ZooKeys 820: 51–70 (2019)
doi: 10.3897/zookeys.820.27258
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

# Description of *Triatoma huehuetenanguensis* sp. n., a potential Chagas disease vector (Hemiptera, Reduviidae, Triatominae)

Raquel Asunción Lima-Cordón[1], María Carlota Monroy[2], Lori Stevens[1], Antonieta Rodas[2], Gabriela Anaité Rodas[2], Patricia L. Dorn[3], Silvia A. Justi[1,4,5]

**1** *Department of Biology, University of Vermont, Burlington, Vermont, USA* **2** *The Applied Entomology and Parasitology Laboratory at Biology School, Pharmacy Faculty, San Carlos University of Guatemala, Guatemala* **3** *Department of Biological Sciences, Loyola University New Orleans, New Orleans, Louisiana, USA* **4** *Walter Reed Biosystematics Unit, Smithsonian Institution Museum Support Center, Maryland, USA* **5** *Walter Reed Army Institute of Research, Silver Spring, MD, USA*

Corresponding author: *Raquel Asunción Lima-Cordón* (raqueasu7@gmail.com)

Academic editor: *G. Zhang* | Received 6 June 2018 | Accepted 4 November 2018 | Published 28 January 2019

*http://zoobank.org/14B0ECA0-1261-409D-B0AA-3009682C4471*

**Citation:** Lima-Cordón RA, Monroy MC, Stevens L, Rodas A, Rodas GA, Dorn PL, Justi SA (2019) Description of *Triatoma huehuetenanguensis* sp. n., a potential Chagas disease vector (Hemiptera, Reduviidae, Triatominae). ZooKeys 820: 51–70. https://doi.org/10.3897/zookeys.820.27258

## Abstract

A new species of the genus *Triatoma* Laporte, 1832 (Hemiptera, Reduviidae) is described based on specimens collected in the department of Huehuetenango, Guatemala. *Triatoma huehuetenanguensis* **sp. n.** is closely related to *T. dimidiata* (Latreille, 1811), with the following main morphological differences: lighter color; smaller overall size, including head length; and width and length of the pronotum. Natural *Trypanosoma cruzi* (Chagas, 1909) infection, coupled with its presence in domestic habitats, makes this species a potentially important vector of *Trypanosoma cruzi* in Guatemala.

## Keywords

Chagas disease vector, *Triatoma dimidiata* s.l., *Trypanosoma cruzi*

## Introduction

As of 2010, more than a million cardiomyopathy cases in Latin America were caused by Chagas disease (WHO 2015) due to the parasite *Trypanosoma cruzi* (Chagas, 1909).

This illness is mainly transmitted through the infected feces of insects of the subfamily Triatominae (Hemiptera: Reduviidae). Known colloquially as kissing bugs, the group is currently divided into five tribes and 15 genera (Justi and Galvao 2017; World Health Organization 2015; Schofield and Galvao 2009). Among these, *Triatoma* Laporte, 1832, *Panstrongylus* Berg, 1879 and *Rhodnius* Stål, 1859 (the first two belonging to the tribe Triatomini and the third genera belonging to Rhodniini) are the most epidemiologically relevant for Chagas transmission (WHO 2002).

The genus *Triatoma* is the most diverse, comprising over half of the described Triatominae species (Justi and Galvao 2017; Schofield and Galvao 2009). *Triatoma dimidiata* (Latreille, 1811), the most important Chagas disease vector in Central America, is in fact a species complex including at least three independently evolving lineages initially identified by sequences of nuclear (internal transcribed spacer, ITS-2) and the mitochondrial marker cytochrome b (cytb) (Bargues et al. 2008; Dorn et al. 2016), and later confirmed by phylogenetic studies using SNPs and species delimitation, obtained by a reduced representation genome genotyping by sequencing (GBS) approach (Justi et al. 2018). These studies recovered *T. dimidiata* as four linages: groups 1 – 4, which appeared to include at least three species: groups 1 and 2 – *T. dimidiata* s. str., group 3 – *Triatoma* sp. aff *dimidiata*, and group 4 – *Triatoma* sp. aff *dimidiata* cave (Bargues et al. 2008, Dorn et al. 2016, Justi et al. 2018). The last of these taxa, *Triatoma* sp. aff *dimidiata* cave (= group 4) was recently described as a new species, *Triatoma mopan* Dorn, Justi & Dale, 2018.

In this study, we formally describe *Triatoma* sp. aff *dimidiata* (group 3) based on morphological and molecular data and name it *Triatoma huehuetenanguensis* sp. n., after the type locality in Guatemala.

## Materials and methods

### Sampling

A total of 39 *Triatoma* specimens was obtained between April 2015 and May 2016 through community participation in the department of Huehuetenango, Guatemala and given to personnel from the Ministry of Health of Huehuetenango who shipped them to the Applied Entomology and Parasitology Laboratory (LENAP), at San Carlos University in Guatemala City. At LENAP the specimens were preserved in 95% ethanol and 5% glycerol and stored at room temperature. Specimens were identified as *T. dimidiata* using the taxonomic key for the genus *Triatoma* published by Lent and Wygodzinsky (1979).

Three females and three males were left intact to comprise the type series used for the morphological description of the new species. DNA was extracted from the remaining 20 by Justi et al. (2018) and tested for infection with *T. cruzi*. Justi et al. (2018) previously recovered all 20 specimens within the same highly supported monophyletic clade named *Triatoma* sp. aff. *dimidiata* based on genome SNP phylogenies.

## Morphological characterization

Since there is no known holotype for *T. dimidiata* (Latreille, 1811), the characterization of the new species was done following the same methodology as Dorn et al. (2018) for the description of *T. mopan*.

Based on (Lent and Wygodzinsky 1979), 17 morphological traits were measured from the type series and an additional 13 specimens and from 15 female and ten male *Triatoma dimidiata* s. str. (group 1–2) from Huehuetenango, Jutiapa and Chiquimula in Guatemala. These 25 *Triatoma dimidiata* s. str. specimens were preserved under the same conditions as the new species (95% ethanol and 5% glycerol). Measurements of the morphological traits were performed using a Nikon stereoscope Model SMZ-1B (see Suppl. material 1: morphological measurements). The morphological traits were:

| | | | |
|---|---|---|---|
| (1) | total length, | (7) | ante-ocular region, |
| (2) | width of the pronotum, | (8) | post-ocular region, |
| (3) | width of the abdomen, | (9) | width of the eye, |
| (4) | head length, | (10) | synthlipsis, |
| (5) | width across eyes, | (11–14) | each of the four antennomeres, and |
| (6) | length of the pronotum, | (15–17) | each of the three labial articles. |

Because of unequal sample sizes for each group (*T. dimidiata* s. str. and the new species), an unpaired t test was used to compare the means of each of the 17 morphological traits in the two groups (JMP Pro version 13.0.0).

Insects were photographed using a Visionary Digital BK Laboratory System, a Canon 5D camera, 65 mm macro zoom lens. Photo stacks of 25–45 slices were compiled using Helicon Focus 5.3 and the image edited to balance light quality, remove background blemishes, and provide a scale on Photoshop CS6.

## *Trypanosoma cruzi* infection

Natural infection by *T. cruzi* was tested by PCR on genomic DNA extracted from the last three segments of the specimens' abdomen. DNA was extracted using Qiagen DNeasy blood and tissue kit, following the manufacturer's tissue protocol for the first two steps, blood protocol for subsequent steps and an additional incubation (65 °C for 10 min, followed by 95 °C for 5 min.). Primers and PCR assay conditions were used as previously described (Moser et al. 1989).

## Molecular phylogenetic analysis

In order to: (a) keep the type series intact, (b) confirm that any specimens that share the same phenotype with the type series belong to *Triatoma* sp. aff *dimidiata,* and (c)

to determine the relationship with the other groups of *T. dimidiata* s.l., ITS-2 and cytB were sequenced for two out of the 20 *Triatoma* sp. aff. *dimidiata* specimens studied by Justi et al. (2018). Sequencing was performed as previously described by Dorn et al. (2016). For comparison, ITS-2 and cytB sequences including representatives from all *T. dimidiata* s.l. groups were retrieved from GenBank (Table 1) and aligned using the algorithm Q-INS-I implemented in the online MAFFT version 7 (Katohet al. 2017) and ClustalW (Larkin et al. 2007) implemented on MEGA v. 6 (Tamura et al. 2013), respectively. Maximum likelihood phylogenies were reconstructed independently for each of the genes using PhyML v.3.1 (Guindon and Gascuel 2003), with 100 bootstrap replicates. The best fit model for each gene, according to the AIC criterion, estimated using JModeltest (Darriba et al. 2015), were model HKY+I for ITS-2 and HKY + G for cytB. *Triatoma infestans* (Klug, 1834) was used as the outgroup. Both phylogenies were reconstructed using the same specimens for both markers, as indicated in the original studies. Phylogenies were plotted as mirror images using the function cophyloplot, from the package ape (Paradis et al. 2004), in R (R Development Core Team, 2013). Specimens photos and clade highlights were inserted using Adobe Photoshop CC 2108.

