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MORPHOLOGICAL STUDIES ON THE LARVAL STAGES OF THE SHEEP BLOWFLY *LUCILIA CUPRINA* WIEDEMANN (*DIPTERA: CALLIPHORIDAE* .)

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SUMMARY

Light and Scanning Electron Microscope (SEM) were used in the description of Lucilia cuprina larvae . The mouth hooks are poorly developed in the 1st instar larva and well developed in the 2nd and 3rd larval stages , the antennal lobes and maxillary lobes are poorly developed in the 1st instar , but generally showed a higher degree of development in the 2nd and 3rd instar larvae . 1st instar larvae has cuticular sensilla represented by spines arranged specifically in the dorsal and ventral locations. The distribution of fleshy processes and tiny spines at the body segments of st, 2nd and 3rd instar larvae are described in details. The morphological characteristics of the cephalic segment, anterior and posterior spiracles of le three larval instars are also described .

INTRODUCTION

Lucilia cuprina ,the sheep blowfly , is the major cause of a considerable number of sheep death in Australia due to fly strike . *L.cuprina* was originally described by Wiedemann in (1830) and Zumpt (1965) gave the first complete description for all larval stages .

The aim of this study was to make a comparative study on the external morphology of the three larval stages of the blowfly *L. cuprina* witch will pave the way for further investigations on this fly species .

The larvae of Lucilia live in urine - soaked wool , proteinaceous sera and blood from the skin lesions, (Makerras and Freney,1933). Lewis (1955) stated that a few Lucilia larvae have been found in various animals .

L.cuprina and L.sericata larvae were collected from camel's mouth and from wool of sheep respectively . The last mentioned species caused sheep strike in some countries .

Monzu (1979) classified the insects which were collected on sheep as primary , secondary and tertiary flies. He recorde 641 strikes in sheep (480 L.cuprina Wied., 125 Calliphora albifrontalis Mall. And 117 C. nociva as primary flies.

Woodburn and Vogt (1982) found L.cuprina came to merino sheep before and after death.

Amin et.al (1997 and 1998) reported that members of family Calliphoridae were involved in specific myiasis or obligatory myiasis producers.
Colwell . et.al (2000) gave scanning Electron Microscope (SEM) observation on second instars of unidentified sarcophagid maggots recovered from the foot of a 2- month old child.

This description may be the first one in Egypt .
In the present work , SEM and light microscope studies were conducted to clarify certain morphological features of 1st , 2nd and 3rd instar larvae of *L.cuprina.*

MATERIALS AND METHODS

Larvae of L.cuprina (which previously identified according to Fatma Adham *) were taken from the running stock culture reared in laboratory of The Department of Entomology , Faculty of Science Cairo Univ., Giza, Egypt since 1993 ,As that used by (Omar ,1974).in rearing of the blowfly Chrysomyia albiceps Wiedemann.

The breading stock of adults was maintained in 35 x 35 x 50 cm. Cages . These cages were made with a wooden floor and two glass sides . the other two sides , together with the roof were made of wire guaze . Emerged adult flies were supplied with sugar, water and meat . water was supplied by dipping a piece of cotton as a wick in a bottle filled with water . The meat was introduced in a Petridish and was changed daily to stimulate egg maturation and oviposition.

The adult cages were examined once daily for egg deposition. The fresh eggs were transferred to the larval breeding plastic dishes (12cm. In diameter) containing fresh meat and were covered with muslin and fastened with rubber bands.

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Light Microscope Studies:

Permanent specimens mounted in Canada balsam were prepared according to Soulsby (1982) for *L.cuprina* larvae. 1st instar larvae were cleared in lactic acid, while 2nd and 3rd instars in 5% caustic soda (NaOH) at laboratory temperature of 25-30 C. The larvae were evacuated, washed several times with distilled water to remove excess of

lactic acid or caustic soda, dehydrated through ascending serial concentration of ethanol, transferred to a mixture of equal volumes of absolute ethanol and xylene and then mounted in Canada balsam .All larval stages were carefully examined, described and photographed.

Scanning Electron Microscope (SEM) studies: Freshly collected larvae were used .1st instar was taken as a whole, 2nd and 3rd instars were cut into anterior and posterior portions ,The anterior portion is composed of the cephalic segment ,the three thoracic segments and one or two abdominal segments the posterior one is the rest of the body .The larvae were fixed in 2.5% glutaraldehyde (PH 7.2) for 24 h. at 4°C, then post fixed in 1% osmium tetroxide for 1 h. at room temperature(Harley and Fergusen , 1990).

