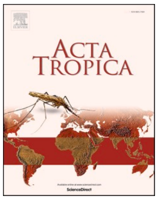
Acta Tropica 273 (2026) 107948

Contents lists available at ScienceDirect

Acta Tropica

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



The dynamic of *Trypanosoma cruzi* transmissibility in field-caught *Mepraia spinolai* (Hemiptera: Reduviidae) of contrasting seasons: A developmental stage-dependent study

Nicol Quiroga [a,†], Francisca Farías [a,b,†], Angélica López [b], Carezza Botto-Mahan [a,c,**], Aldo Solari [b,*]

[a] *Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile*
[b] *Núcleo Integrativo de Biología y Genética, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile*
[c] *Research Ring in Pest Insects and Climate Change (PIC^2), Chile*

A R T I C L E I N F O

Keywords:
Wild triatomines
Chagas disease
Season-dependent risk
Trypanosoma cruzi load
Mepraia spinolai
Vectorial competence

A B S T R A C T

A competent vector can harbor a pathogen and infect a high number of hosts. Scarce information is available on how triatomine vectors accomplish this task in nature, particularly without knowing the number of parasites they excrete. We study the dynamics of *Trypanosoma cruzi* transmissibility in the triatomine *Mepraia spinolai* collected in summer (December 2018) and winter (July 2019) of an austral endemic area. The insects received two feedings in the laboratory, the day after arrival and 45 days after the first feeding. Excreta samples were collected, DNA was extracted, and parasite loads were quantified by qPCR. After feeding, the parasite loads of the first and second excreta samples revealed differences between summer and winter insects. Overall, parasite load reductions and increases were detected in summer (51.2% and 32.5%) and in winter (9.1% and 15.2%) infected insects. The number of times the parasite load was reduced in summer insects revealed that early stage nymphs accomplished it at the highest ratio (× 740), middle stage nymphs (× 14), and late developmental stages with the lowest ratio (× 6.7). Therefore, fifth instar nymphs and adults have the maximal capacity to harbor *T. cruzi*, and those with the highest competence to transmit it, and early nymphs are the ones with minimal vectorial capacity. About 7% of the winter insects and 58.5% of the summer excreted quantifiable parasite loads right after collection. The amount of *T. cruzi* excreted in summer by *M. spinolai* reached medians between 950 and 2597 parasite equivalents/excreta, considering all developmental stages. Finally, molting during processes of *T. cruzi* load reduction and increase suggests that the exoskeleton shedding does not affect the excretion of *T. cruzi*.

1. Introduction

The etiological agent of Chagas disease is the protozoan *Trypanosoma cruzi*, which is distributed in Chile in endemic rural areas with arid and semiarid Mediterranean climates (18°S to 34°S), and transmitted by triatomine insects. Among the four triatomine species circulating in sylvatic and domestic transmission cycles in Chile, *Mepraia spinolai* is the most relevant given its distribution in central latitudes (Canals et al., 2017). This sylvatic triatomine feeds on several rodent species and other sources as well (San Juan et al., 2023). Human dwellings and peridomiciliary invasions with *M. spinolai* have been reported (Frías-Lasserre et al., 2017), as well as detecting that human blood is part of its diet (Sierra-Rosales et al., 2023). Therefore, the presence of *M. spinolai* colonies near human settlements or in tourist areas represents a threat to people.

Triatomines are ectotherms and affected by low temperatures (Botto-Mahan et al., 2005). Studies of *T. cruzi* infection in *M. spinolai* collected during warm seasons detected a high fraction of infections using conventional PCR after laboratory feeding (Egaña et al., 2014, 2016). These infections were minimal in insects collected in winter when dispersion, feeding, metabolism, and development are reduced or interrupted (Botto-Mahan et al., 2005; Botzotz et al., 2023). The taxon

---

[*] Corresponding author.
[**] Corresponding author at: Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.
E-mail addresses: cbotto@uchile.cl (C. Botto-Mahan), asolari@uchile.cl (A. Solari).

[†] These authors contributed equally to this work.

https://doi.org/10.1016/j.actatropica.2025.107948
Received 17 September 2025; Received in revised form 11 December 2025; Accepted 13 December 2025

T. cruzi is composed of several discrete typing units (DTUs), named TcI to TcVI. TcI and TcII have smaller genome sizes. Meanwhile, TcV and TcVI exhibit hybrid characteristics, with higher genome sizes (Vargas et al., 2004). *Mepraia* spp. have been studied in different endemic areas of Chile, with TcI being the most prevalent (Coronado et al., 2009; Campos et al., 2016; Saavedra et al., 2022). After laboratory feedings, mixed *T. cruzi* DTU infections prevailed in *Mepraia* spp., suggesting that residual *T. cruzi* DTUs were still present in the insect gut as reservoirs, and required feeding to be amplified and detected (Egaña et al., 2016).

The life cycle of *T. cruzi* inside the invertebrate host is complex. When the triatomine *Rhodnius prolixus* is infected with blood trypomastigotes, they are extensively killed after the first day of feeding in the stomach or anterior midgut (Dias et al., 2015; Ferreira et al., 2016). However, the migration of *T. cruzi* out of the anterior midgut may be affected by strain and/or infective parasite form (Paranaiba et al., 2021). The time course of the remaining flagellates in the posterior midgut is intermediary amastigote-like forms, which differentiate into non-infective and proliferative epimastigotes (Ferreira et al., 2016).

According to one study monitoring the parasite load by means of qPCR in different gut segments after experimental infection of *R. prolixus*, on the seventh day of infection, no remnants of *T. cruzi* DNA exist in the stomach, and the maximum was obtained in the last portion of the hindgut or rectum (Dias et al., 2015). Experimental infections showed that epimastigotes proliferate when attached to the hindgut wall via their flagella terminus (Zeledon et al., 1984). Approximately 10 days post-feeding, the first remains of blood appear in the rectum, and later, when insects starve, they lose body mass, and nutritional stress triggers metacyclogenesis (García et al., 2010). In infections, approximately two weeks post-infection and during feeding, the highest number of flagellates are found in the hindgut (Dias et al., 2015). The fraction of metacyclic trypomastigotes in the rectal population can increase up to 50 % of total excreted flagellates, and more than 750,000 can be found in experimentally infected fifth instar nymphs of *Triatoma infestans* (Schaub, 1989). However, differences in colonization abilities of *T. cruzi* among triatomine species have been reported (Schaub, 2025).

