Unraveling the diversity of *Trypanosoma* species from Central Mexico: Molecular confirmation on the presence of *Trypanosoma dionisii* and novel Neobat linages

Javier Juárez-Gabriel [a,b], Daniela Alegría-Sánchez [c], Damaris Yáñez-Aguirre [c], Estefania Grostieta [b], Lucía Álvarez-Castillo [d], Marco Torres-Castro [e], Nidia Aréchiga-Ceballos [f], David A. Moo-Llanes [g], Fernanda Moreira Alves [h], Carlos D. Pérez-Brígido [i], Gabriela Aguilar-Tipacamú [j], Carlos A. López González [j], Ingeborg Becker [b], Juan M. Pech-Canché [k,*], Pablo Colunga-Salas [b,l,*], Sokani Sánchez-Montes [b,m,*]

[a] *Maestría en Ciencias del Ambiente, Facultad de Ciencias Biológicas y Agropecuarias región Tuxpan, Universidad Veracruzana, Tuxpan de Rodriguez Cano, Veracruz, México*
[b] *Centro de Medicina Tropical, División de Investigación, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, México*
[c] *Facultad de Ciencias Biológicas y Agropecuarias región Peñuela, Universidad Veracruzana, Amatlán de los Reyes, Veracruz, México*
[d] *Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Av. Ciudad Universitaria 3000, C.P. 04510, Coyoacán, Ciudad de México, México*
[e] *Laboratorio de Zoonosis y Otras Enfermedades Transmitidas por Vector, Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán, Mérida, Yucatán, México*
[f] *Dirección de Diagnóstico y Referencia, Instituto de Diagnóstico y Referencia Epidemiológicos Dr. Manuel Martínez Báez, Mexico City, México*
[g] *Grupo de Arbovirosis y Zoonosis, Centro Regional de Investigación en Salud Pública, Instituto Nacional de Salud Pública, Tapachula, Chiapas, México*
[h] *Laboratory of Trypanosomatid Biology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Rio de Janeiro, Brazil*
[i] *Hospital Veterinario, Facultad de Ciencias Biológicas y Agropecuarias, región Tuxpan, Universidad Veracruzana, Tuxpan de Rodríguez Cano, Veracruz, México*
[j] *CA. Ecología y Diversidad Faunística, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Querétaro, México*
[k] *Laboratorio de Vertebrados Terrestres, Facultad de Ciencias Biológicas y Agropecuarias región Tuxpan, Universidad Veracruzana, Tuxpan de Rodríguez Cano, Veracruz, México*
[l] *Instituto de Biotecnología y Ecología Aplicada, Universidad Veracruzana, Xalapa de Enríquez, Veracruz, México*
[m] *Laboratorio de Diagnóstico, Facultad de Ciencias Biológicas y Agropecuarias región Tuxpan, Universidad Veracruzana, Tuxpan de Rodríguez Cano, Veracruz, México*

ARTICLE INFO

ABSTRACT

Bats are one of the groups of mammals with the highest number of associated *Trypanosoma* taxa. There are 50 *Trypanosoma* species and genotypes infecting more than 75 species of bats across five continents. However, in Mexico, the inventory of species of the genus *Trypanosoma* associated with bats is limited to only two species (*Trypanosoma vespertilionis* and *Trypanosoma cruzi*) even though 140 species of bats inhabit this country. Specifically, 91 bat species have been recorded in the state of Veracruz, but records of trypanosomatids associated with this mammalian group are absent. Due to the complex *Trypanosoma*–bat relationship, the high diversity of bat species in Veracruz, as well as the lack of records of trypanosomatids associated with bats for this state, the aim of this work was to analyze the diversity of species of the genus *Trypanosoma* and their presence from a bat community in the central area of the state of Veracruz, Mexico. During the period of January to August 2022 in the Tequecholapa Environmental Management Unit where bats were collected using mist nets and blood samples were obtained from their thumbs. We extracted genetic material and amplified a fragment of 800 bp of the 18S ribosomal gene of the genus *Trypanosoma* by conventional PCR. The positive amplicons were sequenced, and phylogenetic reconstruction was performed to identify the parasite species. A total of 285 bats (149♀, 136♂) belonging to 13 species from 10 genera and a single family (Phyllostomidae) were collected. Twenty-three specimens from six species tested positive for the presence of *Trypanosoma dionisii, Trypanosoma* sp. Neobat 4,

---

* Corresponding authors at: Centro de Medicina Tropical, División de Investigación, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, México.
E-mail addresses: jmpech@uv.mx (J.M. Pech-Canché), pcolunga@uv.mx (P. Colunga-Salas), danisanchez@uv.mx (S. Sánchez-Montes).

and a potential novelty species provisionally named as *Trypanosoma* sp. Neobat 6. The results of the present work increase the number of species of the genus *Trypanosoma* infecting bats in Mexico and in the Neotropical region.

1. Introduction

The genus *Trypanosoma* encompasses more than 500 species of protozoan with a flagellum attached to an undulating membrane (responsible for locomotion and the union of the protozoan to the surface of the host cells), a nucleus located in the central part of the parasite, and a specialized region of the mitochondria that contains a modified genome known as the kinetoplast. They are heteroxenous parasites of a wide range of vertebrates, including mammals, that are transmitted by a wide range of blood-sucking invertebrates, mainly arthropods (Hoare 1972; Thompson et al., 2014; Kaufer et al., 2017).

Various species of *Trypanosoma* cause important diseases in animals, such as *Trypanosoma evansi* and *Trypanosoma vivax*, the etiological agents of surra and nagana, respectively, affecting cattle and wildlife, causing substantial economic losses in cattle and wildlife population across Africa, Asia, and South America (Monzón et al., 2010; Austen and Barbosa 2021; Auty et al., 2015). In addition, some species affect public health, such as *Trypanosoma cruzi*, the causative agent of Chagas Disease, a neglected tropical disease in Latin American countries, including Mexico (World Health Organization, 2015).

One of the groups of mammals with the highest number of associated trypanosomatid species are the members of the order Chiroptera. Approximately, 50 species and several genotypes of *Trypanosoma* have been reported to infect more than 75 bat species across the five continents (Turmelle and Olival, 2009; Austen and Barbosa, 2021). In Mexico, the inventory of species of the genus *Trypanosoma* associated with bats is scarce and limited to two species: *Trypanosoma vespertilionis*, associated with one species of the family Mormoopidae (*Pteronotus fulvus*) and another specie of the family Phyllostomidae (*Macrotus waterhousii*) in Chiapas and Colima (Mazzotti, 1946). In contrast, *T. cruzi* has been recorded in eight phyllostomid species (*Artibeus jamaicensis, Artibeus lituratus, Carollia perspicillata, Chiroderma villosum, Dermanura phaeotis, Glossophaga mutica, Sturnira hondurensis*, and *Sturnira parvidens*), one mormoopid species (*Pteronotus mesoamericanus*), and two vespertilionid species (*Myotis keaysi* and *Rhogeessa aeneus*) in Campeche, Morelos and Yucatan (Villegas-García and Santillán-Alarcón, 2001; López-Cancino et al., 2015; Torres-Castro et al., 2021; Simmons and Cirranello, 2023). Despite the recording of 140 bat species in Mexico, which represents 10 % of the world's diversity of bats (Ceballos, 2014), only 15 species (10.86 % of the recorded species) have been screened for the identification of members of the genus *Trypanosoma* (Mazzotii, 1946; Villegas-García and Santillán-Alarcón, 2001; López-Cancino et al., 2015; Torres-Castro et al., 2021; Becker et al., 2023; Izeta-Alberdi et al., 2023; Simmons and Cirranello, 2023). Specifically, in the state of Veracruz, 91 species of bats have been recorded, representing 65 % of the diversity of this mammalian order in the entire country (Coates et al., 2017). However, records of trypanosomatids associated with this mammalian group are non-existent in this state.

Due to the complex *Trypanosoma*–bat relationship, reflected in the high diversity of infected bat species, the high diversity of bat species in Veracruz, and the lack of records of trypanosomatids associated with bats in this state, this work aimed to analyze the diversity of species of the genus *Trypanosoma*, the prevalence of infection and the ecological factors driving such interaction in a highly diverse bat community in the central zone of the state of Veracruz, Mexico.

2. Material and methods

2.1. Study area

The study was performed at the Tequecholapa Management Unit for Wildlife Conservation (MUWC) in the municipality of Naranjal, located at the coordinates 18°48′0.5′′N and 96°56′58′′ W, and an altitude of 704 m above sea level (Fig. 1). The pedological characterization consists mainly of luvisol (59 %), followed by vertisol (18 %) and acrisol (16 %). This type of soil allows the accumulation of clays, making them very fertile.

