T. cruzi*, as described (Botto-Mahan et al., 2005). A sample was considered positive when at least two out of the three assays showed amplifications. Samples with only one positive assay were considered negatives or doubtful, and repeated three additional times. A sample of 5 μL of the elution of the extracted mammalian blood or crude pre-boiled extract of triatomine faeces was used as DNA template in 50 μL of final volume. Each experiment included a blank that contained water instead of DNA, and a positive control that contained purified kDNA of *T. cruzi*. The minicircles' hypervariable region PCR product of 330 bp was analyzed by electrophoresis in a 2% agarose gel and visualized by ethidium bromide staining. A second PCR assay directed to nuclear DNA (nDNA) was performed twice in each kDNA based PCR positive sample with unknown DTUs (see Section 2.5). This PCR method is highly specific, with results showing a better performance than kDNA based PCR (Schijman et al., 2011).

2.5. Trypanosoma cruzi *genotyping*

For genotyping, we performed DNA blot analyses by using 10 ul of each kDNA-PCR product. Four *T. cruzi* clones (sp 104 cl 1, CBB cl 3, NR cl 3, and V195 cl 1), corresponding to TcI, TcII, TcV and TcVI, respectively, were used to generate DTU-specific probes. Construction of minicircle probes and radiolabeling was performed as described (Veas et al., 1991). The PCR products were subjected to electrophoresis, transferred onto Hybond N+ nylon membranes (Amersham, Piscataway, NJ, USA), and cross-linked by ultraviolet light for DNA fixation. After transferring PCR products, four membranes were pre-hybridized for at least 2 h at 55 °C. Each membrane was then hybridized with a lineage-specific probe labeled with P^{32} (5×10^6 cpm/membrane) (Solari et al., 2001). After hybridization, membranes were washed under high stringency conditions and then exposed in the Molecular Imager FX (Bio-Rad Laboratories, Hercules, CA). Previous studies have validated this genotyping method with the analysis of minicircles of kDNA, and different *T. cruzi* clones as reference DTUs to construct DNA probes (Veas et al., 1991; Breniere et al., 1998; Arenas et al., 2012).

2.6. Statistical analyses

Genotype distributions among years were compared using chi-square or likelihood-ratio *G*-tests of goodness of fit depending on the comparison (Sokal and Rohlf, 1995).

Table 1
Number of *Octodon degus* individuals per year (male/female/unknown), mean body weight (±SE), mean body total length (±SE) and percentage of *T. cruzi* infection.

Year	Sample size	Body weight (g)	Body length (cm)	*T. cruzi* infection (%)
2010	48/48/2	134.7 (±2.5)	27.3 (±0.2)	70.4
2011	43/43/0	139.3 (±3.1)	27.9 (±0.3)	24.4
2012	110/101/0	131.3 (±2.0)	28.1 (±0.2)	18.0
2013	26/39/0	138.8 (±3.2)	28.6 (±0.3)	64.6

3. Results

A total of 599 *O. degus* were captured between 2010 and 2013, and in 460 individuals blood samples were successfully collected and submitted to DNA extraction. Overall, *T. cruzi* infection was detected in 37.0% of the rodents, with strong differences among years ($\chi^2 = 91.89$, d.f. = 3, $P < 0.001$). *O. degus* individuals also significantly differed among years in body length but not in body weight (body length: $F_{3,445} = 3.92$, $P = 0.009$, Tukey HSD a posteriori test: 2010 ≠ 2013, $P = 0.007$; body weight: $F_{3,443} = 2.30$, $P = 0.077$; see Table 1 for more details).

All samples from infected *O. degus* were submitted to *T. cruzi*-DTU composition analyses based on hybridization tests with the panel of four specific probes. This assay allows detection of single or mixed infections when only one, two or more hybridization signals with the DNA blots are obtained. The ratio of single/mixed infections is conserved over the years ($\chi^2 = 5.04$, d.f. = 3, $P = 0.170$), and interestingly the combination of TcII and TcV is the highest in all the years in which this combination was present (2010, 2012 and 2013; see Table 2 for more details). However, these results need to be examined carefully because of the presence of several unknown DTUs that may be present in single or mixed form.

The kDNA-PCR positive samples with unknown DTUs submitted to nDNA-PCR showed that 51 out of 52 samples analyzed resulted positive by this alternative assay, discarding the possibility of false positives. Therefore, these *O. degus* were really infected with *T. cruzi*.

