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Orijinal araştırma (Original article)

Morphology of spermatheca and eggs of *Coptosoma putoni* Montandon, 1898 (Hemiptera: Plataspidae)

Coptosoma putoni Montandon, 1898 (Hemiptera: Plataspidae) yumurta ve spermateka morfolojisi

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Summary

Spermatheca and eggs of *Coptosoma putoni* Montandon, 1898 were viewed with both light and scanning electron microscopes. The egg batch characteristically has two rows of spike-shaped eggs attached to substrate, with an anterior pole bearing aero-micropylar processes always turned outward. In an egg cluster there are 13-14 eggs that average 0.81 mm in length and 0:48 mm in width. Each side of the surface of the eggs is a slight falling away.The egg surface is covered with irregular chorionic protrusions as revealed by the scanning electron microscope. Micropyles (26-29) are arranged in a circle around the operculum. The spermatheca of *C. putoni* is characterized by a spherical spermathecal bulb, a pumping region, pump flanges, spermathecal duct and a genital chamber with associated sclerites.

Key words: Egg, spermatheca, morphology, SEM, *Coptosoma putoni*

Özet

Bu çalışmada *Coptosoma putoni* Month. (Hemiptera: Pentatomidae)' nin yumurta ve spermatekası ışık ve taramalı elektron mikroskobuyla (SEM) incelenmiştir. *Coptosoma putoni* yumurtaları genellikle başak şeklinde ve iki sıra halinde bırakılmaktadır. Bir yumurta kümesinde 12-16 arasında yumurta bulunmaktadır. Yumurtaların uzunluğu ortalama 0.81 mm, genişliği ise 0.48 mm.dir. Yumurtaların her iki yan yüzeyi hafif içeri çöküktür. Yumurta yüzeyi koryonik çıkıntılılarla kaplıdır. Mikropiller operkulum çevresine dairesel olarak dizilmiştir ve sayıları 26-29 arasında değişmektedir. *C. putoni* de spermateka siferikal spermatekal rezervuar, pompalama bölgesi, pompalama flangeleri, spermatekal kanal, bir genital oda ve skleritler ile karakterize edimektedir.

Anahtar sözcükler: Yumurta, spermateka, morfoloji, SEM *Coptosoma putoni*

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Introduction

This paper is a continuation of a series of reports on egg morphology and structure of the spermatheca of species of Hemiptera. Morphology and surface structure of eggs of a variety of Hemiptera species have been reported by many authors, however accurate knowledge is still lacking in many taxonomic groups (Hinton, 1981; Salkeld, 1983, 1984; Margaritis, 1985; Grigorov, 1988; Suludere et al., 1999; Bundy & McPherson, 2000; Baker & Chandrapatya, 2001; Wolf & Reid, 2000, 2001, 2004, Ubero-Pascal et al., 2005; Candan & Suludere, 1999a, b, 2003, 2006; Doğanlar et al., 2007; Matesco et al., 2009; Vilímová,& Rohanová, 2010; Sandoval et al., 2011). The eggs of Plataspidae, including some *Coptosoma* species, have been investigated by many authors (Southwood, 1956; Puchkova, 1961; Cobben, 1968; Shuzhi, 1985; Davidová-Vilímová, 1987; Shuzhi et al., 1990), but more work needs to be done.

The spermatheca of insects are, depending on the species, differently shaped attachments of the female gonoduct. They serve for the uptake and storage of the spermatozoa, which are transfered during mating, as well as their release for fertilization of egg cells (Lay et al., 1999). In Hemiptera, the structure of the spermatheca shows a great and often highly complex diversity and has been found to exhibit many important characters for classification, taxonomy and phylogeny (Pendergrast, 1957). The first study of the spermatheca of Hemiptera 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 reseptaculum seminis) in Pentatomomorpha. Three fundamental works on the structure of the female genitalia in Hemiptera were published by Dupuis (1955), Pendergrast (1957), and Scudder (1959). Two very important works on the female and male genitalia of Pentatomoidea were published by Kumar (1962) and McDonald (1966). In recent years, morphological studies of Hemiptera spermatheca have been conducted by many investigators (Servadei, 1964; Vavrinova, 1988; Kim & Lee, 1994; Gschwentner & Tadler, 2000; Brailovsky & Barrera, 2001; Kocorek & Danielczok-Demska, 2002; Candan et al., 2007; Candan, 2008; Pluot-Sigwalt & Lis, 2008; Candan et al., 2010, 2011; Stacconi & Romani, 2011).

