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Morphology of the terminal abdominal segments in females of Triatominae (Insecta: Hemiptera: Reduviidae)

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ABSTRACT

Triatominae (Insecta: Hemiptera: Reduviidae) includes 151 extant species that are all potential vectors of Chagas disease. The relationships among these species have been recently studied based on molecular and cytogenetical approaches, and although the morphology of these insects is fairly described, wide comparative studies and cladistics analysis based on this feature are lacking. A comparative study of the terminal abdominal segments in females of 26 species of Triatominae was performed in order to evaluate their importance for the distinction of genera and species, and obtain characters to be used in cladistics analyses. The results showed that the short combined segments IX and X in dorsal view is not diagnostic for *Rhodnius*, and the elongated trapezoidal dorsal shape of combined segments IX and X is not exclusive of *Panstrongylus*. There are diagnostic features at the generic level for *Rhodnius* and *Dipetalogaster*, but not for the other genera studied. Among the structures examined, tergite IX is the most valuable for distinguishing species of Triatominae. The female terminalia is useful for the specific identification of Triatominae, but cannot be used to diagnose most genera or to directly assess supraspecific relationships. These can only be unveiled by using additional morphological and/or molecular data in broad cladistics analyses.

1. Introduction

Kissing bugs (Insecta: Hemiptera: Reduviidae: Triatominae) are widely distributed in the Neotropical Region and represent 151 extant species classified in 18 genera and five tribes (Galvão and de Paula, 2014; Mendonça et al., 2016; Rosa et al., 2017). They are strictly hematophagous during all postembryonic life cycle and vectors of *Trypanosoma cruzi* (Chagas, 1909), the etiological agent of Chagas disease. Between six and seven million people are infected by this parasite, most from Latin America, and more than ten thousand people die each year due to clinical manifestations of the disease (WHO 2017a,b).

The genera *Panstrongylus* Berg, *Triatoma* Laporte and *Rhodnius* Stål are the most diverse of the subfamily and the most significant epidemiologically, due to their synanthropic habits and domiciliation capacity (Schofield 1994). The first two are included in Triatomini and the last in Rhodniini, which are tribes supported by morphometric and molecular analyses (De Paula et al., 2005; Patterson 2007), with wide morphological and physiological disparities (Schofield and Galvão 2009).

Characteristics commonly used to distinguish genera and species of Triatominae include the general color of the body and legs, and morphological aspects of the head, scutellum and male genitalia (Lent and Wygodzinsky 1979). The female genitalia was described for most species of the subfamily (Lent 1948; Abalos and Wygodzinsky 1951; Sherlock and Serafim 1967; Lent and Jurberg 1968, 1969, 1975), but was considered uniform by previous authors and, therefore, not appropriate for specific identification (Lent and Wygodzinsky 1979).

Rosa et al. (2010) were the first to analyze the terminal abdominal segments in females of Triatominae using scanning electron microscopy, and showed that they in fact have diagnostic value. Subsequently, Rosa et al. (2014) studied the genus *Rhodnius* in detail, demonstrating that even species very similar in general morphology can be distinguished based on this feature. Having proven useful, this technique was used in the descriptions of *R. montenegrensis* Rosa et al. (2012), *R. marabaensis* Souza et al. (2016) and *R. taquarussuensis* Rosa et al. (2017). Recently, Rivas et al. (2017) compared this feature in six

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Table 1
Species, collection (CTIOC) or colony number, and origin of the triatomines used in this study.

Species	Collection/colony number	Origin
***Panstrongylus chinai* (Del Ponte, 1929)**	CTIOC 1409	Ecuador, Loja, 2200m
***P. diasi* (Pinto & Lent, 1946)**	CTIOC 1426	Brazil, Espírito Santo
***P. geniculatus* (Latreille, 1811)**	CTIOC 1295	Brazil, Espírito Santo, Alegre
	CTIOC 1333	Brazil, Rio de Janeiro, Angra dos Reis, Mambucaba
***P. guentheri* Berg, 1879**	CTIOC 1812	No locality data
***P. humeralis* (Usinger, 1939)**	CTIOC 1826	Argentina, Córdoba
	CTIOC 1835	Argentina, Córdoba
***P. lignarius* (Walker, 1873)**	CTIOC 6570	Peru, Lima, Jesus Maria
	"2984"	No locality data
***P. lutzi* (Neiva & Pinto, 1923)**	CTIOC 1424	Brazil, Ceará, Itatira, Lagoa do Mato
***P. megistus* (Burmeister, 1835)**	CTIOC 1610	Brazil, Pernambuco, Recife, Nazaré
	Colony 119	Brazil, Minas Gerais, Piranguinho, Esmeril
***P. rufotuberculatus* (Champion, 1899)**	CTIOC 1422	Bolivia
***P. tupynambai*Lent, 1942**	CTIOC 1755	Brazil, Rio Grande do Sul, Cachoeira do Sul
***Dipetalogaster maxima* (Uhler, 1894)**	CTIOC 2715	Mexico, Baja California Sur
***Eratyrus mucronatus* Stål, 1859**	CTIOC 6538	No locality data
***Meccus phyllosomus* (Burmeister, 1835)**	No collection number	Mexico
	No collection number	Mexico
***Nesotriatoma bruneri* Usinger, 1944**	Colony 129	Cuba
***Triatoma barberi* Usinger, 1939**	CTIOC 4813	Mexico, Puebla
***T. brasiliensis* Neiva, 1911**	Colony 04	Brazil, Ceará, Sobral, Boa Vista
***T. dimidiata* (Latreille, 1811)**	Colony 147	Central America
***T. infestans* (Klug, 1834)**	Colony 26	Argentina, Santa Fé, Santa Fé
***T. lecticularia* (Stål, 1859)**	Colony 32	U.S.A., Oklahoma, Waurika
***T. maculata* (Erichson, 1848)**	CTIOC 4581	Venezuela, Mérida
***T. matogrossensis* Leite & Barbosa, 1953**	Colony 38	Brazil
***T. rubrofasciata* (De Geer, 1773)**	Colony 145	Brazil, Pará, Belém
***T. rubrovaria* (Blanchard, 1843)**	Colony 143	Brazil, Rio Grande do Sul, Caçapava do Sul, Serra da Picada
***T. sordida* (Stål, 1859)**	Colony 59	Argentina, Corrientes, San Miguel, zona rural
***T. tibiamaculata* Pinto, 1926**	Colony 68	Brazil, Santa Catarina, Navegantes
***Rhodnius brethesi* Matta, 1919**	Colony 78	Brazil, Amazonas, Rio Acará, afluente do rio Negro

species of the genus *Meccus* Stål.

The objective of the present study is to further test the value of the terminal abdominal segments of females in the distinction of genera and species of Triatominae through a comparative analysis of images generated by scanning electron microscopy, and to identify characters to be used in cladistics analyses.

2. Material and methods

We studied 26 species of Triatominae included in the genera *Panstrongylus* (10 species), *Dipetalogaster* Usinger (1), *Eratyrus* Stål (1), *Meccus* Stål (1), *Nesotriatoma* Usinger (1), *Triatoma* (11) and *Rhodnius* (1) (Table 1). Specimens examined were obtained from the Triatomines Collection of the Oswaldo Cruz Institute (CTIOC) and from the colonies kept by the National and International Reference Laboratory in Taxonomy of Triatomines (LNIRTT), Oswaldo Cruz Institute (IOC), Rio de Janeiro, Brazil. One or two females of each species were metalized (Table 1) and the search for intraspecific variation included 10 additional specimens of each deposited in the CTIOC, except for *P. tupynambai* (5).

Specimens from the colonies were killed by freezing and washed with detergent, whereas those from CTIOC were softened in a relaxing chamber and equally washed. Then, transversal cuts were made between abdominal tergites II and III. The dissected abdomens were dehydrated in alcoholic series, placed in a drying oven at 50° C, and attached to aluminum stubs by their proximal portion.

Subsequently, the structures were metallized in the "Rudolf Barth" Scanning Electron Microscopy Platform (IOC) or in the Chemistry Institute of the São Paulo State University (UNESP, Araraquara, Brazil). Micrographies were obtained in the same institutions by using the microscopes JEOL JSM-6390LV (IOC) and Topcon SM-300 (UNESP). The most evident cases of intraspecific variation were photographed with a digital camera attached to a Leica M205C stereomicroscope and processed using automontage software (LNIRTT).

