

# Nuptial feeding and genital coupling of *Neopanorpa* scorpionflies (Insecta: Mecoptera: Panorpidae) with notal organs of various lengths

*Xin Tong*

Key Laboratory of Plant Protection Resources and Pest Management, Ministry of Education, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China

*Bao-Zhen Hua*

Key Laboratory of Plant Protection Resources and Pest Management, Ministry of Education, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China  
*huabzh@nwfau.edu.cn*

## Abstract

*Neopanorpa*, the second largest genus in Panorpidae, is mainly characterized by the well-developed notal organ on male tergum III. However, it remains largely unknown how the length of the notal organ influences the nuptial feeding behaviour of *Neopanorpa*. Here, we investigated the nuptial feeding by comparing the morphology of mating-related structures and the genital coupling of a) *Neopanorpa lui* Chou & Ran, 1981 with a weakly-developed notal organ, b) *N. carpenteri* Cheng, 1957 with a medium-sized notal organ, and c) *N. longiprocessa* Hua & Chou, 1997 with an extremely elongated notal organ. The couples of *N. lui* and *N. carpenteri* maintain an intermittent mouth-to-mouth mode but do not exchange any edible food. After that the males secrete a salivary mass onto the surface as a nuptial gift, which is distinctly larger in *N. carpenteri* than in *N. lui*. Correspondingly, the male salivary glands are more developed in *N. carpenteri* than in *N. lui*. Males of *N. longiprocessa* bear very short salivary glands corresponding to a coercive mating tactic. The genital couplings are similar among the three species of *Neopanorpa*. The paired hypovalves of males are used to control the cerci of females. The prominent basal processes of male gonostyli grasp the posterior portion of the female medigynium across the intersegmental membrane. The male aedeagus physically couples with the female medigynium to ensure the male phallosome to connect to the female copulatory pore. The influence of the notal organ length on the nuptial feeding behaviour of *Neopanorpa* is briefly discussed.

## Keywords

copulation – copulatory mechanism – mating behaviour – nuptial gift – scorpionfly

## Introduction

Nuptial feeding is an interesting behaviour in animal mating systems and is a bridge to connect the resources between a male and a female (Thornhill & Alcock, 1983; Boggs, 1990). Most nuptial gifts are provided by the male and exhibit multitudinous forms such as glandular secretions, preys, and even the body parts (Vahed, 1998; Lewis & South, 2012). In general, the edible gifts are predicted to help the males attract a female, reduce the female resistance, and prolong the copulation duration to increase the quantity of sperm transferred (Vahed, 1998; Gwynne, 2008; Lewis & South, 2012; Vahed et al., 2014). Notably, the gift-giving behaviour shows surprising plasticity among insect groups (Vahed, 1998; Preston-Mafham, 1999; Stutt & Siva-Jothy, 2001; Lehmann, 2012; Lewis & South, 2012), including Panorpidae (Insecta: Mecoptera) (Thornhill, 1981; Sauer et al., 1998; Engqvist, 2007).

Panorpidae usually attract the interests of entomologists for their diverse courtships and nuptial feeding behaviours (Thornhill, 1980, 1981; Thornhill & Sauer, 1991; Sauer et al., 1998; Zhong & Hua, 2013b). The genus *Panorpa* Linnaeus, 1758 is often treated as the model animal to investigate the mating systems of insects because most males of *Panorpa* can alternatively provide salivary secretions or preys as a nuptial gift according to their nutritional state (Byers & Thornhill, 1983; Engles & Sauer, 2006; Engqvist, 2007, 2009). Males of several Asian species of *Panorpa*, however, only provide a prey prior to copulation (Thornhill, 1992; Ma & Hua, 2011b). Males of *Cerapanorpa* Gao, Ma & Hua, 2016 and *Dicerapanorpa* Zhong & Hua, 2013 provide one or two salivary masses (Zhong et al., 2015a; Tong et al., 2018), whereas the males of *Furcatopanorpa* Ma & Hua, 2011 continuously provide liquid salivary secretions through a mouth-to-mouth mode (Zhong et al., 2015b).

Salivary glands are the structure of male scorpionflies to produce salivary secretions (Byers & Thornhill, 1983; Ma et al., 2011). The salivary glands of male *P. liui* Hua, 1997 are a short bifurcated structure, thereby unable for the males to offer a salivary mass. The male instead provides a prey to the female as a nuptial gift (Ma & Hua, 2011b; Ma et al., 2011). The salivary glands of *D. magna* (Chou, 1981) are well-developed and octofurcated, so that the males can secrete another salivary mass as a nuptial gift when the first mass is lost (Ma et al., 2011; Zhong et al., 2015a). The hexafurcated secretory tubes are extremely elongated for male *P. vulgaris* Imhoff & Labram, 1836, which can provide multiple salivary masses to the female (Potter, 1938; Sauer et al., 1998). The salivary glands of *F. longihypovalva* even branch twice in a dichotomous way to form 24 distal secretory tubules to support the males to continuously provide liquid salivary secretions during copulation (Zhong et al., 2015b). Based on the morphology of salivary glands, we are able to estimate the ability of male scorpionflies to secrete salivary secretions as a nuptial gift.

