International Journal for Parasitology: Parasites and Wildlife 27 (2025) 101073

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Trypanosoma cruzi in wild mammals from an endemic area of Chagas disease on the coast of Ecuador

Ana Soto-Vivas [a,*], Alexander Nicolalde [a], María Mercedes Gavilanez [a], Juan Carlos Benalcázar [a], Camila Acosta-López [a], Jhocelyn Chiluiza [a], María Isabel Calvopiña [a], Édison Encalada [b], Germán Jacóme [c], Jonathan Liria [d,e]

[a] Universidad Central del Ecuador, Facultad Ciencias Biológicas, Carrera Biología, Av. Yaguachi y Numa Pompillo, 170403, Quito, Pichincha, Ecuador
[b] Universidad Central del Ecuador, Facultad de Medicina Veterinaria y Zootecnia, Carrera Medicina Veterinaria y Zootecnia, Jerónimo Leiton s/n y Gatto Sobral, 70521, Quito, Pichincha, Ecuador
[c] Centro Agrícola Cantonal de Quevedo, Ciudad de Quevedo, Los Ríos, 120305, Ecuador
[d] Universidad Regional Amazónica Ikiam, Grupo de investigación en Población y Ambiente, kilómetro 7 vía a Alto Tena, Parroquia Muyuna, 150101, Ecuador
[e] Grupo de Investigación en Biodiversidad, Zoonosis y Salud Pública, Universidad Central del Ecuador, Ecuador

ARTICLE INFO

Keywords:
Hosts dynamics
Chiroptera
Didelphidae

ABSTRACT

Trypanosoma cruzi is a protozoan parasite that causes Chagas disease, endemic in Ecuador. In the province of Los Ríos, where the vector *Triatoma dimidiata* is present, vector-borne transmission is considered the primary route of infection. Many mammalian are involved in the transmission cycle of *T. cruzi*. Nonetheless, significant gaps remain regarding mammalian reservoirs along the Ecuadorian coast, especially in ecologically altered areas. To investigate the role of wild and domestic mammals as hosts, we assessed the presence of *T. cruzi* in two localities of Quevedo canton. Sampling focused on wild, domestic, and peridomestic mammals using live capture techniques such as mist nets for bats and Sherman and Tomahawk traps for terrestrial mammals. Blood samples were collected from all individuals, and DNA was extracted using a commercial kit. PCR was performed targeting three regions of the *T. cruzi* genome. In total, 383 mammals were sampled: 66 domestic animals, 6 peridomestic, and 317 wild individuals. Of the wild mammals, 216 were captured in La Virginia 2 and 95 in the Jacome Forest. Four wild individuals tested positive for *T. cruzi*, including three *Glossophaga soricina* and one *Marmosa simonsi*, all collected from La Virginia 2, a peri-urban community. None of the domestic or peridomestic animals were infected. This study reports, for the first time, the infection of *M. simonsi* with *T. cruzi*. The presence of positive cases exclusively in an area close to human settlements raises concern about the risk of parasite transmission in transitional landscapes. Transmission cycles of the parasite are known to be influenced by land-use change, deforestation, and host community composition. These factors may alter ecological interactions between vectors and hosts, potentially favoring transmission. Further research is needed in Ecuador to understand how ecosystem alterations shape the sylvatic cycle of *T. cruzi*, particularly the role of bats in disease maintenance.

1. Introduction

Chagas disease, also known as American trypanosomiasis, is a zoonotic disease characterized by the parasite *Trypanosoma cruzi*, host vertebrates, and vector insects. It is considered one of the most severe parasite infections in Latin America. According to the Pan American Health Organization, the disease is currently affecting 6 million individuals, and is endemic in 21 nations, posing a threat to 70 million people (PAHO, 2020). In South America, over 30,000 new cases are documented annually via diverse transmission pathways, resulting in approximately 12,000 fatalities each year (PAHO, 2020). Vector-borne transmission is the primary route of Chagas disease infection. In Latin America, over 90 % of patients live in rural areas, where housing conditions and daily practices often favor close contact with triatomine vectors in domestic and peridomestic settings.

Vector-borne transmission is the primary route of *Trypanosoma cruzi* infection. In Ecuador, this transmission mode is strongly influenced by the proximity of human populations to natural habitats of triatomine

* Corresponding author.
E-mail address: aysoto@uce.edu.ec (A. Soto-Vivas).

https://doi.org/10.1016/j.ijppaw.2025.101073
Received 4 February 2025; Received in revised form 17 April 2025; Accepted 17 April 2025
Available online 18 April 2025

vectors, the presence of mammalian hosts, and local living conditions. *Rhodnius ecuadoriensis* and *Triatoma dimidiata* have been identified as the main vectors in the country, with a total of seventeen triatomine species documented (Soto-Vivas et al., 2018; Morales-Viteri et al., 2021; Caiza-Jumbo, 2022).

Alterations in natural environments impact the dynamics of *T. cruzi* transmission as they result in modifications of bioecological patterns of vectors and hosts, occasionally enhancing human-vector interactions and thereby increasing the entomological risk of disease transmission (Vaz et al., 2007). A host is characterized as a species or assemblage of organisms that sustain the life cycle of a parasite in its natural environment (Ashford, 1997); *T. cruzi* is a parasite with several hosts, each contributing distinctively to the infection's transmission network within its habitats and biomes. Host attributes, including parasitemia and infectivity, affect the dynamics of transmission; however, there is little research assessing this capability, particularly in wild animal species in highly sensitive regions (Jansen et al., 2018). Furthermore, multiple studies have proposed that the host diversity might influence the emergence and spread of infectious diseases (Keesing et al., 2006), because higher species diversity may reduce disease transmission through a dilution effect.

Jansen et al. (2015, 2018), in studies conducted in Brazil, found that *T. cruzi* infections were more frequent in generalist mammal species, based on their habitat use and diet. These findings suggest that, in certain ecosystems, mammalian community diversity and composition may serve as indicators of the transmission intensity of *T. cruzi*. Additionally, domestic animals, particularly dogs, have been shown to contribute to the transmission cycle in peri-urban and rural areas in various endemic regions (De Oliveira et al., 2018; Pineda et al., 2021). Moreover, the biomes richness and diversity occupied by *T. cruzi* are essential factors affecting its transmission patterns, particularly in extremely diverse areas like the tropics. Longitudinal studies in Brazil reveal that mammals in the Amazon exhibit higher infection rates compared to those in other biomes (Jansen et al., 2018). These variations are likely due to differences in host population dynamics, vector behaviors, and local ecological characteristics. Understanding these traits is crucial for developing effective control strategies that consider the enzootic complexity and diversity of hosts involved in the transmission of *T. cruzi*. This study investigates the dynamics of *T. cruzi* hosts in an endemic Chagas disease region in coastal Ecuador, improving the understanding of the parasite's transmission dynamics.

2. Methods

2.1. Study area

The study was conducted in the canton of Quevedo, Los Ríos province, Ecuador. The study sites included the locality of La Virginia 2 (−1.054725S, −79.513869W), in the parish of El Guayacán, located at Km 7 on the road to El Empalme (INIAP, 2015). The main economic activities in the area are livestock and agriculture, particularly cacao and banana production. The second site was the Natural Forest Reserve of Mr. Gerardo Jácome (−1.037508S, −79.446122W), located in the parish of San Camilo. This site includes three households in a forest patch with 0.6269 ha. Both localities are separated by 24.7 km, crossing through the urban center of the city of Quevedo (Fig. 1). The sampling of potential hosts covered a gradient from anthropogenic environments to preserved natural habitats.

