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Original article (Orijinal araştırma)

Spermathecae morphology of some Tephritinae (Diptera: Tephritidae) species: A scanning electron microscope study[1]

Bazı Tephritinae (Diptera: Tephritidae) türlerinin spermateka morfolojisi: Bir taramalı elektron mikroskop çalışması

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Abstract

Spermathecal structure of five species [*Campiglossa producta* (Loew, 1844)*, Campiglossa tessellata* (Loew, 1844)*, Euaresta bullans* (Wiedemann, 1830)*, Tephritis formosa* (Loew, 1844)*, Tephritis nigricauda* (Loew, 1856)] from subfamily Tephritinae were examined using light and scanning electron microscopy. The specimens were collected between 1999 and 2013 from various provinces of Turkey. Spermathecae were coated in gold/palladium with a Emitech SC 7620 Sputter Coater and examined with a Jeol 6390 LV scanning electron microscope (SEM) operated at 10 kV. Spermathecal structures were characterized as spermathecal bulb, pumping region and spermathecal channel. Descriptions of the spermathecal structures, size of spermathecal bulb, aspect ratio of spermathecal bulb and SEM micrographs of spermathecae are presented for each species.

Keywords: SEM, spermathecae, Tephritidae, Tephritinae

Öz

Tephritinae altfamilyasına ait beş türün [*Campiglossa producta* (Loew, 1844)*, Campiglossa tessellata* (Loew, 1844)*, Euaresta bullans* (Wiedemann, 1830)*, Tephritis formosa* (Loew, 1844)*, Tephritis nigricauda* (Loew, 1856)] spermateka yapıları ışık ve taramalı elektron mikroskopu (SEM) kullanılarak incelenmiştir. Türler 1999 ve 2013 yılları arasında Türkiye'nin çeşitli illerinden toplanmıştır. Spermateka örneklerine Emitech SC 7620 Sputter Coater ile altın/paladyum kaplaması yapılarak Jeol 6390 LV SEM ile 10 kV' da incelendi. Spermateka yapıları spermatekal bulb, pompalama bölgesi ve spermateka kanalı olarak karakterize edilmiştir. Makalede, her bir türün spermateka yapılarının tanımlamaları, spermatekal bulbun boyutları ve en-boy oranı ve SEM mikrografları sunulmuştur.

Anahtar sözcükler: SEM, spermateka, Tephritidae, Tephritinae

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Introduction

Worldwide, fruit flies (Diptera: Tephritidae) include 4792 species in 497 genera and many additional taxa are still to be discovered (Freidberg, 2006; Pape et al., 2011). Tephritinae is the most specialized subfamily of the Tephritidae. Predominantly, the larvae of Tephritinae infest the inflorescences of the Asteraceae, the largest, and the most advanced and widespread angiosperm family. With few exceptions, the tephritines are small to medium-sized flies, often with whitish, thickened post ocular setae, dark wing pattern with hyaline spots, oval epandrium and two spermathecae (Korneyev, 1999).

Sperm storage organs allow females to temporally separate insemination from fertilization, manipulate ejaculates and control fertilization. In the reproductive tract of female fruit flies, sperm are found in two different organs a pair or triplet of spermathecae, and a fertilization chamber (Twig & Yuval, 2005).

Generally, insects have multiple sperm storage organs and it has been suggested these structures provide a means for females to influence paternity by differential filling or emptying to favor sperm of one male over another (Hellriegel & Ward, 1998; Pitnick et al., 1999; Hellriegel & Bernasconi, 2000; Fritz & Turner, 2002).

For a long time, researchers have tried to understand the reproductive structures and reproductive systems of insects due to their important role in insect biodiversity and evolution. In this study, we aimed to describe surface morphology of the spermathecal structures of five species in two tribes (Tephritini and Euarestini) of the subfamily Tephritinae. Spermathecae were examined in scanning electron microscopy (SEM) micrographs. Spermathecal structures, aspect ratio of spermathecal bulb and spermathecal duct were defined. Also, the similarities and differences between these species were considered.

