RESEARCH ARTICLE

ZooKeys

A peer-reviewed open-access journal

Launched to accelerate biodiversity research

Mini-exon gene reveals circulation of TcI *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae) in bats and small mammals in an ecological reserve in southeastern Mexico

Eliza F. Gómez-Sánchez[1], Héctor Ochoa-Díaz-López[2],
Eduardo E. Espinoza-Medinilla[1], D. Daniel Velázquez-Ramírez[2],
Nancy G. Santos-Hernández[1], Christian Ruiz-Castillejos[1], Dolores G. Vidal-López[3],
Adriana Moreno-Rodríguez[4], Any Laura Flores-Villegas[5],
Eduardo López-Argueta[1], José A. De Fuentes-Vicente[1]

1 *Laboratorio de Investigación y Diagnóstico Molecular, Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas, Tuxtla Gutierrez, Mexico* **2** *Departamento de Salud, El Colegio de la Frontera Sur, Tuxtla Gutierrez, Chiapas, Mexico* **3** *Laboratorio Multidisciplinario Experimental y Bioterio, Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas, Tuxtla Gutierrez, Mexico* **4** *Laboratorio 16, Facultad de Ciencias Químicas, Universidad Autónoma Benito Juárez de Oaxaca, Oaxaca, Mexico* **5** *Laboratorio de Biología de Parásitos, Facultad de Medicina, Universidad Nacional Autónoma de Mexico, Ciudad de México, Mexico*

Corresponding authors: José A. De Fuentes-Vicente (jose.defuentes@unicach.mx),
Nancy G. Santos-Hernández (nancy.santos@unicach.mx)

Abstract

A wide variety of mammals are involved in the sylvatic cycle of *Trypanosoma cruzi*, the causative agent of Chagas disease. In many areas in Latin America where *T. cruzi* is endemic, this cycle is poorly known, and its main reservoirs have not been identified. In this study we analyzed *T. cruzi* infection in bats and other small mammals from an Ecological Reserve in southeastern Mexico. From January through March 2021, we captured wild individuals to extract cardiac and peripheral blood, and infection was detected by PCR of the mini-exon gene. In bats, the prevalence of infection was 16.36%, while in small mammals the prevalence was 28.57%. All of the samples that were positive for *T. cruzi* were identified as the

TCI genotype. Our findings suggest that this zone, situated at the periphery of urban zones might have epidemiological relevance in the sylvatic cycle of *T. cruzi* and needs to be monitored. The infection of bats in this area is particularly concerning since the flight pattern of this populations overlaps with human settlements. Despite being subject to conservation protections, there continue to be anthropogenic actions that disturb the study area, which could exacerbate risks to public health.

Keywords
Chagas disease, molecular epidemiology, reservoirs, sylvatic cycle

Introduction

The protozoan *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae) is the causative agent of Chagas disease, a neglected tropical infection affecting ~6 million people (Krats 2019). This disease typically occurs in rural areas of Central and South America, but urban areas are not exempt. In humans, chronic *T. cruzi* infection leads to heart failure and death in 20–30% of infected patients (WHO 2014; Krats 2019). Chagas disease is difficult to diagnosis, and only two drugs, Nifurtimox and Benznidazole, are currently available to treat it, both of which have severe side effects (Vallejo et al. 2020).

Trypanosoma cruzi exhibits high genetic variability and has recently been classified into six discrete typing units (DTUs; TCI–TCVI) and an additional unit named TC Bat (see Zingales et al. 2018). Under natural conditions, *T. cruzi* is transmitted by blood-sucking insects of the subfamily Triatominae (Hemiptera, Reduviidae) known as kissing bugs (De Fuentes-Vicente and Gutiérrez-Cabrera 2020). *Trypanosoma cruzi* transmission cycles are well defined into domestic, peridomestic, and sylvatic cycles, each with epidemiological and ecological differences. The domestic and peridomestic cycles involve humans, pets (dogs and cats), and farmyard animals. In sylvatic cycles in wild habitats, marsupials, edentates, and rodents are important reservoirs, but *T. cruzi* can infect more than 100 different species of wild mammals (Noireau et al. 2009; Alvarado-Otegui et al. 2012). This heterogeneity suggests a highly variable ecology of *T. cruzi*, and each area may have a unique set of conditions underlying the occurrence of the parasite (Moreira-Alves et al. 2016).