The specimens were then dehydrated with acetone, critical point dried, and finally sputter coated with gold.

The examination and photographing were done through a Jeol Scanning Elactron Microscope (JSM-T330A) equipped with image recording and processing system (Sem Afor) in Ain Shams University Faculty of Agriculture, AGRICULTURAL STADIES AND CONSULTATION CENTER.

RESULTS

The differences between instars morphologically studied by SEM can be correlated directly with light microscope observations (O'Flynn and Moorhouse,1980).

First Instar Larva : The L.cuprina 1st instar larva is cylindrical pointed in front and gradually increasing in diameter towards the caudal blunt end. and creamy white in colour ranging from 2-3.5 mm in length and 1.7 mm in width(average of 10 larvae). The body is truncated behind obliquely. So that the posterior extremity exhibits a concave surface which looks upwards and backwards and within which the posterior spiracles are situated.

It consisted of twelve visible segments (Fig.1).The nine posterior ones having processes on the ventral side which described as foot pads.

The first segment (Cephalic segment) with a pair of slightly ventrally elevated small mouth hooks, cephaloskeleton 0.55 mm in length(average of 10 larvae) two prominent oral ridges around the mouth opening resembles the pseudotracheae of adult fly in its morphological structure, and a pair of small antennal lobes, spines were found on the dorsal edges of segments 2-7 and 2-3 rows of small spines at the ventral anterior margin of segments 3-9. The posterior extremity of the segments 10 and 11 were provided with dorsal and ventral bands of spines.

On segment 12 the posterior spiracles are located , which have peritreme slightly chitinized, the latter

was surrounded by sensory papillae which are provided with spines.(fig.1A).

SEM observation of the 1st instar larvae gave detailed demonstration of the situation and shape of the spine groups(cuticular sensilla) than observed by the light microscope. The spines were generally tapered with relatively enlarged base, and situated at the dorsal and ventral aspects of segments (Figs. 2,3).

The ventro-anterior footpad groups are in a medially situated and are longer than the spines. (Fig.4). On the dorsal side prothoracic spiracles are present with typical digit - like protrusions, six in number (Fig.5).

Posterior spiracles are located on the dorsal side of the last segment (Fig.6).

The posterior spiracles are enclosed within a deep stigmal cavity.

Second Instar Larva : The L.cuprina 2nd instar larva, is cylindrical, pointed anteriorly and blunt posteriorly , creamy white in colour and ranges from 4-10 mm in length and 2 mm in width (average of 10 larvae).It consists of twelve visible segment. Cephalic segment (Fig.7) is bilobate with prominent oral ridges (Fig.7A). It also consists of a pair of large mouthhooks, a pair of large antennal lobes, cephaloskeleton is 1.23 mm in length.

Second segment possesses 8 rows of tiny spines at the anterior dorsal margin around the cephalic segment.

The body segments are provided with dorsal and ventral transverse bands of several rows of tiny spines.

The distribution of spines is similar to that described in the first stage, but segments number 8&9 show complete bands at the anterior margins.

Well developed fleshy processes in the form of small and blunt tubercles situated posteriorly to the ventral transverse bands of spines were found at segments 4-12 (Fig.8).

Two pairs of spiracles are found, an anterior pair located dorsally on the first thoracic segment, characterized by the presence of digit-like protrusion each with a slit like opening (Fig.9) . Each spiracle has six of these protrusions. The posterior spiracle are closed and kidney - shaped (fig.9A) lie in a depression (respiratory cavity) at the last segment. The number of spiracular opening are 3 slits per peritreme. The peritremeis surrounded by a number of dorsal and ventral tubercles (Fig.8) .

SEM micrographs of 2nd instar larva clearly indicated that the mouth opening is ventrally located with an apparent labial lobe (Fig.10).

Each of the antennal lobe is composed of a large

central cone- shaped sensillum surrounded by a cuticular segment (Fig.10A).

There are two prominent oral ridges and maxillary papilla (Fig.10B).

The peritreme of the posterior respiratory spiracle is oval in shape and surrounded by tiny spines arranged in a cresentic shape anteriorly and posteriorly (Figs.11,11A) and are covered by dorsal tubercles (Figs 12,12A).

