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Morphology and identification of fly eggs: application in forensic entomology

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Abstract. Fly eggs found in corpses can be used as entomological evidence in forensic investigation. This study aims to investigate the morphology of forensically important fly eggs. Eggs of *Chrysomya rufifacies*, *Chrysomya megacephala*, *Chrysomya pinguis*, *Chrysomya nigripes*, *Hypopygiopsis tumrasvini*, *Lucilia cuprina*, *Lucilia porphyrina* and *Musca domestica* were examined using 1% potassium permanganate solution for 1 min. Morphometric analysis revealed that the mean length of *Hy. tumrasvini* (1.63 mm) and *C. pinguis* (1.65 mm) eggs was the longest, followed by that of *L. porphyrina* (1.45 mm), *C. rufifacies* (1.34 mm). The egg length, width of median area and darkness staining of hatching pleats were distinctive features. Four categories of median area were proposed, based on width; (1) distinctly wide (*Megaselia scalaris*, *Synthesiomyia nudiseta*); (2) wide (*C. nigripes*, *M. domestica*); (3) slightly widening (*Hy. tumrasvini*, *L. cuprina*, *L. porphyrina*); and (4) narrow (*C. rufifacies*, *C. albiceps*, *C. megacephala*, *C. pinguis*). Four species were examined using SEM, i.e., *C. megacephala*, *C. pinguis*, *Hy. tumrasvini* and *L. porphyrina*. The eggs of *C. megacephala* demonstrated swollen hatching pleats. Inside, the hexagon of the chorion appeared as a sponging bumpy feature. The egg of *C. pinguis* was similar to *C. megacephala*, except for the sponging bumpy feature on the outer surface of the hatching pleats. Regarding *Hy. tumrasvini* and *L. porphyrina*, their island structure was apparent at the inner surface of the upright hatching pleats. The key for identifying these eggs together with other reported species in Thailand has been updated.

INTRODUCTION

Blow fly specimens found at death scenes are well-known currently as entomological evidence in forensic investigations. They are not only used in estimating post-mortem interval (PMI) (Goff *et al.*, 1988; Introna *et al.*, 1998), but also analyzing toxic substances in corpses consumed by fly larvae (Gunatilake & Goff, 1989). Of the four stages in life cycle – egg, larva, pupa and adult – the first three immature stages are used most frequently as entomological evidence. Although the larva stage is most often found to associate with corpses, eggs also have been recorded in some cases. In such cases, dissection of egg samples, and analysis of the embryonic stage of development, may outline the time of fly colonization, and thus enhance PMI estimation (Anderson, 1999; 2004).

In forensic entomology, the correct identification of fly egg specimens is a step primarily needed to further investigations. Attention has been paid to determine the morphological characteristics of fly eggs of forensic importance in many parts of the world (Erzinçlioglu, 1989; Liu & Greenberg, 1989; Greenberg & Singh, 1995; Sukontason *et al.*, 2004b). Many methodologies have been employed to investigate the morphology of

fly eggs, by either using light microscopy (Sukontason *et al*., 2004b) or scanning electron microscopy (SEM) (Liu & Greenberg, 1989; Sukontason *et al*., 2004a; 2008). The purpose of this research was to provide updated information on forensically important fly eggs in Thailand, based on analysis through morphometric and morphology descriptions under light microscopy and SEM. In addition, it aimed to facilitate species identifications and a key was revised for comparisons of egg morphology.

MATERIALS AND METHODS

Fly egg

Seven species of blow fly eggs – *Chrysomya rufifacies*, *Chrysomya megacephala*, *Chrysomya pinguis*, *Chrysomya nigripes*, *Hypopygiopsis tumrasvini*, *Lucilia cuprina*, *Lucilia porphyrina* and the house fly, *Musca domestica* – were employed for light microscopic study. All species, except for *Hy. tumrasvini*, were obtained from the laboratory colonies maintained at the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand. Eggs of *Hy. tumrasvini* were obtained from gravid females that oviposited inside a tube trap baited with fresh pork liver, in the forest of Suthep mountain (18º48'20"N 18º54'34"E, 952 m), Muaeng Chiang Mai district, Chiang Mai province, Thailand. The procedure used for fly-rearing was fresh pork liver provided as an oviposition site previously described by Bunchu *et al*. (2008). Regarding SEM investigation, three species of fly eggs were examined, i.e., *Chrysomya pinguis*, *Hy. tumrasvini* and *Lucilia porphyrina*, due to the lack information on their ultrastructure. Eggs of *C. megacephala* were also examined to compare specifically with *C. pinguis*.

Egg measurement and staining procedure

Around 30 to 100 of each egg species were collected from the fresh pork liver, rinsed using normal saline solution, and kept in an eppendorf tube with 70% ethanol for at least 1 d. They were then transferred onto a glass slide for staining and separated individually using a small toothpick. The length and width of the eggs were measured under a light microscope (Olympus CH®, Japan) with a 4x ocular micrometer. The egg length measurement was carried out from the anterior to posterior ends; whereas the widest point of the egg was measured in the middle. Data were analyzed statistically using analysis of variance (ANOVA), with a P value of <0.05 considered significant.

Regarding the staining procedure, the measured eggs were selected randomly for staining by transferring them into a petri dish containing 1% potassium permanganate solution for 1 min (Sukontason *et al*., 2004b). They were then mounted onto a glass slide with a few drops of permount (Neo-shigaral®, Japan), and covered with a cover slip before examining under light microscope (Olympus CH®, Japan).

