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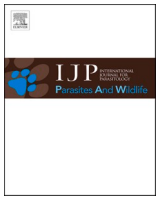

Redefining infections with trypanosomatids in Neotropical primates: Case study of the white-footed tamarin (*Oedipomidas leucopus*)

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ABSTRACT

Trypanosomes are blood parasites capable of infecting nearly any vertebrate. Many Neotropical primates frequently host trypanosomes and are considered potential reservoirs for *Trypanosoma cruzi* and other human-pathogenic trypanosomatids. However, diagnostic methods originally developed for detecting these trypanosomatids in humans and domestic species must be validated to reliably diagnose infections in non-human primates. Without such validation, taxonomic biases and incorrect assignments of wildlife reservoirs can occur. The white-footed tamarin (*Oedipomidas leucopus*), a primate endemic to northwestern Colombia, is classified by the World Health Organization as a reservoir of *T. cruzi*. However, this classification is based on studies with small sample sizes, ambiguous diagnostic methods, and questionable geographic records. In this study, the 18S ribosomal RNA gene was amplified via PCR and sequenced to estimate trypanosome infection rates and identify species in natural populations of *O. leucopus* across a wide geographic range, as well as in (*ex situ*) specimens. This molecular approach was also compared with traditional microscopy diagnosis using blood smears. The molecular diagnosis revealed that over 60% of the tested specimens were infected, whereas traditional microscopy resulted in 58% false negatives compared to the molecular method. A Bayesian phylogeny of the 18S gene identified *T. minasense* as the sole trypanosomatid species present in *O. leucopus*, with no detections of *T. cruzi* or other trypanosomatids of concern to human or domestic animal health. This study highlights the risk of overestimating the presence of human-infecting trypanosomes, such as *T. cruzi*, in tamarins and other vertebrates, and underscores the importance of validating diagnostic methods to accurately assess the zoonotic potential of wild species. Accurate identification of wildlife reservoirs is essential for understanding parasite life cycles and implementing effective management and conservation strategies for primates and other potential reservoirs.

1. Introduction

Zoonotic pathogens, transmitted either through direct infection or via an intermediate host, account for 60% of human infections (Akritidis, 2011; Bueno-Marí et al., 2015; Rahman et al., 2020; Shaheen, 2022). Vector-borne diseases such as Chagas disease and sleeping sickness are caused by the trypanosomatids *Trypanosoma cruzi* and *Trypanosoma brucei*, respectively (World Health Organization, 2019). However, other trypanosomatid-caused anthropozoonoses, including *Trypanosoma congolense*, *Trypanosoma evansi*, *Trypanosoma lewisi*, *Trypanosoma vivax*, and *Trypanosoma rangeli*, have minor or no effect on human health (Desquesnes et al., 2022; Herrera et al., 2022; Schijman et al., 2011; Truc et al., 2013). In the Neotropics, several cases of co-infection by *T. cruzi* and *T. rangeli* have been detected, where one parasite can mask the presence of the other, complicating disease diagnosis (Herrera et al., 2022). The diversity of trypanosomatids and their contrasting impacts on human and animal health underscore the need for reliable diagnostic tools.

Detection and diagnosis of trypanosomatids have historically been conducted using microscopy or serological tests on blood samples from

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presumed infected individuals (Gomes et al., 2009). Microscopy-based methods often require cell cultures and have proven unreliable for diagnosis, as their sensitivity is influenced by parasite loads, leading to false negatives (Schijman et al., 2011). High parasite loads in the bloodstream are necessary for accurate detection, a condition typically met only during the acute phase of the infection when the parasite is in the trypomastigote stage (Gomes et al., 2009).

Additionally, the morphological identification of trypanosomes is unreliable due to substantial changes throughout their life cycle (Gibson, 2003). The trypomastigote morphology can also vary between hosts (Ziccardi and Lourenço-de-Oliveira, 1999) and even between strains of the same species, such as *T. cruzi* (Devera et al., 2003; Martín-Escolano et al., 2022). For example, in *Trypanosoma minasense* inoculated into the Neotropical primates *Saimiri sciureus* and *Callithrix penicillata*, the length, width, and distance from the nucleus to the kinetoplast were up to four times smaller in the former host (Ziccardi and Lourenço-de-Oliveira, 1999). Furthermore, serological and biochemical tests in the Neotropics often use conjugates and chemical parameters optimized for *T. cruzi*, without verifying their specificity for other, less-studied trypanosomes infecting Neotropical primates and other vertebrates (Gomes et al., 2009; Lisboa et al., 2015; Monteiro et al., 2006; Rodrigues Coura, 2013).

