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Distribution of *Triatoma* (*Meccus*) *phyllosoma* and *Triatoma* (*Meccus*) *longipennis* as vectors of *Trypanosoma cruzi* in the state of Aguascalientes, Mexico and surroundings

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ABSTRACT

Chagas disease is transmitted by hematophagous triatomine insects of the *Reduviidae* family. These vectors tend to hide in dark and humid places in homes in the endemic areas, mainly rural in Latin America. The Mexican territory is considered a critical endemic area, in recent years; more than 34 species of triatomines have been described as carriers of the disease, mainly the genera *Rhodnius*, *Paratriatoma*, *Eratyrus*, *Dipetalogaster*, *Belminus*, *Panstrongylus*, *Meccus*, and *Triatoma*. In Aguascalientes and Zacatecas's states, the observationand capture of the *Triatoma phyllosoma* and *Triatoma longipennis* species have been reported over three decades. Recent studies show that both species belong to the genera *Meccus*. This work summarizes the capture, identification, and analysis of *Triatoma* (*Meccus*) *phyllosoma* and *Triatoma* (*Meccus*) *longipennis* species as vectors of Chagas disease in the towns of Palo Alto, El Terrero de la Labor, Temazcal, Piedras Chinas, La Labor, Ojocaliente, Malpaso, Las Cabras and the center of the municipality of Calvillo in the state of Aguascalientes, Apulco and Jalpa in the state of Zacatecas, during March to August of the year 2019. Of a total of 252 collected bed bugs, 44% presented positivity as a carrier of *Trypanosoma cruzi*.

Simultaneously, the morphological identification showed that 66.66% corresponds to the *M. longipennis* species, and the remaining 33.34% is for the *M. phyllosoma* species.

Keywords: Chagas, *Meccus phyllosoma*, *Meccus longipennis*, Triatomines, *Trypanosoma cruzi*, Vector.

RESUMEN

La enfermedad de Chagas es transmitida por insectos triatominos hematófagos de la familia *Reduviidae*. Estos vectores tienden a esconderse en lugares oscuros y húmedos en los hogares de las zonas endémicas, principalmente rurales de América Latina. El territorio mexicano es considerado un área endémica crítica, en años recientes; se han descrito más de 34 especies de triatominos como portadores de la enfermedad, principalmente los géneros *Rhodnius*, *Paratriatoma*, *Eratyrus*, *Dipetalogaster*, *Belminus*, *Panstrongylus*, *Meccus* y *Triatoma*. En los estados de Aguascalientes y Zacatecas, la observación y captura de las especies *Triatoma phyllosoma* y *Triatoma longipennis* se han reportado durante tres décadas. Estudios recientes muestran que ambas especies pertenecen al género *Meccus*. Este trabajo resume la captura, identificación y análisis de especies de *Triatoma* (*Meccus*) *phyllosoma* y *Triatoma* (*Meccus*) *longipennis* como vectores de la enfermedad de Chagas en las localidades de Palo Alto, El Terrero de la Labor, Temazcal, Piedras Chinas, La Labor, Ojocaliente, Malpaso, Las Cabras y el centro del municipio de Calvillo en el estado de Aguascalientes, Apulco y Jalpa en el estado de Zacatecas, durante marzo a agosto del año 2019. De un total de 252 chinches recolectadas, 44% presentó positividad como portador de *Trypanosoma cruzi*. Simultáneamente, la identificación morfológica mostró que el 66,66% corresponde a la especie *M. longipennis* y el 33,34% restante corresponde a la especie *M. phyllosoma*.

Palabras clave: Chagas, *Meccus phyllosoma*, *Meccus longipennis*, Triatomines, *Trypanosoma cruzi*, Vector.

INTRODUCTION

Chagas disease, also known as trypanosomiases, is a life-threatening anthropozoonosis caused by the blood parasite *Trypanosoma cruzi*. It has been estimated that 8 million people are infected worlwide, and more than 65 million living in risk areas. This disease is transmitted mainly to humans and some

mammals (dogs, cats, rodents, marsupials, and primates) through the feces or urine of blood-sucking triatomine *Reduviidae* family insects. However, human infections can also occur in non-endemic areas due to blood transfusions, congenital route, organ transplantation, international migration, laboratory accidents, and the ingestion of beverages or food contaminated by the feces urine of the Triatoma [1, 2, 3, 4].

