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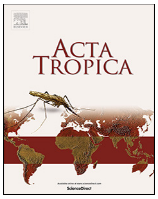

Trypanosoma cruzi could affect wild triatomine approaching behaviour to humans by altering vector nutritional status: A field test

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ABSTRACT

Hematophagous insects exhibit complex behaviour when searching for blood-meals, responding to several host stimuli. The hematophagous insect *Mepraia spinolai* is a wild vector of *Trypanosoma cruzi*, causative agent of Chagas disease in humans, in the semiarid-Mediterranean ecosystem of Chile. In this study, we evaluated the association between the approaching behaviour to a human host, with *T. cruzi* infection status and nutritional condition of *M. spinolai*. To this end, we captured 501 individuals in six consecutive 10 min-timespan, using a human as bait. Captured vectors were weighed, photographed and measured to calculate their nutritional status by means of a Standardized Body Mass Index. *Trypanosoma cruzi* infection was assessed in the intestinal content by using a real-time PCR assay. Ordinal logistic regressions were performed separately for infected and uninfected groups to evaluate if the nutritional status was associated with the approaching behaviour to a human host, recorded as the time-span of capture. Nutritional status of uninfected triatomines was higher than that from infected ones ($p < 0.005$). Among the infected, those with higher nutritional status approached first ($p < 0.01$); there was no effect of nutritional status in the uninfected group. *Trypanosoma cruzi* infection might affect the foraging behaviour of *M. spinolai* under natural conditions, probably deteriorating nutritional status and/or altering vector detection abilities.

1. Introduction

One of the most relevant activities in the life of a hematophagous insect is to find its next meal, especially after a prolonged starvation period. Search for a blood-meal responds to an integrated behaviour, which is divided into three phases: appetite search, activation and orientation, and attraction (Lehane, 2005). Search for appetite occurs when the insect is directed by hunger without an orientated behaviour. Activation and orientation are generated after receiving host stimuli, changing their behavioural patterns to one orientated. Attraction corresponds to the establishment of contact with the potential host (Sutcliffe, 1987; Lehane, 2005). In the case of the strictly blood-sucking hemipterans (Reduviidae: Triatominae) the search activity is a complex behaviour, triatomines present a high number of antennal receptors associated with this activity, given their status of temporary ectoparasites, which visit their hosts only when feeding on their blood (Lehane, 2005). Triatomines are able to detect the regular location of their hosts to increase the probability of finding food sources and thus complete their life cycle (Lehane, 2005). Host location is achieved by receiving stimuli emitted by potential preys, such as CO_2 emanations, heat and shape; although depending on the time elapsed since the last blood intake, CO_2 emanation can attract or repel the insect (Bodin et al., 2009). Even though some of the mechanisms behind host location have been described (Guerenstein and Lazzari, 2009; Moreno et al., 2006), little is known on the extrinsic factors altering host seeking behaviour in triatomines (Ramírez-González et al., 2019).

Triatomines (kissing bugs) are important biological vectors, given the role they play in the transmission of the protozoan parasite *Trypanosoma cruzi* to several mammalian species, including humans; in them it causes Chagas disease (Noireau et al., 2009). In Chile, *Mepraia spinolai* is the main wild vector of *T. cruzi*, distributed between 26° and 33° S (Frías-Lasserre, 2010; Garrido et al., 2019). The habitat of this species includes bird nests, rock crevices, rock piles, caves and bromeliads, as well as abandoned houses (Lent and Wygodzinsky, 1979;