### Distribution map

Reported confirmed distributions of *T. huehuetenanguensis* sp. n. and specimens previously identified as *Triatoma* sp. aff. *dimidiata*, by molecular means were compiled (Table 2, Fig. 1) and GPS coordinates and altitudes were inferred using Google Maps. Localities were plotted on a map of Central America, using the packages plyr (Wickham 2011), raster (Hijmans 2012) and maps (Becker 2017) available in R (R Development Core Team 2008). The script is available in a supplementary file (Suppl. material 2: Script Huehuetenango map).

## Taxonomy

**Family Reduviidae Latreille, 1807**
**Subfamily Triatominae Jeannel, 1919**
**Genus *Triatoma* Laporte, 1832**

***Triatoma huehuetenanguensis* Lima-Cordón & Justi, sp. n.**
http://zoobank.org/F785B1DC-4946-4FAE-BA5D-D36CA413C67B

**Material. Holotype**: Male. GUATEMALA: Huehuetenango, La Democracia, Aldea Chamuxu, coordinates: 15.6333/-91.8667, 2 May 2016, C. Monroy and A. Rodas, National Museum of Natural History, Smithsonian Institution (voucher: USNMENT01241940). **Paratypes**: One female. GUATEMALA: Huehuetenango, San Pedro Necta, Caserio San Juan, coordinates 15.5/-91.76667, 1 June 2016, C. Mon-

**Table 1.** *Triatoma* specimens used to reconstruct the phylogeny, including collection location, sample identification, and GenBank accession numbers.

| Taxon | Locality | Sequence ID | ITS-2 | Cyt b |
|---|---|---|---|---|
| ***T. dimidiata*** | 1 | Sta. Theresa, Toledo, Belize | DQ871354 | FJ197155 |
| | 10 | Caserío la Bendición, Santa Ana, El Salvador | AM286693 | JN585881 |
| | 11 | Caserío la Bendición, Santa Ana, El Salvador | AM286693 | JN585881 |
| | 12 | Caserío la Bendición, anta Ana, El Salvador | AM286693 | JN585881 |
| | 13 | Caserío la Bendición, Santa Ana, El Salvador | AM286695 | JN585881 |
| | 14 | Caserío la Bendición, Santa Ana, El Salvador | KT874438 | JN585881 |
| | 15 | Sto. Tomás, Sto. Domingo, Heredia, Costa Rica | AM286693 | JN585893 |
| | 16 | Sto. Tomás, Sto. Domingo, Heredia, Costa Rica | AM286693 | JN585894 |
| | 17 | Sto. Tomás, Sto. Domingo, Heredia, Costa Rica | AM286693 | JN585894 |
| | 18 | Angeles, San Rafael, Heredia, Costa Rica | KF192843 | JN585894 |
| | 19 | Sto. Tomás, Sto. Domingo, Heredia, Costa Rica | KT874433 | JN585895 |
| | 2 | Mérida, Yucatán, Mexico | FJ197146 | FJ197157 |
| | 20 | Colombia | AM286703 | KT998309 |
| | 21 | Colombia | AM286703 | KT998309 |
| | 22 | Colombia | AM286704 | KT998309 |
| | 23 | Colombia | KF192845 | KT998310 |
| | 24 | Lanquin, Alta Verapaz, Guatemala | AM286702 | KT998313 |
| | 25 | Lanquin, Alta Verapaz, Guatemala | AM286702 | KT998314 |
| | 26 | El Lodo Negro, Intibuca, Honduras | AM286694 | KT998315 |
| | 27 | El Masical, San Antonio, Copán, Honduras | AM286694 | KT998316 |
| | 28 | El Masical, San Antonio, Copán, Honduras | AM286695 | KT998316 |
| | 29 | Caserío la Bendición, Santa Ana, El Salvador | AM286693 | KT998317 |
| | 3 | Lanquin, Alta Verapaz, Guatemala | AM286694 | JN585861 |
| | 30 | Caserío la Bendición, Santa Ana, El Salvador | AM286696 | KT998318 |
| | 31 | El Lodo Negro, Intibuca, Honduras | AM286695 | KT998319 |
| | 32 | El Masical, San Antonio, Copán, Honduras | AM286694 | KT998320 |
| | 33 | El Lodo Negro, Intibuca, Honduras | AM286693 | KT998321 |
| | 34 | El Lodo Negro, Intibuca, Honduras | KT874435 | KT998321 |
| | 35 | El Lodo Negro, Intibuca, Honduras | KT874437 | KT998321 |
| | 36 | El Masical, San Antonio, Copán, Honduras | AM286693 | KT998322 |
| | 37 | El Masical, San Antonio, Copán, Honduras | KT874436 | KT998322 |
| | 38 | El Lodo Negro, Intibuca, Honduras | AM286693 | KT998325 |
| | 39 | El Lodo Negro, Intibuca, Honduras | AM286694 | KT998325 |
| | 4 | Lanquin, Alta Verapaz, Guatemala | AM286702 | JN585861 |
| | 40 | El Lodo Negro, Intibuca, Honduras | AM286695 | KT998325 |
| | 41 | El Masical, San Antonio, Copán, Honduras | KT874434 | KT998325 |
| | 42 | Caserío la Bendición, Santa Ana, El Salvador | AM286693 | KT998327 |
| | 43 | Angeles, San Rafael, Heredia, Costa Rica | AM286693 | KT998328 |
| | 44 | Sto. Tomás, Sto. Domingo, Heredia, Costa Rica | KT874432 | KT998330 |
| | 45 | Angeles, San Rafael, Heredia, Costa Rica | KF192844 | KT998331 |
| | 46 | San Pedro Columbia, Toledo district, Belize | FJ197153 | FJ197154 |
| | 5 | Caserío la Bendición, Santa Ana, El Salvador | AM286693 | JN585881 |
| | 6 | Caserío la Bendición, Santa Ana, El Salvador | AM286693 | JN585881 |
| | 7 | Caserío la Bendición, Santa Ana, El Salvador | AM286693 | JN585881 |
| | 8 | Caserío la Bendición, Santa Ana, El Salvador | AM286693 | JN585881 |
| | 9 | Caserío la Bendición, Santa Ana, El Salvador | AM286693 | JN585881 |
| ***T. huehuetenanguensis*** | A10227 | Caserío San Pedro, Huehuetenango, Guatemala | MG947606 | MG951755 |
| | A10058 | Comunidad El Rosario, Huehuetenango, Guatemala | MG947605 | MG951754 |
| ***T.* sp. aff *dimidiata*** | 1 | Calla Creek, Cayo District, Belize | FJ197152 | FJ197156 |
| | 2 | Mérida, Yucatán, Mexico | FJ197150 | FJ197158 |
| | 3 | Mérida, Yucatán, Mexico | FJ197147 | FJ197159 |
| | 4 | Teya, Yucatán, Mexico | KT874439 | KT998296 |
| ***T. mopan*** | 1 | Río Frio Cave, Cayo District, Belize | KF192846 | JN585883 |
| | 2 | Río Frio Cave, Cayo District, Belize | KF192847 | JN585884 |
| ***T. infestans*** | | | AJ576054 | JN006799 |

**Table 2.** Localities where *T. huehuetenanguensis* (*Triatoma* sp. aff. *dimidiata*) was reported and corresponding longitude, latitude and altitude (meters above the sea level [m a.s.l.]).

