

## Morphology of immatures of *Euschistus (Mitripus) grandis* (Insecta: Hemiptera: Pentatomidae)

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**ABSTRACT.** Immatures are important sources of information about characters, thus potentially improving classification and phylogenies. *Euschistus (Mitripus) grandis* Rolston, 1978 occurs in southern and southeastern Brazil. There are no references in the literature about this species other than the original description and distribution records. Our aim is to describe the egg and five nymphal instars of *E. (M.) grandis*, addressing the ontogeny of the external scent efferent system. Specimens of *E. (M.) grandis* were collected on *Solanum pseudocapsicum* Linnaeus (Solanaceae) in São Francisco de Paula, Rio Grande do Sul, Brazil, and reared in the laboratory. The egg of *E. (M.) grandis* is white and barrel-shaped, chorion translucent bearing spines connected by thin sheets forming polygons. Aero-mycropilar processes translucent and slightly clubbed. The nymphs bear anterior dorsal abdominal scent glands (DAG) with slit-like ostiole continued by a short groove; from the second instar onward median and posterior DAGs with rounded ostiole bearing closing cuticular valve. Spout peritreme flat and curved anteriorly, evaporatorium network-shaped surrounding ostiole plus peritreme; alveoli surface with trabeculae. Nymphs of *E. (M.) grandis* can be distinguished from other species of *Euschistus* by the color pattern and morphological features. The most striking morphological characteristic of 4<sup>th</sup> and 5<sup>th</sup> instar nymphs of *E. (M.) grandis* is the broad, deflected projection of humeral angles.

**KEY WORDS.** Brown stink bug; Carpocorini; chorion sculpturing; dorsal abdominal glands; nymph.

Studies on autoecology, behavior and morphology of immatures of insects are relevant for several fields in entomology (BRAILOVSKY *et al.* 1992). For taxonomic purposes, the immatures are important sources of information about characters, thus potentially improving classification and phylogenies (MATESCO *et al.* 2009). Characters of immatures, both eggs and nymphs, can be useful to identifying families, genera and species (SCHWERTNER *et al.* 2002). In ecological studies the use of immatures to evaluate the local diversity allows a better description and understanding of the ecological dynamics (MENDONÇA *et al.* 2009), although nymphs are commonly excluded from such studies because of the difficulty or even impossibility of identification. Also, early identification of crop pests in agroecosystems enables a faster adoption of control strategies (BRAILOVSKY *et al.* 1992).

Most pentatomids are phytophagous and some species are crop pests (GRAZIA *et al.* 1999). This economic aspect stimulated studies on immature stages of the Pentatomidae and the family is the most thoroughly studied for biological aspects among the Heteroptera (BRAILOVSKY *et al.* 1992, YONKE 1991). Nevertheless, only 146 out of almost 5,000 species of pentatomids have the eggs or nymphs described (POLLO *et al.* 2012). The number of morphological descriptions has increased in the last two decades (see MATESCO *et al.* 2009) and most recent studies focus on the

evaluation of neglected structures, potentially bearing phylogenetic information (e.g., dorsal abdominal scent glands, DAGs). Recent studies also employ scanning electron microscopy (SEM) techniques that allow more detailed descriptions, with relevant morphological features found (e.g., BIANCHI *et al.* 2011, POLLO *et al.* 2012, GILIO-DIAS *et al.* 2013). The phylogenetic importance of the egg stage was demonstrated in cladistic analyses (HASAN & KITCHING 1993, WHEELER *et al.* 1993, HENRY 1997, GRAZIA *et al.* 2008), while the relevance of DAGs in phylogenies was not explored.

*Euschistus* Dallas, 1851 is among the largest genera of Pentatomidae, comprising about 76 species distributed in the Neotropical and Nearctic regions. *Euschistus (Mitripus)* Rolston, 1978 is composed by ten species, with available information about immature stages for *Euschistus (M.) convergens* (Herrich-Schäffer, 1842) and *Euschistus (M.) hansii* Grazia, 1987 (MARTINS & CAMPOS 2006, MATESCO *et al.* 2009).