Historically, the domestic and peridomestic cycles have been the most studied, and little is known about the sylvatic cycle, especially in the southeastern region of Mexico (e.g., Jimenez-Coello et al. 2012). The climatic and biodiversity conditions of this region, in addition to poverty and marginalization, create scenarios for increased occurrence of Chagas disease (see Cruz-Reyes and Pickering-López 2006). In the current study, we sought to determine the infection by *T. cruzi* in wild mammals from the "El Zapotal" Ecological Reserve using multiplex PCR amplification of the mini-exon gene. "El Zapotal" is located in the state of Chiapas in southeastern Mexico, and we believe that this area may have epidemiological importance in the sylvatic cycle of *T. cruzi* in the region and that the proximity of human settlements may make it relevant to public health. In fact, the circulation of *T. cruzi* in small mammals in this area has previously been reported (Domínguez-Vázquez et al. 1990; Solís-Franco et al. 1997; Camacho-

Sierra 2016). In addition, this area has high bat species richness, including synanthropic species (Velazquez-Pérez et al. 2010; López-Argueta 2021). Although bats have played a key role in the evolution of *T. cruzi* (Hamilton et al. 2012), their importance in the transmission dynamics has been poorly studied in many regions.

Although "El Zapotal" is subject to conservation protections, anthropogenic actions may have already caused irreversible damage (Fernández-Moreno 2010). Large-scale changes in land use and habitat fragmentation can affect wild transmission cycles of *T. cruzi* (Vaz et al. 2007), mostly because habitat loss restricts the area and food resources available to wild mammals, which can increase their contact with humans. All these factors support the need to conduct new studies to better understand the dynamics of *T. cruzi* transmission in wild ecotopes.

Methods

Study site

The "El Zapotal" Ecological Reserve, decreed as an Ecological and Recreational Park, is located 2 km southeast of Tuxtla Gutiérrez, Chiapas (Fig. 1). It is a natural protected area measuring approximately 200 ha. The geology is largely karstic,
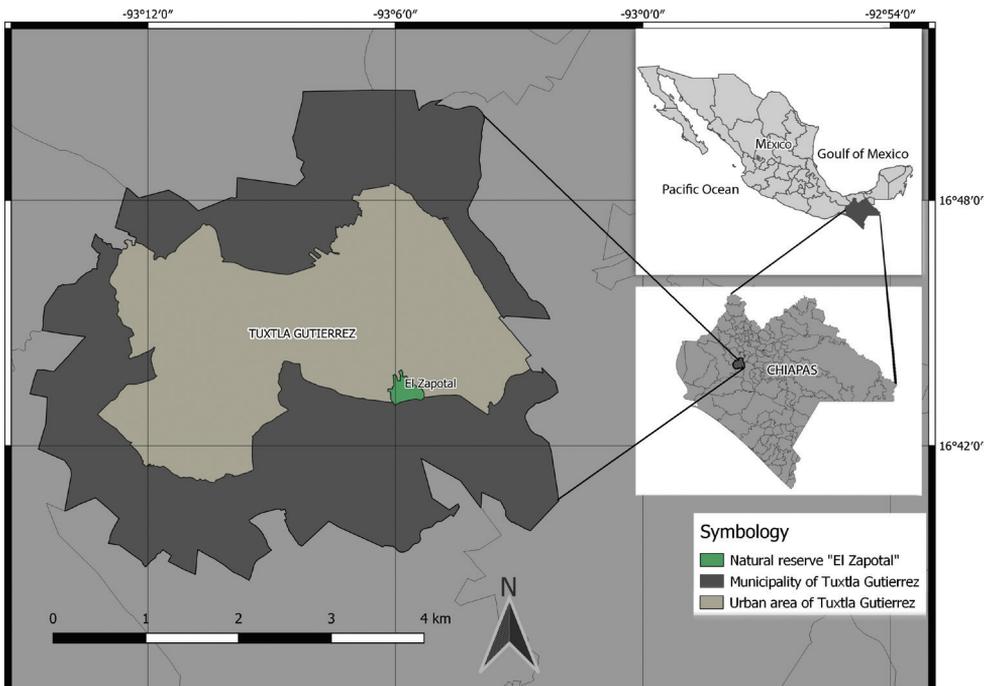

Figure 1. Location of the "El Zapotal" Ecological Reserve in southeastern Mexico, note the border of the reserve with the urban area.

with abundant caves and springs. The altitudinal range is from 600 to 850 m above sea level and the vegetation is medium sub-evergreen forest and low deciduous forest.

Mammal capture and blood sampling

Wild mammals were captured from January through March 2021 in areas of the "El Zapotal" Ecological Reserve near bodies of water and fruit trees. To capture bats, we deployed three 12 × 2.5 m mist nets from dusk to dawn (eight sampling hours per net) for five consecutive nights. The captured specimens were deposited in canvas bags for identification and blood sampling. The identification was performed as described by Díaz (2021). Meanwhile, the blood sample was obtained by intracardiac puncture (100 μL) and deposited in microcentrifuge tubes with 500 μL 3.8% sodium citrate pH 7.2 for their transportation to the laboratory. Finally, the bats were marked on the wings with ink and released on site.

