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ORIGINAL ARTICLE

Ultrastructure of the vasa deferentia of *Terrobittacus implicatus* and *Cerapanorpa nanwutaina* (Insecta: Mecoptera)

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Abstract

The fine structures of vasa deferentia and postvesicular vasa deferentia were investigated in the hangingfly *Terrobittacus implicatus* (Cai et al. 2006) and the scorpionfly *Cerapanorpa nanwutaina* (Chou 1981) using light and transmission electron microscopy, and schematic diagrams were drawn accordingly. The vasa deferentia of both species comprise muscular layers, a basal lamina, and a mono-layered epithelium, but the postvesicular vasa deferentia contain muscular layers, a basal lamina, a single-layered epithelium, a subcuticular cavity, and an inner cuticle respectively. The vas deferens releases secretions into the lumen directly, probably by means of merocrine production. On the contrary, the cells of the postvesicular vas deferens correspond to class I glandular cells, discharging secretions into the subcuticular cavity first, and then into the lumen through an inner cuticle. The epithelium in both structures of Bittacidae is well developed and contains more microvilli, organelles, and more types of secretions than in Panorpidae. In Panorpidae, the spine of the postvesicular vas deferens may serve as a barricade for the reflow of the sperm and to protect the extraordinarily long structure from being collapsed or injured.

Keywords Vas deferens · Male reproductive system · Morphology · Bittacidae · Panorpidae

Introduction

The vasa deferentia of insects refer to the tubes leading from the testes and usually open to the ejaculatory duct, transporting and providing nutrients for sperm (Snodgrass 1935; Chapman 2013). They are primarily a pair of simple tubes, mainly consisting of a thick epithelium, a basement membrane, and a muscular coat, from inner luminal surface to outer basal surface (Bissell 1937; Riemann and Giebultowicz 1992). The vas deferens performs functions of storing sperm and regulating transport of semen in most insects (Brito et al. 2012; Özyurt et al. 2015), influencing the semen composition and sperm development in the Mediterranean flour moth *Ephestia kuehniella* (Riemann and Thorson 1976) and contributing to form a spermatophore in the ground beetle *Pterostichus nigrita* (Krüger et al. 2014) and the blister beetle *Lytta nuttalli* (Gerber et al. 1971).

The vas deferens is usually of the mesodermal origin (Riemann and Thorson 1976; Karakaya et al. 2012; Özyurt et al. 2013) but may more or less be ectodermal in the posterior part in Ephemeroptera (Brito et al. 2011), some Coleoptera (Paoli et al. 2014), and Diptera (Friele 1930; Snodgrass 1935). The bilateral ducts unite with the median ejaculatory duct in most insects but open separately to the exterior in Ephemeroptera and some Dermaptera (Wheeler and Krutzsch 1992; Brito et al. 2011). The vas deferens is occasionally coiled to form an epididymis anteriorly (Sinclair et al. 2007), but is frequently dilatated posteriorly to from a sperm-storage organ, or seminal vesicle (Dallacqua and Landim 2003; Zhang et al. 2016). Both the epididymis and seminal vesicle are absent in some insects (Huang et al. 2007; Wieczorek and Świątek 2008; Paoli et al. 2014), but they both are present in Mecoptera (Xie and Hua 2010).The vas deferens has only been investigated in *Panorpa liui* at the histological level (Shen and Hua 2013). However, the ultrastructure of vas deferens has not been documented in Mecoptera.

Panorpidae and Bittacidae are the two most species-rich families in Mecoptera, a small order in Antliophora of

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Holometabola (Penny and Byers 1979; Byers and Thornhill 1983). The males have long abdominal segments and maintain a V-shaped mating position in most species of Panorpidae (Byers and Thornhill 1983; Zhong et al. 2015) but have relatively short abdominal segments and sustain a belly-to-belly mating position in Bittacidae (Setty 1940; Chen et al. 2013; Gao and Hua 2013). Correspondingly, the vas deferens of Panorpidae is longer than that of Bittacidae (Miyaké 1913; Potter 1938; Grell 1942). However, the ultrastructural differences of the vas deferens between the two families have not been elucidated to date. Therefore, we investigated and compared the ultrastructures of the vasa deferentia of the hangingfly *Terrobittacus implicatus* (Cai et al. 2006; Tan and Hua 2009) (Bittacidae) and the scorpionfly *Cerapanorpa nanwutaina* (Chou 1981) (Chou 1981) (Panorpidae) using light and transmission electron microscopy.

Material and methods

Specimen collection

Adults of *T. implicatus* were captured from the Liping Forest Park (32°51′N, 106°35′E, elev. 1300 m) in the Micang Mountains, China in July 2016. Adults of *C. nanwutaina* were collected from the Huoditang Forest Farm (33°25′N, 108°27′E, elev. 1600 m) in the Qinling Mountains, China in June 2016.

