Institute of Parasitology, Biology Centre CAS
Folia Parasitologica 2025, 72: 011
doi: 10.14411/fp.2025.011

Folia Parasitologica
http://folia.paru.cas.cz

Research Article OPEN ACCESS

Variability in *Trypanosoma cruzi* susceptibility among species of kissing bugs (Hemiptera: Reduviidae: Triatominae) in Mexico

Alberto Antonio-Campos[1,2], **Ernesto Ramírez-Moreno**[2], **Víctor Sánchez-Cordero**[3], **Nancy Rivas**[1] and **Ricardo Alejandre-Aguilar**[1]

[1] Laboratory of Medical Entomology, Departamento de Parasitología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, Mexico;

[2] Laboratory of Protozoology, Departamento de Parasitología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, Mexico;

[3] Departamento de Zoología, Instituto de Biología. Universidad Nacional Autónoma de México, Ciudad Universitaria, Ciudad de México, Mexico

Abstract: Chagas disease, caused by the kinetoplastid *Trypanosoma cruzi* (Chagas, 1909), and transmitted by triatomine bugs, poses a significant public health challenge. Variability in the susceptibility of different triatomine species to *T. cruzi* infection can profoundly influence disease transmission dynamics and control measures. In this study, we assessed the susceptibility to *T. cruzi* infection in the first and third nymphal stages across eight triatomine species to *T. cruzi* infection using experimental inoculation with the NINOA strain and optical microscopy. The evaluated species were *Dipetalogaster maximus* (Uhler), *Triatoma bassolsae* (Alejandre-Aguilar, Nogueda-Torres, Cortéz-Jiménez, Jurberg, Galvão, Carcaballo), *T. infestans* (Klug), *T. lecticularia* (Stål), *T. mexicana* (Herrich-Schaeffer), *T. pallidipennis* (Stål), *T. phyllosoma* (Burmeister) and *T. picturata* (Usinger). The results indicated that *T. bassolsae* exhibited the highest susceptibility to infection, followed by *T. pallidipennis* and *D. maximus*. Our analysis also revealed that *T. cruzi* (NINOA) infection was significantly associated with triatomine species rather than nymphal stage ($p < 0.0001$), with substantial variability observed in susceptibility among species ($p < 0.001$). We ranked triatomine species susceptibility to *T. cruzi* infection as follows: *T. bassolsae* > *D. maximus* = *T. pallidipennis* = *T. picturata* = *T. mexicana* > *T. phyllosoma* = *T. lecticularia* = *T. infestans*. These findings enhance our understanding of *T. cruzi* transmission dynamics and offer valuable insights for the development of effective control strategies against this neglected tropical disease.

Keywords: Chagas disease, triatomine infections, vector competence, nymphal stages, Chagas disease

Chagas disease, or American trypanosomiasis, is caused by the parasite *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida: Trypanosomatidae) and is transmitted in the faeces of vector insects (kissing bugs) belonging to the subfamily Triatominae. The World Health Organisation estimates that there are currently 6–7 million infected people worldwide. In the American continent, 30,000 cases, 12,000 deaths and 8,600 neonatal infections have been reported each year. Currently, there is no effective vaccine, and the treatment is partially effective with severe side effects (WHO 2024).

There are 156 identified extant triatomine species along with three fossil species, categorised into 18 genera and 5 tribes (Gil-Santana et al. 2022, Téllez-Rendón et al. 2023). Within Mexico, records indicate the presence of 35 triatomine species, distributed across 8 genera (Ramsey et al. 2015, Martínez-Hernández et al. 2022, Téllez-Rendón et al. 2023). Nineteen of these 35 triatomines have been reported inside human dwellings, and all have been found to be naturally infected with *T. cruzi* (Ramsey et al. 2015). The genus *Triatoma* Laporte, which includes 24 species (six previously classified as *Meccus*), is the most prevalent and extensively dispersed, of which *Triatoma barberi* Usinger, *Triatoma dimidiata* (Latreille) and *Triatoma pallidipennis* (Stål) are considered the most important vectors (Salazar-Schettino et al. 2010, Ramsey et al. 2015). Risk factors associated with *T. pallidipennis* infestation have been documented in domestic and peridomestic settings, notably in states of Morelos and Michoacán, Mexico, where *T. cruzi* infection prevalence was high, exceeding 50% of samples tested (Salazar-Schettino et al. 2010, Martínez-Ibarra et al. 2011).

Recent studies have highlighted the epidemiological relevance of *Triatoma mexicana* (Herrich-Schaeffer), particularly in central states of Mexico such as Hidalgo, Querétaro and Guanajuato, where cryptic species have been identified

---

*Address for correspondence: Ricardo Alejandre-Aguilar, Laboratory of Medical Entomology, Departamento de Parasitología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Prolongación de Carpio y Calle Plan de Ayala s/n, Santo Tomás, Miguel Hidalgo, C. P. 11340, Ciudad de México, Mexico. E-mail: rialejandre@yahoo.com.mx; ORCID-ID: 0000-0003-4930-2440

