

Short Communication

Trypanosoma cruzi DNA in Desmodus rotundus (common vampire bat) and Histiotus montanus (small big-eared brown bat) from Chile

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ABSTRACT

The protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease, is transmitted by infected feces or consumption of blood-sucking triatomine insects to several mammalian orders including Chiroptera. In Chile, the distribution of several insectivorous and one hematophagous bat species overlaps with those of triatomine vectors, but the *T. cruzi* infection status of local chiropterans is unknown. In 2018, we live-captured bats from two protected areas in Chile to collect plagiopatagium tissue, feces and perianal swab samples, in search for *T. cruzi*-DNA by real time PCR assays using species-specific primers. In Pan de Azúcar island (~26°S), we examined a roost of *Desmodus rotundus* (common vampire bat) and sampled tissue from 17 individuals, detecting *T. cruzi*-DNA in five of them. In Las Chinchillas National Reserve (~31°S), we examined two roosts of *Histiotus montanus* (small big-eared brown bat), collecting feces or perianal swab samples from eight individuals, detecting *T. cruzi*-DNA in four of them. This is the first report of *T. cruzi*-DNA evidence in bat species from Chile. Both vector-borne and oral transmission are potential infection routes that can explain our results. Further investigation is needed for a better understanding of the role of bats in the *T. cruzi* transmission cycle.

Trypanosoma cruzi, the causal agent of Chagas disease, is a protozoan parasite transmitted by blood-sucking insects of the subfamily Triatominae (Hemiptera: Reduviidae) (Jansen et al., 2018). Triatomine vectors become infected by feeding on *T. cruzi*-infected mammalian hosts; the parasite multiplies inside their guts and later on, it is transmitted from vectors to hosts by their infected feces (Kollien and Schaub, 2000). An alternative transmission route to mammals is by consumption of infected triatomines or their feces, known as oral transmission (Jansen et al., 2018). In Chile, several mammal species have been found infected with *T. cruzi*, including rodents, carnivores, marsupials, among others (Correa et al., 2020). Even though several species of chiropterans from America have been described as infected by *T. cruzi* (e.g., Marcili

et al., 2009; Pinto et al., 2015; Argibay et al., 2016; Nichols et al., 2019; Bergner et al., 2021; Torres-Castro et al., 2021), the infection status of bat species present in Chile has not been evaluated.

In Chile, 14 species of bats have been described, including 12 insectivores, one nectarivore, and one hematophagous species (Rodríguez-San Pedro et al., 2016; Ossa et al., 2018), all classified as protected species (MMA, 2020). Two bat species that inhabit in the same geographical range of triatomine vectors in many countries of America are *Desmodus rotundus* (Phyllostomidae; common vampire bat) and *Histiotus montanus* (Vespertilionidae; small big-eared brown bat). *Desmodus rotundus* is a gregarious hematophagous bat distributed in Chile from 18–33°S, present in coastal and insular areas, where it feeds on sea

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lions (Rodríguez-San Pedro and Allendes, 2017; Iturra-Herrera et al., 2019). *Histiotus montanus* is a gregarious insectivore bat distributed in Chile from 18–54°S, inhabiting caves and roosts associated with native forest. This species feeds on moths, mosquitos and beetles, which are pests of agricultural crops in Chile (Rodríguez-San Pedro et al., 2016).

There are four species of triatomine vectors inhabiting coastal and interior valleys of Chile between 18–33°S, all naturally infected by *T. cruzi* (Botto-Mahan et al., 2008; Bacigalupo et al., 2010; Rives-Blanchard et al., 2017; Botto-Mahan et al., 2020). *Mepraia* is a triatomine genus endemic to Chile, with three diurnal species inhabiting rocky outcrops, rock walls, and bromeliads (Ihle-Soto et al., 2019; San Juan et al., 2020). The aim of this study was to examine the presence of *T. cruzi* DNA in samples of bats collected from two protected wild areas of Northern Chile, Pan de Azúcar National Park (26°09'S, 70°39'W; PANP hereafter) and Las Chinchillas National Reserve (31°30'S, 71°06'W; LCNR hereafter) (Fig. 1).