For the capture of smalls mammals 20 Tomahawk type traps and 15 Sherman traps were used (Romero-Almaraz et al. 2000). As bait, a mixture of oats with vanilla extract was used. The traps were set at dusk and removed eight hours later for five nights. Captured individuals were marked and identified as described by Reid (2009), and a blood sample was taken by puncture in the tail vein, after disinfecting the area with 70% alcohol. The samples obtained (100 μL) were treated as mentioned above.

Bioethical guidelines

Animal handling was carried out in accordance with the provisions of Mexican Animal Welfare Law. The capture of animals was approved by the Mexican Secretariat of the Environment and Natural Resources (Secretaría de Medio Ambiente y Recursos Naturales, SEMARNAT (minute 07 / K6-0095 / 10/189)). No individuals were sacrificed or removed from the site.

Extraction of DNA and mini-exon gene amplification

Total DNA was extracted using a modified phenol-chloroform isoamyl alcohol protocol (Espinoza and García 2003). For the amplification of the mini-exon gene, we used a pool of three oligonucleotides reported by Souto et al. (1996): [5'-GTGTCCGC-CACCTCCTTCGGGCC (TCI, group 1-specific), 5'-CCTGCAGGCACACGT-GTGTGTG (TCII, group 2-specific), and 5'-CCCCCCTCCCAGGCCAC ACTG (TC, common to groups TCI and TCI)]. We used the previously characterized strains Querétaro (TCI) and strain Y (TCII), which amplify at 350 and 300 base pairs (bp), respectively, as controls (Espinoza et al. 2010). Amplification reactions were performed in a final volume of 25 μL, containing 12 μL of Go Taq Green Master Mix 2X, 10 μL of nuclease-free water, 0.4 μM of each primer, and 20 ng of Trypanosoma DNA. Cycle amplification was performed using a MyGene MG96G thermal cycler (Hangzhou

LongGene Scientific Instruments Co. Ltd, Hangzhou, China) under the following conditions: 5 min at 94 °C, followed by 27 cycles of 40 s at 94 °C, 40 s at 61 °C, and 1 min at 72 °C, and a final elongation of 5 min at 72 °C. Amplified products were visualized on 2% W/V agarose gels stained with ethidium bromide under UV light.

Results

A total of 152 mammals were captured: 110 bats and 42 small mammals. Among bats, eight species were identified, and *Artibeus jamaicensis* Leach, 1821 (Chiroptera, Phyllostomidae) was the most common species. Only two hematophagous individuals (*Desmodus rotundus* É. Geoffroy, 1810) (Phyllostomidae) were captured. We captured four species of small mammals, of which *Didelphis marsupialis* Linnaeus, 1758 (Didelphimorphia, Didelphidae) was the most common (Table 1).

Table 1. Bats and smalls mammals captured in "El Zapotal" ecological reserve and infected individuals.

Bats			
Family	**Species**	**# individuals**	**Infected indivuals (% prevalence)**
Phyllostomidae	*Artibeus jamaicensis*	64	10 (15.6)
	Artibeus lituratus	16	3 (18.7)
	Sturnira lillium	3	2 (66.6)
	Centurio senex	2	0
	Leptonycteris yebabuenae	2	0
	Carollia perspicillata	7	2 (28.5)
	Desmodus rotundus	2	0
	Glossophaga soricina	8	1 (12.5)
	Pteronotus davyi	1	0
Mormoopidae	*Pteronotus parnelli*	3	0
	Mormoops megallophyla	2	0
Total		**110**	**18 (16.3)**
Small mammals			
Didelphidae	*Didelphis marsupialis*	18	6 (33.3)
Cricetidae	*Peromyscus mexicanus*	7	4 (57.1)
Heteromyidae	*Heteromys desmarestianus*	10	1 (10)
Dasyproctidae	*Dasyprocta mexicana*	7	1 (14.2)
Total		**42**	**12 (28.5)**

Of the total bat samples examined, 18 were positive for *T. cruzi* infection (16.36%). *Sturnira lilium* É. Geoffroy, 1810 had the highest prevalence among the bat species (66.6%), though only three individuals were captured. Meanwhile, the most commonly captured bat species, *A. jamaicensis*, had a prevalence of 15.62% (10/64) (Table 1). All PCR products amplified at 350 bp, indicating that they belonged to the TCI group (Fig. 2).

