

2. การนำผลงานวิจัยไปใช้ประโยชน์

• เชิงสาธารณะ

งานวิจัยครั้งนี้ได้เกิดความร่วมมือกับภาควิชานิติเวชศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ และต่างประเทศคือ Department of Entomology, Texas A&M University (USA), Department of Biology, Hardin-Simmons University (USA), International Department of Dipterology (Japan) และ CSIRO Livestock Industries (Australia)

ผลงานวิจัยที่เกิดจากโครงการนี้ (เรื่องที่ 1.1 และ 1.2) ได้ถูกขอ reprint โดยนักวิจัยจากหลายประเทศได้แก่ USA, Spain, Brazil, Thailand

• เชิงวิชาการ

ผลที่ได้จากการวิจัยครั้งนี้ได้ถูกนำไปใช้ประโยชน์ทางวิชาการคือ ข้อมูลอัตราการเจริญเติบโตของตัวอ่อนแมลงวันหัวเขียว *C. megacephala* และ *C. rufifacies* ที่อุณหภูมิธรรมชาติของจังหวัดเชียงใหม่ ถูกนำไปช่วยแพทย์ผู้ชันสูตรศพ ภาควิชานิติเวชศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ในการช่วยประมาณระยะเวลาหลังการตายของศพ (ประมาณ 2-4 ศพ) ในกรณีที่พบตัวอ่อนของแมลงวันในศพ

มีการนำผลความรู้ที่ได้ไปใช้ในการพัฒนาการเรียนการสอน ทั้งในหลักสูตรแพทยศาสตรบัณฑิตและระดับบัณฑิตศึกษา ภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ โดยบรรจุความรู้ที่ได้รับในหัวข้อบรรยายเรื่อง "Flies"

การวิจัยครั้งนี้มีส่วนช่วยในการสร้างความรู้และประสบการณ์แก่นักวิจัยในภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ทั้งหัวหน้าโครงการวิจัย ผู้ร่วมวิจัย นอกจากนี้ยังช่วยสร้างความรู้และประสบการณ์แก่นักศึกษาระดับบัณฑิตวิทยาลัยที่เข้ามาสังเกตการณ์การทำวิจัยครั้งนี้ จำนวน 5 คน

3. การนำผลงานวิจัยไปใช้ประโยชน์ในด้านอื่นๆ

• การเสนอผลงานในที่ประชุมวิชาการ

3.1 Surface ultrastructure of the third-instar larvae of *Chrysomya rufifacies* (Diptera: Calliphoridae), a fly species of forensic importance

นำเสนอผลงานแบบโปสเตอร์ ในการประชุมวิชาการ The XVIIIth Annual Conference on Electron Microscopy วันที่ 17-19 มกราคม พ.ศ. 2544 ที่จังหวัดขอนแก่น และได้รับรางวัลที่ 1 ในการนำเสนอผลงานโปสเตอร์ ประเภท Biological Science: SEM และได้รับรางวัลชมเชยในการประกวดผลงานแบบ Micrograph: SEM (Abstract และเอกสารดังที่ได้แนบมาด้วย)

3.2 Differentiation of forensically-important fly eggs using a potassium permanganate staining technique

ได้รับการตอบรับให้นำเสนอผลงานแบบโปสเตอร์ ในการประชุมวิชาการระดับนานาชาติ The 5th International Congress of Dipterology ในวันที่ 29 กันยายน – 4 ตุลาคม พ.ศ. 2545 ที่เมือง Brisbane ประเทศออสเตรเลีย (ใบตอบรับและ Abstract ดังที่ได้แนบมาด้วย)

ภาคผนวก

ภาคผนวก

- reprint 2 เรื่อง
- manuscript 3 เรื่อง
- abstract ในการประชุมวิชาการ 2 เรื่อง
- บทความสำหรับการเผยแพร่ 1 เรื่อง

The First Documented Forensic Entomology Case in Thailand

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ABSTRACT The forensic entomology case described herein is the first such case documented in Thailand. A mummified corpse of a 32-yr-old man was discovered in a forested habitat, with the larvae of six species of flies (Diptera) found in association with the corpse at the time of its discovery, i.e., those of *Hydrotaea* (= *Ophyra*) *spinigera* Stein (family Muscidae), *Piophilidae casei* (L.) (family Piophilidae), *Megaselia scalaris* (Loew) (family Phoridae), *Sargus* sp. (family Stratiomyidae), and larvae of two unidentified flesh fly species (family Sarcophagidae). The presence and age of the larval specimens of *P. casei*, *M. scalaris*, and *H. spinigera* gave entomological evidence that the postmortem interval for the corpse was 3-6 mo. This report also documents some of the forensically important fly species that occur in Thailand.

KEY WORDS forensic entomology, fly larvae, first documented case, Thailand

FORENSIC ENTOMOLOGY, THE study of insects and arthropods as a tool in criminal investigations (Hall 1990), is an established field of the forensic sciences. Entomological information is useful for estimating postmortem interval (PMI), determining manner of death, supporting drug-related death investigations, as well as improving other kinds of criminal investigations (Smith 1986, Lee 1989, Lord 1990, Goff and Flynn 1991, Catts 1992, Greenberg and Wells 1998, Introna et al. 1998, Anderson 1999, Carvalho et al. 2000).

In Thailand there are no reports documenting the use of entomological evidence in the investigation of human deaths, even though there are numerous human corpses found each year in Thailand. We report herein the first forensic entomology case to be documented in Thailand, focusing on the species of insects involved in the case, which may be used for future forensic investigations in this country.

Case History. On 13 February 2000, the partially skeletonized and mummified body of a 32-yr-old male was found under a tree, which had nylon rope tied to one of its branches, in the forest near the main highway running through the Doi-Sa-Ket District, Chiang Mai Province, Thailand (~60 km from downtown Chiang Mai). The corpse was transferred to the Forensic Medicine Department, Faculty of Medicine, Chiang Mai University, on 14 February 2000 for investigation.

The head of the corpse was completely skeletonized exposing the skull (Fig. 1). The victim was last seen on 4 October 1999, indicating a maximum PMI of ~4.5 mo after his disappearance. There was a dry wound at the

neck, corresponding with the victim possibly being killed by hanging. Both feet were absent. The body was clothed with a long sleeve shirt and trousers. Inside one of the trouser pockets, an identification card was found. According to the superimposition method used in forensic investigations, the corpse was determined to be the same person as indicated by the identification card found in the trouser pocket. Numerous insect larvae were found and collected from both the interior and exterior regions of the corpse.

Entomological Collections. Collection of insect larvae from the corpse was accomplished during forensic pathological investigations held on 14 February 2000 at the Department of Forensic Medicine, Chiang Mai University, Thailand. All of the specimens collected were larvae of various species of Diptera. Some larval specimens of each species were placed in rearing cages containing vermiculite and fresh pork liver (used as the rearing medium) and were raised to the adult stage for species identification. Some specimens were killed immediately in near-boiling water to fix their proteins and prevent darkening of the specimens in alcohol. These specimens were then stored in 70% alcohol for further identification.

Six species of flies were found to be represented among the larval specimens collected from the corpse and identified using scanning electron microscopy and light microscopic techniques. These species included *Hydrotaea* (= *Ophyra*) *spinigera* Stein (family Muscidae), *Piophilidae casei* (L.) (family Piophilidae), *Megaselia scalaris* (Loew) (family Phoridae), *Sargus* sp. (family Stratiomyidae), and larvae of two unidentified Sarcophagidae species (family Sarcophagidae). The anterior spiracles of the larva of one the sarcophagid species had 24 lobes, arranged in two regular rows, whereas that of the other species had 16 lobes, arranged in three irregular rows. Other fly larval specimens, as collected from the corpse, were found to be

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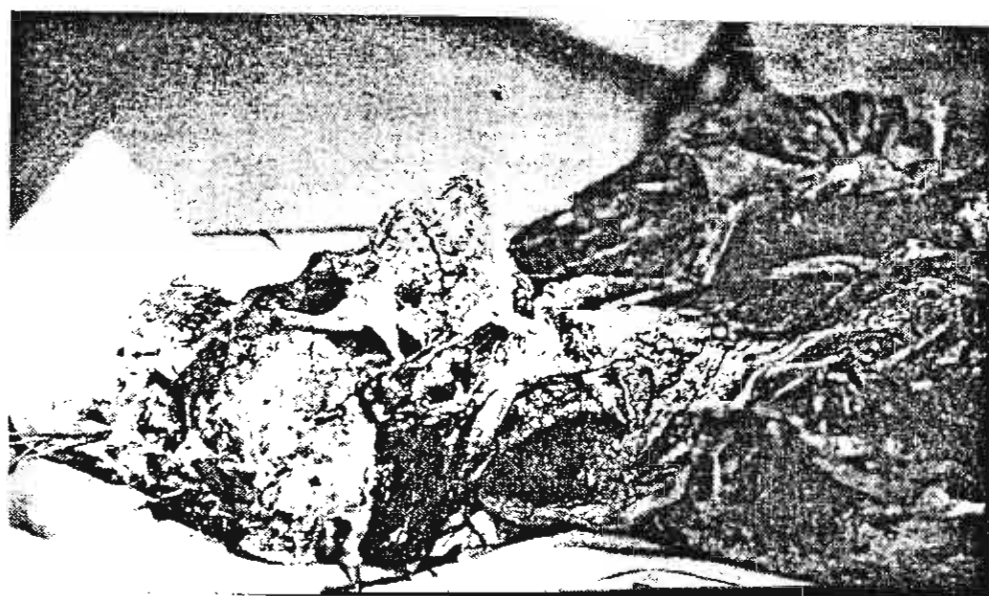


Fig. 1. A mummified corpse with long sleeve shirt and trousers.

in the third instar, and the stage of the *Sargus* larva was in the second instar. The predominant species represented by the maggots collected from the corpse was *M. scalaris*. The adults reared from a sample of these particular larvae were used to confirm the identification. Maggots representing the other species of flies found in association with the corpse were much fewer. Of note here also is that the "skipping" behavior of the *P. casei* larvae (Smith 1986) was observed during the taking of entomological collections from the corpse. Because the corpse had not been investigated by forensic entomologists at the site where it was originally found, soil and litter samples were not taken. This may have resulted in missing some of the other arthropods that might have been associating with the corpse at the scene of the death.

Meteorological Information. Information regarding the maximum and minimum temperatures occurring during the period that the victim had been missing was obtained from the meteorological station of Northern Regional Meteorological Center, Chiang Mai, Thailand, the nearest to the site of the death scene. The prevailing ambient temperatures during the 4.5-mo period extending from the time when the victim disappeared and when his body was found are summarized in Table 1. The period covered is the winter season in Thailand.

Discussion

This is the first documented report of entomological evidence from a human corpse in Thailand, even though there are many corpses found infested with insect larvae each year in this country. The goal of this study was not to estimate the PMI for a corpse, because it was documented by other forensic evidence that the time between when the victim was last seen and when his corpse was discovered spanned ≈ 4.5 mo.

The primary purpose of this study was to document the fly species found in association with the corpse as representative of the types of flies that come in association with human corpses in Thailand, particularly in Chiang Mai Province.

Four of the fly species collected as larvae, i.e., *P. casei*, *M. scalaris*, *H. spinigera*, and *Sargus* sp., were in accordance with those found in mummified corpses (Méglin 1894; Reed 1958; Erzinçlioglu 1983, 1985; Smith 1986; Lord 1990; Goff and Flynn 1991; Greenberg and Wells 1998; Carvalho et al. 2000). Some species, however, were found in a corpse early, e.g., *P. casei* larvae ≈ 35 d postmortem in Hawaii (Goff and Flynn 1991), adult phorids during the first day of traps in Panama (Tanaka 1993) and Thailand (K.S., unpublished data). The early presence of phorids may be due to such factors as the warmer, humid tropical conditions that support higher population levels of these particular flies (Greenberg and Wells 1998), thus increasing the probability of finding larvae in a corpse.

Morphologies of flies in the genus *Hydrotaea* are extremely similar to those occurring in the genus *Ophyra*. According to Pont (1989, 1991), *Ophyra* cannot be maintained as a distinct genus when the world fauna is taken into consideration. For this reason, the

Table 1. Monthly average minimum and maximum temperatures for 4 October 1999 to 14 February 2000, in degree celcius for the Doi-Sa-Ket District, Chiang Mai Province, Thailand

Time	Min (range)	Max (range)
Oct 1999	22.0 (19.0–23.4)	30.8 (26.1–34.1)
Nov 1999	19.8 (15.6–22.4)	30.4 (26.3–33.4)
Dec 1999	13.9 (3.8–20.1)	26.4 (20.4–31.4)
Jan 2000	15.2 (10.3–18.1)	31.0 (28.2–33.2)
Feb 2000	14.1 (12.5–15.8)	31.1 (26.8–36.4)

Source: Northern Regional Meteorological Center, Chiang Mai Province, north Thailand.

species of *Ophyra* has been transferred to *Hydrotaea*. Species of *Ophyra* were recorded as being found after the butyric fermentation phase and during the caseic fermentation phase of decomposition of human corpses; i.e., 3–6 mo PMI (Smith 1986). The presence of *H. spinigera* larvae in the mummified corpse described herein corresponded with the previous reports summarized by Smith (1986); and, thereby, also corroborated other evidence gathered on this particular corpse as to its estimated PMI.