Light microscopy

Live adults were anesthetized with diethyl ether, and the abdomen was fixed in Ringer's solution (Lyu et al. 2018). The male reproductive systems were dissected under a Nikon SMZ168 stereomicroscope (Nikon, Tokyo, Japan). Photographs were taken with a QImaging Retiga 2000R Fast 1394 digital camera (QImaging, Surrey, Canada) mounted on the microscope. Schematic diagrams were drawn with Adobe Illustrator CS4 (Adobe, San Jose, CA, USA).

Transmission electron microscopy

The vasa deferentia can be divided into three parts (the apical, middle, and basal parts) to compare their structures. The dissected vasa deferentia were fixed in a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffered saline (PBS, 0.1 M, pH 7.2) at 4 °C. The fixed samples were rinsed ten times with PBS, post-fixed with phosphate-buffered 1% osmium tetroxide (OsO4) for 1 h at 4 °C, and rinsed ten times in PBS. After dehydration with graded acetone series, the specimens were infiltrated with three mixtures of acetone and Epon 812 resin (3:1 for 2.5 h, 1:1 for 4.5 h, and 1:3 for 12 h) and pure Epon 812 resin for 24 h twice. The samples were embedded in Epon 812 resin and polymerized at 30 °C for 24 h and 60 °C for 48 h.

Ultrathin sections (60 nm) were cut with a diamond knife on the Leica ULTRACUT ultramicrotome (Leica, Nussloch, Germany), double stained with uranyl acetate for 10 min and lead citrate for 8 min, and observed in a Tecnai G2 Spirit Bio Twin transmission electron microscope (FEI, Hillsboro, USA).

Results

Gross morphology of the male reproductive system

The gross structures of the male reproductive system are similar between *T. implicatus* and *C. nanwutaina*, both consisting mainly of a pair of testes, a pair of vasa deferentia, and an ejaculatory duct (Figs 1 and 2). Each testis consists of four brown testicular follicles in *T. implicatus* (Fig. 1) but three whitish follicles in *C. nanwutaina* (Fig. 2). The testis opens into the epididymis, which is formed by a highly coiled distal portion of the vas deferens (Figs. 1 and 2). The paired testes and epididymides are enclosed by a hyaline common sheath to form a testis-epididymis complex in *T. implicatus* (Fig. 1), but the testis and the epididymis on each side are enclosed by a brown sheath in *C. nanwutaina* (Fig. 2). The paired vasa deferentia run anteriorly along the midline of the paired seminal vesicles after leaving the sheath and enter the seminal vesicles at the cephalic end of the latter (Figs. 1 and 2). The

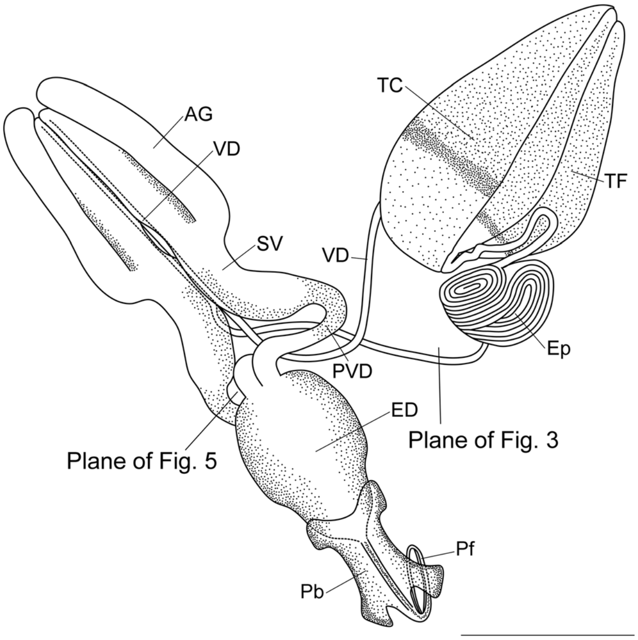

Fig. 1 Schematic diagram of male reproductive system of *Terrobittacus implicatus* with sheath of testis-epididymis complex with the right half removed. *AG* accessory gland, *ED* ejaculatory duct, *Ep* epididymis, *Pb* phallobase, *Pf*, penisfilum, *PVD* postvesicular vas deferens, *SV* seminal vesicle, *TC* testis-epididymis complex, *TF* testicular follicle, *VD* vas deferens. Scale bar 1 mm

