

BRIEF COMMUNICATION

SCANNING ELECTRON MICROSCOPY OF THIRD-INSTAR SARCOPHAGID (DIPTERA: SARCOPHAGIDAE) RECOVERED FROM A MUMMIFIED HUMAN CORPSE IN THAILAND

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SUMMARY

The third-instar of an unidentified sarcophagid, recovered from a mummified body of a 32-yr-old Thai male was examined using scanning electron microscopy (SEM). Although the morphological features of this larva are similar to the other sarcophagid larvae, some features could be helpful for species identification, which is a basic requirement for estimation of postmortem interval in forensic investigation. These features included number and arrangement of papillae on the anterior spiracle, structure of spines, size of circumspiracular tubercles at caudal segment and branching peculiarity of the posterior spiracular hairs. This information could benefit future identification of the sarcophagid larvae that exist in Thailand.

KEYWORDS: Forensic entomology; Scanning electron microscopy; Sarcophagid; Third-instar larva.

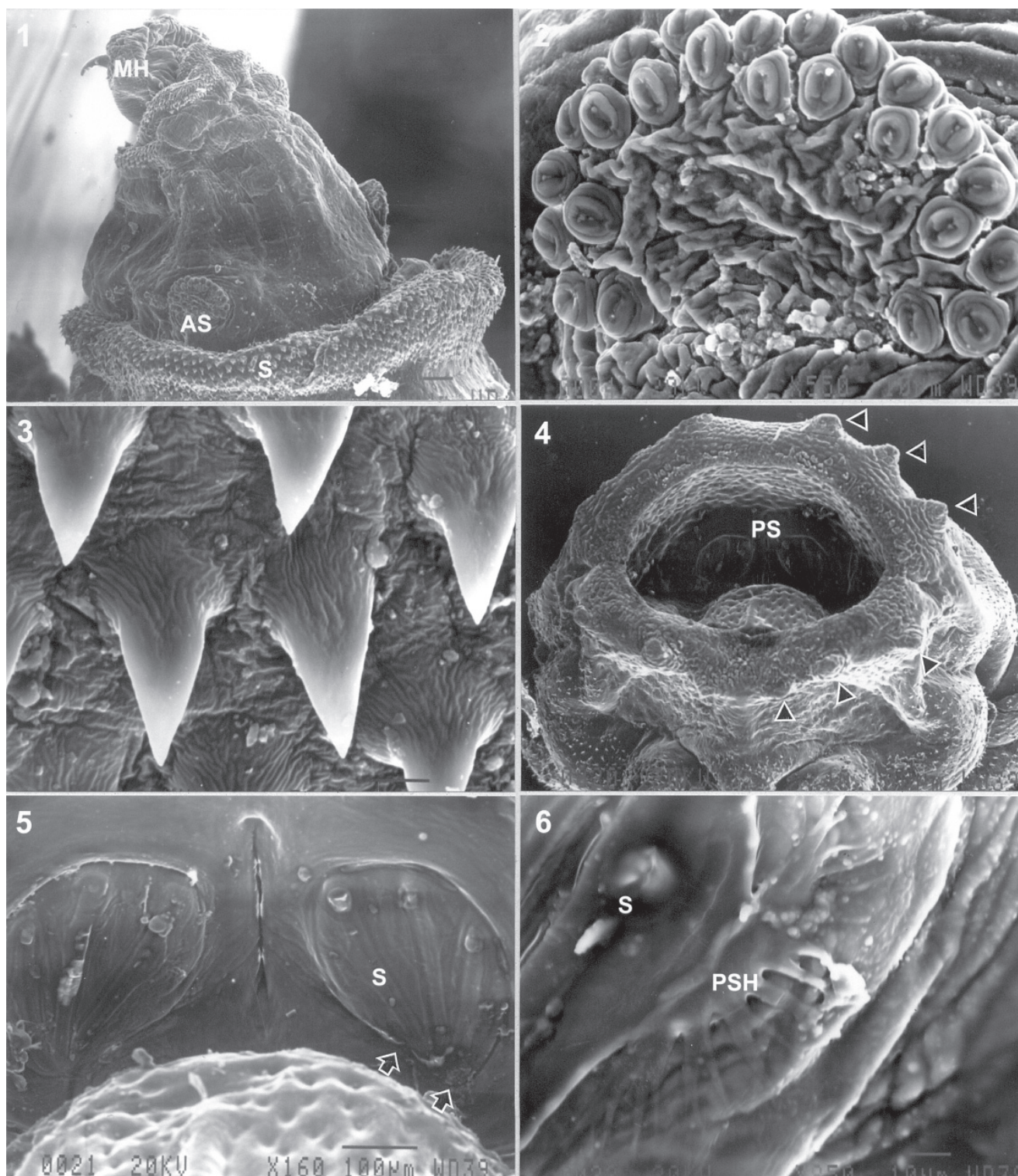
Flies of the family Sarcophagidae (flesh flies) are frequently involved as entomological evidence in forensic investigations. Larvae of several sarcophagid species have been found mostly during the first and/or second wave of insects associates with decomposing human corpses and/or animal carcasses^{1,3,4,9,14-16,18}. The major contribution made by such entomological evidence is an estimate of the duration of the postmortem interval (PMI). Some added contributions include indicative of movement of the corpse and possibly the manner of death⁶. For example, the flesh-fly species, *Parasarcophaga* (= *Sarcophaga*) *argyrostoma* (Robineau-Desvoidy), was shown to be a species highly indication that a corpse found in a more or less closed environment invaded by this fly had probably been lying outside for at least some time⁴.