| Locality | Longitude | Latitude | Altitude (m a.s.l.) | Reference |
|---|---|---|---|---|
| **Barrio La Unión, Huehuetenango, Guatemala** | -91.771511 | 16.261333 | 918 | Justi et al. 2018 |
| **Calkiní, Campeche, Mexico** | -90.0505156 | 20.3707299 | 16.61 | Dorn et al. 2016 |
| **Calla Creek, Cayo District, Belize** | -89.1338271 | 17.1257479 | 69.55 | Dorn et al. 2016 |
| **Caserío San Pedro, Cuilco, Huehuetenango, Guatemala** | -91.966667 | 15.4 | 1144.82 | This study |
| **Chablekal, Merida, Yucatan, Mexico** | -89.5756987 | 21.0961842 | 8.9 | Bargues et al. 2008 |
| **Chapalá, Cuilco, Huehuetenango, Guatemala** | -91.966667 | 15.4 | 1144.82 | This study |
| **Comunidad El Rosario, Huehuetenango, Guatemala** | -91.47826 | 15.31871 | 1889.08 | Justi et al. 2018 |
| | -91.966667 | 15.4 | 1144.82 | This study |
| **Cozumel island, Quintana Roo, Mexico** | -86.9223432 | 20.4229839 | 14.02 | Bargues et al. 2008 |
| **El Escondido, La Democracia, Huehuetenango, Guatemala** | -91.887456 | 15.622212 | 895.51 | Justi et al. 2018 |
| **El Paraiso, Yoro, Honduras** | -87.016667 | 14.983333 | 1753.10 | Bargues et al. 2008 |
| **El Triunfo, Poptún, Petén, Guatemala** | -89.4221967 | 16.3279338 | 521.54 | Dorn et al. 2016 |
| | -89.4221967 | 16.3279338 | 521.54 | Dorn et al. 2016 |
| **Holbox island, Quintana Roo, Mexico** | -87.2866995 | 21.5308421 | 3 | Bargues et al. 2008 |
| **Ixchehuex, Jacaltenango, Huehuetenango, Guatemala** | -91.759167 | 15.722778 | 959.91 | Justi et al. 2018 |
| **Izamal, Yucatan, Mexico** | -89.0227126 | 20.9299997 | 14 | Bargues et al. 2008 |
| **Las Galeras, San Antonio, Huehuetenango, Guatemala** | -91.771362 | 15.651625 | 1031.98 | Justi et al. 2018 |
| | -91.771362 | 15.651625 | 1031.98 | Justi et al. 2018 |
| **Los Chucles, La Democracia, Huehuetenango, Guatemala** | -91.88062 | 15.62151 | 895.51 | Justi et al. 2018 |
| **Los Encuentros, Poptún, Petén, Guatemala** | -89.4221967 | 16.3279338 | 512.87 | Dorn et al. 2016 |
| **Mérida, Yucatán, Mexico** | -89.59258 | 20.96737 | 10.72 | Dorn et al. 2016 |
| **Palenque, Chiapas, Mexico** | -91.9930466 | 17.5109792 | 67.08 | Bargues et al. 2008 |
| **Paraıso, Yucatan, Mexico** | -89.638845 | 21.0108241 | 9.97 | Bargues et al. 2008 |
| **Sabener, Cuilco, Huehuetenango, Guatemala** | -91.966667 | 15.4 | 1144.82 | Justi et al. 2018 |
| **San Luis, Petén, Guatemala** | -89.4399388 | 16.2000032 | 381.78 | Dorn et al. 2016 |
| **Subirama, Yoro, Honduras** | -87.4480115 | 15.2021628 | 843.60 | Bargues et al. 2008 |
| **Teya, Yucatán, Mexico** | -89.5220406 | 20.9358411 | 12.87 | Dorn et al. 2016 |
| **Yaxha, Peten, Guatemala** | -89.4024797 | 17.0734395 | 258.80 | Bargues et al. 2008 |
| **Yaxkukul, Yucatan, Mexico** | -89.4204 | 21.0615692 | 14.78 | Bargues et al. 2008 |

roy and A. Rodas, National Museum of Natural History, Smithsonian Institution, (voucher: USNMENT01241941). Two males. GUATEMALA: Huehuetenango, La Democracia, Aldea Chamuxu, coordinates: 15.6333/-91.8667, 2 May 2016, C. Monroy and A. Rodas, and GUATEMALA: Huehuetenango, San Antonio Huista, Canton Reforma, coordinates: 15.65/-91.7667, May 2016, C. Monroy and A. Rodas, Applied Entomology and Parasitology Laboratory- LENAP (ID: A10723 and A10685, respectively). Two females. GUATEMALA: Huehuetenango, La Democracia, Aldea Chamuxu, coordinates: 15.6333/-91.8667, 28 May 2016, C. Monroy and A. Rodas, and GUATEMALA: Huehuetenango, La Democracia, Aldea Chamuxu, coordinates: 15.6333/-91.8667, 28 May 2016, C. Monroy and A. Rodas, Applied Entomology and Parasitology Laboratory- LENAP (ID: A10800 and A10673, respectively).

**Etymology.** The name *Triatoma huehuetenanguensis* is in reference to the locality (Department of Huehuetenango, Guatemala) where the holotype and paratype specimens were collected.

**Differential diagnosis.** Specimens of *T. huehuetenanguensis* are classified as *T. dimidiata* following the key published by Lent and Wygodzinsky (1979). On closer examination, *T. huehuetenanguensis* differs from *T. dimidiata* in the following diagnos-

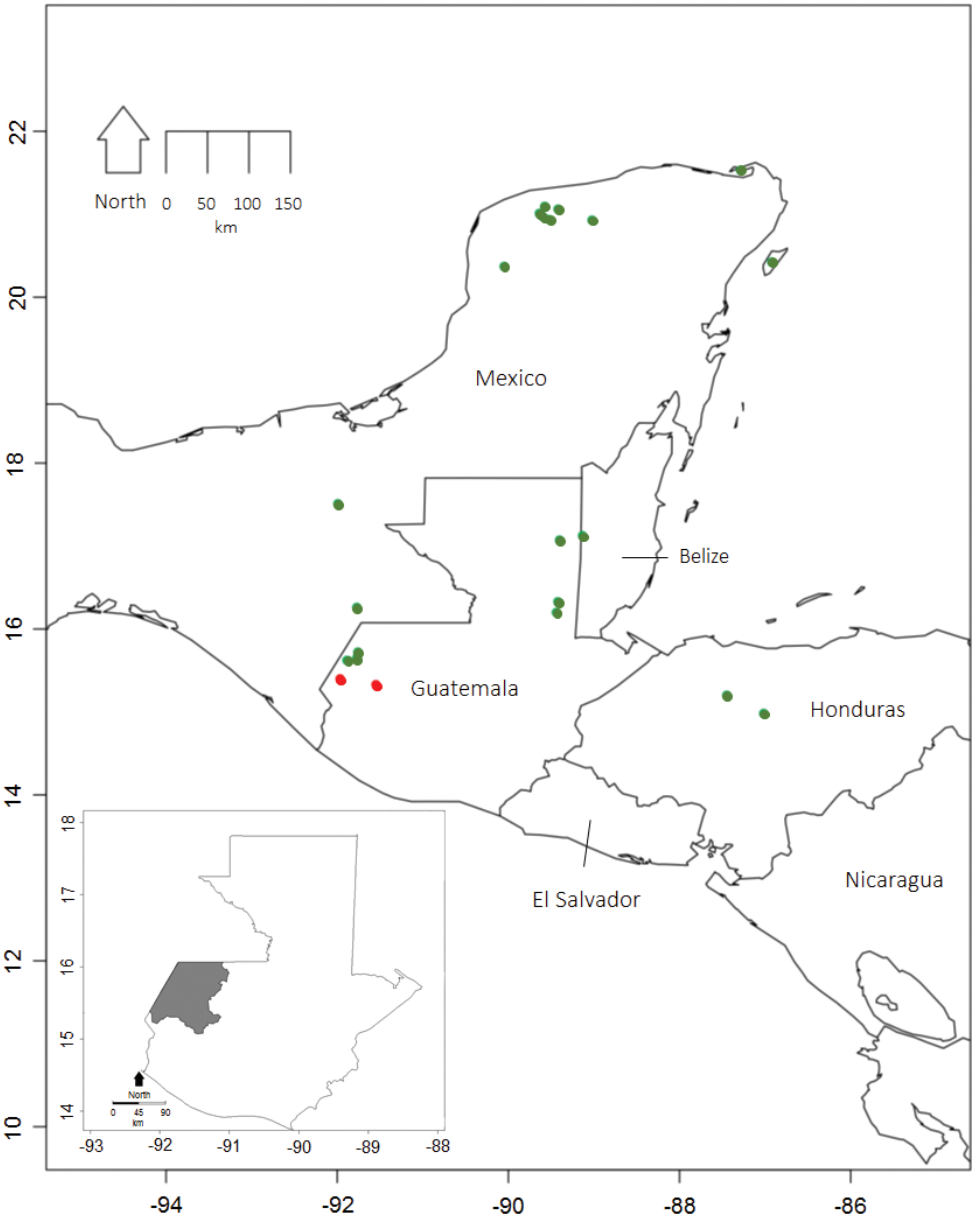


**Figure 1.** *Triatoma huehuetenanguensis* distribution map based on Bargues et al. (2008), Dorn et al. (2016), Justi et al. (2018) and this study. The red spots designate the places where the holotype and paratypes were collected, the green points refer to the locations where *T.* sp. aff. *dimidiata* was previously reported. Map insert highlights the department of Huehuetenango, where *T. huehuetenanguensis* holotype and paratypes were collected.

tic characters: overall color of connexivum, color of head pilosity, ocelli, setae in the second antennomere, anterolateral angles, labial articles joints, setae in the abdomen, spiracles, and female and male terminalia.

