

# Observations on morphology of immature *Lucilia porphyrina* (Diptera: Calliphoridae), a fly species of forensic importance

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**Abstract** *Lucilia porphyrina* (Walker) is a blow fly of forensic importance, and shares its geographical distribution with a related forensically important species, *Lucilia cuprina* (Wiedemann). The immature stages of both species are similar in general appearance; therefore, correct identification should be given special consideration. This study highlighted the main features of *L. porphyrina* larvae, as observed under light microscopy and scanning electron microscopy. Particular attention is given to the anterior and posterior spiracles, cephalopharyngeal skeleton, and characteristics of the dorsal spines between the prothorax and mesothorax. In the third instar specifically, morphological information on *L. porphyrina* showed several features that are shared by *L. cuprina*, and therefore need certain identification to distinguish between them. Such key features are (1) greater posterior spiracle, (2) apparent inner projection between the middle and lower slits of the posterior spiracle, and (3) strongly sclerotized peritreme. The number of papillae on the anterior spiracle may be a supplement, five to nine and three to six in *L. porphyrina* and *L. cuprina*, respectively. The key for identifying third instar of forensically important flies in Thailand has been updated to include *L. porphyrina*.

## Introduction

Blow flies of the genera *Lucilia* are well known to forensic entomologists since their immature stages are found to associate with human corpses, thus being flies of forensic importance. Research works have been documented extensively pertaining to specimens of *Lucilia* in human corpses and/or animal carcasses, e.g., *Lucilia sericata* Meigen (Bonacci et al. 2011; Gunn and Bird 2011; Voss et al. 2011), *Lucilia coeruleiviridis* (Macquart) (Bugajski et al. 2011), *Lucilia caesar* (L.) (Bonacci et al. 2011), *Lucilia cuprina* (Sukontason et al. 2007; Wang et al. 2008), and *Lucilia porphyrina* (Xinghua et al. 2010; Chen 2011).

In Thailand, four *Lucilia* species were recorded, i.e., *L. porphyrina*, *L. cuprina*, *Lucilia papuensis* Macquart, and *Lucilia sinensis* Aubertin (Kurahashi and Bunchu 2011). Based on the literature, searches have mentioned that *L. porphyrina* and *L. cuprina* were of the most forensically important in this taxon. *L. porphyrina* is common throughout the Asian continent, Australasia, and Palearctic regions (Verves 2005), and prevails in higher forested areas at elevations ranging from 335 to 2,444 m, based on collections in Southeast Asian countries (Tumrasvin et al. 1978; Kurahashi et al. 1997; Kurahashi and Magpayo 2000). On the other hand, *L. cuprina* is more urbanized at elevations ranging from 0 to 1,850 m, according to the database of forensically important fly survey (unpublished data of KLS). Mixed populations resulting from adaptation of these species to their environment need correct identification, particularly for forensic investigation purposes.

Many researches have focused on the morphology of immature blow flies, particularly those of forensic importance. Immature stages of blow flies (egg, three instars, and pupal stages) are found commonly to associate with corpses. Knowledge regarding morphology of the immature stage of fly larvae that associate with

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corpses is important before the use of specimens in forensic investigations. Although the morphology of third instar *L. porphyrina* has been described (Ishijima 1967), information of the first and second instars, and puparial stage is limited. The major aim of this study was to present the important characteristics of *L. porphyrina* larvae for potential use in identification.

## Materials and methods

### Fly colony

A laboratory colony of *L. porphyrina* was established in July 2011 using adults collected with a sweep net after baiting with 1-day-tainted beef viscera at an elevation of 950 m, on Suthep Mountain, northern Thailand. The adults were brought to the laboratory at the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand. They were provided with sugar and water as a food source. A few pieces of fresh pork liver were provided both as food and oviposition medium. Flies were maintained in the laboratory at ambient temperature, relative humidity, and natural photoperiod.

### Light microscopy observation

All instars and puparia were obtained from F<sub>1</sub> progeny. The instars were transferred from the rearing trays and sacrificed by placing in near boiling water for a few minutes. The bodies of first and second instars were cut at two sites using a sharp blade in order to obtain three body portions. The first cutting position was across the mesothorax (the third segment) to view the anterior spiracle and intersegmental dorsal spines between the pro- and mesothorax. The second cutting position was across the 11th body segment, in order that morphology of the posterior spiracle could be viewed clearly. The anterior and posterior parts were mounted by transferring them onto a clean glass slide, on which a few drops of Neo-Shigal Permout® (Japan) had been placed. A cover slip was placed over the specimens, and the examination of morphological characters was made using magnification of ×4 to ×40 under a light microscope (Olympus®, Japan) equipped with a calibrated eyepiece micrometer. The micrographs were recorded with a digital camera (Canon®, Japan).

Regarding the third instar, specimens were prepared in the same manner as previously described, except for adding one more procedure by transferring into a clean well containing 10 % KOH for 5 min in order to digest a large amount of body tissue. Then, the specimens were mounted using Neo-Shigal Permout®.

### Morphometric measurement

Measurements were made of the posterior spiracle width and distance between both posterior spiracles of the mounted specimens. The width of the posterior spiracle was measured along the button margin to the posterior spiracle rim in the middle spiracular slit. Distance measurement was performed between the buttons of each posterior spiracle.

### Scanning electron microscopy observation

All instars and puparia were obtained from F<sub>1</sub> progeny. Larval specimens were sacrificed by placing in near boiling water for a few minutes, and kept in 70 % alcohol. Once processed, they were transferred from 70 % alcohol and washed in phosphate buffer at pH 7.4 while shaking slightly for 5 min. The phosphate buffer was removed using a pipette, and the specimens were subjected to 2.5 % glutaraldehyde mixture with phosphate buffer at 4 °C for 24 h. According to their large size, the second and third instars were cut on a paraffin stand by using a sharp blade. The first cut was made in the middle of the mesothorax, while the second was made in the middle of the seventh abdominal segment, in order to view suitably under a scanning electron microscope (SEM). All specimens were rinsed twice with phosphate buffer at 10-min intervals. Larvae were postfixed with 1 % osmium tetroxide at room temperature for 1 to 2 days depending on the size of the larvae. One day was required for the first and second instars, while 2 days was needed for the third instar. Then, all instars were washed twice with phosphate buffer at 10-min intervals or until translucent buffer was obtained. The specimens were then dehydrated with 30, 50, 70, 80, 90, and 100 % alcohol consecutively, each for 12 h, and 100 % alcohol only for a further 12 h. Lastly, they were dehydrated twice in acetone, 12-h periods. All specimens were processed by critical point drying, attached to double-stick tape on aluminum stub, coated with gold in sputter-coating apparatus, and viewed under a JEOL-JSM6610LV scanning electron microscope.