According to Smith (1986), sarcophagids have been reported in the first or second wave of arthropods coming to associate with exposed, decomposing human corpses. In the current case, two species of sarcophagid flies were found in the mummified corpse, giving indications that sarcophagids may occur in human corpses at irregular intervals in tropical regions. Species identification could not be made in the sarcophagid larvae collected in this case. Sarcophagid flies are difficult to identify as larvae (Smith 1986), thus they might be difficult to use in making PMI estimates; a conclusion similar to that reported by Goff and Flynn (1991).

Several species of flies that may be forensically important were collected from a human corpse in Thailand. The entomological evidence, by the nature of the presence of the larvae of *P. casei*, *M. scalaris*, and *H. spinigera* on the corpse, suggested a PMI between 3 and 6 mo. However, the exact time when these fly species occur on a decomposing corpse in tropical conditions such as occur in Thailand is not yet certain. Such factors as higher tropical temperatures and humidity may facilitate more rapid corpse decompositions, shorter life cycles for the flies involved, or greater fly population densities. Thus, more precise entomological information is needed in Thailand for flies that may be of forensic importance. This information includes development rates of such fly species at different given temperature regimens and association behavior of these flies at different stages of corpse decomposition. Such information can then be used to increase accuracy of PMI estimates for conditions that exist most specifically in Thailand.

Acknowledgments

The authors are indebted to Adrian C. Pont of British Museum (United Kingdom) for his suggestions on the current taxonomic status of *Hydrotaea* (= *Ophyra*) *spinigera*. Thanks are extended to Khankham Chaiwan for the technical assistance. The work was supported by the Faculty of Medicine Endowment Fund of Medical Research, Faculty of Medicine, Chiang Mai University, and the Thailand Research Fund (PDF/45/2543).

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Surface ultrastructure of the third-instar larvae of *Hydrotaea spinigera* Stein (Diptera: Muscidae), a fly species of forensic importance

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ABSTRACT: The surface ultrastructure of third-instar larvae of *Hydrotaea* (= *Ophyra*) *spinigera* Stein was observed with scanning electron microscopy. The number of papillae in each anterior spiracle ranges from 5-7. Abdominal segments 4-12 possess ventral welts. Each posterior spiracular disc bears 3 nearly straight slits, with the lower and middle slits closer together than the middle and upper slits. Although the larvae of *H. spinigera* are generally similar to two other closely-related species in Thailand, *Hydrotaea* (= *Ophyra*) *leucostoma* (Wiedemann) and *Hydrotaea* (= *Ophyra*) *chalcogaster* (Wiedemann), some of the morphological features can help in differentiating these species, thus allowing more accurate identification of species of fly larvae in forensic investigations. *Journal of Vector Ecology* 26 (2):191-195. 2001.

Keyword Index: *Hydrotaea* (= *Ophyra*) *spinigera*, surface ultrastructure, fly larvae, forensic entomology, scanning electron microscopy.

INTRODUCTION

Hydrotaea (= *Ophyra*) *spinigera* Stein is a forensically-important fly species in Thailand. Larvae of this species have been found in exposed human corpses as early as 1 week up to 4 ½ months postmortem in Chiang Mai, the largest province in northern Thailand (K. Sukontason, unpublished data). This fly is known from the Palearctic region of the world (Skidmore 1985) and adults have been collected from many parts of Thailand (Tumrasvin and Shinonaga 1982). Taxonomically, species placed in the genus are extremely close to those in *Hydrotaea* and, according to Dr. Adrian C. Pont (pers. comm.), *Ophyra* cannot be maintained as a distinct genus when the world fauna is taken into consideration. For this reason, the species of *Ophyra* have been transferred to *Hydrotaea* (Pont 1989, 1991a,b). Some morphological features of *Hydrotaea* (s.s.) larvae have been illustrated by Skidmore (1985). Our report presents a detailed description of the third-instar larva of *H. spinigera* using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Third-instar larvae of *H. spinigera* were collected from two male human corpses discovered on April 6, 2000, in a forest near the railroad of the Mae Tang District in the Chiang Mai province of northern Thailand. The site where the bodies were found is approximately 35 miles from downtown Chiang Mai. Both male decedents had been tied together with rope and burned. The Crow-Glassman Scale (CGS), which is used to describe the extent of burn injury to human remains (Glassman and Crow 1996) was determined to be level #4 for the bodies in this case. The remains were transferred to the Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, for further investigation. Based on the presence of third-instar larvae of several calliphorid species and the effects of temperature on development of those species during the beginning of April in Chiang Mai, the postmortem interval (or amount of elapsed time since death) was determined to be approximately 7 days.

Larvae collected from the remains were killed by placing them in hot water (~70 °C) and then fixed with 2.5% glutaraldehyde at 4 °C for 24 h. The fixed larvae were then postfixed with 1% osmium tetroxide and with

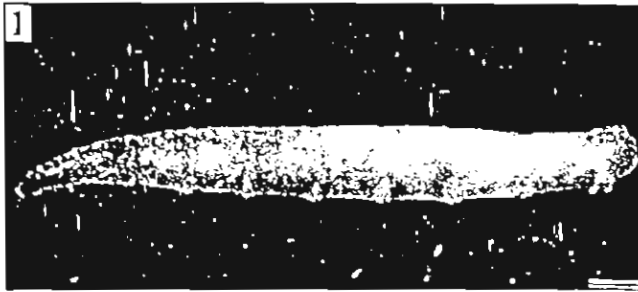


Figure 1. Third-instar larva of *Hydrotaea* (=Ophyra) *spinigera*, lateral view. Bar = 1 mm.

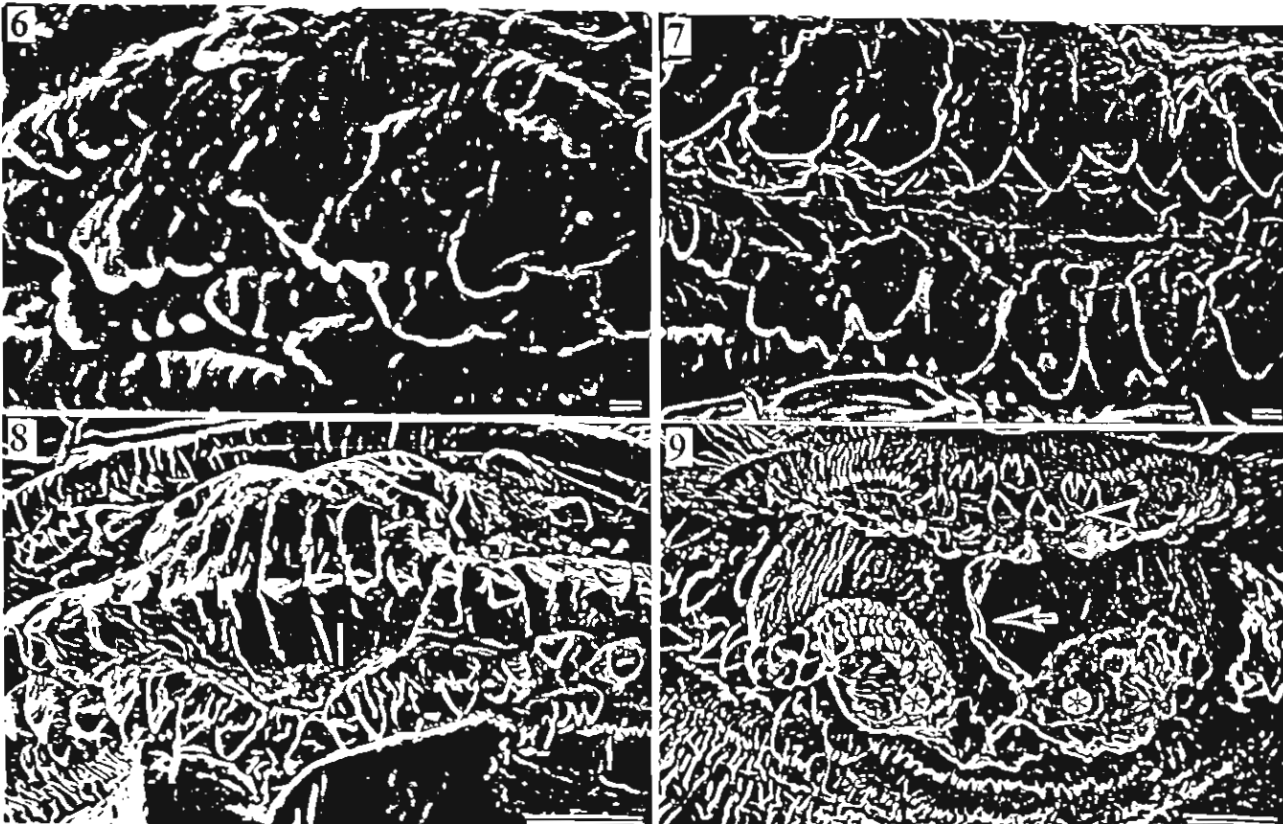
gold, and viewed with a JEOL-JSM840A scanning electron microscope (SEM; Tokyo, Japan) at an accelerating voltage of 20 kV. Photomicrographs were made using Kodak® Verichrome Panchromatic film VP 200 (New York, USA).

RESULTS AND DISCUSSION

The 3rd-instar larva of *H. spinigera* (Figure 1) is muscoid-shaped and more slender than calliphorid larvae. Body size ranges from ~1.3-1.6 cm in length and is creamy white in color. The base of the cephalic segment can be inverted into the prothoracic segment. A pair of antennae, designated as dorsal organs on *Musca domestica* L. (Muscidae) in an electron microscopy study conducted by Chu-Wang and Axtell (1971), are located dorsolaterally on the cephalic segment. Results obtained from use of a transmission electron microscope (TEM) by Chu-Wang and Axtell (1971) indicated that this dome-shaped structure was most likely involved with contact, olfactory, and mechanical stimuli. This is probably true with *H. spinigera* as well. Located ventral to the antenna is the maxillary palp complex or terminal organ (Figures 2 and 3). Prominent papillary sensillae were found on each maxillary palp complex. Four



Figures 2-5. Scanning electron micrographs of morphological features of the 3rd-instar larva of *Hydrotaea* (=Ophyra) *spinigera*. (2) Ventral view of anterior end of the larva illustrating features of the cephalic segment. Bar = 100 μ m. (3) Left maxillary palp complex in apical view showing 6 papillary sensillae (arrowheads). Bar = 1 μ m. (4) Ventral view of oral grooves showing the trace of a groove. Bar = 10 μ m. (5) Anterior spiracle in ventral view showing 6 papillae arranged in a single row. Bar = 10 μ m. A, antenna (or dorsal organ); G, groove; MO, mouth opening; MPC, maxillary palp complex (or terminal organ).



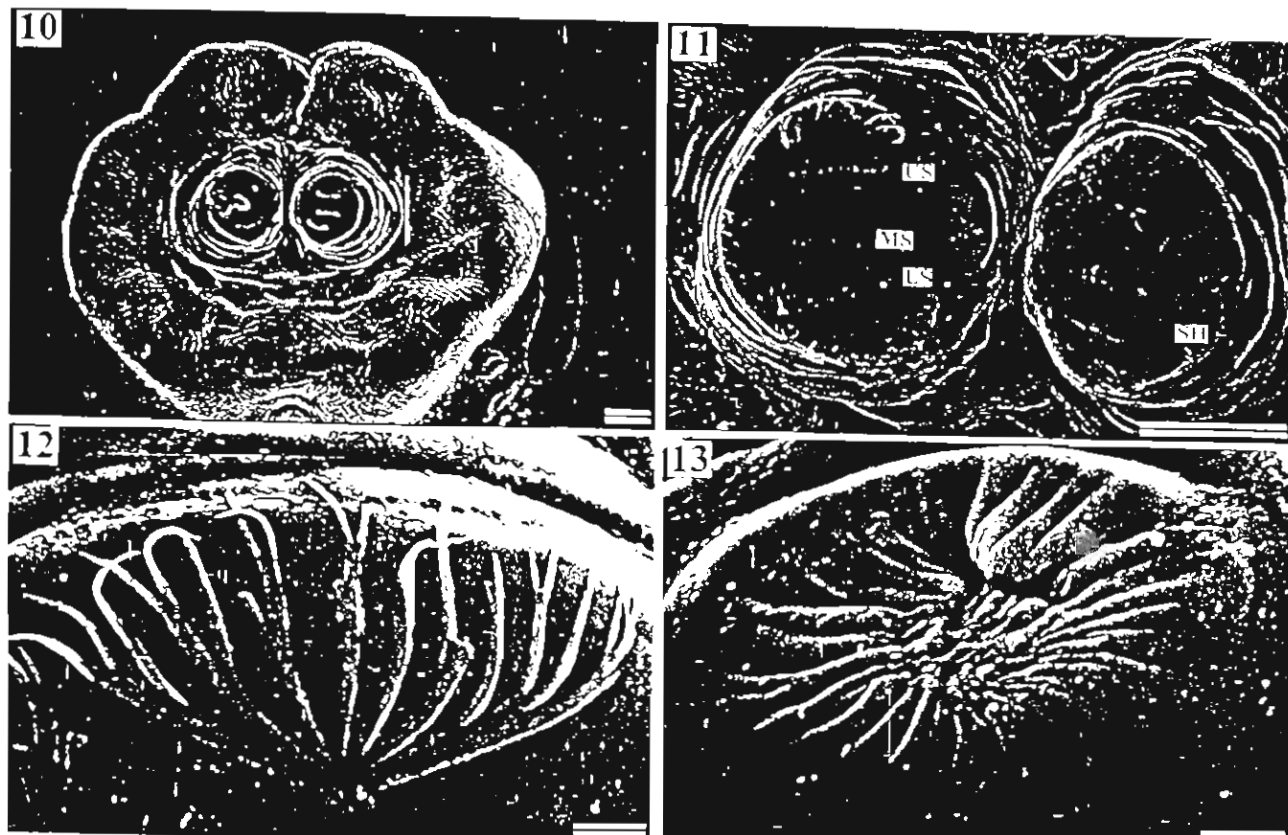
Figures 6-9. Scanning electron micrographs of the creeping welts of 3rd-instar larva of *Hydrotaea* (= *Ophyra*) *spinigera* in ventral view. (6) Welt of the 4th abdominal segment. Bar = 10 µm. (7) Welt of the 5th abdominal segment. Bar = 10 µm. (8) Welt of the 7th abdominal segment. Bar = 100 µm. (9) Welt of the 12th abdominal segment (arrowhead) and anal region showing anal opening (arrow), subanal papillae (asterisk). Bar = 100 µm.

papillary sensillae were clustered in a group apically, whereas the other two papillary sensillae were located laterobasally on the maxillary palp complex. On the basis of ultrastructural evidence from *M. domestica*, the papillary sensillae may function in both chemo- and mechano-reception by contact (Chu-Wang and Axtell 1972). In general, the morphology of the antenna and maxillary palp complex of *H. spinigera* is similar to those observed in other fly species such as *M. domestica* (Muscidae) (Chu-Wang and Axtell 1971), *Oxysarcodexia thornax* (Walker) (Sarcophagidae) (Lopes and Leite 1986), *Oxysarcodexia confusa* Lopes (Sarcophagidae) (Lopes and Leite 1987), *Oxyvinia excisa* (Lopes) (Sarcophagidae) (Leite and Lopes 1987), *Cochliomyia hominivorax* (Coquerel) (Calliphoridae) (Leite and Guevara 1993), *Gasterophilus nasalis* (L.) (Oestridae) (Leite and Scott 1999) and *Piophilus casei* L. (Phoridae) (Sukontason et al. unpublished data).

