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SEXUAL DISTINCTION BETWEEN 5TH INSTAR NYMPHS OF SIX SPECIES OF TRIATOMINAE (HEMIPTERA, REDUVIIDAE)

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The aim of this paper is to present the analysis of sexual morphological differences observed in 5th instar nymphs of the following species: Panstrongylus megistus; Rhodnius neglectus; Triatoma brasiliensis; T. infestans; T. matogrossensis *and* T. tibiamaculata. *Male and female nymphs were examined and photographed with a Scanning Electron Microscope. The 9th segment dimensions of dorsal and ventral faces were determined through a Profile Projector. Results and statistical analysis showed significant differences: the 9th sternite is significantly broader in male than in female nymphs, while in five species, tergites in female nymphs are broad and in male are narrow.*

Key words: sexual distinction – scanning – nymphs – Triatominae

Due to the epidemiological importance of Triatominae, various researchers have studied different aspects of these hemiptera. Researches on external genitalia are of great taxonomic interest.

Galliard (1935) was the first to verify the morphological aspects of the sexual distinction in nymphs of *Triatoma protracta, T. dimidiata,* and *Rhodnius prolixus.* His research was followed by Corrêa (1954), Corrêa et al. (1964) Espínola (1966), Ramirez Perez (1969), Lent & Jurberg (1969), Perlowagora-Szumlewicz & Cruz (1972), Salgado et al. (1979), Martin & Davila (1981), Gonçalves et al. (1985) and Jurberg et al. (1986).

There are many publications showing the sexual differences between nymphs of Triatominae through sketches, schemes and photographs taken by optical microscopes. The present paper discusses the sexual characteristics detected through a Scanning Electron Microscope (SEM), and the measurements of segments that are different in each sex, made with a Profile Projector.

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MATERIALS AND METHODS

The fifth instar nymphs of *Panstrongylus megistus* (Burmeister, 1835); *Rhodnius neglectus* Lent, 1954; *Triatoma brasiliensis* Neiva, 1911; *T. infestans* (Klug, 1834); *T. matogrossensis* Leite & Barbosa, 1953 and *T. tibiamaculata* (Pinto, 1926) were obtained in the insectary of the Department of Epidemiology, Public Health College, University of São Paulo, Special Health Service in Araraquara (SESA).

The drawings of terminal tergites of 5th instar male and female nymphs were made using a stereomicroscope and a Wild, clear camera, M7A.

Preparation of nymphs for examination through the SEM – The 5th instar nymphs were sexually identified using a stereomicroscope.

Six male nymphs and six female nymphs were separated and killed with chloroform vapor. Afterwards they were one by one cut obliquely in the 7th segment. The abdominal side of terminal parts were fixed through the 7th segment to metallic supports. An angle of 50° was formed between the base and the portion examined, to facilitate the visualization of the ventral 9th segment.

The twelve nymphs were metallized with 24 carat gold, in vacuum vaporizing metallizer (JEOL JEE-SS), using a pressure of 10^{-6} tor. After metallization, the nymphs were observed and photographed using a SEM (JEOL model, JSM-P15).

Measurement of nymphs – Fifteen male and female 5th instar nymphs of the six species were separated and killed. The specific mass was determined using an analytic balance (Mettler H51). The breadth of the 9th ventral and dorsal segments were determined using a Profile Projector (Nikon model 6c).

Statistical Analysis

Size of the samples – The size of the samples was determined with the probability of error of the first and second kind in 5%, for the distance between the real values of averages stipulated terms of standard deviation. Operational curves related to average tests were used to determine this size (see Berquó et al., 1981).

The Average tests – Tests of two monocaudal averages were made at a 5% level of significance to verify if averages of ventral and dorsal segments measurements in 5th instar female nymphs were smaller or bigger than the male ones. The specifications indicated by Berquó et al. (1981) were followed for average tests of two non-correlate populations with supposedly different standard deviation.

RESULTS AND DISCUSSION

The measurement of the 9th abdominal ventral segment by the Profile Projector and the statistical analysis made in 15 samples of both sexes in the six species (Tables I, II, III, IV, V, VI), showed significant difference in breadth between the 9th urosternite of the female nymph (narrow) and male one (broad).

Measurements of the 9th abdominal dorsal segment of both sexes, in the six species studied, were also made. The comparative results between males and females were not significant only in the case of *T. brasiliensis* nymphs.

Although the statistical analysis indicated possibility of distinction between male and female nymphs in measurements of the 9th abdominal dorsal segment in the five species (Tables I, II, III, IV, V, VI), in practice this differentiation is very difficult, because no distinctions were noted in the shape of this tergite (Figs 3, 4, 7, 8, 11, 12, 15, 16, 19, 20, 23, 24).

Description of the 9th sternite – The 9th sternite of P. megistus male nymphs shows a longitudinal furrow in the central area (Fig. 1); in *T. infestans,* it has a longitudinal depression (Fig. 13) and in *T. matogrossensis,* it presents a short furrow near the limit of the 10th segment (Fig. 17). The presence of furrows in *P. megistus* and *T. infestans* was already mentioned by Espínola (1966).

TABLE I

Average mensuration and statistical results of *Panstrongylus megistus* of 15 male and female 5th instar nymphs (age not determined)

Sex	9th segment Ventral (mm)	9th segment Dorsal (mm)	Weight (mg)
Male			
Mean	0.333	0.479	344 983
Standard deviation	0.025	0.039	47.017
Female			
Mean	0.154	0.513	362.017
Standard deviation	0.022	0.050	87.923
Comparison test between male and female 5th instar nymphs			
T critic	1.699	1.701	1.714
t observed	20.34	2.05	–0.66
degree of freedom	29	28	23
Result	significant	significant	non significant

TABLE II

Average mensuration and statistical results of *Rhodnius neglectus* of 15 male and female 5th instar nymphs. (Age: six days in the 5th instar)

Sex	9th segment Ventral (mm)	9th segment Dorsal (mm)	Weight (mg)
Male			
Mean	0.244	0.248	35.380
Standard deviation	0.026	0.017	7.088
Female			
Mean	0.132	0.270	31.785
Standard deviation	0.021	0.030	8.831
Comparison test between male and female 5th instar nymphs			
T critic	1.699	1.711	1.699
t observed	12.81	−2.48	1.23
degree of freedom	29	24	29
Result	significant	significant	non significant

TABLE III

Average mensuration and statistical results of *Triatoma brasiliensis* of 15 male and female 5th instar nymphs (age not determined)

Sex	9th segment Ventral (mm)	9th segment Dorsal (mm)	Weight (mg)
Male			
Mean	0.268	0.313	205.970
Standard deviation	0.028	0.024	67.003
Female			
Mean	0.118	0.309	188.418
Standard deviation	0.015	0.029	56.431
Comparison test between male and female 5th instar nymphs			
T critic	1.714	1.699	1.699
t observed	18.34	0.50	0.77
degree of freedom	23	29	29
Result	significant	non significant	non significant

TABLE IV

Average mensuration and statistical results of *Triatoma infestans* of 15 male and female 5th instar nymphs (age not determined)

Sex	9th segment Ventral (mm)	9th segment Dorsal (mm)	Weight (mg)
Male			
Mean	0.281	0.321	331.524
Standard deviation	0.029	0.024	53.047
Female			
Mean	0.130	0.372	395.593
Standard deviation	0.018	0.038	65.137
Comparison test between male and female 5th instar nymphs			
T critic	1.708	1.708	1.699
t observed	16.94	−4.30	−2.95
degree of freedom	25	25	29
Result	significant	significant	significant

TABLE V

Average mensuration and statistical results of *Triatoma matogrossensis* of 15 male and female 5th instar nymphs (age not determined)

