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Biological parameters of interbreeding subspecies of *Meccus phyllosomus* (Hemiptera: Reduviidae: Triatominae) in western Mexico

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Abstract

Understanding the biological parameters of some triatomine subspecies of *Meccus phyllosomus* (Burmeister) is a crucial first step in estimating the epidemiological importance of this group. Biological parameters related to egg eclosion, egg-to-adult development time, number of blood meals to moult, percentage of females at the end of the cycle, number of laid eggs, and the accumulative mortality for each instar of three *M. phyllosomus* subspecies [*Meccus phyllosomus pallidipennis* (Stål), *Meccus phyllosomus longipennis* (Usinger), and *Meccus phyllosomus picturatus* (Usinger)] as well as their laboratory hybrids were evaluated and compared. No significant differences ($P > 0.05$) were recorded among the experimental hybrids (*M. p. longipennis* × *M. p. pallidipennis*, *M. p. longipennis* × *M. p. picturatus*, *M. p. pallidipennis* × *M. p. picturatus*) and reciprocal cohorts. In five of the six studied parameters (egg eclosion, egg-to-adult development time, number of blood meals to moult, number of laid eggs and accumulative mortality), with the exception of the non-significant percentage of females obtained among all the studied cohorts, at least one of the parental cohorts in each set of crosses exhibited better fitness results than by those of their hybrid descendants. The lack of hybrid fitness in our study indicates the maintenance of reproductive isolation of parental genotypes. Moreover, the results lead us to propose that an incipient speciation process by distance is currently developing among the three studied subspecies, increasing the differences between them that modify the transmission efficiency of *Trypanosoma cruzi* to human beings in Mexico.

Keywords: Triatomines, hybrids, laboratory conditions, *Trypanosoma cruzi* transmission, Chagas disease

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Introduction

More than 30 species of triatomines have been collected in Mexico, including six *Meccus* species (Carabarin-Lima *et al.*,

2013). Together with the four other *Meccus* species (Hemiptera: Reduviidae), *Meccus pallidipennis* (Stål), *Meccus longipennis* (Usinger) and *Meccus picturatus* (Usinger), are thought to be the dominant Chagas disease vector species in Mexico, accounting for 74% of the vectorial transmission of *Trypanosoma cruzi* (Trypanosomatida: Trypanosomatidae), the causative agent of Chagas disease (Ibarra-Cerdeña *et al.*, 2009). Stål placed the first representatives of the group in the genus *Meccus*. However, in the middle of the 20th century, all the five described species were moved to the *Triatoma* genus, which currently includes additional Triatominae species (Lent & Wygodzinsky, 1979). Some years later, Carcavallo *et al.* (2000) revalidated the genus *Meccus*, taking into account morphological characteristics such as the structure and shape of the testicles. Recent molecular evidence (Martínez *et al.*, 2005, 2006; Bargues *et al.*, 2008, 2014; Espinoza *et al.*, 2013) has supported that revalidation, and all groups in this study will be considered members of the genus *Meccus*. Furthermore, another discussion involving these species focuses on their proper taxonomic range. More than 60 years ago, most species except for the sixth, then un-described member *Meccus bassolsae* (Alejandre-Aguilar, Nogueda-Torres, Cortez-Jiménez, Jurberg, Galvão, Carcavallo) were ranked as subspecies of *Meccus phyllosomus* (Mazzotti & Osorio, 1942). However, Lent & Wygodzinsky (1979) reinstated the other five as *bona fide* species based entirely on morphological characters. Since then, an argument about their proper taxonomic rank has persisted. Mayr & Diamond (2001) stated that 'subspecies are local populations that are recognizably different from each other but, nevertheless, are 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'. This definition fits with some studies on the reproductive behaviour of *M. longipennis*, *M. picturatus*, and *M. pallidipennis* (Martínez-Ibarra *et al.*, 2009). Because recent biological, morphological, and molecular evidence (Martínez *et al.*, 2006; Bargues *et al.*, 2008, 2014; Martínez-Ibarra *et al.*, 2008*b*, Espinoza *et al.*, 2013) has supported the rank of subspecies for *M. pallidipennis*, *M. longipennis*, and *M. picturatus*, they will be considered subspecies in this study. For many years, arguments about the differences between *Meccus phyllosomus pallidipennis*, *Meccus phyllosomus longipennis*, and *Meccus phyllosomus picturatus* have continued. These three groups are important vectors of *T. cruzi* in Mexico, however they have shown important differences among them in studied biological parameters related to percentage of egg eclosion, fecundity, egg-to-adult development time, feeding, and defecation patterns (Martínez-Ibarra *et al.*, 2012, 2013, 2015*d*). Those differences lead to important differences in their capacity as vectors of *T. cruzi* to human beings and, as a consequence, on their epidemiological importance in Mexico. Therefore, previously studied biological parameters have shown that *M. p. pallidipennis* is a more effective transmitter of *T. cruzi* than *M. p. longipennis* and *M. p. picturatus*, whereas this last species is the least effective of these three.

