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Morphology of the nymphs of *Euschistus convergens* (Hemiptera: Pentatomidae)

Kim R. Barão^{1,2,*}, Kayan V. Mostardeiro¹, Viviana C. Matesco¹, Anne G. Cohen¹, and Jocelia Grazia^{1,2}

Abstract

Nymphs of *Euschistus (Mitripus) convergens* (Herrich-Schaffer) (Hemiptera: Pentatomidae) are described using light and scanning electron microscopy. In the 1st instar, the integument and the lateral margins of the body are smooth, the dorsal abdominal scent glands bear a rounded peritreme with cuticular valve, and the evaporatorium surface bears pointed projections. From the 2nd to the 5th instars, the integument surface is granulated, the lateral margins of the body are serrated, the dorsal abdominal scent glands bear a spout-shaped peritreme with a cuticular valve, and the evaporatorium surface is reticulated. The overall morphology of *E. convergens* nymphs resembles the morphology of nymphs of other *Euschistus* species.

Key Words: Carpocorini; coloration; dorsal abdominal gland; stink bug

Resumen

Se describen las ninfas de *Euschistus (Mitripus) convergens* (Herrich-Schaffer) (Hemiptera: Pentatomidae) utilizando microscopía de luz y de electrónica de barrido. En el primer estadio, el tegumento y los márgenes laterales de su cuerpo son lisos, las glándulas odoríferas abdominales dorsales tienen un peritrema redondeado con válvula cuticular, y la superficie del evaporatorium tiene proyecciones agudas. Desde el 2 al 5 estadios, la superficie del tegumento es granulada, los márgenes laterales de su cuerpo son dentados, las glándulas odoríferas abdominales dorsales tienen un peritrema en forma de pico, con una válvula cuticular y la superficie del evaporatorium es reticulada. La morfología general de las ninfas de *E. convergens* asemeja a la morfología de las ninfas de otras especies de *Euschistus*.

Palabras Clave: Carpocorini; coloración; glándula abdominal dorsal; chinche

Characters of immature stages, both eggs and nymphs, can be useful for identifying families and genera (Schwertner et al. 2002). Most pentatomids (Hemiptera: Heteroptera: Pentatomidae) are phytophagous and some species are important crop pests (Grazia et al. 1999). Studies on immature stages of the Pentatomidae were initially triggered by their economic relevance and, as a result, the family is one of the most thoroughly studied with regard to biological aspects among the Heteroptera (Yonke 1991; Brailovsky et al. 1992). Secondarily, the increasing availability of morphological data of immatures (Matesco et al. 2009a, 2014) has drawn attention to their potential as source of phylogenetic information.

Of Neotropical fauna, *Euschistus* Dallas is one of the best-studied genera. Commonly known as brown stink bugs, some *Euschistus* species are important crop pests in the Neotropics (Panizzi et al. 2000). *Euschistus* comprises about 70 species and 4 subgenera and has been revised in recent decades (Rolston 1974, 1978, 1982, 1984). Its phylogenetic relationships are being studied (Weiler 2011; A.G.C., unpublished data) and immature stages of 6 species have been described (McPherson & Paskewitz 1984; Munyaneza & McPherson 1994; Martins & Campos 2006; Matesco et al. 2009a; Biasotto et al. 2013).

The subgenus *Mitripus* Rolston comprises 10 species, of which immature stages are known for 2 species (Martins & Campos 2006; Matesco et al. 2009a; Biasotto et al. 2013). *Euschistus (Mitripus) con-*

vergens (Herrich-Schaffer) occurs in Argentina, Bolivia, southeastern and southern Brazil, and Paraguay, but there is no record of its host plants. This species has been studied only by Matesco et al. (2009a), who described the egg chorionic ultrastructure and compared it with the morphology of eggs known for other *Euschistus* species.

We describe the 5 instars of *E. (M.) convergens*, based on light and scanning electron microscopy, addressing the ontogenetic changes in morphological ultrastructure. This study is part of a series of papers on the systematics and morphology of the Carpocorini (Pentatomidae: Pentatominae) including the phylogeny of *Euschistus*, which is under development in the Laboratório de Entomologia Sistemática, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil.

