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Antennal phenotype of Mexican haplogroups of the *Triatoma dimidiata* complex, vectors of Chagas disease

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ABSTRACT

Triatoma dimidiata (Latreille) is a species complex that spans North, Central, and South America and which is a key vector of all known discrete typing units (DTU) of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. Morphological and genetic studies indicate that *T. dimidiata* is a species complex with three principal haplogroups (hg) in Mexico. Different markers and traits are still inconclusive regarding if other morphological differentiation may indicate probable behavioral and vectorial divergences within this complex. In this paper we compared the antennae of three Mexican haplogroups (previously verified by molecular markers ND4 and ITS-2) and discussed possible relationships with their capacity to disperse and colonized new habitats. The abundance of each type of sensillum (bristles, basiconics, thick- and thin-walled trichoids) on the antennae of the three haplogroups, were measured under light microscopy and compared using Kruskal–Wallis non-parametric and multivariate non-parametric analyses. Discriminant analyses indicate significant differences among the antennal phenotype of haplogroups either for adults and some nymphal stages, indicating consistency of the character to analyze intraspecific variability within the complex. The present study shows that the adult antennal pedicel of the *T. dimidiata* complex have abundant chemosensory sensilla, according with good capacity for dispersal and invasion of different habitats also related to their high capacity to adapt to conserved as well as modified habitats. However, the numerical differences among the haplogroups are suggesting variations in that capacity. The results here presented support the evidence of *T. dimidiata* as a species complex but show females and males in a different way. Given the close link between the bug's sensory system and its habitat and host-seeking behavior, AP characterization could be useful to complement genetic, neurological and ethological studies of the closely related Dimidiata Complex haplogroups for a better knowledge of their vectorial capacity and a more robust species differentiation.

1. Introduction

Triatoma dimidiata (Latreille) (Reduviidae: Triatominae) is an important species complex of Chagas disease vectors in Latin America. This species complex is distributed in Mexico, Guatemala, Belize, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Venezuela, Guinée Française, Ecuador, and Peru (Schofield, 2002; Bargues et al., 2008; Gourbière et al., 2012). Its populations are morphologically and morphometrically variable along their distribution range and all haplogroups are highly tolerant to habitat modification (high tolerance for secondary vegetation and human presence), in tropical evergreen and seasonal dry landscapes (Ibarra-Cerdeña et al., 2009; Grisales et al., 2010).

Taxonomists have recently suggested that, due to chromatic and size variations classical morphometry, sexual dimorphism, and head and wing character analyses, *T. dimidiata* should be considered a species complex (Jurberg et al., 2005; Galvão and Justi, 2015). Molecular differentiation based on analyses of the second internal transcribed spacer (ITS2), cytochrome oxidase subunit *b* (Cyt*b*), nicotinamide adenine dinucleotide dehydrogenase 4 (ND4), and large ribosomal subunit gene (LSU) also support division of the species complex (Harris and Beard, 2003; Calderón-Fernández et al., 2005; Lehmann et al., 2005; Panzera et al., 2006; Dorn et al., 2007; Bargues et al., 2008; Ibarra-Cerdeña et al., 2009; Monteiro et al., 2013; Ibarra-Cerdeña et al., 2014; Gómez-Palacio et al., 2015).

In Mexico, there are three haplogroups (hg) of *T. dimidiata* (Marcilla et al., 2001; Lehmann et al., 2005; Ibarra-Cerdeña et al., 2014;

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May-Concha et al., 2015). These are haplogroup 1 (hg1) originally described and named from the Yucatan Peninsula (Marcilla et al., 2001; Lehmann et al., 2005; Calderón-Fernández et al., 2011), haplogroup 2 (hg2) collected along the Gulf of Mexico coast and all Pacific populations north of the Isthmus of Tehuantepec, and haplogroup 3 (hg3) from the Pacific coast of Chiapas, which is now considered hg3B subclade along with two Colombian clades (Ibarra-Cerdeña et al., 2009; Calderón-Fernández et al., 2011; Bargues et al., 2008; Gomez-Palacios and Triana, 2014). Phylogenetic analyses indicate that populations identified as hg1 can be found in two distinct regions in Chiapas, that hg2 can be found in the Yucatan Peninsula (Dorn et al., 2009; López-Cancino et al., 2015), and that populations of hg3 recently found in the high regions of Chiapas are from the Central American subclade hg3A (López-Cancino et al., 2015).