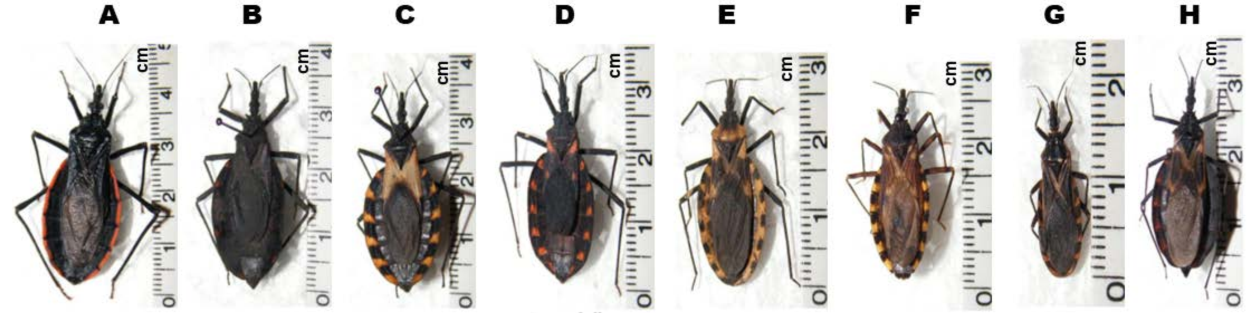

Fig. 1. Adult specimens of the triatomine species included in the study. **A** – *Dipetalogaster maximus*; **B** – *Triatoma bassolsae*; **C** – *T. pallidipennis*; **D** – *T. phyllosoma*; **E** – *T. picturata*; **F** – *T. infestans*; **G** – *T. lecticularia*; **H** – *T. mexicana*.

(Rivas et al. 2022). *Triatoma lecticularia* (Stål), another effective *T. cruzi* vector, was found infected in peridomestic areas in Nuevo León, Mexico, and Texas, USA (Grant-Guillén et al. 2018). *Dipetalogaster maximus* (Uhler), *Triatoma bassolsae* (Alejandre-Aguilar, Nogueda-Torres, Cortéz-Jiménez, Jurberg, Galvão, Carcavallo), and *Triatoma phyllosoma* (Burmeister), all limited to specific regions in Mexico, pose public health concerns (Meraz-Medina et al. 2022, Zumaquero-Rios et al. 2022, Domínguez-Cruz et al. 2024).

Furthermore, *T. phyllosoma*, endemic to southern Oaxaca, emerges as a significant Chagas disease vector (Domínguez-Cruz et al. 2024). *Triatoma picturata* (Usinger) in western Mexico shows infestation rates from 10.8% to 51.9%, with infection rates fluctuating between 18.1% and 35.2% (Meraz-Medina et al. 2023). *Triatoma infestans* (Klug), a species traditionally native to South America, has been passively introduced into Mexico. This was demonstrated in a study conducted in Manzanillo, Colima, Mexico, where 59 specimens were found in newly constructed residences (Martínez-Hernández et al. 2022).

The susceptibility of triatomines to *T. cruzi* infection can vary depending on the species of insect and environmental factors. Some triatomine species are more susceptible to infection than others, and certain environmental conditions, such as temperature and humidity, can also affect the infection likelihood (Araújo et al. 2014, de Abreu et al. 2022a,b). In addition, it has also been described that distinct species of triatomines show differences in susceptibility to infection by circulating strains of *T. cruzi.* This variability has been associated with the geographical area of both the triatomine and the strain of the parasite, although other studies show discrepancies with this hypothesis (Padilla-Valdez et al. 2021).

The variation in susceptibility of triatomine species to *T. cruzi* infection may have several negative consequences, including increased difficulty in controlling the spread of Chagas disease, an increased risk of disease transmission, and complications in disease surveillance efforts. Moreover, it can also limit treatment options for infected individuals and may require additional research to better understand the factors that contribute to differences in infection rates (de Fuentes-Vicente et al. 2018). Furthermore, variations in susceptibility may also lead to the emergence of more virulent strains of *T. cruzi* that are better adapted to infecting certain triatomine species, which could further increase the risk of disease transmission to humans and animals (Araújo et al. 2014, de Abreu et al. 2022a,b).

Understanding the susceptibility of triatomines to *T. cruzi* infection is pivotal for effective disease management. Variability in susceptibility among species and geographical areas underscores the complexity of Chagas disease transmission dynamics. In this study, we aim to systematically evaluate the susceptibility of eight triatomine species (first and third nymphal instars) to *T. cruzi* infection.

MATERIALS AND METHODS

Laboratory animals

The use, maintenance and care of the New Zealand rabbits used to feed the triatomines in the present study were conducted in accordance with the guidelines of the Official Mexican Standard (NOM-062-ZOO-1999), which governs the technical specifications for production, care and use of laboratory animals in Mexico.

Species of triatomine used and their maintenance

A total of 960 specimens (480 first-instar and 480 third-instar nymphs) from the following triatomine species were used: *Dipetalogaster maximus* (Fig. 1A), collected in Baja California Sur (collection year 2009); *Triatoma bassolsae* (Fig. 1B), Xayacatlán, Puebla (1999); *Triatoma pallidipennis* (Fig. 1C), Jiutepec, Morelos (2009); *Triatoma phyllosoma* (Fig. 1D), Tehuantepec, Oaxaca (2007); *Triatoma picturata* (Fig. 1E), Tepic, Nayarit (2008); *Triatoma infestans* (Fig. 1F), Rio Grande do Sul, Brazil (2005); *Triatoma lecticularia* (Fig. 1G), General Terán, Monterrey (2009); and *Triatoma mexicana* (Fig. 1H), Guanajuato (2010). All specimens used in this study were obtained from insectary colonies at the Medical Entomology Laboratory of the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. The insects were maintained under controlled conditions of temperature (28 ± 1 °C), relative humidity (60 ± 5%), and a 12/12 h light/dark cycle, and were regularly fed on New Zealand rabbits.

Table 1. The average amount of blood ingested by triatomine species during the development of two instar nymphs: first instar nymphs and third instar nymphs.

