

Infección por *Trypanosoma cruzi*, unidades discretas de tipificación y preferencias alimenticias de *Psammolestes arthuri* (Reduviidae: Triatominae) colectados en el Este de Colombia

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Trabajo presentado como requisito para optar por el título de Bióloga

Facultad de Ciencias Naturales y Matemáticas

Universidad del Rosario

Bogotá D.C., 2019

Trypanosoma cruzi infection, Discrete Typing Units and feeding sources among *Psammolestes arthuri* (Reduviidae: Triatominae) collected in eastern Colombia

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Abstract

Background: Chagas disease (CD) is caused by the protozoan parasite *Trypanosoma cruzi*, being transmitted by hematophagous insects of the family Reduviidae. *Psammolestes arthuri* is a sylvatic triatomine distributed in Colombia and Venezuela which feeds on birds and there are few studies that report *Ps. arthuri* naturally infected with *T. cruzi*. In Colombia, *Ps. arthuri* have been found in dwellings, making important to evaluate the presence of *T. cruzi* and their feeding sources. We aimed to evaluate the presence of *T. cruzi* and feeding sources of *Ps. arthuri* as a first approach to elucidate new possible sceneries of *T. cruzi* transmission in the country.

Methods: A total of 60 *Ps. arthuri* were collected in Arauca and Casanare, Colombia. We detected and genotyped *T. cruzi* in collected specimens and identified its feeding sources. Presence of *T. cruzi* was evaluated and compared with different eco-epidemiological variables. Multiple Correspondence Analysis was conducted to explore associations between eco-epidemiological variables and the presence of *T. cruzi* and with those results, a logistical regression was made to determine statistical associations.

Results: *T. cruzi* was detected in 70% (42/60) of *Ps. arthuri* individuals. Regarding Discrete Typing Units (DTUs), TcI was found in 54.8% (23/42) of samples, TcII-TcVI in 14.3% (6/42) and mixed infection in 9.5% (4/42). In samples with TcI and mixed infection, TcI_{Dom}-Sylv was detected in 44.4% (12/27), TcI_{Dom} in 25.9% (7/27) and single infection with TcI_{Sylv} was not detected. A total of 16 feeding sources were identified, and the most frequent were: *Cranioleuca baroni* (35.85%), *Homo sapiens* (26.42%), *Thraupis episcopus* (11.32%) and *Serinus albogularis* (3.77%).

Conclusions: Despite *Ps. arthuri* feeding preferences are specially birds, they may be feeding on other animals that can be infected by *T. cruzi* which may explain parasite presence in them. Further studies with molecular techniques and wider sampling are needed to improve information regarding infection rates and feeding sources with the aim to evaluate whether *Ps. arthuri* could have the potential to transmit *T. cruzi*.

Keywords: Chagas disease, Trypanosoma cruzi, Psammolestes arthuri, Colombia.

Background

Trypanosoma cruzi is a flagellated protozoan that causes Chagas Disease (CD), and is mainly transmitted by insects of the Reduviidae (Order: Hemiptera) family through their feces [1]. CD affects about 6-7 million people around the world and is especially important in Latin America, where it is considered a public health problem [2]. Based on 2010 estimates, in Colombia there are 437,960 infected people approximately, with an annual estimated of 5,274 new cases due to vectorial transmission [3]. CD has been reported in different geographic regions of the country, with the departments of Casanare, Arauca, Santander, Boyacá, Norte de Santander and Cundinamarca being endemic for the disease [4]. Due to the high genetic diversity of *T. cruzi*, the parasite has been subdivided into discrete typing units (DTUs): TcI to TcVI [5, 6] and a genotype associated to bats (TcBat) [7, 8, 9]. Each DTU presents different characteristics based on its geographical distribution, clinical manifestations of the disease and epidemiological associations. TcI is the most widely distributed DTU in the Americas, and because of its genetic diversity, it has been divided into domestic and sylvatic genotypes (TcI_{Dom} and TcI_{Syl}) [10].