Sex	9th segment Ventral (mm)	9th segment Dorsal (mm)	Weight (mg)
Male			
Mean	0.246	0.335	281.804
Standard deviation	0.024	0.029	58.399
Female			
Mean	0.141	0.359	312.883
Standard deviation	0.013	0.022	121.790
Comparison test between male and female 5th instar nymphs			
T critic	1.714	1.701	1.721
t observed	14.95	−2.58	−0.89
degree of freedom	23	28	21
Result	significant	significant	non significant

TABLE VI

Average mensuration and statistical results of *Triatoma tibiamaculata* of 15 male and female 5th instar nymphs (age not determined)

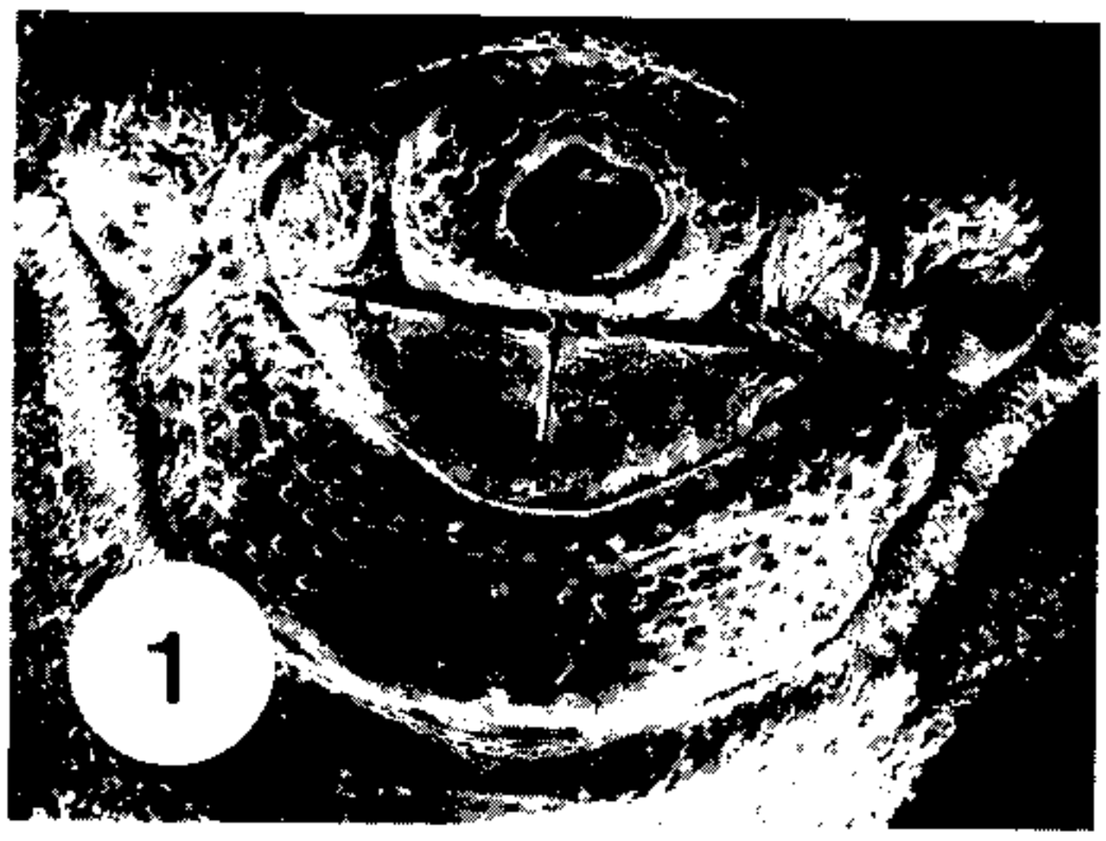
Sex	9th segment Ventral (mm)	9th segment Dorsal (mm)	Weight (mg)
Male			
Mean	0.289	0.500	312.520
Standard deviation	0.036	0.043	50.706
Female			
Mean	0.142	0.564	335.209
Standard deviation	0.016	0.083	90.234
Comparison test between male and female 5th instar nymphs			
T critic	1.725	1.717	1.714
t observed	14.47	−2.64	−0.85
degree of freedom	20	22	23
Result	significant	significant	non significant

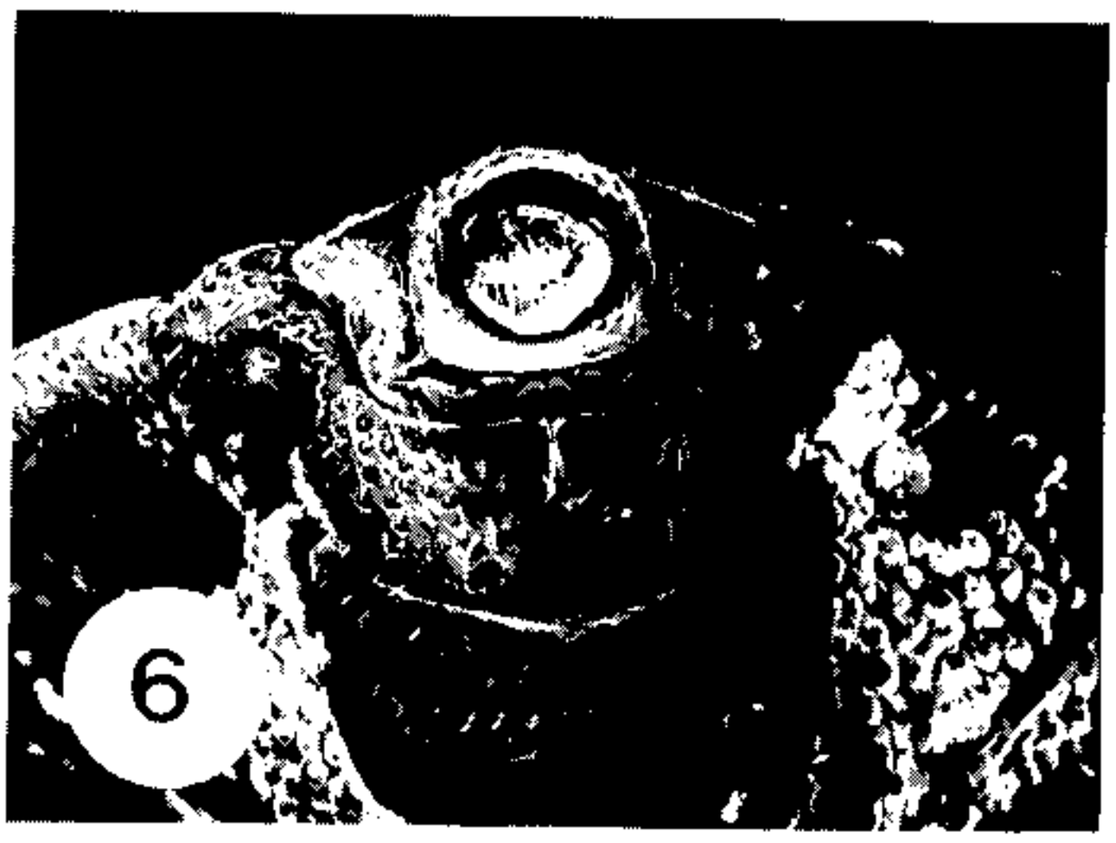
Fifth instar female nymphs of *P. megistus* present a small concavity in the shape of an open "U" in the central longitudinal line, at the limit between the 9th and the 10th sternites, where they have a small furrow situated between two small slitted salience (Fig. 2).