*Euschistus (M.) grandis* occurs in southern and southeastern Brazil, recorded from sites between 800 and 1,700 m of altitude (ROLSTON 1978). There are no references in the literature about the species other than the original description and distribution records. Our aim is to describe the egg and five nymphal instars of *E. (M.) grandis*, addressing the ontogeny of the external scent efferent system using light and scanning electron microscopy.

## MATERIAL AND METHODS

Adults of *E.(M.) grandis* were collected on *Solanum pseudocapsicum* Linnaeus (Solanaceae) in São Francisco de Paula, Rio Grande do Sul, Brazil (29°25'44.99"S, 50°23'36.49"W), from April to December 2011. Couples of specimens were maintained in plastic pots until oviposition. Eggs were transferred to Petri dishes with a moistened cotton pad. From eclosion to fifth instar the specimens were reared in pots, and fed on branches of *S. pseudocapsicum* with fruit and a pod of *Phaseolus vulgaris* Linnaeus (Fabaceae). Petri dishes were inspected daily; water and food were replaced whenever necessary.

Measurements, given in millimeters, were obtained according to CAMPOS *et al.* (2010), from ten eggs and ten individuals of each instar, maintained in 70% ethanol (Table I). Description of color pattern was carried out *in vivo*. Terminology for eggs and nymphs follows respectively MATESCO *et al.* (2009) and MATESCO *et al.* (2006, 2007). Terminology for the cuticular structures of dorsal abdominal scent glands (DAG)

of nymphs follows KMENT & VILÍMOVÁ (2010) as proposed by BIANCHI *et al.* (2011), and VILÍMOVÁ & KUTALOVÁ (2012). For scanning electron microscopy (SEM) analysis of the chorion and nymphs we adopted the protocol described by BIANCHI *et al.* (2011).

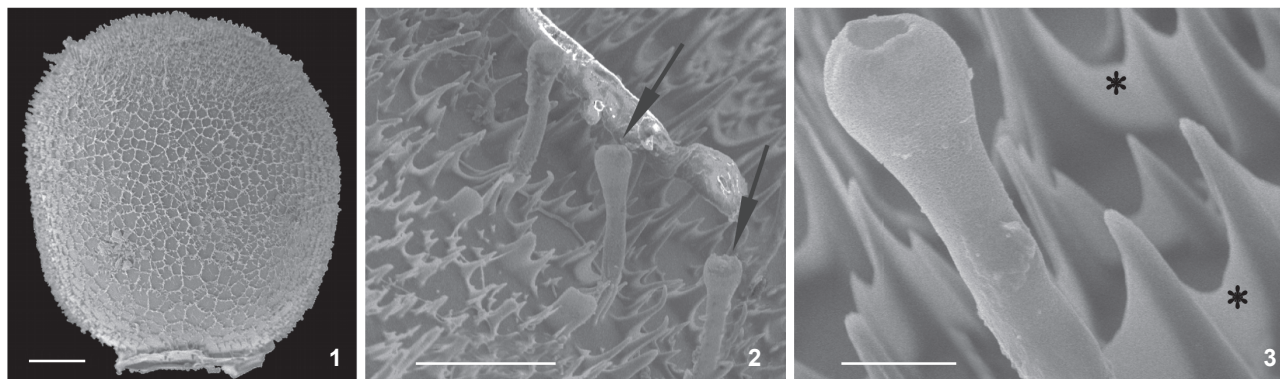
Voucher specimens were deposited in the Coleção Entomológica do Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRS), Porto Alegre.

## RESULTS

Egg (Figs 1-3). White, barrel-shaped, operculum convex, length  $1.47 \pm 0.08$  (1.40-1.68), width  $1.26 \pm 0.03$  (1.22-1.32) (Fig. 1). Chorion translucent, red eyes and brownish *ruptor ovis* visible in developing embryo. Chorion spinose under SEM, spines connected by thin sheets forming irregular polygons. Aero-mycopilar processes (AMP) in number of  $39.3 \pm 4.7$  (31-47), translucent, slightly clubbed (Fig. 2); surface spongy under SEM; bearing apical opening (Fig. 3).