We evaluated the prevalence of each *T. cruzi*-DTU over the years. The prevalence of TcI did not change over the years ($\chi^2 = 6.26$, d.f. = 3, $P = 0.10$; single and mixed infections combined); however, the prevalence of TcII, TcV and TcVI did significantly vary in time ($\chi^2 = 7.91$, d.f. = 3, $P = 0.048$; $\chi^2 = 19.79$, d.f. = 3, $P = 0.002$; $\chi^2 = 32.27$, d.f. = 3, $P < 0.001$, respectively; singles and mixed infections combined). Overall, the *T. cruzi*-DTU most frequently found in *O. degus* blood was TcV, followed by TcII, and TcI/TcVI as the least represented (Table 2). This tendency is detected in three out of the four years.

Table 2
Number of *Octodon degus* individuals with single and mixed *Trypanosoma cruzi* DTUs in for consecutive years (2010–2013).

	Year			
DTU	2010	2011	2012	2013
Single				
TcI	3	1	2	0
TcII	0	0	2	1
TcV	7	2	3	4
TcVI	6	0	3	0
Mixed				
TcI-TcII	0	1	1	1
TcI-TcV	1	0	3	2
TcI-TcVI	0	0	0	1
TcII-TcV	7	0	8	3
TcII-TcVI	3	0	0	0
TcI-TcII-TcV	0	0	1	1
TcII-TcV-TcVI	0	0	4	0
TcI-TcII-TcV-TcVI	0	0	1	1
Unknown	25	2	10	15

Table 3
Number of *Mepraia spinolai* individuals and percentage of *Trypanosoma cruzi* infection by nymphal stage (2013).

Nymphal stage	Sample size	*Trypanosoma cruzi* infection (%)
I	237	18.1
II	66	15.2
III	135	26.7
IV	109	30.3
V	47	25.5
Adults	18	16.7

In 2013, a total of 612 *M. spinolai* individuals were collected in the study site; 22.4% was *T. cruzi*-infected and prevalence ranged from 15.2 to 30.3% depending on the nymphal stage examined (see Table 3 for details). A random sample of 37 infected insects was used to compare vector DTU composition with DTU composition of *O. degus* collected in 2013. Mostly single infections were detected in *M. spinolai* (Single: TcI = 10, TcII = 4, TcV = 16; mixed: TcI + TcV = 3, TcII + TcV = 4), which significantly differed from *O. degus* ratio of single and mixed infections ($\chi^2 = 9.31$, d.f. = 1, $P = 0.002$). Overall, DTU distributions differed between *O. degus* and *M. spinolai* ($G = 16.50$, d.f. = 3, $P < 0.001$), probably given by the complete absence of TcVI in the vector. We evaluated the prevalence of each *T. cruzi*-DTU between host and vector, and the prevalence of TcI, TcII and TcV did not differ ($\chi^2 = 0.01$, d.f. = 1, $P = 0.92$; $\chi^2 = 3.70$, *d.f.* = 1, $P = 0.054$; $\chi^2 = 0.99$, *d.f.* = 1, $P = 0.318$, respectively).

4. Discussion

Overall *T. cruzi*-infection prevalence in *O. degus* was high but varied strongly among years, from 70% in 2010 to 18% in 2012. These results are consistent with a previous report of temporal variation in *T. cruzi* infection in small mammals (Botto-Mahan et al., 2010). However, because *T. cruzi*-DNA detection depends on the amount of collected blood (Schijman et al., 2003; Arenas et al., 2012), some infections in *O. degus* with low parasitemia might not be detected. Interestingly, the proportion of single and mixed infection did not vary among years. Even though, the *T. cruzi*-DTU composition and prevalence may change among years, the proportion of single or mixed infections did not. Probably *O. degus* individuals are being infected with several *T. cruzi*-DTUs at the same time or in a sequential manner, maintaining all DTUs in time. It is necessary to address this observation in an individual level of analysis, performing sequential blood sampling in *O. degus* under field conditions to examine the consistency of *T. cruzi*-DTUs through time (Campos et al., 2010).

The prevalence of TcI-DTU did not vary during the 4 years of this study. A plausible explanation to this observation is that TcI is highly adapted to its host *O. degus*. According to this, Herrera (2010) stated that hosts are biological filters for DTUs, and this could probably depend on the replication and surviving time of the DTU inside the host, once it becomes infected (Araújo et al., 2014). It is necessary to explore the interaction patterns of DTUs in an individual basis, due to this stability and relative low prevalence when compared to the other DTUs. On the other hand, the prevalence of TcII, TcV and TcVI-DTUs significantly varied among years. However, it is important to mention that the significant variation might be caused by an overall small number of infected *O. degus* individuals in 2011. Probably, some ecological factors acting on a higher scale, such as fluctuating climatic conditions, could explain at least in part our results (Botto-Mahan et al., 2010).