Similarly, genetic methods intended to identify *Trypanosoma* species in humans can lead to conflicting or ambiguous results when applied to wild vertebrates without prior validation. In the primates *Saimiri* and *Saguinus* (recently split into the genera *Tamarinus*, *Leontocebus*, *Oedipomidas*, and *Saguinus*; Carvalho et al., 2022; Rylands and Mittermeier, 2024), trypanosome diagnosis by PCR targeting the glycosomal glyceraldehyde-3-phosphate dehydrogenase gene (gGAPDH) revealed infection with *T. cruzi* and *T. rangeli*. However, sequence analysis of the Small Subunit (SSU) of the 18S rRNA gene concluded the presence of *T. minasense* (Sato et al., 2008). Similarly, an analysis of the satellite DNA locus in the howler monkey *Alouatta caraya* in Argentina concluded infection with *T. cruzi*, while amplification of the 24S alpha rRNA produced an amplicon whose size was interpreted as compatible with *T. rangeli*, but its sequence shared high identity with *T. minasense* (Martínez et al., 2016). If such cases corresponded to coinfections with different trypanosomatid species, overlapping peaks would be expected in the electropherogram profiles of the sequenced PCR products of these parasites. It could be argued that coinfecting trypanosomatids share high sequence identity, but this argument demands comprehensive DNA sequence databases, especially when comparing sequences of well-represented species such as *T. cruzi* and *T. rangeli*, and others whose amplification profiles or sequences are still poorly characterized.

This is problematic considering the wide diversity of *Trypanosoma* species parasitizing Neotropical primates, including *Trypanosoma devei*, *Trypanosoma lambrechti*, *T. minasense*, *Trypanosoma mycetae*, and *Trypanosoma saimirii* (D'Alessandro et al., 1986; Lanham et al., 1984; Lisboa et al., 2007; Maia da Silva et al., 2008; Marinkelle, 1966; Sato et al., 2008; Sousa and Dawson, 1976; Ziccardi et al., 2000). However, diagnostic tests in these primates are often applied assuming their specificity for trypanosomatids such as *T. cruzi*, *T. rangeli*, *T. brucei*, *T. vivax*, or others prevalent in humans or domestic species (e.g., Burgos et al., 2007; Duffy et al., 2013; Geysen et al., 2003), without considering the potential presence of other unrecognized or rare species and the unforeseen consequences of cross-reactions with such species (Lisboa et al., 2015; Maia da Silva et al., 2008; Marcili et al., 2009; Monteiro et al., 2006).

It is necessary to implement sensitive and specific methodologies (McManus and Bowles, 1996) to assess the diversity, distribution, and abundance of the *Trypanosoma* community in Neotropical wildlife. One alternative is the molecular identification of species through the characterization of species-specific polymorphisms in variable genetic regions such as SSU and GAPDH (de Arruda et al., 1990; Sato et al., 2008), which has substantially improved the sensitivity and specificity in the taxonomic identification of a wide range of trypanosomatids (Dunlop et al., 2014).

One Neotropical primate species with dubious reports about its infection status with trypanosomatids is the white-footed tamarin (*Oedipomidas leucopus*, previously named *Saguinus leucopus*) (Baker, 1972; Hoare, 1972; Nunn and Altizer, 2005; World Health Organization, 2002). Although part of this primate's geographic range coincides with endemic areas for Chagas disease, the reports of *T. cruzi* in *O. leucopus* are contentious. Despite being endemic to northwestern Colombia (Cuartas-Calle, 2001), the individuals analyzed by Baker (1972) were apparently captured in Brazil. Additionally, Hoare (1972) diagnosed *T. cruzi* after sampling only one tamarin and acknowledged that species identification of trypanosomatids of the subgenus *Schizotrypanum* (including *T. cruzi*) in South American monkeys is difficult in most cases.

Considering the deficiencies in the diagnosis of *T. cruzi* in Neotropical primates, their role as reservoirs of this parasite, and the potential epidemiological risk they pose to human populations, we employed an unbiased method to evaluate the presence of *T. cruzi* and other trypanosomatids in *O. leucopus*. This study aimed to: (1) estimate the level of trypanosome infection in natural and *ex situ* populations of *O. leucopus* through PCR of SSU and compare its sensitivity with traditional microscopy methods, and (2) detect the diversity of trypanosomatid species in this primate.