Table I. Measurements in millimeters (mean  $\pm$  deviation, minimum – maximum, n = 10) of morphometric parameters of nymphs of *Euschistus (Mitripus) grandis* reared under controlled conditions. (AL, AW) Abdominal length and width, (HL, HW) head length and width, (ID) interocular distance, (I, II, III, IV) 1<sup>st</sup> to 4<sup>th</sup> antennal segments, (RL) rostrum length, (THL, THW) thorax length and width, (TL) total length.

|     | 1 <sup>st</sup> instar           | 2 <sup>nd</sup> instar           | 3 <sup>rd</sup> instar           | 4 <sup>th</sup> instar           | 5 <sup>th</sup> instar            |
|-----|----------------------------------|----------------------------------|----------------------------------|----------------------------------|-----------------------------------|
| TL  | $1.83 \pm 0.11$<br>(1.7 – 2.04)  | $2.96 \pm 0.16$<br>(2.79 – 3.24) | $4.20 \pm 0.39$<br>(3.64 – 5.08) | $6.30 \pm 0.57$<br>(5.62 – 7.50) | $9.20 \pm 0.54$<br>(8.20 – 10.00) |
| HL  | $0.62 \pm 0.07$<br>(0.48 – 0.74) | $0.77 \pm 0.81$<br>(0.65 – 0.92) | $1.05 \pm 0.08$<br>(0.92 – 1.16) | $1.37 \pm 0.12$<br>(1.25 – 1.56) | $1.88 \pm 0.21$<br>(1.60 – 2.37)  |
| HW  | $0.79 \pm 0.02$<br>(0.76 – 0.82) | $0.96 \pm 0.02$<br>(0.92 – 1.00) | $1.21 \pm 0.06$<br>(1.08 – 1.28) | $1.51 \pm 0.18$<br>(1.25 – 1.75) | $2.08 \pm 0.06$<br>(2.00 – 2.20)  |
| ID  | $0.58 \pm 0.01$<br>(0.56 – 0.60) | $0.60 \pm 0.06$<br>(0.44 – 0.68) | $0.75 \pm 0.03$<br>(0.68 – 0.80) | $0.95 \pm 0.05$<br>(0.87 – 1.04) | $1.30 \pm 0.12$<br>(1.10 – 1.50)  |
| RL  | $0.97 \pm 0.05$<br>(0.90 – 1.06) | $1.70 \pm 0.27$<br>(1.00 – 1.92) | $2.49 \pm 0.13$<br>(2.32 – 2.68) | $3.29 \pm 0.31$<br>(2.60 – 3.50) | $4.50 \pm 0.32$<br>(4.00 – 5.00)  |
| I   | $0.14 \pm 0.03$<br>(0.10 – 0.20) | $0.21 \pm 0.03$<br>(0.15 – 0.25) | $0.26 \pm 0.02$<br>(0.24 – 0.32) | $0.39 \pm 0.04$<br>(0.35 – 0.45) | $0.53 \pm 0.05$<br>(0.50 – 0.64)  |
| II  | $0.17 \pm 0.01$<br>(0.14 – 0.20) | $0.42 \pm 0.09$<br>(0.34 – 0.67) | $0.62 \pm 0.05$<br>(0.52 – 0.72) | $1.07 \pm 0.06$<br>(0.96 – 1.15) | $1.69 \pm 0.06$<br>(1.60 – 1.80)  |
| III | $0.19 \pm 0.01$<br>(0.18 – 0.22) | $0.39 \pm 0.03$<br>(0.35 – 0.45) | $0.57 \pm 0.04$<br>(0.48 – 0.64) | $0.88 \pm 0.04$<br>(0.80 – 0.95) | $1.30 \pm 0.06$<br>(1.20 – 1.40)  |
| IV  | $0.44 \pm 0.03$<br>(0.40 – 0.48) | $0.63 \pm 0.06$<br>(0.50 – 0.74) | $0.85 \pm 0.07$<br>(0.72 – 0.96) | $1.12 \pm 0.09$<br>(0.88 – 1.20) | $1.46 \pm 0.09$<br>(1.30 – 1.60)  |
| THL | $0.60 \pm 0.02$<br>(0.56 – 0.64) | $1.00 \pm 0.03$<br>(0.95 – 1.05) | $1.36 \pm 0.06$<br>(1.28 – 1.48) | $2.17 \pm 0.17$<br>(1.93 – 2.50) | $3.94 \pm 0.27$<br>(3.20 – 4.00)  |
| THW | $0.90 \pm 0.04$<br>(0.84 – 0.98) | $1.54 \pm 0.40$<br>(1.00 – 2.01) | $2.41 \pm 0.28$<br>(1.92 – 3.00) | $3.94 \pm 0.23$<br>(3.62 – 4.43) | $6.38 \pm 0.29$<br>(6.00 – 6.90)  |
| AL  | $0.61 \pm 0.13$<br>(0.40 – 0.86) | $1.19 \pm 0.15$<br>(0.96 – 1.44) | $1.69 \pm 0.24$<br>(1.32 – 2.04) | $2.82 \pm 0.46$<br>(2.19 – 3.44) | $3.74 \pm 0.52$<br>(2.90 – 4.50)  |
| AW  | $1.53 \pm 0.08$<br>(1.42 – 1.66) | $2.00 \pm 0.15$<br>(1.67 – 2.20) | $2.92 \pm 0.30$<br>(2.48 – 3.44) | $4.4 \pm 0.46$<br>(3.81 – 5.04)  | $6.32 \pm 0.48$<br>(5.20 – 7.00)  |