The puparia were transferred from the colony, collected in a small gauze bag, and submerged in a beaker containing distilled water, which was placed onto a Cimarec™ stirring hotplate (IA, USA). They were then cleaned in the gauze bag with a magnetic stirrer. This cleaning process took 3 h to ensure detachment of debris from the puparial integument. The puparia were kept in 70 % alcohol and then processed in the same manner to that previously described for larvae.

## Results

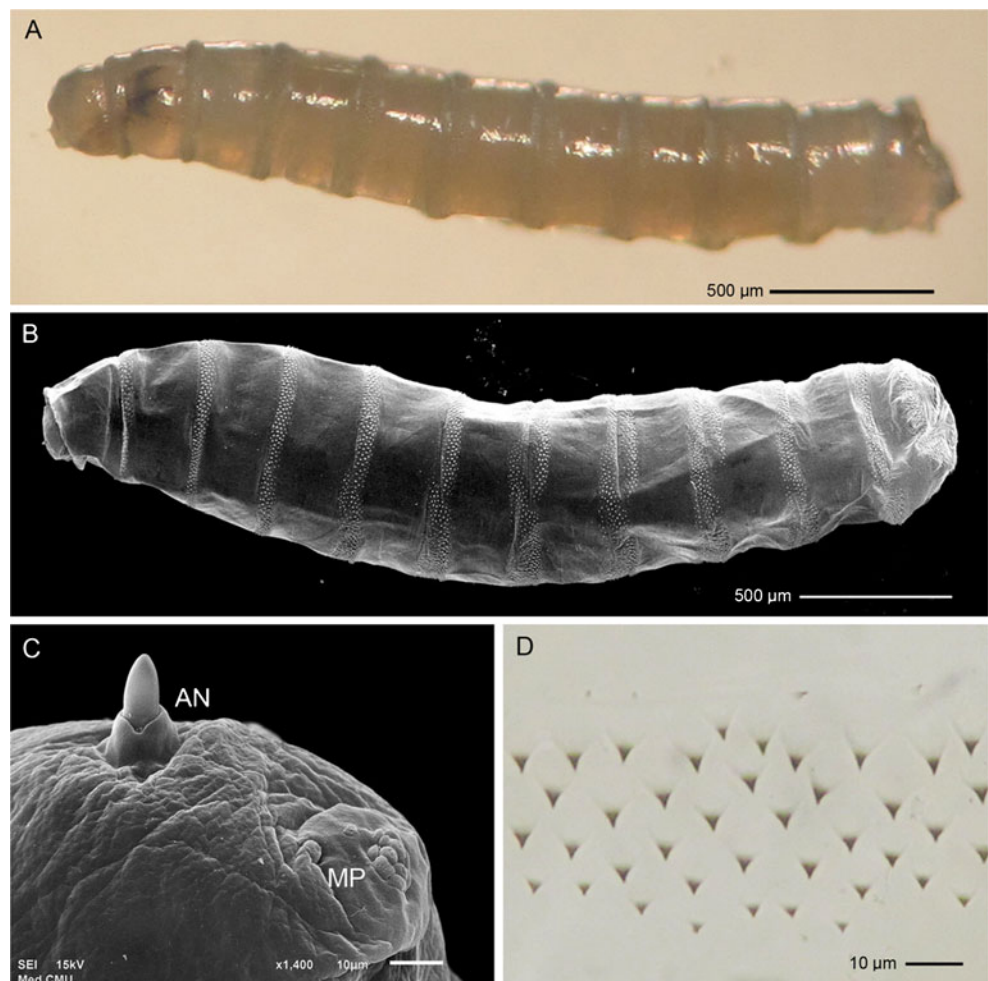
*The first instar* Body length  $2.64 \pm 0.24$  mm ( $n=21$ ). The first instar is smooth and has 12 segments, with the last

abdominal segment being truncated (Fig. 1a, b). A minute single dome-shaped antenna protrudes anteriorly from the dorsal region of the head, while maxillary palpus is located latero-posteriorly (Fig. 1c). The structure of the intersegmental dorsal spines is rather simple between the prothorax and mesothorax, and represented by minute triangular shapes with constricted darker tips that are projected posteriorly (Fig. 1d). The anterior spiracle at the dorso-posterior margin of the prothorax is unnoticeable. The posterior spiracle bears two stout spiracular slits that have their ends joined ventrally, and are encircled by a slightly sclerotized peritreme (Fig. 2a). When observed under SEM, the peripheral rim of the caudal segment bears patches of densely fine setae (Fig. 2b). Under magnification, each posterior spiracle contains two stout spiracular slits interspaced with multi-branched, palm-formed spiracular tufts (Fig. 2c). Internally, the cephalopharyngeal skeleton is skeletonized, with the tip of the mouthhook bending sharply backwards and downwards (Fig. 2d).

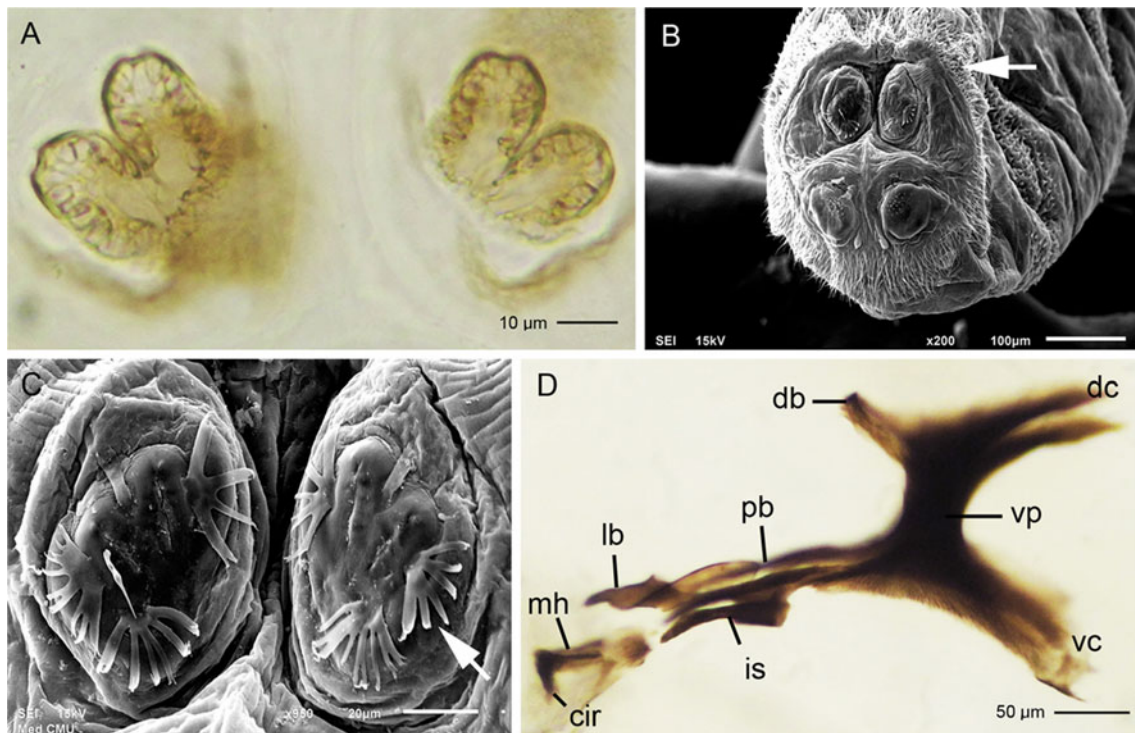
*The second instar* Body length  $4.21 \pm 0.53$  mm ( $n=10$ ). The second instar is  $1.6\times$  as long as the previous one, and its

