Morphological diversity of male salivary glands in Panorpidae (Mecoptera)

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Abstract. The morphology of the male salivary glands of eighteen species of Panorpidae from China was studied using light microscopy. The results show that the male salivary glands differ markedly both at generic and specific levels. In Neopanorpa, the salivary glands consist of only two simple long secretory tubes extending to the fifth or sixth abdominal segment, whereas in Sinopanorpa, the salivary glands are composed of six extremely elongated secretory tubes. In Panorpa, the salivary glands are quite diverse, comprising two simple short secretory tubes only extending to the prothorax in the P. amurensis group (P. liui and P. jilmensis), six long tubes in the P. centralis group, eight to twelve in the P. dicerca group and of a very variable number in the P. davidi group (especially in P. bifasciata and P. subambra). Morphology of the male salivary glands should be included in future studies on the systematics and phylogeny of the Panorpidae.

INTRODUCTION

Panorpidae, the most species-rich family in the Mecoptera, currently consist of over 420 species in four genera (Byers & Thornhill, 1983; Cai et al., 2008). The genus Panorpa L., 1758 occurs throughout the northern continents and includes 274 species (Penny & Byers, 1979; Hua & Cai, 2009). The Oriental Neopanorpa Weele, 1909, ranging from India through southern China and Indochina and southward to Indonesia, consists of 134 species (Cheng, 1957; Rust & Byers, 1976; Cai & Hua, 2009). Leptopanorpa MacLachlan, 1875, with only 13 species, is claimed to be restricted to Java, Indonesia (Lieftinck, 1936; Chau & Byers, 1978). The Oriental Sinopanorpa Cai & Hua in Cai et al., 2008 comprises three species in China.

The genus Panorpa is a large and widely distributed group and its constituent species are categorized into different species groups based on morphological criteria for local faunas (Carpenter, 1931a, 1938; Issiki, 1933; Cheng, 1957; Willmann, 1977; Byers, 1993). Based on the structure of the sixth abdominal segment of males – whether the anal horn is present, absent, or represented by two similar processes, the Chinese Panorpa fauna is divided by Carpenter (1938) into three species groups: P. dicerca group (with double anal horns), P. centralis group (with single anal horn) and P. davidi group (without anal horn). The Northeast Asian fauna of Panorpa is divided by Issiki (1933) into nine species groups including the P. communis and P. amurensis groups. Panorpa is considered paraphyletic based on morphological and molecular data (Willmann, 1989; Misof et al., 2000). However, the paraphyletic problem of Panorpa has not yet been resolved. More new characters are needed for a phylogenetic analysis of the Panorpidae.

Salivary glands of most insects are labial glands, which are remarkably variable at the level of species, family and even order (Chapman, 1998; Walker, 2003). For the Mecoptera, Potter (1938) has described salivary glands of eight species representing six families and records that salivary glands of each family differ considerably in structure. In Panorpidae the salivary glands exhibit considerable sexual dimorphism, the males with well-developed salivary glands and the females with very small and undeveloped salivary glands in P. communis (Dufour, 1841; Potter, 1938) and P. obtusa (Liu & Hua, 2010). This sexual dimorphism in the salivary glands is related to mating behaviour in that the salivary secretions of males are involved in the production of the nuptial gift offered to females during courtship and copulation (Byers & Thornhill, 1983). However, the salivary glands in P. liui are not sexually dimorphic and those of males and females are very similar, both being composed of a common duct and only two short sac-like glands (Ma & Hua, 2011). In this paper, the morphology of the male salivary glands of 12 species of Panorpa, two of Sinopanorpa and four of Neopanorpa are compared and their potential significance in future systematic studies on Panorpidae is highlighted.

MATERIAL AND METHODS

Insects included in this study, localities and dates of collection are presented in Table 1. Specimens from 12 species of Panorpa, two of Sinopanorpa and four of Neopanorpa were investigated, representing all the three genera in the Chinese fauna of Panorpidae. Based on morphological criteria, the species of Panorpa examined are assigned to four species groups sensu Carpenter (1938) and Issiki (1933): P. dicerca group (P. magna and Panorpa sp.), P. centralis group (P. byersi, P. dubia, P. nanwutaina and P. obtusa), P. davidi group (P. bifasciata, P. kunmingensis, P. sexspinosa and P. subambra), and P. amurensis group (P. liui and P. jilmensis).

