Larval ultrastructure of *Parasarcophaga dux* (Thomson) (Diptera: Sarcophagidae)

Kom Sukontason*a,*, Kabkaew L. Sukontasona, Somsak Piangjia, Tarinee Chaiwonga, Noppawan Boonchua, Hiromu Kurahashib, Roy C. Vogtsberger c

aDepartment of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
bDepartment of Medical Entomology, National Institute of Infectious Diseases, Tokyo 162-8640, Japan
cDepartment of Biology, Hardin-Simmons University, Abilene, TX 79698-6165, USA

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Abstract

Ultrastructure of all larval instars of *Parasarcophaga dux* (Thomson), a common flesh fly species in Thailand, is presented using scanning electron microscopy. Special attention is given to the structure of anterior and posterior spiracles since these are important features used to differentiate between other sarcophagids. Each anterior spiracle in second and third instars has a single row of papillae varying in number from 14 to 17. The posterior spiracular discs have incomplete peritremes, with a prominent inner arc. Three long, narrow spiracular slits are oriented more or less vertically in each spiracular disc of third instar. Posterior spiracular hairs lack extensive branching and emanate approximately midway down the length of each slit. Microscopic morphology of the mouthhooks markedly differs between the first and second instars. The structure of these mouthhooks supports this fly species as being necrophagous or capable of producing myiasis.

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1. Introduction

Larvae of flies in the family Sarcophagidae (flesh flies) are one group of insects that are commonly found in investigations of human remains. Death scenes from which flesh fly larvae have been recovered vary from ones with relatively fresh remains to much older mummified corpses (Benecke, 1998; Introna et al., 1998; Goff, 2000; Sukontason et al., 2001; Greenberg and Kunich, 2002). According to Introna et al. (1998), the postmortem interval (PMI) of charred remains was calculated based on third instar of *Bercaea africa* (= *Sarcophaga haemorrhoidalis*). Presence of *Liopygia argyrocoma* (= *Parasarcophaga argyrocoma*) may also be used as an indicator species to determine if a corpse has been lying outside for extended periods of time (Benecke, 1998).

Due to the relatively large size of flesh fly larvae, collection of specimens by forensic investigators at death scenes is easy. However, the collected larvae from a corpse may not be useful in forensic investigations if their specific identification cannot be determined. Generally, identification of fly species is most easily made when they are in the adult stage, but in some cases, identification of third instar can be made almost immediately when taxonomic keys for larvae of fly species in a given area are available. Flesh flies comprise over 2000 species worldwide, and most species occur in either tropical or warm temperate regions (Byrd and Castner, 2001). In a prior survey of flies in Thailand by Tumrasvin and Kano (1979), 48 species of flesh flies were reported. In order to provide more information on a flesh fly species that may be involved in future forensic investigations in Thailand, we are reporting herein some morphological characteristics of all the larval stages of *P. dux*. This species was chosen for study because it is the most common flesh fly species found in the urban area of Chiang Mai City, as well as in rural areas. This fly is known to frequently enter human dwellings and larviposit on carrion and garbage. This behavior justifies investigation and research of this fly species for its possible medical and forensic importance.

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* Corresponding author. Tel.: +66-53-945342; fax: +66-53-217144. E-mail address: ksukonta@mail.med.cmu.ac.th (K. Sukontason).
2. Materials and methods

All larval stages of *P. dux* were obtained from a laboratory colony maintained at the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand. Larvae were first collected from the colony and washed several times with normal saline solution to remove surface artifacts. Then they were pre-fixed with a 2.5% glutaraldehyde mixture in phosphate buffer solution (PBS) with a pH of 7.4 at 4 °C for 24 h. In addition, larvae were rinsed twice with PBS at 10-min intervals, and postfixed with 1% osmium tetroxide at room temperature for 3–4 days. Specimens were then rinsed twice with PBS and dehydrated with alcohol. The dehydration process involved the larvae being sequentially subjected to the following increased alcohol concentrations: 30, 50, 70, 80 and 90%. Larvae remained in each concentration of alcohol for 12 h during each step of the dehydration process. After treatment in the alcohol concentrations, they were placed in absolute alcohol for another two 12 h periods followed by treatment in acetone for two 12 h periods. Finally, the larvae were subjected to critical point drying, attached to double-stick tape on aluminum stubs and coated with gold in a sputter-coating apparatus in order for them to be viewed under a JEOL-JSM840A (JEOL, Japan) scanning electron microscope (SEM).

The whole body of the first instar was processed as mentioned above, but due to their much larger sizes, second and third instars were primarily cut into three portions (head, body and caudal regions) before initial chemical treatment. This enabled them to be more easily processed and examined under SEM.