Although the general biology and ecology of the *T. dimidiata* complex species have been studied (Monroy et al., 2003; Zeledon et al., 2008; Ibarra-Cerdeña et al., 2009; Abad-Franch et al., 2010), phenotype differences among haplogroups, including antennal phenotype, is only currently emerging (Catalá et al., 2005; Arroyo et al., 2007; May-Concha et al., 2015). The antennal phenotype (AP) has been widely used to analyze genetic diversity, as well as environmental influences on populations. In certain species or complexes, AP analysis complements other phenotypic and genetic characteristics (Catalá and Schofield, 1994; Catalá, 1997; Carbajal de la Fuente et al., 2008; Hernández et al., 2008) or provides evidence for species´ differentiation (Catalá and Torres, 2001; Martínez-Hernández et al., 2010). The number of sensilla on antennae has been associated with the habitat range, specific for each species (Catalá, 1997). Given the close link between the bug's sensory system and its habitat and host-seeking behavior, AP characterization could be useful to complement genetic, neurological and ethological analyses of the closely related Dimidiata Complex haplogroups for a better knowledge of their vectorial capacity and a more robust species differentiation. In this paper we compare the antennae of three Mexican haplogroups (previously verified by molecular markers ND4 and ITS-2) and discuss possible relationships with their capacity to disperse and occupy new habitats.

2. Materials and methods

2.1. Insects

T. dimidiata individuals used in this study were collected in domestic sites from the Yucatan and Chiapas states in Mexico, where the three haplogroups had been consistently isolated. Molecular identification of populations analyzed was confirmed using ITS2 and nicotinamide adenine dinucleotide dehydrogenase 4 (ND4) (nuclear and mitochondrial markers) (as in Monteiro et al., 2013; Richards et al., 2013). The nomenclature used herein corresponds to that first used to designate Mexican haplogroups (Marcilla et al., 2001; Lehmann et al., 2005). The antennae of 198 specimens were examined (Table 1). Field collected bugs were transferred to the insectary of the Centro Regional de Investigación en Salud Pública of the INSP (CRISP/INSP). They were reared and maintained separately for four generations at 27 ± 1 °C, 70 ± 5% RH, a photoperiod of 12: 12 (L: D) h, and were allowed to feed every week on rabbit blood (New Zealand White).

2.2. Antennal preparations

We examined all nymphal stages and adults from the three Mexican haplogroups. One antenna per individual was removed using fine forceps and scissors. Antennae were processed with sodium hydroxide 4% for 6 h at 60 °C and then neutralized with glacial acetic acid 5% for 2 min. This procedure allowed cuticle diaphanization and allowed the identification and counting of the sensilla using a stereo microscope DME Leica ® at 400×. The number of receptors on antennal segments was counted using a leukocyte count CT-270 Citocon®. The antennae were mounted "in toto" using glycerin (Hernández et al., 2008). The ventral side of the three distal segments of the antennae (pedicel, flagellum 1 and flagellum 2) was evaluated, identifying and counting the following sensilla: bristles (BR) thin walled trichoid (TH), thick walled trichoid (TK), and basiconica (BA) (nomenclature according to Catalá and Schofield, 1994).

2.3. Data analysis

Differences on the antennal phenotype between insects belonging to different haplogroups were explored using univariate and multivariate techniques. Means and standard deviations of abundance were calculated for each type of sensilla (BR, TH, TK and BA) and antennal segment (adults, 4 variables in the pedicel, 4 in the flagellum 1 and 4 in the flagellum 2.) For nymphs, 4 variables were measured in the flagellum 2, since nymphs had no chemo-receptors on the pedicel and flagellum 1, as in other Triatominae nymphs, Catalá (1997).

Since the variables showed heteroscedascity, the Kruskall–Wallis non-parametric test was used for univariate analysis. The software PAD version 60 was used to estimate functions that identify study groups using discriminant multivariate analysis (Dujardin, 2004) (Wilks and Mahalanobis distance values) and 1000 permutation tests. It offers cross-checked classification tests to validate the re-classification of discriminant analysis. The discriminant analysis was carried out using the 4 variables for nymphs and 12 variables for adults.