Morphology of the salivary glands of some European species of Panorpa reared in the laboratory is claimed to be influenced by age and nutritional status of the adults (Engqvist & Sauer, 2001; Engels & Sauer, 2008). The material used in this study was collected from natural populations in mountainous areas. In these habitats the scorpionflies should be well fed and have no nutritional problems, and their salivary glands should have developed normally. Based on the dissection of many individuals (see Table 1), the morphology of salivary glands of individuals of the same species is very similar.

The specimens in these eighteen species were prepared using three methods of fixation. The live adults of P. obtusa, P.
byersi, P. kunmingensis, P. jilinensis, Panorpa sp., Neopanorpa pulchra, N. penda and N. hainanica were fixed in aqueous Bouin’s solution (picric acid saturated aqueous solution: 40% formalin : glacial acetic acid = 15 : 5 : 1 v/v) for 12 h, washed in 50% ethanol and then in 70% ethanol several times until the washing fluid was no longer stained yellow and stored in 70% ethanol at 4°C before dissection. The freshly collected specimens were fixed in Carnoy’s solution (95% formalin : glacial acetic acid : distilled water = 6 : 15 : 1 : 80 v/v), which was heated until it began to give off vapour but was not bubbling and then for fixation left to stand for 12 h under hood. Then they were washed in 50% ethanol and stored in 70% ethanol at 4°C before dissection. The freshly collected specimens were fixed in Dietrich solution (formalin : 95% ethanol : glacial acetic acid = 3 : 1 v/v) for 12 h before being washed in 50% ethanol and then in 70% ethanol several times until the washing fluid was no longer stained yellow and stored in 70% ethanol at 4°C before dissection. The freshly collected specimens were fixed in aqueous Carnoy’s solution (95% ethanol : glacial acetic acid : distilled water = 6 : 15 : 1 : 80 v/v), which was heated until it began to give off vapour but was not bubbling and then for fixation left to stand for 12 h under hood. Then they were washed in 50% ethanol and stored in 70% ethanol at 4°C. Specimens fixed in Dietrich solution were more easily dissected and in a better state than samples fixed in the other two solutions.

The fixed male specimens were partly embedded in melted wax to reduce movement (Schönitzer & Seifert, 1990) and their salivary glands were dissected out in 70% ethanol under Nikon SMZ168-BL and SMZ1500 stereo microscopes. Photographs were taken with a Nikon CoolPix5000 digital camera attached to the microscope. Only pictures of the salivary glands of 13 species are shown. The other five species are not illustrated because of the poor quality of their preservation.

RESULTS

Gross morphology of the male salivary glands

The male salivary glands of scorpionflies are tubular labial glands, opening at the bottom of the salivarium between the labium and hypopharynx. Posterior to the hypopharynx, there is a pair of dilator muscles, the salivary pump, which is attached ventrally to a dark sclerotized plate. The salivary pump is connected ventrally with the labium and is easily recognized due to the dark coloration of its cuticle. The common salivary duct emerges from the salivary pump and passes posteriorly through the head and bifurcates into two branches (salivary ducts) below the suboesophageal ganglion just anterior to the tentorial bridge. In the anterior part, each branch expands into a sac and the two sacs frequently press up against each other. The subsequent parts of salivary glands lie around the gut and each generally is divided into three distinct regions along the length of the gland: the anterior constricted region, the middle dilated region (or salivary reservoir) and the posterior (or apical) thin secretory region (Fig. 1). The salivary glands vary greatly in their relative size, position and number of secretory tubes both at the specific and generic levels.

Salivary glands in the Panorpa centralis group

The male salivary glands in P. byersi, P. dubia, P. nanwutaina and P. obtusa are very similar in gross morphology (Figs 1A–D). The common salivary duct emerges from the base of the salivary pump below the pharyngeal trough and bifurcates below the suboesophageal ganglion into two salivary ducts. The salivary ducts run up on either side of the nerve cord to the osphagus, where each expands into a sac and then is divided into three secretory tubes, two dorsal and one ventral to the sac and gut. The secretory region leading from the reservoir gradually becomes thinner as it passes backwards and extends as far down the abdomen as the testes in the sixth segment, where its free end bends upwards or downwards.

Although similar in gross morphology, the secretory tubes differ in these four species, especially in the length of the salivary reservoirs. In P. byersi the six secretory tubes are very similar in size, shape and length (Fig. 1A). In P. dubia the two ventral tubes are longer than the four dorsal tubes and have a considerably longer salivary reservoir (Fig. 1B). In P. nanwutaina the two ventral tubes are longer than the four dorsal tubes and have thicker salivary reservoirs (Fig. 1C). The secretory tubes of P. obtusa are the shortest among the four species and their salivary reservoirs are the thickest (Fig. 1D).

