Larval morphology of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) using scanning electron microscopy

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ABSTRACT: The larval morphology of *Chrysomya megacephala* (Fabricius) is presented using scanning electron microscopy (SEM). Extreme similarity of this species to *Chrysomya rufifacies* (Macquart), a species usually found concurrently inhabiting decomposing human corpses in Thailand, is seen only in the first-instar larvae. The relative thickness of the branches of the posterior spiracular hairs in these species could be used to differentiate them in this developmental stage. In contrast, the “hairy” appearance of *C. rufifacies* allows second- and third-instar larvae to be easily distinguished. Results of this study should help in future endeavors to differentiate *C. megacephala* from other larvae found in decomposing human corpses in Thailand. *Journal of Vector Ecology* 28(1): 47-52. 2003.

Keyword Index: *Chrysomya megacephala*, larval morphology, identification, scanning electron microscopy, forensic entomology.

INTRODUCTION

*Chrysomya megacephala* (Fabricius), the Oriental latrine fly, is a fly species of medical importance. Its distribution is throughout the Oriental, Australasian and Oceanian regions, and it was recently introduced to Africa, South America and Central America (Kurahashi and Magpayo 2000). This species is not only known for causing myiasis, mechanically transmitting various pathogens, and being a general domestic nuisance (Zumpt 1965, Greenberg 1971, Sukontason et al. 2000), but more recently *C. megacephala* has been identified as playing an important role in forensic cases (Smith 1986). The larvae of this species have been used as entomological evidence for estimating the postmortem interval (PMI) of a corpse (Lord 1990, Goff and Flynn 1991), as well as for detecting organophosphate poisoning in a putrefying human body (Gunatilake and Goff 1989). In Thailand, the existence of this species is widespread in both urban and forested areas. Larval specimens have been collected from corpses at quite diverse death scenes including forested, urban and suburban areas up to elevations as high as =1500 m above sea level in mountainous areas in the northern part of the country (Sukontason et al. unpublished data).

Specific identification of insects collected from a corpse is crucial before they can be reliably used for forensic investigation. Some morphological structures of *C. megacephala* larvae that could be used in identifying the species have already been described using light microscopy (Zumpt 1965, Wells et al. 1999) and scanning electron microscopy (SEM) (Kitching 1976). However, further treatment was warranted, so we therefore report the morphological features of all instars of *C. megacephala* through SEM, thereby providing several additional important structures to identify this species.

MATERIALS AND METHODS

*C. megacephala* larvae were obtained from a laboratory colony at the Department of Parasitology, Faculty of Medicine, Chiang Mai University. They were washed several times in normal saline solution to remove...
any foreign debris that might obstruct the view of important structures during microscopic examination. The larval specimens were chemically treated with 2.5% glutaraldehyde mixed in phosphate buffer solution (PBS) at a pH of 7.4 at 4 °C for 24 hr for primary fixation. They were then rinsed twice with PBS at 10-min intervals. Rinsed larvae were then treated with 1% osmium tetroxide at room temperature for 3-4 d to accomplish post fixation of the larval tissues. Post fixation was followed by rinsing twice with PBS and dehydrating the specimens with alcohol. To replace the water in larvae with alcohol, larvae were sequentially subjected to the following increasing concentrations of alcohol: 30%, 50%, 70%, 80% and 90%. Larvae remained in each concentration of alcohol for 12 hr during each step of the dehydration process. They were then placed in absolute alcohol for another two 12 hr periods followed by acetone for two 12 hr periods. Finally, the larvae were subjected to critical point drying to complete the dehydration process.

Following the dehydration process, the larvae were attached to double-stick tape on aluminum stubs in order to be coated with gold in the sputter-coating apparatus to enable viewing under a JEOL-JSM840A scanning electron microscope. The first and second-instars were processed as mentioned above, but the third-instars, which were quite large (=1.2 cm), were cut into three portions (head, body and caudal portions) before the initial chemical treatment. Due to their large size, this additional procedure was necessary for them to be easily processed and examined under SEM.

**RESULTS AND DISCUSSION**

The first-instar is muscoid-shaped and composed of 12 segments (Figure 1). The cephalic segment possesses a pair of dorsal organs (Figure 2), a pair of terminal organs (Figures 2 and 3), a pair of multi-branched mouthhooks (Figures 2 and 4) and three oral grooves at