The study area corresponds to a Lowland Evergreen Seasonal Forest of the Ecuadorian Chocó (MAE, 2013) and features a transitional

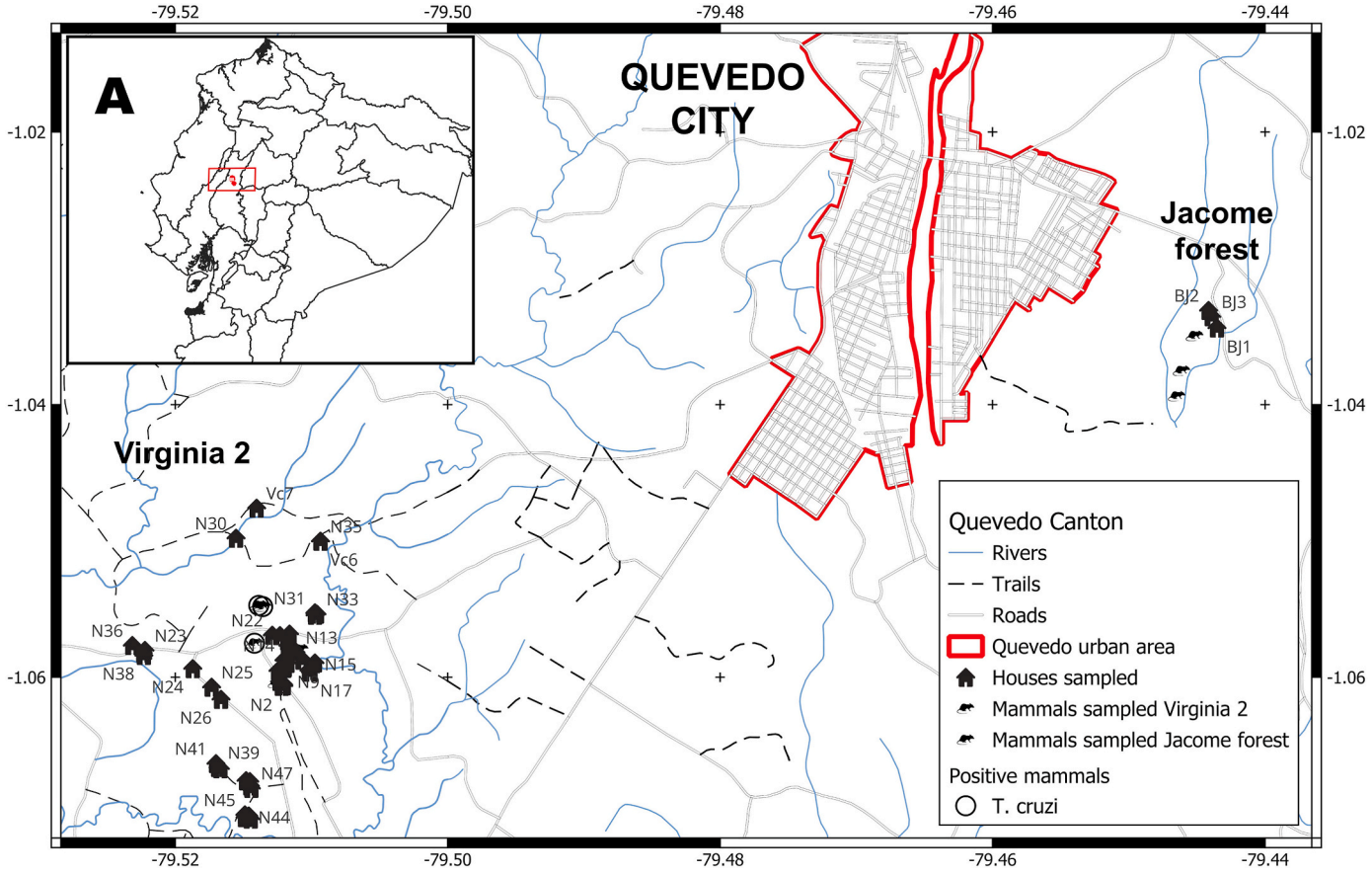

Fig. 1. Study Area in Los Ríos Province (A), showing the location of surveyed households in the La Virginia 2 Community and Jacome Forest localities, near Quevedo City.

tropical monsoon climate, according to the Köppen climate classification. The area is characterized by high relative humidity, summer rainfall, and dry winter seasons, with an average temperature of 25 °C (Beck et al., 2018). The altitude of the study sites ranged between 100 and 120 m above sea level. Sampling for hosts was conducted between September 2022 and July 2023.

2.2. Collection and blood sampling of wild mammals

Sampling was focused on wild small (bats and rodents) and medium-sized mammals (didelphids) using live capture methods. For bats, mist nets of various sizes (6, 9, and 12 m in length, 2.5 m in height) were placed near fruiting trees, water bodies, along flight paths, and, when possible, near clearings. Seven nets were deployed each night during the sampling period, starting at twilight (around 6 p.m.), and were checked every 15 min for approximately 5 h (Sánchez and Giannini, 2014).

For rodents, terrestrial transects were established along forest edges (adjacent to crops in La Virginia or within natural forests (Jacome forest). Each transect comprised ten sampling stations distributed along a 1-km trail. Each station was equipped with ten Sherman traps set for three to four consecutive nights during each sampling period. Traps were checked and rebaited each morning. Sherman traps were baited with a mixture of oats, peanut butter, vanilla, and coconut extract. For medium-sized terrestrial mammals, two linear transects were established at each site. Each transect included 10 Tomahawk traps set at 20-m intervals. These traps were baited with canned sardines and a mixture of oats, peanut butter, and bacon to attract different sets of species (Rocha et al., 2013). All captured individuals—including bats, rodents, and medium-sized mammals—were taxonomically identified, measured, weighed, and sexed. Following blood sample collection, the puncture site was disinfected and compressed until bleeding ceased, after which the animals were released at the site of capture.

The sampling effort at La Virginia 2 included 839 net hours, 1100 Sherman trap nights, and 220 Tomahawk trap nights, while Jacome forest accounted for 810 net hours, 1000 Sherman trap nights, and 200 Tomahawk trap nights. Each site was sampled over three periods, with 3–4 effective sampling nights per period.

Following established protocols (Vaz et al., 2007), blood samples were obtained from all captured individuals. Capillary blood samples were collected using heparinized capillary tubes and emptied onto Whatman No. 1 filter paper. For bats, blood was drawn by puncturing the cephalic vein. A large drop of blood was allowed to accumulate on the propatagium, collected using a glass capillary tube, and immediately used to prepare a smear on a glass slide. For opossums, individuals were restrained by the neck and hind limbs while the veterinarian administered 0.23 mg/kg of intramuscular xylazine hydrochloride. In moderate sedation, the skin at the tip of the tail or ear (1 mm) was disinfected and cut with clean scissors to obtain a thick drop of blood. This was applied directly to glass slides for immediate smear preparation, and another drop was placed on filter paper for molecular analysis. The small wound was sealed with a cauterizer. Blood samples were collected only from adult individuals; juveniles and pregnant females were released.