head is more tapered (Fig. 3a, b). A large peg-shaped antenna projects anteriorly (Fig. 3c). Regarding the maxillary palpus, six sensory papillae are grouped in the same plate, while two are adjacent to the main group (Fig. 3d). The location of the ventral organ is lower than the maxillary palpus and nearer to the medial. The ventral organ is characterized by a raised coronate rim around a central opening (Fig. 3e). Examined at the intersegmental dorsal spines between the prothorax and mesothorax, this structure is more complicated where it demonstrates more spines that tend to be lined in a group of 2 to 4 (Fig. 4a). The anterior spiracle is represented clearly by a single row of 5–9 papillae (Fig. 4b). SEM observation of the dorsal spines showed that they tended to be grouped in a similar way to that seen under light microscopy (LM; Fig. 4b). Changing characteristics across the second instar are apparent in the posterior spiracle, and each one bears two separate spiracular slits that are encircled by a slightly sclerotized peritreme (Fig. 4c). Magnified observation revealed two separate spiracular slits, interspaced with fine multibranching spiracular tufts (Fig. 4d). The cephalopharyngeal skeleton was much developed and differed from the first instar. Based on examination

**Fig. 1** The first instar of *L. porphyrina*. **a** Lateral view, anterior end on the left (LM). **b** Lateral view (SEM). **c** Dome-shaped antenna (AN) and maxillary palpus (MP). **d** Intersegmental dorsal spines between the prothorax and mesothorax displaying minute triangular shapes with constricted dark tips (online figure in color)







**Fig. 2** The first instar of *L. porphyrina*. **a** Posterior spiracle with slightly sclerotized peritreme. **b** Caudal segment bears patches of densely fine setae (arrow) at the peripheral rim. **c** Magnification of the posterior spiracle indicating spiracular slits interspaced with

multibranching, palm-formed spiracular tufts (arrow). **d** Cephalopharyngeal skeleton. *cir* cirri, *db* dorsal bridge, *dc* dorsal cornua, *is* intermediate sclerite, *lb* labrum, *mh* mouthhook, *pb* parastomal bar, *vp* vertical plate (online figure in color)

of the initial second instar, this structure was therefore not fully sclerotized (Fig. 4e).

**The third instar** Body length robust (Fig. 5a, b), lengthening to  $12.04 \pm 1.77$  mm ( $n=36$ ). The head segment is much smaller than the thoracic and abdominal segments. Sensory structures (antenna, maxillary palpus, and ventral organs) at the tip of the head are morphologically similar to those in the second instar (Fig. 5c–e). Morphological features of the intersegmental dorsal spines change substantially between the prothorax and mesothorax, showing large triangular-shaped spines that all tend to be lined three to seven in a group (Fig. 6a, b). There are six to nine papillae on the anterior spiracle, which is similar to the number in the previous instar (Fig. 6c). SEM results revealed that each papilla is shielded by an oval plate, which is dorso-ventrally perforated (Fig. 6c). A broken oval plate displays a chamber patched with antler-like projections (Fig. 6d).

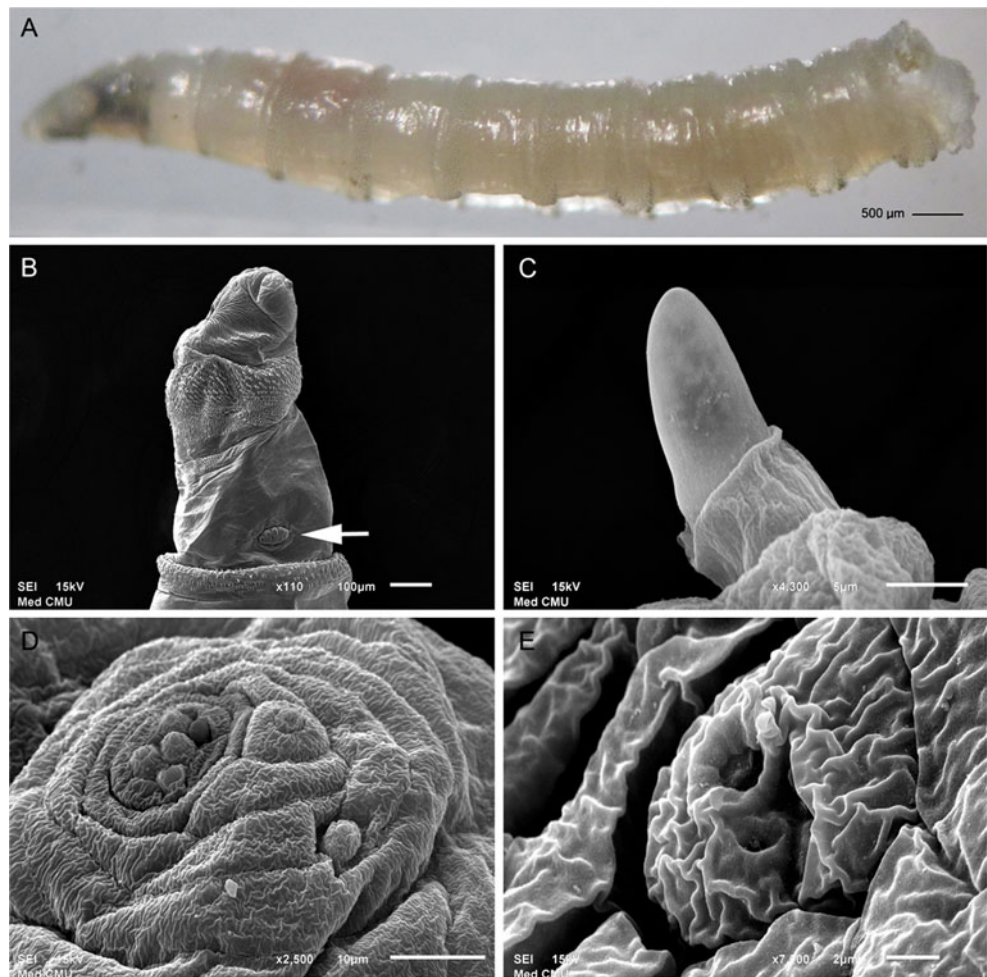
As seen from a caudal view, six pairs of tubercles (i.e., inner, middle, and outer dorsal tubercles and inner, middle, and outer ventral tubercles) are prominent when examined by both light microscopy (Fig. 7a) and SEM observations (Fig. 7b). Notably, the posterior spiracle is relatively large and heavily sclerotized (Fig. 7a, c), measuring  $275.9 \mu\text{m}$ , which was significantly larger than that in *L. cuprina* ( $184 \mu\text{m}$ ) ( $P=0.00$ ; Mann–Whitney  $U$  test; Table 1). In