In PANP, the vector *Mepraia parapatrica* can be found associated to rocky coastal areas and its *T. cruzi* prevalence reaches 61.5% (Rives-Blanchard et al., 2017). In the summer of 2018, we sampled a roost of *D. rotundus* located in the Pan de Azúcar island at PANP. This island is approximately 2 km from mainland, and the vector *M. parapatrica* and rodents that inhabit in there have been reported infected by *T. cruzi* (Rives-Blanchard et al., 2017; Campos-Soto et al., 2020). In a roost of *D. rotundus* with 26 individuals inhabiting a fissure in a rock wall by the sea, we captured them using hand-nets (1 m × 1 m diameter) and mist-nets (7 m × 2.5 m) (Fig. 2A–B). The mist-nets were placed at the cave entrance for three hours after sunset, and at the same time the researchers entered into the cave and captured bats with hand nets. We collected tissue samples from the plagiopatagium of each captured individual using a sterile 4-mm biopsy punch (n = 17 sampled bats) as previously reported (Greville et al., 2018; Nichols et al., 2019). In LCNR, the vector *Mepraia spinolai* can be found mainly associated to rocky outcrops and bromeliads. *Trypanosoma cruzi* prevalence in this wild vector species reaches 55.1% (Estay-Olea et al., 2020) and several

rodent species coexisting with *M. spinolai* have been found infected by *T. cruzi* (Oda et al., 2014; Botto-Mahan et al., 2020). In the summer of 2018, we sampled two bat roosts close from *M. spinolai* colonies using the same capture method previously described (Fig. 2C–D). We obtained three perianal swab samples and five feces' samples from *H. montanus* (n = 8 sampled bats). All bat specimens were released in their roosts after sampling. Bats were captured under the permit number 7612/2017 granted by The Chilean Bureau for Agriculture and Environment (SAG) to JL Allendes. We were unable to obtain other types of biological samples due to logistical and sampling difficulties (blood sample), or due to lack of permission (sample of visceral organs or blood in some species). All procedures were supervised by a veterinarian and conformed to the guidelines for the care and use of wild animals in research reported by the American Society of Mammalogists (Sikes et al., 2016). The investigation in protected areas was authorized by The National Forest Corporation (CONAF) under permit 14/2016 and 18/2018 to JL Allendes. The bat species were identified following Díaz et al. (2016). All samples were stored in absolute ethanol at -20 °C until processing.

For the plagiopatagium samples, ethanol was evaporated and then the tissue cut using a sterile scalpel blade. All the tissue sample was used for DNA isolation. Up to 20 mg of feces and the whole perianal swab were used for DNA isolation. Prior to DNA extraction, the swab sample was submerged and gently shaken in the ATL buffer (Tissue Lysis Buffer, QIAGEN, CA, USA), to allow the elution of the biological material present in the sampled bat. The DNA of all samples was isolated using the DNeasy® Blood & Tissue Kit (QIAGEN, CA, USA), following the manufacturer instruction with an elution volume of 100 µl. We performed real time PCR directed to a nuclear segment of a genomic repetitive DNA sequence of *T. cruzi*-DNA using the primers *Cruzi1* and *Cruzi2* (Piron et al., 2007). The reaction was performed with Hot FIREPol® Eva-Green® qPCR Mix (ROX) (Solis Biotec, Estonia), primers at 0.4 µM, including 5 µl of template in a final volume of 20 µl. Cycling conditions were 15 min at 95°C followed by 50 cycles at 95°C for 15 s, 65°C for 20 s, and 72°C for 20 s, finishing with a default melting curve. We used water