These three subspecies have been inter-crossed with each other under laboratory and wild conditions, and fertile hybrids have been obtained (Martínez-Ibarra *et al.*, 2008*b*, 2009). Some triatomine laboratory hybrids have displayed intermediate characteristics or have shown outstanding biological parameters that may confer higher fitness than their parental species (Almeida *et al.*, 2012; Chávez-Contreras *et al.*, 2013, Martínez-Ibarra *et al.*, 2015*b*, *c*). Wild hybrids have also been associated with resistance to insecticides or have shown higher entomological indices than 'pure' species collected (Martínez-Ibarra *et al.*, 2008*a*; Mas-Coma & Bargues, 2009). In contrast, some hybrids have had reduced fitness or viability compared with their parental lines (Herrera-Aguilar *et al.*, 2009).

Anthropogenic change and landscape heterogeneity may modulate *T. cruzi* transmission risk. These new ecological scenarios might facilitate endemic disease emergence, and create new suitable environments for integration and mating between species, which may potentially result in natural hybrids. Because the consequences of this natural hybridization are unknown, the need for further evaluation of hybrid fitness is imperative (Correia *et al.*, 2013).

In Mexico, the geographical distribution of the subspecies of *M. phyllosomus* currently in the domestic environment would be the result of the colonization of the sylvatic populations present in the natural surrounding environment (Breniere *et al.*, 2007). Some subspecies of *M. phyllosomus* have been sympatrically collected in different areas of Mexico because of anthropogenic and environmental changes. In western and central Mexico, *M. longipennis*, *M. p. picturatus*, and *M. p. pallidipennis* have been repeatedly sympatrically collected (including some hybrids) colonizing agro-pastoral environments recently created by deforestation. The most frequently colonized micro-habitats have been chicken roosts, pigsties and stone fences used habitually as borders for fields by rural inhabitants (Espinoza-Gómez *et al.*, 2002; Magallón-Gastélum *et al.*, 2004, 2006; López-Cárdenas *et al.*, 2005; Martínez-Ibarra *et al.*, 2001, 2008*a*, 2009; Bosseno *et al.*, 2009). In southern Mexico *Meccus phyllosomus mazzottii*, *M. p. pallidipennis* and *M. p. phyllosomus* have been sympatrically collected in stone and wood fences, in firewood piles and inside brick and cement houses (Ramsey *et al.*, 2000; Rodríguez-Bataz *et al.*, 2011). All those studied subspecies of *M. phyllosomus* have been reported as feeding primarily on the abundant human beings, dogs, and rodents (Bosseno *et al.*, 2006; Mota *et al.*, 2007; Rabinovich *et al.*, 2011; Ibáñez-Cervantes *et al.*, 2013). These findings fit with the hypothesis that rather than innate preferences for host species, host use by kissing bugs is influenced by the habitats they colonize. Therefore, it has been established that host accessibility is a major factor that shapes the blood-foraging patterns of kissing bugs (Rabinovich *et al.*, 2011).

This study comparing the biological characteristics of *M. p. pallidipennis*, *M. p. longipennis*, and *M. p. picturatus* hybrids and their parental lines, was conducted as a first step in the assessment of the epidemiological importance of these distinct groups.

