

Description of spermatheca and eggs of *Eurygaster austriaca* (Schrank, 1778) (Heteroptera: Scutelleridae), based on optical and scanning electron microscopy

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Abstract: Spermatheca and egg morphology of *Eurygaster austriaca* (Schrank, 1778) were studied by optical and scanning electron microscopy (SEM). The spermatheca of *E. austriaca* is characterized by a spermathecal bulb, a pumping region, distal and proximal flanges and ducts, and a genital chamber. Each female was shown to deposit 14 green eggs on average in mass. The spherical eggs averaged 1.05 ± 0.05 mm in diameter. The first external evidence of embryonic development was the appearance of 2 red eye spots opposite each other beneath operculum followed by the appearance of a blackish T-shaped egg-burster between the eye spots. The thickened highly sclerotized egg-burster has sucker-shaped structures on the both sides of the tail. Egg surfaces are covered with clearly marked polygon patterns with tubercles. There were 17-19 aero-micropylar processes shaped like truncated cones scattered among the polygons.

Key words: Scanning electron microscope, egg, chorion, spermatheca, *Eurygaster austriaca*, Heteroptera

Eurygaster austriaca (Schrank, 1778) yumurta ve spermatekasının ışık ve taramalı elektron mikroskopuyla tanımı (Heteroptera: Scutelleridae)

Özet: *Eurygaster austriaca* (Schrank, 1778) yumurta ve spermateka morfolojisi ışık ve taramalı elektron mikroskopuyla incelendi. *E. austriaca*'nın spermatekası spermateka haznesi, pompalama bölgesi, distal ve proksimal yaka ve kanallar ve genital oda ile karakterize edilir. Her bir dişi bir yumurta kümesinde genellikle yeşil renkli 14 yumurta bırakır. Yuvarlak şekilli yumurtalar ortalama $1,05 \pm 0,05$ mm çapındadır. Embriyonik gelişimin ilk belirtisi operkulumun altında karşılıklı yer alan iki kırmızı leke ve bunu takiben bu lekelerin arasında görünen T şeklindeki siyahımsı yumurta kırıcısının görünüşüdür. Kalın ve oldukça sklerotize olan yumurta kırıcısı kuyruk kısmının her iki tarafında vantuz şeklinde yapılara sahiptir. Yumurta yüzeyi kolaylıkla fark edilen tüberküllü poligonal yapıyla kaplıdır. Koryonik poligonların arasında kesik koni şeklinde 17-19 aero-mikropil bulunur.

Anahtar sözcükler: Taramalı elektron mikroskobu, yumurta, koryon, spermateka, *Eurygaster austriaca*, Heteroptera

Introduction

Sunn pests (*Eurygaster* spp. including *Eurygaster austriaca*) are one of the most dangerous pests of

wheat and other small grains, not only in Turkey, but throughout the Middle East as far as Middle Asia as well as Bulgaria and Romania in the Balkans.

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Wheat bugs (*Eurygaster* spp. and *Aelia* spp.) have reduced both wheat yields and quality in Turkey and its neighbours for years (Javahery et al., 2000). Wheat bug damage sometimes appears intensively in certain parts of Turkey (Lodos, 1986; Lodos and Kavut, 1991; Öncüler and Kivan, 1995; Olanca et al., 2009). Therefore, it is important to detect their presence before major damage occurs; consequently, classification based on eggs left on plants can be very useful.

The pattern and sculpturing of the surface of insect eggs are useful taxonomic characters for identifying species. The taxonomic and phylogenetic importance of eggshell structure in pterygote insects has been demonstrated in various orders at different levels (Hinton, 1981; Salkeld, 1983, 1984; Margaritis, 1985). In some groups the egg characteristics are of great taxonomic value at species level, but not so much at generic level (Studemann and Landolt, 1997; Ubero-Pascal and Puig, 2009).

Research on eggs of Heteroptera has been reviewed by Southwood (1956), Cobben (1968), and Hinton (1981). Subsequently, chorionic structures of Heteroptera species including Scutelleridae have been reported by many authors (Esselbaugh, 1946; Grigorov, 1988; Simiczjew, 1994; Bundy and McPherson, 2000; Candan and Suludere, 2003, 2006a; Wolf and Reid, 2004; Matesco et al., 2007, 2008, 2009). The egg-burster can also have taxonomic importance (Puchkova, 1966; Hinton, 1981). Breaking the chorion is accomplished by means of the egg-burster, a heavily chitinized ridge on the head of the nymph or larva, which remains attached to embryonic cuticle after larvae have emerged from eggs (Southwood, 1956). Egg-bursters occur widely in the Heteroptera, Neuroptera, Coleoptera, and in other insects (Emden, 1946; Puchkova, 1956; Suludere et al., 1999; Möller et al., 2006).

