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Structures of Spermatheca and Eggs of the Red Firebug, *Pyrrhocoris apterus* (L. 1758) (Heteroptera: Pyrrhocoridae), Based on Optical and Scanning Electron Microscopy

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Highlights

- Spermatheca and eggs of *P. apterus* were examined by the light and scanning electron microscopies.
- Newly laid eggs turn from white to yellow as soon as embryonic differentiation.
- The spermatheca of *P. apterus* consists of a bulb, a pump, a flange of pump, and duct.

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Abstract

In this study, spermatheca and eggs of *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae were examined by light microscope and SEM. The eggs have ~1.30 mm length and 0.94 mm width. Newly laid eggs turn from white to yellow as soon as embryonic differentiation. *P. apterus* egg surface shows polygonal (usually hexagonal and pentagonal) shapes. The eggs have pipe shaped 6-7 micropylar projections. The egg-burster which is a dark T-shaped or triangular pattern, explosive becomes visible when the embryo is well developed. The nimph, which has completed its embryonic development, comes out of the egg with peristaltic movements. The spermatheca of *P. apterus* consists of a bulb, a pump, a flange of pump, and duct. The morphology of the egg and spermatheca is useful for classification, because they show a great diversity among species and genera.

1. INTRODUCTION

The firebug, *Pyrrhocoris apterus* (L., 1758) species *Tilia* spp. (Tiliaceae) feeding on the seeds of Malvaceae (s. Str.) and *Robinia pseudacacia* (Fabaceae), is a common and enterprising species found in the margins of Afrotropical and Eastern regions [1-4].

Although there are many studies on the morphology and surface structure of eggs of various Heteroptera species, many taxonomic groups still need research [5-24].

In insect, the spermatheca is in the form of differently shaped appendages of the female gonoduct, depending on the species. They serve to retrieve and store the spermatozoa transferred during mating and release egg cells for fertilization [25]. Spermatheca, whose structure is large and highly complex in Heteroptera, exhibits an important character for systematic, taxonomy and phylogeny [26]. This organ was mistakenly identified as the sebaceous gland in the first study of Hemiptera spermatheca in 1833 [27]. The correct description of a spermatheca (as receptaculum seminis) in Pentatomomorpha was made in 1837 [28]. Three major studies have been published on the structure of the female genitalia in Hemiptera [26,29,30]. Two very important studies were published in 1962 and 1966 on the male and female reproductive organs of Pentatomoidea [31,32].

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In recent years, many studies have been conducted on the morphological studies of spermatheca belonging to different species of the order Heteroptera [17,18,33-49].

In this study, the egg structure and spermatheca morphology of *P. apterus* were examined and described in detail by light microscope and SEM.

2. MATERIAL METHOD

2.1. The Eggs

Adults of *Pyrrhocoris apterus* were collected from Safranbolu, Karabük (May 2018). Some of the fresh eggs obtained from the species kept under laboratory conditions were examined by Olympus SZX7 stereomicroscope and JEOL JSM 6060 LV scanning electron microscope (SEM) operated at 5-10 kV, 30 of them were measured and photographed.

2.2. The Spermatheca

For light microscope examinations, the spermatheca dissected from female were fixed in Bouin's fixative and then washed in 70% alcohol and passed from 70% to 100% alcohol. After removing the excess of alcohol in xylol, they were embedded in paraffin wax. Sections cut at 6-7 µm were stained with hematoxylin-eosin (H&E). The stained slides were photographed under BX51 Olympus light microscope.

Ten spermathecae were carefully removed from the abdomen softened in 10% KOH for 5-10 minutes and then examined under a stereomicroscope (Olympus SZX7).

Spermatheca, cleaned with a graded alcohol series and air dried to be examined in SEM, was attached to the SEM stubs with double-sided tape and covered with gold (Polaron SC 502 Sputter Coater) and examined with SEM (Jeol JSM 6060 LV). Several terminologies have been used for Spermatheca [26,30,32,42].