The mouth opening lies mid-ventrally between the transversely-oriented trace grooves (Greenberg 1973) (Figure 4). This groove has been referred to by several

names, including oral groove (Skidmore 1985, Baker 1987), oral ridge (de Filippis and Leite 1997) and facial mask in flies of the family Sepsidae (Meier 1996). No taxonomically distinct structures were found in the mouth region. The anterior spiracles are located on each latero-posterior edge of the prothorax (Figure 5). The number of papillae in each anterior spiracle ranges from 5-7, with 6 papillae being the most common (48%; 12/25), followed by 7 (28%; 7/25), and 5 (24%; 6/25). All papillae are arranged in a single row. This number of papillae is taxonomically important when trying to distinguish third-instar larva of *H. spinigera* from those of the closely related species, *Hydrotaea* (= *Ophyra*) *leucostoma* (Wiedemann), which have only 4 papillae in each anterior spiracle (Smith 1986).

Overall, the body of *H. spinigera* has a smooth integument devoid of hairs or spines, but some of the abdominal segments possess creeping welts on their ventral surfaces that aid in locomotion of the larva. The creeping welts first appear on the 4th abdominal segment, are not well-developed, and are composed of simple



Figures 10-13. Scanning electron micrographs of the caudal end of the 3rd-instar larva of *Hydrotaea* (= *Ophyra*) *spinigera*. (10) Posterior view of caudal segment with posterior spiracular discs each bearing 3 nearly straight slits. Bar = 100 μ m. (11) Posterior spiracular discs on raised prominences. Branched spiracular hairs are located peripherally around the 3 slits. Bar = 100 μ m. (12) Spiracular hair containing secondary or tertiary branches. Bar = 10 μ m. (13) Button or ecdysial scar. Bar = 10 μ m. LS, lower slit; MS, middle slit; SH, spiracular hair; US, upper slit.

overlapping curled plates (Figure 6). Welts of the 5-11th abdominal segments are well-developed as two rows of closed projections separated by a deep groove between them (Figures 7, 8). The posterior row of projections is strongly bent. The creeping welt of the last abdominal segment has many rows of unequal projections. The anal region is densely spiculate and subanal papillae are conspicuous (Figure 9).

The caudal segment is abruptly truncated. A deep sagittal groove is present in the swollen dorsal region (Figure 10). A pair of posterior spiracular discs are located upon thick, conical prominences of the caudal segment and are tilted mesally. Each spiracular disc bears 3 nearly straight slits with the upper and lower slits slightly convergent to each other (Figure 11). The lower and middle spiracular slits are arranged closer to each other than the middle and upper slits. This arrangement of the spiracular slits of *H. spinigera* is distinctive and can be used to separate this species from larvae of *Hydrotaea* (= *Ophyra*) *aenescens* (Wiedemann),

Hydrotaea (= *Ophyra*) *chalcogaster* (Wiedemann) and *Hydrotaea* (= *Ophyra*) *capensis* (Wiedemann) (Skidmore 1985). Arranged around the lateral periphery of each spiracular disc are the multi-branched spiracular hairs (Figure 11). Each hair has 6-8 branches from the base, with each branch containing secondary or tertiary branches (Figure 12). A button, or ecdysial scar, is located on the inner edge of the peritreme of each spiracular disc (Figure 11). It appears as a circular depression with radial grooves extending from the edge of its rim toward the center (Figure 13).

Morphological features revealed by SEM provide detailed information to differentiate the third-instar larvae of *H. spinigera* from *H. leucostoma* and *H. chalcogaster*, which are all closely related fly species known to occur in Thailand (Tumrasvin and Shinonaga 1982). Hence, the use of these distinctive features to accurately identify this species of fly is of taxonomic significance. This could help in forensic investigations and estimation of the postmortem interval, especially in

sympatric areas of closely related species.

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May 21, 2002

Dr. Donald R. Barnard, Co-Editor
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Dear Dr. Barnard:

Enclosed please find manuscript submitted for consideration for publication in the *Journal of Medical Entomology*. The paper is entitled "Surface ultrastructure of *Chrysomya rufifacies* (Macquart) larvae (Diptera: Calliphoridae)".

I hope you will find the paper interesting and suitable for publication. It has not been submitted or published elsewhere, and I agree that, if accepted, the copyright will be assigned to the *Journal of Medical Entomology*.

Sincerely yours,

K. Sukontason.

Kabkaew L. Sukontason, Ph.D.

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Sukontason et al.: Surface ultrastructure of *Chrysomya rufifacies* larvae

Surface ultrastructure of *Chrysomya rufifacies* (Macquart) larvae

(Diptera: Calliphoridae)

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ABSTRACT The surface ultrastructure of all larval instars of *Chrysomya rufifacies* (Macquart) is described by means of scanning electron microscopy (SEM). Morphological changes were greatest from the first to the second-instar, but less from the second to the third-instar. Most of these changes involved the structure of the anterior spiracle, posterior spiracle, integument of the body and mouthhooks. Modification of the mouthhooks, especially in the third-instar, are helpful in explaining the ferocious feeding ability of the older maggots. The common name of "hairy-maggot" for *C. rufifacies* is only appropriate for the second and third-instars due to their elongated tubercles along the body, while this name is not descriptive of the first-instars that lack tubercles.

KEY WORDS *Chrysomya rufifacies*, larval morphology, ultrastructure, scanning electron microscopy, forensic entomology

Chrysomya rufifacies (Macquart) is one of the most forensically-important fly species worldwide. Larvae of this species have been reported in association with human corpses in several case situations (Smith, 1986; Gunatilake and Goff, 1989; Lee, 1989; Lord, 1990; Goff and Flynn, 1991). Not only have specimens of *C. rufifacies* been used to estimate the postmortem interval (PMI) in cases (Goff and Flynn, 1991; Sukontason et al., 2001), but also in detecting the organophosphate poisoning in a putrefying body through larval analysis (Gunatilake and Goff, 1989). In Thailand, *C. rufifacies* was the primary species of fly found at death scenes involving exposed, burned, hanging or floating corpses (Sukontason et al., 2001; unpublished data). The types of environment where corpses were found were quite varied, including mountainous area as high as ≈ 1500 m above sea level and forested, urban and suburban areas in the northern part of the country.

Very few research experiments of importance to the science of forensic entomology have been conducted in Thailand, even though many decomposing bodies are found in the country each year. One of the most important aspects of using insect specimens in forensic investigations is being able to determine the species of insects found inhabiting a corpse. Currently, most taxonomic keys used for the identification of flies are only available for third-instars and adults. Keys for any of the earlier developmental stages of flies such as first-instars or second-instars cannot be found. In cases such as this, rearing to the third-instar or adult stage would be required for identification to species. Problems can also arise in rearing early developmental instars. Sometimes, too few specimens are collected, or specimens are immediately preserved from the body at a death scene. Other times, rearing to later instars is simply unsuccessful. Thus, studies on the morphological structures that are useful for identifying all larval stages of *C. rufifacies* is needed. Research utilizing examination by scanning electron microscopy (SEM) is thus being conducted in order to eventually be able to differentiate each stage of *C. rufifacies* from other species.

Materials and Methods

A colony of *C. rufifacies* was established from numerous third-instar larvae collected from a human corpse that was transferred for forensic investigation to the Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, on 16 April 2000. The decomposed body used as the source of larvae for establishing the colony was that of a male estimated to be 40 years of age who was found in the Muang District of Lampang Province, northern Thailand. The procedure utilized for fly-rearing was a modification of the technique by Haskell (1990), with fresh pork liver being provided as a larval food source.

Thirty larvae of the first, second and third-instar of *C. rufifacies* were ultrastructurally investigated using SEM. They were washed several times using normal saline solution (NSS) to remove any pork liver tissue residue. 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 for post-fixation of the larval tissues. Post-fixation was followed by rinsing twice with PBS and dehydrating with alcohol. To replace the water in larvae with alcohol, larvae were 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. Larvae were then placed in absolute alcohol for 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. In order to view the larvae, they were first attached with double-stick tape to aluminum stubs so that they could be coated with gold in a sputter-coating apparatus before being viewed with a JEOL-JSM840A scanning electron microscope (Japan). Photomicrographs were made using Kodak® Verichrome panchromatic film VP 200 (New York, USA).

The first and second-instars were processed as mentioned above, but third-instars, which were quite large (≈ 1.4 cm), were primarily cut into 3 portions (head, body and caudal regions) before initial chemical treatment. This enabled them to be easily processed as well as examined under SEM.

Results

The first-instar of *C. rufifacies* is muscoid-shaped (Fig. 1). The prominent features of the cephalic region are a pair of dome-shaped dorsal organs (Fig. 2), a pair of terminal organs (Fig. 3) and a pair of mouthhooks (Fig. 4). Two distinct types of sensillae (papillary sensillae and knobbed sensillae) were found on each terminal organ. Two papillary sensillae were located apart from a cluster of sensillae (Fig. 3). The mouthhooks are situated mid-dorsally of the rudimentary transverse oral grooves (Fig. 4). Each mouthhook is expanded in its apical half and contains several rows of pointed margins giving it a multi-hooked appearance (Fig. 5). The anterior spiracle is apparent only as a small depression and is located at the posterior margin of the prothorax (Fig. 6). Approximately eight rows of single-pointed spines are seen between the prothorax and mesothorax (Fig. 7). A pair of tricoid sensillae bearing three setae each is present on the anterior edge of the mesothorax (Fig. 8). The integument of the body is devoid of prominent tubercles (Fig. 9). The anterior and posterior margins of each segment bear a few rows of posteriorly-projecting, single-pointed spines (Fig. 10). A pair of posterior spiracular discs is located in a deep depression in the caudal segment (Fig. 11). The rim of the caudal segment bears small tubercles. Each posterior spiracular disc contains two straight spiracular slits that coalesce ventrally and are interspaced with four bundles of broad and multi-branched spiracular hairs (Fig. 12).

General morphology of the second-instar is similar to that of the first-instar. The body is muscoid-shaped and the dorsal and terminal organs are prominent. However, many distinct

differences from the first-instar can be seen in features of the second-instar. A pair of large, robust mouthhooks with entirely smooth surfaces is present (Figs 13, 14). The labium is comprised of three large, round lobes (Fig. 15). The oral groove area is well developed as an array of ridges between oral grooves used for channelling liquid into the mouth opening (Fig. 16). The prothorax contains many pit sensillae encircling the base of the segment (Fig. 17). The presence of trichoid sensillae is similar to that seen in the first-instar. Intersegmental spines of the second-instar have 2-4 points at their ends compared to the single points of the first-instar (Fig. 18). Another distinctive feature is the morphology of the anterior spiracles. The number of papillae on each anterior spiracle ranges from 9-12, with 10 papillae being the most common (64%; 16/25), followed by 11 or 12 (16%; 4/25), and 9 being the least common (4%; 1/25). All papillae are arranged in a single row with each having a centrally-located, longitudinal slit for gaseous exchange (Fig. 19). Distinct, stout tubercles are found encircling each body segment (Fig. 20), with the tip of each having ≈ 6 robust spines (Fig. 21). The whole body integument is covered with numerous dome-shaped papillae (Fig. 22). The caudal segment bears well-developed tubercles (Fig. 23). The posterior spiracular discs are more developed than in the first-instar, each having two slits that are entirely separated from each other (Fig. 24). The dorsal slit is shorter in length than the ventral one. Posterior spiracular hairs are present as four groups of more highly-branched, thinner structures (Fig. 24) than that seen in the first-instar. A button, or ecdysial scar, is located on the medial edge of the peritreme of each spiracular disc and appears as a circular depression.