Scanning Electron Microscopy

The eggs of *C. megacephala*, *C. pinguis*, *Hy. tumrasvini* and *L. porphyrina* were washed several times using normal saline solution to remove any pork liver tissue residue. They were fixed with 2.5% glutaraldehyde mixed in phosphate-buffer at a pH of 7.4 at 4ºC for 24 h, and rinsed twice with phosphate-buffer at 10-min intervals. The rinsed eggs were treated with 1% osmium tetroxide at room temperature for 1 day, and then rinsed twice with phosphate-buffer and dehydrated with alcohol. To replace water with alcohol in the eggs, the eggs were subjected to increasing ethanol concentrations of 30, 50, 70, 80 and 90%; 12 h each. Thereafter, specimens were subjected to critical point drying, attached to aluminum stubs with double-stick tape, coated with gold in a sputter-coating apparatus and viewed with a JEOL-JSM6610LV SEM (Japan), which was operated at 15kV. Terminology of the eggshell followed Hinton (1981), and the revision of Grzywacz *et al.* (2012).

RESULTS

Egg measurement and staining

The eggs of blow flies and house fly were on average 1.10-1.70 mm in length and 0.27-0.45 mm across their central region. They were

elliptical in contour, creamy-white in color, and the anterior pole was somewhat tapered, consisting of micropyle extremity. The median area was expanded slightly at the anterior pole, appearing as Y-shaped in some species, and extending along the egg at the midline. In this light microscopic study, the entire egg surface appeared smooth at the resolution applied.

Differences in dimensions of the eight fly species eggs examined are presented in Table 1. Morphometric analysis revealed that the egg of *Hy. tumrasvini* was the longest (mean ± SD, 1.65 ± 0.10), followed by that of *C. pinguis*, with no significant difference (1.63 ± 0.04) (P<0.05; ANOVA). In consequence, the length of *L. porphyrina* (1.45 ± 0.04) was longer statistically than that in *C. rufifacies* (1.34 ± 0.03), *C. megacephala* (1.29 ± 0.02), *M. domestica* (1.23 ± 0.02) and *C. nigripes* (1.21 ± 0.04). By contrast, the eggs of *L. cuprina* were the shortest (1.15 ± 0.03).

The morphological characteristics of fly eggs after staining for 1 min using 1% potassium permanganate solution are displayed in Fig. 1. Although the freshly laid eggs were creamy-white and slightly glossy at first, they changed to yellow-brown after staining. Distinctions observed were based on the degree of clarity of the median area and hatching pleats which was determined under light microscope. The differences of median area can be categorized into 3 groups; (1) narrow in *C. rufifacies* (Fig. 1A), *C. megacephala* (Fig. 1B) and *C. pinguis* (Fig. 1C), occupying a small portion of the total width; (2) slight widening in *Hy. tumrasvini* (Fig. 1D), *L. cuprina* (Fig. 1E), and *L. porphyrina* (Fig. 1F); and (3) wide in *C. nigripes* (Fig. 1G) and *M. domestica*, occupying ca. one-third of the total width (Fig. 1H). Appearance of the hatching pleats under light microscopic investigation can be categorized into 2 groups; (1) slightly swollen in *C. rufifacies*, *C. megacephala*, *C. pinguis* and *M. domestica*, displaying as narrow dark brown line (Figs. 1A, 1B, 1C and 1H, respectively), and (2) upright in *Hy. tumrasvini*, *L. cuprina*, *L. porphyrina* and *C. nigripes*, displaying as thick dark brown line (Figs. 1D, 1E, 1F, and 1G, respectively).

Egg surface sculpturing

The eggs of *C. megacephala* were elliptical, with the anterior pole slightly more tapered than the posterior one (Fig. 2A). The median area started from the anterior pole, and formed a Y-shape that surrounded the micropyle and extended dorso-medially for almost the entire length of the egg. The median area was quite narrow, averaging 0.009±0.003 mm (n = 10) (Fig. 2B). The hatching pleats were smooth and slightly swollen (Fig. 2B, asterisk). The surface of the chorion was smooth and covered by a hexagonal pattern formed by imprints of the follicular cells. When viewed externally, the profile of the hexagonal pattern boundary appeared relatively smooth (Fig. 2C). SEM observation throughout the egg displayed different features inside the hexagonal pattern, of which the major portion showed an irregular perforated surface (Fig. 2D); while some minor areas, particularly at the posterior end, exhibited large, round perforated configuration (Fig. 2E).

Egg sculpturing of *C. pinguis* is illustrated in Figure 3, demonstrating a similar appearance in ultrastructure to *C. megacephala*, in that the median area started from the anterior pole and forms a Y-shape that surrounds the micropyle and extends dorso-medially for almost the entire length of the egg. The median area also was quite narrow, averaging 0.004±0.002 mm (n = 7) (Fig. 3B). The hatching pleats were slightly swollen, but had a perforated surface (Figs. 3B, 3C, asterisk). The ultrastructure inside the hexagonal pattern displayed an irregular perforated surface, as observed in *C. megacephala* (Fig. 3D). Likewise, some areas, particularly at the posterior end, exhibited large perforated configuration, with their rims encircled by tiny protruding setae, which resembled those of *C. megacephala* (Fig. 3E). The inner surface was examined in some *C. pinguis* eggs (Fig. 3F), displaying a perforated hexagonal pattern, with a fairly smooth boundary (Fig. 3G). Higher magnification of such a pattern showed a sculptured network formed by interconnecting polygonal ridges, with a perforated surface underneath (Fig. 3H).