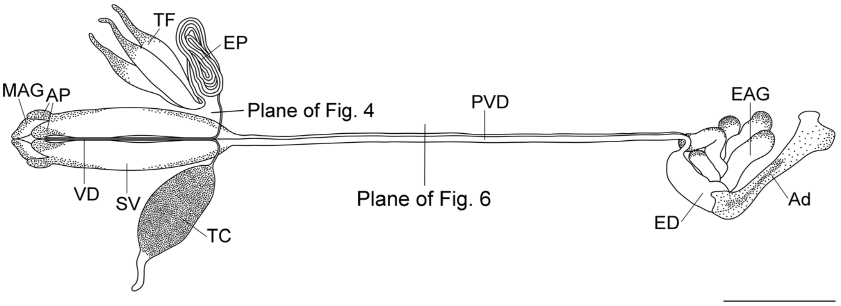

Fig. 2 Schematic diagram of male reproductive system of *Cerapanorpa nanwutaina* with the right testicular capsule removed. *Ad*, aedeagus, *AP* appendices, *EAG* ectodermal accessory gland, *ED* ejaculatory duct, *Ep* epididymis, *MAG* mesodermal accessory gland, *PVD* postvesicular vas deferens, *SV* seminal vesicle, *TC* testis–epididymis complex, *TF* testicular follicle, *VD* vas deferens. Scale bar 1 mm

seminal vesicle connects caudally to the postvesicular vas deferens, which runs posteriorly into the median ejaculatory duct.

For the accessory gland complexes, *T. implicatus* has a pair of mesadenia arising on the lateral side of the seminal vesicles (Fig. 1). The male of *C. nanwutaina*, however, possesses a pair of mesadenia and two pairs of appendices on the anterior end of the seminal vesicles, and five pairs of ectadenia on both sides of the ejaculatory duct (Fig. 2).

Ultrastructure of the vas deferens

The vasa deferentia have no significant differences among various regions at the ultrastructural level; therefore, any section can represent the vasa deferentia.

The vas deferens of *T. implicatus* consists of five muscular layers, a basal lamina, and a mono-layered epithelium (Fig. 3a). In the developed muscular layers, numerous mitochondria are scattered in the cytoplasm (Fig. 3b). The non-cellular basal lamina closely squeezes the epithelium (Fig. 3a, b). The columnar secretory epithelial cells occupy most of the volume of the vas deferens and exhibit almost straight courses (Fig. 3a). In the basal region, the cells attach to the basal lamina by hemidesmosome plaques (Fig. 3c). The nuclei occupy almost half volume of the cells in the basal and medial regions, with irregular patches of heterochromatin (Fig. 3c). The whole cytoplasm has numerous mitochondria and cisterns of rough endoplasmic reticulum (Fig. 3c–e). Short desmosomes are visible between epithelial cells in the apical region (Fig. 3e). Some electron-dense secretory granules and a few multivesicular bodies are mainly distributed in the medial and apical regions (Fig. 3d, e). Packed microvilli of various lengths are visible in the apical region with several granules dispersed among them (Fig. 3e). The granules accumulate into large ones in the central lumen (Fig. 3f).

The vas deferens of *C. nanwutaina* has a similar structure to that of *T. implicatus*. The outermost muscular layers have several irregular nuclei (Fig. 4a, b). The basal lamina loosely encloses the epithelium (Fig. 4b). The mono-layered epithelial cells are small, with cytoplasm considerably scarce (Fig. 4a). In the basal region, intercellular spaces are large between adjacent cells (Fig. 4a, b). Some flat nuclei in the medial region have small patches of heterochromatin (Fig. 4b, c). The apical cytoplasm has numerous mitochondria (Fig. 4c, d). The whole cells contain some rough endoplasmic reticulum and several large electron-lucent vesicles, some of which have irregular electron-dense material (Fig. 4b–d). Some short sparse and occasionally elongated packed microvilli bound the epithelium (Fig. 4d).

Ultrastructure of the postvesicular vas deferens

The postvesicular vas deferens of *T. implicatus* consists of developed muscular layers, a basal lamina, an epithelium, a subcuticular cavity, and an inner cuticle (Fig. 5a). Abundant mitochondria and a few tracheoles are distributed in the muscle bundles (Fig. 5b). The basal lamina is infolded in most regions but smooth in a small portion (Fig. 5b, c). The epithelial cells are pressed together and exhibit straight courses (Fig. 5a). The nuclei consist of distinct nucleoli and several patches of irregular heterochromatin and occupy the basal half of the cell volume (Fig. 5c). The cytoplasm possesses abundant mitochondria mainly in the apical region and rough endoplasmic reticulum everywhere, with a few secretions (Fig. 5c, d). In the apical region, the plasma membranes have numerous infoldings and form a membranous labyrinth (Fig. 5e). Long packed microvilli exhibit a brush border (Fig. 5d, e), with some microtubules scattered (Fig. 5e). Two types of secretions are visible. Electron-dense granules are mainly distributed in the basal region near the basal lamina (Fig. 5b, c), and electron-translucent vesicles are mainly in the apical region (Fig. 5d, e). The vesicles contain more or less electron-dense material. The subcuticular cavity occupies most of the remaining volume, with abundant secretions (Fig. 5a, f). The lumen is irregular and narrow and surrounded by a thick inner cuticle (Fig. 5f).