2. Materials and methods

2.1. Distribution of Oedipomidas leucopus

The geographic range of *O. leucopus* extends between the right bank of the Cauca River and the left bank of the Magdalena River in the departments of Bolívar, Antioquia, Caldas, and Tolima, below 1800 m above sea level in Colombia (Cuartas-Calle, 2001). This species occurs in a wide variety of habitats, including tropical humid forests, tropical dry forests, premontane rainforests, primary and secondary forests, and forested urban areas (Soto-Calderón et al., 2016).

2.2. Study area and sample collection

Between 2009 and 2017, blood samples from 129 wild individuals across 22 social groups were collected in eight municipalities in the department of Antioquia (Medellín, San Carlos, San Rafael, El Carmen de Viboral, San Luis, Caucasia, El Bagre, and Cáceres) and two municipalities in the department of Caldas (Samaná and Norcasia). Between one and four groups per locality were sampled. The three sites in Medellín correspond to urban forest patches (Fig. 1; Table 1). Blood samples were also collected from 35 trafficked individuals of unknown origin (*ex situ* populations), confiscated by four environmental authorities: Cornare (12), Corantioquia (1), Corpocaldas (9), and the Área Metropolitana del Valle de Aburrá (AMVA) (13).

Approximately 1.5–2.0 ml of blood was collected by direct puncture from the femoral vein of each monkey ($\leq$1% of body weight). One drop was used to make a thick blood smear (TBS) and for genetic analysis. DNA was extracted using either a phenol/chloroform technique (Green and Sambrook, 2012) or the QIAamp® DNA Blood Mini Kit (QIAGEN, Valencia, California, USA). DNA concentration was estimated with a NanoDrop™ 1000 Spectrophotometer (ThermoFisher, Waltham, Massachusetts, USA). The detailed methods explaining the baiting, capture, immobilization, sample collection, and DNA extraction protocols are described in Acevedo-Garcés et al. (2014) and Soto-Calderón et al. (2016).

2.3. Infection diagnosis

A fragment of approximately 2,200bp of the SSU was amplified by PCR to estimate *Trypanosoma* infection levels in natural populations (Table 2). Primers SSU-fw/SSU-rv, specifically designed for this study, targeted conserved regions in trypanosomatids identified from GenBank

