Morphological study of the thorax of the five nymphal instars of *Triatoma arthurneivai* Lent & Martins (Hemiptera, Reduviidae, Triatominae)

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ABSTRACT. Morphological study of the thorax of the five nymphal instars of *Triatoma arthurneivai* Lent & Martins (Hemiptera, Reduviidae, Triatominae). Scanning electron microscope (SEM) was done in order to study dorsal, ventral and lateral sides of 1st, 2nd, 3rd, 4th and 5th nymphal instars of *Triatoma arthurneivai*. The five nymphal instar can be recognized based on different pronotum, mesonotum and metanotum shapes and characteristics. In the 1st instar collar, hairless areas and tubercles are absent. The 2 nd instar presents collar, hairless areas and tubercles. In the 3rd instar occurs the development of wing pads. In the 4 th instar the four wing pads are expanded, but do not reach the abdomen and in the 5th instar the anterior wing pads almost overlap the posterior ones. At the ventral side, two metasternal glands openings (1+1) were found in all five nymphal instars. Brindley's gland evaporation areas (1+1) are located at the mesopleuron, as well as an evaporation area is located at the propleuron in all nymphal instars (1+1).

KEYWORDS. Distinction; gland; instar; nymph; *Triatoma arthurneivai.*

RESUMO. Morfologia do tórax do quinto estádio ninfal de *Triatoma arthurneivai* Lent & Martins (Hemiptera, Reduviidae, Triatominae). Foi feito estudo morfológico da face dorsal, ventral e lateral do tórax de ninfas de 1º, 2º, 3º, 4º e 5º estádios de *Triatoma arthurneivai* por meio de microscopia eletrônica de varredura. Os cinco estádios ninfais podem ser reconhecidos pelos caracteres morfológicos observados no pronoto, mesonoto e metanoto. No 1º estádio nota-se ausência do colar, áreas glabras e tubérculos, que estão presentes no 2º estádio. No 3º estádio inicia-se a diferenciação das aletas alares. No 4º estádio ocorre expansão das quatro aletas alares, que, no entanto, não alcançam o abdome. No 5º estádio as aletas alares anteriores praticamente recobrem as posteriores. Na face ventral dos cinco estádios ninfais percebe-se duas (1+1) aberturas das glândulas metasternais. As áreas de evaporação das glândulas de Brindley localizam-se na mesopleura. Também foram observadas duas (1+1) áreas de evaporação na propleura dos cinco estádios ninfais.

PALAVRAS-CHAVE. Distinção; estádios; glândula; ninfas; *Triatoma arthurneivai.*

Triatominae are *Trypanosoma cruzi* vectors, causing agent of Chagas disease, that occurs in 17 countries of Latin America, counting 100 million people of contamination risk and about 16 to 18 million infected people (WHO 1991; Schmuñis 2000; Dias 2002).

Now Triatominae have about137 current valid species, classified in 19 genera (Galvão *et al.* 2003). The five nymphal instars, males and females, can transmit *T. cruzi* through their excrement. This demonstrates the epidemiological importance of these nymphal instars.

Correa *et al.* (1952) showed that the main morphological differences from the first to the fourth instars of *Triatoma infestans* nymphs are in the thorax structure. Southwood (1956) was able to distinguish, in two Heteroptera species, each one of the five nymphal instars by studying the thorax structural modification. Jurberg *et al.* (1986,1993, 2002) described the five nymphal instars of *T. brasiliensis*, *Dipetolagaster maxima and T. jurbergi* using optic microscopy.