2.4. Ethics statement

The Ethics Comission of the National Institute of Public Health (INSP) approved all human communication, collaboration, and sampling protocols under annual renewal of the permits #727 and #1063. All bugs collected with the assistance of community members or by searching in or around houses were approved via verbal consent (following collective workshops and individual interview). The Biosafety Comission of the INSP (Comision de Bioseguridad) reviewed and approved the animal care and use protocol with permit numbers CB08-209, and renewed as CB12-020. Mexican national guidelines (NORMA Oficial Mexicana NOM-062-ZOO-1999, http://www.fmvz.unam.mx/fmvz/principal/archivos/062ZOO.PDF) were adhered to for all animal (NZW rabbits) care and use. This care involved continuous review by competent professionals, diet supplements for iron and multivitamins, and a

Table 1
Collection sites and number de specimens of the *T. dimidiata* complex individuals used in this study. hg1: haplogroup Yucatan-Peninsula; hg2: haplogroup Gulf of Mexico; hg3: haplogroup Pacific-Cost.

Haplogroups	Community	County	State	Longitude (deg/min/s)	Latitude	No. of specimens						
					(deg/min/s)	F	M	1st	2nd	3rd	4th	5th
hg1	Eknakan	Cuzamá	Yucatan	89°22′15″	20°45′31″	10	10	10	10	10	10	10
	San Pedro Chacabal	Motul de Carrillo Puerto	Yucatan	89°13′00″	21°07′06″							
	Kantunil	Kantunil	Yucatán	89°02′04″	20°47′45″							
hg2	Rio Blanco	Berriozabal	Chiapas	93°10′10″	16°25′26″	5	5	10	10	10	10	10
	Montecristo	Berriozabal	Chiapas	93°09′56″	16°25″27″							
hg3	Los Mangos Manacal, Lomas de Chiapas	Tuzantán	Chiapas	92°14′38″	14°54′59″	8	10	10	10	10	10	10

programmed use based on insect populations. This study did not collect endangered or protected species.

3. Results

3.1. Antennal phenotype of the three Mexican haplogroups of T. dimidiata complex

The general antennae characteristics of *T. dimidiata* adults support previous results (Catalá et al., 2005). The three types of chemoreceptors (BA, TH and TK) and one type of mechanoreceptor (BR) were present on both the pedicel and the flagellar segments of all adult insects. There is sexual dimorphism due to an increase of TH on male antennal pedicel ($p < 0.05$ Kruskal–Wallis test). In nymphs, the chemo-sensilla TH, TK and BA were present only on flagellum two (F2) following the general pattern in Triatominae (Catalá, 1997). The numbers of the different antennal sensilla types are presented in Table 2 (N = 198 specimens of the *T. dimidiata* complex).

The number of BR, TH, TK and BA receptors gradually increased during nymph development, with more receptors on the fifth nymphal stage ($p < 0.0001$ Kruskal–Wallis test). There were significant differences in the number of three types of chemoreceptors (BA, TH, TK) among nymph stages ($p < 0.0001$ Kruskal–Wallis test), while the number of the mechanoreceptor BR (F2-BR) was not significantly different among stages ($p > 0.05$ Kruskal–Wallis test) (see Table 2).

3.2. Comparison of the phenotypic expression of the T. dimidiata haplogroups

3.2.1. Univariate analysis

The number of the different sensilla types changed according to haplogroups compared, both in the case of adults as nymphs. There were significant differences in the TH and BA sensilla of the three segments of the antennae ($p < 0.05$ Kruskal–Wallis test) among females of different haplogroups. The number of BR, TH and BA on the pedicel of hg2 females (P-BR: $p = 0.0062$, P-TH: $p = 0.0002$, P-BA: $p = 0.003$ Kruskal–Wallis test), and that of TK on flagellum two (F2-TK: $p = 0.0035$ Kruskal–Wallis test) was lower than in hg1 and hg3. However, the TH on the first segment of the flagellum (F1-TH: $p = 0.006$ Kruskal–Wallis test) was less abundant in the hg1 female than in the hg2 or hg3. There were no significant differences for P-TK, F1-BR, F1-TK, and F2-BR among females of different haplogroups ($p > 0.05$ Kruskal–Wallis test) (Fig. A1–D1, Supplementary data). In contrast, there were differences in the P-BR, P-TK, F1-BR, F1-TH, F1-TK, F1-BA, F2-TH and F2-BA ($p < 0.05$ Kruskal–Wallis test) of males among haplogroups (Fig. A2-D2, Supplementary data).

Table 2
Number of chemoreceptor sensilla on the antennae of adults and nymphs of the *T. dimidiata* complex. Data shown are means and standard deviation. *N* = 198. BR: Bristles; TH: thin-walled trichoids; TK: thick-walled trichoids; BA: basiconics. The number between parentheses represents the number of specimens analyzed. The number between clasps represents the standard deviation of the data. F = female, M = male.

