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Notas Científicas

First report of the bacterial microbiota in the gut of *Panstrongylus chinai* vector of Chagas disease in Southern Ecuador

Primer reporte de la microbiota bacteriana en el intestino de *Panstrongylus chinai* vector de la enfermedad de Chagas en el sur de Ecuador

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Abstract.- Chagas disease (CD), caused by the *Trypanosoma cruzi* parasite and primarily transmitted by blood-sucking insects of the Triatominae subfamily. Endemic in 21 countries across the Americas, CD is found mostly in rural areas but is increasingly spreading to urban regions due to migration. In Ecuador, 16 triatomine species have been identified, including *Panstrongylus chinai*, which acts as a secondary vector in Loja province. While insecticides have been the primary method for controlling CD, there is a need for improved strategies to prevent its spread. This study examined the bacterial microbiota of *P. chinai* (lab-raised and wild insects) from three rural communities in Loja province. A total of 63 domiciliary units (DUs) were examined, with an infestation index of 7.9 %. The study analyzed 12 *P. chinai* individuals collected from the DUs and 50 from laboratory colonies for bacterial composition. The intestine and DNA were extracted, performing molecular detection of *T. cruzi* by PCR and sequencing the bacterial 16S region. The results showed the presence of *T. cruzi* in a collected sample and *Staphylococcus* genus, specifically *S. saprophyticus* (75 %) and *S. equorum* (25 %). These findings improve our understanding of the *P. chinai* microbiota and offer valuable insights for developing new strategies to control CD.

Keywords: Control, Ecuador, microbiota, parasite, triatomines

Resumen.- La Enfermedad de Chagas (ECh) es causada por el parásito *Trypanosoma cruzi* y transmitida, principalmente, por insectos hematófagos de la subfamilia Triatominae. Endémica en 21 países de América la ECh se encuentra principalmente en áreas rurales, sin embargo, se está extendiendo a regiones urbanas debido a la migración. En Ecuador se han identificado 16 especies de triatominos, entre ellos *Panstrongylus chinai*, vector secundario en la provincia de Loja. Si bien los insecticidas han sido el método principal para controlar la ECh, es necesario mejorar las estrategias para prevenir su propagación. Este estudio examinó la microbiota bacteriana de *P. chinai* (insectos criados en laboratorio y recolectados) de tres comunidades rurales de la provincia de Loja. Se examinaron un total de 63 unidades domiciliarias (UD), con un índice de infestación del 7.9 %. El estudio analizó 12 individuos de *P. chinai* recolectadas en las viviendas y 50 de colonias de laboratorio para determinar su composición bacteriana. Se extrajo el intestino y el ADN, realizándose la detección molecular de *T. cruzi* mediante PCR y secuenciando la región bacteriana 16S. Los resultados mostraron la presencia de *T. cruzi* en una muestra colectada y el género *Staphylococcus*, específicamente *S. saprophyticus* (75 %) y *S. equorum* (25 %). Estos hallazgos nos permiten mejorar la comprensión de la microbiota de *P. chinai* y ofrecer información valiosa para desarrollar nuevas estrategias de controlar la ECh.

Palabras clave: Control, Ecuador, microbiota, parásito, triatominos

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Introduction

Panstrongylus chinai is distributed in Ecuador and Peru (Patterson et al. 2009). In Ecuador, this species is found in domestic and peridomestic environments in Loja and El Oro provinces (Grijalva et al. 2015). *Panstrongylus chinai* is a competent vector of *T. cruzi*, transmitting the parasite via defecation during or after feeding at all life stages. This species takes approximately 12 months to complete its development (Mosquera et al. 2016). Chagas disease, also known as American trypanosomiasis, is caused by the parasite *Trypanosoma cruzi*, and primarily transmitted through the feces of blood-feeding insects from the Reduviidae family, subfamily Triatominae (Coura 2013). A total of 151 triatomine species have been identified worldwide (Justi and Galvão 2017), with 16 species documented in Ecuador (Abad-Franch et al. 2001). *Triatoma dimidiata* and *Rhodnius ecuadoriensis* are the main vectors in Ecuador, however, the genus *Panstrongylus* has become increasingly important due to its spread into human habitats (Villacís et al. 2020).