Triatomine species	**Blood ingested by first instar nymphs (CI 99%) (in milligrams)**	**Blood ingested by third instar nymphs (CI 99%) (in milligrams)**
Dipetalogaster maximus	104.2 ± 0 (± 0.06%) ^{A}	1335.1 ± 12.2 (± 0.91%) ^{A}
Triatoma bassolsae	13.58 ± 0.115 (± 0.85%) ^{B}	145.6 ± 3.70 (± 2.54%) ^{B}
Triatoma pallidipennis	13.4 ± 0.116 (± 0.87%) ^{B}	143.6 ± 3.76 (± 2.62%) ^{B}
Triatoma phyllosoma	13.5 ± 0.122 (± 0.90%) ^{B}	146.7 ± 3.62 (± 2.47%) ^{B}
Triatoma picturata	13.47 ± 0.122 (± 0.91%) ^{B}	146.8 ± 4.09 (± 2.79%) ^{B}
Triatoma infestans	10.85 ± 0.126 (± 1.17%) ^{C}	71.9 ± 4.85 (± 6.75%) ^{C}
Triatoma lecticularia	10.83 ± 0.137 (± 1.26%) ^{C}	66.07 ± 4.75 (± 7.20%) ^{C}
Triatoma mexicana	10.93 ±0.183 (± 1.68%) ^{C}	65.80 ± 4.77 (± 7.24%) ^{C}

Mean ± standard error of the mean (SEM), with a 99% confidence interval. Groups that do not share the same letter show statistical differences ($p < 0.05$)

Trypanosoma cruzi strain

For this study, the widely employed NINOA strain, extensively used in parasitological research, was employed. This strain, belonging to the DTU TcI (Padilla-Valdez et al. 2021) was isolated from a girl in the acute phase of Chagas disease who became infected in the state of Oaxaca, and maintained in biphasic NNN, LIT medium, as well as in colonies of infected triatomines (*T. pallidipennis*).

Triatomine infection

Triatomine infection was performed by artificial feeding according to Padilla-Valdez et al. (2021). Briefly, a concentration of 5 × x10^3 trypomastigotes/ml obtained from the faeces of infected triatomines (*T. pallidipennis*) was mixed in defibrinated and decomplemented sheep blood. This mixture was placed in a container covered with a latex membrane and incubated in a water bath at 37 °C for 30 min. Subsequently, the container was then inverted on a glass bottle with paper support inside, allowing triatomines to reach the blood. The amount of blood used to infect each triatomine was weighed before and after feeding. After artificial feeding, triatomines were fed every 15 days with blood from New Zealand rabbits.

Quantification of blood volume and parasite ingestion

The nymphs were weighed individually in 12 groups of 40 insects for each nymphal stage, with an analytical balance (BOECO, Hamburg, Germany) before and after the blood meal. Nymphs exhibiting a weight increase of ≥ 1.0 mg following a feeding event were considered successfully fed. The triatomines were kept under controlled conditions of temperature (28 ± 1 °C), relative humidity (60 ± 5%) and photo regimen (12/12-h light/dark), in the Laboratory of Medical Entomology of the Escuela Nacional de Ciencias Biológicas. After artificial feeding, triatomines were fed every 15 days with blood from New Zealand rabbits. Triatomines that died during molting when transitioning to the next nymphal stage were removed from the study.

Focecal analysis for determining parasite density

Triatomine faeces were examined every 15 days for up to 100 days using parasitological methods, including fresh examination and a differential count with Giemsa-stained smears. Samples testing positive for *Trypanosoma cruzi* infection were assessed for parasite density. This was done by examining ten fields at 400 × magnification using a LEICA DM500™ microscope, with parasite density categorised into three ranges: a) fewer than one parasite per field, b) two to ten parasites per field, and c) more than ten parasites per field.

Statistical analysis

A one-way ANOVA and Tukey pairwise comparison test was performed to analyse the differences between the amount of infected blood feeding by each triatomine species (Minitab® 19.1, State College, PA, USA), the number of infected triatomines per species in first and third instar nymphs (GraphPad Prism 8.0.1, San Diego, CA, USA, Minitab® 19), and the parasite density in those samples with a density > 10 parasites per field. Likewise, a two-way ANOVA and Tukey pairwise comparison test was performed to analyse if there exists any relationship between the infection with *T. cruzi*, triatomine species and nymphal stage (GraphPad Prism 8, Minitab® 19). The chi-square test ($\alpha = 0.01$) was used to determine if *T. cruzi* infection is independent of the triatomine species (Minitab® 19).

RESULTS

Trypanosoma cruzi infection in triatomine species

By analysing the amount of blood ingested by the different triatomine species, we observed that the eight triatomine species were grouped into three different statistical groups ($p < 0.001$), as follows: one consisting entirely of *Dipetalogaster maximus*, a second group included *Triatoma bassolsae*, *T. pallidipennis*, *T. phyllosoma* and *T. picturata*, and a third group included the smallest species *T. infestans*, *T. lecticularia* and *T. mexicana* (Table 1).

The infection with *Trypanosoma cruzi* in the eight (first instar nymphs) triatomine species showed four levels of statistical significance (ANOVA; $p < 0.001$). It is clearly observed that *T. bassolsae* (60 infected), *T. pallidipennis* (59 infected), *D. maximus* (56 infected), *T. picturata* (55 infected) and *T. mexicana* (52 infected) showed the same pattern of infection with the NINOA strain. A second group included *T. phyllosoma* (50 infected), *T. lecticularia* (44 infected), and *T. infestans* (43 infected) (Fig. 2A).

