



Natural infection by *Trypanosoma cruzi* in *Rhodnius robustus* I infesting *Attalea butyracea* palms in El Zulia, Norte de Santander, Colombia

Infección natural por *Trypanosoma cruzi* en *Rhodnius robustus* I asociados a palmas de *Attalea butyracea* en El Zulia, Norte de Santander, Colombia

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Highlights

- *Attalea butyracea* palms in El Zulia, Norte de Santander, Colombia, showed a 30% infestation rate by *Rhodnius*, with abundant immature stages suggesting an active *Trypanosoma cruzi* transmission cycle.
- The presence of *Rhodnius robustus* I, a taxonomically challenging lineage within a genetically connected prolixus-group assemblage, reinforces the need for genetic surveillance of wild vector populations.
- The detection of *Trypanosoma cruzi* infection in 40% of the collected specimens highlights a persistent risk of transmission from wild ecotopes to nearby human communities.

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ABSTRACT

Introduction. Palms such as *Attalea butyracea* function as key sylvatic ecotopes for species of the genus *Rhodnius*, providing shelter, suitable microclimatic conditions, and access to vertebrate hosts, thus contributing to the maintenance of *Trypanosoma cruzi* transmission cycles in disturbed landscapes. **Objective.** To evaluate the infestation of *Attalea butyracea* palms by triatomines and to determine natural infection by *T. cruzi* using parasitological and molecular methods in El Zulia, Norte de Santander, Colombia. **Materials and methods.** Between August and September 2021, 100 palms were sampled using modified Angulo traps. The collected insects were identified morphologically and through sequencing of the mitochondrial cytochrome *b* gene. Infection was assessed by microscopic examination of intestinal contents and PCR detection of *T. cruzi* DNA. **Results.** A total of 64 specimens of the genus *Rhodnius* were collected, with an infestation index of 30% of the sampled palms. Natural infection was 31% by microscopy and 40% by PCR. Molecular analysis confirmed the presence of *Rhodnius robustus* I. **Conclusions.** The detection of *R. robustus* I highlights the importance of integrating molecular surveillance to improve the accuracy of vector identification and to monitor their dispersal and adaptation capacity. Continuous entomological surveillance in natural ecotopes is essential to prevent reinfestation and to consolidate the achievements of control programs.

RESUMEN

Introducción. Palmas como *Attalea butyracea* funcionan como ecotopos silvestres clave para especies del género *Rhodnius*, al proporcionar refugio, condiciones microclimáticas adecuadas y acceso a hospedadores vertebrados, contribuyendo así al mantenimiento de los ciclos de transmisión de *Trypanosoma cruzi* en paisajes intervenidos. **Objetivo.** Evaluar la infestación de palmas de *Attalea butyracea* por triatóminos y determinar la infección natural por *T. cruzi* mediante métodos parasitológicos y moleculares en El Zulia, Norte de Santander, Colombia. **Materiales y métodos.** Entre agosto y septiembre de 2021 se muestrearon 100 palmas utilizando trampas Angulo modificadas. Los insectos recolectados fueron identificados morfológicamente y mediante la secuenciación del gen mitocondrial citocromo *b*. La infección se evaluó mediante examen microscópico del contenido intestinal y detección por PCR de ADN de *T. cruzi*. **Resultados.** Se recolectaron 64 ejemplares del género *Rhodnius*, con un índice de infestación del 30 % de las palmas muestreadas. La infección natural fue del 31 % por microscopía y del 40 % por PCR. El análisis molecular confirmó la presencia de *Rhodnius robustus* I. **Conclusiones.** La detección de *R. robustus* I resalta la importancia de integrar la vigilancia molecular para identificar con mayor precisión los vectores y monitorear su capacidad de dispersión y adaptación. La vigilancia entomológica continua en ecotopos naturales es esencial para prevenir la reinfestación y consolidar los logros de los programas de control.