It features humid evergreen and humid forest vegetation, and is surrounded by farms producing coffee, orchids and zapotes (*Pouteria sapota*). Only a small part is used for planting bananas and coffee. Most of the land within the MUWC has remained untouched, with minimal human intervention, limited to activities by its owners.

2.2. Bat collection

Specimens were collected by convenience sample during two nights per month between January and August 2022 (avoiding full moon nights), using three twelve-meter mist nets in a horizontal position that remained in place between 19:00 to 23:00 h, which corresponds to a total capture effort of 2304 net-meters/hours. The mist nets were constantly monitored every 30 min for the presence of bats. The specimens were handled in accordance with recommended strategies to minimize the risk of *SARS-CoV-2* transmission from humans to bats of the International Union for Conservation of Nature Species Survival Commission (IUCN SSC) and Bat Specialist Group (BSG), as outlined by Frick et al. (2021). Bats were collected under permit SGPA/DGVS/03821/22 from the Secretaría del Medio Ambiente y Recursos Naturales. All bats were unrestricted from the nets with caution and care and transported in rag sacks to facilitate species-level identification using specialized keys (Medellín et al., 2008; Simmons and Cirranello, 2023). We collected blood samples from the thumb of the captured bats and placed on Whatman FTA cards (Whatman Inc., Brentford, UK) under the guidelines described by Eshar and Wienberg (2010) and Parasuraman et al. (2017). After these procedures were completed, all captured specimens were released in situ. The samples were dried and deposited in small individual sterile hermetic plastic bags (INTERSCIENCE, Mourjou, France) for later transport to the laboratory (Gulas-Wroblewski et al., 2021).

To analyze the diversity of bat species, rank-abundance curves of the captured bats were made, with the purpose of observing changes in the presence and abundance of species at the collection site following the procedures of Loayza and Larrea-Alcázar (2006) and Novoa et al. (2011).

2.3. Molecular and bioinformatic detection of Trypanosoma

DNA extraction was performed using the chelating resin CHELEX 100 (Bio-Rad, Hercules, CA, US). The blood sample from the Whatman FTA card of each specimen was introduced into a 1.5 ml conical tube, to which 500 μl of a 10 % CHELEX solution and 20 μl of proteinase K were added. The samples were incubated at 56 ºC for 12 h. Subsequently, the samples were subjected to a temperature shock at 100 ºC for 15 min to intensify the denaturation of the proteins (García-González et al., 2004). The samples were then centrifuged at 14,000 rpm for 15 min, and finally, the supernatant was recovered and stored at −20ºC until its use. The DNA quantification was performed using a ThermoFisher Nano-Drop™ 2000 spectrophotometer.

To confirm the quality and integrity of the extracted DNA, we amplified a fragment of 450 bp of the endogenous mammalian gene Cytochrome Oxidase Subunit I (COI) gene with oligonucleotides L6625 (5′-CCGGATCCTTYTGRTTYTTYGGNCAYCC-3′) and H7005 (5′-CCGGATCCACNACRTARTANGGTRTCRTG-3′) and thermal conditions

previously reported (Hafner et al., 1994). For the *Trypanosoma* detection, DNA from the blood samples was analyzed by conventional PCR by pooling three to five specimens of the same species from the same collection period. A fragment of 800 bp of the 18S small subunit of the ribosomal gene (SSU rDNA) was amplified using oligonucleotides 609F (5′-CACCCGCGGTAATTCCAGC-3′) and 706R (5′-CTGAGACTGTAACC TCAA-3′) (Sgroi et al., 2021). PCR was performed under the following conditions: an initial cycle of 3 min at 94 ℃, followed by 30 cycles of 1 min at 94 ℃, 2 min at 48 ℃ followed by 2 min at 72 ℃, and a final cycle of 10 min at 72 ℃ (Borghesan et al., 2013). The positive pools were screened individually to identify the positive specimens and to establish the prevalence of the infection. In both cases, the PCR reaction mix was performed in a final volume of 25 μl per sample, containing 12.5 μl of GoTaq® Green Master Mix, 2X (Promega Corporation, Madison, WI, USA), 1 μl of each primer (at a concentration of 2 μM), 5 μl of DNA (approximately 100–500 ng), and 5.5 μl of nuclease-free water.

Positive and negative controls were included in each reaction. For COI amplification, the positive control consisted of rodent BALB/c DNA, whereas for amplification of the SSU rDNA of trypanosomatids, a DNA from the *Trypanosoma cruzi* strain NINOA was used. For the negative control, the DNA was replaced by reaction mix with nuclease-free water.

PCR products were analyzed by electrophoresis on 2 % agarose gels stained with 4μl of 6X SmartGlow™ Pre-Stain (Accuris™ Instruments, Edison, NJ, USA) using a 100 bp molecular marker (Cleaver Scientific™ DNA-100BPH). Gels were visualized under an omniDOC Gel Documentation System (Cleaver Scientific™). Positive amplicons were sent for sequencing at ©Macrogen, Inc. Seoul, Republic of Korea.

The recovered sequences were compared with reference sequences using the BLAST-n tool in GenBank ® (www.ncbi.nlm.nih.gov/genbank/). Subsequently, a global alignment was carried out in the MEGA 11 program, using the Muscle algorithm (Edgar, 2004). For this alignment, the sequences recovered from this study were used together with

sequences available from GenBank. The best substitution model was determined using the ModelFinder Algorithm program (Kalyaana-moorthy et al., 2017). Subsequently, a Maximum Likelihood (ML) analysis was performed in the IQ-TREE program (Nguyen et al., 2015), evaluating clades support with 10,000 bootstraps (Hoang et al., 2018).

Finally, pairwise distances were calculated using the K80 model and all sites with the *ape* package in the R environment. The results were then plotted on a heatmap using *ggplot2* to evaluate the distribution of genetic distances for each species (Grostieta et al., 2023). These analyses were carried out using R v. 4.2.2 (R Core Team, 2022).

2.4. Bat species traits

To evaluate the host's traits relevant to *Trypanosoma* infection, information about bat species were obtained from published studies (Supplementary 1). Three feeding guilds were defined: nectarivory ($n = 3$ species), insectivory ($n = 1$), and frugivory ($n = 9$) according to González-Salazar et al. (2014). Roost type was defined as closed (e.g., tunnels, hollows; $n = 8$) or open (e.g., above canopy; $n = 3$), while roost flexibility was defined as single ($n = 3$) or multiple ($n = 8$), as previously defined by Becker et al. (2020). Finally, maximum colony sizes were defined as small (<1000 individuals; $n = 10$) and large (>1000; $n = 2$) following Becker et al. (2020).

2.5. Prevalence analysis and analysis of species infection status of bats

The global prevalence of *Trypanosoma* species, the prevalence per bat species, and the prevalence per *Trypanosoma* species with their 95 % confidence intervals were calculated using the *Prevalence* package in the R environment and following the operational definition of Bush et al. (1997). The calculations utilized the Agresti-Coull interval, considering the sample size (Brown et al., 2001).
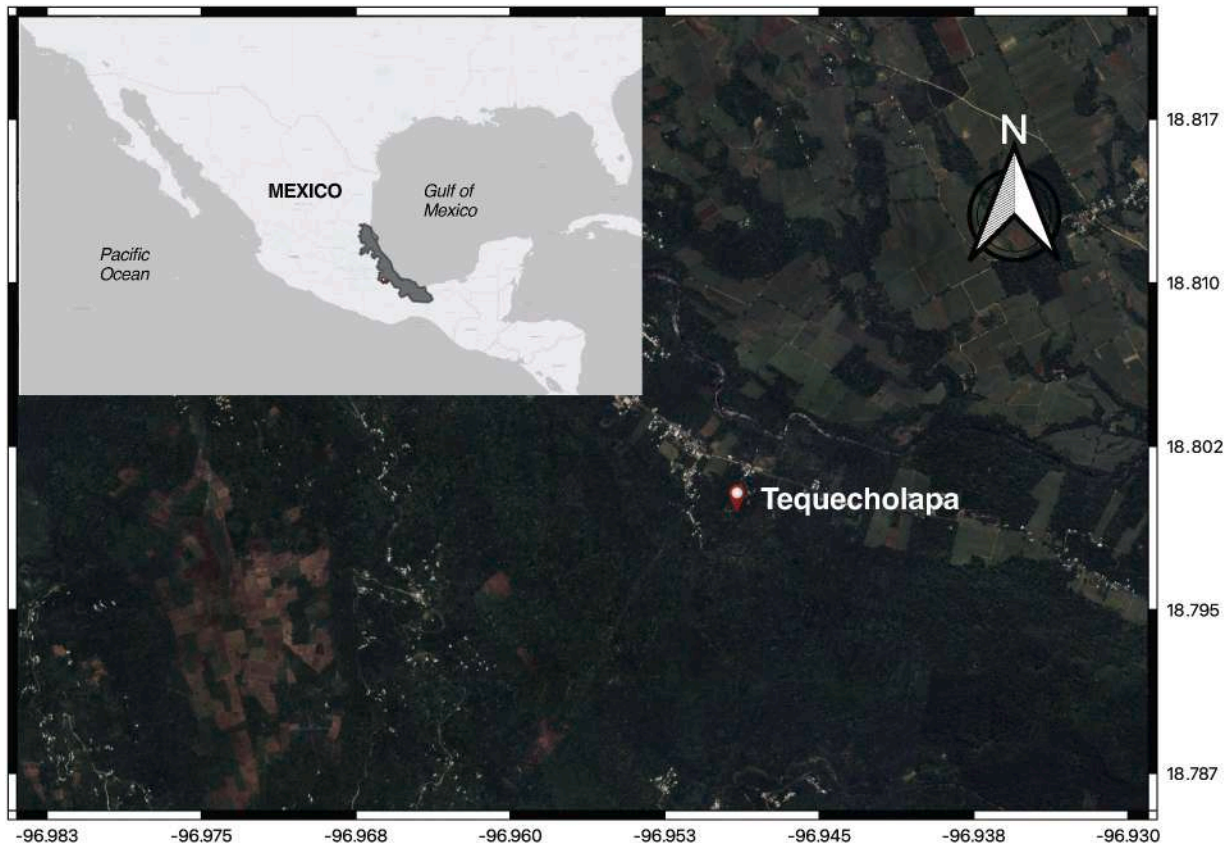