Triatomines of the family Reduviidae are widely distributed in the Americas, mainly in neotropical regions at different altitudes, giving them the ability to be adaptive and feeding on different sources, especially sylvatic and domestic mammals [11, 12]. There are about 180 species of synantropic, sylvatic and domestic animals that have been reported as potential *T. cruzi* reservoirs, mainly rodents, marsupials (didelphimorphos) [13], bats, dogs, chickens, cows, armadillos and other mammals such as anteaters and humans [13, 14, 15, 16]. In Colombia there are 26 triatomine species registered, of which 15 have been reported as naturally infected by *T. cruzi* [17, 18]. *Rhodnius prolixus* and *Triatoma dimidiata* are the main species in the country that transmit *T. cruzi* to humans and are considered primary vectors of the parasite, therefore, surveillance programs focus mainly in these two species [15, 18]. Recently, some species such as *Panstrongylus geniculatus, Rhodnius pallescens* and *Eratyrus mucronatus*, have begun to be considered

important in the parasite transmission, because of their domiciliation process due to deforestation and urbanization [18, 19] that disturb triatomine ecology [11, 15]. Also, defaunation caused by ecological disturbance processes such as anthropogenic deforestation and land-use conversion, directly impacts biotic interactions [20]. In this case, fauna migration and extinction can affect indirectly triatomine populations due to their hematophagic habits, forcing them to find other ecosystemic units and feeding sources to establish in.

Some sylvatic triatomine species are considered as non-risky for transmission of *T. cruzi* to humans, which include *Psammolestes arthuri, Panstrongylus lignarius, Cavernicola pilosa, Belminus rugulosus, Rhodnius colombiensis, Triatoma dispar* and *Microtriatoma trinidadensis. Psammolestes arthuri* is a sylvatic triatomine distributed in Colombia and Venezuela which mainly feed on birds, being frequently found in the nests [21]. There are a few studies that report *Ps. arthuri* naturally infected with *T. cruzi* [22, 23, 24]. One study conducted in Venezuela by Cruz-Guzmán and colleagues in 2014 reported *Ps. arthuri* naturally infected by *T. cruzi* and it is still not been considered important in the transmission of *T. cruzi* for humans [25, 26]. Despite of this, it is not dismissed the possibility that *Ps. arthuri* is feeding on *T. cruzi* reservoirs, contributing to the transmission of *T. cruzi* [25, 27]. In Colombia, *R. prolixus, E. mucronatus* and *Ps. arthuri* have been reported inside the houses, associated to the presence of *Attalea butyracea* nearby them [28, 29]. This fact is relevant because there is a possibility that *Ps. arthuri* could be participating in the domestic cycle of *T. cruzi*. It must be considered the ability of the triatomines to modulate their feeding behavior depending on the feeding sources available, because this is the reason why some sylvatic triatomines habituate to domicile [30].

Therefore, in the light of the current absence of studies in Colombia about the plausible presence of *T. cruzi* in sylvatic triatomines, we evaluated the presence of the parasite and feeding sources of *Ps. arthuri* collected in the departments of Arauca and Casanare, as a preliminary evaluation which may leads to the

execution of future studies that allows truly evaluate if *Ps. arthuri* could be participating in the transmission of *T. cruzi*.