3. RESULTS AND DISCUSSION

3.1. Description of Eggs of *Pyrrhocoris apterus*

The number of eggs laid varies among insect species. Each female in *P. apterus*, generally lays her eggs one by one in the environment (Figures 1a, b). *Mecidea major* Sailer, 1952 (Pentatomidae) female deposits total 10 eggs (double rows) on leaves [12]. The egg batches of *Odontotarsus purpureolineatus* (Rossi 1790) (Scutelleridae) generally consist of 13-14 eggs [17]. *Eurygaster austriaca* (Schrank, 1778) (Scutelleridae) egg mass generally consists of 14 eggs [18].

The shape and appearance of the egg differs from species to species. The oval-shaped eggs of *P. apterus* are 1.30 mm long and 0,94 mm wide on average (Figures 1a, 1b, 2a). In *Euschistus obscurus* (Palisot) (Pentatomidae), egg is subelliptical and length is 1.00-1.14 mm; diameter, 0.88-0.98 mm [11]. In *Euschistus servus* (Say) (Pentatomidae), egg is subelliptical and length is 0.98-1.18 mm; diameter, 0.86-1.10 mm [11]. In *Euschistus tristigmus* (Say) (Pentatomidae), the length of subelliptical shaped egg is 0.98-1.14 mm; diameter, 0.86-1.02 mm [11]. The egg of *Piezodorus guildinii* (Westwood) (Pentatomidae) is cylindrical and, 0.88-1.08 mm length; 0.58-0.80 mm diameter [11]. *Thyanta custator accerra* McAtee (Pentatomidae), egg is subcylindrical and 0.88-1.04 mm length; 0.76-0.88 mm diameter [11]. Eggs *of O. purpureolineatus* are about 1.35 mm long and 1.09 mm wide [17]. The eggs of *E. austriaca* are diameter of 1.05 mm [18]. The egg of *Lethocerus delpontei* (Belostomatidae) is 3-4 mm long, 2-2.5 mm wide, *Kirkaldyia deyrolli* (Vuillefroy, 1864) (Belostomatidae) egg is 4-5 mm length and 2.5-3 mm width [19]. In *Chorosoma schillingi* (Schilling, 1829) (Rhopalidae), the egg is 1.23 length and 0.56 width. The egg in *Brachycarenus tigrinus* (Schilling, 1829) (Rhopalidae) is 1.11 length and 0.37 width. Egg measurement of *Rhopalus* (*Aeschyntelus*) *maculatus* (Fieber, 1837) (Rhopalidae) is 1.0 length and 0.50 width [21]. The eggs of *Brontocoris tabidus* (Signoret, 1863) and *Supputius cincticeps* (Stål) (Pentatomidae) are subglobose to oval and have 2.8 and 2.05 mm height, 2.03 and 1.80 mm diameter [24]. The eggs of *Podisus distinctus* (Stål) (Pentatomidae) are 1.09 mm height and 0.90 mm diameter [45].

The color and patterning of eggs laid in the external environment vary widely among insect species. In *P. apterus*, newly laid eggs turn from white to yellow as soon as embryonic differentiation (Figures 1a-c). In *Acrosternum hilare* (Say) (Pentatomidae), egg color is lemon yellow or pea green [11]. *Podisus maculiventris* (Say) (Pentatomidae) egg color is pale yellow to metallic blue [11] While *M. major* eggs were white when they were first laid, they turned to cream color after 1-3 days [12]. The eggs of *O. purpureolineatus* are whitish when newly released, but turn into a light yellow color when embryonic development begins [17]. The newly laid eggs of *E. austriaca* are green, then the color slightly changed [18]. In *K. deyrolli*, the anterior half of egg is pale yellow and the dorsal side has 10–12 dark longitudinal stripes [19]. *C. schillingi* egg is brown after oviposition, darkening to brown to blackish. In *B. tigrinus*, the egg is green after oviposition, becoming dark green-brown. In *R. maculatus*, egg is goldish after oviposition, becoming yellow-brown to brown, due to embryo showing through [21]. The eggs of *B. tabidus* and *S. cincticeps* show a light or whitish appearance when first laid, and then turn into a graphite color [24]. The color of the newly laid eggs of *P. distinctus* is pearl-like, but four hours after oviposition, the eggs became gray [45].

As with other Pentatomidae, the first evidence of embryonic differentiation is the appearance of two contrasting red eye spots beneath the operculum [11,18]. Red eye spots and egg burster in the final stage of embryonic development are also seen in *P. apterus* (Figure 1c). In the final stage of embryonic development, nymphs emerge from the eggs (Figures 1d-f).