In the present study, the morphology of spermatheca and of eggs of *C. putoni* were examined in detail utilizing both light and scanning electron microscopes.

Materials and Methods

Coptosoma putoni specimens were obtained from field collections in Turkey (Adana, Pozantı, 20.7.2006) and fed *Trifolium* sp. (Fabaceae) under laboratory conditions. Females deposited eggs on cotton batting when placed in plastic jars. Many eggs were examined and 30 of them were measured and photographed using both a Leica EZ4D stereomicroscope and a scanning electron microscope.

The spermathecae were dissected from dried females. Six spermatheca were prepared by first softening the abdomen in 10% KOH for 5–10 minutes. 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, eggs were cleaned and air dried; the spermathecae were dehydrated with an ascending alcohol series and air dried eggs were mounted using a 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 followed for designating parts of the spermatheca were those of Pendergrast (1957), Scudder (1959), McDonald (1966), and Pluot-Sigwalt & Lis (2008).

Results

Description of eggs of *Coptosoma putoni* Month. (Figs. 1-12)

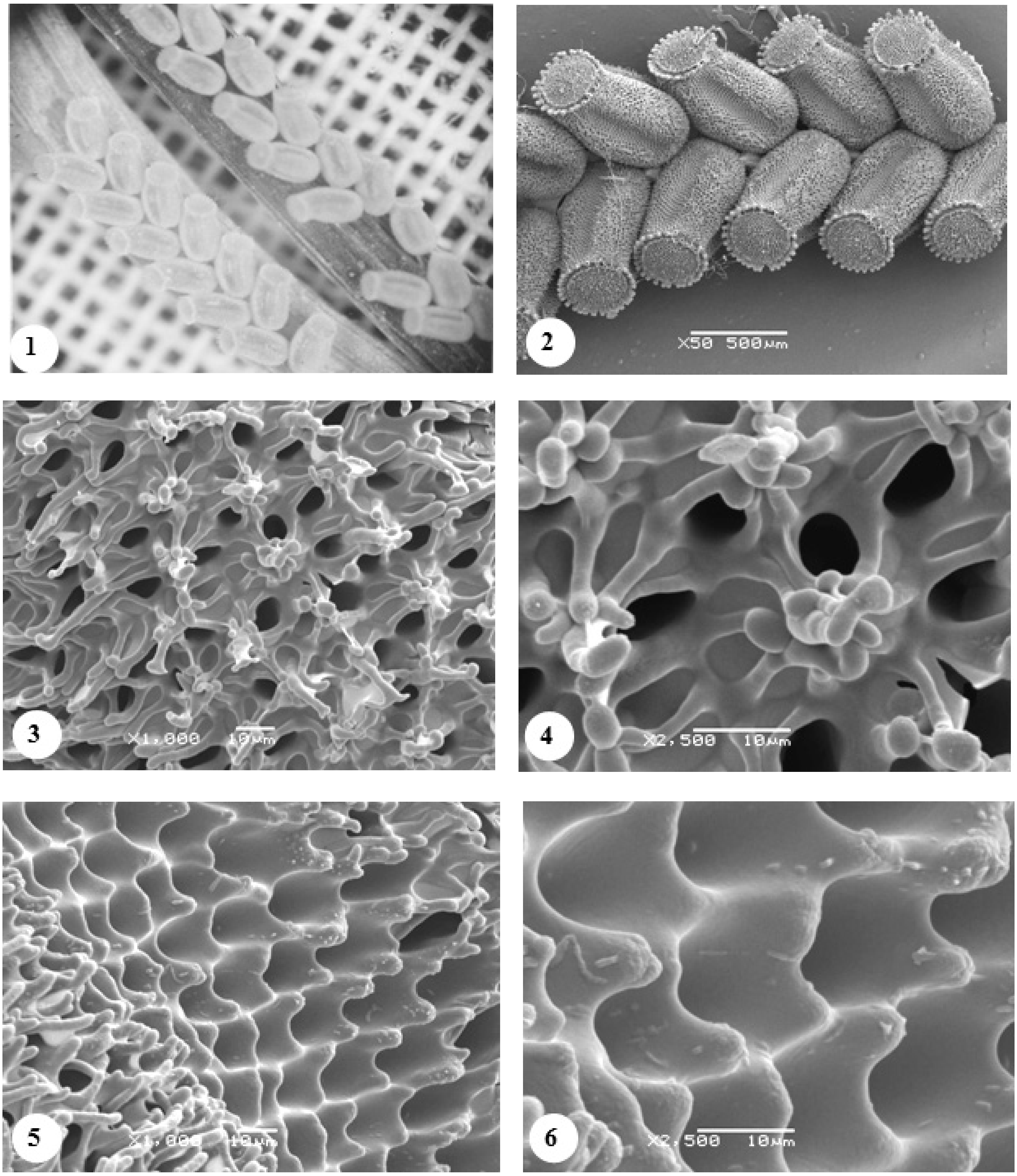
Eggs are barrel-shaped, and moderately cylindrical. The egg batch is spike-shaped when deposited on *Trifolium* sp. (Fabaceae) appearing as two orderly lines containing a variable number of eggs (13–16). The eggs average 0.81 mm in length, and 0.48 mm in width. (Fig. 1). Newly laid eggs are light yellow in color which turns to reddish or rosy with embryonic development. Following hatching the egg shells are clear white. Eggs are moderately dilated towards the posterior pole and moderately narrowed towards the anterior pole (Figs. 1–2). In lateral view the longitudinal axis of the eggs is curved; and the ventral surface is convex, the anterior and posterior poles being raised above the substrate. The dorsal surface is slightly concave. The visible broad depressed grooves on the dorsal surface run parallel with the longitudinal axis from anterior to posterior pole, where they are moderately divergent (Figs. 1–2).