Human infections by *T. cruzi* present two phases of development; phase one, it is also known as acute phase, lasting up to 3 months, presenting parasitemia, fever, Romagna's sign and headaches. On the other hand, phase two, also known as chronic phase, develops even 10 years after infection (30-35% of infected cases), the parasite produces amastigote nests in various organs and peripheral nervous system, causing cardiac, nervous and digestive disorders [5, 6].

Triatomines hide in holes and slots of poorly built houses; this is one reason why Chagas disease is endemic to rural areas of Latin America. Triatomines are active at night, and is when they feed. Transmission occurs primarily through contact with the feces or urine of triatomines infected with the parasite [7], causing health problems in several countries of the American continent [4], mainly in Mexico, Brazil, and Argentina [2, 8].

Mexico is considered a country inside the endemic zone for the Chagas disease, with an estimate of more than one million infected people. Likewise, various species of mammals stand out as carriers of the parasitosis, these include dogs, cats and domestic rodents, which makes it an essential zoonotic disease and increases the risk to human health. [9, 10]. In different states of Mexico such as San Luis Potosí, Jalisco, Querétaro, Estado de Mexico, Oaxaca, and Chiapas, higher seroprevalence has been reported to humans; however, cases of seropositivity to *T. cruzi* have been described in all of Mexico [3].

In two-thirds of the Mexican territory, around 34 species of triatomines transmitting Chagas disease have been described, and these belong to the genera *Rhodnius*, *Paratriatoma*, *Eratyrus*, *Dipetalogaster*, *Belminus*, *Panstrongylus*, *Meccus*, and *Triatoma*; of which the most reported species belong to the genus *Meccus* and *Triatoma* [10, 11, 12, 13].

Concerning the genus *Meccus*, the most important species as transmitters are *M. bassolsae*, *M. longipennis*, *M. mazzottii*, *M. pallidipennis*, *M. phyllosomus*, and *M. picturata* [11, 13]. Within these vectors, the Phyllosoma complex comprises six species of the genus *Meccus*; *M. longipennis*, *M. mazzottii*, *M. pallidipennis*, *M. picturatus*, *M. bassolsae*, and *M. phyllosomus* [14]. On the

other hand, of the genus *Triatoma*, the most relevant species as transmitters of the parasite are *T. gerstaeckeri*, *T. mexicana*, *T. rubida*, *T. barberi*, and *T. dimidiata* [10, 11].

For the year 2019, the Mexican government reports that the species that transmit Chagas disease in Aguascalientes and Zacatecas are *Meccus longipennis* and *Meccus phyllosoma* (Supplementary figure 1) [15]. In previous years, the triatomine species *Triatoma* (*Meccus*) *phyllosoma* and *Triatoma* (*Meccus*) *longipennis* have been reported in the state of Aguascalientes and Zacatecas [11, 16, 17, 18]. The synonymy for the genera is explained in 2010, when Licón-Trillo *et al*. [19] reported the presence of *Meccus phyllosomus longipennis* = *Triatoma longipennis* in Mexican territory, and Rodríguez-Bataz *et al*., in 2011 [20] reported the same species as a carrier of *T. cruzi* in San Tadeo, Presa de Los Serna, Malpaso, Jatiche de Arriba and La Labor towns, in Aguascalientes, Mexico. In the same year, *Meccus longipennis* was reported as a species distributed in Aguascalientes and Zacatecas [11, 21]. For the case of *Triatoma phyllosoma*, Cortés-Jiménez *et al*. in 1996 [22], reported his findings for Aguascaliente's' state and in 2006 Cruz-Reyes and Pickering-Lopez [23] reported presence of *T. longipennis* and *T. phyllosoma* in the states of Aguascalientes and Zacatecas.

This work's objective was to determine the distribution of *Triatoma* (*Meccus*) *phyllosoma* and *Triatoma* (*Meccus*) *longipennis* in various localities of Aguascalientes and Zacatecas and to establish the relevance of these triatomines as vectors of Chagas disease.

METHODOLOGY

Collection areas

Triatomine collections were carried out in 9 towns in Calvillo's municipality, Aguascalientes (Figure 1); this geographical location has an average annual temperature of approximately 19.3 °C, with an average temperature increase to 23 °C between May and June [24]. It is known that the state of Aguascalientes has three types of climate: semi-dry temperate, semi-dry warm, and mild sub-humid with rains in summer. In these climates, the average annual temperature is 17.1, 20.1, and 14.5 °C, respectively, and the mean annual rainfall is 488, 579.1 and 688.3 mm, respectively. Particularly in Calvillo region (Aguascalientes) including all others Aguascalientes collection zones, the average annual temperature is 14-22 °C, and the mean annual average annual rainfall is 500-800 mm, the Apulco (Zacatecas) region the average annual temperature is 16-20 °C, and the mean annual average annual rainfall is 600-800 mm and finally the Jalpa (Zacatecas) region the average annual temperature is 14-24 °C, and the

mean annual average annual rainfall is 600-1000mm [25, 26, 27]. The state's arid zone's vegetation comprises secondary scrub and grasslands; the temperate zone is home to different oak forests or mixed forests (oak-conifers). The tropical area is mostly covered by secondary subtropical scrub [28].