Salivary glands in the Panorpa diceras group

The salivary glands of the P. diceras group (P. magna and Panorpa sp.) are easily distinguished as they have numerous secretory tubes and a particular configuration. The number of secretory tubes is eight in P. magna (Fig. 1E) and twelve in an

<table>
<thead>
<tr>
<th>Genera and species groups</th>
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<th>Specimens dissected</th>
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</thead>
<tbody>
<tr>
<td>Panorpa centralis group</td>
<td>P. byersi Hua &amp; Huang in Li et al., 2007</td>
<td>16</td>
<td>Taibai Co., Shaanxi</td>
<td>vi.2008</td>
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<td></td>
<td>P. nanwutaina Chou in Chou et al., 1981</td>
<td>20</td>
<td>Mt. Taibaishan, Shaanxi</td>
<td>v.2008</td>
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<tr>
<td>Panorpa diceras group</td>
<td>P. magna Chou in Chou et al., 1981</td>
<td>8</td>
<td>Huoditang Forest Farm, Shaanxi</td>
<td>vii.2010</td>
</tr>
<tr>
<td></td>
<td>Panorpa sp.</td>
<td>18</td>
<td>Mt. Taibaishan, Shaanxi</td>
<td>v.2008</td>
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<tr>
<td></td>
<td>P. sexspinosa Cheng, 1949</td>
<td>7</td>
<td>ZHUQUE FOREST PARK, SHANXI</td>
<td>vii.2010</td>
</tr>
<tr>
<td></td>
<td>P. subambra Chou &amp; Tong in Chou et al., 1987</td>
<td>3</td>
<td>QIZIMEISHAN NAT. RESERVE, HUBEI</td>
<td>vii.2008</td>
</tr>
<tr>
<td></td>
<td>P. kunmingensis Fu &amp; Hua, 2009</td>
<td>8</td>
<td>Mt. Xishan, Yunnan</td>
<td>vii.2010</td>
</tr>
<tr>
<td></td>
<td>P. jilinensis Zhou, 2000</td>
<td>10</td>
<td>Linjiang, Jilin</td>
<td>vii.2006</td>
</tr>
<tr>
<td>Sinopanorpa</td>
<td>S. tincta (Navás, 1931)</td>
<td>21</td>
<td>ZHUQUE FOREST PARK, SHANXI</td>
<td>vii.2010</td>
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<td></td>
<td>S. nangongshana Cai &amp; Hua in Cai et al., 2008</td>
<td>9</td>
<td>Mt. Nangongshan, Shaanxi</td>
<td>vii.2010</td>
</tr>
<tr>
<td>Neopanorpa</td>
<td>N. pulchra Carpenter, 1945</td>
<td>10</td>
<td>Mt. Diaoluooshan, Hainan Island</td>
<td>iv.2008</td>
</tr>
<tr>
<td></td>
<td>N. pendula Qian &amp; Zhou, 2001</td>
<td>7</td>
<td>Zhongdian Co., Yunnan</td>
<td>vii.2010</td>
</tr>
</tbody>
</table>

TABLE 1. List of species of Panorpidae examined, locations and collection dates.
undescribed species (Fig. 1F). The tubes are similar to each other in length, size and shape. In addition, each of them is just a narrow tube extending along the whole length of the gland, lacking both a constricted region and dilated salivary reservoir (Fig. 1F).

Salivary glands in the Panorpa davidi group

The salivary glands in the P. davidi group are quite diverse morphologically. In general, they can be divided into three subgroups.

In the first subgroup represented by P. sexspinosa and P. kunmingensis, the male salivary glands are similar to those of the P. communis group in general morphology. The common salivary duct bifurcates below the suboesophageal ganglion into two sacs, each of which in turn divides again into three secretory tubes. The two ventral tubes bear prominently thicker and longer salivary reservoirs than the four dorsal tubes (Fig. 2C). The only difference between these two species is the secretory tubes being markedly shorter in P. kunmingensis.
Representing the second subgroup, *P. bifasciata* is unique in *Panorpa* in that the male salivary glands only have four secretory tubes. After the sac each salivary duct only branches once, resulting in a total of four long secretory tubes, two dorsal and two ventral (Fig. 2B). Each secretory tube is constricted slightly at the base, and then gradually inflates into a stomach-like salivary reservoir, the diameter of which is wider than in all the other species of Panorpidae examined. The two ventral reservoirs are larger than the two dorsal reservoirs. The secretory region is much thinner than the reservoir, with a sharp delimitation between them. From the reservoir, the ventral secretory tubes bend abruptly forward for a short distance and then turn backwards convolutedly and extend to the end of the fifth abdominal segment. The two dorsal secretory tubes extend backwards directly from the reservoir.

