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Spermathecae morphology in Four Species of *Eurydema* Laporte, 1833 (Heteroptera: Pentatomidae) from Turkey: A Scanning Electron Microscope Study

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ABSTRACT
The spermathecae of four species of *Eurydema* (*E. fieberi* Schummel 1837, *E. oleraceum* (Linnaeus 1758), *E. ornatum* (Linnaeus, 1758), and *E. spectabilis* Horváth, 1882) were compared using both light and scanning electron microscope (SEM) images. All the spermathecae which are examined species are contains spermathecal bulb (reservoir), a pumping region, distal and proximal flanges, proximal and distal spermathecal ducts, dilation of spermathecal duct and a genital chamber with two ring sclerites. Spermathecal bulb shape is changed from oval or oblong (*E. fieberi)* semi –oblong (*E. oleraceum, E. ornatum*) ,and semi-spherical *(E. spectabilis).* Distal and proximal flanges of four species of *Eurydema* have strongly sclerotized and median spermathecal dilations a resemble balloons and sclerotized rods are present. Generally, in all species proximal spermathecal duct is long with muscular surface.

Keywords: Heteroptera, Pentatomidae, *Eurydema*, Spermatheca, light and scanning electron microscope (SEM).

1. Introduction

The spermatheca is an accessory female reproductive organ that occurs in all orders of insects except for Protura and Collembola [29]. It is a complex organ and it varies greatly shape and histology in the insect female reproductive system [32]. The spermatheca that open into the anterior tract of the common oviduct plays a significant role in many functions such as sperm storage, nourishment of the spermatozoa, fertilization and oviposition [8, 9, 17-20, 25, 28, 31]. In the vast majority of insects, spermatozoa are transferred to the female genital area during copulation. Thereafter, spermatozoa are stored in the spermatheca where they remain until they are used for the fertilization of eggs [10, 11, 41]. The period of storage ranges in different insects, from hours to months and in exceptional cases such as the honey bee, sperm may be stored in the spermatheca for years.

In most Heteroptera, the structures of the spermatheca show a varied and often highly complex diversity and has been found to exhibit many important characters used in taxonomy and phylogeny [32]. Conversely, in some Heteroptera the spermatheca has been completely lost, while in others the spermatheca has lost its primary function of storing sperm [14, 15, 35]. The spermatheca is present in all Pentatomoidea (Pentatomorpha), and usually consists of a spermathecal duct leading from the vagina to dilated spermathecal bulb (seminal receptacle or bulb), and is characterized by a well marked pumping region (intermediate part) with proximal and distal flanges [30, 32, 33]. Three fundamental works on the structure of the female genitalia in Hemiptera have been published by Dupuis and others, Pendergrast and Scudder [13, 32, 36]. Servadeı [37] provided a detailed description of spermathecae of Acanthosomatidae, Pentatomidae and Scutelleridae, and included an original key to subfamilies and genera based on spernathecal structures. Mcdonald [30], in his work on the genitalia of North American Pentatomoidae, explained that the Podopinae and Asopinae were phylogenetically very recent specializations of the main Pentatominae stock. The spermathecae of 25 central European species of Coreidea were studied by Kım Lee [21], and Vavrınová, [40] compared the spermathecae of eleven species belonging to subfamilies Podopinae and Asopinae from Korea, and was able to establish inter-subfamily relationships based on the structure of the spermathecae. Kocorek and Danıelczok-Demska [22] studied the comparative morphology of the spermathecae of

eleven genera in Dinidoridae. The spermathecae of 25 species in Coreoidea from central Europa were studied by Vavrınová [40] and some other Coreidae species were described by Braılovsky, Barrera [2], and Candan [3]. As a result of all these studies, it is safe to state that the structure of the spermatheca in the Heteroptera is complex and diverse, being the source of valuable systematic characters. The morphology of the spermatheca is useful for classification, because they show a great diversity among species.

Herein we describe for the first time the structure of the spermatheca in species of *Eurydema* (Pentatomidae) by using both light and scanning electron microscopy (SEM).