Further, the infection with *T. cruzi* in the eight (third instar nymphs) triatomine species showed five levels of statistical significance (ANOVA; $p < 0.001$), where it is clearly observed that *T. bassolsae* (60 infected), *T. pallidipennis* (58 infected), *D. maximus* (54 infected), *T. mexicana* (53 infeted) and *T. picturata* (51 infected) showed the same pattern of infection with the NINOA strain. The second

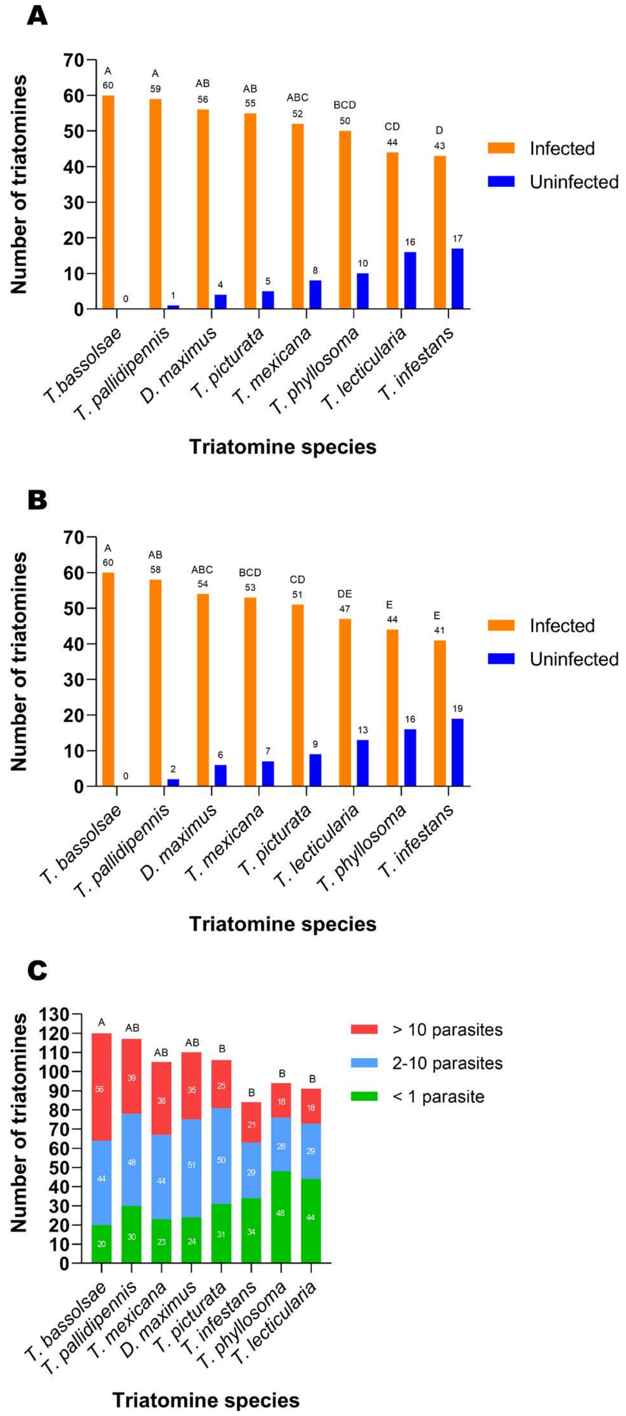

Fig. 2. Number of infected triatomines per species in the first instar nymphs (n = 480) (A). Number of infected triatomines per species in the third instar nymphs (n = 480) (B). Parasite density observed by microscopic field in first and third instar nymphs (n = 827) (C). Bars that do not share the same letter show statistical differences ($p < 0.001$).

group included *T. lecticularia* (47 infected), *T. phyllosoma* (44 infected), and *T. infestans* (41 infected) (Fig. 2B).

Trypanosoma cruzi density

Regarding the parasite density, we observed that the species showing the highest susceptibility to infection with the NINOA strain also showed the highest parasite densities. The densities > 10 parasites per field obtained two levels of statistical significance (ANOVA; $p = 0.003$); one group included *T. bassolsae*, *T. pallidipennis*, *T. mexicana* and *D. maximus*, and a second group included *T. picturata*, *T. infestans*, *T. phyllosoma* and *T. lecticularia* (Fig. 2C). In the association analysis, at the first instar nymphs of the eight triatomine species analysed, the Pearson chi-square statistic was 42.950 ($p < 0.0001$) and the likelihood-ratio chi-square statistic was 49.281 ($p < 0.0001$). Thus, at this first stage of development, the infection with *T. cruzi* appeared associated with the triatomine species. Regarding the third instar nymphs, the Pearson chi-square statistic was 40.261 ($p < 0.0001$), and the likelihood-ratio chi-square statistic was 48.072 ($p < 0.0001$). Thus, at this third stage of development, the infection with *T. cruzi* also appeared associated with the triatomine species.

Correlation among *T. cruzi*, triatomine species and nymphal stage

There was a low correlation between the infection of *T. cruzi* related to the triatomine species and the nymphal stage (two-way ANOVA; *F*-value = 2.06, $p = 0.1$). Thus, there appears to be a weak interaction between the triatomines of each species and the nymphal stages that influence the average infection with *T. cruzi*. Further, there appeared to be a strong effect of some triatomine species on the average infection with *T. cruzi* (two-way ANOVA; *F*-value = 41.73; $p < 0.0001$), and a weak effect of the nymphal stage of the triatomine on the average infection with *T. cruzi* (two-way ANOVA; *F*-value = 3.90, $p < 0.06$). Lastly, a Tukey's multiple comparison test showed that the most susceptible triatomine species to infection with *T. cruzi* were *T. bassolsae* > *D. maximus* = *T. pallidipennis* = *T. picturata* = *T. mexicana* > *T. phyllosoma* = *T. lecticularia* = *T. infestans* (*F*-value = 41.73, $p < 0.0001$), respectively (Fig. 2 A,B).