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Schofield et al., 1982; Canals et al., 1997; Ihle-Soto et al., 2019). Several mammal species serve as blood-meals of *M. spinolai*, acting as hosts of *T. cruzi*, including rodents, carnivores, marsupials and introduced lagomorphs (Botto-Mahan et al., 2009 2010; Oda et al., 2014; Chacón et al., 2016; Ihle-Soto et al., 2019). In some areas, *M. spinolai* populations can reach over 70% of *T. cruzi* infection, with spatial and temporal variation in their abundances and infection frequency (Coronado et al., 2009; Correa et al., 2015; Ihle-Soto et al., 2019; San Juan et al., 2020). Variables directly or indirectly associated with this variation operate at different scales. For example, some abiotic variables such as temperature or precipitation have been linked to variations in abundance or infection of *M. spinolai* populations (Ihle-Soto et al., 2019; San Juan et al., 2020). Vegetation coverage has been linked to variations both in infection and abundance (San Juan et al., 2020), as also variables associated to the host communities' structure, such as abundance or richness of some vertebrate species (*Oryctolagus cuniculus, Phyllotis darwini*, reptiles or domestic mammals) (Botto-Mahan et al., 2020; San Juan et al., 2020). *Mepraia spinolai* exhibits a diurnal behaviour, using its antennal receptors to detect and approach hosts (Canals et al., 1997; Moreno et al., 2006). Triatomines present sensilla on their antennae, structures with sensory cells, mainly olfactory ones to detect host odour (Guerenstein and Lazzari, 2009). Also, they respond to contrasting moving objects, as hosts or predators, using a lateral fixation response mediated by their compound eyes (Barrozo et al., 2017).

Parasites have developed mechanisms that can affect the appearance, behaviour, and physiology of their hosts. These changes in the host could increase their probability of transmission (Poulin et al., 1994; Lefevre and Thomas, 2008; Poulin and Maure, 2015). The evolutionary dynamics between triatomines and trypanosomatid parasites indicate a potential manipulation by the parasite, as suggested for other vector borne diseases (Lefevre and Thomas, 2008). Scarce information has been reported on the effect of *T. cruzi* infection on host detection ability by triatomines. One laboratory study detected that the triatomines *Triatoma pallidipennis* and *Triatoma longipennis* infected with *T. cruzi* are more active, and orientate more times towards human odour than a control odour (distilled water; Ramírez-González et al., 2019). Furthermore, a study in *M. spinolai* individuals experimentally infected with *T. cruzi* detected and orientated towards their hosts faster and exhibited a 45% higher biting rate compared to uninfected *M. spinolai* individuals (Botto-Mahan et al., 2006). Another study showed that the infection by *T. cruzi* in *Rhodnius prolixus* decreases its locomotory activity during maximum activity hours, and the infection by *T. rangeli* increases the expression of a gene that modulates locomotion of the insect (Marliére et al., 2015). Another important variable to be considered regarding the detection and approaching behaviour by vectors is their nutritional status, estimated considering their weight and length (Schofield, 1980). Variation in nutritional status may affect population density, capacity or probability of flight initiation in *Triatoma infestans* (Schofield et al., 1992), and in other cases, sensory response alterations in host searching by *R. prolixus* (Castillo-Neyra et al., 2015).

Notwithstanding, to our best knowledge, it is unknown if the same kind of alterations occur under field conditions, especially considering the complexity of natural environments and the variable *T. cruzi* load of free-ranging triatomines. In this study, we examined if the approaching behaviour to humans of the triatomine *M. spinolai* is associated with its infection and nutritional status on an individual basis.

2. Materials and methods

2.1. Study site

This study was carried out in a protected area, Las Chinchillas National Reserve (31° 30′ S, 71° 06′ W; Chile, Fig. 1), a hyper-endemic zone of Chagas disease (Botto-Mahan et al., 2010). The climate is semi-arid Mediterranean-type with most rainfall concentrated between June and August. The mean annual precipitation is 185 mm, alternating long droughts and unusual years of high rainfall seemingly associated with El Niño events (Di Castri and Hajek, 1976). Vegetation is thorny and mainly represented by shrub and cactus species (Luebert and Pliscoff, 2006).

2.2. Kissing bug collection

In January 2018, we captured individuals of *M. spinolai* from 12 different capturing points (colonies, hereafter (Fig. 1). In each colony, the same trained researcher, acting as human bait, manually captured kissing bugs as they approached during six consecutive 10 min-time-span (total time: 1-hour), in sunny days between 11:00 and 13:00 h, the time of day with maximum activity of this species (Canals et al., 1997). Given that under field conditions we are unable to know when the triatomines started their approaching movement after perceiving the cues emitted by human bait, the approaching behaviour was evaluated using the 10 min-time-span capture interval in which each triatomine was captured.

