

# Ultrastructure of male accessory glands in the scorpionfly *Sinopanorpa tincta* (Navás, 1931) (Mecoptera: Panorpidae)



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## ABSTRACT

The ultrastructure of male reproductive accessory glands was investigated in the scorpionfly *Sinopanorpa tincta* (Navás, 1931) (Mecoptera: Panorpidae) using light and transmission electron microscopy. The male accessory glands comprise one pair of mesodermal glands (mesadenia) and six pairs of ectodermal glands (ectadenia). The former opens into the vasa deferentia and the latter into the ejaculatory sac. The mesadenia consist of a mono-layered elongated columnar epithelium, the cells of which are highly microvillated and extrude secretory granules by means of merocrine mechanisms. The epithelium of ectadenia consists of two types of cells: the large secretory cells and the thin duct-forming cells. These two types of cells that join with a cuticular duct constitute a functional glandular unit, corresponding to the class III glandular cell type of Noirot and Quennedey. The cuticular duct consists of a receiving canal and a conducting canal. The secretory granules were taken up by the receiving canal and then plunged into the lumen through the conducting canal.

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## 1. Introduction

The male accessory glands of insects are abdominal gland systems associated with the reproductive system and are commonly called mesadenia if they arise from the vasa deferentia, or ectadenia if they are associated with the ejaculatory sac (Leopold, 1976; Chapman, 2013; Gullan and Cranston, 2014). These glands are both mesodermal and ectodermal in many insect orders, such as Thysanoptera (Sharga, 1933), Hemiptera (Freitas et al., 2010, 2011; Chiang et al., 2012; Özyurt et al., 2015), Coleoptera (Kölsch, 2000; Paoli et al., 2014), Lepidoptera (Lai-Fook, 1982), Mecoptera (Potter, 1938), and Diptera (Chen, 1984), but are mesodermal in Orthoptera (Marchini et al., 2009), and ectodermal in Collembola (Dallai et al., 1999), Zoraptera (Dallai et al., 2014), Hymenoptera (Gomes et al., 2012), and many other insect orders (Happ, 1984; Kaulenas, 1992; Chapman, 2013). The secretions of male accessory glands have a variety of functions, such as sperm inactivation (Harshman and Prout, 1994), sperm activation (Leopold, 1976; Chen, 1984; King et al., 2011), modification of sperm bundles (Viscuso et al., 2001), and contributions to building of spermatophores and mating plugs (Leopold, 1976; Chen, 1984; Colonello and

Hartfelder, 2005; Marchini et al., 2009; Freitas et al., 2011; Krüger et al., 2014). Moreover, these glands also have some effects on many aspects of female reproductive physiology and behavior, including eliciting egg-laying and reducing sexual receptivity (Raabe, 1986; Avila et al., 2011; Baldini et al., 2012; Naccarati et al., 2012; Hentze et al., 2013; Alfonso-Parra et al., 2014; Hayashi and Takami, 2014; Markow, 2015; Carmel et al., 2016), as in the fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae) (Wolfner, 1997; Gillott, 2003; Gligorov et al., 2013), the seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) (Yamane et al., 2015), and the moth *Spodoptera litura* (Lepidoptera: Noctuidae) (Yu et al., 2014).

The male accessory glands of insects vary not only in function, but also in size, shape, location, and number from group to group and species to species (Chen, 1984; Happ, 1984; Kaulenas, 1992; Chiang et al., 2012; Gomes et al., 2012; Marchini et al., 2012; Paoli et al., 2013; Dallai et al., 2014; Gullan and Cranston, 2014). The morphology of their epithelial cells and types of secretions also vary considerably among taxa (Lai-Fook, 1982; Dallai et al., 1999; Marchini et al., 2003, 2009; Sukontason et al., 2009; Freitas et al., 2010; Moreira et al., 2012; Krüger et al., 2014; Paoli et al., 2014; Özyurt et al., 2015). In Collembola the accessory glands are symmetrical structures flanking the ejaculatory sac, and consist of a series of secretory units that are arranged in parallel (Dallai et al.,

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1999). In Calliphoridae of Diptera a pair of slender and sac-like accessory glands opens into the ejaculatory sac, and consists of capsular cells and glandular cells surrounding the lumen (Sukontason et al., 2009). The accessory glands of Coleoptera are a pair of long S-shaped tubes opening into the ejaculatory sac, and have two types of epithelia in distal and proximal regions, respectively (Krüger et al., 2014). Morphological studies of male accessory glands across species may provide valuable information on the physiological functions of these glands and the types of secretions (Chen, 1984; Kaulenas, 1992). However, structural comparisons between the mesadenia and ectadenia have not been conducted for the male accessory glands in Mecoptera so far (Willmann, 1987, 1989; Penny, 2006).