The filter paper was labeled with sample information, allowed to dry for a few minutes, wrapped in aluminum foil, and placed in a plastic bag. Samples were transported to Laboratorio de Variabilidad Fenotípica y Genotípica from Facultad de Ciencias Biológicas, Universidad Central del Ecuador where they were refrigerated at 4 °C before undergoing PCR analysis.

Additional care measures were applied to ensure animal welfare after sampling. Bats were hydrated with a sugared water solution and released from nearby trees. Rodents and medium-sized mammals were returned to traps with cotton and food and later released at the site of capture. Sedated animals recovered within 30 min and were monitored before release. All procedures followed ethical guidelines approved by the animal bioethics committee of Universidad Central del Ecuador and were conducted under the standards of Erazo et al. (2017). Reference specimens were collected at each site and deposited in the mammal collection of Instituto Nacional de Biodiversidad (INABIO) under research permit given by Ministerio de Ambiente, Agua y Transición Ecológica (MAATE-ARSFC-2022-2673). Taxonomic identification followed the classifications proposed by Eisenberg and Redford (1989), Tirira (1999, 2007), and Díaz et al. (2021).

2.3. Blood sampling of domestic and peridomestic animals

Prior to sampling, all households were visited and informed consent was obtained from the owners. Blood samples from domestic animals were collected only when explicit authorization was granted. Domestic animals and peridomestic mammals were not sedated for sampling. Dogs and cats were restrained with the help of their owners and biologist assistants. Animals were placed in a sphinx position, and a fabric strap was used as a muzzle. The skin of the forelimbs was cleaned with cotton soaked in 60 % ethanol. The veterinarian applied a rubber tourniquet above the elbow joint and collected 0.5 ml of blood from the cephalic vein using an insulin syringe. This volume was sufficient for preparing multiple smears on glass slides and reserving another portion for genetic analysis. For pigs, individuals were restrained with a snare and supported by their owners. The ear area was cleaned with alcohol, and a manual tourniquet was applied at the base of the ear. The veterinarian collected 0.5 ml of blood from the marginal vein using a 3-ml syringe, applying a few drops directly onto glass slides for smears. Another portion was deposited on filter paper for genetic analysis. Pigs were immediately released after sampling.

2.4. Wild mammals community data analysis

Assessing community diversity is crucial when analyzing potential hosts of Chagas disease due to the complex interactions between vectors, hosts, and the environment that influence disease transmission dynamics. Considering the main goal is to assess the overall diversity of potential *T. cruzi* hosts, sampling effort was pooled for all wild mammals and estimated using species accumulation curves separated by site, as these were far enough to be considered independent. Given we had abundance data for species at each site, estimated richness was assessed using the nonparametric estimator Chao 1, using packages Vegan (Oksanen et al., 2024) and BiodiversityR (Kindt and Coe, 2005) in R (R Core Team, 2024). Communities were characterized using rank abundance curves created using package rasterdiv (Rocchini et al., 2021) that allow us to compare community structure across sites. The diversity of potential wild mammalian hosts was calculated using Hill numbers Q0, Q1, and Q2, as well as Pielou's test for evenness. For diversity indexes, we used permutation tests to determine whether communities were significantly different across sites.

2.5. DNA extraction

DNA was extracted at Laboratorio de Variabilidad Fenotípica y Genotípica from Facultad de Ciencias Biológicas, Universidad Central del Ecuador. Extraction was conducted using Column-Pure Blood Genomic DNA kit (abm® Richmond, Canada) with the following modifications. From the droplets deposited in the Whatman filter paper during collection, the blood taint was cut and placed in a 2 ml tube with 100 µL TE buffer (Tris 10 mM, pH 8.0 and 0.1 mM EDTA) and incubated at 56 °C for 15 min. The pieces of paper were then pressed several times and taken away. The solution was then incubated at 86° for 15 min and centrifuged at 10000 rpm for 3 s (Mota et al., 2007). After this, the manufacturer's recommendations were followed. Finally, DNA was quantified with the dsDNA BR Qubit™ kit (ThermoScientific, Waltham, USA). The obtained DNA was stored at −20 °C when not used immediately.

2.6. Detection of T. cruzi

For *T. cruzi* detection a 50 μL PCR reaction was prepared, 33 μL nuclease free water, 5 μL 10X PCR buffer, 2 μL (100 pmol/μL) of each primer, 6 μL (25 mM) MgCl2, 1 μL (10 mM) dNTPs, 1 μL (5U/μL) Taq DNA polymerase and 3 μL of the extracted DNA. The PCR began with a 94 °C denaturation for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, concluding with a 72 °C final extension for 5 min (Burgos et al., 2007; Mota et al., 2007). The PCR products were analyzed using 2 % agarose gel electrophoresis, together with a 100 bp Opti-DNA molecular marker. Positive control was included in all reactions, using DNA from a previously analyzed triatomine, positive for the parasite (Rodríguez-Dos-Santos et al., 2018). The primers used for the identification of *T. cruzi* in animal blood samples target three regions. For the spliced leader-intergenic region, primers 5′-CGT ACC AAT ATA GTA CAG AAA CTG-3′ and 5′-CTC CCC AGT GTG GCC TGG G-3′ were used as described by Burgos et al. (2007). For kDNA, primers 5′-TGG TTT TGG GAG GGG SSK TCA AMT TT-3′ and 5′-TAT ATT ACA CCA ACC CCA ATC GAA CC-3′ were utilized following Mota et al. (2007). Additionally, satellite DNA repetitive sequences were amplified using primers 5′-ASTCGGCTGATCGTTTTCGA-3′ and 5′-AATTCCTCCAAGCAGCGGATA-3’.

2.7. Ethical considerations

The Animal Research Ethics Committee of the Universidad Central del Ecuador, after verifying that the research protocol complied with national and international regulations regarding the use of animals in research, granted the Ethical Viability Certificate No. CEIA-AE-005-2024. The research was conducted with the authorization for the collection of specimens of biological diversity species No. 2673, code MAATE-ARSFC-2022-2673, issued by Ministerio de Ambiente, Agua y Transición Ecológica. The researchers declare their knowledge and understanding of the “Universal Declaration of Animal Rights,” proclaimed by UNESCO in 1978.

3. Results

A total of 383 mammals were collected (Table 1), including 72 domestic animals (6 of which corresponded to pigs in the peridomestic area), and 311 wild mammals (303 bats, 6 didelphids, and 2 rodents), with 216 individuals captured at La Virginia 2 and 95 at Jacome forest. The most diverse and well-represented group at both sites were phyllostomid bats, with 21 species, followed by vespertilionids (2 species) and molossids (1 species). Only one species of rodent and two species of didelphids were recorded, primarily near human settlements in La Virginia. Within bats, the most common species for both sites was *Glossophaga soricina,* followed by *Sturnira erythromos* at Comunidad la Virginia and *Carollia* spp. at Jacome forest.