Once captured, the insects were maintained in a dark and cold place to avoid stress, mortality and weight losses; then, they were classified by stage of development and weighed (precision: ± 0.1 mg). In addition, an ordinal categorization of the abdomen shape was performed to describe extended starvation to recently engorged individuals, as shown in Fig. 2. These measurements were performed during the same day of capture, between 18:00 and 24:00, and the engorgement was assessed by the same researcher. Finally, triatomines were individually stored and euthanized with a 48-h cold shock at −20 °C.

2.3. Processing of captured *M. spinolai* individuals and nutritional status estimation

At the laboratory, captured insects were photographed on graph paper. Total body length and maximum abdomen width were measured on the photographs using the software Image J (version 1.x, Schneider et al., 2012). With these values and the weight measured in the field, we estimated the nutritional status for each insect as a Body Mass Index (*BMI*), calculated using the following equation (Peig and Green, 2010):

$$BMI = \frac{M}{(L_{tb} * W_{ma}) * 10000}$$

where M is the mass of the individuals (in g), L_{tb} is total body length, and W_{ma} is maximum abdomen width (both in cm). To obtain a *BMI* value independent from the stage of development, this value was standardized with the equation (Yu et al., 2009):

$$SBMI = \frac{BMI - V_{min}}{V_{max} - V_{min}}$$

where *SBMI* is the standardized body mass index, V_{min} is the minimum value of *BMI* in that specific age (nymph and adults) and V_{max} is the maximum value of *BMI* in that specific age.

2.4. DNA extraction from intestinal content of captured triatomines

Captured triatomines were subjected to abdominal extrusion to obtain both intestinal content and intestine samples. An aliquot of a maximum of 25 mg from each sample was mixed with 20 µl of nuclease-free water. Whole DNA was isolated from the samples using the DNeasy® Blood & Tissue Kit (QIAGEN, CA, USA). An internal amplification control (IAC) was added to each sample to assess the presence of inhibitors, consisting in 100 pg of *Arabidopsis thaliana* DNA (Duffy et al., 2009). The manufacturer's recommendations were followed, but the samples were centrifuged for 4 min at 17,000 ·g to dry the DNeasy Mini spin column, and the final elution volume was 100 µl. Samples were stored at −20 °C until molecular analysis.