Fig. 2. Salivary glands of males unless specified otherwise. A – *Sinopanorpa tincta*; B – *Panorpa bifasciata*; C – *P. sexspinosa*; D – *P. subambra*; E – *P. liui*, male and female; F – *Neopanorpa pulchra*; G – *N. minuta*. CR – constricted region; CSD – common salivary duct; Sac – salivary sac; SeR – secretory region; SP – salivary pump; ST – secretory tube; SR – salivary reservoir; Td – dorsal secretory tube; Tv – ventral secretory tube. Scale bars: A–D, F, G = 1 mm; E = 0.5 mm.
The male salivary glands in *P. subambra*, the representative of the third subgroup, are extraordinary in their unique configuration with extremely elongated sacs. The thin common salivary duct divides posteriorly below the suboesophageal ganglion to form two extremely elongated sacs lying at the bottom of the body cavity and extending to the end of the third abdominal segment (Fig. 2D). Posterior to the sac, each secretory tube branches several times successively (mostly in a dichotomous way) into 8–9 thin tubes which are convoluted in the fourth and fifth abdominal segments (Fig. 2D). In this case the three different regions of the tube as indicated above are not distinguishable. The reservoirs are not prominent and are very short, closely connected with the distal end of the sac and without a constricted region.

**Salivary glands in the Panorpa amurensis group**

The *P. amurensis* group is represented in China by *P. liui* and *P. filinensis*. The most surprising result is that the male salivary glands of these two species, unlike other species with large salivary glands and several long secretory tubes that extend into the abdomen and display various configurations, are very short and simple, merely consisting of a salivary pump, a common salivary duct and two very short secretory tubes, which are about 1 mm long and only extend to the prothorax (Fig. 2E). The female salivary glands are very similar to those of the males, i.e., there is no sexual dimorphism in the salivary glands in these two species (Fig. 2E), which is quite different from the situation in the other species groups of *Panorpa* examined.

**Salivary glands in Sinopanorpa**

*Sinopanorpa tincta* and *S. nangongshana* were dissected. The salivary glands of these two species are similar. The common salivary duct passes backward through the head and divides below the suboesophageal ganglion into two short salivary ducts, each of which widens into a sac. From the sac three secretory tubes arise, two dorsal and one ventral. The salivary glands of *S. tincta* are exceptional in having the longest secretory tubes and longest secretory reservoirs of all the species examined. Each secretory tube is about 17–18 mm long, slightly constricted at the base and gradually narrowing in diameter as it extends convolutely backwards as far as the accessory glands of the reproductive system in the sixth abdominal segment and then turns back on itself. It is noticeable that in *S. tincta* the number of secretory tubes varies among specimens, six in most individuals (Fig. 2A) and seven in a few, with four on one side and three on the other. The salivary tube (including the salivary reservoir and secretory region) in *S. nangongshana* is also very long and slightly thicker.

**Salivary glands in Neopanorpa**

*Neopanorpa pulchra*, *N. minuta*, *N. hainanica* and *N. pendula* were examined. The male salivary glands of these four species are very similar, with only two elongated secretory tubes lying on the ventral side of the gut. In general, the tube is divided into an anterior thick part and posterior apical thin part. The posterior portion of the thick tube is convoluted and is the same diameter as the anterior straight portion. The twisted thin tube extends to the end of the fifth abdominal segment in *N. pulchra* and the sixth segment in *N. minuta*, *N. hainanica* and *N. pendula*.

In *N. pulchra* the slightly expanded basal part of the secretory tube extends to the end of the third abdominal segment and then gradually narrows into a long thin convoluted tube that extends to the end of the fifth abdominal segment (Fig. 2F). The apical thin tube is much longer in this species than in its congeners.

In *N. minuta* the secretory tubes are thick for almost their whole length, with only the short apical part much thinner (Fig. 2G). The thick tube extends to the end of the third abdominal segment and from there on is convoluted to the sixth segment. Unlike in *N. pulchra*, where the secretory tube has a long thin apical tube, *N. minuta* has a very short apical tube (Fig. 2G).

The secretory tubes in *N. hainanica* and *N. pendula* are similar to those in *N. minuta*, but are slightly longer and more convoluted and difficult to distinguish.