Fleshy processes in the form of blunt tubercles are situated posteriorly to the ventral transverse bands of spines are recorded on segments 4 - 12 (Figs. 13,13A).

Third instar larva : The L.cuprina third instar larvae are creamy white in colour and range from 11 -14 mm in length and 3.3 mm in width(average of 10 larvae) .

It consists of twelve visible segments. The first segment (cephalic segment) is morphologically identical with those of the second instar larvae except that the cephaloskeleton is 1.58 mm in length (Fig.14) (average of 10 larvae).

A pair of anterior spiracles on the prothoracic segment are characterized by the presence of digit - like protrusions (six in number) each with a slite - like opening (Fig. 14).

Usually the girdles of spinules are complete on segments 2 -8 , on 10 and 11 , they are interrupted dorsally on segment number 12 and usually also on segment number 9 .

Well developed fleshy processes appeared in a posterior situation to the spine bands at segment 4-12 , these processes may be aid the larvae in crawling and burrowing through the soil.

The posterior peritremes are semicircular in shape, hardly chitinized , with 3 respiratory pores (Fig.15) , the former is surrounded by fleshy tubercles with sensory hairs .

SEM micrograph of the 3rd instar larva revealed a well developed bilobate cephalic segment (Fig.16) and showed antenno-maxillary lobes.
Antennal lobes are composed of a large central cone-shaped sensillum surrounded by a cuticular segment.

The maxillary sensory complex are sensory papillae.
The shape of the spines on body segments are triangular with a broad base attached to the body segment and free pointed end (Fig. 17).

Moreover, SEM observation demonstrated the correct situation of fleshy processes and spine bands on each segment. The fleshy processes are in anterior situation and the transverse band of spines are in posterior situation (Fig.18). The last segment carries dorsal fleshy tubercles and several rows of spines. The respiratory depression is deep and surrounded by fleshy tubercles which might work as sense organs and had a pair of semicircular-shaped peritremes (Figs.19,19A).

Fig.(1): 1st instar larva of Lucilia cuprina (lateral view) (CS) cephaloskeleton (al) antennal lobe (or) oral ridges. x40 .

Fig.(1A): . (Ps) peritreme with posterior spiracle (SP) sensory papilla.x100.

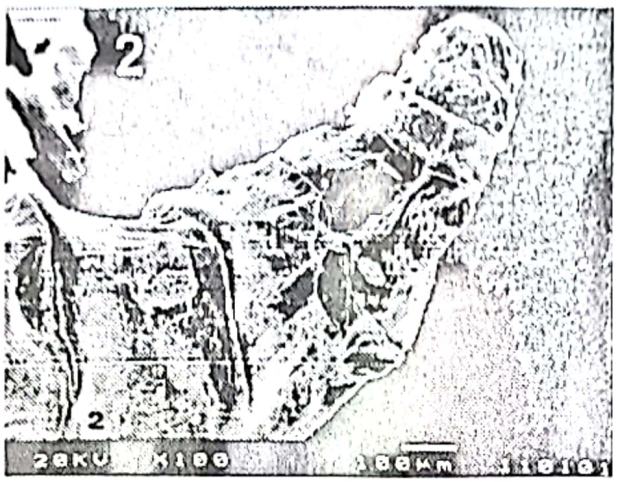


Fig.(2):SEM micrograph of Lucilia cuprina 1st instar larva . x100

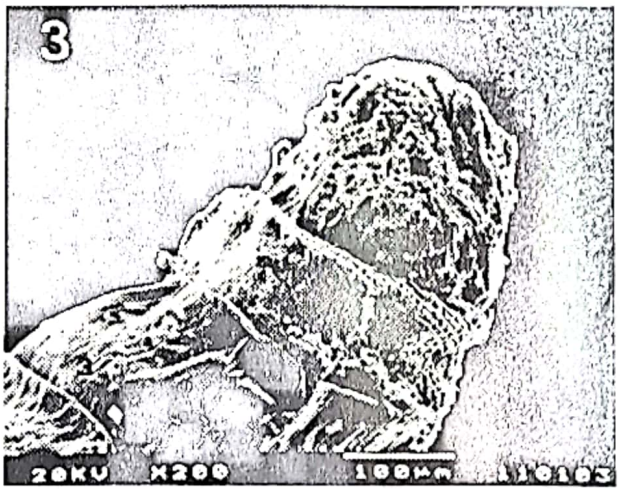


Fig.(3):.showing tapered spines with enlarged base (SP) spines x200.

