| REVIEW ARTICLE |

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Trypanosoma spp. and its association with mammals: a literature review and case studies for the Andes and Orinoquia regions of Colombia

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Abstract

Within *Trypanosoma*, some species are of public health concern, such as *Trypanosoma cruzi* which is the causative agent of Chagas disease, also known as American trypanosomiasis. This disease is endemic in 17 countries across the Americas and primarily affects mammals, including humans. In certain countries such as Colombia, there are still gaps in understanding the associations between *Trypanosoma* and mammals. In this context, this study aimed to consolidate the current state of knowledge regarding the association between *Trypanosoma* and mammals, while providing both morphological and molecular data on trypanosomes circulating in wild mammals in two departments of Colombia—Arauca and Caldas—located in the Orinoquia and Andes regions, respectively. To compile historical records of natural infections involving *Trypanosoma* species in wild and exotic mammals (introduced rats and mice) in Colombia, a literature review using the PRISMA method was conducted. To complement this, morphological and molecular detection and analysis were performed on samples from 109 wild mammals, including blood smears, organ impressions, and amplification of fragments of the mini-exon, Cytb, ITS, SSUrRNA, and gGAPDH genes. Additionally, a morphometric analysis of blood trypomastigotes was conducted. The review yielded 40 scientific articles that reported the presence of eight *Trypanosoma* species in wild and exotic mammals in Colombia, with the highest prevalence observed in bats and rodents. The analysis of samples from Arauca (n = 64) and Caldas (n = 45) revealed a *Trypanosoma* sp. prevalence of 37.6% (n = 41). These findings provide a comprehensive overview of the current knowledge on *Trypanosoma* and its association with mammals in Colombia. Furthermore, the results highlight the presence of

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Trypanosoma **in different wild hosts in the Andes and Orinoquia regions, underscoring the need for enhanced research on wildlife within a One Health framework.**

Key words: *Public health; Systematic review; Trypanosomatida; Vector-borne diseases*

Introduction

Approximately 60% of the emerging pathogens responsible for human illnesses are zoonotic, with wildlife serving as the primary reservoir for these pathogens (Brooks et al., 2014; Ramírez et al., 2014; Gonzalez-Astudillo et al., 2016; Ricardo-Caldera et al., 2024). The family Trypanosomatidae (Euglenozoa, Kinetoplastida) is represented by 19 genera, with *Trypanosoma* containing pathogenic species (Gruby, 1843; Kaufer et al., 2017). *Trypanosoma* sp. are responsible for parasitic diseases of medical and veterinary relevance (e.g., *Trypanosoma brucei* and *Trypanosoma cruzi*) (Rubio-Ortiz et al., 2020). Chagas disease or American trypanosomiasis is a zoonotic infection caused by *T. cruzi*, transmitted mainly by conenose bugs (Triatominae). This disease affects approximately six million people in the Americas and results in around 12,000 deaths per year (Bern and Montgomery, 2009; González-Rugeles, 2018; Pérez-Molina and Molina, 2018; World Health Organization, 2020; Ramos-Sesma et al., 2021). *Trypanosoma cruzi* circulates in different habitats, affecting both domestic and wild species (Jansen et al., 2020). In the wild cycle, triatomine species and wild mammal hosts are involved, while the domestic cycle includes triatomines, domestic and synanthropic mammals, and humans (Jansen et al., 2015; Rodríguez-Monguí et al., 2019).

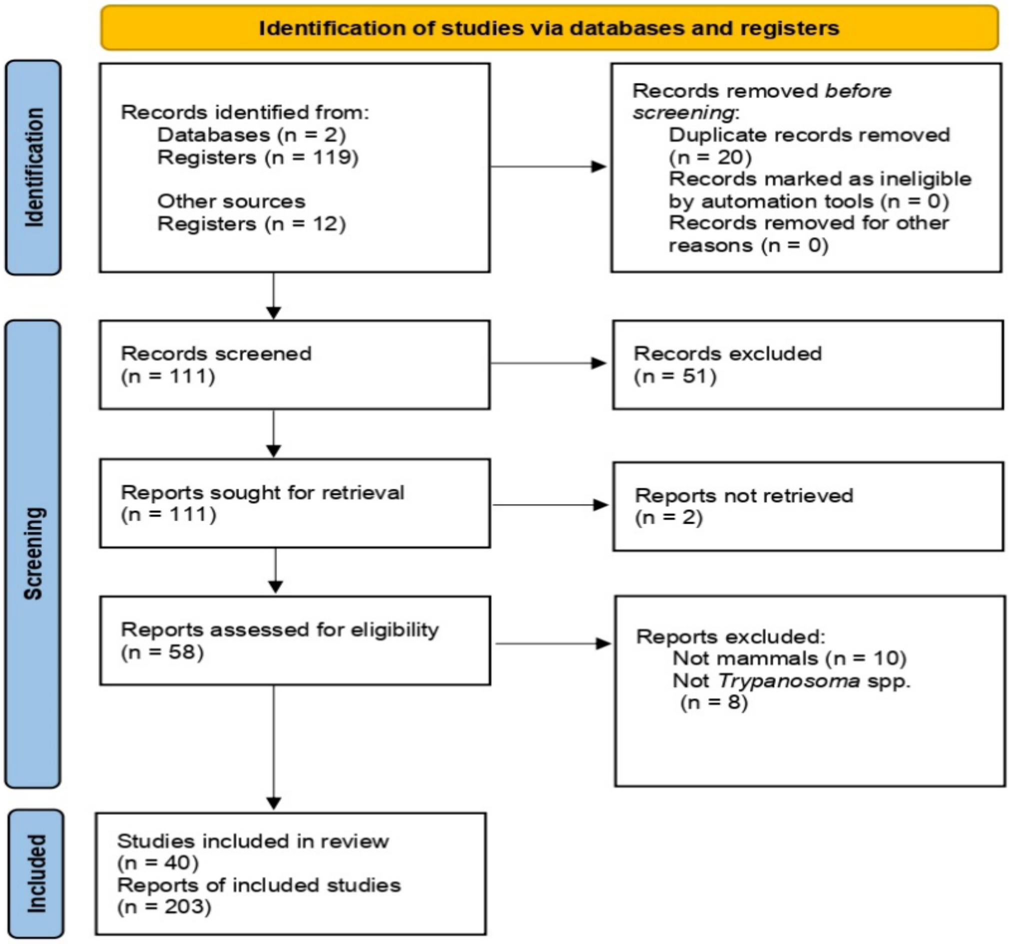
The classification of *Trypanosoma* has been debated over the years. Hoare (1964) divided the genus into two sections: Stercoraria and Salivaria, differing in morphology, development, and vector transmission (Hoare, 1964; Simpson et al., 2006). Salivaria encompasses species whose vector development cycle ends in a salivary medium, resulting in continuous proliferation in the mammal host. This group contains the subgenus *Trypanozoon*, which includes the species that infects humans, *Trypanosoma* (*Trypanozoon*) *brucei*. Stercoraria includes species whose vector development cycle ends in faecal matter, leading to discontinuous proliferation in the mammal host. The Stercoraria group exhibits a wide range of species, forms, and life cycles, and includes the subgenus *Schizotrypanum* and contains one pathogen, *Trypanosoma* (*Schizotrypanum*) *cruzi*. (Chalmers, 1918; Hoare, 1964; Fraga et al., 2016; de Freitas et al., 2024; Kazim et al., 2024).

The life cycle of *T. cruzi* involves three distinct forms, morphologically and physiologically different in their mammal hosts and triatomines vectors: amastigotes, epimastigotes, and trypomastigotes (broad, slender or metacyclic) (Hoare, 1964; Tyler and Engman, 2001; De-Simone et al., 2022). The trypomastigotes found in mammalian blood are characterized by a "C" or "S" shape (length 16–25 µm), with a kinetoplast located at the posterior end, a free flagellum, and a waving membrane (De-Simone et al., 2022). Currently, *T. cruzi* is classified into seven discrete typification units (DTU): *T. cruzi* I (*Tc*I), *T. cruzi* II (*Tc*II), *T. cruzi* III (*Tc*III), *T. cruzi* IV (*Tc*IV), *T. cruzi* V (*Tc*V), *T. cruzi* VI (*Tc*VI), and *Tc*Bat, which is specific to bats (Velásquez-Ortiz et al., 2022). These DTUs exhibit a histotropic clonal model in Chagas disease, where each DTU has a distinct tropism and is associated with different clinical manifestations of the disease (Macedo et al., 2002; Macedo and Segatto, 2010).

In Colombia, approximately one million people have been affected by Chagas disease, with an additional eight million at risk of contracting the infection (Oliveira et al., 2021; Gómez Ortega et al., 2022; Instituto Nacional de Salud, 2024). *Trypanosoma cruzi* infection has been detected in wildlife, domestic animals, and humans across the country (Oliveira et al., 2021; Gómez Ortega et al., 2022; Instituto Nacional de Salud, 2024). Several regions in Colombia are endemic for *T. cruzi,* including the Magdalena River valley, the Cundiboyacense high plateau, and the Catatumbo in the Andean region; the Sierra Nevada of Santa Marta in the Caribbean region, and the Llanos foothills and Serranía de la Macarena in the Orinoquia and Amazon regions. Among these, Orinoquia stands out as the endemic area with the highest mortality rate (Guhl, 2007; Oliveira et al., 2021; Gómez Ortega et al., 2022).

In Colombia, most studies on *Trypanosoma* have focused on humans and domestic mammals, with few examining wild mammals. Among the latter, the majority of the reports on *T. cruzi* infection have been associated with marsupials such as *Didelphis marsupialis* (Guhl and Ramírez, 2013; Reyes et al., 2017). Other mammalian orders identified as hosts include Cingulata (e.g., *Dasypus novemcinctus*), Rodentia (e.g., *Akodon* sp. and *Dasyprocta* spp.), Chiroptera (e.g., *Carollia perspicillata* and *Artibeus obscurus*), and Primates (e.g., *Ateles* spp. and *Cebus* spp.) (Jansen and Roque, 2010). Given

Figure 1. PRISMA diagram selection of articles for Colombia that summarises the sequence of selection of information for the case studies



that Colombia is recognised as one of the world's biodiversity hotspots for mammal species, it is crucial to continue research efforts to enhance our understanding of the epidemiology scenario of *T. cruzi* across the different and diverse natural regions of the country. The objective of this study was to assess the current state of knowledge and provide new insights into the association between *Trypanosoma* and wild mammals in Colombia, with the aim of contributing to future control and prevention strategies within the framework of the One Health approach.

Materials and Methods

Literature review of *Trypanosoma* associated with wild mammals in Colombia

To compile the available information on *Trypanosoma* in wild mammals and small, introduced, exotic commensal rodents (i.e., genera *Mus* and *Rattus*) in Colombia, a literature review was performed using the Scopus and Web of Science databases. The following key words combination were used in the search: *"Trypanosoma AND mammals OR Tripanosomiasis AND mammals OR Chagas AND mammals".* The search was limited to scientific articles, with no restrictions on publication date or language.

The initial search yielded a total of 119 articles. After removing duplicates, 111 papers remained. The articles were reviewed to determine if they met the following inclusion criteria: 1) information on wild mammal species; 2) identification of the *Trypanosoma* species infecting the mammal host; 3) natural *Trypanosoma* infections, and 4) mention of the technique used to identify *Trypanosoma* (e.g., serological, molecular, or morphological). To minimise reviewer bias, the review was conducted by one person (MJNM) following the guidelines outlined in the PRISMA statement (Page et al., 2021). Ultimately, 40 articles met the inclusion criteria, of which 12 were obtained through free searches (Page et al., 2021) (Figure 1).