Currently, the main method to control triatomine population is through the using of insecticides. However, it has been observed that several triatomine have developed resistance (Engel et al. 2013), making it necessary to find a new method to control this disease. One of these alternatives is the use of the indigenous intestinal microbiota of these vectors. Furthermore, there is increasing evidence that the insect gut microbiota may affect the ability of pathogens to colonize and persist in the vector or alter the vector's competence to transmit pathogens to the human host (Waltmann et al. 2019). The microbiota includes a wide variety of commensal, pathogenic, or mutualistic species that affect the reproduction, nutrition and immune system of the vector (Kieran et al. 2019). The gut microbiota in triatomines is crucial for the transmission and virulence of *T. cruzi* to humans, with the parasite potentially affecting the microbiota's composition (Da Mota et al. 2012). Understanding this microbiota is vital for developing new research and control strategies, which requires molecular diagnostic methods to accurately identify and analyze the microorganisms present in triatomines.

Triatomines acquire their intestinal microbes primarily through coprophagy, both in laboratory and in the wild (Waltmann et al. 2019), where they also acquire non-symbiotic or host-associated microbes from blood meals (Engel and Moran 2013). Studying the microbiota of both lab-raised and wild triatomines is important for understanding their behavior and ecological roles.

Culture-dependent methods often underestimate the diversity of bacterial species in natural conditions, as they focus mainly on cultivable bacteria while overlooking non-cultivable taxa (Oliveira et al. 2018). Recent non-culture-based studies have begun to explore the gut microbiota of triatomines, but the bacterial microbiota of *P. chinai* has not been documented in either lab-raised or wild insects. This study aims to describe the gut bacterial microbiota of *P. chinai* (lab-raised and wild insects) using molecular identification techniques.

Materials and Methods

Study area and collection of *Panstrongylus chinai.-* Fieldwork for *Panstrongylus chinai* collection occurred in three rural communities in Calvas county, Loja province: Guara (1064–1450 masl), Chaquizhca (888–1323 masl), and Bellamaría (1000–1384 masl) (Figure 1). Triatomines were collected from domestic and peridomestic habitats (DUs) using the one-man-hour method described by Grijalva et al. (2015) under permit MAAE-DBI-CM-2021-0185, then transported to the CISeAL insectary, PUCE,

Quito under permit MAAE-CMARG-2020-0178. Specimens were identified and classified by Lent and Wygodzinsky's (1979) key. Entomological indices such as: i) infestation index, density, crowding, and colonization rate were calculated (WHO 2002).

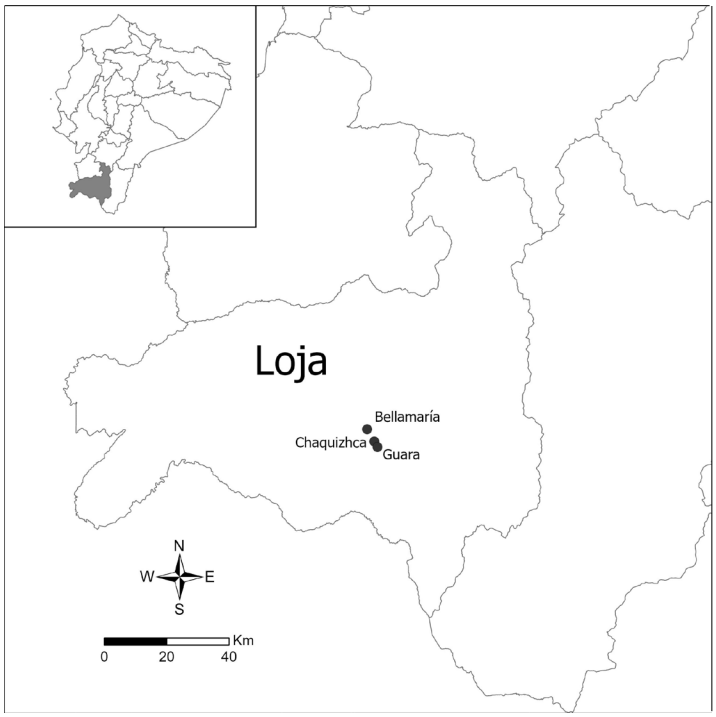

Figure 1. Map of the three rural communities where *Panstrongylus chinai* were collected (Guara, Chaquizcha and Bellamaria), Calvas, Loja province (grey points).