The spermatheca is part of insect female reproductive system where spermatozoa are stored. In some insects (Hymenoptera) it is formed by the reservoir containing spermatozoa, the duct connecting the reservoir to the vagina, the gland related to spermatozoa maintenance, and the muscular pump involved in the spermatozoa releasing (Wheeler and Krutzsch, 1994). After mating, it is filled with spermatozoa, which can be

stored there for long periods of time until they are used to fertilize eggs (Davey, 1965). The spermatheca is provided with a prominent glandular element producing nourishment for spermatozoa. The first study on spermatheca of Heteroptera was carried out by Dufour (1833), who erroneously regarded this organ as a sebaceous gland in which oil may have been produced. Siebold (1837) published the earliest correct description of a spermatheca (as receptaculum seminis) of Pentatomorpha. Afterwards, 3 fundamental works on the structure of the female genitalia in Heteroptera were published by Dupuis (1955), Pendergrast (1957), and Scudder (1959). A very important work on the female and male genitalia of Pentatomoidea was published by Kumar (1962, 1965) and McDonald (1966). Servadei (1964) gave a detailed description of the spermathecae of Acanthosomatidae, Pentatomidae, and Scutelleridae, with an original key to subfamilies and genera. Later, the spermathecae of 11 species belonging to 7 genera of Korean Podopinae and Asopinae (Pentatomidae) were compared morphologically by Kim and Lee (1994). Kocorek and Danielczok-Demska (2002) also studied the comparative morphology of the spermathecae of 11 genera of the family Dinidoridae. The spermathecae of 25 central European species of Coreoidea were studied by Vavrinova (1988) and some other Coreidae species were described by Bravilovsky and Barrera (2001) and Candan (2008). Recently, the spermatheca of *Odontotarsus purpureolineatus* (Rossi, 1790) (Scutelleridae) was described by Candan et al. (2007). The morphology of the spermatheca is useful for classification because they show great diversity among species in Heteroptera.

Although the egg mass and egg-burster of *E. austriaca* (as *E.austriacus*) were illustrated by Puchkova (1959), the present investigation is the first of its kind that examines the egg structure and spermatheca morphology of *E. austriaca* in detail by means of both optical and scanning electron microscope (SEM).

Materials and methods

Adults of *Eurygaster austriaca* (Schrank, 1778) were collected from Edirne (13-15 May 2009) and Kırklareli, Paşayeri village (6 May 2009). Fresh eggs

also were obtained from a colony maintained in breeding cages under laboratory conditions. Females were kept on graminaceous plants in plastic jars until they deposited eggs. Several eggs were examined and 30 of them were measured and photographed with a Leica EZ4D stereomicroscope and scanning electron microscope.

The spermathecae were dissected from dried material. Six spermathecae were prepared by first softening the abdomen in 10% KOH for 5-10 min. Thereafter, tissues were carefully removed and the spermathecae were placed in glycerin. Observations were made using a stereomicroscope (Olympus SZX12 photomicroscope).

For scanning electron microscopy, cleaned eggs and spermathecae dehydrated with ascending alcohol series and air dried were mounted using double-sided tape on SEM stubs, coated with gold using a Polaron SC 502 Sputter Coater, and examined with a Jeol JSM 6060 SEM operated at 5-15 kV.

The terminologies used for the spermathecae followed those of Pendergrast (1957), Scudder (1959), McDonalds (1966), and Pluot-Sigwalt and Lis (2008). The following morphological characters of the spermathecae were examined: shape of the spermathecal bulb (apical receptacle) and the pump, size of the flange of the pump (located between spermathecal pump and spermathecal duct), shape and size of the distal part of the spermathecal duct, shape and size of the proximal part of the spermathecal duct, and shape of the ring sclerites (genital chamber).

Results and discussion

The eggs of Heteroptera are deposited in an upright position and are attached to each other as well as to the substrate with an adhesive secreted by the female (Southwood, 1956; Cobben, 1968; Hinton, 1981; Javahery, 1994; Candan and Suludere, 2006a; Matesco et al., 2009). The spherical *E. austriaca* eggs were mostly laid in 2 rows and glued in a mass around broken stems or on stems of living plants at ground level or glued to the cotton cover of containers in the laboratory (Figures 1a-f).