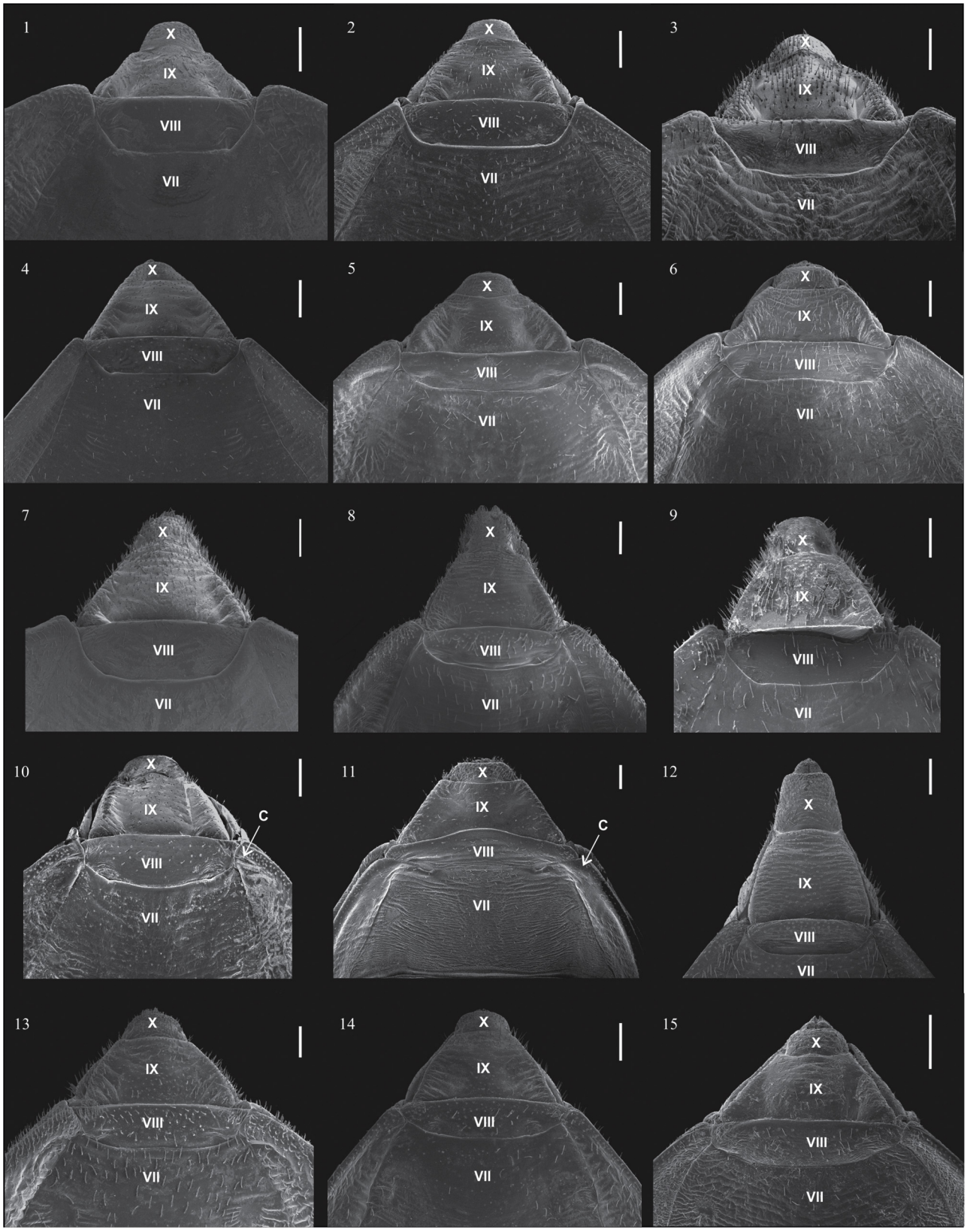
3. Results

3.1. Dorsal view (Figs. 1–26, Figs.79 and 82; Table 2)

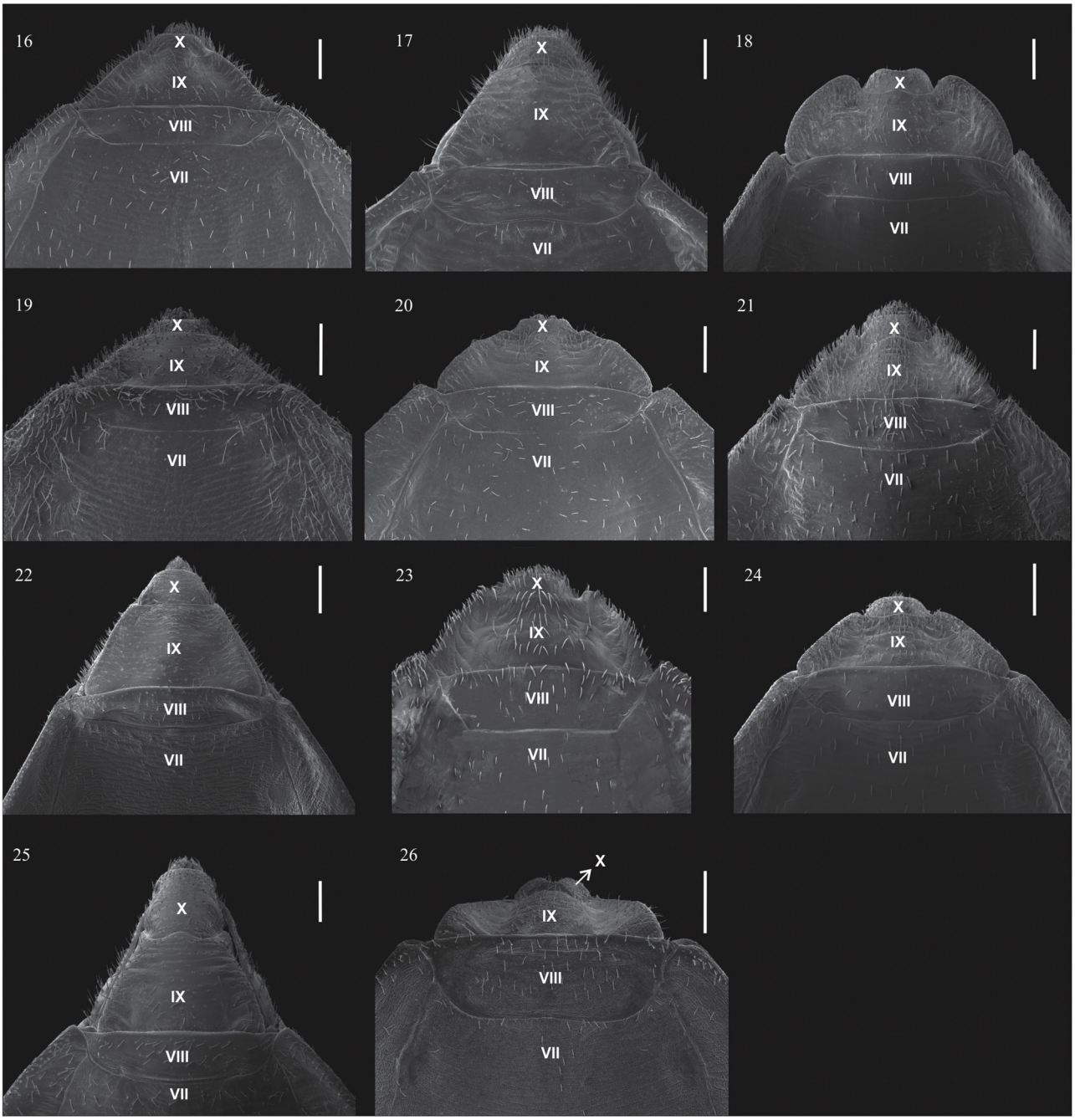
Tergite VII clearly separated from VIII, except for the median portion in *Dipetalogaster maxima* (Uhler, 1894) (Fig. 11), in which the division is only clear under higher magnifications. Posterior margin of tergite VII usually straight (Figs. 1–7, 12–13, 16, 18, 20, 23–24), with the following variation: slightly concave (*P. tupynambai* Lent, 1942, *Nesotriatoma bruneri* Usinger, 1944, *Triatoma barberi* Usinger, 1939, *T. lecticularia* (Stål, 1859), *T. maculata* (Erichson, 1848), *T. rubrofasciata* (De Geer, 1773) and *T. tibiamaculata* Pinto, 1926; Figs. 10, 14–15, 19, 21–22, 25), straight to slightly concave (*Panstrongylus megistus* (Burmeister, 1835) and *P. rufotuberculatus* (Champion, 1899); Fig. 8, 9), and straight to slightly sinuous (*T. dimidiata* (Latreille, 1811) and *Rhodnius brethesi* Matta, 1919; Figs. 17, 26). Apex of connexivum reaching or surpassing posterior margin of tergite VIII, except in *D. maxima*, where it is much shorter (compare Figs. 10 and 11).