Nuptial feeding behaviour is also greatly associated with the grasping structures of male scorpionflies, especially the notal organ. The notal organ, a clamp-like process on the posterior margin of male tergite III, is used to seize the anterior edge of the female's wings from one side (Mickoleit, 1971; Thornhill & Sauer, 1991; Kock et al., 2009; Krzemiński & Soszynska-Maj, 2012). The male of *F. longihypovalva* continuously provides salivary secretions to the male. This behaviour is believed to be related to the male lacking a notal organ to control the female (Zhong et al., 2015b). In *P. vulgaris* the notal organ is regarded helpful to prolong copulation and to obtain a coercive mating (Thornhill & Sauer, 1991). Although Kock et al. (2009) contend the view that the notal organ does not prolong the copulation duration, there is no doubt that

the notal organ can enhance male control of copulation and benefit the male scorpionflies to save the nuptial gift. The male of *Neopanorpa longiprocessa* Hua & Chou, 1997 bears an extremely elongated notal organ that extends to the posterior portion of the fifth abdominal segment. Owing to the firm grasping of the notal organ, *N. longiprocessa* males even no longer need to provide any edible gift during the mating process (Zhong & Hua, 2013b).

*Neopanorpa* van der Weele, 1909, the second largest genus of Panorpidae with over 170 species, is mainly characterized by the bifurcated salivary glands and the notal organ more developed than in other genera of Panorpidae (Cai & Hua, 2008; Ma & Hua, 2011a; Ma et al., 2011; Zhong & Hua, 2013a; Gao et al., 2016; Wang & Hua, 2018a, b). However, how these associated structures influence the nuptial feeding and mating behaviours of *Neopanorpa* has not been satisfactorily clarified.

In *Neopanorpa* only *N. longiprocessa* has been reported for the mating behaviour (Zhong & Hua, 2013b). It is even presumed that all *Neopanorpa* species adopt the coercive mating tactic because the elongated notal organ can force the female and the simple bifurcated salivary glands lack the ability to secrete a salivary mass (Jiang & Hua, 2015; Wang & Hua, 2018b). In fact, not all *Neopanorpa* males bear an elongated notal organ as in *N. longiprocessa*. The notal organs extend merely to the middle portion of the fourth abdominal segment in the males of most *Neopanorpa* species, and even only to the anterior portion of the fourth abdominal segment in a few species. It has not been evaluated whether these *Neopanorpa* males with a moderately or weakly developed notal organ can provide salivary masses as nuptial gifts to the female.

The genitalia of *Neopanorpa* are distinctively different from those of other genera in Panorpidae (Cai & Hua, 2008; Ma & Hua, 2011a; Zhong & Hua, 2013a; Gao et al., 2016;

Wang & Hua, 2018a, 2019a). The basal processes on male gonostyli are usually prominent. The hypovalves are elongate and bear two processes. The parameres fused with the aedeagus bear acute lateral processes. The aedeagus is stout, with the paired ventral valves longer than the paired dorsal valves. The female medigynium (or genital plate) is simple with a narrow main plate and a short axis (Wang & Hua, 2018a, b). However, only *N. longiprocessa* has been described for the copulatory mechanism in this genus based on the male and female genitalia (Zhong & Hua, 2013b). The genital coupling during copulation has not been revealed in *Neopanorpa*.

In this study, we comparatively investigated the courtship, nuptial feeding behaviour, the morphology of mating-related structures, and the genital coupling of a) *N. lui* Chou & Ran, 1981 with a weakly-developed notal organ, b) *N. carpenteri* Cheng, 1957 with a medium-sized notal organ, and c) *N. longiprocessa* Hua & Chou, 1997 with an extremely elongated notal organ, based on the behavioural observation and the freeze-fixed pairs in copula. The influence of the notal organ length on the nuptial feeding behaviour of *Neopanorpa* is briefly discussed.

## Material and methods

### *Insect collection*

Adults of *N. lui* and *N. longiprocessa* were collected from the Tiantaishan Forest Park (34°13' N, 106°59' E, elev. 1500–1800 m) and the Huoditang Forest Park (33°25' N, 108°27' E, elev. 1500–1700 m) in the Qinling Mountains, Shaanxi Province in central China from early June to July of 2017 and 2019. Adults of *N. carpenteri* were captured from the Yinnashan Forest Park (24°23' N, 116°24' E, elev. 500–1200 m), Meixian County, Guangdong Province in south China in late April 2018.

### *Insect rearing*

The live adults collected were transported to the laboratory and were reared following Thornhill & Sauer (1991) and Zhong & Hua (2013b). Ten males and 10 females were maintained in a gauze cage (40 cm × 40 cm × 60 cm). Each cage was supplied with five dead mealworm larvae (*Tenebrio molitor*), damp absorbent cotton, and twigs of plants with leaves every day to prevent low nutritional status and simulate the habitat of the adults.

### *Mating behaviour observation*

Adults of each species were observed 24 h a day by people who checked the circadian rhythms of mating activities in turn for a week. Then these adults were observed every 30 min from dawn to midnight or from dusk to dawn to document the courtship behaviour, mating tactic, mating process, mating duration, and the activities of the mating contacted structures. Photographs were taken with a Nikon D7100 digital camera. The length of nuptial gifts was estimated based on the photographs.

### *Freeze-fixation of pairs in copula*

Copulating pairs were frozen through carbon dioxide aerosol spray compressed in hydraulic cans, and were immediately fixed in Carnoy's solution (100% ethanol: glacial acetic acid = 3:1, v/v) at room temperature for 24–48 h to stabilize the interactions of their genital structures and preserved in 75% ethanol afterwards (Tong et al., 2017, 2018).