On the other hand, collections were made in the municipalities of Apulco and Jalpa, belonging to Zacatecas (Figure 1). These collection areas were selected due to the records and capture of triatomines in central and northern Mexico and the sighting reported by its inhabitants with the help of the community participation method [11, 19]. It should be noted that the state of Zacatecas has a mainly dry climate and has an average annual temperature of 16 °C and moderate variations maximum 35 °C and minimum 6 °C; the average yearly rainfall is 510 mm, with a maximum of 910 mm and a minimum of 314 mm [29, 30]. In its territory, six vegetation types are distinguished: thorn forest, tropical deciduous forest, Quercus forest, coniferous forest, xerophilous scrub, and grassland [31, 32]. The collections were carried out from March to August 2019; besides, they were carried out at a specific time from 20:00 to 24:00; this was due to tonight's behavior feeding triatomines [33, 34]. The capture was carried out under a security protocol, which consists of avoiding direct contact, this using gloves and tweezers [35, 36]. The places where the searches were carried out were in stone fences, barns, chicken coops, abandoned houses, and near corrals and resting places for cattle [37].

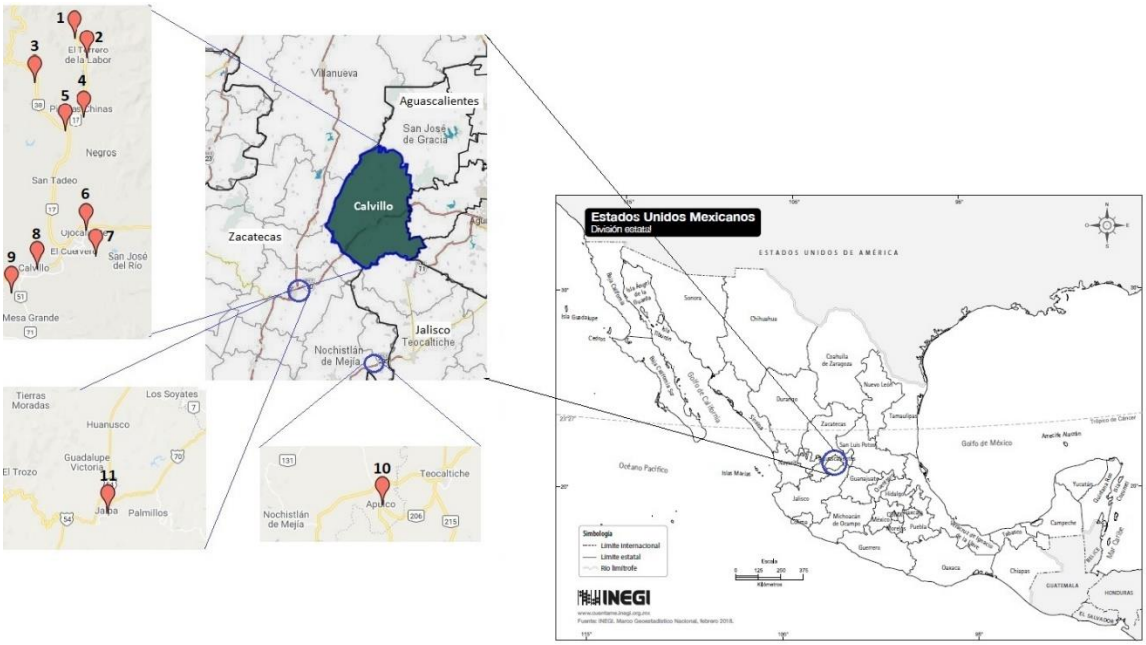

Figure 1. Collection locations in the states of Aguascalientes and Zacatecas, Mexico, the numbers correspond to location code in table 1.

