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# Morphology of Immature Stages of *Dohrniphora cornuta* (Bigot) (Diptera: Phoridae)

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*KEY WORDS* postmortem interval; larvae; puparia; phoridae

*ABSTRACT* Morphology of all larval instars and puparium of *Dohrniphora cornuta* (Bigot), a most common phorid fly species indoors in China, is presented using scanning electron microscopy. The first instar larva was composed of 12 segments, each of segments 3–11 with six slender tubercles situated dorsally, dorsolaterally, and laterally in transverse row. These tubercles divided into two segments, of which the basal one was smooth, and the brush-shaped distal one was comprised of a cluster of fine spines. Antennae and maxillary palp complex were visible. Two slits could be seen at the posterior spiracle. Besides the presence of anterior spiracle, the tubercles of second instar became stouter than those of first instar and were covered by numerous long bristles from the base to top. The posterior spiracle contained four slits. Third larval instar was similar to second instar. The bubble membrane comprised of ≈120 globules with a pointed tip on their top presented at the segment 5 of third instar larvae. Puparia showed a retracted cephalic region and a pair of distinct pupal respiratory horns on the dorsum. The respiratory horns were long and bore numerous branches from base to apex. The apex of branch with two longitudinal slits was relatively broad and curled dorsally. *Microsc. Res. Tech. 75:1528–1533, 2012.* 

## INTRODUCTION

Phorid flies are the most diversified group within the Diptera. Several genera (e.g., *Megaselia*, *Aneurina*, *Conicera*, *Diplonevra*, *Dohrniphora*, *Metopina*, *Triphleba*) were found to develop in vertebrate carrion (Smith, 1986). Thus far, larvae or pupae of these species, *Conicera tibialis* Schmitz, *Megaselia rufipes* (Meigen), *Megaselia abdita* Schmitz, *Megaselia scalaris* (Loew), *Megaselia spiracularis* Schmitz, *Megaselia curtineura* (Brues), Diplonevra florea (*Fabricius*), *Dohrniphora cornuta* (Bigot), *Triphleba opaca* (Meigen), and *Triphleba nudipalpis* (Becker) had been reported from human corpses (Boehme et al., 2010; Campobasso et al., 2004; Disney, 1994, 2006; Disney and Manlove, 2005, 2009; Greenberg and Wells, 1998; Manlove and Disney, 2008; Martín-Vega et al., 2011; Reibe and Madea, 2010; Thevan et al., 2010; Velásquez et al., 2010).

In forensic entomology, the determination of a minimum postmortem interval (PMI) often relies on the determination of the age of blow flies (Calliphoridae), since they are generally among the first colonizers of a corpse. But in indoor cases, due to their relatively small size, Phoridae can enter rooms with closed doors and windows (Disney, 1994) and deposit their offspring prior to Calliphoridae (Boehme et al., 2010; Manlove and Disney, 2008). So, age determination of Phoridae could give much more accurate estimates of the minimum PMI than from larvae of Calliphoridae, at closed places (Reibe and Madea, 2010).

*Dohrniphora cornuta* (Bigot) is one of the more conspicuous and widespread species of Phoridae. Its larvae were found in many kinds of decaying plant and animal tissues. This species is characterized as a secondary invader to carrion, appearing after certain sarcophagids, calliphorids, and *M. scalaris* (Barnes, 1990).

The identification of the fly larva or puparium to species is mandatory to improve the accuracy of a forensic investigation, if this larva or puparium is present in a corpse and could be used as entomological evidence. *D. cornuta*, a most common phorid fly species, is collected frequently when using pork as bait in room in Northeast China. Life history of *D. cornuta* was reported by Barnes (1990). The egg, third instar larva, cephalopharyngeal skeleton and puparium were described based on light microscopy (LM) (Disney, 1994; Kaneko and Furukawa, 1977; Peterson, 1987; Schmitz, 1941, 1949; Teskey, 1981). Moreover, Kloter et al. (1977) illustrated the sternal plate and lateral tubercles of first, second, and third instars. Scanning electron microscopy (SEM) can provide fine details of morphological features. The description of structures by SEM is indispensable for an effective correlation of larval morphology of one species in its three instars. For these reason, SEM is increasingly being adopted by forensic entomologist as a new device to obtain precise information about larval body morphology, not only in eggs and first and second instars, but also for recharacterizing third instar and pupae (Méndez-Vilas and Díaz, 2010). To provide more information on a phorid fly species that may be involved in future indoor forensic investigations in China, we are reporting herein some morphological characteristics of immature stages of *D. cornuta* with the aid of SEM.