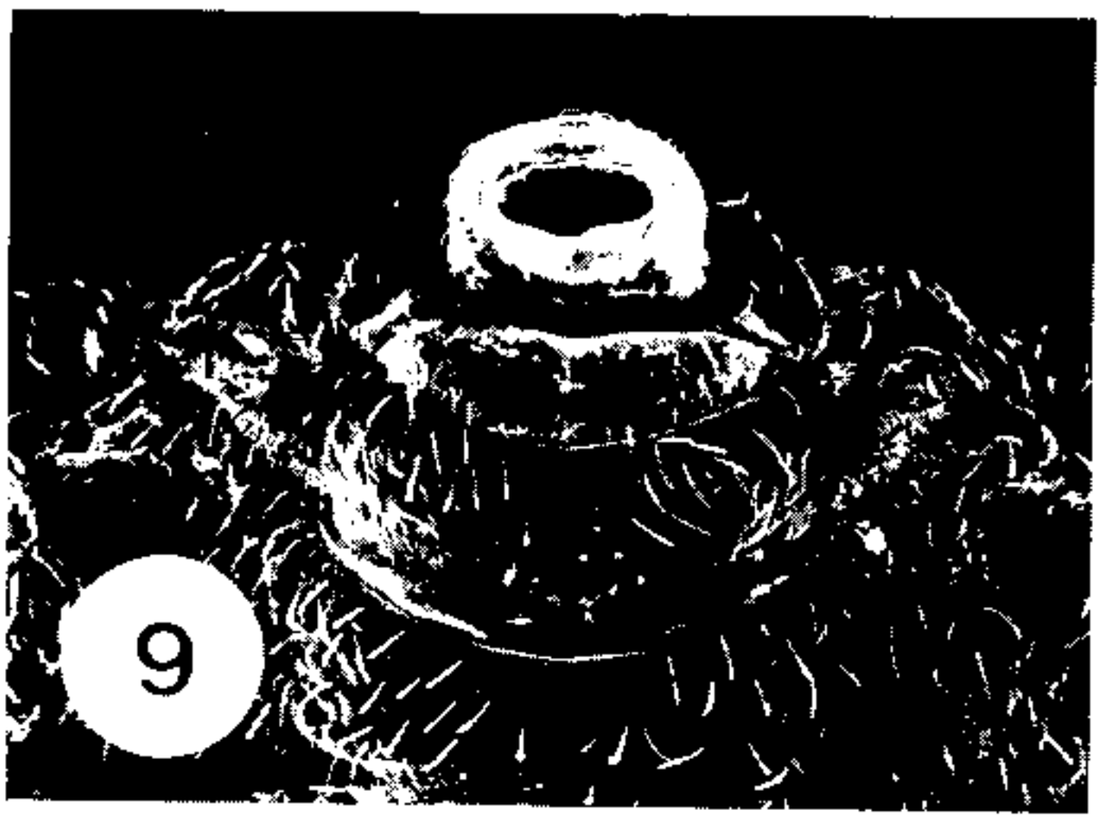
In the 9th ventral segment of *R. neglectus* female nymphs there is a furrow in the longitudinal medial line in the front part, which joins the 8th and 9th sternites (Fig. 6).

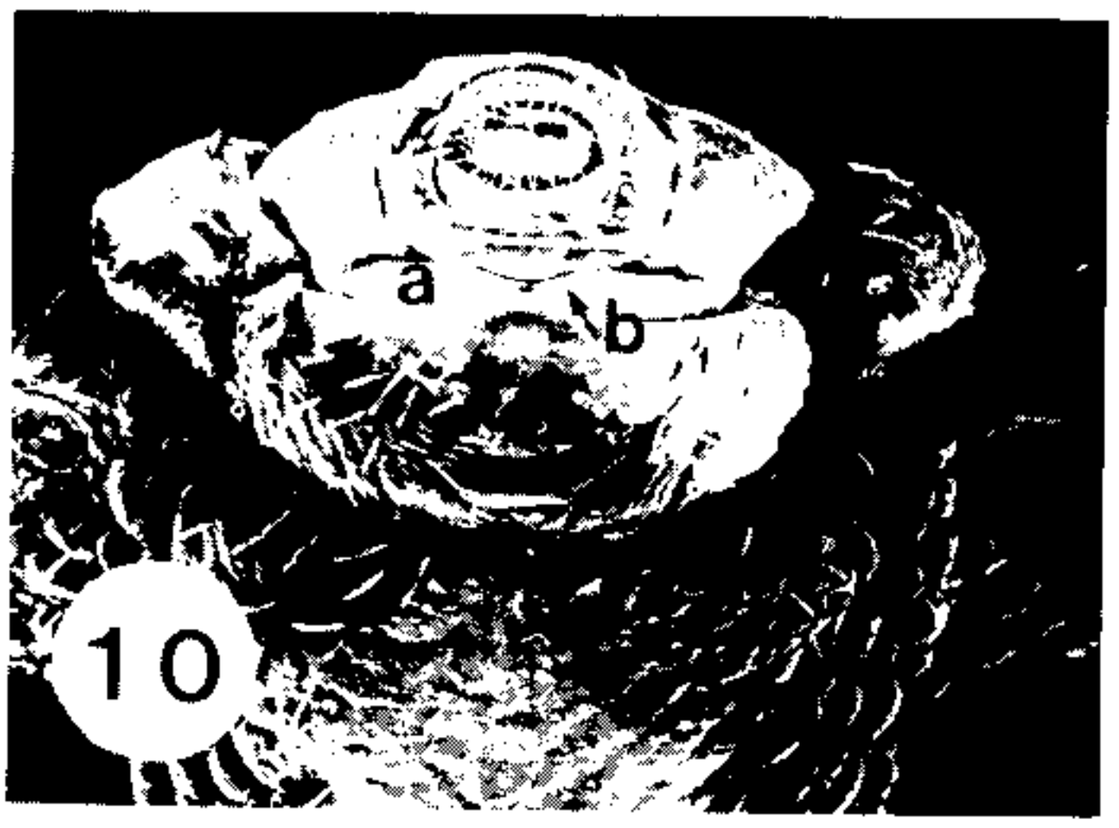
The 5th instar female nymphs of *T. brasiliensis* have a concavity in the central area of the 9th ventral metamere, at its junction with the 10th (Fig. 10). Female nymphs of *T. infestans* have a concavity with a "V" shape in the same area (Fig. 14).

Triatoma matogrossensis female nymphs have a slit in the shape of a "V" in the central limit between the 9th and 10th sternites (Fig. 18). The 9th ventral segments of *T. tibiamaculata* female nymphs show a crumpled aspect and they have two (1+1) short and oblique furrows close to a central area, in the limit between the 9th and 10th sternite (Fig. 22).







