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Inferences about antennal phenotype: the "*Triatoma maculata* complex" (Hemiptera: Triatominae) is valid?

Ana L. Carbajal de la Fuente[a,*], François Noireau[b], Silvia S. Catalá[c]

[a] *Depto Entomologia, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil 4365, CEP 21045-900, Rio de Janeiro, Brazil*
[b] *Institut de Recherche pour le Développement (IRD), UR016, Av. Agropolis, BP 64501, 34394 Montpellier, France*
[c] *CRILAR, Entre Rios y Mendoza s/n, CP 5301, Anillaco, La Rioja, Argentina*

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Abstract

The "*Triatoma maculata* complex" is presently formed by two epidemiologically important species of Triatominae, *Triatoma maculata* and *Triatoma pseudomaculata*, which share morphologic and chromatic characteristics. In order to clarify the systematic status and infer the evolutionary relationships of these vectors of Chagas disease, we performed a comparative analysis of their antennal phenotype, taking also into account *Triatoma wygodzinskyi*, a possible sister species. The comparison was based on *sensilla* arrays on the three distal segments of the antenna. Our results show a close similarity between *T. pseudomaculata* and *T. wygodzinskyi* antennal phenotypes, and significant differences with *T. maculata*. The inter-population study reinforces the idea that *T. pseudomaculata* (arboricolous species) and *T. wygodzinskyi* (rupicolous species) would originate from a common ancestor.

Keywords: *Triatoma maculata* complex; *Triatoma pseudomaculata*; *Triatoma maculata*; *Triatoma wygodzinskyi*; Antennal phenotype; *Sensilla*; Brazil

1. Introduction

Triatominae are the vectors of Chagas disease, which is caused by the protozoan parasite *Trypanosoma cruzi* (Chagas, 1909) and is ranked as one important parasitic disease for Latin America (WHO, 2006). In Brazil, the eradication program focused on *Triatoma infestans* (Klug, 1834), had as consequence the occurrence in dwellings of new potential vectors originally restricted to the sylvatic environment (Dias et al., 2002). In the northeast of Brazil, *Triatoma pseudomaculata* (Corrêa and Espínola, 1964), one of these potential vectors, is exhibiting a quick synanthropic process (Dias et al., 2000; Diotaiuti et al., 2000; Noireau et al., 2005). Because of their similar morphologic and chromatic characteristics, *T. pseudomaculata* was misidentified for many years as *Triatoma maculata* (Erichson, 1848), a species found in the Roraima State of Brazil, Venezuela, Colombia, Surinam, Guyana, French Guiana, and some Caribbean islands (Carcavallo et al., 1998a, 2000). Finally, *T. pseudomaculata* was formally described in 1964 from specimens collected in Sobral, Ceará State. According to the Schofield (1988) hypothesis about their origin, *T. maculata* and *T. pseudomaculata* would be the result of the evolution of two geographic populations issuing from a common ancestor by passive dispersion of nymphs associated to migratory birds.

Because they share many morphologic and chromatic characteristics, both species form the "*T. maculata* complex" (Carcavallo et al., 2000) or "*maculata* subcomplex" (Dujardin et al., 2000). A third Brazilian species, *Triatoma wygodzinskyi* (Lent, 1951), exhibits close morphological similarities with *T. pseudomaculata* (Carcavallo et al., 2000). *T. wygodzinskyi* has been described from a small number of specimens (5) obtained from a single sampling in the south of Minas Gerais State, Brazil.

T. maculata is commonly found in the wild environment in palms trees, associated with birds, bats, rodents and marsupials (Carcavallo et al., 1998b), but exhibits epidemiological importance when it colonizes synanthropic structures (Feliciangeli et al., 2003; Luitgards-Moura et al., 2005). *T. pseudomaculata* is not only found in hollow trees but also in rodent, marsupial and "armadillo" shelters (Lent and Wygodzinsky, 1979;

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[*] Corresponding author. Tel.: +55 21 2598 4320; fax: +55 21 2573 4468.
E-mail address: carbajal@ioc.fiocruz.br (A.L. Carbajal de la Fuente).

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Espínola, 1985; Dias-Lima et al., 2003). It is currently reported to be colonizing artificial structures (Silveira and Vinhaes, 1998) and may be considered as a synanthropic vector candidate. Finally, *T. wygodzinskyi* occurs exclusively in the cracks of stone located in southern Minas Gerais and northern São Paulo (Lent and Wygodzinsky, 1979; Carbajal de la Fuente, unpublished data).

According to Hypša et al. (2002) and Santos et al. (2007), *T. maculata* and *T. pseudomaculata* would pertain to distinct evolutionary lineages whereas *T. pseudomaculata* and *T. wygodzinskyi* would be closely related species. In order to confirm this hypothesis and to clarify the systematic status, we carried out an inter- and intra-specific comparative study of the antennal phenotype observed for *T. maculata*, *T. pseudomaculata* and *T. wygodzinskyi*. We also explored the existence of sexual dimorphism in the antenna of these species.

