Revalidation and redescription of *Triatoma brasiliensis macromelasoma* Galvão, 1956 and an identification key for the *Triatoma brasiliensis* complex (Hemiptera: Reduviidae: Triatominae)

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Triatoma brasiliensis macromelasoma *is revalidated based on the results of previous multidisciplinary studies on the* Triatoma brasiliensis *complex, consisting of crossing experiments and morphological, biological, ecological and molecular analyses. These taxonomic tools showed the closest relationship between* T. b. macromelasoma *and* Triatoma brasiliensis brasiliensis. T. b. macromelasoma *is redescribed based on specimens collected in the type locality and specimens from a F1 colony. The complex now comprises* T. b. brasiliensis, T. b. macromelasoma, Triatoma melanica, Triatoma juazeirensis *and* Triatoma sherlocki. *An identification key for all members of the complex is presented. This detailed comparative study of the morphological features of* T. b. macromelasoma *and the remaining members of the complex corroborates results from multidisciplinary analyses, suggesting that the subspecific status is applicable. This subspecies can be distinguished by the following combination of features: a pronotum with 1+1 narrow brownish-yellow stripes on the submedian carinae, not attaining its apex, hemelytra with membrane cells darkened on the central portion and legs with an incomplete brownish-yellow ring on the apical half of the femora. Because the* T. brasiliensis *complex is of distinct epidemiological importance throughout its geographic distribution, a precise identification of its five members is important for monitoring and controlling actions against Chagas disease transmission.*

Key words: Brazil - Chagas disease vector - Heteroptera - kissing bug - morphology - taxonomy

Chagas disease is an anthropozoonosis caused by the protozoan parasite *Trypanosoma cruzi*, which is mainly transmitted by haematophagous bugs of the subfamily Triatominae (Hemiptera: Heteroptera: Reduviidae), which contains 137 registered valid species (Galvão et al. 2003). Following this work, a few species were added and, currently, 147 species are included in the group (Forero et al. 2004, Poinar 2005, Costa et al. 2006, Galvão & Angulo 2006, Bérenger & Blanchet 2007, Costa & Felix 2007, Martinez et al. 2007, Sandoval et al. 2007, Jurberg et al. 2009, Rosa et al. 2012, Gonçalves et al. 2013).

Triatoma brasiliensis Neiva, 1911 is currently the main Chagas disease vector in the semiarid areas of northeastern Brazil (Silveira & Vinhaes 1999, Costa et al. 2003a) and was originally described from the municipality of Caicó, state of Rio Grande do Norte (RN). Neiva and Lent described a different chromatic form of *T. brasiliensis* from Espinosa, state of Minas Gerais (MG), as subspecies *Triatoma brasiliensis melanica* Neiva & Lent, 1941. Therefore, the nominotypical subspecies *Triatoma brasiliensis brasiliensis* Neiva, 1911 was established.

doi: 10.1590/0074-02761080620130l6
Financial support: CNPq
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Received 13 December 2012
Accepted 22 February 2013

Galvão (1956), based on specimens from the municipalities of Petrolina [state of Pernambuco (PE)] and Curaçá [state of Bahia (BA)], illustrated and characterised a new subspecies in a taxonomic key: *Triatoma brasiliensis macromelasoma* Galvão, 1956. Later, Lent and Wygodzinsky (1979) suggested a synonymy to the subspecies of *T. brasiliensis*, stating that intermediate forms could be found in nature.

Several field captures were then carried out throughout the geographic region of the *T. brasiliensis* complex from 1994-2002. Approximately 2,060 specimens were compared according to their chromatic patterns and geographic distribution. A characteristic darker colour pattern was found in specimens from BA. Multidisciplinary studies were carried out to analyse the distinct *T. brasiliensis* morphotypes based on their morphology (Costa et al. 1997a, 2009), biology (Costa & Marchon-Silva 1998), ecology (Costa et al. 1998, 2002) and isoenzymatic profile (Costa et al. 1997b), as well as crossing experiments (Costa et al. 2003b) and sequence analyses of the cytochrome *b* gene fragments from their mitochondrial DNA (mtDNA) (Monteiro et al. 2004); these studies confirmed the existence of a species complex.