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INTRODUCTION

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a zoonosis of major epidemiological relevance in Latin America, where it affects approximately six million people⁽¹⁾. This parasite is transmitted mainly through contact with the feces of infected triatomines, hematophagous insects belonging to the subfamily Triatominae (Hemiptera: Reduviidae). Although several triatomine species have adapted to domestic environments, many others remain associated with sylvatic habitats, where they play a fundamental role in maintaining the enzootic cycle of *T. cruzi*⁽²⁾.

Among these vectors, species of the genus *Rhodnius* are notable for their close association with palms, particularly *Attalea butyracea*, which constitutes a natural ecotope widely distributed across the American continent⁽³⁾. Recent studies in Colombia have confirmed the epidemiological importance of these palms as habitats for infected triatomines, even showing higher levels of infestation by *Rhodnius prolixus* in *A. butyracea* than in other palm species occurring in transformed productive environments⁽⁴⁾. In Colombia, despite significant progress in vector control, acute cases continue to be reported in rural areas, some of them associated with oral transmission of the parasite⁽⁵⁾. This transmission route, increasingly documented, involves the accidental ingestion of *T. cruzi* through food or beverages contaminated with the feces of infected triatomines and constitutes an emerging risk, even in municipalities officially certified for the interruption of intradomiciliary vector-borne transmission attributed to *Rhodnius prolixus*⁽⁶⁻⁷⁾.

Environmental change and landscape transformation favor the transmission dynamics of *Trypanosoma cruzi* by facilitating triatomine expansion and dispersal, increasing contact among sylvatic, peridomestic, and domestic cycles, and maintaining stable reservoirs of infection in natural ecotopes. These processes allow sylvatic transmission cycles to persist and potentially act as sources of reinfestation, even in areas where conventional vector control strategies have been implemented⁽⁸⁾. In agreement with this, recent studies in Latin America have shown that deforestation and other forms of anthropogenic disturbance modify the distribution of palms of the genus *Attalea* and the ecology of the *Rhodnius* populations associated with them, with possible effects on the persistence of sylvatic cycles and the risk of contact with human populations⁽⁹⁾.

In Colombia, the distribution of *T. cruzi* vectors includes both primary and secondary species with broad geographic coverage. *Rhodnius prolixus*, the main domiciliated vector, is distributed mainly in the eastern region of the country, including the Llanos Orientales, Santander, Norte de Santander, and Boyacá. Other species, such as *Triatoma dimidiata* and *Triatoma maculata*, occur in the Caribbean region and northeastern Colombia, whereas *Triatoma venosa* has been reported in Andean areas of Boyacá and Cundinamarca. In addition, *Rhodnius* species associated with sylvatic ecotopes, such as *R. robustus* and *R. pallescens*, are present in the Amazon, Orinoquía, and Caribbean regions, where they maintain sylvatic transmission cycles of the parasite. This heterogeneous distribution highlights the complexity of Chagas disease transmission in Colombia and the epidemiological importance of sylvatic cycles⁽¹⁰⁾.

The municipality of El Zulia, in Norte de Santander, was certified for the interruption of intradomiciliary vector-borne transmission of *T. cruzi* attributed to *Rhodnius prolixus*⁽⁷⁾, which is considered the main vector of Chagas disease in Colombia⁽⁴⁾. Although this species is predominantly domestic across much of its distribution, its presence has also been documented in sylvatic habitats, where it may overlap with *Rhodnius robustus*, a morphologically similar species⁽¹¹⁻¹²⁾. However, the frequent colonization of houses by sylvatic populations of *R. prolixus*, together with its morphological similarity to *R. robustus*, complicates surveillance and control actions⁽¹²⁾. The persistence of sylvatic cycles associated with natural ecotopes such as *A. butyracea* palms underscores the need to maintain continuous epidemiological and entomological surveillance^(6,13).