Figures 1-3. Egg of *Euschistus (Mitrivirus) grandis* under scanning electron microscopy: (1) lateral view; (2) magnification of anterior pole showing aero-mycopilar processes (arrow); (3) aero-mycopilar process; spines connected by thin sheets (asterisk). Scale bars: 1 = 200  $\mu\text{m}$ , 2 = 50  $\mu\text{m}$ , 3 = 100  $\mu\text{m}$ .

First instar (Figs 4, 6 and 12). Body oval, dorsally convex (Fig. 12); light brown just after hatching, changing to red and dark brown after a few minutes. Short setae uniformly distributed over body. Head declivous, subtriangular, dark brown with concolorous punctures. Tylus longer than juga, rounded at apex. Eyes red, ocelli absent. Antennal segments light brown, translucent, intersegmentar areas unpigmented; proportion of antennal segments:  $I = II = III < IV$ , segment IV two times longer than each of the previous segments. Rostrum light brown, reaching metacoxae. Thorax brown, punctures concolorous; intersegmentar lines conspicuous; lateral margins smooth, convex, with few setae. Legs light brown, tarsi darker. Sternum reddish. Abdomen red, black punctures denser than on thorax, absent on sternites; intersegmentar lines inconspicuous; four median dorsal plates, brown, subrectangular, the anterior three bearing 1+1 ostioles. Anterior DAG with slit-like ostiole continued by a short groove; cuticle surrounding ostiole smooth, lacking ornamentation. Lateral plates dark brown. Median and posterior DAGs with ear-shaped ostiole; cuticular process projected toward ostiole (Fig. 6). Groove behind ostiole transposing a granulated area. Ventral median plates brown, conspicuous on segments V to VIII. Spiracles on segments II to VII; 1+1 trichobotria on segments III to VI placed mesial to spiracles line (Fig. 4).