Species accumulation curves indicate (see Fig. 2) that sampling effort effectively captured most of the expected diversity at Jacome Forest, achieving 93 % sample completeness. However, at La Virginia 2, only 52 % of the expected richness was recorded, suggesting that further sampling may be needed to improve the representation of potential wild hosts for *Trypanosoma* in this area. The Chao estimator suggests an expected richness of 16 ± 1.8 species at Jacome Forest and 46 ± 17.4 at La Virginia 2, with the larger uncertainty at the latter site reinforcing the need for additional sampling. Regarding wild host community diversity as a factor influencing disease transmission, Hill diversity indices revealed variation in community structure. Jacome Forest exhibited lower species richness (Q0 = 15) compared to La Virginia 2 (Q0 = 24), yet had slightly higher Shannon diversity (Q1 = 9.587 vs. 8.32) and lower Simpson diversity (Q2 = 1.158 vs. 1.31). This indicates that while La Virginia 2 harbored more species, Jacome Forest maintained a more even species distribution, as reflected by its higher Pielou’s evenness index (0.308 vs. 0.209). The combination of lower diversity and higher

Table 1
Community composition of potential wild mammalian reservoirs, showing *T. cruzi* positive/total number of sampled individuals positive for *T. cruzi* at the two study sites Jacome forest (Forest remnant) and La Virginia 2 community (Cacao plantation).

Ecotopes	Order	Family	Species	Jacome forest (*T. cruzi*+/Total)	La Virginia 2 (*T. cruzi*+/Total)	% Prevalence
Domestic	Carnivora	Canidae	*Canis lupus familiaris*	0/3	0/50	0
		Felidae	*Felis catus*	0/3	0/10	0
Peridomestic	Artiodactyla	Suidae	*Sus scrofa domesticus*	–	0/6	0
Wild	Rodentia	Cricetidae	*Transandinomys talamancae*	–	0/1	0
		Echymidae	*Proechymis decumanus*	0/1	–	0
	Didelphimorphia	Didelphidae	*Philander melanurus*	0/3	–	0
			Marmosa simonsi	–	1/3	33.33
	Chiroptera	Phyllostomidae	*Artibeus* cf. *aequatorialis*	0/5	0/1	0
			Artibeus cf. *ravus*	0/1	–	0
			Artibeus cf. *fraterculus*	0/3	0/1	0
			Artibeus cf. *lituratus*	0/6	0/6	0
			Carollia cf. *castanea*	0/8	0/1	0
			Carollia cf. *brevicauda*	0/19	0/13	0
			Carollia cf. *perspicillata*	0/16	0/10	0
			Carollia sp.	–	0/71	0
			Gardnerycteris crenulatum	–	0/3	0
			Glossophaga soricina	0/21	3/67	4.47
			Lophostoma occidentalis	–	0/2	0
			Phyllostomus discolor	0/3	0/8	0
			Platyrrhinus helleri	–	0/1	0
			Platyrrhinus matapalensis	–	0/1	0
			Sturnira bidens	–	0/1	0
			Sturnira cf. *bakeri*	0/2	0/3	0
			Sturnira cf. *lilium*	0/4	0/3	0
			Sturnira erythromos	–	0/15	0
			Sturnira ludovici	–	0/1	0
			Sturnira cf. *luisi*	–	0/1	0
			Vampyressa thyone	0/1	–	0
	Molossidae		*Molossus molossus*	–	0/2	0
	Vespertilionidae		*Myotis nigricans*	0/2	0/1	0

Total: 4 positive cases from 9 species tested at Jacome forest and 30 species at La Virginia 2 community. Prevalence: 0/95 (Jacome forest), 4/216 (La Virginia 2), 1.85 % overall.

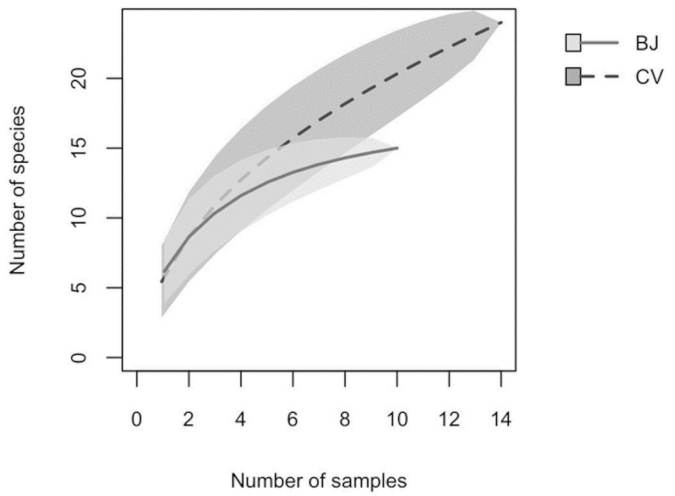

Fig. 2. Species accumulation curves for Jacome forest (BJ) and La Virginia community (CV) based on rarefaction of sampling days and captures at each site.

dominance in La Virginia 2 may facilitate pathogen transmission, supporting the results regarding infection rates in this community. While permutation tests indicated no statistically significant differences in diversity parameters among sites, these ecological patterns provide a relevant framework for understanding differences in infection rates across sites.

Positive *T. cruzi* samples were detected only in wild mammals at cacao plantations near La Virginia 2. Specifically, one an adult male *Marmosa simonsi*, and three female *Glossophaga soricina* individuals. It is important to note that *G. soricina* was the most abundant species at both sites. The overall prevalence of *T. cruzi* in wild mammalian hosts was low (1.85 %), with only four out of 216 individuals showing positive results. In contrast, none of the domestic hosts in the area tested positive for *T. cruzi*, and no vectors were detected during the study period. Additionally, all individuals collected from the remnant forest patch (Jacome forest) tested negative.

Specific data for the positive individuals can be found in Table 1. PCR results amplified the corresponding satellite DNA fragment confirming the presence of *T. cruzi* kinetoplastid DNA (Fig. 3).

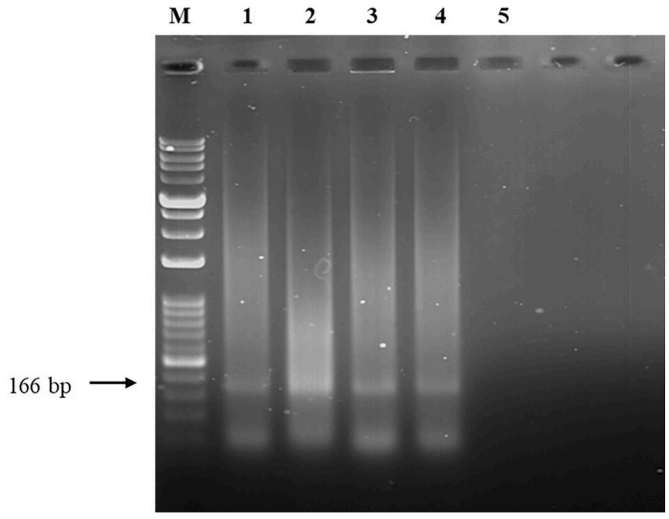

Fig. 3. Agarose gel electrophoresis 2 % for the amplified satellite DNA fragment from *T. cruzi* kinetoplastid. M: 100bp Opti-DNA Ladder, 1: *Marmosa simonsi* positive sample, 2–4: *Glossophaga soricina* positive samples, 5: Negative control (no template). A single 166 bp band (indicated) was observed in all the cases.