The species identification of insect specimens collected from a corpse is a basic requirement in using entomological information forensically, such as to estimate PMI. Rearing of fly larvae to be adults for identification may be met with failure due to reason, e.g. only a few specimens are collected, the specimens are killed during collection, or an unsuitable rearing procedure is used. As for flesh-flies *per se*, they are very similar in appearance to another species (both as larvae and adults) and difficult to identify, often requiring the sending the adult male specimens to specialists¹⁵. In Thailand, 48 sarcophagid species have been reported¹⁷, and neither information on the larval morphology nor keys for larval identification of these particular species as found in Thailand has been located in the literature. The study of ASPOAS², using scanning electron microscopy (SEM), provided useful data to distinguish

larvae between four Afrotropical *Sarcophaga* species. The objective of this study was, therefore, to describe some important features of unidentified third-instar sarcophagid larva, collected from a human corpse in Thailand, which may prove useful for species identification in the future.

The third-instar sarcophagid larva was obtained from a mummified body of a 32-yr-old Thai male found under a tree on 13 February 2000. The tree, found in the forest near the main highway running through Doi-Sa-Ket District, Chiang Mai Province, northern Thailand, had a nylon rope tied to one of its branches. The corpse was transferred for investigation to the Forensic Medicine Department, Faculty of Medicine, Chiang Mai University, on 14 February 2000. A maximum PMI of ca. 4½ months was estimated after his disappearance. The larva was killed in near-boiling water and processed for SEM by placing in 2.5% glutaraldehyde at 4 °C for 24 h. It was then subjected to postfixation in 1% osmium tetroxide and dehydrated in a graded alcohol series. This was followed by immersion in acetone before applying a critical-point drying procedure. The larva was mounted on stubs, coated with gold, viewed with a JEOL-JSM840A Scanning Electron Microscope (Tokyo, Japan) and photographed with Kodak® Verichrome Panchromatic film VP120 (New York, USA).

The general muscomorph features of this unidentified sarcophagid larva were similar to those previously reported for other sarcophagid species. In the cephalic segment is a pair of strong mouthhooks (Fig. 1).



Figs 1-6 - Scanning electron micrographs of the unidentified third-instar sarcophagid larva removed from the corpse. (1) Dorso-lateral view of the cephalic segment illustrating the mouthhook (MH), and the first thoracic segment shows anterior spiracle (AS) and rows of spines (S). (2) Anterior spiracle shows 24 papillae arranging in an irregular double row. (3) Higher magnification of spines at the first thoracic segment. (4) Posterior view of the caudal segment showing 6 pairs of moderate circumspiracular tubercles (arrowheads) and a pair of posterior spiracles (PS) in a deep cavity. (5) Higher magnification of a pair of posterior spiracles, with each spiracular disc bearing 3 straight spiracular slits (S), enclosed with an incomplete peritreme (arrows indicate their ends). (6) Higher magnification of the posterior spiracular hairs (PSH) adjacent to the slit (S).