Table 1. Departments, municipalities and localities of wild mammals captured in the Andean and Orinoquia region of Colombian. Locality numbers are shown in Fig. 2

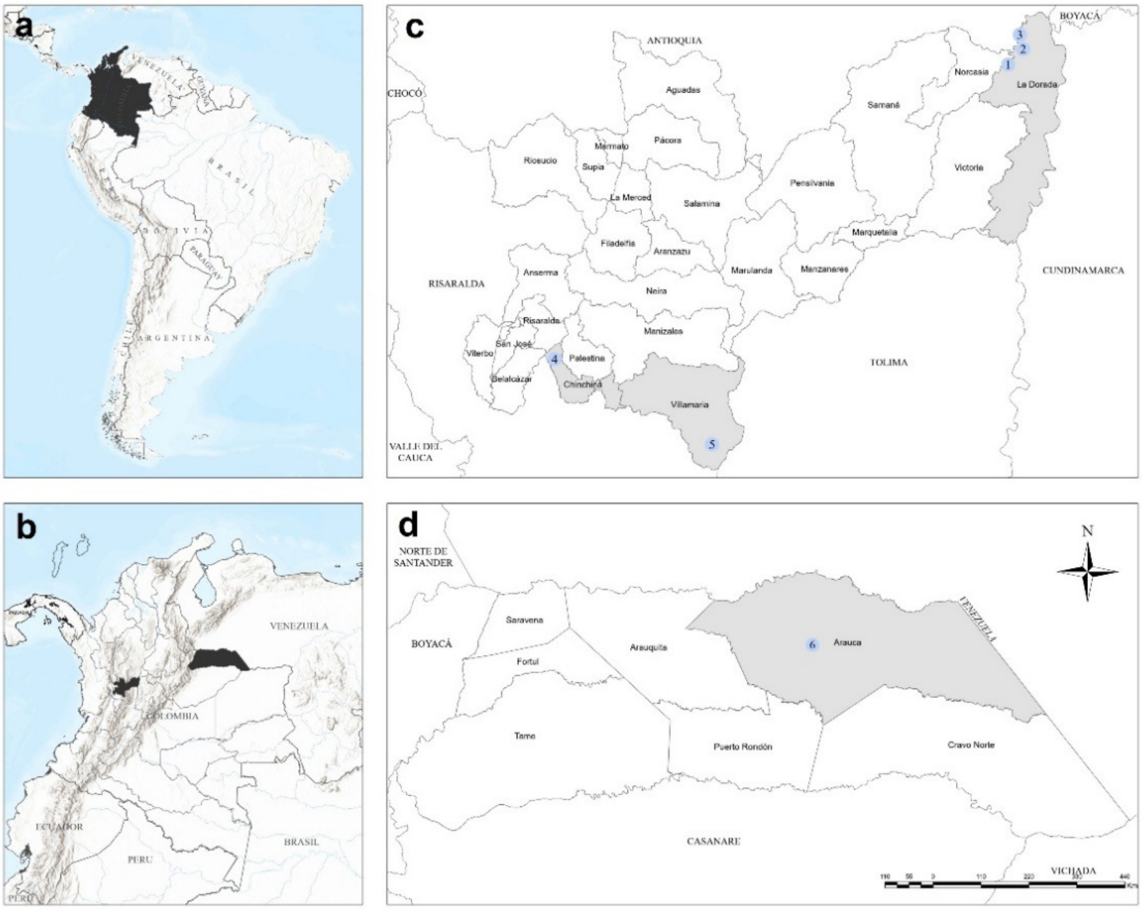
Department	Municipality	Locality	Locality number	Latitude	Longitude	Elevation (meters above sea level)	Habitat type
Caldas	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	1	5.710056	−74.727444	224	Macro-basin with agroforestry matrix and fishing zone
		Vereda La Atarraya, Posada turística Brisas del Río La Miel	2	5.710152	−74.727425	197	Pasture cover, pastures for livestock and agricultural crops
		Vereda La Atarraya, Río la Miel	3	5.720171	−74.726988	178	Agroforestry and livestock matrix in dry forest
	Chinchiná	La Esmeralda CHEC Colonos San Francisco	4	5.027873	−75.7334722	134	Private peri-urban forest reserve
	Villamaría	Sector Santa Bárbara	5	4.844899	−75.3955877	3903	Mountainous topography, livestock and coffee farming area
Arauca	Arauca	Los Trompillos	6	6.779556	−70.716667	120	Livestock grazing area in flooded savannah

Detection and morphological, morphometric and molecular analysis of *Trypanosoma* associated with wild mammals in the Orinoquia and Andes regions of Colombia

To gather additional information on *Trypanosoma* and wild mammals in Colombia, field surveys were conducted in two departments (Figure 2) representing contrasting natural regions: Arauca (Orinoquia region) and Caldas (Andes region). In Arauca, samples were collected between October and November 2021, from different localities within the Arauca municipality (Table 1). This municipality has an agricultural, livestock, and oil exploitation-based economy (Rangel-Ch et al., 2017; Instituto de Hidrología Meteorología y Estudios Ambientales, 2019). In Caldas, samples were collected during March, September, and November 2022 (Figure 2; Table 1). The sampling localities in Caldas are located within the inter-Andean basin of the middle Magdalena and Cauca Rivers, at elevations ranging from 180 to 3903 m. This area supports coffee cultivation and livestock farming (Gobernación de Caldas, 2017; Cardona et al., 2018; Zapata-Torres, 2018), and is home to diverse ecosystems that provide a wide array of environmental services (Corporación Autónoma Regional de Caldas, 2020).

To capture bats (Chiroptera), four mist nets (12 x 2.5 m, mesh 15 mm) were set up. For terrestrial mammals, 60 Sherman and 10 Tomahawk traps were used, placed on the ground and above the vegetation at sampling locations. The traps were baited with sardines and a mixture of granola, banana, and vanilla essence (Voss and Emmons, 1996). Morphological measurements were taken to identify the captured mammals (e.g., tail length, ear, foot, and weight), and all animals were photographed. All animal handling procedures followed the guidelines of the Institutional Animal Care and Use Committee (Sikes et al., 2016; Institutional Animal Care and Use Committee Guidelines and Policies, 2018). Taxonomic identification was based on taxonomic keys proposed by Gardner (2008); Patton et al. (2015), and Díaz et al. (2021). Animal collection was conducted with a permit from the Autoridad Nacional de Licencias Ambientales (ANLA) granted to the Universidad de Caldas according to resolution N° 02497 of 31 December 2018, updated by resolution N° 000026 of 9 January 2024, and the Comité de Bioética de la Facultad de Ciencias Exactas y Naturales - Universidad de Caldas endorsement (2 June 2017 and 20 September 2019). No species registered in the wild threatened species of Colombian biological diversity, recorded in resolution N° 1912 of 2017, updated by resolution

Figure 2. Study area a) South America; b) Colombia; c) Department of Caldas and d) Department of Arauca. The locality numbers are detailed in Table 1



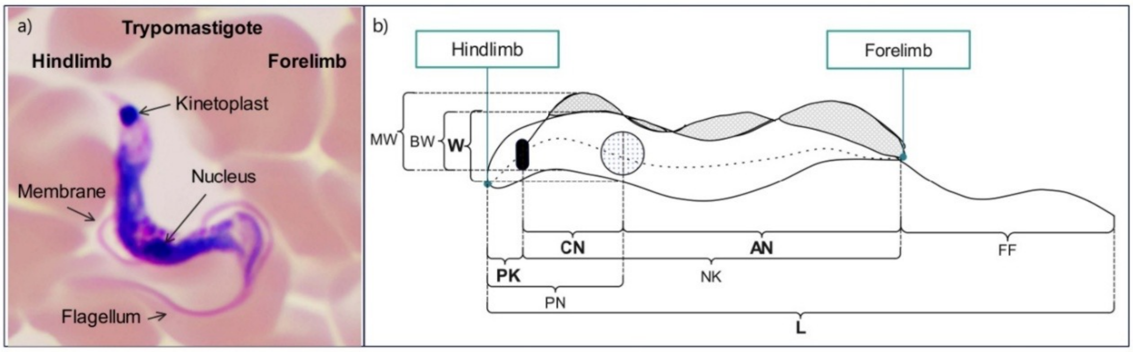
N° 0126 of 6 February 2024 were collected. All collected samples and specimens of interest were deposited in the Colección de Mamíferos, Museo de Historia Natural, Universidad de Caldas (MHN-UCa-M).

Sample collection and morphological identification of *Trypanosoma*

For each captured mammal, a capillary or cardiac puncture blood sample was taken, with the skin sterilised beforehand using cotton soaked in 70% alcohol. A fraction of the blood was used to prepare blood smears (two slides per individual), while the remaining sample was stored in Eppendorf tubes with absolute ethanol for preservation and subsequent molecular analysis. In bats, capillary blood samples were obtained by puncturing the brachial vein, while for terrestrial mammals, such as marsupials and rodents, blood samples were obtained by cutting the posterior part of the tail (Cavazzana et al., 2010; Barbosa et al., 2017; Trujillo-Betancur, 2021). Cardiac puncture was performed immediately following euthanasia, following the Pan American Health Organization (2017) protocol, using a sterile 25G to 27G hypodermic needle, depending on animal size. To assess organ infection (i.e., spleen, brain, heart, liver, spinal cord, lungs, and kidney), organs of interest were extracted. Organ imprints were made on slides (Mancilla-Agrono et al., 2022). The organs were placed individually in sterile Petri dishes, washed with Phosphate Buffered Saline (PBS) (Farbehi et al., 2021), and then washed again with PBS. A longitudinal or transverse cut was made, excess blood was removed with sterile Wypall towels, and the organ was pressed several times onto the slide (multiple cuts were made when necessary). The organs were then stored individually stored in absolute ethanol at 4°C for subsequent molecular analysis. For paired organs, such as kidneys and lungs, a sample was taken from each organ.

Slides were fixed with absolute methanol for three minutes for blood smears and five minutes for organ imprints. They were then stained with 4% Giemsa solution for 40 minutes for blood smears and 45 minutes for organ imprints. Each slide was

Figure 3. ***Trypanosoma cruzi*** **trypomastigote stage with the measurements used for classical morphometry**



a) General structure of the trypanosomatids (kinetoplast, nucleus, undulating membrane and flagellum); b) Measurements were taken for the distance between the following points: L = body length (with flagellum); FF:L = flagellum free length; W = body width (at the middle of the core); BW = Maximum width without membrane-bounded; MW = Maximum width membrane-bounded; CN = distance from the centre of the kinetoplast to the centre of the nucleus; PK = distance from the posterior end of the body to the centre of the kinetoplast, NK = distance from the centre of the kinetoplast to the front end; PN = distance from the posterior end of the body to the centre of the nucleus; AN = distance from the forelimb of the body to the centre of the nucleus; representative measures in bold (Modified from Hoare, 1972)

examined under a light microscope (Olympus BX43) at 100X magnification in bright field in the Molecular Biology Laboratory at the Universidad de Caldas.

Morphological and morphometric analysis of *Trypanosoma*

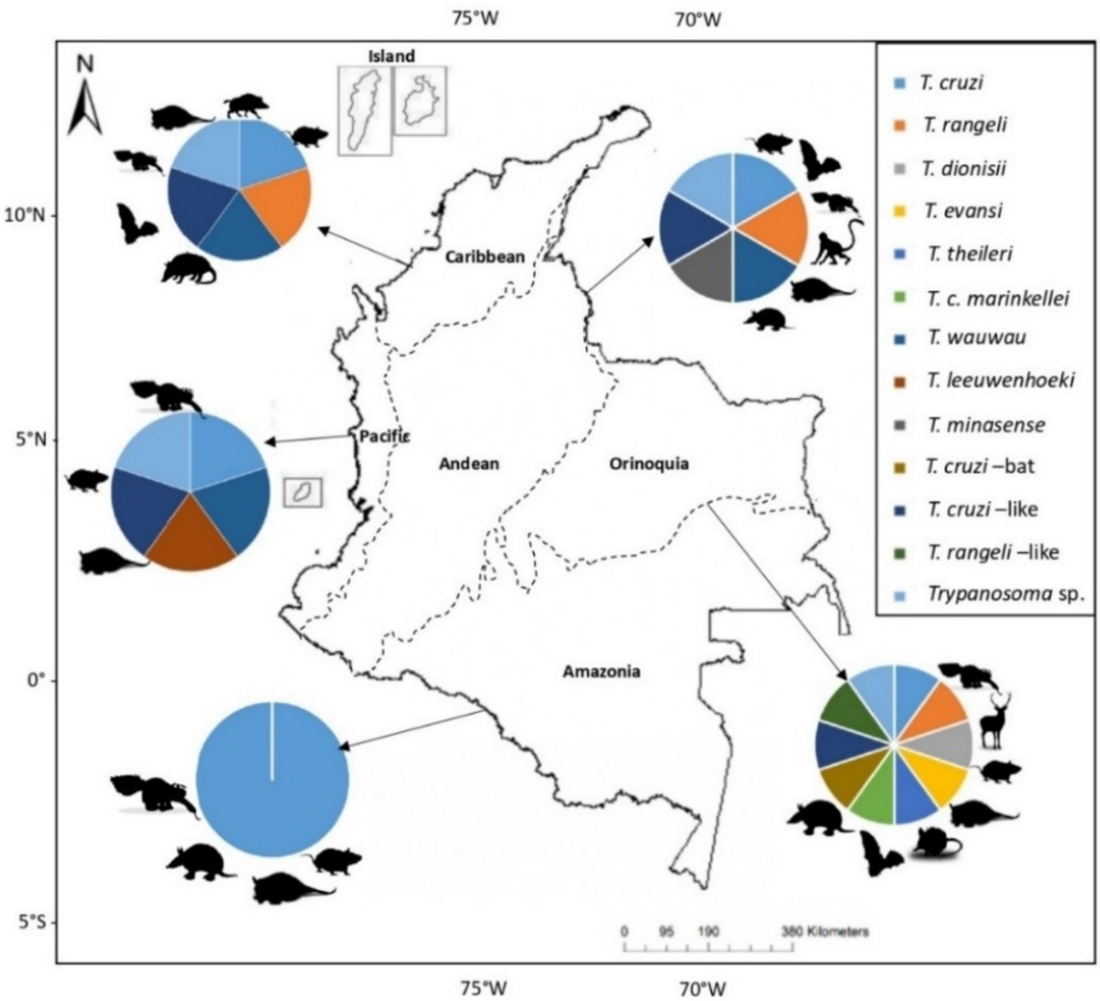
Slide analyses were complemented by morphometric analysis of each *Trypanosoma* in the trypomastigote stage detected in blood smears and organ imprints. Measurements were taken following the guidelines of Dias and Freitas (1943), Hoare (1964 and 1972), and Abràmoff et al. (2004), using the ImageJ image analysis program (version 1.53t, 2022). A constant scale of 20 µm was set, and ten measurements were taken: total length (including the flagellum) (L), width over the nucleus (W), maximum width delimited by the membrane (MW), maximum width without delimitation by the membrane (BW), free flagellum length (FF), length of the kinetoplast with the anterior extremity (NK), distance from the kinetoplast to the posterior extremity (PK), distance to the nucleus [kinetoplast to nucleus] (CN), distance from the anterior extremity of the body to the centre of the nucleus (AN), and distance from the posterior extremity of the body to the centre of the nucleus (PN) (Figure 3).