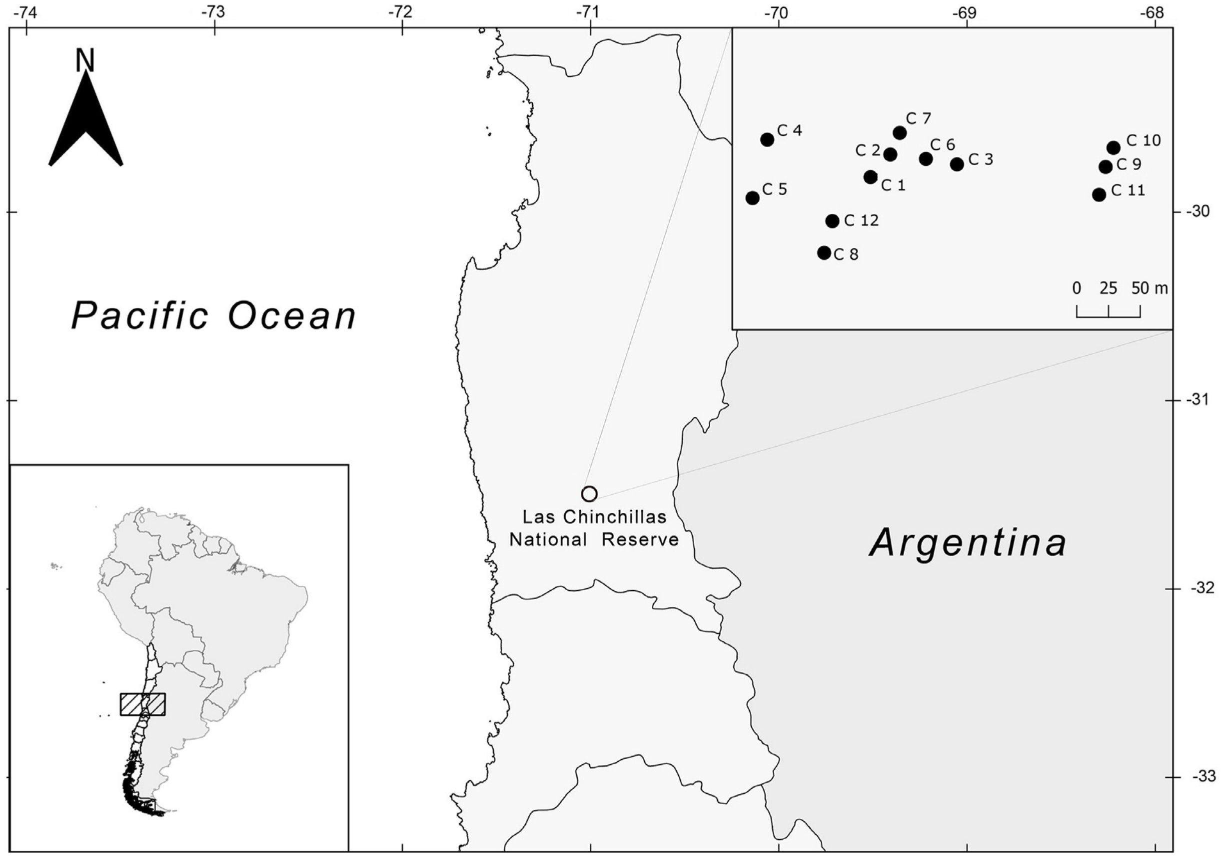

Fig. 1. Map of the study site, Las Chinchillas National Reserve, Chile. Open circle corresponds to the study site. Black dots correspond to the *Mepraia spinolai* colonies prospected.

2.5. Real-Time PCR assays of *Trypanosoma cruzi* satellite and IAC DNA

PCR assays were performed using 0.4 µM of *T. cruzi* nuclear satellite DNA primers Cruzi 1 and Cruzi 2 (Piron et al., 2007), 1 × HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Taru, Estonia) and 5 µl of DNA template, with a final volume of 20 µl. 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. To test for false negative samples, a PCR assays amplifying IAC DNA were performed using primers IAC Fw and IAC Rv, 0.4 µM each (Ramírez et al., 2015). The rest of the master mix components were the same as described above. 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. A triatomine was considered positive when the IAC was efficiently amplified, and when the threshold cycle (Ct) for *T. cruzi* was $<$ of 42; samples with a Ct value $>$ 42 were submitted to an electrophoresis looking for an amplicon of 166 bp (Piron et al., 2007). All the assays were run in a QuantStudio 3 Real-Time PCR System (Thermofisher, USA) with each sample in duplicate, using DNA extracted from *T. cruzi* culture as a positive control and nuclease-free water as not template control in each assay.

2.6. Statistical analyses

To compare differences in the age structure of uninfected and infected triatomines, we used a Kolmogorov-Smirnov test. To assess whether the categorization of the shape of the abdomen was associated with the *SBMI*, we constructed a Generalized Linear Model (GLM) with a quasibinomial distribution. A Kruskal-Wallis analysis was performed to detect the effect of infection on the *SBMI*. Then, we used an ordinal logistic regression to evaluate if *SBMI* (explanatory variable) was associated with the approaching behaviour (response variable), recorded as the 10 min-time-span capture interval of that individual (timespan: 1 to 6). All the analyses were performed with the R software (version 3.6.0, R Development Core Team 2019) and JMP-Pro (version 14).