The molecular studies and crossing experiments were decisive to the elevation of *T. b. melanica* to species status (Costa et al. 2006) and to the description of the new species *Triatoma juazeirensis* Costa and Felix 2007, both identified as independent evolutionary units. Recently, phylogenetic studies using cytochrome b and 16S rRNA sequencing identified *Triatoma sherlocki* Papa et

al. 2002 as a sister species of *T. melanica* and a member of the *T. brasiliensis* complex (Mendonça et al. 2009). These studies also suggest that *T. b. brasiliensis* and *T. b. macromelasoma* are sufficiently distinct from other members of the group and thus, their subspecies status requires revalidation.

In this paper, we provide a detailed redescription of *T. b. macromelasoma*, mainly following the characteristics proposed by Lent and Wygodzinsky (1979) for the Triatominae group. Its subspecies status, as well as that of *T. b. brasiliensis*, is revalidated based on a morphological comparative study of all members of the *T. brasiliensis* complex, which corroborates the results from the above cited multidisciplinary approaches. An identification key for all members of the complex is also provided.

MATERIALS AND METHODS

The material studied herein is deposited in the Entomological Collection of Oswaldo Cruz Institute (CEIOC), Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. The type specimens of *T. b. macromelasoma* and *T. melanica* have been lost; therefore, these specimens were identified based on the literature and on comparisons with previously determined material deposited in the CEIOC, including the holotypes of *T. juazeirensis* (Costa & Felix 2007) and *T. brasiliensis* (Gonçalves et al. 1993).

The redescription of *T. b. macromelasoma* is based on two specimens from its type locality (municipality of Petrolina, PE), as well as 15 males and 15 females from an F1 colony reared from insects collected in this locality.

The manner of description and morphological terminology mainly follow the style of Lent and Wygodzinsky (1979). Measurements were taken using a stereoscopic microscope with an ocular micrometre.

RESULTS

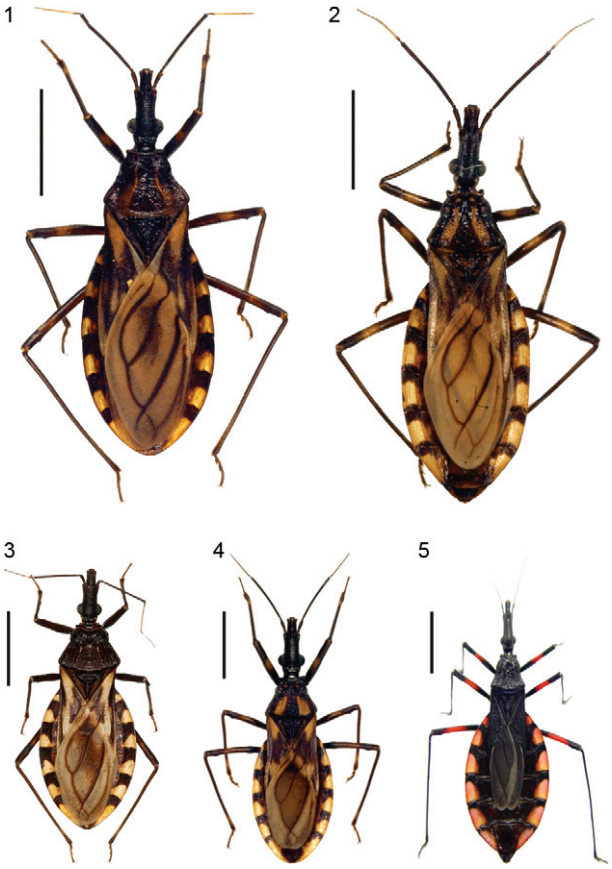
T. b. macromelasoma Galvão, 1956, revalidated
(Fig. 1)
T. b. macromelasoma Galvão (1956)
T. brasiliensis Lent and Wygodzinsky (1979)

Length: males 19.4-24.3 mm, females 21.5-25.5 mm; width of pronotum (posterior lobe): males 3.9-5.4 mm, females 4.4-5.3 mm; width of abdomen: males 6.0-7.6 mm, females 6.6-9.1 mm. All measurements are listed in the Table.

Overall colour: dark brown with yellow to brownish-yellow marks.

