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Morphology and ultrastructure of the male accessory gland of *Spilostethus pandurus* (Scopoli) (Hemiptera: Lygaeidae)

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Abstract

Spilostethus pandurus is a phytophagous bug, which is widely distributed in tropical and subtropical areas. The reproductive system of the males of *Spilostethus pandurus* includes two testes, two seminal vesicles, two vasa deferentia, and one pair of accessory glands. The male accessory glands of *Spilostethus pandurus* are termed mesadenia and open into the vas deferens. The accessory glands of males (mesadenia) take an irregular shape like a sack (with multiple lobes). The mesadenia accessory glands comprise one mono-epithelial layer of secretory cells lying on a thin basal lamina and encompassed in a muscular layer. This research describes the structure and ultrastructure of the accessory glands of males using scanning and transmission electron microscopy.

Keywords: *Spilostethus pandurus*; mesadenia accessory gland; SEM; TEM

Introduction

Hemiptera is a broadly spread group of insects with economic significance because it contains predators (beneficial insects) in addition to pests (harmful ones) (**Schuh and Slater, 1995**). Hemiptera bugs are one of the significant pests that infest the seeds of numerous plants and have long been established in Egypt (**Schaefer and Panizzi, 2000**). Lygaeidae is phytophagous with several agricultural pests. *Spilostethus pandurus (S. pandurus)* (milkweed bug) spreads in subtropical and tropical places. It may result in severe harm (**Kugelberg, 1973**). Additionally, *S. pandurus* infests several crops, such as sunflower seeds, sesame, pecans, wheat, cantaloupe, squash, and watermelon (**Thangavelu, 1979**).

The hemipteran insect's male reproductive system is composed of two testes, two vasa deferentia, a medium ejaculatory duct (ED) that opens in the aedeagus, and accessory glands (**Chapman, 2013**; **Adams, 2001**; **Lemos *et al.*, 2005**). In the reproductive system of males, accessory glands aid in transporting sperm to the females (**Robert and Hiromu, 1984**). All stages of the mated females' reproductive biology, from the sperm getting inserted in the reproductive tract to laying the eggs, are affected by the compounds secreted by the accessory glands (**Gillott, 2003**). Those influences relate to sperm storage, activation, and protection; competition between sperms; female behavior; fecundity; oviposition; ovulation; protection of deposited eggs (**Friedel and Gillot, 1977**; **Chen, 1984**; **Gillott, 2003**).

The ectadenia and mesadenia refer to the male accessory glands, depending on whether they are of ectodermal or mesodermal origin. When insects reach

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the adult stage, the accessory glands start to work. Their secretions modify female behavior and physiology and form the spermatophore, which feeds and protects the sperm (**Chapman, 2013**).

Using a scanning and transmission electron microscope, this work attempts to demonstrate the morphology and ultrastructure of the accessory gland of male *S. pandurus*.

Materials and methods

Insect rearing and adult male reproductive system dissection

The *S. pandurus* colony was reared and maintained at the Entomology Lab, Faculty of Science, Cairo University. In 20 cm x 30 cm polyethylene cylindrical containers, the insects were maintained. At 25° ± 3°C and 65 ± 5% RH, muslin covers were used to cover all containers from the top completely. According to **Elelimy** (**2012**), *Helianthus annuus* (dried, raw sunflower seeds) were used to feed the insects.

Pins were used to fix the adult male insects to a paraffin petri dish through the pronotum region. The abdominal tergites were removed, followed by the reproductive system, by cutting longitudinally along the body on both sides. To avoid drying the internal structure, a saline solution (0.1 M NaCl, 0.1 M KCl) was applied to insects. Using a Leica EZ4D stereomicroscope, the researchers could examine and photograph the gross morphology of the reproductive system in males.

Preparing the tissues for SEM examination

For examination by scanning electron microscopy (SEM), the accessory gland samples were fixed in 2.5% glutaraldehyde (pH 7.2, phosphate buffer), rinsed three times with phosphate buffer, dehydrated in increasing concentrations of ethyl alcohol (70, 80, 90, and 100%), dried with a Polaron CPD 7501 Critical Point Dryer, and mounted with double-sided tape on SEM stubs. Next, they were coated with gold in a Polaron SC 502 sputter coater. After that, digital photos were obtained, and examining the stubs was conducted using a JEOL JSM 6060 LV SEM at 5 to 10 KV accelerating voltage.

Preparing the tissues for transmission electron microscopy

The male accessory glands of 7 days old adult insects were dissected and fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.4 at 4 °C for 2 hrs.). The samples were dehydrated in a graded ethanol series and embedded in Epoxy resin. Examining the ultra-thin sections was performed using a transmission electron microscope, JEOL (JEM-1400 TEM).