2. Material and methods

The species of *Eurydema* (*Eurydema fieberi*, *E. oleraceum*, *E. ornatum* and *E. spectabilis*) used in this study were collected from different regions of Turkey. For light microscopy examination the spermathecae was prepared by first softening the insect's abdomen in 10% KOH for 5-10 minutes. Then the remaining tissues were removed manually and the spermatheca were exposed and cleaned by fine dissection. The spermathecae of each species were placed in glycerine. They were subsequentially stained with Eozin and photographed with a digital camera mounted on a stereomicroscope (Olympus SZX12 photomicroscope). For scanning electron microscopy, cleaned spermatheca dehydrated with ascending alcohol series and air dried, 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 10 kV.

The parts of the spermatheca were labeled following Dupıus [13], Pendergrast [32], Scudder [36], Mcdonald [30] and Pluot-Sigwalt, Lis [33].

The following morphological characters of the spermatheca were examined: shape of spermathecal bulb, shape of the spermathecal pump, size of the flanges (located between spermathecal pump and spermathecal duct), shape and size of the dilation of the spermathecal duct, shape and size of the distal and proximal parts of the spermathecal duct, and structure of the genital chamber and sclerites.

3. Results

3.1 *Eurydema fieberi* Schummel, 1837 (Figs. 1a-h)

The spermathecal bulb is oval or oblong (Figs. 1a-b), the surface of spermathecal bulb has many pores (Fig. 1c), the pumping region with distal and proximal flanges is short and rod-shaped.

The distal and proximal flange is sclerotized (Fig. 1d); the distal spermathecal duct is short and its surface is smooth (Fig. 1e), both median spermathecal dilations and sclerotized rods are present. The proximal spermathecal duct is long, with muscular surface (1a, f), and is connected to the genital chamber. The exterior edges of genital chamber are strongly sclerotized (Fig. 1g-h), two ring sclerites, one at each side of the genital chamber (Fig. 1a) are present.

3.2 *Eurydema oleraceum* (Linnaeus, 1758) (Figs. 2a-h)

The spermathecal bulb is semi-oblong (Fig. 2a) and pores on the spermathecal bulb are present only on the anterior of the bulb (Fig. 2b), observed sperm tails appear very long (Fig. 2c), the pumping region has a short duct, distal flange resembles a plate while the proximal flange is extended and strongly sclerotized (Fig. 2d), the median spermathecal dilations and a sclerotized rod are present. Distal spermathecal duct is short and folded (Fig. 2e), proximal spermathecal duct is long and with a muscular surface (Fig. 2f), proximal spermathecal duct is connected to the genital chamber (Figs. 2gh), there are two ring sclerites, one at each side of the genital chamber (Fig. 2a).

3.3 *Eurydema ornatum* (Linneus, 1758) (Figs. 3a-h)

The spermathecal bulb is semi-oblong (Figs. 3a-b), its surface with multible pores (Fig. 3c), the distal and proximal flanges of the pumping region are long as a tube (Fig. 3d), median spermathecal dilations a resemble balloons and a sclerotized rod is present (Fig. 3a). Distal spermathecal duct is short and its surface smooth (Fig. 3e), proximal spermathecal duct is long with muscular surface (Fig. 3f), genital chamber look like plate (Fig. 3g), spermathecal duct connect to the apical part of genital chamber (Figs. 3a, h), there are two ring sclerites, one at each side of the genital chamber (Fig. 3a).

3.4 *Eurydema spectabilis* Horvart, 1882 (Figs. 4a-h)

Spermathecal bulb is semi-spherical (Figs. 4a-b), the surface of half of spermathecal bulb with many pores (Fig. 4c), pumping region very short, distal flange plate-like and wider than the proksimal (Fig. 4d), proximal flange is convoluted (Fig. 4a), distal spermathecal duct is very short and its surface wrinkled (Fig. 4e), median spermathecal dilations and sclerotized rod present. Proksimal spermathecal duct is long and with muscular surface (Fig. 4f), proximal spermathecal duct is connected to the apical part of genital chamber. (Figs. 4g, h), there are two ring sclerites, one at each side of the genital chamber (Fig. 4a).

