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Morphology of Spermathecae and Eggs of *Stenozygum coloratum* (Klug, 1845) (Heteroptera: Pentatomidae)

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ABSTRACT
The spermathecae and egg morphology of *Stenozygum coloratum* (Klug, 1845) were examined with light and scanning electron microscopy, and their external morphology was characterized in detail (egg chorion surface, operculum, and aero-micropylar processes, and spermathecae morphology). Eggs are barrel-shaped; operculum is round and the hatching line can be easily distinguished in the scanning electron photograph. The egg batches usually consist of 12 eggs. Because of the thick egg chorion, embrionic development was not visible from the outside. The eggs of *S. coloratum* are usually laid in two rows and are glued to the *Capparis spinosa* (Capparacea) plants. The egg burster is highly sclerotized, thick and T shaped. It is clearly observed in the hatched egg. Egg-burster is remains attached with the inside lateral surface of the egg. The well-marked operculum intersects the ring of 18–20 short pipe shaped micropylar projections that are found between the tubercles around the hatching line. In S. *coloratum*, the spermathecae has a semi-oblong spermathecal bulb, a pumping region, intermediate flanges, a dilation of spermathecal duct and distal and proximal ducts. The pumping region has distal and proximal flanges. The balloon-like median spermathecal dilation has sclerotized rod and is narrowed distally and tipped. The proximal area of the spermathecal duct is close to the vagina opening. Two V-shaped sclerites and two ring sclerites are localized on the genital chamber (vagina) wall in to which opens the spermathecae. Egg and spermathecae structures in insects differ from species to species. In this study, spermateca and egg structures of this species, which have not been studied before, were examined in detail, and it was aimed to contribute to systematic and taxonomic studies.

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Stenozygum coloratum (Klug, 1845) (Heteroptera: Pentatomidae)'un Spermateka ve Yumurta Morfolojisi

ÖZET
Stenozygum coloratum'un (Klug, 1845) spermateka ve yumurta morfolojisi ışık ve taramalı elektron mikroskobu ile incelenmiş ve dış morfolojileri ayrıntılı olarak karakterize edilmiştir (yumurta koryon yüzeyi, operkulum ve aero-mikropilleri ve spermateka morfolojisi). Yumurtalar fıçı şeklindedir, operkulum yuvarlaktır ve açılma hattı taramalı elektron mikroskobu fotoğrafında kolaylıkla ayırt edilebilir. Yumurta kümesi genellikle 12 yumurtadan oluşmaktadır. Yumurta koryonunun kalın olması nedeniyle embriyonik gelişim dışarıdan görünmemektedir. *S. coloratum* yumurtaları genellikle iki sıra halinde *Capparis spinosa* (Capparacea) bitkilerine yapıştırılır. Yumurta kırıcısı oldukça sklerotize, kalın ve T şeklindedir. O, açılan yumurtada açıkça görülmektedir. Yumurta kırıcısı, açılan yumurtanın iç yan yüzeyinde kalır. Belirgin operkulum, açılma hattı çevresinde tüberküllerin arasında bulunan 18-20 kısa boru şeklindeki mikropil çıkıntılarının halkası ile kesişir. *S. coloratum*'da, spermateka, yarı oblong bir spermateka haznesi, spermatekal kanal,

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pompalama bölgesi, spermatekal kanalın dilasyonu, distal ve proksimal kanallara sahiptir. Pompalama bölgesi distal ve proksimal yakalara sahiptir. Balon benzeri medyan spermatekal dilasyon sklerotize çubuğa sahiptir ve o distal ve uc kısımda daralmıştır. Spermatekal kanalın proksimal bölgesi vajina açıklığına yakındır. İki V şeklindeki sklerit ve iki halka sklerit, spermatekaya açılan genital oda (vajina) duvarında yer almaktadır. Böceklerde yumurta ve spermateka yapıları türden türe farklılık göstermektedir. Bu çalışmada daha önce çalışılmamış olan bu türün spermateka ve yumurta yapıları detaylı olarak incelenmiş, sistematik ve taksonomik çalışmalara katkı sağlanması amaçlanmıştır.

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INTRODUCTION

Stenozygum coloratum (Klug, 1845), which is called the variegated caper bug, is common in the Eastern Mediterranean region. *S. coloratum* is a small agricultural pest and generally thrives on wild caper-bush, *Capparis spinosa* L. Capparaceae, but sometimes migrates to thrive on a variety of cultivars, including avocados. The damage to the fruit is accompanied by heavy persein secretion and the appearance of black spots (Izhar et al., 1990).

The surface pattern and shape of insect eggs, are taxonomic characters which are useful in identification of species. In pterygote insects, the taxonomic and phylogenetic significance of eggshell structure has been demonstrated at different levels in different orders (Hinton, 1981; Salkeld, 1983; Margaritis, 1985). The color, size and shape of the eggs and the number, size and shape of the micropylar processes of Pentatomidae family can be easily distinguished and constitute significant characters for the identification of species and genera (Esselbaugh, 1946; Hinton, 1981; Izhar et al., 1990; Bundy and McPherson, 2000; Candan et al., 2001). At the egg stage, despite available information regarding the diversity of taxa, there are no cladistic analyzes at lower taxonomic levels in Pentatomidae, including egg characteristics.