Material and Methods

Specimens (Table 1) from Entomology Museum of Gaziantep University that had been collected between 1999 and 2013 from different regions of Turkey were examined. The specimens were boiled for 30-35 min in 10% KOH and dissected to obtain spermathecae for examination under a light microscope (Olympus SZX12, Tokyo, Japan). These spermathecae were cleaned in 96% alcohol and stored in glycerin. The preparation of the specimens followed Candan & Erbey (2006).

Table 1. Taxa, collection locations and number of specimens examined

Species	Collection location	Specimens
Campiglossa producta (Loew, 1844)	Demirtaş, Alanya, Antalya, 36º26' N, 32º12' E, 80 m, 16.V.1999	3 ♀♀
Campiglossa tessellata (Loew, 1844)	Besni, Adıyaman, 37º42' N, 38º00' E, 687 m, 07.VI.2009	2 ♀♀
Euaresta bullans (Wiedemann, 1830)	Boyalı, Eğirdir, Isparta, 38º03' N, 30º50' E, 950 m, 12.V.2001	4 ♀♀
Tephritis formosa (Loew, 1844)	Sarız, Kayseri, 38º27' N, 36º28' E, 1610 m, 08.VII.2005	5 ♀♀
Tephritis nigricauda (Loew, 1856)	Güzelyurt, Aksaray, 38º15' N, 34º25' E, 1789 m, 27.V.2013	4 ♀♀

For SEM observation, spermathecal structures were dried with air for about 10 min then placed on SEM stubs. These samples were coated in gold/palladium with an Emitech SC7620 Sputter Coater (Quorum Technologies Ltd, Laughton, East Sussex, UK) and examined with a Jeol 6390LV SEM (Joel Ltd, Tokyo, Japan) operated at 10 kV, at Gaziantep University Entomology Laboratory and Electron Microscopy Unit.

The spermathecal terminology used follows that of Mcalpine (1981). The spermathecae consists of spermathecal bulb, valve, pumping region and spermathecal duct. In addition, during the designation process the aspect ratio was determined as it is having diagnostic value.

Results

Spermathecal structures of five species in three genera of Tephritinae were photographed using SEM and compared. Characteristic features of spermathecal morphology, surface of spermathecal bulb, glands and pores on the bulb, spermathecal channel and valves were identified for each species. Width, length and aspect ratio are given in the Table 2.

Table 2. Width, length and aspect ratio of spermathecal bulb

Species	Spermathecal bulb		
	Width (µm)	Length (µm)	Aspect ratio
Campiglossa producta	77.80	124.83	0.62
Campiglossa tessellata	55.48	90.70	0.61
Euaresta bullans	58.03	130.81	0.44
Tephritis formosa	63.64	330.42	0.19
Tephritis nigricauda	46.55	181.25	0.25

Campiglossa producta (Loew, 1844)

Spermathecal structure consists of three parts; spermathecal bulb, spermathecal channel and pumping region (valve). Spermathecal bulb papillose, intense papillose shape and formed like pyriform. In addition, spermathecal channel is formed thin and long. Size of spermathecal bulb is 77.80/124.83 (width/length, µm); Aspect ratio of spermathecal bulb is 0.62. Glands located on spermathecal surface one by one or clustered. Spermathecal channel is cylindrical and consist many lateral muscle fibers (Figure 1).