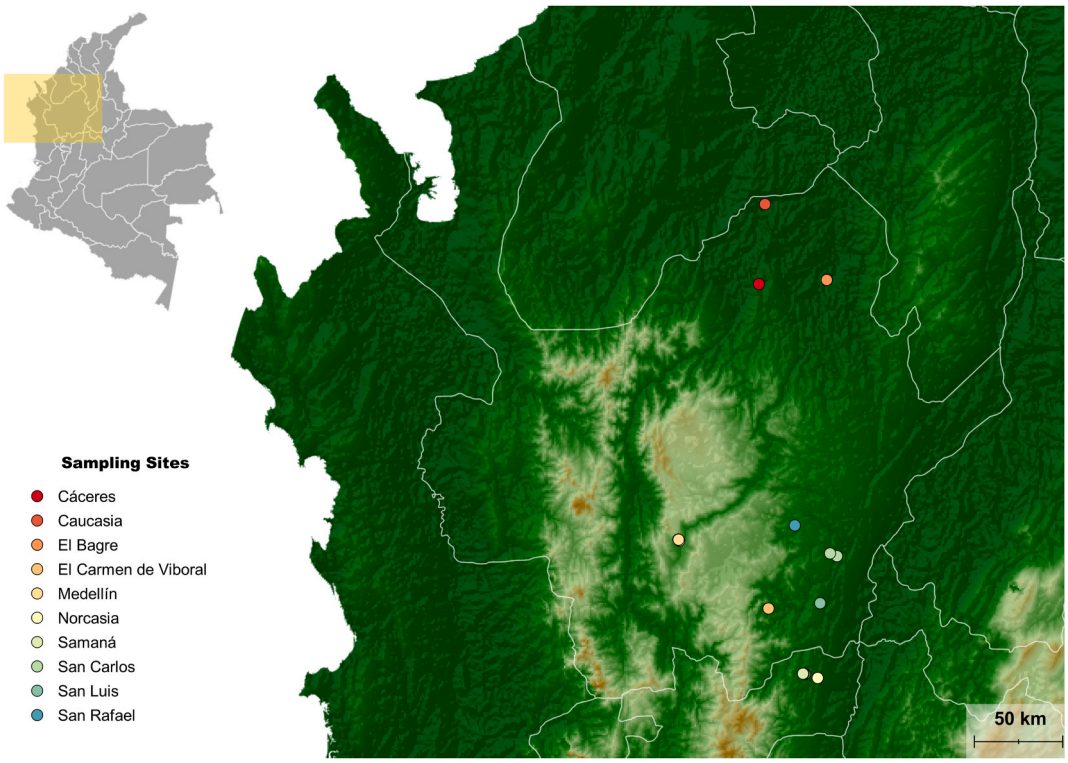

Fig. 1. Sampling sites of *Oedipomidas leucopus* in northwestern Colombia.

Table 1
Sampling sites in 10 localities and animal refuges (*ex situ* populations). The total number of individuals tested for *Trypanosoma* through PCR and microscopy with their corresponding results are indicated for each locality.

Municipality	Sampling site code (number of sampled groups)	n	Microscopy (+/total)	PCR (+/total)
Medellín	UDA (1), NBK (1), SSP (1)	18	0/18	0/18
San Carlos	SC (1), SCA (3)	25	11/13	22/25
San Rafael	JAG (2)	9	2/9	9/9
Carmen de Viboral	CVB (1)	5	5/5	5/5
San Luis	SL (1)	8	0/0	8/8
Caucasia	CCS (2)	7	3/7	7/7
El Bagre	BAG (2)	12	0/0	12/12
Cáceres	ATS (3)	14	0/0	14/14
Samaná	BFP (1), BFA (1), BSJ (1)	25	0/20	14/25
Norcasia	NCM (1)	6	0/2	4/6
Ex situ populations	EM (1), CAV (1), COA (1), CRC (1)	35	0/16	21/35
Total		**164**	**21/90**	**116/164**

Table 2
Primers used to amplify the ribosomal SSU region of *Trypanosoma* spp.

Primer pairs	Sequence 5′-3′	Amplicon size (pb)	Reference
S-762	GACTTTTGCTTCCTCTAWTG	2219	Maslov et al. (1996)
S-763	CATATGCTTGTTTCAAGGAC		
SSU-fw	GATCTGGTTGATTCTGCCAGTAGTC	2285	This study
SSU-rv	AAATGATCCAGCTGCAGGTTCAC		

sequences (Clark et al., 2016). In case of failed amplification, a nested PCR was performed using the internal primers S-762/S-763, originally designed for five orders of Kinetoplastea, including Trypanosomatids (Maslov et al., 1996).

Amplification reactions were performed in a final reaction volume of 40 μL mixing 20–50 ng of genomic DNA (first PCR cycle) or 2.0 μL of PCR product (second cycle), 1X buffer, 0.26 mM dNTPs, 0.4 μM of each primer, 1.0 U of Taq polymerase, 1.8 mM $MgCl_2$ and 5% Dimethyl Sulfoxide (DMSO). The reactions were carried out at an initial temperature of 95 °C for 3 min, followed by 35 amplification cycles of 94 °C for 30s, annealing for 30s and 72 °C for 2min, with a final extension step of 72 °C for 15 min. The annealing temperatures were 65.5 °C and 55 °C for the primers SSU-fw/SSU-rv and S-762/S-763, respectively. Strains MG10 of *T. cruzi* (Mejía-Jaramillo et al., 2012), *T. theileri*, *T. evansi*, *T. brucei, and T. rangeli* were used as positive controls (Jaimes-Dueñez et al., 2018).

The relative sensitivity of PCR and traditional methods (TBS) for parasite detection was evaluated in 90 individuals (Table 3). Two TBS were prepared on separate glass slides and screened for trypomastigotes of *Trypanosoma* using Giemsa stain or Field's technique. These techniques allow visualization of parasite morphology, including the flagellum and the location of the nucleus relative to the kinetoplast. The screenings were performed at a magnification of 1,000X using a light microscope (Soto-Calderón et al., 2016).

2.4. Taxonomic identification of Trypanosoma

Among the individuals with positive PCR results, amplicons of 32 wild individuals were randomly selected from each locality (between two and five individuals per locality) and sequenced. Since all the DNA

Table 3
Two-by-two table of *Trypanosoma* infection status of 90 individuals simultaneously tested by microscopy and PCR.