3. Results

The body of the first instar is the typical muscoid-shaped vermiciform larva which is pointed anteriorly and blunt in the posterior end (Fig. 1(A)). In the cephalic region, a pair of terminal organs (maxillary palp complex) are located beneath the dorsal organs or antenna (Fig. 1(B)). The ventral organs are situated obliquely over the pair of strong mouthhooks (Fig. 1(B)). At higher magnification, each terminal organ can be seen to consist of a group of papillae (Fig. 1(C)). A pair of ventrally curved mouthhooks protrude from the oral area (Fig. 1(B)). A broadly rounded, raised medial ridge spans the length of each mouthhook, but the lateral edges are flattened in the same plane (Fig. 1(D)). On the ventral side of all three thoracic segments, three types of receptor sensillae were found: trichoid sensillae bearing three setae, trichoid sensillae with a single seta, and pit sensillae that were located between the trichoid sensillae (Fig. 1(E)). A pair of posterior spiracular discs is located within a deep terminal cavity in the caudal segment. Each of these posterior spiracular discs bears two straight slits for gaseous exchange that coalesce ventrally (Fig. 1(F)).

The size of the second instar increases, but the typical muscoid-shape is preserved. Similar to those seen in the first instar, dorsal organs, terminal organs and ventral organs persist in the second instar (Fig. 2(A)). The ventral organ appears as a globular structure having one strong spine and few smaller spines located near the apex (Fig. 2(B)). In comparison to the first instar, the surface of the robust mouthhooks is more or less flattened and contains some shallow grooves and depressions (Fig. 2(C)). Each anterior spiracle contains a single row of papillae that number from 14 to 17 (Fig. 2(D)). Out of 40 specimens examined, 16 papillae was the number most commonly found (50%; 20/40). This was followed in prevalence by 15 papillae (27.5%; 11/40), 17 papillae (17.5%; 7/40) and 14 papillae (5%; 2/40), respectively. The posterior spiracular discs were also located in a deep terminal cavity as was seen in the first instar (Fig. 2(E)). Each posterior spiracular disc also contains two spiracular slits, but unlike the first instar, the inner slit is straight whereas the outer slit is curved medially (Fig. 2(F)).

The overall morphology of the third instar is similar to that of the second instar, but is much larger in size. The number and shape of papillae on the anterior spiracles are similar to that of the second instar (figure not shown). Another character used to differentiate species of flesh flies are the intersegmental spines (Aspoas, 1991). When viewing the intersegmental spines of *P. dux* at higher magnification, their surfaces are covered with little wrinkles (Fig. 3(A)). This feature may be distinctive from many other species of flesh flies. Situated around the periphery of the deep terminal cavity of the caudal segment are six pairs of relatively large caudal tubercles (Fig. 3(B)). The posterior spiracular discs appear D-shaped with the width of each being shorter than their length (Fig. 3(C)). The peritreme encircling each set of spiracular slits is incomplete, being open in the ventro-medial 2/3 of its height. An inner arc is quite pronounced. Three long, narrow spiracular slits are oriented more or less vertically in each spiracular disc while the posterior spiracular hairs (PSH) emanate from the margin of each slit near the middle. Each posterior spiracular hair lacks extensive branching (Fig. 3(D)).

4. Discussion

Species of flesh fly larvae are very similar in appearance and traditionally have been difficult to identify (Smith, 1986). Therefore, accurate identification of either larvae or adults has had to be made by taxonomic specialists (Zumpt, 1965). Taxonomically, *P. dux* has been confused with *Parasarcocephaga misera* by many previous authors (Zumpt, 1965), but more recent research has clarified classification of these two species as distinct species and separated them based on adult males (Sugiyama et al., 1988a,b; Kano and Shinonaga, 1994; Greenberg and Kunich, 2002).