		Pedicel				Flagel 1				Flagel 2			
Haplogroup	Life stage	BR	TH	TK	BA	BR	TH	TK	BA	BR	TH	TK	BA
hg1	F (10)	53.90 [7.62]	184.2 [12.15]	106.2 [21.31]	19.7 [6.55]	23.30 [6.43]	93.1 [7.11]	103.6 [13.36]	41.1 [8.91]	14.40 [2.91]	38.9 [9.78]	107.5 [22.19]	33.8 [7.57]
	M (10)	68.10 [8.40]	342.3 [33.81]	82.7 [10.34]	24.5 [6.04]	24.30 [3.80]	170.8 [10.25]	79.2 [18.04]	68.6 [9.65]	15.30 [3.86]	71.3 [6.40]	133 [10.12]	47.9 [7.25]
	1st (10)									13.70 [1.34]	9.2 [1.40]	45.2 [3.61]	10.9 [0.99]
	2nd (10)									15.50 [2.64]	10.4 [1.26]	45.5 [4.28]	10.8 [2.25]
	3rd (10)									17.50 [2.51]	14.5 [2.92]	82.2 [4.34]	23 [2.16]
	4th (10)									16.00 [2.79]	15.6 [3.17]	105.5 [13.46]	20.3 [5.10]
	5th (10)									16.20 [1.69]	15.9 [3.03]	109.5 [8.10]	26.7 [3.95]
hg2	F (5)	40.80 [5.17]	156.4 [12.82]	104.4 [3.91]	7.8 [3.56]	19.60 [3.58]	98.6 [10.33]	115.8 [12.03]	28.8 [4.38]	11.80 [3.11]	65.6 [6.19]	66.8 [6.38]	20.8 [2.77]
	M (5)	53.40 [12.50]	352.6 [65.41]	112.6 [21.05]	20.6 [4.04]	20.60 [4.04]	126.8 [13.50]	136.2 [29.84]	39.6 [8.29]	17.40 [2.19]	54.2 [7.79]	149 [24.82]	30.2 [8.79]
	1st (10)									14.10 [1.97]	8.5 [1.65]	49.2 [9.47]	11.3 [2.83]
	2nd (10)									16.10 [1.60]	8.8 [2.15]	50 [4.67]	12.1 [3.57]
	3rd (10)									14.10 [1.91]	12 [3.62]	69.2 [3.74]	15.6 [2.59]
	4th (10)									15.0 [1.94]	13.6 [2.12]	92.5 [6.19]	19.1 [3.98]
	5th (10)									14.40 [2.59]	14.5 [4.06]	87.6 [5.82]	22.1 [2.81]
hg3	F (8)	60.50 [11.26]	218.375 [14.80]	88.5 [12.33]	23.75 [5.99]	20.13 [4.05]	115.625 [18.70]	115.875 [15.12]	32.125 [3.87]	14.00 [2.51]	44.5 [15.10]	107.625 [27.68]	31.5 [7.54]
	M (10)	52.50 [8.81]	339.4 [32.35]	69.3 [20.36]	17.9 [5.04]	20.20 [2.82]	120.9 [26.55]	105 [16.52]	31.2 [10.14]	17.00 [3.53]	42.5 [14.42]	123.7 [18.23]	39.5 [7.60]
	1st (10)									14.80 [2.87]	10.8 [3.05]	44.3 [7.75]	12.5 [1.78]
	2nd (10)									15.40 [2.01]	8.5 [2.42]	46.3 [3.09]	10 [2.58]
	3rd (10)									14.60 [1.71]	11.6 [2.37]	59.8 [3.82]	17 [2.83]
	4th (10)									13.10 [3.25]	16.3 [4.81]	97.3 [5.79]	22.3 [3.65]
	5th (10)									14.10 [2.28]	17.5 [3.57]	96.1 [11.04]	23.8 [3.97]

In nymphs, there was no difference in the number of TH sensilla between haplogroups (F2-TH: $p > 0.05$ Kruskal–Wallis test), while some differences were evident for the mechanoreceptor F2-BR (F2-BR: $p < 0.05$ Kruskal–Wallis test), the chemoreceptor TK sensilla (F2-TK: $p < 0.01$ Kruskal–Wallis test), and the chemoreceptor BA (F2-BA: $p < 0.05$ Kruskal–Wallis test) (Fig. A3–D3, Supplementary data).