Fig.(4):(fp) foot pad group in a ventral median situation longer than the spines X200

Fig.(5):SEM micrograph of Lucilia cuprina (as) anterior spiracle X1500

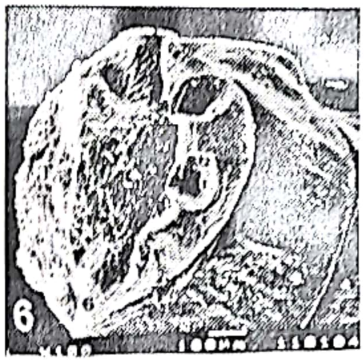


Fig.(6): SEM micrograph of Lucilia cuprina (sc) stigmal cavity.x100

Fig.(7): 2nd instar larva of L.cuprina , lateral view , (bc) bilobed cephalic segment .x40

Fig.(7A): 2nd instar larva with (or) oral ridges (mh) mouth- hooks .x100.

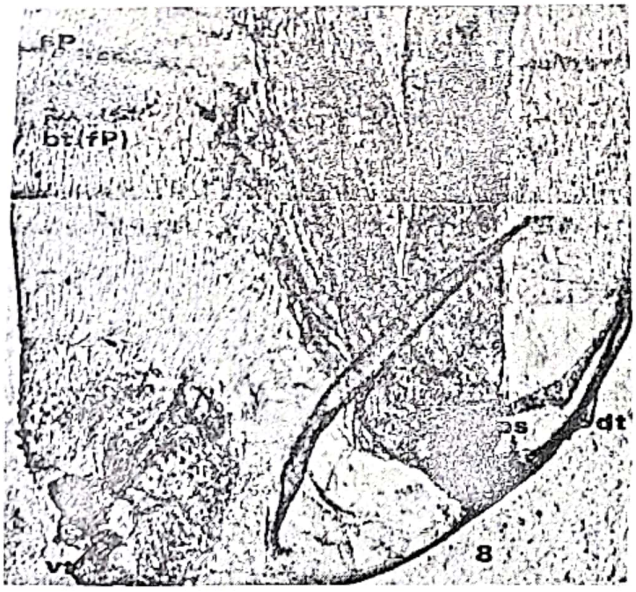


Fig.(8): posterior segments of 2nd instar larva of L.cuprina showing bt(blunt tubercles (fp) foot pads) and (sp)spines, (ps) posterior spiracle surrounded by (dt) dorsal tubercles and (vt)ventral tubercles .x100.

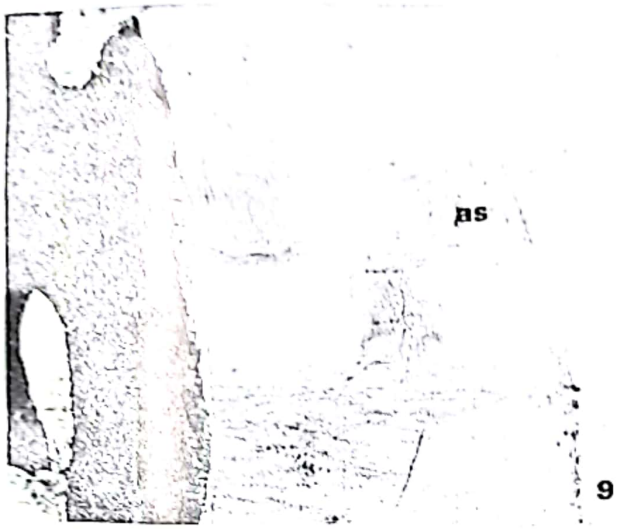
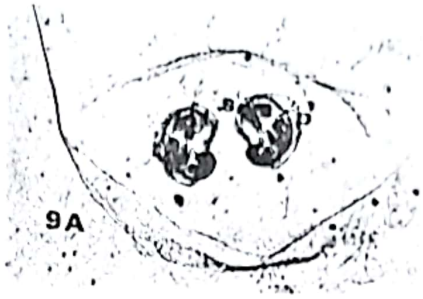


Fig.(9): lateral view of 2nd instar larva of L.cuprina showing (as) anterior spiracle of prothoracic segment x100.



Fig(9.A):posterior end of 2nd instar larva of L.cuprina showing the (p) peritreme with the (so) spiracular opening x40.