Panorpidae is the most species-rich family in Mecoptera (Penny and Byers, 1979) with their adults commonly called scorpionflies because the ninth abdominal segment of their males are enlarged and recurved upward to form a genital bulb, which superficially resembles the stinger of scorpions. The male accessory glands exhibit evidently morphological diversity among the genera of Panorpidae (Miyaké, 1913; Potter, 1938; Grell, 1942). The studies of male accessory glands in Panorpidae have been mainly concentrated on morphological and histological aspects to date. An ultrastructural study has been only involved in one species, *Dicerapanorpa magna* (Chou in Chou et al., 1981) (Mecoptera: Panorpidae) (as *Panorpa* in Xie and Hua, 2010; Zhong and Hua, 2013).

The objective of this study was to investigate the ultrastructure of the male accessory glands in the scorpionfly *Sinopanorpa tincta* (Navás, 1931; Cai et al., 2008) using light and transmission electron microscopy, focusing on the ultrastructural comparison between the mesodermal and ectodermal accessory glands.

## 2. Material and methods

### 2.1. Specimen collection

The male adults of *S. tincta* (Navás, 1931) were captured in the Taibaishan National Nature Reserve (34°09'N, 107°70'E, elev. 1140 m), Qinling Mountains, Shaanxi Province, central China in early July 2016. Live adults were reared in plastic jars filled with 4–5 cm of humid soil to keep humidity (Jiang and Hua, 2013; Jiang et al., 2014).

### 2.2. Light microscopy (LM)

Live male adults were anesthetized with diethyl ether, and their reproductive system was immediately dissected in cold Ringer's solution (Xie and Hua, 2010) under a Nikon SMZ168 stereomicroscope (Nikon, Tokyo, Japan). Pictures were taken with a QImaging Retiga 2000R Fast 1394 Digital camera (QImaging, Surrey, Canada) equipped on the microscope and were stacked with Syncroscope Auto-Montage software.

### 2.3. Transmission electron microscopy (TEM)

The dissected accessory glands were first fixed with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffered saline (PBS, pH 7.2) for 12 h at 4 °C, rinsed ten times in PBS. The samples were post-fixed with 1% osmium tetroxide (OsO<sub>4</sub>) in PBS for 1 h at 4 °C, rinsed ten times in PBS, and dehydrated through a graded ethanol series (30%, 50%, 70% for 10 min each, 80% for 15 min, 90% for 20 min, and 100% for 30 min twice). Then the samples were infiltrated with the graded mixture of acetone and Epon 812 resin (3:1 for 2 h, 1:1 for 4 h, and 1:3 for 12 h), subsequently with Epon 812 resin for 24 h twice. The samples were eventually embedded in pure Epon 812 resin, polymerized at 30 °C for 24 h and 60 °C for 48 h.

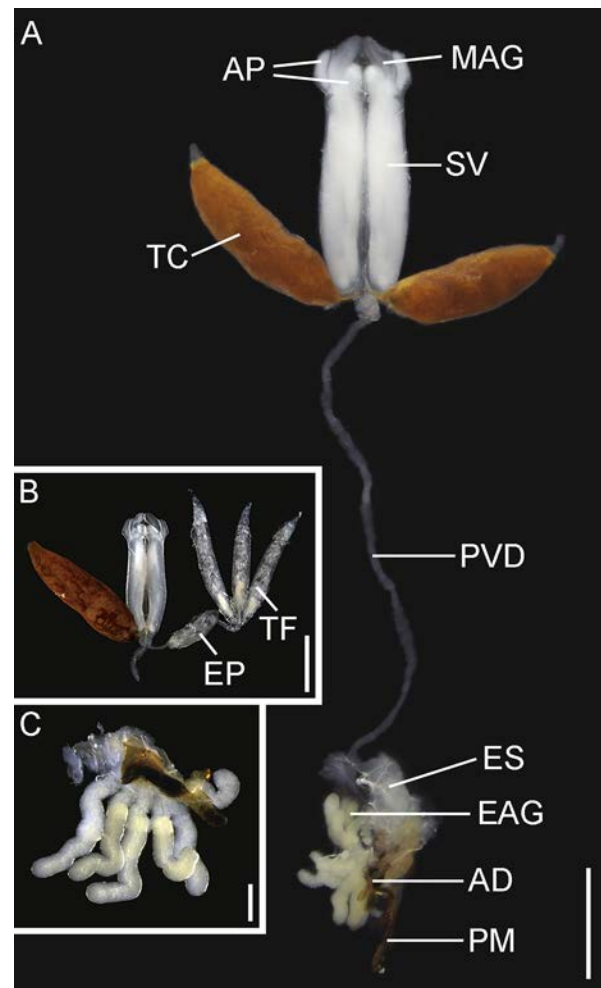
The polymerized samples were cut into ultrathin sections of 60 nm with a glass knife on a Leica ULTRACUT ultramicrotome (Leica, Nussloch, Germany). After being double stained with uranyl acetate and lead citrate, the ultrathin sections were examined in a Hitachi HT7700 transmission electron microscope (Hitachi, Tokyo, Japan) at 80 kV.

