Development of Genital Plates in Nymphs of *Triatoma pallidipennis*, Stål 1872, (Hemiptera: Reduviidae) and its Application as Sexing Method

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Searching for morphometric differences between sexes in immature forms, the development of genital plates in the exuviae of Triatoma pallidipennis *Stål 1872 nymphs was studied. Differences were found in the form and size of the 9th genital urosternite, it is larger and wider in males as compared to females. This difference is reported in several South American* Triatoma *species. From our results it is possible to sex early stages from microscopic observation of genital plates in whole insects.*

Key words: *Triatoma pallidipennis* - sexing methods - genital plates - urosternites

The interest on the studies of development of genital plates in immature forms in triatomines has increased due to its application for sexing and taxonomic value (Perlowagora-Szumlewics & Nigri da Cruz 1972, Da Rosa et al. 1992, Jurberg et al. 1998). Galliard (1935) was first to report morphological evidence from genital plates as useful for sexing 5th nymphal stage in *Triatoma protracta* (Uhler 1894), *T. dimidiata* (Latreille 1811) and *Rhodnius prolixus,* Stål 1872. Further research conducted by Corrêa (1954), Corrêa et al. (1964), Espínola (1966), Ramírez Pérez (1969), Lent and Jurberg (1969), Salgado et al. (1979), Martín and Dávila (1981), Gonçalvez et al. (1985), Jurberg et al. (1986), and Galindez-Giron et al. (1999), include the use of optic and scanning electronic microscopy (SEM).

Most studies were conducted on South American species such as *T. maculata* (Erichson 1848), *T. pseudomaculata* Corrêa and Espínola 1964, *T. brasiliensis* Neiva 1911, *T. infestans* (Klug 1834), *Psammolestes coreodes* Bergroth 1911, and *Panstrongylus megistus* (Burmeister 1835). Previous works by Galliard (1935), Ryckman (1962) and Martin and Dávila (1981) were done on Mexican *Triatoma* species, yet further research is required on the subject. The present work was conducted on *T. pallidipenis,* a species of the "*phyllosoma* complex", widely distributed in México. Because of its peridomestic behavior in some regions, it is an interesting species for epidemiology (Lent & Wygodzinsky 1979, Zárate & Zárate 1985, Beltrán & Carcavallo 1985, Velasco-Castrejón 1991, Martínez-Ibarra & Katthain-Duchateau 1999). We studied the development of genital plates through the life cycle and looked for morphological differences that could allow for sexing on 1st to 5th instar nymphs.

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MATERIALS AND METHODS

T. pallidipennis collected from El Progreso, in Jiutepec, Morelos, México were the original source of a laboratory colony, 120 uninfected nymphs were used. They were individually placed in a glass vial 95 x 15 mm, each containing a piece of folded cardboard, they were numbered and kept under controlled temperature and humidity (28 ± 2°C and 60 ± 2% RH). The bugs were fed weekly on rabbit. Exuviae from each specimen was kept in a glass vial for chemical treatment and microscopic studies. Sex was later confirmed as they got to adult stage.

Treatment of biological material - The exuviae were placed in hot water (80 ± 2ºC) for 10 min, the genital plates were separated discarding the remaining tissues, and we conducted the following procedures: (a) KOH (10%) during 10 min; (b) washing with distilled water for 15 min; (c) neutralization with water-acetic acid for 10 min; (d) dehydration with 70% ethanol for 15 min; (e) dehydration with 90% ethanol for 15 min. Finally glass slide mounting with synthetic resin (60% in toluene, Sigma 7986, lot 43 248), drying at 37°C for 20 days.

Microscopic analyses - We compared genital urosternites between sexes considering: form and size, separation, vestiture, color pattern, and the presence of cuticular structures like lobules, foldings, holes, etc., in or around the genital plate area. Measurement of urosternites were done with an ocular micrometer; microphotographs of the genital plates were taken with a microscope Nikon Labophot 2, equipped with a camera Nikon FX-35Dx.

Statistical analyses - Data for 9th sternite width and length were transformed by $x' = \log(x+1)$ to remove heterocedasticity (Zar 1999), then analyzed by one-way analysis of variance and the Ryan-Einot-Gabriel-Welsch multiple range test or "REGWQ" test (Schlotzhauer & Littell 1987). In all cases $\alpha = 0.05$. We employed SAS computer software (SAS Institute 1990).