	PCR (+)	PCR (−)	Total
Microscopy (+)	21	0	**21**
Microscopy (−)	40	29	**69**
Total	**61**	**29**	**90**

sequences shared a high identity with the same *Trypanosoma* species and were representative of the geographical range of *O. leucopus*, we considered it unnecessary to sequence further samples. Amplicons (25 μL) were purified using a solution containing 10 μL of ExoSAP (20 U/μL Exonuclease (New England Biolabs, Massachusetts) and 1 U/μL Alkaline Phosphatase (Sigma-Aldrich, St. Louis, Missouri, USA) and incubating at 37 °C for 30 min and 95 °C for 5 min. Both strands of the purified products were sequenced using the traditional Sanger method by a commercial laboratory (Wallis and Morrell, 2011). The sequences were edited and aligned using ClustalW implemented in Geneious Prime v2019.2 (Kearse et al., 2012). Possible co-infections were evaluated by examining for overlapped peaks, which would indicate the presence of multiple amplification products. The sequences were aligned with 106 SSU sequences of *Trypanosoma* and one *Paratrypanosoma confusum* (outgroup) retrieved from GenBank (Table S1).

Stretches with indels or ambiguous alignment were excluded (positions 1–43, 167–219, 256–350, 735–835, 1061–1300, 1663–2059, 2092–2147 and 2515–2557 in the reference sequence of *T. minasense* AB362411) resulting in a final alignment of 1684 bp. The total number of unique haplotypes in the study sample was identified using DNAsp (Rozas et al., 2017). The raw sequences generated in this study can be found in the Genbank with Accession numbers PQ490328 - PQ490360.

A Bayesian phylogeny (BI) was inferred in BEAST v2.4.7 (Suchard et al., 2018) with a GTR + I + G mutational model selected using jModelTest implemented in CIPRES (Darriba et al., 2012; Guindon and Gascuel, 2003). This process ran for 80 million generations of a Markov Chain Monte Carlo (MCMC), sampling every 1000 generations and discarding 20% of the samples as burn-in. Tracer v1.7.2 (Rambaut et al., 2018) was used to verify that the MCMC stabilized and reached an optimal value. Finally, a consensus tree was built using TreeAnnotator v2.4.7 (Suchard et al., 2018), which was then visualized and edited with FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

3. Results

3.1. Trypanosoma detection

A total of 73.6% (95/129) of free-range monkeys and 60% (21/35) of monkeys in *ex situ* populations were positive for *Trypanosoma* using SSU PCR (Table 1). *Trypanosoma* was detected in all rural wild groups (19) but absent in all urban groups (3) (Table 1). All individuals from El Carmen de Viboral, San Luis, Caucasia, El Bagre, and Cáceres were positive by PCR. Of the 90 individuals tested for *Trypanosoma* infection using both microscopy and PCR, 23.3% (21) and 67.8% (61) were positive, respectively (Fig. 2, Table 3), where all the specimens positive by microscopy were also positive by PCR, with significant differences between the two methods (Fisher Exact test $p < 0.001$).

3.2. Trypanosoma taxonomy

A total of 32 SSU sequences were generated from *O. leucopus* specimens previously diagnosed by PCR, revealing only two different haplotypes. There was no evidence of coinfection with multiple strains or species, as the sequencing profiles showed unique and discrete peaks for each nucleotide position. The highest identity values were with *Trypanosoma minasense* (Accession Code AB362411), ranging from 98.5% to 99.8%. A sequence from San Carlos (SCA3-7) had a duplication of 180 bp at position 1,751, but once excision of this section, the sequence identity and coverage in reference to AB362411 were 99.8% and 99%, respectively.

The IB phylogeny recovered a monophyletic group (PP > 0.99) for the *Trypanosoma* sequences obtained from *O. leucopus* and the reference sequence for *T. minasense* (AB362411). The sister clade in the phylogeny was conformed by *Trypanosoma irwini* and *Trypanosoma bennetti*, with sequence identities between this and the *T. minasense* clade below 96.3% (Fig. 3). This equates a phylogenetic gap between *T. minasense* and other species over 3%, leaving a clear association between *T. minasense* and the SSU sequences generated from *O. leucopus* blood samples in the present study. Furthermore, this clade was fully differentiated from the *T. cruzi* cluster (PP > 0.99), which also included the MG10 control for this species sequenced in the present study (PQ490350), and from other trypanosomatids of medical interest for humans and domestic mammals (Fig. 3).