Materials and Methods

Eggs, nymphs, and adults were collected in Maquiné, Rio Grande do Sul, Brazil, at the Centro de Pesquisa Litoral Norte of Fundação Estadual de Pesquisa Agropecuária. Specimens were kept in plastic vials and reared at 24 ± 1 °C, 70 ± 10% RH, and 12:12 h L:D photoperiod. Nymphs and adults were offered ad libitum green pods of *Phaseolus vulgaris* L. (Fabales: Fabaceae). Voucher specimens were deposited at the Coleção de Entomologia, Departamento de Zoologia, UFRGS, Brazil.

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Measurements and morphological data were obtained from 10 nymphs of each instar preserved in 70% ethanol. The description of color pattern was carried out from images taken in vivo. Measurements (mean \pm standard deviation, minimum and maximum values, Table 1), given in millimeters, were obtained according to Biasotto et al. (2013).

Drawings were made with the aid of a camera lucida coupled to a stereomicroscope, and then digitally scanned and edited with Adobe Illustrator (Adobe Systems, Inc., San Jose, California, USA). Specimens of each instar were prepared for scanning electron microscopy (SEM) following the cleaning protocol described in Barão et al. (2013). After cleaning, specimens were dehydrated by an increasing ethanol series, transferred to absolute acetone, dried in a critical point drier, sputter coated with gold, and observed by SEM at the Centro de Microscopia Eletrônica (CME) of UFRGS.

Terminology for general morphology follows Matesco et al. (2007, 2008) and that for the external structures of the dorsal abdominal scent glands follows Bianchi et al. (2011a) and Vilímová & Kotalová (2012).

Results

FIRST INSTAR

Body bright yellow after eclosion. Head and thorax become dark brown to black some hours after eclosion; eyes red; antennae and labium reddish-brown. Thorax and abdomen with longitudinal median line pale; thorax red ventrally; legs dark brown, apical half of tibiae and tarsi pale. Abdomen red dorsally and ventrally; dorsal median and lateral plates dark brown.