RESULTS

From the initial 120 nymphs, 58 females and 53 males developed into adults, this is 92.5% survival under laboratory conditions.

Microscopic analysis of genital plates did not show clear differences in several morphological characters observed. We found differences in both width and length of the 9th sternite; in general it appears bigger in males (Table). Also there is morphological difference as a result of more marked lateral projections of the 9th sternite in females. Figs 1-5 show comparative microphotographs and simplified schemes of the genital plates in both sexes for 1st to 5th instars. Comparative development of the 9th sternite per instar, on both males and females is shown in Fig. 6.

DISCUSSION

A good nymphal survival of 92% was observed during the study (111 adults from 120 nymphs), better than 66.7% observed by Martínez and Katthain-Duchateau (1999) when fed on hens every three days, and better than 84.6% by Mena-Segura et al. (1994) fed every 10-12 days.

Microscopic analysis of the genital plates indicates differences between sexes both in form and size of the 9th urosternite. In general, for females it appears as a biconvex disc, narrow in the middle with curved pointed ends; in the case of males it is a wider plate curved towards the 10th sternite and pointless ends. We also observed that the 7th and 8th sternites have a rectangular shape in females, and a trapezoidal shape in males, short side pointing cefalad. During nymphal development the 9th sternite of the genital plates differentiates between sexes (Fig. 1), average growth of the 9th sternite increases gradually up to 4 times in width and 6.8 times in length, in male nymphs from 1st instar until 5th instar; for females it grows 4 times in width and 2.9 times in length (4th instar), as it changes to 5th instar length is reduced 2.1 times its size for 1st instar nymphs.

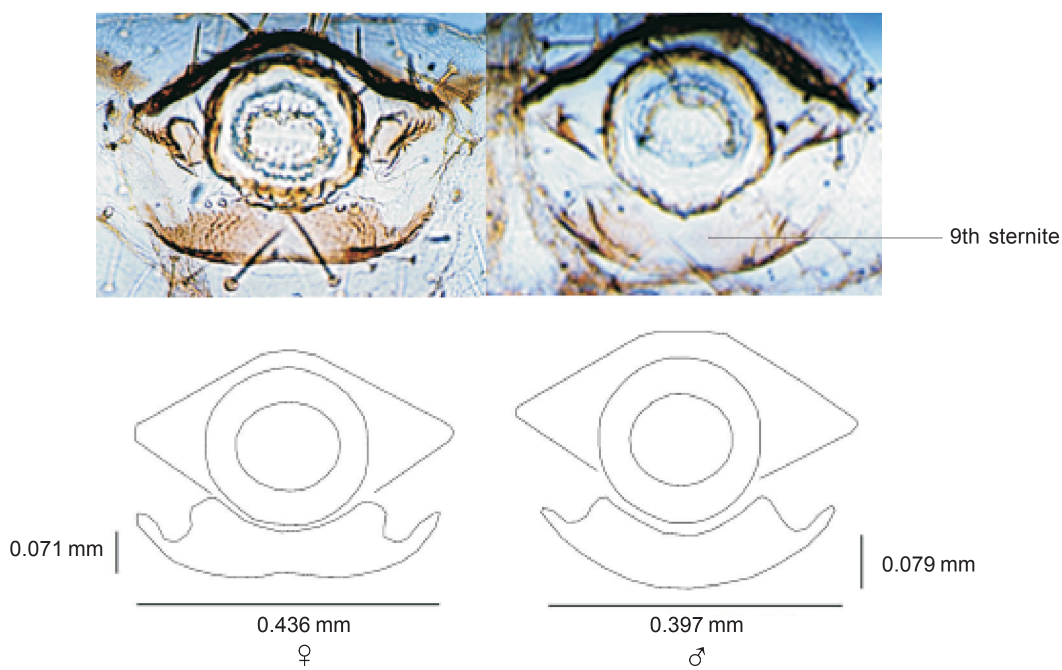

Fig. 1: *Triatoma pallidipennis* Stål 1872, genital plates of 1st instar nymphs

TABLE

Genital plates of *Triatoma pallidipennis* Stål 1872, nymphs, recorded sizes of the 9th sternite