DISCUSSION

Research conducted over the years has yielded valuable insights into the parasite's ability to establish infection, replicate efficiently and develop infective forms (metacyclic trypomastigotes) in infected triatomines (Araújo et al. 2014, de Abreu et al. 2022a,b). Key parameters such as the volume of ingested blood, the proportion of infected triatomines, parasite concentration in faeces and the percentage of metacyclic trypomastigotes discharged in faeces play a crucial role in assessing the susceptibility of triatomine species to *Trypanosoma cruzi* infection (de Fuentes-Vicente et al. 2018, Schaub 2021). Studies have indicated that the predominant genotype of *T. cruzi* in Mexico is TcI (Izeta-Alberdi et al. 2023), represented by the NINOA strain, as confirmed by Padilla-Valdez et al. (2021), which was utilised in this investigation. However, recent studies have identified additional lineages such as TcII, TcIV and TcVI within the country (Izeta-Alberdi et al. 2023, Domínguez-Cruz et al. 2024).

The amount of blood consumed by triatomines serves as a commonly used parameter for evaluating susceptibility to *T. cruzi* infection. Comparative analyses have revealed a correlation between higher blood intake and elevated *T. cruzi* infection rates (Dworak et al. 2017, Padilla-Valdez et al. 2021). Nonetheless, this relationship may not always be evident due to variations in triatomine susceptibility to infection across different strains and genotypes of *T. cruzi* (Araújo et al. 2014, de Abreu et al. 2022b). We documented differences in the susceptibility of different triatomine species to *T. cruzi* infection, both in the initial stages of development (first instar nymphs) and later stages (third instar nymphs).

The categorisation of triatomine species based on the volume of blood ingested suggests that body size influences the infection, since larger triatomine species consume more blood, and thus potentially leading to a higher parasite load. Consequently, in this study, the three largest species that ingested the most blood also showed higher infection rates with the NINOA strain of *T. cruzi*. This trend was not strictly in decreasing body size order, since *Dipetalogaster maximus* is the largest species by far even in nymphal stages and remained below *Triatoma bassolsae* and *T. pallidipennis* in terms of the number of infected specimens. However, Padilla-Valdez et al. (2021) reported that *T. lecticularia* and *T. barberi*, which are relatively small species, were more susceptible to infection with five strains of *T. cruzi* than *T. pallidipennis,* which is a larger species. This suggests that while body size may influence blood meal intake but other underlying factors contribute to a triatomine's susceptibility to *T. cruzi* infection to a greater or lesser degree (de Fuentes-Vicente et al. 2018).

Most studies focus on the fourth and fifth nymphal stages. We selected first and third instar nymphs because early developmental stages provide valuable insights into how initial exposure to the parasite influences its establishment and the subsequent progression of infection. While the fifth instar may offer more information regarding blood intake and its relationship to infection, particularly for transmission, our aim was to assess susceptibility to *T. cruzi* infection in earlier instars, acknowledging that future studies could benefit from including all nymphal stages. In this context, regression models for *Triatoma infestans* suggest that the probability of infection with *T. cruzi* is consistent across all nymphal stages. Additionally, research indicates that parasite transmission is not necessarily linked to the volume of blood ingested, but rather to the frequency of feeding events (Tustin et al. 2020).

The type of blood used for triatomine feeding in infection studies is a critical factor that can significantly influence outcomes, as different blood sources affect feeding behaviour, the triatomine life cycle, parasite acquisition, and overall infection dynamics (Sant'Anna et al. 2017, de Bona et al. 2023, Martínez-Ibarra et al. 2023). In our study, defibrinated and decomplemented sheep blood was used due to its widespread availability and effectiveness in maintaining triatomine colonies in laboratory settings (Padilla-Valdez et al. 2021). However, research has demonstrated that triatomine species exhibit varied feeding preferences and susceptibility to *T. cruzi* infection depending on the blood source, whether it be from mammals, birds, or reptiles (Quiroga et al. 2022, de Bona et al. 2023, Urbano et al. 2024). This variation can influence parasite development, transmission efficiency and infection rates, underscoring the necessity for further investigations into how different blood types affect *T. cruzi* infection dynamics in triatomines under experimental conditions (de Bona et al. 2023). Future studies should explore the use of diverse blood sources and *T. cruzi* strains to better understand their impact on infection outcomes and vector competence.

Although it has been reported that all triatomine species are susceptible to *T. cruzi* infection, it is important to evaluate the susceptibility of distinct triatomine species in different geographical areas of Mexico, to assign their relative risk. Additionally, the quantity of infected triatomines is considered a key parameter for assessing susceptibility, as this characteristic, along with other parameters, is associated with triatomine species considered to be efficient transmitters of *T. cruzi* (Dworak et al. 2017, Padilla-Valdez et al. 2021, de Abreu et al. 2022b).

In our experiments, a statistically significant difference in *T. cruzi* infection was observed among the different triatomine species, with *T. bassolsae* showing 100% (60/60) of specimens infected, followed by *T. pallidipennis* with 98.3% (59/60) of specimens infected. The result observed in *T. bassolsae* had not been previously detailed, as it is a species confined to a specific geographic region in the State of Puebla, Mexico. However, the result observed in *T. pallidipennis* is consistent with previous studies (Padilla-Valdez et al. 2021).

