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

Qi-Hui Lyu, Bao-Zhen Hua\*

State Key Laboratory of Crop Stress Biology for Arid Areas, Key Laboratory of Plant Protection Resources and Pest Management of the Education Ministry, Entomological Museum, Northwest A&F University, Yangling, Shaanxi 712100, China

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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.,

\* Corresponding author.

E-mail address: [huabzh@nwfau.edu.cn](mailto:huabzh@nwfau.edu.cn) (B.-Z. Hua).

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.

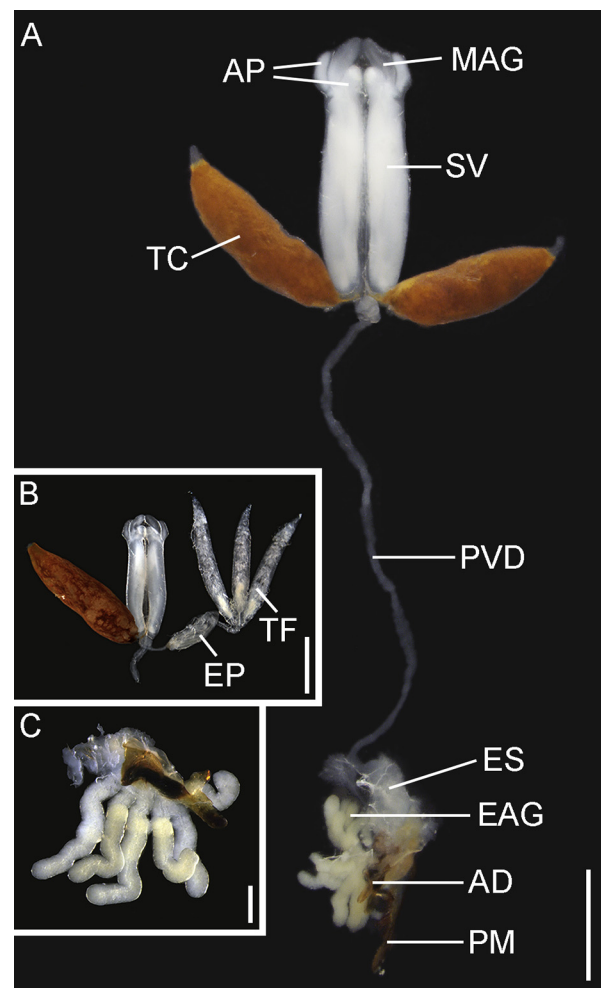
The polymerized samples were cut into ultrathin sections of 60 nm with a glass knife on a Leica ULTRACUT ultramicrotome (Leica, Nussloch, Germany). After being double stained with uranyl acetate and lead citrate, the ultrathin sections were examined in a Hitachi HT7700 transmission electron microscope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

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