		N1		N2		N3		N4		N5	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Width (mm)	min.	0.309	0.379	0.468	0.476	0.563	0.619	0.712	0.855	1.448	1.401
	máx.	0.436	0.420	0.547	0.587	0.722	0.746	0.950	0.997	1.686	1.710
	mean	**0.399**	**0.392**	**0.495**	**0.528**	**0.621**	**0.674**	**0.855**	**0.939**	**1.596**	**1.583**
Length (mm)	min.	0.055	0.071	0.071	0.095	0.126	0.150	0.190	0.237	0.087	0.451
	máx.	0.087	0.103	0.119	0.134	0.182	0.190	0.261	0.308	0.213	0.641
	mean	**0.075**	**0.082**	**0.089**	**0.106**	**0.144**	**0.165**	**0.220**	**0.277**	**0.160**	**0.563**

N1: 1st instar nymphs; N2: 2nd instar nymphs; N3: 3dr instar nymphs; N4: 4th instar nymphs; N5: 5th instar nymphs

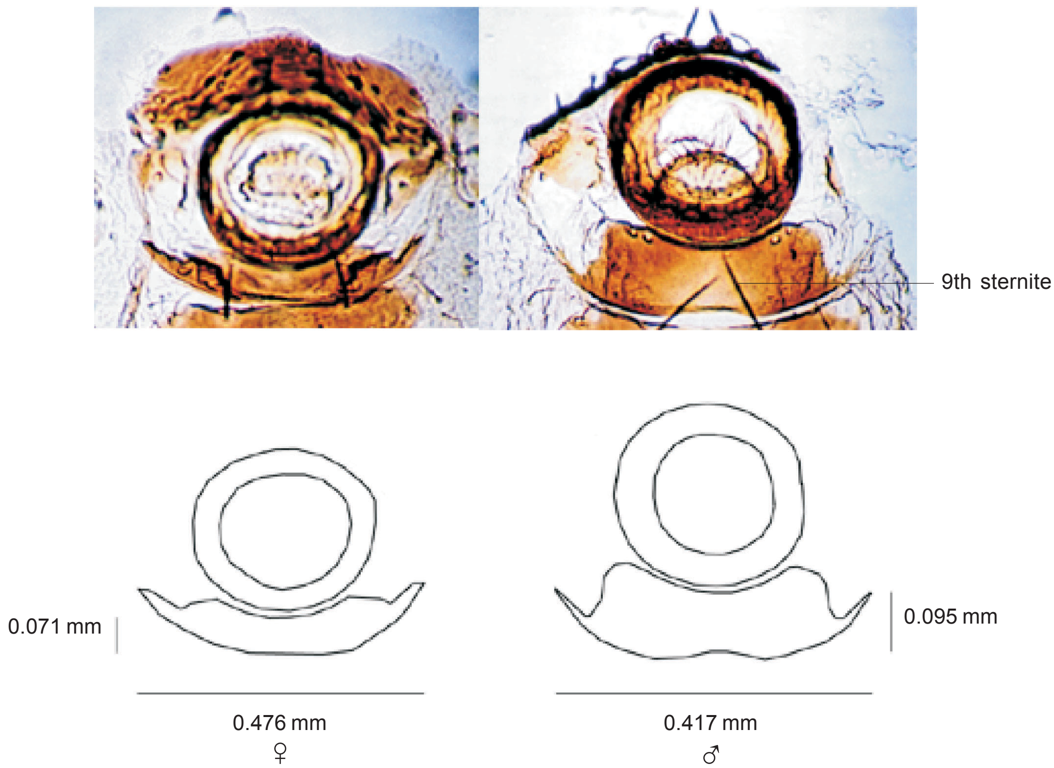

Fig. 2: *Triatoma pallidipennis* Stål 1872, genital plates of 2nd instar nymphs

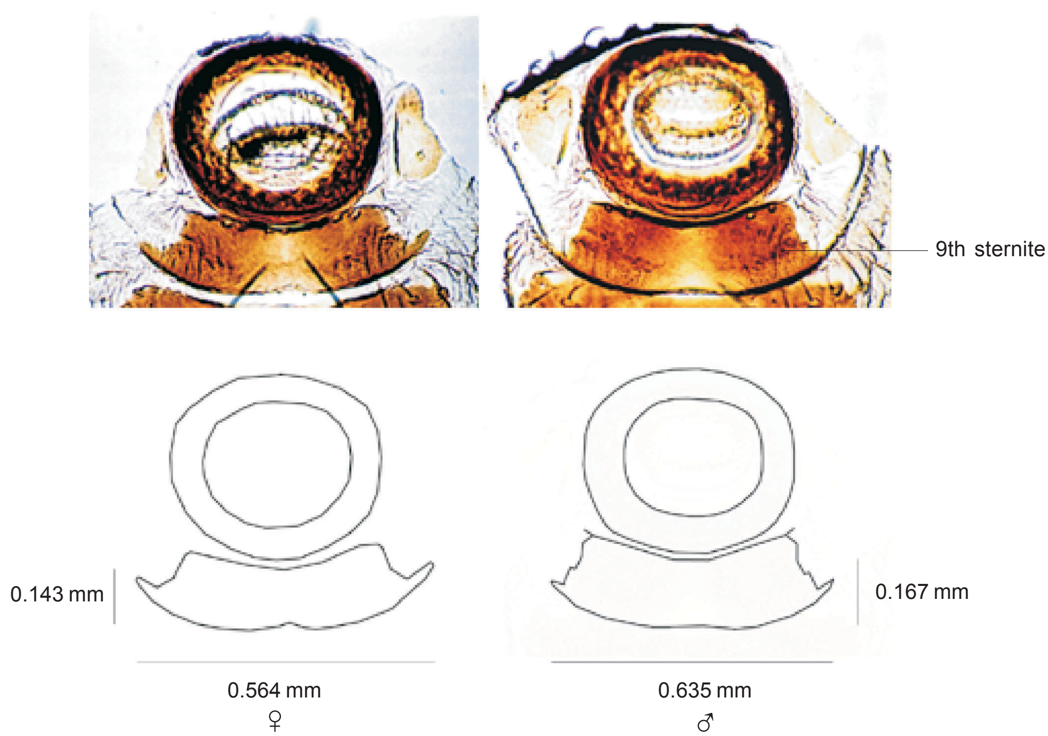

Fig. 3: *Triatoma pallidipennis* Stål 1872, genital plates of 3rd instar nymphs

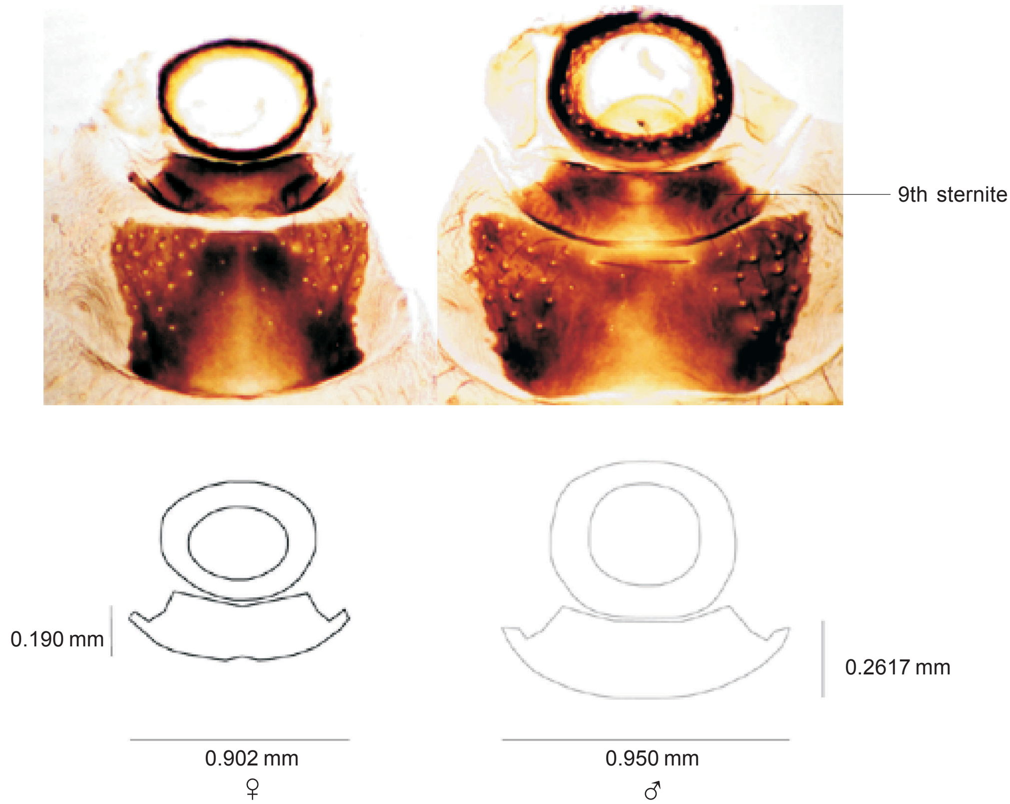

Fig. 4: *Triatoma pallidipennis* Stål 1872, genital plates of 4th instar nymphs

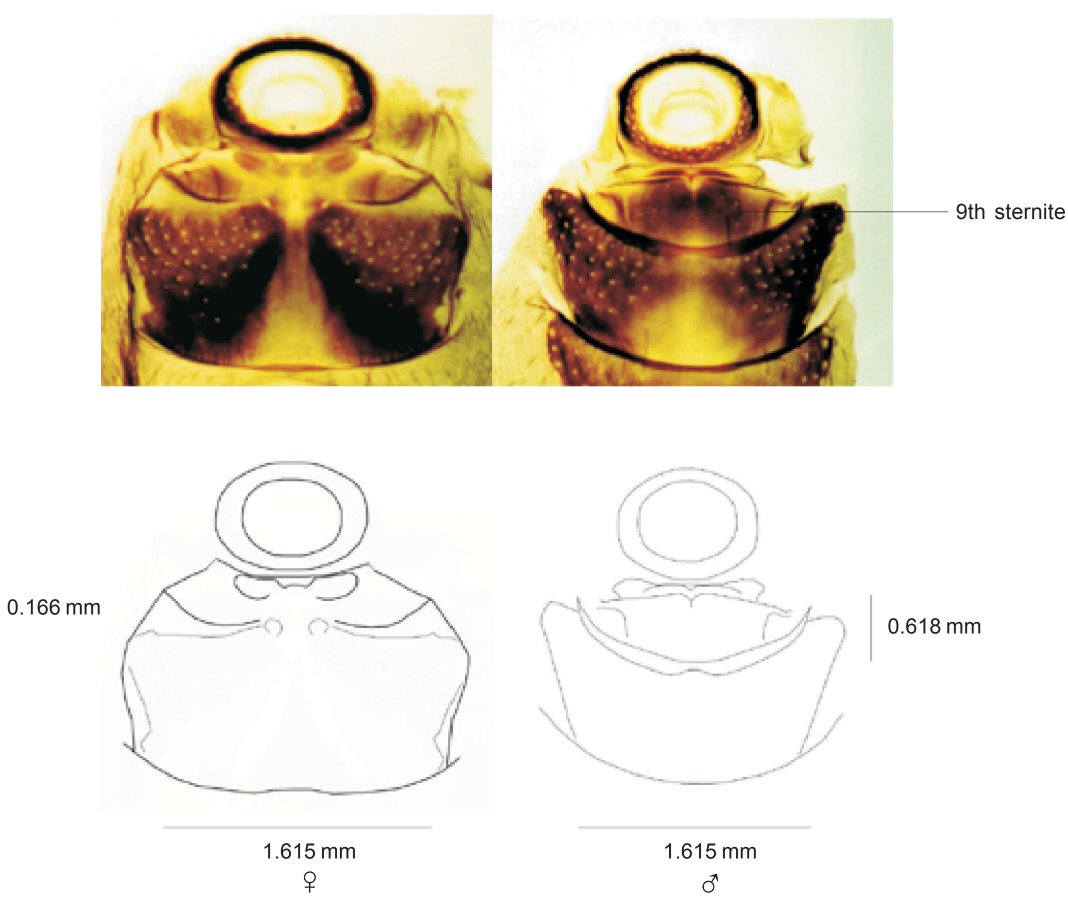

Fig. 5: *Triatoma pallidipennis* Stål 1872, genital plates of 5th instar nymphs

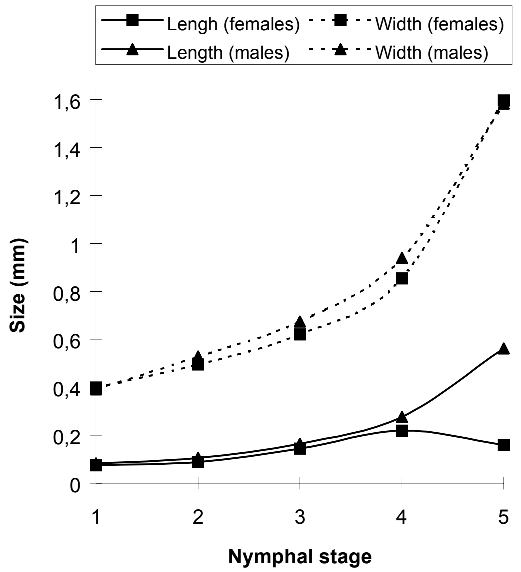

Fig. 6: *Triatoma pallidipennis* Stål 1872, nymphs, males and females, development of the 9th sternite in the genital plate