Results

According to Figure (1A), the *S. pandurus* male reproductive system comprises two testes, two seminal vesicles, two vasa deferentia, and two mesodermal accessory glands (mesadenia). After fixation, mesadenia take the shape of an irregular sack (multiple lobes) and become transparent. They change from transparent white to opaque white. They are tubular structures that envelope the posterior parts of the vasa deferentia and neighbor the seminal vesicles (Figs. 1A, 1B, 1C, 2A). The mesadenia accessory glands comprise one mono-epithelial layer of secretory cells lying on a thin basal lamina and encompassed with a thin muscular layer (Figs. 1D, 2B, 2C, 2D). The gland epithelial layer is cuboidal (Fig. 2B). The cytoplasm of secretory cells includes ribosomes that can be free or bound to the endoplasmic reticulum membranes, round nucleus, secretory vesicles, and mitochondria (Figs. 2C, 2D). The secretions of the mesadenia accessory gland appear in the lumen, as shown in Figure (2C). The mesadenia gland runs parallel to the vas deferens, as shown in figures (2E, 2F). The muscle layer between the mesadenia gland and vas deferens consists mostly of circular fibers with scattered longitudinal fibers (Fig. 2E, 2F). Vas deferens comprise a simple epithelium layer with an ovoid nucleus (Figs. 2E, 2F).