contrast, no statistically significant difference was found in median distance between each posterior spiracle,  $143.1 \mu\text{m}$  and  $163.5 \mu\text{m}$  for *L. porphyrina* and *L. cuprina*, respectively ( $P=0.86$ ; Mann–Whitney  $U$  test). When viewed specifically, each spiracle is composed of a complete heavily sclerotized peritreme encircling three spiracular slits (Fig. 7c). Prominent projections between the posterior spiracular slits are observed, particularly the middle and lower ones. The button is positioned centrally together with the peritreme (Figs. 7c and 8a), while its positioning is either at the center or inside the peritreme of *L. cuprina* (Fig. 8b). Further examination of *L. porphyrina* specimens, using SEM, demonstrated that three separated spiracular slits were interspaced with fine multibranching spiracular tufts (Fig. 7d). To view the structure of the internal cephalopharyngeal skeleton, the picture displayed vast development of such an organ. Accessory sclerite, located beneath the curved and strong mouthhook, was faintly visible (Fig. 7e).

The key used for identifying the third instar of forensically important fly species in Thailand has been published (Moophayak et al. 2011), with the following updates that include *L. porphyrina* presentation:

1. Hairy body (with large tubercles along the body) . . . . 2
- Non-hairy body (without large tubercles along the body) . . . . . 3

**Fig. 3** The second instar of *L. porphyrina*. **a** Lateral view, anterior end on the left. **b** Lateral view showing anterior spiracle at the end of the prothorax (arrow). **c** Antenna. **d** Maxillary palpus. **e** Ventral organ (online figure in color)

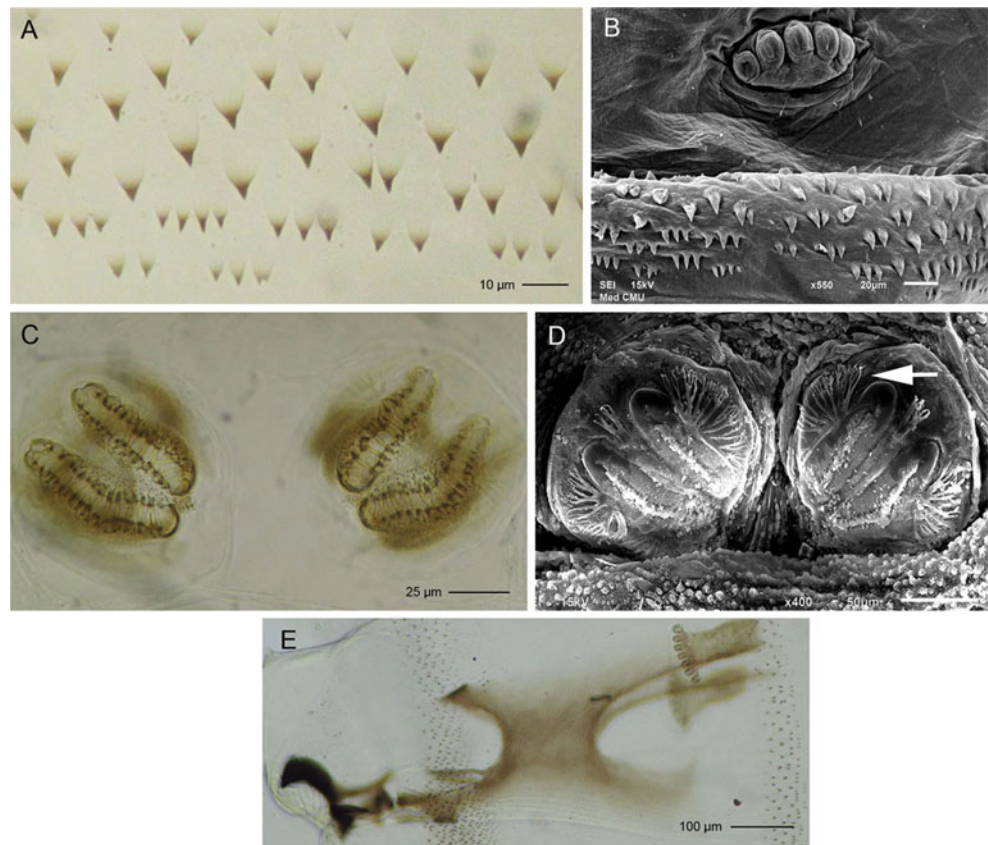


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| <p>2. Small spines only at the tips of the tubercles; 9–12 papillae on each anterior . . . . .<i>Achoetandrus ruffacius</i> (Macquart)<br/>Small spines along the entire tubercles; 13–15 papillae on each anterior . . . . .<i>Achoetandrus villeneuvei</i> (Patton)</p> <p>3. Posterior spiracle with incomplete peritreme ventromedially. . . . . 4<br/>Posterior spiracle with complete peritreme ventromedially. . . . . 5</p> <p>4. With a large dark brown patch on the dorsal surface; with the end of the upper peritreme slightly expanded . . . . . <i>Ceylonomyia nigripes</i> (Aubertin)<br/>Without a large dark brown patch on the dorsal surface; with the end of the upper peritreme not expanded . . . . . <i>Chrysomya megacephala</i> (F.) or <i>Chrysomya pinguis</i> (Walker)</p> <p>5. Sinuous spiracular slits; peritreme “D-shaped”, dark and heavily sclerotized. . . . . 6<br/>Straight spiracular slits; peritreme not D-shaped, dark brown . . . . . 7</p> <p>6. Sinuous spiracular slits “S-shaped” . . . <i>Synthesiomysia nudisetata</i> van der Wulp<sup>a</sup><br/>Sinuous spiracular slits “M-shaped” . . . . . <i>Musca domestica</i> L.<sup>a</sup></p> | <p>7. Slender larval shape; posterior spiracles close together; peritreme . . . . . <i>Hydrotaea spinigera</i> Stein<sup>a</sup><br/>Normal larval shape; posterior spiracles far apart; peritreme dark brown . . . . . 8</p> <p>8. Spines between the prothorax and mesothorax large. . . 9<br/>Spines between the prothorax and mesothorax small . . 10</p> <p>9. Inner projection of the peritreme between the middle and lower spiracular slits prominent; five to nine papillae on the anterior spiracle; median width of posterior spiracle 275.9 µm; strongly sclerotized peritreme . . . . . <i>L. porphyrina</i> (Walker)<br/>Inner projection of the peritreme between the middle and lower spiracular slits not prominent; three to six papillae on anterior spiracle; median width of posterior spiracle 184 µm; slightly sclerotized peritreme . . . . . <i>L. cuprina</i> (Wiedemann)</p> <p>10. Cephalopharyngeal skeleton with accessory sclerite; posterior spiracles far apart; 5–11 papillae on the anterior spiracle . . . . . <i>Hemipyrellia ligurriens</i> (Wiedemann)<br/>Cephalopharyngeal skeleton without accessory sclerite; posterior spiracles close together; 8–11 papillae on the anterior spiracle . . . <i>Hypopygiopsis tumrasvini</i> Kurahashi</p> |
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<sup>a</sup>Flies of the family Muscidae



