Importance of Hybrids of *Meccus phyllosomus mazzottii*, and *M. p. pallidipennis*, and *M. p. phyllosomus* to the Transmission of *Trypanosoma cruzi* in Mexico

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**SUMMARY:** The time interval before beginning feeding, feeding time, and defecation delay for 3 Triatominae subspecies, *Meccus phyllosomus mazzottii* (Ma), *M. p. pallidipennis* (Pa), and *M. p. phyllosomus* (Phy) and their laboratory hybrids were evaluated. The mean time interval for beginning feeding was between 0.1 and 10.1 min for all nymphal instars in each cohort, with significant ($P < 0.05$) differences among hybrids and parental cohorts. Four (both MaPa and MaPhy) hybrid cohorts had similar mean feeding times to that recorded for one of their parental subspecies, but shorter than the other, whereas the remaining hybrid cohorts (both PaPhy) had longer feeding times than did both of their parental subspecies. The specimens of MaPa defecated later than the respective instars on their parental subspecies, whereas most instars of the remaining 4 hybrid cohorts (MaPhy and PaPhy) defecated earlier than the respective instars of *M. p. phyllosomus*. Between 40% and 50% of the defecation events occurred when feeding in MaPhy and PaPhy hybrid cohorts. Given these results, the hybrid cohorts were more effective vectors of *Trypanosoma cruzi* than their parental subspecies, which could indicate a potentially higher risk of transmission of *T. cruzi* to reservoir hosts.

**INTRODUCTION**

In Mexico, Chagas disease, which is caused by *Trypanosoma cruzi* Chagas, is considered one of the more important vector-borne diseases. It is estimated that more than five and one-half million Mexicans are infected with *T. cruzi* and that there are 69,000 new infections annually (1). Among the more than 30 species collected in Mexico, 6 belonging to the recently revalidated genus *Meccus* (2–4) are responsible for 74% of the *T. cruzi* vectorial transmission to humans (5). Among these 6 *Meccus* species, 3 are notable because of their high *T. cruzi* infection indices (6). *Meccus mazzottii* (Usinger) is distributed in 2 states in southern Mexico, *M. pallidipennis* is distributed in 9 states throughout western, central, and southern Mexico (6,7), and *M. phyllosomus* (Burmeister) is currently found in only 1 state in southern Mexico where it is considered an important vector (1,6). *Meccus phyllosomus mazzottii*, *M. p. pallidipennis*, and *M. p. phyllosomus* have interbred with each other under laboratory conditions, and fertile hybrids have been obtained (8,9). In addition, these 3 species are sympatric and have been repeatedly collected from the stone and wooden fences, stacks of firewood, and brick and cement houses (7,10,11) in the southern states of Oaxaca and Guerrero (Fig. 1). In those states, it has been estimated that more than 1,000,000 inhabitants are infected by *T. cruzi* (1). All of the species have been involved in a longstanding debate related to their proper taxonomic range. Historically, they were considered subspecies of *M. phyllosomus* (12). However, 37 years later, Lent and Wygodzinsky (13) reinstated their status as *bona fide* species, based entirely on morphological characters. The debate continued, and recent biological, morphological, and molecular evidence (3,4,9,14–16) lead to them being considered subspecies again, based on the concept that “subspecies are local populations that are recognizably different from each other but they are nevertheless considered to belong to the same species because they are observed to interbreed in nature or because it is inferred that they are likely to interbreed” (17). Consequently, *M. mazzottii*, *M. pallidipennis*, and *M. phyllosomus* will be considered subspecies of *M. phyllosomus* during this study. Laboratory hybrids of triatomine species have exhibited intermediate characteristics or have shown outstanding biological parameters provide them greater fitness than their parental species. This has been demonstrated for laboratory-bred hybrids of *Triatoma sherlocki* Papa, Jurberg, Carcavallo, Cerqueira, and Barata × *T. juazeirensis* Costa and Felix, and *M. phyllosomus × M. mazzottii* (Usinger) (18,19). Wild hybrids have been associated with resistance to insects, such as introgressed hybrid specimens of *T. platensis* Neiva from Uruguay and *T. delponteri* Román and Abalos from Argentina and Bolivia, all with some
sequences of ITS-1 and ITS-2 of *T. infestans* (Klug) (20). Wild hybrid specimens have been collected with higher entomological indices than "pure" species collected at the same time, including hybrids of the *M. p. pallidipennis* and *M. p. picturatus* from western Mexico and those of distinct ITS-2 groups of *T. dimidiata* Latreille (21,22). Human modifications to the environment may increase epidemiological risks, facilitate endemic disease emergence, and create suitable environments for integration and mating between different populations, potentially resulting in natural hybrids. Because the consequences of this natural hybridization are unknown, the need for further studies regarding hybrid behavior is imperative (23).