For smalls mammals there was an overall prevalence of 28.57% (12/42), when combining all four mammal species. *Peromyscus mexicanus* (Saussure, 1860) (Rodentia,
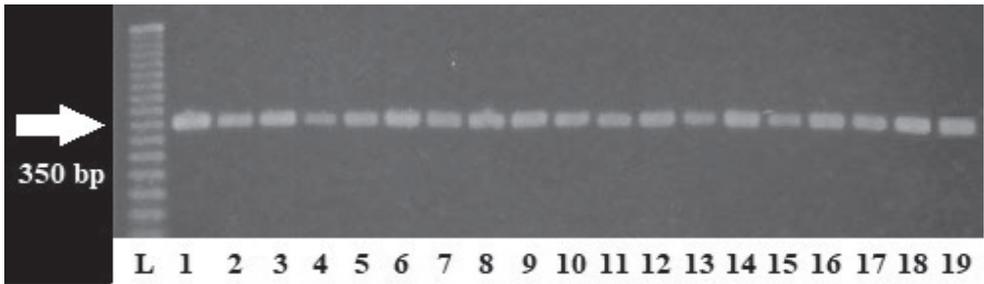
Figure 2. PCR products of the mini-exon gene in blood of bats from the "El Zapotal" Ecological Reserve. Amplification resulted in a PCR product of 350 bp and this confirms that these parasites belong to the TCI group. L: Ladder; Samples: 1 positive control (Qro. strain); 2–11 *A. jamaicensis*; 12–14 *A. lituratus*; 15–16 *C. perspicillata*; 17–18 *S. lilium*; 19 *G. soricina*.
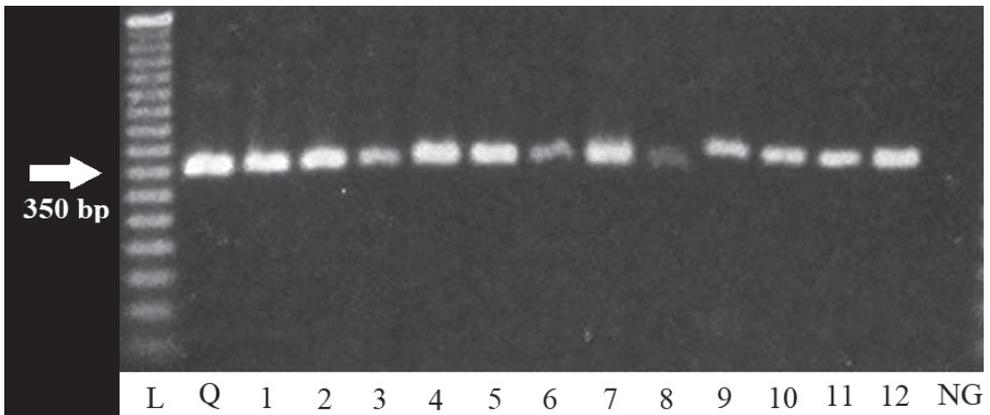

Figure 3. PCR products of the mini-exon gene in blood of smalls mammals from the "El Zapotal" Ecological Reserve. Amplification resulted in a PCR product of 350 bp and this confirms that these parasites belong to the TCI group. L: ladder; samples: Q positive control (Qro. strain); 1–6 *D. marsupialis*; 7–10 *P. mexicanus*; 11 *H. desmarestianus*; 12 *D. mexicana*.

Cricetidae) presented the highest prevalence with 57.14% (4/7), while the most commonly captured species, *D. marsupialis*, had a prevalence of 33.33% (6/18) (Table 1). Here too, all PCR products amplified at 350 bp indicating the TCI group of *T. cruzi* (Fig. 3).

Discussion

We show evidence of the circulation of *T. cruzi* in wild mammals from an ecological reserve in southeastern Mexico. Although other studies have demonstrated the presence of the parasite in small mammals from "El Zapotal" (Domínguez-Vázquez et al. 1990; Solís-Franco et al. 1997; Camacho-Sierra 2016), the present study is the first to report infection in bats. TCI was the only genetic group detected. This genetic group is

the most prevalent in Mexico (Bosseno et al. 2002; Dorn et al. 2017) and is associated with *Triatoma dimidiata* Latreille, 1811 (Hemiptera, Triatominae), the main *T. cruzi* vector in Central and North America (López-Cancino et al. 2015). Some *T. cruzi* genotypes have close evolutionary relationships with specific triatomine species, possibly favoring parasite transmission (De Fuentes-Vicente et al. 2019).