Fig. 1. Map of sampling sites at Tequecholapa Management Unit for Wildlife Conservation, Veracruz, Mexico.

To explore the patterns in *Trypanosoma* species prevalence according to the host's traits, a table of presence/absence of *T. dionisii, Trypanosoma* sp. Neobat 4, and *Trypanosoma* sp. was generated including all host traits and prevalence. With this information, a Principal Coordinates Analysis (PCoA) was performed to explore the variation in *Trypanosoma* species prevalence among 12 bat species. *Micronycteris microtis* was excluded from the analysis because the scatter plot showed this species as an outlier, since the single individual was positive. The analysis was based on a Euclidean distance resemblance matrix. To visualize the results, a two-dimensional scatter plot of the first two principal coordinates was generated.

Furthermore, a one-factor permutational multivariate analysis of variance (PERMANOVA) was conducted to test for significant differences in the prevalence patterns of *Trypanosoma* species among bat species (excluding *Micronycteris microtis*). This analysis used Type III (partial) sum of squares, unrestricted permutation of raw data, and 999 permutations, and used the Euclidean distances resemblance matrix as the response variable. All statistical analyses were run using Primer version 7 software (Primer-E, 2020).

3. Results

3.1. Diversity of bats

A total of 285 bats (149♀, 136♂) belonging to 13 species from 10 genera of a single family (Phyllostomidae) were captured (Table 1). The bat species with the highest number of specimens collected were *Carollia sowellii* at 42.1 % (*n* = 120), followed by *A. jamaicensis* at 14.7 % (*n* = 42), and *S. hondurensis* at 10.8 % (*n* = 31). The species with the lowest richness was *Anoura geoffroyi*, with five specimens (1.7 %). The species with singletons collected were *Centurio senex, M. microtis*, and *Platyrrhinus helleri*.

The rank-abundance analysis showed four dominant frugivorous species (*C. sowelli, A. jamaicensis, S. hondurensis*, and *A. lituratus*). Nectarivorous species were concentrated in the middle part of the curve (*G. mutica, C. godmani*, and *A. geoffroyi*). The rarest species were insectivorous and fruit-eating bats (*Platyrrhinus helleri, M. microtis*, and *C. senex*) and were concentrated in the decreasing part of the logarithmic values (Fig. 2).

3.2. Trypanosoma diversity in bats from Veracruz

DNA was successfully extracted from all samples, with concentrations ranging from 20 ng/μl to 95 ng/μl. DNA quality was evaluated by the amplification of the endogenous COI gene in all samples. *Trypanosoma* DNA was only detected in the blood of 23 bats from six species (Table 1). The alignment consisted of 23 sequences (this study) and 15 sequences from several *Trypanosoma* species retrieved from GenBank. The alignment consisted of a total of 840 positions, 622 with invariant sites (74.04 %), 115 parsimony informative sites (13.69 %), and 76 distinct sites (9.04 %). The best substitution model for the ML was TIM3e+R2.

The molecular identification from the recovered sequences confirmed the presence of three well-defined groups. One group comprised all sequences of *T. dionisii*, four positive *C. sowelli* bats, with a single positive individual of *A. jamaicensis* and *G. mutica* (branch support = 100). Another group comprised two positive *C. sowelli* individuals, with a single individual of *C. godmani* and *C. perspicillata*, highly related to *Trypanosoma* sp. Neobat 4 (branch support = 93). The third group is comprised by a putative novel *Trypanosoma* species, detected in 11 *C. sowelli* individuals, and one individual of each of the following species: *C. perspicillata*, and *M. microtis* (branch support = 100) (Fig. 3). This division into three groups was also observed with the pairwise genetic distances (Fig. 4).

3.3. Prevalence of Trypanosoma species

Twenty-three bats, constituting 8.07 % of the total 285 individuals (95 % CI: 5.38 %–11.86 %) tested positive for *Trypanosoma* DNA. All these bats were infected with a single species of *Trypanosoma* so none of them exhibited the presence of coinfection. The species with the highest prevalence of *Trypanosoma* was *M. microtis* (single sampled individual being positive), followed by *C. sowelli* with 18 positives out of 120, and *C. perspicillata* with one positive out of eight. *Artibeus jamaicensis* had the lowest *Trypanosoma* prevalence, with one positive in 42 sampled individuals (Table 1).

Among the 285 sampled bats, *Trypanosoma* sp. Neobat 6 exhibited the highest prevalence at 4.56 % (95 % CI: 2.61 % – 7.71 %), followed by *T. dionisii* at 2.1 % (95 % CI: 0.85 % – 4.63 %), and *Trypanosoma* sp. Neobat 4 at 1.05 % (95 % CI: 0.21 % – 3.19 %).

3.4. Ecological factors and Trypanosoma infection

The Principal Coordinates Analysis (PCoA) analysis was used to compare the community similarities regarding the prevalence of *T. dionisii, Trypanosoma* sp. Neobat 4, and *Trypanosoma* sp. Neobat 6 between bat species. The scatter plot shows that the first and second principal coordinate axes represented 79.8 % (54 % and 25.8 %, respectively) of the total variation accounted for by the PCoA (Fig. 5). PERMANOVA analyses revealed significant differences related to the host trait of colony size (PERMANOVA test, *p* = 0.002). Therefore, among all the tested host traits, large colony size was associated with an increased probability of infection by any of *T. dionisii, Trypanosoma* sp. Neobat 4 or *Trypanosoma* sp. Neobat 6 (Fig. 5). Specifically, species with colonies up to 1000 individuals had statistically lower probabilities of being infected by any *Trypanosoma* reported in this study than species with colonies larger than 1000 individuals.

4. Discussion

In the Neotropical region, there is an inventory of at least six validated *Trypanosoma* species (*Trypanosoma cruzi, T. dionisii, Trypanosoma madeirae, Trypanosoma rangeli, Trypanosoma theileri*, and *Trypanosoma wauwau*) associated with bats of the families Mormoopidae and Phyllostomidae (Ramírez et al., 2014; Barros et al., 2019; Jaimes-Dueñes et al., 2020; Alves et al., 2021). However, in recent years the use of bioinformatic tools and molecular techniques focused on the amplification and sequencing of genes such as 18S-rDNA and GAPDH has made it possible to unravel the complexity of the trypanosomatid community in this vast group of mammals. This work contributes to our understanding of *Trypanosoma* species in wildlife, particularly in bats, by reporting the first occurrences of *T. dionisii* and *Trypanosoma* sp. Neobat 4 in Mexico.

In this study, we detected the presence of three species of phyllostomid bats (*A. jamaicensis, C. sowelli*, and *G. mutica*) infected with *T. dionisii*. This parasite exhibits a global distribution (Austen et al., 2020) and has been reported with high frequency in bats from Brazil and Australia, and less frequently in the United Kingdom, Czech Republic, Bulgaria, Poland, China, and the United States of America (Cavazzana et al., 2010; García et al., 2012; Ramírez et al., 2014; Hodo et al., 2016; Dario et al., 2017; Dos Santos et al., 2018; Bento et al., 2018; Mafie et al., 2018; Wang et al., 2019; Austen et al., 2020; Barros et al., 2020; Linhart et al., 2020).