Egg surface appearance varies from species to species. The *P. apterus* egg surface shows polygonal (usually hexagonal and pentagonal) shapes (Figure 2e). The chorion of *A. hilare* is coarsely reticulate, foveate. The chorion of *Nezara viridula* (Linnaeus 1758) (Pentatomidae) and *O. pugnax* are nearly smooth. *T. custator accerra* has rough chorion [11]. Chorion surface of *Cyrtocoris egeris* Packauskas & Schaefer (Pentatomidae) has smooth, lateral and superior surfaces with gross circular elevations. The *O. purpureolineatus* egg surface is covered by polygonal ridges and tiny chorionic tubercles [17]. The egg of *E. austriaca* is covered with a polygonal reticulated shape [18]. The chorion of *L. delpontei*, *K. deyrolli*, and *Horvathinia pelocoroides* Montadon, 1911 (Belostomatidae) is covered by a hexagonal pattern delimited by ridges in the three studied species [19]. *Euschistus* (*Mitripus*) *convergens* (Herrich-Schaffer), *E. hansi* Grazia, 1987, and *E. picticornis* Stål, 1872 (Pentatomidae) have spinose chorion surface. In *Chinavia erythrocnemis* (Berg), *C. longicorialis* (Breddin), *C. obstinata* (Stål, 1860), and *C. pengue* (Rolston, 1983) (Pentatomidae), egg surface is reticulated sculpture pattern and polygonal. The chorion of *Chinavia musiva* (Berg, 1878) (Pentatomidae) eggs shows a granulated sculpture pattern. *Grazia tincta* (Distant, 1890) (Pentatomidae) chorion pattern is sharply reticulated. *Loxa deducta* chorion surface is spinose. In *Pallantia macunaim* Grazia (Pentatomidae), chorion surface is covered by long and thin spines [20]. The chorion in *C. schillingi* is tetragonal and *B. tigrinus* has distinct low rounded tubercles [21].