During metacyclogenesis, the initial events include a decrease in the size of the reservosome of lipids, followed by autophagy (Vanrell et al., 2017). These processes involve significant degradation of proteins and organelles, ultimately leading to the complete remodeling of subcellular architecture and ultra-structural changes in *T. cruzi,* resulting in a slimmer appearance (Cardoso et al., 2011; Pérez-Morales et al., 2017). Metacyclic trypomastigotes with the new flagella detach from the hindgut wall and reach the rectal lumen to be excreted together with free epimastigotes on the vertebrate host, along with the urine flush a few minutes after the blood meal (Zeledón et al., 1988). This rapid diuresis response represents an important factor for a competent vector to transmit a stercorarian pathogen. When feeding exceeds 5–10 times their weight, *M. spinolai* nymphs trigger molting between 18–23 days post feeding in early nymphal instars, and 72–95 days in the late instars (Botto-Mahan, 2009). Interestingly, triatomines extend their cuticle to the excretory system. The rectal cuticle of triatomines has an ectodermal origin and shares the same architecture as the external integuments of insects, including the waxy hydrophobic superficial layer that protects insects from dehydration (Wigglesworth et al., 1985; Kleffmann et al., 1998). This portion of the rectum, then, is a site where *T. cruzi* epimastigotes can be retained by hydrophobic interactions and colonize the insect hindgut for some time. Therefore insect molting may represent a leak of *T. cruzi* epimastigotes during the molting process, which occurs after full engorgement and under circadian control (Steel and Vafopoulou, 2006).

Trypanosoma cruzi infections in triatomines have been studied using various classical methods, some of which employ fresh samples; however, these have low sensitivity. Others are more sensitive, as is conventional PCR, which also has some limitations. The latter detects pathogen DNA, which has limitations since *T. cruzi* might die in the hindgut after long periods of fasting. Therefore, it is crucial to determine whether the excreta of a triatomine sample contains alive *T. cruzi* or just *T. cruzi* remnants and/or DNA fragments, especially among samples with low *T. cruzi* load. This inconvenience can be addressed by examining different fecal samples collected after subsequent feedings, thereby improving the accuracy of molecular determination of parasite load. In this study, we performed successive laboratory feedings with non-infected blood to amplify hindgut resident *T. cruzi* populations in field-caught *M. spinolai.* We used qPCR to evaluate *T. cruzi* transmissibility by assessing: (i) whether *T. cruzi* load in excretions changes between successive laboratory feedings across different insect developmental stages; (ii) the impact of insect molting on *T. cruzi* excretion; and (iii) whether the *T. cruzi* load in excretions differs in insects collected during contrasting seasons.

2. Materials and methods

2.1. Permits

Mus musculus (2-month-old) used to feed triatomines were obtained from the vivarium facilities of the Faculty of Medicine, University of Chile. All procedures involving animal handling carried out in this study were performed in accordance with the rules and with the permission of the Animal Ethics Committee of the University of Chile (CBA#0987-FMUCH-2018).

2.2. Triatomine capturing site, fecal sample collection, and molting

Mepraia spinolai were manually captured at Quebrada El Cuyano (31°29′01″S, 71°03′40″ W; Chile), an area characterized by a semiarid-Mediterranean climate with widely variable temperatures throughout the year, scarce plant cover, large areas of rock piles, and with domestic (goats, sheep, dogs), and sylvatic small mammals (several rodent species and the introduced European rabbit). A total of 154 individuals were collected in December 2018 (austral summer; mean min T° ± SD = 7.36 ± 1.01 °C, mean max T° ± SD = 29.38 ± 1.67 °C) and 122 individuals in July 2019 (austral winter, mean min T° ± SD = 2.70 ± 0.52 °C, mean max T° ± SD = 20.90 ± 2.28 °C) (Fig. 1). The insects were transported to the laboratory - within 2 days - in a container with paper as a refuge. During the study, triatomines were maintained in a climatic chamber at 26 °C, 73 % relative humidity, and under a 14:10 h light:dark (L:D) cycle. Three to five days later, the collected individuals were individually fed to full engorgement with uninfected *M. musculus* anesthetized with 2 % sodium thiopental. Next, each insect was placed separately into a plastic cage with small compartments (3.2 × 3.6 × 1.5 cm) for individual identification. Thirty minutes after each feeding, spontaneous excreta samples were collected naturally without pressuring the abdomen, thereby avoiding harm to the insects. The samples were collected with 100 μl of distilled water and stored at −20 °C for follow-up evaluation. This process was repeated twice after a new feeding, 45 days later, to obtain a second excreta sample from summer and winter insects. These samples were used to extract DNA. Dead insects and ecdysis were recorded 45 days after each feeding. At this point, the remaining alive insects were fed again, as previously described.

2.3. DNA extraction and T. cruzi satellite DNA real time PCR assays

Each excreta sample was extracted in a final volume of 200 μl with conditions described in the EZNA Blood DNA Mini Kit (OMEGA BIO-TEX, Norcross, GA, USA). Additionally, to assess for inhibitors and avoid false negatives, an internal amplification control (IAC) was added in each sample during DNA extraction following Duffy et al. (2009). *T. cruzi* infection was tested in a QuantStudio® 3 real-time polymerase chain reaction (PCR) system (Thermo Fisher, USA). Samples were analyzed in duplicate using 0.4 μM of *T. cruzi* nuclear satellite DNA primers Cruzi 1 and Cruzi 2 (Piron et al., 2007; Moreira et al., 2013), 1 × HOT FIREPol® EvaGreen qPCR Mix Plus (Solis BioDyne, Tartu, Estonia),

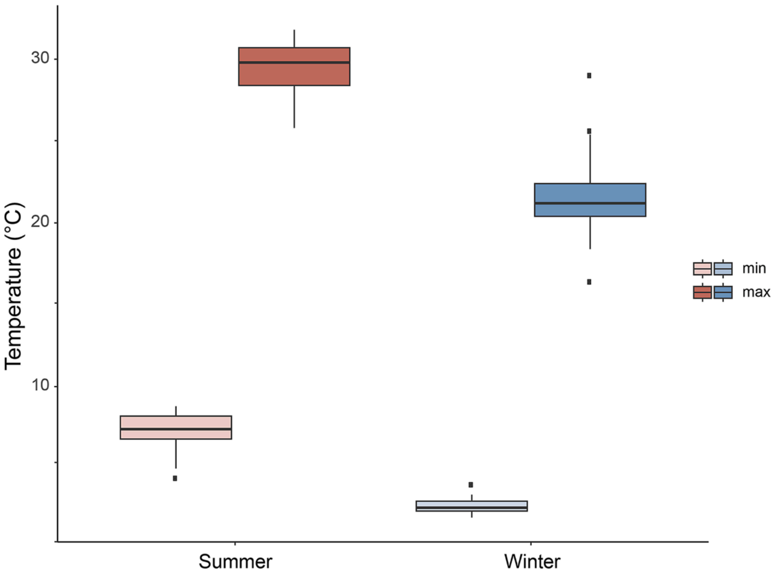


Fig. 1. Boxplot of minimum (light red and light blue boxes) and maximum (red and blue boxes) temperatures of December 2018 and July 2019, respectively, at Quebrada El Cuyano, Coquimbo Region, Chile.