**Fig. 4** The second instar of *L. porphyrina*. **a** Intersegmental dorsal spines between the prothorax and mesothorax demonstrating spines tend to be lined in a group of two to four. **b** Anterior spiracle with five to nine papillae. **c** Posterior spiracle. **d** Posterior spiracle showing two spiracular slits, interspaced with fine multibranched spiracular tufts (arrow). **e** Cephalopharyngeal skeleton and anterior spiracle (online figure in color)



*Puparia* Length,  $7.13 \pm 0.49$  mm; width,  $3.11 \pm 0.22$  mm ( $n=21$ ); tapered anteriorly.

To view the gradual coloration changes of puparia, further examination was performed under a stereomicroscope. Immediately after pupariation, the integument was creamy-white (hour 1), but became dull orange brown (hour 3), light brown (~hour 4–5), dark red brown (~day 2–5) and eventually almost black (~day 6–9) before emergence (Fig. 9). Based on rearing at  $27.1 \pm 1.2$  °C and  $70.5 \pm 7.7$  % RH in the laboratory, the total developmental time until emergence was 9 days.

SEM observation of puparia demonstrated the coarctate form, of which the first four segments of the anterior region are gradually rounded anteriorly (Fig. 10a), while the eighth abdominal segment of the posterior region is rounded (Fig. 10b). A pair of anterior spiracles is located laterally on the prothorax (Fig. 10a). Broken anterior spiracles in some specimens revealed antler-like projections lined within a chamber (Fig. 10c). A pair of respiratory horns is visible as minute projections at the dorso-lateral margin of the fifth segment (Fig. 10a, d). Numerous minute, oblique openings are observed throughout the whole respiratory horn (Fig. 10d). Some broken specimens of the respiratory horn displayed antler-like projections lined within a chamber (Fig. 10e). A pair of posterior spiracles was situated at the

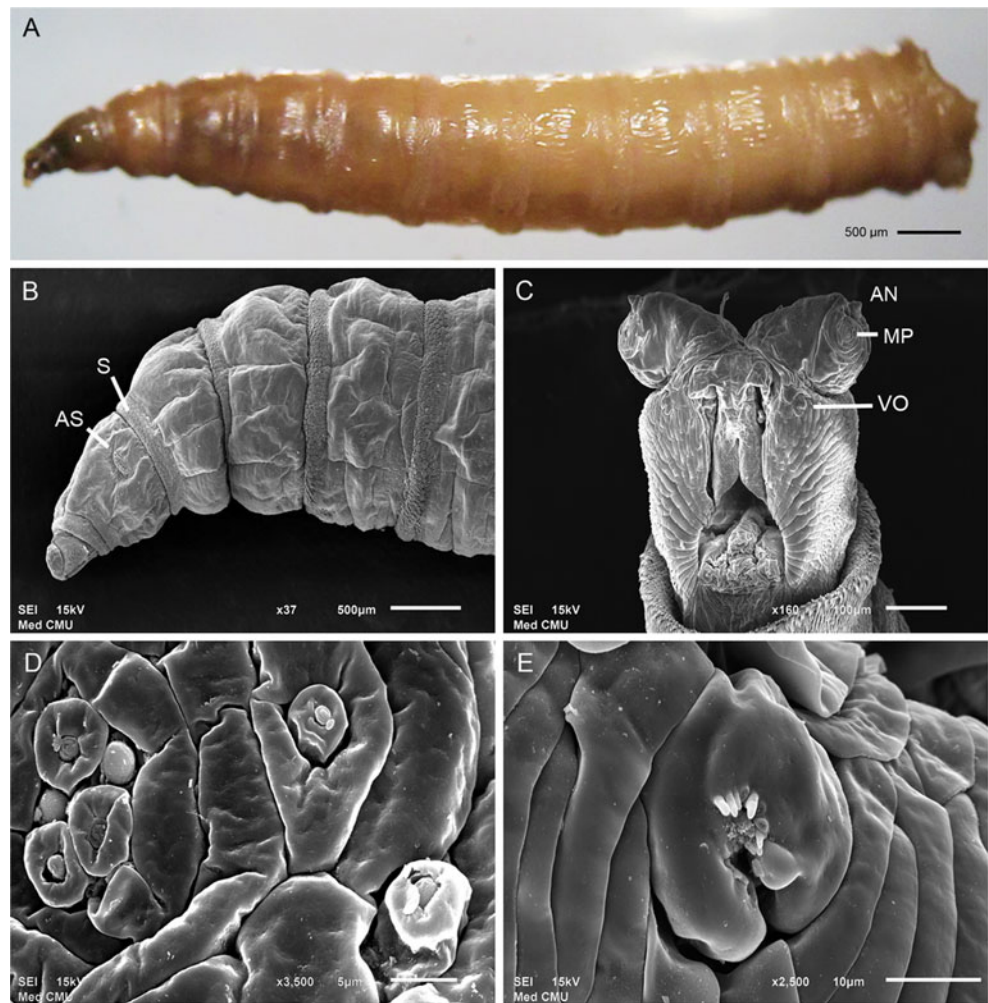
caudal end, showing a similar structure to that in the third instar (Fig. 10f).