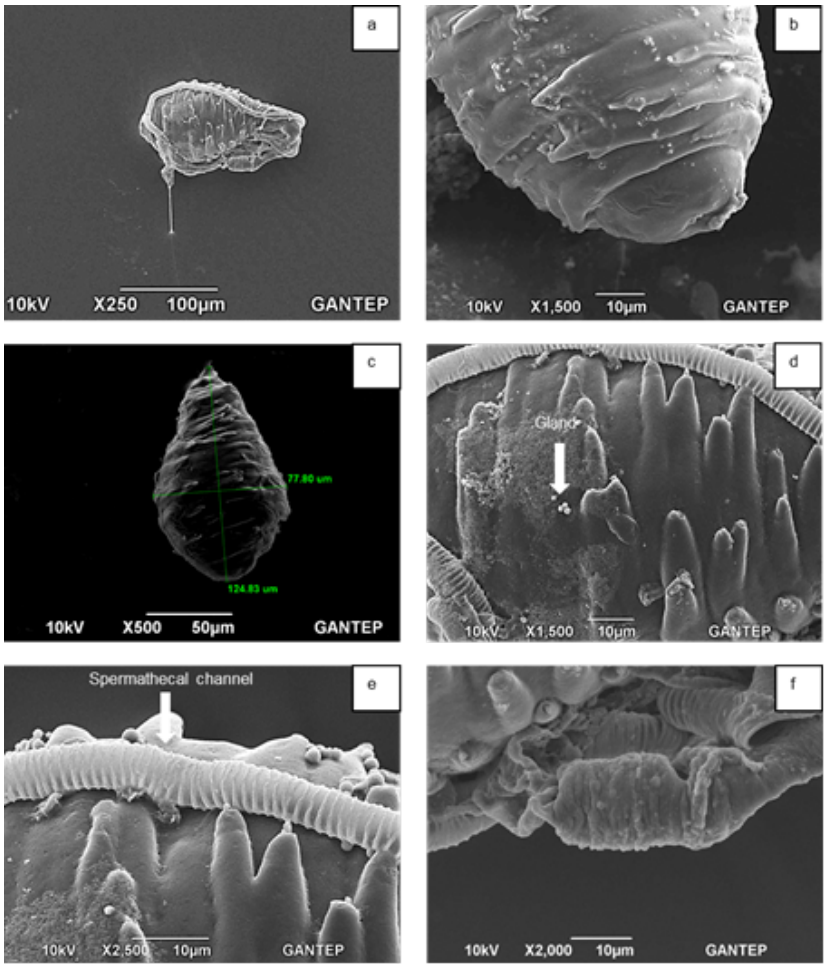

Figure 1. SEM micrographs of the spermathecae of *Campiglossa producta*: a) SEM photo of spermathecal bulb and distal flange of pump; b) apical part of spermathecal bulb; c) length/width of spermathecal bulb; d) spermathecal gland; e) spermathecal channel; and f) pumping region.

Campiglossa tessellata (Loew, 1844)

Spermathecae consists of three parts; spermathecal bulb, spermathecal channel and pumping region (valve). Spermathecal bulb generally seems oval and size is 55.48/90.70 (width/length, µm) and aspect ratio is 0.61. Spermathecal bulb has thin papillose structure. Apex of spermathecal bulb is flat not papillose. Spermathecal channel distinct and consist many lateral muscle fibers and turbinated (Figure 2).