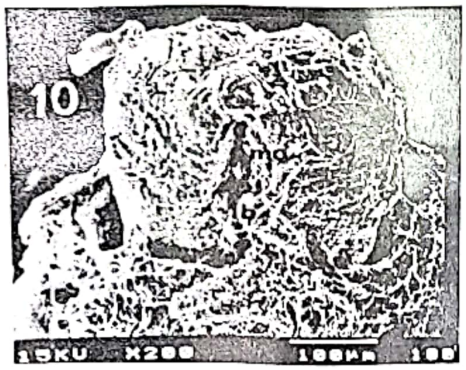


Fig.(10):SEM micrograph of L.cuprina 2nd instar larva showing (mo)mouth opening with (Lb)labial lobe x200

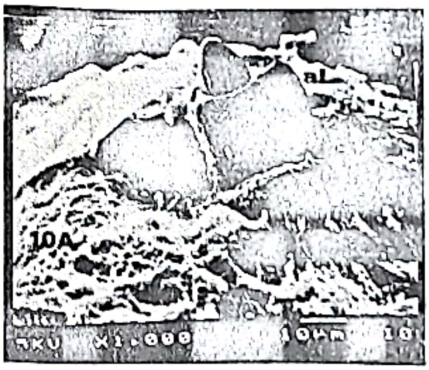


Fig.(10A): SEM micrograph of L.cuprina 2nd instar larva (al) antennal lobe x1000.

Fig.(10B): SEM micrograph of L. cuprina 2nd instar larva (anterior view) showing (or) oral ridges and (mp) maxillary papilla x200.

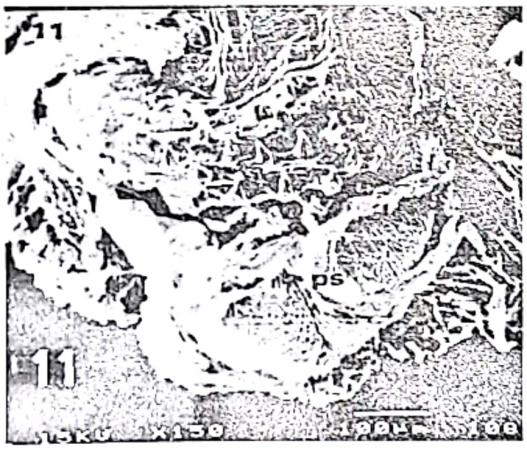


Fig.(11):SEM micrograph of 2nd instar larva showing (ps) posterior spiracles surrounded by (ts) tiny spines x150 .

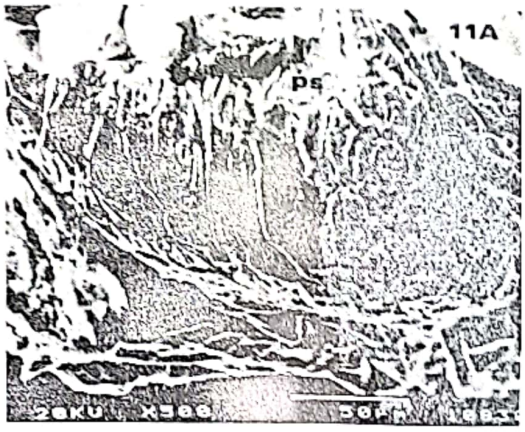


Fig.(11A): SEM micrograph of higher magnification (ps)posterior spiracle X 500.

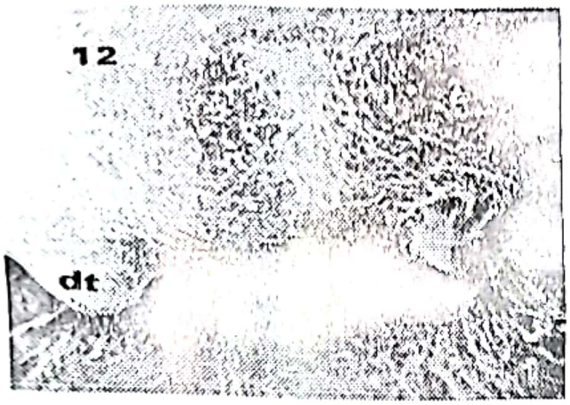


Fig.(12): SEM micrograph of 2nd instar larva , (dt) dorsal tubercle covered the posterior spiracle x100.