3. Results

3.1. *Trypanosoma cruzi* infection and population age structure

A total of 501 insects were captured (capture rate mean ± SE: 41.6 ± 0.9 insects/hour), including 457 nymphs and 44 adults (detailed individual based information with the complete dataset of the variables used in this study can be found in Estay-Olea et al., 2020). A total of 276 insects (55.1%) were PCR-positive (mean Ct ± SD = 33.4 ± 7.66); all samples amplified in the IAC PCR assays. When assessing age structure in both uninfected and infected groups, first instar nymphs were overall the most represented age stage captured ($N = 161$, and $N = 122$, respectively; Fig. 3; Supplementary data - Table 1). The age structure in the uninfected and infected groups differed (Kolmogorov-Smirnov test, $p < 0.001$), with a higher relative frequency of first instar nymphs in the uninfected group than in the infected group (71.6% versus 44.2%, respectively).

3.2. Nutritional status and infection status

The *SBMI* was significantly related to the abdomen shape categorization (GLM, $p < 0.001$), where extremely concave abdomen (1) obtained the lowest *SBMI* and extremely convex abdomen (5) the higher *SBMI* (Fig. 4). The *SBMI* was significantly higher in uninfected triatomines (median = 0.583) compared to those infected (median = 0.462)

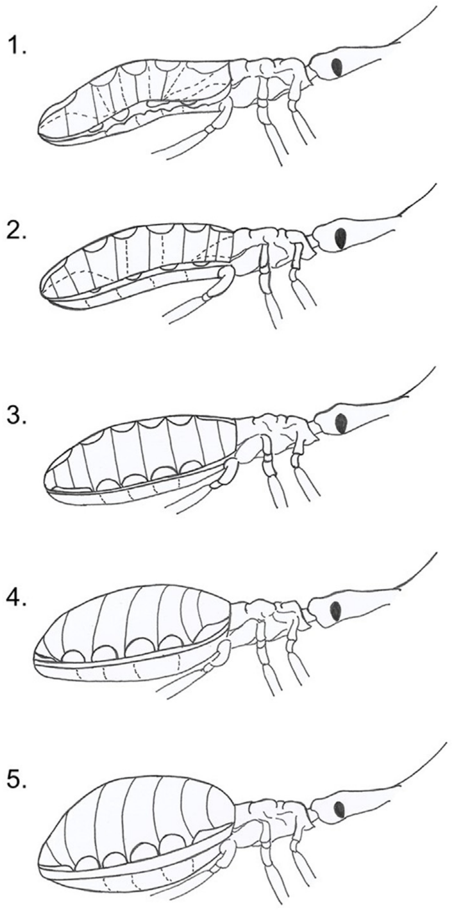

Fig. 2. Categories of abdomen shape of *Mepraia spinolai* individuals. Categories run from extremely concave abdomen (1), moderately concave abdomen (2), flat abdomen (3), moderately convex abdomen (4), and extremely convex abdomen (5).

(Kruskal-Wallis test, $p < 0.005$; Fig. 5).

3.3. Arrival time according to nutritional and infection status

Because of the significant association between *T. cruzi* infection and *SBMI*, we ran the ordinal logistic regression for the uninfected and infected group separately, testing the association between the former variables and the arrival time. In the infected group mostly individuals with higher SBMI arrived in the first-time interval of capture (ordinal logistic regression, $p < 0.01$; Fig. 6). We did not detect an effect of the SBMI on the arriving time of uninfected triatomines ($p = 0.09$; Fig. 6).

4. Discussion

We found that the approaching behaviour to a human host by *M. spinolai* is related to *T. cruzi* infection status and nutritional status, in which infected insects with higher nutritional status approach first to a human host compared to those infected with lower nutritional status. This pattern is not detected in uninfected insects. The attraction of triatomines to hosts is mediated by several cues emitted by hosts including heat, shape, humidity or odour. Even though in our field study we did not evaluate which human cues were the ones that attracted triatomines, they are probably above the threshold described for other triatomine species in laboratory settings (Barrozo and Lazzari, 2004).

