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LIGHT AND SCANNING ELECTRON MICROSCOPY OF THIRD-INSTAR LARVA OF *LUCILIA SERICATA* (DIPTERA: CALLIPHORIDAE) RECOVERED FROM A CASE OF HUMAN INTESTINAL MYIASIS

(With 4 Plates)

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وصف الطور الثالث ليرقة الليوسيليا سيريكاتا (ديبترا: كاليفوريدى) بالميكروسكوب الضوئى والإلكتروني الماسح وجدت فى حالة تدويد معوى بالإنسان

مها سيد ابراهيم شاهين ، أمانى ابراهيم حمزة ، سلمى محمد عبد الرحمن

يوصف هذا العمل حالة تدويد بيرقات الطور الثالث لذبابة ليوسيليا سيريكاتا بالأمعاء التى تم الحصول عليها من عينة براز لطفل، حيث تم فحصها بالميكروسكوب الضوئى والالكترونى الماسح. ووجد ان الأوصاف الشكلية لهذه اليرقات تتشابه مع البعض الأخرمن عائلة كاليفوريدى. وتم تشخيص اليرقات من نوع الليوسيليا سيريكاتا. وقد أضاف هذا العمل أوصاف جديدة ساعدت فى التعرف على النوع. وهذه الأوصاف شملت دد وترتيب النتوءات على فتحة التنفس الأمامية، تركيب الأشواك الموجودة على جسم اليرقة، شكل وترتيب النتوءات المحيطة بفتحة التنفس الخلفية، وكذلك التفرعات الخاصة بالشعيرات المحيطة بفتحة التنفس. وهذه الاضافات قد تفيد فى التشخيصات المستقبلية ليرقة الليوسيليا سيريكاتا المتواجدة فى مصر.

SUMMARY

The current work describes a case of intestinal myiasis, where the third-instar of unidentified larvae, recovered from a stool specimen of a child, were examined using scanning electron microscopy (SEM). Morphological features of these larvae were similar to larvae of family Calliphoridae. They were identified as *Lucilia sericata*. The present work added new features that could be helpful for species identification. These features included number and arrangement of papillae on the

anterior spiracle, structure of spines, shape and arrangement of circumspiracular papillae at caudal segment and branching peculiarity of the posterior spiracular hairs. These new additions could benefit future identification of the *Lucilia spp.* larvae that exist in Egypt.

Key words: *Intestinal myiasis, 3rd. instsr larva, SEM, Lucilia sericata.*

INTRODUCTION

Lucilia sericata flies belong to family Calliphoridae, which are ectoparasites found in corpse of animals, and cause myiasis in humans and domestic herbivorous animals (Granz *et al.,* 1975; Mateos *et al.,* 1990 and Morsy *et al.*, 1991).

Members of this genus are responsible for the condition known as "blowfly strike" of sheep in a number of countries. The adult flies are metallic green or coppery green and are therefore known collectively as greenbottles. Female *Lucilia species* lay their eggs on carcasses in neglected suppurating wounds and in particular, on the wool of sheep soiled with urine, faeces or blood (Zumpt, 1965; Smith, 1986).

Myiasis caused by *Lucilia sericata* was reported in 1826 by Meigen who separated larvae for the first time from mouth, eyes, and paranasal sinuses of a hospital patient (Daniel *et al.,* 1994). *Lucilia sericata* is not only a cause of myiasis but also it is important in forensic medicine (Bourel *et al.,* 1999 and *Erzinçlioglu, 2003*). Moreover, it can be used as a therapeutic agent in treatment of debriding nonhealing foot and leg ulcers in diabetic patients (Ronald & Sherman, 2003).

The present work aimed at identification of the obtained larvae, providing possible additional important features of the third instar through scanning electron microscopy (SEM) for accurate species identification.

Case Report:

A 12 years old boy was admitted to Assiut University Hospital with a history of colic and irritability. The patient frequently observed worm-like maggots in his stool. He was treated with piperazine by the physician for a presumptive diagnosis of pinworm infection. However, the patient continued to see "worms" in his stool. Larvae were seen in each of two stool specimens collected on different days. The specimens were brought to the Department of Parasitology, Faculty of Medicine, Assiut University for identification.