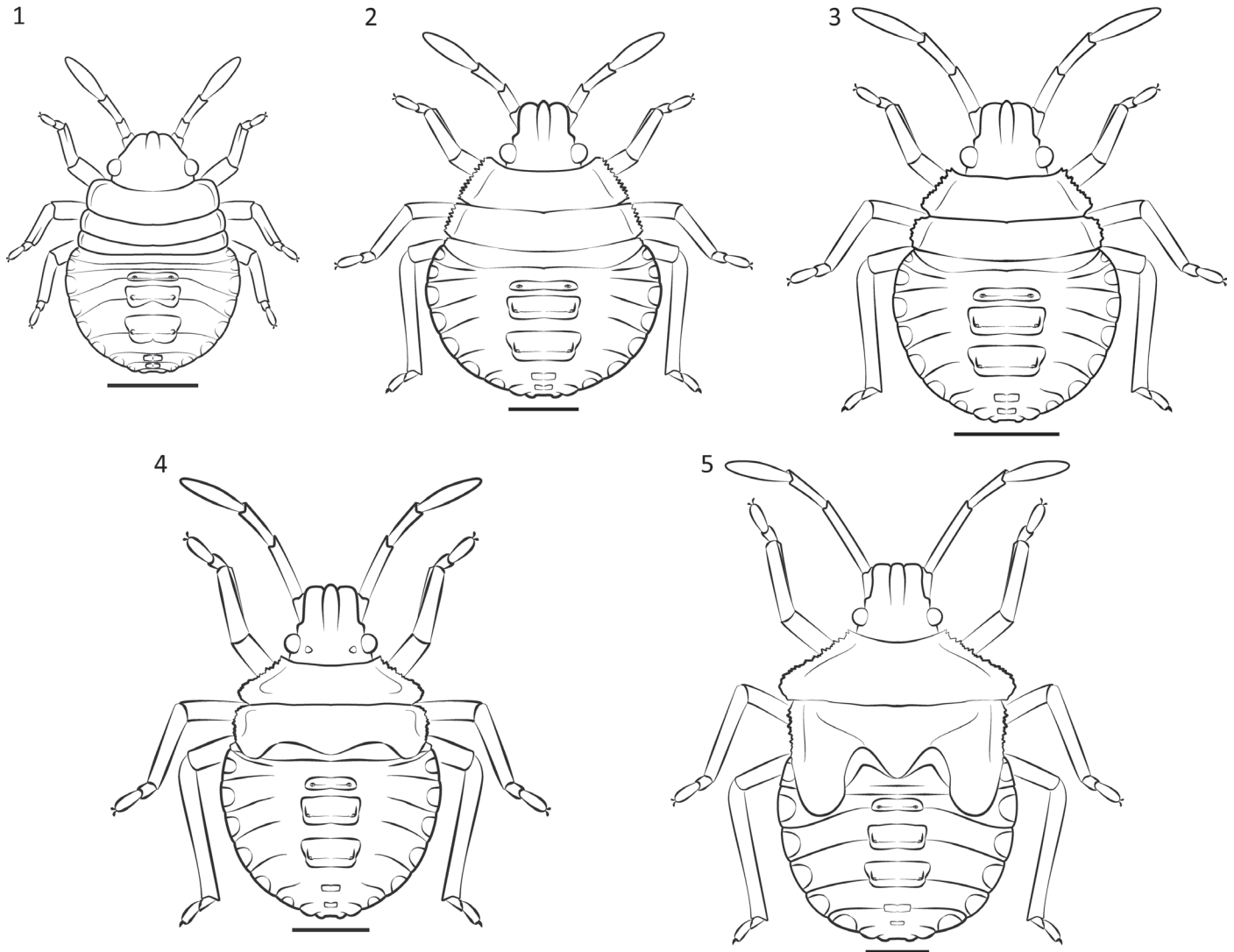
Body circular, strongly convex (Fig. 1). Lateral margins of body bearing setae (Fig. 6). Head rounded; apex of clypeus and mandibular plates rounded; mandibular plates shorter than clypeus; ocelli absent; antennae covered by setae, proportion of antennal segments I < II = III < IV; labial apex reaching 3rd abdominal segment. Posterior margin of each thoracic segment bearing small hemispheric integumental projections (Fig. 6). Dorsal surface of abdominal segments smooth, with 5 pairs of dorsal median and 8 pairs of dorsolateral sclerotized plates (Figs. 1 and 7). First 3 dorsal median plates placed between abdominal segments 3–4, 4–5, and 5–6 (Fig. 8), bearing a pair of ostioles of the dorsal abdominal scent glands (Figs. 9–11); dorsal median plates of abdominal segments 7 and 8 not bearing dorsal abdominal glands. Ostioles of dorsal abdominal scent glands attended by operculum; surface of evaporatorium sculptured with pyramidal projections (Figs. 10 and 11). Abdominal ventral plates present on abdominal segments 5–8, covered by comb-like projections (Fig. 12). Spiracles on abdominal segments 2–7; a pair of trichobothria on abdominal segments 3–6, mesad of spiracular line (Fig. 13).

SECOND INSTAR

General body color light brown; punctures dark brown. Antennal segments brown, apex of antennal segments I, II, and III pale. Legs dark brown; coxae, basal half of femora, and tarsi pale. A pair of dark brown, submedian, longitudinal stripes on thorax dorsally. Abdominal margins reddish dorsally; dorsal median plates dark brown, with light brown spots medially and adjacent to ostioles of dorsal abdominal scent glands; dorsolateral plates pale; abdomen reddish ventrally; ventral plates dark brown.

Table 1. Measurements (mean \pm standard error [minimum–maximum]), in millimeters, of morphometric characters of *Euschistus convergens* nymphs.