The spermathecae, which is insect female reproductive system region, is responsible for sperm storage, fertilization, copulation, and oviposition (Gaffour-Bensebbane, 1991, 1994; Pascini, 2017).

The egg and spermathecae structures differ among insect species and these structures are systematically and taxonomically important. Therefore, in this study, the egg and spermathecae structure of *S. coloratum* was examined in detail and compared with other Heteroptera species.

MATERIAL AND METHOD

Preparation of Eggs

Adults of *Stenozygum coloratum* were collected from Adana, Turkey (June 2013). At room temperature, fresh eggs were collected from a colony kept in rearing cages. Insects were kept on *Capparis spinosa* plants in plastic jars until they deposited eggs.

The eggs were observed with stereomicroscopy (Olympus SZX12). Length and width measures were taken and aero-micropylar processes were counted.

The freshly laid eggs were cleaned, prepared and air dried for SEM examination. The eggs were mounted with double sided tape on SEM stubs, coated with gold using a sputter coater (Polaron SC 502). Then, the eggs were viewed with SEM (Jeol JSM 6060 LV) at 5 kV and taken photograph.

Preparation of Spermathecae

The abdomen regions of six female samples were prepared by first softening for 5-10 minutes in 10% KOH. Then spermathecae parts were carefully removed under a dissection stereomicroscope (Olympus SZX7). Then the spermathecae were placed in glycerin. The examinations were made and photographed with light microscope (Olympus BX51).

For scanning electron microscopy (SEM), six spermathecae were cleaned. After being dehydrated in a graded ethanol series and air dried were mounted using double-sided tape on SEM stubs, gold coated by a sputtering device Polaron SC 502 Sputter Coater. Then, sample examinations were made and photographed using a Jeol JSM 6060 LV SEM.

For the structure of the spermathecae, we follow the terminology proposed by Pendergrast (1957), Salkeld (1983), Margaritis (1985) and Pascini (2017). Abbreviation list is seen in Table 1.

RESULTS

Description of Eggs of *Stenozygum coloratum*

The eggs of *S. coloratum* are usually laid in two rows (Figures 1a-d) and are glued to the *C. spinosa* plants. Each female of *S. coloratum* was deposited 12 eggs on average in mass (Figures 1a-d). The barrel-shaped eggs averaged 1.05 ± 0.05 mm in diameter. The chorion and the egg microsculpture prevent observation of the embryonic development. There are two black bands or rings around each egg, one lower and one higher. The

Table 1. The spermathecae abbreviations used in figures.
Çizelge 1. Şekillerde kullanılan spermateka kısaltmaları.

Spermathecae abbreviations (Spermateka kısaltmaları)	Descriptions (Tanımlar)
Sb	Spermathecal bulb (apical receptacle)
Df	Distal flange of pump
Pf	Proximal flange of pump
Pr	Pumping region
Dsd	Distal part of spermathecal duct
Dpd	Proximal part of spermathecal duct
Dl	Dilation of spermathecal duct
Dr	Dilation rod