3.2.2. Multivariate analysis

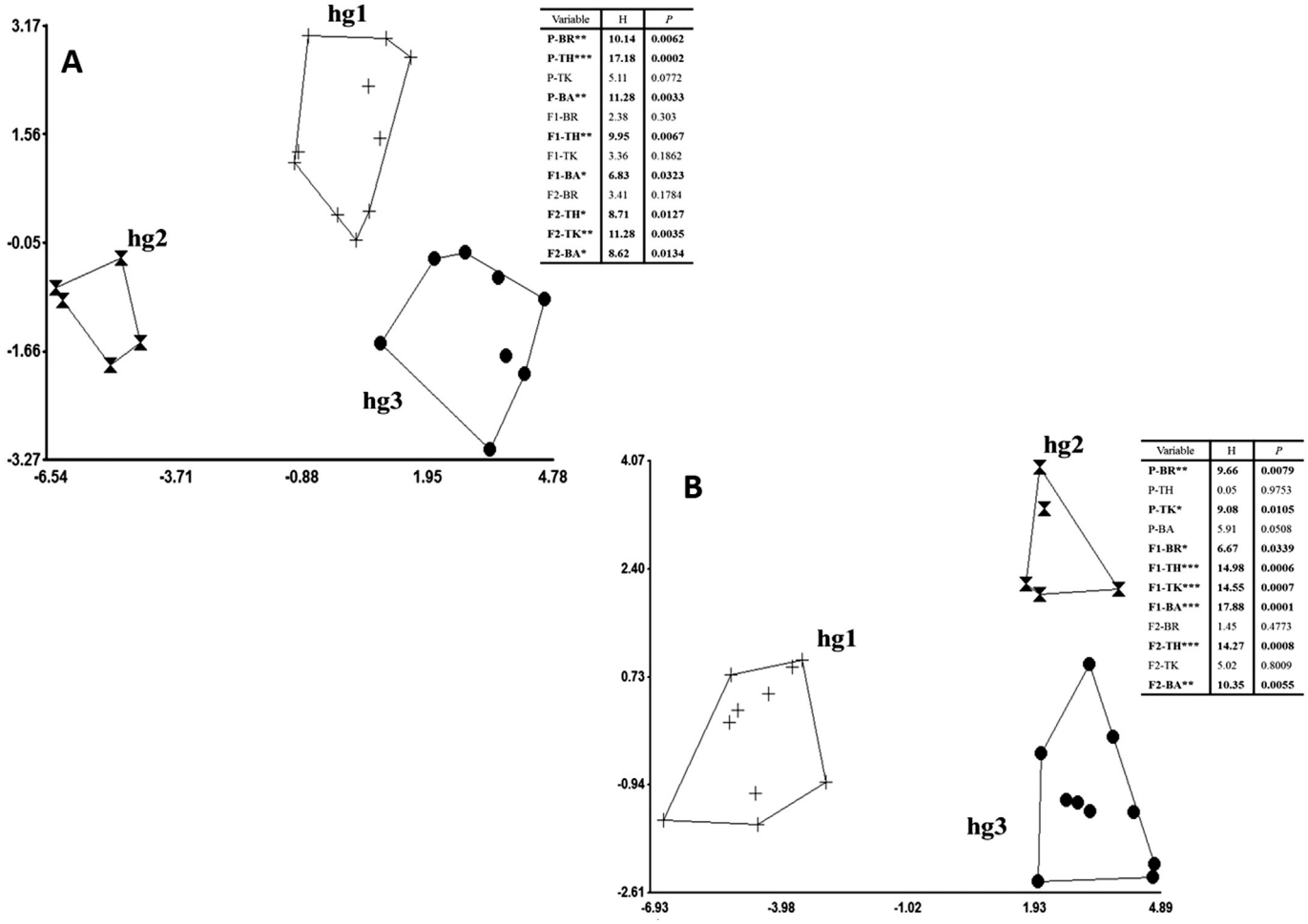
The antennae of both sexes of the haplogroups were significantly discriminated using the 12 antennal variables. For the females, the first function explained 84.02% of the total variation, while the second function explained 15.98%. Eight variables were the most important to differentiate among females using discriminant multivariate analysis (Fig. 1A). There were significant differences between females of hg2 with hg1 ($p < 0.007$) and hg3 ($p < 0.002$), but not between hg1 and hg3 ($p > 0.01$). In discriminant space (Fig. 1A), females of hg2 cluster separately from hg1 and hg3. The group's centroid distances indicate that hg2 is more distant from hg3 (D2 = 8.63) than from hg1 (D2 = 6.28), while hg1 was less distant from hg3 (D2 = 4.09).