Furthermore, this result, together with the natural infection rates (51.4%) of *T. pallidipennis* documented in endemic states (Martínez-Ibarra et al. 2011), as well as the report of new areas colonised by this species of triatomine with infection rates of 17.8%, highlights its importance as a vector of *T. cruzi* (Alejandre-Aguilar et al. 2023). In *D. maximus*, 93.3% (56/60) of the bugs got infected. Although there is no statistical difference compared to *T. bassolsae* and *T. pallidipennis*, it is worth noting the size difference between these species and *D. maximus*. Even though it showed the same infection rate and has been used for xenodiagnosis, it is important to mention that these species have only been found in the State of Baja California Sur, Mexico (Meraz-Medina et al. 2022).

In the first nymphal stage, the fourth, fifth and sixth highest rates of infection were found in *Triatoma picturata* with 92% (55/60), *T. mexicana* with 87% (52/60), and *T. phyllosoma* with 83% (50/60) respectively. However, in the third nymphal stage, the fourth, fifth and sixth highest rates were observed in *T. mexicana* with 88% (53/60), *T. picturata* with 85% (51/60), and *T. lecticularia* with 78% (47/60) respectively. In the first-stage nymphs of *T. picturata*, *T. mexicana*, and *T. phyllosoma*, there was not a clear difference in *T. cruzi* infection.

Similarly, in the third-stage nymphs, there was not much variation in infection rates for *T. mexicana*, *T. picturata*, and *T. lecticularia*. However, when it comes to *T. phyllosoma*, there was a noticeable difference. This could be due to each species' unique traits and the strain of *T. cruzi* used in our study (Araújo et al. 2014). Interestingly, although *T. mexicana* (Rivas et al. 2022), *T. picturata* (Meraz-Medina et al. 2023), and *T. lecticularia* (Grant-Guillén et al. 2018) are widespread in Mexico and play a role in public health, there have not been many studies on how susceptible they are to *T. cruzi* infection. On the other hand, *T. phyllosoma*, found exclusively in Oaxaca, Mexico, appears to be the primary vector of *T. cruzi* in the Isthmus of Tehuantepec region, where recent research has reported a high infection rate of 43.6% (Domínguez-Cruz et al. 2024).

One of the most widespread vectors of *T. cruzi* in South America is *T. infestans*. Its reappearance in Mexico after a 50-year absence was documented by Martínez-Hernández et al. (2022). In our study, this species had the lowest infection rates, both in its first-stage nymphs (72%; 43/60) and the third-stage nymphs (68%; 41/60). Recent reviews suggest that *T. infestans* can be infected by various *T. cruzi* strains, notably genotypes TcI, TcV and TcVI (Brenière et al. 2016). However, there are reported conflicting results in specimens infected with the YuYu and Dm28c strains, both TcI genotypes. YuYu showed higher parasite levels than Dm28c, indicating varying susceptibility to this triatomine species (Paranaiba et al. 2021).

Triatoma infestans and *Rhodnius prolixus* Stål have been the most extensively studied species in terms of infection with various *T. cruzi* DTUs, primarily due to their ease of handling, rearing and reproduction in laboratory environments. Several studies have shown that specific TcI strains can infect and complete their developmental cycle in *R. prolixus*, while the TcII genotype is eliminated by this triatomine species, suggesting a degree of selective susceptibility.

In contrast, *T. infestans* can sustain infection with both TcI and TcII genotypes, and additional research has demonstrated its ability to carry other DTUs, such as TcV and TcVI, which are more prevalent in the Southern Cone of South America. These observations support the theory that certain triatomine species function as biological filtres for different *T. cruzi* DTUs, with their capacity to harbour or eliminate particular lineages potentially influencing local transmission dynamics. Furthermore, these differences may affect parasite load, vector competence and the epidemiology of Chagas disease in distinct geographical regions (Zingales and Bartholomeu 2022).

Regarding parasite density, two groups with statistical differences were determined, where *T. bassolsae*, *T. pallidipennis*, *T. mexicana* and *D. maximus* comprised the first group with the highest density. It is noteworthy that this order is like that observed in the number of infected triatomines per species. Parasite density, combined with the infection percentage of each species, are some of the parameters used to define an efficient vector of *T. cruzi* (de Fuentes-Vicente et al. 2018). Interestingly, it has been documented that a high parasite burden in triatomines shortens detection time, increases the number of bites during feeding and reduces defecation time, which could enhance their vectorial capacity (Chacón et al. 2022).

The second group, composed of *T. picturata*, *T. infestans*, *T. phyllosoma*, and *T. lecticularia*, exhibited a lower parasitic density in faeces compared to the first group. This finding aligns with the observed number of infected triatomines per species within this group. It is worth noting the comparatively low parasitic density of *T. infestans* when contrasted with endemic species in Mexico.

We acknowledge some shortcomings in our study. For example, the characteristics assessed represent only a subset of the criteria necessary for a triatomine species to qualify as a competent vector of *T. cruzi*. Additional factors, including domiciliation patterns, defecation timing, natural infection rates, crowding index and geographic distribution, must also be considered. Second, it is necessary to consider the intrinsic biological behaviour of each of the triatomine species studied (de Fuentes-Vicente et al. 2018).

In addition to the optical microscopy employed in this study, alternative approaches such as conventional PCR or real-time PCR (qPCR) could have been used to detect *T. cruzi* with a higher sensitivity and specificity. Real-time PCR allows not only for the detection but also for the quantification of parasite load, providing a more accurate measurement of infection intensity. This method could offer insights into the dynamics of *T. cruzi* replication in different triatomine species, potentially revealing differences in parasite burden that may not be evident using traditional detection methods. Furthermore, reverse transcription PCR (RT-PCR) could be employed to quantify active parasitic gene expression, offering additional layers of information on parasite activity within vectors, which might influence the interpretation of vector competence and transmission risk (Duffy et al. 2013).