The lateral and dorsal choronic surfaces of *C. putoni* eggs are covered with rod like protrusions which extend outward and intermixed with numerous follicular pits (Figs. 3-4). Some protrusions are singular while others combine and take the shape of bow. On the egg’s ventral surface, these protrusions are replaced by numerous deep pits (Figs. 5-6). The operculum is surrounded by a ring of distinct aero-micropylar processes (Fig. 7). The operculum surface is covered by rod shaped protrusions with deep pits (Fig. 18). These rod-shaped protrusions extend from the center to the hatching line (Fig. 9.)

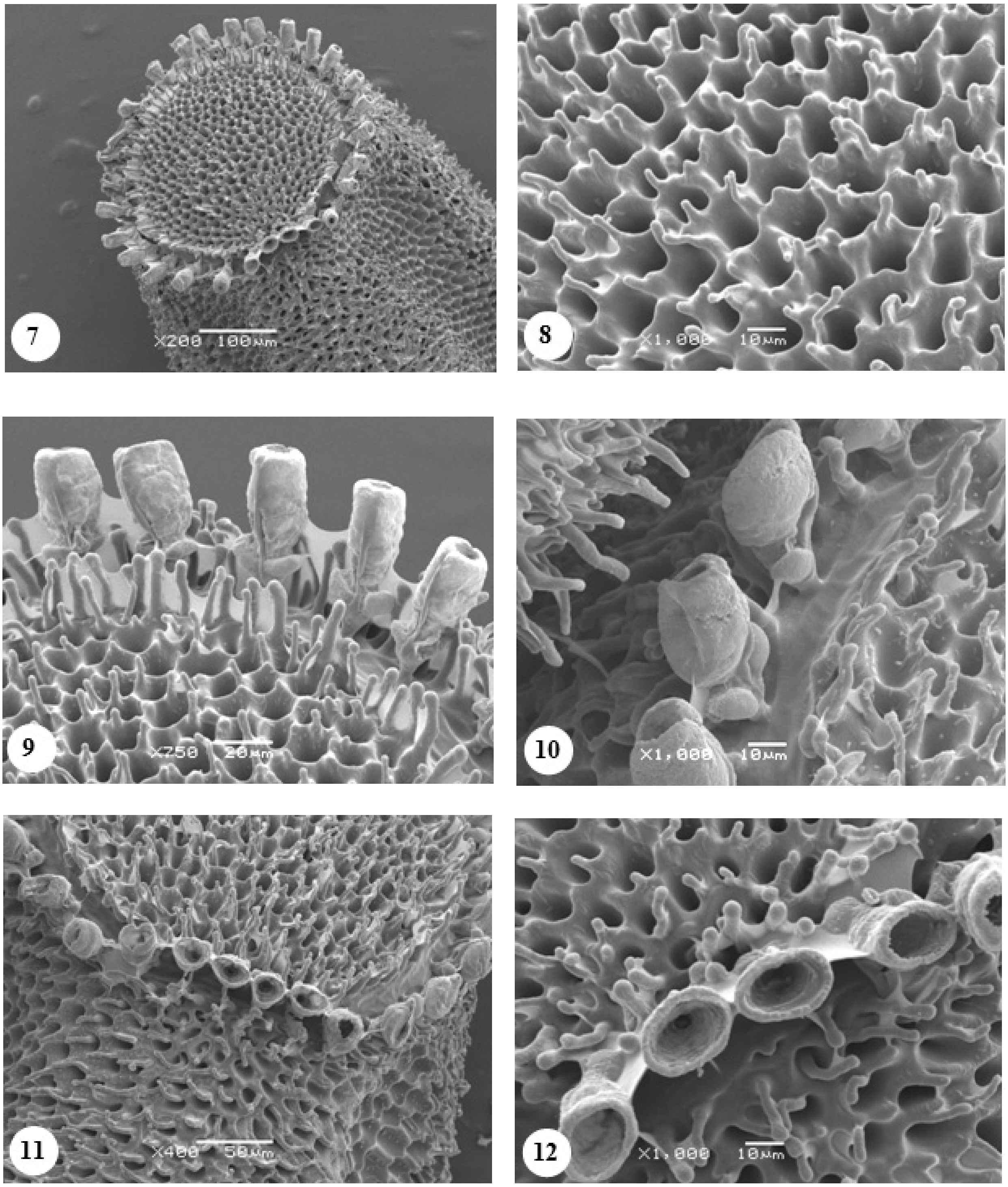
Aero-micropylar processes (26-29 in number) surround the anterior pole and are whitish in color (Figs. 9-12). The basal parts of the aero-micropylar processes are narrowed while the apical parts are widened. There is one well developed aero-saccule on each side of the aero-micropylar processes at the base (Fig. 10).

