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RESEARCH

Trypanosomatids in Costa Rican Bats: First Molecular Evidence of the *Leishmania (Viannia) guyanensis* Complex and Evidence of a Broader Host Association for *Trypanosoma minasense*

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Abstract

Purpose Molecular data on wildlife trypanosomatids in Central America remain limited, constraining our understanding of host associations and sylvatic transmission cycles in the region. We characterized *Leishmania* and *Trypanosoma* infections in Costa Rican bats and assessed contemporary circulation of *Trypanosoma minasense* in howler monkeys within a comparative phylogenetic framework.

Methods Whole blood from 98 bats across 11 Costa Rican localities (2013–2014) was screened by PCR for *Leishmania* kDNA (120 bp), ITS-1 (~330 bp), and *Trypanosoma* 18 S SSU rRNA (nested PCR) with species-level identification based on sequence-confirmed amplicons. ITS-1 and 18 S amplicons were Sanger-sequenced and assigned by BLAST. For context, 18 S sequences from howler monkeys (*Alouatta palliata*) sampled in Costa Rica (2011–2025), including 20 animals from 2025, were included in phylogenetic analyses.

Results *Leishmania* kDNA was detected in 4/98 bats (4.1%), but only one (*Sturnira parvidens*) yielded ITS-1 and clustered within the *Leishmania (Viannia) guyanensis* complex. *Trypanosoma* DNA was detected in 9/98 bats (9.2%): *T. cruzi* ($n=3$), *T. minasense* ($n=3$), and undetermined *Trypanosoma* spp. ($n=3$). In 2025, 17/20 howler monkeys were PCR-positive for *T. minasense*; two were successfully sequenced and clustered within the Costa Rican *T. minasense* clade alongside bat-derived sequences.

Conclusion We report the first ITS-1- confirmed molecular evidence of the *L. (V.) guyanensis* complex in Costa Rican bats, supporting evidence of a broader host association and long-term local persistence of *T. minasense* across bats and primates.

Keywords *Leishmania (Viannia) guyanensis* complex · *Trypanosoma minasense* · *Trypanosoma cruzi* · Chiroptera · *Alouatta palliata* · Costa Rica

Introduction

Trypanosomatid hemoflagellates, including species of *Trypanosoma* and *Leishmania*, are widely distributed across the Neotropics and infect a broad range of mammalian hosts. Bats and non-human primates are of particular interest because they are ecologically widespread, frequently exposed to hematophagous vectors, and may contribute to the maintenance of sylvatic transmission cycles [1–3]. Although South American surveys have generated substantial molecular data on wildlife trypanosomatids [4, 5], Central America remains comparatively underrepresented despite its high biodiversity and potential for zoonotic spillover.

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Bats are increasingly recognized as hosts of diverse trypanosomatids, including *Trypanosoma cruzi*, *T. dionisii*, *T. pessoai*, and several *Leishmania* species [6, 7], although their epidemiological role varies among species and remains unresolved in many systems. However, prevalence estimates and species composition vary widely among regions, ranging from low or undetectable infection in some large colonies to moderate rates in cave-roosting and urban assemblages [8, 9]. Detection is strongly influenced by host community structure, ecological context, and, critically, the molecular targets applied [2]. For *Leishmania*, confirmatory sequencing of ITS-1 or HSP70 is considered essential to avoid misclassification based solely on kinetoplast DNA screening [2, 10].

In Costa Rica, most information on wildlife trypanosomatids derives from studies in marsupials and rodents, with only occasional reports in bats [11]. Species-level molecular confirmation remains limited, constraining inferences about host–parasite associations and local transmission cycles. Studies have documented *Leishmania panamensis* in sylvatic settings, and *T. cruzi* infection in several mammalian hosts [12], but the contribution of bats to these cycles remains poorly characterized. Moreover, other taxa such as *T. minasense*, traditionally associated with primates, have been only sporadically reported in Costa Rica [13]. Recent evidence of high *T. minasense* prevalence in South American primates suggests that its host range and ecological distribution may be broader than previously recognized [14].