In contrast to the connexivum and corium color of *T. dimidiata* (pale yellow to orange yellow), *T. huehuetenanguensis* is brown, with connexivum and corium from yellow to pale yellow. The ventral color in *T. huehuetenanguensis* is light yellow, while in *T. dimidiata* it is piceous or black (Fig. 2). The setae around the abdomen are less dense in *T. huehuetenanguensis* when compared to *T. dimidiata*. In *T. huehuetenanguensis*, the spiracles are adjacent to the connexival suture, while in *T. dimidiata*, the spiracles are close but not adjacent to the connexival suture. In addition, spiracles are surrounded by a dark spot in *T. dimidiata*, while the spot is absent in *T. huehuetenanguensis* (Fig. 5). The first antennomere in *T. huehuetenanguensis* does not reach the apex of the head, whereas in *T. dimidiata*, it does. The setae in the second antennomere of *T. huehuetenanguensis* are not as dense as in *T. dimidiata* (Fig. 3). Anterolateral angles are laterally oriented in *T. huehuetenanguensis*, while in *T. dimidiata* they are anterolaterally oriented. The three labial articles of *T. huehuetenanguensis* are light colored while in *T. dimidiata* they are dark, similar to the dark body color. The joint of each of the labial articles are pale yellow only in *T. huehuetenanguensis* (Fig. 3). The collar is relatively thicker in *T. huehuetenanguensis* compared with *T. dimidiata*. The scutellum is rugose in both, *T. dimidiata* and *T. huehuetenanguensis*. However, the central scutellum area in *T. huehuetenanguensis* is more depressed as compared with *T. dimidiata* (Fig. 4). Legs in *T. huehuetenanguensis* with 1 + 1 subapical denticles, sometimes with a very small apical denticle on fore and/or mid-femora. Females sometimes with only one subapical denticle or a callosity, slightly lighter than the tegument on its proximal side. Fore and mid-femora of both males and females with lighter ventral subapical band, ranging from almost imperceptible to yellow.

The terminalia in males of *T. huehuetenanguensis* is almost square-shaped and darker than the rest of the tegument, presenting sparse dark pilosity, while in *T. dimidiata* it is ovoid and dark, presenting abundant dark pilosity. Posterior margin of urosternite VIII convex on *T. dimidiata* and almost straight in *T. huehuetenanguensis*. Posterior margin of urosternite IX slightly sinuous and not exceeding the abdomen on *T. huehuetenanguensis*, convex and exceeding the abdomen on *T. dimidiata*. Female terminalia in both species is triangle-shaped with very dark and dense pilosity. However, in *T. huehuetenanguensis* it is pale and very pointed while, in *T. dimidiata* it has rounded apex and is dark colored. Posterior margin of sternite VII sinuous on *T. dimidiata* and very sinuous in *T. huehuetenanguensis*; gonocoxite VIII (Gc8) pointed on *T. huehuetenanguensis* and rounded on *T. dimidiata*; gonapophysis VIII (Gp8) is wider than long in *T. huehuetenanguensis* compared to *T. dimidiata*. Gonocoxite IX (Gc9) strongly expanded exceeding the abdomen in *T. huehuetenanguensis* compared to *T. dimidiata* (Fig. 6).

**Description.** Overall color brown, connexivum, and corium yellow to light yellow. Pilosity short, distinctively yellow, covering whole body, except male and female terminalia, where pilosity is brown.

Total length, male 22.5–26.5 mm, female 22.2–29.3 mm; pronotum width, male 4.9–6.2 mm, females 4.9–6.4 mm; pronotum length, male 3.7–4.2 mm, female 3.4–4.5 mm (Table 3).

**Table 3.** Means and standard deviation (in parenthesis) of 17 morphological traits taken from *T. huehuetenanguensis* sp. n. and *T. dimidiata*.

| Morphological character | | *T. dimidiata* | | *T. huehuetenanguensis* | |
|---|---|---|---|---|---|
| | | ♀ (mm) | ♂ (mm) | ♀ (mm) | ♂ (mm) |
| **Total length**† | | 33.7 (1.42) | 32.6 (0.97) | 26.2 (2.29) | 25.2 (1.23) |
| **Width of pronotum**† | | 7.4 (0.51) | 7.5 (0.45) | 5.7 (0.42) | 5.7 (0.39) |
| **Width of abdomen**† | | 12.8 (1.31) | 11.9 (1.03) | 8.7 (0.97) | 8.4 (0.54) |
| **Head length**† | | 5.4 (0.33) | 5.3 (0.19) | 4.5 (0.33) | 4.5 (0.18) |
| **Width across eyes** | | 2.5 (0.23) | 2.6 (0.10) | 2.2 (0.15) | 2.1 (0.10) |
| **Length of pronotum**† | | 4.9 (0.32) | 5.1 (0.25) | 3.9 (0.39) | 3.9 (0.16) |
| **Ante ocular region** | | 3.0 (0.25) | 2.8 (0.08) | 2.5 (0.07) | 2.3 (0.14) |
| **Post ocular region** | | 0.8 (0.13) | 0.8 (0.08) | 0.8 (0.06) | 0.8 (0.10) |
| **Width of eye** | | 0.7 (0.11) | 0.8 (0.04) | 0.6 (0.07) | 0.6 (0.07) |
| **Synthlipsis** | | 1.0 (0.09) | 0.9 (0.06) | 0.9 (0.11) | 0.9 (0.10) |
| **Antennae** | 1st antennomere | 1.4 (0.14) | 1.4 (0.11) | 0.9 (0.08) | 1.1 (0.08) |
| | 2nd antennomere | 4.4 (0.42) | 4.8 (0.36) | 4.0 (0.46) | 4.3 (0.16) |
| | 3rd antennomere | 3.8 (0.16) | 3.6 (0.27) | 3.6 | 3.3 |
| | 4th antennomere | 3.0 (0.31) | 3.0 (0.30) | 0.0 | 2.8 |
| **Labium** | 1st article | 1.9 (0.23) | 1.8 (0.17) | 1.9 (0.14) | 1.8 (0.24) |
| | 2nd article | 3.5 (0.17) | 3.5 (0.25) | 2.8 (0.20) | 2.8 (0.17) |
| | 3rd article | 0.97 (0.05) | 0.9 (0.11) | 0.8 (0.03) | 0.9 (0.10) |
| | **n** | **15** | **10** | **8** | **11** |

†Statistically significant difference between *T. huehuetenanguensis* and *T. dimidiata* s. str. ($p < 0.01$, unpaired t-test). Standard deviation is in parenthesis.

Head dark brown with scarce yellow pilosity and overall smooth surface; central band with very shallow rugosity. Head length, male 4.1–4.7 mm, female 4.0–4.8 mm. Ocelli large, lighter than tegument, placed on a pronounced tubercle. Antenniferous tubercles subcylindrical, very short, situated in the middle of the anteocular region; first antennomere not attaining apex of head. Second antennomere lighter than first, with long setae. Ratio of antennomeres I–IV 1:3.9-4.4:3-4:2.5. Apex of clypeus distinctively lighter than rest of head. Labium (Fig. 3) slender, first article reaching level of apex of antenniferous tubercle; second article exceeding posterior border of head, attaining neck; third article light brown, with interarticular areas light yellow (Fig. 3). Ratio of labial articles 1: 1.6:0.5. Most labial setae short, not very numerous on first and second article. Third labial article reaching first third of stridulatory sulcus on males, half on females. Neck dark brown, with very smooth surface, and a pair of lateral yellowish spots.

Pronotum brown, with humerus blunt and pointed, usually lighter in color. Anterolateral angles short, almost round, laterally oriented, almost perpendicular to neck. Submedian carinae very pronounced, with tubercle aspect. Scutellum triangular, shallowly rugose, with the central area distinctly depressed, apical process sometimes lighter in color. (Fig. 4).