The postvesicular vas deferens of *C. nanwutaina* has the same structure with that of *T. implicatus* (Figs. 5a and 6a). A few flat nuclei and mitochondria are

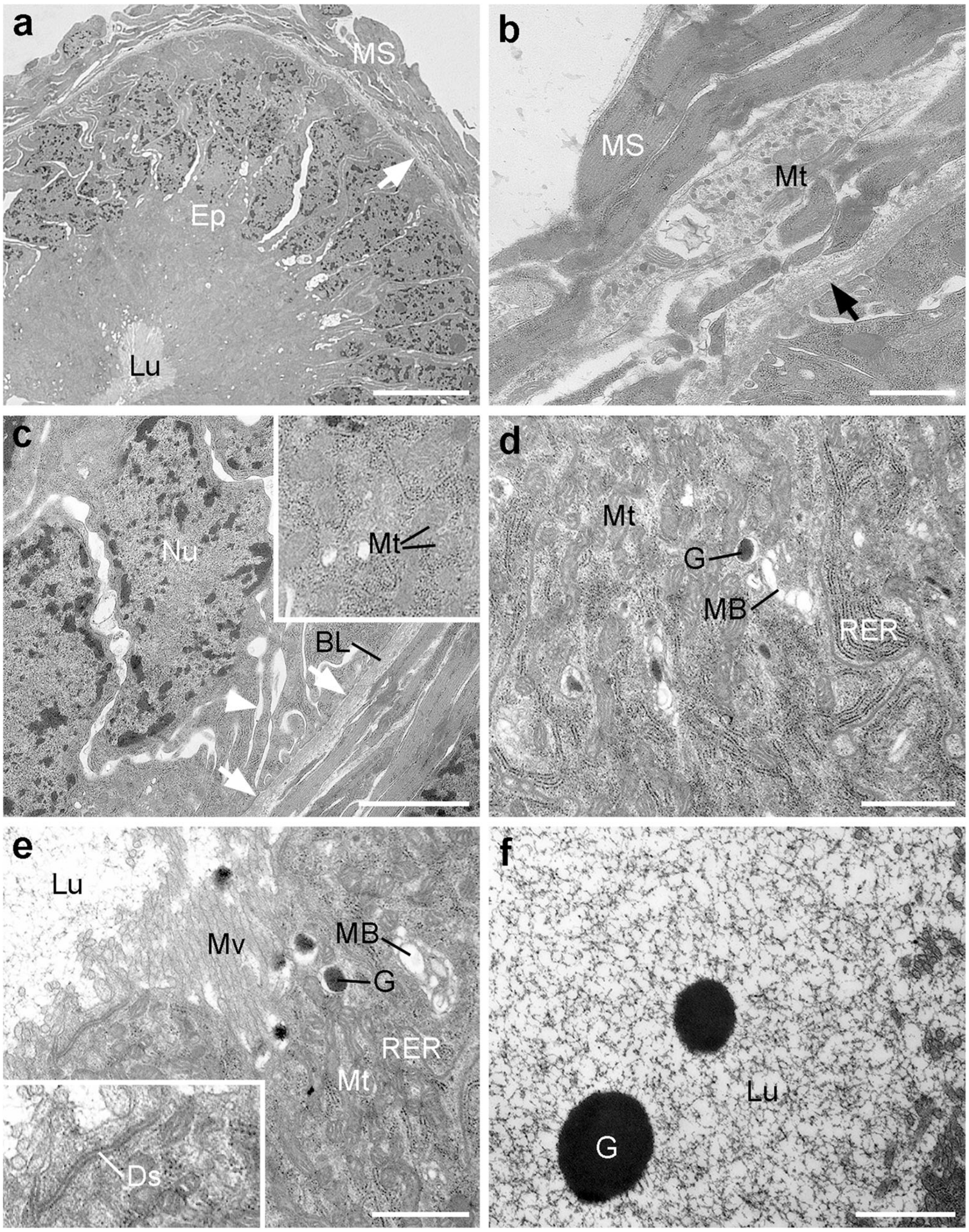

Fig. 3 TEM micrographs of vas deferens of *Terrobittacus implicatus, cross-section*. **a** Muscular layers, epithelium and lumen. Arrow points to basal lamina. **b** Circular muscular sheath surrounds basal lamina (Arrow). Some mitochondria are present in muscle cells. **c** Nuclei in basal cytoplasm. Arrows point to hemidesmosome plaques. Arrowhead points to intercellular space. **d** Organelles and secretions in medial cytoplasm. **e** Apical part of epithelium. **f** Scattered secretions and some granules in lumen. *BL* basal lamina, *Ds* desmosome, *Ep* epithelium, *G* granule, *Lu* lumen, *MB* multivesicular body, *MS* muscular sheath, *Mt* mitochondrion, *Mv* microvilli, *Nu* nucleus, *RER* rough endoplasmic reticulum. Scale bars **a** 5 μm, **b** and **d**–**f** 1 μm, and **c** 2 μm