Material and methods

Biological material

The individuals used in crossing experiments were obtained from the third generation of previously established Triatominae colonies that originated in two non-overlapping areas. A laboratory colony of *M. p. pallidipennis* that was established in 2012 from 41 specimens collected in Amilcingo (18°50′N, 98°49′W), Morelos, Mexico was used. A colony of *M. p. longipennis* established in 2012 from 27 specimens from El Jocuixtle, Durango (23°25′N, 105°34′W) and a colony of *M. p. picturatus* established in 2012 from 26 specimens from Juan Gil Preciado (19°36′N, 105°02′W) also were used. When initially collected, founders of each colony were identified

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following the Lent & Wygodzinsky (1979) keys, the revalidation of *Meccus* also was taken into account (Carcavallo *et al*., 2000; Bargues *et al*., 2014) and the specimens corresponded to the typical morphological characteristics of each species. Colonies were maintained at 27 ± 1°C and 75 ± 5% relative humidity (RH) and under a 12/12 h (light/dark) regimen, similar to the laboratory conditions used in previously published studies that investigated the biology of the three subspecies (referred to as *M. pallidipennis*, *M. longipennis*, and *M. picturatus*) (Martínez-Ibarra *et al*., 2012, 2013, 2015*a*). Specimens were fed on immobilized and anesthetized (using 0.25 ml kg^{-1} of ketamine that was injected intramuscularly) New Zealand rabbits for a 1-h period on a fortnightly basis. Rabbits were maintained under laboratory conditions (of space, food, water, and cleanliness) and were handled and anaesthetized following Norma Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio (Technical guidelines for production, care, and use of laboratory animals) regulations (SAGARPA, 1999). Observance of the NOM-062-ZOO-1999 was fulfilled by the head of the Committee of Ethical Behaviour of the Centro Universitario del Sur.

Crossing experiments

To conduct the reciprocal experimental crosses in this study, 10 pairs from the following sets were placed in plastic jars (5 cm diameter × 10 cm height): (1) *M. p. pallidipennis* female and *M. p. longipennis* male, (2) *M. p. longipennis* female and *M. p. pallidipennis* male, (3) *M. p. longipennis* female and *M. p. picturatus* male, (4) *M. p. picturatus* female and *M. p. longipennis* male, (5) *M. p. pallidipennis* female and *M. p. picturatus* male, and (6) *M. p. picturatus* female and *M. p. pallidipennis* male. The three parental lineages involved in the study also were used as controls: (7) *M. p. pallidipennis* female and *M. p. pallidipennis* male, (8) *M. p. longipennis* female and *M. p. longipennis* male, and (9) *M. p. picturatus* female and *M. p. picturatus* male. 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' (Mayr & Ashlock, 1991).

Differences among subspecies

Specimens were maintained as previously described. To record fecundity, all crosses were checked daily for spermatophore elimination and copulation events. To assess egg fertility, eggs from each cross were collected for 30 days and incubated under previously described laboratory conditions.

Eggs from couples in each cohort were grouped by the date of oviposition for 1 week to initiate a cohort of 200 eggs each. Following eclosion, groups of first instar nymphs were separated by cohort individually into plastic containers (5.5 cm diameter × 10.5 cm height) with a centre support of absorbent cardboard. Three days after eclosion and every 2 weeks after, each cohort of nymphs were fed individually on New Zealand rabbits as previously described. The bugs were maintained in a dark incubator at 27 ± 1°C and 75 ± 5% RH under a 12/12 h (light/dark) regimen, and were checked daily for ecdysis or death. From the insects that completed development into adults, 10 adult couples from each cohort were placed in individual containers (5 cm diameter × 10 cm height), and were maintained as previously described to determine oviposition patterns.

In order to estimate biological fitness (individual health), all biological parameters of each hybrid cohort were compared with those obtained from the reciprocal cross and with those from the two parental lines.

Statistical analyses

Variables with a normal distribution were compared using Student's *t*-test or an analysis of variance. A non-parametric Kruskal–Wallis test was used to compare the developmental cycle periods and the number of blood meals to moult in the cohorts. Moreover, the Holm-Sidak method was used to compare the number of eggs laid per female. Pairwise comparisons were performed for comparisons among the studied subspecies using Dunn's method, and the chi-square test was used to compare frequencies. Sigma Stat 3.1 software (version 3.1 for Windows, Systat Software Inc., San Jose, CA) was used for statistical analysis. Results were considered to be statistically different when $P < 0.05$.