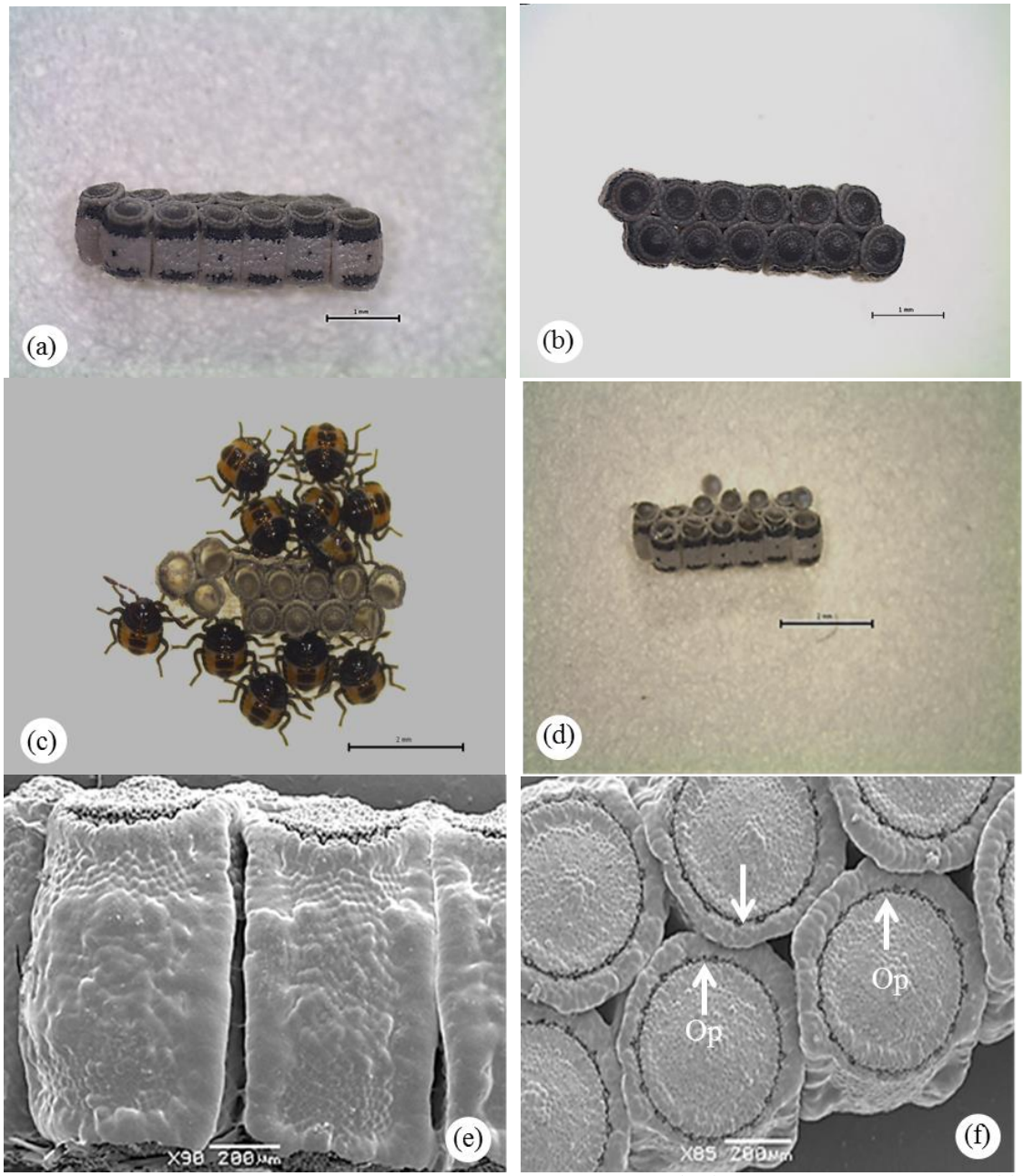

Figure 1. Light and SEM photos of eggs of *Stenozygum coloratum*. (a-b) Egg mass of *S. coloratum*. (a) Side view (b) Dorsal view (c) Hatched egg and nymps (d) Dorsolateral view of hatched eggs (e) SEM photos of lateral surface of eggs. (f) SEM photos of dorsal view of unhatched eggs, hatching line (→) and operculum (Op)

Şekil 1. Stenozygum coloratum yumurtalarının ışık ve SEM fotoğrafları. (a-b) S. coloratum'un yumurta kümesi. (a) Yandan görünüm (b) Üsten görünümü (c) Açılmış yumurta ve nimfler (d) Açılmış yumurtaların dorsolateral görünümü (e) Yumurtaların yan yüzeyinin SEM fotoğrafları. (f) Açılmamış yumurtaların operkulum hattının (→) ve operkulumun (Op) dorsal görünümünün SEM fotoğrafları

lower band is narrower than the upper one and the middle of the outside surface of each egg has a blackish spot. The operculum is almost entirely white with only the margin of the central white spot slightly black. The egg shells are clear white following hatching (Figures 1a-d). In light and SEM microscopic examination, the operculum and egg surface of *S. coloratum* is generally rough, and there is no pattern on the surface (Figures 1e, f), but when the surface of the egg chorion (exochorion) is removed, the egg surface is found to be covered with irregular polygonal shapes and deeply buttressed walls (Figures 2a-d). The polygons are mostly hexagonal and tightly connected to each other. Their edges are different in length. Among these polygons there are large voids and chambers in the form hexagonal (Figures 2a-d).