Methods

Sampling, DNA extraction and detection of T. cruzi

A total of 60 Ps. arthuri individuals were collected in the departments of Arauca (2 individuals) and Casanare (58 individuals) (Fig. 1A). In Casanare, individuals were collected in the municipalities of Mani (12/60) (Mararabe 4.8333, -72.35), Monterrey (14/60) (Marenao 4.884472, -72.7553), Paz de Ariporo (4/60) (Caño chiquito 5.75, -71.48333), Pore (12/60) (El verde 5.6761665, -72.0752884) and Tamara (16/60) (La picacha 5.84462, -72.16366). In Arauca, the individuals were collected in Arauquita (2/60) (Arauca 6.9682918, -71.1965589). Casanare individuals were collected in trees near human houses (Fig. 1B) and captured in Cacicus cela (15/60) (Yellow-rumped Cacique) and Phacellodomus rufifrons (43/60) (Rufousfronted Thornbird) nests using manual capturing (Fig. 1C). Furthermore, Arauca insects were collected in domestic ecotope (inside the houses). All specimens were stored and conserved in ethanol until processing. DNA extraction of insect's gut was conducted using the Qiagen Dneasy Blood & Tissue kit. Detection of T. cruzi was conducted by end-point qPCR using Master Mix Roche 2x (Roche Diagnostics GmbH Corp, Mannheim, Germany), water and the primers cruzi1 (10 µM) (5'-AST CGG CTG ATC GTT TTC-3'), cruzi2 $(10 \,\mu\text{M})$ (5'-AAT TCC TCC AAG CAG CGG ATA -3') and a cruzi3 probe (5 μ M) (FAM-CAC ACA CTG GAC ACC AA-NFQ-MGB) to detect the satellite tandem repeat DNA of the parasite (166 pb) following the conditions previously reported [31]. Cts value < 38 were considered as positive amplification [15]. Then, for insects with positive results by qPCR, a conventional PCR for kinetoplast DNA amplification was conducted using a Buffer Taq 10x, MgCl₂ 50 mM, dNTPs 25 mM, Taq Platinum 5 U/µL, water and the primers 121 50 pmol/µL (5'- AAA TAA TGT ACG GGK GAG ATG CAT GA -3') and 122 (5'- GGT TCG ATT GGG GTT GGT GTA ATA TA -3') 50 pmol/µL to discriminate between T. cruzi (330 pb) and T. rangeli (400-450 pb) as reported elsewhere [32].

T. cruzi genotyping

Parasite genotyping was accomplished by the amplification of the spliced leader intergenic region of miniexon gene (SL-IR), dividing DTUs in two groups: TcI (350 bp) and TcII-TcVI (300 bp). Reaction mix consisted of Go Taq Green Master Mix 2x, water and primers TCC 10 nM (5⁻ CCC CCC TCC CAG GCC ACA CTG - '3), TC1 10 nM (5⁻ GTG TCC GCC ACC TCC TTC GGG CC - '3) and TC2 10 nM (5⁻ CCT GCA GGC ACA CGT GTG TGT G - '3) [33]. Then, amplicons were submitted to Sanger sequencing using the TCC primer as reported elsewhere [34]. PCR products were cleaned using ExoSAP-IT® Express PCR Product Cleanup 75001/75002 (Affimetrix, USB, USA) and then submitted to Sanger sequencing. Sequences were aligned in MEGA X software [35] and submitted to BLASTn for similarity search.

Feeding sources characterization

A 215 bp fragment of the 12S gene fragment was amplified using Go Taq Green Master Mix, water and primers L1085 10 nM (5'- CCC AAA CTG GGA TTA GAT ACC C -3') and H1259 10 nM (5'- GTT TGC TGA AGA TGG CGG TA -3') as reported by Dumonteil and colleagues [35]. PCR products were cleaned using ExoSAP-IT® Express PCR Product Cleanup 75001/75002 (Affimetrix, USB, USA) and then submitted to Sanger sequencing. Resulting sequences were edited in MEGA X software [36] and submitted to BLASTn for similarity search.

Statistical analysis

We determined the frequency of *T. cruzi* infection, as well as DTUs and feeding sources, regarding variables such as ecotopes (sylvatic or domestic), insect stages (adult or nymph), sex (male and female) and bird nests (*P. rufifrons* and *C. cela*) to find any plausible associations among them. We carried out a multiple correspondence analysis (MCA) using 3 dimensions to explore the variables proximity with the presence of *T. cruzi*. MCA was made with 51 samples, given that 9 samples were excluded due to their lack of information for the evaluated variables, including both Arauca individuals because no nest information was

available. Cronbach's alpha coefficient and inercy were calculated to establish dimension consistency in the model and to determine the proportion of total variability contributed by each variable in the matrix, respectively.

A binary logistic regression, without including the intercept, was made to determine the statistical associations between *T. cruzi* infection and the explicative variables: locality, ecotope, insect stage, sex, bird nest and feeding source (x_1 , x_2 , x_3), previously identified in the MCA. Logistic regression was selected between five multivariate models that adjusted to type of data (Additional file 1: Figure S1). Statistical analyses were performed in Statistical Package for the Social Sciences (SPSS) version 25 and p-value < 0.05 was established as statistically significant.