Tergite VIII always clearly separated from IX. Tergite VIII of *R. brethesi* (Fig. 26) much larger than in other species. Posterior margin of tergite VIII usually straight (Figs. 1–7, 10, 13, 15–19, 24–26, 79, 82), except for *P. megistus, P. rufotuberculatus, T. maculata, T. matogrossensis* Leite & Barbosa, 1953, *T. rubrofasciata, T. rubrovaria* (Blanchard, 1843), *T. sordida* (Stål, 1859) (slightly convex; Figs. 8–9, 20–23), *Eratyrus mucronatus* Stål, 1859, *N. bruneri* (straight to slightly convex; Figs. 12, 14) and *D. maxima* (convex and swollen; Fig. 11).

Combined abdominal segments IX and X usually trapezoidal (Figs. 1–2, 4–7, 9–11, 13–17, 19, 21, 79). Differently shaped in *P. megistus, Eratyrus mucronatus, T. dimidiata, T. rubrofasciata* and *T. tibiamaculata* (elongated trapezoidal; Figs. 8, 12, 17, 22, 25); *T. maculata* and *T. sordida* (semi-oval; Figs. 20, 24); *T. infestans* (Klug, 1834) and *T. rubrovaria* (semi-oval with expanded posterolateral angles; Figs. 18, 23); and *R. brethesi* (short, much wider than long; Fig. 26). Usually trapezoidal, in *P. geniculatus* (Latreille, 1811) (Fig. 82), but semi-oval in



Figs. 1–15. Female external genitalia examined by scanning electron microscopy, dorsal view: 1, *Panstrongylus chinai*. 2, *P. diasi*. 3, *P. geniculatus*. 4, *P. guentheri*. 5, *P. humeralis*. 6, *P. lignarius*. 7, *P. lutzi*. 8, *P. megistus*. 9, *P. rufotuberculatus*. 10, *P. tupynambai*. 11, *Dipetalogaster maxima*. 12, *Eratyrus mucronatus*. 13, *Meccus phyllosomus*. 14, *Nesotriatoma bruneri*. 15, *Triatoma barberi*. (VII and VIII, tergites; IX and X, segments). Scale bars = 500 μm.



Figs. 16–26. Female external genitalia examined by scanning electron microscopy, dorsal view: 16, *Triatoma brasiliensis*. 17, *T. dimidiata*. 18, *T. infestans*. 19, *T. lecticularia*. 20, *T. maculata*. 21, *T. matogrossensis*. 22, *T. rubrofasciata*. 23, *T. rubrovaria*. 24, *T. sordida*. 25, *T. tibiamaculata*. 26, *Rhodnius brethesi*. (VII and VIII, tergites; IX and X segments). Scale bars = 500 μm.

some specimens (Fig. 3).

Tergite IX clearly separated from X (Figs. 2–15, 17–26), except for *P. chinai* (Del Ponte, 1929) and *T. brasiliensis* Neiva, 1911 with tenuous median portion of division (Figs. 1, 16, 79). *Panstrongylus lignarius* (Walker, 1873) with anterolateral angles more projected than in other species (Fig. 6). Lateral margins of tergite IX straight (Figs. 1–12, 14–17, 19, 21–22, 25–26, 79, 82), straight to slightly convex (*Meccus phyllosomus* (Burmeister, 1835); Fig. 13); or weakly to strongly expanded (*T. infestans, T. maculata, T. rubrovaria, T. sordida*; Figs. 18, 20, 23–24). Posterior margin of tergite IX usually straight to slightly convex or slightly concave (Figs. 1–14, 17, 19, 22, 79, 82); weakly to strongly sinuous, forming three lobes, with lateral angles more or less prominent and elevated (Figs. 10, 15–16, 18, 20–21, 23–25); or straight with median region elevated and rounded (*R. brethesi*; Fig. 26).

Posterior margin of tergite X not narrowed, straight to curved (Figs. 1–17, 19–25, 79, 82); *T. infestans* and *R. brethesi* with median notch (Figs. 18, 26). Posterior margin straight to curved in most *P. geniculatus*, but semi-oval in some specimens (Fig. 3).

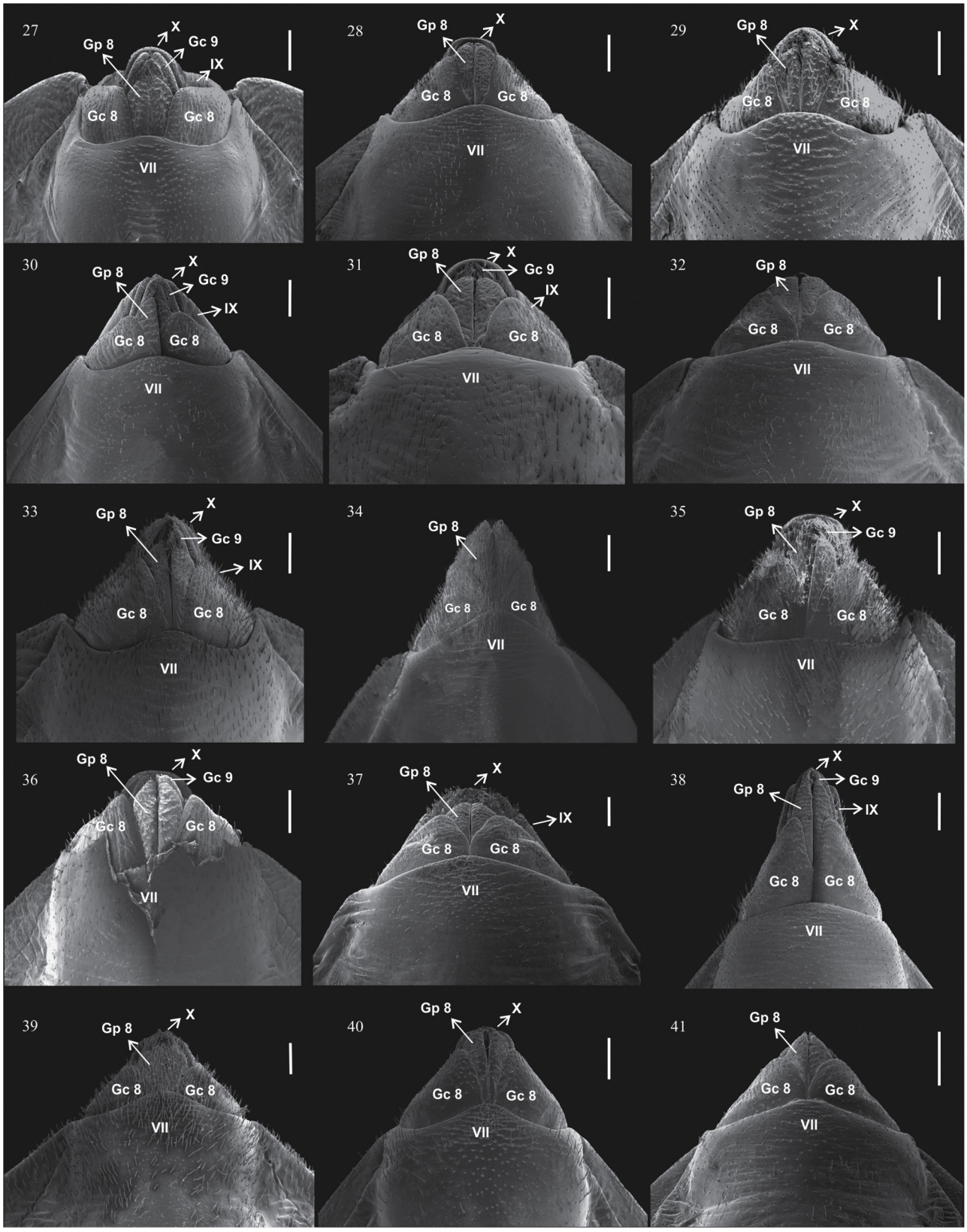
3.2. Ventral view (Figs. 27–52, 80, 83; Table 3)

Posterior margin of sternite VII variably sinuous (Figs. 27–30, 32–40, 42, 44–47, 49–52) or straight and centrally projected (*P. humeralis* (Usinger, 1939), *P. megistus, T. barberi, T. dimidiata* and *T. rubrofasciata*; Figs. 31, 34, 41, 43, 48).