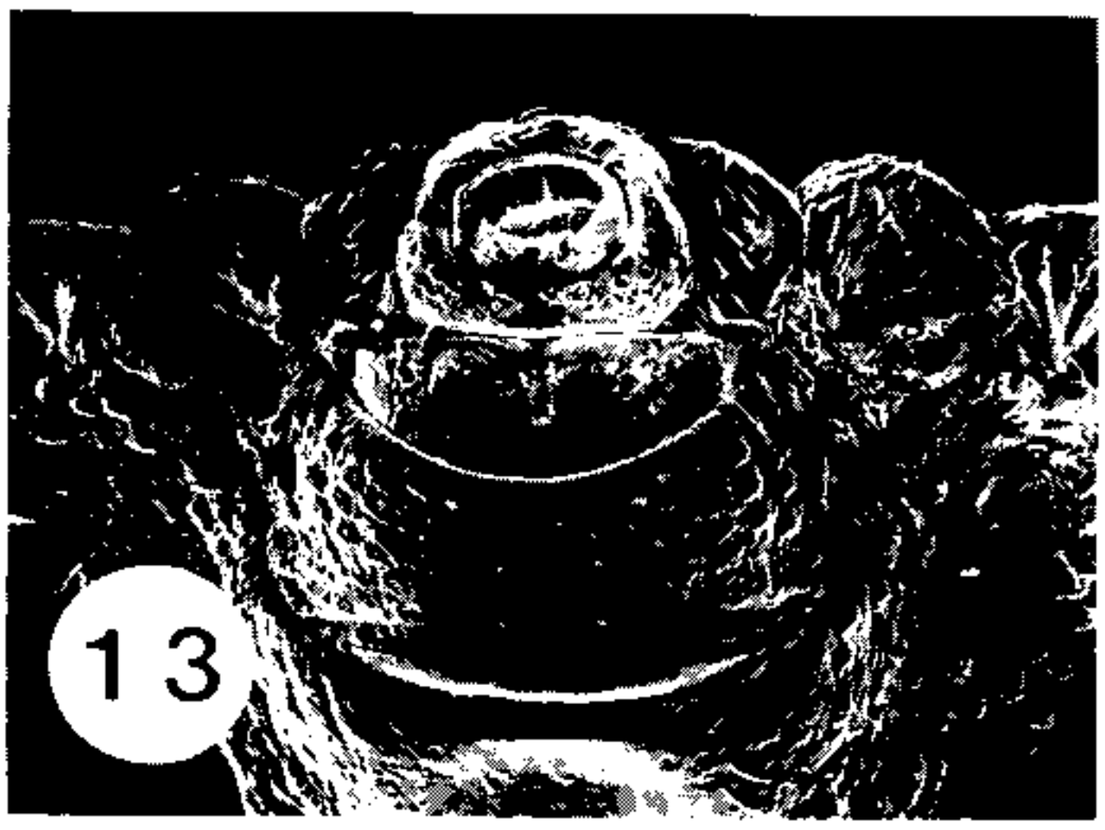


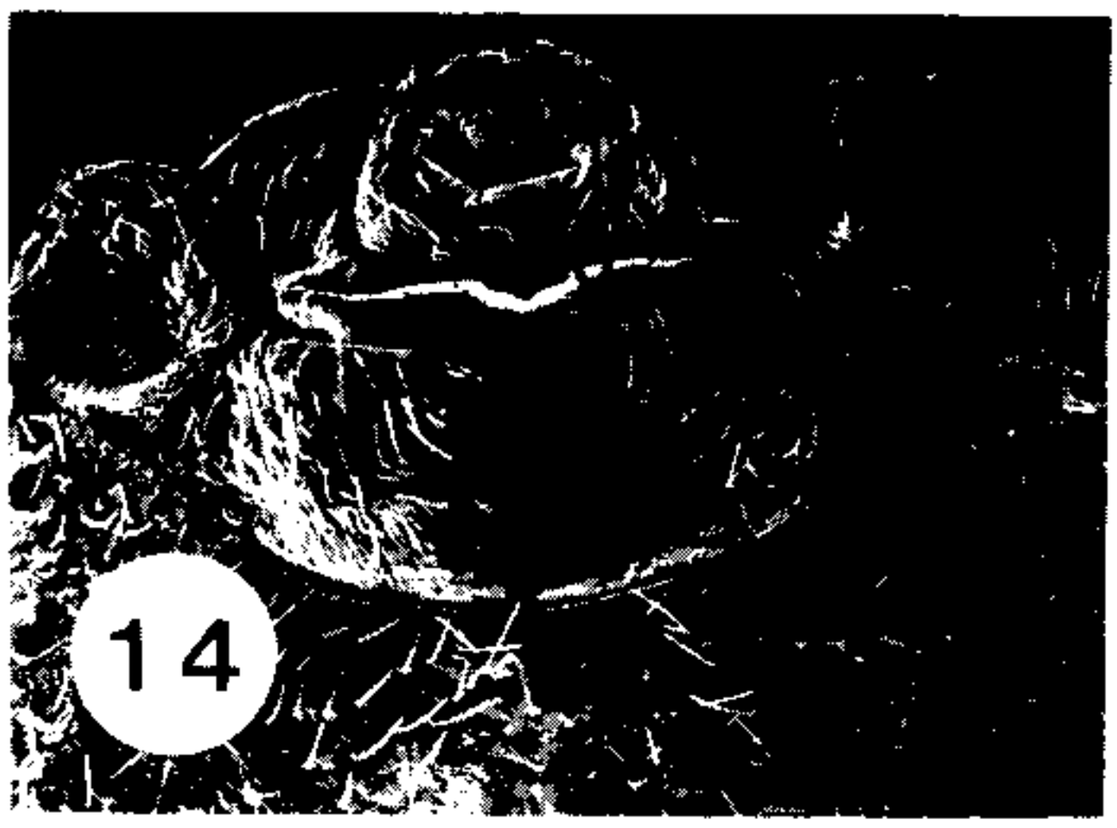
Ventral face, terminal segments, of 5th instar nymphs SEM 50X – (a = 9th segment). Fig. 1: *Panstrongylus megistus* male nymph (b = central longitudinal furrow; c = cavity). Fig. 2: *P. megistus* female nymph (b = depression in the shape of an "U"; c = triangular structure; d = slitted salience). Fig. 5: *Rhodnius neglectus* male nymph. Fig. 6. = *R. neglectus* female nymph (b = furrow; c = pore). Fig. 9: *Triatoma brasiliensis* male nymph (b= concavity). Fig. 10: *T. brasiliensis* female nymph (b = concavity).

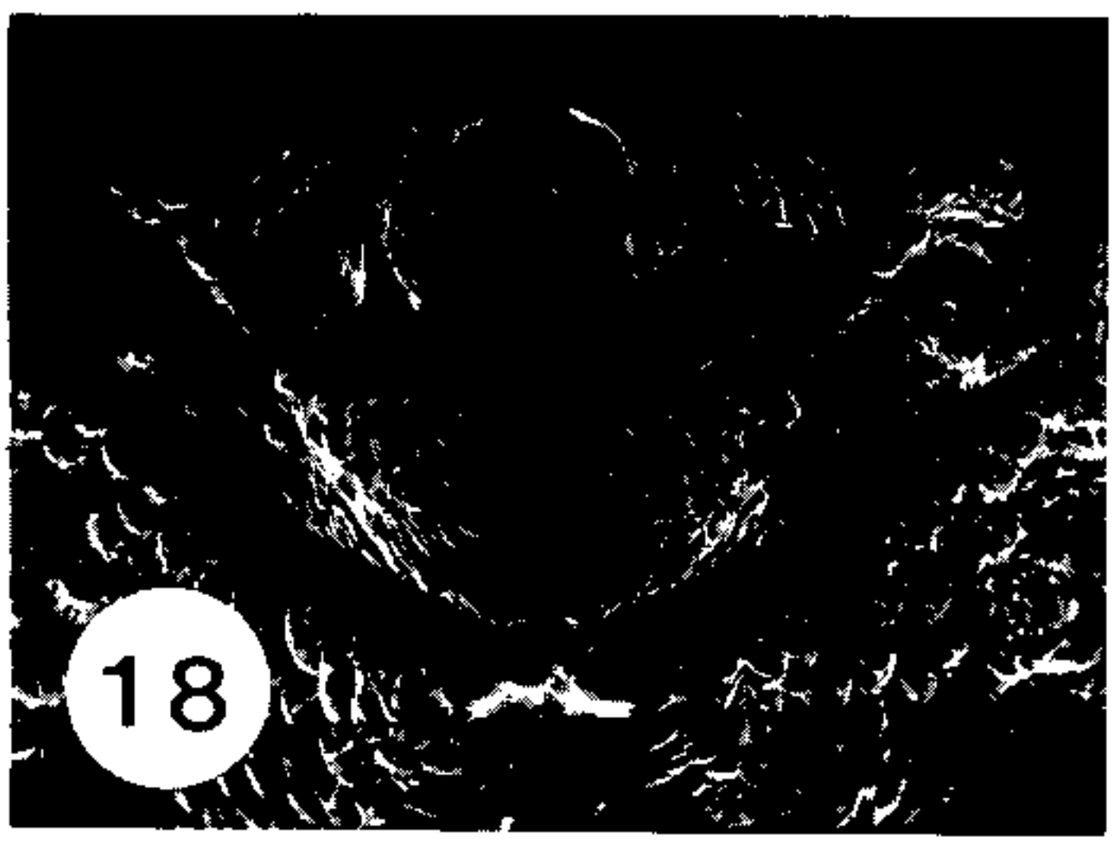
Description of the 8th sternite – The area without bristles of the 8th ventral metamere has several conformations: in *P. megistus* female nymphs it is triangular, with the lateral lines of the triangle failing to meet; it shows a small aperture from the apex towards the 9th segment; on the lateral side two (1+1) obliquely slited salience can be observed (Fig. 2).