***Panstrongylus chinai* colonies.-** *Panstrongylus chinai* colonies were maintained under controlled conditions temperature (24 ± 6 °C), humidity (70 ± 10 %), and photoperiod (12 [L:D] h) as described Villacís et al. (2008), and periodically fed defibrinated human blood via the Hemotek system (Durden et al. 2023). The colonies, originating from 2009 and 2011, corresponded to 11 and 13 generations, respectively, as described by Moquera et al. (2016) in relation to the *P. chinai* life cycle.

Intestinal extraction of *Panstrongylus chinai.-* Intestinal extractions were performed on 12 individuals (6 males, 1 female, 4 nymph V, and 1 nymph IV), as well as 50 individuals from colonies (10 individuals per stage, ranging from nymph III to nymph V, and adults, both female and male), following the sterilization procedure outlined by Gumiel et al. (2015), which incorporated a flow chamber, micropipettes, and filter tips to minimize contamination. Extracted intestines in 200 µL of sterile PBS were stored at - 20 °C until DNA extraction.

Microbial cultures.- During the extraction process, cultures were performed on blood agar with the only purpose to confirm the presence of bacteria within the intestinal content, not for identification purposes. The incubation temperature was 37 ± 2 °C and the growth was checked after 24 and 48 hours.

DNA extraction.- For the DNA extraction, DNAzol reagent from the MRC brand was used according to the manufacturer's instructions. Subsequently, the DNA samples were subjected to quantification and purity evaluation using a NANO2000 (Thermo Scientifc) drop instrument.

Molecular detection of *T. cruzi* and Amplification of the 16s region.- *T. cruzi* detection employed PCR with TCZ1/TCZ2 primers (Moser et al. 1989). The Promega kit was utilized for creating the reaction mixture, and the thermocycler settings were: initial denaturation at 95 °C for a duration of 3 minutes; followed by 35 cycles at 95 °C for 30 seconds, 52 °C for 30 seconds, and 72 °C for 30 seconds each, finally, there was a concluding extension step at 72 °C for 10 minutes. Followed by electrophoresis on 2 % agarose gels stained with SYBR Safe. In the same way the 16s region was amplified using primers 27F/R1492 (Heuer et al. 1997). The amplification process involved an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation for 1 minute at 95 °C, annealing for 1 minute at 57 °C, and extension for 1 minute at 72 °C. A final extension step of 10 minutes at 72 °C was performed (Da Mota et al. 2012). The positive samples in the electrophoresis gel were Sanger sequencing.

Bioinformatic analysis.- The samples were sent to Biosequence S.A.S in Quito, Ecuador, for Sanger amplicon sequencing. The sequences were then analyzed using Geneious Prime 2023.2.1 and compared with BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the closest matches based on sequence similarity.

Results

Entomological indexes and natural infection with *Trypanosoma cruzi*.- In the three communities, Guara, Chaquizcha, and Bellamaria, a total of 63 domestic units (DUs) were examined. Among them, 5 DUs were infested with *Panstrongylus chinai*, resulting in an infestation index of 7.9 %. A total of 12 bugs were collected (density = 0.2 bugs / DUs searched, crowding = 2.4 bugs / infested DUs, colonization index = 20 % DUs with nymphs) (Table 1). In total, 12 P*. chinai* specimens from these communities were analyzed for *T. cruzi* infection using PCR, with only one sample testing positive, representing 8.33 %.

Table 1. Entomological indices of *Panstrongylus chinai* in three rural communities (Guara, Chaquizhca and Bellamaria) in the southern region of Ecuador. DUs: Domiciliary Units.