In a broader perspective, the field evidence documented in our study suggests that *O. degus* could be considered a competent reser-

voir for Chagas disease transmission. However, future research should quantify the impact of *O. degus* as a reservoir in the wild cycle of Chagas disease in semiarid and Mediterranean ecosystems (Oda et al., 2014). For instance, it would be important to estimate the proportion of infected vectors produced by an *O. degus* infected individual, i.e., its infectivity, to quantify its reservoir competence.

Our results concerning *O. degus* and *M. spinolai* infection in 2013 indicated that *T. cruzi*-prevalence in rodent blood was significantly higher than in vector feces (64.6% versus 22.4%, respectively). This result could be explained by the longer life-span of *O. degus* individuals; therefore, they have a higher chance to become infected and a cumulative effect when mixing rodent individuals from different reproductive seasons (Botto-Mahan et al., 2012). Vector feces samples showed significantly more single DTU infections than rodent blood samples. We detected the opposite for *O. degus* individuals in 2013. This result could be explained by the conditions that *M. spinolai* provides to the different *T. cruzi*-DTUs, making a mixed configuration less sustainable. The *T. cruzi*-DTU global distributions significantly differed between *O. degus* and *M. spinolai*. The high prevalence of TcV and the absence of TcVI in *M. spinolai* feces samples could explain this finding. We cannot discard that in this case the vector is working as a biological filter for TcVI-DTU (Herrera, 2010). Similarly *Rhodnius prolixus* individuals experimentally infected with a specific TcII strain (Y strain) eliminated the parasite from their digestive system 11 days after infection (Mello et al., 1996). Therefore, this could explain at least in part why in some years this DTU is completely absent from *O. degus* population.

Acknowledgments

The authors thank A. Bacigalupo, R. Cares, J.P. Correa, M. Espinoza, M.F. González, C. Jiménez, N. Lártiga, A. López, V. Manríquez, L. Moreno, E. Oda, N. Peña, T. Poch, M. Puebla, P. Ramírez and A.P. Yáñez for helping during field data collection. We thank CONAF-Illapel for logistic support. Financial support: FONDECYT1140521 (CBM), FONDECYT1120122 (AS). CONICYT-Scholarship for PhD studies and CONICYT-Becas Chile for Master studies supported GR and FP, respectively.

References

Araújo, C.A., Waniek, P.J., Jansen, A.M., 2014. TcI/TcII co-infection can enhance *Trypanosoma cruzi* growth in *Rhodnius prolixus*. Parasit. Vectors 7, 94.

Arenas, M., Campos, R., Coronado, X., Ortiz, S., Solari, A., 2012. *Trypanosoma cruzi* genotypes of insect vectors and patients with Chagas of Chile studied by means of cytochrome b gene sequencing, minicircle hybridization, and nuclear gene polymorphisms. Vector-Borne Zoon. Dis. 12, 196–205.

Botto-Mahan, C., Ortiz, S., Rozas, M., Cattan, P., Solari, A., 2005. DNA evidence of *Trypanosoma cruzi* in the Chilean wild vector *Mepraia spinolai* (Hemiptera:Reduviidae). Mem. Inst. Oswaldo Cruz 100, 237–239.

Botto-Mahan, C., Campos, R., Acuña-Retamar, M., Coronado, X., Cattan, P.E., Solari, A., 2010. Temporal variation of *Trypanosoma cruzi* infection in native mammals in Chile. Vector-Borne Zoon. Dis. 10, 317–319.

Botto-Mahan, C., Bacigalupo, A., Correa, J.P., Oda, E., Solari, A., 2012. Field assessment of *Trypanosoma cruzi* infection and host survival in the native rodent *Octodon degus*. Acta Trop. 122, 164–167.

Breniere, S.F., Bosseno, M.F., Telleria, J., Bastrenta, B., Yacsik, N., Noireau, F., Alcazar, J.-L., Barnabé, C., Wincker, P., Tibayrenc, M., 1998. Different behavior of two *Trypanosoma cruzi* major clones: transmission and circulation in young Bolivian patients. Exp. Parasitol. 89, 285–295.

Campos, R., Acuña-Retamar, M., Botto-Mahan, C., Ortiz, S., Cattan, P., Solari, A., 2007. Susceptibility of *Mepraia spinolai* and *Triatoma infestans* to different *Trypanosoma cruzi* strains from naturally infected rodent hosts. Acta Trop. 104, 25–29.

Campos, R., Botto-Mahan, C., Ortiz, S., Coronado, X., Solari, A., 2010. Temporal fluctuation of infection with different *Trypanosoma cruzi* genotypes in the wild rodent *Octodon degus*. Am. J. Trop. Med. Hyg. 83, 380–381.