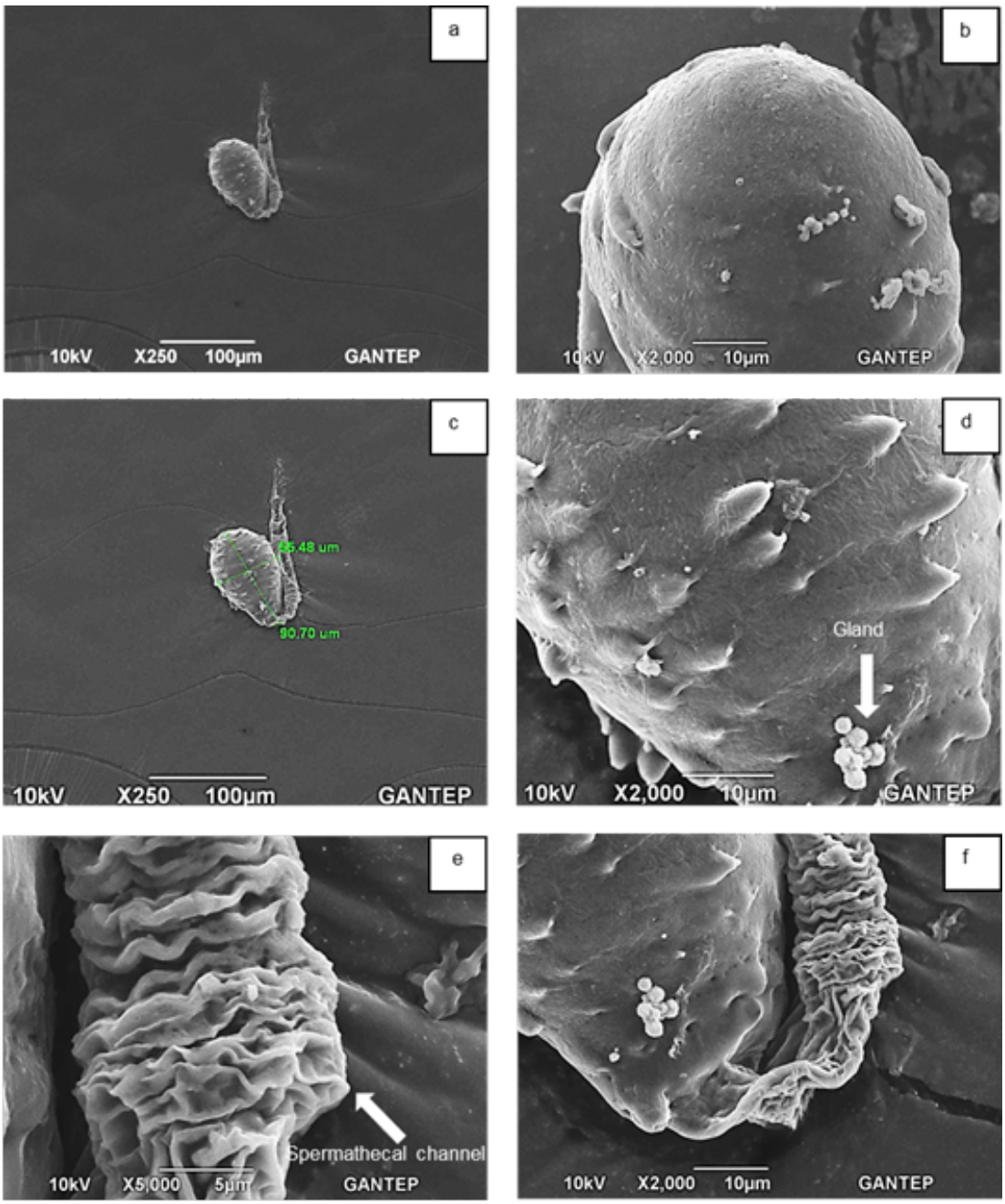

Figure 2. SEM micrographs of the spermathecae of *Campiglossa tessellata*: a) SEM photo of spermathecal bulb and distal flange of pump; b) apical part of spermathecal bulb; c) length/width of spermathecal bulb; d) spermathecal gland; e) spermathecal channel; and f) pumping region.

Euaresta bullans (Wiedemann, 1830)

Spermathecae consist of three parts; spermathecal bulb, pumping region (valve) and spermathecal channel. Spermathecal bulb is regular from basal to apical and formed as a saccate. Size of spermathecal bulb is 58.03/130.81 (width/length, µm) and aspect ratio is 0.44. Surface morphology of spermathecal bulb is dense papillose form and it has rare ducts. Papillose structure is spiral form. Spermathecal channel distinct and consist many lateral muscle fibers (Figure 3).