Although *T. dionisii* has previously been detected in *C. perspecillata* and *A. lituratus* in Brazil (Cavazzana et al., 2010), it has not been detected in *C. sowelli* or *A. jamaicensis*, so this study represents the first report of *T. dionisii* infection in these bat species. The prevalence of *T. dionisii* reported in this work (2.1 %), is similar to that in previous studies carried out in the USA, in which nine out of 593 (1.5 %) individuals belonging to three bat species (*Tadarida brasiliensis, Parastrellus hesperus*, and *Antrozous pallidus*) tested positive for this parasite

Table 1

Sample size and prevalence (95 % CI) per bat species collected at Tequecholapa Management Unit for Wildlife Conservation, Veracruz, Mexico between January to August 2022. Analyses were stratified by *Trypanosoma* species and sex. Td: prevalence for *T. dionisii*, T4: prevalence for *Trypanosoma* sp. NeoBat 4, and T6: prevalence for *Trypanosoma* sp. NeoBat 6.

| Species	n	♀	♂	*Trypanosoma dionisii*			*Trypanosoma* sp. NeoBat 4			*Trypanosoma* sp. NeoBat 6		
				Td (95 % CI)	Td♀ (95 % CI)	Td♂ (95 % CI)	T4 (95 % CI)	T4♀ (95 % CI)	T4♂ (95 % CI)	T6 (95 % CI)	T6♀ (95 % CI)	T6♂ (95 % CI)
Anoura geoffroyi	5	5	0	0	0	0	0	0	0	0	0	0
Artibeus jamaicensis	42	22	20	2.38 (0–13.44) [1/42]	4.54 (0–23.51) [1/22]	0	0	0	0	0	0	0
Artibeus lituratus	20	15	5	0	0	0	0	0	0	0	0	0
Carollia perspicilata	8	5	3	0	0	0	0	0	0	12.5 (0.11–49.22) [1/8]	20 (2.03–64.03) [1/5]	0
Carollia sowelli	120	54	66	3.33 (1.02–8.54) [4/120]	3.70 (0.3–13.25) [2/54]	3.03 (0.21–1.1) [2/66]	1.66 (0.08–6.25) [2/120]	1.85 (0–10.69) [1/54]	1.51 (0–8.88) [1/66]	10 (5.68–16.8) [12/120]	11 (4.83–22.55) [6/54]	9.09 (3.9–18.77) [6/66]
Centurio senex	1	1	0	0	0	0	0	0	0	0	0	0
Choeroniscus godmani	9	7	2	0	0	0	11.11 (0–45.67) [1/9]	14.28 (0.53–53.34) [1/7]	0	0	0	0
Dermanura tolteca	19	12	7	0	0	0	0	0	0	0	0	0
Glossophaga mutica	15	10	5	6.66 (0–31.84) [1/15]	0	20 (2.03–64.03) [1/5]	0	0	0	0	0	0
Micronycteris microtis	1	0	1	0	0	0	0	0	0	100 (22.35–100) [1/1]	0	100 (22.35–100) [1/1]
Platyrrhinus helleri	1	0	1	0	0	0	0	0	0	0	0	0
Sturnira hondurensis	31	10	21	0	0	0	0	0	0	0	0	0
Sturnira parvidens	13	8	5	0	0	0	0	0	0	0	0	0
	285	149	136	2.10 (0.85–4.63) [6/285]	2.01 (0.42–6.01) [3/149]	2.20 (0.46–6.57) [3/136]	1.05 (0.21–3.19) [3/285]	1.34 (0.05–5.07) [2/149]	0.73 (0–4.45) [1/136]	4.56 (2.61–7.71) [13/285]	4.02 (1.66–8.69) [6/149]	5.14 (2.32–10.42) [7/136]
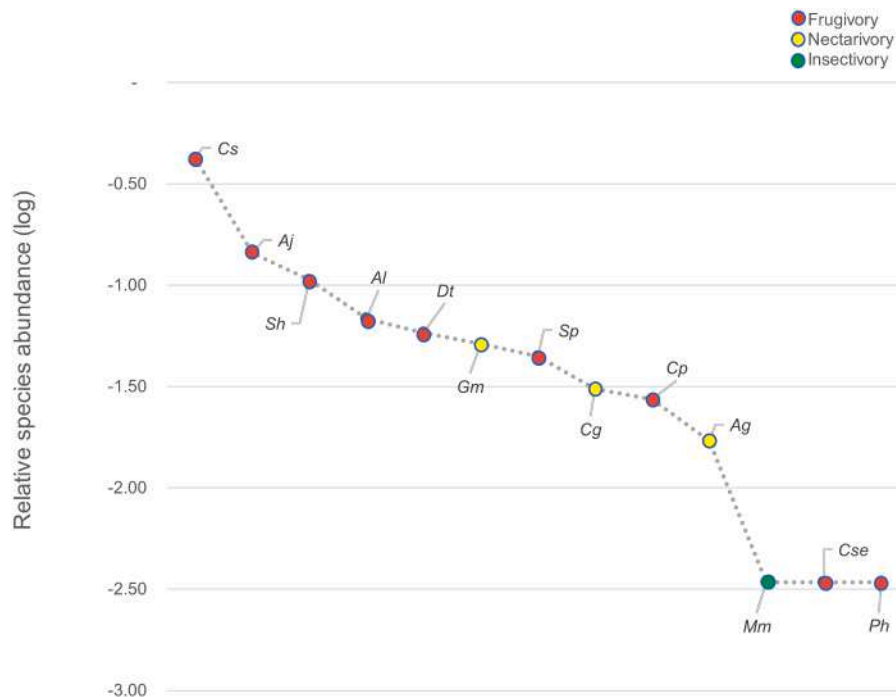
Fig. 2. Rank-abundance curve from bat species captured in this study. Colors represent the trophic guild of each species. Ag, *Anoura geoffroyi*; Aj, *Artibeus jamaicensis;* Al, *Artibeus lituratus*; Cp, *Carollia perspicilata*; Cs, *Carollia sowelli*; Cse, *Centurio senex*; Cg, *Choeroniscus godmani*; Dt, *Dermanura tolteca*; Gm, *Glossophaga mutica*; Mm, *Micronycteris microtis*; Ph, *Platyrrhinus helleri*; Sh, *Sturnira hondurensis*; Sp, *Sturnira parvidens*.

(Hodo et al., 2016). Another study carried out in Japan reported a prevalence of *T. dionisii* infection of 2.1 % (2/94) in *Miniopterus fuliginosus* (Mafie et al., 2018). Higher prevalence, ranging from 9.6 to 20 %, have also been reported in bats from the United Kingdom, Czech Republic, China, Brazil, and Australia (Cavazzana et al., 2010; Hamilton et al., 2012; Dos Santos et al., 2018; Bento et al., 2018; Wang et al., 2019; Austen et al., 2020).

Until today, the only potential group of biological vectors for *T. dionisii* are bat bugs of the family Cimicidae, a group of blood-sucking insects (vectorial transmission) with a worldwide distribution and approximately 110 known species, most of which are biologically and ecologically associated with bats (Ossa et al., 2019; Najera-Cortazar et al., 2023). However, knowledge of the transmission of *T. dionisii* by cimicids is limited (Bolaños-García et al., 2018). Regarding the study area, there are no reports of Cimicidae, which highlights our limited knowledge about these ectoparasites as well as the urgency of carrying out studies to determine if these insects are involved in the transmission cycle of *T. dionisii* in the region.

In the last 10 years, the concept of molecular operational taxonomic unit (MOTU) has been established for the delimitation of new lineages of the genus *Trypanosoma*, which has a genetic divergence ≥ 0.01 with previously recorded sequences of the 18S-rDNA gene (Cottontail et al., 2014; Rodrigues et al., 2019; Alves et al., 2021; 2023). This alternative classification arises because conventional methods for the study of this group of parasites such as blood culture have been unsuccessful and it may be challenging to study and isolate in the laboratory (Alves et al., 2023). Additionally, the increasing use of molecular tools in the surveillance and systematic study of trypanosomes in vertebrates in Brazil, Colombia, and Panama (mainly bats and marsupials) has allowed us to increase the bank of available sequences for this ribosomal gene (Cottontail et al., 2014; Lima et al., 2015; Rodrigues et al., 2019; Alves et al., 2021; 2023). Until today the mention of at least five potential new species, *Trypanosoma* sp. NeoBat 1–5, being discovered in various bat species within the family Phyllostomidae, with prevalence rates ranging between 10 – 20 % in the populations studied, underscores the diversity and complexity of *Trypanosoma* parasites in bat populations, particularly

those of the genus *Carollia* (Rodrigues et al., 2019; Alves et al., 2021; 2023).