Communities	Code of the communitites	DUs	Infested DUs	DUs with nymphs	N of bugs collected	Infestation index (%)	Density	Crowding	Colonization index (%)
Guara	GA	11	1	1	8	9.1	0.7	8	100
Chaquizhca	CQ	23	2	0	2	8.7	0.1	1	0
Bellamaria	BM	29	2	0	2	6.9	0.1	1	0
TOTAL		63	5	1	12	7.9	0.2	2.4	20

Presence of bacteria in the culture media.- In the culture media, the growth of whitish, round, creamy-looking colonies were observed at 24 hours. At 48 hours the growth of these same colonies and the appearance of punctate white colonies was observed (data no show).

Bacterial composition of *Panstrongylus chinai*.- A total of 12 samples collected in the DUs and 50 individuals from laboratory colonies were analyzed. A total of 13 samples were selected to amplify the 16S gene on the agarose gel. Of these amplified samples, 8 samples were from the field and 5 samples were from the laboratory. These selected samples went through Sanger sequencing and bioinformatic analysis. After bioinformatic analysis, only four samples showed corresponding

alignments *Staphylococcus* genus. The dominant species was *S. saprophyticus* with 75 % and *S. equorum* with 25 % (Table 2). We observed that *S. saprophyticus* predominates in both, field and laboratory insects, and it predominates in both, adults and nymphs (NIV) (Table 2). Unfortunately, the *T. cruzi* sample presented no amplification in the 16S region, thus it was not possible to analyze the microbiota.

Table 2. BLAST sequences percent identity of the bacterial microbiota species within *Panstrongylus chinai* collected in field and laboratory-reared, and percentage of *Staphylococcus* genus.

Community	Habitat	Field / Laboratory-raised	Stage	Bacterial microbiota species	BLAST sequence percent identity	Percentage of *Staphylococcus* genus (%)
Guara	Domestic	Field	Male	*Staphylococcus saprophyticus*	99	
Chaquizcha	Domestic	Field	Male	*Staphylococcus saprophyticus*	99	75
Bellamaria	Domestic	Laboratory	Nymph IV	*Staphylococcus saprophyticus*	99	
Bellamaria	Domestic	Field	Male	*Staphylococcus equorum*	99	25

Discussion

The research identified a low infestation of *Panstrongylus chinai* in DUs across three communities in Calvas County. Only adult specimens of this species were found in domestic environments, suggesting that these adults are likely attracted to lights in the DUs, similar to the behavior of *P. howardi* in Manabí province (Villacís et al. 2015). Our findings showed that certain bacterial species were predominant in triatomines, suggesting that their microbial communities are adapted to their specific insect hosts, however, the predominant bacterial species vary by species (Da Mota et al. 2012). While triatomines consume sterile blood, their intestines are quickly colonized by bacteria, likely acquired through contact with vertebrate skin during feeding (Guarneri & Schaub 2021). Cannibalism and coprophagy are the primary ways these insects transmit their microbiota and *T. cruzi*, which may be essential for maintaining the microbiota in laboratory insects and could also play a key role for field insects (Díaz et al. 2016).

In this study, we found that *Staphylococcus* sp. was the only bacterial genus present, in contrast to another research that identifies *Staphylococcus*, *Serratia*, *Enterobacter*, *Burkholderia-Caballeronia-Paraburkholderia*, and *Bacteroides* as the five most significant genera in triatomine intestinal microbiota (Hu et al. 2020; Villacís et al. 2024). The predominance of *Staphylococcus* suggests it may play crucial roles in the insect's physiology, particularly in survival and reproduction (Oliveira et al. 2018). This genus has also been found in other triatomine species like *Panstrongylus megistus* (Schaub 2020) and *Meccus pallidipennis* (Jiménez et al. 2021), and isolated from *Triatoma* spp. (Mann et al. 2020). One of the *Staphylococcus* species identified was *Staphylococcus equorum*, initially isolated from horses (Schleifer et al. 1984) and subsequently found in cow's milk with mastitis as well as in goats (Meugnier et al. 1996). It has also been detected in clinical materials (Alcaráz et al. 2003). Understanding the origins of these microorganisms is significant because some *P. chinai* were collected from communities where cattle are present in the peridomestic environment (Grijalva et al. 2015).