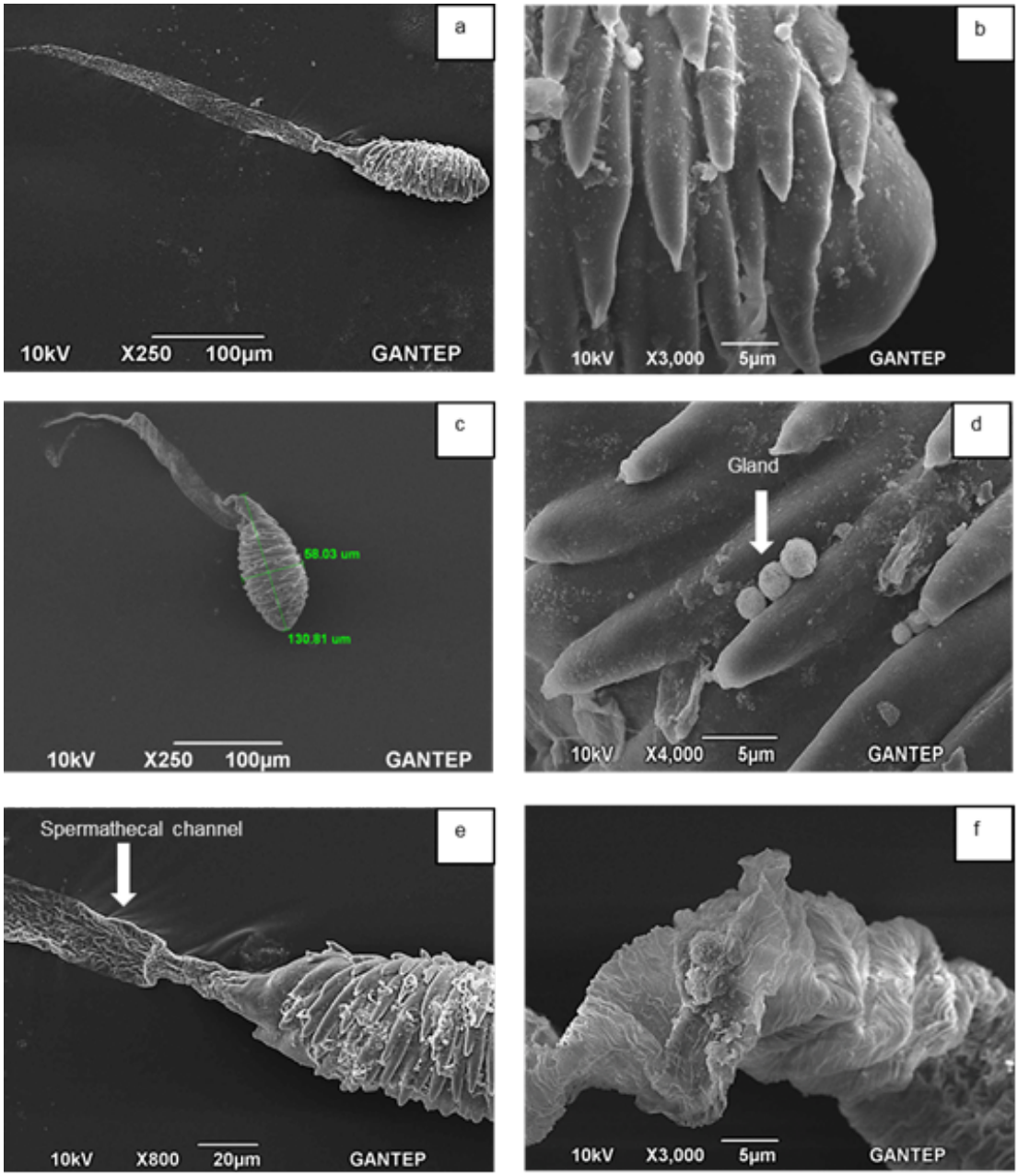

Figure 3. SEM micrographs of the spermathecae of *Euaresta bullans*: a) SEM photo of spermathecal bulb and distal flange of pump; b) apical part of spermathecal bulb; c) length/width of spermathecal bulb; d) spermathecal gland; e) spermathecal channel; and f) pumping region.