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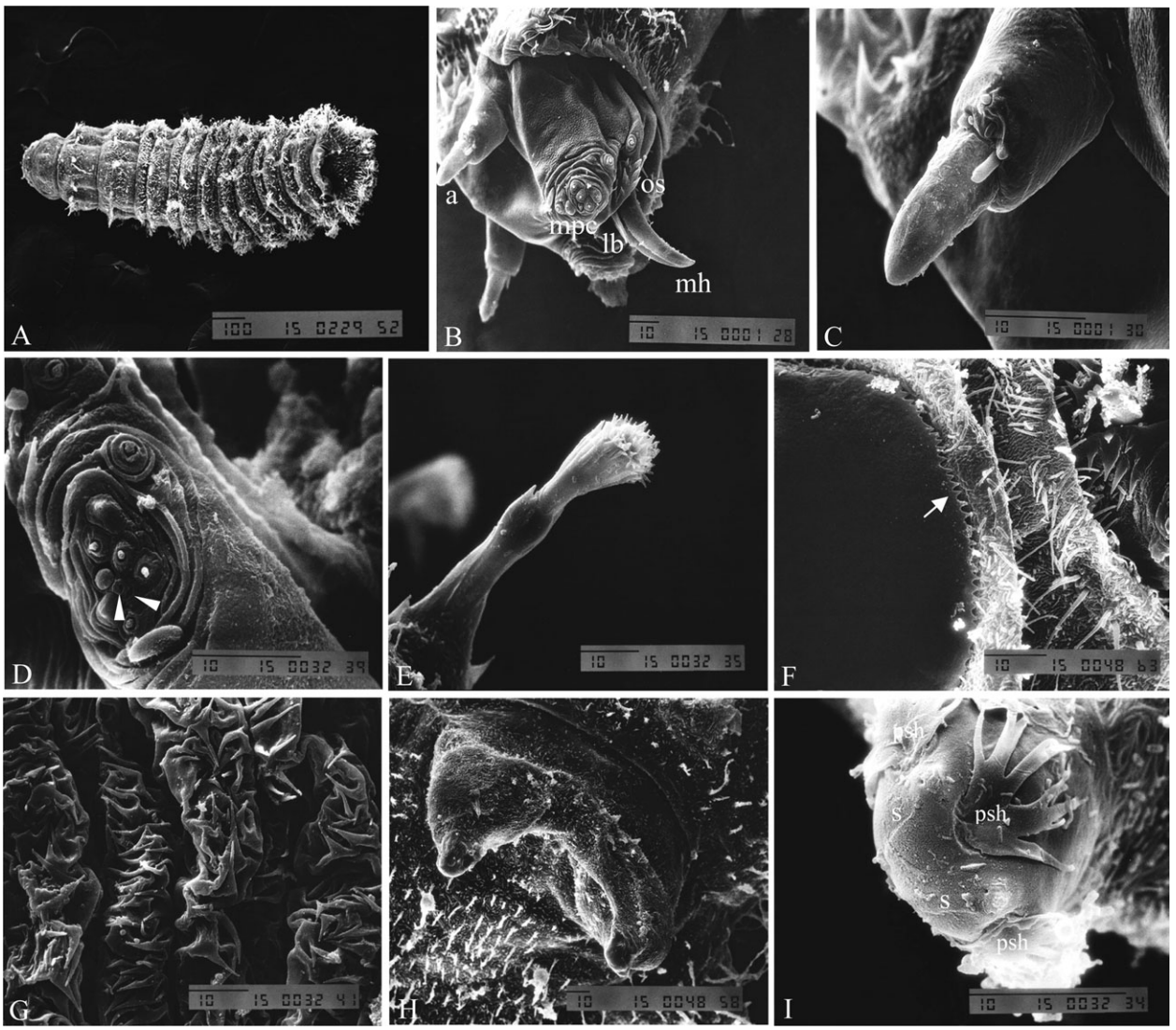