Description of the spermatheca *Coptosoma putoni* Month. (Figs. 13-22)

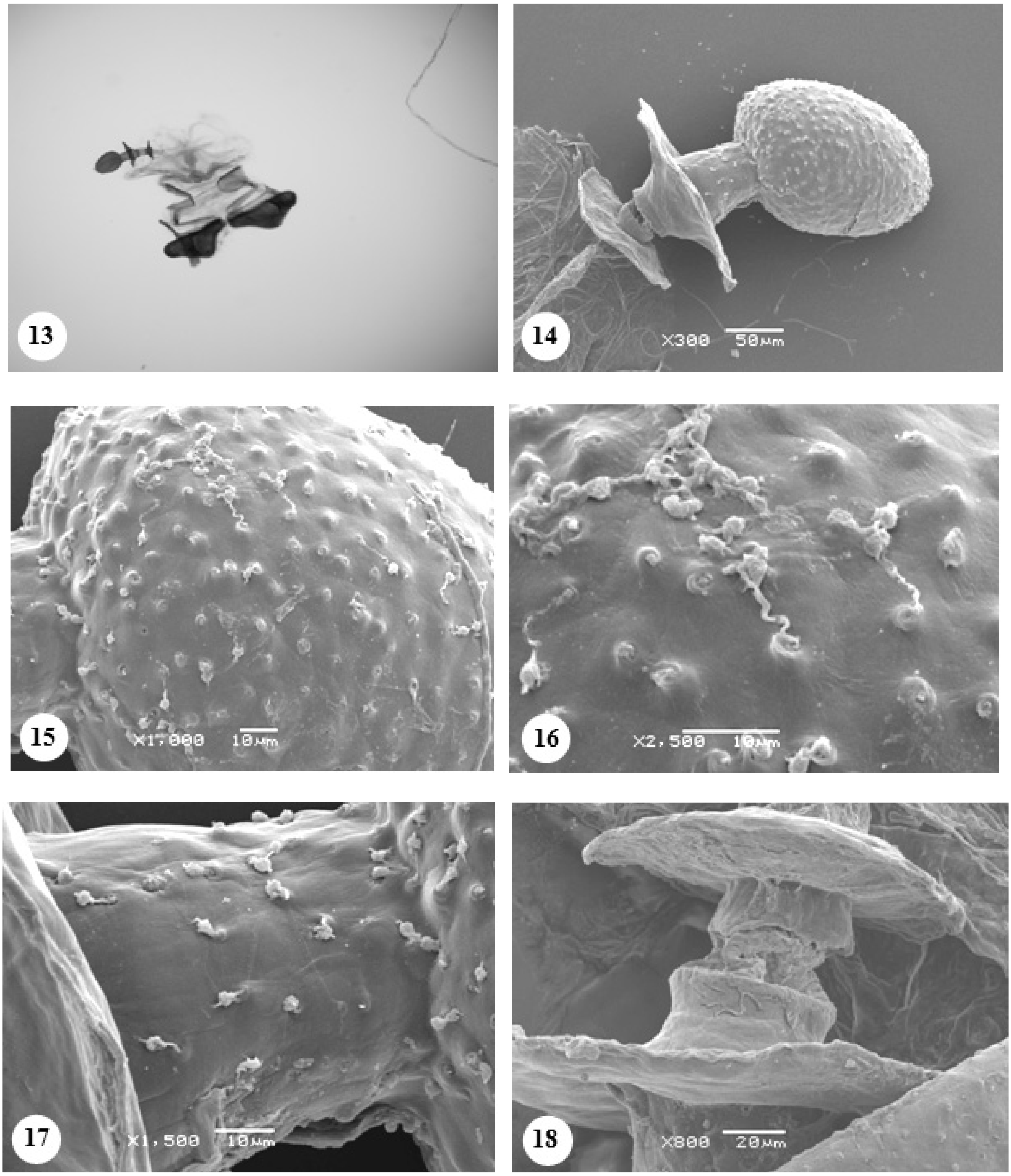
The spermatheca of *C. putoni* consists of the spermathecal bulb, a spermathecal pump, intermediate flanges associated with the pump, the spermathecal duct, and a genital chamber with associated sclerites (Figs. 13). The spermathecal bulb is light yellow in color, and is oval-shaped and sclerotized (Fig. 14). The surface of the bulb is covered with many pores through which a spermatozoon may enter. The spermatozoa are stored and nourished in the bulb (Figs. 15-16). The spermathecal pump is lightly sclerotized and contains many pores. The spermathecas have two pumping flanges (distal and proximal) and are plate shaped (Figs. 14, 18). The pumping flanges are connected to the distal portion of the spermathecal duct which is relatively short and quite muscular (Fig. 20) and is closely associated with the opening into the vagina (Fig. 21). There are two V shaped ring sclerites, with one at each side of the genital chamber. (Fig. 22).