4. Discussion

A spermatheca is present in all Heteropteran Pentatomoidea, including Pentatomidae and it is usually consist of a spermathecal duct, leading from the vagina to a dilated spermathecal bulb (seminal receptacle, distal bulb) as in *Eurydema*. In these insects, the spermatheca is characterized by a well marked pump in the intermediate part with both distal and proximal flanges [32, 33]. Other researchers state that the spermatheca in Pentatomidae are composed of a spermathecal bulb, a pump with two flanges, a median spermathecal dilation with sclerotized rod, and one or two sclerites [23, 24, 30].

In some Pentatomidae, the spermathecal morphology is different. In the Podopinae (Pentatomidae), the spermathecal bulb may be spherical (*Scotinophara lurida, S. scotti, S. horwathi*), semioblong (*Graphosoma rubrolineatum*) or oblong ovate (*Dybowskyia reticulata*). Also in the Asopinae (Pentatomidae), the spermathecal bulb may be spherical (*Arma chinensis, Picromerus bidens, P. lewisi*), semioblong (*Pinthaeus sanguinipes*) or semioval (*Zicrona caerulea*). In our investigation, the spermatheca of *Eurydema* (Pentatomidae-Pentatominae) vary considerably, especially the spermathecal bulb;which is oval-oblong in *Eurydema fieberi* (Fig. 1b), semi-oblong in *E. oleraceum* (Fig. 2b), semi-oblong in *E. ornatum* (Fig. 3b) and semi-spherical in *E. spectabilis* (Fig. 4b). All species of the Asopinae and these four species of *Eurydema* lack spermathecal processes. The function of these processes is not clear but the presence or absence of spermathecal processes is an important taxonomic character used at the generic level [21, 27]. We have clearly shown with SEM that the surface of the spermathecal bulb in *Eurydema* is covered with multiple pores. The spermatozoa move out of these pores (Figs. 1-4c) into the spermathecal duct.