## 3. Results

### 3.1. Gross morphology of the male reproductive system and accessory glands

The male reproductive system of *S. tincta* mainly comprises a pair of testes, a pair of long vasa deferentia, and an ejaculatory sac (Fig. 1A). The male reproductive gland complexes consist of a pair of mesadenia and six pairs of ectadenia. The former opens into the vasa deferentia and the latter into the ejaculatory sac.

Each testis comprises three tubular testicular follicles, which fuse at the distal end and open basally into the end of the thin vas deferens. The vas deferens is highly coiled for its distal part to form an epididymis, and greatly expanded for its middle part to form a



**Fig. 1.** Light micrographs of the male reproductive system of *Sinopanorpa tincta*. (A) Gross morphology. (B) Testicular capsule of one side has been removed to show three testicular follicles and epididymis. (C) A magnification of the ectodermal accessory glands. AD, aedeagus; AP, appendices; EAG, ectodermal accessory gland; EP, epididymis; ES, ejaculatory sac; MAG, mesodermal accessory gland; PM, paramere; PVD, postvesicular vas deferens; SV, seminal vesicle; TC, testis-epididymis complex; TF, testicular follicle. Scale bars: (A) and (B) = 1 mm; (C) = 0.2 mm.

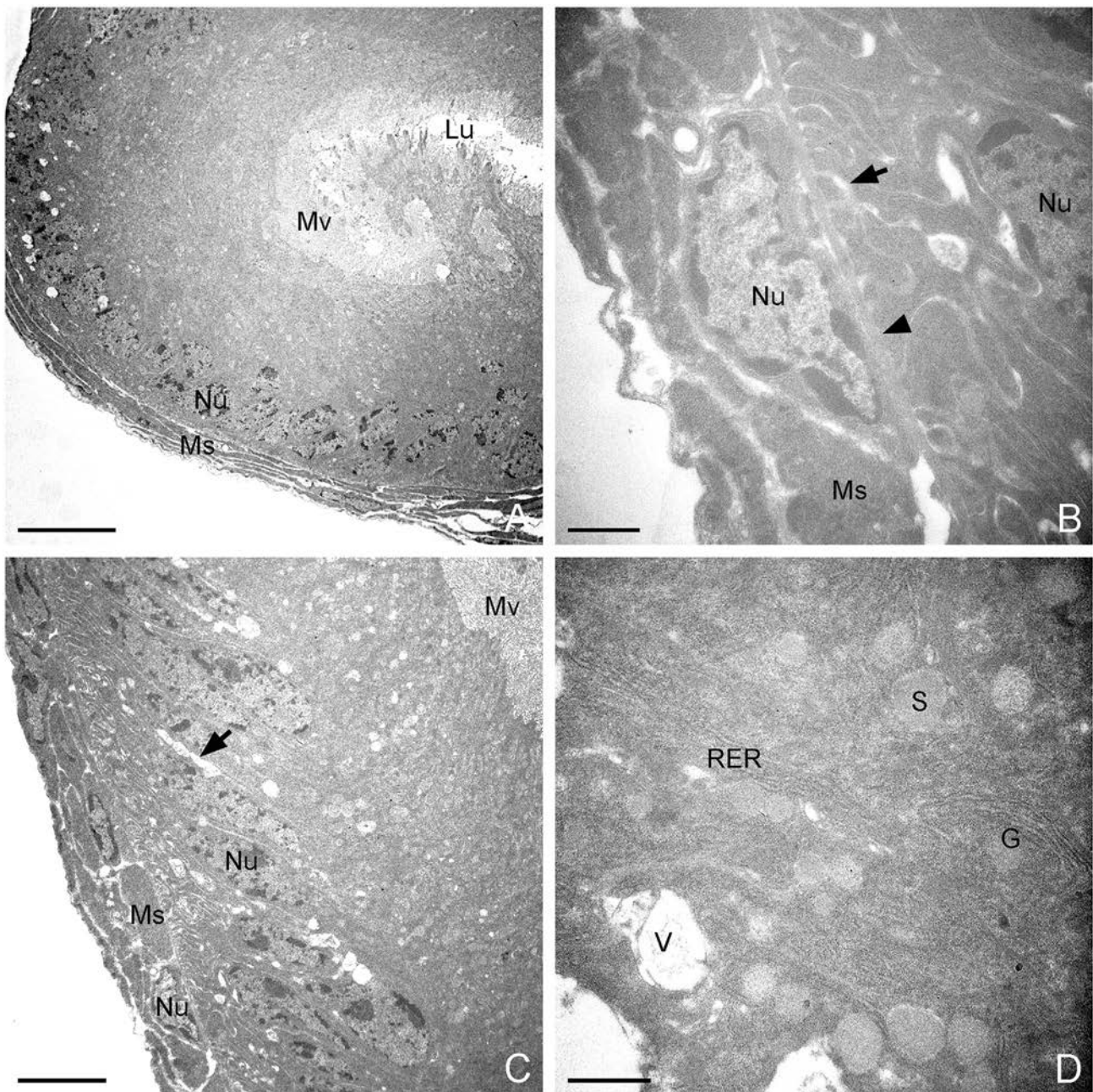


well-developed seminal vesicle. The epididymis and the three testicular follicles are enclosed together by a common brown peritoneal sheath to form a testis-epididymis complex (Fig. 1B). The vas deferens runs forward along the ventral line of the seminal vesicle for a short distance after leaving the sheath, and enters the seminal vesicle at the anterior end of the latter. On the anterior end of the seminal vesicles arise a pair of mesodermal accessory glands (mesadenia) and two pairs of appendices. The seminal vesicles posteriorly open into the thick postvesicular vasa deferentia, which eventually run into the anterior part of the ejaculatory sac (Fig. 1A).