Results

Frequency of T. cruzi infection and DTUs

Of the 60 *Ps. arthuri* collected, we found that 70% (42/60) were positive by end-point qPCR for *T. cruzi*. Of these, TcI was found in 54.8% (23/42) of samples, TcII-TcVI in 14.3% (6/42), mixed infection in 9.5% (4/42) and 21.4% (9/42) were not able to be typed. In samples with TcI and mixed infection, TcI_{Dom}- $_{Sylv}$ was detected in 44.4% (12/27), TcI_{Dom} in 25.9% (7/27), single infection with TcI_{Sylv} was not detected and 29.6% (8/27) were unable to typed. Table 1 shows the eco-epidemiological information evaluated by ecotope, insect stages, sex and bird nests. As it is observed in Table 1, 97.6% of the *Ps. arthuri* were positive for *T. cruzi* presence. No nymphs were infected with *T. cruzi* and 70% of the insects were found in *Phacellodomus rufifrons* nests. No insects were found infected with *T. rangeli*.

Feeding sources of Ps. arthuri individuals

A total of 15 feeding sources of *Ps. arthuri* were found (Fig. 2). The frequencies were: *Cranioleuca baroni* (Baron's Spinetail) (35.85%, n=19), *Homo sapiens* (human) (28.30%; n=15), *Thraupis episcopus* (Blue-gray Tanager) (11.32%; n=6), *Serinus albogularis* (White-throated canary) (3.77%, n=2) and the other 11 species correspond to 1.89%: *Arremon aurantiirostris* (Orange-billed Sparrow), *Chrysolophus amherstiae* (Lady Amherst Pheasant), *Geospiza magnirostris* (Large Ground-finch), *Icterus mesomelas* (Yellow-tailed Oriole), *Melospiza melodia* (Song sparrow), *Phasianus colchicus alaschanicus* (Common Pheasant), *Pipilo maculatus* (Spotted Towhee), *Pan paniscus* (Bonobo), *Pyrrhula pyrrhula* (Common Bullfinch), *Serinus canaria* (Wild Canary) and *Varanus flavescens* (Yellow Monitor).

Additionally, we found that the majority of insects feeding on different bird species were *T. cruzi* positive, especially those who fed on *C. baroni*, and mostly presents TcI DTU. Also, we found that insects that were *T. cruzi* positive fed on humans presented TcI DTU (6/14) and just one insect had TcII. Mixed infection was found when *Ps. arthuri* fed on 2 species: *C. baroni* (2) and *S. albogularis* (1). Two species: *Arremon aurantiirostris* and *Phasianus colchicus alaschanicus* presents only TcII. Some feeding sources were *T. cruzi* positive but no information about DTUs was available (Fig. 3).

Proxy associations between T. cruzi infection and eco-epidemiological variables

Two subgroups of variables were identified on the MCA. The first one conformed by the variables ecotope and locality, and the second one contains the variables *T. cruzi* infection, insect stage, sex, bird nest and feeding sources. Additionally, in dimension 2 we identified a geometric relation between the variable ecotope and *T. cruzi* presence (Table 2). Once MCA values were obtained, a proximity bidimensional plot (Bi-plot) was made to elucidate relations between variables by representing the geometric distribution in three dimensions of evaluated variables (Fig. 4). It can be observed that, generally, *T. cruzi* presence is mostly associated with insect stage, sex, bird nest and feeding source. Logistic regression results show that

Pore municipality, adult insect stage, male *Ps. arthuri*, *C. cela* bird nest and *C. baroni* feeding preference are related the most with *T. cruzi* presence (Table 3). This analysis is consistent with results obtained in MCA (Fig. 4).