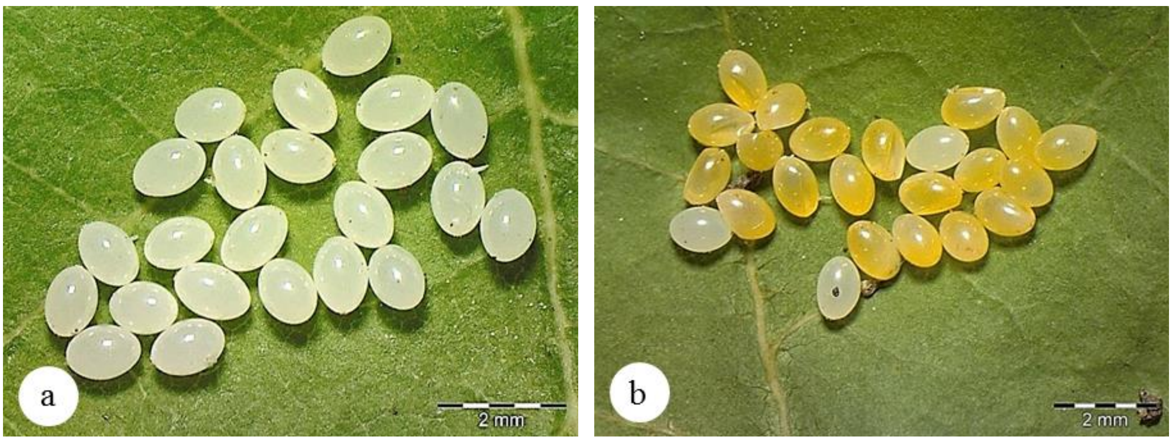
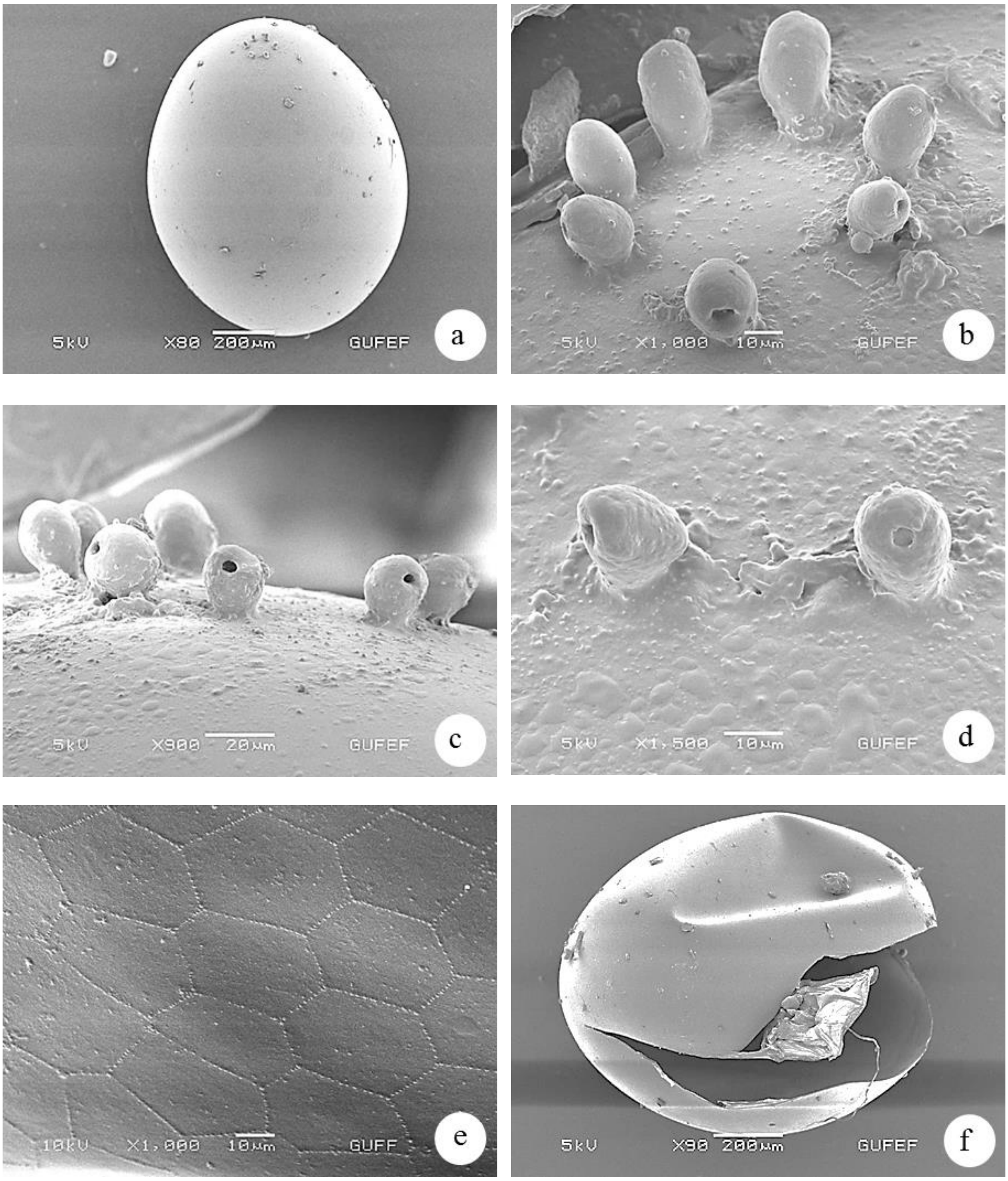


Figure 1. *Light micrographs of different phases of eggs masses of P. apterus a. Newly laid egg mass. b. Embryonic development color change on the 3-4. day. c. The appearance of red eye spots. d-e. The emergence of nymphs from the egg in the final phase of embryonic development and the empty egg casings. f. The newly hatched nymph*