The kinetoplast position index (KI) was calculated in relation to the nucleus and the posterior end of the body, the nucleus position index (NI) in relation to the posterior body end, and the flagellar index (FF:L), which defines the proportion of the free flagellum to body length (Pereira, 1965; Karbowiak and Wita, 2004; Barros et al., 2019). Principal Component Analysis (PCA) was performed using seven measurements (L, W, PK, NK, MW, BW, PN, and CN), before checking the normality for each of the variables using the Kolmogorov–Smirnov test (with Lilliefors correction), using the “lillie.test” v27.0 package to identify the variables that explained the greatest variability among the measurements and to visualise group formation in the morphometric space (Jolliffe, 2002; Balzarini et al., 2011). Analyses were conducted with the “cluster” v2.1.6, “NbClust” v3.0.1, and “vegan” v2.6-4 packages in RStudio 2023.03.1v (R Core Team, 2023).

Molecular Detection and Identification of *Trypanosoma*

DNA extraction from each tissue was performed using the Wizard® Genomic DNA Purification Kit (Promega Corporation) according to the manufacturer's standard protocol. Pure water (negative control) and *T. cruzi* and *Trypanosoma rangeli* (positive controls, donated by the Laboratorio de Parasitología Tropical, Universidad del Tolima, Colombia) were used as controls for DNA extraction. The extracted DNA was subjected to PCR amplification of six fragments: 1) Kinetoplast DNA (kDNA); 2) intergenic region of the mini-exon gene; 3) Cytochrome b gene (Cyt b); 4) small subunit rRNA gene (SSUrRNA); 5) glyceraldehyde-3-phosphate dehydrogenase gene (gGAPDH); and 6) internal transcribed spacer (ITS) (Table S2). PCR reactions were performed in a final volume of 25 µL (6 µL 5X

Figure 4. Regions of Colombia in which studies on *Trypanosoma* have been conducted. The circles refer to the *Trypanosoma* species that have been reported in each region of Colombia, excluding the insular region where there are no records of *Trypanosoma* infection in wild mammals. The silhouettes of the animals represent the taxonomic groups of wild mammals in which infection by *Trypanosoma* spp. has been reported



Buffer, 2.5 µL 10 mM dNTP mix, 0.5 µL each primer at 25 µM, 0.24 µL 5 U/µL Taq polymerase, and 3 µL DNA).

PCR products were visualised by horizontal electrophoresis in 1% agarose gels stained with ethidium bromide in a TBE 1X pH 8.0 running buffer at 100V/50mA, using a GelDoc-It®2310 Image photodocumenter (UVP), (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Some PCR products were purified using the Wizard® SV Gel and PCR Clean-Up Start-Up Kit (Promega Corporation). All amplification products, including purified ones, were sent for sequencing by Sanger's method at Macrogen Inc. (South Korea). The obtained sequences were evaluated and edited using Genious Prime® v. 2022.2.2 software (Drummond et al., 2009), and species confirmation was performed using Nucleotide BLAST on the National Center for Biotechnology Information (NCBI).

Results

Literature review of Trypanosoma associated with wild mammals in Colombia

A total of 40 scientific articles met the inclusion criteria, encompassing 203 case studies involving 90 wild mammal species, grouped into 50 genera, 23 families, and eight orders across five regions of Colombia (Table 2, Figure 4). The literature review, covering studies up to August 2024, denotes the presence of eight *Trypanosoma* species in Colombia: *T. cruzi, Trypanosoma dionisii, Trypanosoma evansi, Trypanosoma leeuwenhoeki, Trypanosoma minasense, T. rangeli, Trypanosoma theleiri,* and *Trypanosoma wauwau*. In 46 of the case studies in six papers, the presence is reported of *T. cruzi*-bat, *Trypanosoma* sp.,

Table 2. Records of trypanosomes in Colombia from 1966 to 2024 with 203 case studies. For each department and species of *Trypanosoma*, the reference to the 40 articles is presented as a subscript. Authors of the articles indicate up to more than one species of *Trypanosoma* for a species.

Region	Department	Mammals				Trypanosoma species
		Orden	Family	Species	Number of infected individuals cases	
Amazon	Amazonas[22]	Didelphimorphia	Didelphidae	*Caluromys lanatus*[a]	1	*T. cruzi*
	Caquetá[12]	Cingulata	Dasypodidae	*Dasypus novemcinctus*[a]	1	*T. cruzi*
	Putumayo[12]	Pilosa	Myrmecophagidae	*Tamandua tetradactyla*[a]	1	*T. cruzi*
Andean	Antioquia[6, 32], Tolima[12, 6, 7, 9], Cundinamarca[13], Santander[10, 28]	Didelphimorphia	Didelphidae	*Didelphis marsupialis*[a,b,c,e]	10	*T. cruzi*
	Norte de Santander[12], Santander[12]	Chiroptera	Emballonuridae	*Rhynchonycteris naso*[a]	2	*T. cruzi*
	Tolima[23]		Phyllostomidae	*Artibeus lituratus*[a]	1	*T. cruzi*
	Boyacá[12], Tolima[23]			*Artibeus planirostris*[a]	2	*T. cruzi*
	Tolima[23, 35]			*Carollia perspicillata*[a]	1	*T. cruzi bat*
				Glossophaga soricina[a]	1	*T. cruzi bat*
				Micronycteris megalotis[a]	1	*T. cruzi*
				Phyllostomus hastatus[a]	1	*T. cruzi*[23, 35] - *T. rangeli*[35]
	Cundinamarca[12]		Vespertilionidae	*Myotis oxyotus*[a]	1	*T. cruzi*
	Tolima[23]			*Myotis riparius*[a]	1	*T. cruzi*
	Santander[10], Boyacá[26]	Cingulata	Dasypodidae	*Dasypus* sp. [a,b]	2	*T. cruzi*
	Cundinamarca[13], Santander[14]	Pilosa	Myrmecophagidae	*Tamandua tetradactyla*[a]	2	*T. cruzi*
	Boyacá[1]	Primates	Atelidae	*Ateles geoffroyi* [a,c,d]	1	*T. cruzi* - *T. minasense*
				Ateles hybridus [a,c,d]	1	*T. cruzi*
	Santander[14]			*Alouatta seniculus*	1	*T. cruzi*

Region	Department	Mammals				Trypanosoma species
		Orden	Family	Species	Number of infected individuals cases	
	Cundinamarca[1]		Cebidae	*Cebus albifrons*[a,c,d] *hypoleucus* [a,c,d]	1	*T. minasense*
	Tolima[1]			*Cebus albifrons unicolor*[a,c,d,f,g]	1	*T. cruzi* - *T. minasense* - *T. cruzi-like*
	Cundinamarca[1]			*Cebus capucinus*[a,c,d,f,g]	1	*T. cruzi* - *T. cruzi-like* -*T. minasense* - *T. rangeli*
	Boyacá[1]			*Saguinus leucopus* [a,c,d]	1	*T. cruzi*
	Boyacá[1]			*Saimiri cassiquiarensis*[a,c,d]	1	*T. minasense*
	Tolima[1]			*Sapajus apella* [a,c,d]	1	*T. cruzi* - *T. minasense* - *T. cruzi*-like
	Boyacá[22]	Rodentia	Cricetidae	*Oryzomys* sp. [a]	1	*T. cruzi*
	Santander[14]		Echimyidae	*Proechimys* sp. [a]	1	*T. cruzi*
	Santander[10], Boyacá[14]		Muridae	*Rattus rattus* [a,b]	2	*T. cruzi*
Caribbean	Córdoba[5, 40], Sucre[11, 25], Cesar[12 17, 34], Guajira[16], Bolívar[18, 21, 36, 39], Magdalena[19]	Didelphimorphia	Didelphidae	*Didelphis marsupialis*[b,c,d,e]	14	*T. cruzi*[5, 11, 16, 17, 18, 19, 34, 36, 39, 40] - *T. cruzi*-like[5] - *T. wauwau*[5] - *T. rangeli*[36, 39]
	Córdoba[5]			*Philander melanurus* [a]	1	*Trypanosoma* sp.
				Didelphis sp.[a]	1	*T. wauwau*
	Bolívar[18]			*Marmosa cf. robinsoni* [a,b]	2	*T. cruzi*
	Guajira[16]			*Metachirus myosuros* [a,c]	1	*T. cruzi*
	Bolívar[12]	Chiroptera	Phyllostomidae	*Carollia perspicillata* [a]	1	*T. cruzi*
	Córdoba[40]			*Artibeus planirostris* [a]	1	*T. cruzi*
	Magdalena[15]			*Phyllostomus* sp. [a,b]	1	*T. cruzi*
	Bolívar[15]	Cingulata	Dasypodidae	*Dasypus fenestratus* [a,b]	1	*T. cruzi*
	Córdoba[5]	Pilosa	Bradypodidae	*Bradypus variegatus* [a,b]	1	*T. cruzi*

Region	Department	Mammals				Trypanosoma species
		Orden	**Family**	**Species**	**Number of infected individuals cases**	
	Sucre[12]		Myrmecophagidae	*Tamandua mexicana* [a]	1	*T. cruzi*
	Magdalena[15]			*Tamandua mexicana* [a]	1	*T. cruzi*
	Bolívar[1]	Primates	Cebidae	*Saguinus oedipus* [a,c,d]	1	*T. minasense*
	Bolívar[18]	Rodentia	Cricetidae	*Oecomys* sp. [a,b,c]	2	*T. cruzi*
	Córdoba[5]			*Tylomys mirae* [a]	1	*T. cruzi* - *Trypanosoma* sp.
	Bolívar[18]			*Zygodontomys brunneus*[a,b,c]	1	*T. cruzi*
	Magdalena[19]		Dasyproctidae	*Dasyprocta punctata* [a,b]	1	*T. cruzi*
	Magdalena[15]			*Dasyprocta punctata* [a,b]	1	*T. cruzi*
	Bolívar[18]		Echimyidae	*Diplomys* sp. [a,b,c]	2	*T. cruzi*
	Córdoba[5], Magdalena[8, 19]			*Proechimys semispinosus* [a,b,c]	3	*T. cruzi*
	Córdoba[40], Magdalena[8, 19]		Heteromyidae	*Heteromys anomalus* [a,b]	3	*T. cruzi*
	Bolívar[21]		Muridae	*Mus musculus* [a,b,e]	1	*T. cruzi*
	Sucre[6]			*Mus musculus* [a,e]	1	*T. cruzi*
	Bolívar[21]			*Rattus norvegicus* [a,b,e]	1	*T. cruzi*
	Córdoba[5], Sucre[11, 30], Magdalena[12], Bolívar[22, 15, 18]			*Rattus rattus* [b,c]	7	*T. cruzi*[5, 11, 18, 12, 22, 30] - *Trypanosoma* sp. [5]
	Bolívar, Sucre[25]			*Rattus* sp. [a,b]	2	*T. cruzi*
Orinoquia	Meta[3, 6, 27], Arauca[22], vichada[37], Casanare[6, 9, 11, 14, 24, 25, 27, 29, 31, 33, 38]	Didelphimorphia	Didelphidae	*Didelphis marsupialis,*[b,c,d,e]	17	*T. cruzi*[3, 6, 9, 11, 14, 22, 25, 27, 29, 31, 33, 37, 38] - *T. rangeli*[3]
	Meta[3], Casanare[24,33]			*Marmosa* sp. [a]	3	*T. cruzi*[3, 24,33] - *T. cruzi*-like[3]
	Casanare[31]			*Marmosa murina*[a]	1	*T. cruzi*[31]
	Meta[1]	Chiroptera	Emballonuridae	*Peropteryx m. macrotis*[a,c,e]	1	*T. cruzi* - *T. cruzi*-like