distributed in the developed circular muscular cells (Fig. 6b). The basal lamina encloses the mono-layered epithelium loosely (Fig. 6c, d). A quarter of epithelium forms a spine occupying one-third volume of the vas deferens, while the remaining epithelium is half in thickness of the former (Fig. 6a, c, d). The basal and medial plasma membranes have conspicuous invagination. The cytoplasm of these parts is considerably scarce, with numerous large intercellular spaces (Fig. 6c, d). The cytoplasm is mainly concentrated in the apical part and contains irregular nuclei, rough endoplasmic reticulum, abundant mitochondria, and a few electron-translucent vesicles (Fig. 6e). Numerous clustered microvilli project into the subcuticular cavity, causing the epithelium to appear crescent (Fig. 6a, c, d). The subcuticular cavity is wide, situated between the epithelium and the inner cuticle, with abundant secretions (Fig. 6f). The zigzag inner cuticle encloses the lumen, in which no marked secretions were observed (Fig. 6f).

Discussion

In Mecoptera, the vasa deferentia have been investigated in five families (Choristidae, Boreidae, Meropeidae, Bittacidae, and Panorpidae) (Steiner 1937; Potter 1938; Setty 1940; Grell 1942; Cooper 1972). Among these families, Panorpidae bear

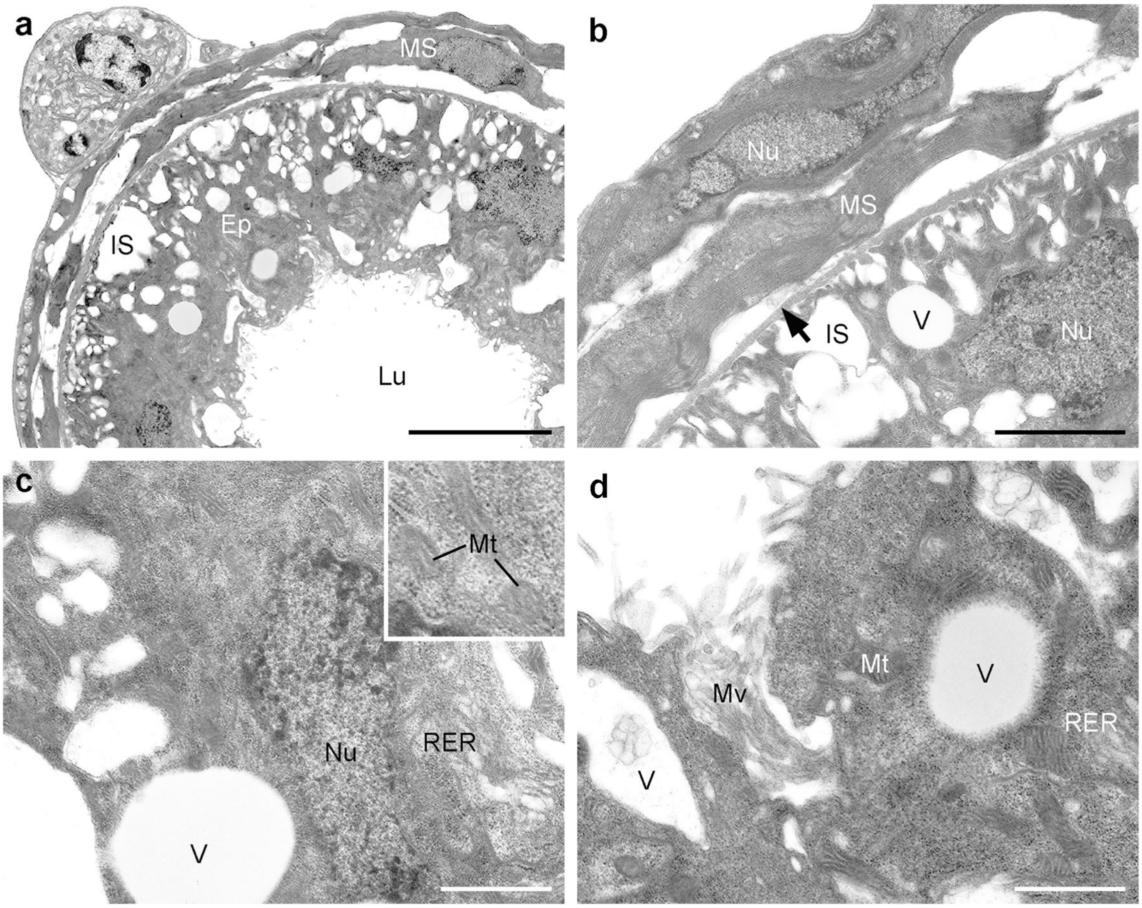

Fig. 4 TEM micrographs of vas deferens of *Cerapanorpa nanwutaina*, cross-section. **a** Muscular and epithelial layers. Some glial cells are visible outside the two layers. **b** Nuclei of muscular sheath. Arrow points to basal lamina. **c** Nucleus and organelles of epithelium. **d** Organelles and secretions in apical region of epidermis. *Ep* epithelium, *IS* intercellular space, *Lu* lumen, *MS* muscular sheath, *Mt* mitochondrion, *Mv* microvilli, *Nu* nucleus, *RER* rough endoplasmic reticulum, *V* vesicle. Scale bars **a** 5 μm, **b** 2 μm, and **c** and **d** 1 μm

prominently elongated vasa deferentia in contrast with short ones in other families including Bittacidae (Potter 1938; Grell 1942).