**DISCUSSION**

The salivary glands differ considerably in structure among the six families of Mecoptera (Potter, 1938). They are tubular in Panorpidae and Boreidae, botryose with many globular secretory acini in Meropidae and intermediate between tubular and acinous in Bittacidae. The salivary glands of Meropidae are similar to those of lower neopteran insects, suggesting that Meropidae might be one of the basal taxa in Mecoptera (Potter, 1938). However, according to Friedrich & Beutel (2010), Meropidae clearly belong to the Pistillifera, whereas Boreidae and Nannochoriistidae do not.

The size of male salivary glands in some European *Panorpa* is dependent on the age of the adults (Mercier, 1915). Although the salivary glands are not developed in newly emerged males whose glandular tubes might be as short as 2 or 3 mm, they reach their mature and relatively constant size after 4 days (Carpenter, 1931b; Engels & Sauer, 2008). The specimens used in this study were all collected from natural populations over several years from the same or different localities and in different months. A large number of specimens were dissected to check the stability of the male salivary glands. For example, over 40 specimens collected over several years were dissected of *P. liui*, the secretory tubes of which were only 1 mm long (Fig. 2E), even shorter than the 2 or 3 mm of the newly emerged male adults of European *Panorpa* (Carpenter, 1931b), and the salivary gland morphology was very stable (Ma & Hua, 2011). Hence, it is very likely that the salivary glands of the male Panorpidae examined reflected their natural state.

The salivary glands of only a limited number of species of insects have been investigated in detail and they show great range of variation (Walker, 2003). In Panorpidae only the male salivary glands of the European *P. communis* and *P. germanica* and Asian *P. obtusa* and *P. liui* have been previously studied (Potter, 1938; Liu & Hua, 2010; Ma & Hua, 2011). The present paper is the first attempt to compare the morphology of male salivary glands in Panorpidae both at the specific and generic levels. The male salivary glands were found to vary remarkably among the genera and even the species in Panorpidae.

In the four species of *Neopanorpa* examined, the male salivary glands consist of only two long secretory tubes extending to the fifth or sixth abdominal segment. The two elongated secretory tubes might be the generic character of the salivary glands in *Neopanorpa*.

In *Sinopanorpa* the salivary glands are extraordinary in having extremely elongated secretory tubes. Their salivary reservoir is the longest of the species of the Panorpidae investigated.

In *Panorpa* the salivary glands are variable. The European *P. communis*, the type species of the genus, has six tubular glands, which are uniform in diameter and lack a salivary reservoir (Potter, 1938). In the Chinese species of *Panorpa* the number and configuration of secretory tubes vary greatly among species; they are remarkably constant within the *P. amurensis*, *P. centralis* and *P. diceras* groups, but very variable in the *P. davidi* group. In the *P. amurensis* group the male salivary glands are peculiar in that they are very small and consist of only two short secretory tubes that only extend to the prothorax rather than to the abdomen. In general, the salivary glands exhibit a
distinct sexual dimorphism in *Panorpa* (Brants, 1839; Dufour, 1841; Brauer, 1855). However, in *P. liui* and *P. jilinensis* the salivary glands are very similar in the two sexes, showing that sexual dimorphism of the salivary glands is not universal in the genus *Panorpa* as currently accepted (the genus may be paraphyletic, as proposed by Willmann, 1989, or Misof et al., 2000).

In the *P. centralis* group the male salivary glands are quite similar in their relative size, configuration and number of secretory tubes. Each of the six secretory tubes is distinctly differentiated into three regions – a constricted region, a dilated reservoir and a thin apical secretory region. In the *P. dicerca* group the number of secretory tubes is much greater than in other species groups, varying from eight in *P. magna* to twelve in an undescribed species. In the *P. davidi* group the male salivary glands vary greatly among species. They are similar to those of the *P. centralis* group in *P. sexpinosa* and *P. kummingensis*, but quite unique in *P. bifasciata* and *P. subambra*. The salivary glands have only four secretory tubes in *P. bifasciata*, but are extraordinary for the two elongated salivary ducts bifurcating several times into eight or nine tubes in *P. subambra*.

Based on this investigation, characters of male salivary glands can be used to distinguish genera of Panorpidae, and might provide additional evidence in future comprehensive phylogenetic studies. The *P. centralis*, *P. dicerca*, and *P. amurenensis* groups are easily distinguished from each other and it may be reasonable to raise these species groups to (sub)generic levels if supported by other characters. The morphology of the salivary glands of the *P. davidi* group is very variable and needs further research.


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