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Male reproductive system and sperm ultrastructure of *Furcatopanorpa longihypovalva* (Hua and Cai, 2009) (Mecoptera: Panorpidae) and its phylogenetic implication

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ABSTRACT

The male reproductive system and sperm ultrastructure of the scorpionfly *Furcatopanorpa longihypovalva* (Hua & Cai) were investigated using light and transmission electron microscopy. A peculiar feature of the male reproductive system is the position of the epididymis, separately from the base of testis within the peritoneal sheath, unlike the species of other genera of Panorpidae pressed against the lateral base of testis. The mature spermatozoon is filiform, approximately 1600 μm in length. It has largely maintained a mecopteran groundplan condition, with a bilayered acrosome, an elongate nucleus, a neck region, and a long flagellum. As for the sperm features, similarly to other species of Panorpidae, the nucleus has two lateral grooves and a helical appearance. The neck region shows a sheath-shaped centriolar adjunct, beneath the nucleus. The flagellum is helical and consists of a 9 + 2 axoneme, two mitochondrial derivatives of unequal size, one accessory body and two extra-axonemal accessory structures, as in the genus *Panorpa* of Panorpidae. The uniqueness of spermatozoa of *F. longihypovalva* lies in the specifically hammer-shaped mitochondrial derivative along most of the flagellum length. The phylogenetic position of the genus *Furcatopanorpa* in Panorpidae is briefly discussed based on our findings and literature in this respect.

1. Introduction

Furcatopanorpa Ma and Hua, 2011, a monotypic genus of Panorpidae in Mecoptera, was erected with *Panorpa longihypovalva* Hua and Cai, 2009 as its type species. The genus is distinguishable by several morphological characters (Hua and Cai, 2009; Ma and Hua, 2011; Zhong et al., 2015a), especially the axis of the female genital plate forked distally, and the hypovalvae of male genitalia extremely elongated and parameres extraordinarily developed with complicated lobes. The eggs are elongate and oval in shape, with low, dispersed raised protuberances and irregular loose sculpturing on the polar areas (Ma et al., 2009). Regarding mating behavior, the well-developed and multi-furcated male salivary glands (Zhong et al., 2015a) provide liquid secretion through a mouth-to-mouth mode for the female. The male maintains copulation mainly by continuous provision of salivary secretion rather than by seizing the female with grasping devices as in other species in Panorpidae (Thornhill, 1981; Zhong et al., 2015b).

The phylogenetic position of *Furcatopanorpa* in Panorpidae, however, is still a controversial issue. Based on morphological characters, Ma et al. (2012) concluded that the genus *Furcatopanorpa* branches off from a basal node and is the sister taxon to all the other species of Panorpidae . On the basis of molecular data, however, Hu et al. (2015) regarded *Furcatopanorpa* as the sister group of *Panorpa liui* Hua, 1997. It is obvious that additional characters are needed to solve the problem of the phylogenetic position of *Furcatopanorpa*.

Spermatozoa are highly specialized male gametes in sexually reproductive animals, and characterized by patterns of rapid and divergent morphological evolution (Birkhead et al., 2009). Examination of a single sperm cell can be used diagnostically to determine the phylum, order, family, genus, and species of the male from which the cell comes. In addition, variation in sperm morphology, especially the ultrastructure, can provide valuable characters for the assessment of evolution and the reconstruction of phylogenies in various groups (Baccetti, 1972; Jamieson, 1987; Jamieson et al., 1995, 1999; Dallai, 2009, 2014; Dallai et al., 2016; Gottardo et al.,

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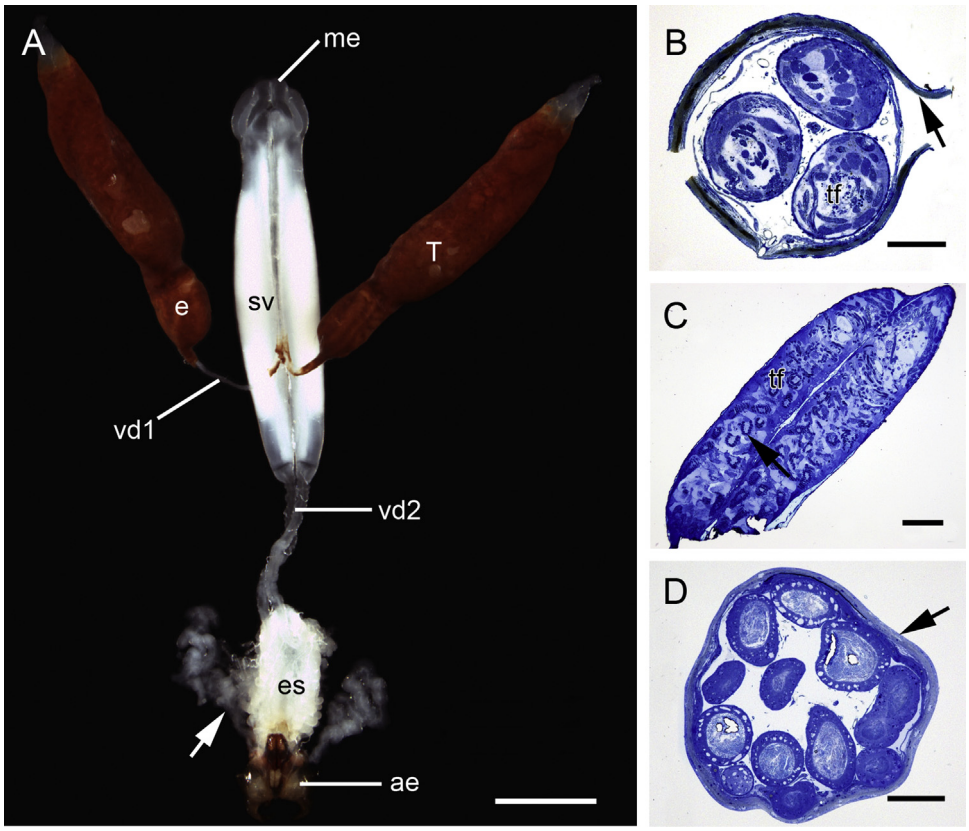