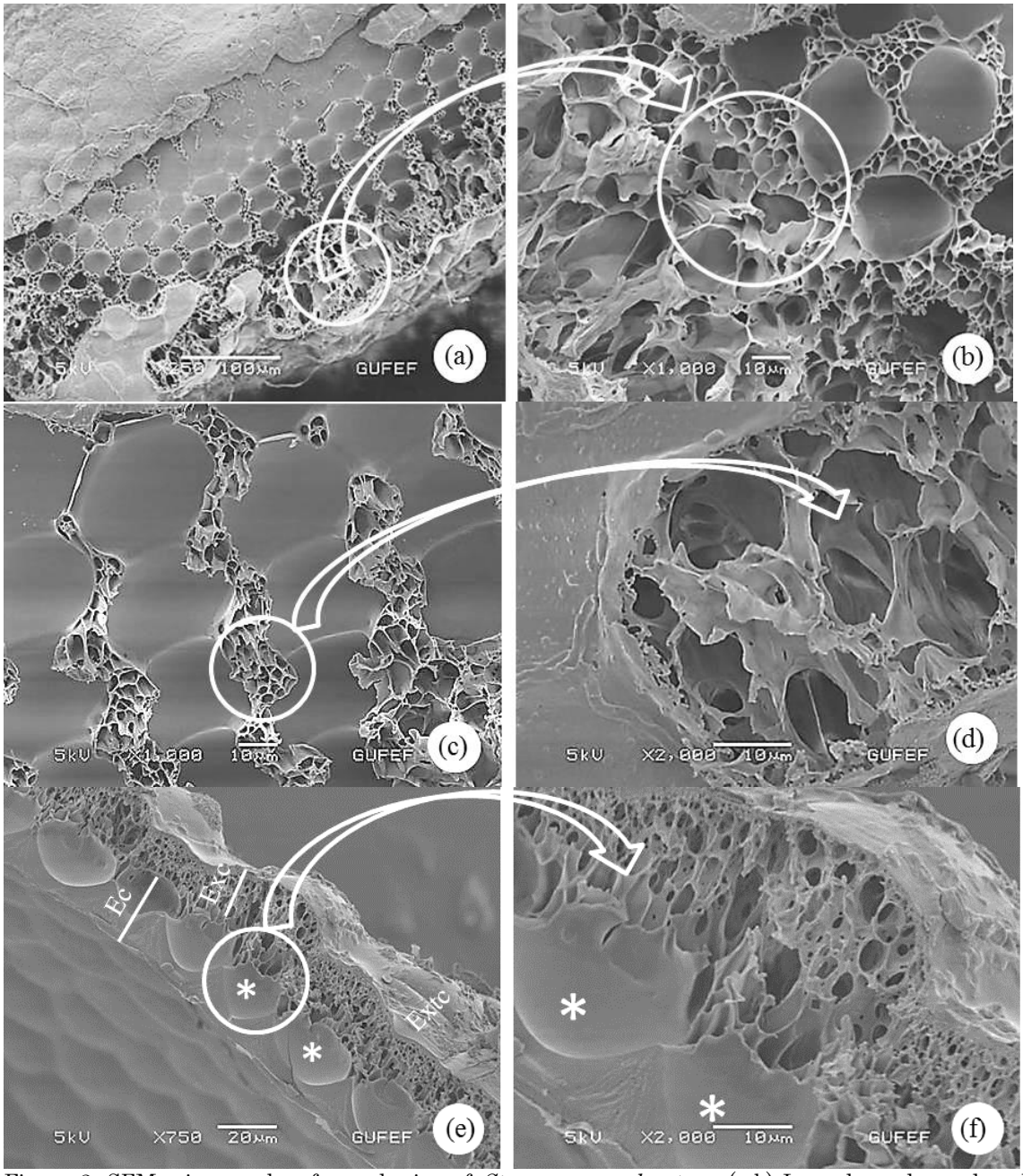

Figure 2. SEM micrographs of egg chorion of *Stenozygum coloratum*. (a-b) Irregular polygonal sculptures outlined by the extrachorion. (c-d) The chorionic network with perforated polygons in middle of the egg exochorion. (e) Chorion layers in egg cross sections. Ec: Endochorion, Exc: Exochorion, Extc: Extrachorion (f) Perforated layer in exochorion and air chamber in endochorion (*).

Şekil 2. Stenozygum coloratum'un yumurta koryonunun SEM mikrografları. (a-b) Ekstrakoryon tarafından ana hatları çizilen düzensiz poligonal şekiller. (c-d) Yumurta ekzokoryonunun ortasında delikli çokgenlere sahip koryonik ağ. (e) Yumurtanın enine kesitinde koryon tabakaları. Ec: Endochorion, Exc: Exochorion, Extc: Extrachorion (f) Ekzokoryonda delikli katman ve endokoryonda hava odası (*)

In cross sections of the egg, the chorion is shown to consist of three different layers. These are extrachorion, exochorion and endochorion. The exochorion has a perforated layer; the endochorion also contains large air chambers (Figures 2e, f).

The operculum consists of irregular polygonal swollen tubercles. They vary in shape and size, and some of them have pores in the middle (Figures 3a-c). In unhatched eggs, the eclosion line is hardly discernible among the tubercles (Figure 2f). In hatched eggs, the eclosion line appears in a circular shape around the operculum (Figure 2f). Around the eclosion line, similar tubercles with irregular shapes and deeply buttressed walls are dispersed (Figures 3a-e). The gap among the tubercles is very large and perforations are very distinct (Figures 3b, c, e).