Results

Differences among subspecies

The egg eclosion rate was variable, mostly over 69%, except for *M. p. longipennis* × *M. p. picturatus* cohorts and *M. p. longipennis* parents. Non-significant differences ($X^2 = 0.06$, df = 2, $P > 0.81$) were recorded when the *M. p. longipennis* × *M. p. pallidipennis* cohort egg eclosion rate and that of its reciprocal cross were compared. Similarly, non-significant differences ($P > 0.05$) were recorded when each set of crosses was compared with its reciprocal cross. On the other hand, both *M. p. longipennis* × *M. p. pallidipennis* cohorts from reciprocal crosses had significantly ($X^2 = 38.87$, df = 2, $P < 0.001$) higher egg eclosion rates than *M. p. longipennis* parental line, but significantly ($X^2 = 36.05$, df = 2, $P < 0.001$) lower egg eclosion rates than *M. p. pallidipennis*. Significantly ($X^2 = 40.41$, df = 2, $P < 0.001$) lower egg eclosion rates also were detected when both sets of *M. p. longipennis* × *M. p. picturatus* cohorts were compared with the *M. p. longipennis* and *M. p. picturatus* parental lines ($X^2 = 43.37$, df = 2, $P < 0.0000001$). Likewise, significantly lower ($X^2 = 37.80$, df = 2, $P < 0.001$) rates were detected when *M. p. pallidipennis* × *M. p. picturatus* cohorts were compared with the *M. p. pallidipennis* parental line, but no significant differences ($P > 0.05$) were found when the hybrid was compared with the *M. p. picturatus* parental line (table 1). The average incubation period was approximately 19 days for all cohorts (data not shown).

The average egg-to-adult development time was highly variable, but was generally longer in the hybrid cohorts. Non-significant differences ($Q = 1.66$, df = 2, $P > 0.05$) were recorded when the *M. p. longipennis* × *M. p. pallidipennis* cohorts and reciprocal crosses were compared (table 1). Furthermore, non-significant differences ($P > 0.05$) were found when each set of crosses was compared with its reciprocal cross. Significantly longer average egg-to-adult development times ($Q = 6.03$, 4.99, df = 2, $P < 0.05$) were recorded for *M. p. longipennis* × *M. p. pallidipennis* cohorts compared with the *M. p. longipennis* and *M. p. pallidipennis* parental crosses. Similarly, significantly longer average egg-to-adult development times ($Q = 6.71$, 5.56, df = 2, $P < 0.05$) were found when *M. p. longipennis* × *M. p. picturatus* cohorts were compared

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Table 1. Percentage of egg eclosion, time of development (mean ± SD) from egg to adult, blood meals to moult (mean ± SD), percentage of accumulative mortality, percentage of obtained females and number of laid eggs (mean ± SD) of *Meccus phyllosomus longipennis*, *Meccus phyllosomus picturatus*, *Meccus. phyllosomus pallidipennis* and their hybrids.

Biological parameter	LoPa[1]	LoPa[2]	LoPi[2]	LoPi[3]	PaPi[1]	PaPi[3]	Pa	Lo	Pi
Egg eclosion rate (%)	73.96[a]	69.23[a]	34.19[b]	32.36[b]	72.01[a]	69.95[a]	87.18[c]	53.50[d]	76.81[a]
Time of development (days)	216.97 ± 57.75[a]	214.88 ± 49.97[a]	227.47 ± 57.89[a]	229.32 ± 62.78[a]	151.14 ± 26.48[b]	153.15 ± 31.76[b]	162.37 ± 21.51[b]	153.51 ± 18.74[b]	164.61 ± 19.39[b]
Average blood meals	11.33 ± 0.7[a,c]	10.97 ± 0.7[a,c]	10.4 ± 0.8[a,b]	10.7 ± 0.6[a,b]	9.27 ± 0.6[b]	10.1 ± 0.8[b]	11.97 ± 0.6[c]	14.87 ± 0.9[d]	8.38 ± 0.8[b]
Accumulative mortality (%)	36.66[a]	34.84[a]	60.60[b]	63.29[b]	29.76[a,c]	32.25[a,c]	21.34[c]	36.98[a]	48.78[b]
Biological parameter	LoPa[1]	LoPa[2]	LoPi[2]	LoPi[3]	PaPi[1]	PaPi[3]	Pa	Lo	Pi
Females (%)	65.78[a]	67.44[a]	46.15[a]	48.27[a]	49.15[a]	52.38[a]	54.28[a]	56.52[a]	52.38[a]
Average eggs laid	1.52 ± 0.3[a]	1.49 ± 0.3[a]	1.21 ± 0.4[a]	1.19 ± 0.5[a]	1.06 ± 0.3[a]	1.1 ± 0.6[a]	2.8 ± 1.0[b]	1.19 ± 0.6[a]	1.42 ± 0.7[a]