Fig. 1. Light micrographs of the male reproductive system of *F. longihypovalva*. (A) Male reproductive system, testis (T), epididymis (e), thin and thick vas deferens (vd1, vd2), seminal vesicle (sv), mesadenia (me), ejaculatory sac (es), aedeagus (ae). Arrow indicates the ectadenia. (B) Cross-section of testis consisting of three testicular follicles (tf). Arrow indicates the peritoneal sheath. (C) Longitudinal section of testicular follicles (tf), showing coiled sperm bundles (arrow). (D) Cross-section of the epididymis. Arrow indicates the peritoneal sheath. Scale bars: A = 1 mm; B and D = 100 μm; C = 50 μm.

2016). Although Dallai et al. (2003) used sperm ultrastructure to investigate the monophyly of Mecoptera, the characters of sperm ultrastructure have not been used in the phylogenetic analysis of Panorpidae.

Sperm structure has been observed in several species of *Panorpa* in Panorpidae (Baccetti et al., 1969; Gassner et al., 1972; Dallai et al., 2003), but the spermatozoon ultrastructure has not been examined in *Furcatopanorpa* hitherto. In this study, we investigated the male reproductive system and sperm ultrastructure of *F. longihypovalva* (Hua and Cai, 2009) using light and transmission electron microscopy, in order to provide eventual additional characters useful for its phylogenetic placement in Panorpidae.

2. Materials and methods

2.1. Insect collection

Adult males of *F. longihypovalva* were obtained from the Tiantaishan Forest Park (34°13′N, 106°59′E, elev. 1500–1600 m), Qinling Mountains, Shaanxi Province in early June 2015.

2.2. Light microscopy (LM)

Live adult males were anaesthetized in diethyl ether (Zhang and Hua, 2014), and their reproductive system was rapidly dissected in Ringer's solution under a Nikon SMZ1500 stereo microscope (Nikon, Tokyo, Japan). Pictures were taken with a QImaging Retiga 2000R Fast 1394 Digital CCD equipped on the microscope.

The testicular follicles were opened at the basal part and the sperm bundles were spread onto a clean microslide. To determine the size of sperm nucleus, the slides were stained with 4,6-diamino-2-phenylindole (DAPI, Beyotime) for 5 min, and rinsed in distilled water. Photographs were taken with a Nikon DS-Ril digital camera attached to a Nikon Eclipse 80i epifluorescence microscope (Nikon, Tokyo, Japan).

For histological observation, the samples, embedded in polymerized Epon 812 resin, were cut into semi-thin sections (1000 nm) with a Leica EM UC7 ultramicrotome (Leica, Nussloch, Germany), stained with 0.5% toluidine blue, and examined under a microscope.

2.3. Transmission electron microscopy (TEM)

Live adult males were anaesthetized in diethyl ether. The testes and epididymides were dissected rapidly in Ringer's solution, and were fixed at 4 °C overnight in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.2), in which 3% sucrose was added.

The fixed samples were rinsed six times with PB, and post-fixed in phosphate-buffered 1% osmium tetroxide (O_5O_4) for 1.5 h at 4 °C. After rinsing six times with PB, the samples were dehydrated through a graded series of ethanol (30%, 50%, 70% for 10 min each, 80% for 15 min, 90% for 20 min, and 100% for 30 min twice). The samples were infiltrated successively through three mixtures of acetone/Epon 812 resin (3:1 for 2 h, 1:1 for 4 h, and 1:3 for 12 h) and pure Epon 812 resin for 24 h twice at room temperature, and were embedded in pure Epon 812 resin, polymerized at 30 °C for 24 h and 60 °C for 48 h.

Ultra-thin sections (70 nm) were cut with a diamond knife on the ultramicrotome, stained with uranyl acetate and lead citrate for