MATERIALS and METHODS

I. Light microscopic examination: Fresh specimens of the obtained larvae were examined by light microscope and photographed with the aid of Digital camera.

II. Scanning Electron Microscopy: The obtained larvae were put in glutraldehyde 2.5% and processed for SEM according to Colwell & O'Connor (2000).

RESULTS

I. Light microscopic examination:

The maggots are creamy white in color and cylindrical in shape. The length of live maggots is 11–11.5 mm. The head end is tapering to a point (Plate I Fig.1). There are hook-like mouth parts with an accessory oral sclerite (Plate I Fig. 2) and non pigmented hypopharyngeal sclerite. They possess anterior spiracles. The posterior end is thick and surrounded by 12 conical pigmented papillae (Plate I Fig. 3).

II. Scanning Electron Microscopy:

The body is composed of an attenuated head and 12 segments that have broad encircling bands of spinules. Anterior spiracles are located on each latero-posterior edge of the prothorax (Plate II Fig.1) with digit like protrusions, which are respiratory branchiae. The number of these protrusions in each anterior spiracle is 12, being arranged in a fan-shaped row (Plate II Fig. 2). By higher magnification, the protrusions of the anterior spiracles appear rounded with a raised elongated central structure (Plate II Fig. 3).

The head is bulbous in shape and carries a pair of mouth hooks (Plate II Fig.4). On either side of the midline there is antennomaxillary lobes composed of antennal complex and maxillary complex on each lobe (Plate II Fig.5). The maxillary compex has a rosette-shaped appearance (Plate II Fig.6).

The segments:

In the cephalic segment, there are cephalic senella in the form of spines arranged in rows and ended in single tips, being completely different in shape from those adjacent spines of the first thoracic segment (Plate III Fig.1). An accessory sclerite is arising from the posterior part of the cephalic segment and directed anteriorly, it has an elongated tapering shape.

In the thoracic segments, bands of spines are located on the anterior edges of both ventral and dorsal surfaces of thoracic segments while the rest of the segment is devoid of spines (Plate III Fig.2). Cuticular sensellae are distributed along the ventral surface of thoracic segments. They are trichoid sensellae characterised by the presence of three setae (Plate III Fig. 3).

The abdominal segments are broad, covered with sharp triangular spines, which are denser and sharper on the upper edge of segments. Each segment has a central row of elongated papillae on the dorsal and ventral surfaces (Plate III Fig.4). These papillae end without hairs or spines (Plate III Fig.5). All spines distributed on the abdominal segments are located singly with a wrinkled base and end in a single tip (Plate III Fig.6) except for the last segment; on the dorsal surface the spines are arranged in pairs or in triads and some of them end in double tips (Plate III Fig.7) while on the ventral surface the spines surrounding anal papillae are dense, sharp and forked (Plate III Fig.8).

The caudal segment (segment 12) is composed of shallow cavity surrounded by 6 pairs of conical circumspiracular papillae and one pair of anal papillae, which ends with terminal spine (Plate III Fig.8). The conical-shaped circumspiracular papillae are arranged as three dorsal and three ventral ones (internal, middle and external papillae), (Plate IV Fig.1).

A pair of posterior spiracles is situated in this shallow cavity, with spiracular discs protruding out of the cavity. Each spiracular disc bears 3 spiracular openings (slits) enclosed by a peritreme (Plate IV Fig.2). The two peritrema are surrounded by a thick, pitted wall. The innermost slits are the longest and tilt centrally while the outermost slits are the shortest (Plate IV Fig.3). The peritrema are closed and enclosing a button (Plate IV Fig.4). A tubercle situated between the two peritrema is noticed for the first time in this instar, a structure which seems to be of spiracular support (Plate IVFig.5). Near the rim of slits are spiracular hairs having secondary and tertiary branches (Plate IVFig.5,6).

