

Distribution and infection of triatomines (Hemiptera: Reduviidae) by *Trypanosoma cruzi* in the state of Michoacán, Mexico

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An entomological study of triatomine species was carried out to assess their prevalence in 10 localities of the state of Michoacán, Mexico. Entomological indices were calculated to estimate the risk for vector-borne transmission of Trypanosoma cruzi to the human population in this area. Four triatomine species (Triatoma barberi, Triatoma dimidiata, Meccus pallidipennis and Meccus longipennis) were collected from the study area. This is the first report of M. longipennis and T. dimidiata in Michoacán. M. pallidipennis was significantly ($p < 0.05$) more abundant than any of the other species collected in the study area. Infection indices were greater than 50% for each of the four collected triatomine species. Significantly more triatomines were collected from intradomiciliary areas than from peridomiciliary or sylvatic areas. Infestation, crowding and density indices were low, whereas colonisation indices were high in five localities. The current vectorial conditions in the study area require continuous entomological and serological surveillance to diminish the risk of T. cruzi transmission to human populations.

Key words: Triatominae - Chagas disease - Michoacán - Mexico

In Mexico, at least 1.5 million inhabitants are infected with *Trypanosoma cruzi*, and this number rises by almost 70,000 new cases each year. The primary (96%) route of infection for *T. cruzi* in humans is vectorial transmission. There are more than 70 million people living in endemic areas who are at risk of becoming infected and at least 20 million more are put at risk by visiting these areas. Eight Mexican states are the source of 69% (931,643) of all serologically positive human cases of Chagas disease in Mexico and, of these, the state of Michoacán has almost 100,000 of those cases (Ramsey et al. 2003). In Michoacán, the presence of *Meccus* (former *Triatoma*) *pallidipennis* (Stål) and *Meccus mazzottii* (Usinger), which are of the Phyllosoma complex and proposed subspecies of *Meccus phyllosomus* (Burmeister) (Bargues et al. 2008, Martínez-Ibarra et al. 2008b, 2009, Martínez et al. 2010), are responsible for 67% of vectorial transmissions of *T. cruzi* to humans in Mexico (Zárate & Zárate 1985). There also exists *Triatoma barberi* Usinger, which is responsible for 3% of vectorial transmissions of *T. cruzi* to humans in Mexico (Ramsey et al. 2003).

Although Michoacán is a state with endemic *T. cruzi* infections, no recent entomological data on triatomines has been gathered. However, this is not the case for each of the neighbouring and western states (Colima, Guanajuato, Querétaro, Guerrero, Jalisco, Nayarit, Aguascalientes and Estado de México), where the study of vectors

for *T. cruzi* is considered important (Espinoza-Gómez et al. 2002, López-Cárdenas et al. 2005, Villagrán et al. 2008, Licón-Trillo et al. 2010, Martínez-Ibarra et al. 2010a, b, Medina-Torres et al. 2010, Rodríguez-Bataz et al. 2011). These studies have led to a clear epidemiological picture of Chagas disease in western and central Mexico, with the exception of Michoacán. Therefore, this entomological study was carried out in Michoacán to determine the current distribution of triatomines and their rates of infection with *T. cruzi* in this area.

MATERIALS AND METHODS

The study was performed during 2009. The following 10 localities from 10 municipalities were selected for the study: Tiquicheo (18°54'N 100°47'W) from the municipality of Tiquicheo, Tuzantla (19°32'N 100°37'W) from Tuzantla, Turicato (19°05'N 101°27'W) from Turicato, Taretan (19°23'N 101°57'W) from Taretan, Nuevo Urecho (19°12'N 101°50'W) from Nuevo Urecho, Nuevo Capirio (19°48'N 102°12'W) from Mugica, Crucero de Parácuaro (19°35'N 101°30'W) from Parácuaro, Los Limones (19°03'N 102°16'W) from Los Reyes de Salgado, San Isidro (19°06'N 102°51'W) from Tepalcatepec and La Palma (20°12'N 101°44'W) from the municipality of Venustiano Carranza (Figure). La Palma is located on the shores of Lake Chapala, which is the largest lake in Mexico. This area is known as the Ciénega region and it is composed of 16 municipalities epidemiologically relevant for Chagas disease (Gómez-Hernández et al. 2008). Approximately 80% of this area belongs to the state of Jalisco, while the remainder is within the Michoacán (SEGOB 2010). Interestingly, prior to this study, the region of La Palma in the Michoacán was the only area that had not been evaluated for the presence of triatomines.