Character	First instar	Second instar	Third instar	Fourth instar	Fifth instar
Total length	1.20 \pm 0.04 (0.86–1.37)	1.62 \pm 0.04 (1.42–1.89)	3.00 \pm 0.08 (2.58–3.32)	4.52 \pm 0.10 (3.93–5.00)	5.50 \pm 0.09 (5.03–5.96)
Head length	0.30 \pm 0.02 (0.17–0.44)	0.45 \pm 0.02 (0.33–0.55)	0.69 \pm 0.02 (0.55–0.80)	0.94 \pm 0.03 (0.71–1.13)	1.07 \pm 0.05 (0.80–1.29)
Head width	0.50 \pm 0.01 (0.45–0.53)	0.65 \pm 0.01 (0.61–0.70)	0.85 \pm 0.01 (0.80–0.97)	1.14 \pm 0.02 (1.09–1.19)	1.44 \pm 0.02 (1.32–1.51)
Interocular distance	0.35 \pm 0.01 (0.30–0.40)	0.40 \pm 0.01 (0.34–0.44)	0.52 \pm 0.01 (0.45–0.55)	0.67 \pm 0.02 (0.58–0.90)	0.85 \pm 0.01 (0.80–0.93)
Rostrum length	0.58 \pm 0.02 (0.48–0.67)	1.20 \pm 0.02 (1.09–1.26)	1.72 \pm 0.05 (1.45–1.90)	2.46 \pm 0.03 (2.32–2.64)	3.09 \pm 0.07 (2.77–2.48)
Length of 1st antennal segment	0.09 \pm 0.01 (0.06–0.11)	0.15 \pm 0.004 (0.12–0.17)	0.23 \pm 0.001 (0.19–0.29)	0.28 \pm 0.01 (0.19–0.35)	0.38 \pm 0.01 (0.32–0.48)
Length of 2nd antennal segment	0.11 \pm 0.01 (0.08–0.14)	0.25 \pm 0.01 (0.22–0.30)	0.46 \pm 0.02 (0.35–0.61)	0.72 \pm 0.02 (0.55–0.77)	1.13 \pm 0.04 (0.97–1.32)
Length of 3rd antennal segment	0.13 \pm 0.08 (0.09–0.19)	0.26 \pm 0.01 (0.23–0.30)	0.42 \pm 0.01 (0.35–0.48)	0.55 \pm 0.01 (0.48–0.61)	0.88 \pm 0.02 (0.77–0.97)
Length of 4th antennal segment	0.29 \pm 0.01 (0.26–0.33)	0.43 \pm 0.01 (0.36–0.48)	0.60 \pm 0.01 (0.51–0.64)	0.72 \pm 0.03 (0.48–0.84)	0.94 \pm 0.02 (0.84–1.03)
Pronotum length	0.18 \pm 0.01 (0.14–0.25)	0.27 \pm 0.01 (0.23–0.31)	0.46 \pm 0.01 (0.39–0.52)	0.71 \pm 0.02 (0.58–0.84)	1.08 \pm 0.02 (0.96–1.19)
Pronotum width	0.71 \pm 0.01 (0.64–0.80)	1.03 \pm 0.02 (0.93–1.12)	1.57 \pm 0.03 (1.45–1.77)	2.44 \pm 0.04 (2.16–1.74)	3.81 \pm 0.05 (3.55–4.16)
Abdominal width	0.90 \pm 0.02 (0.79–1.03)	1.35 \pm 0.04 (1.17–1.64)	2.06 \pm 0.05 (1.77–2.26)	3.01 \pm 0.06 (2.71–3.32)	3.92 \pm 0.06 (3.71–4.35)



Figs. 1–5. Dorsal view of nymphs of *Euschistus convergens*. **1.** First instar. **2.** Second instar. **3.** Third instar. **4.** Fourth instar. **5.** Fifth instar. Scale bars = 0.5, 0.5, 1, 1, and 1 mm, respectively.

