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Short Report: Wild Ecotopes and Food Habits of *Triatoma longipennis* Infected by *Trypanosoma cruzi* Lineages I and II in Mexico

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Abstract. The control of wild triatomine populations that can invade dwellings is a major challenge for Chagas disease control in Mexico, but a better knowledge of the biology of these populations is required to develop appropriate control methods. We describe a new terrestrial ecotope of *Triatoma longipennis*, a principal vector in the occidental part of Mexico, in addition to its previously identified niche in rock pile boundary walls. Analysis of feeding hosts in the two ecotopes showed that this species is able to diversify its food sources outside of the principal hosts, Dasypus *novemcinctus* and *Procyon lotor*, and to disperse in search of new meals. Moreover, *T. longipennis* are strongly infected not only by the *Trypanosoma cruzi* I lineage found in the domestic cycle, but also by *T. cruzi* lineage II. The impact of *T. cruzi* II on human infection remains to be determined.

In Mexico, at least 31 primarily wild (sylvatic) triatomine species have been described, of which 21 are found naturally infected with *Trypanosoma cruzi*.[1] Among these species, *Triatoma longipennis* is the principal vector in the state of Jalisco and this vector infests villages where peridomestic colonization can be high.[2–4] However, few studies are available on the ecology, geographic distribution, and behavior of the vector in the natural environment of *T. longipennis*. Invasion or incursion in villages by wild populations is a permanent threat in the region. We previously reported on the colonization by *T. longipennis* of rock pile boundary walls that separate the culture fields in the Ameca Valley, whereas here we describe a new natural ecotope.[5] Also, we identified blood meal origins and strains of *T. cruzi* in the two ecotopes, and provided evidence of the presence of the *T. cruzi* II lineage in addition to *T. cruzi* I, which is the only lineage identified in the domestic cycle.[6]

Triatomines were collected in the Ameca Valley (20°22′55.46″ N, 103°53′15.78″ W, altitude = 1,345 meters) in the municipality of San Martin de Hidalgo. The site was composed of large rocks at the top of a small hill located in an agro-pastoral area. In surrounding area, the main agricultural crops were corn (*Zea mays*) and Maguey tequilero (*Agave tequilana*); grazing of animals is also common (Figure 1A). The climate in this region is semi-arid, with an average annual temperature of 20.9°C and an rainfall between 829 mm and 964 mm. The rainy season is between June and September; winter and spring are very dry. Many mammals have disappeared from this region but raccoons, armadillos, opossums, rabbits, hares, squirrels, and other species of small rodents are still abundant (www.e-local.gob.mx/work/templates/enciclo/jalisco/mpios/14077a.htm).

Collections were made by using a modified Noireau mice bait-trap (Figure 1B).[7] Traps were placed in small caves and cracks between the rocks for four nights at the beginning of June 2006 and for one night at the end of August 2007 (Figure 1C). Mice enclosed in a trap that contains food and bedding and are generally removed alive the next day. A total of 254 triatomines were captured at this site. The percentage of positive traps was 24.3% (total of traps = 485) with a mean ± SD of 2.1 ± 1.1 triatomines per positive trap. *Triatoma longipennis* was the dominant species (95.7%); other species were *T. pallidipennis* (3.4%) and *T. picturata* (0.9%). Age groups of the triatomines consisted of adults (46%), fourth and fifth stages (40.6%), and first and second nymphs (13.4%). The sex ratio was slightly biased toward females (1:1.2), but the difference was not significant (degrees of freedom = 1, $\chi^2 = 0.5$, $P > 0.05$). The infection rate determined by microscopic observations of feces ranged from 25% in first and second nymphs to 66.7% in adults; no significant differences were observed between sexes. We did not observe differences in infestation and infection rates between 2006 and 2007 triatomine collections.