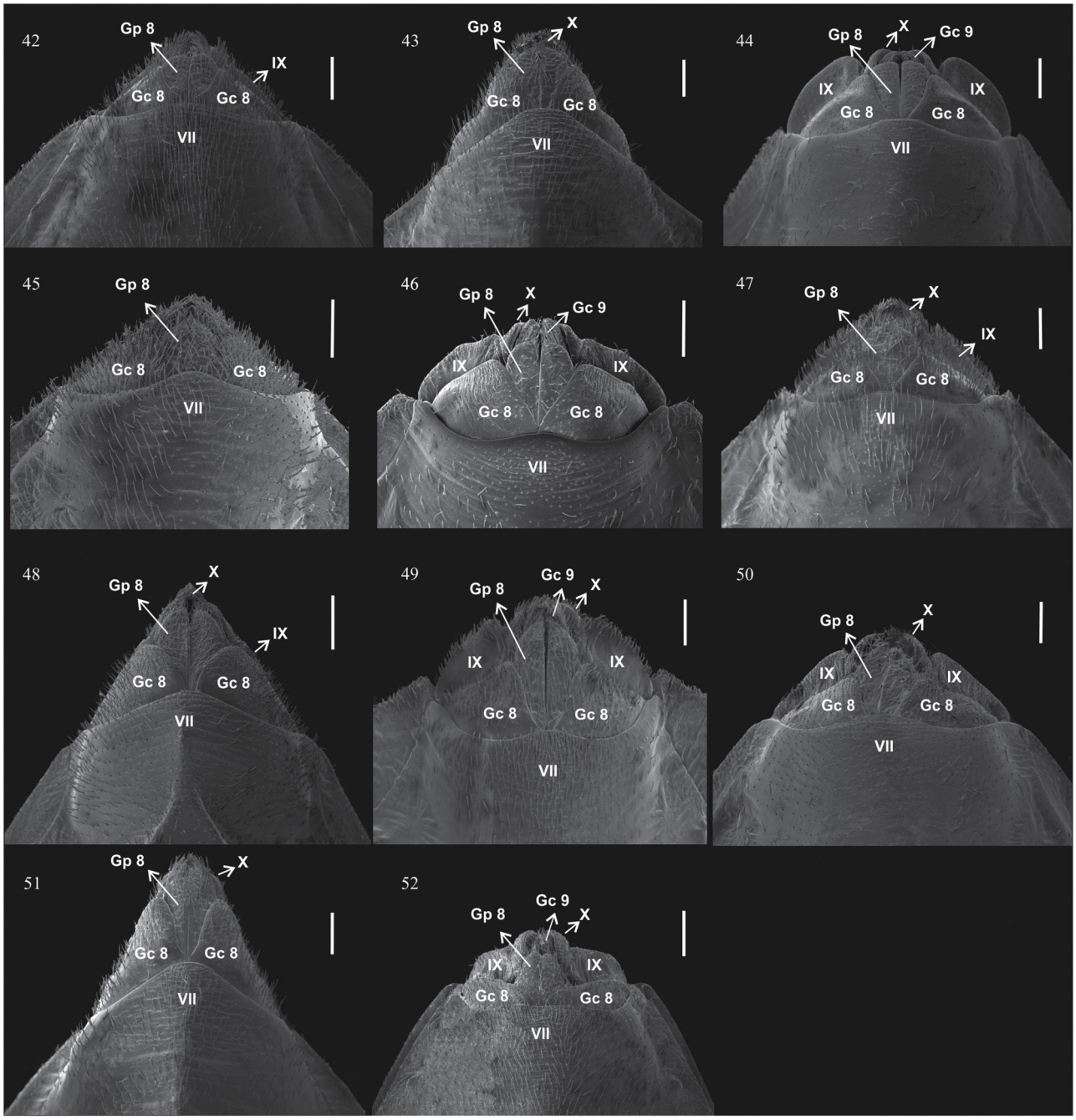
Combined abdominal segments VIII–X wider than long (Figs. 27–33,

Table 2
Variable features of the female external genitalia in 26 species of Triatominae (dorsal view).

Species	Tergite VII posterior margin	Tergite VIII posterior margin	Combined abdominal segments IX–X	Tergite IX posterior margin	Tergite IX lateral margins	Tergite X posterior margin
Panstrongylus chinai	Straight	Straight	Trapezoidal	Straight to slightly convex; some with tenuous median portion	Straight	Straight to curved
P. diasi	Straight	Straight	Trapezoidal	Slightly convex	Straight	Straight to curved
P. geniculatus	Straight	Straight	Trapezoidal; semi-oval in some specimens	Convex	Straight	Straight to curved; semi-oval in some species
P. guentheri	Straight	Straight	Trapezoidal	Straight to slightly concave	Straight	Straight to curved
P. humeralis	Straight	Straight	Trapezoidal	Concave	Straight	Straight to curved
P. lignarius	Straight	Straight	Trapezoidal	Slightly concave	Straight with anterolateral angles more projected	Straight to curved
P. lutzi	Straight	Straight	Trapezoidal	Slightly convex	Straight	Straight to curved
P. megistus	Straight to slightly concave	Slightly convex	Elongated trapezoidal	Straight	Straight	Straight to curved
P. rufotuberculatus	Straight to slightly concave	Slightly convex	Trapezoidal	Straight to slightly convex	Straight	Straight to curved
P. tupynambai	Slightly concave	Straight	Trapezoidal	Sinuous, forming three lobes, with prominent and elevated lateral angles	Straight	Straight to curved
Dipetalogaster maxima	Median portion not clearly separated from VIII	Convex and swollen	Trapezoidal	Concave	Straight	Straight to curved
Eratyrus mucronatus	Straight	Straight to slightly convex	Elongated trapezoidal	Slightly convex	Straight	Straight to curved
Meccus phyllosomus	Straight	Straight	Trapezoidal	Slightly convex	Straight to slightly convex	Straight to curved
Nesotriatoma bruneri	Slightly concave	Straight	Trapezoidal	Slightly convex	Straight	Straight to curved
Triatoma barberi	Slightly concave	Straight	Trapezoidal	Weakly sinuous, forming three lobes	Straight	Straight to curved
T. brasiliensis	Straight	Straight	Trapezoidal	Weakly sinuous, forming three lobes; tenuous median portion	Straight	Straight to curved
T. dimidiata	Straight to slightly sinuous	Straight	Trapezoidal	Straight	Straight	Straight to curved
T. infestans	Straight	Straight	Semi-oval with expanded posterolateral angles	Sinuous, forming three lobes, with prominent and elevated lateral angles	Strongly expanded	With median notch
T. lecticularia	Slightly concave	Straight	Trapezoidal	Straight to rounded	Straight	Straight to curved
T. maculata	Straight	Slightly convex	Semi-oval	Slightly concave with lateral angles slightly elevated	Weakly expanded	Straight to curved
T. matogrossensis	Slightly concave	Slightly convex	Trapezoidal	Sinuous, forming three lobes, with prominent and elevated lateral angles	Straight	Straight to curved
T. rubrofasciata	Slightly concave	Slightly convex	Elongated trapezoidal	Straight	Straight	Straight to curved
T. rubrovaria	Straight	Slightly convex	Semi-oval with expanded posterolateral angles	Straight to slightly sinuous with prominent lateral angles	Expanded	Straight to curved
T. sordida	Straight	Slightly convex	Semi-oval	Sinuous, with slightly elevated lateral angles	Weakly expanded	Straight to curved
T. tibiamaculata	Slightly concave	Straight	Elongated trapezoidal	Sinuous, with slightly elevated lateral angles	Straight	Straight to curved
Rhodnius brethesi	Straight to slightly sinuous	Straight; tergite much larger than other species	Very wide and short	Straight with median region elevated and rounded	Straight	With median notch



Figs. 27–41. Female external genitalia examined by scanning electron microscopy, ventral view: 27, *Panstrongylus chinai*. 28, *P. diasi*. 29, *P. geniculatus*. 30, *P. guentheri*. 31, *P. humeralis*. 32, *P. lignarius*. 33, *P. lutzi*. 34, *P. megistus*. 35, *P. rufotuberculatus*. 36, *P. tupynambai*. 37, *Dipetalogaster maxima*. 38, *Eratyrus mucronatus*. 39, *Meccus phyllosomus*. 40, *Nesotriatoma bruneri*. 41, *Triatoma barberi*. (Gc8, gonocoxite VIII; Gc9, gonocoxite IX; Gp8, gonapophysis VIII; Gp9, gonapophysis IX; VII, sternite; IX and X, segments). Scale bars = 500 μm.