This study documented the feeding and defecation behaviors of hybrids of *M. p. mazzottii*, *M. p. pallidipennis*, and *M. p. phyllosomus* and compared them with those from parental lines. It was conducted as a first step in the estimation their efficacy of hybrids as transmission vectors of *T. cruzi* to human populations in Mexico and the assessment of the epidemiological importance of these distinct groups.

**MATERIALS AND METHODS**

**Biological material (Triatomines):** The individuals used in crossing experiments were third generation (F3) and were obtained from colonies previously established from Triatominae originating in 3 non-overlapping areas. A laboratory colony of *M. p. pallidipennis* that was established in 2013 from 41 specimens collected in Amlícingo (18°50′N, 98°49′W), Morelos, Mexico was used. A colony of *M. p. mazzottii* established in 2013 from 31 specimens from El Parotal, Guerrero (17°35′N, 100°59′W) and a colony of *M. p. phyllosomus* established in 2013 from 34 specimens from Santo Domingo Tehuantepec (16°17′N, 95°25′W) were also used. When initially collected, founders of each colony were identified following the Lent and Wygodzinsky (13) keys, taking into account the revalidation of the genus *Meccus* (2) and the corresponding morphological characteristics specific to each species. Colonies were maintained at 27 ± 1°C and 75 ± 5% relative humidity (rh), similar to the laboratory conditions in previously published studies on the biology of *M. p. mazzottii*, *M. p. pallidipennis*, and *M. p. phyllosomus* (24–26). They fed every two weeks on immobilized and anesthetized New Zealand rabbits. Rabbits were maintained under laboratory conditions (of space, food, water, cleanliness), handled, and anesthetized following Norma Oficial Mexicana NOM-062-ZOO-1999 (Technical guidelines for production, care, and use of laboratory animals) (27).

**Crossing experiments:** Reciprocal experimental crosses were achieved by placing 10 pairs from each set in plastic jars (5-cm diameter × 10-cm height) as follows: (A) *M. p. mazzottii* male and *M. p. pallidipennis* female, (B) *M. p. pallidipennis* male and *M. p. mazzottii* female, (C) *M. p. mazzottii* male and *M. p. phyllosomus* female, (D) *M. p. phyllosomus* male and *M. p. mazzottii* female, (E) *M. p. pallidipennis* male and *M. p. phyllosomus* female, and (F) *M. p. phyllosomus* male and *M. p. pallidipennis* female. The 3 parental lineages involved in the study were used as controls: (G) *M. p. mazzottii* male and *M. p. mazzottii* female, (H) *M. p. pallidipennis* male and *M. p. pallidipennis* female, and (I) *M. p. phyllosomus* male and *M. p. phyllosomus* female. Offspring of interspecific crosses were considered hybrids based on the definition of a hybrid "as the product of the crossing of individuals belonging to two unlike natural populations, principally different species" (28). The hybrids are hereafter referred by the following acronyms: MaPa (*M. p. mazzottii* × *M. p. pallidipennis*), MaPhy (*M. p. mazzottii* × *M. p. phyllosomus*), and PaPhy (*M. p. pallidipennis* × *M. p. phyllosomus*).

**Differences among subspecies in feeding and defecation behaviors:** Specimens were maintained within incubators at 27 ± 1°C and 75 ± 5% rh and were fed on every 2 weeks on New Zealand rabbits. Eggs from pairs in each cohort were grouped by date of oviposition for a week to initiate a cohort of 200 eggs per pair. After eclosion, groups of first instar nymphs were separated by cohort into individual plastic containers (5.5-cm diameter × 10.5-cm height), with a center support of absorbent cardboard. Three days after eclosion, each cohort of nymphs was fed on immobilized and anesthetized New Zealand rabbits during a 1-h period, subsequently blood meals, they were fed fortnightly. The observation period for nymphs started at the beginning of feeding, had concluded at the end of a 20-min post-feeding period. Feeding and defecation behaviors were recorded. The bugs were maintained in a dark incubator at same condition shown above under a 12/12 h (light/dark) regimen.

**Statistical analysis:** Variables having a normal distribution were compared using Student’s *t*-test or an analysis of variance (ANOVA). A nonparametric Kruskal-Wallis test was used to compare developmental cycle periods and the number of blood meals to molting in the studied cohorts, because the Bartlett’s test revealed unequal variances (*P* < 0 for all comparisons). Pairwise comparisons were performed using Dunn’s method. Sigma Stat 3.1 software (version 3.1 for Windows; Systat Software, San Jose, CA, USA) was used for statistical analysis. The significance of probability levels was set at a maximum of 5%.