The vas deferens of Bittacidae has a reletively larger well-developed epithelium, more microvilli, organelles, and more types of secretions than that of Panorpidae. The numbers of mitochondria and rough endoplasmic reticulum were roughly compared through counting them per area on the plane. The presences of numerous mitochondria and rough endoplasmic reticulum in the vas deferens may imply an intensive secretory activity (Snodgrass 1935; Freitas et al. 2010), and occurrence of extensive rough endoplasmic reticulum usually indicates that most secretions are proteinaceous (Kölsch 2000; Šobotník et al. 2014; Filimonova 2016). We assume that the vas deferens of Bittacidae affords a considerable secretory function. On the contrary, the epithelium of the vas deferens in Panorpidae is weakly developed, with extensive large intercellular spaces and less organelles. The vas deferens also has well-developed muscular layers and is highly innervated, suggesting that the main function of the vas deferens of Panorpidae is transportation of seminal fluid and that the males are able to exert some control over this process (Landim and Dallacqua 2005; Radhakrishnan et al. 2009).

The vas deferens differs markedly from the postvesicular vas deferens in both families, mainly in the overall structure, intensity of secretory activity, and secretory mode. The postvesicular vas deferens has more secretions and two additional structures, a subcuticular cavity and an inner cuticle, compared with the vas deferens. Although the secretory mode is unclear yet at present, the secretions are likely released by a merocrine mode, as in the genital tracts of most other insects (Quennedey 1998; Viscuso et al. 2005; Marchini et al. 2009). The postvesicular vas deferens first releases secretions into the large subcuticular cavity and then discharges them into the lumen through the inner cuticle. This type of cells corresponds to class I glandular cells, having a single structure and directly adjoining the inner cuticle (Noirot and Quennedey 1974; Quennedey 1998).

Abundant mitochondria are present in the apical region of the epithelium of genital tracts except the vasa deferentia of