### *Light microscopy*

Thirty-six live adults (10 males and 10 females of *N. lui*; 5 males and 5 females of *N. longiprocessa*; 4 males and 2 females of *N. carpenteri*) were fixed in Dietrich's fluid (formalin: 95% ethanol: glacial acetic acid: distilled water = 6:15:1:80, v/v) to dissect the salivary glands and genitalia. Photographs were taken with a QImaging Retiga-2000R Fast 1394 Digital Camera

attached to the microscope and were stacked with Syncroscopy Auto-Montage software.

For histological observations, freeze-fixed pairs in copula were rinsed six times in 0.1 M phosphate buffered saline (pH 7.2) and dehydrated through a graded ethanol series. Then the samples were infiltrated in the mixtures of ethanol and LR White resin (3:1 for 2 h, 1:1 for 4 h, and 1:3 for 12 h), subsequently with pure LR White resin for 24 h at 18°C twice. Finally, the samples were embedded in pure LR White resin and polymerized at 55°C for 48 h (Wang & Hua, 2018c; Tong & Hua, 2019).

After complete polymerization, the samples were cut into 1.0 µm thick sections with a glass knife on the Leica EM UC7 ultramicrotome and stained with 0.5% toluidine blue. The sections were observed and photographed under a Nikon Eclipse 80i light microscope attached with a Nikon DS-Fil digital camera.

## **Results**

### *Courtship and nuptial feeding behaviour*

The mating behaviour of *N. lui* mainly occurs from midnight to dawn. In the initial phase, the male stretches the hypandrium to expose the paired sex pheromone glands and swing the genitalia to emit sex pheromones. The sex pheromones can attract the nearby females as in *Panorpa* (Engqvist & Sauer, 2003; Kock et al., 2007). When a female is attracted and approaches the male, he terminates the pheromone emission and moves to one side of the female. At this moment, both sexes stretch their antennae and forelegs to touch each other (fig. 1A), and turn their heads to allow their mouthparts to connect. During this process, the labial and maxillary palps are active (fig. 1B). The whole mouth-to-mouth phase can last for several hours. However, the phase is intermittent. Both sexes can

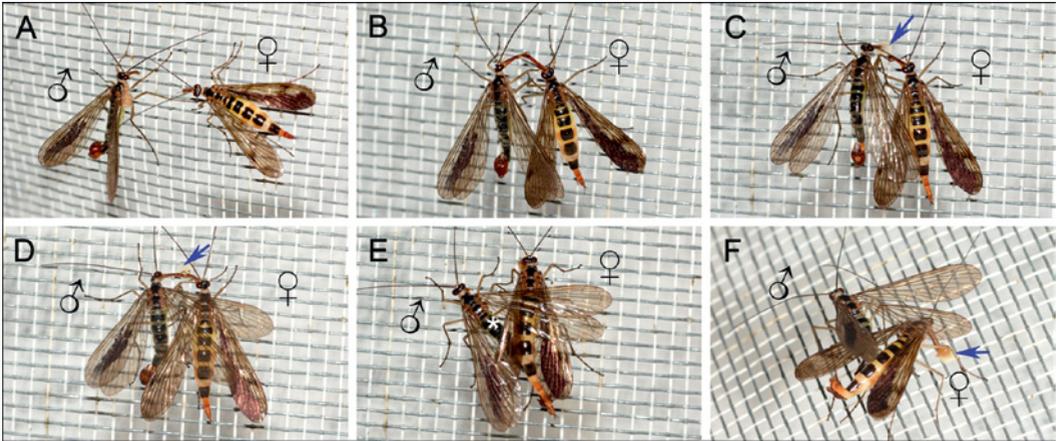


FIGURE 1 Mating process of *Neopanorpa lui*. A, a male and a female touch each other through the forelegs and antennae; B, the mouthparts of both sexes connect to each other to form a mouth-to-mouth mode; C, the male secretes a salivary mass (arrow); D, the male invites the female to feed on the salivary mass (arrow); E, the female starts to feed on the nuptial gift, the male seizes the female's wing by the notal organ (\*); F, the male controls the female and establishes a coupling of genitalia.

tentatively interrupt the connection for several minutes and reestablish connection soon afterwards. In the final phase, the male secretes a small salivary mass ( $1.95 \pm 0.69$  mm in length) onto the surface to entice the female to feed on it (figs. 1C, D). When the female begins to feed on the nuptial gift, the male seizes the anterior edge of the female's wings with his notal organ and grasps the female genital segment by his genitalia to establish genital coupling with the female (figs. 1E, F). The copulation lasts for  $160 \pm 55$  min.

The mating process of *N. carpenteri* is similar to that of *N. lui*. Males and females of *N. carpenteri* also connect their mouthparts with each other to form a mouth-to-mouth mode (fig. 2A). One to two hours later, the male secretes a huge thick salivary mass ( $4.00 \pm 0.95$  mm in length) onto the surface as a nuptial gift (figs. 2B–D). When the female starts to feed on the salivary mass, the male controls the female through the notal organ and establishes genital coupling soon (figs. 2C, D). The copulation duration lasts for  $157 \pm 49$  min.

The mating behaviour of *N. longiprocessa* occurs any time in a day. No edible food is

presented during the mating process. The male tightly grasps the wings of the female for  $121 \pm 41$  min to obtain a coercive copulation, which can last for  $145 \pm 59$  min (Zhong & Hua, 2013b). The mouth-to-mouth mode is lacking in *N. longiprocessa* (supplementary fig. S1).

#### *Morphology of the salivary glands*

The salivary glands of *Neopanorpa* mainly consist of a salivary pump, a common salivary duct, and paired salivary tubes.