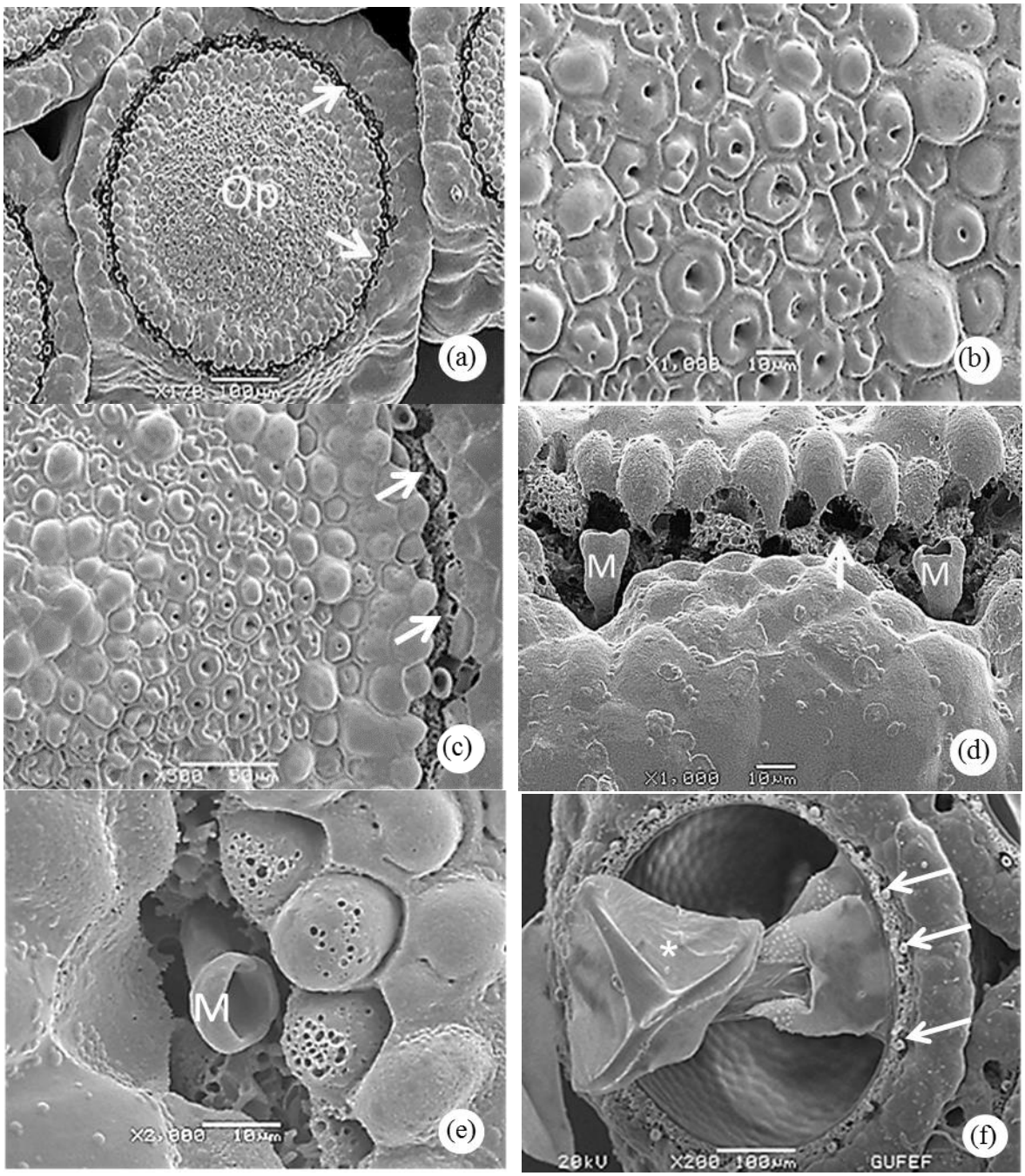

Figure 3. SEM photos of eggs of *Stenozygum coloratum*. (a) Operculum (Op), hatching line (→), and micropylar projections (b) Regular and irregular polygons in operculum surface. (c-e) Eclosion line (→), micropylar projections (M) and perforated polygons in operculum surface. (f) T-shaped egg-burster (*) in the hatched egg and micropylar projections (→)

Şekil 3. Stenozygum coloratum yumurtalarının SEM fotoğrafları. (a) Operkulum (Op), açılma hattı (→) ve mikropilar çıkıntılar (b) Operkulum yüzeyinde düzenli ve düzensiz çokgenler. (c-e) Açılma hattı çizgisi (→), mikropil çıkıntıları (M) ve operkulum yüzeyinde delikli çokgenler. (f) Açılan yumurtadaki T-şekilli yumurta kırıcı (*) ve mikropil çıkıntıları (→)

There are 18-20 short pipe-shaped micropylar projections that are found between the tubercles along the hatching line. Each micropylar projection rises from a large cavity in the chorion (Figures 3c-e). The egg burster, which is inverted T-shaped black and it is responsible for opening the operculum. The egg burster is thick and highly sclerotized (Figure 3f).

Description of spermathecae of *Stenozygum coloratum*

S. coloratum spermathecae has the spermathecal bulb (apical receptacle), a spermathecal pump (pumping region), intermediate flanges (distal and proximal) associated with the pump, the spermathecal ducts, spermathecal dilation with sclerotized the ring sclerites are localized on the wall of the genital chamber (vagina) into which opens the spermathecae (Figures 4a, b).