The eggs of *E. austriaca* were almost spherical with average diameter of 1.05 ± 0.05 mm (SE) and the

egg mass generally consisted of 14 eggs (Figures 1a-f, 2a-b). In the other *Eurygaster* species, *E. alternata* (Say, 1828), *E. integriceps* Puton, 1881, and *E. maura* (Linnaeus, 1758) eggs are spherical or barrel shaped and generally have 14 eggs in a mass (Javahery, 1994; Candan and Suludere 2006b). Under lower magnification or observation with the naked eye, the chorionic surface of the eggs of *E. austriaca* is smooth and shiny like that of *E. alternata*, *E. integriceps*, and *E. maura* (Javahery, 1994; Candan and Suludere, 2006b). Newly laid eggs were green (Figure 1a); then their color slightly changed and pigmentation started to occur under the chorion (Figure 1b). Red eye spots and the egg-burster appear in the last phase of embryonic development (Figures 1c-f). It has been reported that the changing of egg color is normal during embryogenesis in insects including most of the Scutelleridae and Pentatomidae (Hinton, 1981; Javahery, 1994; Suludere et al., 1999; Candan and Suludere, 1999, 2006b).

The egg of *E. austriaca* is covered with a polygonal reticulated pattern covering both the operculum and egg surface as seen in *E. maura* (Figures 2c-f). While one or more dome-shaped granules are situated in the central area of some polygons, some of them lack granules on the egg surface (Figures 2d-f). Although similar granules are sparsely distributed on the chorion of *Psacasta exanthematica* Scopoli, 1763 (Scutelleridae), no polygonal pattern was present (Candan and Suludere, 2003).

In *E. austriaca*, there is a ring of widely separated aero-micropyles around the anterior pole. The operculum intersects the ring of 17-19 aero-micropyles (Figures 2c and 3a). Their shapes are similar to a truncated cone with an orifice at the apex (Figure 2d). According to Hinton (1981), the number of micropylar processes differs among such *Eurygaster* species as *E. austriaca* (as *E. austriacus* in Hinton) (16-19), *E. integriceps* (16-18), *E. testudinarius* (Geoffroy, 1785) (20-23) and *E. maura* (20-22) [Candan and Suludere (2006b)].

Micropylar structures arise from the chorion around the cap in Pentatomidae, but they tend to project from the inner side of the shell in Acanthosomidae, Cydnidae, Scutelleridae, and Thyrocoridae (Javahery, 1994). The aero-micropylar process has a central canal for the passage of sperm