Hemelytra not reaching apex of the abdomen, darker at membrane, with scarce light yellow pilosity, dark brown spots around the intersection of the claval suture and

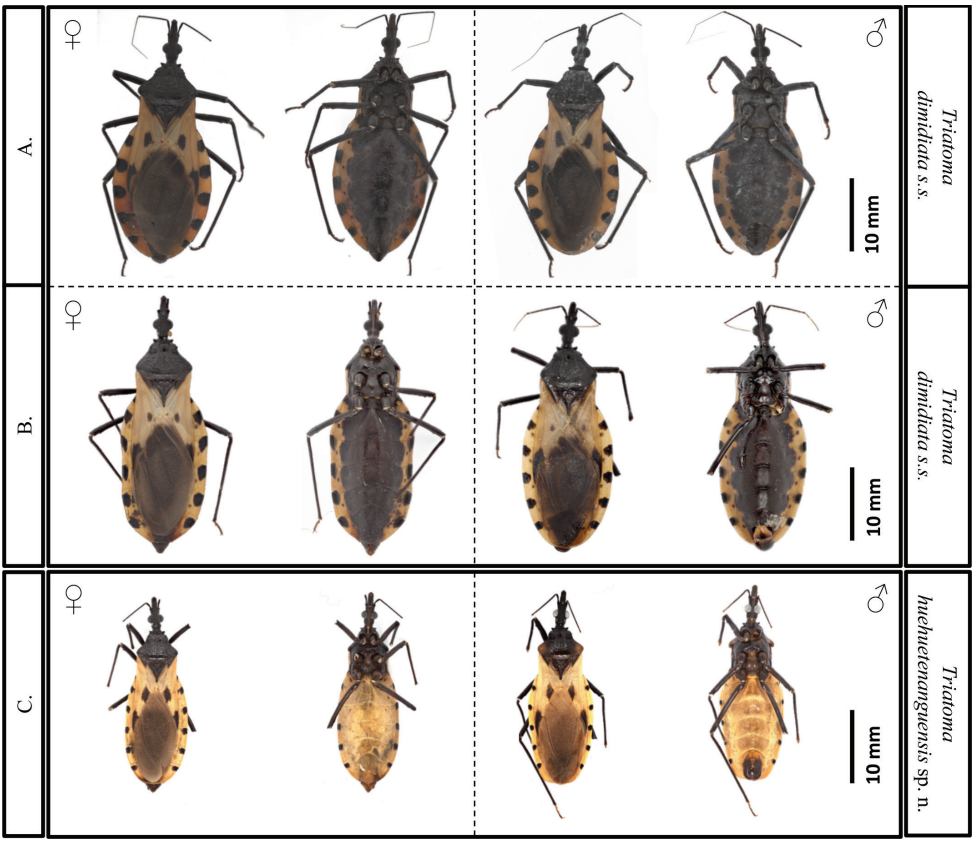


**Figure 2.** Comparison between *T. dimidiata* s. str. and *T. huehuetenanguensis* sp. n. **A** *T. dimidiata* female (left) and male (right) from Jutiapa (dorsal and ventral view) **B** *T. dimidiata* female (left) and male (right) from Huehuetenango (dorsal and ventral view) and **C** *T. huehuetenanguensis* sp. n. female (left) and male (right) from Huehuetenango (dorsal and ventral view). Photograph credits: RL and SJ.

the cubital vein, and two larger dark brown spots: one covering the posterior portion of the cubital and medial veins, and the other (largest) covering the joining of the radial and subcostal veins.

Abdomen ventrally convex, shortly pilose, yellow to light yellow (Fig. 3). Spiracles adjacent, but not touching connexival suture, not very pronounced, concolorous with rest of tegument. Connexival segments with a piceous or black spot covering the entire width of the anterior third, yellow posteriorly (Fig. 5).

Males terminalia almost square-shaped, darker than the rest of the body, with scarce dark pilosity. The posterior margin of urosternite VIII almost straight. Posterior margin of urosternite IX slightly sinuous and not exceeding the abdomen. Female external terminalia triangle-shaped, pale, with very dark, dense pilosity (Fig. 6). Posterior margin of sternite VII very sinuous; gonocoxite VIII (Gc8) pointed; gonapophysis VIII (Gp8) is wider than long. Gonocoxite IX (Gc9) strongly expanded exceeding the abdomen in *T. huehuetenanguensis* (Fig. 6).

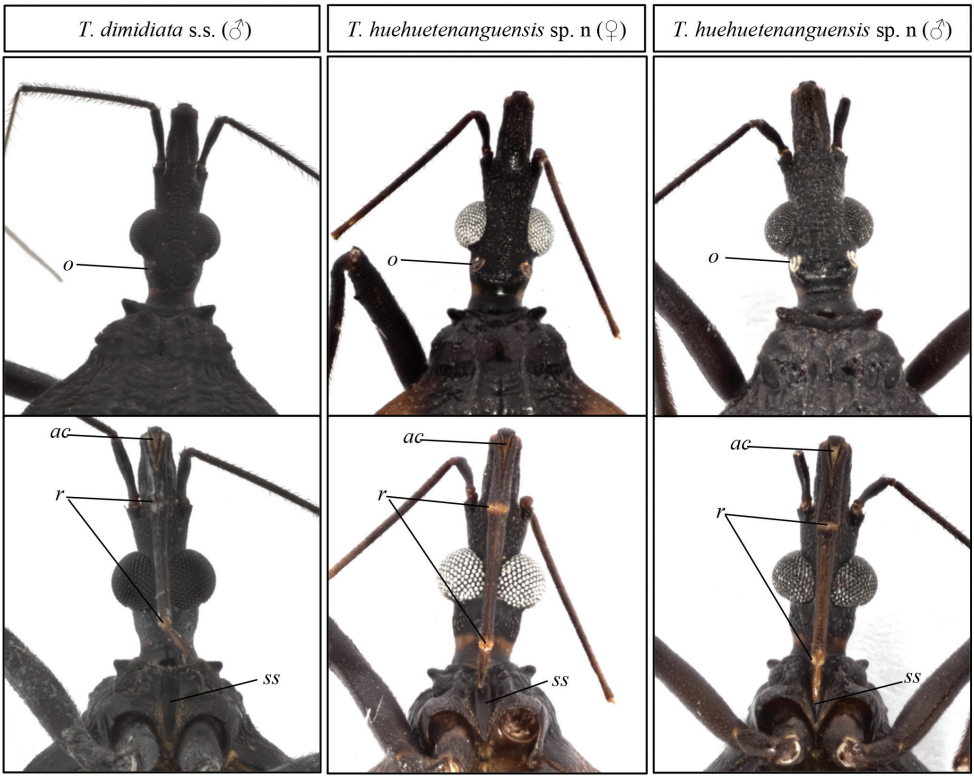


**Figure 3.** Heads of *T. dimidiata* s. str. and *T. huehuetenanguensis* sp. n. Top panel, dorsal view of the head. Bottom panel, ventral view of the head. Abbreviations: **o** ocelli, **ac** apex of clypeus, **ss** stridulatory sulcus, **r** connections between rostral segments. Photograph credits RL and SJ.

**Distribution.** Holotype and paratypes specimens of *T. huehuetenanguensis* were obtained by community participation and reported to be found in domestic environments, near to tropical forest. Huehuetenango is at the northwest of Guatemala and is characterized by pine forest. The altitude ranges from 300 to >3,000 m above sea level. Other localities (Table 1 and Fig. 1) were inferred from specimens collected for previously published molecular phylogenetics studies (Bargues et al. 2008, Dorn et al. 2016, Justi et al. 2018).

**Host-parasite data.** 18 out of the 20 specimens tested were found to be infected with *Trypanosoma cruzi*.

**Discussion.** The ecological diversity within the subfamily Triatominae (>150 species) and its wide distribution through the Americas, and particularly Latin America, have made it difficult to control vector-borne transmission of Chagas disease (WHO 2002). *Triatoma dimidiata* is one of the main vector taxa involved in Chagas transmission in Latin America, specifically the main vector for Central America and a secondary vector in Mexico and Colombia. Its broad geographic range and phylogenetic diversity have posed taxonomic challenges for many years (Dorn et al. 2007). Therefore, understanding the taxonomy, phylogenetic and ecological diversity of the *T. dimidiata* complex is important for understanding *T. cruzi* transmission.

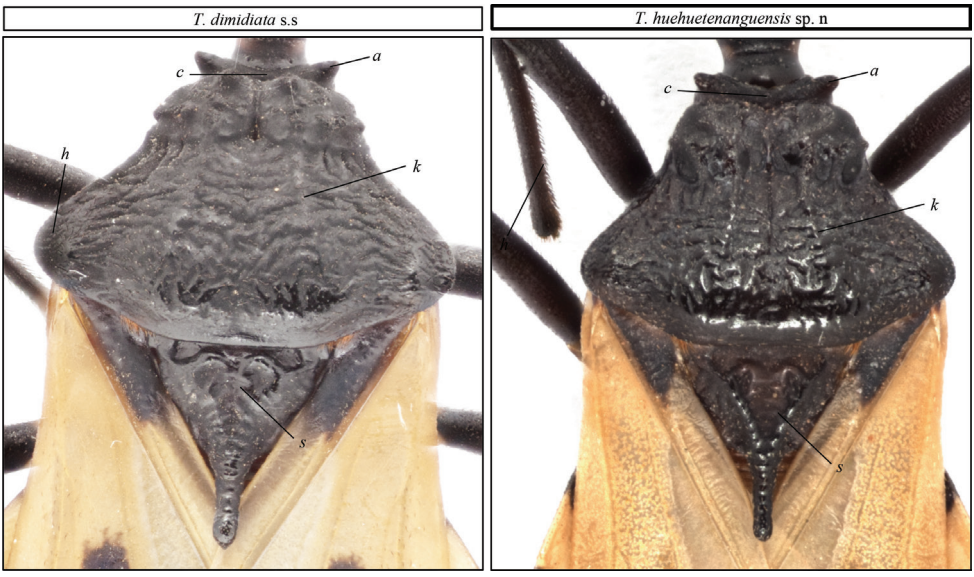


**Figure 4.** Pronotum of *T. dimidiata* s. str. (left), and *T. huehuetenanguensis* sp. n. (right). Abbreviations: **c** collar, **a** anterolateral angles, **h** humerus and **s** scutellum. Photograph credits RL and SJ.