LoPa = *M. p. longipennis* × *M. p. pallidipennis*, LoPi = *M. p. longipennis* × *M. p. picturatus*, PaPi = *M. p. pallidipennis* × *M. p. picturatus*, Pa = *M. p. pallidipennis*, Lo = *M. p. longipennis*, Pi = *M. p. picturatus*.
Means in rows followed by the same letters are not significantly different ($P < 0.05$).
[1]♀*M. p. pallidipennis*.
[2]♀*M. p. longipennis*.
[3]♀*M. p. picturatus*.

with *M. p. longipennis* and *M. p. picturatus* parental crosses. However, non-significant differences ($Q = 2.80$, 2.66, df = 2, $P > 0.05$) were recorded when *M. p. pallidipennis* × *M. p. picturatus* cohorts and the reciprocal crosses were compared with the *M. p. picturatus* and *M. p. pallidipennis* parental cohorts (table 1).

The average number of blood meals to moult to the next instar did not differ significantly ($Q = 1.12$, df = 2, $P > 0.05$) when the hybrid cohorts from each cross and the reciprocal crosses were compared. Interestingly, hybrid cohorts from crosses involving *M. p. longipennis* (*M. p. longipennis* × *M. p. pallidipennis* and *M. p. longipennis* × *M. p. picturatus*) required a significantly lower total number of meals ($Q = 6.29$, 4.25, df = 2, $P < 0.05$) to moult through each nymphal instar until the adult stage as compared with the *M. p. longipennis* parental cohort. In contrast, non-significant differences ($Q = 2.46$, 1.34, df = 2, $P > 0.05$) were recorded between the *M. p. pallidipennis* and *M. p. picturatus* parental lines. Regarding the *M. p. pallidipennis* × *M. p. picturatus* cohorts, they were only significantly ($Q = 6.71$, 6.66, df = 2, $P < 0.05$) lower in blood meals to moult to the next instar compared with the *M. p. pallidipennis* parental cohort (table 1).

Accumulative mortality was similar (X^2 = from 0.001 to 0.01, df = 2, $P > 0.05$) when each hybrid cohort from reciprocal crosses was compared. The percentage mortality of *M. p. longipennis* × *M. p. pallidipennis* cohorts was significantly ($X^2 = 4.22$, 4.24, df = 2, $P < 0.05$) lower than the *M. p. pallidipennis* parental line, but those hybrid cohorts were similar ($X^2 = 0.001$, df = 2, $P > 0.05$) to the *M. p. longipennis* parental line. Additionally, *M. p. longipennis* × *M. p. picturatus* cohorts had significantly higher mortality percentages ($X^2 = 7.75$, 8.1, df = 2, $P < 0.05$) than the *M. p. longipennis* parental line, and did not differ significantly ($X^2 = 2.60$, 2.69, df = 2, $P > 0.05$) from the *M. p. picturatus* parental line. Lastly, *M. p. pallidipennis* × *M. p. picturatus* cohorts ($X^2 = 1.61$, 1.7, df = 2, $P > 0.05$) were similar to the *M. p. pallidipennis* parental line, but showed significantly lower mortality percentages ($X^2 = 5.9$, 6.3, df = 2, $P < 0.05$) than the *M. p. picturatus* parental line (table 1).

Non-significant differences (X^2 = from 0.1 to 1.34, df = 2, $P > 0.05$) were found when the percentages of obtained females at the end of the life cycles of all cohorts were compared (table 1).