Overall, the prevalence of *T. cruzi* infection was 19.73% in all captured individuals (30/152). The overall infection prevalence in small mammals (28.57%) was similar to previous findings in a recent study (26.66%) in the same area (Camacho-Sierra 2016) and higher than in bats. We found a higher prevalence of infection in *P. mexicanus* (Mexican mouse) than in *D. marsupialis* (common opossum). The fact that small rodents are an important food source for several predators could maintain the transmission of *T. cruzi* among mammals through predation. In addition, vertical or congenital transmission has been demonstrated in these animals (Alarcón et al. 2009). Other studies in southern Mexico have also reported high prevalence of *T. cruzi* circulation in terrestrial mammals (e.g., Ruiz-Piña and Cruz-Reyes 2002; Martínez-Hernández et al. 2014), including in livestock (sheep, pigs, and horses) and urban and rural dogs in Yucatán (Jiménez-Coello et al. 2008; Ruiz-Piña et al. 2018).

To date, it is largely unknown how the sylvatic cycle interacts with the peridomestic and domestic cycles, but it is inferred that some synanthropic animals may be the link between them. For example, some synanthropic rodents captured in Yucatán have shown histological lesions associated with *T. cruzi* infection (Torres-Castro et al. 2016; Ucan-Euan et al. 2019). "El Zapotal" is surrounded by urbanized human settlements, and infected rodents might represent a public health risk due to their ability to invade and colonize human dwellings, where they could interact with domestic animals and parasite transmission could occur. For example, in the neighboring city of Tuxtla Gutiérrez, a prevalence of 4.5% of *T. cruzi* infection in stray dogs has been reported (Jiménez-Coello et al. 2010), and recently the first report of an infected triatomine bug in the urban area was published (De Fuentes-Vicente et al. 2020).

In Mexico, the dynamics of *T. cruzi* in bats in the sylvatic cycle has been little studied, even though bats have wide distributions that may overlap with urbanized environments (Krauel and Lee Buhn 2016), as occurs in the populations analyzed here (López-Argueta 2022). The synanthropic condition of bats has made them the transmitters of several pathogens including Ebola virus, rabies, and hantaviruses (Calisher et al. 2006; Kasso and Balakrishnan 2013). Currently, they are the focus of increased attention because of their possible relationship with the origin of the novel SARS-COV-2 coronavirus that causes COVID-19 (Lau et al. 2020; Córdoba-Aguilar et al. 2021). In particular, bats play a role of interest in the evolution of *T. cruzi* because, according to some hypotheses, *T. cruzi* evolved from a larger clade of bat trypanosomes (Hamilton et al. 2012). The importance of bats as reservoirs of *T. cruzi* may be enhanced by their ability to fly, gregarious social structure, and longevity (Luis et al. 2013). In bats, we found a prevalence of infection of 16.36%, a value much higher than that found in the only previous study in Chiapas, which sampled bats from the Selva Lacandona (1.60%) (Víquez-Rodríguez 2015). Interestingly, they reported a higher prevalence of

infection by *Leishmania mexicana* in the same individuals (8.84%) and only one bat infected by both (Víquez-Rodríguez 2015). Although bats are known to be associated with a wide range of zoonotic pathogens, the effects of competition between parasites in the same reservoir remains virtually unknown (Bashey 2015).

High prevalence of *T. cruzi* infection in bats have been previously reported in southern Mexico: Torres-Castro et al. (2021) reported a 30.2% prevalence of infection in bats from Campeche and Yucatan, mostly in non-hematophagous species. We only collected two hematophagous individuals (*D. rotundus*), neither of which was infected. Non-hematophagous species may acquire the parasite by ingesting infected insects or by vector transmission, but we did not find any triatomine insects at the study sites. Vertical transmission of *T. cruzi* has also been demonstrated in bats (Añez et al. 2009), so this mechanism may also favor the permanence of the parasite in these animals. Another interesting fact in this group is that *T. cruzi* was detected in the salivary glands of a hematophagous bat specimen in Peru (Villena et al. 2018), suggesting that the importance of bats in the dynamics of *T. cruzi* may be greater than previously thought, since they may be able to transmit the parasite directly through biting.

Maintaining biodiversity has been shown to be an important – if not the most important – action to prevent the spread of zoonotic parasites (Córdoba-Aguilar et al. 2021), and *T. cruzi* is no exception (Keesing and Ostfeld 2021). As such, we must continue to explore how ecosystem fragmentation affects sylvatic transmission cycles of *T. cruzi*, a topic which is further complicated by heterogeneity in the reservoirs, vectors, and genetic structure of the parasites. Further studies of all of these topics are necessary in order to construct effective interventions that prevent the sylvatic cycle from connecting with the peridomestic or domestic cycle and further exposing humans. Finally, future research should emphasize the role of bats in the dynamics of *T. cruzi* to determine their role in the epidemiology of Chagas disease and to inform health authorities about this potential danger.

Acknowledgements

References

Alarcón M, Pérez MC, Villarreal J, Araujo S, Goncalves L, González A, Lugo-Yarbuh A (2009) Detección de ADN de *Trypanosoma cruzi* en la placenta y fetos de ratones con infección chagásica aguda. Investigación Clínica 50: 335–345.