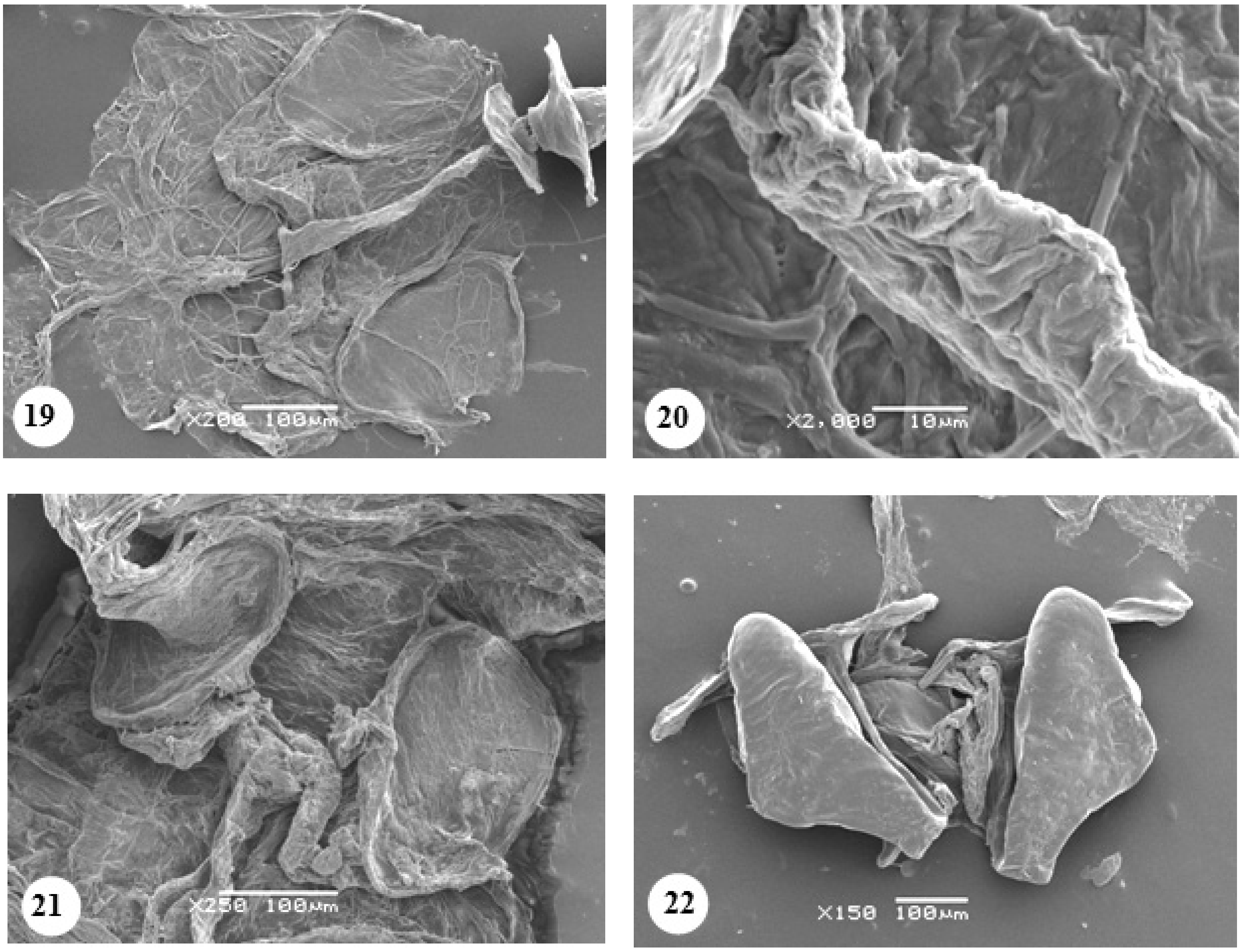
Figures 1-6. Light and SEM views of eggs of *Coptosoma putoni* Month. 1. Egg Clusters (Light microscopy) 2-6. SEM micrographs of general view of egg cluster. 3-4. Protrusions on the surface of the egg chorion and aeropyles. 5-6. Irregular pits on the surface of each side of the egg chorion.