Ongoing eco-epidemiological changes in Mesoamerica, including climate and land-use shifts, are expected to affect vector distributions and contact rates among wildlife, humans, and domestic animals, potentially altering trypanosomatid transmission dynamics [15]. In this context, improving the molecular characterization of trypanosomatids circulating in wildlife is important to clarify ecological roles and to better interpret potential links with human disease risk [16, 17].

Here, we combined retrospective (2013–2014) molecular screening of bats with targeted contemporary (2025) sampling of non-human primates in Costa Rica. Our objectives were to (i) characterize trypanosomatid diversity in bats at the species level using sequence-confirmed ITS-1 and 18 S markers, (ii) report molecular evidence of the *Leishmania (Viannia) guyanensis* complex in bats, and (iii) evaluate the current circulation of *Trypanosoma minasense* in howler monkeys using PCR screening and phylogenetic placement of sequence-confirmed isolates (two of 17 PCR-positive animals). By integrating historical and recent data, this study provides a baseline for assessing persistence and host associations of wildlife trypanosomatids in Central America.

Materials and Methods

Study Area and Sampling Design

Between June 2013 and August 2014, we conducted a descriptive cross-sectional study of bats captured along forest edges and woodland areas across 11 localities representing distinct ecological life zones in Costa Rica, providing a historical baseline for comparison with contemporary data. Local climatic conditions were characterized by using the Holdridge life zone system, which assigns sites based on geographic coordinates that reflect ranges of biotemperature and annual precipitation [18]. The sampling localities included Talamanca (Këkoldi Reserve; 9°38′16″N, 82°47′47″W), Turrialba (Wagelia Reserve; 9°56′45″N, 83°41′24″W), Puerto Viejo de Sarapiquí (Pozo Azul Reserve, 10°23′56″N, 84°07′41″W; Finca Starke, 10°26′19″N, 84°00′02″W; Ara Ambigua, 10°27′22″N, 84°01′32″W), Guápiles (Finca Corbana, 10°09′52″N, 83°46′33″W), Orosí (Finca Navarro, 9°49′33″N, 83°52′37″W), Tapantí National Park (9°46′52″N, 83°48′44″W), Carara National Park (9°46′48″N, 84°36′19″W), Santa Rosa National Park (10°50′04″N, 85°36′43″W), and Barra Honda National Park (10°11′14″N, 85°19′07″W).

Bat Capture, Identification, and Blood Sampling

Bats were captured using four Japanese mist nets (type BWF, 50 denier/2 ply, 1½-inch mesh openings; 12 m × 2.5 m) set at ground level. Captured individuals were identified to species based on external morphology following Timm et al. [19]. For each bat, body weight, sex, reproductive status, and age class were recorded. Blood was collected by venipuncture of the propatagial or patagial vein using 27- or 30-gauge needles, following Schinnerl et al. [20]. After sampling, bats were assessed for physical condition, provided an oral glucose solution, and released at the capture site. Blood was collected into 1 mL EDTA-treated MiniCollect® tubes, kept at 4 °C during transport, and stored at − 20 °C until DNA extraction. Molecular analyses were performed in 2014, shortly after completion of field sampling.

Contemporary Sampling of Howler Monkeys

To assess whether trypanosomatids detected in the retrospective bat survey were also circulating in non-human primates, we opportunistically analyzed blood samples from 20 howler monkeys (*Alouatta palliata*) collected in 2025 during routine wildlife health surveillance activities in Costa Rica. Whole blood was obtained by peripheral venipuncture using sterile techniques and collected into

EDTA-treated tubes. Samples were kept at 4 °C during transport and stored at −20 °C until molecular analysis. Screening for *Leishmania* spp. and *Trypanosoma* spp. followed the same PCR protocols used for bat samples. For *T. minasense*, PCR screening results from 2025 were used to support current circulation, and a subset of PCR-positive samples (2 of 17 PCR-positive animals) was confirmed by Sanger sequencing and incorporated into phylogenetic analyses.