Figs. 42–52. Female external genitalia examined by scanning electron microscopy, ventral view: 42, *Triatoma brasiliensis*. 43, *T. dimidiata*. 44, *T. infestans*. 45, *T. lecticularia*. 46, *T. maculata*. 47, *T. matogrossensis*. 48, *T. rubrofasciata*. 49, *T. rubrovaria*. 50, *T. sordida*. 51, *T. tibiamaculata*. 52, *Rhodnius brethesi*. (Gc8, gonocoxite VIII; Gc9, gonocoxite IX; Gp8, gonapophysis VIII; Gp9, gonapophysis IX; VII, sternite; IX and X, segments). Scale bars = 500 μm.

35–37, 39–42, 44–47, 49–50, 52, 80, 83), except for *P. megistus*, *E. mucronatus*, *T. dimidiata*, *T. rubrofasciata* and *T. tibiamaculata* (longer than wide; Figs. 34, 38, 43, 48, 51).

Gonocoxites VIII usually triangular and convergent (Figs. 28 –33, 35–42, 44–50, 83), but sometimes not convergent, elongated (*P. megistus, E. mucronatus, T. dimidiata, T. tibiamaculata*; Figs. 34, 38, 43, 51), or transversal with straight posterior margins (*R. brethesi*; Fig. 52). The last condition is an exception in *P. chinai* (Fig. 27), because most specimens have triangular, convergent plates (Fig. 80). Another character that is variable in *P. chinai* is the apex of the connexivum, which is wide and surpasses gonocoxite VIII in some specimens (Fig. 27), a condition not seen in other species of *Panstrongylus*. Gonapophysis VIII never elongated, but variable intraspecifically; usually longer in *E. mucronatus*, *T. rubrovaria* e *T. tibiamaculata* (Figs. 38, 49, 51).

Sternites IX and X, and gonocoxite IX visible or not depending on the position of the genitalia in the specimen. Sternite IX usually narrow, except for *T. infestans*, *T. maculata*, *T. matogrossensis*, *T. rubrovaria*, *T. sordida* and *R. brethesi* (Figs. 44, 46–47, 49–50, 52), in which it is expanded as can be seen in dorsal view (Figs. 18, 20–21, 23–24, 26).

3.3. Posterior view (Figs. 53–78, 81, 84; Table 4)

Lateral sclerites (appendices) between tergite VIII and gonocoxites VIII present in all species, but visible or not depending on the position and shape of tergite IX. Appendices never visible in species with tergite IX enlarged at base: *P. humeralis*, *P. lignarius*, *T. brasiliensis*, *T. infestans*,

Table 3
Variable features of the female external genitalia in 26 species of Triatominae (ventral view).

Species	Sternite VII posterior margin	Combined abdominal segments VIII–X	Gonocoxites VIII	Sternite IX
Panstrongylus chinai	Sinuous	Wider than long	Triangular and convergent or transversal with straight apical margins	Narrow or not visible
P. diasi	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
P. geniculatus	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
P. guentheri	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
P. humeralis	Straight, centrally projected	Wider than long	Triangular and convergent	Narrow or not visible
P. lignarius	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
P. lutzi	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
P. megistus	Sinuous	Longer than wide	Not convergent, elongated	Narrow or not visible
P. rufotuberculatus	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
P. tupynambai	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
Dipetalogaster maxima	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
Eratyrus mucronatus	Sinuous	Longer than wide	Not convergent, elongated	Narrow or not visible
Meccus phyllosomus	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
Nesotriatoma bruneri	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
Triatoma barberi	Straight, centrally projected	Wider than long	Triangular and convergent	Narrow or not visible
T. brasiliensis	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
T. dimidiata	Straight, centrally projected	Longer than wide	Not convergent, elongated	Narrow or not visible
T. infestans	Sinuous	Wider than long	Triangular and convergent	Strongly expanded
T. lecticularia	Sinuous	Wider than long	Triangular and convergent	Narrow or not visible
T. maculata	Sinuous	Wider than long	Triangular and convergent	Expanded
T. matogrossensis	Sinuous	Wider than long	Triangular and convergent	Expanded
T. rubrofasciata	Straight, centrally projected	Longer than wide	Triangular and convergent	Narrow or not visible
T. rubrovaria	Sinuous	Wider than long	Triangular and convergent	Strongly expanded
T. sordida	Sinuous	Wider than long	Triangular and convergent	Expanded
T. tibiamaculata	Variably sinuous	Longer than wide	Not convergent, elongated	Narrow or not visible
Rhodnius brethesi	Sinuous	Wider than long	Transversal with straight posterior margins	Expanded

T. maculata, *T. matogrossensis*, *T. rubrovaria* and *T. sordida* (Figs. 57–58, 68, 70, 72–73, 75–76).

Gonocoxites VIII elongated and narrow (Figs. 53, 55, 59–62, 81, 84); slightly wider (Figs. 54, 56, 63–78); or distinctly wider (Figs. 57, 58). Gonapophysis VIII and gonocoxites IX visible in all species.

Abdominal segments IX–X turned down, perpendicular to the body, longer than wide (Figs. 53, 55, 59–62, 81, 84); in the same plane of the body or slightly turned down, wider than long (Figs. 57–58, 63, 68, 70–76) or as wide as long (Figs. 54, 56, 64–67, 69, 77). *R. brethesi* has segments IX–X perpendicular to the body, but short and wide (Fig. 78).

Posterior margin of tergite IX medially tenuous or fused with tergite X in *P. chinai*, *P. diasi* (Pinto & Lent, 1946), *P. geniculatus*, *T. brasiliensis* and *T. maculata* (Figs. 53–55, 68, 72, 81, 84); clearly separated from tergite X in other species (Table 4).