Region	Department	Mammals				Trypanosoma species
		Orden	Family	Species	Number of infected individuals cases	
	Casanare[20]			*Rhynchonycteris naso* [a]	1	*T. cruzi - T. rangeli - T. dionisii - T. c. marinkellei*
	Meta[3], Casanare[33]		Molossidae	*Molossus rufus* [a]	2	*T. cruzi*[3, 33] - *T. cruzi*-like[3]
	Meta[1, 6]			*Molossus molossus* [a,c,d,e]	2	*T. cruzi*[1, 6] - *T. cruzi*-like[1]
	Meta[1]		Noctilionidae	*Noctilio albiventris* [a,c,d]	1	*T. cruzi - T. cruzi*-like
	Arauca[12], Casanare[20]		Phyllostomidae	*Artibeus obscurus* [a]	2	*T. cruzi*[12, 20] - *T. rangeli*[20] - *T. dionisii*[20]
	Meta[1, 3]			*Artibeus jamaicensis* [a,c,d]	2	*T. cruzi*[1, 3] - *T. cruzi*-like[1, 3]
	Meta[1], Casanare[24]			*Artibeus lituratus* [a,c,d]	2	*T. cruzi*[1] - *T. cruzi-like*[1] - *T. cruzi bat*[24] - *T. rangeli*[1]
	Casanare[20]			*Artibeus planirostris* [a]	1	*T. cruzi - T. rangeli - T. c. marinkellei*
	Meta[3]			*Artibeus* sp. [a]	1	*Trypanosoma* sp.
	Meta[1], Casanare[20, 38]			*Carollia perspicillata* [a,c,d]	3	*T. cruzi*[1, 20, 38] - *T. cruzi*-like[1] - *T. rangeli*[20] - *T. rangeli*-like[20] - *T. dionisii*[20] -*T. evansi2*[0] - *T. theileri*[20] - *T. c. marinkellei*[20]
	Casanare[33]			*Carollia* sp. [a]	1	*T. cruzi*
	Meta[1], Casanare[12, 20, 38]			*Desmodus rotundus* [a,c,d]	4	*T. cruzi*[1, 12, 20, 38] - *T. cruzi*-like[1] - *T. rangeli*[20] - *T. evansi*[20] - *T. theileri2*[0] - *T. c. marinkellei*[20]
	Meta[1], Casanare[38]			*Glossophaga soricina* [a,c,d]	2	*T. cruzi1, 38 - T. cruzi*-like[1] - *T. rangeli1*
	Meta[1, 3]			*Phyllostomus discolor* [a,c,d]	2	*T. cruzi*[1, 3] - *T. cruzi*-like[1] - *Trypansoma* sp. [3]
	Meta[1], Casanare[38]			*Phyllostomus hastatus* [a,c,d]	2	*T. cruzi*[1, 38] - *T. cruzi*-like[1]
	Casanare[33]			*Phyllostomus* sp. [a]	1	*T. cruzi*
	Meta[1]			*Uroderma bilobatum* [a,c,d]	1	*T. cruzi - T. cruzi*-like
	Casanare[24]			*Uroderma* sp. [a]	1	*T. cruzi - T. cruzi bat*
	Meta[1]		Vespertilionidae	*Lasiurus villosissimus* [a,c,d]	1	*T. cruzi - T. cruzi*-like

Region	Department	Mammals				Trypanosoma species
		Orden	Family	Species	Number of infected individuals cases	
	Meta[3]			*Neoeptesicus furinalis* [a,c,d]	1	*T. cruzi* - *T. cruzi*-like
	Casanare[38]			*Myotis brandtii* [a]	1	*T. cruzi*
	Casanare[38]			*Myotis* sp. [a]	1	*T. cruzi*
	Meta[1]			*Myotis n. nigricans* [a,c,d]	1	*T. cruzi* - *T. cruzi*-like
	Casanare2[0]			*Myotis oxyotus* [a]	1	*T. cruzi*
	Meta[3]	Artiodactyla	Cervidae	*Odocoileus virginianus* [a]	1	*T. cruzi* - *Trypanosoma* sp.
	Meta[3], vichada[37]	Carnivora	Procyonidae	*Potos flavus* [a,b]	2	*T. cruzi*[3, 37] - *T. rangeli*[3]
	Casanare[29]			*Procyon cancrivorus* [a]	1	*T. cruzi*
	Meta[3]	Cingulata	Chlamyphoridae	*Priodontes maximus* [a]	1	*Trypanosoma* sp.
			Dasypodidae	*Dasypus pastasae* [a]	1	*T. cruzi* - *Trypanosoma* sp.
				Dasypus sabanicola [a]	1	*T. cruzi*
	Meta[6], Casanare[26]			*Dasypus* sp. [a,c,d]	2	*T. cruzi*
	Meta[3], Casanare[24]	Pilosa	Myrmecophagidae	*Tamandua tetradactyla* [a]	2	*T. cruzi*[24] - *T. rangeli*[3]
	Meta[1]	Primates	Aotidae	*Aotus trivirgatus* [a,,c,d]	1	*T. cruzi* - *T. cruzi*-like
			Atelidae	*Lagothrix lagotricha* [a,,c,d]	1	*T. cruzi* - *T. cruzi*-like
			Cebidae	*Cebus albifrons* [a,,c,d]	1	*T. minasense*
				Saimiri sp. [a,,c,d]	1	*T. cruzi* - *T. cruzi*-like
	Casanare[33]	Rodentia	Cricetidae	*Microryzomys minutus* [a]	1	*T. cruzi*

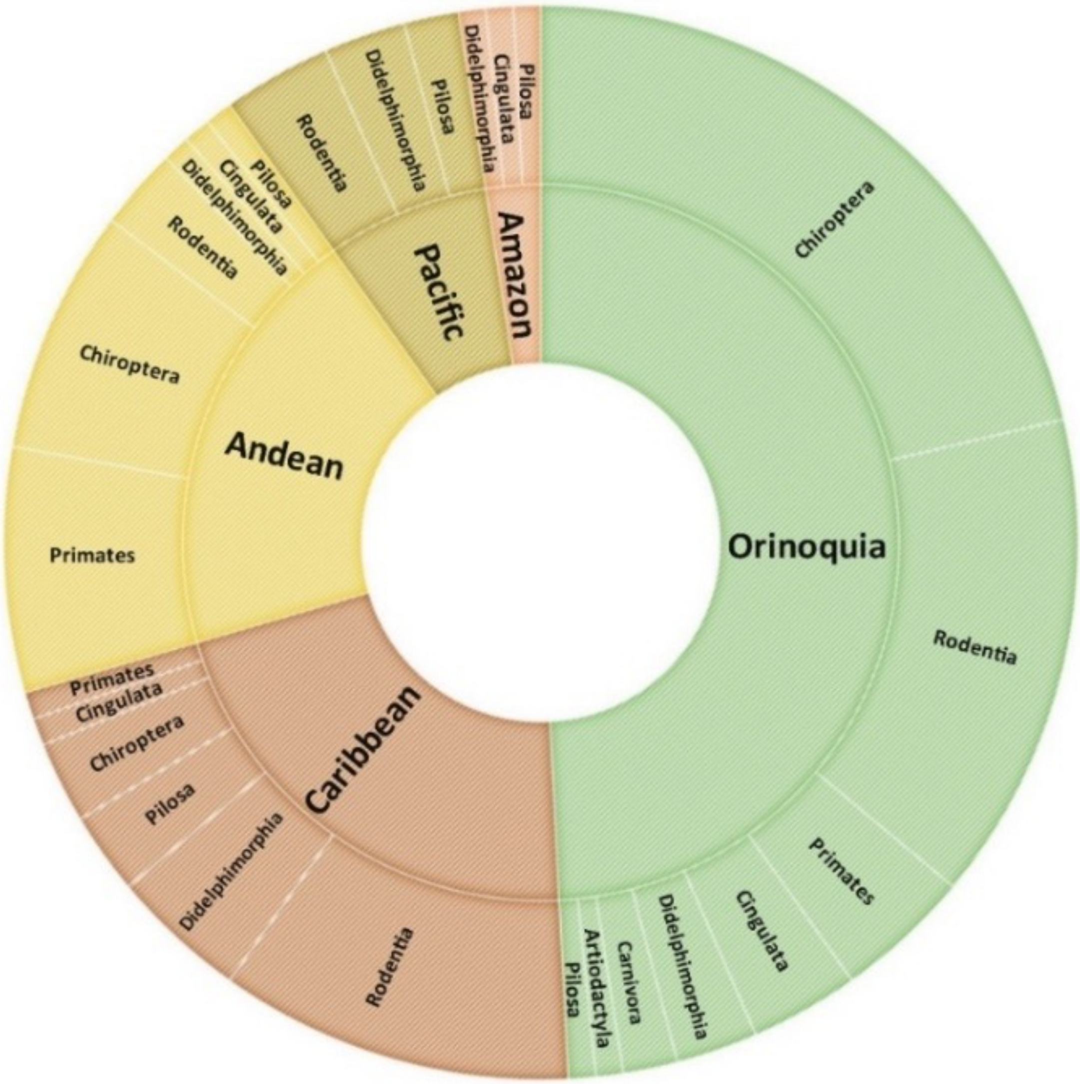
Region	Department	Mammals				Trypanosoma species
		Orden	Family	Species	Number of infected individuals cases	
				Oecomys trinitatis [a]	1	*T. cruzi*
	Meta[3]			*Oligoryzomys fulvescens* [a]	1	*T. cruzi* - *Trypanosoma* sp.
	Casanare[29]			*Oligoryzomys fulvescens* [a]	1	*T. cruzi*
	Meta[3]			*Oecomys concolor* [a]	1	*T. cruzi*
	Casanare[33]			*Sigmodon alstoni* [a]	1	*T. cruzi*
	Casanare[29, 33]			*Zygodontomys brevicauda* [a]	2	*T. cruzi*
	Meta[3]		Cuniculidae	*Cuniculus paca* [a]	1	*T. cruzi*
	Meta[3, 6]		Dasyproctidae	*Dasyprocta fuliginosa* [a,c,d]	2	*T. cruzi*
	Casanare[24]		Echimyidae	*Proechimys oconnelli* [a]	1	*T. cruzi*
	Vichada[37]			*Proechimys semispinosus* [a,b]	1	*T. cruzi*
	Meta[3], Casanare[34]			*Proechimys* sp. [a]	3	*T. cruzi*[3, 34] - *T. rangeli*[3, 34] - *Typanosoma* sp. [3]
	Casanare[24]		Heteromyidae	*Heteromys anomalus* [a]	1	*T. cruzi*-like
	Meta[2, 3], Vichada[2, 12]		Hydrochaeridae	*Hydrochoerus hydrochaeris* [a,d]	4	*T. cruzi*[12] - *T. evansi*[2] - *Trypanosoma* sp. [2, 3]
	Casanare[6]		Muridae	*Molossus molossus* [a,c,d]	1	*T. cruzi*
	Casanare[33]			*Mus musculus* [a]	1	*T. cruzi*
	Vichada[37]			*Rattus rattus* [a,b]	1	*T. cruzi*
	Meta[12]			*Rodentia* [a]	1	*T. cruzi*
Pacific	Nariño[4, 5]	Didelphimorphia	Didelphidae	*Didelphis marsupialis*[a]	2	*T. cruzi*[5] - *T. cruzi*-like[5] - *T. wauwau*[5] - *T. leeuwenhoeki*[4]
				Didelphis sp. [a]	1	*T. wauwau*[5]
				Philander melanurus[a]	1	*Trypanosoma* sp. [5]

Region	Department	Mammals				Trypanosoma species
		Orden	Family	Species	Number of infected individuals cases	
		Pilosa	Choloepodidae	*Choloepus hoffmanni*[a]	1	*T. leeuwenhoeki*[4]
			Bradypodidae	*Bradypus variegatus*[a]	1	*T. wauwau*[5]
		Rodentia	Cricetidae	*Tylomys mirae*[a]	1	*Trypanosoma* sp. [5]
			Echimyidae	*Proechimys semispinosus*[a]	1	*T. cruzi*[5]
			Muridae	*Rattus rattus*[a]	1	*Trypanosoma* sp. [5]
	Chocó[12]		Hydrochaeridae	*Hydrochoerus isthmius*[a]	1	*T. cruzi*

Methods: a. Molecular techniques; b. Serological methods; c. Serodiagnosis; d. Morphological methods; e. Blood cultures; f. Morphometry; g. Histology

Marinkelle, 1966[1]; Morales, et al., 1976[2]; D'alessandro et al., 1984[3]; Travi et al., 1989[4]; Travi et al., 1994[5]; Marquez et al., 1998[6]; Falla et al., 2009[7]; Rodríguez, et al., 2009[8]; Cura et al., 2010[9]; Farfán-García & Angulo-Silva, 2011[10]; Mejía-Jaramillo et al., 2012[11]; Gulh and Ramirez, 2013[12]; Ramírez et al., 2013a[13]; Ramírez et al., 2013b[14]; Vásquez et al., 2013[15]; Peña-García et al., 2014[16]; Soto et al., 2014[17]; Cantillo-Barraza et al., 2014[18]; Mejía-Jaramillo et al., 2014[19]; Ramírez et al., 2014[20]; Cantillo-Barraza et al., 2015[21]; León et al., 2015[22]; Lima et al., 2015[23]; Rendón et al., 2015[24]; Gómez-Palacio et al., 2016[25]; Messenger et al., 2016[26]; Hernández et al., 2016[27]; Reyes et al., 2017[28]; Erazo et al., 2019[29]; Gómez-Hernández et al., 2019[30]; Wehrendt et al., 2019[31]; Cantillo-Barraza et al., 2020[32]; Erazo et al., 2020[33]; Cantillo-Barraza et al., 2021[34]; Patiño et al., 2021[35]; Ardila et al., 2022[36]; Cantillo-Barraza et al., 2022[37]; Castillo-Castañeda et al., 2022[38]; Ardila et al., 2023[39]; Ricardo-Caldera et al., 2024[40]

Figure 5. Regions of Colombia with reports of natural infection by *Trypanosoma* spp. for each mammal order. The inner circle indicates five of the six Colombian regions where cases of *Trypanosoma* studies in mammals have been reported; specifically, the outer circle shows the orders of wild and exotic mammals in which infections by some of the species have been reported of *Trypanosoma*



T. cruzi-like and *T. rangeli*-like species, as well as the *Trypanosoma cruzi marinkellei* (Table 2, Figure 4). The highest number of mammals infected by *Trypanosoma* species were from Chiroptera (28 species) and Rodentia (28 species), followed by Primates (14 species), Didelphimorphia (eight species), Cingulata (five species), Pilosa (four species), and Artiodactyla, Carnivora (two species) (Table 1). Chiroptera exhibited the greatest diversity of associated *Trypanosoma* species, including five species (*T. cruzi, T. dionisii, T. evansi, T. rangeli,* and *T. theleiri*), as well as *T. c. marinkellei, T. cruzi*-like, *T. rangeli*-like, and *T. cruzi* bat.