## Discussion

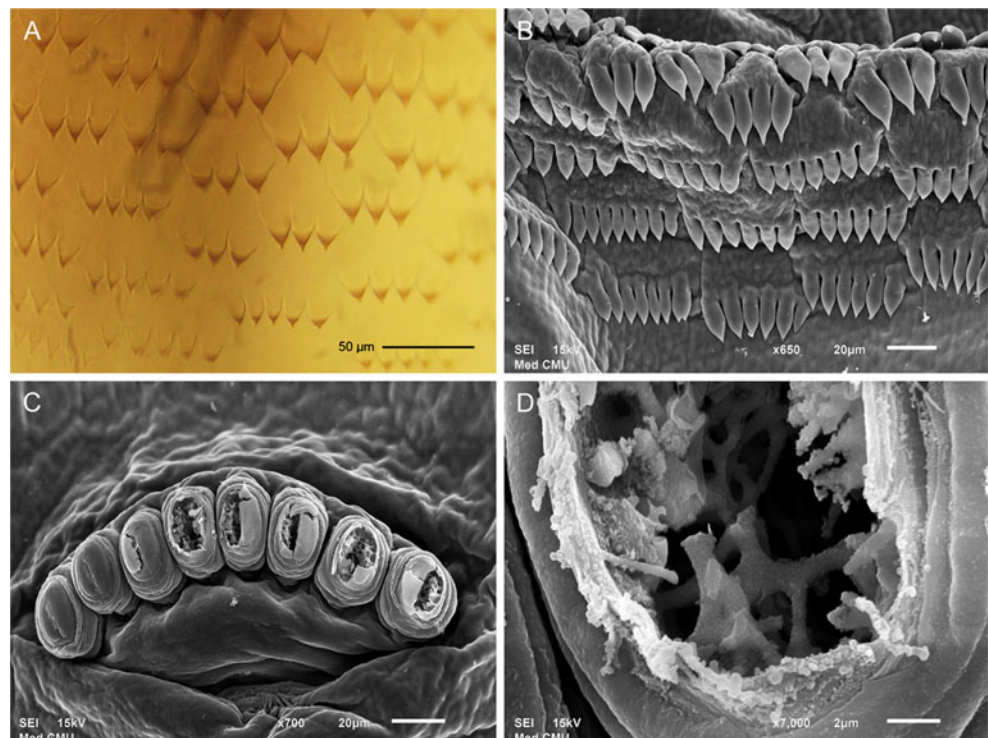
*L. porphyrina* is a forensically important blow fly species, and identification of larvae, which associates with corpses, further its use as a primary and essential step in forensic investigations. The aim of this study was to observe the morphology of all instars of *L. porphyrina* using LM and SEM. Early taxonomists relied heavily on the external characteristics of the third instar for species identification (Ishijima 1967).

Sculpture of the dorsal spines between the prothorax and mesothorax of the third instar displays relatively unique features in the same sister taxon of the tribe Luciliini, particularly under LM observation (see Fig. 6a). The spines of *L. porphyrina* are indistinguishable from those of flies in the same genus, as demonstrated in *L. cuprina* (Sukontason et al. 2004). Those species have a larger size than that observed in *Hemipyrellia* or *Hypopygiopsis*, which are taxonomically grouped in the same tribe. Fewer spines in *Lucilia* than *Hemipyrellia* or *Hypopygiopsis* are apparent. However, the spines of *H. ligurriens* (Wiedemann)

**Fig. 5** The third instar of *L. porphyrina*. **a** Lateral view. **b** Lateral view showing the anterior spiracle (*AS*) and intersegmental dorsal spines between the prothorax and mesothorax (*S*). **c** Ventral view of the head displaying the antenna (*AN*), maxillary palpus (*MP*), and ventral organ (*VO*). **d** Maxillary palpus. **e** Ventral organ (online figure in color)

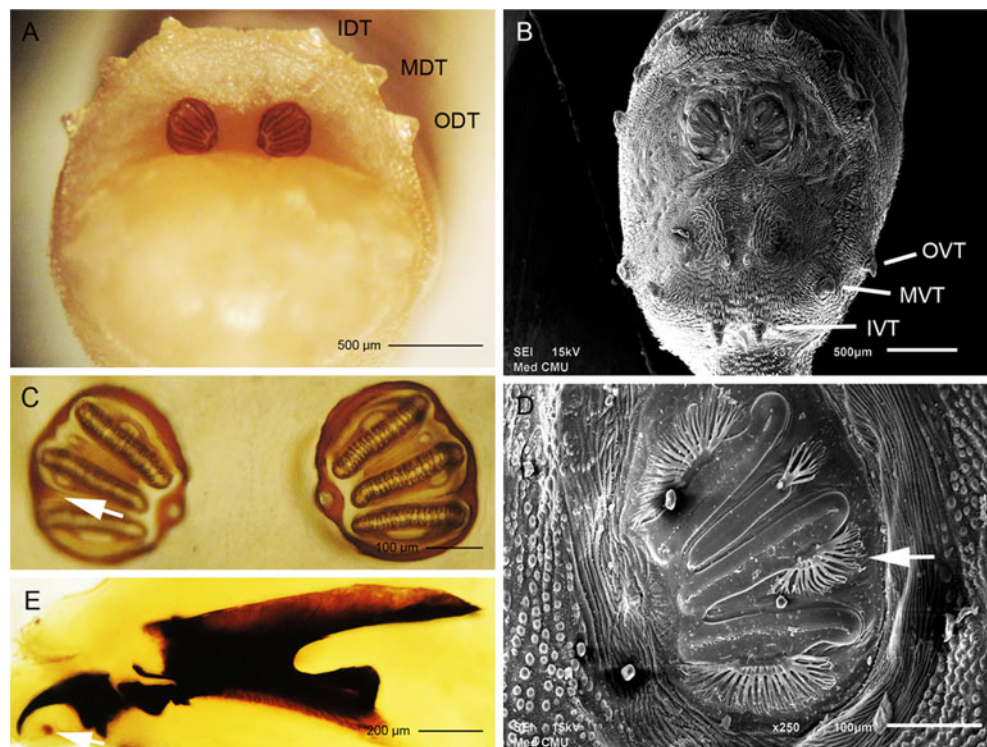


**Fig. 6** The third instar of *L. porphyrina*. **a** Intersegmental dorsal spines between the prothorax and mesothorax showing large triangular-shaped spines that tend to be lined three to seven in a group. **b** Group of dorsal spines between the prothorax and mesothorax. **c** Anterior spiracle. **d** Broken oval plate of the anterior spiracle presenting antler-like projections lined within a chamber (online figure in color)





**Fig. 7** The third instar of *L. porphyrina*. **a** Caudal segment displaying the prominent three pairs of dorsal tubercles: *IDT* inner dorsal tubercle, *MDT* middle dorsal tubercle, *ODT* outer dorsal tubercle. **b** Caudal segment displaying the prominent three pairs of ventral tubercles; *IVT* inner ventral tubercle, *MVT* middle ventral tubercle, *OVT* outer ventral tubercle. **c** Posterior spiracle with complete peritreme, which is heavily sclerotized. *Arrow* indicates prominent projections between the middle and lower slits. **d** Three spiracular slits interspaced with fine multibranching spiracular tufts (*arrow*). **e** Cephalophyngal skeleton with faint accessory sclerite (*arrow*; online figure in color)



(Sukontason et al. 2008) are indistinguishable from *H. tumrasvini* Kurahashi (Moophayak et al. 2011).