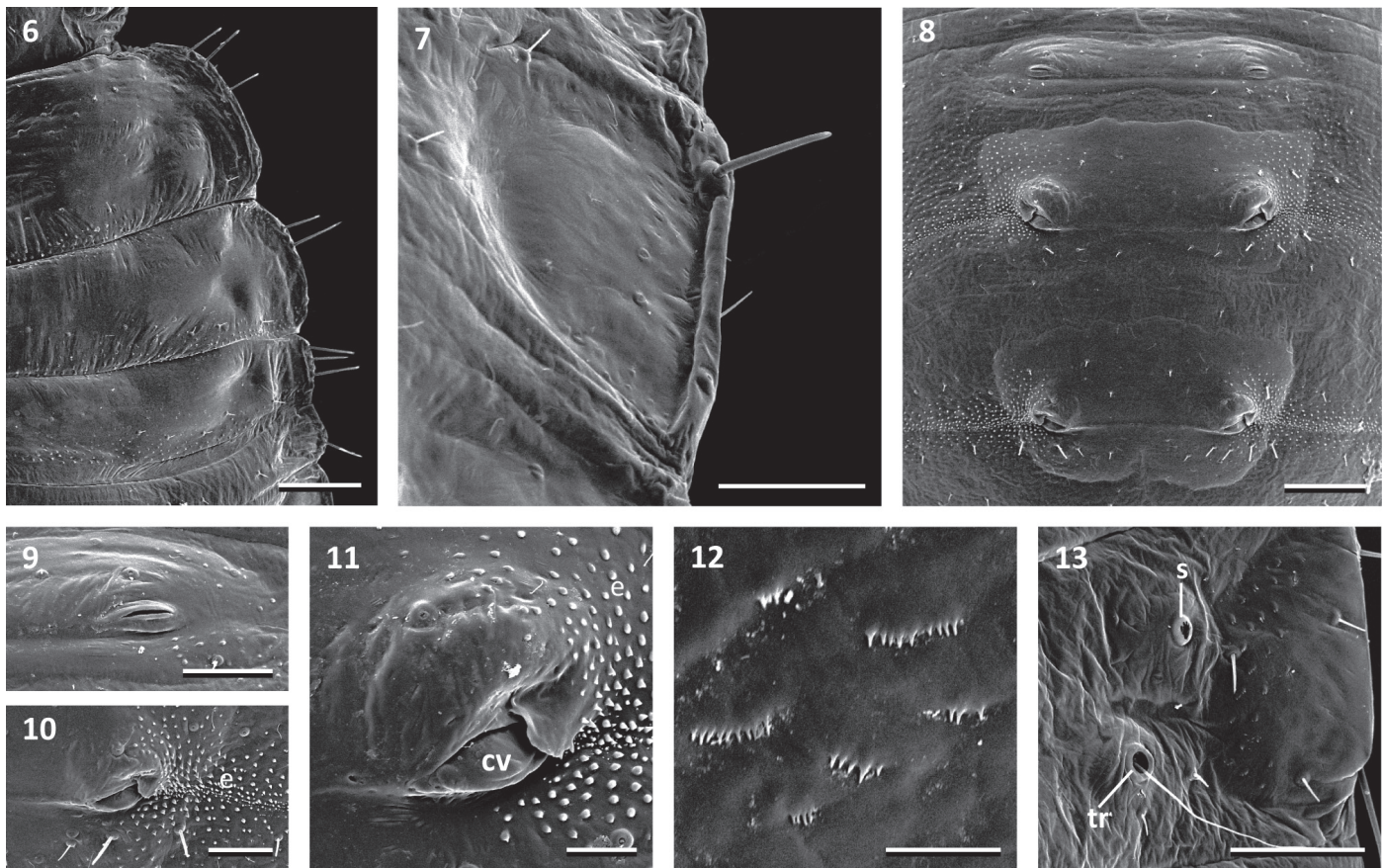
Body oval (Fig. 2), less convex than in the 1st instar; dorsal surface punctured (Fig. 14). Apex of mandibular plates rounded; lateral margins of head with a rounded projection anterior to the eyes; labium surpassing abdominal segment 3. Lateral margins of thoracic segments punctured, explanate, and serrated (Fig. 14); prothorax trapezoidal; tibiae flattened dorsally. Abdominal surface wrinkled (Fig. 18); lateral margins of abdomen slightly serrated (Fig. 20); dorsal median plates on abdominal segments wrinkled (Fig. 15); ostioles of dorsal abdominal scent glands on abdominal segments 3–4 elongate, not attended by peritreme, evaporatorium inconspicuous (Fig. 15); dorsal abdominal scent glands on abdominal segments 4–5 and 5–6 with rounded ostioles, bearing closing cuticular valve, ostiolar peritreme spout shaped (Fig. 16), evaporatorium reticulate, surface of alveoli with trabeculae (Fig. 17), periostiolar groove wrinkled. Abdominal ventral plates present on abdominal segments 5–9, covered by comb-like projections (Fig. 19); 2 pairs of trichobothria on abdominal segments 3–7, one aligned to the spiracle and other mesad of spiracular line (Fig. 20). Other characters as described for the preceding instar.