and 5 µl of DNA template in a final volume of 20 µl. DNA from TcI-CDMC *T. cruzi* strain (provided by Dr. Gonzalo Cabrera, ICBM, Faculty of Medicine, University of Chile, Chile) was used as a positive control, and water instead of DNA as a no-template control. Cycling conditions were 15 min at 95 °C followed by 50 cycles at 95 °C for 15 s, 65 °C for 20 s, and 72 °C for 20 s, finishing with a default melting curve. A real-time PCR assay was conducted, amplifying IAC using specific primers with a concentration of 0.4 (Ramírez et al., 2015; Duffy et al., 2009) and the same conditions previously described. The PCR conditions were 12 min at 95 °C followed by 40 cycles at 95 °C for 15 s, 64 °C for 15 s, and 72 °C for 15 s, finishing with a default melting curve. Individuals were considered infected when *T. cruzi* and IAC amplified, and the cycle threshold value (Ct) was lower than 45.

2.4. Parasite standard calibration curve and T. cruzi load quantification

To perform absolute quantification, a standard curve was generated by mixing DNA from cultured *T. cruzi* (TcI-CDMC) and elution buffer from the kit EZNA Blood DNA Mini Kit (OMEGA BIO-TEX, Norcross, GA, USA). The *T. cruzi* DNA was diluted six times to obtain a curve from 10^6 to 10° par-eq/excreta, and the last concentration was taken as the limit of quantification (Moreira et al., 2013). The standard curve parameters were Ct = 0.283, efficiency = 100.749 %, and linear regression coefficient R^2 = 0.993. The qPCR analysis was performed on the same equipment. Since DTU I is the main circulating *T. cruzi* DTU in *M. spinolai* (Egaña et al., 2016), the estimated calculation of parasite equivalents/satellite DNA in each analyzed sample would correspond to that DTU according to Vargas et al. (2004) and Duffy et al. (2009).

2.5. Reduction/increase ratio of T. cruzi load in excreta samples of M. spinolai

Parasitic loads were classified into two categories: (i) *T. cruzi* load ≥1.0 par-eq/excreta in any of the collected excreta samples were considered as quantifiable loads; (ii) *T. cruzi* load <1.0 par-eq/excreta in any of the collected excreta samples were considered as non-quantifiable loads. Reduction/increase ratio was calculated for each insect only when both *T. cruzi* loads were ≥1.0 par-eq/excreta after feeding, by dividing the larger over the smaller *T. cruzi* load.

2.6. Statistical analyses

We performed chi-square tests to compare: (i) *T. cruzi* infection frequency, and (ii) the proportion of insects with quantifiable loads, non-quantifiable loads, and not infected, between the first and the second excreta of summer as well as winter insects. The same test was used to evaluate the association between molting and load reduction. Mann-Whitney tests were used to compare the overall parasite loads of the first and second excreta from summer- and winter-infected insects (all developmental stages combined) with quantifiable loads. These same tests mentioned above were used to compare summer and winter insects.

We performed chi-square tests to evaluate the association between *T. cruzi* infection frequency in the first excreta and the developmental stage of summer and winter insects. To this end, insects were grouped into three developmental stage categories: early (first and second instar nymphs), middle (third and fourth instar nymphs), and late (fifth instar nymphs and adults). In addition, we tested for differences in parasite loads among the three developmental stage groups using Kruskal-Wallis tests in both the first and second excreta of summer insects, considering the nymph developmental stage at arrival. Wilcoxon Signed-Rank tests were used to test for differences between the first and second excreta of each developmental stage group in the summer for individuals with two quantifiable loads. Parasite load reduction ratios were compared among the three summer developmental stage groups by a Kruskal-Wallis test. The significance level (or alpha level) considered statistically significant in this study was 0.05. Analyses were performed using R version 4.5.1, R Development Core Team 2025 (R Core Team, 2025).

3. Results

3.1. Infection frequency, parasite load, and molting of summer insects

During the summer, a total of 154 individuals arrived to the laboratory (Sample size (N): first instar nymph=18, second instar nymph=52, third instar nymph=55, fourth instar nymph=10, fifth instar nymph=7, and adults=12); however, one individual died upon arrival (one first instar nymph) and six between the first and the second feeding trial to obtain the excreta (three second instar nymph, one third instar nymph, and two adults). Therefore, 147 individuals of the summer group completed the study and were included in the following analyses.

The overall infection frequency calculated from the first excreta, i.e., after the first feeding in the laboratory, reached 83.67 % (123 out of 147 individuals), with a similar figure detected in the second excreta, 86.40 % (127 out of 147) (χ^2=0.428, $p = 0.513$). In the first excreta, 58.50 % of the individuals ($N = 86$) presented quantifiable loads, 25.17 % ($N = 37$) non-quantifiable loads, and 16.33 % no *T. cruzi* infection ($N = 24$) (Fig. 2A), and these figures did not statistically differ from the second excreta, 66.67 % quantifiable loads ($N = 98$), 19.73 % non-quantifiable loads ($N = 29$), and 13.60 % with no *T. cruzi* infection ($N = 20$) ($\chi^2 = 2.116$; $p = 0.347$). However, the median of *T. cruzi* quantifiable loads of the first excreta in all insects (2050.6 par-eq/excreta; range: 1.0–44,479.2), statistically differed from the median load of the second excreta (104.9 par-eq/excreta; range: 1.0–75,819.0) (Mann-Whitney z=−5.116; $p < 0.001$).

Considering all summer insects, between the first and the second excreta, 30 individuals changed their infection status (20.41 %; 17 from non-infected to infected and 13 vice versa). Among infected insects, 63 individuals reduced, 40 increased, and seven maintained their parasite loads.