The salivary glands of *N. lui* are bifurcated and exhibit distinct sexual dimorphism (fig. 3A). In general, the male secretory tube is thin and straight in the anterior portion, thick and convoluted in the middle portion, and gradually constricted in the posterior portion. The salivary glands of male *N. lui* extend to the fifth or sixth abdominal segment.

The salivary glands (fig. 3B) of *N. carpenteri* is very similar to those of *N. lui* in gross morphology, but the convoluted middle portion of the male secretory tubes of *N. carpenteri* is longer than those of *N. lui*. Furthermore, the salivary glands of *N. carpenteri* can extend to the sixth abdominal segment.

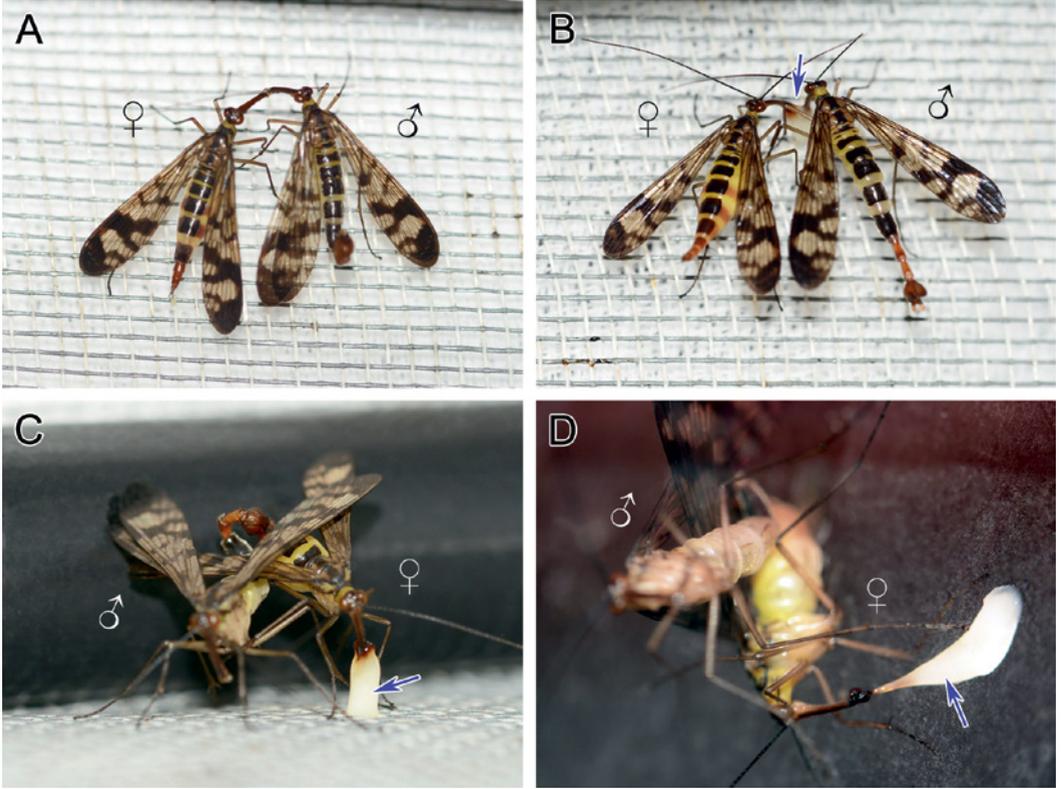


FIGURE 2 Mating process of *Neopanorpa carpenteri*. A, a male and a female maintain a mouth-to-mouth mode; B, the male secretes a salivary mass (arrow) and invites the female to feed on the nuptial gift; C, the female starts to feed on the salivary mass (arrow), the male establishes the coupling of genitalia; D, the salivary mass (arrow) provided by the male is huge.

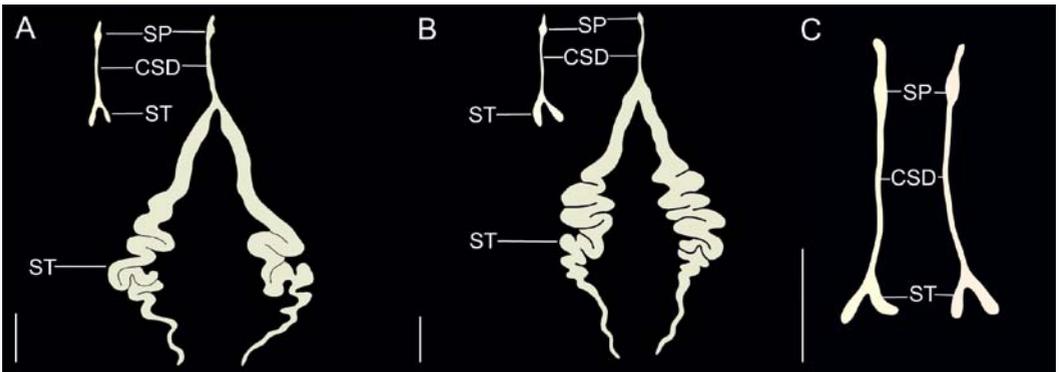


FIGURE 3 Schematic diagram of male and female salivary glands of *Neopanorpa* (for all, the female salivary glands are situated on the left, the male on the right). A, *N. lui*; B, *N. carpenteri*; C, *N. longiprocesa*. CSD, common salivary duct; SP, salivary pump; ST, secretory tube. Scale bars = 1.0 mm.

The salivary glands of *N. longiprocessa* are devoid of sexual dimorphism (fig. 3C). The male salivary glands are simple bifurcated and very short, only extending to the prothorax.