Staphylococcus saprophyticus was detected in both field and laboratory insects, indicating that this species may have a persistent association with *P. chinai*. Additionally, *S. saprophyticus* has been isolated from the intestinal tracts and feces of nymphs and adults of *Triatoma dimidiata* (López-Ordoñez et al. 2018). Similar to our findings, *S. saprophyticus* was also identified in a laboratory-raised *NIV*. However, López-Ordoñez et al. (2018) isolated two bacterial species, *S. saprophyticus* and *S. gallinarum*, in both field and laboratory insects. The bacterial diversity in *P. chinai* is low but consistent, with *Staphylococcus* as the dominant genus, similar to other triatomine species. This suggests that *Staphylococcus* may serve as a symbiont for the insect. However, further research is needed, especially given *P. chinai's* role as a secondary vector of Chagas disease in the southern Andean region of Ecuador. Studying the impact of *S. saprophyticus* and *S. equorum* on *P. chinai* could be important for exploring the potential of its microbiota as a biological control for *T. cruzi*.

Understanding the gut bacterial microbiota of *Panstrongylus chinai* offers promising avenues for developing targeted and sustainable vector control strategies. These include: i) Paratransgenesis, where genetically modified symbiotic bacteria express molecules that block *Trypanosoma cruzi* development (Beard et al. 2001). ii) Microbiota manipulation, through the introduction of probiotic strains that compete with the parasite (Weiss & Aksoy 2011). iii) Use of selective antibiotics or natural compounds, which alter microbiota composition and reduce vector fitness (Wang et al. 2024). iv) Identification of microbial biomarkers to monitor infection or physiological status in field populations (Oliveira et al. 2018). v) Disruption of essential symbiosis, targeting bacteria critical for vector survival or reproduction (Sassera et al. 2013). These approaches demonstrate how microbiota-based strategies can complement traditional control methods for Chagas disease vectors.

This study had several limitations: i) Unknown blood sources of field-collected *P. chinai*, as host details were not available despite most specimens being gathered from domestic environments. ii) Only 12 *P. chinai* individuals were collected, limiting the sample size. iii) Insufficient data on triatomine insects from other provinces in Ecuador and Peru. iv) The study used Sanger sequencing for bacterial 16S rRNA, however next-generation sequencing could offer a more comprehensive analysis of the microbiota species present. Despite these limitations, their potential influence on the results is outweighed by the consistency of the findings, which offer valuable insights—especially as this is the first study of the gut microbiota of *Panstrongylus chinai*, a secondary vector in the southern north of Ecuador.

Conclusion

This research, investigated *Panstrongylus chinai*, a vector of *Trypanosoma cruzi*, in the context of Chagas disease transmission in Ecuador. Utilizing molecular techniques, the study characterized the gut bacterial microbiota of *P. chinai* specimens collected from domestic and peridomestic environments in Loja province. Results demonstrated a low infestation rate and revealed a microbiota composition dominated by *Staphylococcus* species, particularly *S. saprophyticus* and *S. equorum*. These findings suggest a potential role of these bacterial species in the physiological processes and survival of the insect vector. This study emphasizes the importance of vector microbiota in shaping innovative strategies for controlling Chagas disease.

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Authors contributions

MCC: Conception and design of the study, experimental design, data acquisition/collection, data analysis and interpretation, drafting the initial version of the manuscript, manuscript review. ALR: Technical support in the study, data analysis and interpretation, manuscript review. JJB: Technical support in the study, manuscript review. JFV: Technical support in the study, manuscript review. CAY: Technical support in the study (Figure 1), manuscript review. MJG: Technical support and manuscript review. AGV: Conception and design of the study, technical support, financial management, data acquisition and review, writing and manuscript review.

Conflict of interest

The author declares that the research was conducted independently and without any conflict of interest. The study's funding sources or third-party entities had no influence on the design, implementation, analysis, or interpretation of the findings. All conclusions drawn in this work are solely the responsibility of the author.

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