Fig. 1. Scanning electron micrographs of the first instar larva of *D. cornuta*. **A**: Dorsal view of the entire body, bar = 100 μm, ×100; **B**: anterolateral view of cephalic segment showing antenna (a), maxillary palp complex (mpc), labrum (lb), mouthhook (mh), oral spines (os), bar = 10 μm, ×1,000; **C**: details of antenna, bar = 10 μm, ×3,500; **D**: details of maxillary palp complex. Arrowheads indicate the pores, bar = 10 μm, ×3,500; **E**: details of tubercle, bar = 10 μm, ×2,000; **F**: sternal plate on segment 3. Arrow indicates the saw-like frontal edge, bar = 10 μm, ×2,000; **G**: higher magnification of transverse rows of small spines on ventral surface, bar = 10 μm, ×2,000; **H**: dorsal view of the caudal segment showing a pair of posterior spiracles, bar = 10 μm, 750×; **I**: posterior spiracle bearing two straight slits (s) and interspaced with groups of posterior spiracular hairs (psh), bar = 10 μm, ×5,000.

## MATERIALS AND METHODS

All immature stages of *D. cornuta* utilized in this study were obtained from a laboratory breed maintained at the Liaoning Key Laboratory of Urban Integrated Pest Management and Ecological Security, Shenyang University, China. The adults of *D. cornuta* were identified morphologically based on Liu (2001).

Larvae were washed several times with normal saline solution. They were then prefixed with 2.5% glutaraldehyde mixed in phosphate buffer solution (PBS) at a pH of 7.4 at 4°C for 24 h, rinsed twice with PBS at 15-min intervals, and postfixed with 1% osmium tetroxide at room temperature for 1–2 days. Specimens were then rinsed twice with PBS and dehydrated with alcohol. The dehydration process sequentially subjected the larvae to the following increased alcohol concentrations: 30, 50, 70, 80, and 90%. They were placed in acetone for another two 30-min periods followed by tertiary butanol for two 30-min periods. Finally, the larvae were subjected to vacuum drying, attached to double-stick tape on aluminum stubs and coated with gold in the sputter-coating apparatus to be viewed under a JEOL-T300 scanning electron microscope. Puparia were refrigerated at −20°C for 5 min, and then they were placed onto double-stick tape on aluminum stubs, coated with gold, and examined with the same SEM. A total of 110 specimens were observed: 40 specimens of first instar larvae, 30 specimens of second