### Morphology of the notal organ

The notal organ on the posterior margin of tergite III functions in cooperation with the postnotal organ on the middle portion of tergite IV. The notal organ is semicircular, extending to the anterior portion of the fourth abdominal segment in *N. lui* (fig. 4A); is elongate, extending to the middle portion of the fourth abdominal segment in *N. carpenteri* (fig. 4B); and is extremely elongate, extending to the posterior margin of the fifth abdominal segment in *N. longiprocessa* (fig. 4C).

### Morphology of the genitalia

The male genitalia of *Neopanorpa* consist of a dorsal epandrium (tergum IX), a ventral hypandrium (sternum IX), a pair of lateral gonopods, and a median aedeagus. The gonopod consists of a basal gonocoxite and a distal gonostylus. Each gonostylus bears a promi-

nent lobe-like basal process and a blunt median tooth. The aedeagus is composed of a pair of ventral valves and a pair of dorsal valves, with a central phallotreme situated between the valves.

In *N. lui* the epandrium is long and broad. The hypandrium consists of a broad basal stalk and a pair of oval hypovalves (supplementary fig. S2). The aedeagus is stout and bears a pair of acute lateral processes. The ventral valves of aedeagus are longer than the dorsal valves (fig. 4D).

In *N. carpenteri* the aedeagus is similar to that of *N. lui* (fig. 4E). However, the hypovalves are quadrangular, with a pair of distinct sclerotized lateral processes (figs. 5A, B). The sex pheromone glands are well-developed (fig. 4E).

In *N. longiprocessa* the aedeagus is similar to that of *N. lui*. The hypovalves are oblong, with a pair of distinct blunt dorsad-directing processes (fig. 4F).

Table 1 summarizes differences of mating behaviour and the mating-related structures among three species of *Neopanorpa*.

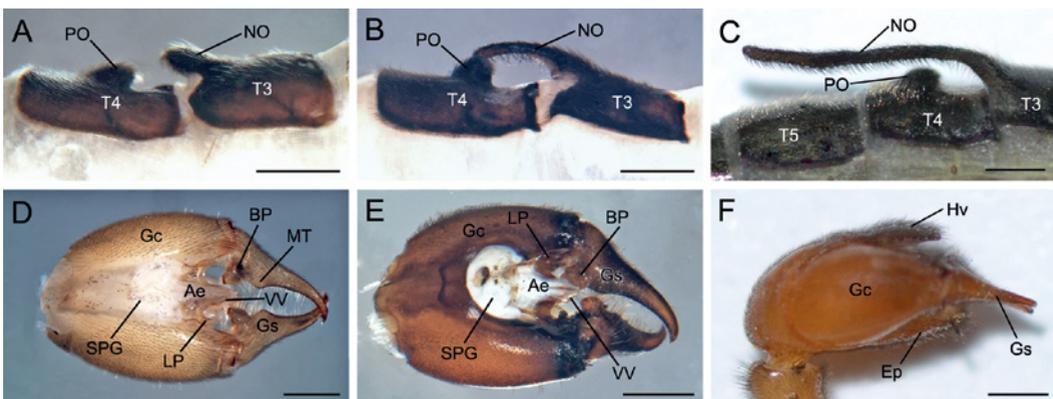


FIGURE 4 Male notal organs and genitalia of *Neopanorpa*. A, the notal organ of *N. lui*; B, *N. carpenteri*; C, *N. longiprocessa*; D, male genitalia of *N. lui* in ventral view with the epandrium and hypandrium removed; E, the same of *N. carpenteri*; F, male genitalia of *N. longiprocessa* in lateral view. Ae, aedeagus; BP, basal process; Ep, epandrium; Gc, gonocoxite; Gs, gonostylus; Hv, hypovalve; LP, lateral process; MT, median tooth; NO, notal organ; PO, postnotal organ; SPG, sex pheromone glands; VV, ventral valve; T<sub>3</sub> – T<sub>5</sub>, tergites III – V. Scale bars = 0.5 mm.

TABLE 1 Comparisons of mating behaviour and mating-related structures among three species of *Neopanorpa*

	<i>N. lui</i>	<i>N. carpenteri</i>	<i>N. longiprocessa</i>
Mating time	From midnight to dawn	From dusk to dawn	Any time in a day
Courtship mode	Mouth-to-mouth	Mouth-to-mouth	None
Copulation duration	160 ± 55 min	157 ± 49 min	145 ± 59 min
Mating tactic	Nuptial gift providing	Nuptial gift providing	Coercive mating
Nuptial gift	A salivary mass of 1–2 mm in length	A huge salivary mass of 3–5 mm in length	None
Salivary glands	Extending to A5 or A6	Extending to A6	Extending to prothorax
Notal organ	Extending to the anterior portion of A4	Extending to the middle portion of A4	Extending to the posterior portion of A5
Hypovalve	Oblong, without process	Quadrangular, with process	Oblong, with process

### *Coupling of the male and female genitalia*

The terminal genital ends of male and female *N. carpenteri* tightly couple with each other (figs. 5A, B). The paired hypovalves with sclerotized processes grasp the female cerci and cause the cerci to bend dorsally. The inflated sex pheromone glands support the female cerci from the ventral side. The paired well-developed basal processes seize the lateral membranes of the female genital segment (figs. 5A, B).

The genital coupling of *N. lui* is almost identical with that of *N. carpenteri* (supplementary fig. S2).

The basal processes of *N. longiprocessa* gonostyli grasp the posterior portion of the female medigynium across the lateral membranes from the ventral side (fig. 5C). On the dorsal side, the basal processes seize the female subgenital plate to allow the intersegmental membrane of abdominal segments VIII and IX to be narrowed (fig. 5D).