Additionally, the morphological identification of some *Rhodnius* species may be challenging due to the presence of cryptic taxa within the *Rhodnius robustus* complex⁽¹²⁻¹⁶⁾. In this context, molecular techniques, including the amplification and sequencing of genetic markers, have become essential tools for the accurate identification of these vectors and for understanding their role in the transmission of *T. cruzi*⁽¹³⁻¹⁶⁾. Given this background and the epidemiological importance of sylvatic cycles in areas certified for vector control, the aim of the present study was to evaluate the infestation of *Attalea butyracea* palms by Chagas disease vectors and to determine natural infection by *Trypanosoma* spp. in the municipality of El Zulia, Norte de Santander, using molecular techniques.

MATERIALS AND METHODS

Study population and sampling

An observational, descriptive, cross-sectional study was conducted using a non-probabilistic convenience sampling approach. Sampling was carried out during August and September 2021. The target population consisted of sylvatic triatomines of the genus *Rhodnius* associated with *Attalea butyracea* palms, collected in the rural area of La Macarena, specifically at La Ceiba farm (coordinates: 8°08'41.75"N – 72°37'10.87"W), located in the municipality of El Zulia, Norte de Santander Department, in the Andean region of Colombia (**Figure 1**).

The farm is primarily dedicated to cattle production, and the palms present on the property provide shade for livestock. The municipality of El Zulia is located in the eastern part of the department, is part of the Cúcuta Metropolitan Area, and lies near the border with the Bolivarian Republic of Venezuela⁽¹⁷⁾. The region has an average temperature of 27 °C and an approximate elevation of 204 m above sea level.

The study area covered approximately 900 m² and included an estimated 200 *Attalea butyracea* palms distributed within a cattle-production landscape interspersed with secondary vegetation. **Figure 2** presents photographs of the sampled sylvatic ecotope and the surrounding landscape, including the degree of anthropogenic disturbance.

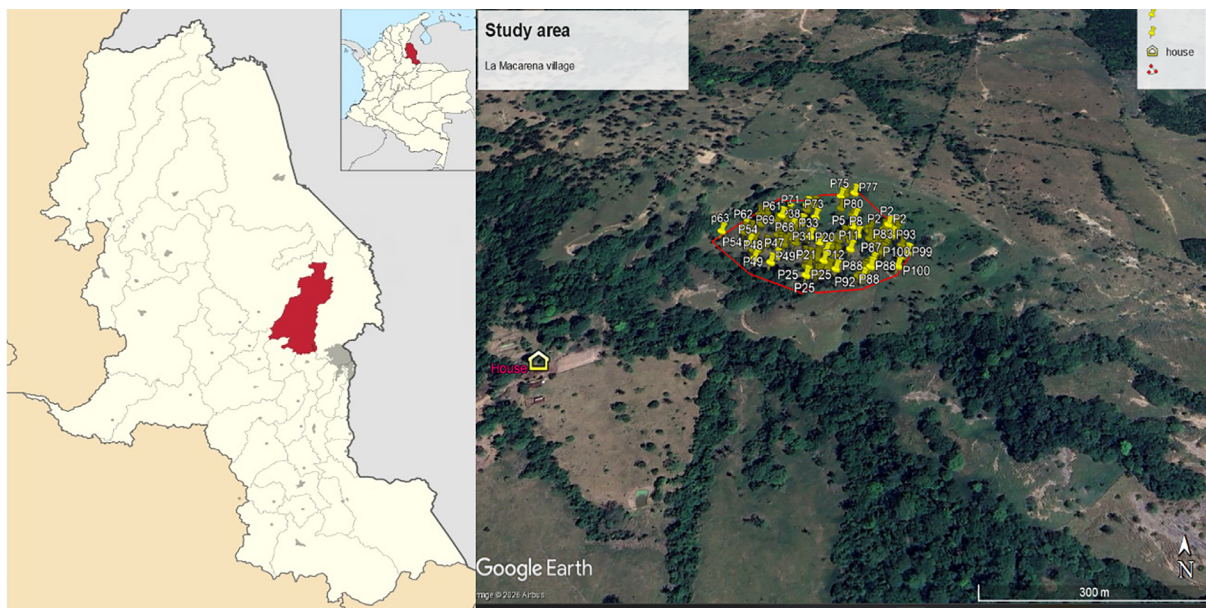


Figure 1. Geographic location of the study area, La Macarena, municipality of El Zulia, Norte de Santander.