To identify mammal reservoirs in large rocks and in rock pile boundary walls, mammals were captured at these sites (the boundary wall was previously studied and was 8.2 km away from large rocks; 20°23′56.73″ N, 103°58′00.30″ W).[5] At each site, 100 traps (live traps, 8 cm × 9 cm × 23 cm; H. B. Sherman Trap Co., Tallahassee, FL) were set. Similar numbers of mammals were captured in the two ecotopes (16 at the rock site and 17 in the boundary wall). *Baiomys* sp. (Pygmy mouse) was the dominant species (28 animals of which only six were adults); the others were *Liomys irroratus* (Mexican spiny pocket mouse, two adults) and *Reithrodontomys fulvescens* (fulvous harvest mouse, three adults). Blood samples were taken on site after animals were anesthetized using gloves, a protective shield, and mask. Blood smears and thick drops were microscopically negative for parasites. However, *T. cruzi* was detected in blood by a polymerase chain reaction (PCR) in 45.5% of the animals by using primers that amplified specifically *T. cruzi* minisatellites.[8] The three species were positive by PCR.

Blood meal origins were identified in 47 triatomines captured in large rocks (35 samples) and in several rock pile boundary walls in the Ameca Valley (12 samples) by using the cytochrome b heteroduplex essay and sequencing of PCR products (Genome Express, Meylan, France) directly or after cloning (TOPO TA kit; Invitrogen, Cergy-Pontoise, France).[9] A total of 14 heteroduplex patterns were observed. The most frequent pattern (57.5%) corresponded to a sequence best

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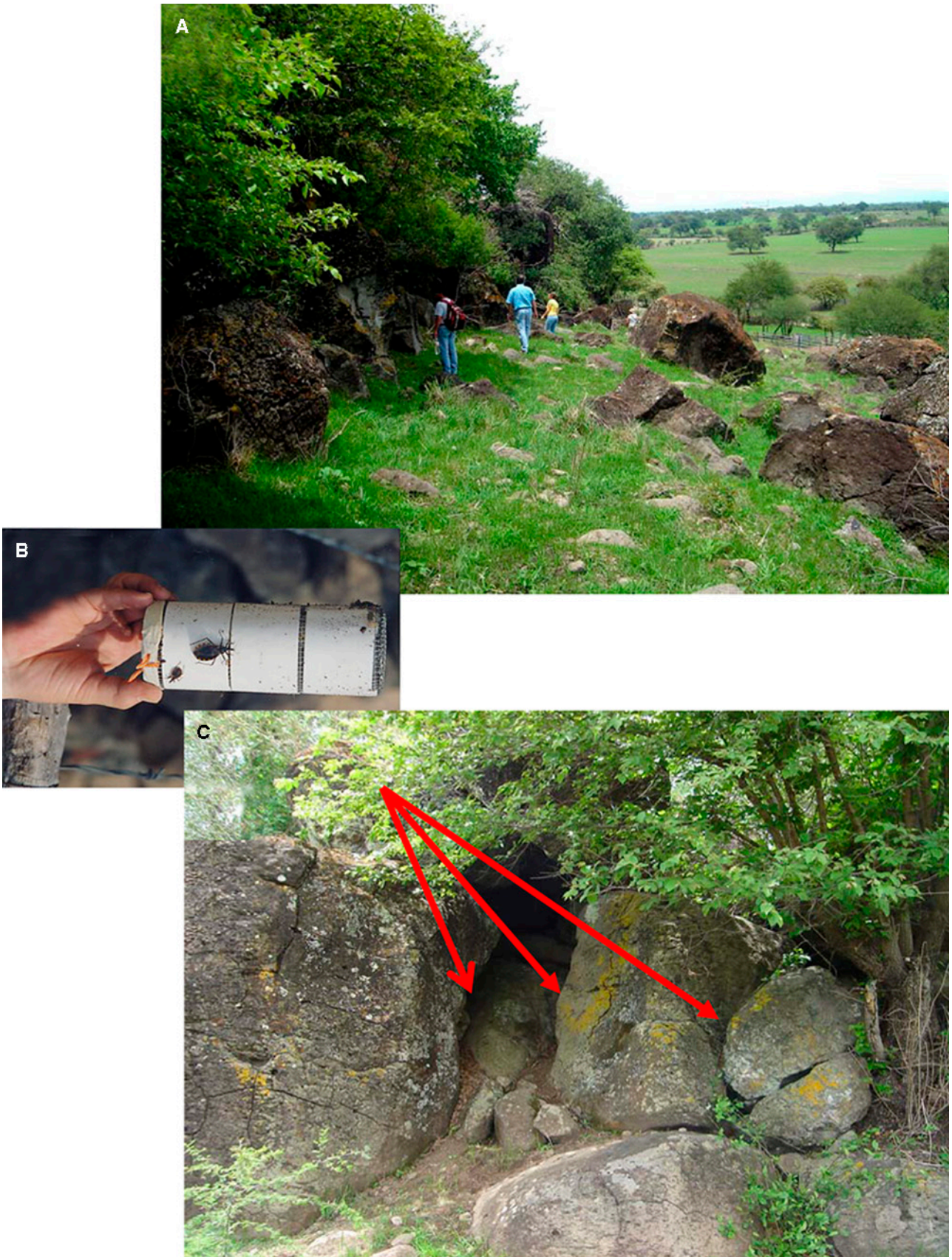