**RESULTS**

The mean time interval between the presentation of
the blood meal and the beginning of feeding varied from 0.1 to 12.4 min with significant differences (P < 0.05) among the studied cohort. Both MaPa cohorts exhibited significantly (P < 0.05) shorter periods to the beginning of feeding than did their parental cohort, M. p. pallidipennis. Similarly, both cohorts of MaPhy and PaPhy began feeding in less time than did their parental cohort, M. p. phyllosomus. In pairwise comparisons, no significant differences (P ≥ 0.05) were recorded when MaPhy and PaPhy were compared with their parental cohorts of M. p. mazzottii and M. p. pallidipennis, respectively (Table 1).

Mean feeding times were longer than 10 min (10.9–12.4 min) for all instars of MaPa cohorts, as well as in their parental cohorts of M. p. mazzottii and M. p. pallidipennis, with differences significant (P < 0.05) only between the MaPa cohorts and M. p. mazzottii. For MaPhy cohorts, instar comparisons revealed that both MaPhy cohorts required significantly (P < 0.05) less time for feeding than all instars of M. p. mazzottii. In contrast, feeding times were similar (except for adults of both sexes) to those of M. p. phyllosomus. Finally, both PaPhy cohorts had significantly (P < 0.05) longer feeding times than both of their parental cohorts, except for adults of both sexes. Instars of M. p. mazzottii (except for adults) were significantly different (P < 0.05) than those of M. p. pallidipennis and M. p. phyllosomus, however, no significant differences (P ≥ 0.05) occurred between M. p. pallidipennis and M. p. phyllosomus (Table 2).

Mean defecation delay was less than 10 min for all instar nymphs and adults for 2 parental (M. p. mazzottii and M. p. pallidipennis) and 4 hybrid (both sets of MaPhy and PaPhy) cohorts. In contrast, both MaPa cohorts, as well as M. p. phyllosomus, required more than 13 min before defecating. Significantly (P < 0.05) shorter defecation delays recorded only for young (1st to 3rd instar) nymphs in the MaPhy and PaPhy cohorts relative to young nymphs of M. p. mazzottii and M. p. phyllosomus. Furthermore, significantly (P < 0.05) shorter defecation delays occurred for all instars of MaPhy and PaPhy cohorts compared with those of M. p. phyllosomus (Table 3). More than 50% of nymphs and adults in 4 of the hybrid cohorts (MaPhy and PaPhy) defecated when feeding or immediately thereafter, compared with 15–20% in both parental cohorts (data not shown).

**DISCUSSION**

The mean time interval before feeding began was similar or shorter for all nymphal instars of the 6 hybrid cohorts in comparison to the 3 parental cohorts. This suggests that the hybrid nymphs are as fast, or faster in many cases, begin feeding as the “pure” cohorts. Hybrid adult female and male descendants of crosses of the 3 subspecies exhibited a shorter time lapse before beginning to feed than did the M. p. pallidipennis and M. p. phyllosomus parental lines, which indicates an advantage for hybrid individuals. The shorter interval before feeding is an efficient feeding behavior, which increases the probability population survival.

### Table 1. Time (min) for starting a blood meal (Mean ± SD) in M. p. mazzottii, M. p. pallidipennis, M. p. phyllosomus, and their hybrids