Figures 7–12. SEM micrographs of operculum and aero-micropylar processes. 7. General view of operculum and aero-micropylar processes. 8. Rod-shaped protrusions and deep pits on operculum surface. 9–12. Aero-micropylar processes and aero-succules (➚) and chorionic protrusions.



Figures 13-18. Light and SEM view of spermatheca of *Coptosoma putoni*. 13. Spermatheca: general view (Light microscopy) 14. SEM micrographs of general view of spermathecal bulb and spermathecal flanges 15–16.Surface of spermathecal bulb illustrating pores (➔) and visible sperm tails.17. Spermathecal pump and sperm tails.18. Distal and proximal flanges.



Figures 19–22. SEM view of spermatheca of *Coptosoma putoni.* 19. Distal and proximal flanges and spermathecal duct. 20. Spermathecal duct. 21. The spermathecal duct is closely associated with the opening into the vagina and two ring sclerites 22. V-shaped ring sclerites.

Discussion

Nearly 500 species belonging to the family Plataspidae have been described worldwide; however, only 50 are recorded from the Palearctic region. All members are phytophagous. Adult and nymphs are recorded to feed on crops, especially Fabaceae, but they are very rarely seen in nature. *Coptosoma* species, which are found in most of the Palearctic Region, are usually represented by 1-2 species in Europe, North Africa, West Russia and Middle East. Lodos & Önder, 1978 and Önder et al. (2006) produced a list of 6 species*; Coptosoma scutellatum* (Geoffroy), 1785); *C.mucronatum* Scidenstrücker, 1963; *C. maurum* Vidal, 1938; *C. putoni* Montandon,1898; *C. costale* Stal,1876) *C. anatolicum* Horwardt, 1883 from Turkey with their distribution records, and Doğanlar et al., (2007) added *C. oenderi* Doğanlar, Karsavuran and Demirel, 2007 and *C. lodosi* Doğanlar, Karsavuran and Demirel, 2007 to the list.

Coptosoma females deposit their eggs on leaves or stems of Leguminosae**.** Newly laid eggs are white to yellowish. They turn darker some days before eclosion as rosy color of embryo shines through. Following hatching, the eggs are clear white with a silver shine. (Cobben, 1968; Davidová-Vilímová, 1987; Davidová-Vilímová & Stys, 1982).

The eggs of most Plataspidae, including *Coptosoma,* are deposited in exposed positions. The egg batch has a characteristic form of two rows of spike-shaped eggs attached to the substrate, with the anterior pole bearing aero micropylar processes always turned outwards. The number of eggs in one batch may range from 3 to 16; however, Ramakrishna (1913) reported up to 40 eggs/batch for *Megacopta cribriria***.** Davidová-Vilímová & Stys (1982) found that odd numbers of eggs in batches of all size clusters prevail over even numbers in *Coptosoma mucronatum* and *C. scutellatum.* In all species, almost all the ventral surfaces of eggs are glued to each other and to the substrate with a secretion secreted by the female (Southwood, 1956; Davidová-Vilímová, 1987). Several authors have stated that the eggs of other Hemiptera are deposited upright and attached to each other as well as to the substrate with an adhesive secreted by female (Southwood, 1956; Cobben, 1968; Hinton, 1981; Javahery, 1994; Suludere et al., 1999).