The male aedeagus and female medigynium of *N. lui* physically couple with each other (figs. 5E, F). The acute lateral processes of the aedeagus support the prolonged posterior arms of the medigynium from the ventral side. The well-developed ventral valves touch

the middle portion of the medigynium to allow the male phallotreme to connect to the female copulatory pore (figs. 5E, F).

The elongate ventral valves of *N. longiprocessa* also keep down the female medigynium (fig. 6A). The lateral processes of the aedeagus couple with the posterior arms of medigynium (figs. 6B, C). The parameres are located between the male ventral valves and the female posterior arms (fig. 6B). The basal processes firmly control the female medigynium across the intersegmental membrane of abdominal segments VIII and IX to establish a connection of the male phallotreme to the female copulatory pore (fig. 6C).

### Discussion

In the present study, both male *N. lui* with a weakly-developed notal organ and male *N. carpenteri* with a medium-sized notal organ provide a salivary mass as the nuptial gift, whereas male *N. longiprocessa* with an extremely elongated notal organ continuously grasps the female wings and adopt a coercive mating tactic. Prior to providing a salivary mass, both couples of *N. lui* and *N. carpenteri*

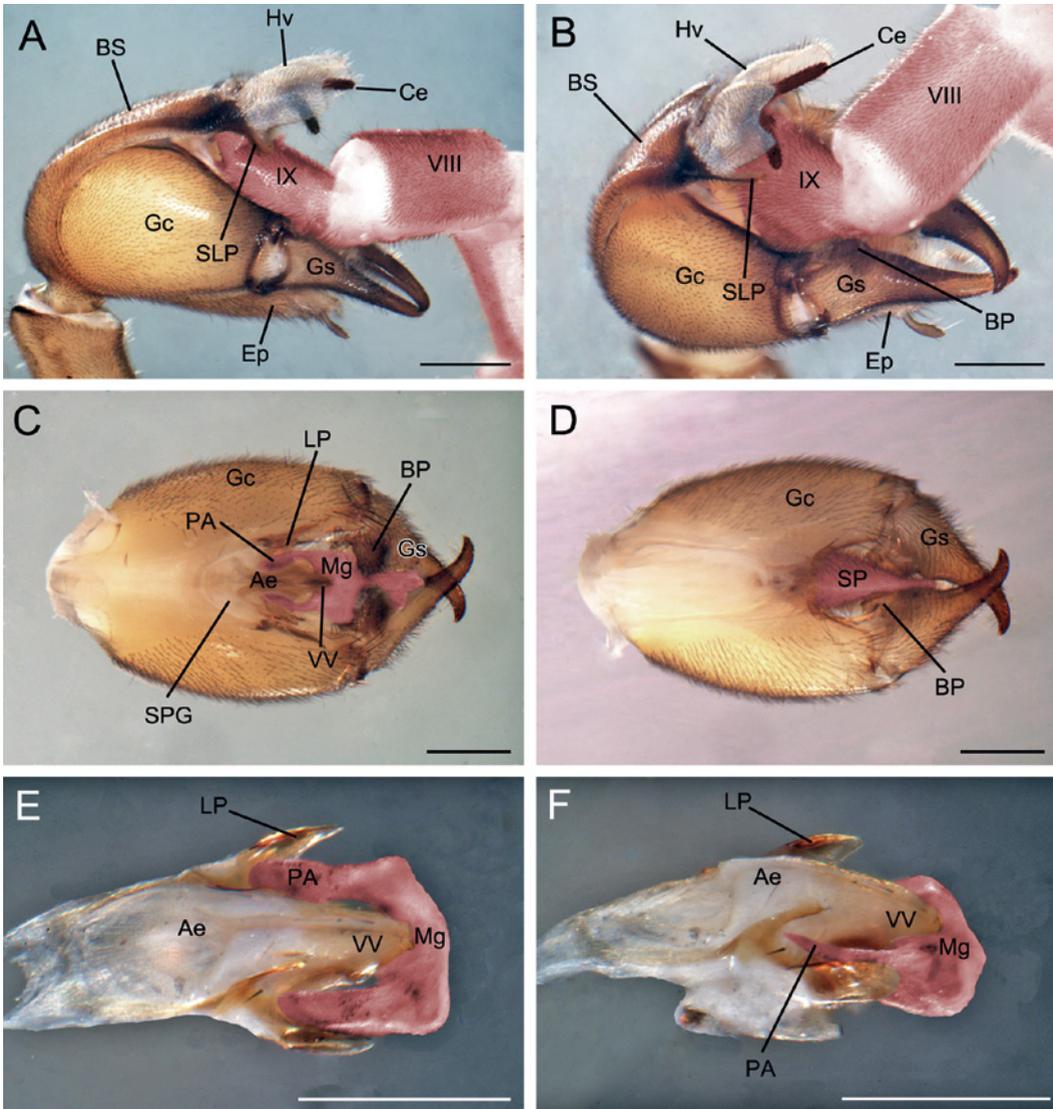


FIGURE 5 Coupling genitalia in *Neopanorpa* (for all, the male genital structures are situated on the left, the female on the right; terminal end of female is colored in pink). A, B, genital coupling of *N. carpenteri* to show the hypovalves control the female cerci and the basal processes grasp the female lateral membranes of the genital segments; C, D, genital coupling of *N. longiprocessa* with the epandrium and hypandrium removed to show the basal processes grasp the female medigynium and subgenital plate, and the ventral valves of aedeagus keep down the female medigynium; E, F, simulating copulation of the female medigynium and the male aedeagus of *N. lui* to show the ventral valves of the aedeagus keep down the female medigynium. Ae, aedeagus; BP, basal process; BS, basal stalk; Ce, cercus; Ep, epandrium; Gc, gonocoxite; Gs, gonostylus; Hv, hypovalve; LP, lateral process; Mg, medigynium; PA, posterior arm; SP, subgenital plate; SLP, sclerotized lateral process; SPG, sex pheromone glands; VV, ventral valve. Scale bars = 0.5 mm.