Coronado, X., Rozas, M., Botto-Mahan, C., Ortiz, S., Cattan, P.E., Solari, A., 2009. Molecular epidemiology of Chagas disease in the wild transmission cycle: the evaluation in the sylvatic vector *Mepraia spinolai* from endemic area of Chile. Am. J. Trop. Med. Hyg. 81, 656–659.

Herrera, L., 2010. Una revisión sobre reservorios de *Trypanosoma* (Schizotrypanum) *cruzi* (Chagas, 1909), agente etiológico de la Enfermedad de Chagas. Bol. Malariol. Salud Amb. 50, 3–15.

Jekl, V., Hauptman, K., Knotek, Z., 2011. Diseases in pet degus: a retrospective study in 300 animals. J. Small Anim. Pract. 52, 107–112.

Jiménez, C., Fontúrbel, F., Oda, E., Ramírez, P., Botto-Mahan, C., 2015. Parasitic infection alters rodent movement in a semiarid ecosystem. Mamm. Biol. 80, 255–259.

Johnson-Delaney, C.A., 2006. Common procedures in hedgehogs, prairie dogs, exotic rodents, and companion marsupials. Vet. Clin. Exot. Anim. 9, 415–435.

Junqueira, A.C.V., Degrave, W., Brandao, A., 2005. Minicircle organization and diversity in *Trypanosoma cruzi* populations. Trends Parasitol. 21, 270–272.

Kollien, A.H., Schaub, G.A., 2000. The development of *Trypanosoma cruzi* in Triatominae. Parasitol. Today 16, 381–387.

Lee, T.M., 2004. *Octodon degus*: a diurnal, social, and long-lived rodent. ILAR J. 45, 14–24.

Leslie, M., 2011. Drug developers finally take aim at a neglected disease. Science 333, 933–935.

Mello, C.B., Azambuja, P., Garcia, E.S., Ratcliffe, N.A., 1996. Differential in vitro and in vivo behaviour of three strains of *Trypanosoma cruzi* in the gut and hemolymph of *Rhodnius prolixus*. Exp. Parasitol. 82, 112–121.

Morton, D.B., Abbot, D., Barclay, R., Close, B.S., Ewbank, R., Gask, D., Heath, M., Mattic, S., Poole, T., Seamer, J., Southee, J., Thompson, A., Trussell, B., West, C., Jennings, M., 1993. Removal of blood from laboratory mammals and birds: first report of the BVA/FRAME/ RSPCA/UFAW joint working group on refinement. Lab. Anim. 27, 1–22.

Muñoz, A., Yáñez, J., 2000. Mamíferos de Chile. CEA Ediciones, Santiago.

Oda, E., Solari, A., Botto-Mahan, C., 2014. Effects of mammal host diversity and density in the infection level of a sylvatic kissing bug. Med. Vet. Entomol. 28, 384–390.

Previtali, M.A., Meserve, P.L., Kelt, D.A., Milstead, W.B., Gutierrez, J.R., 2009. Effects of more frequent and prolonged El Niño events on life-history parameters of the Degu, a long-lived and slow-reproducing rodent. Conserv. Biol. 24, 18–28.

Rozas, M., Botto-Mahan, C., Coronado, X., Ortiz, S., Cattan, P.E., Solari, 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., Altcheh, J., Burgos, J.M., Biancardi, M., Bisio, M., Levin, M.J., Freilij, H., 2003. Aetiological treatment of congenital Chagas disease diagnosed and monitored by the polymerase chain reaction. J. Antimicrob. Chemother. 52, 441–449.

Schijman, A.G., Bisio, M., Orellana, L., Sued, M., Duffy, T., Mejia Jaramillo, A.M., Cura, C., 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.

Sikes, R.S., Gannon, W.L., 2011. Care and use committee. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J. Mammal. 92, 235–253.

Sokal, R.R., Rohlf, F.J., 1995. Biometry. The Principles and Practice of Statistics in Biological Research. WH Freeman and Co., New York.

Solari, A., Campillay, R., Ortiz, S., Wallace, A., 2001. Identification of *Trypanosoma cruzi* genotypes circulating in Chilean chagasic patients. Exp. Parasitol. 97, 226–233.

Veas, F., Breniere, S.F., Cuny, G., Brengues, C., Solari, A., Tibayrenc, M., 1991. General procedure to construct highly specific kDNA probes for clones of *Trypanosoma cruzi* for sensitive detection by polymerase chain reaction. Cell. Mol. Biol. 37, 73–84.

Whiting, C., 1946. Contribución al estudio de las reservas de parásitos en la enfermedad de Chagas en Chile. I. Primeros hallazgos en Chile de mamíferos silvestres naturalmente infectados por el *Trypanosoma cruzi*. Rev. Chil. Hig. Med. Prev. 8, 69–102.

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.