FIGURE 1. Natural ecotope of *Triatoma longipennis* composed of large rocks in the agro-pastoral area of the Ameca Valley, which is highly infected with *Trypanosoma cruzi.* **A**, General view of the site. **B**, Mice bait-trap with triatomines that consisted of a cylindrical wire-netting bait-trap covered with double-coated adhesive tape 20 cm long and 7 cm diameter. **C**, Example of sites where the traps are hidden. This figure appears in color at www.ajtmh.org.

aligned with that of *Procyon lotor* (raccoon) (GenBank accession no. AB297804.1, 98% identity). The second most frequent pattern (25%) corresponded to a sequence best aligned with that of *Dasypus novemcinctus* (nine-banded armadillo) (Y11832.1, 97% identity). Similarly, other sequences showed the closest similarity with *Philander opossum* (DQ236275.1, 91% identity), *Lynx rufus* (AY499332.1, 89% identity), *Sigmodon mascotensis* (AY041203.1, 97% identity), *Megasorex gigas* (AB175150.1, 79% identity), *Reithrodontomys* fulvescens (AY041206, 96% identity), *Mephitis macroura* (usually called zorrillo) (L27301.1, 88% identity), *Sceloporus occidentalis* (western fence lizard, AB079242, 86% identity), and *Homo sapiens* (AY50968.1, 99% identity).

Four samples had multiple blood meal origins. *Dasypus novemcinctus* was identified in the four samples with other blood meals (*S. mascotensis* [2 samples], *Bos taurus* [cattle, one sample, best alignment with AY819734.1, 99% identity], and *S. occidentalis* [one sample]). Most blood meals for triatomines in rock pile boundary walls were from armadillo (*D. novemcinctus*, 7 of 13 [54%]), whereas the principal feeding host captured in large rocks was the raccoon (*P. lotor*, 25 of 38 [66%]) for those captured in large rocks (Table 1). Among identified species, *P. opossum* and *S. occidentalis* have not been reported in the Jalisco State.

Triatomines with flagellate-positive feces were also processed by using mini-exon, multiplex, PCR-based typing for direct detection of *T. rangeli* and *T. cruzi* sub-groups according to methods based on PCR sizes.[10] Among 129 triatomines (31 from rock pile boundary rocks and 98 from the large rocks site), *T. cruzi* I (200-basepair band) was the major lineage (84.5% of the blood meals), 13.2% corresponded to *T. cruzi* II (150-basepair [bp] band, DTU 2a and 2c), two samples (1%) had two bands (200 bp and 150 bp), which suggested a mixture of the two lineages, and one sample had a band of approximately 300 bp, which was not previously described. Sequencing of PCR products of the entire intergenic region of the mini-exon gene and the NADH dehydrogenase subunit 1 gene from current *T. cruzi* II samples showed the greatest homology with strains from the United States belonging to DTU 2a. The frequencies of *T. cruzi* I and *T. cruzi* II were not significantly different between the two ecotopes (Table 1) ($P > 0.05$). However, *T. cruzi* II was not detected in armadillo blood meals but was found in 27% of raccoon blood meals ($\chi^2 = 3.66$, $P = 0.055$).