4. Discussion

This study demonstrated the circulation of *T. cruzi* in wild mammals from an endemic area of Chagas disease in an ecologically altered region in coastal Ecuador; *Marmosa simonsi* was reported for the first time infected with *T. cruzi* (prevalence 33.33 %). From an ecological perspective, *T. cruzi* is considered a generalist parasite that can affect hundreds of species of mammals in the Americas. This wide range of hosts is an important part of the parasite's life cycle in nature. Wild animals, especially from the genus *Didelphis* and *Philander*, are important in the spread of the parasite because they have high parasitemia and can bioaccumulate different kinds of *T. cruzi* (Jansen et al., 2018; De Oliveira et al., 2018). In the wild, the *T. cruzi* cycle is connected to trophic networks. Felines and canines can get infected by eating small vertebrates or triatomines that are already infected. This way that predators and prey share information is common in carnivores like *Nasua* and some primates (El Saadi et al., 2020). In the study area, we did not report the presence of medium carnivores probably due to the highly fragmented conditions of the area coupled with the specific habitat requirements of these meso predators (Ferreira et al., 2018; Barrera-Vargas et al., 2023)

The species found to be positive in this study exhibit synanthropic behavior, playing a crucial role in the epidemiology of *T. cruzi* by potentially linking sylvatic and peridomestic transmission cycles. All positive samples came from individuals living in Community La Virginia 2, an area highly impacted by human activity. Interestingly, the positive cases were all associated with generalist species, likely reflecting habitat loss for specialist species or the advantage of generalists in adapting to disturbed environments (McCallum and Dobson, 2002). A similar pattern was reported in Cantillo Barraza et al., (2014) in a study of *T. cruzi* transmission from the Colombian Caribbean region, where didelphids had the higher infection rates. This dynamic increases the chances of interactions between hosts, vectors, humans, and domestic animals (Deem et al., 2001).

The ecological studies about this species are scarce, but some authors showed that *M. simonsi* can use transient shelters such as ground burrows, tree hollows, and abandoned bird nests. These marsupials frequently construct their shelters with leaves collected from the area, which could facilitate the movement of immature triatomine (eggs and nymphs), promoting the passive dispersal of these insects in the wild environment (O'Connell, 1979; Delgado-V et al., 2014; Brito et al., 2022). Furthermore, the passive dispersal of these insects plays an important role in *T. cruzi* transmission. The primary vectors of *T. cruzi* in the study region are *R. ecuadoriensis* and *T. dimidiata*, which display arboreal behaviors and intermittently utilize burrows as habitats, thereby potentially increasing the of interactions between triatomines and marmosas (Jansen et al., 2018; El Saadi et al., 2020; Caiza-Jumbo, 2022; Moo-Millan et al., 2023). The ecological interactions of vectors and hosts elevate the risk of parasite transmission in natural ecosystems. In Ecuador, despite their wide distribution which often overlaps with urbanized environments, the role of bats in the *T. cruzi* transmission dynamics remains poorly understood (Gómez-Sánchez et al., 2022). The prevalence of *T. cruzi* in *Glossophaga soricina* was 4.47 %, lower than 25 % reported by Pinto et al. (2015) for the same bat species in Loja Province. These authors observed 36.5 % prevalence in bats from the study area, including 2.5 % for *Myotis* sp. While bats are associated with a wide range of zoonotic pathogens, competition among parasites within the same host remains understudied (Bashey, 2015).

Bats are hosts of multiple pathogens due to their synanthropic nature (Calisher et al., 2006; Kasso and Balakrishnan, 2013). Evidence suggests a close relationship between Neotropical bat trypanosomes and trypanosomes from Australian lineage related to *T. cruzi* clade, suggesting that they likely evolved in bats and dispersed within and between continents (Hamilton et al., 2012; Lima et al., 2015). The flight capabilities, longevity, and gregarious social structure may increase their

significance as *T. cruzi* hosts (Luis et al., 2013.). The prevalence of *T. cruzi* infection in non-hematophagous bats in Mexico was reported to be 30.2 % by Torres-Castro et al. (2021). The parasite may be acquired by non-hematophagous species through vector transmission or by insectivorous bats via the oral ingestion of infected invertebrates. However, triatomines were not discovered at the sampling sites in this investigation. Bats have been demonstrated to transmit *T. cruzi* vertically (Añez et al., 2009), suggesting that their role in the parasite dynamics may be more important than previously understood, as they may directly transfer the pathogen through bites.

The occurrence of *G. soricina* in cacao plantations, and the identification of individuals positive for *T. cruzi*, demonstrated the complex relationships between wildlife and agricultural ecosystems. In these habitats, bats function as pollinators and possible disease hosts, highlighting the importance of understanding animal ecology within agroecosystems to effectively minimize disease risks. From an epidemiological perspective, the discovery of *T. cruzi* in *G. soricina* raises concerns about the possible oral transmission of Chagas disease, a route demonstrated in other areas where food contamination by hosts species occurs (Rueda et al., 2014; Gutiérrez et al., 2023). This transmission pathway is relevant in regions where conventional vector control methods, like targeting domestic triatomines, are less effective due to the presence of secondary vectors and hosts (Cantillo-Barraza et al., 2014; Flores-Ferrer et al., 2019). The transmission dynamics of *T. cruzi* in these environments are related to biodiversity, since heightened mammalian variety may elevate parasite prevalence (Morales-Betancourt, 2016). Moreover, environmental alterations such as deforestation and agricultural expansion may disturb vectors and host interactions, resulting in novel transmission dynamics that necessitate continuous monitoring and adaptive management techniques (Rueda et al., 2014; Santos et al., 2021). Ecological markers, including certain palm tree species that host triatomines, can assist in identifying regions with heightened risk of Chagas disease transmission (Santos et al., 2021).

In this study no domestic or peridomestic animals were infected with *T. cruzi*. Nonetheless, domestic animals, especially dogs, play an important role in transmitting *T. cruzi*, particularly in peri-urban and residential areas. Dogs can act as sentinels for infection because of their regular exposure to infected triatomines and wildlife hosts; their proximity to humans and frequent contact with vectors and infected animals position them as a crucial link between sylvatic and domestic transmission cycles (Ricardo-Caldera et al., 2024).

In line with these observations, although only four individuals tested positive for *T. cruzi*, it is important to acknowledge that the PCR assays used in this study targeted specific regions associated primarily with DTU TcI. Therefore, the low prevalence detected may reflect limitations in detection sensitivity toward other circulating DTUs (Zecca et al., 2020). Given that the region is considered an area of intense transmission, it is plausible that other DTUs may be present but remain undetected. Future studies incorporating broader or multiplex PCR assays capable of detecting multiple DTUs are needed to better assess the full spectrum of *T. cruzi* diversity in this region.

Transmission cycles of *T. cruzi* are substantially influenced by different structural and ecological factors, such as changes in land use, habitat fragmentation and potential host community diversity. Deforestation and the fast development of intensive agriculture have seriously reduced biodiversity in the study area and fragmented natural forests. According to longitudinal research conducted in Brazil, Amazonian mammals show more infections than those in other biomes (Zecca et al., 2020). These variations may reflect changes in local ecological traits, vector behavior, and host population dynamics. Developing efficient control plans considering the enzootic complexity and diversity of hosts engaged in *T. cruzi* transmission depends on an awareness of these elements. Furthermore, in our study, *T. cruzi* was detected exclusively in La Virginia 2, a site with higher species richness but lower evenness compared to Jacome Forest. This pattern aligns with the dilution effect hypothesis, which suggests that high biodiversity in a community can reduce pathogen transmission by diluting the pool of competent hosts (Keesing and Ostfeld, 2021). More even communities may decrease encounter rates between vectors and efficient hosts, thereby reducing pathogen persistence (Rohr et al., 2020). Research on other vector-borne diseases, like Amazonian leishmaniases, supports the dilution effect, showing that higher mammal diversity correlates with lower parasite prevalence (Kocher et al., 2022). While our diversity indices did not show statistically significant differences, La Virginia 2 exhibited lower evenness, suggesting a more dominant species composition that may favor pathogen transmission. The presence of *T. cruzi* exclusively in cacao plantations near human settlements highlights the need for control strategies that integrate ecological factors, such as community structure and species interactions, into disease management (Erazo et al., 2017; Stella et al., 2018; Gurgel-Gonçalves, 2023).