Seba's short-tailed bat, *Carollia perspicillata,* showed the highest number of infections and *Trypanosoma* diversity, while the common opossum, *Didelphis marsupialis*, was reported in all articles as being infected by various *Trypanosoma* species. The common vampire bat, *Desmodus rotundus*, and the black rat, *Rattus rattus*, which were mentioned in 11 of the reviewed articles, with *T. cruzi* (Table 2) being the primary infection agent. Orders with fewer reports of *T. cruzi* or *Trypanosoma* sp. infection included Artiodactyla (1) and Cingulata (4) (Table 2).

The Amazon region presented exclusively *T. cruzi* infections, primarily in marsupials and xenarthrans such as *Caluromys lanatus* (Didelphimorphia), *Dasypus novemcinctus* (Cingulata), and *Tamandua tetradactyla* (Pilosa) (Figure 5, Table 2). In the Andes region, Chiroptera and Primates were the most affected orders, with higher infection rates in Phyllostomidae and Cebidae species (Figure 5, Table 2), caused by *T. cruzi*, *T. cruzi* bat, and *T. rangeli*. The Caribbean region showed a high prevalence of *Trypanosoma* infections in Rodentia families such as Cricetidae, Echimyidae, Heteromyidae, and Muridae (Figure 5, Table 2). In Didelphimorphia, infections by *T. cruzi, T. rangeli,* and *T. wauwau* were documented (Table 2). The Orinoquia region stood out for numerous reports of *T. cruzi and T. cruzi*-like lineage infections in wild mammals. The highest number of infections (Figure 5, Table 2) were recorded in Chiroptera (fami-

Table 3. Mammals infected with *Trypanosoma* sp. in the departments of Arauca and Caldas, Colombia

Host Order	Host Family	Mammal species	No. Species for municipality, locality - (No. infected species/No. examined species) - % prevalence	Infected Tissuea
Didelphimorphia	Didelphidae	*Didelphis marsupialis*	L6 (1/2) 50	Blood, Heart, Kidney, Liver and Lung
		*Metachirus myosurus**	L1 (1/1) 100	Blood, Heart, Kidney, Liver and Lung
Chiroptera	Phyllostomidae	*Phyllostomus discolor*	L3 (1/1) 100 and L6 (1/4) 25	Lung and Spleen L3; Blood L6
		Artibeus lituratus	L4 (1/1) 100	Lung
		Desmodus rotundus	L6 (1/4) 25	Blood, Heart, Kidney, Liver and Lung
		*Uroderma magnirostrum**	L6 (1/4) 25	Blood
		Carollia perspicillata	L3 (1/4) 25	Blood
		*Dermanura andersenii**	L3 (1/1) 100	Blood
		*Dermanura phaeotis**	L4 (1/1) 100	Lung
		*Lophostoma nicaraguae**	L1 (3/3) 100	Blood, Heart, Kidney, Liver and Lung
		*Trinycteris nicefori**	L1 (1/1) 100	Blood and Kidney
		*Platyrrhinus helleri**	L1 (1/2) 50 and L4 (1/1) 100	Blood L1 and Heart L4
		Sturnira cf. *parvidens**	L1 (1/1) 100	Blood
		*Artibeus obscurus**	L1 (1/1) 100	Blood
		*Dermanura anderseni**	L1 (2/4) 50	Blood
	Vespertilionidae	*Neoeptesicus orinocensis**+	L6 (2/9) 22.22	Blood, Heart, Kidney, Liver and Lung
		*Myotis handleyi**	L6 (1/9) 11.11	Blood
	Molossidae	*Molossus pretiosus**	L6 (3/4) 75	Blood and Heart
		Molossus rufus	L6 (1/5) 20	Blood
		*Molossops temminckii**	L1 (1/1) 100 and L6 (1/3) 3.33	Blood L1-6
		*Cynomops planirostris**	L1 (2/10) 20	Blood
		*Cynomops greenhalli**	L1 (1/1) 100	Blood
	Emballonuridae	*Saccopteryx leptura**	L2 (1/1) 100	Blood
	Noctilionidae	*Noctilio albiventris**	L6 (1/6) 16.67	Blood
Artiodactyla	Cervidae	*Odocoileus v. cariacou**	L6 (1/1) 100	Blood
Rodentia	Cricetidae	*Microryzomys altissimus**	L5 (1/1) 100	Lung

* New association between mammals and *Trypanosoma* sp. for Colombia
a Organ infection location when there are more than two locations with infected individuals
+ Report of *T. cruzi*, strain Sylvio X10/1, TcI

lies Phyllostomidae and Vespertilionidae), followed by Rodentia (families Muridae and Cricetidae). In contrast, the Pacific region exhibited the highest incidence of infections in Rodentia and Didelphimorphia, primarily caused by *T. cruzi, T. cruzi*-like, *T. leeuwenhoeki*, and *T. wauwau* (Figure 5). PCR-based techniques were exclusively used in 60% (n = 24) papers for the detection of *Trypanosoma* spp., while 15% (n = 6) relied on serological methods. Additionally, 22.5% (n = 9) of the articles analysed used at least one of the following detection techniques: serodiagnosis, morphological methods, blood cultures, morphometry, or histology.

Detection, morphological, morphometric and molecular analysis of *Trypanosoma* associated with wild mammals in two departments of Colombia

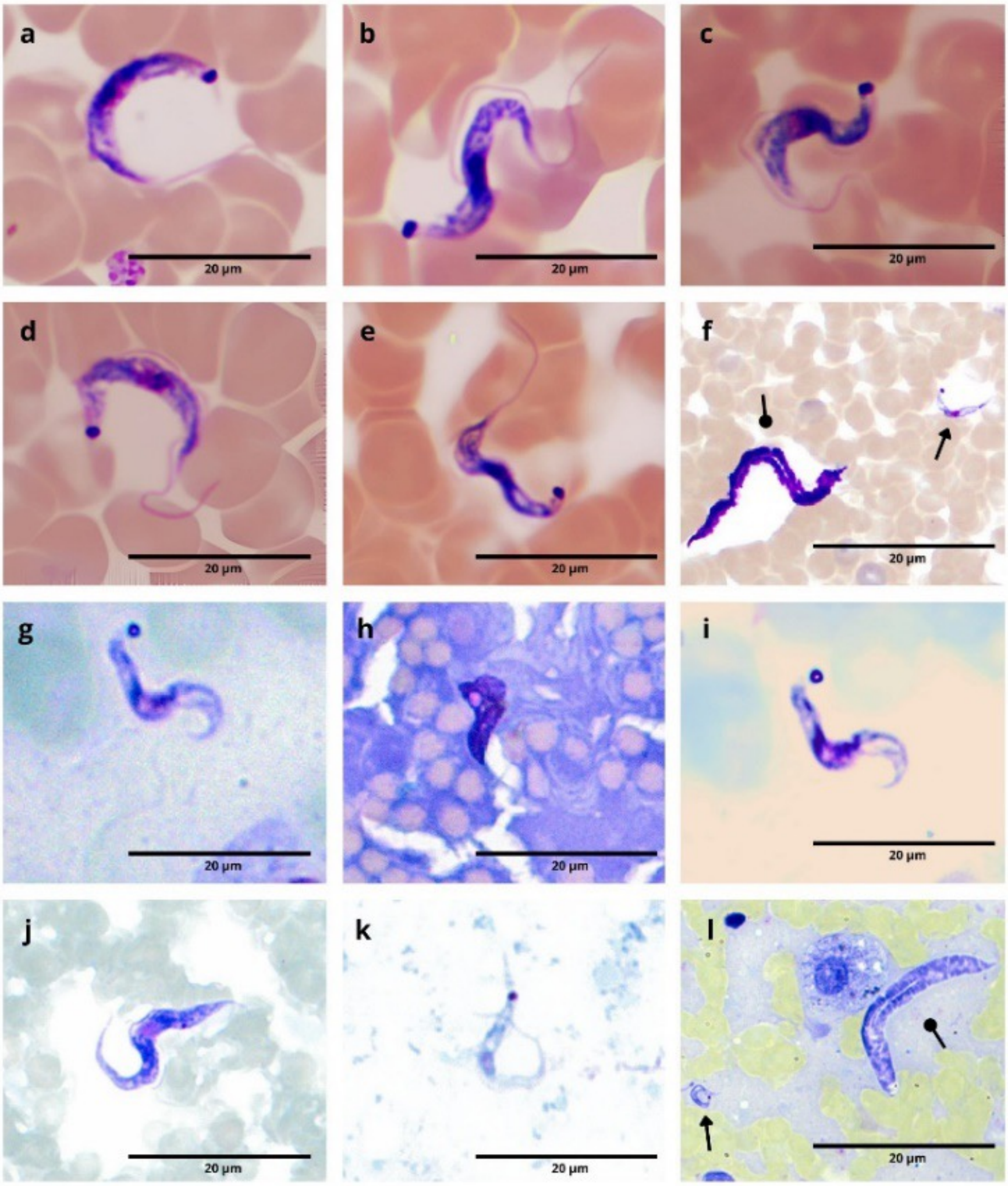
A total of 109 wild mammals were analysed including 45 from Caldas in the Andes region and 64 from Arauca in the Orinoquia region. The individuals studied belonged to four orders (Artiodactyla, Chiroptera, Didelphimorphia, and Rodentia), nine families (Caviidae, Cervidae, Cricetidae, Didelphidae, Emballonuridae, Noctilionidae, Molossidae, Phyllostomidae, and Vespertilionidae), and 46 species (Table S1). This study reports 20 new *Trypanosoma* associations for Colombia (Table 3), including the first report of *T. c. marinkellei* in the marsupial *Metachirus myosuros, T. cruzi* in the bat Orinoco serotine, *Neoeptesicus orinocensis* and *Trypanosoma* sp. in the common vampire-bat *Desmodus rotundus*.

The overall morphological or molecular prevalence of *Trypanosoma* was 37.6% (n = 41). Bat families with the highest prevalence of *Trypanosoma* were Phyllostomidae (52.4%, n = 22), Molossidae (33.3%, n = 10), and Vespertilionidae (16.7%, n = 3). In Arauca, a prevalence of 26.6% (n = 17) was recorded, with the following positive sample distribution: blood 53.3% (n = 16), liver and heart 13.3% (n = 4) each, and lung and kidney 10% (n = 3) each. In Caldas, the prevalence of *Trypanosoma* was 53.3% (n = 24), with the following positive sample distribution: blood 45.9% (n = 17), lung 16.2% (n = 6), liver and heart 13.5% (n=5) each, kidney 8.1% (n=3), and spleen 2.7% (n = 1) (Table 3). Trypomastigotes were identified morphologically in blood smears and impressions from liver, lung, and heart (Figures 6a – 6k). Coinfections with microfilariae were also observed in the blood of two bat species: *Myotis handleyi* and *Phyllostomus discolor* (Figures 6f and 6l).