| Instar | Hybrid | Within subspecies |
|--------|--------|-------------------|
|        | MaPa¹ | MaPa² | MaPhy³ | MaPhy⁴ | PaPhy³ | PaPhy⁴ | M. p. mazzottii | M. p. pallidipennis | M. p. phyllosomus |
|        |       |       |        |        |        |        |            |             |                      |
| NI     | 0.1 ± 0.1³ | 0.1 ± 0.1³ | 1.4 ± 1.2³ | 1.2 ± 1.1³ | 2.1 ± 2.0³ | 1.8 ± 1.6³ | 1.9 ± 0.5³ | 1.4 ± 1.1³ | 4.1 ± 1.3³ |
| NII    | 0.1 ± 0.1³ | 0.1 ± 0.1³ | 1.3 ± 1.8³ | 1.7 ± 1.2³ | 1.9 ± 1.4³ | 1.8 ± 1.7³ | 0.7 ± 0.3³ | 1.8 ± 1.1³ | 5.3 ± 2.7³ |
| NIII   | 0.2 ± 0.1³ | 0.2 ± 0.1³ | 1.1 ± 1.1³ | 0.9 ± 0.8³ | 1.6 ± 1.3³ | 1.8 ± 1.4³ | 1.0 ± 0.8³ | 1.7 ± 1.2³ | 5.7 ± 3.4³ |
| NIV    | 0.1 ± 0.1³ | 0.1 ± 0.1³ | 1.7 ± 1.4³ | 1.8 ± 1.4³ | 2.2 ± 1.7³ | 2.1 ± 1.7³ | 1.1 ± 0.9³ | 4.1 ± 2.1³ | 8.4 ± 5.5³ |
| NV     | 0.2 ± 0.1³ | 0.2 ± 0.1³ | 2.4 ± 1.9³ | 1.9 ± 1.5³ | 2.4 ± 1.9³ | 2.9 ± 2.8³ | 1.5 ± 0.8³ | 5.1 ± 2.1³ | 10.1 ± 6.8³ |
| female| 0.3 ± 0.1³ | 0.2 ± 0.1³ | 2.2 ± 2.9³ | 2.3 ± 2.0³ | 2.6 ± 1.8³ | 2.9 ± 2.5³ | 1.8 ± 0.9³ | 7.1 ± 4.2³ | 11.4 ± 7.7³ |
| male   | 0.5 ± 0.2³ | 0.7 ± 0.2³ | 2.8 ± 2.6³ | 3.1 ± 2.9³ | 2.4 ± 1.9³ | 2.1 ± 2.0³ | 2.1 ± 1.2³ | 8.3 ± 7.9³ | 12.4 ± 5.9³ |

¹³: Similar letters within a line indicate no significant differences (P ≥ 0.05).
1: Female, M. p. pallidipennis; 2: Female, M. p. mazzottii; 3: Female, M. p. phyllosomus.

### Table 2. Feeding times (min) (Mean ± SD) in M. p. mazzottii, M. p. pallidipennis, M. p. phyllosomus, and their hybrids

| Instar | Hybrid | Within subspecies |
|--------|--------|-------------------|
|        | MaPa¹ | MaPa² | MaPhy³ | MaPhy⁴ | PaPhy³ | PaPhy⁴ | M. p. mazzottii | M. p. pallidipennis | M. p. phyllosomus |
|        |       |       |        |        |        |        |            |             |                      |
| NI     | 12.0 ± 5.1³ | 12.3 ± 4.6³ | 11.2 ± 5.1³ | 12.1 ± 3.3³ | 20.1 ± 8.7³ | 20.1 ± 7.9³ | 24.5 ± 0.9³ | 11.1 ± 1.4³ | 11.3 ± 1.4³ |
| NII    | 10.9 ± 7.4³ | 10.8 ± 5.7³ | 11.9 ± 4.9³ | 12.6 ± 5.3³ | 21.1 ± 7.2³ | 22.1 ± 9.4³ | 19.9 ± 0.9³ | 13.1 ± 1.5³ | 10.5 ± 3.2³ |
| NIII   | 12.3 ± 6.8³ | 12.3 ± 4.9³ | 9.5 ± 4.9³ | 10.2 ± 6.7³ | 21.7 ± 8.8³ | 20.3 ± 7.1³ | 22.7 ± 0.8³ | 10.6 ± 3.2³ | 7.5 ± 3.5³ |
| NIV    | 12.4 ± 4.8³ | 11.9 ± 4.8³ | 10.1 ± 6.1³ | 9.9 ± 5.9³ | 20.4 ± 9.7³ | 20.3 ± 8.4³ | 25.4 ± 0.9³ | 11.9 ± 2.1³ | 8.3 ± 4.6³ |
| NV     | 11.9 ± 6.9³ | 12.1 ± 7.8³ | 10.4 ± 5.9³ | 9.8 ± 7.1³ | 19.9 ± 7.7³ | 21.1 ± 8.4³ | 27.7 ± 0.9³ | 14.8 ± 2.2³ | 11.2 ± 5.2³ |
| female| 11.9 ± 5.3³ | 10.9 ± 5.8³ | 10.7 ± 8.7³ | 10.4 ± 6.7³ | 19.7 ± 7.4³ | 20.2 ± 6.4³ | 24.5 ± 0.8³ | 20.1 ± 10.9³ | 20.1 ± 6.9³ |
| male   | 11.4 ± 6.2³ | 10.9 ± 7.2³ | 10.9 ± 9.4³ | 10.8 ± 8.5³ | 19.8 ± 6.4³ | 18.9 ± 6.8³ | 26.8 ± 0.5³ | 19.3 ± 9.6³ | 21.3 ± 4.6³ |