Table 1. Average egg size measured before staining with 1% potassium permanganate solution

Species	*n*	Length (mm)*	Width (mm)*
Chrysomya megacephala	47	1.29±0.02[d]	0.28±0.01[f]
Chrysomya nigripes	64	1.21±0.04[f]	0.32±0.02[d]
Chrysomya pinguis	100	1.63±0.04[a]	0.38±0.02[b]
Chrysomya rufifacies	66	1.34±0.03[c]	0.31±0.01[e]
Hypopygiopsis tumrasvini	78	1.65±0.10[a]	0.41±0.04[a]
Lucilia cuprina	69	1.15±0.03[g]	0.28±0.01[f]
Lucilia porphyrina	31	1.45±0.04[b]	0.36±0.02[c]
Musca domestica	51	1.23±0.02[e]	0.26±0.01[g]

*The data are expressed as mean±SD with a different letter in the same column being significantly different at $P<0.05$ (ANOVA)

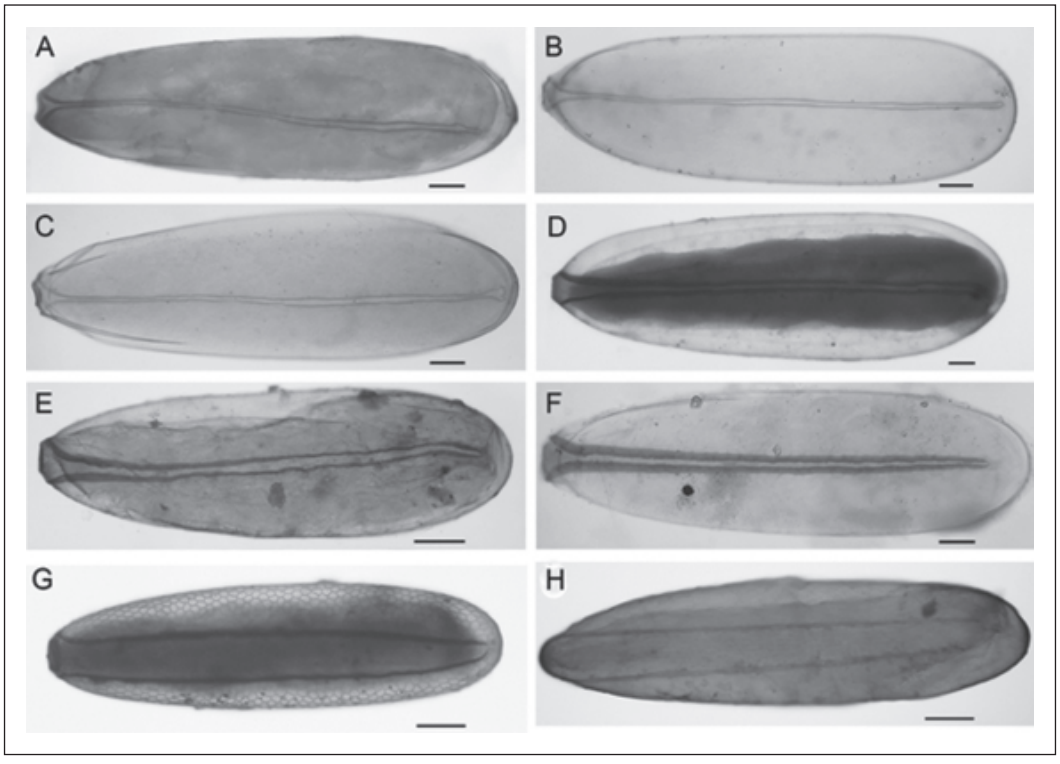

Figure 1. Images of fly eggs after staining with 1% potassium permanganate solution for 1 min. Anterior end on the left. **A:** *Chrysomya rufifacies*, whole egg with long and narrow median area. **B:** *Chrysomya megacephala*, whole egg with long and narrow median area. **C:** *Chrysomya pinguis*, whole egg with long and narrow median area. **D:** *Hypopygiopsis tumrasvini*, whole egg with long and slightly widening median area. **E:** *Lucilia cuprina*, whole egg with long and slightly widening median area. **F:** *Lucilia porphyrina*, whole egg with long and slightly widening median area. **G:** *Chrysomya nigripes*, with long and wide median area, ca. one-third of the total width. **H:** *Musca domestica*, whole egg with long and wide median area, ca. one-third of the total width. Scale bars = 0.1 mm for all figures