The antennae of males were also significantly discriminated using the 12 variables. The first discriminant function explained 87.25% of total variation, whereas the second function explained 12.75%. Eight variables were the most important affecting differentiation patterns for the males using discriminant multivariate analysis (Fig. 1B). There were significant differences between male hg1 with hg2 ($p < 0.0001$), and hg3 ($p < 0.0001$); but not between hg2 and hg3 ($p > 0.01$). The centroid for hg1 males was more distant from hg3 (D2 = 8.01) than from hg2 (D2 = 7.53), while hg2 was less distant from hg3 (D2 = 4.03).

The 1st (Fig. 2A), 2nd (Fig. 2B) and 4th (Fig. 2D) nymphs were not significantly different among haplogroups ($p > 0.01$), while there were significant differences in the distance of 3rd stage nymphs among the three haplogroups ($p < 0.0001$). In the latter case, the first discriminant function explained 93.30% of total variation in nymphs, whereas the second function explained 6.70%. Three variables were the most important affecting differentiation patterns for third stage nymphs (Fig. 2C). The hg1 was separate from hg2 and hg3 in discriminant space (Fig. 2C) and Mahalanobis distance (D2) of group centroids indicates that hg1 is more distant from hg3 (D2 = 6.54) than from hg2 (D2 = 4.53), whereas hg2 was less distant from hg3 (D2 = 2.78). The Mahalanobis distance of 5th stage nymphs (Fig. 2E) was significantly different between hg1 and hg2 ($p < 0.0001$), but was not between hg1 vs hg3 and hg2 vs hg3 ($p > 0.01$).

4. Discussion

The antennae of all adult Triatominii carry a specific number of chemosensorysensilla (as TH, TK and BA) in both segments of the flagellum and pedicel. These have olfactory and/or gustative function for detection of habitats, refuges, hosts and couple (Bernard, 1974; Guerenstein and Lazzari, 2009). It was observed (Catalá, 1997), that the number of these sensilla on the antennal pedicel is associated with the habitat range of species, being more abundant in species adapted to many habitats (e.g. *T. sordida*, Catalá, 1997). The present study



Variable	H	P
P-BR**	**10.14**	**0.0062**
P-TH***	**17.18**	**0.0002**
P-TK	5.11	0.0772
P-BA**	**11.28**	**0.0033**
F1-BR	2.38	0.303
F1-TH**	**9.95**	**0.0067**
F1-TK	3.36	0.1862
F1-BA*	**6.83**	**0.0323**
F2-BR	3.41	0.1784
F2-TH*	**8.71**	**0.0127**
F2-TK**	**11.28**	**0.0035**
F2-BA*	**8.62**	**0.0134**

Variable	H	P
P-BR**	**9.66**	**0.0079**
P-TH	0.05	0.9753
P-TK*	**9.08**	**0.0105**
P-BA	5.91	0.0508
F1-BR*	**6.67**	**0.0339**
F1-TH***	**14.98**	**0.0006**
F1-TK***	**14.55**	**0.0007**
F1-BA***	**17.88**	**0.0001**
F2-BR	1.45	0.4773
F2-TH***	**14.27**	**0.0008**
F2-TK	5.02	0.8009
F2-BA**	**10.35**	**0.0055**

Fig. 1. Principal components analysis of adults, A) females and B) males using twelve antennal sensilla variables. Each point represents one specimen on the canonical axis. Polygons enclose specimens of each haplogroup of the Dimidiata Complex: hg1: haplogroup Yucatan-Peninsula; hg2: haplogroup Gulf of Mexico; hg3: haplogroup Pacific-Coast. Table show statistics for each type of sensilla for each antennal segment. Significant differences between haplogroups (Kruskal–Wallis test) indicated by $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