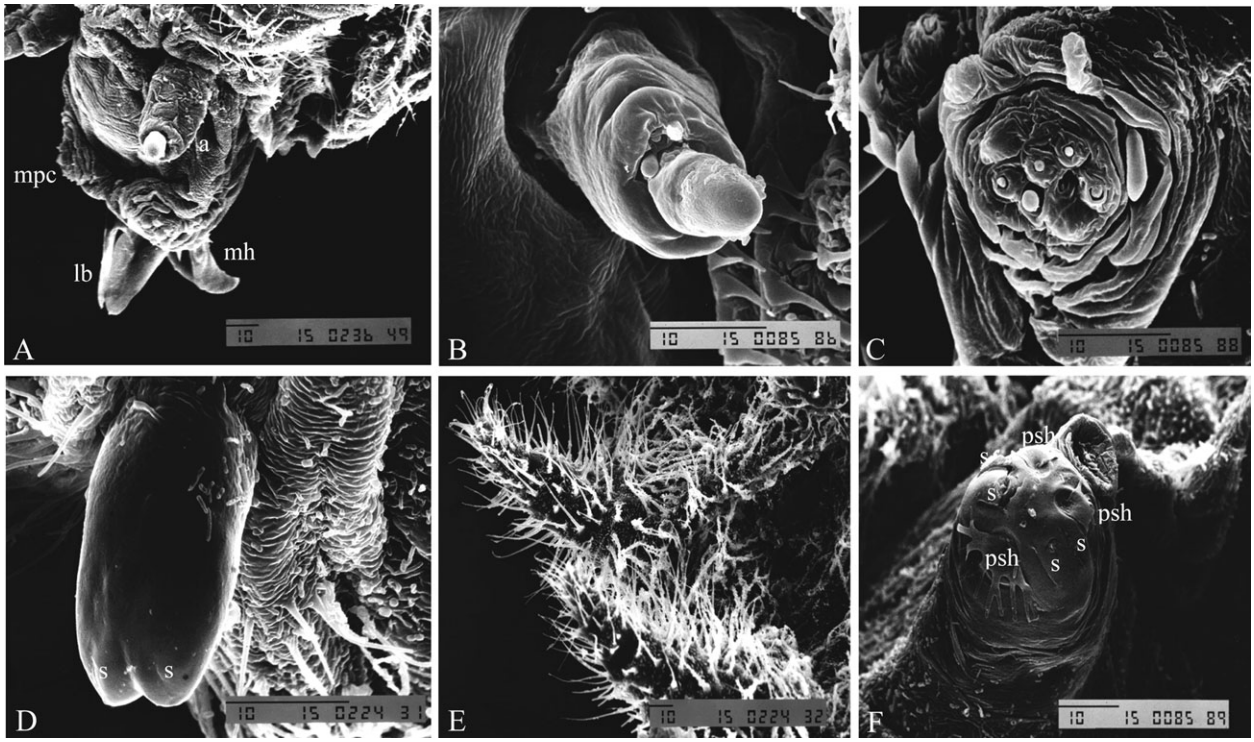


Fig. 2. Scanning electron micrographs of the second instar larva of *D. cornuta*. **A**: Dorsolateral view of cephalic segment showing antenna (a), maxillary palp complex (mpc), labrum (lb) and mouthhook (mh), ×1,000; **B**: details of antenna, ×3,500; **C**: details of maxillary palp complex, ×3,500; **D**: anterior spiracle bearing two straight slits (s), ×3,500; **E**: the stout tubercles situated laterally bearing numerous long bristles from the base to top, ×750; **F**: posterior spiracle showing four straight slits (s) and posterior spiracular hairs (psh), ×2,000. Bar = 10 μm for all figures.

instar larvae, 20 specimens of third instar larvae and 20 specimens of puparia.

## RESULTS

### First Instar Larva

The body of the first instar larva of *D. cornuta* is composed of 12 segments, each of segments 3–11 with six slender tubercles situated dorsally, dorsolaterally, and laterally in transverse row. The caudal segment is margined by six stout tubercles covered by numerous long bristles at the base through the apex (Fig. 1A). The labrum appears as a flattened, bi-lobed structure. The mouthhook curves downward, with a longitudinal bending groove on its dorsum. In the cephalic region, rows of longitudinal oral spines are located at each pseudocephalic lobes (Fig. 1B). Antenna is composed of two structures, a basal ring or socket with four sensory papillae located outside on its top and a distal conic-shaped dome (Figs. 1B and 1C). The maxillary palp complex (or terminal organ) is a flattened protuberance clearly distinguished from the ambient surface of the pseudocephalon. In the central part of the maxillary palp complex is a cluster of five distinct peg sensilla (MPS), four set in swollen sockets and one nonsocketed, plus two swollen dome-shaped sensilla. Two small pores are also observed in the cluster. Another nonsocketed sensillum is situated above the MPS cluster, and this sensillum is strongly elongated. One additional socketed peg sensilla surrounded by a circular ridge is under the MPS cluster (Figs. 1B and 1D). The slender tubercles along the body divide into two segments, of which the basal one is smooth, and the brush-shaped distal one is comprised of a cluster of fine spines (Fig. 1E). The frontal edge of sternal plate on segment 3 appears as sawteeth (Fig. 1F). Transverse rows of small spines are on the ventral surface of segments 4–12 (Fig. 1G). A pair of protruding posterior spiracles is located dorsally on the caudal segment (Fig. 1H). Each of these posterior spiracles contains two straight slits and is interspaced with groups of posterior spiracular hairs (Fig. 1I).

### Second Instar Larva

In the cephalic region of the second instar, with exception of the size, not much has changed in the appearance of the labrum, mouthhook, antenna, and the maxillary palp complex (Figs. 2A–2C). Each anterior spiracle is a circular papilliform structure that contains two straight slits (Fig. 2D). The tubercles become stouter than those of the first instar and are covered by numerous long bristles from the base to top (Fig. 2E). The posterior spiracle contains four straight slits and is interspaced with groups of posterior spiracular hairs (Fig. 2F).