On the other hand it is observed that the 9th sternite had an average increase in males of 1.4 times its width and 1.6 times its length. Females increased 1.4 times both width and length, this is considering only 1st to 4th instars. Males have longer 9th sternite than females: it is 1.09 times longer in 1st instar, 1.19 in 2nd instar, 1.14 times in 3rd instar, 1.25 in 4th instar and 3.51 times in 5th instar nymphs.

Statistical analyses (ANOVA and REGWQ test) determined statistically significant differences between sexes in width of the 9th sternite for 2nd, 3rd and 4th instars (Fig. 7). We found significant differences between sexes for length of the 9th sternite in every instar (Fig. 8).

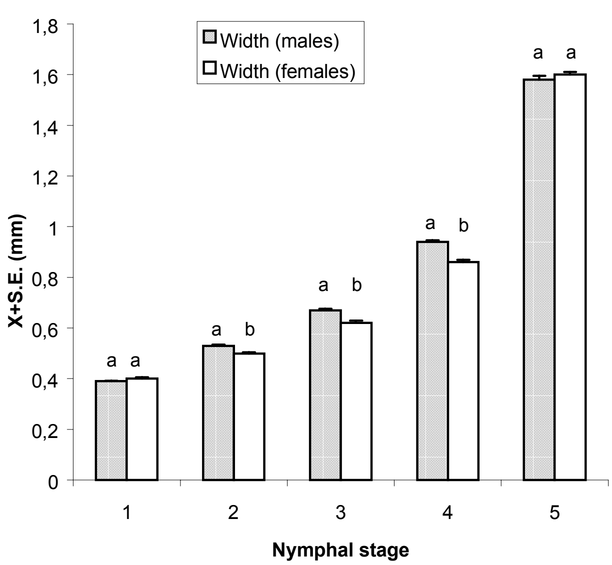