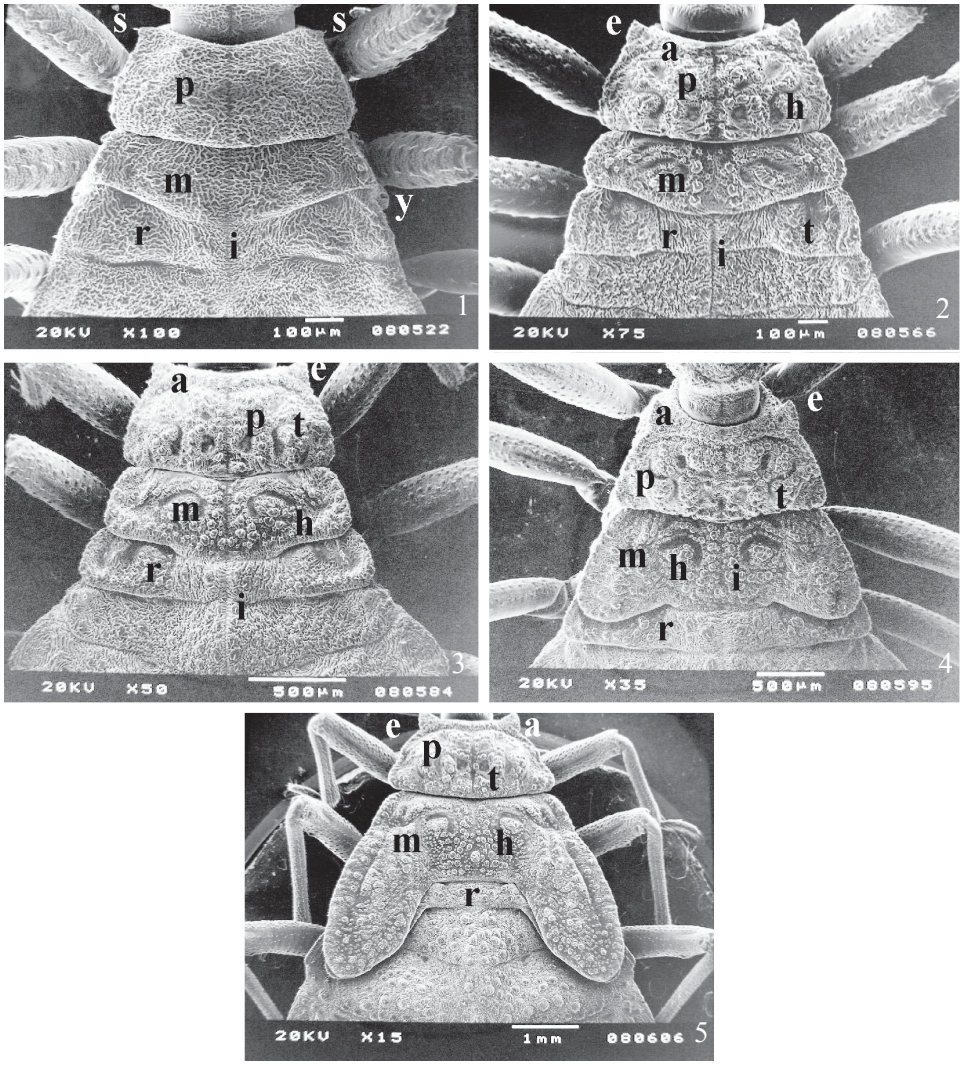
In Brazil, *T. arthurneivai* is widespread in Bahia, Minas Gerais, Piauí and São Paulo states (Galvão *et al*. 2003). The contribution made for the understanding of *T. arthurneivai* morphology was done during its description by Lent & Martins (1940), Lent & Wygodzinsky (1979) and Girón *et al*. (1998).

The present study was done due to the fact that there are no SEM data showing Triatominae nymphal instars differences. Moreover, this study shows the dorsal, ventral and lateral thorax details of the five *T. arthurneivai* nymphal instars. The results presented here increase not only the knowledge about *T. arthurneivai*, but the understanding of the Triatominae nymphal instars distinctions and its morphology as well.

MATERIALS AND METHODS

Specimens of *T. arthurneivai* were originally collected by Dr. Vera Lúcia Cortiço Rodrigues in Santa Tereza farm located in Espírito Santo do Pinhal, São Paulo state, Brazil, in 1981. The laboratory culture is maintained at the Insetário da Faculdade de Ciências Farmacêuticas/UNESP/Araraquara.