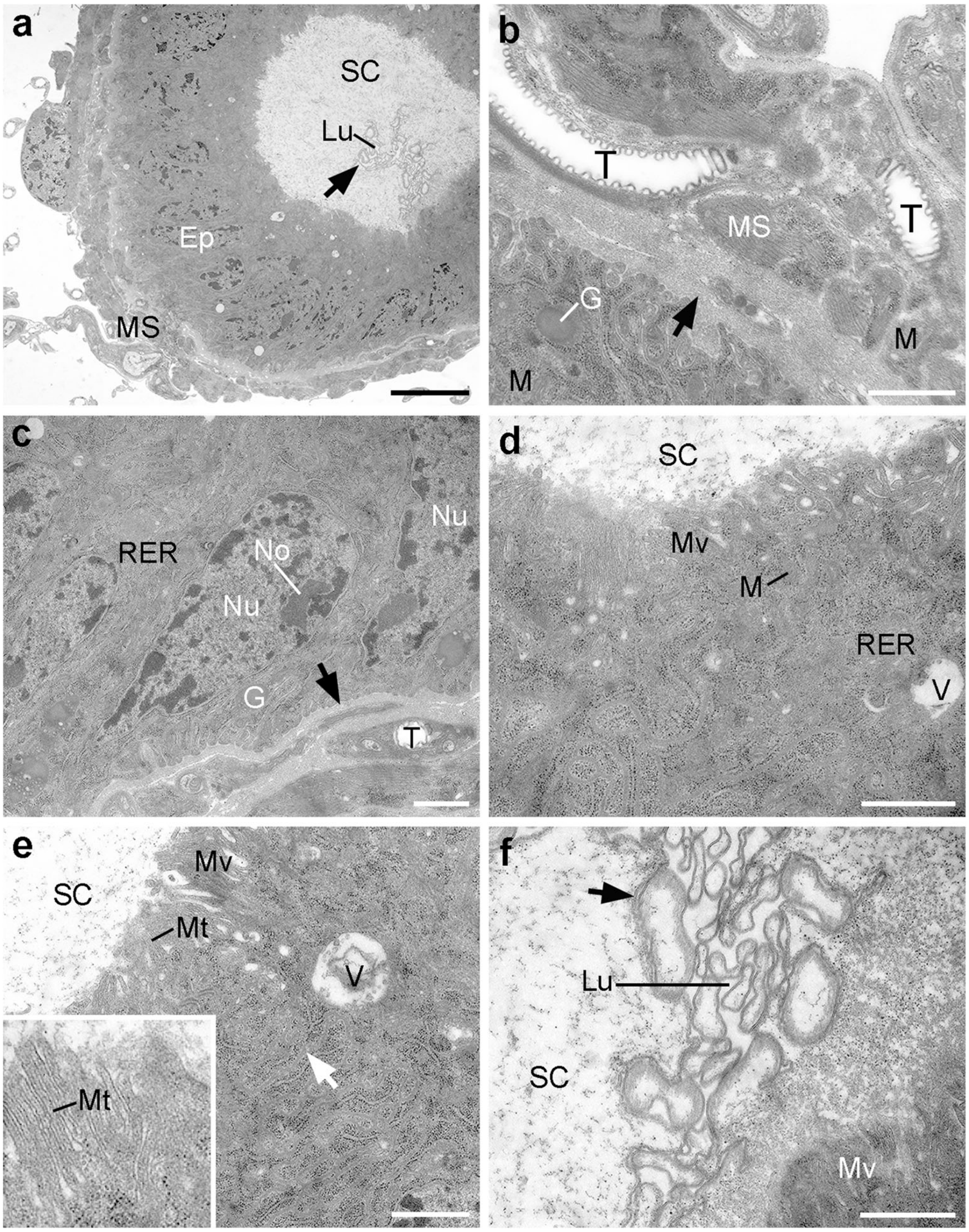

Fig. 5 TEM micrographs of postvesicular vas deferens of *Terrobittacus implicatus, cross-section*. **a** Muscular sheath, epithelium, and subcuticular cavity. Arrow points to inner cuticle. **b** Some tracheoles and mitochondria are present in muscular layers. Arrow points to basal lamina. **c** Nuclei of epithelium. Arrow points to basal lamina. **d** Apical region of epidermis and microvilli. **e** Plasma membranes (arrow) and microtubules in the apical epithelium. **f** Secretions in subcuticular cavity and lumen. Arrow points to inner cuticle. *Ep* epithelium, *G* granule, *Lu* lumen, *M* mitochondrion, *MS* muscular sheath, *Mt* microtubule, *Mv* microvilli, *No* nucleolus, *Nu* nucleus, *RER* rough endoplasmic reticulum, *SC* subcuticular cavity, *T* tracheole, *V* vesicle. Scale bars **a** 5 μm and **b**–**f** 1 μm

Bittacidae. This feature indicates a high metabolic activity in the apical portion of the cells, as in the stingless bees *Melipona bicolor* and *Scaptotrigona xanthotricha* (Dallacqua and Landim 2003; Araújo et al. 2005), the honeybee *Apis mellifera* (Moors et al. 2005), and the jewel wasp *Nasonia vitripennis* (Liu et al. 2017). The abundance of mitochondria in the apical region is likely connected with the regulation of the luminal pH (Araújo et al. 2005).

Numerous microtubules are scattered close to the microvilli in the postvesicular vas deferens of Bittacidae, similar to some other insects (Dallai et al. 1988; Radhakrishnan et al. 2009). These microtubules have diverse functions, such as sequestrating or transporting ions (Ledbetter and Porter 1963), strengthening fibrillar cytoplasm (Satir and Stuart 1965), maintaining the asymmetry of cells (Dustin 1985), and participating in the process of protein secretion (Dallai et al. 1988). Based on the position of microtubules and the intense secretory activity of the cytoplasm, we may assume that these microtubules are associated with the protein secretion in the postvesicular vas deferens of Bittacidae.

For the tube between the seminal vesicle and the ejaculatory duct, the usage of terms is inconsistent in Mecoptera. “Postvesicular vas deferens” follows Gomes et al. (2012). The term “vas deferens” (Potter 1938; Grell 1942; Xie and Hua 2010) was abandoned to avoid confusion with the tube between the epididymis and the seminal vesicle. The very structure was called “ejaculatory duct” by Matsuda (1976) and Sinclair et al. (2007). The tube has a narrow epithelium