In the present study we detected the presence of *Trypanosoma* sp. Neobat 4, a member of the *Trypanosoma wauwau* complex which was initially considered a specialized parasite with high host-parasite specificity within the genus *Carollia* (particularly with *Carollia perspicillata,* in which it was originally described) (Rodrigues et al., 2019). *Trypanosoma* sp. Neobat 4 is expanding its host range within the bat species in Brazil. This expansion of host species from *C. perspicillata* to other bat species, including *Anoura caudifer, Glossophaga soricina, Platyrrhinus recifinus, C. sowelli*, and *C. godmani*, suggests that this *Trypanosoma* species is more versatile and adaptable in terms of host species than previously believed (Alves et al., 2021; 2023). In the present work, the geographical distribution of *Trypanosoma* sp. Neobat 4 is expanded outside of Brazil and represents the most northern record of this taxon for the Neotropical region.

Because the members of the *T. wauwau* complex represent recently described species or taxa, a possible transmitting arthropod species has not been incriminated yet (da Costa et al., 2016). However, the high prevalence of infected bats suggests that this parasite could be transmitted by hematophagous ectoparasites that are commonly found in chiropterans, such as streblids, hypoboscids, and/or soft ticks (Lima et al., 2013; Orta-Pineda et al., 2020). It is important to note that the possibility of transmission by kissing bugs has been ruled out, as laboratory experiments have shown that *T. wauwau* parasites are destroyed in the haemolymph of these arthropods (Lima et al., 2013). Further research and studies will be necessary to explore the biology, behavior, ecological role, and any potential impact of Neobat 4 on bat communities in the neotropical region.

Finally, based on the 18S-rDNA criteria delimitation of Rodrigues et al. (2019) and Alves et al. (2021; 2023), the MOTU detected in *C. sowelli* and *M. microtis* has a divergence of more than 0.02 with other sequences of the genus *Trypanosoma* deposited in GenBank, which is why we propose that it be temporarily named *Trypanosoma* sp. NeoBat 6 following the continuity of the MOTUS described in bats from the neotropical region. Thus, discovery of a putative species, is significant,
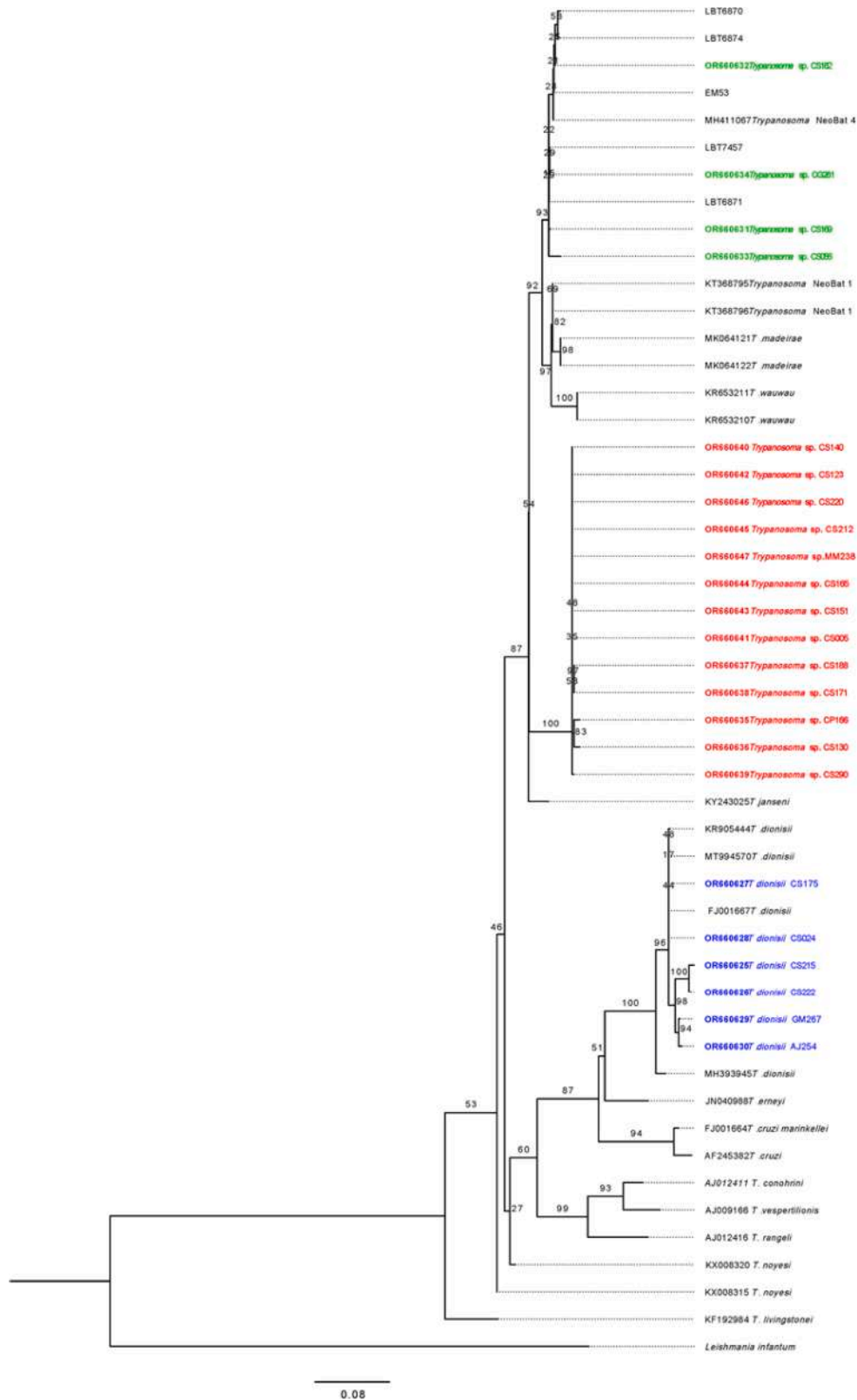
Fig. 3. Maximum likelihood phylogenetic tree of *Trypanosoma* sequences using the 18S SSU rDNA gene detected in bats captured in Tequecholapa Environmental Management Unit, Veracruz state, Mexico. Tree nodes represent bootstrap values. Accession numbers of sequences retrieved from Genbank are shown on tip labels. The scale bar indicates nucleotide substitution per site. Green group corresponds to *Trypanosoma* sp. NeoBat 4, red group to putative *Trypanosoma* sp. NeoBat 6, and blue group to *Trypanosoma dionisii*.
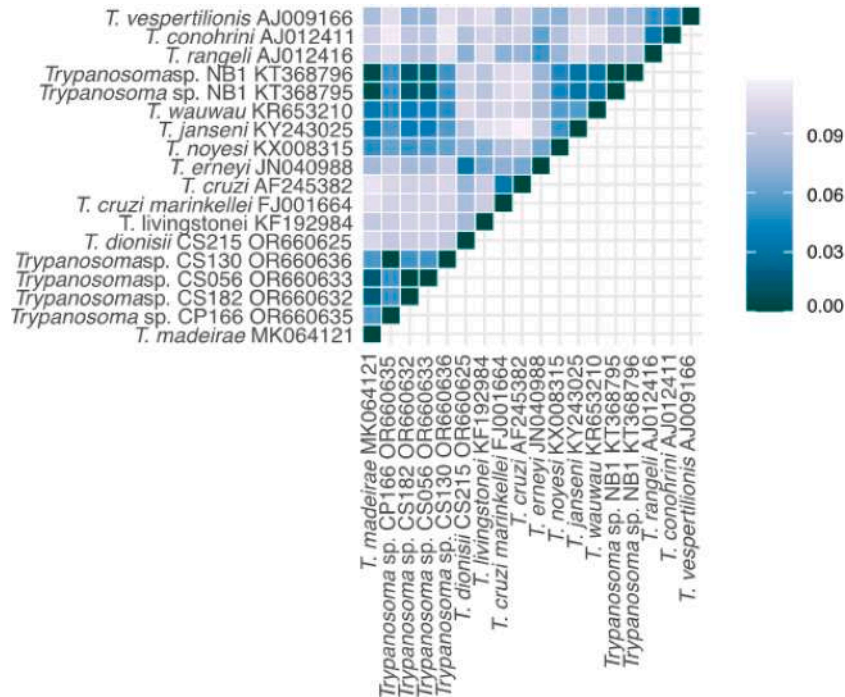
Fig. 4. Heatmap of pairwise genetic distances of the 18S region for *Trypanosoma*. Color gradient represent the differentiation percentage according to the scale bar.
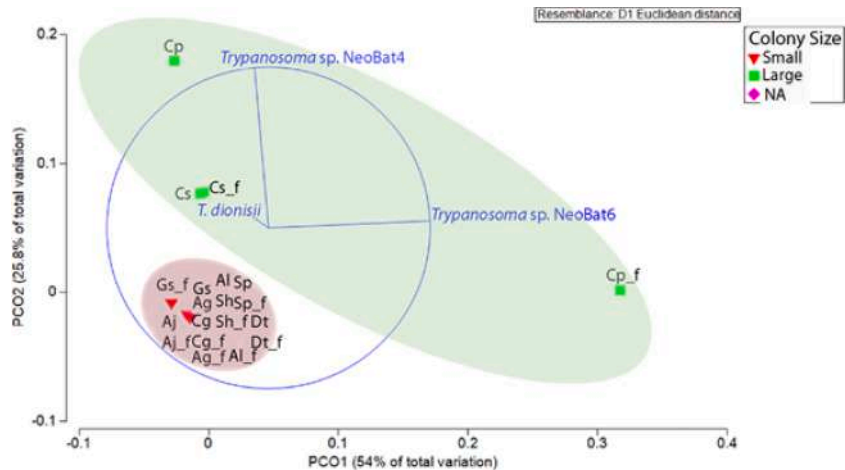