The micropyles in *P. apterus*, which are responsible for the passage of sperm through the egg shell prior to fertilization, are located in the anterior region of the egg that are arranged in a circle, as in *A. hilare* egg [11]. The number and shape of micropiles vary in insect species. The micropyles in *P. apterus* have pipe shaped 6-7 micropylar projections. In *P. apterus*, the openings of all micropiles are facing out (Figures 2a-d). *T. custator accerra* has clavate and slender shaped 18-25 micropylar processes [11]. In *E. obscurus* and *E. servus*, micropylar processes are 26-39 weakly clavate [11]. In *Euschistus quadrator* Rolston, 1874 (Pentatomide), micropylar processes are 29-39, weakly clavate. In *E. tristigmus*, micropylar processes are 28-39, weakly clavate [11]. *P. guildinii* has 31-44 micropylar processes [11]. In *A. hilare* egg, micropylar processes are 47-64, clavate and decurved [11]. *O. pugnax* is 50-79 mushroom-shaped micropylar processes [11]. As in *A. hilare* and *P. guildinii*, in *P. apterus*, micropyle surface is smooth. However, in *E. obscurus*, *E. quadrator*, *E. servus*, *O. pugnax* and *E. tristigmus*, the surface of micropyle is porous [11]. *M. major* egg is surrounded by 7-18 clavate and smooth micropylar processes [12]. In *O. purpureolineatus* egg has 8–10 aero-micropylar processes [17]. *E. austriaca* egg has 17-19 aeromicropyles which are truncated cone shapes [18]. In *K. deyrolli* and *L. delpontei*, there are 8 to 10 micropyles, arranged in an ellipsoid pattern [19]. *P. distinctus*, the aero-micropylar processes varied from 12 to 18 [45]. In *C. obtusus* eggs, the number of the micropylar processes varies from 18 to 19 [50]. *T. marginata* has 9-13 white aero-micropylar processes spaced, very long and slender [51].

The egg-burster begins to appear towards the end of embryogenesis (Figures 2f, 2h). Similar structures have been seen in *E. austriaca* [18]. In *P. apterus*, upon hatching, the pseudo-operculum is cracked through the middle of the micropiles by the egg-burster, which is thick and highly sclerotized (Figures 2g, 2h). Similarly, *C. obtusus* eggs do not have real operculum and operculum hatching line. After the completion of embryonic egg development, it splits into two through the lateral hatching line with the help of the egg burster [50]. The egg-burster of *P. apterus* carries a dark T-shaped or triangular pattern (Figure 2h). The nimph, which has completed its embryonic development, comes out of the egg with peristaltic movements (Figures 1d-1f, 2ı, 2j). The egg-burster remains attached to the inner lateral face of the egg (Figure 2f).