Overall, 77 out of the 137 available nymphs molted after the first feeding (56.2 %), but molting was not statistically associated with significantly more load reduction events than non-molting (χ^2=0.061; $p = 0.806$).

3.1.1. *Stage-dependent frequency of T. cruzi infection and loads in summer insects*

All developmental stage groups exhibited similar and high *T. cruzi* infection frequency in the first excreta (Early: 87.88 %, middle: 76.56 %, and late: 94.12 %; χ^2=4.581, $p = 0.101$; Fig. 3), and this scenario was similar when analyzing the second excreta (early: 83.3 %, middle: 85.94 %, and late: 100.0 %).

T. cruzi loads did not significantly differ among developmental stage groups in the first excreta with quantifiable *T. cruzi* loads (Kruskal-Wallis, $H = 2.713$, $p = 0.258$), nor the second excreta (Kruskal-Wallis, $H = 2.952$, $p = 0.229$). However, early and late developmental groups presented significantly higher *T. cruzi* load in the first over the second excreta (Wilcoxon Signed-Rank; early nymphs: z=−2.837, $p = 0.005$, $N = 20$; late developmental stages: z=−2.845, $p = 0.004$, $N = 11$), but this difference was not detected in middle nymphs (Wilcoxon Signed-Rank; z=−0.5624, $p = 0.562$; $N = 32$) (Fig. 4ABC).

3.1.2. *Reduction/increase ratios of T. cruzi loads in summer insects*

Reduction ratios of *T. cruzi* loads differed among early nymphs (median=740.5 times), middle nymphs (median=14.0 times), and late developmental stages (median=6.7 times) (Kruskal-Wallis, $H = 6.934$, $p = 0.031$). Increase ratios of *T. cruzi* loads of early nymphs (median=30.9 times) tended to be higher than middle nymphs (median=4.2 times), but no statistical analysis was performed due to sample size.

3.2. *Infection frequency, parasite load, and molting of winter insects*

During the winter collection, a total of 122 individuals arrived to the laboratory (N: first instar nymph=20, second instar nymph=49, third instar nymph=16, fourth instar nymph=19, fifth instar nymph=16, and adults=2); however, one individual died upon arrival (one first instar nymph), four between the first and the second feeding trial to obtain the excreta (two first instar nymph, one second instar nymph, and one fifth instar nymph), and in one first instar nymph the IAC did not amplified. Therefore, 116 individuals of the winter group completed the study and were included in the following analyses.

The overall frequency of infection calculated from the first excreta, i.e., after the first feeding in the laboratory, reached 28.45 % (33 out of 116 individuals), with a similar figure detected in the second excreta, 26.72 % (31 out of 116) (χ^2=0.086, $p = 0.769$). In the first excreta, 6.90 % of the individuals ($N = 8$) presented quantifiable loads, 21.55 % ($N = 25$) non-quantifiable loads (Fig. 2B), and 71.55 % no *T. cruzi* infection ($N = 83$), and these figures did not statistically differ from the second excreta, 6.04 % quantifiable loads ($N = 7$), 20.69 % non-quantifiable loads ($N = 24$), and 73.28 % with no *T. cruzi* infection ($N = 85$) (χ^2=0.110; $p = 0.946$). Similarly, the median of *T. cruzi* quantifiable load of the first excreta, 135.8 par-eq/excreta (range: 1.4–8177.4), did not differ from the median load of the second excreta, 554.7 par-eq/excreta (range: 1.2–3810.4) (Mann-Whitney z=−0.752; $p = 0.453$).

Considering all winter insects, between the first and second excreta, 38 individuals changed their infection status (32.76 %; 18 from no infection to infection and 20 vice versa). Among infected insects, five maintained their parasite loads, five increased them, and three individuals reduced their parasite loads.

Overall, 81 out of the 114 available nymphs molted after the first feeding (71.1 %), but given the small number of infected insects with quantifiable *T. cruzi* loads, no posterior analyses were performed.

3.2.1. *Stage-dependent frequency of T. cruzi infection and loads in winter insects*

All developmental stage groups exhibited low and similar *T. cruzi* infection frequency in the first excreta (Early: 28.13 %, middle: 20.00 %, and late: 47.10 %; χ^2=4.123, $p = 0.127$) (Fig. 3), and this scenario was similar when analyzing the second excreta (Early: 26.56 %, middle: 22.86 %, and late: 35.29 %).

Given the small number of infected winter insects with quantifiable *T. cruzi* loads no posterior analyses were performed by developmental stage groups.

3.2.2. *Reduction/increase ratios of T. cruzi loads in winter insects*

Only eight and seven winter insects showed quantifiable *T. cruzi* loads in the first or second excreta, respectively. Among insects with two successive quantifiable loads, two showed a reduction, and four an increase; however, given the small sample size, no further statistical

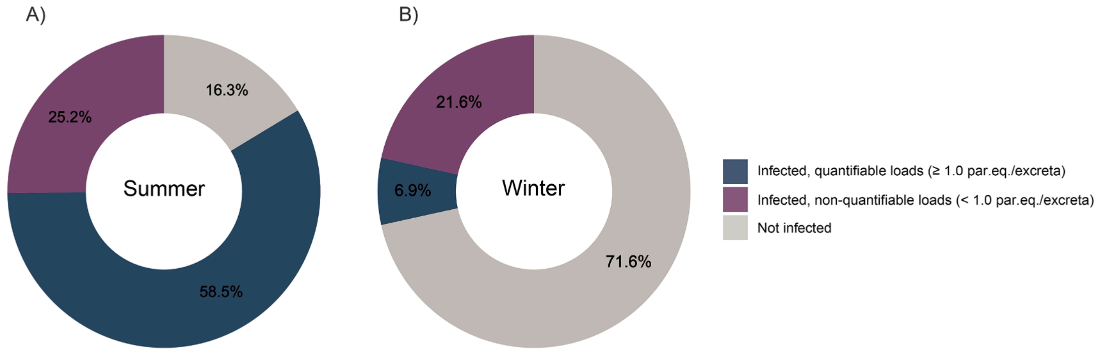


Fig. 2. Percentage of summer (A) and winter (B) insects with quantifiable *T. cruzi* load, non-quantifiable *T. cruzi* load, and non-infected in the first excreta after feeding at arrival.