Alvarado-Otegui JA, Ceballos LA, Orozco MM, Enriquez GF, Cardinal MV, Cura C, Schijman AG, Kitron U, Gurtler RE (2012) The sylvatic transmission cycle of *Trypanosoma cruzi* in a rural area in the humid Chaco of Argentina. Acta Tropica 124: 79–86. https://doi.org/10.1016/j.actatropica.2012.06.010

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

Bashey F (2015) Within-host competitive interactions as a mechanism for the maintenance of parasite diversity. Philosophical Transactions of the Royal Society B 370(1675): e20140301. https://doi.org/10.1098/rstb.2014.0301

Bosseno M, Barnabe C, Magallón E, Lozano F, Ramsey J, Espinoza B, Frédérique S (2002) Predominance of *Trypanosoma cruzi* Lineage I in México. Journal of Clinical Microbiology 40: 627–632. https://doi.org/10.1128/JCM.40.2.627-632.2002

Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T (2006) Bats: important reservoir hosts of emerging viruses. Clinical Microbiology Reviews 19: 531–545. https://doi.org/10.1128/CMR.00017-06

Camacho-Sierra V (2016) Identificación de unidades discretas de tipificación (DTU's) de *Trypanosoma cruzi* en marsupiales (*Didelphis marsupialis*, *Didelphis virginianus*, *Philander oposum*) presentes en la Reserva Ecológica "El Zapotal" en el estado de Chiapas. Master's thesis, Universidad Autónoma del estado de México, México.

Córdoba-Aguilar A, Ibarra-Cerdeña CN, Castro-Arellano I, Suzan G (2021) Tackling zoonoses in a crowded world: lessons to be learned from the COVID-19 pandemic. Acta Tropica 214: 105780. https://doi.org/10.1016/j.actatropica.2020.105780

Cruz-Reyes A, Pickering-López JM (2006) Chagas disease in Mexico: an analysis of geographical distribution during the past 76 years—a review. Memorias do Instituto Oswaldo Cruz 101: 345–354. https://doi.org/10.1590/S0074-02762006000400001

De Fuentes-Vicente JA, Gómez-Gómez A, Santos-Hernández NG, Ruiz-Castillejos C, Gómez-Sánchez EF, Vidal-López DG, Flores-Villegas L, Gutiérrez-Jiménez J, Moreno-Rodríguez A (2021) First report of an infected triatomine bug in an urban area of Tuxtla Gutierrez, Chiapas, México. Biocyt: Biología, Ciencia y Tecnología 14: 1009–1020.

De Fuentes-Vicente JA, Gutiérrez-Cabrera AE (2020) Kissing bugs (triatominae). References Module in Biomedical Sciences. Elsevier. https://doi.org/10.1016/B978-0-12-818731-9.00010-0

De Fuentes-Vicente JA, Vidal-López DG, Flores-Villegas AL, Moreno-Rodríguez A, De Alba-Alvarado MC, Salazar-Schettino PM, Gutiérrez-Cabrera AE (2019) *Trypanosoma cruzi*: a review of biological and methodological factors in Mexican strains. Acta Tropica 195: 51–57. https://doi.org/10.1016/j.actatropica.2019.04.024

Díaz M, Solari S, Gregorin R, Aguirre L, Barquez R (2021) Clave de identificación de los murciélagos neotropicales. Primera edición. Ed Programa de Conservación de los Murciélagos de Argentina, Tucumán, 211 pp.

Domínguez A, Ricárdez J, Espinoza E (1990) Study of *Trypanosoma cruzi* in wild reservoirs in the ecological reserve of El Zapotal, Chiapas, México. Boletín Chileno de Parasitología 45: 3–8.

Dorn PL, McClure AG, Gallaspy MD, Waleckx E, Woods AS, Monroy M, Stevens L (2017) The diversity of the Chagas parasite, *Trypanosoma cruzi*, infecting the main Central American vector, *Triatoma dimidiata*, from Mexico to Colombia. PLoS Neglected Tropical Diseases 11: e0005878. https://doi.org/10.1371/journal.pntd.0005878

Espinoza B, Rico T, Sosa S, Oaxaca E, Vizcaino-Castillo A, Caballero ML, Satoskar AR (2010) Mexican *Trypanosoma cruz*i (TCI) strains with different degrees of virulence induce diverse humoral and cellular immune responses in a murine experimental infection model. Journal of Biomedicine and Biotechnology 2010: e890672. https://doi.org/10.1155/2010/890672

Espinoza E, García E (2003) Manual de laboratorio de genética. ECOSUR-San Cristóbal de las Casas. El Colegio de la Frontera Sur, Tapachula, 37 pp.