Diagnosis:

According to Greenberg (1971) and Greenberg & Szyska (1984) the maggots are identified as the third stage larvae of *Lucilia sericata* Meigen 1826 (Diptera: Calliphoridae) according to the following criteria:

1) Presence of accessory sclerite.
2) Cephalopharyngeal skeleton without pigmented area below posterior extremity of ventral cornua.

3) Posterior spiracle with complete peritreme enclosing a button.
4) Inner papillae of upper margin of anal segment separated by a distance approximately equal to distance between inner and middle papillae.

DISCUSSION

Lucilia sericata is distributed allover the world and are the most known species to cause animal and human infestation. It was found in wounds and natural orifices of human body or animals particularly the mouth, eyes and paranasal sinuses (Daniel *et al.*, 1994). Wound myiasis due to *Lucilia sericata* was reported by Pays & Haas (1976); Kaufman *et al.* (1989) & Daniel *et al.* (1994). Aural myiasis caused by *Lucilia sericata* had been reported by Chigusa *et al.* (1994) and Cho *et al.* (1999).

Recently, Hira *et al.* (2004) reported a nosocomial infection caused by the second and third instars of *Lucilia sericata,* the maggots were observed coming out of the nostrils of a child in the intensive care unit.

As regard intestinal myiasis in human, a case of internal myiasis was reported by Chung *et al.* (1996) where maggots were observed to crawl out from nasogastric tube. In current study, larvae were revealed from a stool specimen where they were identified as *Lucilia sericata* larvae. The diagnosis depended mainly on SEM for species identification. This was in agreement with Colwell & O'Connor (2000) who stated that, under SEM observation, some morphological features of larva are different and could perhaps be used for differentiation at the species level.

The present SEM study revealed some distinctive features of the larva, which were undetectable under light microscopy, so species identification could be accomplished, these observations may be useful to ascertain the identity of *Lucilia* larvae in the future.

The first feature studied was the number and arrangement of papillae on the anterior spiracle. The larva under study has 12 branchiae in each spiracle arranged in a single row. The number of papillae is very close to those larvae of several reported Chrysomyiinae. It may share other Calliphorids in this character where, Ishijima (1967) reported third instar larvae of *Chrysomyia rufifacies* to have an anterior spiracle with 11-12 spiracular branchiae (ramifications); Queiroz & Carvalho (1987) observed that the third instar larvae of *Chrysomyia albiceps* have an

anterior spiracle with 11 branchiae, while Greenberg and Szyska (1984) observed that *Chrysomyia putoria* has an anterior spiracle with 10-12 branchiae and *Chrysomyia macellaria* has 8-12 branchiae while *Lucilia cuprina* has 5-6 branchiae. Although there is an overlap in the number of branchiae among several other calliphorid species, yet studying the shape of branchiae done in the present work was essential for ultimate identification. Thus number of branchiae and their arrangement approximates the situation described for *Lucilia serricata*.

The second feature that may be useful for differentiation of larvae is the spines shape and distribution. In Calliphoridae the structure of spines are important taxonomic features (Erzinçlioglu, 1987; Mangan & Welch, 1990 and Iwasa & Hori, 1990). In the present work, the scanning of spines provided a description of shape and arrangement of spines on the larval cuticle that could be useful as a new morphological criterion for species identification. 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 surfaces. This was in agreement with Kaufman *et al.* (1989) who studied *Lucilia cupriana.*

The third distinctive feature of *Lucilia sericata* larva was the branching peculiarity of spiracular hairs, that were situated adjacent to the posterior spiracular opening. This charcter differentiates it from other calliphorid larvae as those of *Chrysomyia rufifacies* that observed by Liu & Greenberg (1989).