Insect collection and identification

Of the approximately 200 *Attalea butyracea* palms present in the study area, 100 were selected for sampling based on criteria of accessibility, safety, and suitability for trap installation. Insects were collected using modified Angulo traps⁽¹⁸⁾. The trap consisted of an artisanal cylindrical structure measuring 40 cm in height and 25 cm in diameter, constructed from metallic mesh and fitted with a conical metallic lid with a hook for suspension. Its cylindrical shape allowed adaptation to the axillary spaces of palm crowns, facilitating installation within the natural microhabitats of *Attalea butyracea*. A rectangular mesh door (14 × 17 cm) was located in the middle section to allow placement of the animal bait.

The external surface was covered with strips of dried palm leaves, folded to create sheltered spaces that mimic natural palm microhabitats and provide refuge for sylvatic triatomines; these strips were secured with transparent adhesive tape. At the base, the trap included a 5 cm-high refuge chamber made of metallic mesh, with partial lateral openings to allow insertion and removal of dried palm leaves used as substrate. The trap did not include adhesive components for insect retention, in order to promote temporary sheltering and allow the collection of live specimens (**Figure 3**). Traps were installed from 6:00 p.m. to 7:00 a.m. the following day, corresponding to the period of peak nocturnal activity of *Rhodnius* species. Each night, 10 palms were sampled, placing one trap per palm. Sampling continued until the evaluation of the 100 selected palms was completed

Traps were installed on the trunk of the palm near the base of the leaves, and the trap in its fully assembled, ready-to-install condition is shown in (**Figures 4A–B**). GPS coordinates were recorded to determine the location of each sampled palm. The distribution of the sampled palms was visualized on a satellite image with relief representation in Google Earth, allowing a general assessment of their spatial distribution within the study area.



Figure 2. Wild ecotope sampled in La Macarena village, municipality of El Zulia, Norte de Santander, Colombia. *Attalea butyracea* palms are observed within a cattle-production landscape, illustrating the degree of anthropogenic disturbance and the structure of the surrounding vegetation where Angulo traps were installed.

Taxonomic identification of the palms was performed at the species level based on morphological criteria, following the keys and descriptions for Arecaceae proposed by Galeano and Bernal⁽¹⁹⁾. Identification of *Attalea butyracea* was supported by diagnostic characters such as the robust stem, large pinnate leaves with leaflets arranged in multiple planes, persistence of leaf bases forming a dense structure, and the characteristic morphology of the inflorescences and infructescences. These criteria allowed reliable differentiation of *A. butyracea* from other palm species present in the study area⁽¹⁹⁾. Morphological identification of captured insects was performed at Centro Experimental de Diagnóstico Molecular (CEDIMOL), Universidad de Pamplona, Colombia using the taxonomic keys of Lent and Wygodzinsky (1979)⁽²⁰⁾. Infestation was determined by calculating the percentage of palms positive for triatomines relative to the total number of palms evaluated. A palm was considered positive when at least one specimen was captured in the trap.

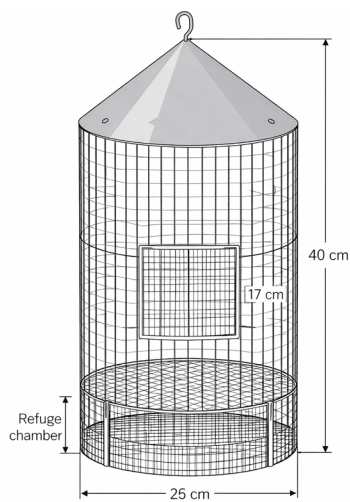


Figure 3. Schematic representation of the modified Angulo trap used for triatomine collection