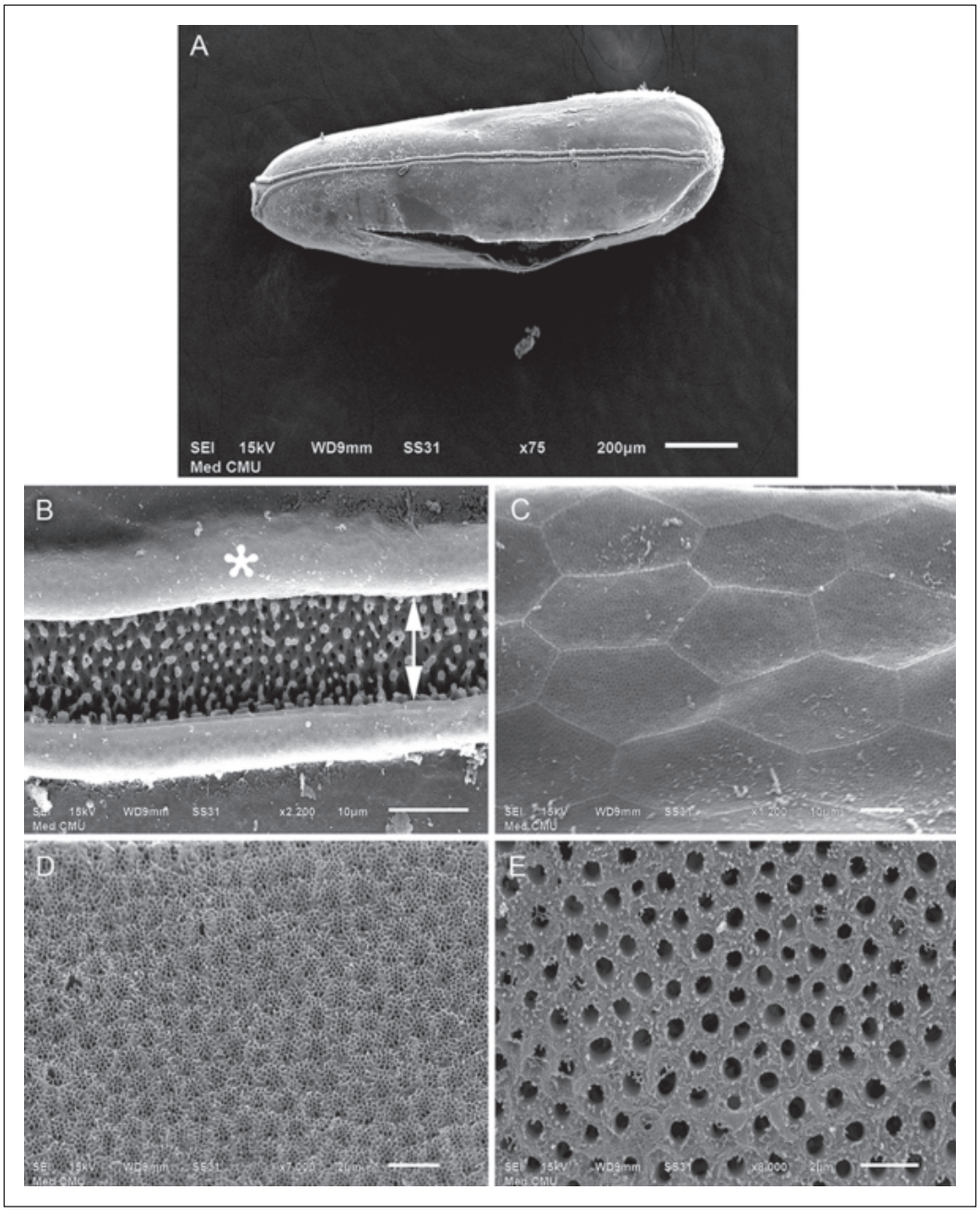

Figure 2. SEM images of the *C. megacephala* egg. **A:** Whole egg with long and narrow median area. **B:** Narrow median area (distance) and smooth, swollen hatching pleats (asterisk). **C:** Chorion displaying hexagonal pattern. **D:** Higher magnification of hexagonal pattern showing irregular perforated surface. **E:** Some areas of the hexagonal pattern appearing as a smooth perforated surface