In the present work, it was observed that each abdomenal segment of third instar larvae of *Lucilia sericata* has long tubercles on the dorsal and ventral surfaces; these tubercles were also observed by Queiroz & Carvalho (1987) in *Chrysomyia albiceps*. Liu & Greenberg (1989) worked on third instar larvae of *Chrysomyia rufifacies*, they observed that the last segment (12th) possessed a posterior margin with pronounced tubercles and conical ones as in the other calliphorids. Also the authors observed that the margins of the 12th segment of first instar larvae of *Chrysomyia rufifacies*, *Cochliomyia macellaria* Fabricius, 1775 and *Lucilia sericata* Meigen, 1826 presented small hairs which were actually small spines, and the peritrema of third instar larvae were incomplete in the first two species and had a spiracular bud in the third. These results agreed with that obtained in the present study, as small spines in third instar larvae of *Lucilia sericata* were visualized. Ishijima (1967) and Prins (1982) observed the presence of an accessory oral

sclerite in *Chrysomyia regalis* Robineau-Desvoidy, 1830, which was club-shaped in *Chrysomyia pinguins* Walker, 1858 and small comma-shaped in *Chrysomyia megacephala.*

A characteristic feature of *Lucilia sericata* is the presence of accessory sclerite having an elongated tapering shape, which was detected in the present work. Erzinçlioglu (1989) observed that the cephalopharyngeal skeleton of *Lucilia cuprina* Wiedemann, 1830 and *Lucilia sericata* Meigen, 1826 differs from that of *Chrysomyia putoria* and *Chrysomyia albicans* by the presence of an accessory oral sclerite in the first two species. Prins (1979) in a study of some specimens of *Chrysomyia megacephala* and *Chrysomyia chloropyga* observed that both have an open peritrema and are easily distinguishable from *Lucilia sericata*, which has a closed peritrema.

Conclusion:

- According to the available data, it seems that the present work is the first record of *Lucilia sericata* as a cause of intestinal myiasis in Egypt.
- With the aid of SEM, the third stage larvae of *Lucilia sericata* can be easily distinguished from other callipforid larvae by the following combination of characters:

  (1) The detailed description and distribution of spines on the cuticular surface of the whole body. (2) The papillae present in the middle of abdominal segments end without hairs. (3) The shape and arrangement of papillae on both of the dorsal and ventral surfaces of the caudal segment. (4) The presence of tubercle inbetween the posterior respiratory discs.
- The described characters can be used as a guide for taxonomic identification for this species.

The authors recommend more studies using SEM to be undertaken in larvae obtained from human and domestic animals myiasis.

LEGEND OF FIGURES

Plate (I): light microscopy:

Fig. 1: Third stage larva with tapering anterior end (A) & broad posterior end (P).

Fig. 2: Lateral view of the larva (Anterior end) showing mouth hooks (MH) & non pigmented hypopharyngeal sclerite (hy).

Fig. 3: Caudal segment showing circumspiracular papillae.

Plate (II): Scanning electron microscopy of cephalic segment:

Fig. 1: Dorsolateral view of anterior end of the larva, showing anterior respiratory spiracles (arrow). X75.

Fig. 2: Anterior respiratory spiracles (AS) on prothoracic segment with 12 spiracular openings. X500.

Fig. 3: Higher magnification of spiracular openings, showing 12 digit-like protrusions. X2000.

Fig. 4: The head showing, mouth hooks (MH)& cephalic sensilla (CS). X200.

Fig. 5: Antennalcomplex (A) & Maxillary complex (M). X350.

Fig. 6: Higher magnification of antennalcomplex (A). X750.

Plate (III): Scanning electron microscopy showing distribution of spines:

Fig. 1: Anterior end showing spines of cephalic segment & accessory scerite (ASc) and a part of prothoracic segment. X500.

Fig. 2: Spines of thoracic segments. X200.

Fig. 3: Trichoid sensilla of thoracic segments with three setae. X 2000.

Fig. 4: Spines of abdominal segment & papillae on the middle of segments. X200.

Fig. 5: Higher magnification of papillae on abdominal segments. X750.

Fig. 6: Higher magnification of spines on abdominal segment. X1500.

Fig. 7: Spines of last abdominal segment (dorsal surface). X1500.

Fig. 8: Spines of last abdominal segment (ventral surface) surrounding anal papilla (arrow). X1500.