Infestation assessment

Detection of *Trypanosoma cruzi*

Parasitological methods: Specimens that arrived alive at the laboratory were subjected to microscopic examination of fecal contents using optical microscopy at 40× magnification to detect the presence of motile trypomastigotes as an indicator of parasite viability. Molecular methods: Specimens that did not survive transport were preserved in absolute ethanol until processing. DNA was extracted from intestinal contents using the phenol–chloroform method⁽²¹⁾. Subsequently, PCR was performed using the primers T1 (5'-AA TAATGTTACGGG(T/G) GAGATGCATGA-3') and T2 (5'-GGTTCGAT'TGGG GTTGGTGAATATA-3')⁽²²⁾, which are specific for the detection of *T. cruzi*. Amplified products were visualized by electrophoresis on 2.5% agarose gels.



Figure 4. Modified Angulo trap used for triatomine collection and its installation on *Attalea butyracea* palms in the study. A. Photograph of the modified trap used in this study for the collection of triatomines. **B.** *Attalea butyracea* palm sampled in the study area showing the modified Angulo trap installed near the base of the leaves. The accumulation of dry leaf bases, organic debris, and associated vegetation provides suitable microhabitats for the establishment of sylvatic triatomine populations. The yellow arrow indicates the location of the trap.

Taxonomic and molecular identification of triatomines

For species-level determination, DNA was extracted from the thorax of each specimen using the High Pure PCR Template Preparation Kit (column-based extraction). The mitochondrial cytochrome *b* gene was then amplified by PCR using primers: 7432F (5'- GGACGWGGWATT TAT'TATGGATC-3') and 7433R (5'-GCWCCAATTCARGTTARTAA-3')⁽²³⁾ in a final reaction volume of 25 µl under the following thermal profile: initial denaturation at 94 °C for 5 min; 40 cycles of 94 °C for 40 s, 52 °C for 40 s, and 72 °C for 60 s; final extension at 72 °C for 5 min; and holding at 10 °C indefinitely. Amplification products were verified by electrophoresis on 2% agarose gels.

Amplicons were sent for Sanger sequencing. Forward and reverse sequences were assembled, chromatograms were inspected, and consensus sequences were generated using the automated package SangerseqR version 4.4.1. Finally, sequences were aligned and compared using BLAST (Basic Local Alignment Search Tool), with species identification defined by >99% identity and an E-value close to zero.

Data availability. The dataset supporting this study is publicly available in: Prieto Silva, T., Blanco Quintana, I., Oviedo Niño, C., & Riaño Gutiérrez, N. (2026). Dataset on Triatomine Presence and Palm Infestation in Norte de Santander, Colombia (Version 1.0) [Data set]. Zenodo. <https://doi.org/10.5281/zenodo.1950104>

RESULTS

Infestation of *Attalea butyracea*

Of the 100 *Attalea butyracea* palms sampled in the study area, sylvatic triatomines were detected in 30 palms, corresponding to an infestation rate of 30%. A total of 64 specimens were collected, 90% of them alive at the time of recovery from the traps, and distributed across different developmental stages (**Table 1**). During the sampling period, evening ambient temperature showed a median of 29.7 °C (range: 26.8–34.5), whereas morning temperature was 26.0 °C (22.0–29.2). Evening relative humidity had a median of 64.6% (55.6–78.9), and morning relative humidity was 76.1% (60.8–92.7) (**Table 2**). Infested palms were distributed across different sectors of the sampled area and were not concentrated exclusively at a single point.

(**Figure 5**).