Fernández-Moreno Y (2010) Percepciones ambientales sobre una Reserva Ecológica Urbana, El Zapotal, Tuxtla Gutiérrez, Chiapas. El Colegio de la Frontera Sur, San Cristóbal de las Casas, 170 pp.

Hamilton PB, Teixeira MM, Stevens JR (2012) The evolution of *Trypanosoma cruzi*: the 'bat seeding' hypothesis. Trends in Parasitology 28: 136–141. https://doi.org/10.1016/j.pt.2012.01.006

Jiménez-Coello M, Acosta-Viana KY, Guzman-Marin E, Gomez-Rios A, Ortega-Pacheco A (2012) Epidemiological survey of *Trypanosoma cruzi* infection in domestic owned cats from the tropical southeast of Mexico. Zoonoses Public Health 59: 102–109. https://doi.org/10.1111/j.1863-2378.2012.01463.x

Jimenez-Coello M, Ortega-Pacheco A, Guzman-Marin E, Guiris-Andrade DM, Martinez-Figueroa L, Acosta-Viana KY (2010) Stray dogs as reservoirs of the zoonotic agents *Leptospira interrogans*, *Trypanosoma cruzi*, and *Aspergillus* spp. in an urban area of Chiapas in southern Mexico. Vector-borne and Zoonotic Diseases 10: 135–141. https://doi.org/10.1089/vbz.2008.0170

Jimenez-Coello M, Poot-Cob M, Ortega-Pacheco A, Guzman-Marin E, Ramos-Ligonio A, Sauri-Arceo C, Acosta-Viana K (2008) American trypanosomiasis in dogs from an urban and rural Area of Yucatan, Mexico. Vector-Borne and Zoonotic Diseases 10: 755–762. https://doi.org/10.1089/vbz.2007.0224

Kasso M, Balakrishnan M (2013) Ecological and economic importance of bats (order Chiroptera). International Scholarly Research Notices 2013: e187415. https://doi.org/10.1155/2013/187415

Keesing F, Ostfeld R (2021) Impacts of biodiversity and biodiversity loss on zoonotic diseases. Proceedings of the National Academy of Sciences of the United States of America 118: e2023540118. https://doi.org/10.1073/pnas.2023540118

Kratz JM (2019) Drug discovery for chagas disease: a viewpoint. Acta Tropica 198: 105107. https://doi.org/10.1016/j.actatropica.2019.105107

Krauel JJ, LeBuhn G (2016) Patterns of bat distribution and foraging activity in a highly urbanized temperate environment. PLoS ONE 11(12): e0168927. https://doi.org/10.1371/journal.pone.0168927

Lau SKP, Luk HKH, Wong ACP, Li KSM, Zhu L, He Z, Fung J, Chan TTY, Fung KSC, Woo PCY (2020) Possible bat origin of Severe Acute Respiratory Syndrome Coronavirus 2. Emerging Infectious Diseases 26: 1542–1547. https://doi.org/10.3201/eid2607.200092

López-Argueta E (2022) Diversidad y gremios alimenticios de los murciélagos en el ambiente urbano de Tuxtla Gutiérrez, Chiapas. Bachelor's thesis, Universidad de Ciencias y Artes de Chiapas, México.

López-Cancino SA, Tun-Ku E, De la Cruz-Felix HK, Ibarra-Cerdeña CN, Izeta-Alberdi A, Pech-May A, Mazariegos-Hidalgo CJ, Valdez-Tah A, Ramsey JM (2015) Landscape ecology of *Trypanosoma cruzi* in the southern Yucatan Peninsula. Acta Tropica 151: 58–72. https://doi.org/10.1016/j.actatropica.2015.07.021

Luis AD, Hayman DT, O'Shea TJ, Cryan PM, Gilbert AT, Pulliam JR, Webb CT (2013) A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? Proceedings of the Royal Society B: Biological Sciences 280: e20122753. https://doi.org/10.1098/rspb.2012.2753

Martínez-Hernández F, Rendon-Franco E, Gama-Campillo L, Villanueva-García C, Romero-Valdovinos M, Maravilla P, Villalobos G (2014) Follow up of natural infection with *Trypanosoma cruzi* in two mammals species, *Nasua narica* and *Procyon lotor* (Carnivora: Procyonidae): evidence of infection control? Parasites & Vectors 7: e405. https://doi.org/10.1186/1756-3305-7-405

Moreira-Alves F, De Lima JS, Rocha FL, Herrera HM, Mourao GD, Jansen AM (2016) Complexity and multi-factoriality of *Trypanosoma cruzi* sylvatic cycle in coatis, *Nasua nasua* (Procyonidae), and triatomine bugs in the Brazilian Pantanal. Parasites & Vectors 9: e378. https://doi.org/10.1186/s13071-016-1649-4

Noireau F, Diosque P, Jansen AM (2009) *Trypanosoma cruzi*: adaptation to its vectors and its hosts. Veterinary Research 40: e26. https://doi.org/10.1051/vetres/2009009

Reid F (2009) A Field Guide of the Mammals of Central America and Southeast México, Second Edition. Oxford University Press, New York, 238 pp.