Generally, eggs of *Coptosoma putoni* are barrel-shaped, (Table 1) and moderately elongate. They are moderately dilated towards the posterior pole and moderately narrowed towards the anterior pole. The longitudinal axis of the egg is curved; from a lateral view; the ventral surface is convex; the anterior and posterior poles are raised above the substrate. The dorsal surface is slightly concave. Visible broadly depressed grooves traverse the dorsal surface parallel with the longitudinal axis from anterior to posterior pole**,** where they are moderately divergent (Davidová-Vilímová, 1987).

The surface morphology of the chorion of *Coptosoma* eggs changes from species to species. For example; in *Coptosoma caribrium*, the chorion of the dorsal and lateral regions of the egg has deep follicular pits (Table 1). The pits are much shallower on the ventral surface of the egg where it is attached to the substrata. A ridge and a groove run down each side of the dorsal surface (Southwood, 1956). The chorion of the eggs of *C. mucronatum* and *C. scutellatum* have an hexagonal structure (Table 1); the centre of each hexagon is depressed. Except at the centre, the surface of each hexagon bears sharp, conical processes of varying size (Davidová-Vilímová, 1987). In *C. xanthogramma,* the upper surfaces of the chorion are densely and finely pitted (Table 1). The lower surfaces are smoother, with relatively weakly developed pitting (Beardsley & Fluker, 1967). Shuzhi (1985) stated that the chorionic surface differs notably between the species of *Coptosoma*. For example, the reticulation of the egg shell may have spinous projections in the basal part, midway or near the posterior pole as in *C. notabilis*, C*. biguttula*, C*. bifaria, C. Munda.* The chorion of *C. variegata* is distinctly different from that of the other four species as it contains numerous follicular pits, and has many small projections around them (Table 1).

Generally, in the eggs of *Coptosoma* species, as in *C. putoni,* aeromicropylar processes surrounding the anterior pole are regularly arranged along the hatching line. Aero-micropylar projections on the eclosion line are pipe shaped and longer than chorionic spins. Aero-micropylar openings are clearly visible on the tips; the basal parts of aero-micropylar processes are narrowed and the apical parts are widened. There is a single well developed aero-saccule on each side of aero-micropylar processes at the base. Similar structures are visible on other *Coptosoma* eggs, but aero-saccules on each side of aero-micropylar processes at the base were not noted by Davidová-Vilímová (1987) for *C. scutellatum* and *C. mucronatum*. The number of aero-micropylar processes vary from 22–38 in *Coptosoma* species (Table 1). As seen from the table, the number of aero-micropylar processes also varies from species to species. They are believed to serve the dual functions of allowing entrance of the sperm at fertilization, and as respiratory organs through which gaseous exchange takes place between the developing embryo and the external environment (Southwood, 1956; Cobben, 1968; Hinton, 1981; Javahery, 1994).

Hatching is accomplished with the aid of an "egg-buster", a pointed sclerotized spine situated dorsally on the head of the mature embryo. The spine is used to help break open the operculum and is shed as the nymph leaves the egg (Beardsley & Fluker, 1967). Such egg busters have been observed in Coptosoma species.

The egg shape, size, chorionic pattern and number of micropylar projections have taxonomic importance (Puchkova, 1966; Hinton, 1981).

Table 1. Comparison of egg characteristics for nine species of *Coptosoma* (Hemiptera: Plataspidae *Coptosoma* species