### Third Instar Larva

In shape, the third instar generally resembles the second instar. The antenna and maxillary palp complex are completely developed (Figs. 3A–3C). The integument of the body is covered by dense hairs ventrally

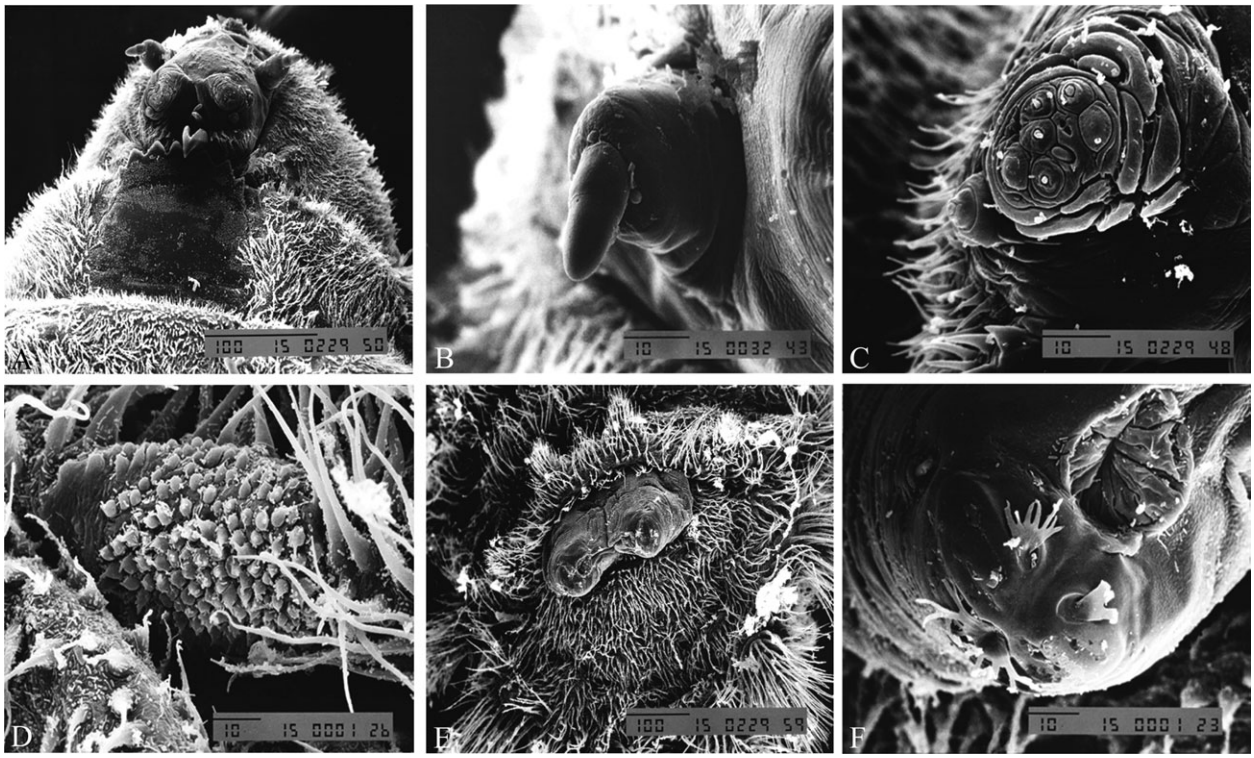

Fig. 3. Scanning electron micrographs of the third instar larva of *D. cornuta*. **A**: Ventral view of anterior region, bar = 100 μm, ×350; **B**: details of antenna, bar = 10 μm, ×2,000; **C**: details of the maxillary palp complex, bar = 10 μm, ×2,000; **D**: higher magnification of the bubble membrane, bar = 10 μm, ×1,500; **E**: dorsal view of the caudal segment showing a pair of posterior spiracles, bar = 100 μm, ×200; **F**: apical view of the left posterior spiracle, bar = 10 μm, ×1,500.

and dorsally (Figs. 3A and 3E). The bubble membrane, on the dorsum of the segment 5, is a group of globular structures with a pointed tip on their top (Fig. 3D). The number of globules is ≈120 in each. The posterior spiracles are similar to those of the second instar, except for the size (Fig. 3F).

### Puparium

The puparia of *D. cornuta* resemble third instars in most respects but bear a pair of distinct respiratory horns on the dorsum of the segment 5 (Fig. 4A). When dorsally observed, a pair of anterior spiracles is found on the segment 2 (Fig. 4B). These spiracles are more or less rounded, bearing two slits (Fig. 4C). The respiratory horns are long and bear numerous branches from its base through the apex (Figs. 4B and 4D). Many setae are located at the base of the branches (Fig. 4D). The dorsal surface of anterior end bears many small, rod-like tubercles (Fig. 4E). The apex of branch with two longitudinal slits is relatively broad and curled dorsally (Fig. 4F). At the ventral view, the cephalic segment is invaginated but the antenna, maxillary palp complex, and mouthhook are still visible (Fig. 4G). At the posterior end, a pair of posterior spiracles is observed on the dorsal surface of the last segment (Fig. 4H). Each spiracular plate is composed of four straight slits, two of each group in parallel. The posterior spiracular hairs can also be seen. A large cavity, in the position most fitted to the ecdysial scar or button, locates near the inner side of spiracular plate (Fig. 4I).