Additionally, fieldwork logistics were constrained by safety concerns in certain areas of the canton of Quevedo. Due to episodes of local insecurity and limitations related to the safety of research personnel, access to some zones was restricted, which may have reduced spatial sampling coverage and affected the full representativeness of mammalian diversity in the region. However, the active support of local community members helped identify safe sampling areas, facilitating the continuation of field activities. Future efforts should consider establishing formal collaborations with local police authorities to enhance field safety and enable broader ecological assessments.

Finally, understanding the enzootic complexity and diversity of hosts involved in *T. cruzi* transmission is essential for developing effective control plans tailored to fragmented and productive landscapes. Future research should prioritize the role of bats in *T. cruzi* dynamics to gain a more comprehensive understanding of their function in Chagas disease epidemiology and to inform public health authorities about this potential risk. Keesing and Ostfeld (2021) have demonstrated that preserving biodiversity is essential for preventing the transmission of zoonotic parasites (Córdoba-Aguilar et al., 2021). Additional research is required in Ecuador to comprehend the impact of ecosystem fragmentation on the sylvatic transmission cycles of *T. cruzi*.

CRediT authorship contribution statement

Ana Soto-Vivas: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Alexander Nicolade:** Methodology, Investigation, Data curation. **María Mercedes Gavilanez:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Juan Carlos Benalcázar:** Methodology, Investigation, Data curation. **Camila Acosta-López:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Jhocelyn Chiluiza:** Methodology, Investigation, Data curation. **María Isabel Calvopiña:** Methodology, Investigation, Data curation. **Édison Encalada:** Methodology, Investigation, Data curation. **Germán Jacóme:** Methodology, Investigation, Conceptualization. **Jonathan Liria:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors utilized OpenAI's ChatGPT to enhance the readability and language of the text. The authors carefully reviewed and edited the content generated by the tool to ensure accuracy and integrity. The authors take full responsibility for the content of this publication.

Research funding

Universidad Central del Ecuador, through the Dirección de

Investigación and the Senior 2021 grant program (Dinámica de transmisión de *Trypanosoma cruzi* Chagas y ciencia ciudadana en dos localidades del cantón Quevedo, como un modelo de estudio de la enfermedad de Chagas en el Ecuador. Grant No. DI-CONV-2021-24), funded this project.

Declaration of competing interest

The authors declare that they have no financial or personal conflicts of interest that could have influenced the work reported in this article.

Acknowledgments

The authors thank the community of La Virginia 2, the Jácome family, and the members of the Cantonal Agricultural Center of Quevedo for their unconditional support during the field phase. We thank the anonymous reviewers for their constructive comments and suggestions, which helped improve the quality of this manuscript.

References

Añez, N., Crisante, G., Soriano, P.J., 2009. *Trypanosoma cruzi* congenital transmission in wild bats. Acta Trop. 109, 78–80. https://doi.org/10.1016/j.actatropica.2008.08.009.

Ashford, R.W., 1997. What it takes to be a reservoir host. Belg. J. Zool. 127, 85–90.

Bashey, F., 2015. Within-host competitive interactions as a mechanism for the maintenance of parasite diversity. Philos. Trans. R. Soc. Lond. B Biol. Sci. 270, e20140301. https://doi.org/10.1098/rstb.2014.0301.

Barrera-Vargas, J., Delgado, C.A., Arias-Alzate, A., 2023. Mesocarnivores activity patterns in the northern Colombian andes. Therya 14 (3). https://doi.org/10.12933/therya-23-1243.

Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future Köppen-Geiger climate classification maps at 1-km resolution. Sci Data 30 (5), 180214. https://doi.org/10.1038/sdata.2018.214.

Brito, G.A., Salas, J.A., Heimpel, G.E., Bulgarella, M., 2022. Use of artificial nest boxes by two species of small, arboreal mammals in Ecuadorian tropical dry forest. Neotrop. Biodivers. 8, 108–111. https://doi.org/10.1080/23766808.2022.2031562.

Burgos, J.M., Altcheh, J., Bisio, M., Duffy, T., Valadares, H.M., Seidenstein, M.E., Piccinali, R., Freitas, J.M., Levin, M.J., Macchi, L., Macedo, A.M., Freilij, H., Schijman, A.G., 2007. Direct molecular profiling of minicircle signatures and lineages of Trypanosoma cruzi bloodstream populations causing congenital Chagas disease. Int. J. Parasitol. 37, 1319–1327. https://doi.org/10.1016/j.ijpara.2007.04.015.

Caiza-Jumbo, S.C., 2022. Modelamiento de nicho ecológico, áreas de endemismo de Triatominae e implicaciones en la enfermedad de Chagas en Ecuador. [Tesis de Licenciatura]. Universidad Central del Ecuador, Quito, pp. 1–50.

Cantillo-Barraza, O., Chaverra, D., Marcet, P., Arboleda-Sánchez, S., Triana-Chávez, O., 2014. Transmission in a Colombian Caribbean region suggests that secondary vectors play an important epidemiological role. Parasites Vectors 7, 1–10. https://doi.org/10.1186/1756-3305-7-381.

Calisher, C.H., Childs, J.E., Field, H.E., Holmes, K.V., Schountz, T., 2006. Bats: important reservoir hosts of emerging viruses. Clin. Microbiol. Rev. 19, 531–545. https://doi.org/10.1128/cmr.00017-06.

Córdoba-Aguilar, A., Ibarra-Cerdeña, C.N., Castro-Arellano, I., Suzan, G., 2021. Tackling zoonoses in a crowded world: lessons to be learned from the COVID-19 pandemic. Acta Trop. https://doi.org/10.1016/j.actatropica.2020.105780.

Deem, S.L., Karesh, W.B., Weisman, W., 2001. Putting theory into practice: wildlife health in conservation. Conserv. Biol. 15 (5), 1224–1233.

Delgado-V, C.A., Arias-Alzate, A., Aristizábal-Arango, S.V., Sánchez-Londoño, J.D., 2014. Uso de la cola y el marsupio en *Didelphis marsupialis* y *Metachirus nudicaudatus* (Didelphiomorphia: Didelphidae) para transportar material de anidación. Mastozool. Neotrop. 21, 129–134.

De Oliveira, G., Santos, F.M., Carvalho, G., Gomes, W., Vilela, J., Campos, A., Meyers, R. M., Perles, L., De Oliveira, C., Das Chagas, S., De Andrade, G., Jansen, A., Herrera, H., 2018. Maintenance of *Trypanosoma cruzi, T. evansi*, and *Leishmania* spp. by domestic dogs and wild mammals in a rural settlement in the Brazil-Bolivian border. Int. J. Parasitol. Parasites Wildl. 7, 398–404. https://doi.org/10.1016/j.ijppaw.2018.10.004.