Discussion

In Colombia 15 of the 26 registered triatomines have been reported as naturally infected by *T. cruzi* and some are considered non-risky for *T. cruzi* transmission [18]. This last group includes *Ps. arthuri*, a triatomine with sylvatic habits [21]. *Ps. arthuri* has been reported in 3 departments of Colombia: Meta, Arauca and Casanare, with no available data about *T. cruzi* presence [18] and it is associated with *Phacellodomus rufifrons* nests [37]. Furthermore, there are some studies that report triatomine species with sylvatic habits in a domiciliation process due to deforestation and ecosystemic fragmentation. Therefore, these triatomines have begun to be considered an important factor in the parasite transmission [18, 19], especially in Arauca and Casanare, where has been found that sylvatic triatomine species such as *Ps. arthuri*, *P. geniculatus*, *T. maculata* and *T. venosa*, are moving to human houses [15, 28].

In this study, the percentage of *Ps. arthuri* infected with *T. cruzi* was high (70%), which might suppose a possibility that *Ps. arthuri* is changing its ornithophilic behavior to feed on other blood sources including human blood (Fig. 2). It is important to highlight that niche changing or accustom to other niche starts with a behavioral change [41], which is relevant in the context of new transmission sceneries of *T. cruzi*. By feeding solely on birds, triatomines cannot get infected with the parasite, because birds are naturally resistant to *T. cruzi* infection due to their innate immune mechanism against the parasite that quickly eliminates infective forms from their system [42]. Schaub [43] showed that chicken blood had a lytic effect for trypanosomes after an incubation for 60 minutes, but transmission could still occur because of lysis-resistant epimastigotes. Otherwise, it is known that specific feeding preferences of triatomines can influence the transmission dynamics of *T. cruzi* [44]. For instance, a study showed that mice infected with *T. cruzi*

isolates, previously exposed to bird blood, presented a high survival rate, suggesting that ornithophilic behavior of triatomines could modulate the parasite and may reduce their effectivity to infect [45].

On the other hand, direct transmission of *T. cruzi* between insects can occur through entomophagy behaviors, such as cannibalism, coprophagy and cleptohaematophagy. Cannibalism in triatomines is rare and poorly demonstrated but coprophagic behavior is associated with the acquisition of microbe symbionts and is a possibility of infection that cannot be excluded [43]. Coprophagy has been described for many insects, such as Isoptera (termites), Hemiptera (true bugs) and Blattaria (cockroaches) as a predominant route of beneficial bacteria transmission [46], and the possibility of *T. cruzi* transmission should be considered as well. Otherwise, some triatomine species are able to feed on hemolymph of other insects if starving [47]. Sandoval and colleagues [48] showed that *Belminus herreri* in early stages was unable to feed on vertebrate hosts but successfully fed on replete *R. prolixus*, showing a cleptohaematophagy behavior. This behavior has been reported too with *R. prolixus* nymphs [49]. Now, this can be considered as another possible way by which *Ps. arthuri* could become infected with *T. cruzi*, taking into account that this triatomine has been found in sympatry with *R. prolixus* [25, 28], but further studies are required to prove this hypothesis.

Furthermore, most of the *T. cruzi* positive insects (70.7%; 29/43) were found in *P. rufifrons* nests (Table 2) just as reported by Cruz-Guzmán and colleagues [25]. However, logistic regression indicates that *C. cela* bird nest is associated with presence of *T. cruzi* but this could be explained by the sample size, because only 15 of the 60 specimens were collected in this nest. Same occurs with the other variables associated with the presence of *T. cruzi*, such as insect stage, where 57 of 60 individuals were adults and feeding on *C. baroni* because it was the most frequent feeding source among specimens (35.8%). In this results, highlight Pore municipality OR value (OR 11.00), which may suggest that the frequency of *Ps. arthuri* positive for *T. cruzi* presence in Pore is 10 times more than in other municipalities. But this also may be due to sample size, because Pore was the only municipality where all *Ps. arthuri* collected were positive for the presence of T. *cruzi* except one (11 of 12 individuals) (Table 3). Otherwise, obtained frequency for

T. cruzi presence in Pore, Casanare, could be explained taking into account other triatomines in there, such as *R. prolixus*, which is the triatomine with the widely distribution in Colombia and the one that presents the highly rates of infection with *T. cruzi* [18].