4. Discussion

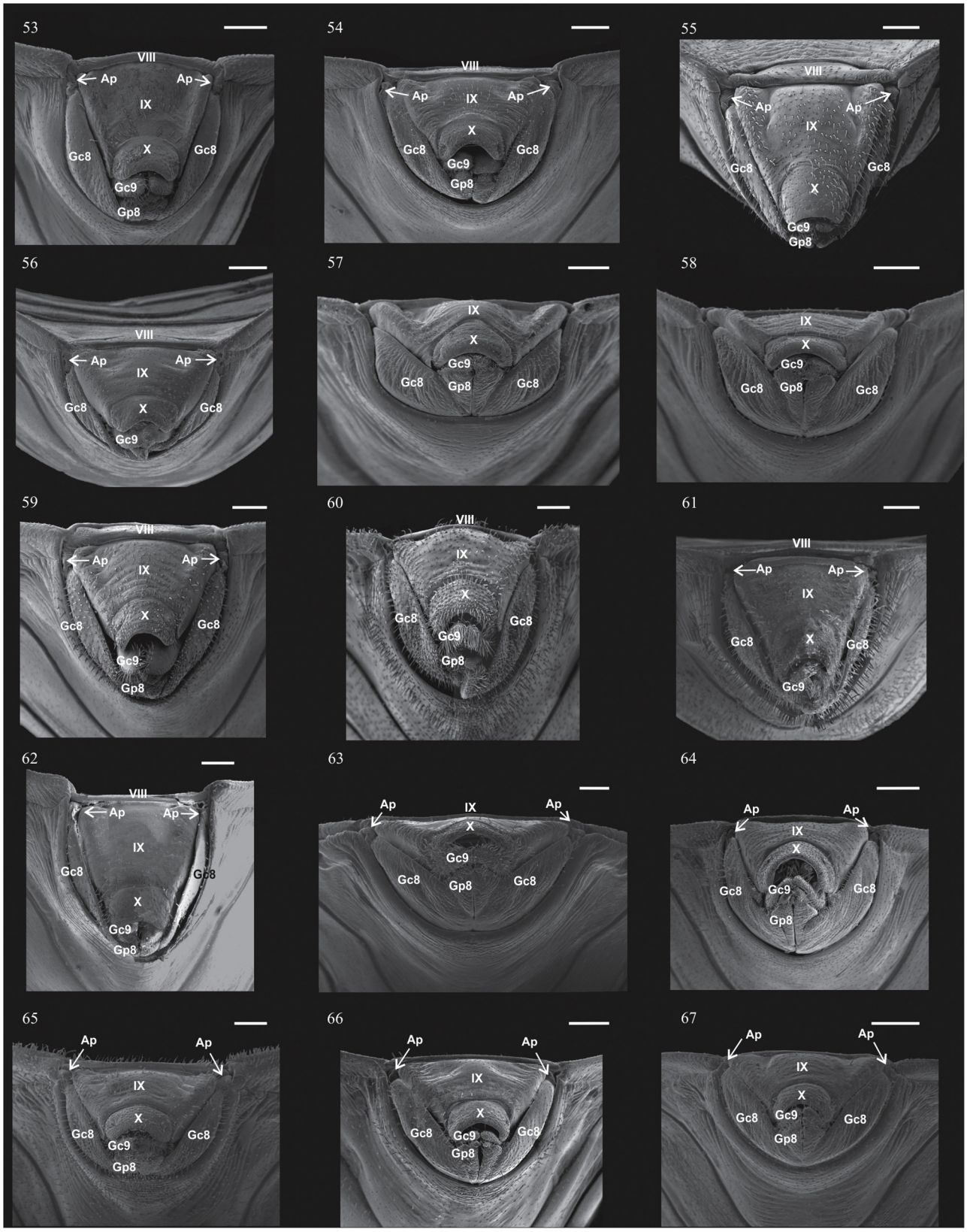
Our results include new morphological data for several species of Triatominae and corroborate most findings of Rosa et al. (2010), with a few noteworthy exceptions: 1) the short combined segments IX and X in dorsal view cannot be considered diagnostic for *Rhodnius*, because this character is shared with non-congeneric species such as *T. lecticularia* and *T. sordida*; 2) the elongated trapezoidal dorsal shape of combined segments IX and X is found not only in species of *Panstrongylus*, but also in *E. mucronatus* and *T. tibiamaculata*; 3) our images show a clear separation between tergites VII and VIII of *P. megistus*, suggesting that either the specimen observed by Rosa et al. (2010) was dirty in this area or there is intraspecific variation (unseen in our material); 4) *T. infestans* displays variation in the posterior margin of tergite VII (with large median inflection as shown by those authors or straight as illustrated here) and in the tergite IX (with callosities near margins vs. without callosities); and 6) unlike stated by Rosa et al. (2010), the lateral margins of tergite IX are in fact slightly elevated in *P. megistus*, as well as in all species studied here.

We found diagnostic features at the generic level for *Rhodnius* and *Dipetalogaster*. The former has tergite VII very large, combined abdominal segments IX–X short, much wider than long, tergite IX short and wide, and posterior margin of tergite X with median notch; whereas the latter can be identified by the median portion of tergite VII not clearly separated from tergite VIII, the apex of the connexivum short, not reaching the posterior margin of tergite VIII, which is convex and swollen.

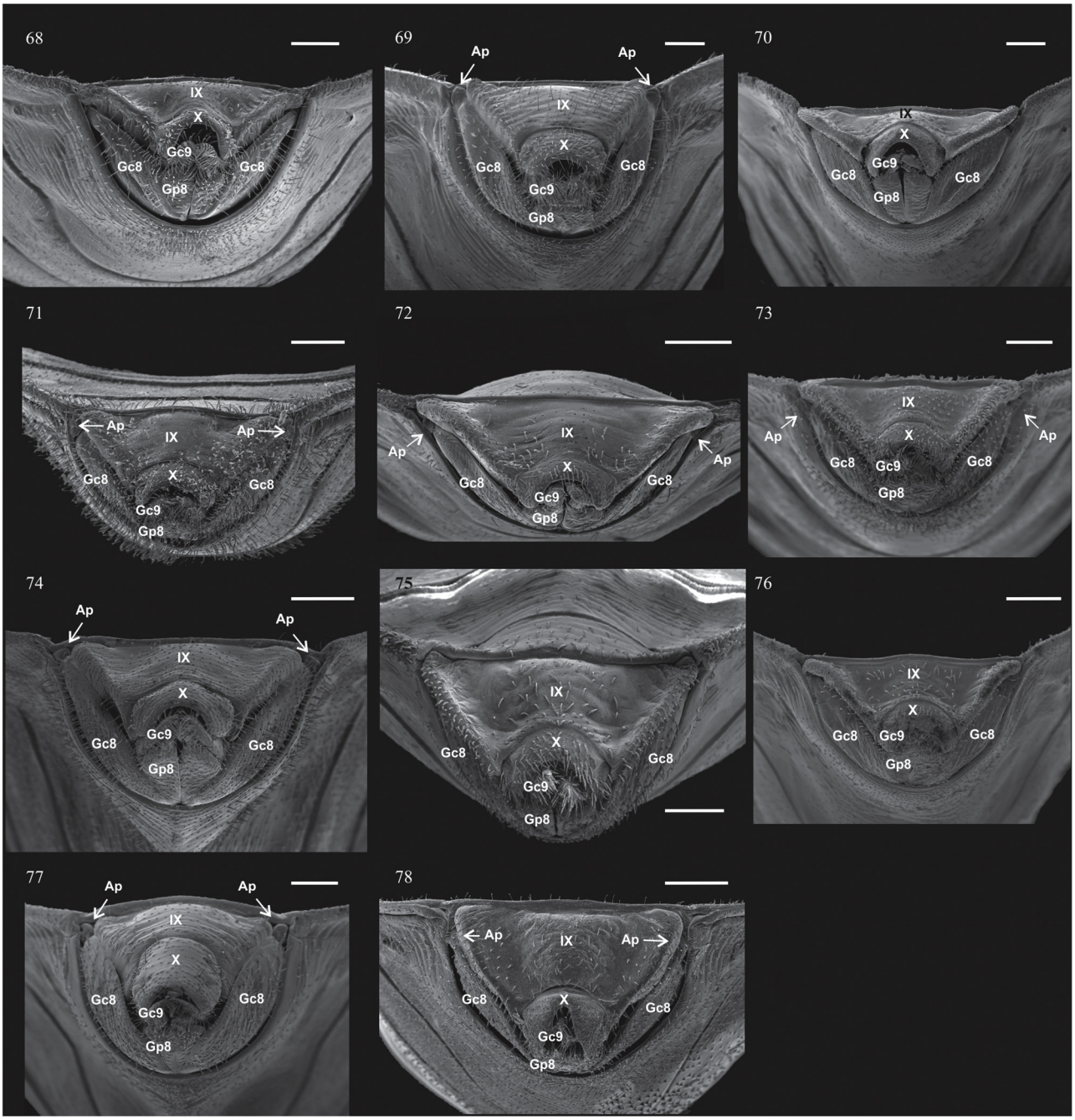
Small groups of species of *Panstrongylus* and *Triatoma* can be formed based on the same character, such as *P. chinai*, *P. geniculatus*, *P. lutzi* (Neiva & Pinto, 1923), *P. megistus, P. rufotuberculatus* and *P. tupynambai* with segments IX and X in posterior view turned down, perpendicular to the body, longer than wide, with similar shapes; or *Triatoma infestans*, *T. maculata*, *T. matogrossensis, T. rubrovaria* and *T. sordida* with tergite IX expanded. However, non-congeneric species also share the same general aspect of the terminal abdomen, e.g. *P. megistus*, *E. mucronatus*, *T. dimidiata*, *T. rubrofasciata* and *T. tibiamaculata* with combined segments IX and X in dorsal view elongated, trapezoidal.