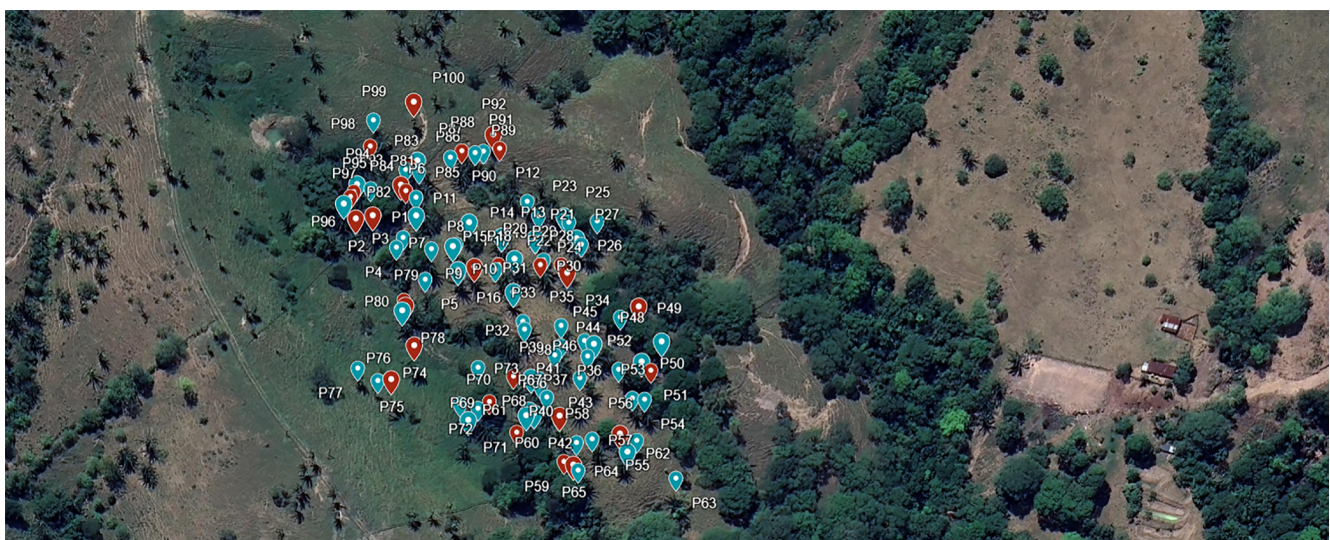


Figure 5. Spatial distribution of non-infested (blue) and infested (red) palms.

Composition by developmental stage

The distribution of developmental stages, including nymphs and adults, is presented in **Table 1**. Nymphal stages predominated (**Figure 6A**), especially third-instar nymphs (45.3%) and second-instar nymphs (23.4%), followed by fourth-instar nymphs (14.1%), fifth-instar nymphs (10.9%), adults (4.7%), and first-instar nymphs (1.6%).

Table 1. *Attalea butyracea* palms positive for triatomines and stage composition of specimens collected in El Zulia, Norte de Santander, Colombia.

Variable	Value
Palms evaluated, n	100
Infested palms, n (%)	30 (30.0)
Non-infested palms, n (%)	70 (70.0)
Insects collected, n	64
Mean number of insects per trap in positive palms	2.3
First-instar nymph, n (%)	1 (1.6)
Second-instar nymph, n (%)	15 (23.4)
Third-instar nymph, n (%)	29 (45.3)
Fourth-instar nymph, n (%)	9 (14.1)
Fifth-instar nymph, n (%)	7 (10.9)
Adults, n (%)	3 (4.7)

Table 2. Environmental variables recorded during the sampling period in the study area.

Variable	Value
Evening temperature (°C)	29.7 (26.8–34.5)
Morning temperature (°C)	26.0 (22.0–29.2)
Evening relative humidity (%)	64.6 (55.6–78.9)
Morning relative humidity (%)	76.1 (60.8–92.7)

Environmental variables are expressed as median (range).

Detection of *Trypanosoma cruzi*

Direct parasitological examination was performed on 13 triatomine specimens, of which 4 were positive for *T. cruzi*, corresponding to an infection rate of 30.8%. A total of 62 specimens, including the 13 examined by direct parasitological methods, were analyzed by polymerase chain reaction (PCR), of which 25 showed positive amplification for *T. cruzi*, representing an infection rate of 40.3% (Figure 6B). Although the two methods were applied to overlapping sets of specimens, molecular detection identified a higher proportion of positive specimens than optical microscopy.