In the third-instar, the overall morphological features observed under SEM are similar to that of the second-instar, but some important distinguishing differences still remain. There is a greatly increased body size overall. In the cephalic region, the lateral margins of the mouthhooks are serrated (Figs. 25,26) and the labium is not tri-lobed. The prothorax contains many well developed pit sensillae encircling the base of the segment (Fig. 27) and the anterior

spiracle at the posterior margin (Fig. 28). A very distinct ultrastructure of the surface integument is present in the third-instar and is covered with several net-like patches (Fig. 29). Each patch is comprised of many denticles, with a single, much larger denticle being more or less centrally located. The elongated tubercles encircling body segments are located along the body and are each slender in shape, with a tip having ≈ 3 circular rows of spines apically (Fig. 30). The stalk of each tubercle has ≈ 3 oblique rows of encircling small knobs. The six peripheral tubercles of the caudal segment (inner dorsal, median dorsal, outer dorsal, outer ventral, median ventral and inner ventral tubercles) are extremely well developed (Fig. 31). Each posterior spiracular disc contains three straight slits and a relatively thick peritreme (Fig. 32).

Discussion

Morphological studies of the immature stages of flies are mandatory for ultimate use in forensic investigations since morphological features are the primary means utilized for species identification of specimens collected from a corpse. The SEM observations of the larvae of *C. rufifacies* revealed in this study provide much detail of the morphology of this species, such as was seen in other species in previous works (Kitching, 1976; Lopes and Leite, 1986; Baker, 1987; Lopes and Leite, 1987; Leite and Lopes, 1987; Iwasa and Hori, 1990; Aspoas, 1991; Leite and Guevara, 1993; Wells et al., 1999; Colwell and O'Connor, 2000). Intricate details provided by this method should help in differentiating species of fly larvae having extremely similar appearances, such as other calliphorid species (Kitching, 1976; Liu and Greenberg, 1989; Iwasa and Hori, 1990; Wells et al., 1999), and sarcophagids (Leite and Lopes, 1987; Lopes and Leite, 1987; Aspoas, 1991).

Prior to this study, some features of the third-instar *C. rufifacies* had been described using SEM (Kitching, 1976; Wells et al., 1999). The present SEM study encompasses all

larval instars and has demonstrated that the metamorphosis of this species of fly larva yielded not only larger sizes with age, but also distinct transformation of many structures of the body. Morphological changes were greatest from the first to the second-instar, but less from the second to the third-instar. These changes are similar to those that occur in larval development of other blowfly species such as *Chrysomya bezziana* Villeneuve and *Cochliomyia hominivorax* (Coquerel) (Kitching, 1976; Leite and Guevara, 1993). One of the biggest changes was in the anterior spiracle. It appeared as only a minute depression in the first-instar, but transformed into a large, fan-shaped structure bearing several papillae in the second and third-instars. The posterior spiracular discs became more developed in later instars as well. The first-instar of *C. rufifacies* or *C. hominivorax* has two spiracular slits in each posterior spiracular disc, but they coalesce ventrally and are interspaced with relatively broad spiracular hairs (Leite and Guevara, 1993). Separated spiracular slits with much thinner branches of the spiracular hairs are observed in the second and third-instars. The increased development in structures of the respiratory system of *C. rufifacies* larvae seems to be conducive to their way of life, such as in causing myiasis. A similar point involving *C. bezziana* was discussed by Kitching (1976).

The other obvious structural change from the first to second and third-instars is in the integument of the body. The common name of “hairy maggot” for *C. rufifacies* is quite appropriate for the second and third-instars due to their possession of elongated tubercles all along the body, which give them a “hairy” appearance. On the contrary, the name is not descriptive of the first-instars that lack these tubercles. The “hairy larvae” are also mentioned in discussing the third-instar of *Chrysomya varipes* (Macquart) by Kitching (1976), but prominent papillae appear only on the dorsal half of body.

This SEM study not only provides details of the morphology of larvae, but also may help in explaining some of their behavior. For example, the feeding ability of *C. rufifacies*

larvae may now be explained from results of this study. The omnivorous feeding behavior, including cannibalism and predation by *C. rufifacies* larvae, has been recorded and reviewed by Goodbrod and Goff (1990) and Baumgartner (1993). The current study reveals the serrate structure of the lateral margins of the mouthhooks of third-instars that is likely to support this type of ferocious feeding. Laboratory experiments by Wells and Greenberg (1992) indicated that second-instars were less inclined than third-instars to serve as predators in the Nearctic region. This may be explained by the lack of serrate lateral margins of the mouthhooks in second-instar *C. rufifacies* larvae.

When a human corpse is discovered, flies inhabiting the body may be found in any stage of development. The accurate identification of these fly specimens to the species level would be beneficial in forensic investigations. Results of this morphological study of *C. rufifacies* using SEM observation will be useful in identification of this species in future forensic cases involving association of this species with a corpse. This information will be especially helpful in areas falling within the geographical distribution of *C. rufifacies*.

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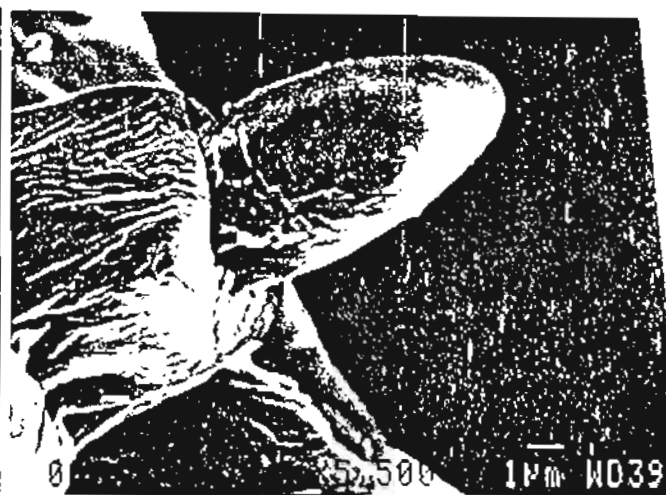
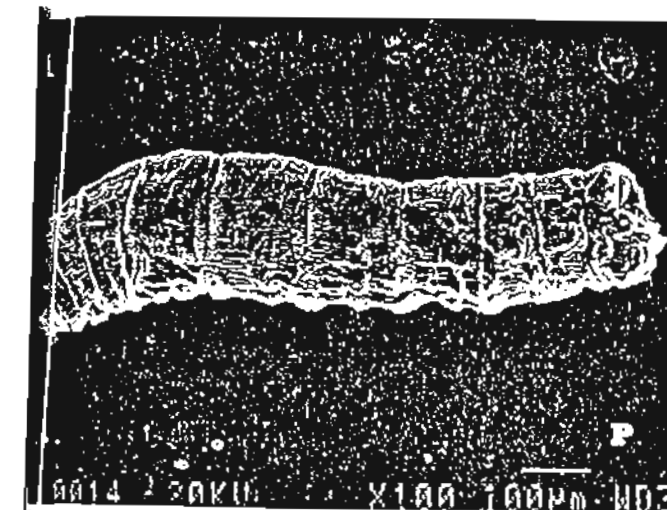
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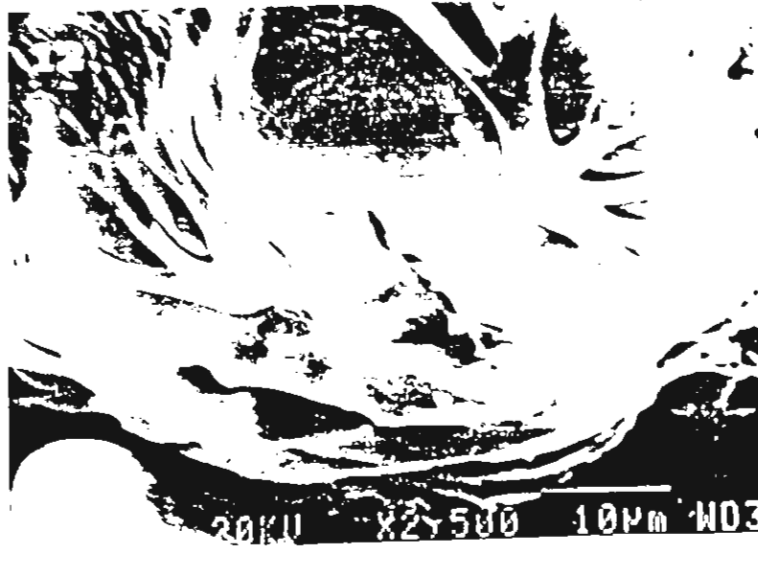
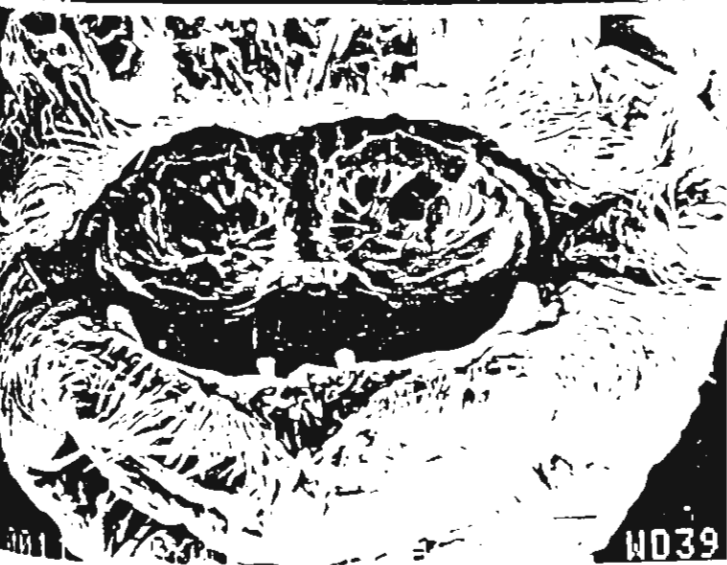
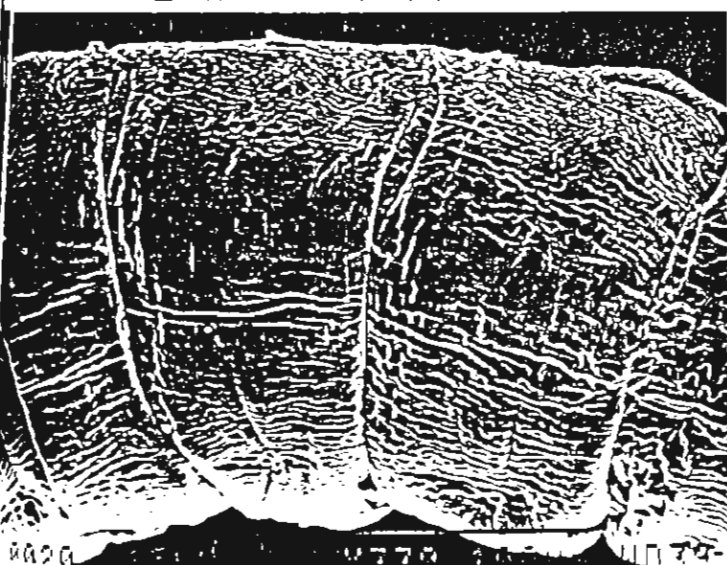
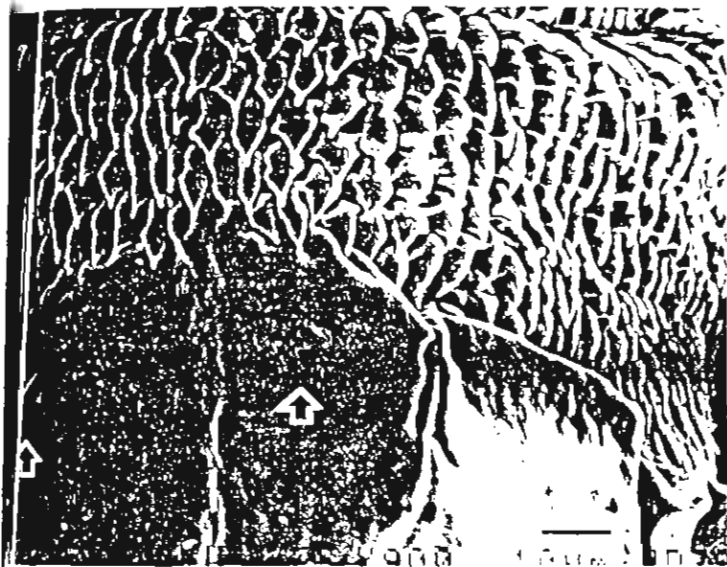
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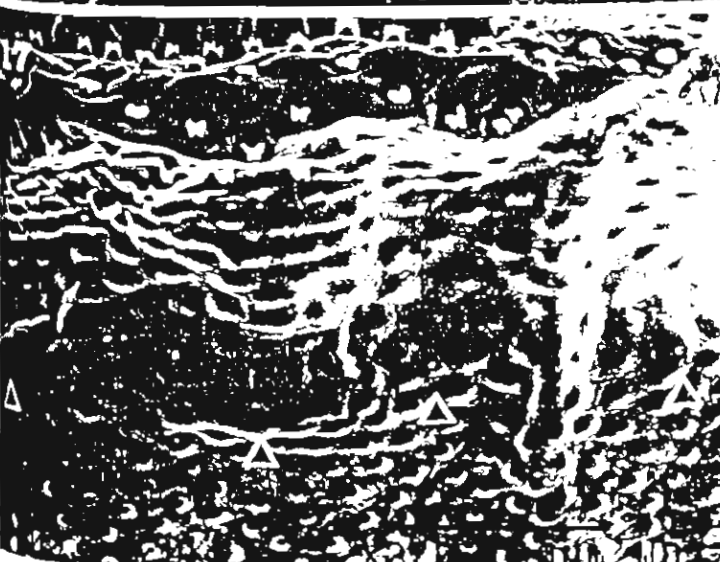
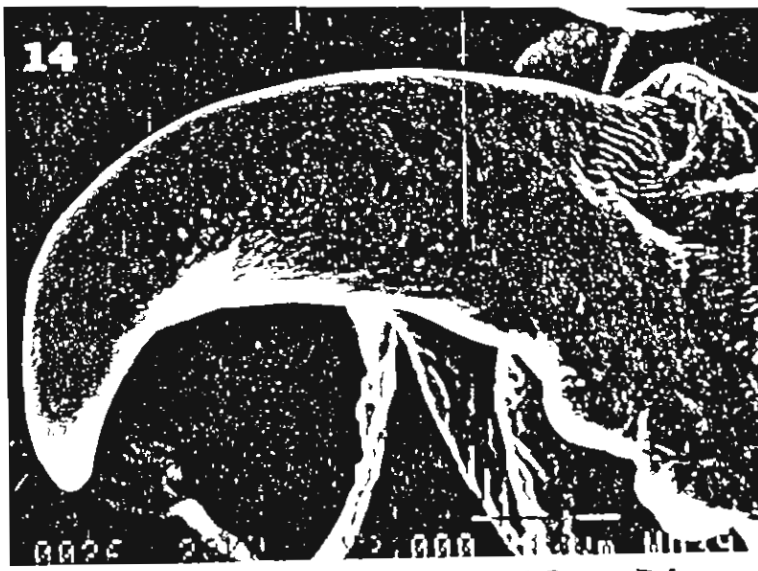
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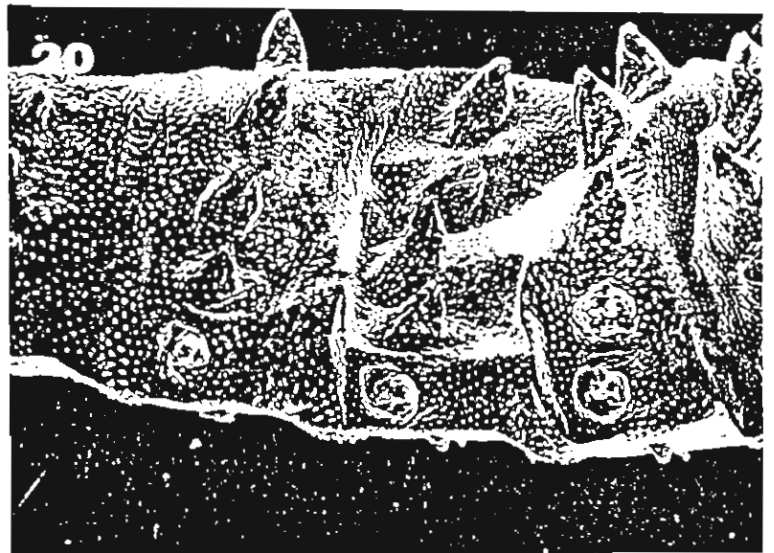
Figs 1-6. SEM micrographs of first-instar *C. rufifacies*. (1) Lateral view of whole larva. Anterior end (A) at left, posterior end (P) at right. (2) Dome-shaped dorsal organ. (3) Terminal organ indicating papillae. (4) Anterolateral view of cephalic segment showing terminal organ (TO), oral groove (OG) and the pair of mouthhooks (MH). (5) Mouthhooks with several rows of spines in their apical halves. (6) Anterior spiracle apparent as a small depression.