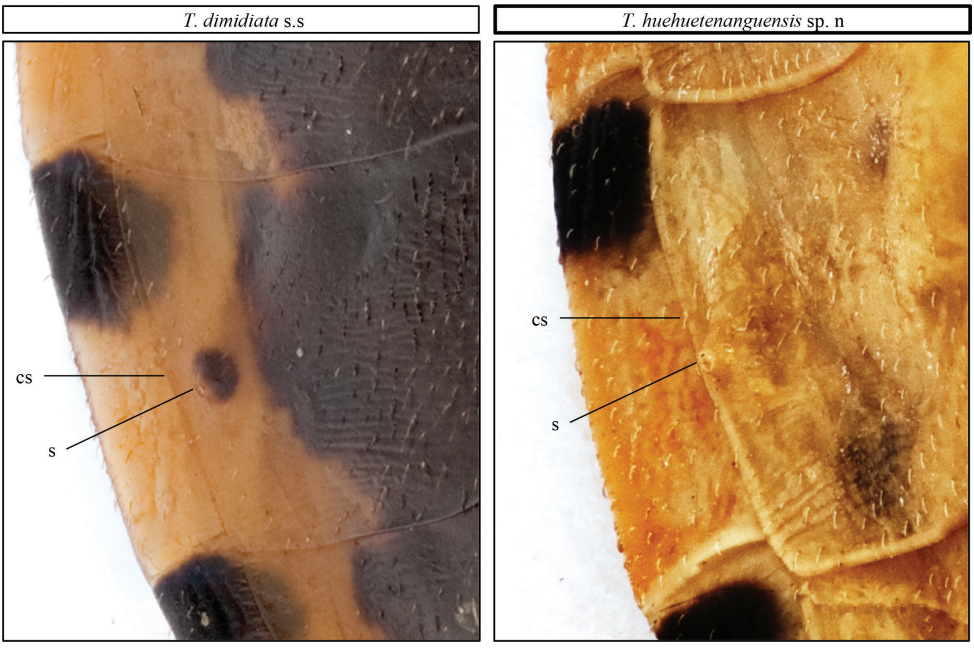


**Figure 5.** Ventral connexival plate and part of 4th urosternite of *T. dimidiata* s. str. (left), and *T. huehuetenanguensis* sp. n. (right). Abbreviations: **cs** connexival suture and **s** spiracles. Photograph credits RL and SJ.

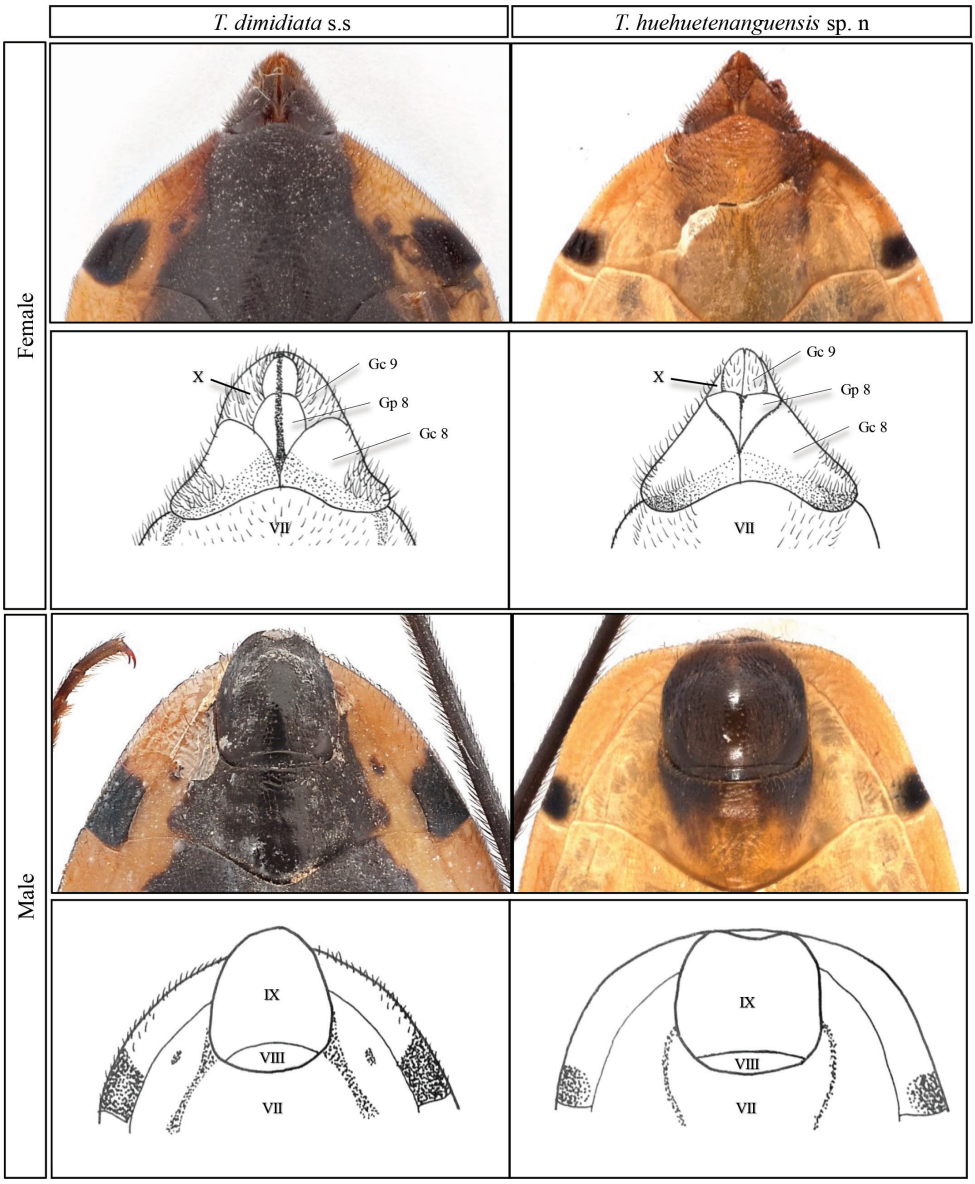


**Figure 6.** Comparison between the external terminalia of *T. dimidiata* s. str. and *T. huehuetenanguensis* sp. n. Abbreviations: **Gc 8** gonocoxite VIII; **Gc 9** gonocoxite IX; **Gp8** gonapophysis VIII; **VII** sternite; **IX** and **X** segments. Drawings RL. Photograph credits RL and SJ.

Here we are presenting three lines of evidence that support *T. huehuetenanguensis* as a distinct species: morphological, nuclear genetic (ITS-2) and mitochondrial genetic (cytB). The morphological characters included in the taxonomic key for *Triatoma* species (Lent and Wygodzinsky 1979) for *T. dimidiata* encompass *T. huehuetenanguensis*, specifically overall size and measurements of the head and