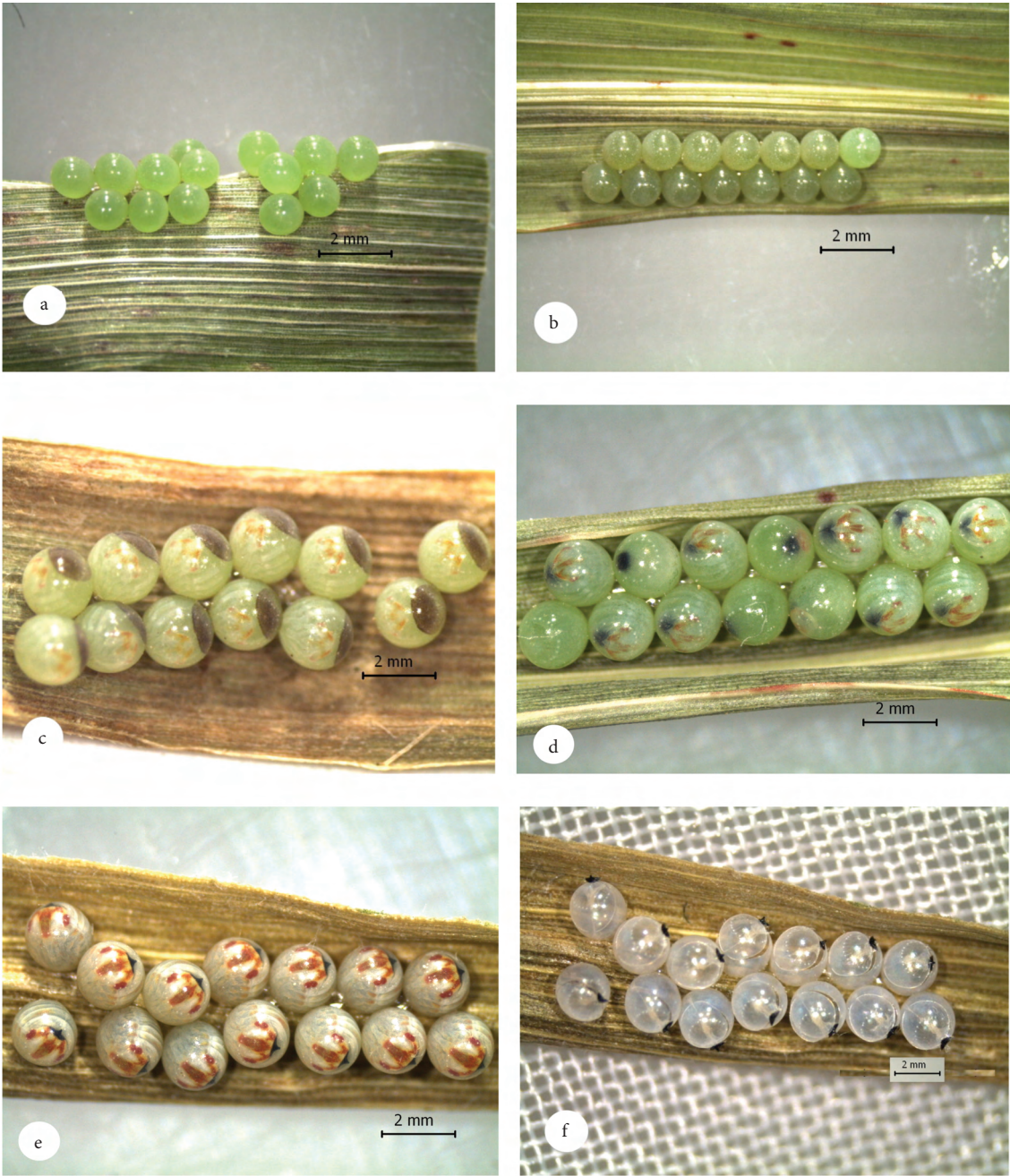


Figure 1. Light micrographs of different phases of eggs masses of *Eurygaster austriaca*. a. Newly laid egg mass, b. Initiation of pigmentation under the chorion in the later phase, c. Initiation of development under operculum, d. Appearance of red eye spots and egg-burster, e. Egg-burster and red eye spots in the last phase of embryonic development, f. Empty egg casings.

and serves for respiratory interchange in many species of Heteroptera including *E. austriaca* (Southwood, 1956; Cobben, 1968; Hinton, 1981).

The egg-burster becomes visible when the embryo is well developed and can be seen through the thin and transparent shell to move during the