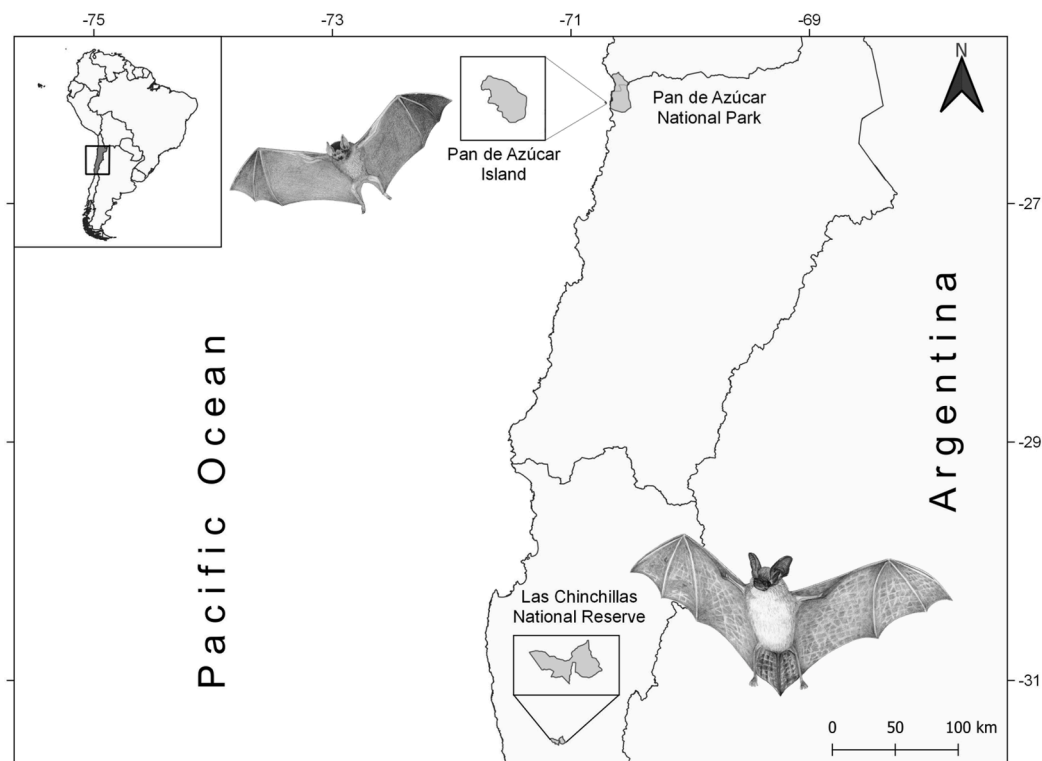


Fig. 1. Map of Chile depicting the location of the two protected areas prospected: Pan de Azúcar island in Pan de Azúcar National Park (PANP), and Las Chinchillas National Reserve (LCNR). Drawings: *Desmodus rotundus* (top), *Histiotus montanus* (bottom). Bat drawings by Daniela Estay-Olea.