Reference Sequences and Comparative Phylogenetic Framework (2011–2025)

To contextualize bat- and howler monkey–derived *Trypanosoma minasense* sequences, additional 18 S SSU rRNA sequences obtained from howler monkeys in Costa Rica in 2011 and 2022 [21, 22] were included in the phylogenetic analyses. These sequences were retrieved from published studies, together with newly generated sequences from the 2025 howler monkey subset confirmed by Sanger sequencing (see above). All sequences were aligned with those generated in the present study and used exclusively for comparative phylogenetic reconstruction. No prevalence or epidemiological inferences were derived from the 2011 and 2022 datasets beyond their use in supporting temporal persistence and phylogenetic placement of *T. minasense* in Costa Rica.

DNA Extraction, PCR, and Sequencing

DNA was extracted from whole blood using the QIAamp® DNA Investigator Kit (QIAGEN, Crawley, UK) according to the manufacturer's instructions. For *Leishmania* spp., samples were first screened by conventional PCR using primers 13 A (5′-GTG GGG GAG GGG CGT TCT-3′) and 13B (5′-ATT TTA CAC CAA CCC CCA GTT-3′), which amplify a 120-bp conserved region of kinetoplast DNA (kDNA) [23]. kDNA-positive samples were subsequently tested using primers LITSR (5′-CTG GAT CAT TTT CCG ATG-3′) and L5.8 S (5′-TGA TAC CAC TTA TCG CAC TT-3′) targeting the ITS-1 region (~ 330 bp) for sequencing [10].

Trypanosoma spp. detection was performed by nested PCR targeting the 18 S SSU rRNA gene following Savani et al. [6]. The first round used primers S4 (5′-GAT CCA GCT GCA GGT TCA CC-3′) and S12 (5′-GGT TGA TTC CGT CCA CGG AC-3′) to amplify a 540-bp fragment, and the second round used primers S17 (5′-CCA AGC TGC CCA GTA GAA T-3′) and S18 (5′-TCG GGC GGA TAA AAC ACC-3′) to yield a 480-bp fragment. In addition, a ~ 667-bp fragment of the 18 S rRNA gene was amplified only for samples that were positive for *Trypanosoma* by the nested 18 S assay and had sufficient DNA, as an additional marker to further characterize *T. cruzi* [24].

Positive controls included DNA from *Leishmania braziliensis* (provided by Dr. Azael Saldaña, Gorgas Memorial Institute, Panama) and DNA from *Triatoma dimidiata* previously confirmed positive for *T. cruzi* (GenBank MH020170). Nuclease-free water was used as a negative control. PCR products were resolved on 2% agarose gels in 1× TBE buffer, stained with GelRed™, and visualized under UV light. Fragment sizes were estimated using the GeneRuler™ 100 bp DNA Ladder Plus (Fermentas®). Amplicons of 330 bp (ITS-1) were interpreted as *Leishmania* positive for confirmatory sequencing, whereas bands of 480 bp (nested 18 S) and 667 bp were interpreted as *Trypanosoma* positive for downstream sequencing when sufficient product was available.

Bidirectional Sanger sequencing was performed at MACROGEN (Seoul, South Korea). Chromatograms were edited in BioEdit v7.2.5, and taxonomic assignment was performed by BLAST against GenBank reference sequences. Sequences generated in this study were deposited in GenBank.

Results

Detection of Trypanosomatids in Bats (2013–2014)

Blood samples were obtained from 98 bats captured across 11 sampling localities. Most individuals belonged to the genera *Carollia*, *Artibeus*, *Sturnira*, and *Glossophaga*.

Leishmania kDNA screening was positive in 4/98 bats (4.1%). However, ITS-1 amplification and sequencing were successful for only one sample, collected from *Sturnira parvidens*. ITS-1 sequencing and BLAST analysis placed this isolate within the *Leishmania (Viannia) guyanensis* complex, with the closest match to *Leishmania panamensis* (98% identity; GenBank MT606232). Phylogenetic analysis of the ITS-1 sequence confirmed that the sequence generated in this study (PX060482) clustered within the *L. (V.) guyanensis* complex (Fig. 1).

PCR targeting the 18 S SSU rRNA gene detected *Trypanosoma* DNA in 9/98 bats (9.2%). Based on sequencing of the 480-bp amplicons, three samples showed 98–99% identity with *Trypanosoma cruzi* (GenBank FJ900241). Three samples matched *Trypanosoma minasense* (98–99% identity; GenBank PQ490328 and PQ490338), and three additional samples yielded *Trypanosoma* 18 S amplicons but could not be assigned to the species level based on BLAST results.