The mean number of eggs laid per female was similar (t = 0.322; df = 2, $P > 0.05$) for each hybrid cohort and its reciprocal. Moreover, *M. p. longipennis* × *M. p. pallidipennis* and *M. p. pallidipennis* × *M. p. picturatus* cohorts shown significantly ($t = 4.75$, 5.69, df = 2, $P = 0.003$, 0.002) lower mean number of eggs when compared with the *M. p. pallidipennis* parental cohort). In contrast, the differences between *M. p. longipennis* × *M. p. pallidipennis* and *M. p. longipennis* were not significant ($t = 0.435$, df = 2, $P > 0.05$) and the same was true for those of *M. p. pallidipennis* × *M. p. picturatus* as compared with *M. p. picturatus* ($t = 1.17$, df = 2, $P > 0.05$). Lastly, when *M. p. longipennis* × *M. p. picturatus* cohorts were compared with both parental cohorts, no significant differences ($t = 0.233$, 0.245, df = 2, $P > 0.940$, 0.915) were detected (table 1).

Discussion

The recorded egg eclosion rates of *M. p. longipennis* × *M. p. pallidipennis* and *M. p. pallidipennis* × *M. p. picturatus* cohorts are comparable with those of various species of triatomines (e.g. *T. ryckmani* Zeledón and Ponce, *T. juazeirensis* Costa and Felix, and *T. patagonica* Del Ponte) (Zeledón *et al.*, 2010;

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Lima-Neiva *et al*., 2012; Nattero *et al*., 2013), as well as to that of *M. p. longipennis* identified in a previous study (Martínez-Ibarra *et al*., 2013). Moreover, the average incubation period was approximately 19 days, reflecting the favourable maintenance conditions for the development of these subspecies and hybrids. In the descendant cohorts of crosses where *M. p. pallidipennis* was involved (*M. p. longipennis* × *M. p. pallidipennis* and *M. p. pallidipennis* × *M. p. picturatus*), egg eclosion rates were lower than that recorded for *M. p. pallidipennis*, which indicates reduced hybrid fitness. In the case of *M. p. longipennis* × *M. p. picturatus* cohorts, reduced hybrid fitness also was recorded, constituting a postzygotic barrier that maintains the reproductive isolation of parental genotypes, which is similar to that observed with *T. dimidiata* (Latreille) hybrids in Yucatán, México (Herrera-Aguilar *et al*., 2009).

The average egg-to-adult development time for the three parental cohorts and the *M. p. pallidipennis* × *M. p. picturatus* cohorts was between five and five and a half months. However, the development time varied from seven to seven and a half months for the *M. p. longipennis* × *M. p. pallidipennis* and *M. p. longipennis* × *M. p. picturatus* cohorts. The longer average egg-to-adult development time for *M. p. longipennis* × *M. p. pallidipennis* and *M. p. longipennis* × *M. p. picturatus* cohorts in comparison with the three parental lines reflects the lower fitness of the hybrid cohorts.

Specimens of the *M. p. longipennis* × *M. p. pallidipennis*, *M. p. longipennis* × *M. p. picturatus*, and *M. p. pallidipennis* × *M. p. picturatus* cohorts required a lower number of blood meals to moult than the *M. p. longipennis* and *M. p. pallidipennis* parental lines, which suggests higher fitness. This would be an advantage for the hybrids since every triatomine may be at risk each time it leaves its shelter to find a host.

Accumulative mortality of the *M. p. pallidipennis* cohort was lower than *M. p. longipennis* × *M. p. pallidipennis* and *M. p. pallidipennis* × *M. p. picturatus* cohorts, but the *M. p. picturatus* cohort had lower mortality than the *M. p. longipennis* × *M. p. picturatus* cohort. This parameter also suggests a lack of hybrid fitness. As reported for *M. p. longipennis* and *M. p. pallidipennis* (Martínez-Ibarra *et al*., 2012, 2013), death in the youngest nymphs seemed to be caused by the feeding incapacity of insects because dead triatomines were generally found without substantial intestinal content. On the other hand, death of older nymphs appeared to occur during moulting.