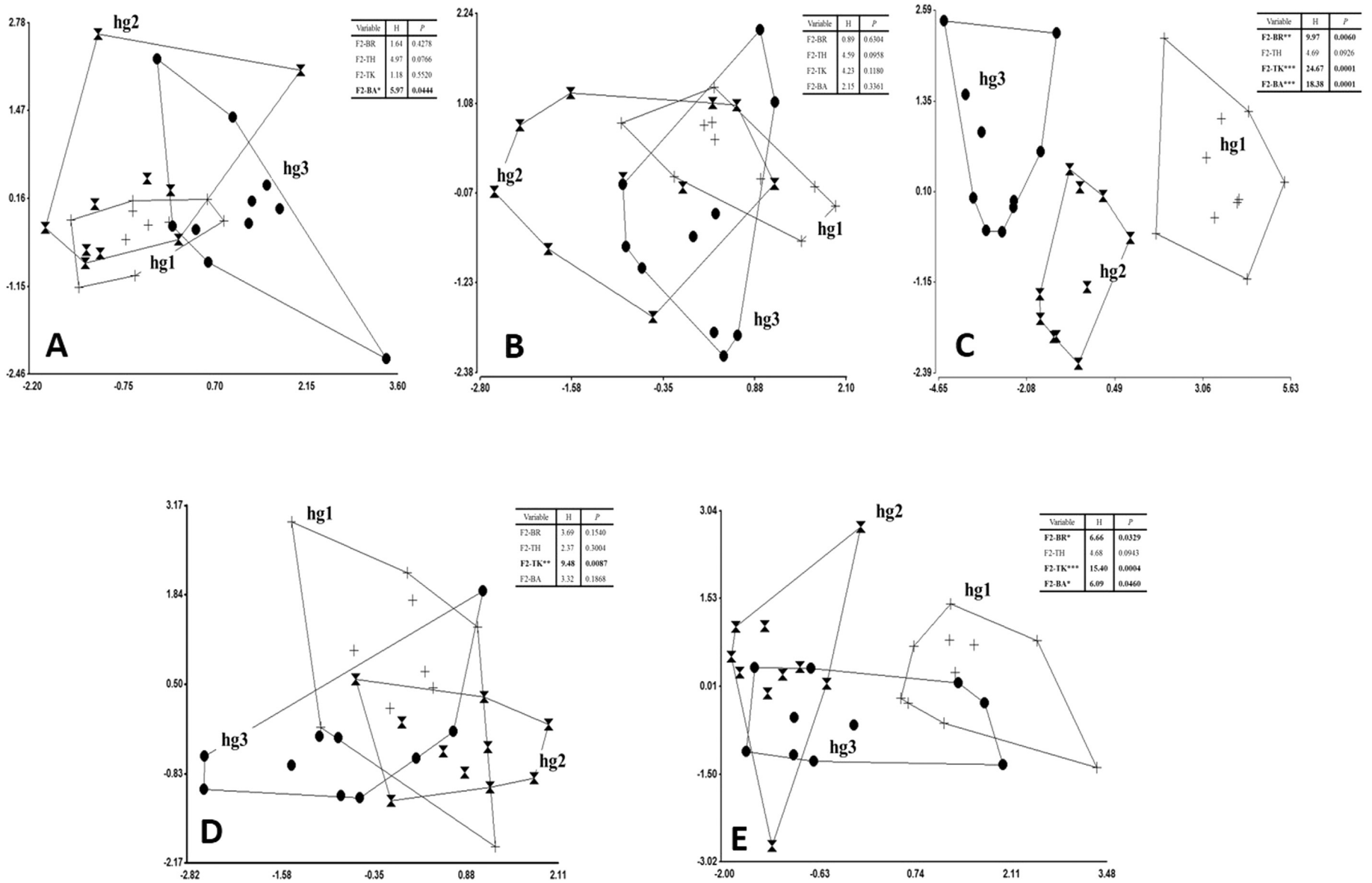


Fig. 2. Principal components analysis of nymph stages, A) 1st, B) 2nd, C) 3rd, D) 4th, and E) 5th instar nymphs using four antennal sensilla variables. Each point represents one specimen on the canonical axis. Polygons enclose specimens of each haplogroup of Dimidiata Complex: hg1: haplogroup Yucatan-Peninsula; hg2: haplogroup Gulf of Mexico; hg3: haplogroup Pacific-Coast. Table show statistics for each type of sensilla on the flagellum two of the antenna. Significant differences between haplogroups (Kruskal–Wallis test) indicated by $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

shows that the adult antennal pedicel of the *T. dimidiata* complex have abundant chemosensory sensilla, according with good capacity for dispersal and invasion of different habitats (Catalá et al., 2005; Arroyo et al., 2007) also related to their high capacity to adapt to conserved as well as modified habitats (Dumonteil et al., 2002; Guzman-Tapia et al., 2007; Ibarra-Cerdeña et al., 2014; López-Cancino et al., 2015). The antennae of the *T. dimidiata* species complex also have many TK sensilla, although their chemosensory function has not been confirmed (Bernard, 1974; Guerenstein, 1999).