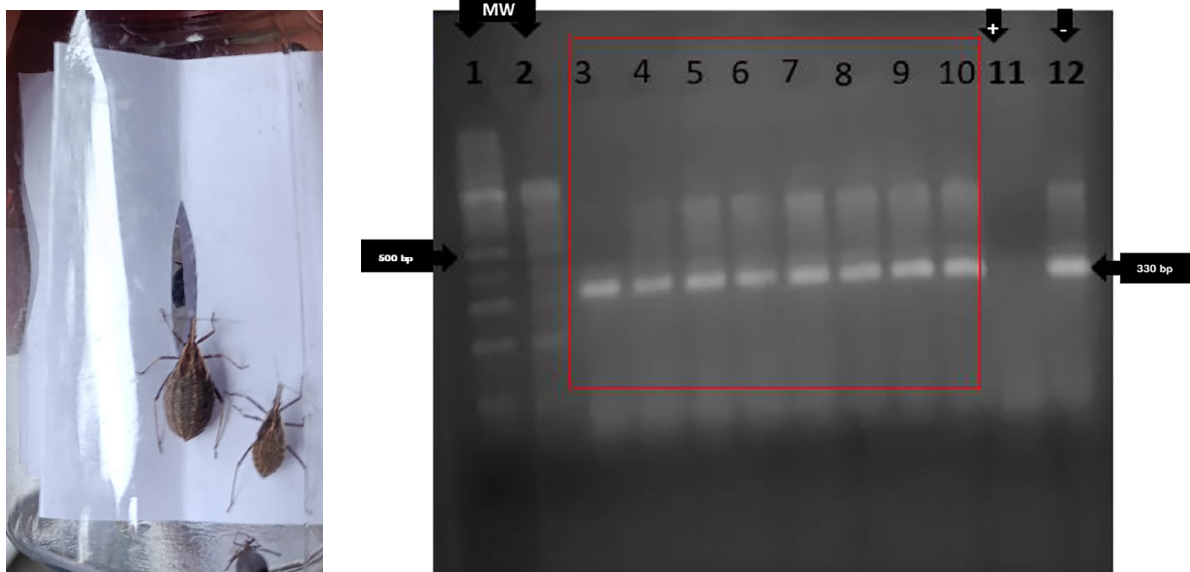


Figure 6 (A). Representative *Rhodnius* specimens collected from *Attalea butyracea* palms. Nymphal stages are shown. Species identification was confirmed as *Rhodnius robustus* I by sequencing of the mitochondrial cytochrome *b* gene. **(B).** Agarose gel electrophoresis (2%) of PCR amplicons obtained from triatomine intestinal contents for *Trypanosoma cruzi* detection. Lanes 1 and 2 show the 100-bp DNA molecular weight marker. Lanes 3–10 correspond to intestinal content samples from PCR-positive insects, in which a specific amplicon of approximately 330 bp was observed in samples ZVLM 18 (lane 3), ZVLM 49A (lane 4), AZVLM 56A (lane 5), ZVLM 63 (lane 6), ZVLM 28A (lane 7), ZVLM 69A (lane 8), ZVLM 68A (lane 9), and ZVLM 30 (lane 10). Lane 11 corresponds to the negative control, whereas lane 12 corresponds to the positive control.

Taxonomic and molecular identification of triatomines

Morphological identification using the taxonomic key proposed by Lent and Wygodzinsky (1979) established that the collected triatomines belonged to the genus *Rhodnius*. Subsequently, amplification of the mitochondrial cytochrome *b* gene and sequence analysis confirmed the presence of *Rhodnius robustus* I, showing greater than 98% identity with reference sequences of *R. robustus* from the Orinoco region of Venezuela. Three sequences were deposited in GenBank under accession numbers PV944418, PV944419, and PV944420.