In a study made in Venezuela, Cruz-Guzmán and colleagues in 2014 [25] found a few *Ps. arthuri* naturally infected with *T. cruzi*, despite these triatomines fed on birds. This feeding preference is not a coincidence, because *Psammolestes* genus is closely related with *Rhodnius* (Rhodniini Tribe) [38], and many species of these genus feed on birds, due to their associations with palm trees in which birds' nests are [39]. *T. maculata*, a sylvatic triatomine with wide distribution in Colombia, whose diet also consist mainly in birds' blood and because of that, is excluded from vector control programs [40]. But recently, Hernández and colleagues in 2016 [15] reported *T. maculata* feeding on humans with a frequency of 75% and also infected with *T. cruzi* with a frequency of 67%. They also found TcI and TcII in some specimens, suggesting a connection between parasites' domestic and sylvatic transmission cycles by this triatomine. Finally, they highlight *T. maculata* as a potential vector for CD and underline the importance of prioritize secondary vectors in vector surveillance due to their capacity of domiciliation. This could show a future behavior that *Ps. arthuri* could develop, but further studies about its potential as a *T. cruzi* vector are needed.

Triatomines are known as nest-dwelling insects, and usual hosts of them are tree-dwelling animals, like birds, reptiles, marsupials, and burrow vertebrates such as bats, rodents and armadillos [50]. Also, their alimentary habits may be influenced by density and availability of new feeding sources [30] which is relevant in the context of human urbanization. Here, we highlight that 28.3% of *Ps. arthuri* positive for *T. cruzi* fed on humans (Fig. 2), being the first study in Colombia and to our knowledge in the region to report it. This is a fact to underline, because there is an option where, besides *Ps. arthuri* is reaching dwellings, human activities near houses put people accessible to these triatomines. Further studies are required to evaluate the possibility of *Ps. arthuri* to effectively transmit *T. cruzi* to humans, case in which these finding become

relevant for CD transmission; particularly in an endemic region for CD as Casanare which shows the highest incidence for this pathology in the country and where most of the individuals were collected.

The wide variety of feeding sources of *Ps. arthuri* found in this study could be explained because other animals often use *P. rufifrons* nests [51], which may explain no-birds feeding sources found in some of the insects evaluated, such as *Varanus flavescens* and *Pan paniscus* (Fig. 2). Additionally, *Ps. arthuri* has been found in other bird nests and under bark of dead trees [52], suggesting they are moving through different niches looking for blood meals. The description of feeding patterns of sylvatic triatomines is relevant for a well-understanding of *T. cruzi* transmission and its circulation among different hosts. Also, the presence of the parasite in a sylvatic vector considered as no-risky for humans is highly relevant for CD and vector control programs, because they may still be important for parasites' transmission cycle. *In vivo* studies of *Ps. arthuri* are required to evaluate the progression and development of *T. cruzi* life cycle in it. Also, it is important to evaluate defecation patterns, insect densities, parasite-triatomine interactions, triatomine microbiota, immune response and ecology, because these are main factors to determine if a triatomine could be a potential vector for *T. cruzi* [44, 53].

It is important to highlight the presence of TcI_{Dom} genotype in 40.4% of *Ps. arthuri* positive for *T. cruzi*, knowing that 97.6% of insects were collected in sylvatic ecotope, indicating the intrusion of domestic DTUs in the sylvatic cycle of the parasite. In contrast, Cruz-Guzmán and colleagues [25] reported the presence of TcIII in an adult specimen of *Ps. arthuri*. This DTU belongs to sylvatic cycle of the parasite and has been found in armadillos and didelphimorphos [54], animals that are frequently found near dwellings. Another study of secondary vectors of CD, reported *T. maculata* and *P. geniculatus* infected with TcI/TcIII and TcI-TcV, respectively, concluding this triatomines represents an important connection between sylvatic and domestic transmission cycles, facilitating the circulation of many DTUs [15]. In this study we did not discriminate DTUs from TcII-TcVI group. Further studies are required to determine truly circulating genotypes. Also, TcI is associated with arboricoral mammals such as *Didelphis marsupialis* and others like