THIRD INSTAR

Color similar to 2nd instar; antennal segments red-brownish, intersegmental membrane pale. Body pyriform; head rectangular (Fig. 3). Mandibular plates as long as clypeus. Proportion of antennal segments $I < II = IV > III$. Dorsal surface of thorax irregularly granulated (Fig. 21). Dorsal surface of abdominal segments minutely wrinkled; ventral surface smooth. Evaporatorium of dorsal abdominal scent glands on abdominal segments 3–4 reticulate. Abdominal ventral plates present on abdominal segments 5–9, covered by comb-like projections and bearing scattered setae. Other characters as described for the preceding instar.

FOURTH INSTAR

Color similar to 2nd instar; antennal segments red-brownish, basal half of 4th segment pale; thorax dark brown, densely punctate; thoracic cicatrices light brown; dorsolateral plates on abdominal segments pale, outlined in dark brown. Body oval (Fig. 4). Proportion of antennal segments $I < II > III = IV$. Mesothorax subrectangular; wing pads reach-



Figs. 6–13. First instar of *Euschistus convergens* under scanning electron microscopy. **6.** Lateral margin of thorax, dorsal view. **7.** Dorsolateral plate on the 3rd abdominal segment. **8.** Dorsal plates on 3rd to 6th abdominal segments, bearing scent glands. **9.** Dorsal abdominal scent gland on 3rd abdominal segment. **10–11.** Dorsal abdominal scent gland on 4th abdominal segment. **12.** Sculpturing of the ventral plate of the 6th abdominal segment. **13.** Spiracle and trichobothrium on the 6th abdominal segment. *cv*, cuticular valve; *e*, evaporatorium; *s*, spiracle; *tr*, trichobothrium. Scale bars = 100, 50, 100, 40, 40, 20, 10, and 50 μ m, respectively.

ing posterior margin of metathorax (Fig. 22). Abdominal segments more densely punctate ventrally than dorsally. Other characters as described for preceding instar.

FIFTH INSTAR

Color similar to 2nd instar; antennal segments red-brownish, basal half of 3rd and 4th segments pale; thoracic cicatrices light brown; dorsolateral plates on abdominal segments pale, outlined in dark brown. Body rectangular (Fig. 5). Wing pads reaching posterior margin of abdominal segment 3; lateral margins of wing pads serrated (Fig. 23). Other characters as described for preceding instar.