The morphometric analysis showed variations in trypomastigote measurements to found on tissues and organs differents: Group 1 (G1) in blood (n = 4) and lung (n = 2) samples compared to Group 2 (G2) in lung (n = 3), heart (n = 1) and blood (n = 1) samples. These suggest variation both within and between groups based on classical morphometric parameters (Table S3). Forms of trypomastigotes observed included Slim "C" or "S" shaped forms (Figures S2a and S2b), as well as elongated intermediate and wide forms (Figures S2c, d, e). The position of the kinetoplast (Table S3, KI) varied; it was located at the posterior terminal end (KI < 2), near the nucleus (KI = 2), or in a subterminal position (KI > 2) (Figures S2e, f, and g). The nucleus was centrally located (NI = 1) (Figures S2a, b, e, f, h, and i), near the posterior end (NI < 1; Figure S2d), or near the anterior end (NI > 1; Figure S2g). A well-developed undulating membrane was observed in some trypomastigotes (Figures S2h, e, i), while others exhibited a flat membrane (Figures S2b and e). The length of the free flagellum (Table S3, FF:L) varied between long (Figures S2a, b, e, g, h, and i), short (Figures S2d and f), or absent (Figure S2c). The length of the free flagellum (Table S3, FF:L) varied between long (Figures S2a, b, e, g, h, and i), short (Figures S2d and f), or absent (Figure S2c). Eight of ten variables were selected using the Kolmogorov–Smirnov test (with Lilliefors correction) with normality significance (α= 0.05) (L, W, PK, NK, MW, BW, PN, and CN). The PCA indicated that the first two components explained 74.6% of the total variation, with PC1 accounting for 53.3% and PC2 contributing 21.3%. The analysis formed two groups (Figure S1), both dominated by bat species. In Group 1 (G1), trypomastigotes with the highest MW values were observed in the common vampire bat, *D. rotundus* (Table S3), while the rodent *Microryzomys altissimus* exhibited the lowest MW values (Table S3). In Group 2 (G2), the highest MW were found in *N. orinocensis*, while *Artibeus lituratus* exhibited the lowest values. Concerning the total length of *Trypanosoma* spp., morphotypes in G1 ranged between 9.3 µm and 16.04 µm, while total length in G2 ranged between 7.9 µm and 30.8 µm (Table S3). The kinetoplast position (PK) was smaller in G1 (0.7 µm and 3.8 µm) compared to G2 (1.6 µm and 7.5 µm). Additionally, the maximum width delimited by the membrane measurement (MW) was longer in G2 than in G1 (Table S3).

Two DNA sequences of *Trypanosoma* from *N. orinocensis* bats captured in the Orinoquia region were obtained (Table 3, Figure S3). The SSUrRNA gene sequences showed 100% identicality with *T. cruzi*, CL Brener strain, TcIIe [XM804040.1]. The sequence obtained for the gGAPDH gene showed 99.8% identicality with *T. cruzi,* matching the strain Sylvio X10/1, TcI [MG471429.1] (GenBank accession: PV147115).

Figure 6. *Trypanosoma* sp. in tissue samples from hosts from the departments of Arauca and Caldas.



Micrographs of trypomastigotes in mammal blood: a) *Cynomops planirostris* (Molossidae)-Arauca, b) *Phyllostomus discolor* (Phyllostomidae)-Caldas, c) *Phyllostomus discolor* (Phyllostomidae)-Arauca, d) *Artibeus lituratus* (Phyllostomidae)-Caldas, e) *Odocoileus v. cariacou* (Cervidae)-Caldas, f) *Myotis handleyi* (Vespertilionidae), trypomastigote (arrow) and coinfection with microfilaria (round arrow)-Arauca. Micrographs of trypomastigotes in organs: g) *Neoeptesicus orinocensis* (Vespertilionidae) in liver-Arauca, h) *Dermanura phaeotis* (Phyllostomidae) in lung-Caldas, i) lung of *Artibeus lituratus* (Phyllostomidae)-Caldas, j) *Microryzomys altissimus* (Cricetidae) in liver-Caldas, k) *Platyrrhinus helleri* (Phyllostomidae) in heart-Caldas, l) *Phyllostomus discolor* (Phyllostomidae), trypomastigote (arrow) with coinfection with microfilaria (round arrow)-Arauca. Bar = 20 µm

Table S1. Primers and amplification conditions for each of the genes used for the detection and identification of *Trypanosoma* spp. in two departments of Colombia

Gene	Spp.	Primers	Sequence 5´-3´	Size (pb)	The amplification cycle	References
KDNA	*T. cruzi and T. rangeli*	S35	AAATAATGTA-CGGGTGGAGATG-CATGA	330 *T. cruzi*; 300-450 y 760 *T. rangeli*	1 cycle: 94°C for 5 min; 29 cycles: 94°C for 1 min. 60°C for 1 seg. 72°C for 1 min. 1 cycle: 72°C for 5 min	Souto. et al. 1996; Guhl et al. 1999; Guhl et al. 2002
		S36	GGGTTC-GATTGGGGTTGGTGT			
Mini-exón	*T. cruzi*	TCC	CCCCCCTCCCAGGC-CACACTG	350 *T. cruzi* I; 300 *T. cruzi* II – *T. cruzi* VI	1 cycle: 94°C for 5 min; 34 cycles: 94°C for 30 seg. 48°C for 45 seg. 72°C for 30 seg. 1 cycle: 72°C for 10 min	Souto. et al. 1996; Velásquez-Ortiz. et al. 2018
		TC1	GTG TCC GCC ACC TCC TTC GGG CC			
		TC2	CCT GCA GGC ACA CGT GTG TGT G			
CytB	*T. cruzi Bat*	p18F	GACAGGATTGAGAA-GCGAGAGAG	~516 *T. cruzi*	1 cycle: 94°C for 5 min; 35 cycles: 94°C for 1 min. 48°C for 45 seg. 72°C for 1:30 min. 1 cycle: 72°C for 10 min	Marcili et al. 2009; Garcia et al. 2012
		P20R	CAAACCTATCA-CAAAAAGCATCTG			
SSUrRNA (V7-V8)	*Trypanosoma* spp.	609F	CACCCGCGGTAATT-CCAGC	900 *T. cruzi*	1 cycle: 94°C for 3 min; 29 cycles: 94°C for 1 min. 48°C for 1 min. 72°C for 1 min. 1 cycle: 72°C for 10 min	Dasilva et al. 2004
		706R	TTGAGGTTACAGT-CTCAG			
gGAPDH	*Trypanosoma* spp.	GAP-tryF	GGNCGCATGGT-STTYCAGG	~853 *T. cruzi*	1 cycle: 94°C for 5 min; 29 cycles: 94°C for 1 min. 55°C for 1 min. 72°C for 1 min. 1 cycle: 72°C for 10 min	Hamilton et al. 2007
		GAP-tryR	CCCCACTCGTTRT-CRTACC			
ITS1	*Trypanosoma* spp.	IR1	GCTGTAGGTGAAC-CTGCAGCAGCTG-GATCATT	~930 *T. cruzi*	1 cycle: 94°C for 5 min; 30 cycles: 94°C for 1 min. 55°C for 1 min. 72°C for 2 min. 1 cycle: 72°C for 10 min	Cupolillo. 1995; Dasilva et al. 2004
		IR2	GCGGGTAGTCCTG-CCAAACACTCAGGT-CTG			

Table S2. Wild mammals sampled in the Departments of Arauca and Caldas, Colombia

Mammals		Municipality	Locality	Elevation	Coordinates
Family	Scientific name				
Department of Arauca					
Molossidae	*Eumops glaucinus*	Arauca	Los Trompillos	129	06° 46' 47.5'' N, 70° 43' 02.3'' W
	Eumops nanus	Arauca	Los Trompillos	126	06° 46' 47.3'' N, 70° 43' 02.3'' W
	Molossops temminckii	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Molossops temminckii	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Cynomops planirostris	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Cynomops planirostris	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Cynomops planirostris	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Cynomops planirostris	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Cynomops planirostris	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Cynomops planirostris	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Cynomops planirostris	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Molossops temminckii	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Cynomops planirostris	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Cynomops planirostris	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Cynomops planirostris	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Molossus rufus	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Molossus rufus	Arauca	Los Trompillos	120	06° 46' 47'' N, 70° 42' 59.8'' W
	Molossus pretiosus	Arauca	Los Trompillos	129	06° 46' 47.5'' N, 70° 43' 02.3'' W
	Molossus pretiosus	Arauca	Los Trompillos	129	06° 46' 47.5'' N, 70° 43' 02.3'' W
	Molossus pretiosus	Arauca	Los Trompillos	134	06° 46' 47'' N, 70° 42' 59.3'' W
	Molossus rufus	Arauca	Los Trompillos	126	06° 46' 47.3'' N, 70° 43' 02.3'' W
	Molossus rufus	Arauca	Los Trompillos	127	06° 46' 46.7'' N, 70° 43' 02.1'' W
	Molossus pretiosus	Arauca	Los Trompillos	121	06° 46' 47'' N, 70° 42' 59.8'' W
	Molossus rufus	Arauca	Los Trompillos	120	06° 46' 43.2'' N, 70° 43' 36.1'' W
	Molossus molossus	Arauca	Los Trompillos	129	06° 46' 47.5'' N, 70° 43' 02.3'' W
	Molossus molossus	Arauca	Los Trompillos	121	06° 46' 47'' N, 70° 42' 59.8'' W
	Molossus molossus	Arauca	Los Trompillos	121	06° 46' 47'' N, 70° 42' 59.8'' W

Mammals		Municipality	Locality	Elevation	Coordinates
Family	Scientific name				
	Molossus coibensis	Arauca	Los Trompillos	129	06° 46' 47.5'' N, 70° 43' 02.3'' W
Noctilionidae	*Noctilio albiventris*	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Noctilio albiventris	Arauca	Los Trompillos	122	06° 46' 47.7'' N, 70° 43' 00.5'' W
	Noctilio albiventris	Arauca	Los Trompillos	127	06° 46' 46.7'' N, 70° 43' 02.1'' W
	Noctilio albiventris	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Noctilio albiventris	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Noctilio albiventris	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
Phyllostomidae	*Phyllostomus discolor*	Arauca	Los Trompillos	134	06° 46' 47'' N, 70° 42' 59.3'' W
	Carollia brevicauda	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Uroderma magnirostrum	Arauca	Los Trompillos	126	06° 46' 47.3'' N, 70° 43' 02.3'' W
	Desmodus rotundus	Arauca	Los Trompillos	135	06° 46' 46.3'' N, 70° 42' 59.2'' W
Vespertilionidae	*Neoeptesicus orinocensis*	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Neoeptesicus orinocensis	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Neoeptesicus orinocensis	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Neoeptesicus orinocensis	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Neoeptesicus orinocensis	Arauca	Los Trompillos	127	06° 46' 46.7'' N, 70° 43' 02.1'' W
	Neoeptesicus orinocensis	Arauca	Los Trompillos		06° 46' 46.7'' N, 70° 43' 02.1'' W
	Neoeptesicus orinocensis	Arauca	Los Trompillos	121	06° 46' 47'' N, 70° 42' 59.8'' W
	Myotis handleyi	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Myotis handleyi	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Myotis handleyi	Arauca	Los Trompillos	134	06° 46' 47'' N, 70° 42' 59.3'' W
	Myotis handleyi	Arauca	Los Trompillos	134	06° 46' 47'' N, 70° 42' 59.3'' W
	Myotis handleyi	Arauca	Los Trompillos	134	06° 46' 47'' N, 70° 42' 59.3'' W
	Myotis handleyi	Arauca	Los Trompillos	134	06° 46' 47'' N, 70° 42' 59.3'' W
	Myotis handleyi	Arauca	Los Trompillos	134	06° 46' 47'' N, 70° 42' 59.3'' W
	Myotis handleyi	Arauca	Los Trompillos	134	06° 46' 47'' N, 70° 42' 59.3'' W
	Neoeptesicus orinocensis	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Neoeptesicus orinocensis	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
	Myotis handleyi	Arauca	Los Trompillos	135	06° 46' 46.4'' N, 70° 43' 00'' W
Cervidae	*Odocoileus v. cariacou*	Arauca	Los Trompillos		06° 46' 47.5'' N, 70° 43' 02.3'' W