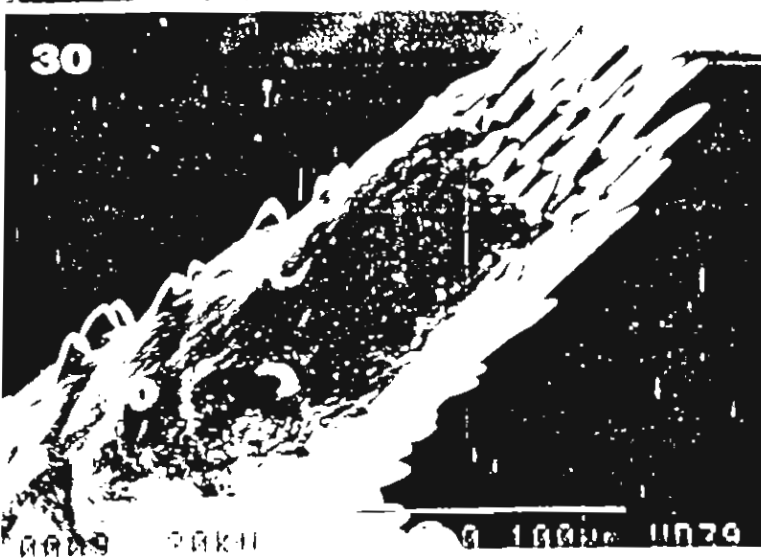
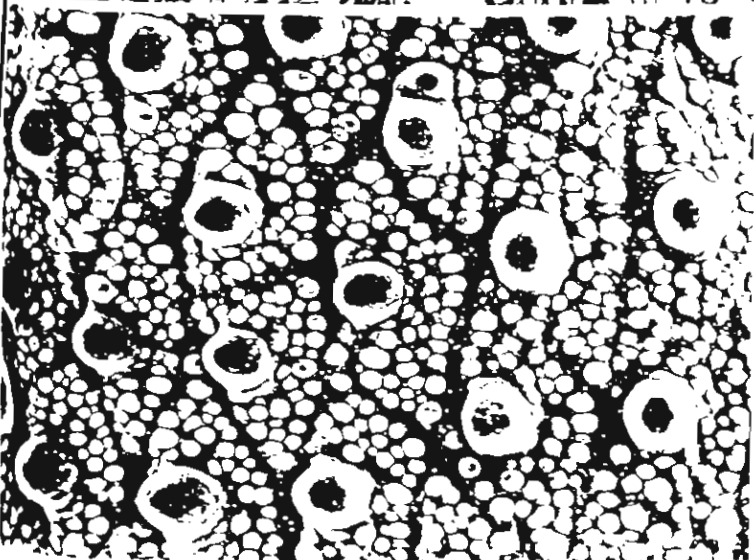
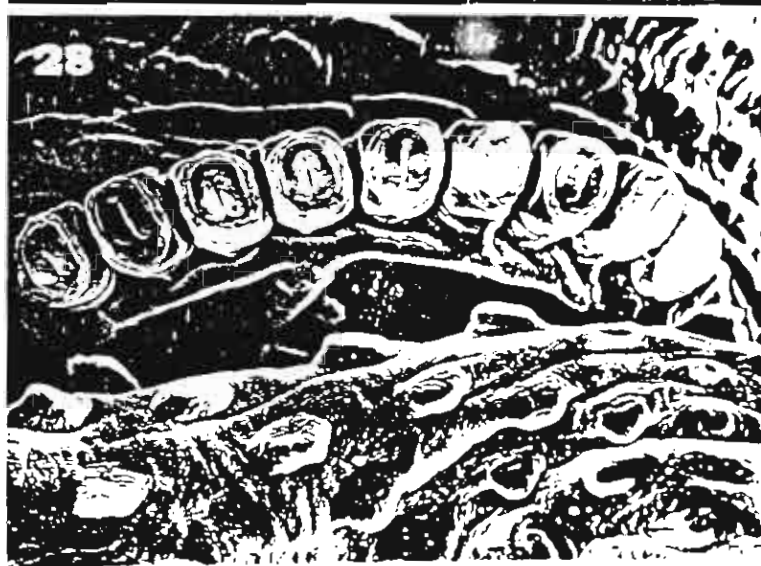
Figs 7-12. SEM micrographs of first-instar *C. rufifacies*. (7) Single-pointed intersegmental spines between pro-and mesothorax. Arrows indicate pit sensillae. (8) A pair of trichoid sensillae (arrowheads) bearing 3 setae on anterior edge of mesothorax. (9) Integument of body. (10) Posteriorly-projecting, single-pointed intersegmental spines. (11) Posterior view of larva showing the pair of posterior spiracular discs (PSD), each having two slits coalescing ventrally. (12) Posterior spiracular disc bearing two slits (S) interspaced with spiracular hairs (PSH).



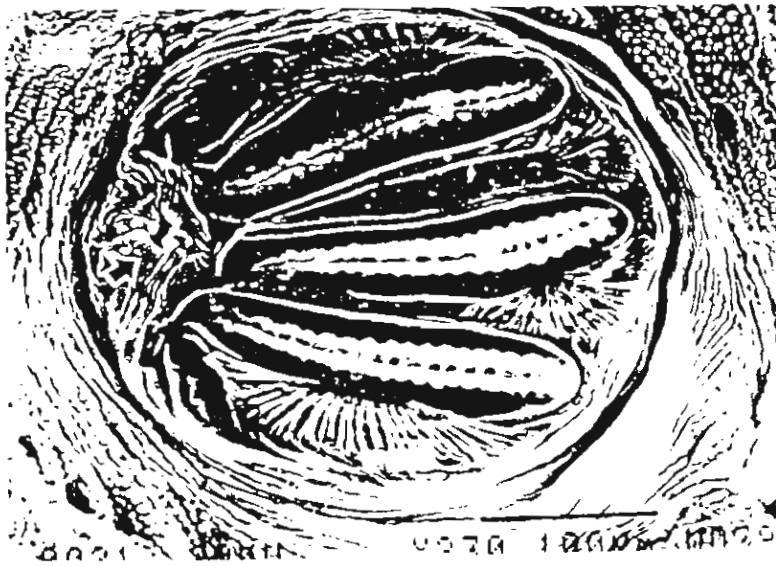
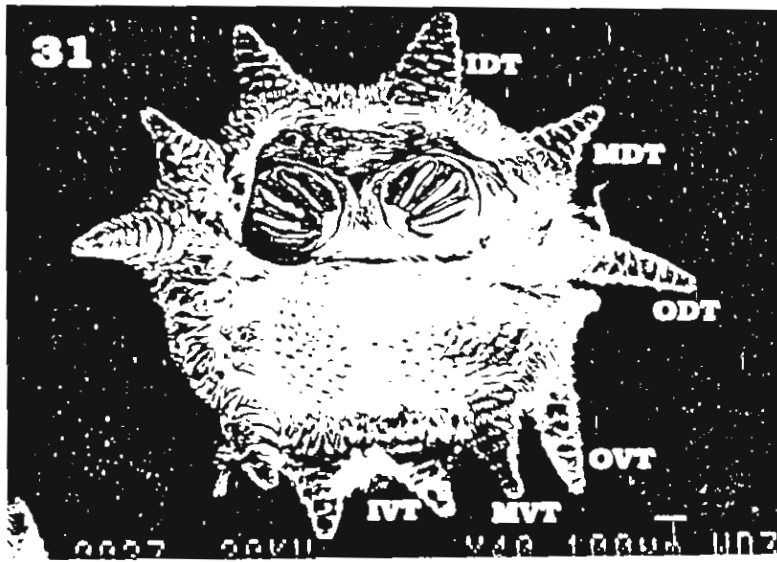
Figs 13-18. SEM micrographs of second-instar *C. rufifacies*. (13) Anterolateral view of cephalic segment showing terminal organ (TO), ventral organ (VO), oral grooves (OG), the pair of prominent mouthhooks (MH) and labium (L). (14) Mouthhook at higher magnification. (15) Labium showing three rounded lobes. (16) Oral grooves at higher magnification. (17) Integument of body. Arrowheads indicate pit sensillae. (18) Intersegmental spines bearing 2-4 points at ends.



Figs 19-24. SEM micrographs of second-instar *C. rufifacies*. (19) Anterior spiracle showing 10 papillae in a single row. (20) Elongated tubercles seen around each body segment. (21) Tubercle of body segment showing stout base and tip bearing ≈ 6 short spines. (22) Integument at higher magnification showing numerous dome-shaped papillae. (23) Posterior view of larva showing the pair of posterior spiracular discs, each having two slits entirely separated. Six pairs of well-developed peripheral tubercles are present. (24) Posterior spiracular disc bearing two slits (S) interspaced with fine-branched of spiracular hairs (PSH). Arrow indicates button.



Figs 25-30. SEM micrographs of third-instar *C. rufifacies*. (25) Anterolateral view of cephalic segment showing dorsal organ (DO), terminal organ (TO), ventral organ (VO), mouthhook (MH) and oral grooves (OG). (26) Mouthhook at higher magnification showing serrate lateral margin. (27) Prothorax showing many pit sensillae (PS) and a pair of trichoid sensillae (arrowhead). (28) Anterior spiracle bearing 10 papillae. (29) Integument of body showing distinct net-like patches. (30) Elongated tubercle of the body showing slender shape and three rows of sharp apical spines. Small knobs are encircling base of tubercle.



Figs 31,32. SEM micrographs of third-instar *C. rufifacies*. (31) Posterior view of larva showing the pair of posterior spiracles, each containing three distinct slits. Six pairs of well-developed peripheral tubercles are present: inner dorsal tubercle (IDT), median dorsal tubercle (MDT), outer dorsal tubercle (ODT), outer ventral tubercle (OVT), median ventral tubercle (MVT) and inner ventral tubercle (IVT). (32) Posterior spiracular disc bearing three slits (S) interspaced with fine-branched spiracular hairs (PSH). Button (arrow) appears as a small hole in medial portion of disc.