Table 1. Locations and coordinates of the sampling carried out

LOCATION CODE	AGUASCALIENTES LOCATIONS	COORDINATES	ELEVATION (masl)
1	**Palo alto**	22°02’30.19’’ N y 102° 40’57.64’’O	1920 m
2	**El Terrero de la Labor**	22°1’28.999’’ N y 102° 40’ 20.20’’ O	1850 m
3	**Temazcal**	22°0’15’’ N y 102° 43’ 01.99’’ O	1950 m
4	**Piedras Chinas**	21°58’29.33’’ N y 102° 40’ 32.76’’ O	1785 m
5	**La Labor**	21°57’50.59’’ N y 102° 41’ 30.13’’ O	1750 m
6	**Ojocaliente**	21°52'51.07"N y 102°40'25.59"O	1705 m
7	**Malpaso**	21°51’34’’ N y 102° 39’ 50.999’’ O	1710 m
8	**Centro Calvillo**	21°50'56.80"N y 102°42'53.15"O	1628 m
9	**Las Cabras**	21°49'37.10"N y 102°44'19.40"O	1669 m
	ZACATECAS LOCATIONS		
10	**Apulco**	21°23'32.64"N y 102°40'31.72"O	1798 m
11	**Jalpa**	21°38'16.39"N y 102°58'29.85"O	1390 m

Following the method of Rubio *et al*. [38], with some modifications, all the individuals obtained were placed in glass jars, which contained folded paper to minimize the triatomines' stress and try to simulate their habitat conditions. Each container was covered with gauze and held firmly with an elastic band to allow the triatomine to breathe. Each bottle was labeled with the corresponding coordinates. Also, it was worked in an approximate radius of 100 meters [39, 40].

Maintenance and feeding

To keep alive and ensure the prevalence of triatomines, they were fed with rabbit blood; for this, it was placed in a dorsal position, and the flasks with the collected individuals were placed on its abdomen. The feeding time lasted approximately 60 min, and the light feeding area was covered to simulate the conditions in which the vectors feed [33, 41, 42].

Microscopic examination of feces

Immediately after feeding, the specimens were handled, taking into consideration all biosecurity measures [35, 36], execute an abdominal-ventral massage to each of them to stimulate the excretion of feces; each sample collected was diluted with 20 µL of PBS (pH of 7.2). Subsequently, the search for the *Trypanosoma cruzi* parasite in its metacyclic trypomastigote stage was carried out through the microscopic review of each sample with the help of a bright-field microscope (Leica DM750) at 40X magnification (Supplementary figure 2) [35, 43].

Morphological identification of triatomines

Various morphological characteristics were considered to identify the species of the collected triatomines, such as length, convex, type of abdomen, hemeliters, the color of the convex bands, and their anal socket [44].

RESULTS

Table 1 shows the localities of the states of Aguascalientes and Zacatecas where the samplings were carried out, the sampled areas present a similar elevation above sea level (masl); above 1,300 m but below 2,000 m. In the same way, these localities are adjacent or close to each other. A total of 252 triatomines were collected between April and August of 2019, of which 87.2% belong to the state of Aguascalientes and 12.8% to the state of Zacatecas (Table 2). Two species of triatomines were identified in the collection areas, being *Triatoma* (*Meccus*) *longipennis* and *Triatoma* (*Meccus*) *phyllosoma*, this with their morphological help characteristics. After microscopic observation (40X) of fecal samples, positives for the *Trypanosoma cruzi* parasite were recorded. According to size (between 15 and 45 µm), shape (flagellate), and stage (metacyclic trypomastigote). It resulted in 110 positive triatomines that correspond to 43.65% of the total individuals collected (Table 2).

Table 2. Number of triatomines and species collected in the different locations

Localities	Number of triatomines	*M .longipennis*	*M. phyllosoma*
Palo alto	21 (5)*	10 (5)	11
El Terrero de la Labor	27 (5)	24 (5)	3
Temazcal	21 (5)	21 (5)	0
Piedras Chinas	17 (0)	17	0
La labor	17 (2)	17 (2)	0
Ojocaliente	28 (21)	20 (15)	8 (6)
Malpaso	27 (11)	27 (11)	0
Centro Calvillo	1 (0)	0	1
Las Cabras	61 (44)	24 (22)	37 (22)
Apulco	29 (15)	23 (14)	6 (1)
Jalpa	3 (2)	1 (1)	2 (1)
TOTAL	252 (110)	184 (80)	68(30)

* ( ) represents the number of individuals positive for *Trypanosoma cruzi* after microscopic examination.

The triatomines identification showed that both species collected and identified are carriers of the *Trypasonoma cruzi* parasite. In *M. longipennis*, a 31.75% positivity was recorded, while for *M. phyllosoma*, it presented 11.90% positivity for the total number. In Las Cabras, a greater number of triatomines were found, being 61 total and 44 positives, and there is a higher prevalence of *M. phyllosoma*. Regarding the morphological identification of triatomines, 73% corresponds to the *M. longipennis*, while 27% corresponds to the *M. phyllosoma*.