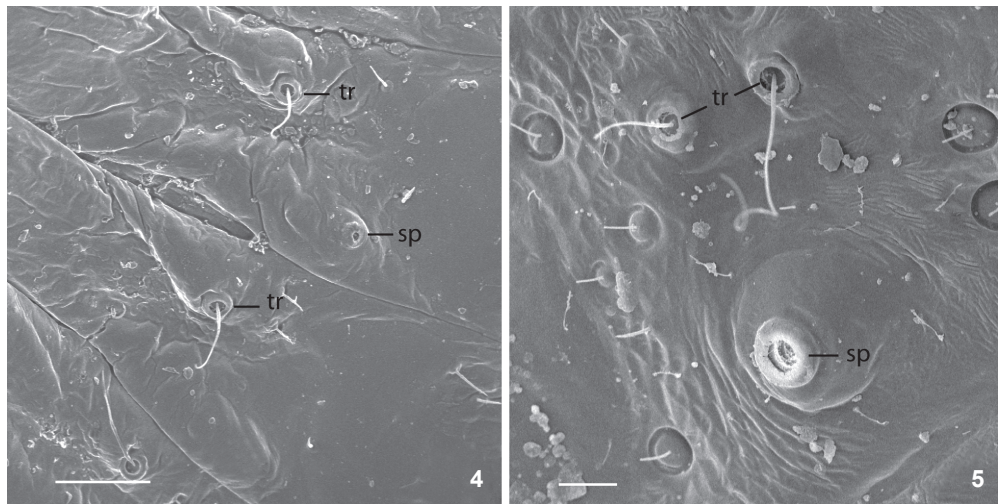
Second instar (Figs 5, 7 and 13). Head subquadrangular, less declivous and denser punctured than first instar; dark spots near eyes and on margins of juga; proportion of antennal segments:  $I < II = III < IV$  (Fig. 13). Rostrum reaching base of abdomen. Ventral surface of head dark brown. Thorax brown to ivory, denser punctured than first instar; pronotum with 1+1 dark brown irregular areas. Lateral margins serrate, compressed and slightly deflected, unpunctured. Ventral surface of thorax dark brown. Abdomen dorsally convex, red to ivory with red striation, denser punctured than first instar. Lateral plates lighter than disc, translucent, and outlined in dark brown; lateral margins minutely serrate to smooth. Dorsal median plates punctured. Anterior DAGs bearing wrinkles around ostiole. Median

and posterior DAGs with rounded ostiole bearing closing cuticular valve (Fig. 7). Spout peritreme flat and curved anteriorly; posterior peritreme with broader apex than median. Evaporatorium network-shaped surrounding ostiole plus peritrema; alveoli surface with trabeculae; periostiolar groove wrinkled. Ventral surface of abdomen sparsely punctured near lateral margins, unpunctured on disc; 2+2 trichobotria on segments III to VII (Fig. 5). Other characters as described for preceding instar.