A JEOL scanning eletronic microscope, model JSM-T330A, of the Instituto de Química/ UNESP/Araraquara, was used. The five days old nymphs were killed with chloroform or ether, washed in distilled water with the aid of a Thorton ultrasound device, model T-314, dehydrated in alcooholic series and dried in oven at 50º C. After the cleaning, dehydration and drying, the nymphs were fixed in metallic base and metallized using an Edward gold sputter coater. Finishing the gold metallizing



Figs. 1-5. *T. arthurneivai* dorsal view of nymphs by SEM: 1, 1st instar; 2, 2nd instar; 3, 3th instar; 4, 4th instar; 5, 5th instar. a: collar; e: anterolateral angle; h: hairless area; i: suture; m: mesonotum; p: pronotum; r: metanotum; s: sensilla; t: tubercles; y: Brindley's gland evaporation area.

process, the nymphs were photographed with the SEM, that operated at 20KV and used Fuji film (Neopan SS 120), according to Rosa *et al*. (1999). The nymphal instars identification was made using optic microscopy, according to Correa *et al.* (1952).

RESULTS

Thorax dorsal view. In the five nymphal instars, it was observed that the pronotum, mesonotum and metanotum are clearly divided, and present a longitudinal suture. Pronotum has a trapezoid form and is the largest one, except in the 4th and 5th instars (Figs. 1, 2, 3 ,4, 5).

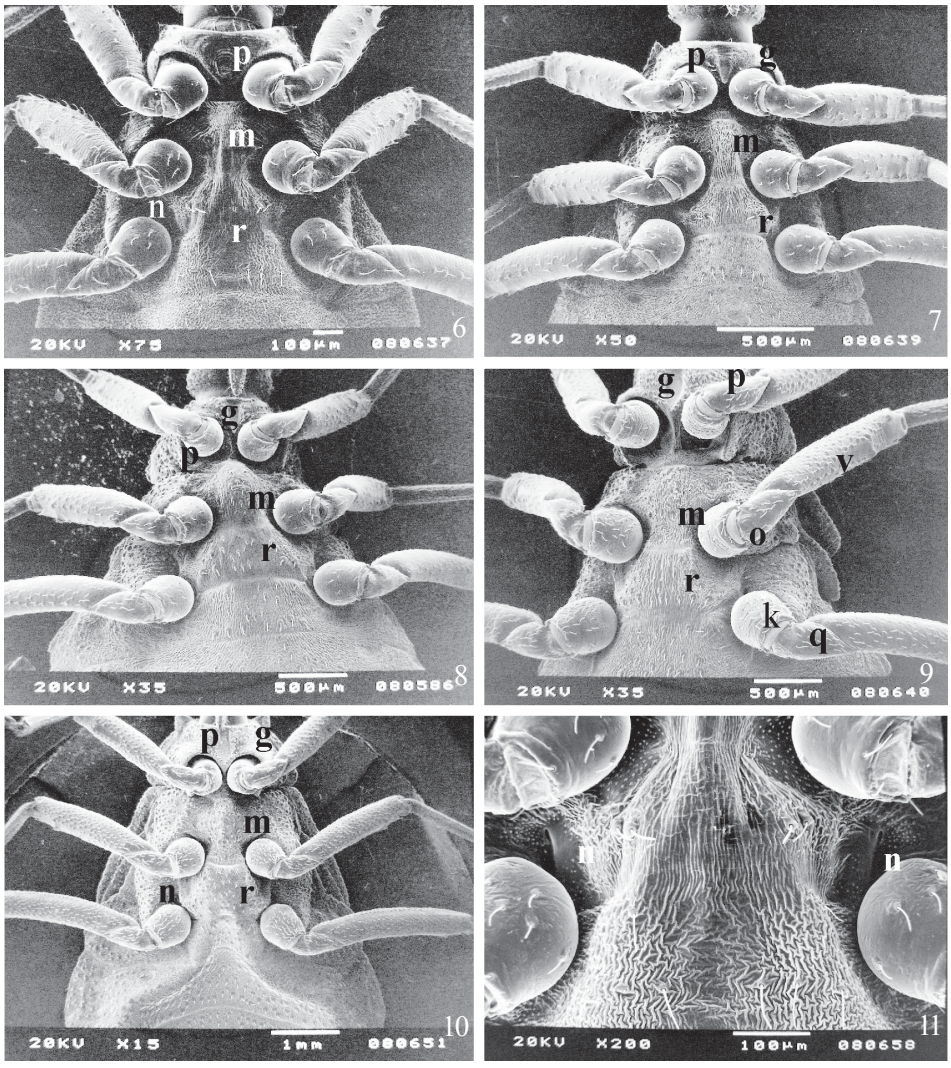
The five nymphal instars present mesonotum clearly separated from the pronotum, but not separated from the metanotum, in the posterior center region of the 1st and 3rd nymphal instars (Figs. 1, 3).