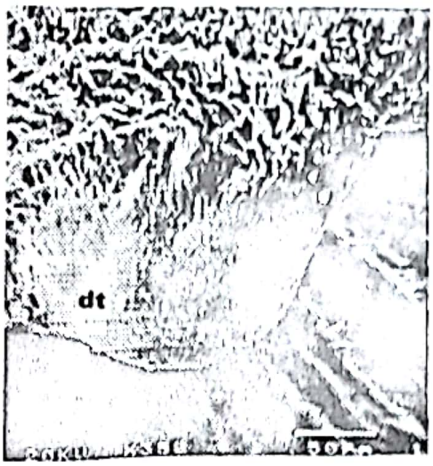
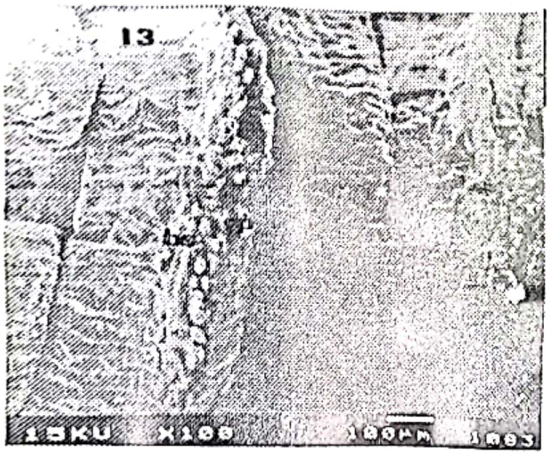
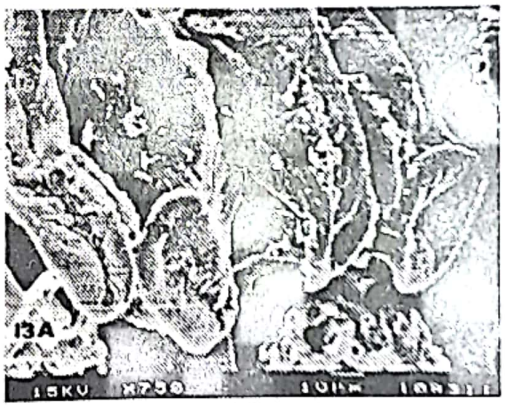


Fig.(12A): SEM micrograph of higher magnification (dt) dorsal tubercle x3 50.



Fig(13):SEM micrograph of 2nd instar larva showing (fp)fleshy processes and (bs) bands of spines on the ventral side x100.



Fig(.13A): SEM micrograph showing higher magnification (fp) fleshy processes on the ventral side x750

Fig.(14): lateral view of 3rd instar larva L.cuprina showing (cs) cephaloskeleton (as) anterior spiracle x40.

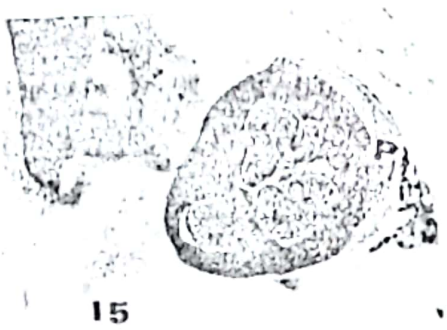


Fig.(15):showing hardly chitinized peritreme with three (so) spiracular opening x100.

Fig.(16):SEM micrograph of 3rd instar larva of L.cuprina cephalic segment bilobate showed (aml)antenno-maxillary lobes x200.

Fig (17): SEM micrograph of 3rd instar larva L.cuprina showing the shape of spines on body segments x350.

Fig.(18):SEM micrograph of 3rd instar larva L.cuprina showing (bs) bands of spines and (fp)fleshy processes on the body segments x350.

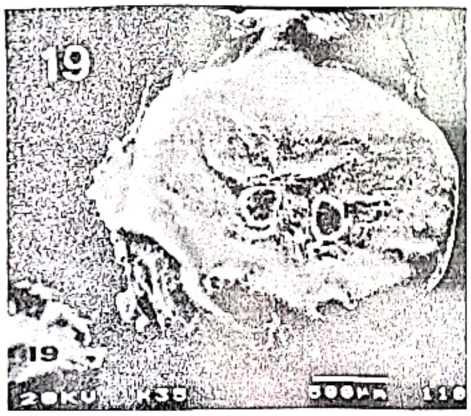


Fig.(19): SEM micrograph of 3rd instar larva L.cuprina showing (ps)posterior spiracles on the last segment in (p)periterme witch surrounded by a number of (t)tubercles x35.