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**Figures 1-4.** Scanning electron micrographs of first-instar *C. megacephala*. (1) Lateral view of whole larva. Anterior end (A) at right, posterior end (P) at left. Bar = 100 µm. (2) Anterolateral view of cephalic segment showing dorsal organ (DO), terminal organ (TO), oral groove (OG) and multi-branched mouthhooks (MH). (3) Terminal organ showing papillae. (4) Mouthhooks with several rows of single, curved spines.
Figures 5-7. Scanning electron micrographs of first-instar *C. megacephala*. (5) A pair of trichoid sensillae (arrows) bearing three setae and a pair of pit sensillae (arrowheads) located on the ventral of anterior edge of all thoracic segments. (6) Posterior view of larva showing the pair of posterior spiracular discs (PSD) and pair of circular, deep depressions ventral to the spiracular discs. (7) Posterior spiracular disc bearing two slits (S) that coalesce ventrally and are interspaced with relatively thin-branched posterior spiracular hairs (PSH). Bar = 10 µm.
Figures 8-12. Scanning electron micrographs of second-instar *C. megacephala*. (8) Anterolateral view of cephalic segment showing dorsal organ (DO), terminal organ (TO), ventral organ (VO), oral grooves (OG) and labium (L). (9) Higher magnification of terminal organ with arrowheads indicating two separated sensillae (10) Higher magnification of ventral organ showing C-shape and the four short spines located on the anterior side of the inner curvature of the organ. Bar = 1 µm. (11) Anterior spiracle consisting of a single row of 11 papillae bearing spiracular openings. (12) Posterior spiracular discs with each bearing two straight slits (S) encircled by thin-branched posterior spiracular hairs (PSH). The button (B), or ecdysial scar, is located ventro-medially on the posterior spiracular discs.
each side (Figure 2). The mouthhooks are situated mid-dorsally of the mouth region (Figure 2). Each mouthhook contains 3-4 rows of single, curved spines with sharp tips, giving them a multi-branched appearance (Figure 4). The anterior spiracle is not apparent in this instar. At the anterior edge of each thoracic segment are a lateral pair of trichoid sensillae bearing three setae and a medial pair of pit sensillae (Figure 5). The prominent features of the caudal segment are a pair of posterior spiracular discs and a pair of circular, deep depressions ventral to the spiracular discs (Figure 6). Each spiracular disc contains two straight spiracular slits that coalesce ventrally and are interspaced with bundles of relatively thin and multi-branched spiracular hairs (Figure 7).

The general morphology of the second-instar is very similar to that of the first-instar. The dorsal and terminal organs still remain with minimal change, but the ventral organ and oral grooves are more extensive and well developed (Figures 8 and 9). The labium appears as a tri-lobed structure. The terminal organ is composed of a group of sensillae, but in the second-instar two sensillae are widely separated from the rest of the cluster (Figure 9). The ventral organ appears as C-shaped with four short spines appearing on the anterior side of the inner curvature of the organ (Figure 10). The prothoracic anterior spiracles become well-developed in this stage, having 11 papillae in a single row and each bearing a spiracular opening (Figure 11). The caudal segment bears the posterior spiracular discs that each have two separated straight slits encircled by four multi-branched spiracular hairs (Figure 12). The button, or ecdysial scar, appears as a hole ventro-medially on the posterior spiracular discs.

There is a large increase in body size of the third-instar, but the overall morphological features are still similar to that of the second-instar. In the caudal segment, the posterior spiracular discs are located in a shallow cavity (Figure 13). A third spiracular slit is added to each of the posterior spiracular discs in this instar (Figure 14).

In forensic entomology, *C. megacephala* is one of the primary flies found in association with decomposing human corpses in areas where this species is present. It may occur either alone or as part of a mixed infestation with other species. If mixed, the most common species in Thailand and/or Malaysia with which it is found cohabiting a body is *Chrysomya rufifacies* (Macquart) (Cheong et al. 1973, Lee 1996, Sukontason et al. unpublished data). The first-instars of both of these species are almost identical and could not be distinguished using light microscopy. The only feature found to be useful in differentiating between them under SEM is the posterior spiracular hairs. Those of *C. megacephala* have relatively thin branches, but the branches are much broader in *C. rufifacies* (Sukontason et al. unpublished data). On the contrary, differentiation of larvae of these two species is easy in the second-instars, third-instars and puparia since those of *C. rufifacies* appear “hairy” (Kitching 1976, Wells et al. 1999, Sukontason et al. unpublished data). Results from this SEM study suggest that when first-instar larvae are collected from a corpse, they should be reared to the second-instar in order to easily differentiate between *C. megacephala* and *C. rufifacies*. However, rearing to the third-instar is required in order to definitely separate these two species from other closely-related blow fly species that may be found in a corpse. In Thailand, 42 calliphorid species were recorded from the country by Tumrasvin et al. (1979). Recently, the significance of the current SEM study was supported when a corpse was...
brought to our laboratory during a forensic investigation. Third-instar larvae collected from the body were determined to be an unknown fly species that is very similar to *C. megacephala* using results from this study. Thus, the larval morphology of *C. megacephala* described in this SEM study should be beneficial for future use, particularly in specific identification of fly larvae in forensic investigations.

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