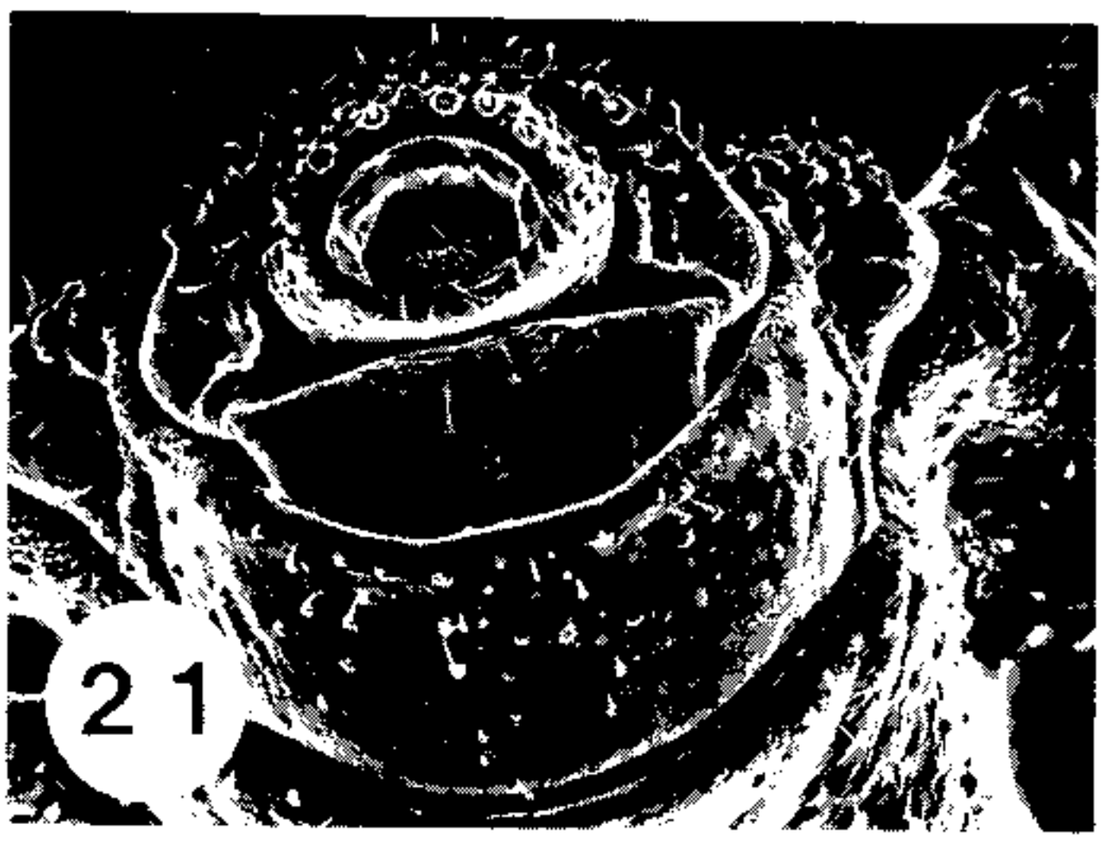
In *R. neglectus* 5th instar female nymphs, the 8th sternite, does not have a triangular shape in its central areas. The principal characteristic is a well-defined furrow in the central part of the back, which extends up to the beginning of the 9th metamere and has two pores, one in the limit with the 9th segment and the other before it (Fig. 6).

Triatoma infestans, 5th instar female nymphs have a central area of the 8th sternite