Phylogenetic reconstruction using 18 S SSU rRNA sequences showed that the *T. minasense* isolates generated

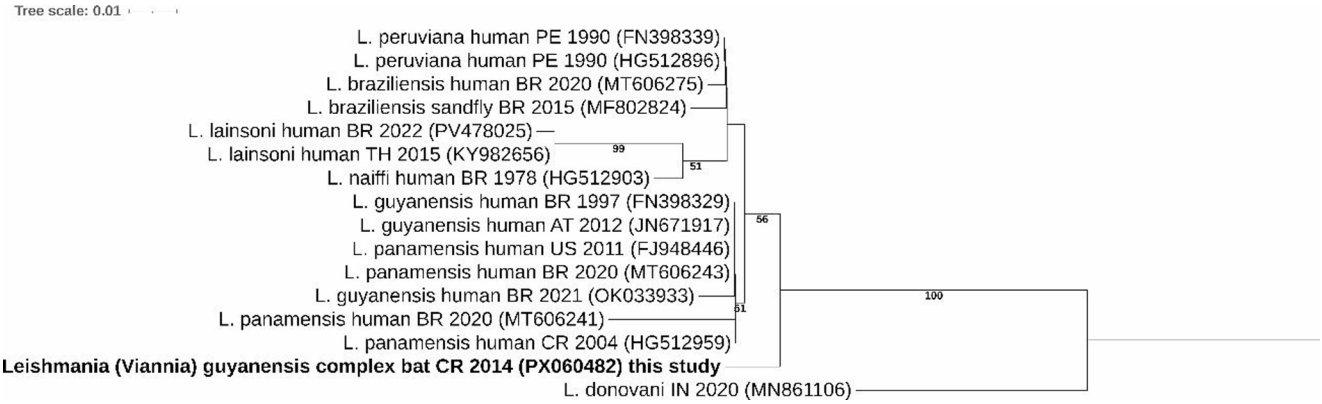

Fig. 1 Maximum-likelihood phylogenetic tree based on ITS-1 sequences of *Leishmania* spp. The *Leishmania (Viannia) guyanensis* complex is indicated, and the sequence obtained in this study (PX060482; labeled "this study") clusters within this group. Other *Viannia* species form distinct clades. *Leishmania donovani* was used as the outgroup. Bootstrap values ≥ 50% are shown. The tree was inferred under the GTR + G + I substitution model using 1,000 bootstrap replicates. Country codes: PE = Peru, BR = Brazil, TH = Thailand, AT = Austria, US = United States, CR = Costa Rica, IN = India