## DISCUSSION

This study clearly showed that the larval stages of *D. cornuta* share many morphological features during their development. Several morphological changes have been observed from the first to the second instar. Far fewer changes have been seen in development from the second to the third instar. With exception of the larger body size seen in transformation to the next larval stage, the most prominent changes observed were in the anterior and posterior spiracles. The anterior spiracles appear to be absent in the first instar larvae of *D. cornuta*, but are quite apparent as circular structures containing two slits in both the second and third instar larvae. Two slits of the posterior spiracles in the first instar are rudimentary while four straight slits arranged as two opposite groups in both the second and third instar larvae. This change was also observed in *M. scalaris* (Boonchu et al., 2004), *M. spiracularis* (Feng and Liu, in press) and *Diplonevra peregrina* (Feng and Liu, in press). Another distinct change is that the slender tubercles along the body segments, when developing to second instar, become stouter and are covered by numerous long bristles from the base to top.

The morphology of maxillary palp complex is considered to be of help in interspecific identification (Kirk-Spriggs, 2003; Singh et al., 2012). The maxillary palp complex of *D. cornuta* with several types of papillae is different from those of *M. scalaris*, *M. spiracularis*, and *D. peregrina* by the number and type of

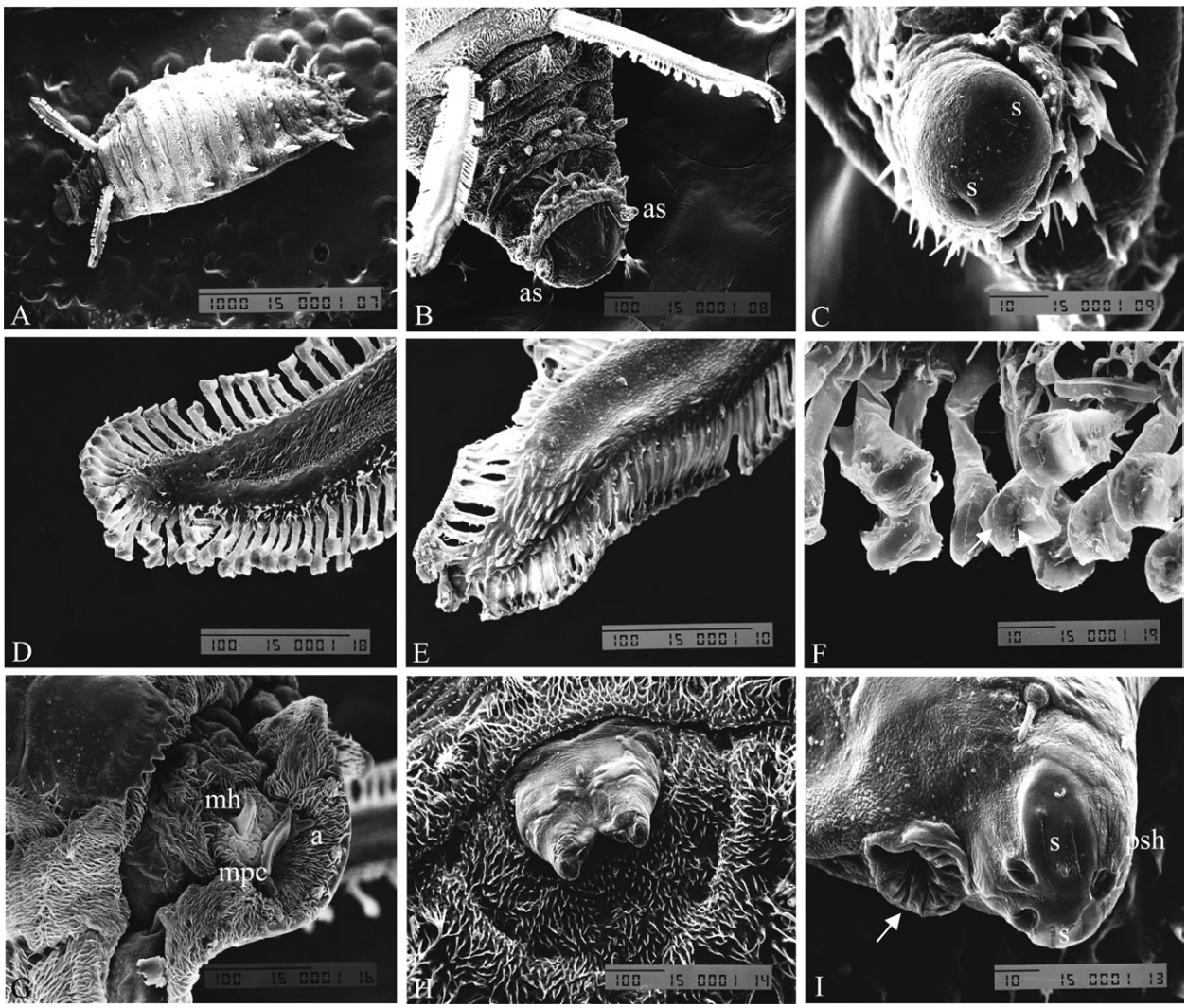


Fig. 4. Scanning electron micrographs of the puparium of *D. cornuta*. **A**: Dorsal view of the entire puparium, bar = 1,000 μm, ×35; **B**: dorsal view of the anterior region showing a pair of anterior spiracles (as) and respiratory horns, bar = 100 μm, ×100; **C**: anterior spiracle bearing two straight slits (s), bar = 10 μm, ×2,000; **D**: ventral view of the apex of respiratory horn, bar = 100 μm, ×500; **E**: dorsal view of the apex of respiratory horn, bar = 100 μm, ×500; **F**: details of branches of respiratory horn showing two longitudinal slits (arrows), bar = 10 μm, ×2,000; **G**: ventral view of the anterior end indicating an invaginated cephalic segment. Antenna(a), maxillary palp complex (mpc) and mouthhook (mh) were observed, bar = 100 μm, ×350; **H**: dorsal view of the posterior end indicating a pair of posterior spiracles, bar = 100 μm, ×200; **I**: right lobe of the posterior spiracle showing four straight slits (s) and posterior spiracular hairs (psh). The arrow probably indicates the ecdysial scar or button, bar = 10 μm, ×1,500.