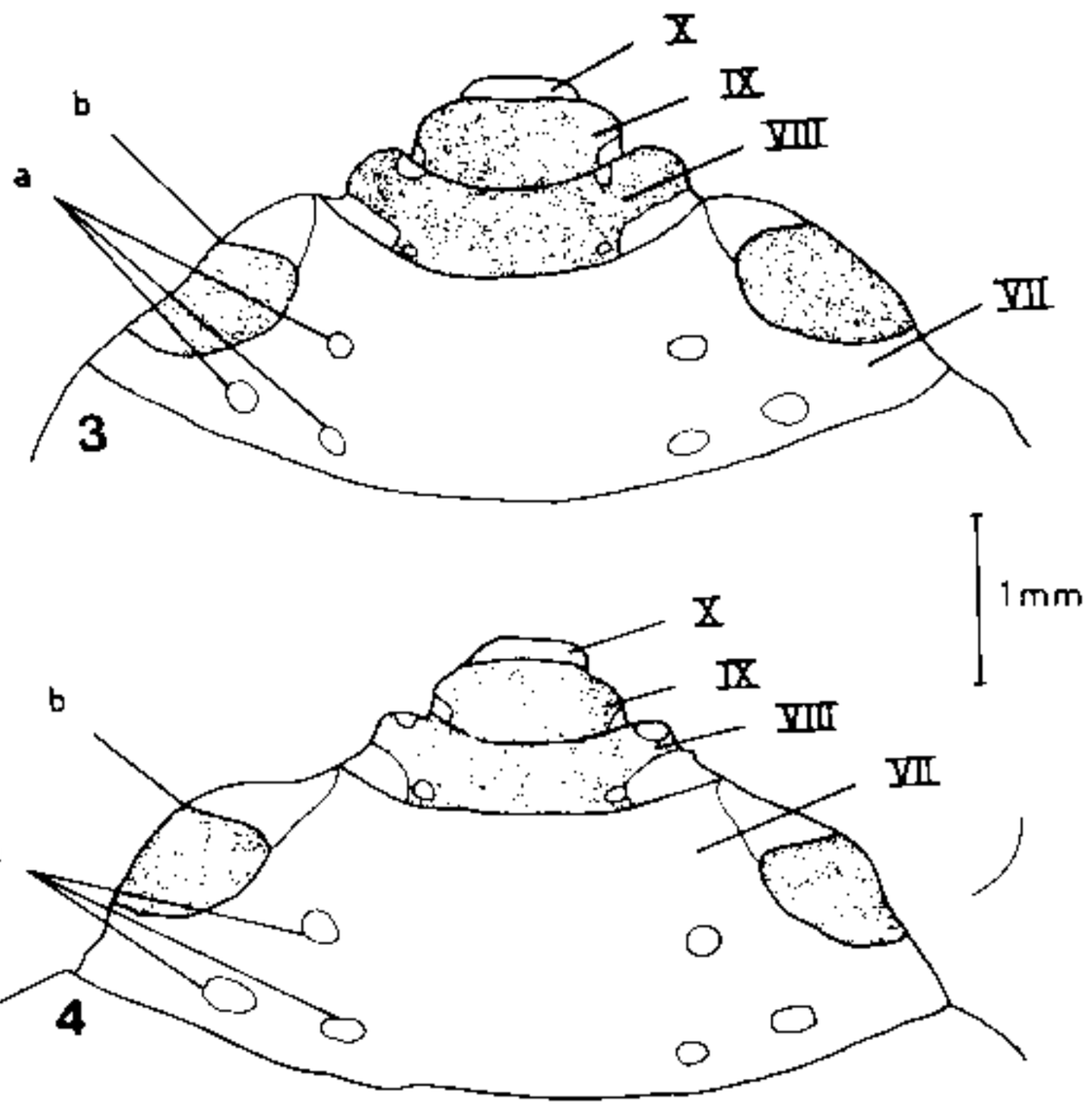


Ventral face, terminal segments, of 5th instar nymphs SEM 50X – (a = 9th segment). Fig. 13: Triatoma infestans male nymph (b = depresssion; c = cavity). Fig. 14: T. infestans female nymph (b = depression; c = slots; d = cavity in "V"). Fig. 17: Triatoma matogrossensis male nymph. Fig. 18: T. matrogrossensis female nymph (b = split; c = triangular structure; d = structure in the shape of a plug). Fig. 21: Triatoma tibiamaculata male nymph. Fig. 22: T. tibiamaculata female nymph (b = slitted salience cofee seed like; c = triangular structure).

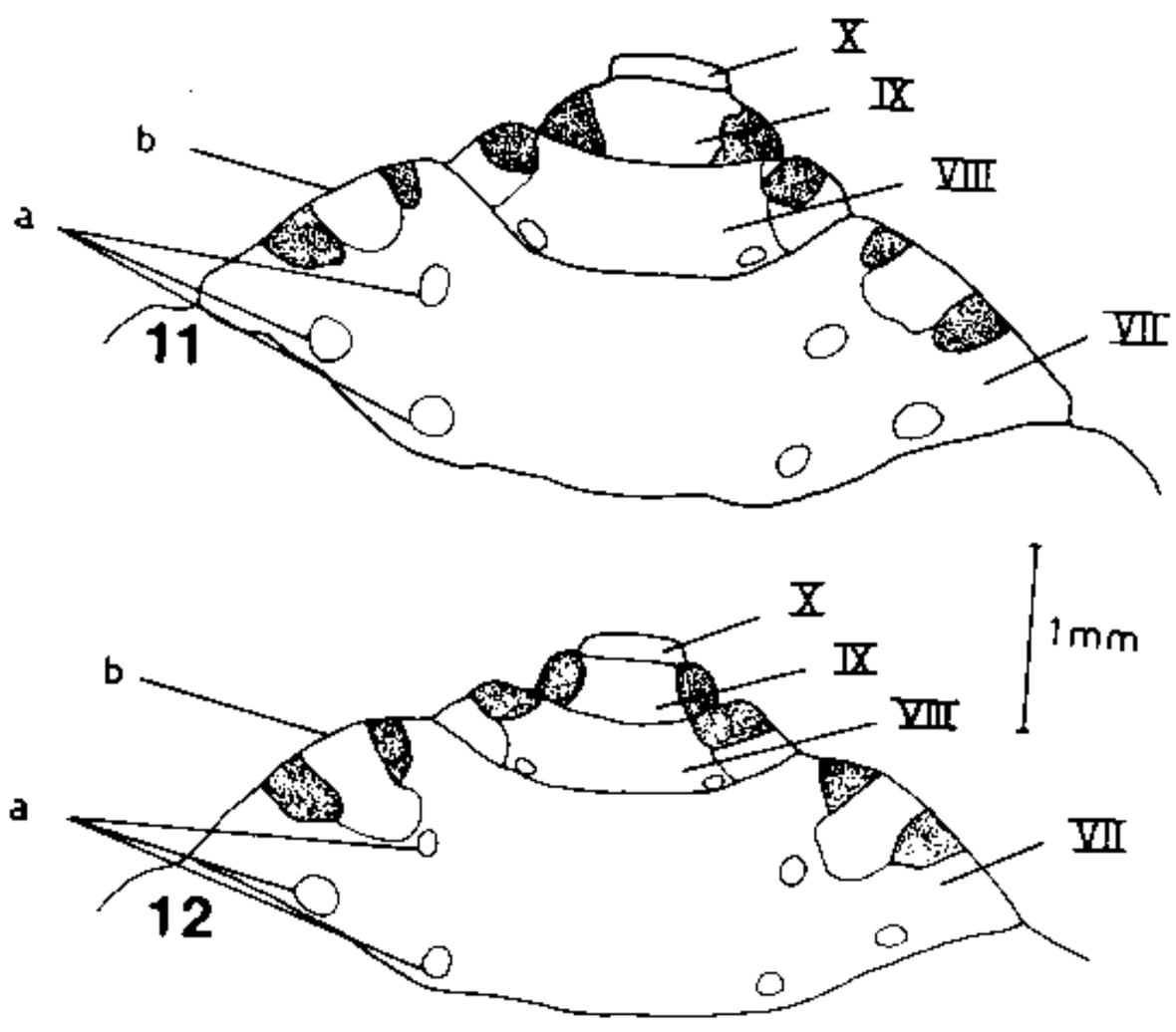
in the drop shape, with a well-defined point, close to the limit of the 9th metamere. A small slit can be seen above the point of the drop, as well as (2+2) oblique slots; the lower slots are bigger than the upper ones (Fig. 14). Corrêa (1954) described such slots as being two callosities. This formation can be observed using a stereomicroscope. The formation of the 8th sternite in the other species were seen only in the SEM.

The front central area of the 8th sternite of the 5th instar female nymphs of *T. matogrossensis* has no bristles and presents a structure like a two pin plug fitted into the 7th segment. There is a structure of triangular shape in the back area with apex pointing to the 9th metamere (Fig. 18).

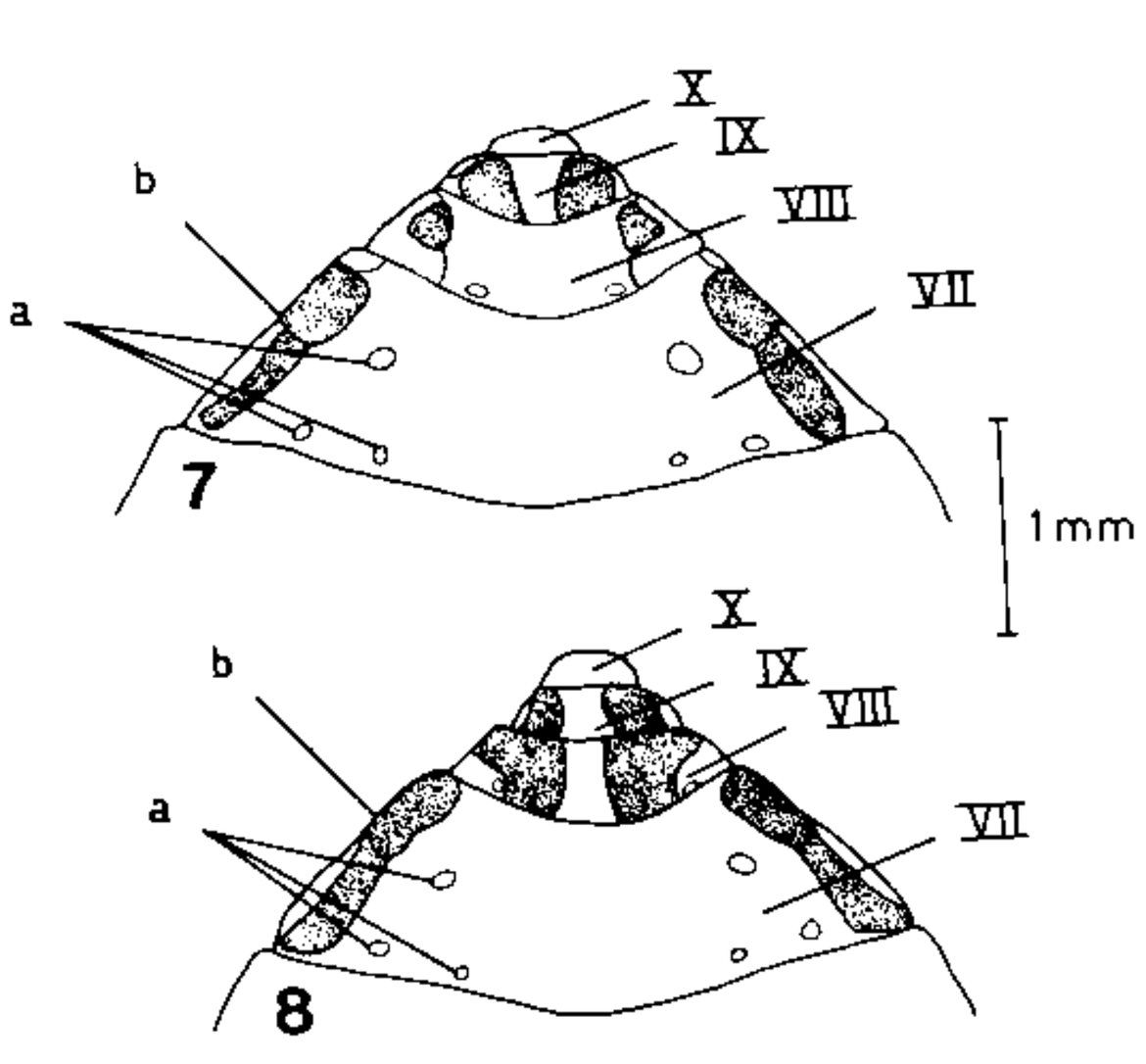
In *T. tibiamaculata* 5th instar female nymphs, the 8th sternite has a region, with no