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Triatomines have been found to exist in many of the study localities (Zárate & Zárate 1985), although the descriptions provided by the local health sanitary authorities were poor (Orozco-Mosqueda 2003). For this study, the localities selected represent the majority of Michoacán territory as well as the many diverse habitats of triatomines (pine forest, arid areas, rural and urban areas) (SEGOB 2010).

In total, 12 field trips (1 per month) lasting three days each were made to each village. Triatomines were searched for using a map of each locality and 8.3–38.1% of the households in each village were selected at random using the Epi Info 6.04 package (CDC, Atlanta, GA, USA). In each selected human dwelling, intradomiciliary and peridomiciliary areas, as well as natural ecotopes, were searched. Sampling was performed in all natural ecotopes of triatomines, which include bird nests, hollow trees and cracks, holes in the ground, railings, rock pile boundary walls, heaps of stones and hollows in caves. The presence or absence of triatomines in each residence was determined by the results of 20 min intradomicile and 20 min peridomicile timed manual collections of adult or nymphal triatomines. Daytime searches were conducted by spraying an irritant insecticide and then using a flashlight to look into cracks and crevices throughout the interior of the buildings, in cupboards, behind pictures on walls and under furniture and bedding. A team of three trained personnel conducted the searches on each house selected from each village. The intradomiciliary (domestic) area was defined as the interior of houses and attached buildings, which included all rooms circumscribed by the main walls of the dwelling where inhabitants normally sleep. The peridomiciliary (peridomestic) area was defined as the area surrounding the homestead, which was usually enclosed by a fence and often contained features such as rocks, mounds of construction materials, animal shelters and agricultural products. Consequently, human dwellings (intradomiciliary and peridomiciliary areas) included the area from the main building of the dwelling to the fences (20–30 m)

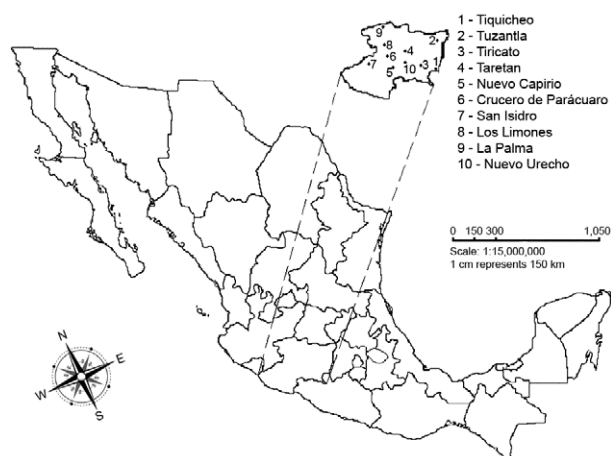
(Cohen et al. 2006). Sites beyond the fences were considered sylvatic habitats. In addition, 30 wire-net bait traps, each containing a Wistar rat, were used. These were similar to Noireau traps (Noireau et al. 2002) but were modified by Martínez-Ibarra et al. (2008a). Traps were placed overnight in 15 sylvatic and 15 peridomestic sites that included natural bug ecotopes described previously. Triatominae were collected by hand during the day, with the aid of flashlights, and were placed inside plastic containers labelled with collection data (place of capture and sex) using tweezers.