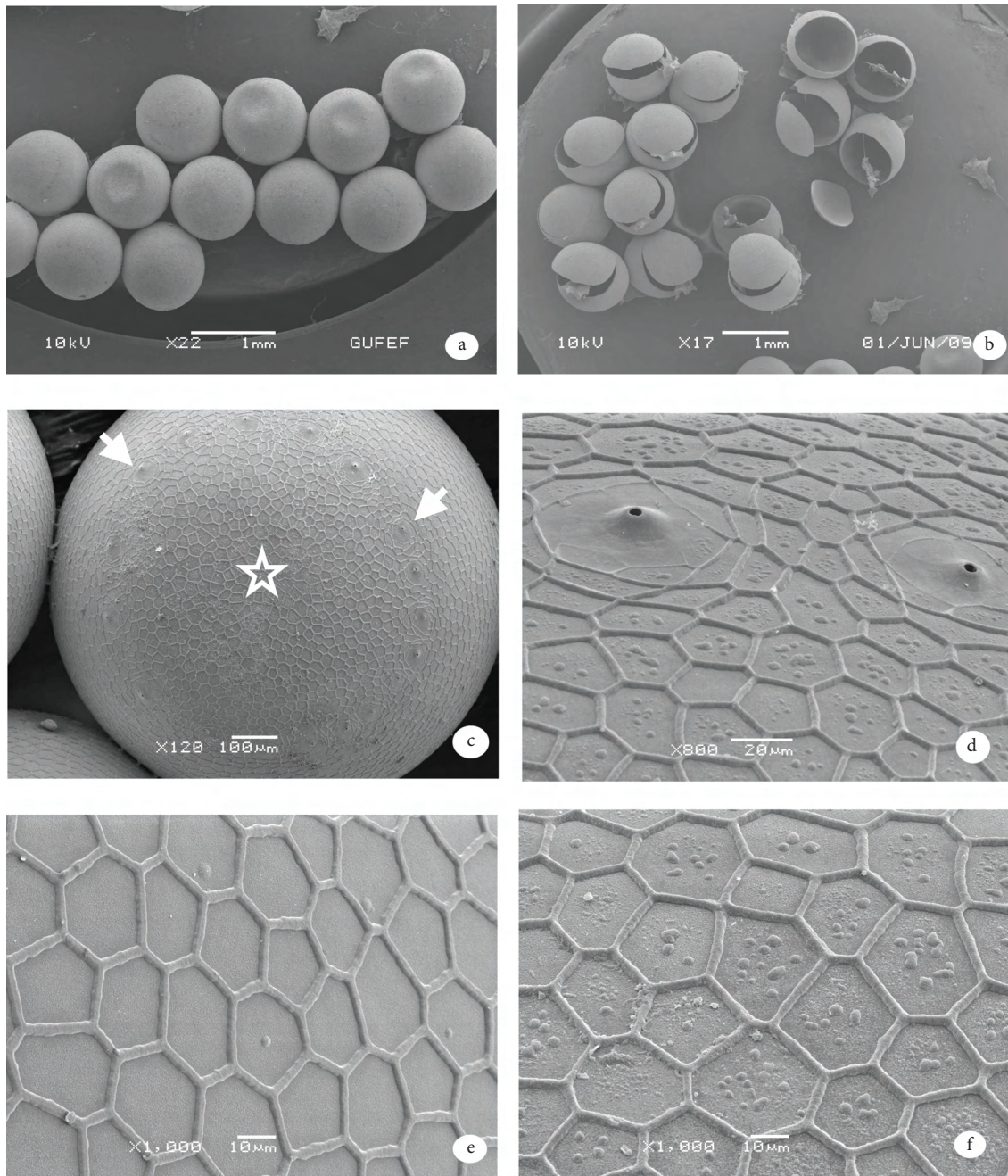


Figure 2. Scanning electron micrographs of eggs of *Eurygaster austriaca*. a. General view of eggs of *E. austriaca*, b. Hatched-egg mass, c. Micropyles (arrows) around operculum region (ê) of egg, d. Micropyles with orifice, e-f. Polygonal reticulated patterns with and without dome-shaped granules.

last day of embryonic development (Figures 1d-f). Although the regular hatching line can be seen between the operculum and micropylar ring in many Pentatomidae (Suludere et al., 1999; Candan and Suludere, 2006a), an irregular split of the chorion

occurs in Scutellerid species such as *E. austriaca* (Figures 2b, 3a) (Candan and Suludere, 2003, 2006b).

The egg-burster is thick and highly sclerotized in *E. austriaca*. It is easily seen as a dark T-shaped or triangular configuration in the hatched egg

(Figures 1d-f, 2b, 3a-c). T-shaped egg-bursters are common in most Scutelleridae and Pentatomidae while a Y-shaped egg-burster is found in the Acanthosomatidae, Cydnidae, and Thyrocoridae (Schumacher, 1917; Southwood, 1956; Puchkova, 1959, 1966; Cobben, 1968; Hinton, 1981; Javahery, 1994).

Hatching begins with the peristaltic contraction of the body of prolarva from the back to the front forcing the sharp sclerotized teeth of the egg-burster against the anterior pole of the egg. The egg-burster on hatched eggs does not separate from them and adheres by its tail to the inner lateral side of the egg (Figures 3a-f). The middle part of an egg-burster's has sucker-shaped structures on the both sides of tail (s 3d-e), not previously noted by other authors. The egg-burster has taxonomical importance in Heteroptera as well as the egg shape, the number of micropylar projections, and the chorionic pattern (Puchkova, 1966; Hinton, 1981).

A spermatheca is present in all Pentatomoidea (Heteroptera), including the Scutelleridae; generally only one spermatheca has been found to be present. The spermatheca consists of a spermathecal duct, leading from the vagina to a dilated spermathecal bulb (seminal receptacle, distal bulb). It is characterized by a well marked pump in the intermediate part with both proximal and distal flanges (Pendergrast, 1957; Kumar, 1965; McDonald, 1966; Pluot-Sigwalt and Lis, 2008). However, in some Heteroptera the spermathecal morphology is different and varies from species to species (Kumar, 1965; McDonald, 1966).