The mesadenia are a pair of whitish auriform tubular secretory tubules, which adhere to each other on the distal ends (Fig. 1A). The tubules are closely adherent to the ventral side of the testes,

dorsally opening into the anterior end of the seminal vesicles. They vary in length from 300 to 500  $\mu\text{m}$ , with a diameter of approximately 150  $\mu\text{m}$ .

The ectadenia consist of six pairs of whitish secretory tubules symmetrically on each side of the ejaculatory sac (Fig. 1C). Some secretory tubules are occasionally bifurcated for a variable length at the distal ends. They are simple blind tubules with the basal end open into the lumen of the ejaculatory sac, and the distal end free in the genital bulb. The tubules of the ectadenia can be categorized into two equal groups by length. The shorter tubules (mean  $\pm$  SD = 543.90  $\pm$  65.46  $\mu\text{m}$ ,  $n = 9$ ) and the longer tubules (811.84  $\pm$  75.17  $\mu\text{m}$ ,  $n = 9$ ) are uniform in the trunk diameter (108.92  $\pm$  8.08  $\mu\text{m}$ ,  $n = 18$ ).



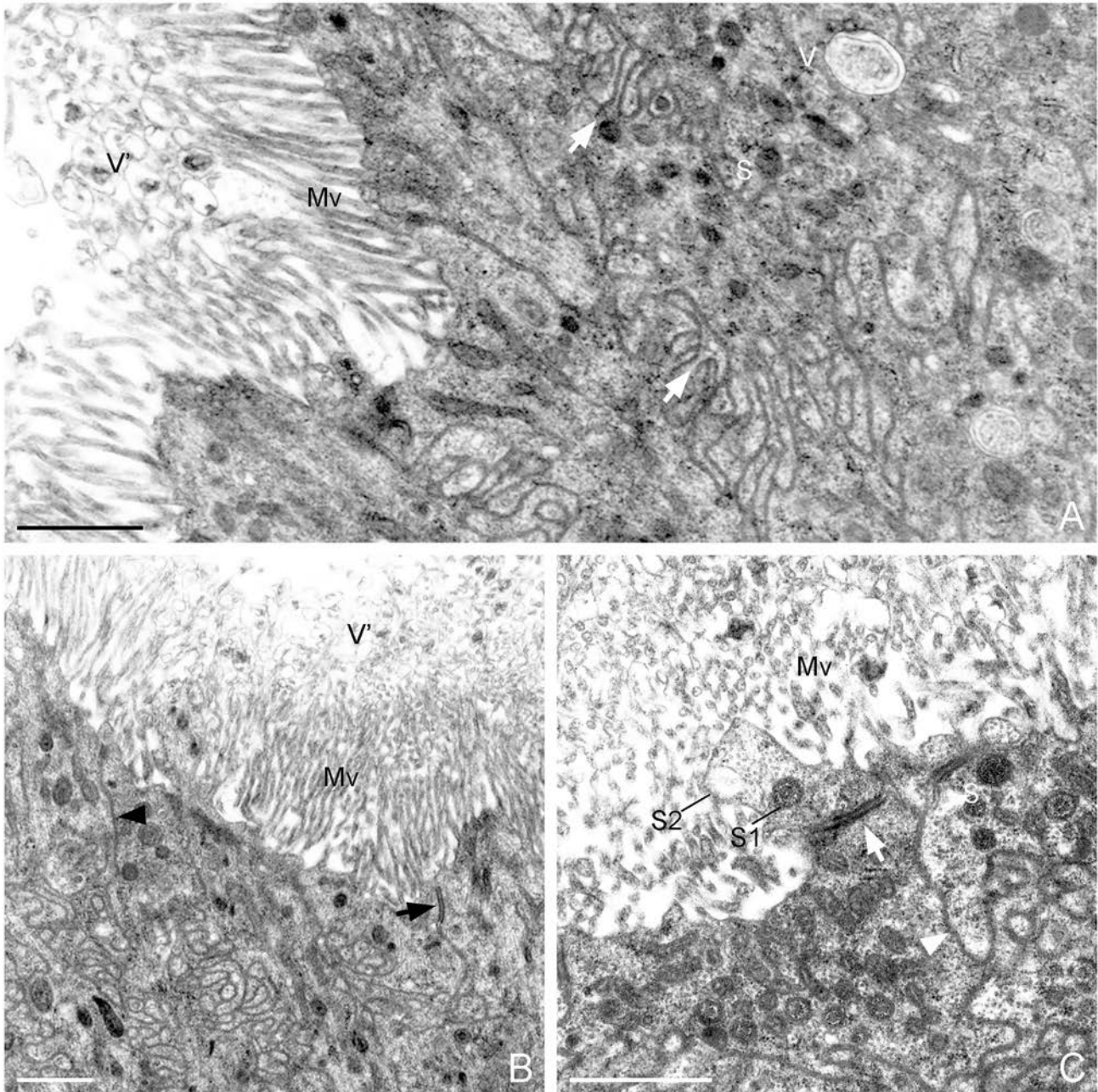
**Fig. 2.** TEM micrographs of the mesodermal accessory glands of *Sinopanorpa tinctoria*. (A) Cross-section, shows muscular and epithelial layer surrounding the small central lumen (Lu). (B) Nuclei (Nu) are present in the muscular layer (Ms). Arrow shows the intracellular spaces of the epithelial cells. Arrowhead shows the basal lamina. (C) In the basal region of the epithelium the plasma membranes expands out toward the periphery (arrow). (D) The intermediate region of epithelium. G, Golgi complex; Ms, muscular layer; Mv, microvilli; Nu, nucleus; RER, rough endoplasmic reticulum; S, secretory granule; V, vesicle. Scale bars: (A) = 10  $\mu\text{m}$ ; (B) and (D) = 1  $\mu\text{m}$ ; (C) = 5  $\mu\text{m}$ .