More than half of the total triatomines captured were infected with *T. cruzi* (55.1%), an infection frequency within the range previously described (39.7% - 76.1%; Coronado et al., 2009; Botto-Mahan et al., 2010; Ihle-Soto et al., 2019). Infection in triatomine bugs exhibits temporal and spatial variation, and detection of infection depends on the starvation levels of insect vectors, in which *T. cruzi* infection is more likely to be detected when vectors have recently fed (Egaña et al., 2014). The capture frequency of developmental stages differed between the groups; the uninfected group was mainly represented by first instar nymphs, with very low representation of other nymphal stages. In the case of the infected group, this difference was less marked. Because triatomines mainly acquire *T. cruzi* from blood-meals on infected mammalian hosts (Garcia et al., 2010) or by coprophagy on infected triatomines' faeces (Lehane, 2005), infection probability increases with the progress of developmental stages. Additional studies should be carried out in other seasons, for example during the cold months of winter, to evaluate how the capture frequency of different developmental stages and *T. cruzi* infection of triatomines change, as described by other studies on natural populations of *M. spinolai,* in which infection rate varies according to season and feeding status (Ihle-Soto et al., 2019; Mc Cabe et al., 2019).

Our results showed that the *SBMI* may be considered an accurate indicator of the nutritional status of a triatomine, due to the strong relationship detected with *M. spinolai's* abdomen shape, especially in

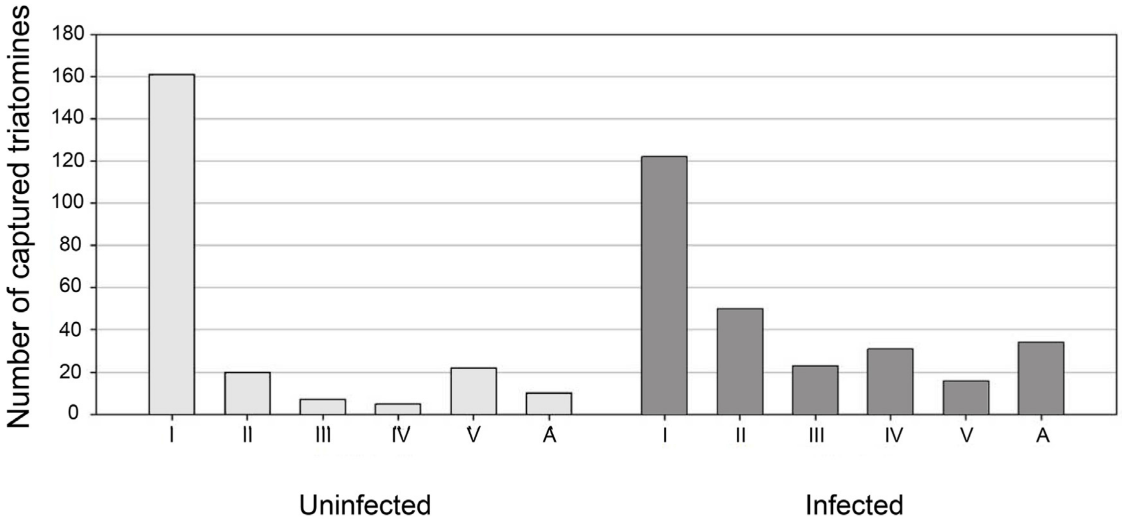

Fig. 3. Total number of captured triatomines by age, in uninfected and infected groups. I, II, III, IV and V correspond to first, second, third, fourth and fifth instar nymphs, respectively. A corresponds to adults (males and females combined).