The spermatheca of *E. austriaca* is of the typical Pentatomid type and has a spermathecal bulb, a pumping region, distal and proximal flanges, and spermathecal ducts (Figures 4a-h). The spermathecal bulb is spherical and sclerotized and its surface is covered with pores (Figures 4a-c). Among various species in the Scutelleridae, the shape of spermathecal bulb shows variety from spherical to elongate. The bulb is spherical in *E. alternata*, *Symphylus caribbeanus* Kirkaldy, 1909, and *O. purpureolineatus*; it is elongate, cylindrical in *Pachycoris torridus* (Scopoli, 1772) and in *Diolcus irroratus* (Fabricius, 1775); and it is elongate and rod-shaped in *Acantholomidea porosa* (Germar, 1839) (McDonald, 1966; Candan et al., 2007).

In some Pentatomoidea including Scutelleridae, the pumping region is well developed and connected to the spermathecal dilation by a short duct and has 1 or 2 flanges (McDonald, 1966; Kocorek and Danielczok-Demska, 2002; Candan et al., 2007). According to Kumar (1965) the female genitalia of Eurygasterinae and Pachycorinae tend to be more specialized than in Scutellerinae. In *E. austriaca*, the pumping region with associated sclerite is also well developed with a gourd-shaped swelling at the posterior end and its surface is covered with pores (Figures 4a and d). The spermathecal processes and median spermathecal dilation with sclerotized rod are missing in other species of Scutelleridae such as *O. purpureolineatus* (Candan et al., 2007). The spermathecal bulb and the pumping region in *E. austriaca* have many pores like those of *O. purpureolineatus*, but this feature is not mentioned for other Scutellerid and Pentatomoid species (McDonald, 1966; Kim and Lee, 1994; Adams 2001; Kocorek and Danielczok-Demska, 2002; Candan et al., 2007).

E. austriaca have 2 sclerotized pump flanges (distal and proximal flanges) and they are distant from the bulb (Figures 4a, d-g). The distal pump flange is collar-shaped and located under the pumping region (Figures 4d-e). The proximal flange is concave plate-shaped and is located between distal and proximal spermathecal ducts (Figures 4f-g). A collar-shaped distal pump flange is seen in some Scutellerid species such as *Graptocoris aulicus* (Germar, 1837), *Chelysoma variabilis* Herrich-Schaeffer (1837), *Homaemus aenifrons consors* (Uhler, 1875), and *Hotea subfasciata* (Westwood, 1837) (Kumar, 1965). Among the Pentatomidae the pumping region varies in size and shape. Two flanges of *Graphosoma rubrolineatum* (Westwood, 1873) and *Dybowskyia reticulata* (Dallas, 1851) are of the same diameter, but the distal flange of *Scotinophara lurida* (Burmeister, 1834) is wider than the proximal one (Kim and Lee, 1994). Within the Scutelleridae, proximal and distal flanges are well developed in *P. torridus*, but the distal one is reduced in *A. porosa* while the proximal flange is reduced in *Chelysomidea guttata* (Herrich-Schaeffer, 1837) (McDonald, 1966). *O. purpureolineatus* has only one distinct distal flange (Candan et al., 2007). In addition, some Scutellerids have well developed pumping regions, but lack

projecting pump flanges such as *Scutellera nobilis* Fabr. and *Odontoscellis fuliginosa* L. (Kumar, 1965).

The spermathecal duct adjacent to the bulb is modified as the intermediate piece or pump, the cuticular lining of which is unsclerotized and

flexible (Lee and Pendergrast, 1983). While the distal spermathecal duct of *E. austriaca* is very thin and sclerotized, the proximal duct is muscular, convoluted, and accordion-shaped (Figures 4a, e-g). The proximal duct is connected to the anterior vagina (Figures 4g-