	Egg form	Egg size	No of micropylar processes	Form and rows of egg	Characteristics of chorion	Citation
C. notabilis Mot.	Barrel-shaped	0.9-0.55 mm	30-32	2rows, spike	Chorion with spinous projections	Shuzhi, 1985
C. variegata Her-Sh.	Barrel-shaped	0.9-0.53 mm	26-32	2rows, spike	Chorion with many projection around the follicular pits	Shuzhi, 1985
C. biguttula Mont.	Barrel-shaped	0.91-0.55 mm	36-38	2rows, spike	Chorion with spinous projections	Shuzhi, 1985
C. bifaria Mont.	Barrel-shaped	0.88-0.50 mm	34-36	2rows, spike	Chorion with spinous projections	Shuzhi, 1985
C. munda Berg.	Barrel-shaped	0.9-0.57 mm	30-32	2rows, spike	Chorion with spinous projections	Shuzhi, 1985
C. mucronatum Seid.	Barrel-shaped	0.80-0.45 mm	28-32	2rows, spike	Chorion hexagonal structure, hexagons bears sharp, conical processes of various size	Davidová-Vilímová, 1987
C. scutellatum Geof.	Barrel-shaped	0.89-0.56 mm	26-31	2rows, spike	Chorion hexagonal structure, hexagons bears sharp, conical processes of various size	Davidová-Vilímová, 1987
			30			Michalk, 1935
			25-30			Poisson, 1933
			29-32			Puchkova, 1961
C. caribrium Fab.	Barrel shaped	——	——	2rows, spike	Chorion with follicular pits	Southwood, 1956
C. xanthogramma (White)	Barrel shaped	0.85 mm	22	2rows, spike	Chorion with densely and finely pitted	Beardsley & Fluker, 1967

The spermatheca is an accessory female reproductive organ which occurs in all orders of insects, except for Protura and Collembola (Matsuda, 1976). In insects, in general, spermatozoa transferred during copulation are stored by the female in a specialized organ, the spermatheca, where they remain viable for a very long time until they are used to fertilize eggs (Davey, 1965).

Spermathecae are present in all Pentatomoidea (Hemiptera), including Plataspidae; generally only one spermatheca has been found. The spermatheca proper consists of a spermathecal duct, leading from the vagina to a dilated spermathecal bulb (seminal receptacle, distal bulb). In these insects, the spermatheca 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 & Lis, 2008). However, in some Hemiptera, the spermathecal morphology is different and varies from species to species (Kumar, 1965; McDonald, 1966). In some Hemiptera, the spermatheca has been completely lost while in others it has lost its primary function of storing sperm (Dupuis & Carvelho, 1956; Dupuis, 1970; Schuh & Slater, 1995).

The spermatheca of *C. putoni* has a spermathecal bulb, a pumping region, distal and proximal flanges and spermathecal ducts. The spermathecal duct is closely associated with the opening into the vagina. There are two V shaped ring sclerites, one at each side of the genital chamber. In some Hemiptera, the shape of spermathecal bulb changes from spherical to elongate. In some Pentatomoidea, including Plataspidae, the pumping region is well developed and connected to the spermathecal dilation by a short duct and has one or two flanges The transfer of sperm can be achieved directly with the aid of the sperm pump (McDonald, 1966; Kocorek & Danielczok-Demska, 2002; Candan et al., 2007).

Coptosoma putoni have two sclerotized pump flanges (distal and proximal flanges) which are distant from the bulb. The distal pump flange is collar shaped and located under the pumping region. A collar shaped distal pump flange is seen in some other Scutelleridae and Pentatomidae (Kumar, 1965). The proximal flange is concave, plate shaped and is located between distal and proximal spermathecal ducts. The spermathecal processes and the median spermathecal dilation with sclerotized rod are missing in other families of Hemiptera, such as Scutelleridae and Dinidoridae (Candan et al., 2007; Kocorek & Danielczok-Demska, 2002). The spermathecal bulb of *C. putoni* has many pores but this feature is not mentioned in descriptions of other Scutellerid and Pentatomoid species (McDonald, 1966; Kim & Lee, 1994; Adams, 2001; Kocorek & Danielczok-Demska, 2002). Scanning electron microscopic studies have demonstrated that pores exist on the surface of the spermathecal bulb in some Hemiptera (Candan et al., 2007, 2010, 2011; Candan, 2008).

The spermathecal duct adjacent to the bulb is modified into an intermediate piece or pump, the cuticular lining of which is unsclerotized and flexible (Lee & Pendergrast, 1983). While the distal spermathecal duct of *C.putoni* is very short and sclerotized, the proximal duct is muscular and connected to the anterior vagina. 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, 2010). A muscle has been described at the base of the spermatheca in a variety of insects (Kocorek & Danielczok-Demska, 2002; Candan et al., 2007, 2011). This spermathecal muscle may enable females to control insemination and egg fertilization (Gschwentner & Tadler, 2000).

Since *C. putoni* exhibits distinct morphological characters both in eggs and spermathecae these characters can be important in the higher classification of *Hemiptera*. More SEM work is needed to establish clear trends within the plataspid group

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