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Research Note

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Survey of forensically-relevant fly species in Chiang Mai, northern Thailand

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ABSTRACT: A survey of forensically-relevant fly species was conducted during the summer of the year 2000 in six mountainous areas around downtown Chiang Mai, northern Thailand. Fly species collected in the survey represent at least 12 genera in 4 families of Diptera: Calliphoridae [*Chrysomya megacephala* (Fabricius), *Chrysomya rufifacies* (Macquart), *Hypopygiopsis infumata* (Bigot), *Hemipyrellia ligurriens* (Wiedemann), *Hemipyrellia pulchra* (Wiedemann), *Lucilia papuensis* Macquart and *Lucilia porphyria* (Walker)], Muscidae [*Musca ventrosa* Wiedemann, *Musca sorbens* Wiedemann, *Hydrotaea* sp. and *Atherigona* sp.], Sarcophagidae [*Parasarcophaga misera* (Walker), *Parasarcophaga* sp., *Boettcherisca peregrina* (Robineau-Desvoidy), *Sarcosolomonina harinasutai* Kano et Sooksri and *Pierretia* sp.], and an unidentified species of Platystomatidae. *Chrysomya megacephala* was the predominant species in all collections (74.62%), while *C. rufifacies* ranked second in abundance of collection (19.57%). Other species collected were found in much smaller numbers (0.04 – 0.91%). Ecological factors at the time of collection, such as season, elevation and location, affected the different species and number of flies collected in the survey.

Keyword Index: insect survey, forensic entomology, flies, mountain, Thailand

Aside from being insects of medical and veterinary importance, flies of the families Calliphoridae, Muscidae and Sarcophagidae are currently associated with forensic investigations. Various stages of flies found in human corpses have been used in determining postmortem interval (PMI), determining manner of death, supporting drug-related death investigations, and revealing movement of a corpse from one locality to another (Smith 1986, Lee 1989, Lord 1990, Goff and Flynn 1991, Catts 1992, Greenberg and Wells 1998, Introna et al. 1998, Anderson 1999, Carvalho et al. 2000). Regarding such investigations as involving movement of a corpse, information of indigenous insect fauna is needed in all habitats in order to determine whether a corpse has been moved. Evidence of relocation of a corpse can be revealed by identifying insect species from the corpse that are foreign to the area where the body was discovered.

In Thailand, there are many corpses found in various stages of decay each year. Although many surveys of medically-important flies have been reported in this country (Sucharit et al. 1976, Tumrasvin et al. 1978, 1979, Tumrasvin and Shinonaga 1978, 1982, Tumrasvin and Kano 1979, Sucharit and Tumrasvin 1981), no research of forensically-important flies has been carried out. Therefore, the objective of this study was to conduct the first survey of forensically-relevant fly species in mountainous areas of Chiang Mai province, northern Thailand, during summer conditions in the year 2000. This information will provide baseline data that can be further used for forensic investigations in Thailand when fly specimens are present in a corpse.

This survey was carried out at six mountainous sites in Chiang Mai province (17-21 °N and 98-99 °E), representing different areas around downtown Chiang Mai (Fig. 1). The date of collections and location of each specific site from downtown Chiang Mai are shown in Table 1.

Two fly traps (30 × 30 × 30 cm) were placed at each site where trees were abounding for 3 h (0900-1200 a.m.). Approximately 300 g of beef liver that was allowed to decompose for 24 h was used as bait for adult flies in each trap. In order to transport traps to the laboratory of the Department of Parasitology, Faculty of Medicine, Chiang Mai University, a large plastic bag was used to plug the hole of each trap to prevent flies from escaping. These traps were then placed in a large refrigerator set at 4 °C for 5-10 min to anesthetize the flies. All flies from each specific site were pooled into separate transparent plastic bags, fitted with rubber bands and killed by placing in a freezer at -70 °C for approximately 1 h. The specimens were then dried overnight in an incubator set at 50 °C, counted, sexed and identified using taxonomic keys from Tumrasvin and Shinonaga (1978, 1982), and Tumrasvin et al. (1979).

A total of 2,754 flies were collected in 6 mountainous areas of Chiang Mai province during the summer of 2000 (Table 2). Collection site 1 near the Chiang Mai Zoo was the site where the greatest abundance of flies was collected (84.28%), followed by collection site 5 (7.59%). Fly species collected in the survey represent at least 12 genera in 4 families of Diptera: Calliphoridae [*Chrysomya megacephala* (Fabricius), *Chrysomya rufifacies* (Macquart), *Hypopygiopsis infumata* (Bigot), *Hemipyrellia ligurriens* (Wiedemann), *Hemipyrellia pulchra* (Wiedemann), *Lucilia papuensis* Macquart and *Lucilia porphyrina* (Walker)], Muscidae [*Musca ventrosa* Wiedemann, *Musca sorbens* Wiedemann, *Hydrotaea* sp. and *Atherigona* sp.], Sarcophagidae [*Parasarcophaga misera* (Walker), *Parasarcophaga* sp., *Boettcherisca peregrina* (Robineau-Desvoidy), *Sarcosolomonina harinasutai* Kano et Sooksri, and *Pierretia* sp.], and an unidentified species of Platystomatidae. *Chrysomya megacephala* was the predominant species in all collections (74.62%), while *C. rufifacies* ranked second in abundance of collection (19.57%). Other species collected were found in much smaller numbers (0.04 – 0.91%).

Since *C. megacephala* and *C. rufifacies* were the two most abundant fly species collected, the sex ratio of these species was also determined. Number of females was higher than males in all collections, with the female:male ratio of *C. megacephala* ranging from 2.2-30:1 and in *C. rufifacies*, ranging from 1.5-7.5:1.

Although all of the collections were performed during the summer, the species and numbers of flies collected from the six different areas around downtown Chiang Mai were quite diverse. Ecologically, various environmental factors were undoubtedly involved in establishing this diversity. Altitude at each site of collection is one such factor. Some species from the present study (i.e., *C. megacephala*, *H. infumata*, *H. ligurriens* and *M. ventrosa*) resemble the fauna collected at relatively low altitude (< 500 m above sea level) on Doi Indhanondh Mountain in the southern part of Chiang Mai by Tumarasvin et al. (1978). However, at higher elevations of >2,600 m, blow fly species such as *Calliphora vomitoria* (Linnaeus), *Calliphora pattoni* Aubertin, and *L. porphyrina* could be found. Moreover, different species can still be found during different seasons at the same high altitude.

Other factors pertaining to specific collection sites can be important in determining the species present and their abundance. The site of collection must be considered. Numerous and abundant species of flies were found at collection site 1 near the zoo (Table 2). This reflects various conditions of this particular area that are conducive to the development of several different fly species. These conditions include ample supply of animal feces and/or food from zoo animals and garbage in the zoo. This serves as oviposition substrate and as a source of high quality protein for egg maturation and larval food (Norris 1965).

Since this survey was restricted to collection in the summer of 2000, many species of forensically-relevant flies from other seasons may yet be reported from mountainous areas around downtown Chiang Mai. Martínez-Sánchez et al. (2000) reported that the changing pattern of abundance of calliphorid and muscid flies occurred within, and between years in a

Mediterranean ecosystem; whereas a study by Avancini and Silveira (2000) showed the difference in site preferences of some muscid flies during various hours of daylight (i.e., morning, noon, and late afternoon) in Brazil.

The present study provides accounts of various species of indigenous flies in Chiang Mai province, which may be encountered in future forensic cases. This helps begin establishment of a database that can be used to confirm relocation of a corpse to this particular area. Establishment of the presence of these species in mountainous areas near Chiang Mai is merit for further study on their bionomics so that this information may be used to aid in forensic investigations in this country.

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TABLE 1. Date and location of forensically-relevant fly collections in mountainous areas around downtown Chiang Mai, northern Thailand, during the summer of 2000.

Collection site	Date	Direction of site from downtown
		Chiang Mai ($\approx$ km)
1	March, 14	West (3)
2	March, 24	North-west (8)
3	March, 27	North-east (40)
4	March, 30	South-west (40)
5	April, 5	West (8)
6	May, 1	East (25)

TABLE 2. Species and number of flies collected from 6 mountainous areas around downtown Chiang Mai, northern Thailand, during the summer of 2000.

Species	Collection site						(%)
	1	2	3	4	5	6	
Family Calliphoridae							
<i>Chrysomya megacephala</i>	1,797	76	31	8	133	10	74.62
<i>Chrysomya rufifacies</i>	449	17	8	2	62	1	19.57
<i>Hemipyrellia ligurriens</i>	14	2	1	1	-	-	0.65
<i>Hemipyrellia pulchra</i>	1	1	2	-	-	7	0.40
<i>Hypopygiopsis infumata</i>	6	-	-	-	3	-	0.33
<i>Lucilia papuensis</i>	-	-	-	-	3	10	0.47
<i>Lucilia porphyrina</i>	1	-	-	-	-	-	0.04
Family Muscidae							
<i>Atherigona</i> sp.	23	-	-	-	-	2	0.91
<i>Hydrotaea</i> sp.	3	-	-	2	-	-	0.18
<i>Musca sorbens</i>	-	-	-	-	-	2	0.07
<i>Musca ventrosa</i>	2	5	1	-	-	7	0.54
Family Sarcophagidae							
<i>Boettcherisca peregrina</i>	5	2	2	-	4	2	0.54
<i>Parasarcophaga misera</i>	5	2	1	1	1	6	0.58
<i>Parasarcophaga</i> sp.	5	3	5	-	3	2	0.65
<i>Pierretia</i> sp.	3	-	1	1	-	-	0.18
<i>Sarcosolomonina harinasutai</i>	6	-	-	-	-	-	0.22
							0.04
Family Platystomatidae	1	-	-	-	-	-	
Total	2,321	108	52	15	209	49	2,754
(%)	(84.28)	(3.92)	(1.89)	(0.54)	(7.59)	(1.78)	(100)

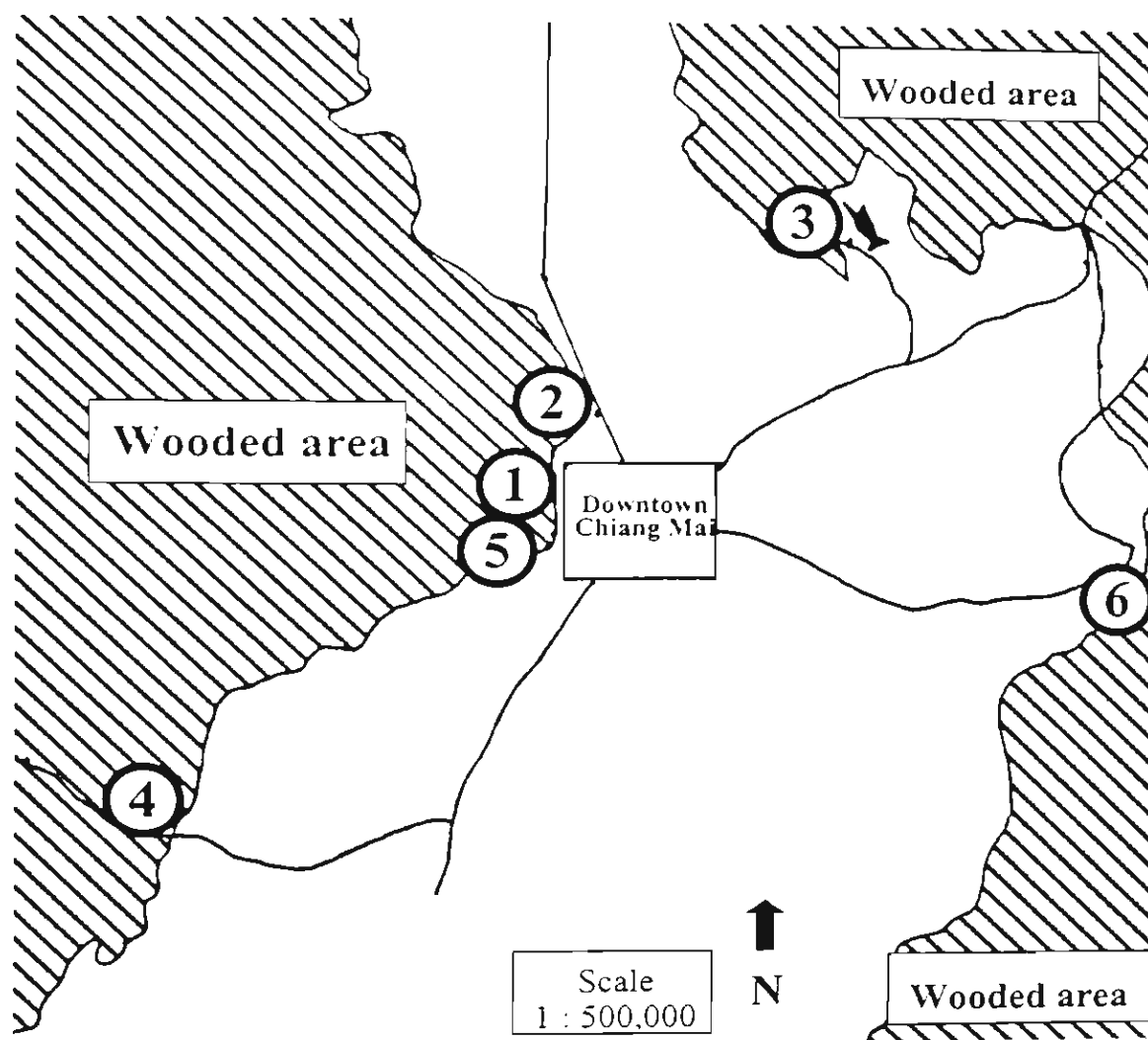


Figure 1. Mountainous areas around downtown Chiang Mai, northern Thailand, surveyed for forensically-relevant flies in the summer of 2000. Numbers in circles correspond to collection sites listed in Table 1.