In the 3rd nymphal instar, the mesonotum and metanotum start to expand to originate the wing pads (Fig. 3). These wing pads are more evident in the 4th and 5th instars. In the 5th instar the metathoracic wing pads are overlapped by the mesothoracic ones (Figs. 4, 5).

Thorax ventral view. Prosternum, mesosternum and metasternum are regions clearly defined, as well as the pronotum, mesonotum and metanotum. Prosternum is the narrowest region in which there are a stridulatory sulcus and articulated legs near each other (Figs. 6, 7, 8, 9, 10).

Mesosternum is wider than the prosternum and has one pair of articulated legs. Metasternum is the widest one, with one pair of articulated legs. The distance between the legs in metasternum is wider than the mesosternum legs distance, wich is wider than in the protosternum legs distance. This is observed in the five nymphal instars (Figs. 6, 7, 8, 9, 10). In this region, it is possible to notice two metasternal glands openings (1+1) (Figs. 6, 9, 10, 11).

Thorax lateral view. In the five nymphal instars, propleuron and mesopleuron present grooves that divided these regions (Figs. 14, 15).



Figs. 6-11. *T. arthurneivai* ventral view of nymphs by SEM: 6, 1st instar; 7, 2nd instar; 8, 3th instar; 9, 4th instar; 10, 5th instar; 11, 1st instar metasternum. g: stridulatory sulcus; k: coxa; m: mesosternum; n: metasternal gland opening; p: prosternum; q: trochanter; r: metasternum; o: articular membrane; v: femur.

In the five nymphal instars, the medial portion of the posterior limit of the propleuron contains a (1+1) glandular evaporation area (Figs. 12, 13, 14, 15, 16, 17).

The Brindley's gland evaporation areas (1+1) are located in the superior portion of the mesopleuron, near the limit of the metapleuron (Figs. 12, 13, 14, 15, 16, 17).

DISCUSSION

Dorsal side

The results have showed that, besides the size, the five nymphal instars can be distinguished by the morphological characters observed in the pronotum, mesonotum and metanotum (Figs. 1, 2, 3, 4, 5). Such modifications were first verified by Correa *et al*. (1952) in *T. infestans* using optical microscopy.