Rattus rattus and *Canis lupus familiaris* [5, 15, 16], while TcIII and TcIV are related with armadillos [55]. *Ps. arthuri* may be feeding on these reservoirs associated to domestic cycle, explaining the presence of TcI and TcII-TcVI DTUs in it. Furthermore, presence of domestic DTUs may be explained considering that collection sites are located near human settlements (Fig. 1B) and that *Ps. arthuri* could be moving through domestic and sylvatic transmission cycles, as reported in vectors and synanthropic reservoirs for the parasite [10]. These triatomines could be circulating between sylvatic and domestic ecotope not only because of the need to find new feeding sources, but through active dispersal, because they might be attracted to artificial light of human dwellings [56]. This phenomenon has important epidemiological significance, because if they are truly attracted to this light, the probability of triatomines to arrive to dwelling increases [47]. Jácome-Pinilla and colleagues [57] made a study about the associated risks among dispersive nocturnal flights of sylvatic triatomines because of artificial lights in northeastern Colombia, in which they reported *Ps. arthuri*, T. maculata, P. geniculatus and R. prolixus being attracted to lights traps, highlighting a potential risk of active dispersion of sylvatic triatomines and their implications in the introduction of sylvatic DTUs into the domestic transmission cycle. Moreover, a study in Brazil about attraction of Chagas disease vectors to artificial light found that almost all of the known vectors of CD in the zone were attracted by artificial light sources, and they propose this as a possible route by which triatomines can reach dwellings and become involved in T. cruzi transmission [58].

Conclusions

We presented the first *Ps. arthuri* study in Colombia regarding *T. cruzi* infection and its feeding preferences. Our findings indicate that *Ps. arthuri* could be feeding on potential reservoirs for *T. cruzi* aside from birds, which may explain the presence of the parasite in them. Also, finding that these triatomines are feeding on humans may be of high relevance for the epidemiology and control of CD, but further studies are needed to evaluate *Ps. arthuri* as a potential vector for *T. cruzi*; considering factors such as the biology of the parasite inside the triatomine, changes that the parasite can trigger in the host and feeding and defecation

behavior, because these are important factors to better understand *T. cruzi* transmission. Moreover, studies using other molecular techniques such as deep sequencing are required to improve feeding sources detection and *T. cruzi* genotyping. Also, a wider and robust sampling is needed to determine true associations of *Ps. arthuri* and the presence of *T. cruzi*. Although, we encourage the scientific community to keep including sylvatic triatomine species into CD eco-epidemiological studies for better understanding of transmission dynamics of the parasite. We also encourage the government to pay attention to *Ps. arthuri* in Colombia, considering they are distributed in some endemic departments for CD and the findings of the present study where these insects had a positive result for *T. cruzi* presence and are feeding on humans.

Additional file 1

File name: Additional file 1

File format: .tiff

Title of data: Figure S1

Description of data: Model selection using SPSS software modeler.

List of abbreviations

CD: Chagas disease; DTU: Discrete typing unit; PCR: Polymerase chain reaction; qPCR: real-time PCR; SL-IR: Spliced leader- Intergenic region; MCA: Multiple correspondence analysis; OR: Odds ratio

Declarations

Acknowledgments

We thank the personnel from the Secretaria Departamental de Arauca and the bachelor students from Unitropico that helped in the collection of the individuals.

Funding

This work was funded by DIRECCIÓN DE INVESTIGACION E INNOVACIÓN from Universidad del Rosario.

Availability of data and materials

The data supporting the conclusions of this article are included within the article and its additional file.

Authors' contributions

NVO wrote the manuscript. JDR, GH, CH and NVO analyzed the data. AT carried out statistical tests. GH, LMAG, AH, LCS and NVO carried out experimental procedures. AC and PU made the insects collection in Arauca and Casanare, respectively. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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Figure Legends

Figure 1. A) Geographical distribution of 60 Ps. arthuri collected in the departments of Arauca and Casanare, Colombia, B) collection sites in Casanare, C) capturing methods in field.

Figure 2. Frequency of the 15 feeding sources found in the evaluated Ps. arthuri.

Figure 3. *T. cruzi* presence, DTUs and feeding sources of 53/60 *Ps. arthuri* collected. Red color means presence of the characteristic in each individual.

Figure 4. Proximity Bi-plot between T. cruzi and the eco-epidemiological variables evaluated.

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