Tergite IX is the most valuable structure for distinguishing species. It can be: 1) longer than wide (*P. megistus*, *E. mucronatus*, *T. dimidiata, T. rubrofasciata* and *T. tibiamaculata*), very wide and short (*R. brethesi*), or wider than long (all other species); 2) posterior margin weakly to strongly sinuous, forming three lobes, with lateral angles more or less prominent and elevated (*P. tupynambai*, *T. barberi*, *T. brasiliensis*, *T. infestans*, *T. matogrossensis*, *T. rubrovaria* and *T. tibiamaculata*), straight with median region elevated and rounded (*R. brethesi*), or straight to slightly convex or slightly concave (all other species); and 3) lateral margins weakly to strongly expanded (*T. infestans, T. maculata, T. matogrossensis, T. rubrovaria* and *T. sordida*) or straight to slightly convex (all other species).

Finally, the comparative study allowed us to identify, describe and encode 10 characters, five binary and five multistate, to be used in



Figs. 53–67. Female external genitalia examined by scanning electron microscopy, posterior view: 53, *Panstrongylus chinai*. 54, *P. diasi*. 55, *P. geniculatus*. 56, *P. guentheri*. 57, *P. humeralis*. 58, *P. lignarius*. 59, *P. lutzi*. 60, *P. megistus*. 61, *P. rufotuberculatus*. 62, *P. tupynambai*. 63, *Dipetalogaster maxima*. 64, *Eratyrus mucronatus*. 65, *Meccus phyllosomus*. 66, *Nesotriatoma bruneri*. 67, *Triatoma barberi*. (Ap, appendix; Gc8, gonocoxite VIII; Gc9, gonocoxite IX; Gp8, gonapophysis VIII; Gp9, gonapophysis IX; VIII, tergite; IX and X, segments). Scale bars = 500 μm.



Figs. 68–78. Female external genitalia examined by scanning elecron microscopy, posterior view: 68, *Triatoma brasiliensis*. 69, *T. dimidiata*. 70, *T. infestans*. 71, *T. lecticularia*. 72, *T. maculata*. 73, *T. matogrossensis*. 74, *T. rubrofasciata*. 75, *T. rubrovaria.* 76, *T. sordida*. 77, *T. tibiamaculata*. 78, *Rhodnius brethesi*. (Ap, appendix; Gc8, gonocoxite VIII; Gc9, gonocoxite IX; Gp8, gonapophysis VIII; Gp9, gonapophysis IX; VIII, tergite; IX and X, segments). Scale bars = 500 μm.

cladistics analyses (available as supplementary data).

5. Conclusion

The female terminalia proved useful for the specific identification of Triatominae, but cannot be used to diagnose most genera or to directly assess supraspecific relationships. These can only be unveiled by using additional morphological and/or molecular data in broad cladistics analyses.

Conflict of interest

The authors declare no conflict of interests.

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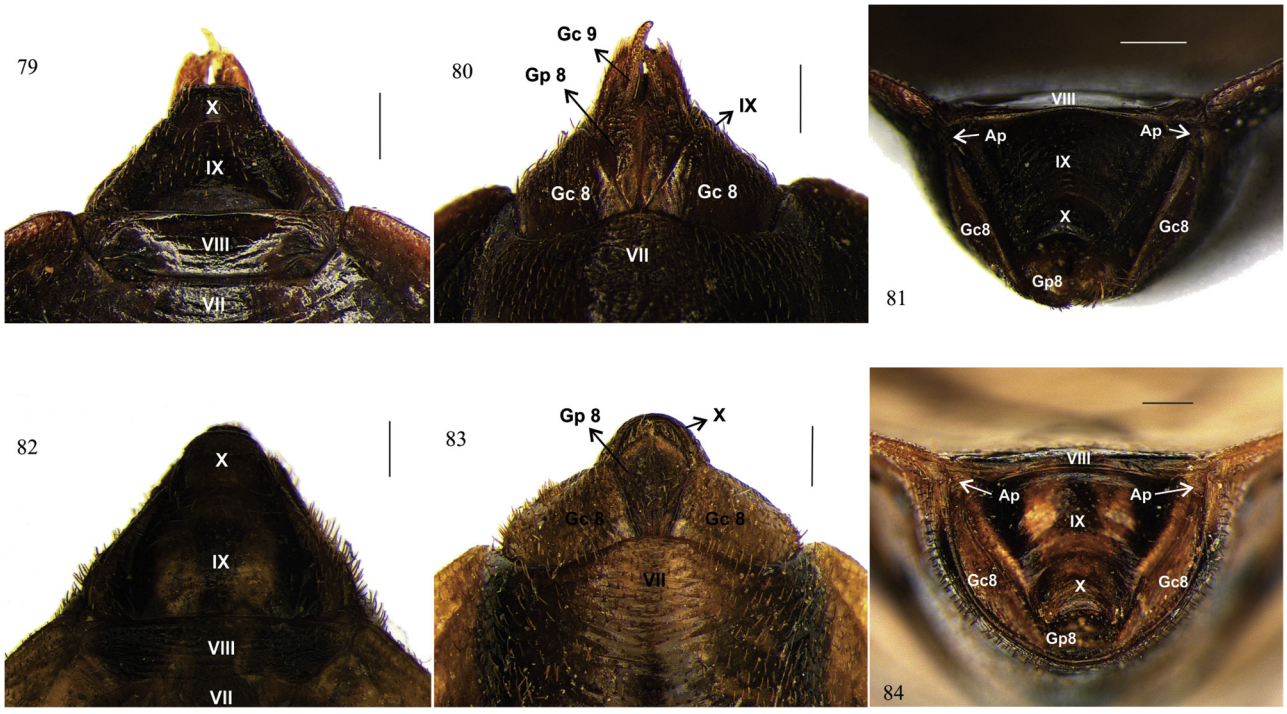

Figs. 79–84. High resolution digital photographs of the female external genitalia. 79–81, *Panstrongylus chinai*. 79, dorsal view. 80, ventral view. 81, posterior view. 82–84, *P. geniculatus*. 82, dorsal view. 83, ventral view. 84, posterior view. (Ap, appendix; Gc8, gonocoxite VIII; Gc9, gonocoxite IX; Gp8, gonapophyse VIII; Gp9, gonapophyse IX; VII and VIII, tergite; IX and X, segments). Scale bars = 0.5 mm.

Table 4
Variable features of the female external genitalia in 26 species of Triatominae (posterior view).