Seasonal and Spatial Distribution of the Two Most Forensically-Important Fly Species in Thailand

In Thailand, the two most forensically-important fly species are the blow flies *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifacies* (Macquart). This rating is due to the common collection of the larvae of these species from human corpses (Sukontason *et al.*, 2001). Bodies from death scenes investigated in this study ranged from being fresh up to ones in the black putrefaction stage. In addition, the bodies presented in various different situations such as exposed, burned, submerged and hanging. Utilizing elapsed time during larval development under natural ambient temperature in Chiang Mai province, northern Thailand, the postmortem interval (PMI) could often be estimated in cases when these larvae were present.

These two fly species are also of forensic importance worldwide (Smith, 1986; Gunatilake and Goff, 1989; Lord, 1990; Goff and Flynn, 1991). In Thailand, many corpses are found in various stages of decay each year. Although many surveys of medically-important flies have been reported in this country (Sucharit *et al.*, 1976; Sucharit and Tumrasvin, 1981), little research on forensically-important flies has been carried out to date. The purpose of this study was to determine the seasonal fluctuations and geographical distribution of the two most forensically-important fly species in the country in mountainous areas in the central area of Chiang Mai. This information will provide baseline data that can be used in future forensic investigations in Thailand that involve the presence of these fly species in a corpse.

Fly collections were conducted monthly from March 2000 through February 2001 at three mountainous sites in the central area of Chiang Mai ranging from 17-21 ° N and 98-99 ° E (Fig. 1). Site 1 is located close to the Chiang Mai Zoo, situated at foothill of the mountain “Doi Suthep”, Muang District, ≈3 km west of downtown. Numerous types of animals including mammals, reptiles, amphibians, and birds are maintained at the zoo. Site 2 is an area close to the Mae-Kuang Dam, Doi Sa Ket District, ≈40 km northeast of downtown, and site 3 is an area near the railroad that passes through the Mae-On Subdistrict, San Kham Phaeng District, ≈30 km southeast of downtown.

Two fly traps measuring 30 × 30 × 30 cm each were placed at each site in areas with abundant trees for 3 h intervals (0900-1200 a.m.). Approximately 300 g of beef liver that had been allowed to decompose for 24 h was used as bait to lure adult flies into each trap. Traps were in place for all four characteristically different times of the year at each site, resulting in representative collections of flies for each of the seasons in Thailand (summer, early rainy, late rainy, and winter). In order to transport the traps to the laboratory of the Department of Parasitology, Faculty of Medicine, Chiang Mai University for processing, a large plastic bag was used to plug the entrance hole of each trap to prevent flies from escaping. These traps were then placed in a large refrigerator set at 4 °C for 5-10 min to anesthetize the flies. All flies from each specific site were pooled into separate transparent plastic bags fitted with rubber bands and killed by placing in a freezer at -70 °C for approximately 1 h. The specimens were then dried overnight in an incubator set at 50 °C, counted, sexed and identified using taxonomic keys from Tumrasvin and Shinonaga (1978; 1982), Tumrasvin *et al.* (1979), Tumrasvin and Kano (1979) and Kurahashi *et al.* (1997).

A total of 3,754 adult fly specimens representing four families were collected in this study (Table 1). Flies of the family Calliphoridae comprised 95.99 % of the total insects collected. Among them, *Chrysomya megacephala* was the most predominant species collected (78.02%), while *Chrysomya rufifacies* ranked the second most abundant (16.80%). These results deem these two species as the two most forensically-important species in the study sites. The number of females collected was higher than males in all collections. The female:male ratio of *C. megacephala* ranged from 1.5-30 : 1 and in *C. rufifacies*, ranged from 1-52 : 1 (data not shown). In addition to these two species, another five species of blow flies were also found: *Hemipyrellia ligurriens*, *Hemipyrellia pulchra*, *Hypopygiopsis infumata*, *Lucilia papuensis* and *Lucilia porphyryna*. A total of five species of Muscidae were collected: *Atherigona* sp., *Hydrotaea spinigera*, *Musca domestica*, *Musca sorbens* and *Musca ventrosa*. Several species of Sarcophagidae were also captured and collectively represented the lowest number of specimens collected in the study. These included *Boettherisca nathani*, *Boettherisca peregrina*, *Parasarcophaga brevicornis*, *Parasarcophaga dux*, *Parasarcophaga idmais*, *Parasarcophaga misera*, *Parasarcophaga* sp., *Pierretia* sp., *Sarcorohdendorfia inextricata*, *Sarcosolomonina harinasutai* and 17 individual unidentified sarcophagids.

The two most abundant species in this study, *C. megacephala* and *C. rufifacies*, were recorded throughout the year. Their peak occurrence was in the summer followed in prevalence by the late rainy season (Fig 2). As for prevalence at each specific site, 90.15% (3,384/3,754) of the total fly specimens collected was from site 1, followed by 7.27% (273/3,754) from site 2 and 2.58% (97/3,754) from site 3 respectively.

The present study clearly confirms that *C. megacephala* and *C. rufifacies* are the two most forensically-important fly species in Chiang Mai due to their abundance in this area. This is supported by the fact that the larvae of these two species are normally found in cases involving human corpses in several areas of Chiang Mai (Sukontason *et al.*, 2001). The presence of both species is year-round, with their peak abundance occurring in the summer when ambient temperatures are highest. Incidentally, temperature is the primary environmental factor that influences the rate of larval development and accelerates the growth of fly populations in nature.

The species and numbers of flies collected in this study from the three different areas around downtown Chiang Mai represented by the study sites were quite diverse. Ecologically, various environmental factors were undoubtedly involved in establishing this diversity. Altitude at each site of collection is one such factor. Some species from the present study (i.e. *C. megacephala*, *H. infumata*, *H. ligurriens* and *M. ventrosa*) were the same as what was collected at relatively low altitude (< 500 m above sea level) on Doi Indhanondh Mountain in the southern part of Chiang Mai by Tumarasvin *et al.* (1978).

The present study provides accounts of various species of indigenous flies to Chiang Mai that may be encountered in future forensic cases. This information helps begin establishment of a database that can be used for various purposes such as to confirm relocation of a corpse to this particular area. Establishment of the presence of these species in mountainous areas near Chiang Mai justifies further study on their bionomics so that this new information may be used to aid in future forensic investigations in this country.

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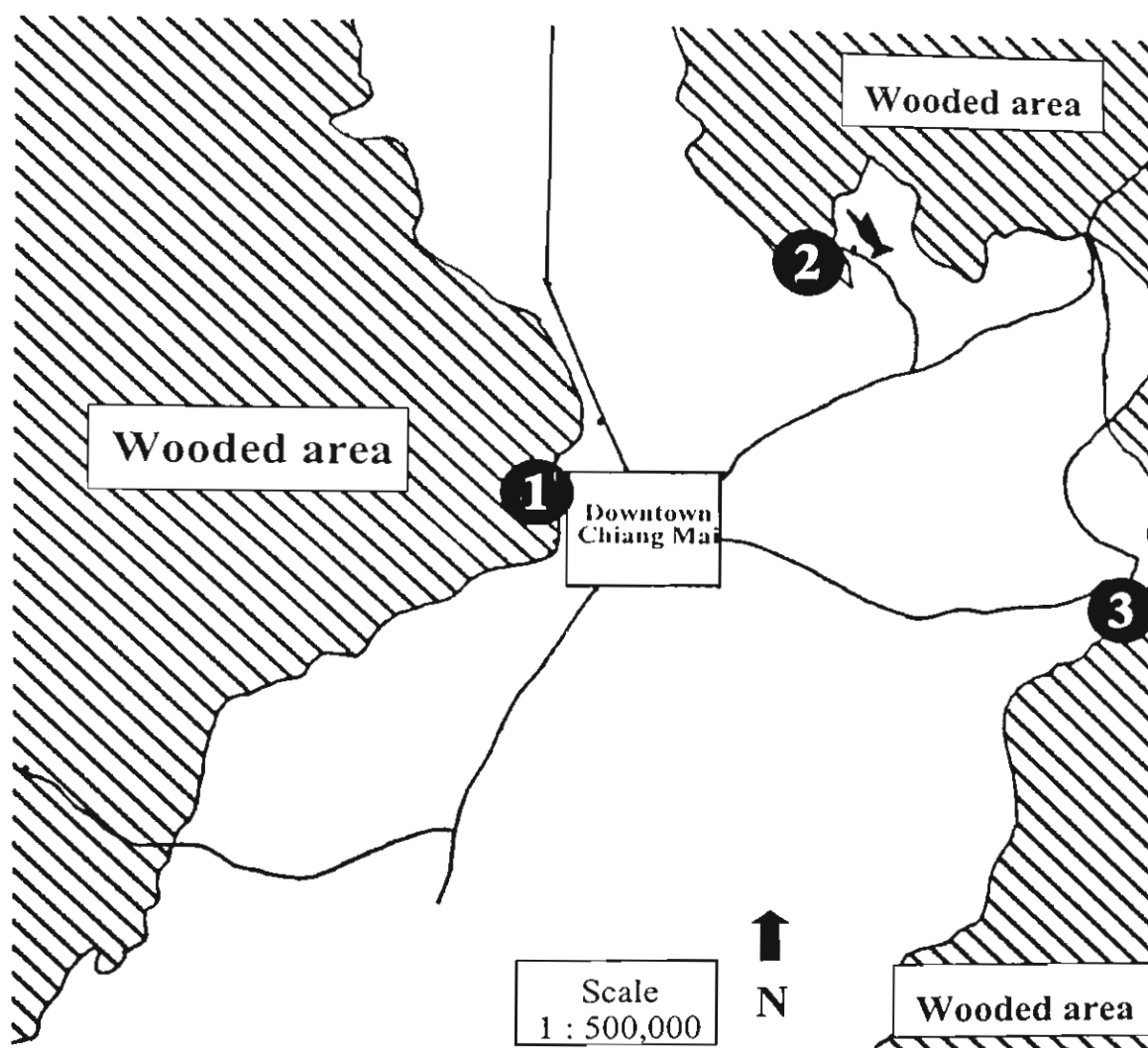


Figure 1. Mountainous sites in the central area of Chiang Mai, northern Thailand, surveyed for the two most forensically-important fly species. Numbers 1, 2 and 3 in black circles indicate site close to the Chiang Mai Zoo of Muang Distract, site close to Mae-Kuang Dam of Doi Sa Ket District and site near the railroad that passes through the Mae-On Subdistrict, San Kham Phaeng District, respectively.



Figure 2. Seasonal abundance of *Chrysomya megacephala* and *Chrysomya rufifacies*, the two most forensically-important fly species in mountainous areas around downtown Chiang Mai, northern Thailand, from March 1999 to February 2000.

Sukontason
Sukontason
Lertthamnongtham
Kuntalue
Tichug
Piangjai
Boonchu

Surface Ultrastructure of the Third-Instar Larvae of *Chrysomya rufifacies* (Diptera: Calliphoridae), A Fly Species of Forensic Importance