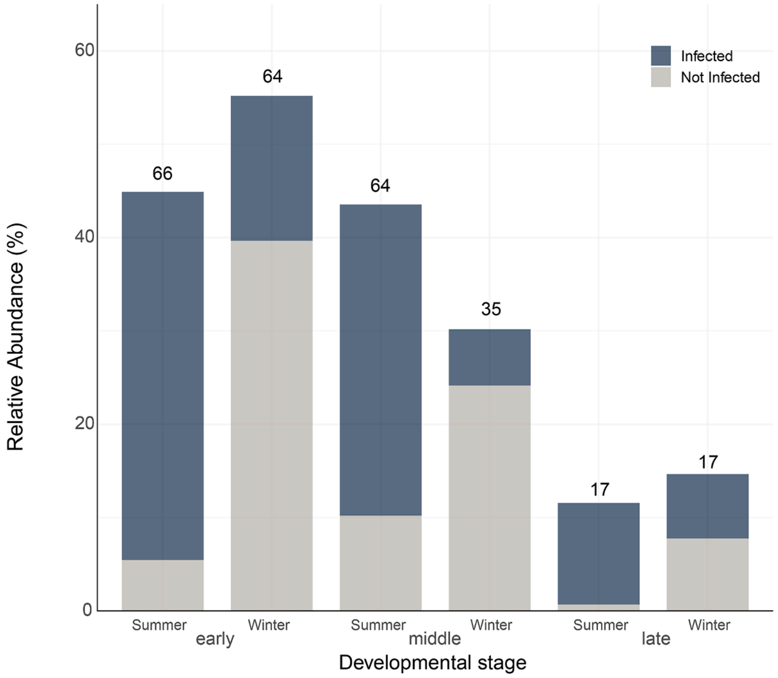


Fig. 3. Relative abundance of developmental stage groups (early: first and second instar nymphs, middle: third and fourth instar nymphs, and late: fifth instar nymphs and adults) captured in summer and winter. In each bar, the proportion of *T. cruzi*-infected insects with quantified plus non-quantified parasite loads is shown.

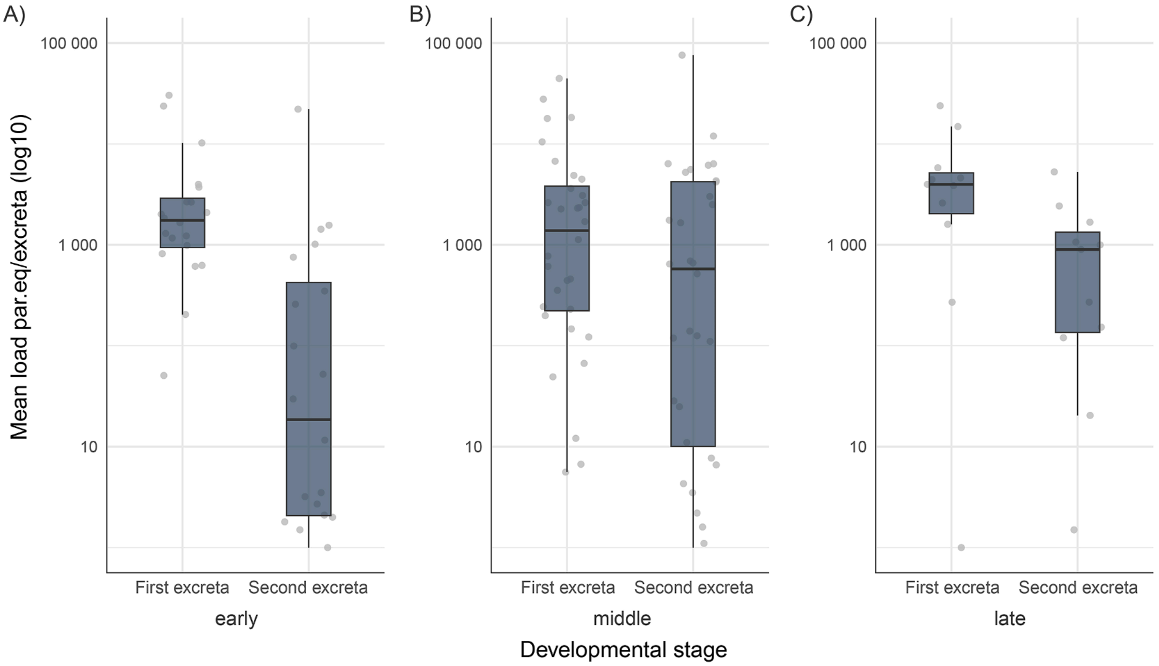


Fig. 4. Boxplot of the *T. cruzi* loads (par-eq/excreta) in the first and second excreta of summer insects, shown by developmental stage groups. (A) early: first and second instar nymphs, (B) middle: third and fourth instar nymphs, and (C) late: fifth instar nymphs and adults.

analyses were performed.

3.3. Comparing infection and loads between summer and winter insects

Overall, *T. cruzi* infection frequency at arrival (i.e., first excreta after feeding) was significantly higher in summer than in winter *M. spinolai* ($\chi^2 = 81.940$, $p < 0.001$). In addition, between the first and the second excreta, a higher proportion of winter insects changed their infection status compared to summer insects (Summer: 20.41 %, Winter: 32.76 %; $\chi^2 = 5.16$, $p = 0.023$).

In the first excreta of infected summer insects, 69.92 % ($N = 86$) presented quantifiable loads and 30.08 % ($N = 37$) non-quantifiable loads, and these figures statistically differed from winter insects with 24.24 % quantifiable loads ($N = 8$) and 75.76 % non-quantifiable loads ($N = 25$) ($\chi^2 = 22.670$; $p < 0.001$). Similarly, *T. cruzi* loads differed significantly between summer and winter insects for the first excreta

(Mann-Whitney, $z = 2.419$, $p = 0.016$), but not in the second excreta (Mann-Whitney, $z = -0.642$, $p = 0.522$).

The number of infected insects with load reduction, increase, and maintenance between the first and second excreta differed depending on the season of collection. Specifically, summer insects mostly reduced their loads (53.85 % of the individuals), while winter insects maintained their loads (89.74 % of the individuals) ($\chi^2 = 115.940$; $p < 0.001$).