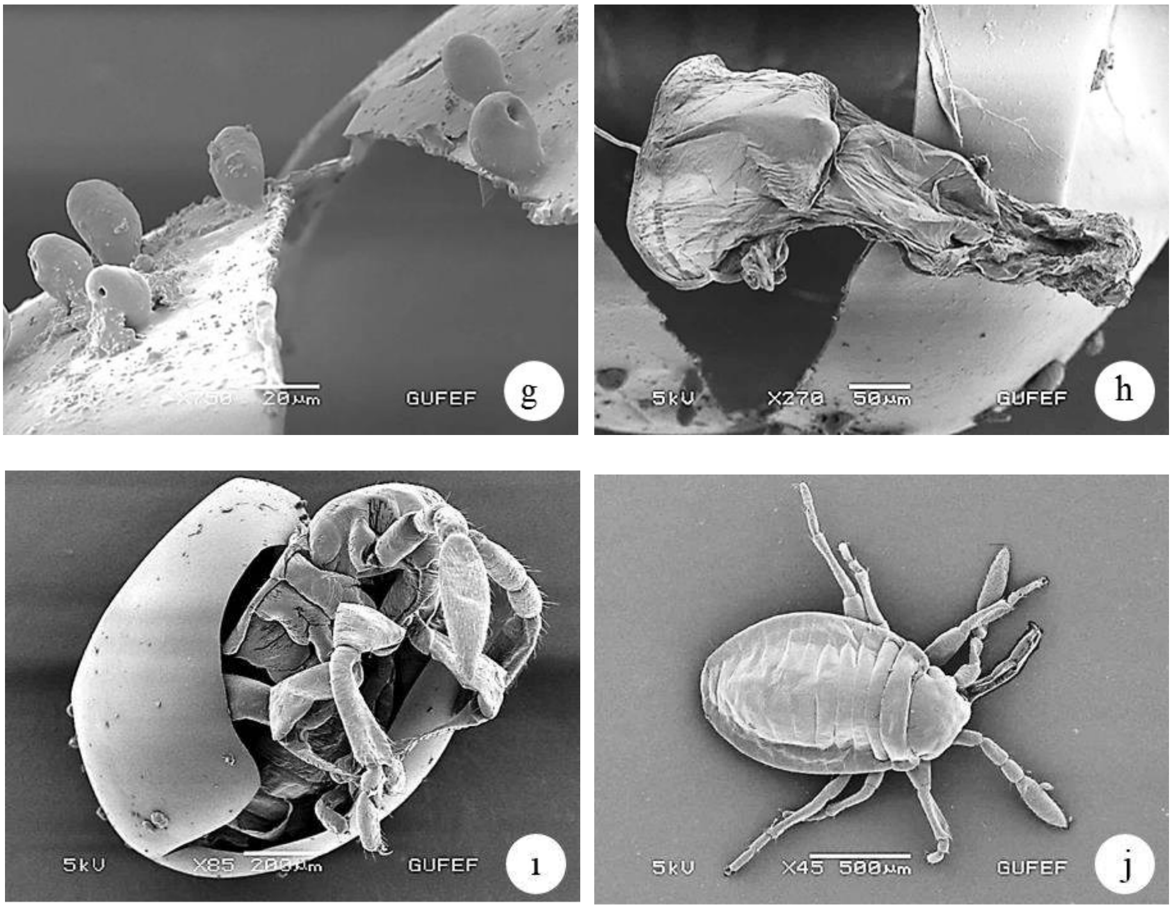


Figure 2. *SEM micrographs of the eggs of Pyrrhocoris apterus. a. General view of the eggs of P. apterus, b-d. Micropyles and microplar opening. e. Polygonal with reticulated patterns on egg surface. f-g. Hatching line of egg and egg burster. h. The egg-burster on hatched eggs ı. The emergence of nymph from the egg in the final phase of embryonic development. j. The newly hatched nymph*

3.2. Description of Spermatheca of *Pyrrhocoris apterus*

The number and morphology of the spermatheca, an ectodermal organ responsible for receiving, protecting and releasing the sperm to fertilize the eggs in the female insect, varies according to the insect species [48].

In Heteroptera, spermatheca structure is different between families and even between species. The spermatheca of *P. apterus* consists of a bulb, a pump, a flange of pump, and duct. Spermathecal processes and a median spermathecal dilation with sclerotized rod are missing (Figures 3a-c). The spermatheca of *O. purpureolineatus* consists of a bulb, a pump, a flange, and dilation [17]. Spermatheca of *Lygaeus simulans* Deckert, 1985 (Lygaeidae) consists of the proximal part in convoluted tube shape and the distal part in irregularly coiled canal shape [37]. The spermatheca *of E. austriaca*, *Rhaphigaster nebulosa* (Poda 1761) (Pentatomidae), *Palomena prasina* (Linnaeus 1761) (Pentatomidae), *Piezodorus lituratus* (Fabricius 1794) (Pentatomidae), *Graphosoma lineatum* (Linnaeus 1758) (Pentatomidae), *Graphosoma semipunctatum* (Fabricius 1775) (Pentatomidae), *Aelia albovittata* (Fieber 1868) (Pentatomidae), *Codophila varia* (Fabricius 1787) (Pentatomidae), *Ancyrosoma leucogrammes* (Gmelin 1790) (Pentatomidae), *Nezara viridula* (Linnaeus 1758) (Pentatomidae) have a spermathecal bulb, a pumping region, distal and proximal flanges, and spermathecal ducts [18,39]. The spermatheca in *Murgania histrionica* (Hahn) (Pentatomidae) and *Leptoglossus zonatus* (Coreidae) consists of three main regions: the distal region, the median region and the proximal region [46,47]. The spermatheca consist of bulb and intermediate part which has distal, middle, and proximal regions [49].