Species	Appendices	Gonocoxites VIII	Abdominal segments IX and X	Tergite IX posterior margin
Panstrongylus chinai	Present and visible	Elongated and narrow	Turned down, perpendicular to the body, longer than wide	Medially tenuous or fused with tergite X
P. diasi	Present and visible	Elongated, slightly wider	Slightly turned down, perpendicular to the body, longer than wide	Medially tenuous or fused with tergite X
P. geniculatus	Present and visible	Elongated and narrow	Turned down, perpendicular to the body, longer than wide	Medially tenuous or fused with tergite X
P. guentheri	Present and visible	Elongated, slightly wider	Slightly turned down, perpendicular to the body, longer than wide	Clearly separated from tergite X
P. humeralis	Not visible	Distinctly wider	In the same plane of the body, wider than long	Clearly separated from tergite X
P. lignarius	Not visible	Distinctly wider	In the same plane of the body, wider than long	Clearly separated from tergite X
P. lutzi	Present and visible	Elongated and narrow	Turned down, perpendicular to the body, longer than wide	Clearly separated from tergite X
P. megistus	Present and visible	Elongated and narrow	Turned down, perpendicular to the body, longer than wide	Clearly separated from tergite X
P. rufotuberculatus	Present and visible	Elongated and narrow	Turned down, perpendicular to the body, longer than wide	Clearly separated from tergite X
P. tupynambai	Present and visible	Elongated and narrow	Turned down, perpendicular to the body, longer than wide	Clearly separated from tergite X
Dipetalogaster maxima	Present and visible	Elongated, slightly wider	In the same plane of the body, wider than long	Clearly separated from tergite X
Eratyrus mucronatus	Present and visible	Elongated, slightly wider	Slightly turned down, as wide as long	Clearly separated from tergite X
Meccus phyllosomus	Present and visible	Elongated, slightly wider	Slightly turned down, as wide as long	Clearly separated from tergite X
Nesotriatoma bruneri	Present and visible	Elongated, slightly wider	Slightly turned down, as wide as long	Clearly separated from tergite X
Triatoma barberi	Present and visible	Elongated, slightly wider	Slightly turned down, as wide as long	Clearly separated from tergite X
T. brasiliensis	Not visible	Elongated, slightly wider	Slightly turned down, as wide as long	Medially tenuous or fused with tergite X
T. dimidiata	Present and visible	Elongated, slightly wider	Slightly turned down, as wide as long	Clearly separated from tergite X
T. infestans	Not visible	Elongated, slightly wider	Slightly turned down, wider than long	Clearly separated from tergite X
T. lecticularia	Present and visible	Elongated, slightly wider	Slightly turned down, wider than long	Clearly separated from tergite X
T. maculata	Not visible	Elongated, slightly wider	Slightly turned down, wider than long	Medially tenuous or fused with tergite X
T. matogrossensis	Not visible	Elongated, slightly wider	Slightly turned down, wider than long	Clearly separated from tergite X
T. rubrofasciata	Present and visible	Elongated, slightly wider	Slightly turned down, wider than long	Clearly separated from tergite X
T. rubrovaria	Not visible	Elongated, slightly wider	Slightly turned down, wider than long	Clearly separated from tergite X
T. sordida	Not visible	Elongated, slightly wider	Slightly turned down, as wide as long	Clearly separated from tergite X
T. tibiamaculata	Present and visible	Elongated, slightly wider	Slightly turned down, as wide as long	Clearly separated from tergite X
Rhodnius brethesi	Present and visible	Elongated, slightly wider	In the same plane of the body, short and wide	Clearly separated from tergite X

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.actatropica.2018.04.021.

References

Abalos, J.W., Wygodzinsky, P., 1951. Las Triatominae Argentinas (Reduviidae, Hemiptera). An. Inst. Med. Reg. Tucumán. Monogr. 2, 1–179.

De Paula, A.S., Diotaiuti, L., Schofield, C.J., 2005. Testing the sistergroup relationship of the Rhodniini and Triatomini (Insecta: Hemiptera: Reduviidae: Triatominae). Mol. Phylogenet. Evol. 35, 712–718. http://dx.doi.org/10.1016/j.ympev.2005.03.003.

Galvão, C., de Paula, A.S., 2014. Sistemática e evolução dos vetores. In: Galvão, Vetores, C. (Eds.), Vetores da Doença de Chagas no Brasil. Sociedade Brasileira de Zoologia, Curitiba, pp. 26–32. http://dx.doi.org/10.7476/9788598203096.

Lent, H., Jurberg, J., 1968. Estudo morfológico comparativo de "Panstrongylus geniculatus" (Latreille, 1811) e "Panstrongylus megistus" (Burmeister, 1835) e suas genitálias externas (Hemiptera, Reduviidae, Triatominae). Rev. Bras. Biol. 28 (4), 499–520.

Lent, H., Jurberg, J., 1969. O gênero Rhodnius Stal, 1859, com um estudo sôbre a genitália das espécies (Hemiptera, Reduviidae, Triatominae). Rev. Bras. Biol. 29 (4), 487–560.

Lent, H., Jurberg, J., 1975. O gênero *Panstrongylus* Berg, 1879, com um estudo sobre a genitalia externa das espécies (Hemiptera, Reduviidae, Triatominae). Rev. Bras. Biol. 35 (3), 379–418.

Lent, H., Wygodzinsky, P., 1979. Revision of the Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas' disease. Bull. Am. Mus. Nat. Hist. 163, 125–520.

Lent, H., 1948. O gênero *Rhodnius* Stål, 1859 (Hemiptera: Reduviidae). Rev. Bras. Biol. 8, 297–339.

Mendonça, V.J., Alevi, K.C.C., Pinotti, H., Gurgel-Gonçalves, R., Pita, S., Guerra, A.L., Panzera, F., Araújo, R.F., Azeredo-Oliveira, M.T.V., Rosa, J.A., 2016. Revalidation of *Triatoma bahiensis* Sherlock & Serafim, 1967 (Hemiptera: Reduviidae) and phylogeny of the T. brasiliensis species complex. Zootaxa 4107 (2), 239–254. http://dx.doi.org/10.11646/zootaxa.4107.2.6.

Patterson, J.S., 2007. Comparative Morphometric and Molecular Genetic Analyses of Triatominae (Hemiptera: Reduviidae). [Ph.D. Thesis]. 253 pp. University of London.

Rivas, N., Sánchez-Cordero, V., Camacho, A.D., Alejandre-Aguilar, R., 2017. External female genitalia of six species of the genus *Meccus* (Hemiptera: Reduviidae: Triatominae). J. Vect. Ecol. 42 (2), 271–278. http://dx.doi.org/10.1111/jvec.12267.

Rosa, J.A., Mendonça, V.J., Rocha, C.S., Gardim, S., Cilense, M., 2010. Characterization of the external female genitalia of six species of Triatominae (Hemiptera: Reduviidade) by scanning electron microscopy. Mem. Inst. Oswaldo Cruz 105 (3), 286–292. http://dx.doi.org/10.1590/S0074-02762010000300007.

Rosa, J.A., Rocha, C.S., Gardim, S., Pinto, M.C., Mendonça, V.J., Ferreira Filho, J.C.R., Carvalho, E.O.C., Camargo, L.M.A., Oliveira, J., Nascimento, J.D., Cilense, M., Almeida, C.E., 2012. Description of *Rhodnius montenegrensis* n.sp. (Hemiptera: Reduviidae: Triatominae) from the state of Rondônia. Brazil. Zootaxa 3478, 62–76.

Rosa, J.A., Mendonça, V.J., Gardim, S., Carvalho, D.B., de Oliveira, J., Nascimento, J.D., Pinotti, H., Pinto, M.C., Cilense, M., Galvão, C., Barata, J.M., 2014. Study of the external female genitalia of 14 *Rhodnius* species (Hemiptera, Reduviidae, Triatominae) using scanning electron microscopy. Parasit Vectors 7, 17.

Rosa, J.A., Justino, H.H.G., Nascimento, J.D., Mendonça, V.J., Rocha, C.S., Carvalho, D.B., Falcone, R., Azeredo-Oliveira, M.T.V., Alevi, K.C.C., Oliveira, J., 2017. A new species of *Rhodnius* from Brazil (Hemiptera, Reduviidae, Triatominae). ZooKeys 675, 1–25. http://dx.doi.org/10.3897/zookeys.675.12024.

Schofield, C.J., Galvão, C., 2009. Classification, evolution, and species groups within the Triatominae. Acta Trop. 110 (2–3), 88–100.

Schofield, C.J., 1994. Triatominae – Biología y Control. Eurocommunica Publications, West Sussex (80 pp).

Sherlock, I.A., Serafim, E.M., 1967. *Triatoma lenti* sp.n., *Triatoma pessoai* sp.n. e *Triatoma bahiensis* sp.n. do estado da Bahia, Brasil (Hemiptera: Reduviidae). Gaz Med Bahia 67, 75–92.

Souza, E.S., Von Artzingen, N.C.B., Furtado, M.B., Oliveira, J., Nascimento, J.D., Vendrami, D.P., Gardim, S., Rosa, J.A., 2016. Description of *Rhodnius marabaensis* sp.n. (Hemiptera, Reduviidae, Triatominae) from Pará State, Brazil. Zookeys 621, 45–62. http://dx.doi.org/10.3897/zookeys.621.9662.

WHO – World Health Organization, 2018a. Chagas Disease. (American trypanosomiasis). [homepage on the internet]. [Updated 2017 March; cited 2017 April 26]. Available from: http://www.who.int/mediacentre/factsheets/fs340/en/.ted.

WHO – World Health Organization, 2018b. What Is Chagas Disease? ([homepage on the internet]. [Updated 2017 March; cited 2017 April 26]. Available from: http://www.who.int/chagas/disease/en/).