Head: dark brown. Twice as long as wide across the eyes (males 1:0.35-0.42; females 1:0.35-0.41) and distinctly longer than the pronotum (males 1:0.70-0.86; females 1:0.72-0.89). Anteocular region four times as long as postocular (1:0.25). Clypeus distinctly, but not abruptly widened behind the middle. Genae tapering distally, but with apex narrowly rounded, not pointed, slightly projecting beyond the level of the apex of clypeus. Jugae widely rounded apically. Eyes, in lateral view, approaching, but not attaining the level of the under surface and remote from the level of the upper surface of the head. Ratio width of eye to synthlipsis 1:2.00-2.30 (males) and 1:1.91-2.67 (females). Antenniferous tubercles inserted slightly before middle of anteocular region. First antennal segment attaining the level of the apex of clypeus; second segment subcylindrical, beset with declivous setae shorter than diameter of the segment. Ratio of antennal segments 1:4.0-5.3:2.4-3.8:2.0-2.8 (males) and 1:3.4-4.5:2.4-3.2:2.0-2.8 (females). Rostrum thick, as dark as the head capsule, with medium-sized hairs on the first and on the underside of the second segment, with long and very numerous hairs on the upper surface of the second and on the entire third segment; hairs especially dense dorsally at the junction of the second and third segments. First rostral segment extending to the level of the apex of antenniferous tubercles, second one to the level of the apex of the middle of the eyes. Ratio of rostral segments 1:1.9-2.7:1.1-1.4 (males) and 1:2.1-2.3:1.1-1.3 (females). Neck dark, with a pair of light-coloured spots laterally.

Pronotum: very sparsely granulose. Dark brown. Anterior lobe with distal brownish-yellow tubercles. Posterior lobe with submedian carinae evanescent on posterior fourth; 1+1 narrow brownish-yellow stripes on carinae broadening posteriorly, not attaining pronotal apex. Humeral angles rounded, slightly angular. Scutellum: black, posterior process with small yellow or brownish-yellow point on apex. Scutellum coarsely



Figs 1-5: the five members of the *Triatoma brasiliensis* species complex, dorsal habitus. 1: *Triatoma brasiliensis macromelasoma*, male; 2: *Triatoma brasiliensis brasiliensis*, female; 3: *Triatoma juazeirensis*, male; 4: *Triatoma melanica*, male; 5: *Triatoma sherlocki*, female. Bars = 5 mm.

wrinkled, with or without a distinct central depression. Posterior process as long as the main body of the scutellum, subcylindrical, but slightly compressed laterally; apex slightly elevated, rounded.

Hemelytra: extending to variable levels from the base to the apex of the seventh urotergite. Corium light yellow, with dark areas of variable extension, mainly in veins Cu, m-cu and R+M, attaining Sc. Clavus entirely dark. Membrane fumose, yellowish-brown, as light as light-coloured areas of corium. Veins of membrane black; lumen of cells with a more or less extensive, irregularly shaped sooty spot extending over the central portion, mainly extending to the Cu and Pcu veins.

Legs: dark, with light markings on the trochantera; incomplete brownish-yellow ring on the apical half of the femora and the subapical portion of the tibiae. Legs slender, fore femora six-seven times as long as wide. Fore and mid femora salient below subapically or with one or two weak denticles. Males with spongy fossulae on tibiae of fore and mid legs; absent in females.

Abdomen: slightly flattened below in both sexes, delicately striate transversally, sparsely setose. Spiracles adjoining connexival suture. Abdomen of female very wide, lateral portions of urotergites exposed. Venter brown; spiracles enclosed in minute yellow area. Connexival segments presenting a black spot on anterior region, posterior edge rounded, followed by a larger light yellow spot; wide black spot enclosing intersegmental sutures.

Male genitalia: as described by Lent and Jurberg (1978) [according to Costa et al. (1997a), individual variations in the male genitalia of *T. brasiliensis* and the other members of the complex are not correlated with their different and stable chromatic forms. Therefore, the genital structures are not useful for distinguishing *T. b. macromelasoma*.].

Material examined - *T. b. macromelasoma* - PE: two males, Petrolina, CEIOC; 15 males, 15 females, F1 colony reared from insects from Petrolina, CEIOC. *T. b. brasiliensis* - RN: one female (holotype), Caicó, CEIOC; state of Ceará: five males, five females, Jaguaruana, CEIOC. *T. juazeirensis* - BA: one female (holotype), three males (paratypes), two females (paratypes), Juazeiro, CEIOC; seven males, 12 females, Juazeiro, CEIOC. *T. melanica* - MG: three males, three females, Espinosa, CEIOC. *T. sherlocki* - BA: one male, one female, Gentio do Ouro, CEIOC.