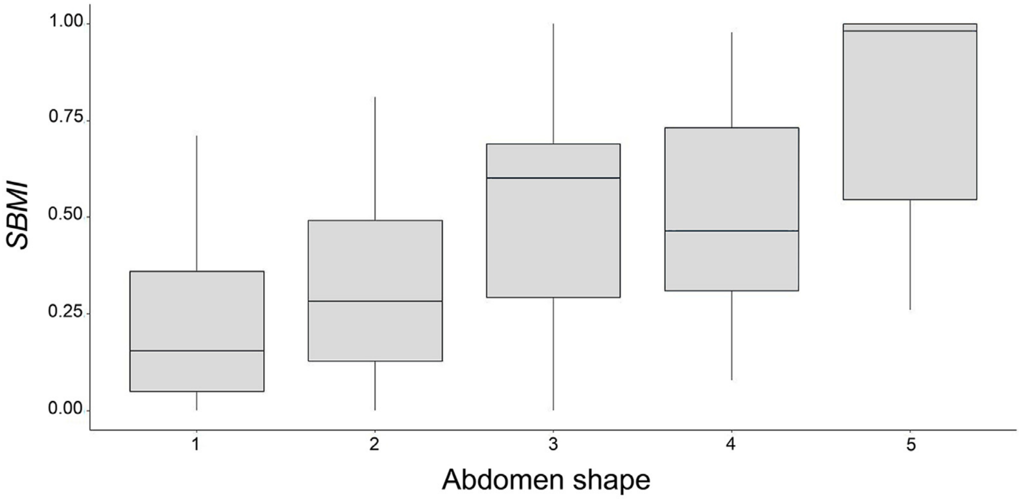

Fig. 4. Nutritional condition (*SBMI*) by abdomen shape. (1) extremely concave abdomen; (2) moderately concave abdomen; (3) flat abdomen; (4) moderately convex abdomen; (5) extremely convex abdomen.

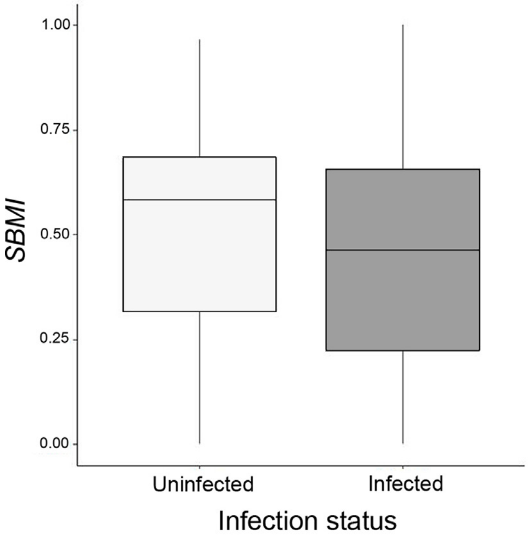

Fig. 5. Nutritional condition of triatomines (*SBMI*) by infection status.

the more extremes categories. A blood meal may increase 5–10 times the insect's body weight, changing the abdomen shape, in which the signal of end-feeding is provided by abdominal stretch mechanoreceptors (Anwyl, 1972; Chiang and Davey, 1988). The uninfected groups presented a higher nutritional status than the infected group, in line with previous studies suggesting that *T. cruzi*-infected triatomines have a lower weight than those uninfected, need more time to reach maturity and present lower survival, especially before the last stage (Botto-Mahan, 2009; Schaub, 1992). Kollien and Schaub (2000) suggested that trypanosomatids may compete for trace nutrients with triatomines. In addition, the acquisition of *T. cruzi* generates an immune response in the triatomine (Garcia et al., 2010), which also may decrease its nutritional status or body condition in general. Nevertheless, the fitness cost of *T. cruzi* infection in triatomines could differ according to the type of infecting parasite, as shown in *Meccus* (*Triatoma*) *pallidipennis* infected with two strains of *T. cruzi* that had different outcomes in relation to size, production and success of eggs, survival, and parasite load (Cordero-Montoya et al., 2019).

The impact of this protozoan parasite on the approaching behaviour of *M. spinolai* was related with the nutritional status. This result, in which infected insects with the best nutritional status arrived during the first interval of capture, could be due to low physiological impact of *T. cruzi* on triatomines with higher nutritional condition. A previous study reported that infected *T. infestans* under optimal nutritional