The anterior spiracles are located on each latero-posterior edge of the prothorax (Fig. 1). The number of papillae in each anterior spiracle is 24, with them being arranged in an irregular double row (Fig. 2). The band of spines appears anteriorly and posteriorly of each segment. Viewed at the dorso-anterior edge of the third segment, the spine band appears as ca. 13 rows of a backwardly projecting single point. At higher magnification, the base of each spine is wrinkle and the tip is a sharp-triangular shape (Fig. 3). The inter-band areas were devoid of spines.

The caudal segment is composed of deep stigmal cavity and 6 pairs of moderate circumspiracular tubercles (Fig. 4). A pair of posterior spiracles is situated in a cavity. Each spiracular disc bears 3 spiracular openings (slits) enclosed by an incomplete peritreme (Fig. 5). The innermost slits are longest and tilt centrally while the outermost slits are shortest. Near the rim of slits are the spiracular hairs having secondary and/or tertiary branches (Fig. 6).

Generally, the morphology of this unidentified sarcophagid larva is similar to that previously described for other larval sarcophagids. Under SEM observation, some morphological features of larva are different and could perhaps be used for differentiation at the species level². The first feature is the number and arrangement of papillae on the anterior spiracle^{2,7}. Our unidentified larva had 24 papillae in each spiracle arranged in an irregular double row. This large number of papillae and their double row arrangement approximates the situation described for *Sarcophaga tibialis* Macquart that had 15-21 papillae² and *Parasarcophaga omega* (Macquart), which had 25 papillae observed on the second-instar⁵. The number of papillae (up to 24) of our species of sarcophagid is much greater than those of several reported sarcophagid larvae^{2,7,11,12}. Although there is an overlap in the number of papillae among several other sarcophagid species^{2,5,7}, this feature is likely to be essential in the ultimate identification.

The second feature that may be useful for differentiation of larvae like one of our particular species of sarcophagid are the spines. In Calliphoridae the structure of spines are important taxonomic features^{8,10,13} while in Sarcophagidae, spine structure and density are important². On the third body segment of the larvae of four Afrotropical sarcophagid species, the spines appear as having single points; however, the morphology and surface of spine as determined using SEM are different². The SEM results for the spines of our unidentified larva agreed with ASPOAS² in terms of both structure and density. However, spine structure at the same particular area of larval segment should be taken into account to determine specific patterns of each species since the shape, density and orientation of spines varies along the length of body and also between dorsal, ventral and lateral surfaces⁷.

The size of the circumspiracular tubercles at the caudal segment of second and third-instar sarcophagid is an important morphological character¹². The tubercles on the larva in this study are moderate and similar in size, except the smallest of inner ventral tubercles as shown in Fig. 4. The study on third-instar *Oxysarcodexia paulistanensis* (Mattos)¹² showed the outer dorsal tubercles to be much more elongated than those of *Oxysarcodexia confusa* Lopes.

The branching peculiarity of spiracular hairs, adjacent to the posterior spiracular opening, has been shown as a distinctive feature of sarcophagid larvae². The spiracular hair of larva in this present study markedly differed

from those of *Sarcophaga cruentata* Meigen, *S. exuberans* Pandelle and *S. tibialis*².

The present SEM study revealed some distinctive features of our unidentified sarcophagid larva, which we were unable to easily discern under light microscopy. Although species identification could not be accomplished, this information may be useful to ascertain the identity of this sarcophagid larva in the future.

RESUMO

Microscopia eletrônica de varredura de sarcófago de terceira fase (Diptera: Sarcophagidae) recuperado de corpo humano mumificado na Tailândia

A terceira fase de um sarcófago não identificado recuperado de corpo mumificado de Tailandês masculino de 32 anos foi examinado usando microscopia eletrônica de varredura (SEM). Embora as características morfológicas desta larva sejam similares a outras larvas sarcófago, algumas características poderiam ser úteis para a identificação da espécie que é requisito básico para a estimativa do intervalo pós-mortal da investigação forense. Estas características incluíram o número e o arranjo das papilas no espiráculo anterior, estrutura das espículas, tamanho dos tubérculos circunspiraculares do segmento caudal e peculiaridade de ramificação dos pelos espiraculares posteriores.

Esta informação poderia beneficiar as futuras identificações das larvas sarcófago que existem na Tailândia.

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