Romero-Almaraz ML, Sánchez-Hernández C, García-Estrada C, Owen RD (2000) Mamíferos pequeños: manual de técnicas de captura, preparación, preservación y estudio. Editorial Las prensas de ciencias UNAM, México, 324 pp.

Ruiz-Piña HA, Cruz-Reyes A (2002) The opossum *Didelphis virginiana* as a synanthropic reservoir of *Trypanosoma cruzi* in Dzidzilché, Yucatán, México. Memórias do Instituto Oswaldo Cruz 97: 613–620. https://doi.org/10.1590/S0074-02762002000500003

Ruiz-Piña HA, Gutiérrez-Ruiz E, Escobedo-Ortegón FJ, Rodríguez-Vivas RI, Bolio-González M, Ucan-Leal D (2018) Prevalence of *Trypanosoma cruzi* in backyard mammals from a rural community of Yucatan, Mexico. Tropical and Subtropical Agroecosystems 21: 367–371.

Solís-Franco R, Romo-Zapata A, Martínez-Ibarra J (1997) Wild reservoirs infected by *Trypanosoma cruzi* in the ecological park "El Zapotal", Tuxtla Gutiérrez, Chiapas, México. Memorias do Instituto Oswaldo Cruz 92: 163–164. https://doi.org/10.1590/S0074-02761997000200006

Souto RP, Fernandes O, Macedo AM, Campbell DA, Zingales B (1996) DNA markers define two major phylogenetic lineages of *Trypanosoma cruzi*. Molecular and Biochemical Parasitology 83: 141–152. https://doi.org/10.1016/S0166-6851(96)02755-7

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

Torres-Castro M, Hernández-Betancourt S, Puerto FI, Torres-León M (2016) Lesiones histológicas asociadas a la posible infección por *Trypanosoma cruzi* (Chagas, 1909) en corazones de roedores sinantrópicos capturados en Yucatán, México. Anales de Biología 38: 29–35. https://doi.org/10.6018/analesbio.38.03

Ucan-Euan F, Hernández-Betancourt S, Arjona-Torres M, Panti-May A, Torres-Castro M (2019) Estudio histopatológico de tejido cardiaco de roedores infectados con *Trypanosoma cruzi* capturados en barrios suburbanos de Mérida, México. Biomédica 39: 32–43. https://doi.org/10.7705/biomedica.v39i3.4192

Vallejo M, Reyes PP, García MM, Garay AG (2020) Trypanocidal drugs for late-stage, symptomatic Chagas disease (*Trypanosoma cruzi* infection). Cochrane Database of Systematic Reviews 12: CD004102. https://doi.org/10.1002/14651858.CD004102.pub3

Vaz VC, D'Andrea PS, Jansen AM (2007) Effects of habitat fragmentation on wild mammal infection by *Trypanosoma cruzi*. Parasitology 134: 1785–1793. https://doi.org/10.1017/S003118200700323X

Vázquez-Pérez EU, Roque-Velázquez JA, Velázquez-Velázquez E (2010) Diversidad alfa y beta en murciélagos cavernícolas de la Depresión Central, Chiapas, México. Lacandonia 4: 47–54.

Villena FE, Gómez-Puerta LA, Jhonston EJ, Del Alcazar OM, Maguiña JL, Albujar C, Ampuero JS (2018) First report of *Trypanosoma cruzi* infection in salivary gland of bats from the Peruvian Amazon. The American Journal of Tropical Medicine and Hygiene 99: 723–728. https://doi.org/10.4269/ajtmh.17-0816

Vízquez-Rodríguez LR (2015) Prevalencia de *Leishmania* y *Trypanosoma cruzi* en los murciélagos *Carollia sowelli* y *Sturnira lilum* bajo dos condiciones distintas de perturbación antropogénica en la Selva Lacandona, Chiapas. Master's thesis, Universidad Nacional Autónoma de México, México.

WHO (2014) A Global Brief on Vector-Borne Disease. World Health Organization, Geneva, 54 pp. https://apps.who.int/iris/handle/10665/111008

Zingales B (2018) *Trypanosoma cruzi* genetic diversity: something new for something known about Chagas disease manifestations, serodiagnosis and drug sensitivity. Acta Tropica 184: 38–52. https://doi.org/10.1016/j.actatropica.2017.09.017