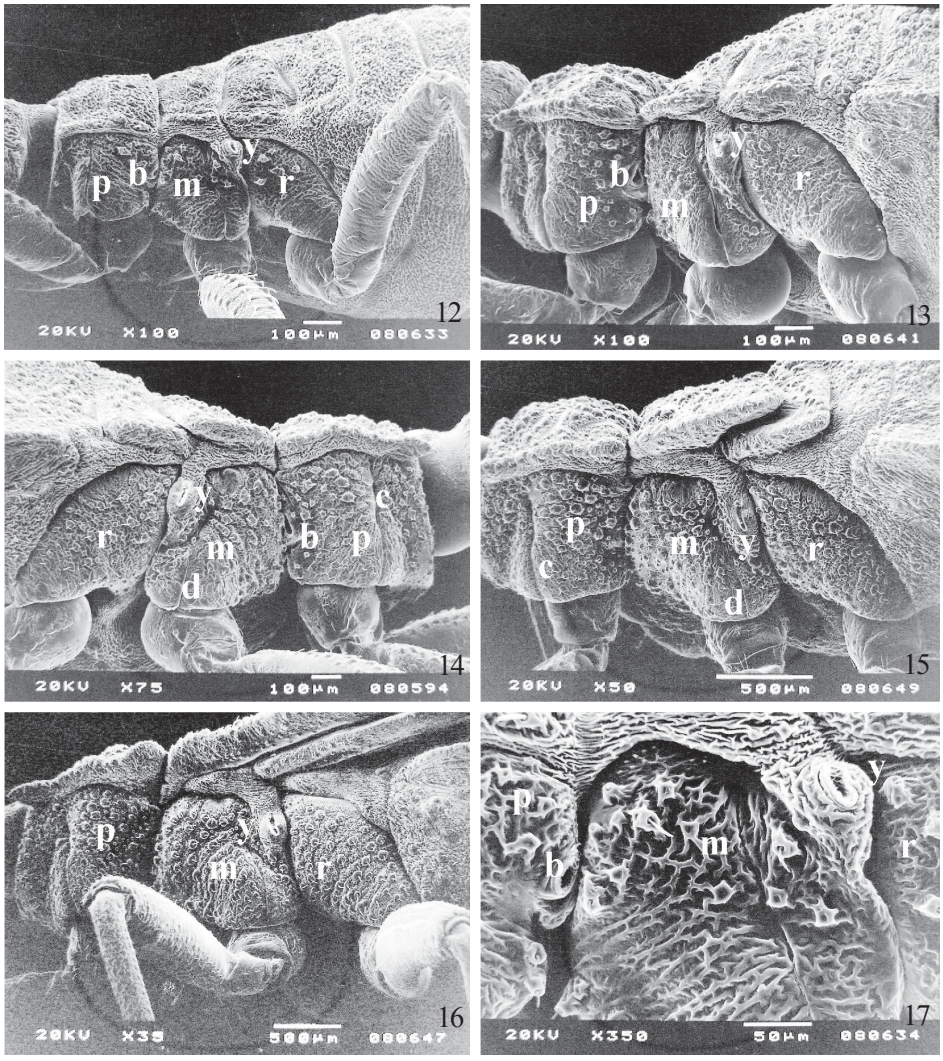
The thorax dorsal surface of the 1st nymphal instar is distinct from the other instars, by the absence of collar, tubercles and hairless areas. The suture at the central longitudinal thorax is more evident in the 1st nymphal instar (Fig. 1).

The 2nd instar of *T. arthurneivai* nymphs distinguishes from the 1st one by the presence of tubercles and hairless area on the pronotum, mesonotum and metanotum. The distinction between the 2nd and 3rd instars is possible due to the wing pads development of the 3rd nymphal instars (Figs. 2, 3).

The 4th nymphal instar can be characterized by the wing pad expansions and by the partial projection of the anterior wing pad over the posterior one (Fig. 4).

The main distinction of the 5th nymphal instar is that the anterior wing pads almost overlap the posterior ones. Due to this fact only vestiges of the internal lateral portions and the tips of the posterior wing pads are observed (Fig. 5).

The wing pads reaching the second abdominal segment is an aspect that allows the distinction between the 4th and 5th instars because in the 4th instar the wing pads do not reach the abdomen, and the metathorax is still visible (Figs. 4, 5).



Figs. 12-17. *T. arthurneivai* lateral view of nymphs by SEM: 12, 1st instar; 13, 2nd instar; 14, 3th instar; 15, 4th instar; 16, 5th instar; 17, 1st instar mesopleuron and metapleuron. b: glandular evaporation area; c: groove of propleuron; d: grouve of mesopleuron; m: mesopleuron; p: propleuron; r: metapleuron; y: Brindley's gland evaporation area.