Tephritis formosa (Loew, 1844)

Spermathecae consists of three parts; spermathecal bulb, pumping region (valve) and spermathecal channel. Spermathecal bulb formed as corncob. Apical part of spermathecal bulb is oval, middle part is swollen and basal part is formed in a J-shape and connected to spermathecal channel. Size of spermathecal bulb is 63.64/330.42 (width/length, µm) and aspect ratio is 0.61. Spermathecal bulb consists dense papillose and glands clustered (Figure 4).

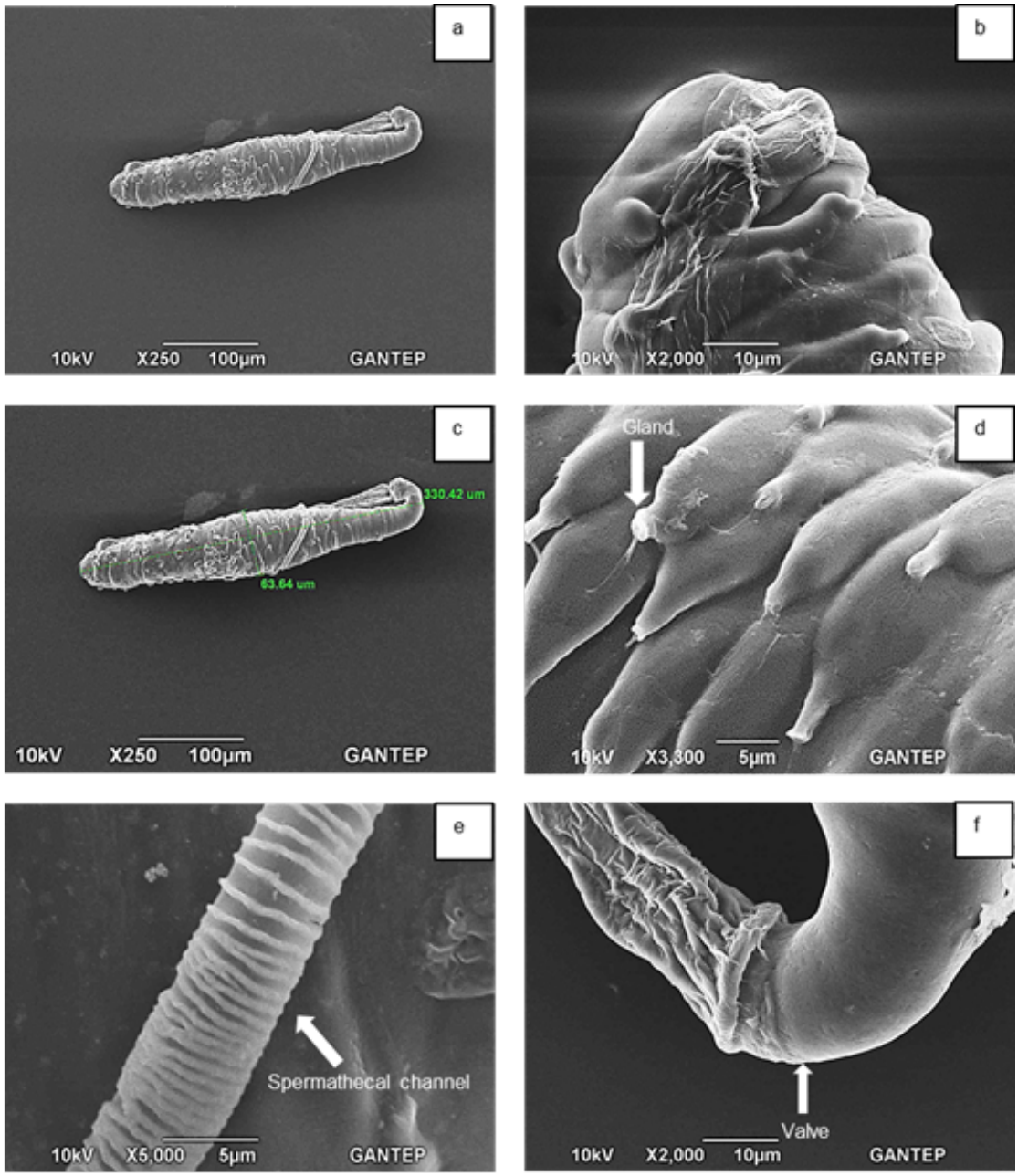

Figure 4. SEM micrographs of the spermathecae of *Tephritis formosa*: a) SEM photo of spermathecal bulb and distal flange of pump; b) apical part of spermathecal bulb; c) length/width of spermathecal bulb; d) spermathecal gland; e) spermathecal channel; and f) pumping region.

Tephritis nigricauda (Loew, 1856)

Spermathecae consist of three parts; spermathecal bulb, pumping region (valve) and spermathecal channel. Spermathecal bulb formed as corncob, there is a stenosis in its central parts towards base from ends, and it becomes thicken and then thinner towards base. General size of spermathecal bulb is 46.55/181.25 (width/length, µm) and aspect ratio 0.25. Gland canaliculus and glands extend outward from pores taking part at the end of digitate bulges. Spermathecal bulb formed in a J-shape at its base and connected to channel. Muscle fibrils in the spermathecal channel are distinct and fibrous in structure (Figure 5).