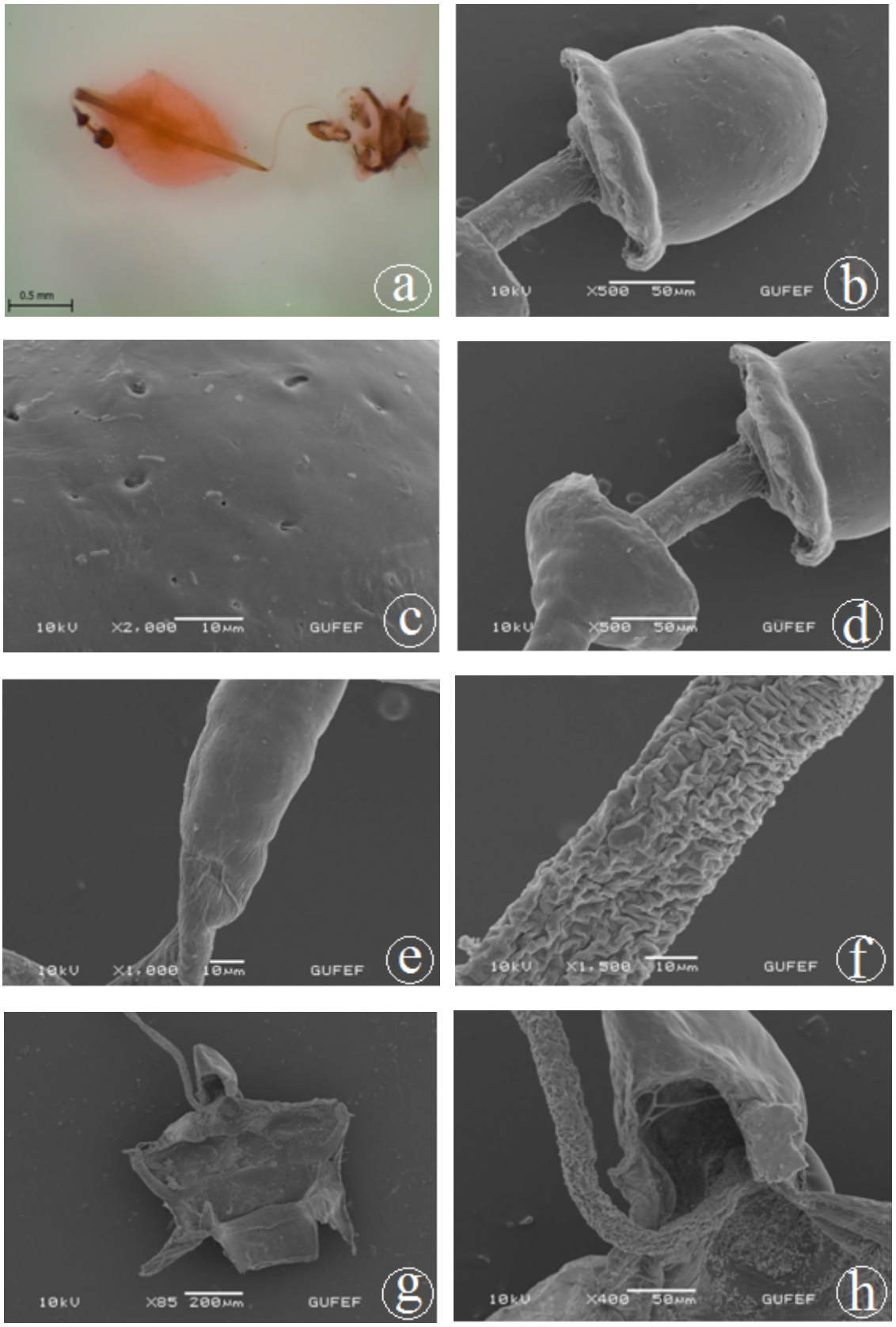

Fig 1: a-h: Light and SEM micrographs of the spermatheca of *Eurydema fieberi*; **a:** Spermatheca, overview utilizing light microscope, **b:** SEM Photo of spermathecal bulb and distal flange of pump, **c:** Pores on the spermathecal bulb, **d:** Pumping region and proximal flange, **e:** Distal part with muscles of spermathecal duct, **f:** Proximal part with muscles of spermathecal duct, **g:** Genital chamber, **h:** Opening of proximal duct and back surface of genital chamber.