DISCUSSION

Carrier vectors of *T. cruzi* (triatomines) are found mainly in Latin America's rural areas, being Mexico one of the countries with the greatest health problems due to these carrier insects [2, 7, 8]. It has been pointed out that the Mexican territory is considered endemic for more than 34 species of triatomines. Being, the genus *Meccus* and *Triatoma* species the most widely reported [10, 13]. Specifically, the Aguascalientes state is considered a place of a high risk of infection by *T. cruzi* due to the triatomes present in the area [45]. It is for the above reasons that it was decided to sample various rural areas of Aguascalientes and some nearby areas in search of triatomines.

Triatoma (*Meccus*) *longipennis* and *Triatoma* (*Meccus*) *phyllosoma* were found in the locations mentioned above. This information corresponds to what was reported in previous years regarding the sighting, capture, and identification of Aguascalientes species mentioned above and its surroundings [11, 16, 18, 19]. From the year 2010 [19], there is mention that the genus *Meccus* is synonymous or that it presents homology to the genus *Triatoma*; this happens again in 2012 [21]. But until 2019, Mexico's government reports *Meccus longipennis* and *Meccus phyllosoma* species in Aguascalientes and Zacatecas regions [15]. On the other hand, the specimens were collected between 1390 and 1950 masl. To this respect, it has been reported that the species different triatomine species carrying *T. cruzi* have been collected between 100 and 2360 masl in Mexican territory [11, 21, 46, 47], biotic characteristics consistent with this work.

It is essential to highlight that the different vectors of the *Hemiptera* family: *Reduviidae*, develop in a great variety of climates and temperatures; within the latter, the annual averages of the states where the collections were made oscillate between 14.5 and 20.1 °C with a record of average variations of up to 35 °C as a maximum [25, 26, 27, 29, 30]. Documents have determined that for the *Triatoma dimidiata* species, the sighting and capture have been reported in Campeche, Mexico. This state has an average annual

temperature of 26 °C [48]; this is higher than that of the previously mentioned areas. For the *Meccus pallidipennis* species, its capture was recorded in Guerrero, Mexico, a state with an average annual temperature of 18 °C [49]. These temperatures are similar to the yearly averages of Aguascalientes and Zacatecas, Mexico.

Before morphological identification of the triatomines, microscopic observations of the feces were made to determine the presence of *T. cruzi*. The results indicate that 43.65% of the total triatomines were positive for *T. cruzi*. Specifically, it was recorded that *Triatoma* (*Meccus*) *longipennis* presented 31.75% positivity. While for *Triatoma* (*Meccus*) *phyllosoma*, there was 11.90% positivity. Molina-Garza [50] report that approximately 59% of the triatomines of the *Triatoma gerstaeckeri* species analyzed were positive for the *T. cruzi* parasite. Likewise, Salazar-Schettino *et al.* [11] mentioned different percentages of triatomines carries *T. cruzi*, 37% and 72% of positivity in *Triatoma Barberi*, 4.1% and 34% in *Triatoma dimidiate*, 25% and 85% in *Meccus longipennis* and, 6.6% and 53% in *Meccus mazzottii*. All these data were obtained from collections and records in various states of the Mexican Republic. The percentages mentioned are similar or higher than those obtained for the two species under study. Finally, it should be noted that in 1993 100% positivity was reported in the *Meccus longipennis* specimens collected by Rubio [51] (n = 46), Calvillo, Aguascalientes; however, our collections indicate a lower percentage of positive triatomines for *T. cruzi*. The latter may be due to the number of samplings carried out, the number of individuals analyzed, the collection schedule, a difference of more than 20 years between both studies and the collection time.

CONCLUSIONS

The 43.65% of triatomines collected in the present investigation were positives for *T. cruzi*. *Triatoma* (*Meccus*) *longipennis* and *Triatoma* (*Meccus*) *phyllosoma* were identified as potentially transmitting Chagas disease vectors. It is recommended that in these areas and their surroundings, these vectors be sampled regularly to establish adequate controls; such as fumigations, specific areas for domestic animals and the construction of houses. Also, carry out analyzes in its human inhabitants to verify seropositivity and carry out more studies of the non-human reservoirs of the areas under research.

INTEREST CONFLICT

The authors of the present work do not present any conflict of interest regarding the project

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