Plate (IV): Scanning electron microscopy of caudal segment:

Fig. 1: Caudal segment showing a shallow cavity and 6 pairs of conical circumspiracular papillae X75:

idp: internal dorsal papilla **ivp:** internal ventral papilla
mdp: middle dorsal papilla **mvp:** middle ventral papilla
edp: external dorsal papilla **evp:** external ventral papilla

Fig. 2: Posterior spiracles with protruding spiracular discs (arrow). X100.

Fig. 3: Higher magnification of posterior spiracles (PS) & wall (W). X200.

Fig. 4: Closed peritrema enclosing a button (B). X750.

Fig. 5: A tubercle (t) between the two peritrema (P). X350.

Fig. 6: Spiracular hairs (SH) showing secondary and tertiary branches. X1500.

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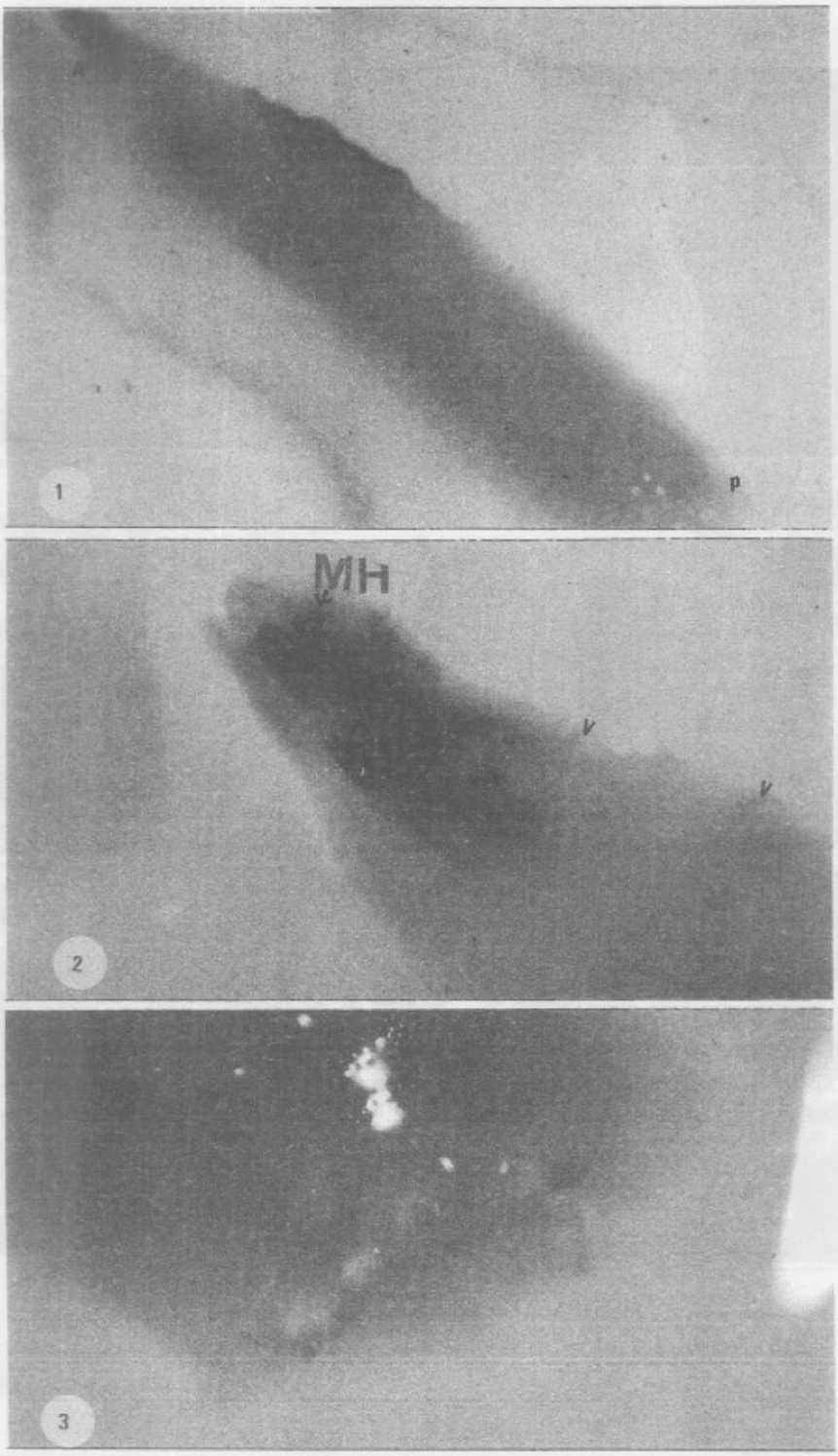
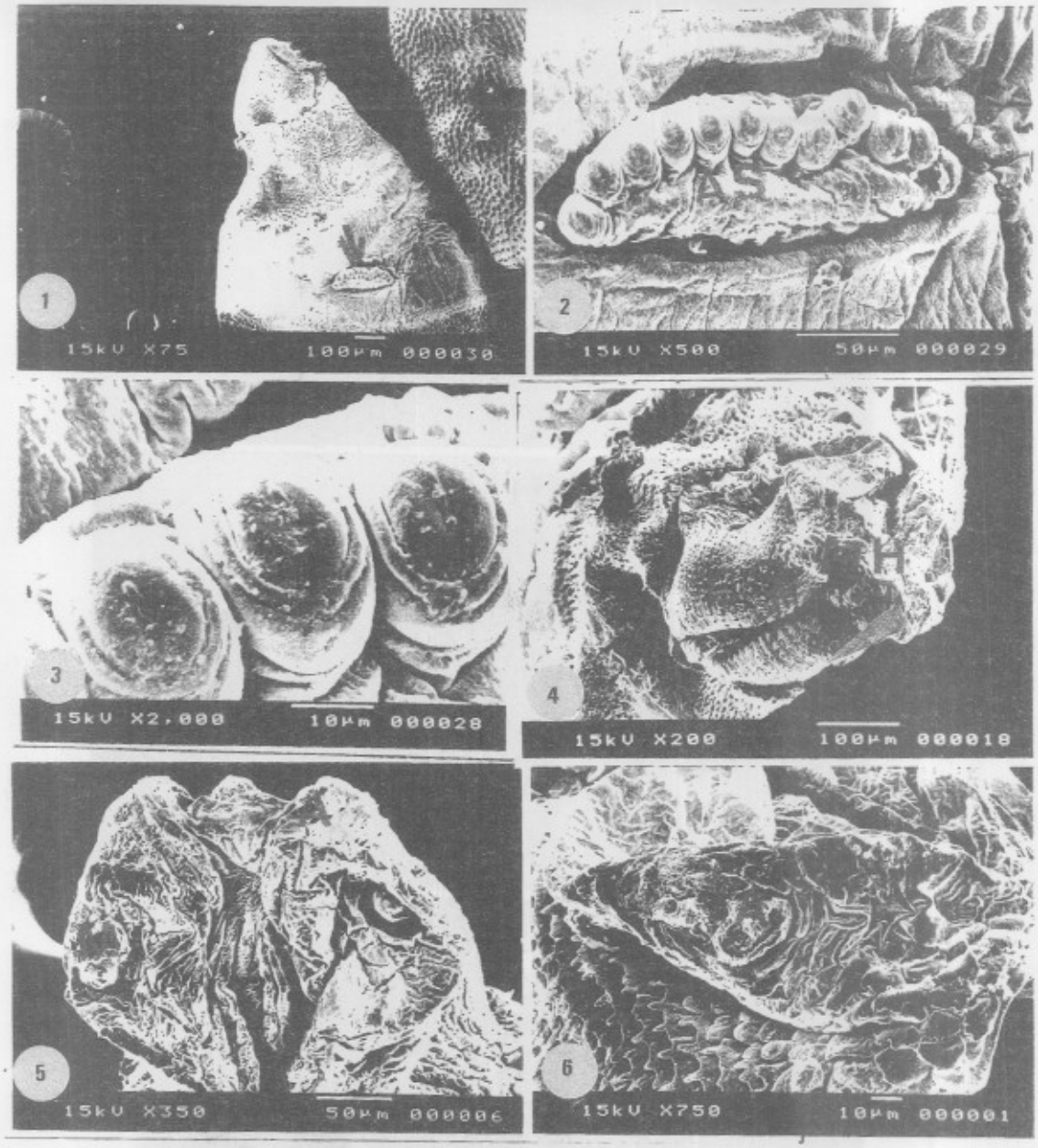