Keys to 29 species of third instar sarcophagids in Japan were provided by Ishijima (1967), emphasizing the number
Fig. 1. SEM micrographs of first instar of *P. dux*. (A) Dorsal view of entire body with anterior end facing left. (B) Lateral view of cephalic segment showing terminal organ (TO) beneath dorsal organ (arrow). Mouthhook (MH) is also indicated. The ventral organs (arrow-head) are situated obliquely over the pair of strong mouthhooks. (C) Group of papillae on terminal organ. (D) Higher magnification of mouthhook showing broadly rounded and raised medial ridge. (E) Ventral view of thoracic segment showing trichoid sensillum bearing three setae (left), pit sensillum (middle), and trichoid sensillum bearing one seta (right). (F) Posterior spiracular disc bearing two straight slits (S) coalescing ventrally.
Fig. 2. SEM micrographs of second instar of *P. dux*. (A) Cephalic segment showing dorsal organ (DO), terminal organ (TO), ventral organ (VO), and mouthhook (MH). (B) Ventral organ at higher magnification. (C) Terminal end of strong, robust mouthhook at higher magnification. (D) Anterior spiracle bearing single row of 17 papillae. (E) Postero-lateral view of caudal segment showing posterior spiracle (PS) in deep cavity. (F) Posterior spiracular disc showing two spiracular slits (S) and posterior spiracular hairs (PSH). Bar = 10 μm.
and arrangement of papillae on the anterior spiracles, as well as morphological features of the posterior spiracles and cephalopharyngeal skeleton. In the present study, the structure of the cephalopharyngeal skeleton was not addressed due to its location being internal. Using SEM techniques, micromorphological features of third instar of four species of Afrotropical Sarcophaga were described by Aspoas (1991), highlighting the structure of the anterior spiracles, spine surface texture, size of circumspiracular tubercles of the caudal segment and the structure of the PSH. These two previous reports showed overlapping of numbers of papillae on anterior spiracles of different species, which agreed with the findings of Colwell and O’Connor (2000). However, the conspicuous reduction in number of papillae occurring on anterior spiracles in P. dux (14–17) differs markedly from the more numerous papillae found on many other sarcophagid species such as Boettcherisca peregrina (24–26), Boettcherisca septentrionalis (28–30), Kramerea shuetzi (32–36), Parasarcophaga albiceps (32–38), Parasarcophaga orchidea (28–34), Parasarcophaga harpax (40–44), Parasarcophaga tsushimae (33–36), Parasarcophaga similis (24–30), Parasarcophaga kawayensis (32–36), Parasarcophaga shiritakaensis (46–49), Parasarcophaga oshimensis (38–46), Kanoa okazakii (38–43), Pierretia horii (34–37), Pierretia pterygota (24–28), Pierretia caudagalii (32–36), Pierretia kanekoi (36–38), Pierretia kagaensis (34–38), Robineauella scoparia (48–54), Tricholioproctia antilope (46–52), and Tricholioproctia flavina (42–46) (Ishijima, 1967). Still, some sarcophagid species are known to have fewer papillae on the anterior spiracles, e.g. Sarcophaga crassipalpis (11–12) (Uni et al., 1999) and Wohlfahrtia magnifica (5–6) (Ruiz-Martinez et al., 1989).

![Fig. 3. SEM micrographs of third instar of P. dux. (A) Intersegmental spines showing wrinkled surface. (B) Caudal segment showing large caudal tubercles around periphery of deep terminal cavity. (C) Pair of D-shaped posterior spiracular discs with incomplete peritreme encircling long, narrow spiracular slits (S). Posterior spiracular hairs (PSH) emanate from the margin of each slit near the middle. Arrow indicates inner arc. (D) Posterior spiracular hair (PSH) at higher magnification emphasizing lack of extensive branching.](image)
The single row of papillae seen in *P. dux* also differs in arrangement from the double rows found in some of the sarcophagid species (e.g. *B. peregrina*, *B. septentrionalis*, *P. orchidea*, *P. similis*, and *P. kagaensis*) reported in the study by Ishijima (1967). Consequently, the number and arrangement of papillae on the anterior spiracles of later instar are still included as one of the distinctive features among sarcophagids.

In general, the posterior spiracular discs of third instar sarcophagids are very similar, often having incomplete peritremes and bearing three nearly vertical and parallel spiracular slits. Aspoas (1991) reported that the pattern of the PSH is distinct, and was one feature that could be used to differentiate among *Sarcophaga cruentata*, *Sarcophaga exuberans*, *Sarcophaga nodosa* and *Sarcophaga tibialis*. The pattern of PSH in *P. dux* presented in this study may be useful in the future to differentiate from other closely related species such as *P. misera*.

Larvae of sarcophagid flies have the most diverse feeding habits of all families of calyptrate flies (Dahlem, 1991). Many are parasitoids of other arthropods, while others are necrophagous, predaceous or saprophagous. This diversity may be construed from the morphological differences in mouthparts of many immature sarcophagids. The blade-like lateral edges of mouthhooks in first instar *P. dux* differ markedly from other sarcophagids such as *Oxysarcodexia confusa*, *Oxysarcodexia thornax*, and *Chaetoravinia almeidai*, whose mouthhooks bear small, sharp teeth (Lopes and Leite, 1986; Leite and Lopes, 1987). Even in comparison to these species, fewer number and more relatively round teeth have been recorded in the mouthhooks of first instar *Oxysarcodexia paulistanensis* (Lopes and Leite, 1987). Based on observations from the SEM used in this study, the ultrastructure of the mouthhooks of *P. dux* could serve the larvae in being successful invaders of carrion and/or wounds of animals (as in cases of myiasis).

Geographically, *P. dux* has a fairly wide distribution and has been collected from several countries in Asia (i.e. China, Taiwan, Indonesia, Philippines, India, Nepal, Myanmar, Thailand, Sri Lanka, Korea, and Japan), Papua New Guinea, Australia, and as far out as Hawaii (Kano and Shinonaga, 1994). Thus, the inclusive ultrastructure of all larval instars of *P. dux* included herein should be useful baseline data for this larval identification after the ultrastructural studies of other related sarcophagid species have been done, which will be helpful in forensic investigations.

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