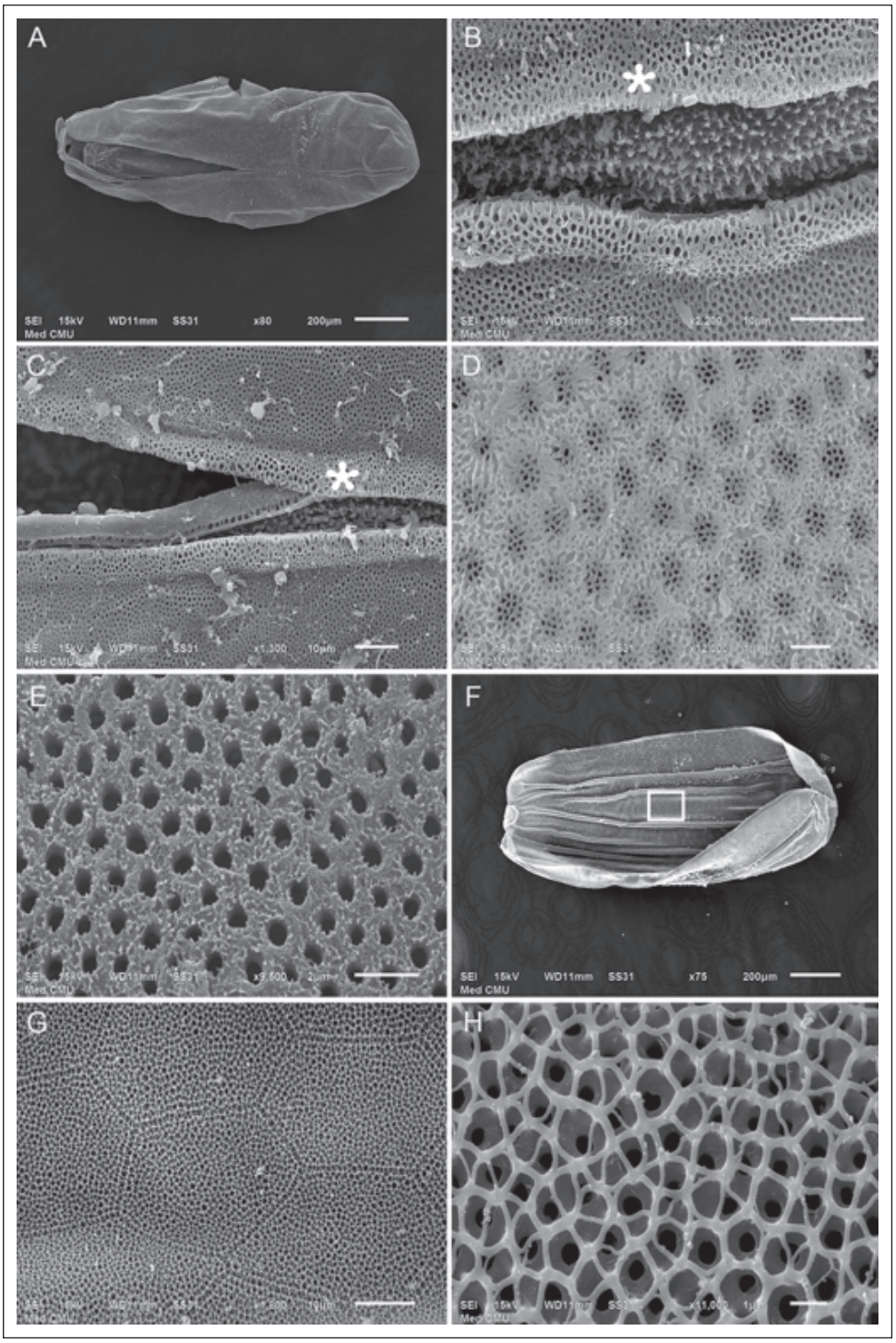

Figure 3. SEM images of the *C. pinguis* egg. **A:** Whole egg with long and narrow median area. **B:** Narrow median area (distance) and slightly swollen hatching pleats, but perforated surface (asterisk). **C:** hatching line. **D:** Irregular perforated surface inside the hexagonal pattern. **E:** Higher magnification of the hexagonal pattern showing irregular perforated surface. **F:** Inner surface. **G:** Hexagonal pattern inside "F". **H:** Magnification of "**G**" inside the hexagonal pattern displaying perforated reticulations

Egg sculpturing of *Hy. tumrasvini* is illustrated in Fig. 4, with a slightly widening median area (Fig. 4A), averaging 0.011±0.002 mm (n = 10) (Fig. 4B). The hatching pleats were upright (Figs. 4B, 4C, arrow) and smooth externally (Fig. 4C, asterisk). However, the inner surface of the hatching pleats was filled with a plastron structure (Figs. 4B, 4C, arrows), of which the inside median area formed in islands pitted with noticeably minute orifices (Fig. 4D). A connecting perforated patch network was observed at the base of these islands (Fig. 4D). Slightly elevated boundaries in a hexagonal pattern were seen at the chorion (Fig. 4E). Some broken egg specimens illustrated a profile of several eggshell layers, showing the outermost exochorion, outer endochorion, layer of vertical pillars between the irregular space of aeropyles, inner endochorion, innermost chorionic layer and distinct vitelline membrane (Fig. 4F).

The ultrastructure of the *L. porphyrina* egg was similar to that of *Hy. tumrasvini* (Fig. 5), in having a slightly widening median area (Fig. 5A), that averaged 0.011±0.002 mm (n = 8) (Fig. 5B). The hatching pleats were upright (Fig. 5B, arrow), and smooth externally; whereas the inner surface was filled with a plastron structure (Fig. 5B, arrow). The profile of the hexagonal pattern boundary was slightly elevated, with no form of perforation inside it (Fig. 5C). The ultrastructure of the eggshell revealed several layers, similar to that of *Hy. tumrasvini* (Fig. 5D).

A key for identifying fly species eggs of potential forensic importance in Thailand has been published (Sukontason *et al.*, 2007), and the following one is an update:

1. Median area wide, more than half of the total width (Figs. 6A, 6B) 2
   Median area narrow, less than half of the total width 3
2. Length > 0.6 mm; hatching pleats with continuous scale-like projection (Fig. 6A) ..... *Megaselia scalaris* (Phoridae)
   Length < 0.6 mm; hatching pleats upright (Fig. 6B)..... *Synthesiomyia nudiseta* (Muscidae)
3. Median area narrow (Figs. 1A, 1B, 1C) ..... *Chrysomya rufifacies*, *Chrysomya megacephala*, *Chrysomya pinguis*
   Median area slightly widening (Figs. 1D, 1E, 1F) or wide (Figs. 1G, 1H) 4
4. Median area slightly widening; boundary of the hexagonal pattern smooth, and indistinct after staining (Figs. 1D, 1E, 1F) ..... *Hypopygiopsis tumrasvini*, *Lucilia cuprina*, *Lucilia porphyrina*
   Median area wide, ca. one-third of the total width 5
5. Boundary of the hexagonal pattern elevated, distinct after staining (Fig. 1G) ..... *Chrysomya nigripes*
   Boundary of the hexagonal pattern smooth, indistinct after staining (Fig. 1H) ..... *Musca domestica*

DISCUSSION

Identification of fly eggs, particularly those of forensic importance, may be difficult due to their similarity in general appearance. For a thorough comparison among species, some features are shown as unique, thereby allowing differentiation among them. Such features observed either under light microscopy or SEM include width of the median area, bifurcation of the median area surrounding the micropyle, hatching pleats, chorionic sculpturing, and thickening of the chorion along the hatching lines (Liu & Greenberg, 1989; Greenberg & Kunich, 2002; Sukontason *et al.*, 2004a). Egg staining with 1% potassium permanganate solution enhanced these features in order to observe clearly them under a light microscope. Staining can distinguish between some groups, but the ultrastructure of *C. rufifacies*, *C. albiceps*, *C. megacephala* and *C. pinguis* eggs appears to be remarkably similar and indistinguishable, when determined by either light microscopy or SEM (Mendonça *et al.*, 2010). For specific comparison between *C. megacephala* and *C. pinguis* eggs, micrographs revealed no clear morphological difference. There were no distinctions between narrow median areas, smooth and