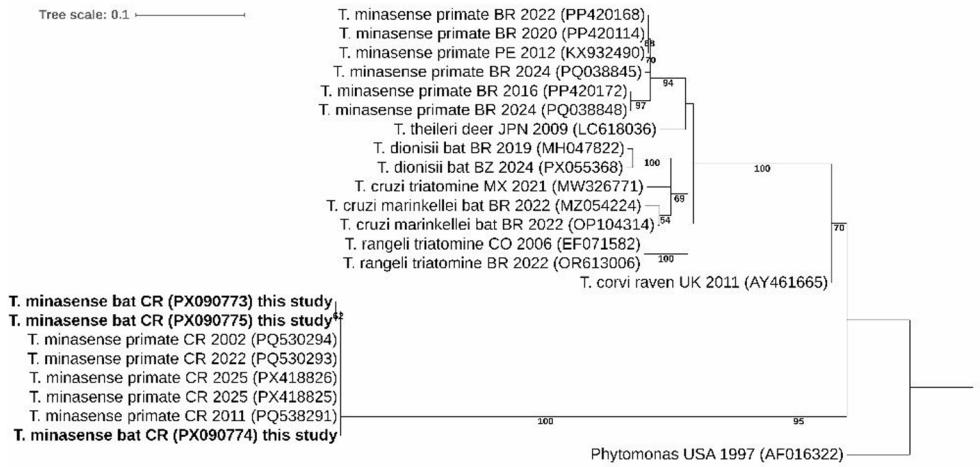

Fig. 2 Maximum-likelihood phylogenetic tree based on 18 S SSU rRNA sequences of trypanosomatids detected in bats from Costa Rica (this study), together with reference sequences from non-human primates, bats, and triatomines across the Neotropics. The dataset includes Costa Rican howler monkey isolates obtained between 2011 and 2022, as well as two sequence-confirmed howler monkey isolates from 2025. The Costa Rican *Trypanosoma minasense* clade is indicated, and sequences generated in this study are labeled "this study." Phytomonas was used as the outgroup. Bootstrap values ≥ 50% are shown. The tree was inferred under the GTR + G + I substitution model with 1,000 bootstrap replicates. Country codes: BR = Brazil, PE = Peru, JP = Japan, BZ = Belize, MX = Mexico, CO = Colombia, CR = Costa Rica

in this study clustered within a well-supported Costa Rican *T. minasense* clade, distinct from South American lineages (Fig. 2). For comparative purposes, bat-derived sequences were analyzed together with reference 18 S sequences from non-human primates collected in Costa Rica between 2011 and 2022. Lineage-level typing of *T. cruzi* was not performed due to limited DNA availability for additional assays.

Table 1 summarizes the bat species, capture sites, host characteristics, and the trypanosomatid sequences generated in this study, including BLAST results and GenBank accession numbers.

Contemporary Detection of *Trypanosoma minasense* in Howler Monkeys (2025)

Among 20 howler monkeys sampled in 2025, *T. minasense* DNA was detected by 18 S PCR in 17 individuals (as determined by PCR screening). A subset of PCR-positive samples (2/17) yielded high-quality 18 S sequences for Sanger confirmation. These two sequence-confirmed isolates clustered within the Costa Rican *T. minasense* clade together with bat-derived sequences generated in this study (Fig. 2). All howler monkey samples were negative for *Leishmania* by PCR.

Table 1 Bat species, capture sites, and individual characteristics, with trypanosomatid PCR screening results and sequencing-based identification (ITS-1 and 18 S rDNA) for confirmed positives, including GenBank accession numbers

Bat species	Capture site	Sex	Weight (g)	Trypanosomatid species (reference accession, coverage %, identity %, E-value, bit score)	GenBank accession number (this study)
Sturnira parvidens	Navarro-Orosí, Cartago	Male	22	*L. (Viannia) guyanensis* complex, highest identity to *L. panamensis* (MT606232, 100%, 98%, 2e−143, 521)	PX060482
Carollia perspicillata	Finca Starke, Sarapiquí, Heredia	Female	40	*Trypanosoma cruzi* (FJ900241, 98%, 99%, 0.0, 959)	PX090777
Artibeus jamaicensis	Pozo Azul, Sarapiquí, Heredia	Male	49	*Trypanosoma cruzi* (FJ900241, 99%, 98%, 0.0, 948)	PX090778
Artibeus jamaicensis	Carara National Park, Puntarenas	Female	48.5	*Trypanosoma cruzi* (FJ900241, 99%, 99%, 0.0, 948)	PX090776
Glossophaga mutica	Guápiles, Limón	Female	11	*Trypanosoma minasense* (PQ490338, 100%, 99%, 0.0, 728)	PX090775
Carollia sowelli	Këkoldi Reserve, Talamanca, Limón	Female	18	*Trypanosoma minasense* (PQ490338, 100%, 98%, 0.0, 680)	PX090774
Glossophaga mutica	Navarro-Orosí, Cartago	Male	12	*Trypanosoma minasense* (PQ490328, 96%, 99%, 0.0, 693)	PX090773
Carollia sowelli	Carara National Park, Puntarenas	Female	18	ND	–
Artibeus jamaicensis	Ara Ambigua Farm, Sarapiquí, Heredia	Female	48	ND	–
Carollia sowelli	Pozo Azul, Sarapiquí, Heredia	Male	20.5	ND	–

ND: Not determined *Trypanosoma* spp. PCR-positive samples (nested 18 S SSU rRNA) with unsuccessful Sanger sequencing; therefore, species-level identification could not be assigned

Discussion

This study provides the first ITS-1 sequence-confirmed molecular evidence of detection of the *Leishmania (Viannia) guyanensis* complex in a bat from Costa Rica and expands current knowledge of trypanosomatid diversity in Neotropical wildlife. By integrating retrospective bat screening (2013–2014) with contemporary PCR screening of howler monkeys in 2025 and phylogenetic placement of sequence-confirmed isolates, our findings contribute to understanding host associations and the potential persistence of trypanosomatids in Mesoamerica.