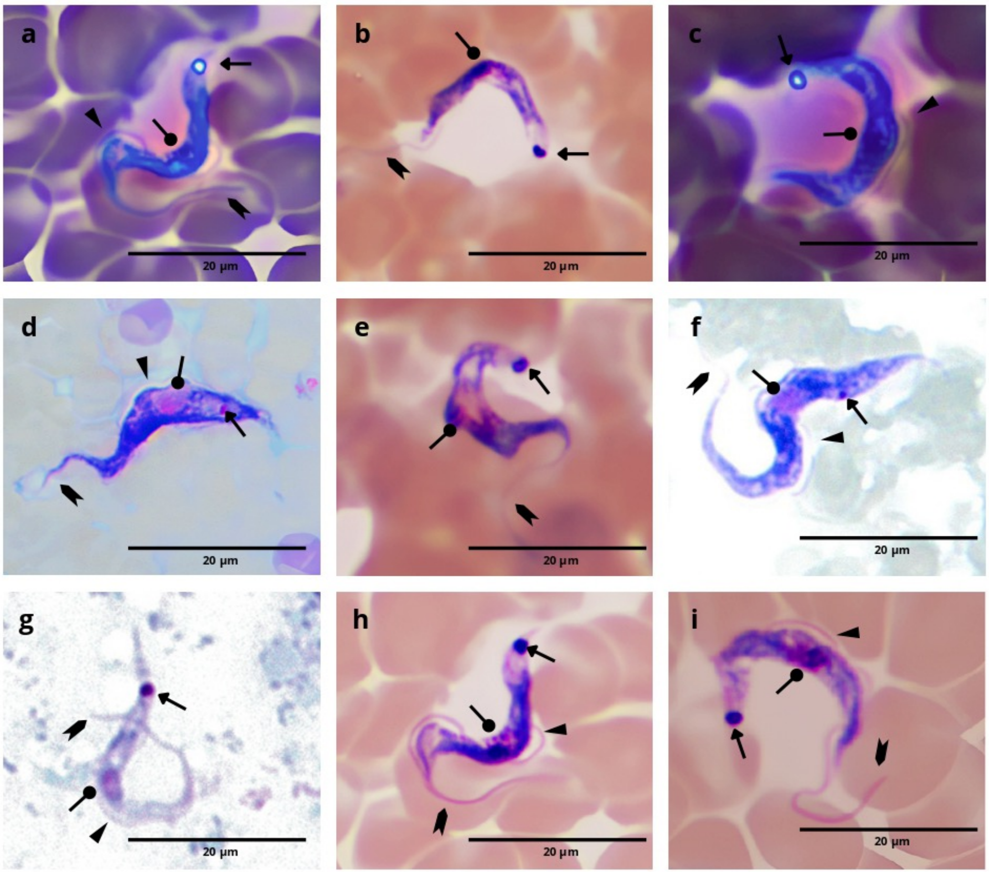
Mammals		Municipality	Locality	Elevation	Coordinates
Family	Scientific name				
Didelphidae	*Didelphis marsupialis*	Arauca	Los Trompillos	127	06° 47' 19.4'' N, 70° 42' 39.1'' W
	Didelphis marsupialis	Arauca	Los Trompillos	130	06° 47' 21.1'' N, 70° 42' 44.7'' W
	Marmosa robinsoni	Arauca	Los Trompillos	127	06° 47' 17.9'' N, 70° 42' 40.9'' W
Caviidae	*Hydrochoerus hydrochaeris*	Arauca	Finca Palmira	121	06° 46' 47'' N, 70° 42' 59.8'' W
	Hydrochoerus hydrochaeris	Arauca	Finca Palmira	121	06° 46' 47'' N, 70° 42' 59.8'' W
Cricetidae	*Oecomys speciosus*	Arauca	Los Trompillos	128	06° 47' 20.9'' N, 70° 42' 44.8'' W
	Oecomys speciosus	Arauca	Los Trompillos	125	06° 47' 20.1'' N, 70° 42' 46.5'' W
Department of Caldas					
Didelphidae	*Metachurus myosurus*	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
Emballonuridae	*Rhynchonycteris naso*	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Saccopteryx leptura	La Dorada	Vereda La Atarraya, cerca de Posada Turística Brisas del Río La Miel	197	05° 42.604' N, 74° 43.646' W
Molossidae	*Molossops griseiventer*	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	197	05° 42' 604" N, 74° 43' 646" W
	Cynomops greenhalli	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	197	05° 40' 371" N, 74° 44' 392" W
Phyllostomidae	*Carollia castanea*	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Carollia perspicillata	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Carollia perspicillata	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Carollia castanea	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Carollia brevicauda	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W

Mammals		Municipality	Locality	Elevation	Coordinates
Family	Scientific name				
	Carollia perspicillata	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Carollia perspicillata	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Carollia castanea	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Carollia castanea	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Carollia brevicauda	La Dorada	Vereda La Atarraya, cerca a Posada Turística Brisas del Río La Miel	197	05° 42.604' N, 74° 43.646' W
	Phyllostomus discolor	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Phyllostumus hastatus	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Lophostoma nicaraguae	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Lophostoma nicaraguae	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392"W
	Lophostoma nicaraguae	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392"W
	Sturnira luisi	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Uroderma convexum	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Sturnira luisi	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Artibeus lituratus	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Dermanura andersenii	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W

Mammals		Municipality	Locality	Elevation	Coordinates
Family	Scientific name				
	Platyrrhinus helleri	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Sturnira sp.	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Sturnira sp.	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Sturnira giannae	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Dermanura andersenii	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Platyrrhinus helleri	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Sturnira luisi	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Artibeus obscurus	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Dermanura anderseni	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Dermanura anderseni	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Micronycteris microtis	La Dorada	Vereda La Atarraya, orillas río La Miel	178	05° 43.209' N, 74° 43.618' W
	Trinycteris nicefori	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392" W
	Glossophaga soricina	La Dorada	Vereda La Atarraya, cerca a Posada Turística Brisas del Río La Miel	197	05° 42.604' N, 74° 43.646' W
	Glossophaga soricina	La Dorada	Vereda La Atarraya, cerca a Posada Turística Brisas del Río La Miel	197	05° 42.604' N, 74° 43.646' W

Mammals		Municipality	Locality	Elevation	Coordinates
Family	Scientific name				
	Dermanura anderseni	La Dorada	Vereda La Atarraya, cerca a Posada Turística Brisas del Río La Miel	197	05° 42.604' N, 74° 43.646' W
	Platyrrhinus helleri	Chinchiná	La Esmeralda CHEC Colonos San francisco detrás ISA	134	05° 01´ 40.42"N-75° 44´ 0,5" W
	Dermanura phaeotis	Chinchiná	La Esmeralda CHEC Colonos San francisco detrás ISA	134	05° 01´ 40.42"N-75° 44´ 0,5" W
	Artibeus lituratus	Chinchiná	La Esmeralda CHEC Colonos San francisco detrás ISA	134	05° 01´ 40.42"N-75° 44´ 0,5" W
Cricetidae	*Microryzomys altissimus*	Villamaría	Sector Santa Bárbara	3903	04° 50' 41.6'' N-75° 23' 44'' W
	Neacomys tenuipes	La Dorada	Vereda La Atarraya, Jardín Botánico del Magdalena	224	05° 40' 371" N, 74° 44' 392"W

Figure S2. Micrographs of the trypomastigote stage of *Trypanosoma* spp. in wild mammals from Colombia.



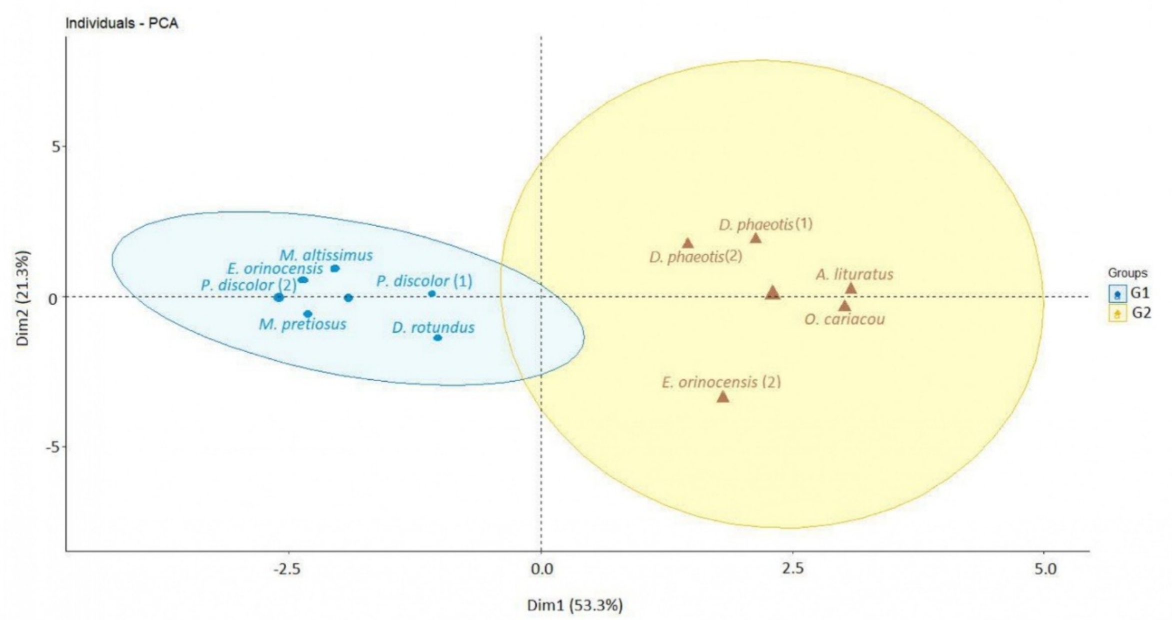
Kinetoplast (long arrow), nucleus (round arrow), membrane (single arrowhead), and free flagellum (triangle). Bar = 20 µm.

Table S3. Measurements of *Trypanosoma* sp. trypomastigote stage for classical morphometry. Measurements of group G1 are shown in blue and the measurements of group G2 obtained from principal component analysis (PCA) are shown in orange

Species	Tissue	Locality	Parameter										Indices		
			L	W	MW	BW	FF	Kinetoplast			Nucleus		KI = (PK + KN)/KN	NI = (PK + KN)/NA	FF*BL = (BL/FF)
								AK	PK	NK	PN	AN			
Group 1															
D. rotundus	Blood	Arauca	11.51 ± 2.64 (9.05-14.20)	2.06 ± 002 (2.05-2.08)	3.58 ± 0.03 (3.56-3.60)	2.17 ± 0.02 (2.15-2.18)	7.81 ± 0.06 (7.77-7.85)	9.52 ± -	4.11 ± -	2.65 ± 0.53 (2.28-3.03)	7.16 ± 0.32 (6.93-7.38)	9.46 ± 5.56 (3.05-12.68)	2.55	2.02	1.47
N. orinocensis (1)	Blood	Arauca	7.34 ± 6.93 (3.31-11.76)	1.19 ± -	1.63 ± 0.02 (1.62-1.64)	1.20 ± 0.04 (1.18-1.23)	9.10 ± 0.29 (8.89-9.30)	11.22 ± -	0.84 ± 0.12 (0.76-0.92)	3.52 ± 0.16 (3.41-3.63)	3.78 ± 0.04 (3.75-3.81)	12.81 ± 0.02 (12.80-12.83)	1.24	1.27	0.81
P. discolor (1)	Blood	Arauca	13.13 ± 1.97 (11.78-16.04)	2.159 ± 0.13 (2.19-2.32)	2.63 ± 0.24 (2.70-3.10)	1.77 ± 0.52 (2.01-2.07)	9.87 ± 0.18 (8.76-10.40)	14.7 ± 0.19 (14.86-15.21)	1.06 ± 0.06 (1.07-1.15)	4.64 ± 0.31 (4.72-5.70)	5.42 ± 0.04 (4.53-5.35)	8.69 ± 0.18 (7.49-7.71)	7.89	2.01	1.46
P. discolor (2)	Lung	Arauca	9.89 ± 0.36 (9.59-9.89)	1.50 ± 0.22 (1.56-1.70)	1.71 ± 0.14 (1.74-1.93)	1.29 ± 0.16 (1.33-1.55)	6.68 ± 0.13 (5.90-6.09)	8.79 ± 0.71 (8.2-9.29)	1.23 ± 0.1 (1.18-1.29)	5.24 ± 0.08 (5.18-5.30)	5.51 ± 0.21 (4.79-4.92)	7.27 ± 0.14 (7.91-9.47)	1.32	1.38	1.31
M. altissimus	Lung	Caldas	7.25 ± 2.99 (5.15-9.33)	1.85 ± 0.086 (1.31-1.44)	1.9 ± 0.11 (1.76-1.91)	1.34 ± 0.12 (1.02-1.2)	7.91 ± 0.34 (10.705-7.81)	7.68 ± 0.81 (5.81-8.08)	4.12 ± 0.11 (3.66-3.83)	6.20 ± 0.07 (6.15-6.26)	5.96 ± 1.37 (2.39-6.48)	11.78 ± 0.01 (11.86-8.22)	1.66	2.02	0.93
M. pretiosus	Blood	Arauca	10.22 ± 0.17 (10.10-10.34)	2.39 ± 0.14 (2.29-2.49)	3.16 ± -	1.24 ± 0.05 (1.20-1.27)	4.38 ± -	9.82 ± 0.17 (9.70-9.94)	2.44 ± 0.08 (2.39-2.50)	7.78 ± 0.24 (7.61-7.95)	8.92 ± 0.19 (8.78-9.05)	4.12 ± 0.17 (4.00-4.24)	1.24	1.29	2.32
Group 2															
D. phaeotis 1	Lung	Caldas	30.69 ± 3.20 (30.55-30.84)	3.22 ± 0.06 (3.18-3.26)	2.87 ± 0.36 (2.61-3.12)	1.44 ± 0.03 (1.42-1.46)	9.04±9.13 (9.09-7.98)	23.6 ± -	5.81 ± -	6.07 ± -	15.13 ± 0.18 (15.01-15.26)	16.21 ± 0.23 (16.04-16.38)	2.13	2.11	3.24
D. phaeotis 2	Lung	Caldas	24.76 ± 0.13 (24.67-24.86)	2.96 ± 0.17 (2.85-3.08)	2.22 v 0.01 (2.21-2.23)	1.60 ± 0.05 (1.56-1.63)	7.98 ± 0.11 (7.89-8.06)	20.94 ± 0.06 (20.90-20.98)	7.17 ± 0.55 (6.78-7.56)	5.56 ± 0.03 (5.54-5.59)	10.69 ± 0.03 (10.67-10.71)	15.79 ± 0.54 (15.41-16.17)	1.96	1.92	3.72
A. lituratus	Heart	Caldas	29.126 ± 0.93 (7.91-9.53)	3.898 ± 0.087 (1.65-1.77)	4.641 ± 0.14 (2.24-2.38)	2.956 ±0.07 (1.37-1.47)	7.58 ± 0.16 (5.8-6.02)	15.796 ± 2.15 (7.20-10.25)	8.981 ± 0.02 (1.61-1.64)	7.06 ± 0.07 (6.25-6.36)	15.918 ± 0.34 (7.21-7.36)	14.768 ± 0.11 (7.20-7.35)	2.27	1.09	3.84
N. orinocensis (2)	Lung	Arauca	21.82 ± 0.1 (12.55-12.68)	3.416 ± 0.07 (1.42-1.52)	5.79 ± 0.16 (1.36-1.59)	4.13 ± 0.15 (1.31-1.53)	9.19 ± 0.07 (7.41-7.51)	16.04 ± 0.06 (11.84-12.21)	3.98 ± 0.12 (1.10-1.27)	2.91 ± 0.67 (4.05-4.15)	8.11 ± 0.03 (4.25-4.29)	13.52 ± 0.13 (10.07-10.25)	2.37	0.51	2.37
O. cariacou	Blood	Arauca	26.79 ± 0.06 (26.75-26.82)	3.51 ± 0.14 (3.41-3.61)	3.91 ± 0.02 (3.90-3.93)	2.70 ± 0.16 (2.59-2.82)	7.36 ±0.06 (7.32-7.40)	17.55 ± 0.05 (17.51-17.58)	9.81 ± -	2.75 ± 3.25 (0.45-5.04)	14.63 ± 0.03 (14.61-14.66)	13.23 ± 0.12 (13.14-13.31)	4.57	2.42	3.64