In conclusion, our study highlights the intricate relationship between triatomine species and susceptibility to *T. cruzi* infection. Despite the absence of a correlation between infection rates and nymphal stages, we observed a significant association between parasite infection and specific triatomine species. This underscores the importance of considering species-specific factors in assessing vector competence. Further research needs to address complex vector-parasite interactions, including a broader range of factors, and extending methodological settings to field conditions. Such investigations are essential for developing effective control strategies and mitigating the transmission of Chagas disease.

Acknowledgements. The authors are grateful to José Manuel Pérez-Rivero for technical assistance.

Authors' contributions. AAC performed the experiments and wrote the paper. ERM and VSC supervised the work and helped with statistics, visualisation and writing of the manuscript. NR and RAA conceived the idea, designed the experimental procedures and obtained the funding for this study.

REFERENCES

de Abreu A.P., Lucas Da Silva H.F., Sarto M.P.M., Iunklaus G.F., Trovo J.V., de Souza Fernandes N., Teston A.P.M., Toledo M.J.O. 2022b: Infection susceptibility and vector competence of *Rhodnius robustus* Larrousse, 1927 and *R. pictipes* Stal, 1872 (Hemiptera, Reduviidae, Triatominae) for strains of *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae) I, II and IV. Parasit. Vectors 15: 239.

de Abreu A.P., Teston A.P.M., Massago M., Lucas da Silva H.F., Ferreira É.C., Fernandes N.S., Araújo S.M., Dale C., Galvão C., Toledo M.J.O. 2022a: Experimental infection of *Rhodnius robustus* Larrousse, 1927 (Hemiptera, Reduviidae, Triatominae) with *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae) IV. Exp Parasitol. 232: 108197.

Alejandre-Aguilar R., Antonio-Campos A., Noguez-García J., Rivas N. 2023: *Triatoma pallidipennis* (Stål, 1872) (Hemiptera: Reduviidae) and its potential for infestation in Tecozautla, Hidalgo state, Mexico. J. Vector Ecol. 48: 1–6.

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

de Bona S., Correa J.P., San Juan E., Estay-Olea D., Quiroga N., Bacigalupo A., Araya-Donoso R., Botto-Mahan C. 2023: Opportunistic or selective? Stage-dependent feeding behavior in a wild vector of Chagas disease. Int. J. Parasitol. 53: 55–64.

Brenière S.F., Waleckx E., Barnabé C. 2016: Over six thousand *Trypanosoma cruzi* strains classified into Discrete Typing Units (DTUs): attempt at an inventory. PLoS Negl. Trop. Dis. 10: e0004792.

Chacón F., Bacigalupo A., Álvarez-Duhart B., Cattan P.E., Solís R., Muñoz-San Martín C. 2022: The parasite load of *Trypanosoma cruzi* modulates feeding and defecation patterns of the Chagas disease vector *Triatoma infestans*. Microorganisms 10: 1003.

Domínguez-Cruz D.C., Velázquez-Ramírez D.D., Olivo-Vidal Z.E., Fuentes-Vicente J.A., Ochoa-Díaz-López H. 2024: First report of mixed *Trypanosoma cruzi* discrete typing units infection in *Triatoma phyllosoma* in the peri-urban environment of Oaxaca, Mexico. Rev. Soc. Bras. Med. Trop. 57: e007032024.

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

Dworak E.S., Araújo S.M., Gomes M.L., Massago M., Ferreira ÉC., Toledo M.J.O. 2017: Sympatry influence in the interaction of *Trypanosoma cruzi* with triatomine. Rev. Soc. Bras. Med. Trop. 50: 629–37.

de Fuentes-Vicente J.A., Gutiérrez-Cabrera A.E., Flores-Villegas A.L., Lowenberger C., Benelli G., Salazar-Schettino P.M., Córdoba-Aguilar A. 2018: What makes an effective Chagas disease vector? Factors underlying *Trypanosoma cruzi*-triatomine interactions. Acta Trop. 183: 23–31.

Gil-Santana H.R., Chavez T., Pita S., Panzera F., Galvão C. 2022: *Panstrongylus noireaui*, a remarkable new species of Triatominae (Hemiptera, Reduviidae) from Bolivia. ZooKeys 1104: 203–225.

Grant-Guillén Y., Nogueda-Torres B., Gascón-Sánchez J., Goicochea-Del Rosal G., Martínez-Ibarra J.A. 2018: Biology of the introduced species *Triatoma lecticularia* (Hemiptera: Reduviidae) to northwestern Mexico, under laboratory conditions. Acta Trop. 177: 194–199.

Izeta-Alberdi A., Pech-May A., Tun-Ku E., Mazariegos-Hidalgo C.J., López-Cancino S.A., Gutiérrez S., Albino-Miranda S., De La Cruz-Felix K., Diaz De La Cruz J.M., Ibarra-Cerdeña C.N., Arellano E., Vallejo R.M., Ocampo M.A., Ramsey J.M. 2023: *Trypanosoma cruzi* in Mexican Neotropical vectors and mammals: wildlife, livestock, pets, and human population. Salud Públ. Mex. 65: 114–126.

Martínez-Hernández F., Villalobos G., Montañez-Valdez O.D., Martínez-Ibarra J.A. 2022: A new record of the introduced species *Triatoma infestans* (Hemiptera: Reduviidae) in Mexico. J. Med. Entomol. 59: 2150–2157.

Martínez-Ibarra J.A., Nogueda-Torres B., Montañez-Valdez O.D., Michel-Parra J.G. 2023: Influence of blood meal source on the biological parameters of *Triatoma pallidipennis* (Heteroptera: Reduviidae) from Mexico. Med. Vet. Entomol. 37: 124–131.