2. Material and methods

2.1. Insects

Brazilian populations of three species were compared: *T. maculata* (one population from Mucajaí, Roraima State); *T. pseudomaculata* (three populations from Itaobim, Minas Gerais; Curaçá, Bahía; and Sobral, Ceará) and *T. wygodzinskyi* (two population from Santa Rita de Caldas, Minas Gerais, and Vargem Grande do Sul, São Paulo) (Table 1). The insects analyzed were field specimens, except for *T. maculata* and the Sobral population of *T. pseudomaculata*, which originated from a first generation, obtained from the insectary of the Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas, Instituto Rene Rachou, FIOCRUZ, Brazil. We currently consider that the triatomine populations from São Paulo State characterized as *Triatoma arthurneivai* (Lent and Martins, 1940), and studied by Hypša et al. (2002) and Paula et al. (2005) pertained in reality to the species *T. wygodzinskyi* (Santos et al., 2007). Unfortunately, we could not include specimens of *T. arthurneivai* in this analysis. The antennas of all the collection type specimens were damaged and recent collecting efforts in Serra do Cipó, Minas Gerais State, were unsuccessful.

Table 1
Characteristics of the different samples analyzed

Species	Code	Locality, State	Habitat	*N*	
				F	M
T. wygodzinskyi	TwyMG	Santa Rita de Caldas, MG	Rocks	5	5
	TwySP	Vargem Grande do Sul, SP		5	5
T. pseudomaculata	TpsCE	Sobral, CE	Peridomestic	5	5
	TpsBA	Curaçá, BA		5	5
	TpsMG	Itaobim, MG		0	5
T. maculata	TmaRR	Mucajaí, RR	Palm tree	5	5

MG: Minas Gerais; SP: São Paulo; CE: Ceará; BA: Bahía; RR: Roraima. *N*: Number of antennas, F: females, M: males.

2.2. Antennal preparations

One antenna per individual was removed using fine forceps, stored in 70% ethanol and diafanized in sodium hydroxide (10%). After neutralization with acetic acid (10%), each antenna was mounted on a slide in glycerine. *Sensilla* identification and counting were made on the ventral side of the three distal segments of the antenna: pedicel (P), flagellum first segment (F1), and flagellum second segment (F2), using optical microscopy (400×) and a drawing chamber (Carbajal de la Fuente and Catalá, 2002). *Sensilla* were classified in Bristles (BR), thin-walled trichoids (TH), thick-walled trichoids (TK) and basiconica (BAS) according to Catalá and Schofield (1994).

2.3. Data analysis

Means and standard deviations were calculated for each type of *sensilla* in each one of the antennal segments. Levene's test was used to check the homogeneity of variances. Variables were analyzed using ANOVA and mean values were contrasted using the LSD (least significant difference) test. Variables with significant differences were used for discriminant analysis between species using PADWIN software, version 81a (J.P. Dujardin, http://www.mpl.ird.fr/morphometrics). For population analysis, Mahalanobis distances and their statistical significance were calculated by permutation tests (1000 runs each) after Bonferroni correction. Cluster analysis based on Euclidean distances was used to build an UPGMA (unweighted pair-group method with arithmetic average) phenogram. This analysis was carried out with 12 variables using PAST software version 1.44 (http://www.folk.uio.no/ohammer/past).

3. Results

3.1. Analysis by species

T. maculata and *T. pseudomaculata* presented the four types of *sensilla* distributed on their pedicel and antennal segments. *T. wygodzinskyi* presented a low quantity (TwySP) or did not present (TwyMG) the P-TK *sensilla* type (Table 2). Interspecific univariate analysis (variables log transformed) showed significant differences in pedicel mechanoreceptor (P-BR) and chemoreceptor (P-TH, P-TK, P-BAS, F2-TH and F2-BAS) densities. The LSD test revealed that P-TH and F2-TH could separate the three species, and P-TK, P-BAS and F2-BAS separated *T. maculata* to the remaining species ($P < 0.01$). P-TK, P-BAS and F2-BAS did not exhibit significant differences between *T. pseudomaculata* and *T. wygodzinskyi*. For discriminant analysis, canonical factor 1 (CF1) separated *T. maculata* from the remaining species and demonstrated close proximity for *T. pseudomaculata* and *T. wygodzinskyi* (Fig. 1). The CF1 explained 92% of the variance while the CF2 explained 8%. The Mahalanobis distances between the three species were highly significant ($P < 0.001$). After 1000 permutations, 80% of *T. wygodzinskyi* specimens were correctly reclassified, while *T. maculata* and *T. pseudomaculata* had reclassification rates of 70% and 72%, respectively.