### 3.2. Ultrastructure of the mesadenia

The mesadenia consist of a developed muscular layer and an epidermal layer, resting on a basal lamina (Fig. 2A). In the muscular layer, nuclei can be occasionally seen distributed in the muscle bundles (Fig. 2B). Near the basal region, the limiting plasma membranes of epithelium cells display extended intracellular space (Fig. 2B and C). The columnar secretory epithelial cells exhibit straight courses, and are pressed closely together (Fig. 2A). They occupy most of the volume of the mesadenia, which have a relatively small lumen. Nuclei occupy the basal region of the epithelial

cells in a uniform line (Fig. 2C). Abundant rough endoplasmic reticulum, Golgi complexes, electron-translucent vesicles, and electron-dense granules are present in the intermediate region (Fig. 2D). The plasma membranes of the epithelial cells are of irregular shape and have numerous infoldings (Fig. 3A). Pronounced septate junctions and zonulae adherentes are observed along the cells in the apical region (Fig. 3B). Along the apical surface of the cells, microvilli release fiber-bounded vesicles occasionally (Fig. 3A and B). Membrane-bound secretory vesicles secrete their secretions through numerous long packed aligned microvilli in an exocytotic process (Fig. 3B and C).



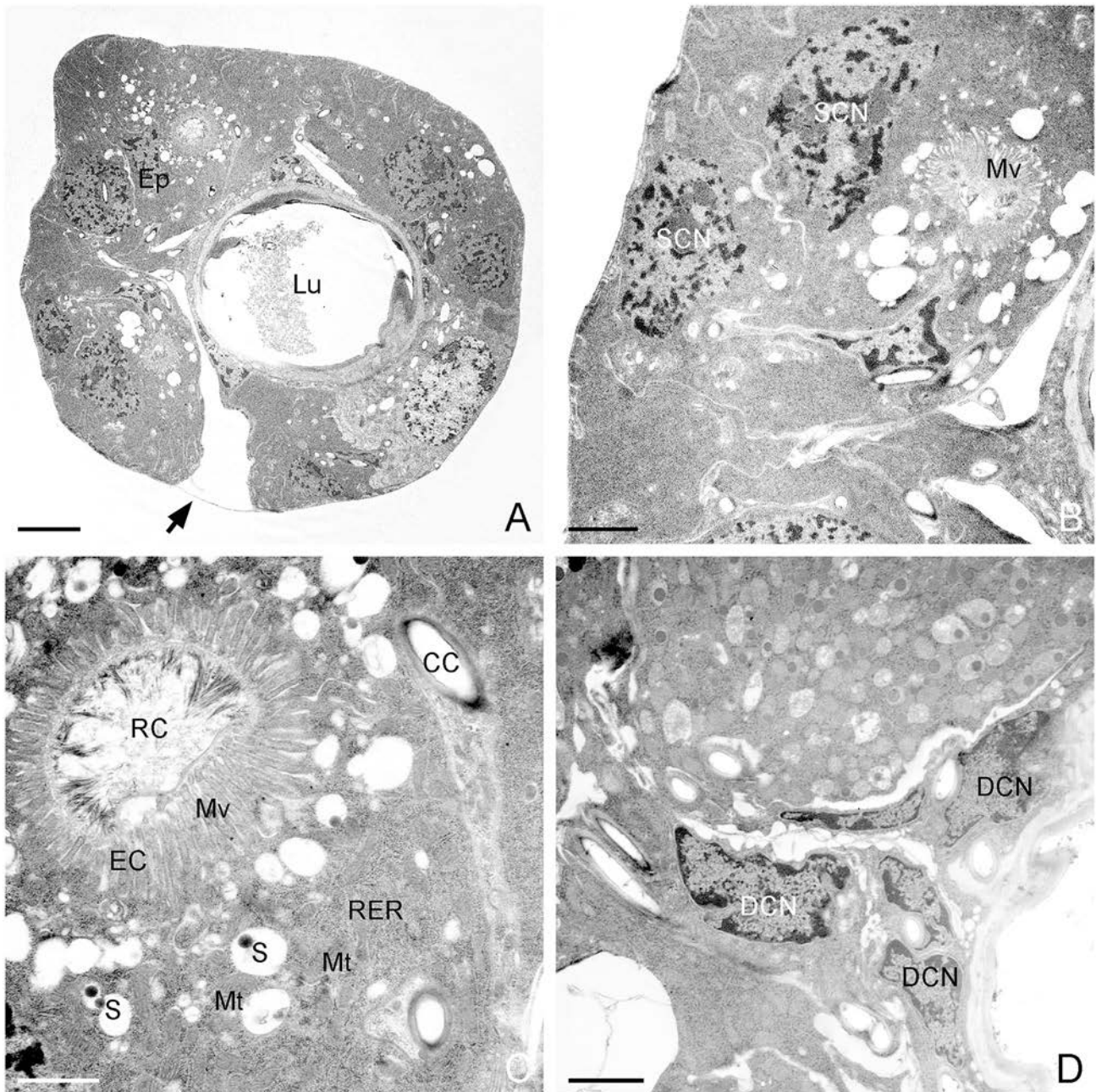
**Fig. 3.** TEM micrographs of the mesodermal accessory glands of *Sinoponorpa tinctoria*. (A) Infolding (arrows) of the plasma membrane. Secretory vesicles (V) and secretory granules (S) scattered in the intermediate region. (B) The epithelial cells are microvillated. Septate junction (arrowhead) and zonula adherens (arrow) in the apical region. (C) The various stages involved in merocrine secretion. First the membrane-bound secretory granule close to the cell membrane (S1), then the