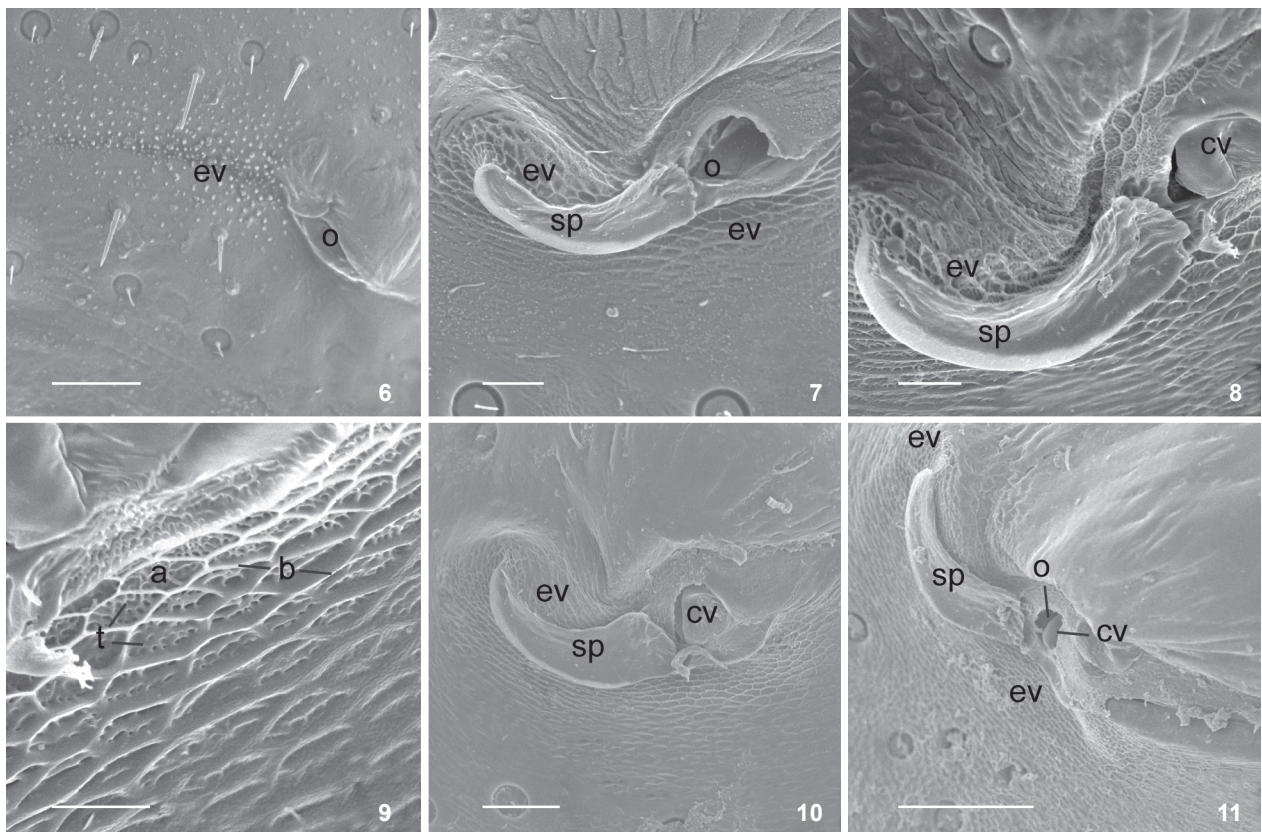
Third instar (Figs 8, 9 and 14). Head ivory with light brown spots; antennal segment III and distal half of IV reddish, remaining areas light grey to white (Fig. 14). Thorax ivory with light brown spots, humeral angles reddish-brown; lateral margins of pronotum deflected. Abdomen red to ivory with red striation, or variegate in green, red, and ivory; lateral plates translucent to greenish, with rare punctures. Median and posterior DAGs with a broader evaporatorium than previous instar (Fig. 8); alveoli covered by trabeculae; periostiolar groove deeper than preceding instar (Fig. 9). Ventral surface of abdomen red with irregular ivory spots. Other characters as described for preceding instars.

Fourth instar (Figs 10 and 15). Head green with brown spots; tylus and juga subequal; antennal segment II lighter (Fig. 15). Thorax greenish; humeral angles with brown spots, margins finely serrate; developing wing pads reaching abdominal segment III. Ventral surface of thorax light brown. Abdomen greenish with small irregular ivory areas; outline of lateral plates interrupted medially. Median and posterior DAGs with broader evaporatorium than previous instar; median peritreme with sharp apex (Fig. 10). Ventral surface of abdomen ivory with reddish spots; spiracles dark brown. Other characters as described for preceding instars.