Leishmania in Bats

The ITS-1–confirmed detection of a member of the *L. (V.) guyanensis* complex in *Sturnira parvidens* suggests that bats in Costa Rica are exposed to sylvatic transmission cycles of cutaneous leishmaniasis. PCR-based detection of *Leishmania* DNA in blood should not be interpreted as evidence of reservoir competence or a role in human transmission; rather, it is consistent with exposure to infected phlebotomine vectors. Although an increasing number of studies report natural *Leishmania* infections in bats across Latin America, their epidemiological relevance for humans remains uncertain and is generally considered secondary. Nevertheless, bats may participate in sylvatic transmission cycles in some ecological contexts, reinforcing their value for zoonotic surveillance and for understanding parasite maintenance in nature. Reports from the Americas indicate that bat–*Leishmania* interactions occur across multiple ecological contexts. For example, natural infection of bats with *Leishmania (Leishmania) mexicana* has been documented in Mexico, supporting the possibility that bats can participate, at least as incidental hosts, in enzootic cycles [25]. A recent global systematic review further highlighted that *Leishmania* detection in bats spans diverse bat families and geographic regions [26]. In this context, our record extends evidence to Central America and to the *Viannia* subgenus.

Importantly, ITS-1 sequencing provides higher specificity than kinetoplast DNA screening alone, and confirmatory sequencing is recommended to reduce misclassification [2, 10]. Accordingly, the ITS-1–based confirmation in this study supports interpretation as a true infection rather than non-specific amplification. Given that *L. (V.) panamensis* is the primary cause of human cutaneous leishmaniasis in Costa Rica [11], further work is warranted to clarify whether bat infections reflect incidental spillover from local transmission cycles or more structured ecological interactions.

Trypanosoma minasense Beyond Primates

We emphasize that molecular detection alone does not confirm active infection, persistence of parasitemia, infectivity to vectors, or host competence. PCR positivity in blood indicates the presence of parasite DNA at the time of sampling, but it cannot distinguish between transient exposure and sustained infection. Therefore, parasite isolation/culture and longitudinal sampling remain essential to clarify transmission dynamics and the contribution of different hosts to sylvatic cycles. Given the scarcity of *T. minasense* isolates, future studies should prioritize isolation/culture efforts and complementary approaches (e.g., multilocus genotyping and quantitative assays).

Trypanosoma minasense has traditionally been associated with Neotropical primates, and recent data from South America suggest it may occur at high prevalence in non-human primates [14]. In this study, detection and sequencing of *T. minasense* in bats (*Carollia sowelli* and *Glossophaga mutica*) supports a broader host range than previously recognized. In addition, PCR screening of howler monkeys in 2025 indicated that *T. minasense* is currently circulating in Costa Rica (17/20 PCR-positive), and two sequence-confirmed detections clustered within the same Costa Rican *T. minasense* clade as the bat-derived sequences. Together with reference sequences spanning 2011–2022, these results suggest long-term local persistence and geographic structuring of *T. minasense* in the region.

The shared phylogenetic clustering of bat- and primate-derived sequences raises questions about transmission ecology, including whether generalist vectors or shared habitats facilitate multi-host circulation. Importantly, this shared clustering does not imply direct bat–primate transmission. Similar 18 S placement across hosts may reflect exposure to shared vectors, overlapping habitats, or circulation of closely related parasite lineages in the same ecological landscape, rather than host-to-host transmission. Confirming transmission links will require parasite isolation and vector-based evidence. Host flexibility can promote parasite persistence across heterogeneous landscapes and may increase opportunities for cross-species transmission in multi-host systems [16].