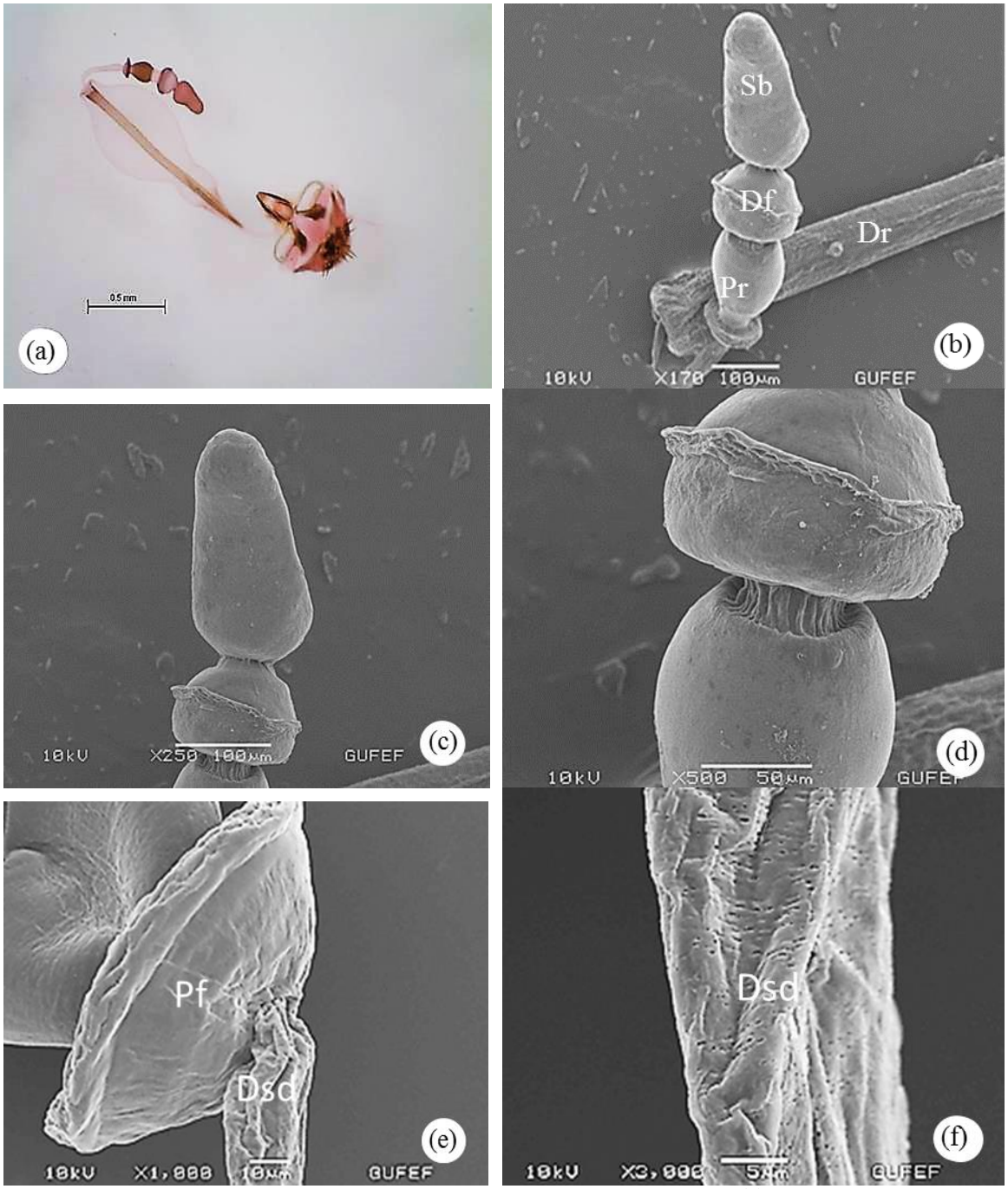

Figure 4. Light and SEM photos of spermathecae of *Stenozygum coloratum*. (a) General view of spermathecae of *S. coloratum*. (b-d) Spermathecal bulb (Sb), distal flange (Df) and pumping region (Pr), sclerotized dilation rod (Dr). (e-f) Proximal flange (Pf) and distal spermathecal duct (Dsd).

Şekil 4. Stenozygum coloratum spermatekal'ın ışık ve SEM fotoğrafları. (a) S. coloratum'un spermatekası'nın genel görünümü. (b-d) Spermatekal hazne (Sb), distal yaka (Df) ve pompalama bölgesi (Pr), sklerotize dilatasyon çubuğu (Dr). (e-f) Proksimal yaka (Pf) ve distal spermathekal kanal (Dsd)

The spermathecal bulb is sclerotized and semi-oblong. The spermathecal pump with distal and proximal flanges is somewhat sclerotized and swollen at the mid-posterior position (Figures4a-c). The distal spermathecal duct is the same length as the proximal duct. The proximal flange is narrower than the distal flange and appears as a plate (Figures 4c-f).

The balloon-like median spermathecal dilation has sclerotized rod and is narrowed distally and tipped (Figure 4a); Spermathecal dilation is join with the proximal part of the spermathecal duct (Figure 5a). The spermathecal dilation has thin muscular walls (Figure 5b). The cuticular wall of proximal spermathecal duct is strongly wrinkled and is closely associated with the genital chamber (vagina) opening (Figures 5c-f). There are two sclerites, one at each side of the genital chamber (Figure 5e).