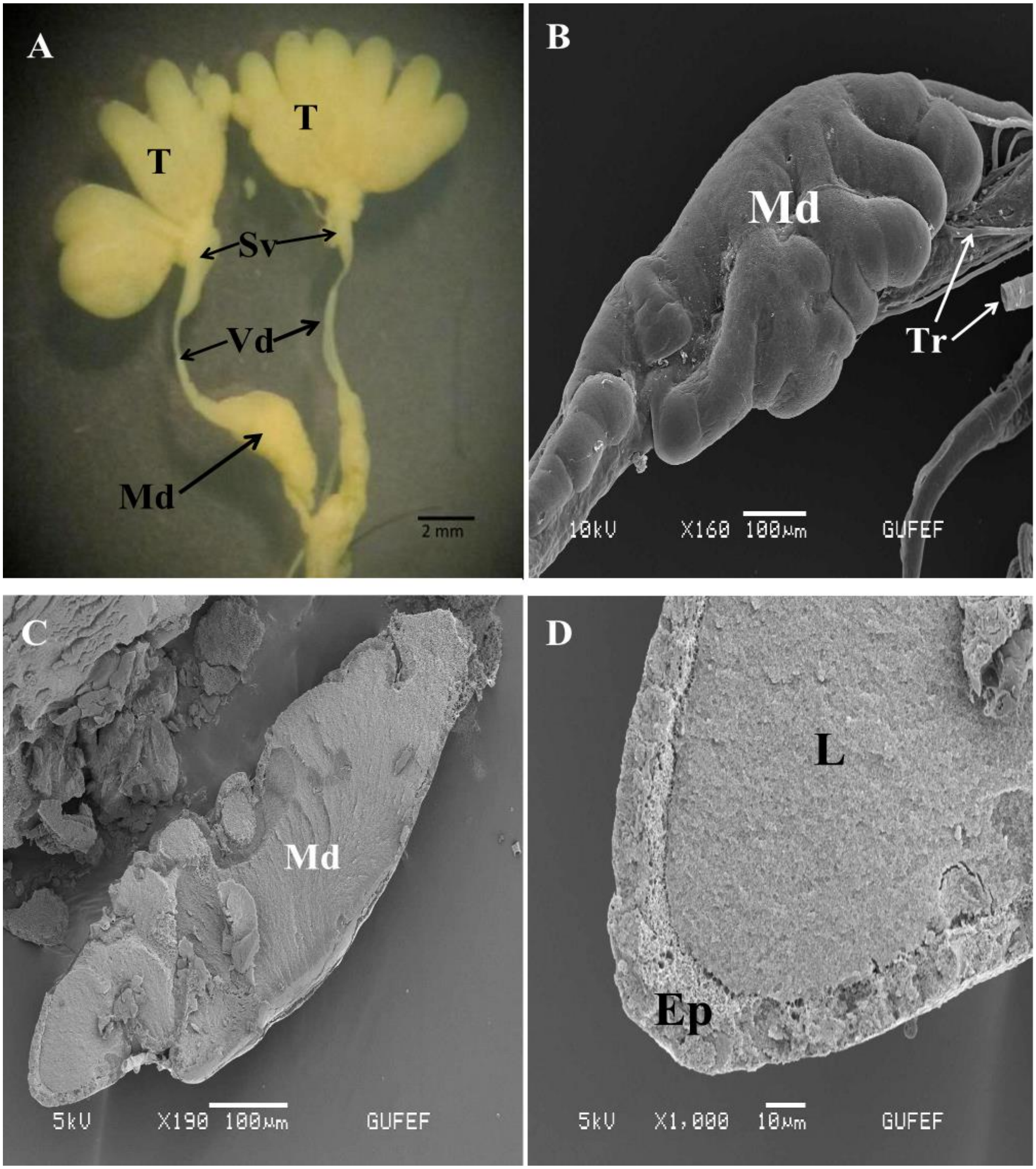

Fig. 1. Light photograph of the male reproductive system and SEM micrographs of the male accessory gland (mesadenia) of *Spilostethus pandurus* adult. A) General view of the male reproductive system showing pair of testes (T), seminal vesicle (Sv), vas deferens (Vd), and mesadenia accessory gland (Md). B) General view of mesadenia accessory gland (Md) and tracheole (Tr). C) Part of the divided mesadenia accessory gland (Md). D) High magnification of mesadenia accessory gland showing epithelial layer (Ep) and its lumen (L).