DISCUSSION

The detection of multiple developmental stages of *Rhodnius robustus* I in *Attalea butyracea* supports the role of this palm species as a stable sylvatic ecotope for triatomine populations^(3,9,24). The coexistence of several nymphal stages together with adults suggests that this microhabitat provides suitable conditions for vector shelter, feeding, and development. In agreement with this, the observation of wildlife in the surrounding area, including mammals recognized as *T. cruzi* reservoirs—bats, primates, squirrels, and foxes—supports the hypothesis of an active enzootic cycle in the study area. Although these observations were not obtained through systematic wildlife sampling, they are consistent with the ecological context expected in sylvatic transmission systems. Within this framework, the infection rates detected by parasitological examination and PCR confirm the circulation of *T. cruzi* among the collected triatomines. These proportions are comparable to those reported in other sylvatic environments in Latin America for triatomines associated with similar ecotopes^(25–28). In

addition, the mean abundance observed in positive palms provides further evidence of triatomine occupation of this ecotope, in agreement with previous studies on *Attalea butyracea* and other sylvatic palm systems^(3,9,25). It is also important to consider that the maintenance of these cycles may be influenced by multiple ecological factors, including the abundance and diversity of vertebrate hosts, climatic conditions, and the degree of anthropogenic disturbance in the landscape^(9,29-30).

The molecular identification carried out in this study showed that the specimens were assigned to the *Rhodnius robustus* I lineage, a taxonomic entity defined on the basis of mitochondrial markers within the *R. robustus* complex/prolixus group, phylogenetically close to *R. prolixus* and generally difficult to distinguish from it using morphological characters alone^(2,13,24). This finding is particularly relevant in the context of the eastern Andean region of Colombia and areas close to the Venezuelan border, where the taxonomic delimitation of species within the genus *Rhodnius* has posed a challenge^(2,12,24). In this context, the use of molecular tools, such as amplification and sequencing of the cytochrome *b* gene, was essential to support the lineage assignment of the *Rhodnius* specimens associated with *A. butyracea* in the study area.

In turn, *R. robustus* I has been regarded as an evolutionary lineage within an assemblage showing high genetic connectivity in the Andean-Orinoquian region⁽¹⁵⁾. However, recent multilocus evidence suggests that boundaries within the prolixus group are not yet fully resolved and that genetic structure may be shaped more strongly by geography and connectivity among populations than by discrete taxonomic boundaries⁽¹⁵⁾. This pattern suggests possible connectivity among populations distributed across different ecotopes, including palms and environments transformed by human activities, although the extent of exchange between these habitats remains to be evaluated specifically. In this sense, the detection of this lineage in *A. butyracea* within a disturbed landscape reinforces its eco-epidemiological relevance and the need to maintain surveillance of sylvatic cycles, particularly in scenarios where interfaces with domestic and peridomestic environments may be established.

In this context, the finding of *R. robustus* I in a rural area of El Zulia, a municipality certified as free of intradomiciliary transmission by *R. prolixus*, acquires particular relevance in light of recent studies showing that secondary triatomine species may participate in transmission cycles⁽³¹⁾. Taken together, these results indicate that entomological surveillance should not be limited to historically dominant vectors, but should also incorporate other sylvatic or secondary species with potential involvement in the transmission of *T. cruzi*.

One limitation of this study is the lack of analysis of the blood-feeding sources of the collected triatomines, which is a key aspect for a more precise understanding of the transmission dynamics of *T. cruzi*. Previous studies in Colombia have shown that triatomines associated with *A. butyracea* may feed on a wide diversity of vertebrates, and that both generalist hosts and ecological factors, such as the microclimate of the palm crown, host availability, and landscape conditions, may influence triatomine abundance and the probability of *T. cruzi* infection⁽²⁵⁻²⁷⁾. Consequently, incorporating blood meal analysis in future studies would allow a more precise characterization of vector-host interactions and strengthen the eco-epidemiological interpretation of these sylvatic transmission cycles.

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ETHICAL CONSIDERATIONS

The University of Pamplona holds the permit granted by the Corporación Autónoma Regional de la Frontera Nororiental (CORPONOR) under Resolution No. 000200 of April 13, 2015, authorizing the collection of specimens of wild species from biological diversity for non-commercial scientific research purposes in Norte de Santander Department.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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