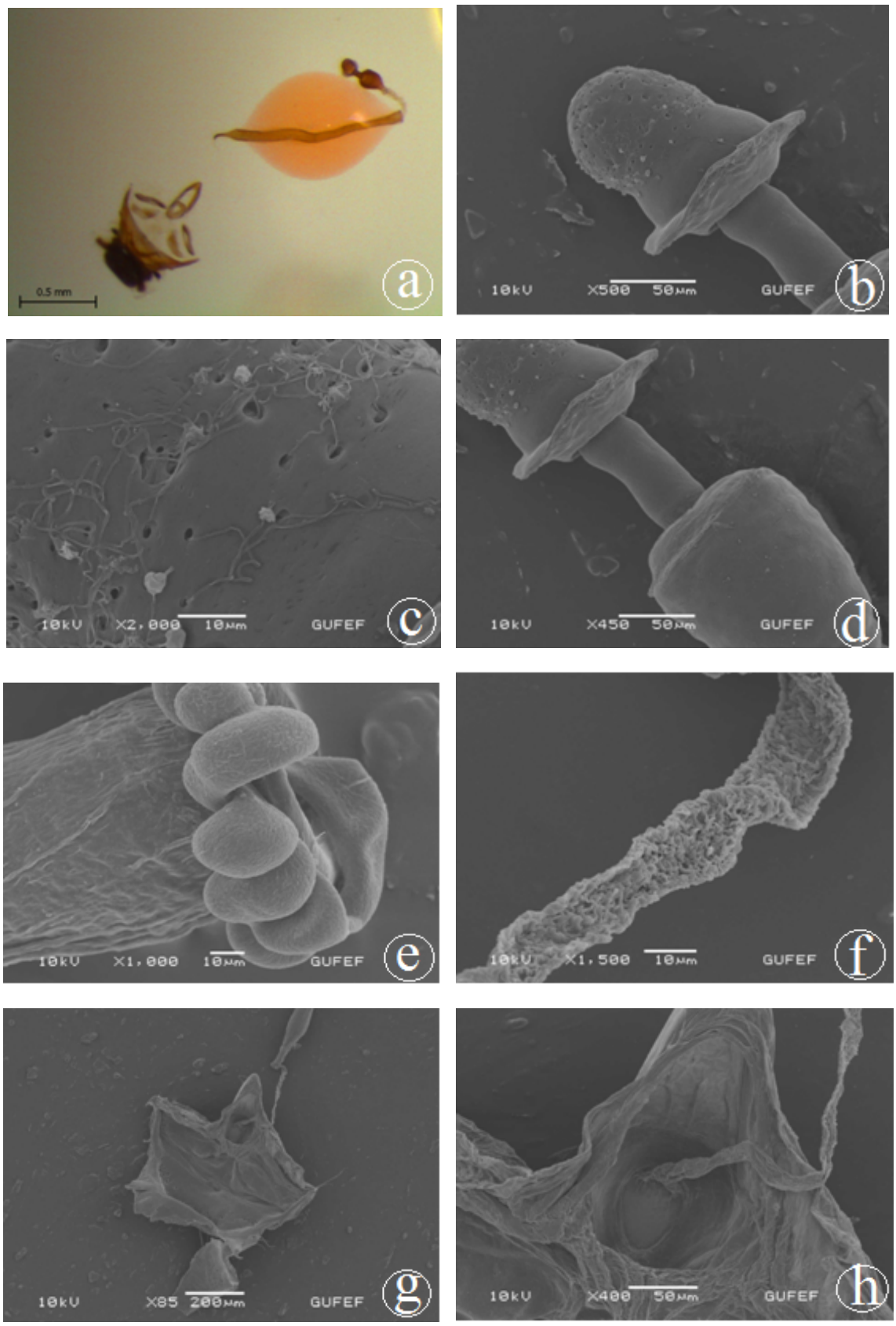

Fig 2: a-h: Light and SEM micrographs of the spermatheca of *Eurydema oleraceum*; **a:** Spermatheca, overview utilizing light microscope, **b:** SEM Photo of spermathecal bulb and distal flange of pump, **c:** Pores on the spermathecal bulb, **d:** Pumping region and proximal flange, **e:** Distal part with muscles of spermathecal duct, **f:** Proximal part with muscles of spermathecal duct, **g:** Genital chamber, **h:** Opening of proximal duct and back surface of genital chamber.