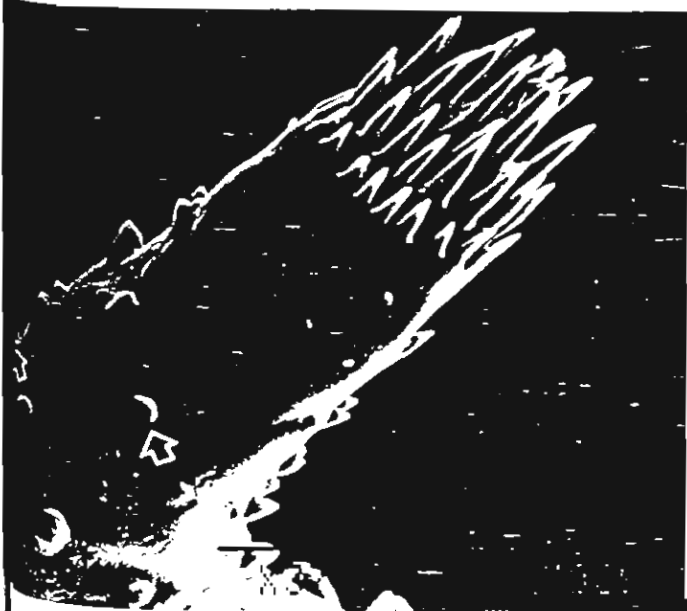
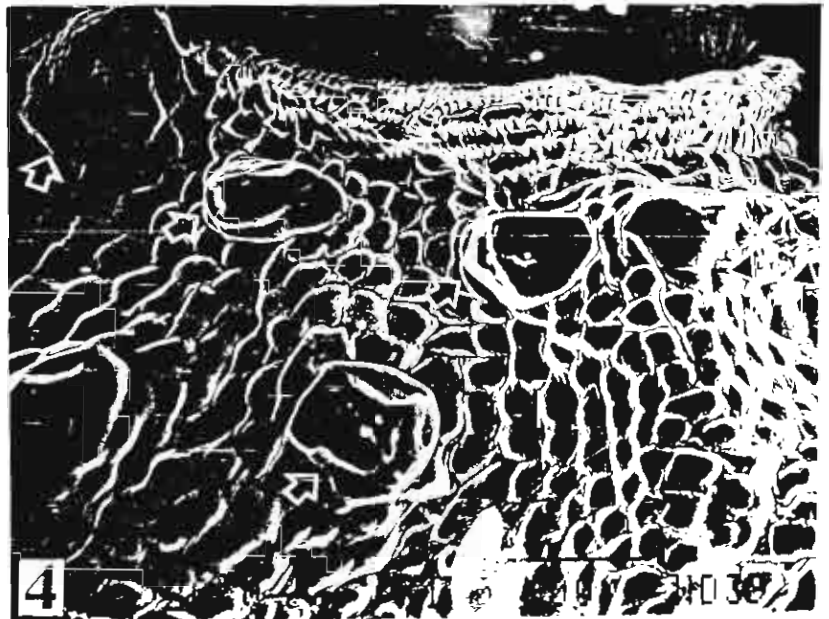
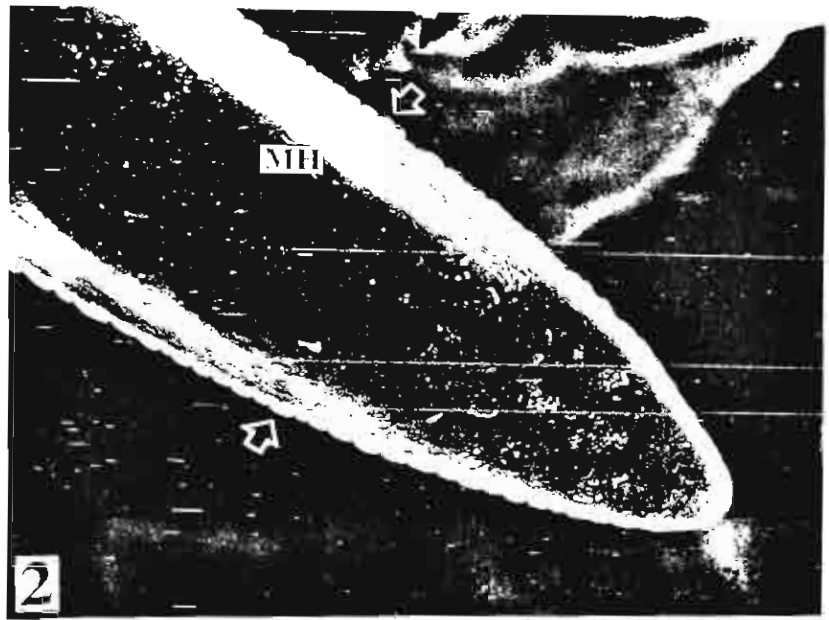
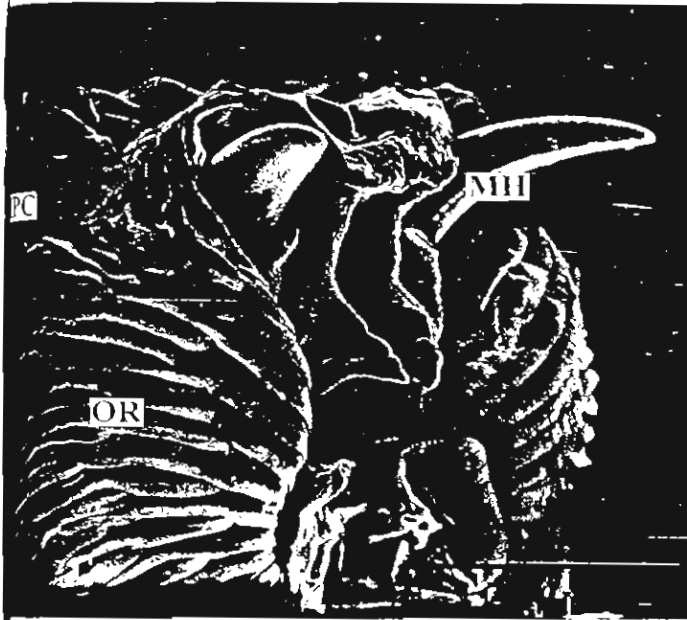
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A scanning electron microscopic study was performed to observe the surface ultrastructure of third-instar larvae of the hairy maggot blowfly, *Chrysomya rufifacies*. The prominent features of cephalic segment are antenna, maxillary palp complex, mouthhook and oral ridge. The dome-shaped antenna is most likely involved with contact, olfactory and mechanical stimuli while the papillary sensillae on each maxillary palp complex may function in both chemo- and mechano-reception by contact. The saw-toothed structure on both lateral margins of mouthhook is capable of supporting predacious and cannibalistic behavior of larvae. The anterior spiracles are located on each latero-posterior edge of the prothorax, with the number of papillae ranging from 9-12. Trichoid and pit sensillae appear on the anterior edge of the prothorax. Whole body integument is covered with numerous dome-shaped sculpturing and composes of rows of conspicuous tubercles having relatively large apical spines and round-shaped knob at base. The last abdominal segment possesses 6 pairs of tubercles and a pair of posterior spiracular discs. Each spiracular disc bears 3 nearly straight slits. Although the third-instar larva of *C. rufifacies* is generally similar to the closely-related species, *C. albiceps*, some of the morphological features can help in differentiating these species, thus allowing more accurate identification of species of fly larvae in forensic investigations.

Acknowledgement

This work was supported by the Faculty of Medicine Endowment Fund for Medical Research, Chiang Mai University, and the Thailand Research Fund (PDF/45/2543).

Figure 1-6. Scanning electron micrographs of morphological features of the 3rd-instar larva of *Chrysomya rufifacies*. (1) Cephalic segment bears antenna (A), maxillary palp complex (MPC), mouthhook (MH) and oral ridge (OR). (2) Serration structure (arrows) on lateral margin of mouthhook. (3) Anterior spiracle showing 10 papillae arranged in a single row. (4) Prothorax bears pit sensillae (arrows) and a pair of trichoid sensillae (triangles). (5) Tubercle showing large apical spines and round-shaped knob at base (arrows). (6) Caudal segment showing inner dorsal tubercles (IDT), median dorsal tubercles (MDT), outer dorsal tubercles (ODT), outer ventral tubercles (OVT), median ventral tubercles (MVT) and inner ventral tubercles (IVT). Posterior spiracular discs (PSD) each bearing 3 slits.



Electron Microscopy Society of Thailand

Best Poster Presentation

Biological Science: SEM

1st Prize

Surface Ultrastructure of the Third-Instar Larvae of *Chrysomya rufifacies*
(Diptera: Calliphoridae) A Fly Species of Forensic Importance

*K. Sukontason, S. Lertthamnongtham, B. Kuntalue, N. Tichu,
S. Piangjai and N. Boonchu*

presented at

**The XVIIIth Annual Conference
on Electron Microscopy**

EMST

on January 17-19, 2001
in Khon Kaen, Thailand

Pramote V

President

Electron Microscopy Society of Thailand

Micrograph Contest Award

Biological Science: SEM

Honorable Prize

Scanning Electron Micrograph of the Third-Instar Larvae of *Chrysomya rufifacies*, A Fly Species of Forensic Importance

Department of Parasitology, Faculty of Medicine, Chiang Mai University

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President



5th International Congress of Dipterology

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4 April 2002

Dr Kabkaew L Sukontason
Department of Parasitology
Faculty of Medicine
Chiang Mai University
Chiang Mai 50200
THAILAND

Dear Dr Sukontason

I would like to welcome you as one of the presenters at the 5th International Congress of Dipterology and would like to thank you for submitting your abstract "*Differentiation of forensically-important fly eggs using a potassium permanganate staining technique*". The Committee is pleased to accept your submission.

The Draft Program will be up on the web site towards the end of June 2002 and I will advise you as soon as it is available so that you can check your session, presentation day and time.

The Committee would like to thank you very much for your contribution to the program. We look forward to meeting you in Brisbane and to an interesting and rewarding conference.

Please do not hesitate to contact me if you have any questions.

Yours sincerely

Sally Brown
Conference Coordinator
On behalf of the Organising Committee SICD

Differentiation of forensically-important fly eggs using a potassium permanganate staining technique

Sukontason, K.L. (1), Sukontason, K. (1), Boonchu, N. (1), Piangjai, S. (1),
Kurahashi, H. (2), Hope, M. (3) & Olson, J.K. (4)

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Fly eggs found in a corpse can be utilized as entomological evidence in forensic investigations if the species of fly and the developmental rate at a temperature similar to the death scene are known. The species identification of fly eggs is primarily needed, and previously scanning electron microscope has been used for this purpose. We report a simple technique, using light microscopy, to differentiate forensically-important eggs from *Chrysomya megacephala*, *Chrysomya rufifacies*, *Chrysomya pacifica*, *Aldrichina grahami*, *Lucilia cuprina*, *Musca domestica* and *Megaselia scalaris*. A one percent potassium permanganate solution was used to stain the eggs surface for 1 min, followed by dehydration in 15%, 70%, 95%, absolute alcohol (each step for 1 min) and permanent mounting. A key for identification of these species was developed based on the morphological features of size, length and width of plastron, morphology of plastron around the micropyle and chorionic sculpture.

บทความสำหรับการเผยแพร่

เรื่อง ความสำคัญของหนอนแมลงวันในการช่วยชันสูตรศพ

ทุกวันนี้มักมีข่าวการพบศพเน่าในที่ลับตาคนเช่นในป่าละเมาะหรือป่าลึกบ่อยครั้ง ไม่ว่าจะจากการฆาตกรรมหรือจากสาเหตุอื่น เนื่องจากสถานที่พบศพเหล่านี้อยู่ห่างจากชุมชน ทำให้กว่าจะพบศพเป็นเวลหลายวันในบางครั้งศพมีสภาพเน่าจนเหลือแต่กระดูกและมีรอยสัตว์แทะ การพบศพดังกล่าวมักจะเป็นการบังเอิญที่มีผู้เดินทางผ่านไปมาในบริเวณนั้นหรือกลิ่นศพที่เหม็น ทำให้ผู้ที่เดินทางผ่านในบริเวณใกล้เคียงต้องออกมาหาแหล่งต้นตอของกลิ่นจนกระทั่งพบศพ การชันสูตรศพเน่าเหล่านี้มีข้อจำกัดหลายอย่างไม่ว่าจะประมาณเวลาการตายหรือการหาสาเหตุการตาย

ในศพเน่ามักจะพบแมลงต่างๆที่กินเนื้อเน่าเป็นอาหารโดยเฉพาะอย่างยิ่งหนอนแมลงวัน ชนิดและขนาดของหนอนแมลงวันสามารถช่วยในการประมาณเวลาหลังการตายในศพเน่าได้ ผศ. ดร.กานต์ สุคนธสรณ์ อาจารย์จากภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ มหาวิทยาลัย เชียงใหม่ ได้ร่วมมือกับนักวิจัยในภาควิชาปรสิตวิทยาและภาควิชานิติเวชศาสตร์ คณะแพทย ศาสตร์ มหาวิทยาลัยเชียงใหม่ และนักวิจัยจากประเทศสหรัฐอเมริกา ญี่ปุ่นและออสเตรเลียทำการ วิจัยเกี่ยวกับหนอนแมลงวันที่พบในศพเน่า จากการศึกษาพบว่าหนอนแมลงวันหัวเขียวสองชนิด สามารถพบได้บ่อยที่สุด นักวิจัยยังพบว่าในป่าละเมาะของจังหวัดเชียงใหม่ก็พบแมลงวันหัวเขียว ทั้งสองชนิดมากเช่นกัน นอกจากนี้ยังสามารถใช้ต่างกับที่มละลายนำมาทำการย้อมไขของ แมลงวันเพื่อแยกชนิดของแมลงวัน ซึ่งผลที่ได้จากการย้อมใกล้เคียงกับผลการศึกษาด้วยกล้องจุลทรรศน์อิเล็กตรอน

แมลงวันหัวเขียวทั้งสองชนิดมีการเจริญเติบโตที่เร็วมาก จากไข่เป็นตัวหนอนจนเริ่มเข้า ดักแด่จะใช้เวลาประมาณ 1 สัปดาห์ การเจริญเติบโตจะเร็วที่สุดในช่วงฤดูร้อน โดยใช้เวลาเพียง 4-6 วัน และช้าที่สุดในฤดูหนาว (5-8 วัน) ส่วนฤดูฝนจะใช้เวลา 5-7 วัน จากความรู้ดังกล่าวนี้ นักวิจัย สามารถประมาณระยะเวลาหลังการตายของศพเน่าจากขนาดของแมลงวันที่พบในศพและเปรียบ เทียบกับขนาดของแมลงวันที่เพาะเลี้ยงไว้ในฤดูต่างๆ ความรู้ที่ได้รับสามารถนำไปประยุกต์ใช้ในการชันสูตรพลิกศพเน่าที่มีไข่และหนอนแมลงวัน โดยผู้ชันสูตรจะสามารถแยกชนิดของไข่และตัว หนอนแมลงวันอย่างง่ายได้ อย่างไรก็ตามในศพเน่าสามารถพบหนอนแมลงวันชนิดอื่นเช่น แมลงวันหลังลาย ในอนาคตจึงมีความจำเป็นที่จะต้องขยายการศึกษาครอบคลุมแมลงวันเหล่านี้ ด้วย