Total length (L), width over the nucleus (W), maximum width delimited by the membrane (MW), maximum width without delimitation by the membrane (BW), length of the free flagellum (FF), length of the kinetoplast with anterior end (NK), distance of the kinetoplast with posterior end (PK), distance kinetoplast to nucleus (CN), distance anterior end of the body to the center of the nucleus (AN), distance posterior end of the body to the center of the nucleus (PN), ratio of the position of the kinetoplast (KI) relative to the nucleus and to the posterior end of the body; position of the nucleus (NI) relative to the posterior end of the body, and the flagellar index (FF:L) which defines the proportion of the free flagellum to the body length.

Figure S1. Principal Component Analysis (PCA).



Sample *Trypanosoma cruzi* trypomastigotes isolated from wild mammals. Group 1 (G1) species: *Neoeptesicus orinocensis* (blood)*, Microryzomys altissimus* (lung)*, Molossus pretiosus* (blood), *Desmodus rotundus* (blood) and *Phyllostomus discolor* (1-blood and 2-lung). Group 2 (G2) species: *Artibeus lituratus* (heart)*, Dermanura phaeotis* (1-lung and 2-lung), *Neoeptesicus orinocensis* (2-lung) and *Odocoileus virginianus cariacou* (blood)

Discussion

The results reveal that in Colombia, wild mammals and commensal rodents are widely infected with *Trypanosoma* species known for their zoonotic relevance (Coura and Dias, 2009; World Organization for Animal Health, 2021). However, the presence of *Trypanosoma* spp. has primarily been documented in human infections, with *T. cruzi* showing the highest number of records (Rodríguez-Monguí et al., 2019). As a result, research on *Trypanosoma* in wild mammals remains limited, and the epidemiological status of trypanosomes infecting mammals endemically is poorly understood (Vásquez et al., 2013).

Records of *Trypanosoma* spp. infections in Colombia are scarce, especially when compared to studies from six biomes of Brazil (Jansen et al., 2018, 2020) and other countries (Fetene et al., 2021), where bats are considered amplifying hosts for various *Trypanosoma* species (da Silva et al., 2009; Matiz-González et al., 2025). The *Trypanosoma* species documented in Colombia include *T. cruzi*, *T. c. marinkelei*, *T. dionisii*, and *T. rangeli*, which are also documented in other countries of the Americas (Cavazzana, et al., 2010; Hamilton et al., 2012; Lima et al., 2015; Pinto et al., 2015; Bento et al., 2018; Barros et al., 2019). In particular, the bat family Phyllostomidae, the most diverse bat family in the Neotropical region (Ramírez-Chaves et al., 2024), plays a crucial role in the zoonotic cycles of these *Trypanosoma* species, with prior reports from Panama, Colombia, Ecuador, and Brazil (Hoare, 1972; Cottontail et al., 2014; Ramírez et al., 2014; Pinto et al., 2015), Similarly, associations between Didelphidae and *Trypanosoma* have been more frequently documented in Brazil, the United States, and Mexico (Sánchez-Cordero et al., 2024). Species such as *D. marsupialis* (Didelphimorpha) stand out as the key reservoir of *T. cruzi*, with circulating forms of the parasite in peripheral blood and infective forms in its anal glands (Tineo-González et al., 2023). These marsupials play an active role in the zoonotic transmission cycles of *Trypanosoma* and are considered an excellent reservoir due to high rates of persistent infections (Noireau et al., 2009; Herrera, 2010; Rodríguez-Monguí et al., 2019; Ardila et al., 2023). It is emphasised that for the Orinoquia region, the species *Myotis brandtii* Eversmann, 1845 (Chiroptera, Vespertilionidae) is not present in Colombia, although it was referenced in an earlier study (Castillo-Castañeda et al., 2022). This species is mainly documented in Europe (Taake, 1984; Piksa et al., 2022). Another taxon reported for the Orinoquia as *Rattus spinosus* (Rodentia) (Gulh and Ramirez, 2013) does not exist and we included as Rodentia.

In Colombia, most studies have concentrated on endemic regions of Chagas disease, such as the Orinoquia region (departments of Casanare and Meta) and the Caribbean region (departments of Bolívar and Magdalena) (Oliveira et al., 2021; Gómez Ortega et al., 2022). While these areas have been the main focus of research due to their high prevalence, there remains a significant gap in studies from other regions, such as the Insular, Amazon and Pacific regions (Figure 4, Table 2). Expanding research encompasses these regions to better understand the circulation of *Trypanosoma* spp. within different ecosystems (Moncayo, 2003), and investigate whether natural reservoirs and triatomine vectors (e.g., *Triatoma dimidiata, Rhodnius prolixus* and *Panstrongylus geniculatus*) are present (Méndez-Cardona et al., 2022), providing valuable insights for generating a more comprehensive epidemiological outlook.

The analyses conducted on the presence of *Trypanosoma* in wild mammals from the Andes and Orinoquia regions of Colombia, based on field data, provided morphological and molecular evidence of the presence of *T. cruzi*. Additionally, *T. c. marinkellei* infection is reported for the first time in the marsupial *Metachirus myosuros,* and *T. cruzi* in the bat *N. orinocensis*. These findings underscore the significant role of marsupials and bats in the circulation of *Trypanosoma* spp., as previously suggested (Travi et al., 1994; Castillo-Castañeda et al., 2022; Ricardo-Caldera et al., 2024). Furthermore, bats are known as potential reservoirs of Chagas disease in Colombia, with an infection rate higher than those observed in other mammals (Jansen et al., 2018).

According to our morphological analyses, *T. cruzi* shows pleomorphisms (Brener and Chiari, 1963; Schottelius and Uhlenbruck, 1983). This is evident in metacyclic trypomastigotes, whose length can range from 12.03 ± 3.03 μm as the smallest size, reaching between 26–34 μm when including the free flagellum. These trypomastigotes show notable differences in shape, size, and position (Navarro et al., 2003; Trujillo-Betancur, 2021) (Figure S2, Table S3). In natural infections, the manifestation of *T. cruzi* can vary, with more slender forms being predominant, and having greater tissue penetration capacity (Schottelius and Uhlenbruck, 1983; Beatty et al., 2024). However, this morphological variability complicates the establishment of a standardised measurement of trypomastigotes, as *T. cruzi* often disperses multifocally in tissues, resulting in significant polymorphism in the peripheral blood of the mammalian host (Rimoldi et al., 2012; Kunii et al., 2022). Since these parasites undergo a transformation process from epimastigotes, intracellular parasites do not differentiate synchronously, allowing for the observation of all transition stages in a single cell (Souza, 2002). Therefore, in diagnostics, it is crucial to consider factors like the infection stage, site, development stages of the parasite, host variability, and immune responses (Hoare, 1972).

This study represents the first morphological and molecular report confirming the presence of *T. cruzi* strains CL Brener, TcIIe, and Sylvio X10/1, TcI in two individuals of the bat *N. orinocensis*. This aligns with reports in other bat species, highlighting their ability to harbour *T. cruzi* DTUs, especially TcI and TcII, as well as representatives of the subgenus *Schizotrypanum*, such as *T. c. marinkellei*, which are exclusive to bats in the Americas (Amórtegui et al., 2022). Furthermore, the association of *Trypanosoma* sp. in the common vampire bat *D. rotundus* aligns with previous studies (Villena et al., 2018; Bergner et al., 2021), which detected *T. cruzi* in the salivary glands and identified the TcI lineage of *T. cruzi* in other vampire bat species like *Diphylla ecaudata*, and *Diaemus youngii*. The findings underscore the important role of bats in the transmission of *T. cruzi* infection to humans (Lazo et al., 2019; Bergner et al., 2020; Acosta-Jamett and Chaves, 2024). It is known that TcI primarily affects humans and can lead to Chagas cardiomyopathy, meningoencephalitis in immunocompromised patients, and affect the central nervous system (Miles et al., 2009; Shelton and Gonzáles, 2024). In this context, bats could serve as both reservoirs and transmitters of the parasite to other mammals, including humans, even in absence of triatomine vectors (Bustamante et al., 2014; Jansen et al., 2015, 2020; Villena et al., 2018). Therefore, it is essential to enhance studies involving wildlife under the One Health framework to better understand the epidemiology and transmission dynamics of *T. cruzi*.

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> *Trypanosoma* spp. i njihova povezanost sa sisavcima: pregled literatura i slučajeva za regije Ande i Orinoquía u Kolumbiji

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Unutar roda *Trypanosoma*, vrste kao Trypanosoma cruzi predstavljaju javnozdravstveni problem. *T. cruzi* je uzročnik Chagasove bolesti, poznate i kao američka tripanosomijaza. Navedena je bolest endemična u 17 zemalja diljem Amerika i prije svega pogađa sisavce, uključujući ljude. U nekim zemljama kao što je Kolumbija povezanosti *Trypanosoma* i sisavaca još je nedovoljno poznato. U tome kontekstu, cilj je ovoga istraživanje sakupljati sadašnja znanja o povezanosti *Trypanosoma* i sisavaca, navodeći i morfološke i molekularne podatke o tripanosomima u divljim sisavcima u dvije Kolumbijske regije - Arauca i Caldas - koje se nalaze u Orinoquiji, odnosno u Andskoj regiji. Kako bi se prikupilo povijesne evidencije prirodnih zaraza vrstama *Trypanosoma* u divljih i egzotičnih sisavaca (uneseni štakori i miševi) u Kolumbiji, proveden je literaturni pregled korištenjem metode PRISMA. Komplementarno navedenome, izvelo se morfološko i molekularno analiza uzoraka od 109 divljih sisavaca, uključujući krvne briseve, otiske organa i pojačavanje fragmenata mini-eksona, Cytb, ITS, SSUrRNA i Ggapdh gena. Dodatno se provela morfometrijska analiza krvnih tripomastigota. Pregled literature pronašao je 40 znanstvenih članaka koji su navodili prisutnost osam vrsta *Trypanosoma* u divljih i egzotičnih sisavaca u Kolumbiji, s najvišom prevalencijom u šišmiša i glodavaca. Analiza uzoraka iz Arauce (n=64) i Caldasa (n=45) otkrila je prevalenciju vrste *Trypanosoma* od 37,6 % (n=41). Navedeni rezultati čine sveobuhvatni pregled sadašnjeg znanja o vrstama *Trypanosoma* i njihovim povezanostima sa sisavcima u Kolumbiji. Nadalje, rezultati ističu prisutnost *Trypanosoma* u različitim divljim domaćinima u regijama Anda i Orinoquije, naglašavajući potrebu za pojačanim istraživanjem divljih životinja u okviru programa One Health.

Ključne riječi: *javno zdravstvo, literaturni pregled, Tripanosomatide, vektorski prenosive bolesti*