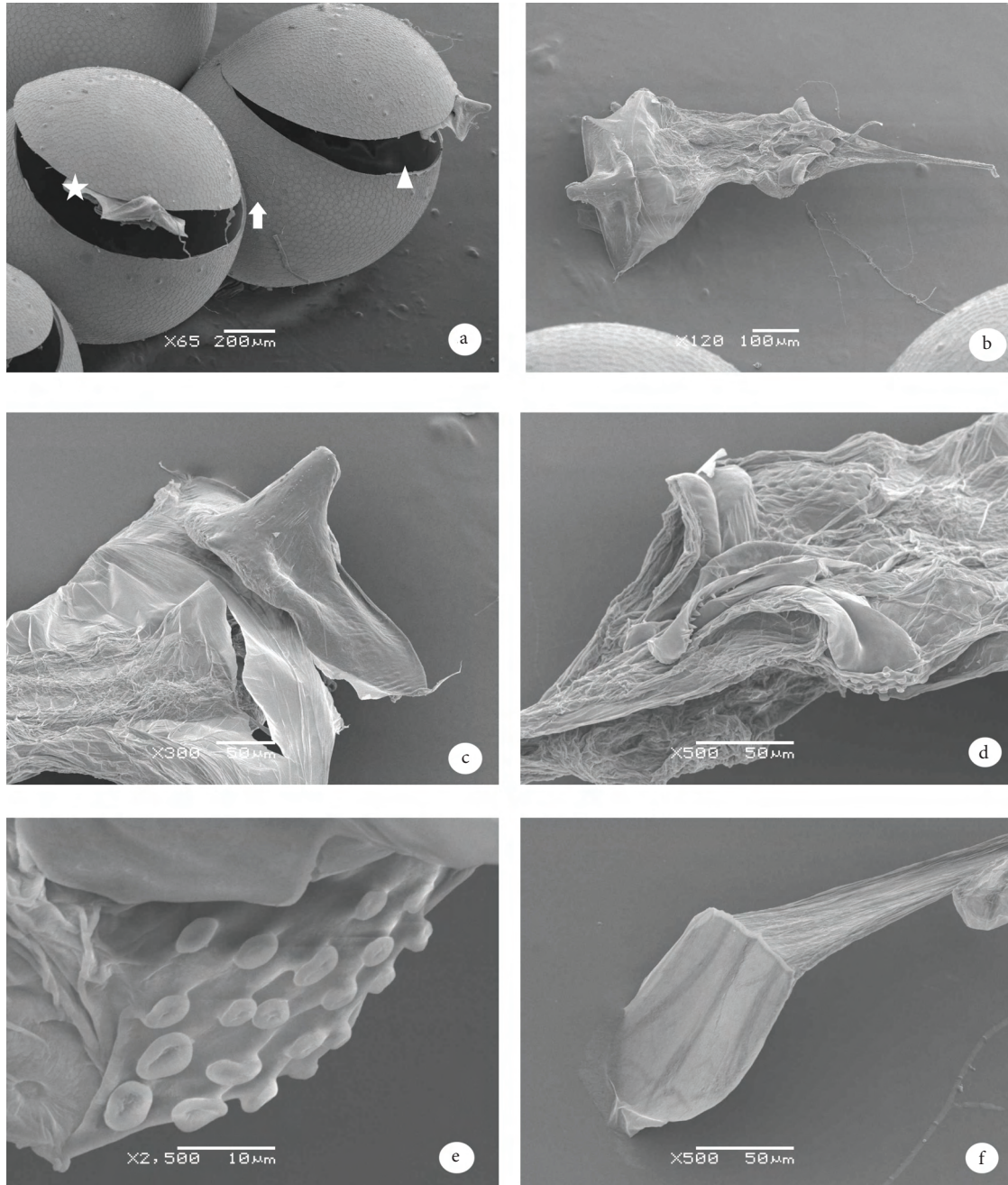


Figure 3. Scanning electron micrographs of eggs-burster of *E. austriaca*. a. Egg-burster still attached to empty eggs, b. Overview of egg-burster; T-shaped egg-burster («), sucker-shaped structure (') and tail (r), c. T-shaped egg-burster, d. Sucker-shaped structures on the egg-burster, e. Close-up of sucker-shaped structures f. Tail of egg-burster.