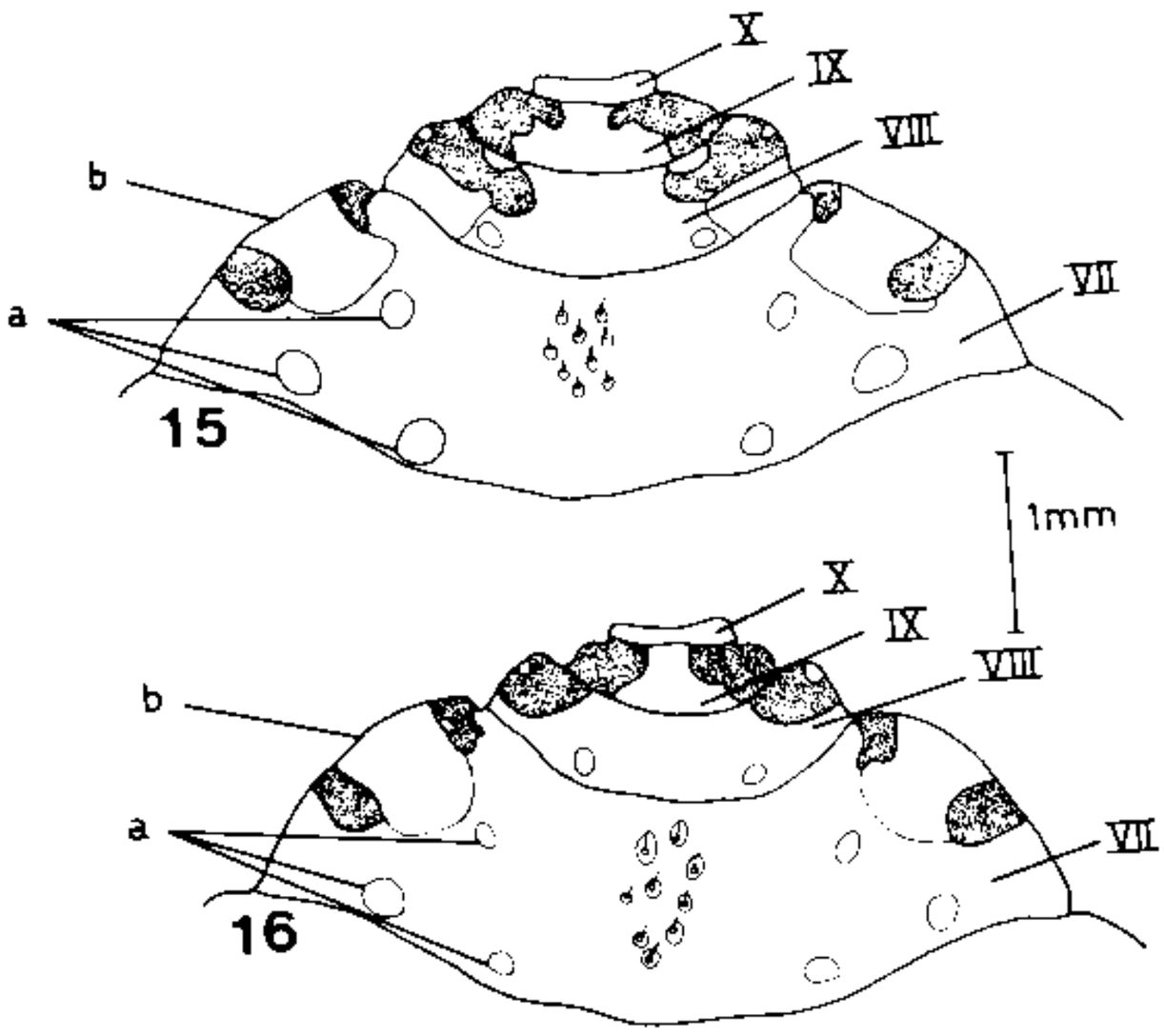
Dorsal face, terminal segments, of *Panstrongylus megistus* 5th instar nymph. Fig. 3: female. Fig. 4: male. a: hairless area; b: connexivum; VII. . . X: abdominal segments.



Dorsal face, terminal segments, of *Triatoma brasiliensis* 5th instar nymph. Fig. 11: female. Fig. 12: male. a: hairless area; b: connexivum; VII. . . X: abdominal segments.



Dorsal face, terminal segments, of Rhodnius neglectus 5th instar nymph. Fig. 7: female. Fig. 8: male. a: hairless area; b: connexivum; VII. . . X: abdominal segments.



Dorsal face, terminal segments, of *Triatoma infestans* 5th instar nymph. Fig. 15: female. Fig. 16: male. a: hairless area; b: connexivum; VII. . . X: abdominal segments.

bristles, in the shape of an isosceles triangle, whose apex extends up to the limit with 9th metamere, where there are two (1+1) slitted salience coffee seed like in oblique position (Fig. 22).

In 5th instar male nymphs, of the six species studied through SEM, the 9th sternite is different in shape and dimension and specific morphological structures are found, characteristic of each species. The 8th sternite did not show characteristic formations for any of the six species (Figs 1, 5, 9, 13, 17, 21).

The 9th sternites of 5th instar female nymphs have a differentiated shape and are smaller than the respective metameres of male nymphs. They present structural details characteristic of each one of the six species studies. In the 8th sternite specific, well-defined formations were verified which male nymphs did not present.