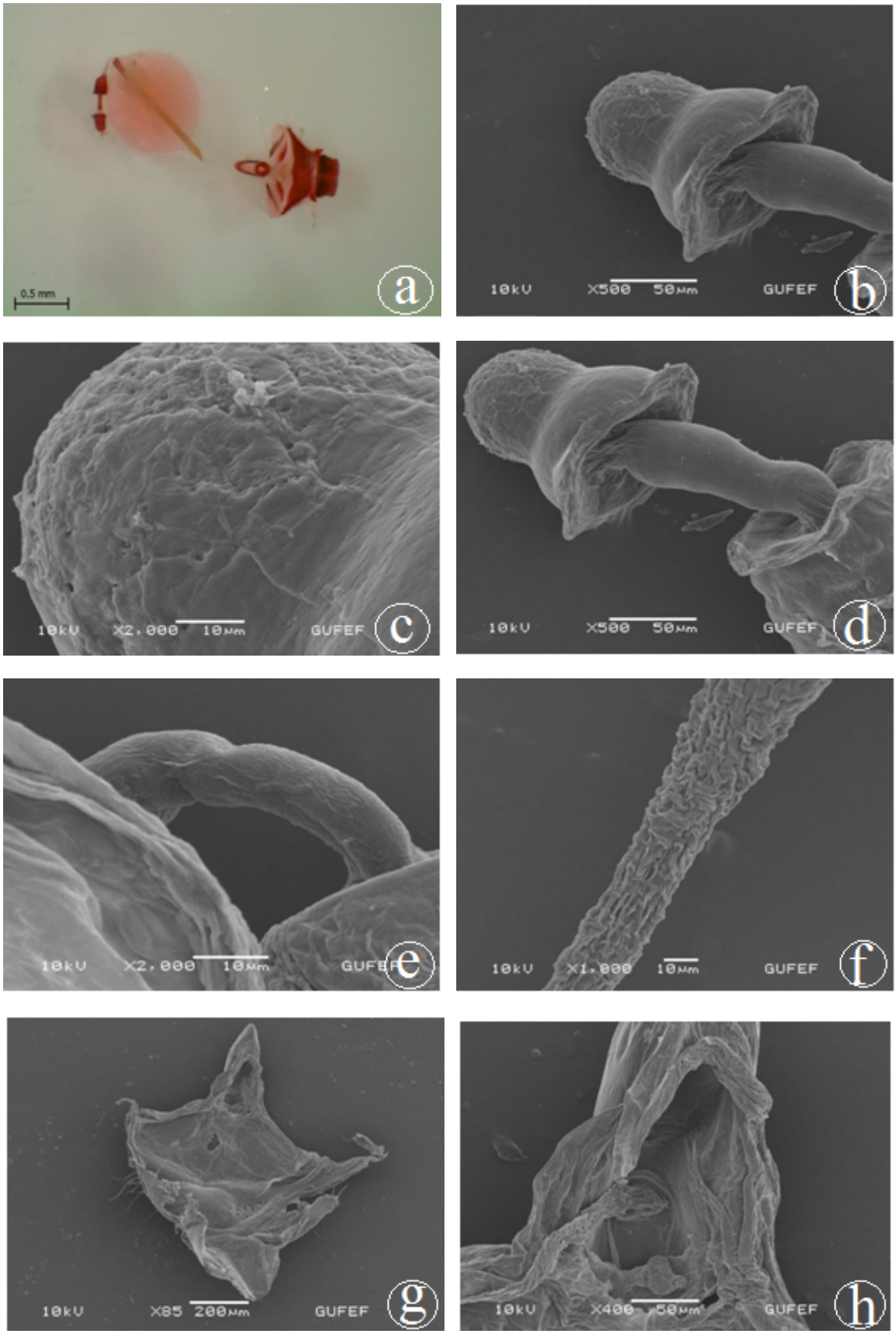

Fig 3: a-h: Light and SEM micrographs of the spermatheca of *Eurydema ornatum;* **a:**Spermatheca, overview utilizing light microscope, **b:** SEM Photo of spermathecal bulb and distal flange of pump, **c:** Pores on the spermathecal bulb, **d:** Pumping region and proximal flange, **e:** Distal part with muscles of spermathecal duct, **f:** Proximal part with muscles of spermathecal duct, **g:** Genital chamber, **h:** Opening of proximal duct and back surface of genital chamber.