Plate I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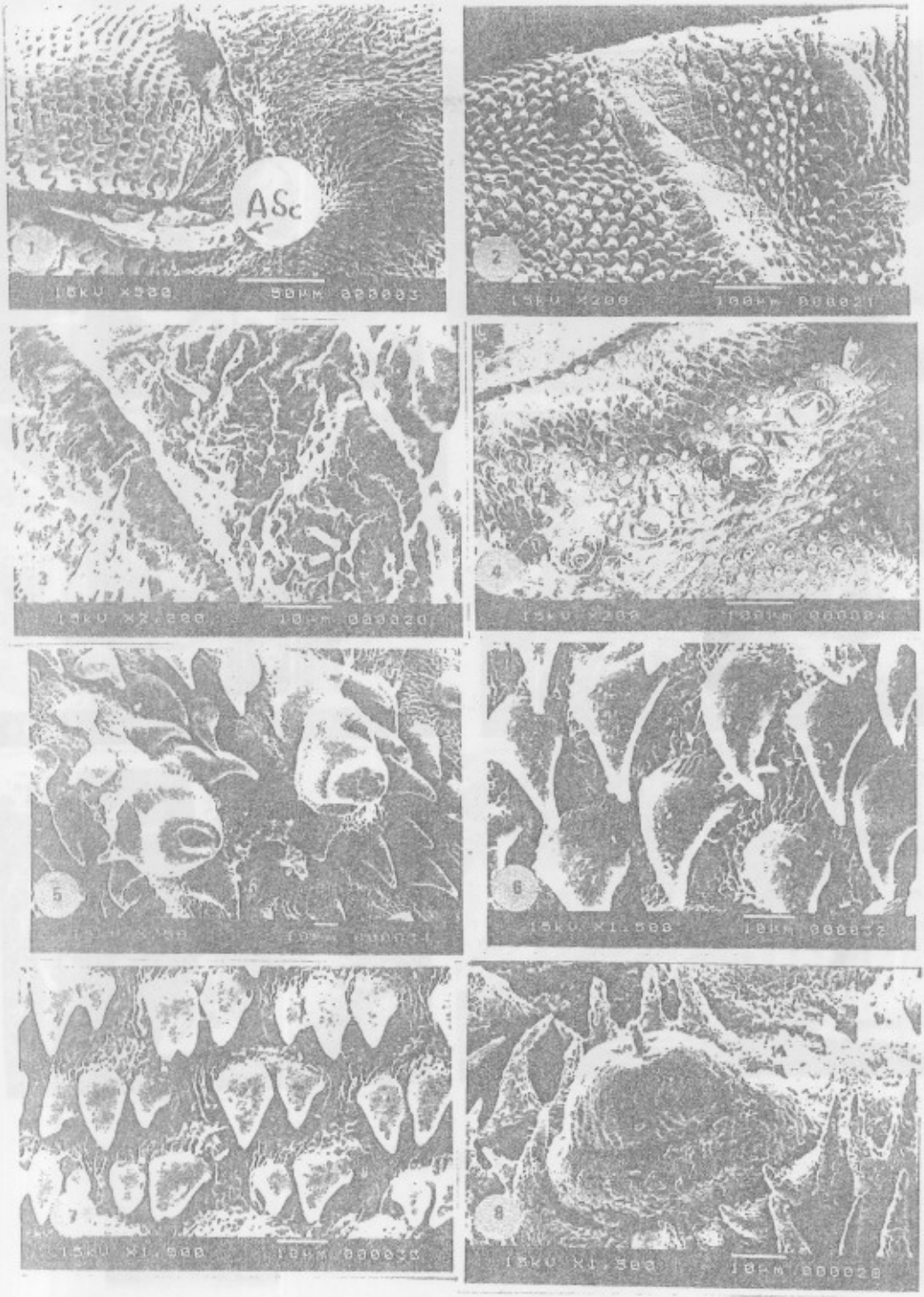