All haplogroups of *T. dimidiata* have a sexual dimorphism regarding antennal sensilla, based on the numerous TH on the pedicel and on flagellum 1 of males (Catalá et al., 2000; Carbajal de la Fuente and Catalá, 2002; Catalá et al., 2005). This sexual dimorphism coincides with antennae of other Triatominae and may be linked to differences in sensing sexual pheromones. May-Concha (2010) suggested the presence of a sexual pheromone in *T. dimidiata*, since fewer mating attempts were observed when the opening of female glands was occluded. Evidence exists for a chemical signal produced during *T. dimidiata* mating which promotes the attraction of males to volatiles emitted by females, to mating pairs, to pairs with males having the MG orifices occluded, and to MG extracts (May-Concha et al., 2013). Some of the TH sensilla, more abundant in males, could be a receptor for a putative sex pheromone.

The expected transitional difference from nymphs to adults in sensilla patterns, has also been noted in other Triatominae (Bernard, 1974; Catalá and Schofield, 1994; Catalá, 1997). There is an increase in the number of receptors in the adult stage, particularly chemoreceptors. These increases reflect additional sensorial requirements in adults such as those related to reproduction and active dispersal by flight (Chapman, 1982; McIver and Siemicki, 1985; Hernández et al., 2015).

Morphometrics, hydrocarbon analysis, pheromone analysis, cytogenetics, and molecular data have provided unambiguous evidence for cryptic taxa and the recognition of *T. dimidiata* as a species complex (Harris and Beard, 2003; Calderón-Fernández et al., 2005; Lehmann et al., 2005; Panzera et al., 2006; Dorn et al., 2007, 2009; Bargues et al., 2008; Ibarra-Cerdeña et al., 2009; Monteiro et al., 2013; Ibarra-Cerdeña et al., 2014; Gómez-Palacio et al., 2015; May-Concha et al., 2015). The results here presented support the evidence of *T. dimidiata* as a species complex but show females and males in a different way. The multivariate analysis of females AP separated hg2 from the other groups. The main characteristic of this hg2 females are a lower number of TH sensilla on pedicel. A similar observation in *T. infestans* (Hernández et al., 2008) was clearly linked with a lower area of expansion and habitat range as previously suggested for Triatominae species in Catalá (1997). It is important to mark that female is the key piece (Goubière et al., 2008) to colonize new habitats because it can spread with eggs and sperm ready for colonization (Abrahan et al., 2011).

A greater proximity was evident between AP from hg2 and hg3 males which may suggest different olfactory capacity respect to hg 1 males.

The ecological niche of haplogroups 2 and 3 is conserved (Ibarra-Cerdeña et al., 2014), and evidence now exists for the sympatry of haplogroups 1 and 2 in the Yucatan Peninsula, Chiapas and Tabasco (Herrera-Aguilar et al., 2009; Garcia et al., 2013; López-Cancino et al., 2015) and haplogroups 1, 2 and 3 are sympatric in northern Chiapas (Pech-May, personal communication), suggesting that macroecological tolerances may not have affected these differences. This however, does not exclude microecological influences, such as landscape modification, its frequency or degree. No study has analyzed multiple populations from within a haplogroup to analyze association of antennal phenotype with habitat modification.

Discriminant and cluster analysis of AP clearly differentiate between sexes and among all three haplogroups, indicating consistency of the character to analyze intraspecific variability within the complex. However, the numerical differences among the haplogroups are suggesting variations in their capacity to adapt to different habitats. Further AP studies on other haplogroups or subclades from Central America and Colombia would further ratify their taxonomic value, along with previously mentioned intra-haplogroup analysis in conjunction with other morphometric and genetic analyses.

If the haplogroups are reproductively isolated (Dorn et al., 2009; López-Cancino et al., 2015), and dispersal is increasing along with population migration and commerce, vector control strategies will need to consider the population dynamics and characteristics of all haplogroups, as well as understand their differences or similarities. The study of AP could be an important tool to analyze populations across their ecological niche and according to anthropic modification within *T. dimidiata* haplogroups. This evidence will be fundamental to design evidence-based control and monitoring strategies.

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