¹³: Similar letters within a line indicate no significant differences (P ≥ 0.05).
1: Female, M. p. pallidipennis; 2: Female, M. p. mazzottii; 3: Female, M. p. phyllosomus.
advantageous feeding behavior in female hybrid descendants could influence the transmission of *T. cruzi* because a greater number of females laying eggs will lead to a larger population of triatomines and, as a consequence, an increased chance of contact with a vector-reservoir host, which could lead to an increase in *T. cruzi* infection rates. Similarly short periods before the initiation of feeding were also observed in *Triatoma protracta* (Uhler), *Triatoma rubida* (Uhler), and *Triatoma recurva* (Stal), which are important vectors of *T. cruzi* in the United States (30–32), and *Eratyrus mucronatus* (Stal), a mainly sylvatic species in Brazil (33).

In only one hybrid cohort (PaPhy), all nymphal instars exhibited longer feeding times than those of both parental cohorts (*M. p. pallidipennis* and *M. p. phyllosomus*). However, mean feeding times were longer than 10 min for most instars in the 9 cohorts. Mean feeding times were similar to those of *T. rubida*, *T. protracta*, and *T. recurva* (30–32). According to Zeledón et al. (34), species that feed for more than 10 min should be considered important potential vectors of *T. cruzi* because prolonged vector-host contact could increase the probability of interrupted feeding, with the consequent risk of contact with vector feces. Therefore, MaPa cohorts should be considered important potential vectors of *T. cruzi*, as *M. p. pallidipennis* currently is, whereas MaPhy cohorts should be considered as important as *M. p. phyllosomus*. Finally, PaPhy hybrids could be more important potential vectors of *T. cruzi* than *M. p. pallidipennis* and *M. p. phyllosomus*.

The mean defecation delay was less than 10 min for all the studied instars in 6 of the 9 cohorts. Similarly short defecation delays were recorded for *T. rubida*, *T. protracta*, and *T. recurva*, and the 5th-instar nymphs of *T. boliviensis* Martínez, Chávez, Sossa, Aranda, Vargas, and Vidaurre (30–32,35). Zeledón et al. (34) proposed that triatomines that defecate before 10 min postfeeding should be considered potentially effective transmitters of *T. cruzi* because they are usually in contact with their host for at least that period. Overall, all instars in the 6 studied cohorts (*M. p. mazzottii*, *M. p. pallidipennis*, and both sets of MaPhy and PaPhy) should be considered effective vectors of *T. cruzi*. However, some specimens (primarily old instar nymphs [4th and 5th] and female and male subjects) of the 4 hybrid cohorts were notable because the specimens tended to defecate before finishing a blood meal or immediately thereafter in approximately 40% (>50% in old nymphs and adults) of the defecation events. In contrast, this only occurred in 15–20% of defecation events in *M. p. mazzottii* and *M. p. pallidipennis*. This behavior increases the *T. cruzi* transmission potential of the hybrid cohorts in comparison to that of the 3 parental cohorts.

The studied parameters led us to the conclusion that the 3 subspecies and their hybrids have potentially different levels of efficacy in transmitting *T. cruzi* to human populations where they currently exist. According to the results for most of the parameters, the 6 hybrid cohorts appeared to be more efficient vectors than their parental species. The greater capacity to transmit *T. cruzi* to reservoir hosts and especially to human populations increases the importance of studying hybrids. Furthermore, as in different countries (Brazil, Argentina, and Mexico, among many others), current geographic barriers for some species and the extent to which human interference in the natural environment favor hybridization are unknown. These factors have been studied in *T. platensis*, *T. delpontei*, and *T. infestans*; *T. brasiliensis* Neiva and *T. juazeirensis*; *M. pallidipennis* (=*M. p. pallidipennis*), *M. picturatus* (=*M. p. picturatus*), and *M. longipennis* (=*M. p. longipennis*) (15,20,36). All these hybrids, in addition to their higher potential efficacy in transmitting *T. cruzi* to reservoir hosts than their parental lineages, may frequently colonize areas unfavorable to the parental cohort, widening the range of environmental parameters that the lineage can tolerate (36).

Studies related to the prevalence of infection by *T. cruzi* on triatomines, human beings, and wild and domestic reservoir hosts are currently being conducted in areas where natural hybrid populations of subspecies of *M. phyllosomus* have been recorded. Similarly, complementary biological studies on closely related species and subspecies of the genus *Meccus* and their hybrids are being developed by our team to elucidate the potential role of triatomine hybrids in the transmission of *T. cruzi* to human populations.

**Conflict of interest**  None to declare.

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