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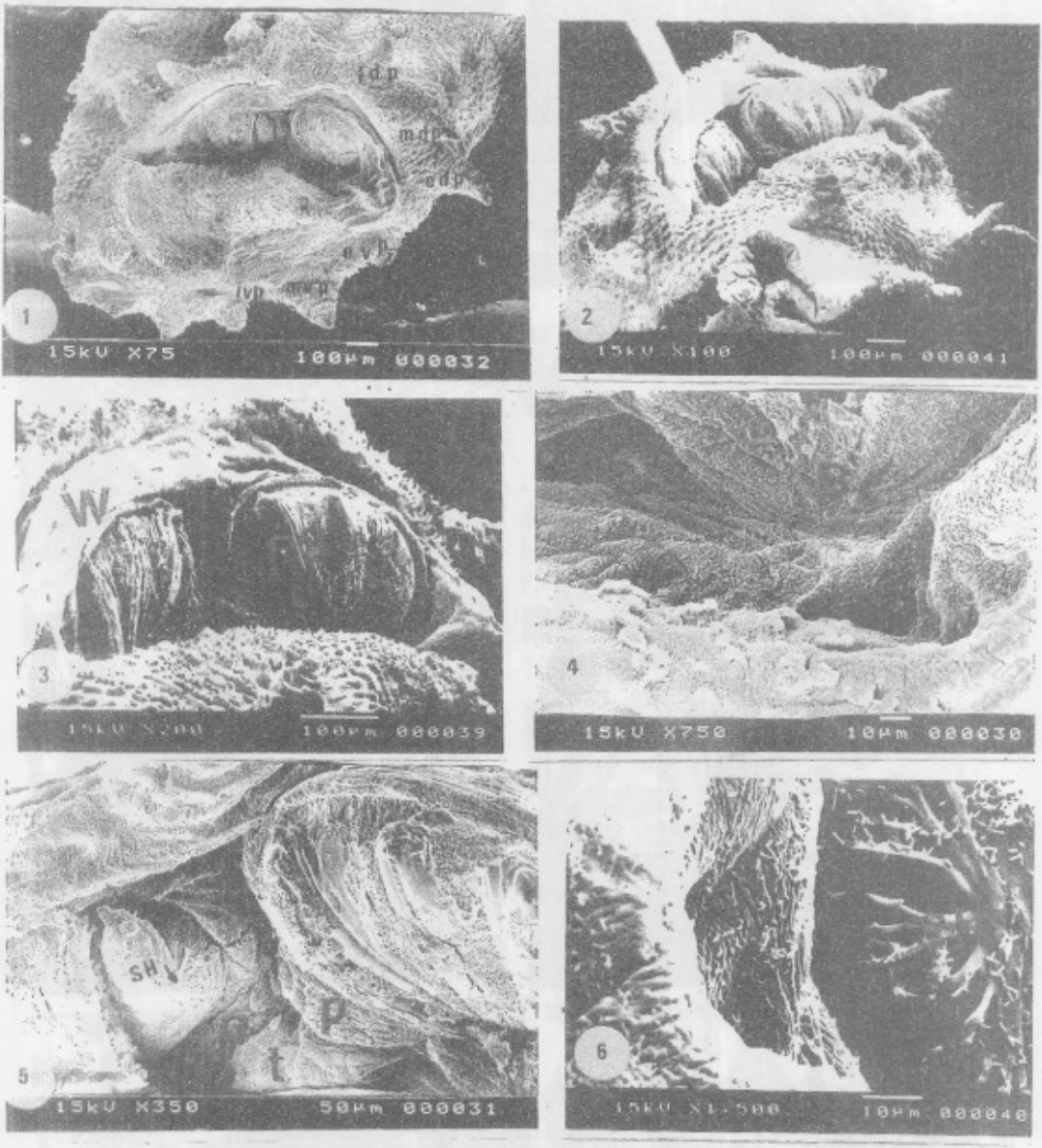
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PlateIV