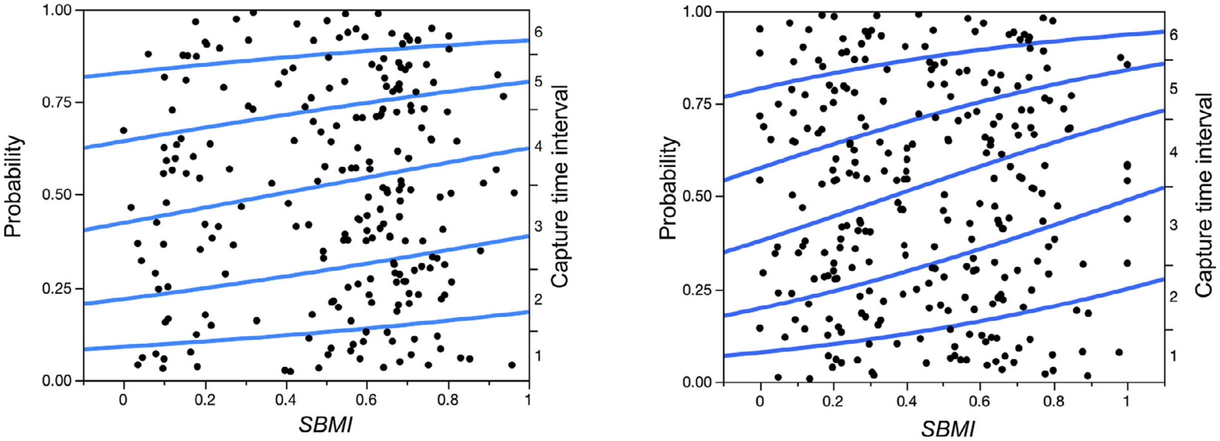

Fig. 6. Scatter plot of the ordinal logistic regression for capture time interval (10 min-time-span: 1 to 6) in relation to the standardized body mass index (*SBMI*). Area under the curve (blue lines, only online version) represents the probability of an individual of a determined value of *SBMI* (black dot) to be captured in each capture time interval. Left panel: Uninfected triatomines; Right panel: Infected triatomines.

condition presented a mortality rate of 9%, a figure only slightly higher than the 4% reported for uninfected *T. infestans* (Schaub, 1988). However, Schaub and Lösch (1989) found that *T. cruzi* infection reduces the starvation resistance of *T. infestans* by about 15% when comparing with uninfected individuals, which could be related to our finding that uninfected triatomines arrive at any capture interval, regardless of their nutritional status. If the SBMI is a proxy of the time elapsed since the last blood-meal, probably the observed differences in the approaching time in infected individuals could be associated with different resistance to starvation translated into different approaching velocities to hosts.

Laboratory experimental evidence shows that *M. spinolai* infected with *T. cruzi* finds hosts almost twice as fast as uninfected individuals (Botto-Mahan et al., 2006), probably indicating that *T. cruzi* alters the feeding behaviour of this triatomine depending on its nutritional status. In addition, infected *R. prolixus* decrease their locomotory activity in the absence of host cues; this could be considered an energy saving mechanism to avoid loss of nutritional resources (Marliére et al., 2015).

In summary, our findings suggest that under natural conditions *T. cruzi* may induce changes in the nutritional status and, in turn, in the approaching behaviour of *M. spinolai*. Even when field studies can improve the understanding of natural processes, such as vector-parasite interactions, in our study some variables could not be established; for example, the time elapsed since a triatomine became infected by *T. cruzi*, or the time since its last meal (i.e., the starvation period). To improve our understanding of the changes *T. cruzi* may produce in triatomines, future studies should evaluate how variation in parasitic loads, DTUs or genotypes, or infection according to triatomine age, could explain part of the large variability detected in the approaching behaviour of infected triatomines. In order to evaluate if the observed pattern is an adaptation of *M. spinolai* to humans, different host species should be tested as baits in future studies.

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CRediT authorship contribution statement

Daniela Estay-Olea: Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Juana P. Correa:** Conceptualization, Methodology, Formal analysis, Resources, Writing - review & editing, Supervision, Funding acquisition. **Sophie de Bona:** Investigation. **Antonella Bacigalupo:** Investigation, Writing - review & editing. **Nicol Quiroga:** Investigation. **Esteban San Juan:** Formal analysis, Investigation, Visualization. **Aldo Solari:** Conceptualization, Writing - review & editing. **Carezza Botto-Mahan:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2020.105574.

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