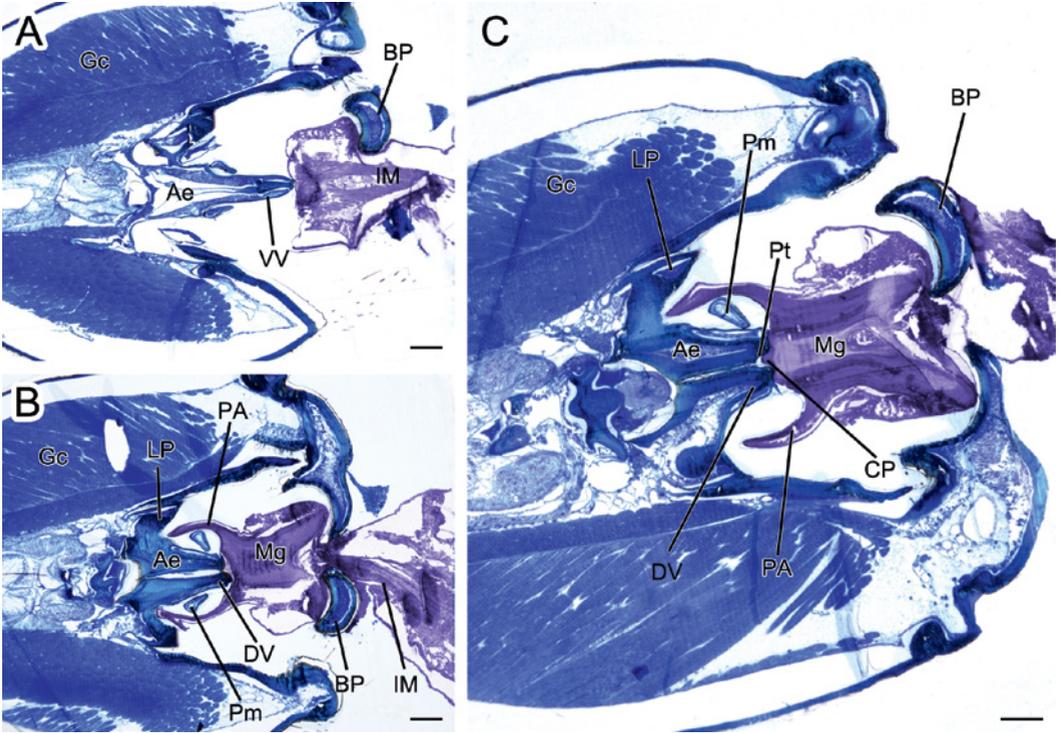


FIGURE 6 Section series of coupling genitalia of *Neopanorpa longiprocessa* (for all, the male genital structures are situated on the left, the female on the right; terminal end of female is colored in purple). A, ventral portion of the coupling genitalia to show the elongated ventral valves of the aedeagus; B, median portion of the coupling genitalia to show the male basal processes grasp the female medigynium and the male aedeagus physically couples with the female medigynium; C, the same to show the male phallosome connects to the female copulatory pore. Ae, aedeagus; BP, basal process; CP, copulatory pore; DV, dorsal valve; Gc, gonocoxite; Gs, gonostylus; IM, intersegmental membrane; LP, lateral process; Mg, medigynium; PA, posterior arm; Pm, paramere; Pt, phallosome; VV, ventral valve. Scale bars = 0.1 mm.

intermittently maintain a mouth-to-mouth mode for approximately two hours, and do not transfer any salivary secretion. This special behaviour is lacking in *N. longiprocessa* and other genera of scorpionflies (Thornhill, 1980, 1981; Thornhill & Sauer, 1991; Sauer et al., 1998; Engqvist & Sauer, 2001; Engles & Sauer, 2006; Zhong & Hua, 2013b; Zhong et al., 2015a, b; Tong et al., 2018). The genital couplings are very similar among the three species of *Neopanorpa* with notal organs of various lengths.

The hypoalves grasp the cerci of the female and move up and down rhythmically, likely to stimulate and pacify the female (Ma et al., 2010; Zhong & Hua, 2013b; Zhong et al., 2015a, b;

Tong et al., 2018). The paired lateral processes on the male hypoalves of *N. carpenteri* may function to help the hypoalves to control the female cerci. Although the hypoalves of *Neopanorpa* are morphologically different from those of other genera (Cai & Hua, 2008; Ma & Hua, 2011a; Zhong & Hua, 2013a; Gao et al., 2016; Wang & Hua, 2018a, 2019), their function may be similar to that of *Cerapanorpa*, *Furcatopanorpa*, and *Panorpa*.