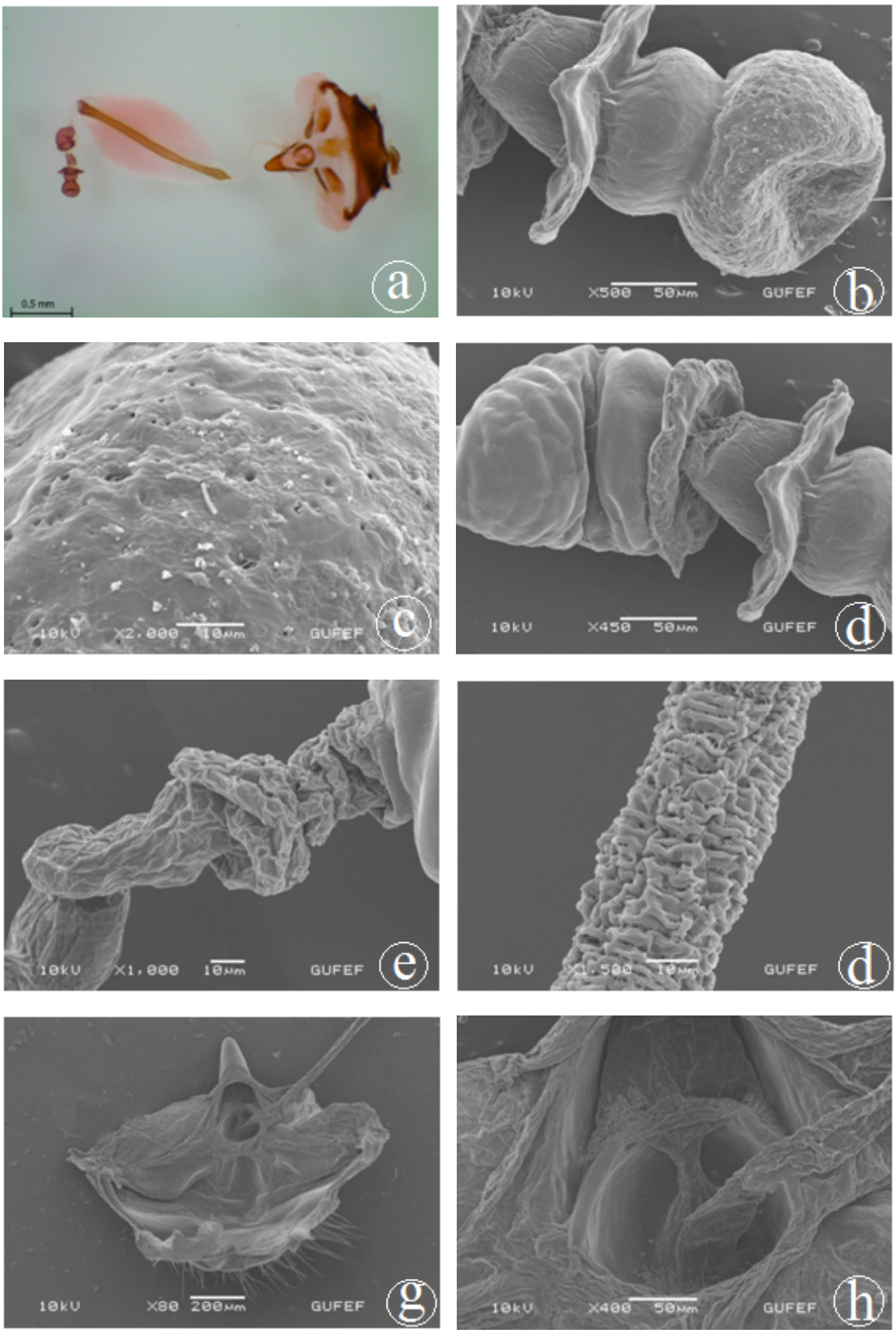

Fig 4: a-h: Light and SEM micrographs of the spermatheca of *Eurydema spectabilis*; **a:** Spermatheca, overview utilizing light microscope, **b:** SEM Photo of Spermathecal bulb and distal flange of pump, **c:** Pores on the spermathecal bulb, **d:** Pumping region and proximal flange, **e:** Distal part with muscles of spermathecal duct, **f:** Proximal part with muscles of spermathecal duct, **g:** Genital chamber, **h:** opening of proximal duct and rear surface of genital chamber.

Generally, in Pentatomidae the pumping region have with two flanges (distal and proximal). Their size and shape can be similar or different. The spermathecal duct in the Pentatomidae varies from short to long. The length of the distal spermathecal duct in the Asopinae (*Arma custos*, *Picromerus bidens*, *Zicrona caerulea*) is much shorter than that of Podopinae (*Graphosoma rubrolineatum*, *Dybowskyia reticulata*, *Scotinophara lurida*), as is also seen in species in *Eurydema*. This is a common feature of pentatomids [1, 16, 21, 26, 30, 32, 34]. In *Eurydema*, the distal spermathecal duct that look like clearly fold in *E. oleraceum* is different from the others species (Fig. 2e). All Pentatomidae as *Eurydema* species have a median spermathecal dilation with sclerotized rod but in some families of Hemiptera, such as Scutelleridae and Dinidoridae are missing [4, 22]. The number of hardening sclerite which opening of spermathecal duct and two ring sclerites are similar in all species of *Eurydema*.

With the use of SEM, I have demonstrated the presence of pores in surface the spermathecal bulb, pumping region, distal and proximal flange, median spermatecal dilation, spermathecal ducts and genital chamber. Thus, we suggest that the characters of spermathecae in *Eurydema* species are valuable to classify pentatomidae and are useful to the classification of the generic level.

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6. References

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