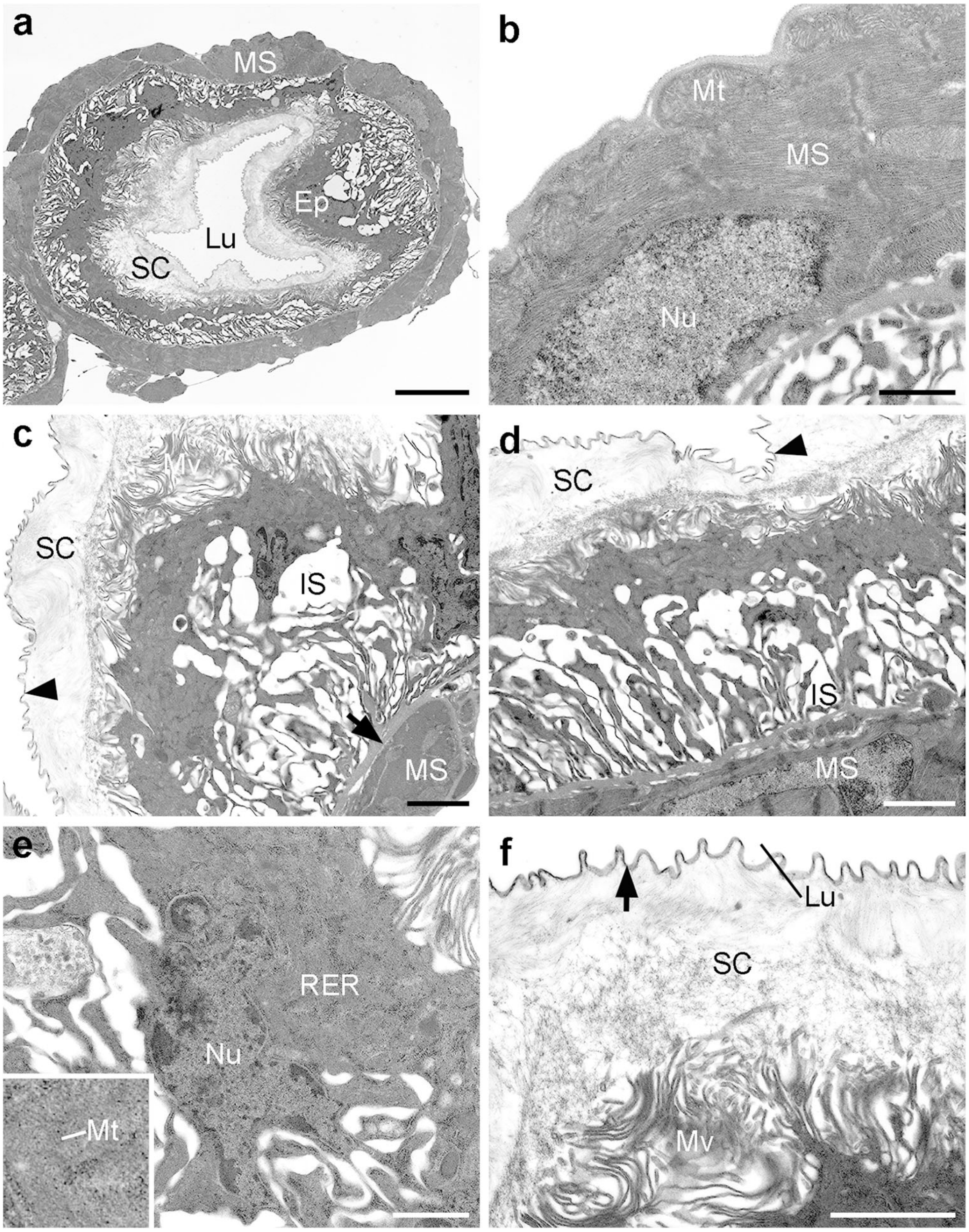

Fig. 6 TEM micrographs of postvesicular vas deferens of *Cerapanorpa nanwutaina*. **a** Cross-section of postvesicular vas deferens. **b** Nucleus and mitochondria in muscular layers. **c** Enlargement of epithelium. Arrow points to basal lamina. Arrowhead points to inner cuticle. **d** Normal part of epithelium. Arrow points to basal lamina. Arrowhead points to inner cuticle. **e** Nuclei and organelles of epithelial cells. **f** Secretions in subcuticular cavity. Arrow points to inner cuticle. *Ep* epithelium, *IS* intercellular space, *Lu* lumen, *MS* muscular sheath, *Mt* mitochondrion, *Mv* microvilli, *Nu* nucleus, *RER* rough endoplasmic reticulum, *SC* subcuticular cavity. Scale bars **a** 5 μm, **b** and **e** 1 μm, and **c**, **d**, and **f** 2 μm

and lacks a strong secretory function, mainly serving transportation, different from the ejaculatory duct that has an intense secretory activity associated with sperm viability.

It is interesting that the postvesicular vas deferens has an inner spine in Panorpidae. This structure is usually a simple tube, with a cylindrical lumen (Snodgrass 1935). The postvesicular vas deferens of Panorpidae has a zipper-like spine and a narrow lumen and may have the same function as in the ductus receptaculi of the cricket *Teleogryllus commodus*, which has a narrow lumen to serve as a barricade for the reflow of sperm during copulation (Sturm 2008). Besides, because the postvesicular vas deferens is extraordinarily long in most panorpids, the spine is likely to serve to protect the structure from collapse or injury during the movement of the terminal abdominal segments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with animals and human participants performed by any of the authors.

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