Fig. 5. Principal Coordinates Analysis (PCoA) analysis based on Euclidean distance of prevalence of *Trypanosoma* species between colony sizes of bat species. Colored ellipses correspond to colony sizes and delimit the 95 % confidence intervals for each trait. Ag, *Anoura geoffroyi*; Aj, *Artibeus jamaicensis*; Al, *Artibeus lituratus*; Cp, *Carollia perspicilata*; Cs, *Carollia sowelli*; Cse, *Centurio senex*; Cg, *Choeroniscus godmani*; Dt, *Dermanura tolteca*; Gs, *Glossophaga mutica*; Ph, *Platyrrhinus helleri*; Sh, *Sturnira hondurensis*; Sp, *Sturnira parvidens*.

and adds to the growing body of knowledge regarding *Trypanosoma* species in bat populations, particularly in the family Phyllostomidae (Alves et al., 2021; 2023). However, the use of other molecular markers such as GAPDH is a priority, as well as the isolation attempt to identify the morphology of the parasite, will allow clarifying the identity and validity of this MOTU at the species level.

Across bats in this area, we observed that the trypanosomatid community is primarily influenced by prevalence of *C. perspicillata* and *C. sowelli*. These species are known for their resilience, characterized by behaviors such as living in large colonies, utilizing various roost types including caves, tree holes, and tunnels, and having a diverse diet that includes fruits and insects (Bernard et al., 2001). Specifically, in the case of *C. sowelli*, its behavior traits and abundance observed in this study could account for the diversity of *Trypanosoma* species associated with this bat species. All three *Trypanosoma* species were found in *C. sowelli*

individuals. Consequently, this species may play a key role in the transmission cycle of *Trypanosoma*, as has been reported for other mammalian hosts of *Trypanosoma* (Oda et al., 2014).

In contrast to previous findings by Cotê and Poulinb (1995), who reported a negative correlation between intensity of infection in vector-borne parasites and host density, our study revealed that larger colony sizes increased the probability of *Trypanosoma* infection. This relation could be translated into more contacts between the vector and the host, and thus, higher probabilities of becoming infected (Oda et al., 2014).

Although, some ecological insights seem to favor the presence of *Trypanosoma* in bats, future studies should investigate how species density and *Trypanosoma* infection patterns may vary across larger geographical regions and within more diverse bat communities. On the other hand, the substantial diversity of *Trypanosoma* species associated

with neotropical bats from central Veracruz, as reported in this study, underscores the necessity for further research aimed at creating inventories of parasites within this highly diverse group of hosts. Additionally, such research could help elucidate the potential effect of these parasites on bat populations, shed light on their life cycles, and confirm the role of host density as a crucial ecological factor influencing *Trypanosoma* infection among bats species. These endeavors will serve as valuable starting points for future parasitological and ecological investigations into trypanosomatid infections.

In conclusion, we highlight the importance of the community of phyllostomid bats associated with a humid evergreen forest in the Mexican neotropics, specifically in central Veracruz, as hosts of three *Trypanosoma* species. We emphasize the first record of *T. dionisii, Trypanosoma* sp. NeoBat 4, and the putative *Trypanosoma* sp. NeoBat 6 species in Mexican bats, which expands the distribution of these trypanosomatid species to central Mexico. Although there are still studies to be carried out, it seems that the ecological characteristics of the colony size can positively impact the presence of *Trypanosoma* in bat colonies.

Ethical approval

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. This study was approved by the Bioethics Committee of the Facultad de Ciencias Biológicas y Agropecuarias, Campus Poza Rica-Tuxpan of the Universidad Veracruzana (UV) (Animals were handled according to National Legislation and Ethics (NOM-012-ZOO-1993).

CRediT authorship contribution statement

Javier Juárez-Gabriel: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **Daniela Alegría-Sánchez:** Data curation, Investigation, Methodology, Writing – original draft. **Damaris Yáñez-Aguirre:** Data curation, Investigation, Methodology, Writing – original draft. **Estefania Grostieta:** Data curation, Investigation, Methodology, Writing – original draft. **Lucía Álvarez-Castillo:** Data curation, Investigation, Methodology, Writing – original draft. **Marco Torres-Castro:** Investigation, Supervision, Writing – original draft, Writing – review & editing. **Nidia Aréchiga-Ceballos:** Investigation, Supervision, Writing – original draft, Writing – review & editing. **David A. Moo-Llanes:** Investigation, Supervision, Writing – original draft, Writing – review & editing. **Fernanda Moreira Alves:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **Carlos D. Pérez-Brígido:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **Gabriela Aguilar-Tipacamú:** Investigation, Supervision, Writing – original draft, Writing – review & editing. **Carlos A. López González:** Supervision, Writing – original draft, Writing – review & editing. **Ingeborg Becker:** Investigation, Supervision, Writing – original draft, Writing – review & editing. **Juan M. Pech-Canché:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing. **Pablo Colunga-Salas:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing. **Sokani Sánchez-Montes:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2023.107113.

References

Alves, F.M., Rangel, D.A., Vilar, E.M., Pavan, M.G., Moratelli, R., Roque, A.L.R., Jansen, A.M., 2021. *Trypanosoma* spp. Neobats: insights about those poorly known trypanosomatids. Int. J. Parasitol. Parasites. Wildl. 16, 145–152. https://doi.org/10.1016/j.ijppaw.2021.09.003.

Alves, F.M., Lisboa, C.V., Dario, M.A., Novaes, R.L.M., Tiepolo, L.M., Moratelli, R., Jansen, A.M., 2023. Old methods, new insights: reviewing concepts on the ecology of trypanosomatids and *Bodo* sp. by improving conventional diagnostic tools. Pathogens 12, 71. https://doi.org/10.3390/pathogens12010071.

Austen, J.M., Van Kampen, E., Egan, S.L., O'Dea, M.A., Jackson, B., Ryan, U.M., Irwin, P. J., Prada, D., 2020. First report of *Trypanosoma dionisii* (Trypanosomatidae) identified in Australia. Parasitol 147, 1801–1809. https://doi.org/10.1017/S0031182020001845.

Austen, J.M., Barbosa, A.D., 2021. Diversity and epidemiology of bat trypanosomes: a one health perspective. Pathogens 10, 1148. https://doi.org/10.3390/pathogens10091148.

Auty, H., Torr, S., Michoel, T., Jayaraman, P., Morrison, L., 2015. Cattle trypanosomosis: the diversity of trypanosomes and implications for disease epidemiology and control. Rev. Sci. Tech. 34, 587–598. https://doi.org/10.20506/rst.34.2.2382.

Barros, J.H.S., Lima, L., Schubach, A.O., Teixeira, M.M.G., 2019. *Trypanosoma madeira* sp. n.: a species of the clade *T. cruzi* associated with the neotropical common vampire bat *Desmodus rotundus*. Int. J. Parasitol. Parasites. Wildl. 8, 71–81. https://doi.org/10.1016/j.ijppaw.2018.12.009.

Barros, J.H.S., Roque, A.L.R., Xavier, S.C.D.C., Nascimento, K.C.S., Toma, H.K., Madeira, M.F., 2020. Biological and genetic heterogeneity in *Trypanosoma dionisii* isolates from hematophagous and insectivorous bats. Pathogens 9, 736. https://doi.org/10.3390/pathogens9090736.

Bento, E.C., Gómez-Hernández, C., Batista, L.R., Anversa, L., Pedrosa, A.L., Lages-Silva, E., Ramírez, J.D., Ramirez, L.E., 2018. Identification of bat trypanosomes from Minas Gerais state, Brazil, based on 18S rDNA and Cathepsin-L-like targets. Parasitol. Res. 117, 737–746. https://doi.org/10.1007/s00436-017-5744-z.