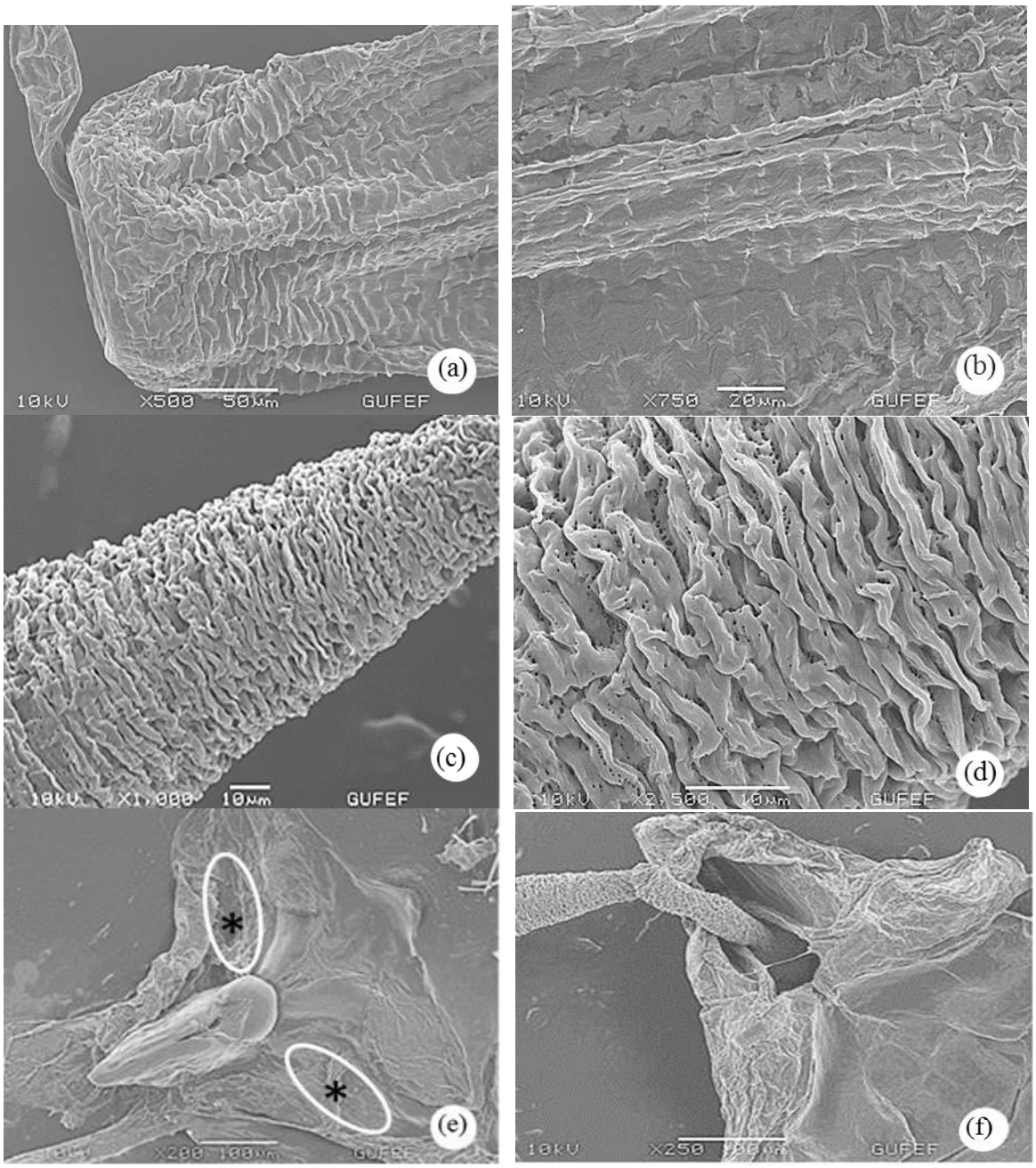

Figure 5. SEM photos of spermathecae of *Stenozygum coloratum*. (a-b) Distal region of median spermathecal dilation and surface. (c-d) The cuticular wall of the proximal duct, strongly wrinkled. (e) Two ring sclerites (*) at both dorsal sides of genital chamber (f) Opening of spermathecal duct, lateral sides of genital chamber and proximal spermathecal duct.

Şekil 5. Stenozygum coloratum'un spermatekal'ının SEM fotoğrafları. (a-b) Orta spermatekal dilatasyonun distal bölgesi ve yüzeyi. (c-d) Proksimal kanalın oldukça kırışık kütiküler duvarı (e) Genital odanın her iki dorsal tarafında iki halka sklerit (*) (f) Spermatekal kanalın, genital odanın yan taraflarının ve proksimal spermathekal kanalın açıklığı

DISCUSSION

The barrel shaped *Stenozygum coloratum* eggs are usually deposited in 2 rows and are glued to host plants (*Capparis spinosa* in the field). The main characters of the microsculpture of the chorion, color, operculum, egg burster and the number of micropylar processes are of great systematic value at generic and family level (Puchkova, 1959, 1961; Cobben, 1968; Hinton, 1981; Javahery, 1994). In insects, the color of eggs changes during embryogenesis (Hinton, 1981; Javahery, 1994). Among Pentatomidae species, some eggs are barrel-shaped with hexagonal pits or shallow depressions, others may resemble those of *S. coloratum*, or fine spines or with chorionic hairs at the reticulation lines (Javahery, 1994). Some eggs are cylindrical or spherical but lack chorionic spines or reticulations (Candan, 1997).

The eggs of stink bugs, studied by various authors so far, differ greatly in their chorion surface morphology. Most of them have a chorion called "spinose" and this chorion is characterized by spines with dotted surfaces. *Euschistus servus* Say, *E. obscurus* Palisot, *E. quadrator* Rolston, *E. tristigmus* Say (Bundy and McPherson, 2000), *Codophila varia* Fabricius (Candan et al., 2001), *Carpocoris pudicus* Poda (Cobben, 1968), *Mormidea* sp. (Javahery, 1994; Wolf and Reid, 2003), *Palomena prassina* Linnaeus (Candan, 1998a), *Piezodorus lituratus* Fabricius (Candan, 1998b), *Eocanthecona furcellata* Wolff (Kumar et al., 2002), *Podisus maculiventris* Say (Lambdin and Lu, 1984), *Euschistus variolarious* Palisot (Candan et al., 2005), *G. semipunctatum* Fabricius (Candan, 1999b) and *Graphosoma lineatum* Müller (Candan and Suludere, 1999) serve as examples. Others are called to as "coarsely reticulate, foveate" (Bundy and McPherson, 2000) and are characterized by the presence of pits arranged hexagonally on the surface. This type of chorion has been stated in *Acrosternum hilare* Say (Javahery, 1994), *Apodiphus amygdali* Germar (Candan, 1997), *Rhaphigaster nebulosa* Poda (Candan, 2001), *Coptosoma siamicum* Walker (Mohan, 1988), *Eurydema rugulosum* Dohrru (Suludere et al., 1999). As well as, a spongy and mushroom shaped chorion type has been seen in the surface of *Vilpianus gali* Wolff (Candan, 1997) and *Ancyrosoma leucogrammes* Gmelin (Candan, 1999a) eggs, a wart-like chorion has been found on *Edessa bifida* Say (Wolf and Reid, 2003) eggs and the presence of polymorphic tubercles have been noted on the chorionic surface of *Eurydema ventrale* Kolenati and *E. blandum* Horvath eggs (Suludere et al., 1999).