The well-developed basal processes on the male gonostyli of *Neopanorpa* firmly control the female medigynium to allow the male aedeagus to couple with the female medigynium, as in *Panorpodes kuandianensis* Zhong,

Zhang & Hua, 2011 (Tong et al., 2017). In *F. longihypovalva* and *C. dubia* (Chou & Wang, 1981), however, the undeveloped basal processes grasp the posterior portion of the female medigynium because the female axis is elongated (Zhong et al., 2015b; Tong et al., 2018). The ventral valves of the male aedeagus of *Neopanorpa* are longer than those of *C. dubia* (Gao et al., 2016; Tong et al., 2018) and also function to keep down the female medigynium. Therefore, the female medigynium of *Neopanorpa* can be ventrally controlled by the ventral valves of the aedeagus, seized dorsally and anteriorly by the basal processes. The males of *Neopanorpa* may control the female medigynium more firmly than other genera of Panorpidae.

The mouth-to-mouth behaviour is an interesting phenomenon in insect mating process. Some social insects (such as bees, ants and wasps) pass food to one another through a mouth-to-mouth trophallaxis (Korst & Velthuis, 1982; Suryanarayanan & Jeanne, 2008; Hamilton et al., 2010). In Mecoptera, the males of *Chorista* (Choristidae) and *Furcatepanorpa* (Panorpidae) transfer liquid salivary secretions through a mouth-to-mouth mode during mating (Byers & Thornhill, 1983; Zhong et al., 2015b). In *N. lui* and *N. carpenteri* the males and females connect their mouthparts to each other prior to copulation but do not transfer any edible food. It is difficult for us to support the hypothesis that *Neopanorpa* female stimulates the male to secrete a nuptial gift through a mouth-to-mouth mode, because the male of *N. setigera* Wang & Hua, 2018 with a greatly elongate notal organ also has the mouth-to-mouth behaviour during the seizing phase but lacks nuptial feeding (supplementary fig. S3) as in *N. longiprocesa*. We infer that *Neopanorpa* males may stimulate and pacify the female through the connection of the mouthparts to benefit them to obtain copulation.

In Mecoptera the nuptial feeding behaviour is widespread in Panorpidae, Panorpididae, Bittacidae, and Choristidae (Byers & Thornhill, 1983; Sauer et al., 1998; Engqvist & Sauer, 2003; Gao & Hua, 2013; Zhong et al., 2015a, b; Tong et al., 2017). It is regarded to help the male scorpionflies to attract a female and prolong the copulation duration (Sauer et al., 1998; Engqvist & Sauer, 2003; Zhong et al., 2015a). The only reported species lacking a nuptial feeding behaviour during mating in *Neopanorpa* is *N. longiprocesa*, whose elongated notal organ can seize the female's wings for approximately two hours to coercively mate with the female (Zhong & Hua, 2013b). During this process, however, an intense physical conflict arises in the male and the female of *N. longiprocesa*. In the present study, the males of *N. lui* and *N. carpenteri* obtain copulation soon afterwards when they secrete a salivary mass. The female shows little resistance when she begins to feed on the nuptial gift. Our results may support the hypothesis that the nuptial feeding behaviour benefits male scorpionflies to obtain copulation easily and reduce the resistance of the female. However, our data hardly provide any evidence that the nuptial gift can prolong copulation, because the copulation duration of three species of *Neopanorpa* have no significant differences.

The insects' glands associated with nuptial feeding behaviours show a diversity according to their various abilities to secrete a nuptial gift, such as the accessory glands of dobsonflies and the salivary glands of scorpionflies (Byers & Thornhill, 1983; Hayashi, 1992; Ma & Hua, 2011b; Ma et al., 2011; Liu et al., 2015). The male dobsonflies of *Neoneuromus*, *Nevromus*, *Neurhermes*, and *Protohermes* can provide larger spermatophores as nuptial gifts due to their well-developed accessory glands (Hayashi, 1992; Liu et al., 2015). The male scorpionflies of *Furcatepanorpa*, *Dicerapanorpa*, *Cerapanorpa*, and most

*Panorpa* can offer abundant salivary secretions as nuptial gifts corresponding to their elongated multi-furcated salivary glands (Potter, 1938; Ma et al., 2011; Zhong et al., 2015b).

The males of *P. liui* and *N. longiprocesa* fail to provide any salivary secretions as a nuptial gift because their salivary glands are very short and only extend to the prothorax (Ma & Hua, 2011b; Ma et al., 2011). In *N. lui* and *N. carpenteri* the males offer salivary masses to the female during mating. Although their salivary glands are also bifurcated, they are elongated to the abdominal segment V or VI. The male of *N. carpenteri* can produce larger salivary mass than the male of *N. lui* because its salivary glands are more developed and longer than the latter. Our results may provide additional evidence to support the hypothesis that the developmental degree of the secretory glands may correspond to a male's ability to produce the salivary secretions as the nuptial gift.

The notal organs of various lengths function to seize the wings of female scorpionflies to enhance the male control of copulation (Thornhill & Sauer, 1991; Kock et al., 2009; Zhong & Hua, 2013b). However, only in *Neopanorpa* with an extremely elongated notal organ can the male firmly grasp the female to primarily adopt a forced mating tactic. The male with a weakly-developed or medium-sized notal organ probably has to evolve a nuptial feeding behaviour to attract the female to obtain copulation with ease. We summarize the lengths of the notal organs of 32 species of *Neopanorpa* in supplementary table S1, according to the phylogenetic tree presented by Miao et al. (2019). Most notal organs of *Neopanorpa* males are short. Only a few males bear the extremely elongated notal organs and they are marked in supplementary fig. S4. It is necessary to add ancestral reconstruction analysis for future studies to assess the evolution of the notal organ lengths and the mating tactic of *Neopanorpa*.

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## Supplementary material

Supplementary material is available online at: <https://doi.org/10.6084/m9.figshare.9192470>

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