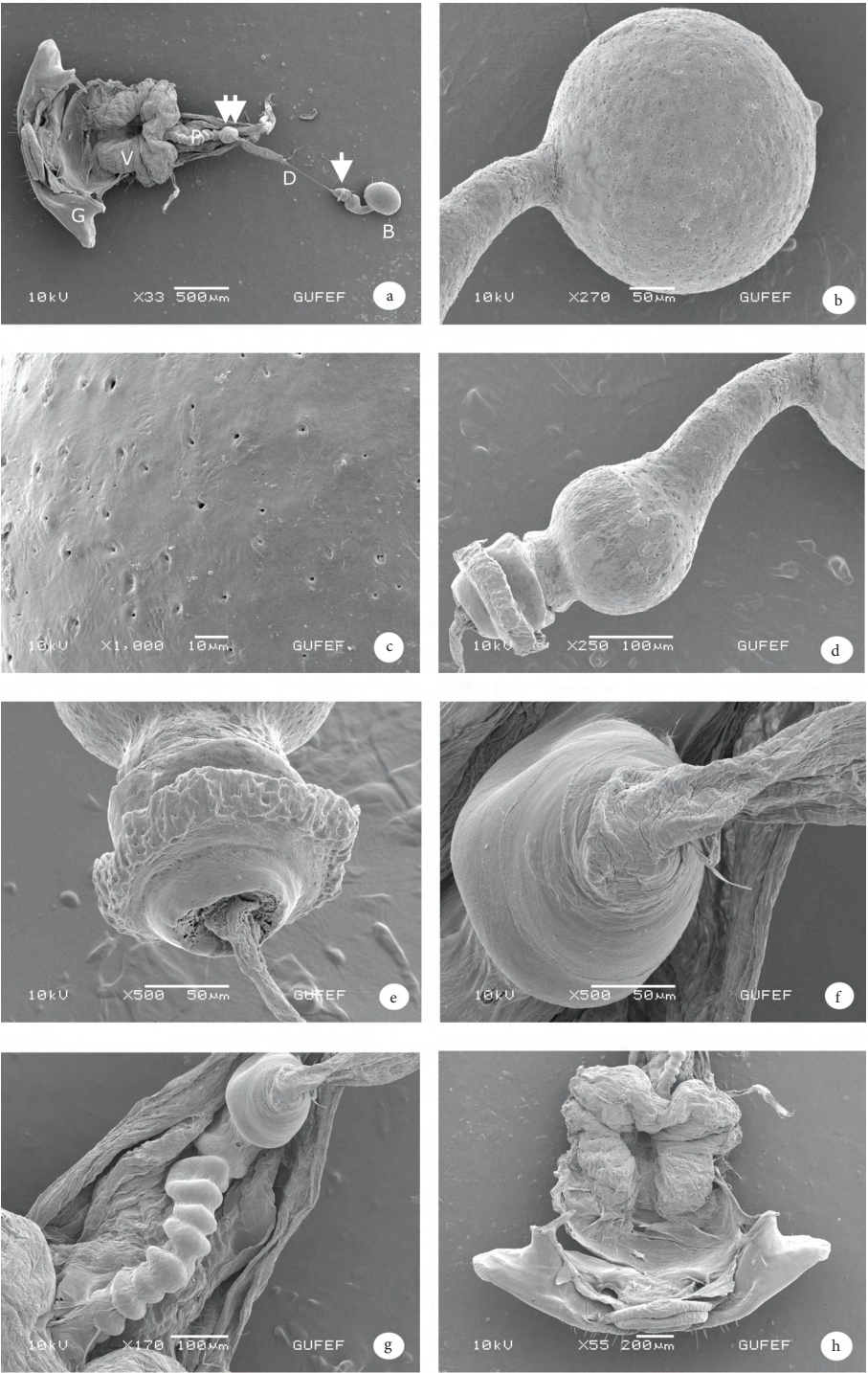


Figure 4. Scanning electron micrographs of spermatheca. a. General view of spermatheca; spermathecal bulb (b), distal flange (“), distal (d) and proximal (p) ducts, proximal flange (l), vagina (v) and genital chamber (g), b. Spermathecal bulb with pores, c. Pores in the surface of spermathecal bulb, d. Pumping region with gour-shaped and distal flange, e. Collar-shaped distal flange and distal spermathecal duct, f. Distal spermathecal duct and proximal flange, g. Convoluted proximal spermathecal duct, h. Vagina and genital chamber.

h). Spermathecal ducts serve as part of the sperm transport system whereby the sperm can be moved within the spermathecal duct from the spermatheca directly to the common oviduct (Chiang, 2009). A muscle at the base of the spermatheca has been described in a variety of female insects (Kocorek and Danielczok-Demska, 2002; Candan et al., 2007).

Morphological characters of eggs and spermathecae are important in the higher

classification of *E. austriaca*. More work involving SEM is needed to establish clear trends within this Scutelleridae group.

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