Díaz, M.M., Solari, S., Gregorin, R., Aguirre, L.F., Barquez, R.M., 2021. Clave de identificación de los murciélagos neotropicales. CONYCET, PCMArg, Publicación Especial No. 4.

Eisenberg, J.F., Redford, K.H., 1989. Mammals of the Neotropics, 3: Ecuador, vol. 3. University of Chicago Press, Bolivia, Brazil.

El Saadi, N., Bah, A., Mahdjoub, T., Kribs, C., 2020. On the sylvatic transmission of *Trypanosoma cruzi*, the parasite causing Chagas disease: a view from an agent-based model. Parasite Epidemiol. Control. 5, e00120. https://doi.org/10.1016/j.ecolmodel.2020.109001.

Erazo, D., Cordovez, J.M., Cabrera, C., Calzada, J.E., Saldaña, A., Gottdenker, N.L., 2017. Modelling the influence of host community composition in a sylvatic Trypanosoma cruzi system. Parasitology 144 (14), 1881–1889. https://doi.org/10.1017/S0031182017001287.

Ferreira, A.S., Peres, C.A., Bogoni, J.A., Cassano, C.R., 2018. Use of agroecosystem matrix habitats by mammalian carnivores (Carnivora): a global-scale analysis. Mamm Rev. 48 (4), 312–327. https://doi.org/10.1111/mam.12137.

Flores-Ferrer, A., Waleckx, E., Rascalou, G., Dumonteil, E., Gourbière, S., 2019. *Trypanosoma cruzi* transmission dynamics in a synanthropic and domesticated host community. PLoS Neglected Trop. Dis. 13 (12), e0007902. https://doi.org/10.1371/journal.pntd.0007902.

Gómez-Sánchez, E.F., Ochoa-Díaz-López, H., Espinoza-Medinilla, E.E., Velázquez-Ramírez, D.D., Santos-Hernández, N.G., Ruiz-Castillejos, C., Vidal-López, D.G., Moreno-Rodríguez, A., Flores-Villegas, A.L., López-Argueta, E., De Fuentes-Vicente, J.A., 2022. Mini-exon gene reveals circulation of TcI *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae) in bats and small mammals in an ecological reserve in southeastern Mexico. ZooKeys 1084, 139–150. https://doi.org/10.3897/zookeys.1084.78664.

Gurgel-Gonçalves, R., 2023. Stronger control-surveillance systems for vector-borne Chagas disease. Memorias Do Instituto Oswaldo Cruz 117. https://doi.org/10.1590/0074-02760210130chgsb.

Gutiérrez, S.A., Jaimes-Dueñez, J., Cruz-Saavedra, L., Hernández, C., Cantillo-Barraza, O., Álvarez, F., Blanco, M., Leal, B., Martinez, L., Medina, M., Medina, M., Valdivieso, S., Ramírez, J.D., 2023. An outbreak of acute Chagas disease possibly spread through oral transmission involving animal reservoirs in Eastern Colombia. Am. J. Trop. Med. Hyg. 110 (1), 36–39. https://doi.org/10.4269/ajtmh.23-0380.

Hamilton, P.B., Teixeira, M.M., Stevens, J.R., 2012. The evolution of *Trypanosoma cruzi*: the 'bat seeding' hypothesis. Trends Parasitol. 28, 136–141. https://doi.org/10.1016/j.pt.2012.01.006.

Instituto Nacional de Investigaciones Agropecuarias (INIAP), Estación Experimental Tropical Pichilingue, Departamento Nacional de Manejo de Suelos y Aguas, 2015. Informe Anual 2015. Mocache, Ecuador.

Jansen, A.M., Xavier, S.C., Roque, A.L., 2015. The multiple, complex, and changeable scenarios of the *Trypanosoma cruzi* transmission cycle in the sylvatic environment. Acta Trop. 151, 1–15. https://doi.org/10.1016/j.actatropica.2015.07.018.

Jansen, A.M., Xavier, S.C., Roque, A.L., 2018. *Trypanosoma cruzi* transmission in the wild and its most important reservoir hosts in Brazil. Parasites Vectors 11, 1–25. https://doi.org/10.1186/s13071-018-3067-2.

Kasso, M., Balakrishnan, M., 2013. Ecological and economic importance of bats (order Chiroptera). Int. Sch. Res. Not. 2013, e187415. https://doi.org/10.1155/2013/187415.

Keesing, F., Ostfeld, R.S., 2021. Impacts of biodiversity and biodiversity loss on zoonotic diseases. Proc. Natl. Acad. Sci. U. S. A. 118 (17), e2023540118. https://doi.org/10.1073/pnas.2023540118.

Kindt, R., Coe, R., 2005. Tree Diversity Analysis. A Manual and Software for Common Statistical Methods for Ecological and Biodiversity Studies. World Agroforestry Centre (ICRAF). ISBN 92-9059-179-X.

Kocher, A., Cornuault, J., Gantier, J.-C., Manzi, S., Chavy, A., Girod, R., Dusfour, I., Forget, P.-M., Ginouves, M., Prévot, G., Guégan, J.-F., Bañuls, A.-L., de Thoisy, B., Murienne, J., 2022. Biodiversity and vector-borne diseases: host dilution and vector amplification occur simultaneously for Amazonian leishmaniases. Mol. Ecol. 32 (8), 1817–1831. https://doi.org/10.1111/mec.16341.

Lima, L., Espinosa-Álvarez, O., Pinto, C.M., Cavazzana Jr., M., Pavan, A.C., Carranza, J. C., Lim, B.K., Campaner, M., Takata, C.S., Camargo, E.P., Hamilton, P.B., Teixeira, M.M., 2015. New insights into the evolution of the *Trypanosoma cruzi* clade provided by a new trypanosome species tightly linked to Neotropical *Pteronotus* bats and related to an Australian lineage of trypanosomes. Parasites Vectors 8, 657. https://doi.org/10.1186/s13071-015-1255-x.

Luis, A.D., Hayman, D.T., O'Shea, T.J., Cryan, P.M., Gilbert, A.T., Pulliam, J.R.C., Mills, J.N., Timonin, M.E., Willis, C.K.R., Cunningham, A.A., Fooks, A.R., Rupprecht, C.E., Wood, J.L.N., Webb, C.T., 2013. A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? Proc. Biol. Sci. 280, e20122753. https://doi.org/10.1098/rspb.2012.2753.

McCallum, H., Dobson, A., 2002. Disease, habitat fragmentation, and conservation. Proc. Biol. Sci. 269 (1504), 2041–2049. https://doi.org/10.1098/rspb.2002.2079.

Ministerio del Ambiente del Ecuador (MAE), 2013. Sistema de clasificación de los ecosistemas del Ecuador continental. Subsecretaría de Patrimonio Natural, Quito, p. 143.

Moo-Millan, J., Hernández-Andrade, A., May-Concha, I., Montalvo-Balam, T., Arnal, A., Talavera-Escalante, T., Amblard-Rambert, A., Martínez-Vega, P., Ramos-Ligonio, A., Ibarra-Cerdeña, C., Hernández-Betancourt, S., Waleckx, E., 2023. Temporal variation of *Triatoma dimidiata* abundance and infection with *Trypanosoma cruzi* in domestic and sylvatic habitats of rural Yucatan, Mexico. Acta Trop. https://doi.org/10.1016/j.actatropica.2023.101456.