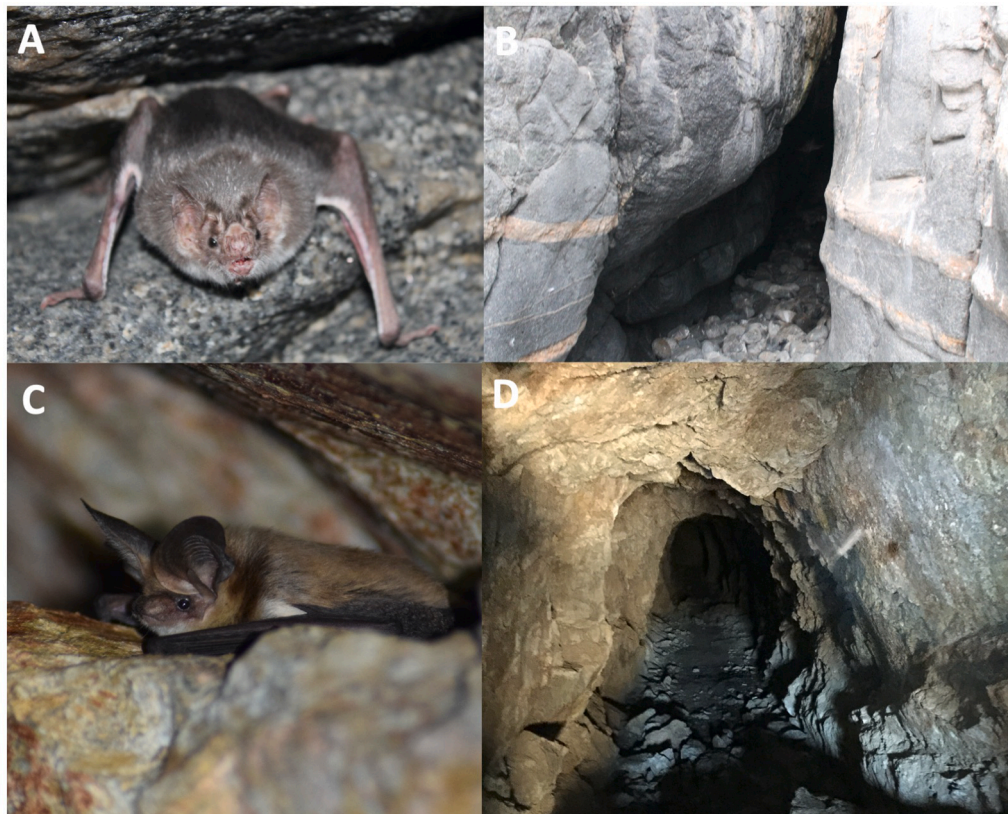


Fig. 2. (A) *Desmodus rotundus* (common vampire bat) and (B) fissure in a rock wall where the roost was located (Pan de Azúcar island; PANP). (C) *Histiotus montanus* (small big-eared brown bat) and (D) abandoned mining cave where roosts are located (Las Chinchillas National Reserve; LCNR). Photographs by R. Campos-Soto (2A-B), A. Rodríguez San-Pedro (2C), and C. Botto-Mahan (2D).

as non-template control and DNA from a culture of *T. cruzi*, obtained from the Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, as positive control. Each sample was analyzed in duplicate, and it was considered positive for *T. cruzi* when at least one of the replicates had a specific amplification. One amplicon per sampled animal ($Ct < 36.6$) was submitted to standard sequencing of both strands (Macrogen, Korea). The quality of the sequences was checked by inspection of each chromatogram and the consensus sequence was obtained using Bioedit 7.0.4.1 (Hall, 1999) and compared to available sequences through BLAST in GenBank.

We detected *T. cruzi*-DNA in 5 out of 17 tissue samples (29.4%) of *D. rotundus* (mean $Ct \pm SD$: 35.7 ± 1.1), and 3 out of 3 samples of perianal swabs (100%) (mean $Ct \pm SD$: 38.3 ± 1.3) and 1 out of 5 fecal samples (20.0%) from *H. montanus* (Ct : 36.5). The results of each bat sampled are available in Table S1- Supplementary material. Results from standard sequencing showed over 96.9% of identity with *T. cruzi* sequences in all samples. Complete BLAST analysis results (i.e., score, query cover, and percentage of identity) can be found in Table S2 – Supplementary material.

These results are the first record of detection of *T. cruzi* DNA in chiropterans from Chile; however, at present there is not enough information on how natural infection in these bats could be occurring, and comparison of possible transmission routes between the two bat species studied in this work is limited given the different type of samples analyzed from each of them. Most bat species present in Chile are insectivores, such as *H. montanus*, and they could become infected by consuming infected vectors (i.e., oral transmission) or by vectorial transmission, when triatomines feed on them. A recent work detected *T. cruzi* DNA in saliva samples of different bat species in Peru, proposing oral transmission by inadvertently consuming vectors while grooming as another hypothesis to be considered, besides directly feeding on

triatomines in the case of insectivore bats (Bergner et al., 2021), like *H. montanus*. In the case of the hematophagous *D. rotundus*, we cannot discard the oral transmission by consuming blood of infected vertebrate hosts as the route of transmission (Jansen et al., 2018). However, vector-borne transmission was also possible in these cases due to the proximity of *H. montanus* and *D. rotundus*' roosts to *M. spinolai* and *M. parapatrica* colonies, respectively. Congenital transmission of *T. cruzi* could be also occurring, as reported in a study on *Molossus molossus* (Chiroptera: Molossidae) (Añez et al., 2009); therefore, further studies are needed to determine the transmission routes in the bat species under study, including other types of samples (e.g., blood, saliva, organs). The *T. cruzi* DNA detected in feces and perianal swab samples from the insectivore *H. montanus* could correspond to remains of infected insects, so it may not necessarily indicate an infection of the sampled bat. Nonetheless, considering the habits and habitats of the bat species examined in this study, and previous reports of *T. cruzi* infection in several bat species present in other American countries, including some species that are also found in Chile, it is likely that bats have a role as hosts in the transmission cycle of *T. cruzi* in Chile. Further studies are needed to evaluate the functional role of bats in the maintenance and transmission of *T. cruzi* in sylvatic and peridomestic habitats.

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Declaration of Competing Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.actatropica.2021.106206](https://doi.org/10.1016/j.actatropica.2021.106206).

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