Becker, D.J., Speer, K.A., Brown, A.M., Fenton, M.B., Washburne, A.D., Altizer, S., Streicker, D.G., Plowright, R.K., Chizhikov, V.E., Simmons, N.B., Volokhov, D.V., 2020. Ecological and evolutionary drivers of haemoplasma infection and bacterial genotype sharing in a Neotropical bat community. Mol. Ecol. 29, 1534–1549. https://doi.org/10.1111/mec.15422.

Becker, I., Miranda-Ortiz, H., Fernández-Figueroa, E.A., Sánchez-Montes, S., Colunga-Salas, P., Grostieta, E., Juárez-Gabriel, J., Lozano-Sardaneta, Y.N., Arce-Fonseca, M., Rodríguez-Morales, O., Meneses-Ruíz, G., Pastén-Sánchez, S., López Martínez, I., González-Guzmán, S., Paredes-Cervantes, V., Moreira, O.C., Finamore-Araujo, P., Canseco-Méndez, J.C., Coquis-Navarrete, U., Rengifo-Correa, L., Alfaro-Cortés, M. M., Falcón-Lezama, J.A., Tapia-Conyer, R., Stephens, C.R., 2023. The low variability of Tc24 in *Trypanosoma cruzi* TcI as an advantage for chagas disease prophylaxis and diagnosis in Mexico. Pathogens 12, 368. https://doi.org/10.3390/pathogens12030368.

Bernard, E., 2001. Vertical stratification of bat communities in primary forests of Central Amazon. Brasil. J. Trop. Ecol. 17, 115–126. https://doi.org/10.1017/S0266467401001079.

Bolaños-García, R., Rodríguez-Estrella, R., Guzmán-Cornejo, C., 2018. Ectoparásitos asociados a polluelos del Búho Cornudo (Aves: strigidae) en un paisaje fragmentado de la península de Baja California, México. Acta Zool. Mex. 34, 1–15. https://doi.org/10.21829/azm.2018.3412142.

Brown, L., Cai, T.T., Das-Gupta, A., 2001. Interval estimation for a binomial proportion. Stat. Sci. 16, 101–133. https://doi.org/10.1214/ss/1009213286.

Borghesan, T.C., Ferreira, R.C., Takata, C.A., Campaner, M., Borda, C.C., Paiva, F., Milder, R., Teixeira, M.M.G., Camargo, P., 2013. Molecular phylogenetic redefinition of *Herpetomonas* (kinetoplastea, trypanosomatidae), a genus of insect parasites associated with flies. Protist 164, 129–152. https://doi.org/10.1016/j.protis.2012.06.001.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al., revisited. J. Parasitol. 83, 575–583.

Coates, R., Ramírez-Lucho, I., González-Christen, A., 2017. Una lista actualizada de los murciélagos de la región de Los Tuxtlas. Veracruz. Rev. Mex. Biodiv. 2, 349–357. https://doi.org/10.1016/j.rmb.2017.03.015.

Cavazzana Jr., M., Marcili, A., Lima, L., da Silva, F.M., Junqueira, A.C., Veludo, H.H., Viola, L.B., Campaner, M., Nunes, V.L., Paiva, F., Coura, J.R., Camargo, E.P., Teixeira, M.M., 2010. Phylogeographical, ecological and biological patterns shown by nuclear (ssrRNA and gGAPDH) and mitochondrial (*Cyt b*) genes of trypanosomes of the subgenus *Schizotrypanum* parasitic in Brazilian bats. Int J Parasitol 40, 345–355. https://doi.org/10.1016/j.ijpara.2009.08.015. Mar 1.

Ceballos, G., 2014. Mammals of Mexico. Johns Hopkins University Press, Baltimore.

Cotê, I.M., Poulinb, R., 1995. Parasitism and group size in social animals: a meta-analysis. Behav. Ecol. 6, 159–165. https://doi.org/10.1093/beheco/6.2.159.

Cottontail, V.M., Kalko, E.K., Cottontail, I., Wellinghausen, N., Tschapka, M., Perkins, S. L., Pinto, C.M., 2014. High local diversity of *Trypanosoma* in a common bat species, and implications for the biogeography and taxonomy of the *T. cruzi* clade. PLoS ONE 9, e108603. https://doi.org/10.1371/journal.pone.0108603.

da Costa, A., Nunes, P., Santos-Leite, B., Ferreira, S., Tonhosolo, R., Ruckert, A., da Rosa, P., da Rocha, C., Solange, M., 2016. Diversity of bats trypanosomes in hydroeletric area of Belo Monte in Brazilian Amazonia. Acta Trop 164, 185–193. https://doi.org/10.1016/j.actatropica.2016.08.033.

Dario, M.A., Lisboa, C.V., Costa, L.M., Moratelli, R., Nascimento, M.P., Costa, L.P., Leite, Y.L.R., Llewellyn, M.S., Xavier, S.C., Roque, A.L.R., Jansen, A.M., 2017. High *Trypanosoma* spp. diversity is maintained by bats and triatomines in Espírito Santo state, Brazil. PLoS One. 27, 12–22. https://doi.org/10.1371/journal.pone.0188412.

Dos Santos, F.C.B., Lisboa, C.V., Xavier, S.C.C., Dario, M.A., Verde, R.S., Calouro, A.M., Roque, A.L.R., Jansen, A.M., 2018. *Trypanosoma* sp. diversity in Amazonian bats (chiroptera; mammalia) from Acre State, Brazil. Parasitol. 145, 828–837. https://doi.org/10.1017/S0031182017001834.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic. Acid. Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340.

Eshar, D., Weinberg, M., 2010. Venipuncture in bats. Lab. Anim. (N.Y.) 39, 175–176.

Frick, W., Kingston, T., Leopardi, S., Medellín, R., Mendenhall, I.H., Racey, P., Shapiro, J. T., Vicente-Santos, A., Viquez-R, L., Worledge, L., 2021. IUCN SSC Bat Specialist Group (BSG) recommendations to reduce the risk of transmission of *SARS-CoV-2* from humans to bats by cavers. Living Document Version 2.0, pp. 1–10.

García-González, L.A., Rodrigo-Tapia, J.P., Sánchez Lazo, P., Ramos, S., Suárez-Nieto, C., 2004. DNA extraction using Chelex resin for oncogenic amplification analysis in head and neck tumours. Acta. Otorrinolaringol. Esp. 55, 139–144. https://doi.org/10.1016/s0001-6519(04)78497-6.

García, L., Ortiz, S., Osorio, G., Torrico, M.C., Torrico, F., Solari, A., 2012. Phylogenetic analysis of Bolivian bat trypanosomes of the subgenus *Schizotrypanum* based on cytochrome B sequence and minicircle analyses. PLoS ONE 7, e36578. https://doi.org/10.1371/journal.pone.0036578.

González-Salazar, C., Martínez-Meyer, E., López-Santiago, G., 2014. Clasificación jerárquica de gremios tróficos para aves y mamíferos de Norteamérica. Rev. Mex. Biodiv. 85, 931–941. https://doi.org/10.7550/rmb.38023.

Grostieta, E., Miranda-Caballero, C.I., Sánchez-Montes, S., Colunga-Salas, P., González, C.A.L., Valderas-Muñoz, K.D., Arciniega-Luna, G., Aguilar-Tipacamú, G., 2023. DNA barcoding and new records of *Ornithodoros yumatensis* from Central Mexico. Vet. Res. Commun. 47, 2339–2350. https://doi.org/10.1007/s11259-023-10164-4.

Gulas-Wroblewski, B., Kairis, R., Gorchakov, R., Wheless, A., Murray, K., 2021. Optimization of dna extraction from field-collected mammalian whole blood on filter paper for *Trypanosoma cruzi* (chagas disease) detection. Pathogens 10, 1040. https://doi.org/10.3390/pathogens10081040.

Hafner, M.S., Sudman, P.D., Villablanca, F.X., Spradling, T.A., Demastes, J.W., Nadler, S. A., 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. Science 265, 1087–1090. https://doi.org/10.1126/science.8066445.

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

Hoare, C.A., 1972. The Trypanosomes of Mammals. Blackwell Scientific Publishing, Oxford, England.

Hoang, T.D., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, S.L., 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol. Biol. Evol. 1 (35), 518–522. https://doi.org/10.1093/molbev/msx281.

Hodo, C.L., Goodwin, C.C., Mayes, B.C., Mariscal, J.A., Waldrup, K.A., Hamer, S.A., 2016. Trypanosome species, including *Tryanosoma cruzi,* in sylvatic and peridomestic bats of Texas, USA. Acta. Trop. 164, 259–266. https://doi.org/10.1016/j.actatropica.2016.09.013.