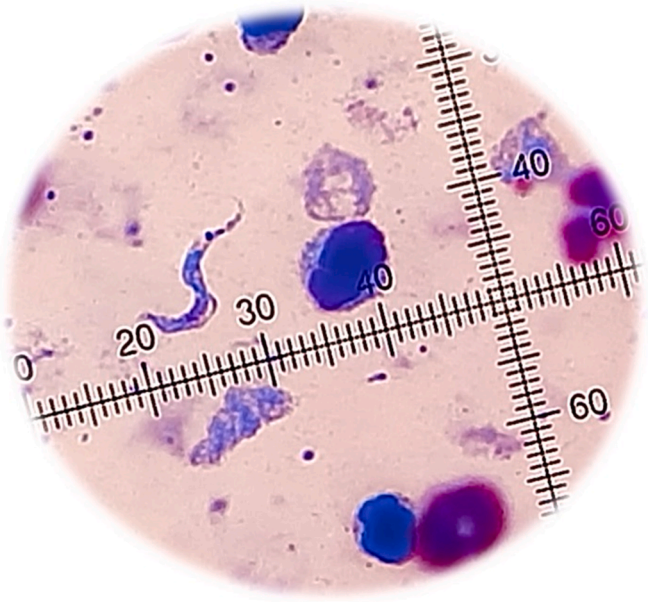

Fig. 2. Giemsa-stained thick blood smear showing a trypomastigote of *Trypanosoma* spp. (1000x) in *Oedipomidas leucopus*.

4. Discussion

The methods used to diagnose trypanosomatid infections in wildlife, particularly in Neotropical primates, have several limitations, especially when it comes to assessing the risk these parasites pose to human health. In this study, we employed PCR and sequencing of the Small Subunit of the 18S rRNA gene, targeting trypanosomatids without any taxonomic bias. Our goal was to evaluate the sensitivity and specificity of this approach in the tamarin *O. leucopus*, a primate suspected to be a reservoir for *T. cruzi* according to the World Health Organization (2002).

Nested PCR and subsequent sequencing of SSU are recommended for infection diagnosis and identification of *Trypanosoma* species in Neotropical primates for several reasons. Firstly, the detection ability of trypanosomes by nested PCR of SSU, with the modifications introduced here, was almost three-fold higher than detection results using microscopy (2.90 = 61/21). This difference in detection capacity is expected to be greater in advanced stages of the infection (i. e. the chronic phase) when there are few parasites (trypomastigotes) in the blood stream and overall, in cases of low parasite load (Gomes et al., 2009).

Secondly, the high phylogenetic information content of SSU and the availability of public genetic databases with ample taxonomic representation have allowed the application of a reliable Molecular Barcode approach (Hutchinson and Stevens, 2018) and successful diagnosis of *Trypanosoma* species without taxonomic bias toward human-infecting species. This approach was successfully applied to SSU sequences recovered from both blood samples of infected *O. leucopus* and a group of controls of medical interest (*T. cruzi*, *T. theileri*, *T. evansi*, *T. rangeli*, and *T. brucei*).

Additionally, the widespread infection of *T. minasense* in *O. leucopus* contrasts with the absence of other *Trypanosoma* species previously reported in the same geographic area, such as *T. cruzi* and *T. rangeli* (Vallejo et al., 2015; Velásquez-Ortiz et al., 2022; World Health