When analyzing inclusive morphological characters, some recognizable features were observed that differentiate the third instar of *L. porphyrina* from that of *L. cuprina*, and both are regarded as flies of forensic importance. Of particular key interest are (1) size of posterior spiracle, (2) inner projection between the middle and lower slits of the posterior spiracle, (3) degree of sclerotization in the peritreme, and (4) number of papillae on the anterior spiracle. Firstly, the size of the posterior spiracle, as designated by the width of *L. porphyrina*, was significantly larger than that of *L. cuprina*. Secondly, specimens of *L. porphyrina* exhibited a more prominent inner projection between the middle and lower slits than those of *L. cuprina*. Thirdly, a strongly sclerotized peritreme was

observed in *L. porphyrina*, in contrast to a weakened peritreme in *L. cuprina*, even after the same treatment with permount. Aside from those features, specimens of *L. porphyrina* comprise more papillae on the anterior spiracle (five to nine) than those of *L. cuprina* (three to six), based on the large number encountered ( $n=50$  and  $60$ ). Five to nine papillae observed in *L. porphyrina* specimens, collected in Thailand for this study, were concordant with the Japanese strain (Ishijima 1967). With regard to *L. cuprina*, three to six papillae of the Thailand strain were observed, but five to seven recorded specimens were collected in Japan (Ishijima 1967), thereby indicating morphological and anatomical variation in this fly species. Therefore, this last characteristic is perhaps a supplement parameter that can be used to differentiate between species.

**Table 1** Comparison of important characteristics in the third instar of *L. porphyrina* and *L. cuprina*

Diagnostic characteristics	<i>L. porphyrina</i>	<i>L. cuprina</i>	Remarks
Number of papillae on AS	5–9 ( $n=50$ )	3–6 ( $n=60$ )	SC
Median (range) width of PS ( $\mu\text{m}$ )	275.9 (204.4 - 306.6) <sup>a</sup>	184 (132.9 - 224.9) <sup>b</sup>	$P=0.00$ MC
Median (range) distance between PS ( $\mu\text{m}$ )	143.1 (92 - 214.6) ( $n=13$ )	163.5 (112.4 - 204.4) ( $n=21$ )	$P=0.86$
Position of button	Central between peritreme	Central or slant inside peritreme	SC
Inner projection between MS and LS	Prominent	Not prominent	MC
Degree of sclerotization in the peritreme	Strongly sclerotized	Weakened sclerotized	MC

Values in the same row followed by a lowercase letter are significantly different (Mann–Whitney *U* test); specimens are measured in a mounted slide AS anterior spiracle, PS posterior spiracle, MS middle slit, LS lower slit, MC main characteristic used for differentiation, SC supplement characteristic used for differentiation