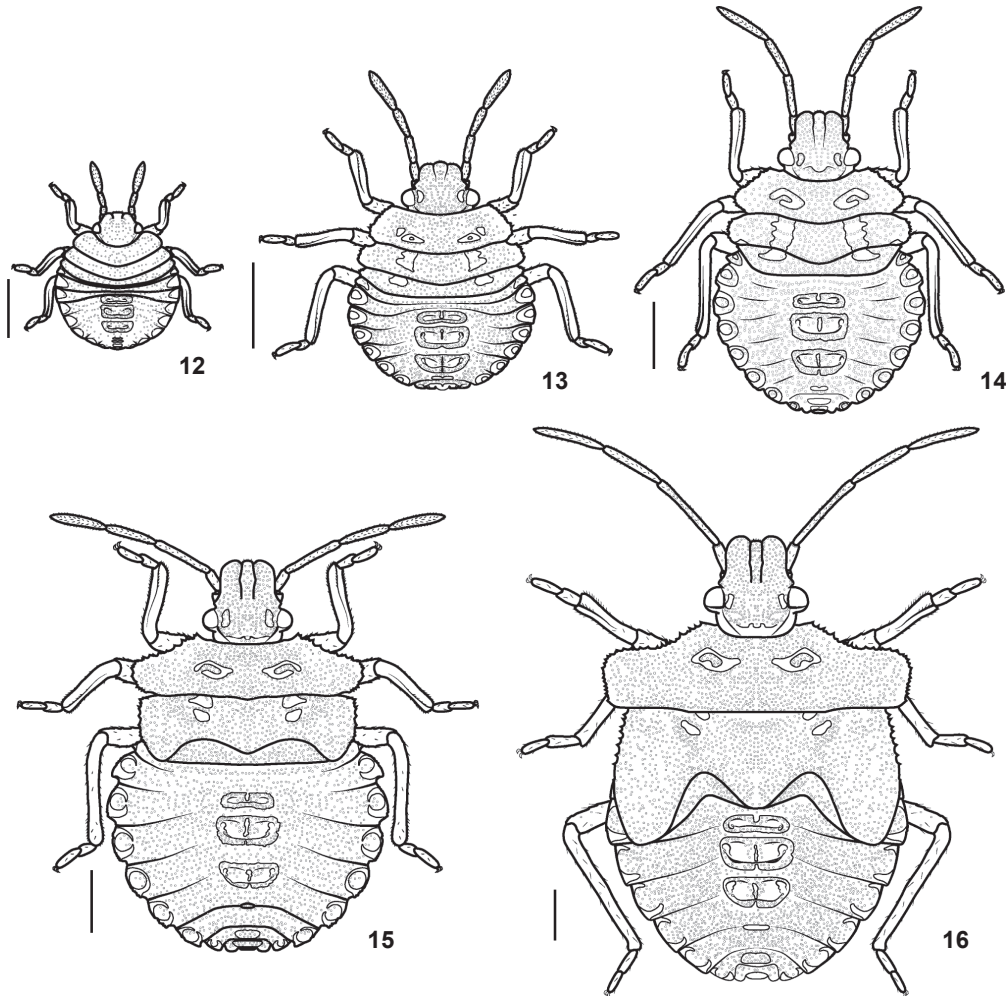
Fifth instar (Figs 11 and 16). Head with tylus and juga outlined in black; lateral margins of juga deflected; proportion of antennal segments:  $I < II > III = IV$  (Fig. 11). Rostrum green at base. Thorax dark brown on humeral angles and apex of wing pads; humeral angles truncate, prominent; wing pads reaching abdominal segment IV. Ventral disc of thorax greenish, includ-



Figures 4-5. Lateral margin of abdominal segments of *Euschistus (Mitripus) grandis* under scanning electron microscopy: (4) first instar, segments IV-V; (5) second instar, segment V. (sp) spiracle, (tr) trichobothrium. Scale bars: 4 = 50  $\mu\text{m}$ , 5 = 20  $\mu\text{m}$ .



Figures 6-11. External cuticular structures of median dorsal abdominal gland of nymphs of *Euschistus (Mitripus) grandis* under scanning electron microscopy: (6) first instar; (7) second instar; (8) third instar; (9) magnification of evaporatorium, third instar; (10) fourth instar; (11) fifth instar. (a) Alveole, (b) bridges, (cv) cuticular valve, (ev) evaporatorium, (o) ostiole, (sp) spout peritreme, (t) trabeculae. Scale bars: 6, 10 = 50  $\mu\text{m}$ , 7-8 = 20  $\mu\text{m}$ , 9 = 10  $\mu\text{m}$ , 11 = 100  $\mu\text{m}$ .