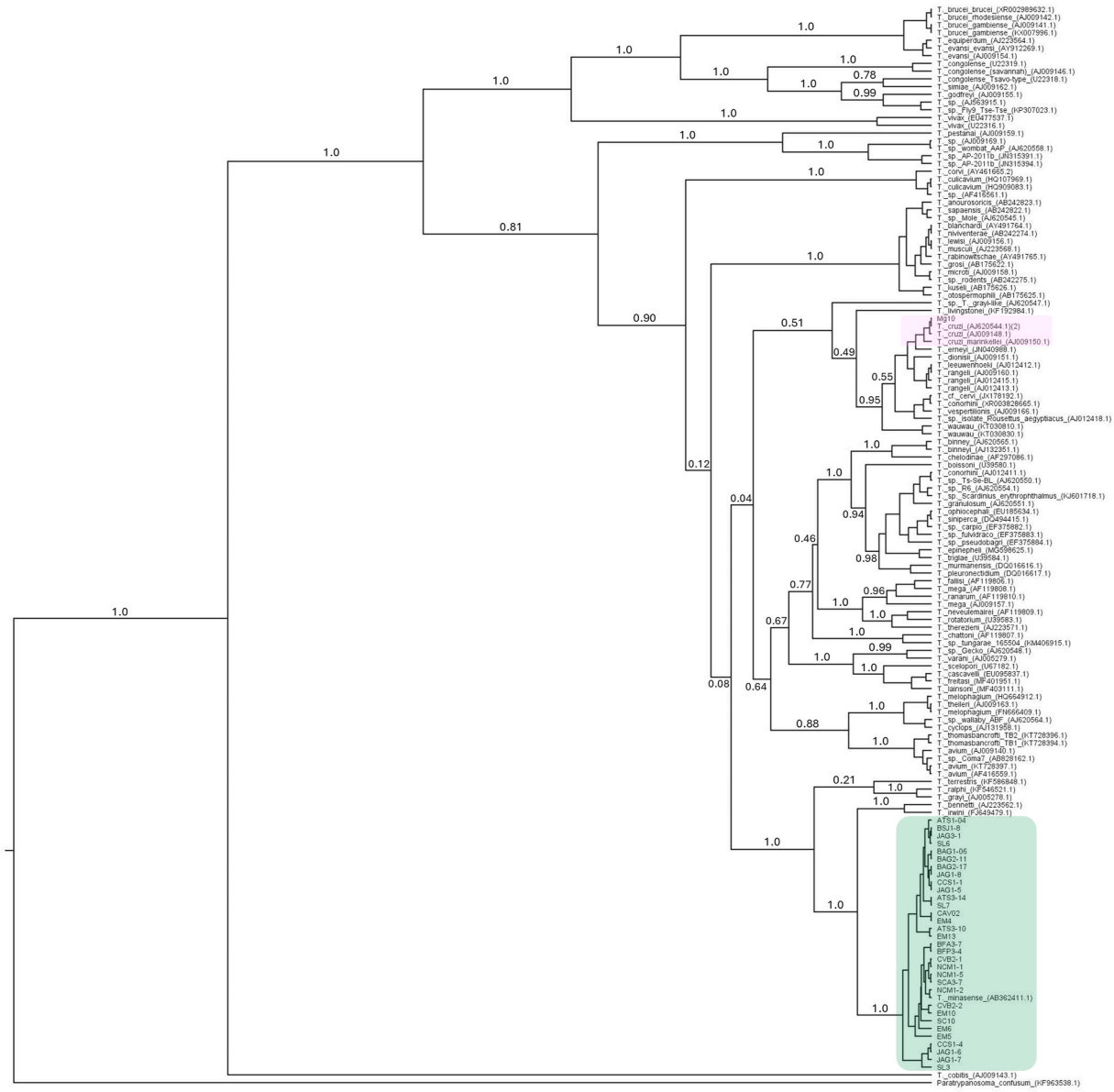

Fig. 3. Bayesian phylogenetic inference of trypanosomatids using the SSU locus. Sequences generated from infected *Oedipomidas leucopus* and the *Trypanosoma cruzi* control are highlighted in green and red boxes, respectively. Posterior probabilities are shown for main branches. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Organization, 2002). The absence of these parasites could be due to their difficulty in completing their life cycles using the white-footed tamarin as a host. Alternatively, a low prevalence of these parasites or sporadic outbreaks in *O. leucopus* could account for the negative results, but this is unlikely given our sampling during a relatively long period of almost nine years and our coverage of an extensive geographic region across the range of *O. leucopus*. This highlights the need for future studies with larger sample sizes in endemic areas for trypanosomiasis not included in the present study (Guimarães et al., 2022).

The high levels of infection and wide distribution of *T. minasense* in populations of *O. leucopus* suggest low or null levels of pathogenicity, as it has been reported in other tamarins (Coimbra et al., 2020). Although the health status of the tamarins infected with *Trypanosoma* was not tested in this work, a previous characterization of the hemoparasite community in several localities also included in this study, such as San Carlos, San Rafael, Carmen de Viboral and Caucasia, concluded that health conditions were normal in adult specimens infected with *Trypanosoma* sp. (Soto-Calderón et al., 2016). All of this is consistent with little or no effect of *T. minasense* on the health of *O. leucopus*. A similar situation could be occurring in other tamarin species (e. g. *Oedipomidas geoffroyi*, *Oedipomidas oedipus*, *Saguinus midas*, *Callithrix* sp., *Leontocebus nigricollis* and *Tamarinus imperator*) and other genera of Neotropical primates, in which *T. minasense* has been reported (Coimbra et al., 2020; Deane and Deane, 1961; Dunn et al., 1963; Guimarães et al., 2022; Hoare, 1972; Sato et al., 2008; Sousa and Dawson, 1976; Ziccardi and Lourenço-de-Oliveira, 1999; Ziccardi et al., 2000).