membrane of granule fuses with the outer cell membrane with contents of granule secreted into the lumen (S2). Arrowhead points at a septate junction. Arrow points at a zonula adherens. Mv, microvilli; S, secretory granule. V', fiber-bounded vesicle. Scale bars = 1  $\mu$ m.



### 3.3. Ultrastructure of the ectadenia

The six pairs of secretory tubules have no remarkable ultrastructure differences at various regions, except for their size. The ectadenia consist of a basal lamina and a layer of epithelial cells, which surround the relatively large lumen (Fig. 4A). The epithelium of the ectadenia consists of glandular units, each of which comprises a large secretory cell, a thin duct-forming cell, and a cuticular duct, corresponding to the class III glandular cell type *sensu* Noirot and Quennedey (1974). In the outer large secretory cells, irregular nuclei occupy most part of the epithelial volume (Fig. 4B). The cytoplasm is rich in mitochondria and rough endoplasmic reticulum (Fig. 4C). In the inner thin duct-forming

cell, the nucleus is wrapped around the cuticular duct, with relatively scarce cytoplasm (Fig. 4D). The cytoplasm contains abundant mitochondria, rough endoplasmic reticulum, and Golgi complexes (Fig. 5A). The cuticular duct consists of two parts, transferring secretory granules from the outer secretory cell to the central lumen (Figs. 4C and 5B–D). The basal receiving canal (i.e. end apparatus) is surrounded by the secretory cell, and the apical conducting canal is formed by the duct-forming cell. The receiving canal, as an elongation of the conducting canal, is formed by the interrupted inner epicuticle arranged in multilayers, bounded by microvilli. The microvilli are located in the irregular extracellular cavity, run tortuous courses and exhibit a loose spongy meshwork (Figs. 4C and 5B).