The spermathecal bulb of *P. apterus* is spherical and sclerotized (Figures 3a, 3c), like in *Thalma secunda* (Dinidoridae), *Dinidor rufocinctus* Stål, 1870 (Dinidoridae), *Byrsodepsus sundanus* Breddin, 1900

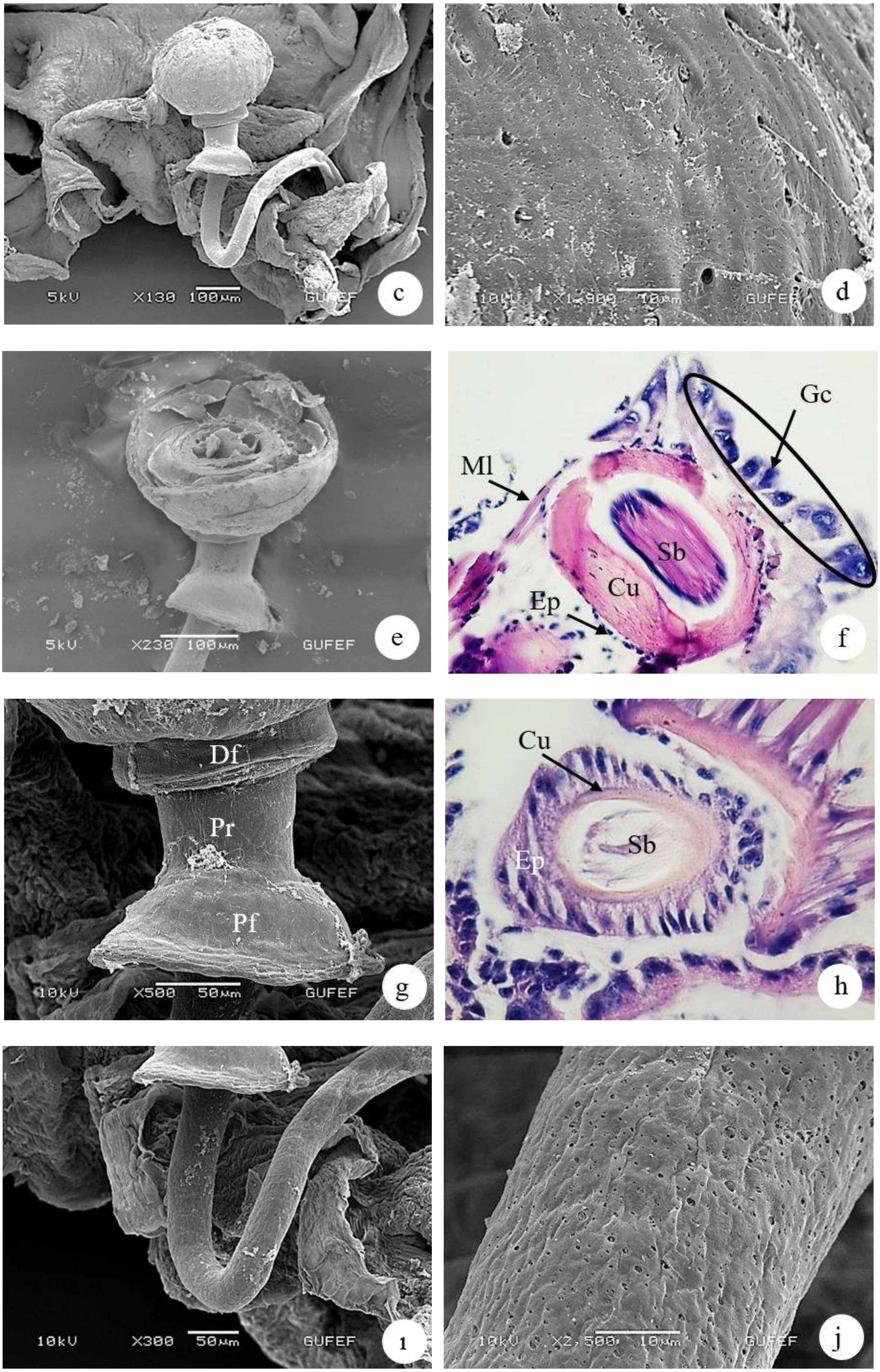
(Dinidoridae), *O. purpureolineatus*, *Amnestus pusio* (Stål, 1860) (Cydnidae), *Pseudostibaropus testaceus* (Walker, 1867) (Cydnidae), *Cydnus aterrimus* (Forster, 1771) (Cydnidae) and *E. austriaca* [17,18,41,42]. However, the bulb in *Aradus australis* Erichson (Aradidae) is flattened [34]. The spermathecal bulb of *Eurydema oleraceum* (Linnaeus, 1758) (Pentatomidae) and *Eurydema ornatum* (Linnaeus, 1758) (Pentatomidae) is semi-oblong [39]. The bulb in *M. histrionica* is a subcylindrical structure [48]. *Coreus marginatus* (Linnaeus, 1758) (Coreidae) has kidney-shaped bulb [49].