Unlike rural areas where a wide distribution of *T. minasense* was found, no cases of infection were detected in urban *O. leucopus*. This pattern is far from being a coincidence since other blood and intestinal parasites such as *Prosthenorchis, Spirudidae, Trichostrongylus, Ancylostomatidae, Endolimax, Entamoeba*, and *Ascaris* have also been found in rural but not urban wild populations of *O. leucopus* (Soto-Calderón et al., 2016). In other primates and some carnivorous species, disruption of the transmission cycle due to the absence of vectors or alternative hosts explains a lower prevalence of parasites in urban environments (Werner and Nunn, 2020). Arboreal insects have been suggested as potential

vectors of *T. minasense*, but also direct transmission among primates has been speculated (Hoare, 1972; Deane et al., 1974; Ziccardi and Lourenço-de-Oliveira, 1999). Despite multiple efforts, this remains an enigma, but the apparent absence of this parasite in urban tamarins is consistent with the lack of intermediate hosts and vectors in these areas.

Finally, it is recommended to review the WHO technical reports regarding the wildlife species presumed reservoirs of *T. cruzi*, since their sympatry is just circumstantial evidence that cannot be understood as an unequivocal condition to assign reservoirs (World Health Organization, 2002). Considering primates as potential reservoirs of infectious agents is understandable from an epidemiological perspective. However, an objective assessment of the actual reservoirs of *Trypanosoma* species of medical interest will allow for determining the actual zoonotic risk for human populations. Failure to do so may result in incorrect species diagnosis, fallacious parasite-host interactions, epidemiological implications, and ultimately stigmatization and extermination of certain primate species (Bicca-Marques and de Freitas, 2010; Khan, 2023).

5. Conclusions

Although the White-footed tamarin (*O. leucopus*) is identified by the World Health Organization as a potential reservoir of *Trypanosoma cruzi*, molecular diagnostics of *Trypanosoma* infections conducted on 129 tamarins from 22 social groups, sampled over a nine-year period across a significant portion of the species' distribution, did not detect any cases of human-infecting trypanosomatids. This includes regions where *T. cruzi* and *T. rangeli* are endemic. These findings call into question the current understanding of the role of *O. leucopus* and other tamarins as reservoirs of human-infecting trypanosomatids.

Conversely, a widespread and prevalent infection with *T. minasense* was observed, with no apparent adverse effects on the health or physical condition of *O. leucopus*.

Finally, the specificity of diagnostic methods targeting trypanosomatids relevant to the health of humans and domestic mammals must undergo rigorous validation through testing on wild species. This validation is essential to avoid the overestimation of pathogenic trypanosomatids, the misidentification of reservoirs, and to design effective strategies for the prevention and control of tropical infectious diseases. Ultimately, it also plays a critical role in preventing the unwarranted stigmatization of wild species.

CRediT authorship contribution statement

Juliana Tabares-Medina: Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Katherinne García-Blandón:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Gisela M. García-Montoya:** Methodology, Conceptualization. **Iván Darío Soto-Calderón:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Ethics statement

This research was granted by the Autoridad Nacional de Licencias Ambientales de Colombia through Research Permit No. 268 (issued on February 1, 2013) and the Ministry of the Environment through the Permit of Access to Genetic Resources No. 1686 (issued on December 5, 2013). The University of Antioquia holds the Non-Commercial Scientific Research Permit issued by the Ministry of Environment (Resolution 0524, May 27, 2014). The capture and sampling procedures implemented in this study were endorsed by the Institutional Ethics Committee for Animal Experimentation (CEEA) of the University of Antioquia on August 22, 2014.

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During the preparation of this work the authors used ChatGPT in order to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Declaration of interest

All co-authors confirm that they have no competing financial interests or personal relationships that could influence the work reported in this manuscript. They also affirm that our manuscript is entirely original, free of plagiarism or self-plagiarism, it is not under consideration for publication elsewhere, and that they have actively contributed to the research presented in the manuscript. All co-authors approve the submission of this manuscript to the *International Journal for Parasitology: Parasites and Wildlife*.

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Appendix A. Supplementary data

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