Triatomines were collected live and transported to the laboratory, where they fed on hens and were placed individually in Petri dishes until defecation. Triatomine infection with *T. cruzi* was determined by microscopic examination of the faeces. Parasites detected in the faeces were collected and intraperitoneally inoculated into Swiss mice. For a detailed description of *T. cruzi* infection in the area and among species, the infections were reported by species and instar. Adults were classified as males and females, younger nymphs were classified as first to third instars and older nymphs as fourth and fifth instars. Identification of triatomine species was made using the keys from Lent and Wygodzinsky (1979) and Alejandro-Aguilar et al. (1999), while taking into account the reclassification of the genus *Meccus* (Carvalho et al. 2000, Bargues et al. 2008).

The entomological indices were calculated for village and species using the methods of Silveira et al. (1984), whereas the crowding and density indices were calculated using a subsequent modification of the method (Pinto-Dias & Gonçalves-Diotaiuti 1998). Data were analysed using the Epi Info 6.04 package. Chi-square tests were used to compare distribution across several categories. Differences were considered significant when $p < 0.05$ and the Bonferroni correction was applied for appropriately sized groups.

RESULTS

In total, 835 triatomines of the genera *Triatoma* [*T. barberi* and *T. dimidiata* (Latreille)] and *Meccus* (*M. longipennis* and *M. pallidipennis*) were collected from the study area. This is the first report of *M. longipennis* and *T. dimidiata* in Michoacán. We were unable to collect *M. mazzottii* specimens, despite previous reports of this species in the study area. The most abundant species was *M. pallidipennis* (83.5%, $n = 835$), whereas the least abundant were *T. barberi* (3.1%) and *T. dimidiata* (1.2%). Rates of infection with *T. cruzi* were greater than 50% in all of the species examined. These were highest in *T. dimidiata* and *T. barberi*, but these differences were not significant (Table I). Significantly more total specimens were collected from intradomiciliary areas than from peridomiciliary or sylvatic areas. However, more specimens of *T. barberi* (85%, $n = 26$) were collected from peridomiciliary areas (Table II). Infestation indices were low (under 10%) in most localities, but were significantly greater in Taretan (23%, $n = 100$). Colonisation indices were approximately 20% or more in most localities, but again, were signifi-



Studied localities in the state of Michoacán, Mexico.

TABLE I

Natural infection (NI) by *Trypanosoma cruzi* by species and instars on triatomines collected in the state of Michoacán along 2009

Species	Presence of <i>T. cruzi</i> in triatomines										NI (%)
	♀		♂		Nymphs				Total		
					Younger		Older				
-	+	-	+	-	+	-	+	-	+		
<i>Meccus pallidipennis</i>	141	173	119	124	31	24	48	37	339	358	51.4
<i>Meccus longipennis</i>	14	30	14	13	6	7	9	9	43	59	57.8
<i>Triatoma barberi</i>	2	6	2	5	0	2	3	6	7	19	73.1
<i>Triatoma dimidiata</i>	2	4	1	3	0	0	0	0	3	7	70
Total	159	213	136	145	37	33	60	52	392	443	53.1

TABLE II

Triatomine species according to the collection area in the state of Michoacán along 2009

Triatomine species	n	Collection area					
		Intradomiciliary		Peridomiciliary		Sylvatic	
		n	%	n	%	n	%
<i>Meccus pallidipennis</i>	697	541	77.6	135	19.4	21	3
<i>Meccus longipennis</i>	102	73	71.6	25	24.5	4	3.9
<i>Triatoma barberi</i>	26	4	15.4	22	84.6	0	0
<i>Triatoma dimidiata</i>	10	10	100	0	0	0	0
Total	835	628	75.2	182	21.8	25	3

cantly greater in Taretan (91%, n = 23). Crowding indices were also low (greater than 8) in most of the communities and were greatest in Turicato (17.7) and Tuzantla (18.3). The density indices were less than 1 in most localities (Table III). Specimens of two sympatric species were collected from Taretan (*M. pallidipennis* and *T. dimidiata*) and La Palma (*M. longipennis* and *T. barberi*).