The diameter of the bulb in *P. apterus* is 218-278 µm. The bulb diameter of *O. purpureolineatus* is 280-310 µm [17]. The bulb of *P. apterus* is surrounded by an inner cuticle (Figures 3e, 3f). A single layer epithelium lines a thick inner cuticle. External to the spermathecal epithelium, there are muscle fibers surround the entire spermathecal bulb (Figure 3f). The bulb of *P. apterus* is covered by glandular cells. The glandular portion of the spermatheca in *P. apterus* consists of modified epithelial cells integrated into the reservoir wall. *P. apterus* has approximately 13-14 bulb glandular cells (Figure 3f). In the bulb lumen, there are sperm bundles (Figure 3f). The spermathecal bulb of *P. apterus* possesses many pores (Figure 3d), as *O. purpureolineatus* and *E. ornatum* [17,39]. However, in *E. oleraceum*, pores on the spermathecal bulb shows on the bulb anterior [39].

In *P. apterus*, the distal (132 µm) and proximal flange (154 µm) of the spermathecal pump are sclerotized and distal flange adheres closely to the bulb (Figure 3g) as in *C. bechynei* [41]. Nonetheless, distal flange is not adhering to the bulb in *T. secunda*, and in *Colpoproctus pullus* (Stål, 1853) (Dinidoridae), flanges are distant from the bulb [41]. In *P. apterus*, the proximal flange is larger than the distal one, as in *T. secunda* and *D. rufocinctus* [41]. However, in *M. histrionica*, the distal flange (255 µm) is larger than the proximal one (175 µm) [46], as in *Isodermus tenuicornis* Usinger and Matsuda (Aradidae), *B. sundanus* and *Eurydema spectabilis* Horváth, 1882 (Pentatomidae) [34,39,41]. In *Doesbergiana borneoensis* (Dinidoridae), distal and proximal flanges are equal in size [41]. The pumping region in *P. apterus* is short and between distal and proximal flange (Figure 3g). The pumping region is about 50.8 µm long. Histologically, the wall of pumping region is surrounded by cuticle and a single layer cylindirical epithelium. In the lumen, sperm bundles are seen (Figure 3h).

The spermathecal duct of *P. apterus* is thin, long and has pores (Figures 3ı, 3j). However, the spermathecal canals of *O. purpureolineatus* has distal, median and proximal portions. The first and third is narrow, second is swollen [17]. In *Carventaptera spinifera* U. & M. (Aradidae), the duct is fairly short, narrow, and with slight basal swelling [34]. The spermathecal duct is responsible for sperm transfer from the spermatheca directly to the common oviduct [44].

More studies on eggs and spermatheca are needed to establish clear trends within the Pyrrhocoridae family. With this study, the morphological characters of the egg and spermatheca of *P. apterus* were defined and contributed to the higher classification of Pyrrhocoridae families.


5kV X130 100µm GUFEF
c
10kV X1,900 10µm GUFEF
d
5kV X230 100µm GUFEF
e
Gc
Ml
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Ep
f
Df
Pr
Pf
10kV X500 50µm GUFEF
g
Cu
Sb
Ep
h
10kV X300 50µm GUFEF
i
10kV X2,500 10µm GUFEF
j

Figure 3. *Light microscope and SEM micrographs of spermatheca. a. General view of spermathecal. b. The longitudinal section of spermathecal bulb (X40) (H&E). c. Spermathecal bulb, distal and proximal flange and spermathecal duct. d. Pores on the spermathecal bulb surface. e-f. The cross section of the spermathecal bulb (X400) (H&E). g. The pumping region between distal and proximal flanges. h. The cross sections of pumping region. ı, j. Spermathecal duct and pores in spermathecal duct surface. Abbreviations: Gc-glandular cell, Sb-sperm bundle, Cu-cuticle, Ep-epithelium, Ml-muscle layer, Df-distal flange, Pr-pumping region, Pf-proximal flange*

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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