4. Discussion

A competent triatomine vector can harbor *T. cruzi* for an extended period. It is epidemiologically associated with the risk of transmission to any vertebrate host. In this study, we evaluated the dynamics of *T. cruzi*-excretions in *M. spinolai* collected during warm and cold seasons by means of qPCR, a quantitative method to measure *T. cruzi* load in excreta samples after successive feedings without reinfection, even though the *T. cruzi* DTU composition was unknown in each insect sample. Considering all the information gathered with the quantifiable and non-quantifiable *T. cruzi* categories, the *T. cruzi* prevalence determined here in *M. spinolai* of the austral summer (December 2018) was 83.7 % and 28.5 % among those collected in the austral winter (July 2019).

The results of *T. cruzi* load analyzed here are different when insects are collected in summer compared with those collected in winter. *Trypanosoma cruzi*-infected insects with a quantifiable *T. cruzi* load category from the summer presented more reductions than increases or maintenance in *T. cruzi* loads after two feedings. Meanwhile, the equivalent insects from winter evaluated after feedings, maintained or presented mainly increases in *T. cruzi* load. The increases in *T. cruzi* loads probably represent residual or old infections, which need one or more feedings to amplify the few *T. cruzi* harboring the insect hindgut due to a long fasting in winter, which can last two or three months, especially considering the reduced vertebrate host availability in cold seasons (Botto-Mahan et al., 2005). A similar dynamic of *T. cruzi* load is observed in a small fraction of insects collected in the summer, which are infected with a quantifiable *T. cruzi* load. The results suggest that insects with this behavior are those that fasted for an undetermined period before being collected in summer.

The results with insects presenting a non-quantifiable *T. cruzi* category probably indicate that they are no longer infected with alive *T. cruzi* but with remnants or dead *T. cruzi* from previous infections. These kinds of infections represent a higher fraction of insects collected in winter than in summer. This was also observed in a few first, second, fourth, and fifth instar nymphs collected in winter, which presented exponential increments of *T. cruzi* burden up to maximums after a longer follow-up with three feedings (Egaña et al., 2016). The residual infections can be reactivated with prolonged feeding, and DTU TcII required three feedings to be amplified and detected by PCR (Egaña et al., 2016). We also detected insects that reduced *T. cruzi* loads from a quantifiable qPCR category to the non-quantifiable or non-infected categories. Nymphs with quantifiable qPCR showing a high reduction of *T. cruzi* load are probably insects that are in the process of losing the infection, especially in the early nymphs. These types of infections have also been described in triatomines with experimental infections (Vargas and Zeledón, 1985). Similarly, previous results from infected *M. spinolai* collected in the summer, mainly second- and third-instar nymphs, showed a reduction in natural *T. cruzi* infections evaluated after successive feedings by conventional PCR (Egaña et al., 2016; García et al., 2019).

In conclusion, excreta reductions of *T. cruzi* load after feeding represent insects that probably have fed recently in the field, increased the *T. cruzi* population in the rectal wall, later released some of them to the rectal lumen, and finally are excreted with the next feeding and concomitant diuresis. Previous studies have determined that only 25 % of flagellates reside in the hindgut lumen of late developmental stages of *T. infestans* with regular feeding in experimental infections (Schaub, 1989), and that after laboratory feeding, they re-populate with epimastigotes residing in the midgut, which do not attach to the extracellular membrane layers (Kollien et al., 1998). The same figure of 25 % was found for metacyclic trypomastigotes in infected *R. prolixus* (Dias et al., 2015).

The dynamics of *T. cruzi* load evaluated in this study after feeding differ according to the developmental stage of *M. spinolai*. In summer, the early nymphs with a quantifiable *T. cruzi* load decreased the amount of parasite excreted after feeding in a very high ratio (median ratio=687 times). Meanwhile, in the middle nymphs collected in summer, reductions were also observed, albeit to a lesser extent (median ratio=14 times). Finally, regarding fifth instar nymphs and adults, they presented mainly reductions in their *T. cruzi* loads, with the minimum ratio (median ratio=6.7 times). These results suggest that middle nymphs and late developmental stages fed frequently, and/or since they are bigger, have a higher capacity to retain epimastigotes. Meanwhile, the early nymphs, even though they can amplify epimastigotes after feeding, do not retain high amounts since they have a small rectum. Recent results in frequently fed *R. prolixus* show *T. cruzi* accumulation in the adult stage (Loshouam and Guarneri, 2024).

The huge reduction in *T. cruzi* excretion in insects collected in summer, after feeding, could be explained as well by an innate immune response triggered following a feeding without reinfection that lysed *T. cruzi*, only in the midgut and hindgut of *M. spinolai* where they reside (Azambuja et al., 2017). One of these responses was described in the midgut of *R. prolixus*. The activation has prophenoloxidase (PPO), leading to melanin that hardens the hindgut cuticle after molting (Ratcliffe et al., 1984). Melanin toxic intermediates might help in killing invading pathogens in the last portion of the gut. Additionally, an immune response of antimicrobial peptides (AMP) might participate as well in controlling *T. cruzi* proliferation in the gut lumen (Salcedo-Porras and Lowenberger, 2019). However, we believe that the reductions in *T. cruzi* loads observed here are not due to lysis by an immune response, as this phenomenon mainly occurs in summer insects. Winter insects can exponentially increase *T. cruzi* loads after a longer follow-up (A. Solari, unpublished data).

Interestingly, in spite of the fact that early nymphs reduce their *T. cruzi* load after feeding at higher rates than in other nymphal stages and adults, all summer-collected insects excreted similar amounts of flagellates/insect after the first feeding (medians between 950–2597 par-eq/insect excretion). Moreover, late developmental stages reduce their *T. cruzi* loads to a lesser extent compared to the early and middle nymphs after a feeding. They have the capacity to harbor flagellates for the next excretion and infect more hosts, thereby improving the vectorial capacity to transmit *T. cruzi*. A study reported the infectivity of *T. cruzi* excreted by the triatomine *Dipetalogaster maximus* in mice, indicating that the inoculum size ranged between 640–1250 parasites for full infections (Kirchhoff and Hoft, 1990). Therefore, our results on the total excreted *T. cruzi* DNA load ensure transmissibility with a fraction of the total excreta, remaining a crucial metric for transmission risk assessment. Notwithstanding, at this point, we cannot discard that *T. cruzi* remnants and/or DNA fragments, instead of alive *T. cruzi,* are being quantified.