Key to *T. brasiliensis* complex

1a. Brachypterous specimens, hemelytra not extending posteriorly beyond the posterior margin of urotergite VI; legs unusually long; ground colour dark brown to black, connexivum and femora with orange to red marks ..... *T. sherlocki* (Fig. 5) (BA)

1b. Macropterous specimens, hemelytra extending posteriorly at least as far as urotergite VII; legs normally long; ground colour brown, hemelytra and connexivum with brownish-yellow marks 2

2a. Pronotum and scutellum dark brown to black, rarely with few inconspicuous brownish-yellow marks; femora entirely dark brown to black, without brownish-yellow rings ..... *T. juazeirensis* (Fig. 3) (BA)

2b. Pronotum with 1+1 elongate or subtriangular broad areas or narrow stripes, brownish-yellow; scutellum with apex of posterior process brownish-yellow; femora with complete or incomplete brownish-yellow rings 3

TABLE

Measurements (in mm) of *Triatoma brasiliensis macromelasoma* based on 15 males and 15 females from a F1 colony reared from insects collected in the type locality (municipality of Petrolina, state of Pernambuco, Brazil)

Gender	Male					Female				
Variables	Min	Max	X	S^2	SD	Min	Max	X	S^2	SD
Total length	19.4	24.3	21.17	1.573	1.254	21.5	25.5	23.04	0.937	0.968
Length of head	3.7	4.7	4.19	0.001	0.027	4.1	4.8	4.40	0.000	0.018
Width of head	1.9	2.4	2.09	0.000	0.012	2.0	2.3	2.13	0.000	0.008
Synthlipsis	1.0	1.2	1.07	0.000	0.007	1.1	1.3	1.15	0.000	0.007
Width of eyes	0.5	0.6	0.50	0.000	0.003	0.5	0.6	0.51	0.000	0.003
Length of 1st antennal segment	0.7	1.0	0.82	0.000	0.007	0.8	1.0	0.93	0.000	0.007
Length of 2nd antennal segment	3.4	4.0	3.70	0.000	0.021	3.4	4.3	3.78	0.001	0.023
Length of 3rd antennal segment	2.3	2.8	2.52	0.000	0.014	2.4	2.9	2.61	0.000	0.017
Length of 4th antennal segment	1.8	2.1	1.95	0.000	0.013	2.0	2.4	2.14	0.000	0.011
Length of 1st rostral segment	0.9	1.1	0.98	0.000	0.006	1.0	1.1	1.04	0.000	0.005
Length of 2nd rostral segment	2.1	2.4	2.24	0.000	0.010	2.2	2.5	2.28	0.000	0.009
Length of 3rd rostral segment	1.0	1.2	1.15	0.000	0.006	1.1	1.4	1.20	0.000	0.006
Length of pronotum	2.8	3.9	3.16	0.001	0.030	3.1	3.9	3.34	0.000	0.022
Anterior width of pronotum	2.4	3.1	2.66	0.000	0.020	2.6	3.2	2.83	0.000	0.016
Posterior width of pronotum	3.9	5.4	4.39	0.001	0.037	4.4	5.3	4.69	0.001	0.027
Width of abdomen	6.0	7.6	6.69	0.003	0.052	6.6	9.1	7.44	0.004	0.062

Max; maxima; Min: minimum; SD: standard deviation; S^2: variance; X: average.

3a. Pronotum with 1+1 narrow brownish-yellow stripes; membrane of hemelytra with lumen of cells partially darkened ..... *T. b. macromelasoma* (Fig. 1) (PE)

3b. Pronotum with 1+1 broad, elongated brownish-yellow areas; membrane of hemelytra with lumen of cells entirely or not darkened 4

4a. Pronotum with 1+1 brownish-yellow areas extending from posterior portion of anterior lobe to posterior lobe; femora with broad brownish-yellow rings; membrane of hemelytra with lumen of cells not darkened; males with spongy fossulae on fore and mid tibiae ..... *T. b. brasiliensis* (Fig. 2) (Northeast Region of Brazil, state of Goiás and Federal District)