Micropile processes originate from the chorion around the cap in all Pentatomidae species, but in Acanthosomidae, Cydnidae, Scutelleridae, and Thyrocoridae, they tend to project from the inner side of the shell. They differ in shape, length and number according to the taxa taken into consideration (Javahery, 1994). The aeromicropile process has a central channel for the passageway of sperm and serves for respiratory exchange in many Heteroptera species, including *S. coloratum* (Southwood, 1956;,Cobben, 1969; Candan 1998a).

In some Pentatomidae species, the first sign of embryonic development in eggs appears long after revolution as two red eye spots with a T-shaped or Y-shaped egg burster between them just below the operculum (Javahery, 1994; Candan, 1998a; 1998b). The egg burster is highly sclerotized and thick in *S. coloratum*. It is difficult to observe the T-shaped egg burster and two eye spots in unhatched eggs because of the sculpturing and the thickness of the chorion. The role of the egg burster in hatching of pentatomid eggs has been noted previously (Southwood, 1956; Puchkova, 1959, 1961; Cobben, 1968; Hinton, 1981) as described below. Hatching begins with the peristaltic contraction of the prolarva body, and a curved incision appears on the burster, extending into a circular incision in the inner margin of the micropile processes (Javahery, 1994). In Pentatomidae species, the incision line or hatching line is a straight longitudinal incision or a circular line. In *S. coloratum*, the hatching line is circular. The egg-burster also has taxonomical importance in Heteroptera as in Pentatomidae (Hinton, 1981; Puchkova, 1966).

One spermathecae is present in all Pentatomoidea (Heteroptera) species, usually only one spermathecae is found to be present. Spermathecae is linked to the vagina (Dupuis, 1955, 1970). 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). In some Pentatomidae, the spermathecal morphology is different. In some subfamilies (Podopinae and Asopinae) the spermathecae has a spermathecal bulb, a pump with two flanges, a median spermathecal dilation with sclerotized rod, and one or two sclerites (Margaritis, 1985; Kocorek and Danielczok-Demska, 2002). In other Podopinae, the spermathecal bulb is [oblong-ovate (*Dybowskyia reticulate* (Dallas), spherical *Scotinophara lurida* (Burmeister), *S. scotti* (Horvath), *S. horvathi* (Distant), or semi-oblong [*Graphosoma rubrolineatum* (Westwood)], and has one to three spermathecal processes. In some Asopinae, the spermathecal bulb is spherical *Picromerus bidens* (Linnaeus), *P. lewisi* (Scott), *Arma chinensis* Fallou, semi-oblong (*Pinthaeus sanguinipes* (Fabricius), or semi-ovoidal *Zicrona caerulea* (Linnaeus) and all species of the Asopinae lack spermathecal processes (Javahery, 1994). Our study shows that the spermathecal bulb of *S. coloratum* is semi-oblong and lacks a process.

Sperm transfer is performed directly with the help of a sperm pump (Margaritis, 1985; Kim and Lee, 1994; Candan et al., 2007, 2014, 2015). In Pentatomidae,

generally, the pumping region has two flanges (distal and proximal) as does *S. coloratum*. The size and shape of the flanges can vary. Also, the spermathecal ducts can also vary according to shape. In some taxa, they are essentially straight; in others they can be very coiled. Also, sometimes there is a swelling or enlargement just below the proximal flange (Javahery, 1994; Candan et al., 2014, 2015). In this SEM study, the distal spermathecal duct and the proximal spermathecal are almost the same length, and muscular structures. These spermathecal ducts are longitudinal muscular fibers and connect with the common oviduct. This is a common feature of pentatomids (Scudder, 1959; Puchkova, 1966; Margaritis, 1985; Javahery, 1994; Pascini and Martins, 2017). The spermathecal duct, where it attaches to the bulb, is also transformed into a pump, the cuticular lining of which is slightly sclerotized and flexible (Lambdin, and Lu, 1984).

Spermathecal ducts are region of the sperm transport system, they are responsible for sperm can be transported directly from the spermathecae to the common oviduct (Puchkova, 1959). The two ring sclerites presence within the genital chamber are common to all species of Pentatomidae previously studied (Javahery, 1994; Candan et al., 2014, 2015).

Based on our study, morphological characters of eggs and spermathecae of *S. coloratum* can be useful in the classification of Pentatomidae. However, further studies are required to establish clear trends within the Pentatomidae family to which *S. coloratum* belongs.

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Author's Contributions

The contribution of the authors is equal.

Conflicts of Interest

No conflict of interest was declared by the authors.

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