DISCUSSION

More than 800 triatomines were collected from the study area. This number is similar to that collected from the neighbouring state of Jalisco, where populations of the same species of triatomines as those studied here have recently been reported (Gómez-Hernández et al. 2008, Martínez-Ibarra et al. 2008a). The most common species found throughout the study area was *M. pallidipennis*, which was collected most often from houses with cement-covered adobe walls and tile roofs. Similar results were obtained from the northwestern neighbouring state of Colima and from the eastern neighbouring states of Estado de México and Guerrero (Espinoza-Gómez et al.

2002, Medina-Torres et al. 2010, Rodríguez-Bataz et al. 2011). *M. pallidipennis* is also present in the northeastern neighbouring state of Querétaro, in the western neighbouring state of Jalisco and in the northern of Guanajuato, where it has been sympatrically collected with *M. longipennis* (López-Cárdenas et al. 2005, Martínez-Ibarra et al. 2009). These three states are considered to be the western and northern limits for the distribution of *M. pallidipennis* and other species [*Triatoma mexicana* (Herrich-Scaeffler) and *M. longipennis*] are more common here (López-Cárdenas et al. 2005, Villagrán et al. 2008, Martínez-Ibarra et al. 2010a). Greater than 50% of the *M. pallidipennis* specimens analysed tested positive for *T. cruzi* infection, which demonstrates the epidemiological importance of this species in the study area. High infection indices for populations of *M. pallidipennis* have also been recorded in the states of Colima, Morelos, Jalisco, Estado de México and Guerrero (Espinoza-Gómez et al. 2002, Villegas-García & Santillán-Alarcón 2004, Martínez-Ibarra et al. 2008a, Medina-Torres et al. 2010, Rodríguez-Bataz et al. 2011).

TABLE III
Entomological indices^a per locality in the state of Michoacán along 2009

Locality	Houses		Indices				NI (%)
	Searched	Total at the locality (%)	Infestation	Colonisation	Crowding	Density	
San Isidro	70	32.7	11	25	7.8	0.9	66
Crucero de Parácuaro	90	35.6	8	29	8.0	0.6	18
Nuevo Urecho	93	34.1	5	20	10.6	0.6	45
Taretan	100	8.3	23	91	7.1	1.6	60
Nuevo Capirio	89	26.4	6	20	9.8	0.6	63
Turicato	93	22.7	8	14	17.7	1.3	50
Tiquicheo	83	13.1	8	29	8.6	0.7	55
Los Limones	94	38.1	10	20	8.6	0.9	44
La Palma	69	9.22	7	20	8.5	1.9	61
Tuzantla	92	18	3	33	18.3	0.6	54

a: Silveira et al. (1984); NI: natural infection.

M. longipennis was found only in the Ciénega region of La Palma and mostly was found inside houses built with cement-covered adobe walls and concrete roofs. Although this species has not previously been reported in Michoacán, its presence is not surprising because it has been collected from other parts of the Ciénega region and from other nearby areas in Jalisco (Gómez-Hernández 2008, Martínez-Ibarra et al. 2009). In contrast, no specimens of *M. mazzottii* were collected from the Ciénega region, where they have been previously reported (Zárate & Zárate 1985). However, some *M. longipennis* specimens collected had an unusual red colour (instead of the typical orange) in the connexivum, which is similar to the typical colouring of *M. mazzottii*. It is therefore possible that the previous identification of *M. mazzottii* in Michoacán (Zárate & Zárate 1985) may have been a mistake that was caused by the misidentification of atypical *M. longipennis* specimens.

M. longipennis was the most common species (79.7%, n = 128) in La Palma. This prevalence is similar to that previously reported for the Ciénega region (55.4%) (Gómez-Hernández 2008) and to other areas in Jalisco (Martínez-Ibarra et al. 2010a). Almost 60% of specimens were infected with *T. cruzi*, which is also similar to those previously studied in the Ciénega area (71.05%) (Gómez-Hernández et al. 2008) and in the neighbouring region of Jalisco (52%) (Breniere et al. 2010).