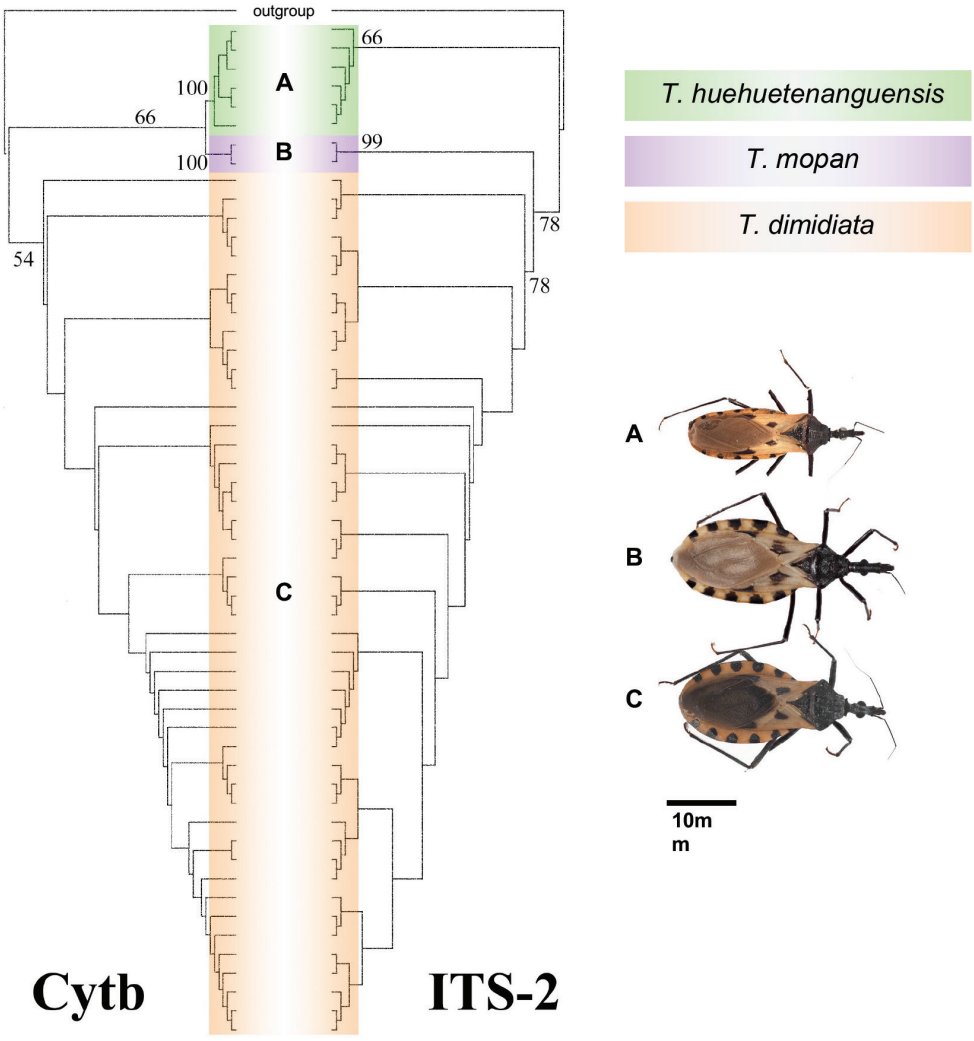


**Figure 7.** Maximum likelihood cytB and ITS-2 phylogenies. Bootstrap support values of the relevant clades are shown. Habitus of *T. huehuetenanguensis* and related species are shown to scale (10 mm).

eyes. However, on closer examination, macroscopic differences, including those summarized in Table 4, reveal *T. huehuetenanguensis* as morphologically different. Color differentiation on connexivum, pilosity, ocelli, labial articles joints, and female and male terminalia separate *T. huehuetenanguensis* from *T. dimidiata*. Differentiation based on the color pattern of the connexivum and other body regions was used in the description of *T. brailovskyi* (Martinez, Carcavallo & Pelaez, 1984), *T. gomeznunezi* (Martinez, Carcavallo & Jurberg, 1994) and most recently *Triatoma mopan* (Dorn et al., 2018). This latter study attributed the diminished pigmentation of *T. mopan* as the result to the cave environment. In our case, where both, *T. huehuetenanguensis* and *T. dimidiata* are found in sympatry and in domestic environments, it would be

**Table 4.** Ten distinguishing features between *T. dimidiata* and *T. huehuetenanguensis* sp. n.

| Feature | *Triatoma dimidiata*† | *Triatoma huehuetenanguensis* **sp. n** |
|---|---|---|
| **Connexivum overall color** | Dorsal part is pale yellow to orange yellow, and the ventral part is brown to black | Dorsal and ventral sides are yellow to light yellow |
| **Color of head pilosity** | Dark colored | Light yellow |
| **Ocelli** | Dark colored | Light colored, implanted in a very pronounced tubercle |
| **Setae in the 2nd** antennomere | Abundant | Scarce |
| **Anterolateral angles** | Anteriorly oriented, short and sub conical | Laterally oriented, almost perpendicular to the neck, very short and rounder |
| **Labial articles** | Joint dark colored | Joint yellow colored |
| **Setae in the abdomen** | Abundant and dark colored | Scarce and yellow colored |
| **Spiracles** | Close but not touching the connexival suture. Surrounded by a dark spot on the tegument. | Adjacent, but not touching the connexival suture. Same color as the tegument. |
| **Female external terminalia** | Triangle-shaped with rounded apex | Triangle-shaped with pointed apex |
| **Male external terminalia** | Ovoid shape | Square-shaped |
| | Limit of the 8th urosternite is curved | Limit of the 8th urosternite is straight |

† *T. dimidiata* features are based on Lent and Wygodzinsky (1979) and *T. dimidiata* s. str. (group 1–2) specimens from Jutiapa, Guatemala.

interesting to determine the process that caused such color differentiation between these two species. Another important morphological character is the location and pigmentation of the spiracles. These structures in *T. huehuetenanguensis* are adjacent to the connexival suture, whereas in *T. dimidiata*, they are close but not adjacent to the connexival suture. Differentiation based on the location and color of the respiratory spiracles was reported in the description of *Rhodnius montenegrensis* (Rosa et al. 2012) and most recently in the description of *Triatoma mopan* (Dorn et al., 2018).

The phylogenetic molecular analysis from the nuclear ITS-2 and the mitochondrial cytB gene, recovered a single monophyletic clade with high support (Fig. 7), and low genetic divergence (<3.1% for cytb and < 0.6% for ITS-2) indicating that *Triatoma dimidiata* auct., non (Latreille, 1811), *Triatoma* sp. aff. *dimidiata* (Bargues et al. 2008), *Triatoma* sp. aff *dimidiata* group 3 (Monteiro et al. 2013, Dorn et al. 2016, Justi et al. 2018) and *T. huehuetenanguensis* are the same species (see Suppl. material 3: cytB maximum likelihood phylogeny and Suppl. material 4: ITS-2 maximum likelihood phylogeny). Previously published molecular phylogenies (Bargues et al. 2008, Monteiro et al. 2013, Dorn et al. 2016, Justi et al. 2018) have thoroughly described the ITS-2 and cybB diversity within and among the four groups that comprise *T. dimidiata* s. l. This allowed us to compare these two regions in two of the specimens used by Justi et al. (2018), and verify that they fall within the monophyletic clade. Two Triatominae species were differentiated based on molecular analysis of the mitochondrial gene (Cytb), *R. montenegrensis* (Da Rosa et al. 2012) and *Rhodnius marabaensis* (Souza et al. 2016).

As supported by our genetic data, we suggest the inclusion of *T. huehuetenanguensis* in the subcomplex *T. dimidiata*. Based on Justi and Galvao (2017), this subcomplex

**Table 5.** Distinguishing features between the species of *T. dimidiata* subcomplex based on Justi and Galvão (2017). This reference was used as the original description is not very detailed, and Lent and Wygodzinsky (1979) provide a much more detailed description of the morphology upon inspection of 160 specimens.

| Species | Reference | Features |
|---|---|---|
| *T. dimidiata* | Lent and Wygodzinsky (1979)* | First antennae segment attaining level of apex of clypeus; anterolateral angles anterolaterally directed; central area of the scutellum not depressed; spiracles close but not adjacent to connexival suture, connexivum dark. |
| *T. hegneri* | Lent and Wygodzinsky (1979) | Labium very short; abdomen flattened below with spiracles distant from connexival suture; venter and connexivum uniformly dark. |
| *T. brailovskyi* | Martinez et al. (1984) | Overall size small with very large eyes and ocelli, pronotum with an evident keel at the border, anterolateral angles short and subconical, fore and mid femora with 1 + 1 subapical denticles. |
| *T. gomeznunezi* | Martinez et al. (1994) | Antenniferous tubercle laterally covered with long setae and dorsally glabrous; neck polished and entirely black; corium dark brown with two basal and distal yellowish spots; venter convex but longitudinally flattened; venter black. |
| *T. mopan* | Dorn et al. (2018) | Pronotum without discal tubercles and presenting a straight latitudinal depression dividing it in half, fore-femora with 1+1 apical, small denticles, 2 +1 subapical denticles in both males and females; and 1+1 apical, small denticles, 2 +2 asymmetrical subapical larger denticles on males and 2 +2 larger, asymmetrical subapical denticles on females, and spiracles close adjacent to connexival suture, surrounded by a spot slightly darker then the tegument |
| *T. huehuetenanguensis* | This study | Short yellow pilosity in the whole body except in the genitalia; connections between each segment of the labium are very visible and light-yellow colored; color of venter light yellow. |

is comprised by: *T. dimidiata*, *T. hegneri*, *T. brailovskyi* and *T. gomeznunezi,* and more recently *T. mopan* has been added (Dorn et al. 2018). The most relevant differences between these six species are summarized in Table 5.