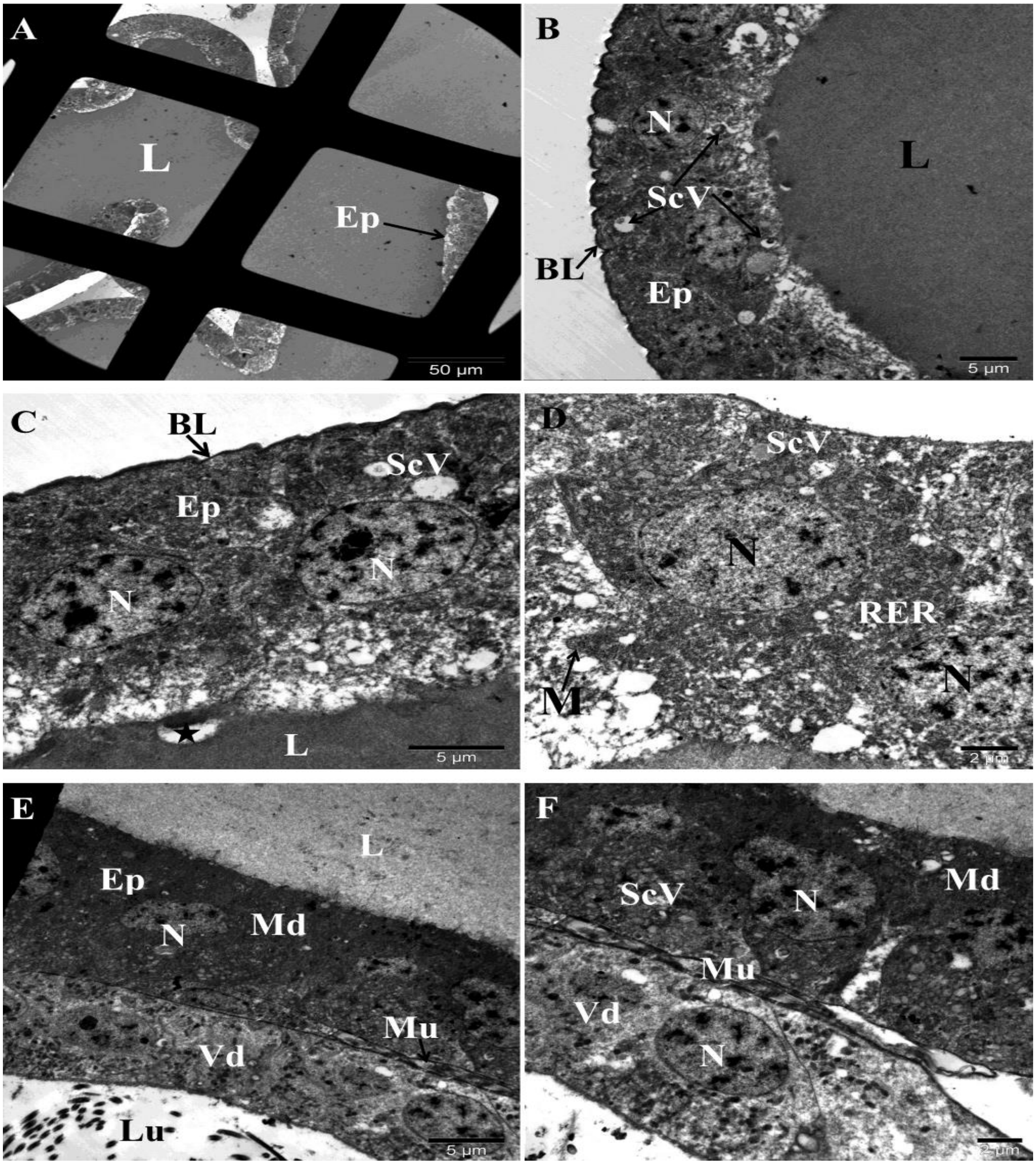

Fig. 2. TEM micrographs of the male accessory gland (mesadenia) of *Spilostethus pandurus* adult. A) General view of the internal structure of the mesadenia gland showing epithelial layer (Ep) and lumen (L). B) Higher magnification of mesadenia gland showing epithelial layer (Ep), nucleus (N), secretory vesicles (ScV), lumen (L), and basal lamina (BL). C) and D) Higher magnification of mesadenia gland epithelial layer (Ep) showing nucleus (N), secretion (star) in the lumen (L), rough endoplasmic reticulum (RER), and mitochondria (M). E) and F) Mesadenia gland (Md) epithelial layer (Ep) with part of vas deferens (Vd) epithelial layer, vas deferens nucleus (N), sperms inside vas deferens lumen (Lu), muscle layer (Mu), mesadenia secretory vesicles (ScV), mesadenia nucleus (N) and its lumen (L).

Discussion

Like other Hemiptera, *S. pandurus*' adult male reproductive system has a similar structure (**Chapman 2013**; **Adams 2001**; **Lemos *et al*. 2005**; **Bonhag & Wick 1953**; **Freitas *et al*. 2010**). Still, testes in some species can be integrated into a mono median mass testis (**Nijhout, 1998**). *Oncopeltus fasciatus* (Heteroptera: Lygaeidae) has a seminal vesicle that is located before the vas deferens, according to **Bonhag & Wick's** (**1953**) observation. The reproductive system of male *S. pandurus* (Hemiptera: Lygaeidae) also shares similarities with this observation.

According to whether they are mesodermal or ectodermal in origin, the accessory glands of males are divided into two categories. The accessory glands are known as mesadenia and open into the vas deferens if they are mesodermal in origin. Ectadenia are ectodermal in origin and open in the ejaculatory duct (**Chapman 2013**). According to **Freitas *et al.*** (**2007**), Hemiptera commonly has male accessory glands. Both ectadenia and mesadenia are found in *Nezara viridula* (Hemiptera: Pentatomidae), just like in other Pentatomidae whose reproductive systems were illustrated (**Pendergrast 1956**; **Chapman 2013**).

The ectadenia is not found in adult male *S. pandurus* reproductive system, but mesadenia is present (**Elelimy 2017**). According to **Leston** (**1953**), the Acanthosominae, which is a subfamily of the Pentatomidae, should be removed from the family because ectadenia is absent in the reproductive system of males. The accessory glands of the male *Adparaproba gabrieli* seem mesodermic in origin and are classified as mesadenia because they lack a cuticle lining (**Uceli *et al.* 2011**). They differ significantly in number, shape, size, as well as embryological origin among male insects (**Adiyodi & Adiyodi 1975**; **Leopold 1976**; **Chapman 2013**). Contributing to the seminal fluid and spermatozoa activation are two functions of these glands' secretions (**Chen 1984**). Hemipteran accessory glands produce spermatophores and certain active peptides that promote ovary activation after mating and reduce female receptivity to mating. Such secretions serve to promote sperm transfer, improve insemination, and modify the behavior of females (**Pendergrast 1956**; **Leopold 1976**; **Adams 2001**; **Kubli 2003**; **Freitas *et al*. 2007**, **2010**).

Like most insect epithelia, the secretory epithelia of accessory glands are simple or monolayers. Every secretory cell works as a separate factory for the production, storage, and export of secretory products. Rough or smooth ER and Golgi zones produce, package, and transfer produced items in membrane-bound vesicles for most of the length of the cell. Secretions release into the lumen at each cell's apex and ejaculate during copulation (**Robert and Hiromu, 1984**). According to **Alberts *et al.*** (**2004**), secretory granules, rough endoplasmic reticulum (RER), Golgi complex, and mitochondria are present in cells with high secretory activity. According to **Uceli *et al.*** (**2011**), *Adparaproba gabrieli's* male accessory glands are lined by a single layer of columnar epithelium, and their nuclei contain decondensed chromatin. However, the mesadenia accessory gland of *S. pandurus* has a cuboidal epithelial layer resting on a basal lamina, and its cytoplasm contains a round nucleus, RER, secretory vesicles, and mitochondria.

Conclusion

In this research, the reproductive system of male *S. pandurus* has a structural organization very similar to other insects. The male accessory gland of *S. pandurus* is termed mesadenia and opens into the vas deferens. The mesadenia glands comprise one single epithelium layer of secretory cells resting on a thin basal lamina. Moreover, their cytoplasm has an RER, round nucleus, secretory vesicles, and mitochondria.

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