Regarding the morphological aspects of the pronotum, it is observed that the 1st instar has two (1+1) sensilla at the anterior extremity, and absence of collar, tubercles and hairless area (Fig.1), contrary to the other instars (Figs. 2,3,4,5).

In the 2nd instar, the pronotum shows two anteroexternal angles also present in the 3rd, 4th, and 5th instars. From the 2nd , 3rd , 4th and 5th instars, the pronotum is clearly divided into two regions: the collar, wich is narrower, anterior and located at the inferior level; and the posterior level, which is larger (Figs. 2,3,4,5).

Ventral side

Morphological differences have not been found in the sternum of the five *T. arthurneivai* nymphal instars. However, two probable (1+1) metasternal gland openings were verified next to the third pair of legs (Figs. 6, 7, 8, 9,10, 11). Such openings are present in the five nymphal instars and were previously described in the 1st and 5th instars of *T. circummaculata* and *T. rubrovaria* nymphs by Rosa (1995).

Schofield & Upton (1978) described the metasternal glands in *Panstrongylus megistus* adults and repported that the Triatominae nymphs do not have this gland.

Carcavallo *et al*. (1997) observed, in Triatomine adults, the presence of foramens next to where the legs are inserted. They also observed the presence of two respiratory estigmas located between the prosternum and the mesosternum, and between the mesosternum and the metasternum in adults.

Considering that the openings found in the five nymphal instars are metasternal glands (Figs. 6, 7, 8, 9, 10, 1) and the observations of other authors in adults (Schofield & Upton, 1978; Carcavallo *et al.* 1997), we conclude that more detailed studies on both the external and the internal parts of these structures are necessary.

Lateral side

This view does not allow to perceive morphological differences in the pleura in order to separate the five instars of *T. arthurneivai* nymphs. (Figs. 12, 13, 14, 15, 16, 17).

However, it was noticed the presence of the Brindley's gland evaporation area in the mesopleuron of all five nymphal instars (Figs. 12, 13, 14, 15, 16, 17). Such structure has been observed before in the 1st and 5th instars of *T. circummaculata* and *T.rubrovaria* nymphs by Rosa (1995). The evaporation area and the Brindley's gland aperture was described in *P. megistus* adults by Santos-Mallet & Souza (1990). Brindley's gland was described as a metaesternal scent-gland present in the Heteroptera (Brindley, 1930).

Kalin & Barret (1975) observed the Brindley's gland in *R. prolixus* adults. Schofield & Upton (1978) described the Brindley's gland in *P.megistus* adults, but they have shown that it is not found in Triatominae nymphs. Millen *et al.* (1979) studied the development of the Brindley's gland in the 5th instar of *R. prolixus* nymphs.

From the data presented in this study, and based on the literature, we can conclude that more studies will be necessary in order to clarify the Brindley's gland development in Triatomine nymphal instars. Another interesting data found in this study was the observation of other evaporation areas (Figs. 12, 13, 14, 15, 16, 17) located at the medial portion of the propleuron, next to the mesopleuron. Such areas have not been described in the literature.

Acknowledgements. We thank Dr Vera Lúcia Cortiço Corrêa Rodrigues from SUCEN for donation of *T. arthurneivai*. João Luis Molina Gil and João Maurício Nóbrega Filho from SESA that made the colony maintenance. Francisco Carlos Rocatelli for digital copy of photograph. Hernany Henrique Garcia Justino, Maria Zenaide Tita Fernandes and Isabel Martinez for their assistance. Mara Cristina Pinto for English version. Dr Edvanda Bonavina da Rosa and Dr Sandro Roberto Valentini for critical revision. This work was supported by FAPESP, Process 96/09905-9.

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Received 18.VI.2004; accepted 04.VII.2005