Table 1
Identification of blood meal origins and *Trypanosoma cruzi* lineage in sylvatic *Triatoma longipennis* according to sites of capture

Triatomine blood meal origin	No. of triatomines: Large rocks	No. of triatomines: Rock pile boundary walls	*T. cruzi* lineage detected in triatomine
Dasypus novemcinctus (armadillo)	1	7	I
Philander opossum	1		I
Sigmodon mascotensis		1	I
		1	Not done
Procyon lotor (mapache)	15		I
	5		II
	1		I and II
	4		Not done
Chrotomys whiteheadi (family muridae)		1	II
Reithrodontomys fulvescens (family muridae)	1		I
Mephitis macroura (zorrillo, carnivore)	1		I
	1		II
Lynx rufus	1		Not done
Human		1	I
Sceloporus occidentalis	1		II
D. novemcinctus and *Bos taurus*	1		I
D. novemcinctus and *Sigmodon* sp.		1	I
	1		Not done
D. novemcinctus and *Sceloporus occidentalis*	1		I
	60	17	I
	8	2	II
Not done	1		I and II
		1	Unknown lineage
Total	104	32	

Our results and those of previous studies show that *T. longipennis* colonizes agro-pastoral environments and can form large colonies in artificial structures such as rock pile boundary walls, but can also colonize the natural ecotope, such as large rock formations that provide shelters for many host feeders.[5] Recent discovery of a second site equally composed of large rocks infested with *T. longipennis* at the edge Guadalajara (20°46′06.36″ N, 103°22′54.15″ W) suggests that such ecotopes are common. The success of the adaptation of *T. longipennis* to these sites may be caused by the abundance of mammals and the capacity of *T. longipennis* to diversify its food source to many species of mammals and reptiles (Iguana family), which has been rarely observed.[11]

These breeding sites, when highly infested, could serve as the spreading origins of triatomines to villages and could maintain transmission of the disease. A blood meal obtained from a man was identified in a *T. longipennis* female captured in a rock pile boundary in a grazing field located 500 meters from the nearest dwelling. Because work in fields is conducted during the day, it is likely that this triatomine entered a house, and then left the house after feeding. Also, the cow blood meal origin shows the capacity of triatomines to get out from their hiding sites and go into the fields.

The sylvan triatomines analyzed in this study were heavily parasitized, which suggests that the two principal blood meal hosts, *D. novemcinctus* and *P. lotor*, are important reservoirs in the region.[12] Also, as shown in our study, several rodent species are heavily infected. Raccoons are important reservoirs in the United States; extensive seroprevalence surveys showed infection rates ranging from 20% to 30%.[13–15] These host species are also able to approach human dwellings and play a role in linking sylvatic and domestic cycles. The previous identification of one armadillo blood meal in the peridomiciliary area also supports this role.[16]

We report circulation of the *T. cruzi* II parasite lineage in Mexico. This lineage had not been previously identified in domestic and peridomestic cycles. Also, no human infection with *T. cruzi* II has been reported in Mexico. However, few human strains have been characterized, especially in the studied area where several cases of mega-esophagus have been described, unlike in other parts of Mexico where only cases of chagasic myocardiopathy were reported.[17] Therefore, the implication of the *T. cruzi* II lineage in Chagas disease remains to be explored. A previous report suggested *T. cruzi* lineage II–raccoon specificity in the United States (Georgia) but other results suggest that these stocks can infect other hosts.[18]

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