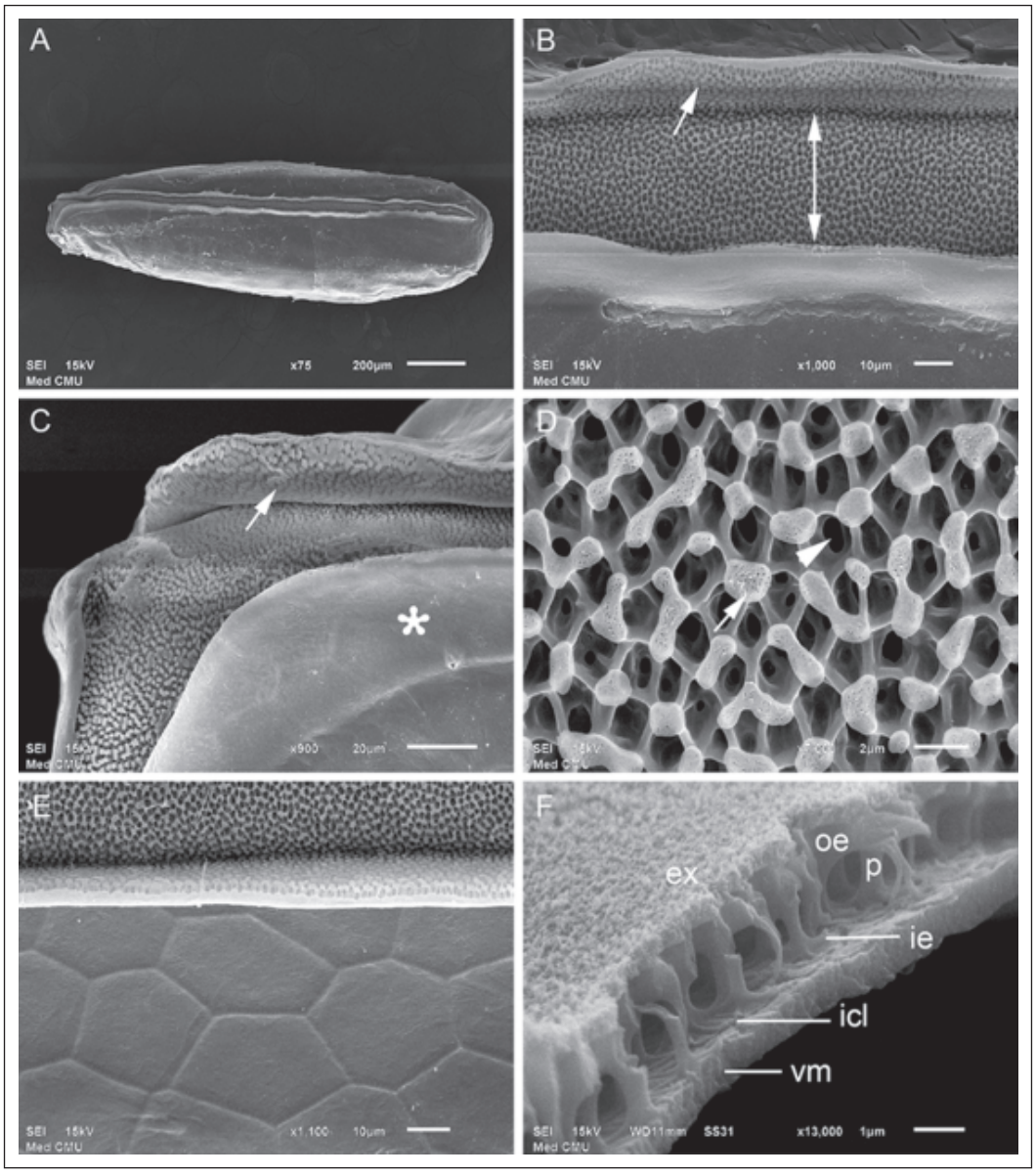

Figure 4. SEM images of the *Hy. tumrasvini* egg. **A:** Whole egg with long and slightly widening median area. **B:** Slightly widening median area (distance) and upright hatching pleats (arrow). **C:** Upright hatching pleats (arrow) with smooth external surface (asterisk). **D:** Plastron pattern inside the median area, illustrating islands pitted with noticeably minute orifices (arrow). Connecting perforated patch network at the base of the islands (arrowhead). **E:** Slightly elevated hexagonal pattern boundaries of the chorion. **F:** Eggshell revealing several layers, outermost exochorion (ex), outer endochorion (oe), layer of vertical pillars (p) between the irregular space of aeropyles, inner endochorion (ie), innermost chorionic layer (icl) and distinct vitelline membrane (vm)