Izeta-Alberdi, A., Pech-May, A., Tun-Ku, E., Mazariegos-Hidalgo, C.J., López-Cancino, S. A., Gutiérrez, J., Albino-Miranda, S., De la Cruz-Felix, K., Diaz de la Cruz, J.M., Ibarra-Cerdeña, C.N., Arellano, E., Vallejo, R.M., Ocampo, M.A., Ramsey, J.M., 2023. *Trypanosoma cruzi* in Mexican Neotropical vectors and mammals: wildlife, livestock, pets, and human population. Salud Publica Mex 65, 114–126. https://doi.org/10.21149/13801.

Jaimes-Dueñez, J., Cantillo-Barraza, O., Triana-Chávez, O., Mejia-Jaramillo, A.M., 2020. Molecular surveillance reveals bats from eastern Colombia infected with *Trypanosoma theileri* and *Trypanosoma wauwau*-like parasites. Prev. Vet. Med. 184, 105159 https://doi.org/10.1016/j.prevetmed.2020.105159.

Kalyaanamoorthy, S., Minh, Q.B., Wong, K.F.T., von Haeseler, A., Jermiin, S.L., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Method. 14, 587–589. https://doi.org/10.1038/nmeth.4285.

Kaufer, A., John, E., Damien, S., Barrat, J., 2017. The evolution of trypanosomatid taxonomy. Parasit. Vect. 10, 287. https://doi.org/10.1186/s13071-017-2204-7.

Lima, L., Espinosa-Álvarez, O., Hamilton, P., Neves, L., Takata, C., Campaner, M., Attias, M., de Souza, W., Camargo, E., Teixeira, M., 2013. *Trypanosoma livingstonei*: a new species from African bats supports the bat seeding hypothesis for the *Trypanosoma cruzi* clade. Parasit. Vect. 6, 221. https://doi.org/10.1186/1756-3305-6-221.

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

Linhart, P., Bandouchova, H., Zukal, J., Votypka, J., Kokurewicz, T., Dundarova, H., Apoznanski, G., Heger, T., Kubickova, A., Nemcova, M., Piacek, V., Sedlackova, J., Seidlova, V., Berkova, H., Hanzal, V., Pikula, J., 2020. Trypanosomes in Eastern and Central European bats. Acta Vet. 89, 69–78. https://doi.org/10.2754/avb202089010069.

Loayza, A., Larrea-Alcázar, D., 2006. Disponibilidad de recurso y dieta de murciélagos frugívoros en la estación biológica Tunquini, Bolivia. Ecol. Boliv. 41, 7–23.

López-Cancino, S.A., Tun-Ku, E., De la Cruz-Felix, H.K., Ibarra-Cerdeña, C.N., Izeta-Alberdi, A., Pech-May, A., Mazariegos-Hidalgo, C.J., Valdez-Tah, A., Ramsey, J.M., 2015. Landscape ecology of *Trypanosoma cruzi* in the southern Yucatan Peninsula. Acta Trop. 151, 58–72. https://doi.org/10.1016/j.actatropica.2015.07.021.

Mafie, E., Rupa, F.H., Susuki, K., Maeda, K., Sato, H., 2018. First record of *Trypanosoma dionisii* of the *T. cruzi* clade from the Eastern bent-winged bat (*Miniopterus fuliginosus*) in the Far East. Parasitol. Res. 117, 673–680. https://doi.org/10.1007/s00436-017-5717-2.

Mazzotti, L., 1946. Hallazgo del *Trypanosoma vespertillionis* en murciélagos mexicanos. Rev. Soc. Mex. Hist. Nat. 7, 49–50.

Medellín, A., Arita, T., Sánchez, H., 2008. Identificación De Los Murciélagos de México. Clave de Campo. Instituto de Ecología. UNAM, Ciudad de México, Mexico.

Monzón, C., Mancebo, O., Jiménez, J., 2010. *Trypanosoma vivax* en Búfalos (*Bubalus bubalis*) en Formosa, Argentina. Rev. Vet. Arg. 27, 1–8.

Najera-Cortazar, L.A., Keen, A., Kitching, T., Stokes, D., Goodman, S.J., 2023. Phylogenetic analyses reveal bat communities in Northwestern Mexico harbor a high diversity of novel cryptic ectoparasite species. Ecol. Evol. 13, e9645. https://doi.org/10.1002/ece3.9645.

Novoa, S., Cadenillas, R., Pacheco, V., 2011. Dispersión de Semillas por Murciélagos frugívoros en Bosques del Parque Nacional Cerros de Amotape, Tumpes. Perú. Mast. Neotrop. 18, 81–93.

Nguyen, L.T., Schmidt, H.A., Haeseler, A.V., Minh, B.Q., 2015. IQ-Tree: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274. https://doi.org/10.1093/molbev/msu300.

Oda, E., Solari, A., Botto-Mahan, C., 2014. Effects of mammal host diversity and density on the infection level of *Trypanosoma cruzi* in sylvatic kissing bugs. Med. Vet. Entomol. 28, 384–390. https://doi.org/10.1111/mve.12064.

Ossa, G., Johnson, J.S., Puisto, A.I.E., Rinne, V., Sääksjärvi, I.E., Waag, A., Vesterinen, E. J., Lilley, T.M., 2019. The Klingon batbugs: morphological adaptations in the primitive bat bugs, *Bucimex chilensis* and *Primicimex cavernis*, including updated phylogeny of Cimicidae. Ecol. Evol. 9, 1736–1749. https://doi.org/10.1002/ece3.4846.

Orta-Pineda, G., Rodríguez-Valencia, V.M., Rico-Chávez, O., Zamora-Bárcenas, D.F., Rodríguez-Moreno, A., Montiel-Parra, G., Arenas-Montaño, M., Galindo-Maldonado, F.A., Suzán-Azpiri, G., Ojeda-Flores, R., 2020. Composición de comunidades y filoespecificidad de ectoparásitos de murciélagos en paisajes agropecuarios de Veracruz, México. Ecosis. Recur. Agropec. 7, e2059. https://doi.org/10.19136/era.a7nl.2059.

Parasuraman, S., Raveendran, R., Kesavan, R., 2017. Blood sample collection in small laboratory animals. J. Pharmacol. Pharmacother. 8, 153–154. https://doi.org/10.4103/0976-500X.72350.

Primer, E., 2020. Primer 7. Primer-E Ltd.

R Core Team, 2022. R: A language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramírez, J.D., Tapia-Calle, G., Muñoz-Cruz, G., Poveda, C., Rendón, L.M., Hincapié, E., Guhl, F., 2014. Trypanosome species in neo-tropical bats: biological, evolutionary and epidemiological implications. Infect. Genet. Evol. 22, 250–256. https://doi.org/10.1016/j.meegid.2013.06.022.

Rodrigues, M.S., Lima, L., Xavier, S.C.D.C., Herrera, H.M., Rocha, F.L., Roque, A.L.R., Teixeira, M.M.G., Jansen, A.M., 2019. Uncovering *Trypanosoma* spp. diversity of wild mammals by the use of DNA from blood clots. Int. J. Parasitol. Parasites. Wildl. 8, 171–181. https://doi.org/10.1016/j.ijppaw.2019.02.004.

Sgroi, G., Latta, R., Lia, P.R., Latrofa, S.M., Samarelli, R., Camarda, A., Otranto, D., 2021. *Trypanosoma* (*Megatrypanum*) *pestanai* in Eurasian badgers (*Meles meles*) and Ixodidae ticks, Italy. Parasitol. 148, 1516–1521. https://doi.org/10.1017/S0031182021001190.

Simmons, N.B., Cirranello, A.L., 2023. Bat Species of the World: a Taxonomic and Geographic Database. Version 1.4. Accessed on 10/11/2023.

Thompson, C., Godfrey, S., Thompson, A., 2014. Trypanosomes of Australian mammals: a review. Int. J. Parasitol. Parasites Wildl. 3, 57–66. https://doi.org/10.1016/j.ijppaw.2014.02.002.

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

Turmelle, A.S., Olival, K.J., 2009. Correlates of viral richness in bats (order Chiroptera). Ecohealth 6, 522–539.

Villegas-García, J.C., Santillán-Alarcón, S., 2001. Sylvatic focus of American Trypanosomiasis in the State of Morelos, Mexico. Rev. Biol. Trop. 49, 685–688.

Wang, L.J., Han, H.J., Zhao, M., Liu, J.W., Luo, L.M., Wen, H.L., Qin, X.R., Zhou, C.M., Qi, R., Yu, H., Yu, X.J., 2019. *Trypanosoma dionisii* in insectivorous bats from northern China. Acta Trop. 193, 124–128. https://doi.org/10.1016/j.actatropica.2019.02.028.

World Health Organization, 2015. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Wkly. Epidemiol. Rec 90, 33–43.