Figures 12-16. *Euschistus (Mitripus) grandis* nymphs, first to fifth instar, respectively, dorsal view. Scale bars = 1 mm.

ing coxae. Abdomen with green median plates; evaporatorium broader than preceding instar (Fig. 16). Intersegmentar lines conspicuous. Lateral margins of segments II and III minutely serrate. Ventral surface of abdomen variegate in green, red and ivory. Other characters as described for preceding instars.

## DISCUSSION

Since most immatures described belong to species of agricultural interest and these are not necessarily closely related species, the information about immature morphology is scattered in the family, making it difficult to recognize patterns within subgroups of Pentatomidae. However, considering that *Euschistus* and Carpororini are the best studied groups, some relevant comparisons can be made here. The eggs of *Euschistus* fit the external morphology commonly found in Carpororini (MATESCO *et al.* 2009), namely translucent eggs with spinose

chorion. Besides these characteristics, the eggs of *E. (M.) grandis* resemble those of other species of *Euschistus* by the barrel shape and convex operculum (BRAILOVSKY *et al.* 1992, MARTINS & CAMPOS 2006, MATESCO *et al.* 2009, CANDAN *et al.* 2005). Similar chorion sculpture as found in *E. (M.) grandis*, with irregular polygons formed by thin sheets connecting spines, was reported for all species of *Euschistus* already studied under SEM (MATESCO *et al.* 2009, CANDAN *et al.* 2005, BUNDY & MCPHERSON 2000). Two species, *Euschistus (E.) ictericus* (Linnaeus, 1763) and *E. (E.) sulcatus* Rolston, 1971, had their chorion described under light microscopy, the first as reticulate with spines (MCPHERSON & PASKIEWITZ 1984) and the second as bearing "many setae" (BRAILOVSKY *et al.* 1992). Further comparisons are not possible because the two species were not studied under SEM.

Few species of Pentatomidae had diagnostic characters described to enable their identification prior to the adult stage (e.g., GILIO-DIAS *et al.* 2013). Nymphs of *E. (M.) grandis* can be

distinguished from other species of *Euschistus* by the color pattern and morphological features. From 1<sup>st</sup> to 3<sup>rd</sup> instar most nymphs of *Euschistus* are morphologically similar (MARTINS & CAMPOS 2006, MCPHERSON & PASKEWITZ 1984, GRAZIA *et al.* 1980, BRAILOVSKY *et al.* 1992, PARISH 1934), but only *E. (M.) hansii* presents a color pattern as seen in *E. (M.) grandis*, although nymphs of the former are about 1/3 smaller than the latter and thus easily distinguishable. In 4<sup>th</sup> and 5<sup>th</sup> instars predominantly greenish nymphs as in *E. (M.) grandis* were described for *E. (E.) variolarius* (Palisot, 1817). The latter can be distinguished from the former by the presence of black and red spots (PARISH 1934). The most striking morphological characteristic of 4<sup>th</sup> and 5<sup>th</sup> instar nymphs of *E. (M.) grandis* is the broad, deflected projection of humeral angles, the latter feature also present in *E. (E.) sulcatus* and *E. (E.) bifibulus* (Palisot de Beauvois, 1805) (BRAILOVSKY *et al.* 1992).

During nymphal ontogenesis the anterior DAG pair changes only by the increment of wrinkles around the ostioles, whereas the median and posterior DAGs are progressively more complex and with a wider evaporatorium. The external ontogeny of DAGs is considered to include only the expansion of the evaporatorium (VILÍMOVÁ & KUTALOVÁ 2012), although our results indicate that the occurrence of fine structural modification should be better investigated. We suggest the description of DAGs under SEM should become a standard practice in future morphological studies of nymphs. Such practice will provide a better understanding of the comparative morphology of the DAGs across different taxonomic levels. We also indicate as character sources for phylogenetic studies the chorion sculpture, the structure of AMP, and the cuticular structure of DAGs, all under SEM.

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