Discussion

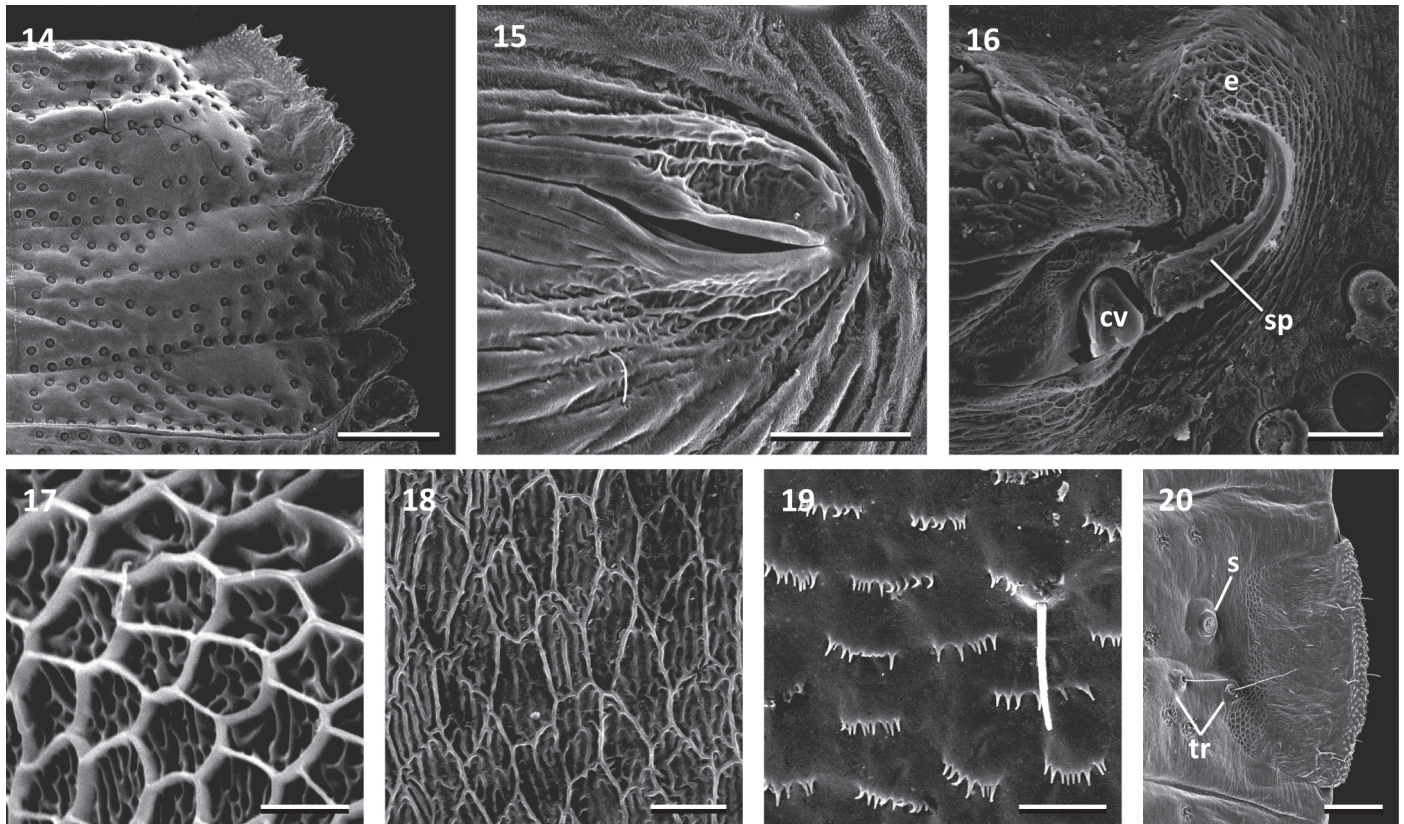
According to several authors, the knowledge on immature stages of pentatomid species associated with crops can help in the early identification of crop pests. However, of the few species whose immatures have been described, even less had diagnostic characters described to enable identification prior to the adult stage. This is probably because of the homogeneity of pentatomid immature morphology.

Identification of immature pentatomids relies on coloration (e.g., Fürstenau et al. 2013). The nymphs of *E. convergens* are character-

ized by a variegated pattern of brown and light yellow throughout all instars, differing from *Euschistus (Mitripus) grandis* Rolston and *Euschistus (Mitripus) hansii* Grazia; the latter species have 2 different sets of coloration, one from the 1st to 3rd instars and the other in the 4th and 5th instars (Martins & Campos 2006; Biasotto et al. 2013). The use of coloration to identify pentatomid species should be done cautiously, as biotic and abiotic factors can alter the color of nymphs and adults (e.g., Schwertner et al. 2002; Musolin & Numata 2003; Niva & Takeda 2003).

From the 1st to 4th instars, the known immatures of *Euschistus* are morphologically similar. With regard to the subgenus *Mitripus*, the anterolateral pronotal margins are slightly convex (Martins & Campos 2006; Biasotto et al. 2013), whereas in the subgenus *Euschistus* the anterolateral pronotal margins are straight (McPherson & Paskewitz 1984; Munyaneza & McPherson 1994). The 5th instars of *Mitripus* species have the humeral angles of the pronotum rounded, well produced, and the anterolateral margins concave (Martins & Campos 2006; Biasotto et al. 2013), characteristics that can distinguish them from species of the subgenus *Euschistus*, in which the humeral angles of the pronotum are rounded, but not produced, and the anterolateral margins are straight or slightly concave (McPherson & Paskewitz 1984; Munyaneza & McPherson 1994).

The use of SEM has allowed the study of neglected integumentary structures, such as dorsal abdominal scent glands and ventral plates.