Morales-Betancourt, J.A., 2016. Measuring the Relationship between Biodiversity, *Trypanosoma Cruzi* Prevalence, and Triatominae Blood Meal Sources in Secondary Forests and Oil Palm Plantations in the Eastern Llanos of Colombia. Universidad de los Andes, Colombia. Master's Thesis.

Morales-Viteri, D., Quinatoa-Tutillo, P., Sánchez-Mackenzie, D., Cagua-Ordoñez, J., 2021. Enfermedad de Chagas en el Ecuador: Una revisión sistemática de los aspectos epidemiológicos y entomológicos. INSPILIP 5, 1–11. https://doi.org/10.31790/inspilip.v5i1.2.

Mota, J., Chacon, J.C., Gutiérrez-Cabrera, A.E., Sánchez-Cordero, V., Wirtz, R.A., Ordoñez, R., Panzera, F., Ramsey, J.M., 2007. Identification of blood meal source and infection with *Trypanosoma cruzi* of Chagas disease vectors using a multiplex cytochrome b polymerase chain reaction assay. Vector Borne Zoonotic Dis. 7, 617–628. https://doi.org/10.1089/vbz.2007.0106.

O'Connell, M.A., 1979. Ecología de marsupiales didélfidos del norte de Venezuela. En. In: Eisenberg, J.F. (Ed.), Ecología de vertebrados en el Neotrópico Norte. Smithsonian Institution Press, Washington, DC, pp. 73–87.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C., Weedon, J., 2024. Package 'vegan': community ecology package. R package version 2.6–6.1. https://CRAN.R-project.org/package=vegan.

Pan American Health Organization (PAHO), 2020. Chagas Disease - PAHO/WHO. Pan American Health Organization, 2022 Sep. 30.

Pineda, V.J., González, K.A., Perea, M., Rigg, C., Calzada, J.E., Chaves, L.F., Vásquez, V., Samudio, F., Gottdenker, N., Saldaña, A., 2021. Surveillance and genotype characterization of zoonotic trypanosomatidae in *Didelphis marsupialis* in two endemic sites of rural Panama. Int. J. Parasitol. Parasites Wildl. 17, 20–25. https://doi.org/10.1016/j.ijppaw.2021.12.002.

Pinto, C.M., Ocaña-Mayorga, S., Tapia, E.E., Lobos, S.E., Zurita, A.P., Aguirre-Villacís, F., MacDonald, A., Villacís, A., Lima, L., Teixeira, M., Grijalva, M., Perkins, S., 2015. Bats, trypanosomes, and triatomines in Ecuador: new insights into the diversity, transmission, and origins of *Trypanosoma cruzi* and Chagas disease. PLoS One 10, e0139999. https://doi.org/10.1371/journal.pone.0139999.

R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Disponible en: https://www.r-project.org.

Ricardo-Caldera, D., Espitia-Pérez, L., Avilés-Vergara, P.A., Benítez, Á.J., Chacón-Pacheco, J., Ballesteros-Correa, J., Negrette-Oquendo, A., Soto-De León, S., Tovar-Acero, C., 2024. *Trypanosoma cruzi* in domestic and wild mammals in the northeast region of Colombia. Int. J. Parasitol. Parasites Wildl. 24, 100940. https://doi.org/10.1016/j.ijppaw.2024.100940.

Rocha, F.L., Roque, A.L.R., Arrais, R.C., et al., 2013. Transmission of TcI and TcII of *Trypanosoma cruzi* among wild carnivores, small mammals, and dogs in a conservation unit and surrounding areas, Brazil. Parasitology 140, 160–170. https://doi.org/10.1017/S0031182012001539.

Rodríguez-Dos-Santos, Í., Melo, M., De Castro, L., Hasslocher-Moreno, A., Do Brasil, O., Silvestre, A., Britto, C., Moreira, O., 2018. Exploring the parasite load and molecular diversity of *Trypanosoma cruzi* in patients with chronic Chagas disease from different regions of Brazil. PLoS Neglected Trop. Dis. 12, e0006939. https://doi.org/10.1371/journal.pntd.0006939.

Rohr, J.R., Civitello, D.J., Halliday, F.W., Hudson, P.J., Lafferty, K.D., Wood, C.L., Mordecai, E.A., 2020. Towards common ground in the biodiversity–disease debate. Nature Ecol.; Evol. 4 (1), 24–33. https://doi.org/10.1038/s41559-019-1060-6.

Rueda, K., Trujillo, J.E., Carranza, J.C., Vallejo, G.A., 2014. Oral transmission of *Trypanosoma cruzi*: a new epidemiological scenario for Chagas disease in Colombia and other South American countries. Biomedica 34, 631–641. https://doi.org/10.1590/S0120-41572014000400017.

Sánchez, M.S., Giannini, N.P., 2014. Altitudinal patterns in two syntopic species of *Sturnira* (Mammalia: chiroptera: Phyllostomidae) in the montane rain forests of Argentina. Biotropica 46, 1–5. https://doi.org/10.1111/btp.12082.

Santos, W.S., Gurgel-Gonçalves, R., Garcez, L.M., Abad-Franch, F., 2021. Deforestation effects on *Attalea* palms and their resident *Rhodnius*, vectors of Chagas disease, in eastern Amazonia. PLoS One 16, e0252071. https://doi.org/10.1371/journal.pone.0252071.

Soto-Vivas, A., Enriquez, S., Villacres, E., Arrivillaga, J., Hinojosa, M., Liria, J, 2018. New kissing bug (Hemiptera: Reduviidae: Triatominae) records from Napo and Morona-Santiago provinces with distribution updates in Ecuador. Journal of Threatened Taxa 10 (11), 12515–12522. https://doi.org/10.11609/jott.4345.10.11.12523-12530.

Stella, M., Selaković, S., Antonioni, A., Andreazzi, C.S., 2018. Ecological multiplex interactions determine the role of species for parasite spread amplification. Elife 7 (32814). https://doi.org/10.7554/ELIFE.32814.

Tirira, D., 1999. Mamíferos del Ecuador. In: Museo de Zoología, Centro de Biodiversidad y Ambiente, vol. 2. Pontificia Universidad Católica del Ecuador, Quito.

Tirira, D., 2007. Guía de campo de los mamíferos del Ecuador. Publicación especial sobre los mamíferos del Ecuador, 6. Ediciones Murciélago Blanco, p. 576. Quito, Ecuador.

Torres-Castro, M., Cuevas-Koh, N., Hernández-Betancourt, S., Noh-Pech, H., Estrella, E., Herrera-Flores, B., Peláez-Sánchez, R., 2021. Natural infection with *Trypanosoma cruzi* in bats captured in Campeche and Yucatán, México. Biomedica 41, 131–140. https://doi.org/10.7705/biomedica.5450.

Vaz, V.C., D'Andrea, P.S., Jansen, A.M., 2007. Effects of habitat fragmentation on wild mammal infection by *Trypanosoma cruzi*. Parasitology 134, 1785–1793. https://doi.org/10.1017/S003118200700323X.

Zecca, I.B., Hodo, C.L., Slack, S., Auckland, L., Hamer, S.A., 2020. *Trypanosoma cruzi* infections and associated pathology in urban-dwelling Virginia opossums (*Didelphis virginiana*). Int. J. Parasitol. Parasites Wildl. 11, 287–293. https://doi.org/10.1016/j.ijppaw.2020.03.004.