The percentage of obtained females at the end of the life cycles was similar among all cohorts. However, the *M. p. pallidipennis* cohort laid about four times as many eggs compared with the *M. p. longipennis* × *M. p. pallidipennis* and *M. p. pallidipennis* × *M. p. picturatus* cohorts. More females laying many eggs indicate a greater possibility of having a larger population of triatomines, which might result in a highly successful population. The results presented here indicate that, for the experimental conditions used, in four of the five studied parameters (with the exception of the percentage of obtained females), at least one of the parental cohorts involved in each set of crosses had better fitness results than their hybrid descendants. Maybe under different experimental conditions (e.g., meal source, feeding frequency) hybrids may have an advantage over parental cohorts because of fitness plasticity, such as has been recorded in different cohorts of *Rhodnius prolixus* Stål (Rodríguez & Rabinovich, 1980; Sulbaran & Chaves, 2006).

Various studies have established that *M. p. longipennis*, *M. p. picturatus*, and *M. p. pallidipennis* subspecies are almost genetically identical (Martínez *et al*., 2005, 2006; Bargues *et al*., 2008, Martínez-Hernández *et al*., 2010; Espinoza *et al*., 2013), and pairwise comparisons of ITS-2 sequences indicated identical *M. p. longipennis* (=*T. longipennis*) and *M. p. picturatus* (=*T. picturata*) sequences and only two nucleotide differences between *M. p. longipennis*, *M. p. picturatus*, and *M. p. pallidipennis* (=*T. longipennis*). In contrast, other studies of the biological parameters and morphological characteristics have shown important differences among the three subspecies (Martínez-Hernández *et al*., 2010, Martínez-Ibarra *et al*., 2012, 2013, 2015*d*; De la Rúa *et al*., 2014; Rivas *et al*., 2014). Our results are consistent with those last cited studies, supporting the proposal that the three subspecies are different enough from one another to be considered subspecies, not a single one.

It has been previously established that hybrid fertility and fitness are key parameters in determining the long-term outcome of the mixture of two different natural populations (Herrera-Aguilar *et al*., 2009; Mendoça *et al*., 2014). A lack of hybrid fitness leads to the maintenance of reproductive isolation of parental genotypes (Herrera-Aguilar *et al*., 2009), and our results fit with this statement. Moreover, based on our results, it can be proposed that an incipient speciation process by distance (Mayr & Ashlock, 1991) is currently developing among non-overlapping populations of each subspecies (*M. p. longipennis*, *M. p. picturatus*, and *M. p. pallidipennis*). This hypothesis is supported by important differences that were detected in the results of morphometric antennal analyses and molecular analyses using ITS-2 when different populations of *M. p. pallidipennis* and of *M. p. longipennis* (by species) from non-overlapping areas were compared (Martínez-Hernández *et al*., 2010; Martínez-Martínez *et al*., 2010). The results from our study and these two latter studies suggest that genetic exchange might not impede or delay the definitive divergence processes needed to reach the species level.

Our hypothesis is also supported by other considerations. For instance, the distribution areas of these three subspecies are based only on specific recent studies (López-Cárdenas *et al*., 2005; Martínez-Ibarra *et al*., 2008*a*; Benítez-Alva *et al*., 2012) avoiding taking compilations of mixed old and new data into account. Therefore, the distribution is accurately delimited, with a few overlapping areas (Mayr & Ashlock, 1991) in western Mexico and some natural hybrids have been recorded (Martínez-Ibarra *et al*., 2009). Those recorded distribution areas match mosaic hybrid zone models (Hewitt, 1989), which are areas involving many independent contacts between 'entities' (subspecies in our case), each with a potentially unique evolutionary trajectory (Harrison & Rand, 1989). The existence of a heterogeneous environment, as recorded in previous studies (Martínez-Ibarra *et al*., 2008a, 2011; Benítez-Alva *et al*., 2012), impedes fusion by favouring alternative types in different areas. Both the heterogeneity of the environment in which they occur and the complex internal structure of these hybrid zones promote the maintenance of diversity (species diversity or allelic diversity) (Harrison & Rand, 1989).

In summary, the results provided in this study show that different subspecies of *M. p. longipennis*, *M. p. picturatus*, *M. p. pallidipennis*, and their hybrids present differences in their biological parameters, and as a consequence, their biological fitness. This is likely due to the intrinsic variation as different groups. Evidence from our study, in conjunction with those from previous field studies, indicates that an incipient process of speciation is occurring among these three

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subspecies, with previously reported important differences in their capacity as vectors of *T. cruzi* to humans in Mexico (Martínez-Ibarra *et al.*, 2012, 2013, 2015*a*).

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