4b. Pronotum with 1+1 brownish-yellow areas only on posterior lobe; femora with narrow brownish-yellow rings; membrane of hemelytra with lumen of cells entirely darkened; males with spongy fossulae only on fore tibiae ..... *T. melanica* (Fig. 4) (BA and MG)

DISCUSSION

Triatoma b. brasiliensis and *T. b. macromelasoma* are the most closely related forms among all members of the *T. brasiliensis* complex. This was determined from an isoenzymatic analysis with nine loci conducted to calculate the values of Nei's genetic distance (Nei 1987, Costa et al. 1997b) and the sequences of the mtDNA cytochrome *b* gene and by applying phylogeographic approaches among 136 specimens representing 16 populations (Monteiro et al. 2004). The latter analysis revealed the existence of three discrete lineages: *brasiliensis+macromelasoma*, *juazeiro* (*T. juazeirensis*) and *melanica*. These clades are separated from each other by more than nine mutational steps, which is the 95% confidence interval for the statistical parsimony process.

Despite this close relationship, the phylogenetic analyses showed that *T. b. brasiliensis* and *T. b. macromelasoma* have significant genetic differentiation, indicating their status as subspecies (Monteiro et al. 2004). In addition, Costa et al. (2009) used geometric morphometric techniques to compare the shape of the wings of the *T. brasiliensis* complex members. These authors proposed that *T. b. macromelasoma* could have originated by homoploid hybrid speciation (Greig et al. 2002, Gross & Rieseberg 2005, Mavarez et al. 2006, Meyer et al. 2006, Mallet 2007) via ancient contact between the neighbouring species *T. brasiliensis* and *T. juazeirensis* and the hybridisation of the putative parental forms most likely occurred in the area of PE. Furthermore, the geometric morphometric analysis showed that *T. b. macromelasoma* is closely related to *T. b. brasiliensis* and is distinct from all other members of the *T. brasiliensis* complex (Costa et al. 2009). The Mahalanobis distances were calculated for all members of the complex based on the shape (conformation) of the wings and these were convergent with the values of the matrices of genetic distances obtained either by isoenzyme analyses or mtDNA sequences (Costa et al. 2009). In addition, despite all morphological, biological, genetic and ecological differences recorded among the members of the complex, they are all able to produce viable hybrids under laboratory conditions (Costa et al. 2003b, Almeida et al. 2012). However, no evidence of natural crosses was observed between the members of the complex, except for *T. b. brasiliensis* and *T. b. macromelasoma*.

These multidisciplinary analyses revealed that *T. b. macromelasoma* has (i) a stable, homogeneous and differentiated colour pattern that is easily distinguished from those of the other members of the complex (Costa et al. 1997a, 2009), (ii) a distinct egg shape and exochorion ornamentation (Costa et al. 1997a) and (iii) a distinct geographic distribution, as it is found only in areas of PE (Costa et al. 1998, 2003a). Therefore, the revalidation of *T. b. macromelasoma* as a subspecies agrees with the results of several independent taxonomic analyses, showing the closest relationship between this taxon and *T. b. brasiliensis* when compared to the other members of the complex.

Morphologically, *T. b. macromelasoma* can be distinguished from all members of the *T. brasiliensis* species complex by the following combination of features: (i) pronotum with 1+1 narrow brownish-yellow stripes on the submedian carinae, not attaining its apex, (ii) hemelytra with darkened membrane cells on the central portion and (iii) legs with an incomplete brownish-yellow ring on the apical half of the femora. The yellow marks on the pronotum are the more consistent feature that differentiates the members of the complex (Lent & Wygodzinsky 1979, Costa 1997). As expected, the females of *T. b. macromelasoma* tend to be slightly larger than the males, a pattern commonly observed in triatomines (Lent & Wygodzinsky 1979) and, more specifically, in other members of the *T. brasiliensis* complex (Costa et al. 2006, Costa & Felix 2007).

A definition of the taxonomic status of the *T. brasiliensis* complex members is important for epidemiological studies and control measures (Costa & Felix 2007) and correct morphological identification is equally important. Thus, the present key, which includes all members of the complex, can be considered a fundamental tool for the identification of specimens, especially in fieldwork.

ACKNOWLEDGEMENTS

To Archibaldo Belo Galvão (*in memoriam*) and Herman Lent (*in memoriam*), for suggestions and incentive, and to the technicians of the SVS and Funasa, Brazil, for valuable help in the fieldworks.

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