**Fig. 4.** TEM micrographs of the ectodermal accessory glands of *Sinopanorpa tincta*. (A) Cross-section of a bifurcated secretory tubule. Arrow shows the basal lamina. (B) Epithelium of the gland. (C) The secretory granules (S) enter the receiving canal through the tortuous microvilli. (D) A thin duct-forming cell with a flattened nucleus. CC, conducting canal; DCN, nucleus of the duct-forming cell; EC, extracellular cavity; Ep, epithelium; Lu, lumen; Mt, mitochondrion; Mv, microvilli; RC, receiving canal; RER, rough endoplasmic reticulum; SCN, nucleus of secretory cell. Scale bars: (A) = 5  $\mu\text{m}$ ; (B) and (D) = 2  $\mu\text{m}$ ; (C) = 1  $\mu\text{m}$ .



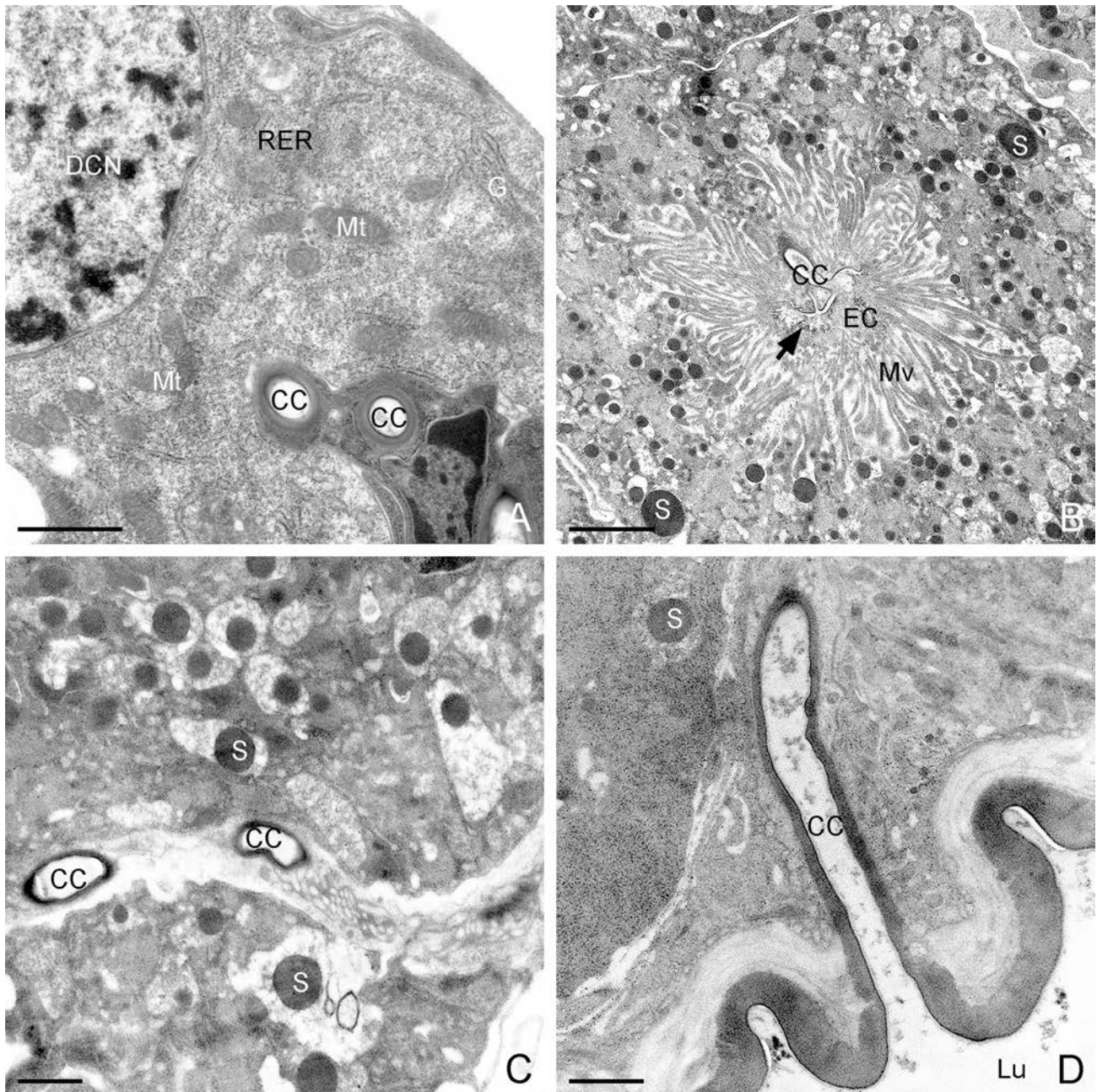
The secretions enter the extracellular cavity through the long slender microvilli (Fig. 5B). Subsequently they are taken up by the receiving canal and then plunge into the lumen through the conducting canal (Fig. 5B–D).