T. barberi was also found only in La Palma and was mainly found in exterior concrete walls and on amassed tiles. This species was not found in the eastern area of Tuzantla, although previous studies have reported its presence here (Zárate & Zárate 1985). *T. barberi* was found to be very scarce throughout the entire study area, which is consistent with previous reports from the western neighbouring state of Jalisco (Martínez-Ibarra et al. 2008a, 2010a). More than 70% (n = 26) of the *T. barberi* specimens were infected by *T. cruzi*. This value is

similar to infection rates found throughout the Ciénega region (87.8%) and from a neighbouring area of Jalisco (53.2%) (Martínez-Ibarra et al. 2010a).

This study is the first to report the identification of *T. dimidiata* and *M. longipennis* in Michoacán. However, *T. dimidiata* was found to be scarce throughout the study area. In Taretan, this species was only collected from two human dwellings made of concrete walls and tile roofs and it had an infection index of 70% (n = 10). The limited presence of *T. dimidiata* in Michoacán is compatible with the proposed existence of “islands” where this species is found that are considered epidemiologically unimportant (Ramsey et al. 2003). These exist in certain western (Colima, Jalisco), central (Guanajuato, Querétaro, Estado de México) and southern (Guerrero) states of Mexico (Espinoza-Gómez et al. 2002, López-Cárdenas et al. 2005, Gómez-Hernández et al. 2008, Villagrán et al. 2008, Medina-Torres et al. 2010, Rodríguez-Bataz et al. 2011). It is likely that the limited number of specimens collected in these areas had been passively transported by sugar cane (*Saccharum officinarum* L), as the sugar mill in Taretan receives sugar cane plants from some very distant locations that have not been evaluated for triatomine species. Similar phenomena have been recorded in Colima and Guanajuato, where *T. dimidiata* was likely transported to human dwellings on firewood (Espinoza-Gómez et al. 2002, López-Cárdenas et al. 2005).

More than 75% (n = 835) of the triatomines in this study were collected inside of human houses. This result is similar to that reported for *M. longipennis* in Jalisco and Aguascalientes and for *M. pallidipennis* in Estado de México (Licón-Trillo et al. 2010, Martínez-Ibarra et al. 2010a, Medina-Torres et al. 2010), which confirms that these species have adapted to exist in human dwellings in some areas of Mexico (Zeledón 1983). Indeed, approximately 100,000 inhabitants were reported to be infected with *T. cruzi* (Ramsey et al. 2003). It is likely that close

vector-host contact inside of human dwellings promoted the increased natural infection indices of triatomines in this study. On the other hand, more than 80% (n = 26) of *T. barberi* specimens were collected from peridomestic areas and this result is similar to the results of previous studies from areas near Colima and Jalisco. This finding confirms the secondary role of this species in the transmission of *T. cruzi* to humans in western Mexico (Espinoza-Gómez et al. 2002, Breniere et al. 2010).

Infestation indices were low in most locations studied, with the exception of Taretan, where more than one-fifth of the houses examined were infested. Crowding and density indices were also low and were similar to those calculated for the same species in Jalisco, with the exception of *T. dimidiata*. Also similarly to reports from Jalisco, the indices in this study were low in most localities because triatomines were generally collected from only a few houses in each location. These low indices could prove to be advantageous for spraying control efforts to effectively and inexpensively eliminate triatomines from houses (Martínez-Ibarra et al. 2008a, Breniere et al. 2010).

Taking into account each of the entomological indices calculated, the inhabitants of Taretan are at the greatest risk for vectorial transmission of *T. cruzi*. However, the presence of *M. pallidipennis*, *M. longipennis*, *T. barberi* and *T. dimidiata* in areas throughout Mexico highlights the potential risk for vectorial transmission elsewhere, as these have been shown to be important vectors for *T. cruzi* transmission (Cruz-Reyes & Pickering-López 2006). These data, together with the high infection indices and the large number of people infected (Ramsey et al. 2003) argue that continuous entomological and serological surveillance is required to better understand the routes of *T. cruzi* transmission to human populations within the study area.

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