Relevant Triatominae species for human *T. cruzi* transmission are those that have evolved to live close to humans and have been found to be infected *T. cruzi* (WHO 2002). *Triatoma huehuetenanguensis* was collected in both peridomestic and intradomestic environments. The high natural infection with *T. cruzi* (> 90% of the specimens) suggests it is a potentially important vector and its role in human Chagas disease should be further evaluated.

## Acknowledgements

Salvador Castellanos at the Applied Entomology and Parasitology Laboratory (LENAP) for insect preservation. Lisa Chamberland and Laura Caicedo at the University of Vermont (UVM) for support and help with the Visionary Digital BK Laboratory System. Sara Cahan (UVM) for use of the Nikon stereoscope Model SMZ-1B. The personnel

of the Ministry of Health of Huehuetenango, especially to Mirna Sosa for their help in field collections. The reviewers who helped greatly improve this manuscript.

This manuscript was prepared in part while SAJ held a Postdoctoral fellowship from the National Science Foundation (NSF) grant BCS-1216193 as part of the joint NSF-NIH-USDA (United States Department of Agriculture) Ecology and Evolution of Infectious Diseases program; and, in part as a National Research Council Research Associate Awardee at the Walter Reed Army Institute of Research. The material published reflects the views of the authors and should not be construed to represent those of the Department of the Army, the Department of Defense or the Department of Agriculture.

Financial Support: This work was supported with a subsidy of the Ecohealth Initiative Program of the Center of Investigations for the Development of Canada (IDRC; http://www.idrc.ca/ecohealth) (Subsidy no 106531) to Carlota Monroy; a grant from the World Health Organization (Tropical Disease Research-World Health Organization grant, TDR –WHO ID# A10249) awarded to CM, by National Science Foundation (NSF) grant BCS-1216193 as part of the joint NSF-NIH-USDA (United States Department of Agriculture) Ecology and Evolution of Infectious Diseases program to CM, PD and LS and grant R03AI26268/1-2 from the National Institute of Allergy and Infectious Disease (NIAID) of the National Institutes of Health (NIH) to LS.

## References

Bargues MD, Klisiowicz DR, Gonzalez-Candelas F, Ramsey JM, Monroy C, Ponce C, Salazar-Schettino PM, Panzera F, Abad-Franch F, Sousa OE, Schofield CJ, Dujardin JP, Guhl F, Mas-Coma S (2008) Phylogeography and genetic variation of *Triatoma dimidiata*, the main Chagas disease vector in Central America, and its position within the genus *Triatoma*. PLoS Neglected Tropical Diseases 2(5): e233. https://doi.org/10.1371/journal.pntd.0000233

Becker RWA, Wilks AR (2018) Package "maps": Draw Geographical Maps. R package version (>=3.0.0). http://CRAN.R-project.org/pachage=maps

Chagas C (1909) Nova tripanozomiaze humana: estudos sobre a morfolojia e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen., n. sp., ajente etiolojico de nova entidade morbida do homem. Memórias do Instituto Oswaldo Cruz 1(2): 159–218. https://doi.org/10.1590/S0074-02761909000200008

Dorn PL, de la Rúa NM, Axen H, Smith N, Richards BR, Charabati J, Suarez J, Woods A, Pessoa R, Monroy C, Kilpatrick W, Stevens L (2016) Hypothesis testing clarifies the systematics of the main Central American Chagas disease vector, *Triatoma dimidiata* (Latreille, 1811), across its geographic range. Infection Genetics and Evolution 44: 431–443. https://doi.org/10.1016/j.meegid.2016.07.046

Dorn PL, Monroy C, Curtis A (2007) *Triatoma dimidiata* (Latreille, 1811): a review of its diversity across its geographic range and the relationship among populations. Infection Genetics and Evolution 7(2): 343–352. https://doi.org/10.1016/j.meegid.2006.10.001

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52(5): 696–704. https://doi.org/10.1080/10635150390235520

Hijmans R, van Etter J (2012) Raster: Geographic analysis and modeling with raster data. R package version 2.0–12.

Justi SA, Cahan S, Stevens L, Monroy C, Lima-Cordon R, Dorn PL (2018) Vectors of diversity: Genome wide diversity across the geographic range of the Chagas disease vector *Triatoma dimidiata* sensu lato (Hemiptera: Reduviidae). Molecular Phylogenetics and Evolution 120: 144–150. https://doi.org/10.1016/j.ympev.2017.12.016

Justi SA, Galvao C (2017) The Evolutionary Origin of Diversity in Chagas Disease Vectors. Trends in Parasitology 33(1): 42–52. https://doi.org/10.1016/j.pt.2016.11.002

Katoh K, Rozewicki J, Yamada KD (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics. https://doi.org/10.1093/bib/bbx108

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23(21): 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Lent H, Wygodzinsky P (1979) Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. Bulletin of the American museum of Natural History 163(3): 123–520.

Martinez A, Carcavallo RU, Jurberg J (1994) *Triatoma gomeznunezi* a new species of Triatomini from Mexico (Hemiptera, Reduviidae, Triatominae). Entomología y Vectores 1: 15–19.

Martinez A, Carcavallo RU, Pelaez D (1984) *Triatoma brailovskyi* nueva especie de Triatominae de Mexico. Chagas 1(2): 39–42.

Monteiro FA, Peretolchina T, Lazoski C, Harris K, Dotson EM, Abad-Franch F, Tamayo E, Pennington PM, Monroy C, Cordon-Rosales C, Salazar-Schettino PM, Gómez-Palacio A, Grijalva MJ, Beard CB, Marcet PL (2013) Phylogeographic pattern and extensive mitochondrial DNA divergence disclose a species complex within the Chagas disease vector *Triatoma dimidiata*. PLoS ONE 8(8): e70974. https://doi.org/10.1371/journal.pone.0070974

Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 20(2): 289–290. https://doi.org/10.1093/bioinformatics/btg412

Da Rosa JA, Rocha CS, Gardim S, Pinto MC, Mendonca VJ, Ferreira Filho JCR, Oliveira Costa de Carvalho E, Aranha Camargo LM, De Oliveira J, Nascimento JD, Cilense M, Almeida CE (2012) Description of *Rhodnius montenegrensis* n. sp. (Hemiptera: Reduviidae: Triatominae) from the state of Rondônia, Brazil. Zootaxa 3478: 62–76.

Schofield CJ, Galvao C (2009) Classification, evolution, and species groups within the Triatominae. Acta Tropica 110(2–3): 88–100. https://doi.org/10.1016/j.actatropica.2009.01.010

Souza ED, Von Atzingen NC, Furtado MB, de Oliveira J, Nascimento JD, Vendrami DP, Gardim S, da Rosa JA (2016) Description of *Rhodnius marabaensis* sp. n. (Hemiptera, Reduvii-

dae, Triatominae) from Para State, Brazil. ZooKeys 621: 45–62. https://doi.org/10.3897/zookeys.621.9662

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/msw054

Team RC (2013) R: A language and environment for statistical computing.

WHO (2002) Control of Chagas disease: Second report of the WHO Expert Committee (Vol. 2): World Health Organization.

WHO (2015) Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Weekly Epidemiological Record / Relevé épidémiologique hebdomadaire 90(06): 33–44.

Wickham H (2011) The split-apply-combine strategy for data analysis. Journal of Statistical Software 40(1): 1–29. https://doi.org/10.18637/jss.v040.i01

## Supplementary material 1

**Morphological measurements**

Authors: Raquel Asunción Lima-Cordón, María Carlota Monroy, Lori Stevens, Antonieta Rodas, Gabriela Anaité Rodas, Patricia L. Dorn, Silvia A. Justi

Data type: species data



Link: https://doi.org/10.3897/zookeys.820.27258.suppl1

## Supplementary material 2

**Script Huehuetenango map**

Authors: Raquel Asunción Lima-Cordón, María Carlota Monroy, Lori Stevens, Antonieta Rodas, Gabriela Anaité Rodas, Patricia L. Dorn, Silvia A. Justi

Data type: occurrence



Link: https://doi.org/10.3897/zookeys.820.27258.suppl2

## Supplementary material 3

**CytB maximum likelihood phylogeny**

Authors: Raquel Asunción Lima-Cordón, María Carlota Monroy, Lori Stevens, Antonieta Rodas, Gabriela Anaité Rodas, Patricia L. Dorn, Silvia A. Justi

Data type: phylogeny data



Link: https://doi.org/10.3897/zookeys.820.27258.suppl3

## Supplementary material 4

**ITS2 maximum likelihood phylogeny**

Authors: Raquel Asunción Lima-Cordón, María Carlota Monroy, Lori Stevens, Antonieta Rodas, Gabriela Anaité Rodas, Patricia L. Dorn, Silvia A. Justi

Data type: phylogeny data



Link: https://doi.org/10.3897/zookeys.820.27258.suppl4