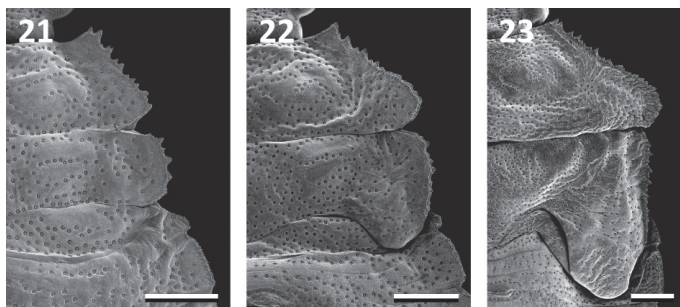
Figs. 14–20. Second instar of *Euschistus convergens* under scanning electron microscopy. **14.** Lateral margin of thorax, dorsal view. **15.** Dorsal abdominal scent gland on the 3rd abdominal segment. **16.** Dorsal abdominal scent gland on the 4th abdominal segment. **17.** Evaporatorium on the 5th abdominal segment. **18.** Cuticular sculpturing of tergum of the 4th abdominal segment. **19.** Sculpturing of the 3rd abdominal ventral plate. **20.** Spiracle, trichobothria, and lateroventral plate of the 4th abdominal segment. *cv*, cuticular valve; *e*, evaporatorium; *s*, spiracle; *sp*, spout peritreme; *tr*, trichobothrium. Scale bars = 200, 20, 20, 5, 10, 10, and 200 μm , respectively.

The dorsal abdominal scent glands of nymphs of the Pentatomoidea are functional (Cassier et al. 1994; Vilimová & Kutalová 2012), playing a role in chemical defense against predators, and they are morphologically and histologically diverse (Lucchi 1993): the 2nd and 3rd pairs are similar but differ from the 1st pair. So far, the 1st pair of dorsal abdominal scent glands of the Pentatomidae has been found to be simple, with a rounded ostiole, unattended by peritreme, and with incipient evaporatorium (Pollo et al. 2012; Biasotto et al. 2013; Gilio-Dias et al. 2013). The 2nd and 3rd pairs of dorsal abdominal scent glands, from the 2nd to 5th instars, are each attended by a spout-shaped peritreme, the ostiole is rounded and is closed by a cuticular valve, and the evaporatorium is developed in a reticulated pattern (Pollo et al. 2012; Biasotto

et al. 2013; Gilio-Dias et al. 2013). In contrast, in the scutellerid *Galeacius martini* Schouteden, the 2nd dorsal abdominal scent gland pair is spout shaped, but the 3rd pair is disk shaped (Bianchi et al. 2011b).

Abdominal ventral plates were first observed recently in Pentatomidae with the aid of SEM, being recorded as strongly sclerotized sclerites, bearing minute comb-like projections (Matesco et al. 2008, 2009b; Pollo et al. 2012; Gilio-Dias et al. 2013). However, the distribution of abdominal ventral plates on abdominal segments has not been recorded. In *E. convergens*, abdominal ventral plates occur on abdominal segments 5–8 in the 1st instar and on abdominal segments 5–9 from the 2nd to 5th instars. The function of the abdominal ventral plates' comb-like projections is unknown. Abdominal ventral plates are found also in Scutelleridae (Hussey 1934; Peredo 2002; Williams et al. 2005; Bianchi et al. 2011b), but are covered by stout projections, allegedly used in vibrational communication between nymphs and adults.

In conclusion, the morphology of nymphs of *E. convergens* agrees with the pattern described for the subgenus *Mitripus*. Coloration is the unique feature that makes the identification of the species feasible in early instars. New descriptions of immature stages of Pentatomidae are needed, especially those that focus on ultrastructural morphology with the aid of SEM, that allow the corroboration of patterns described for the family, and that provide characters useful for phylogenetic analyses. Structures of special interest would pertain to morphology of eggs and nymphs, ontogenesis of the dorso-abdominal scent-efferent system, the ventral abdominal plates, and the trichobothria (Bianchi et al. 2011b).



Figs. 21–23. Dorsal view of lateral margin of thorax in 3rd to 5th instars of *Euschistus convergens* under scanning electron microscopy. **21.** Third instar. **22.** Fourth instar. **23.** Fifth instar. Scale bars = 400, 500, and 500 μm , respectively.

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