T. cruzi epimastigotes attached to the rectal wall, which are shed during molting, could represent a loss of flagellates to generate new metacyclic trypomastigotes. The results of *T. cruzi* load obtained from winter and summer insects suggest that molting does not alter the reduction dynamics of *T. cruzi* loads during post-feeding excretion. Probably during molting and previous ecdysis, the new cuticle behind the reabsorbed old one is re-populated with free-swimming epimastigotes residing in the midgut, mostly free in the periphery of the gut content (Kollien et al., 1998). Previous studies with experimental infections have not found a great decrease in the *T. cruzi* rectal population with recently molting dissected bugs (Patterson and Miles, 1973; Schaub, 1989).

This study shows that triatomines captured in the winter maintain their infection after feeding when reared under optimal conditions. Due to the low temperatures during the winter months, it is likely that vectorial transmission occurs less frequently because of the insects' reduced feeding activity and the lower density of infective *T. cruzi* in their feces. Finally, we demonstrate that a quantitative tool, such as qPCR, is useful for measuring *T. cruzi* loads and evaluating changes in parasite excretion after feeding, to analyze the dynamics of *M. spinolai* transmissibility through parasite excretions in naturally infected kissing bugs collected under different ambient temperatures with unpredictable feeding. However, future studies should consider the *T. cruzi* DTU composition and long-term data in their analysis to assess how climate change, primarily related to overall temperature increases and reductions in winter rainfall, might attenuate the detected differences between triatomine vectors collected in contrasting seasons.

Author statement

The authors declare that no AI has been used in any section of the submitted manuscript.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Nicol Quiroga: Writing – review & editing, Methodology, Data curation. **Francisca Farías:** Writing – review & editing, Methodology, Investigation. **Angélica López:** Writing – review & editing, Methodology, Investigation. **Carezza Botto-Mahan:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Aldo Solari:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We especially thank Nora Peña for her invaluable work collecting *M. spinolai* in the field, Esteban San Juan for compiling the figures and graphical abstract. We are very grateful to Jorge Silva (National Forest Corporation, CONAF) for providing climatic data from Las Chinchillas National Reserve, and José Fernández from the Faculty of Medicine, University of Chile, for providing bibliographic resources. This study was funded by the Fondo Nacional de Desarrollo Científico y Tecnológico (ANID-FONDECYT) grants N° 1190392 (AS) and 1221045 (CB-M), and by ANID-ANILLO-ATE230025 (CB-M).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2025.107948.

Data availability

All raw data are included in this submission as a Supplementary excel file

References

Azambuja, P., Garcia, E.S., Waniek, P.J., Vieira, C.S., Figueiredo, M.B., Gonzalez, M.S., Mello, C.B., Castro, D.P., Ratcliffe, N.A., 2017. *Rodnius prolixus*: from physiology by Wigglesworth to recent studies of immune system modulation by *Trypanosoma cruzi* and *Trypanosoma rangeli*. J. Insect Physiol. 97, 45–65.

Botto-Mahan, C., 2009. *Trypanosoma cruzi* induces life-history trait changes in the wild kissing bug *Mepraia spinolai*: implications for parasite transmission. Vector-borne Zoonotic Dis. 9, 505–510.

Botto-Mahan, C., Cattan, P.E., Canals, M., Acuña, M., 2005. Seasonal variation in the home range and host availability of the blood-sucking insect *Mepraia spinolai* in wild environment. Acta Trop. 95, 160–163.

Botzotz, J., Méndez-Valdés, G., Ortiz, S., López, A., Botto-Mahan, C., Solari, A., 2023. Natural *Trypanosoma cruzi* infection and climatic season influence the development capacity in field-caught *Mepraia spinolai* nymphs. Insects 14 (3), 272.

Campos-Soto, R., Ortiz, S., Cordova, I., Bruneau, N., Botto-Mahan, C., Solari, A., 2016. Interactions between *Trypanosoma cruzi*, the Chagas disease parasite and naturally infected wild *Mepraia* vectors of Chile. Vector-Borne Zoonotic Dis. 16 (3), 165–171.

Canals, M., González, C., Canals, L., Canals, A., Cáceres, D., Alvarado, S., Cattan, P.E., Saavedra, M., Zulantay, I., Apt, W., 2017. What do the numbers tell us about the temporal evolution of Chagas' disease? Rev. Chil. Infectol. 34, 120–127.

Cardoso, J., Lima, C., de, P., Leal, T., Gradia, D.F., Fragoso, S.P., Goldenberg, S., De Sá, R. G., Krieger, M.A., 2011. Analysis of proteasomal proteolysis during the in vitro metacyclogenesis of *Trypanosoma cruzi*. PLoS One 6, e21027.

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

Dias, F., Guerra, B., Vieira, L.R., Perdomo, H.D., Gandara, A.C., Amaral, R.J., Vollú, R.E., Gomes, S.A., Lara, F.A., Sorgine, M.H., Medei, E., de Oliveira, P.L., Salmon, D., 2015. Monitoring of the parasite load in the digestive tract of *Rhodnius prolixus* by combined qPCR analysis and imaging techniques provides new insights into the trypanosome life cycle. PLOS Negl. Trop. Dis. 9 (10), e0004186.

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 (4), e419.

Egaña, C., Vergara, B., Campos, S., Ortiz, C., Botto-Mahan, Solari, A., 2014. *Trypanosoma cruzi* infection in *Mepraia spinolai:* the effect of feeding nymphs from the field. Am. J. Trop. Med. Hyg. 91, 534–536.

Egaña, C., Pinto, R., Vergara, F., Ortiz, F., Campos-Soto, R., Solari, A., 2016. Fluctuations in *Trypanosoma cruzi* discrete typing unit composition in two naturally infected triatomines: *mepraia gajardoi* and *M. spinolai* after laboratory feeding. Acta Trop. 160, 9–14.

Ferreira, R.C., Kellser, R., Lorenzo, M.G., Macedo, M.P.P., Ferreira, L., de, L., Probst, C. M., Alves-Silva, J., Guarneri, A.A., 2016. Colonization of *Rhodnius prolixus* gut by *Trypanosoma cruzi* involves an extensive parasite killing. Parasitology 143, 434–443.

Frías-Lasserre, D., González, C.R., Valenzuela, C.R., de Carvalho, D.B., Oliveira, J., Canals, M., da Rosa, J.A., 2017. Wing polymorphism and *Trypanosoma cruzi* infection in wild, peridomestic, and domestic collections of *Mepraia spinolai* (Hemiptera: reduviidae) from Chile. J. Med. Entomol. 54 (4), 1061–1066.

García, E., Genta, F.A., Azambuja, P., Schaub, G.A., 2010. Interactions between intestinal compounds of triatomines and *Trypanosoma cruzi*. Trends Parasitol. 26, 499–505.