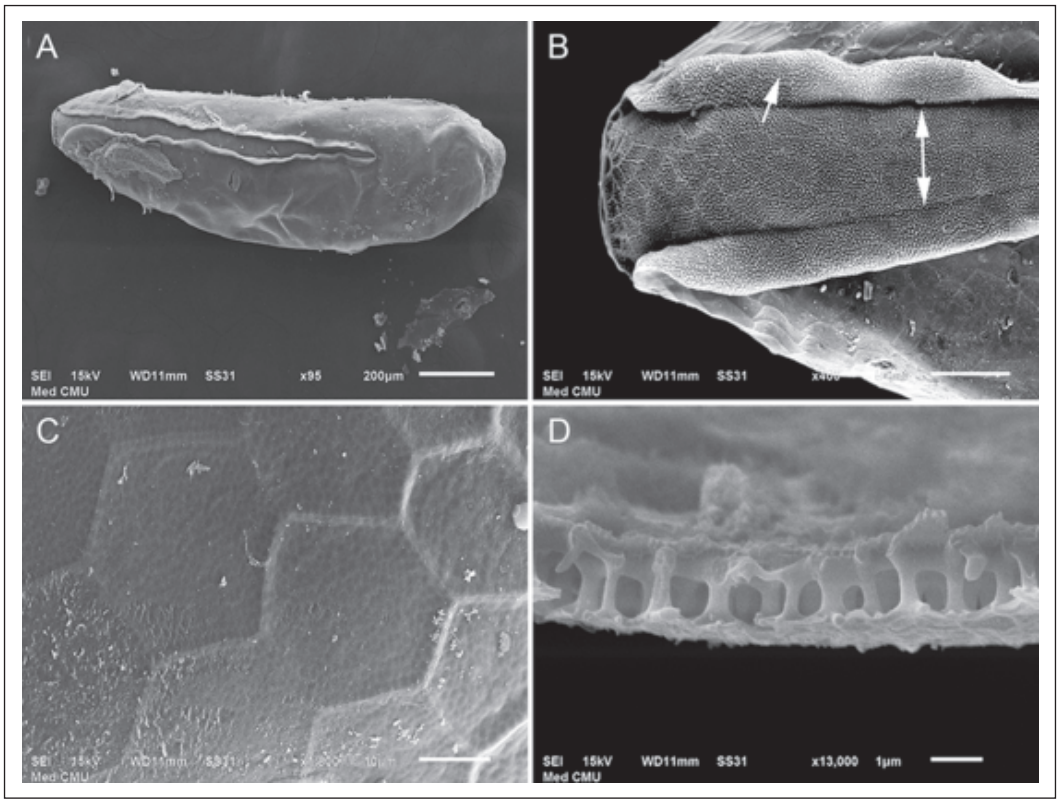

Figure 5. SEM images of the *L. porphyrina* egg. **A:** Whole egg with long and slightly widening median area. **B:** Slightly widening median area (distance) and upright hatching pleats (arrow). **C:** Slightly elevated hexagonal pattern boundaries of the chorion. **D:** Eggshell revealing several layers

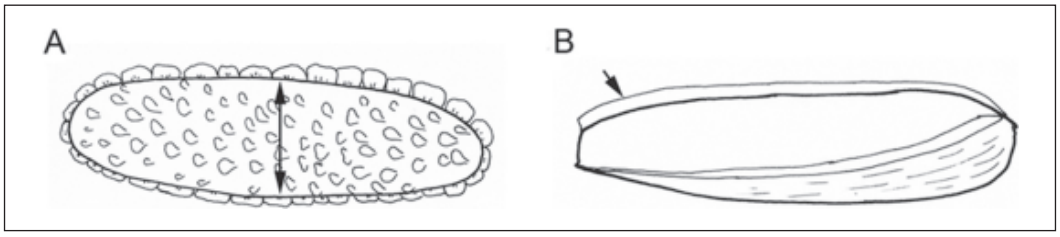

Figure 6. Diagrammatic pictures of fly eggs. **A:** *M. scalaris* displaying a wide median area, occupying more than half of the total width (distance). Hatching pleats with continuous scale-like projection. **B:** *S. nudiseta* showing a wide median area that occupies more than half of the total width, and upright hatching pleats (arrow)

swelling hatching pleats, smooth hexagonal pattern boundaries, irregular perforated surfaces and large, round perforated configurations. Based on SEM observations, the only convincingly different feature from those of *C. megacephala* was the perforated structure of hatching pleats in *C. pinguis*. According to the egg length listed in Table 1, our result showed statistical differences between these two species, but as previously suggested, length of the eggs depends on dietary level, therefore, this feature cannot be used as a primary characteristic for identification (Erzinçlioglu, 1989).

Width of the median area is apparently distinct among groups of fly eggs, and thereby a main criterion for egg identification. Based on this characteristic, we proposed the following four categories of fly eggs examined in this study and in earlier publications (Table 2): (1) distinctly wide (e.g., *M. scalaris*, *S. nudiseta*); (2) wide (e.g., *C. nigripes*, *M. domestica*, *Pollenia* spp.) (Grzywacz *et al*., 2012); (3) slightly widening (e.g., *Hy. tumrasvini*, *L. cuprina*, *L. porphyrina*); and (4) narrow (e.g., *C. rufifacies*, *C. albiceps*, *C. megacephala*, *C. pinguis*). Our results indicate that the eggs of blow flies in the subfamily Luciliinae (genera *Lucilia*, *Hemipyrellia*, *Hypopygiopsis*) (Rognes, 1997), especially flies of forensic importance, are in agreement with other analyses. They display relatively unique characteristics in having a slightly widening median area, and hatching pleats that appear upright, as shown clearly in SEM micrographs (Liu & Greenberg, 1989; Greenberg & Singh, 1995; Mendonça *et al*., 2008; Sukontason *et al*., 2008).