Fig. 7: *Triatoma pallidipennis* Stål 1872, nymphs, comparison between males and females for width of the 9th sternite in the genital plate

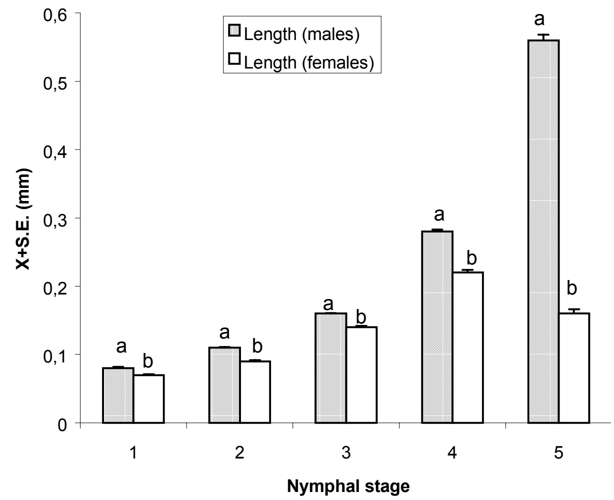

Fig. 8: *Triatoma pallidipennis* Stål 1872, nymphs, comparison between males and females for length of the 9th sternite in the genital plate

Our results agree with other morphometric studies conducted in South American *Triatoma* species (Perlowagora-Szumlewicz & Cruz 1972, Jurberg et al. 1986, Da Rosa et al. 1992). We consider that it is possible to sex *T. pallidipennis* nymphs considering the size and shape of the 9th urosternite.

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