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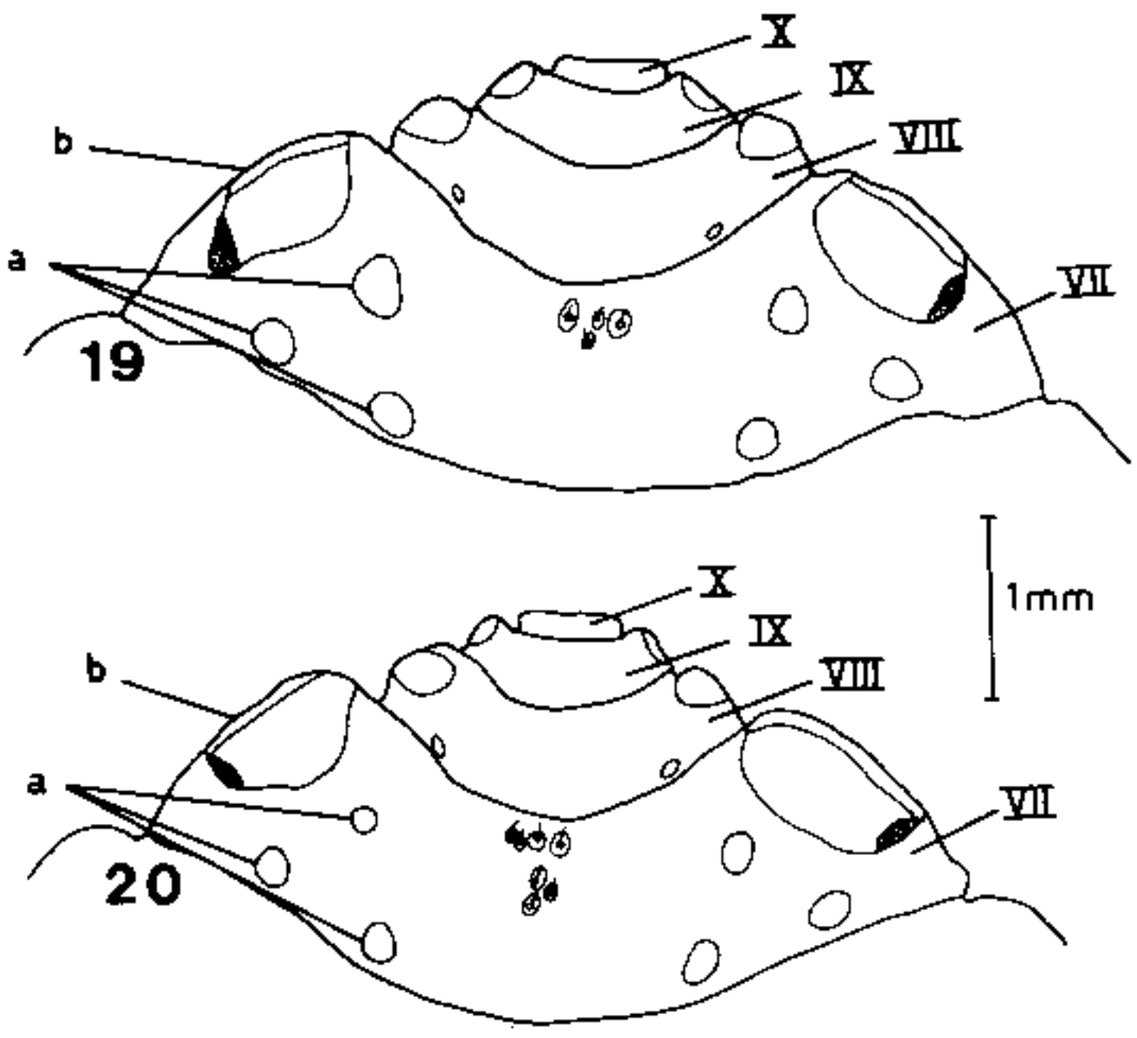
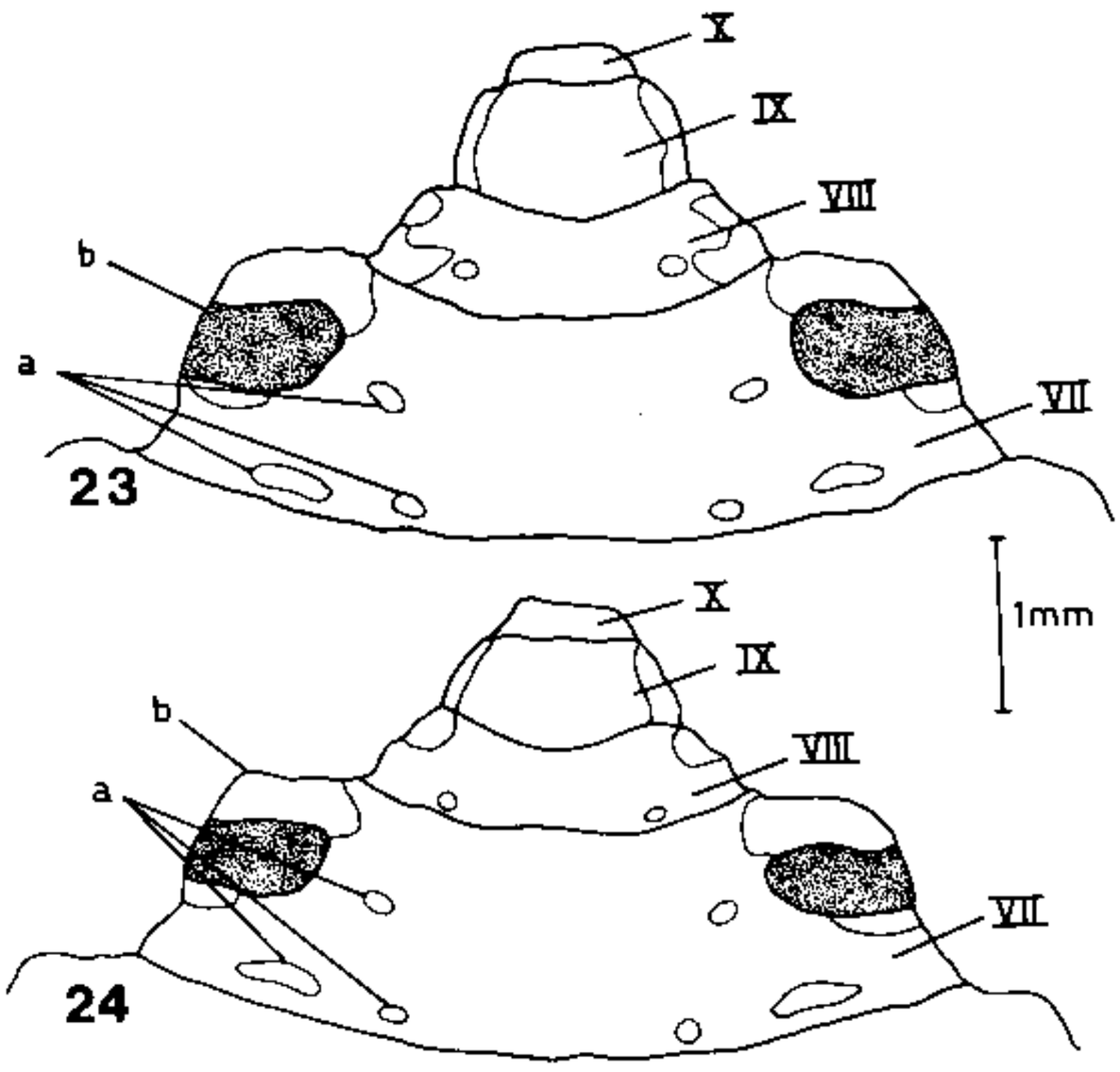


Dorsal face, terminal segments, of *Triatoma matogrossensis* 5th instar nymph. Fig. 19: female. Fig. 20: male. a: hairless area; b: connexivum; VII. . . X: abdominal segments.



Dorsal face, terminal segments, of *Triatoma tibiamaculata* 5th instar nymph. Fig. 23: female. Fig. 24: male. a: hairless area; b: connexivum; VII. . . X: abdominal segments.

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