sensilla and their arrangement. The slender tubercle of the first instar larvae is comprised of two segments, of which the brush-shaped distal one forms a cluster of fine spines, which differ markedly from other phorid flies such as *M. scalaris* and *M. spiracularis* (Sukontason et al., 2002; Boonchu et al., 2004; Feng and Liu, in press). Those revealed in *D. peregrina* also divide into two segments but its distal one looks more like a wheatear, and the basal one is covered by minute spines (Feng and Liu, in press). The bubble membrane is represented as one of characteristics used for differentiating the forensically important flies (Liu and Greenberg, 1989; Sukontason et al., 2006; Sukontason et al., 2008). In this study, the bubble membrane of *D. cornuta*, is a group of globular structures with a pointed tip on their top, which is different from the globular structures of other described flies. The number of globules is ≈120, which is much more than that of another phorid fly, *M. spiracularis* (≈40 globules). It is also higher than that of other flies, *C. macellaria* (absent of globules), *C. vicina* (≈20 globules), *Calliphora livida* Hall (≈25 globules), *Phaenicia sericata* (Meigen) (≈23 globules), and *Lucilia illustris* (Meigen) (≈30 globules), *Phormia regina* (Meigen) (≈40 globules), *Phaenicia coeruleiviridis* (Macquart) (≈50 globules), and *Calliphora peruviana* (Robineau-Desvoidy) (≈70 globules). However, the number of globules in *D. cornuta* is much less than that of *Chrysomya villeneuvi* (≈225 globules).

In addition, previous studies did not provide the fine structures of respiratory horns of *D. cornuta* because of restriction to the resolution. In this study, the respira-

tory horns are long and bear numerous branches from its base through the apex. The apex of branch with two longitudinal slits is relatively broad and curled dorsally, which is markedly different from other described flies.

The hairy *D. cornuta* larvae tend to embed themselves in the food, often becoming completely immersed for long periods while feeding (Barnes, 1990; Kloter et al., 1977). So its body is full of bacteria could not completely be cleared away when observed by SEM which influences the quality of some photographs in this study.

The present study expands the work of previous authors by SEM to describe fine details of the external morphology of immature stages of *D. cornuta* for possible future use, particularly during indoors forensic investigations.

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