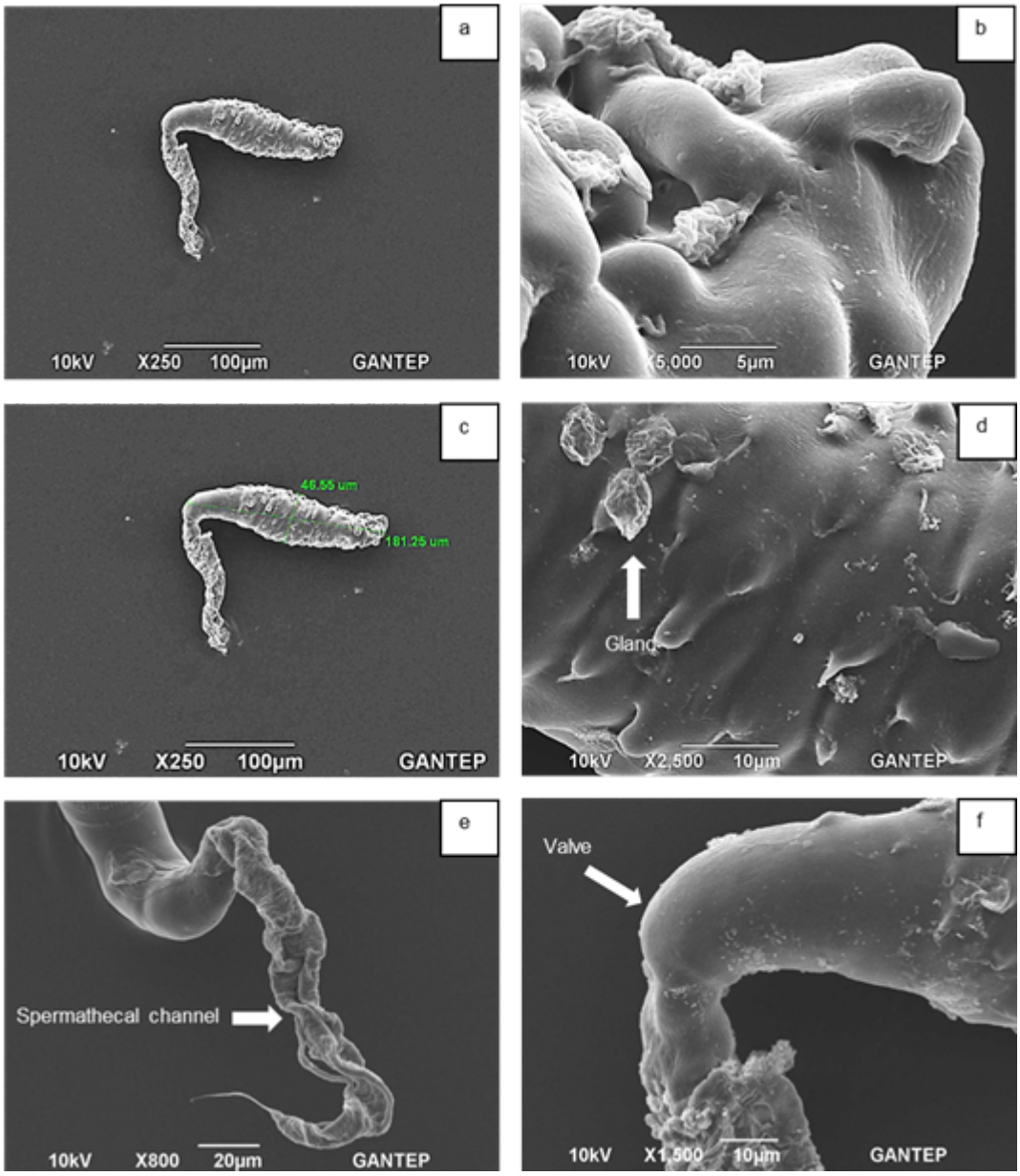

Figure 5. SEM micrographs of the spermathecae of *Tephritis nigricauda*: a) SEM photo of spermathecal bulb and distal flange of pump; b) apical part of spermathecal bulb; c) length/width of spermathecal bulb; d) spermathecal gland; e) spermathecal channel; and f) pumping region.

Discussion

Studies on the morphology of the spermathecae have increased in recent years, however, there are few studies on the spermathecal morphology of fruit flies. Knowledge of spermathecal morphology may be useful to elucidate taxonomic and phylogenetic relationships between genera of Tephritinae.

Spermathecae structures of five species were identified using by SEM micrographs. The spermathecal bulb of *Campiglossa* and *Euaresta* are pyriform and differ from *Tephritis* with this feature. In the two *Tephritis* spp., the spermathecal bulb resembles a corncob in appearance. *Euaresta bullans* clearly differs from *C. producta* and *C. tessellata* with its long papillose shape. Also, the aspect ratio of *E. bullans* was determined to be 0.44 (Table 1). In *C. producta* and *C. tessellata,* the aspect ratio was determined to be 0.61 and 0.62, respectively (Table 1). The spermathecal bulb of *C. producta* is longer than that of *C. tessellata*, being 124.83 and 90.70 µm, respectively. *Campiglossa producta* clearly differs from *C. tessellata* with intense papillose shape. In *C. tessellata*, papillose rare and short. The spermathecal bulb of *Tephritis formosa* has intense and long papillose shape, and *T. nigricauda* has short and rare papillose shape. Also, aspect ratio of spermathecal bulb was determined to be 0.19 and 0.25 in *T. formosa* and *T. nigricauda,* respectively. The spermathecal bulb of *T. formosa* is longer than that of *T. nigricauda* being 330.42 and 181.25 µm, respectively (Table 1).

Based on these observations, it is evident that spermathecae morphology is useful for the identification of species and genera of Tephritinae; especially the size of spermathecal bulb for species and the aspect ratio of the spermathecal bulb for genera. Therefore, the findings of this study make a significant contribution by demonstrating characters that can be used to distinguish similar species and genera.

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