Mixed infections and overlapping enzootic cycles are increasingly recognized in Neotropical mammal communities, where multiple trypanosomatids can circulate sympatrically. Coinfections may influence transmission dynamics, parasite burden, and within-host interactions (e.g., competition or facilitation), and can complicate molecular diagnosis when relying on single targets. These considerations highlight the importance of sequence confirmation and multilocus strategies, ideally integrated with vector investigations, to better resolve parasite–host–vector system complexity [2, 28]. Against this background, we discuss below the detection of *Trypanosoma cruzi* in bats and its potential epidemiological implications.

Trypanosoma cruzi in Bats and Epidemiological Implications

Although bats are unlikely to represent major reservoirs of *T. cruzi* compared with opossums or rodents, their dispersal capacity and roosting ecology may increase exposure to triatomines and enable parasite circulation within multispecies assemblages. Detection of *T. cruzi* in three bat species is consistent with reports from other regions of the Americas, including cave- and urban-associated bat communities [9, 27]. Because DTU typing was not possible due to limited DNA, we cannot infer the genetic structure of the strains detected here. Future studies incorporating DTU typing and vector investigations will be important to determine whether bat infections reflect strictly sylvatic cycles or overlap with peri-domestic transmission involving humans and domestic animals [16, 28].

More broadly, *Trypanosoma* spp. display heterogeneous host specificity, spanning relatively host-associated lineages and more generalist parasites that can circulate across multiple mammal taxa, often shaped by vector ecology and shared habitats. In this context, wildlife reservoirs contribute to parasite persistence in nature by maintaining sylvatic transmission and providing opportunities for spillover where wildlife–human interfaces intensify. Therefore, wildlife surveillance—ideally integrated with vector investigations—remains essential to refine host–parasite associations and to interpret molecular detections within plausible transmission scenarios [1, 28].

Eco-epidemiological Relevance

The detection of *Leishmania* and *Trypanosoma* in bats and primates over a period exceeding a decade supports the presence of persistent sylvatic transmission cycles in Costa Rica. However, environmental changes such as deforestation, agricultural expansion, and climate variability may alter vector distributions and contact rates at the wildlife–human interface, potentially increasing spillover risk [15, 29].

Limitations and Perspectives

These findings should be interpreted cautiously because PCR detection in blood does not confirm active infection, infectivity to vectors, or reservoir competence [1]. The contemporary primate component relied primarily on PCR screening, with sequencing confirmation available for only

a subset of positives, and parasite detection was based on single loci for each taxonomic group, which may limit resolution [17]. Bat sampling relied on ground-level mist nets and may underrepresent canopy and fast-flying/aerial insectivorous species; future surveys should combine understory and canopy nets and include harp traps to reduce capture bias and improve representativeness. Future work should incorporate multilocus approaches, vector incrimination, and longitudinal sampling to clarify transmission pathways, infection duration, and the epidemiological role of bats and primates in regional trypanosomatid cycles.

Conclusions

This study reports the first ITS-1 sequence-confirmed molecular evidence of the *Leishmania (Viannia) guyanensis* complex in a bat from Costa Rica and documents *T. cruzi* and *T. minasense* in bats, with contemporary PCR evidence of *T. minasense* circulation in howler monkeys and phylogenetic support from sequence-confirmed isolates. These findings expand known host associations of wildlife trypanosomatids in Central America and provide a baseline for future eco-epidemiological investigations. Together, they support the integration of wildlife surveillance into One Health–oriented studies to better resolve sylvatic transmission networks and parasite maintenance in natural ecosystems.

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Data Availability All nucleotide sequences generated in this study have been deposited in GenBank under the accession numbers listed in Table 1.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical Approval and Animal Welfare All procedures for the capture and sampling of bats were approved by the Bioethics Committee of the School of Veterinary Medicine, Universidad Nacional (Costa Rica). Fieldwork was conducted under permits granted by CONAGEBIO (National Commission for Biodiversity Management; File No. 163) and SINAC (National System of Conservation Areas). Opportunistic sampling of howler monkeys in 2025 was conducted as part of routine wildlife health surveillance activities, in accordance with the corresponding institutional procedures and permits. No animals were captured specifically for this study.

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