The chorionic sculpture is a distinct characteristic used to differentiate fly eggs, by either a smooth boundary (as seen in the blow flies, *C. megacephala*, *C. pinguis* and *C. rufifacies*) or markedly elevated hexagonal pattern boundary (as seen in *C. nigripes* or the scuttle fly *M. scalaris*). Staining with 1% potassium permanganate solution verifies such features in *C. nigripes* and *M. scalaris*, when displaying a prominent dark brown polygon (Sukontason *et al*., 2004b).

Table 2. Category of median area width recognized in fly eggs

Width of median area	Character of hatching pleats	Family	Species	References
distinctly wide	rims with prominent flanges	Phoridae	*Megaselia scalaris*	Liu & Greenberg, 1989; Sukontason et al., 2007; de Oliveira David *et al*., 2008
	upright	Muscidae	*Synthesiomyia nudiseta*	Sukontason *et al*., 2007
Wide		Muscidae	*Musca domestica*	this study
	?	Calliphoridae	*Pollenia amentaria*	Grzywacz *et al*., 2012
	?	Calliphoridae	*Pollenia angustigena*	Grzywacz *et al*., 2012
	?	Calliphoridae	*Pollenia atramentaria*	Grzywacz *et al*., 2012
	upright	Calliphoridae	*Pollenia labialis*	Grzywacz *et al*., 2012
	?	Calliphoridae	*Pollenia mayeri*	Grzywacz *et al*., 2012
	?	Calliphoridae	*Pollenia pediculata*	Grzywacz *et al*., 2012
	upright	Calliphoridae	*Pollenia rudis*	Grzywacz *et al*., 2012
	?	Calliphoridae	*Pollenia similis*	Grzywacz *et al*., 2012
	?	Calliphoridae	*Pollenia vagabunda*	Grzywacz *et al*., 2012
slightly widening	upright	Calliphoridae	*Aldrichina grahami*	Sukontason *et al*., 2004b
	?		*Calliphora alpina*	Erzinçlioglu, 1989
	?		*Calliphora loewi*	Erzinçlioglu, 1989
	upright		*Calliphora uralensis*	Erzinçlioglu, 1989
	upright		*Calliphora vicina*	Liu & Greenberg, 1989; Greenberg & Singh, 1995
	upright		*Calliphora vomitoria*	Greenberg & Singh, 1995
	smooth, swelling boundary		*Chrysomya nigripes*	Sukontason *et al*., 2004a
	smooth, swelling boundary		*Chrysomya putoria*	Mendonça *et al*., 2008

	smooth		*Cochliomyia hominivorax*	Peterson & Newman, Jr., 1991
	upright		*Cynomya mortuorum*	Erzinçlioglu, 1989
	upright		*Hemipyrellia ligurriens*	Sukontason *et al.*, 2008
	upright		*Lucilia coeruleiviridis*	Greenberg & Singh, 1995
	upright		*Lucilia cuprina*	Liu & Greenberg, 1989; Sukontason *et al.*, 2007; Mendonça *et al.*, 2008
	upright		*Lucilia eximia*	Mendonça *et al.*, 2008
	upright		*Lucilia illustris*	Liu & Greenberg, 1989; Greenberg & Singh, 1995
	upright		*Lucilia porphyrina*	this study
	upright		*Lucilia sericata*	Greenberg & Singh, 1995
	?		*Phormia terraenovae*	Erzinçlioglu, 1989
	smooth, swelling boundary	Muscidae	*Hydrotaea* (= *Ophyra) aenescens*	Mendonca *et al.*, 2008
narrow	smooth, swelling boundary	Calliphoridae	*Chrysomya albiceps*	Greenberg & Singh, 1995; Mendonça *et al.*, 2010
	smooth, swelling boundary		*Chrysomya chloropyga*	Greenberg & Singh, 1995
	smooth, swelling boundary		*Chrysomya megacephala*	Mendonça *et al.*, 2008; this study
	smooth, swelling boundary		*Chrysomya pacifica*	Sukontason *et al.*, 2004b
	smooth, swelling boundary		*Chrysomya pinguis*	this study
	smooth, swelling boundary		*Chrysomya rufifacies*	Liu & Greenberg, 1989; Greenberg & Singh, 1995
	smooth, swelling boundary		*Cochliomyia macellaria*	Liu & Greenberg, 1989; Greenberg & Singh, 1995
	smooth, swelling boundary		*Hemilucilia segmentaria*	Thyssen & Linhares, 2007
	smooth, swelling boundary		*Phormia regina*	Liu & Greenberg, 1989; Greenberg & Singh, 1995

SEM examination of the eggs in this study presented an apparently perforated surface when exposed to the general atmosphere, not only at the major portion, but also in the large, round perforated configuration at the posterior end. Such a characteristic therefore ensures respiration for oxygen supply and gas exchange between the external environment and developing embryo.

SEM observation of *Hy. tumrasvini* and *L. porphyrina* in this study revealed similarity in their complex eggshell structure, which comprised several layers in unison that resemble many previously reported fly species, e.g., blow flies, *C. nigripes*, *L. cuprina* and *Cochliomyia hominivorax* (Peterson & Newman, Jr., 1991; Sukontason *et al.*, 2004a; 2007), flesh fly, *Liosarcophaga dux* (Sukontason *et al.*, 2005) and fruit fly, *Drosophila melanogaster* (Margaritis & Mazzini, 1998). Therefore, the general configuration of eggshell layers is conserved in these groups of flies.

In conclusion, this study aimed to differentiate between fly eggs of forensic importance by comparing their distinct features for identification using light microscopy after staining with 1% potassium permanganate solution and SEM observation. Consequently, the width of median area, characteristic of hatching pleats and chorionic sculpture may be of value in distinguishing between eggs of forensically important fly species.

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