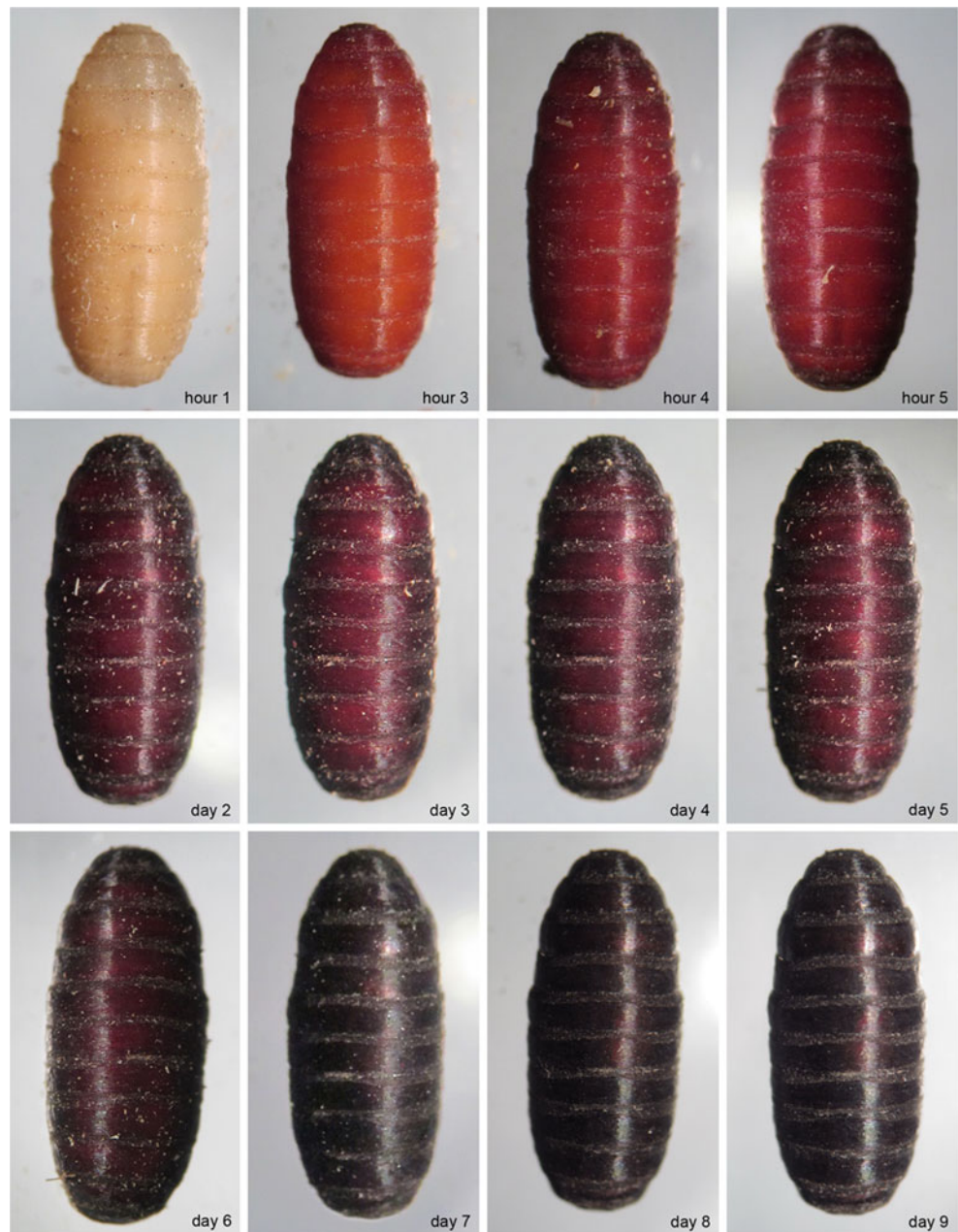




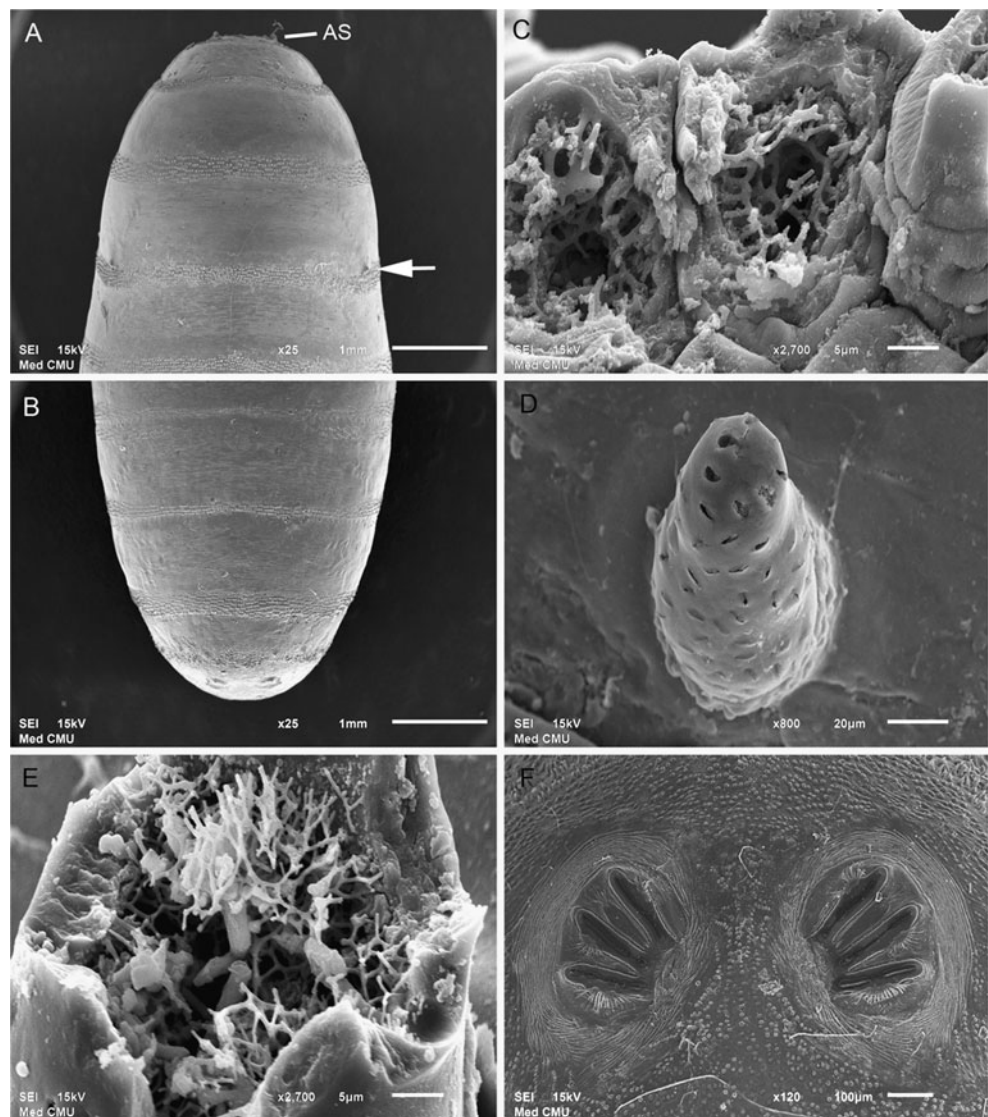
**Fig. 8** Posterior spiracle of the third instar. **a** *L. porphyrina* showing a large spiracle, represented by the width (*W*). Arrow indicates prominent projections between the middle and lower slits. *B* button, *D* distance

between both spiracles. **b** *L. cuprina* showing a small spiracle. Both pictures have the same magnification (online figure in color)

**Fig. 9** Puparia of *L. porphyrina* showing gradual coloration changes from creamy-white (hour 1), to dull orange brown (hour 3), light brown (~hour 4–5), dark red brown (~day 2–5), and eventually almost black (~day 6–9) before emergence (online figure in color)



**Fig. 10** Puparia of *L. porphyrina*. **a** Anterior end revealing anterior spiracle at the lateral margin of the prothorax (AS). Arrow points to a minute respiratory horn at the dorso-lateral margin of the fifth segment. **b** Rounded posterior end. **c** Broken anterior spiracle showing antler-like projections lined within a chamber. **d** Respiratory horn with numerous minute openings throughout. **e** Broken specimens of the respiratory horn displaying antler-like projections lined within a chamber. **f** Posterior spiracle



SEM observation displayed a short tubular respiratory horn at the dorso-lateral margin of the fifth segment of *L. porphyrina* puparia. Such a short respiratory horn resembles that found in *L. cuprina* (Sukontason et al. 2006a) and *H. ligurriens* (Sukontason et al. 2008). Likewise, numerous minute openings are observed throughout the whole respiratory horn in these fly species. It is worth noting that an antler-like projection inside papillae of the anterior spiracle in third instar *L. porphyrina* is morphologically similar to the respiratory horns of the same species, as well as those in puparia of the blow fly, *Chrysomya bezziana* (Villeneuve) (Sukontason et al. 2006b). The oval plate cover internal antler-like projections are well suited as filter apparatus. Therefore, small particles, such as dust, would be prevented from entering into the air chamber by such a structure, thus protecting both third instar and the puparia.

Observations in this study illustrated roughly three phases of puparial coloration. Development of the puparial

color in *L. porphyrina* underwent rapid changes within the first day post-pupariation, or designation of the initial phase. In contrast, coloration changes were relatively similar during the middle (day 2–6) and final phases (day 7–9). This finding suggested that it may be useful to approximate pupal age, at least during different phases. Recently, work published by Davies and Harvey (2012) revealed the potential for pupal age estimation of the blow flies, *Calliphora vicina* Robineau-Desvoidy and *L. sericata* (Meigen) using a histological technique to determine the internal morphology. However, research pertaining to estimate pupal age precisely by other techniques warrants further study.

In conclusion, the third instar of *L. porphyrina* is morphologically distinguishable from previous descriptions of *L. cuprina* third instar, which can be distinguished by the difference in size of the posterior spiracle, prominent inner projection between the middle and lower slits, degree of sclerotized peritreme, and probably the number of papillae on the anterior

spiracle. Such information has contributed important characteristics that can be used for identification not only in Thailand but also in areas where these species exist.

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