García, V., Graterol, J., López, A., Ortiz, S., Solari, A., 2019. Influence of *Trypanosoma cruzi* (*kinetoplastida: trypanosomatidae*) infection on mortality of the sylvatic *triatomine* vector, *Mepraia spinolai* (Heteroptera: reduviidae), under fasting. J. Med. Entomol. 56 (5), 1384–1388.

Kirchhoff, L.V., Hoft, D.F., 1990. Immunization and challenge of mice with insect-derived metacyclic trypomastigotes of *Trypanosoma cruzi*. Parasite Immunol. 12, 65–74.

Kleffmann, T., Schmidt, J., Schaub, G.A., 1998. Attachment of *Trypanosoma cruzi* epimastigotes to hydrophobic substrates and use of this property to separate stages and promote metacyclogenesis. J. Eukaryot. Microbiol. 45 (5), 548–555.

Kollien, A., Schmidt, J., Schaub, G.A., 1998. Modes of association of *Trypanosoma cruzi* with the intestinal tract of the vector *Triatoma infestans*. Acta Trop. 70, 127–141.

Loshouarn, H., Guarneri, A.A., 2024. The interplay between temperature, *Trypanosoma cruzi* parasite load, and nutrition: their effects on the development and life-cycle of the Chagas disease vector *Rhodnius prolixus*. PLoS Negl. Trop. Dis. 18 (2), e0011937.

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

Paranaiba, L.F., Soares, R.P., Guarneri, A.A., 2021. *Triatoma infestans* susceptibility to different *Trypanosoma cruzi* strains: parasite development and early escape from anterior midgut. Parasitology 148, 295–301.

Patterson, J.W., Miles, M.A., 1973. *Trypanosoma* density in the rectum of *Rhodnius prolixus* infected with *Trypanosoma cruzi*. Trans. R. Soc. Trop. Med. Hyg. 67, 442. https://doi.org/10.1016/0035-9203(73)90072-2.

Pérez-Morales, D., Hernández, K.D.R., Martínez, I., Agredano-Moreno, L.T., Jiménez-García, L.F., Espinoza, B., 2017. Ultrastructural and physiological changes induced by different stress conditions on the human parasite *Trypanosoma cruzi*. Cell Stress. Chaperones 22, 15–27.

Piron, M., Fisa, R., Casamitjana, N., López-Chejade, P., Puig, L., Vergés, M., Gacón, J., Gómez i Prat, J., Portús, M., Sauleda, S., 2007. Development of a real-time PCR assay for *Trypanosoma cruzi* detection in blood samples. Acta Trop. 103, 195–200.

R Core Team, 2025. R: A language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ratcliffe, N.A., Leonard, C., Rowley, A.F., 1984. Prophenoloxidase activation: nonself recognition and cell cooperation in insect immunity. Science 226, 557–559.

Ramírez, J.C., Cura, C.I., da Cruz, M.O., Lages-Silva, E., Juiz, N., Velázquez, E., et al., 2015. Analytical validation of quantitative real-time PCR methods for quantification of *Trypanosoma cruzi* DNA in blood samples from Chagas disease patients. J. Mol. Diagn. 17, 605–615.

Saavedra, M., Bacigalupo, A., Barrera, M.V., Vergara, M.J., Alvarez-Duhart, B., Muñoz-San Martin, C., Solis, R., Cattan, P.E., 2022. *Trypanosoma cruzi* infection in the wild Chagas disease vector, *Mepraia spinolai*: parasitic load, discrete typing units, and blood meal sources. Acta Trop. 229, 106365.

Salcedo-Porras, N., Lowenberger, C., 2019. The innate immune system of kissing bugs, vectors of Chagas disease. Dev. Comp. Immunol. 98, 119–128.

San Juan, E., Araya-Donoso, R., Sierra-Rosales, C., Correa, J.P., Quiroga, N., Campos-Soto, C., Solari, A., Llewellyn, M., Bacigalupo, A., Botto-Mahan, C., 2023. Humans as blood-feeding sources in sylvatic triatomines of Chile unveiled by next-generation sequencing. Parasit. Vectors 16, 225.

Schaub, G.A., 1989. *Trypanosoma cruzi*: quantitative studies of development of two strains in small intestine and rectum of the vector *Triatoma infestans*. Exp. Parasitol. 68, 260–273.

Schaub, G.A., 2025. *Trypanosoma cruzi*/Triatomine Interactions-A Review. Pathogens 14, 392.

Sierra-Rosales, C., San Juan, E., Quiroga, N., Araya-Donoso, R., Correa, J.P., Solari, A., Bacigalupo, A., Botto-Mahan, C., 2023. Diet of the sylvatic triatomine *Mepraia spinolai*: Association with *Trypanosoma cruzi* infection near human settlements. Acta Trop. 248, 107039.

Steel, C., Vafopoulou, X., 2006. Circadian orchestration of developmental hormones in the insect, *Rhodnius prolixus*. Comp. Biochem. Physiol. A 144, 351–364.

Vanrell, M.C., Losinno, A.D., Cueto, J.A., Balcazar, D., Fraccaroli, L.V., Carrillo, C., Romano, P.S., 2017. The regulation of autophagy differentially affects *Trypanosoma cruzi* metacyclogenesis. PLoS Negl. Trop. Dis. 11, e0006049.

Vargas, I.G., Zeledón, R., 1985. Effects of fasting on *Trypanosoma cruzi* infection in *Triatoma dimidiate* (Hemiptera: reduviidae). J. Med. Entomol. 22, 683.

Vargas, N., Pedroso, A., Zingales, B., 2004. Chromosomal polymorphism, gene synteny and genome size in *T. cruzi* I and *T. cruzi* II groups. Mol. Biochem. Parasitol. 138, 131–141.

Wigglesworth, V.B., 1985. The transfer of lipid in insects from the epidermal cells to the cuticle. Tissue Cell 17, 249–265.

Zeledón, R., Bolaños, R., Rojas, M., 1984. Scanning electron microscopy of the final phase of the life cycle of *Trypanosoma cruzi* in the insect vector. Acta Trop. 41, 39–43.

Zeledón, R., Bolanos, R., Espejo Navarro, M.R., Rojas, M., 1988. Morphological evidence by scanning electron of excretion of metacyclic forms of *Trypanosoma cruzi* in vector's urine. Mem. Inst. Oswaldo Cruz 83, 361–365.