#### 4. Discussion

In Mecoptera previous studies are mainly focused on the gross morphology of the male accessory glands in five families (Choristidae, Boreidae, Meropeidae, Bittacidae, and Panorpidae) (Steiner, 1937; Potter, 1938; Setty, 1940; Grell, 1942; Cooper, 1972). Panorpidae bear relatively small mesodermal accessory glands in contrast with the elongate glands in Bittacidae (Potter, 1938; Grell,

1942). The long ectodermal accessory glands of *S. tincta* are also prominently different from the short glands in Bittacidae. Ectadenia are absent in males of Choristidae, Boreidae, and Meropeidae (Potter, 1938).

The auricular mesadenia of male *S. tincta* are similar in shape and ultrastructure to the mesadenia in *D. magna* (Mecoptera: Panorpidae) (Xie and Hua, 2010). The cells of these glands are rich in rough endoplasmic reticulum and Golgi complexes. The presence of extensive rough endoplasmic reticulum indicates that proteinaceous secretions are produced (Mercer and Brunet, 1959; Berry, 1968; Kölsch, 2000; Courrent et al., 2008; Šobotník et al., 2014; Filimonova, 2016). The Golgi complex is in close association with rough endoplasmic reticulum, implying that the secretions contain



**Fig. 5.** TEM micrographs of the ectodermal accessory glands of *Sinopanorpa tincta*. (A) Organelles of the duct-forming cell. (B) The cuticular duct consists of a conducting canal (CC) and a receiving canal (RC). Arrow shows the multilayer of the receiving canal interrupting inner epicuticle. (C) Secretory granules being taken up by the receiving canal. (D) A cuticular duct connects the inner intima. CC, conducting canal; DCN, nucleus of the duct-forming cell; EC, extracellular cavity; G, Golgi complex; Lu, lumen; Mt, mitochondrion; Mv, microvilli; RER, rough endoplasmic reticulum; S, secretory granule. Scale bars: (A), (C) and (D) = 1  $\mu$ m; (B) = 2  $\mu$ m.

glycoprotein material (Berry, 1968; Quennedey, 1998). Based on the ultrastructural characters, the route of secretion can be inferred from the rough endoplasmic reticulum through Golgi complex to the cell surface, along with fusing with the cell membranes by means of exocytotic secretion. This pathway of the secretory granules is very common in insect glands (Berry, 1968; Stay and Coop, 1974; Lai-Fook, 1982; Happ, 1984; Fausto et al., 1997; Afshar et al., 2013; Ma et al., 2013; Schierling and Dettner, 2013).

The ectadenia are more complex in structure and secretory activity than the mesadenia. The epithelial structure of the ectadenia is characterized by developed secretory cells, thin canal cells, and cuticular ducts, corresponding to Class III glandular cells *sensu* Noirot and Quennedey (1974). The secretory cells are rich in mitochondria and rough endoplasmic reticulum, indicating an active biosynthetic secretory activity. Similar to the epithelium of mesadenia, the secretory cells have extensive rough endoplasmic reticulum associated with proteinaceous secretions. Compared with the pheromone-producing cells or the defensive secreting cells, the secretory cells of the ectadenia lack smooth endoplasmic reticulum, indicating that the secretory cells scarcely synthesize nonproteinaceous material (Happ et al., 1966; Crossley and Waterhouse, 1969; Kölsch, 2000).

In male *S. tincta* the abdominal segments VII and VIII are greatly elongated, so that the liquid sperm need to be transferred through the elongated vasa deferentia during copulation (Xie and Hua, 2010; Shen and Hua, 2013). To ensure the success of fertilization, the spermatozoa likely need more energy and abundant seminal fluid with nutrients than the species of other insect groups. In this case, the mesadenia hardly can afford such strenuous secretory functions; eventually the seminal vesicles have been modified into a secretory organ, instead of the original sperm storage during the evolutionary course. Alternatively, the function of sperm storage has been taken over by the epididymides, the coiled distal part of the vasa deferentia (Grell, 1942; Sinclair et al., 2007; Xie and Hua, 2010; Özyurt et al., 2013a, 2013b, 2014, 2015). Eventually, the mesadenia are likely reduced gradually in size.

In contrast to the small mesadenia, the ectadenia of *S. tincta* are well-developed. The developed ectadenia may associate with the presence of the sperm pump. The piston of the sperm pump acts as a reciprocating plunger waving at high frequency during copulation (Hünefeld and Beutel, 2005; Shen and Hua, 2013). To prevent the sperm pump from being damaged, the ectadenia need to generate secretions that serve as lubricant. The specific secretions and their functions in copulation of scorpionflies need further research.

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