Martínez-Ibarra J.A., Valencia-Navarro I., León-Saucedo S., Ibáñez-Cervantes G., Bustos-Saldaña R., Montañez-Valdez O.D., Cervantes Díaz O.I., Nogueda-Torres B. 2011: Distribution and infection of triatomines (Hemiptera: Reduviidae) by *Trypanosoma cruzi* in the state of Michoacán, Mexico. Mem. Inst. Oswaldo Cruz 106: 445–450.

Meraz-Medina T., Nogueda-Torres B., Martínez-Ibarra J.A. 2022: Life history data of *Dipetalogaster maxima* (Hemiptera: Reduviidae). J. Med. Entomol. 59: 1519–1524.

Meraz-Medina T., Rúa-Vázquez P.D., Montealegre-Bautista J.F., Martínez-Ibarra J.A. 2023: Infection with *Trypanosoma cruzi* Chagas and characterization of human habitats of *Triatoma picturata* (Usinger) in western Mexico. J. Vector Ecol. J. Soc. Vector Ecol. 48: 66–71.

Padilla-Valdez J.M., Antonio-Campos A., Arias-Del-Angel J.A., Rivas N., Alejandre-Aguilar R. 2021: Susceptibility dynamics between five *Trypanosoma cruzi* strains and three triatomine (Hemiptera: Reduviidae) species. J. Vector Ecol. J. Soc. Vector. Ecol. 46: 82–95.

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.

Quiroga N., Correa J.P., Campos-Soto R., San Juan E., Araya-Donoso R., Díaz-Campusano G., González C.R., Botto-Mahan C. 2022: Blood-meal sources and *Trypanosoma cruzi* infection in coastal and insular triatomine bugs from the Atacama Desert of Chile. Microorganisms 10: 785.

Ramsey J.M., Peterson A.T., Carmona-Castro O., Moo-Llanes D.A., Nakazawa Y., Butrick M., Tun-Ku E., la Cruz-Félix K., Ibarra-Cerdeña C.N. 2015: Atlas of Mexican Triatominae (Reduviidae: Hemiptera) and vector transmission of Chagas disease. Mem. Inst. Oswaldo Cruz 110: 339–352.

Rivas N., Martínez-Hernández F., Antonio-Campos A., Sánchez-Cordero V., Alejandre-Aguilar R. 2022: Genetic diversity in peridomiciliary populations of *Triatoma mexicana* (Hemiptera: Reduviidae: Triatominae) in central Mexico. Parasitol. Res. 121: 2875–2886.

Salazar-Schettino P.M., Rojas Wastavino G.E., Cabrera Bravo M., Bucio Torres M.I., Martínez Ibarra J.A., Monroy Escobar M.C., Rodas-Retana A., Guevara-Gómez Y., Vences-Blanco M.O., Ruiz-Hernández A.L., Torres-Gutiérrez E. 2010: Revisión de 13 especies de la familia Triatominae (Hemiptera: Reduviidae) vectores de la enfermedad de Chagas, en México. J. Selva Andina Res. Soc. 1: 57–81.

Sant'Anna M.R., Soares A.C., Araujo R.N., Gontijo N.F., Pereira M.H. 2017: Triatomines (Hemiptera, Reduviidae) blood intake: physical constraints and biological adaptations. J. Insect Physiol. 97: 20–26.

Schaub G.A. 2021: An update on the knowledge of parasite-vector interactions of Chagas disease. Res. Rep. Trop. Med. 12: 63–76.

Téllez-Rendón J., Esteban L., Rengifo-Correa L., Díaz-Albiter H., Huerta H., Dale C. 2023: *Triatoma yelapensis* sp. nov. (Hemiptera: Reduviidae) from Mexico, with a key of *Triatoma* species recorded in Mexico. Insects 14: 331.

Tustin A.W., Castillo-Neyra R., Tamayo L.D., Salazar R., Borini-Mayorí K., Levy M.Z. 2020: Elucidating the mechanism of *Trypanosoma cruzi* acquisition by triatomine insects: evidence from a large field survey of *Triatoma infestans*. Trop. Med. Infect. Dis. 5: 87.

Urbano P., Hernández C., Ballesteros N., Vega L., Alvarado M., Velásquez-Ortiz N., Martínez D., Barragán K., Ramírez A., Páez-Triana L., Urrea V., Ramírez J.D., González C. 2024: Exploring dietary differences among developmental stages of triatomines infected with *Trypanosoma cruzi* in different habitats. Int. J. Parasitol. 54: 559–568.

World Health Organization (WHO) 2024: Chagas disease (American trypanosomiasis). Available from: https://www.who.int/health-topics/chagas-disease

Zingales B., Bartholomeu D.C. 2022: *Trypanosoma cruzi* genetic diversity: impact on transmission cycles and Chagas disease. Mem. Inst. Oswaldo Cruz 117: e210193.

Zumaquero-Rios J.L., López-Monteon A., López-Domínguez J., Melo-Pérez F.V.R., Torres-Montero J., Ochoa-Martínez P., Ramos-Ligonio A. 2022: *Meccus pallidipennis* and *Triatoma bassolsae* as main vectors of *Trypanosoma cruzi*: the case of Xayacatlán de Bravo, Puebla, Mexico. Southwest Entomol. 47: 45–52.

Received 15 June 2024 Accepted 11 January 2025

Cite this article as: Antonio-Campos A., Ramírez-Moreno E., Sánchez-Cordero V., Rivas N., Alejandre-Aguilar R. 2025: Variability in *Trypanosoma cruzi* susceptibility among species of kissing bugs (Hemiptera: Reduviidae: Triatominae) in Mexico. Folia Parasitol. 72: 011.