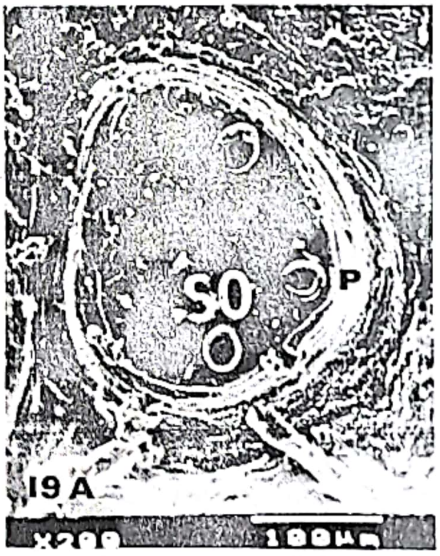


Fig.(19A): SEM micrograph showing a high power of a semicircular (p)peritreme with three (so) spiracular opening x200.

DISCUSSION

This study presents light microscopic and , SEM observations on the 1st , 2nd and 3rd instar larvae of Lucilia cuprina clearly emphasizing most of its important morphological features. As reported by Zumpt (1965) the larval instars of L.cuprina. The cephaloskeleton of the 1st instar larvae are small, anteriorly , the portion of the labial sclerite bent slightly downwards., cephaloskeleton of 2nd & 3rd instar larvae have fully-developed mouth hooks.

The present study demonstrated that the antennal lobes were poorly developed in the 1st instar larvae, but gradually showed a higher degree of development in the 2nd stage & were especially prominent in the 3rd instar larvae . Teskey (1981) concluded that the oral ridges may function like the pseudotracheae of the adult were it directing the liquids by capillary action into the mouth or may be filter out the solid particles.

Chamberlain et.al., (1969) stated that the absence of cuticular oral ridges on the cephalic segments of both Cuterebra fontinella and Hypoderma spp. 1st instar can be considered an adaptation to parasitism and reflect the difference in the mode of feeding between parasitic larvae and their free-living counterparts.

The 1st- instar larvae of Cuterebra and Hypoderma apparently do not ingest particulate matter and it has been suggested that a part of the nutrient requirements of these larvae is acquired across the cuticle.In L.cuprina 1st instar larva adapted to semi-liquid nutrients with oral ridges and folds to guide the fluid to the mouth .

Light microscopic observation presented the distribution of spines on the body segments of the 1st, 2nd and 3rd larval instars of L.cuprina this arrangement was in agreement with that reported by Zumpt (1965) .However, the light microscopic and SEM observations gave more details on the arrangement and situation of these spines and also the fleshy processes on the ventral side of the abdominal segments of the 1st, 2nd and 3rd larval instars . The fleshy processes on the ventral side of segments 4-12 were described previously by Smit, (1931) as footpads. These aid the larvae in crawling and burrowing through the soil.

Fahmy (1991) gave detailed SEM morphological features of 3rd larval instar of Cephalopina titillator demonstrating that each body segment is provided with eight bilobed fleshy processes and creeping welts which carry 3-4 rows of spines.
Abdel-Meguid et.al (1993) used light and SEM to study the morphology of the 1st, 2nd and 3rd larval stages of Cephalopina titillator.

The present SEM investigation clarified that the fleshy processes are anteriorly situated , but the tiny spines are posteriorly located in each segment from 3-12 .

The prothoracic pairs of spiracles of 1st, 2nd and 3rd instar larvae are present with typical digit-like protrusion (six in number) which is agreed with Zumpt (1965).

The last segment carried dorsal and ventral fleshy tubercles with spines which might act as cuticular sensilla. However, the major diagnostic feature of each larval instar are the posterior spiracles.
These are situated on the posterior segment and can be clearly seen by SEM . The differences between instars under the SEM can be correlated directly with light microscope observations (O'Flynn and Moorhouse,1980).

Several authors also have studied and compared the number and type of sensilla on parasitic larvae with Those on free living dipterous larvae.

(Colwell,1986;Chu&Axtell,1971;1972; Ryan& Behan, 1973 a,b: Singh & Singh, 1984;Ajidgaba et.al., 1985; Schmidt J.M,1993). These studies indicated that the increasing number and great morphological diversity of sensilla correlated with increasing requirements for location of a suitable environment in which the larvae feed and develop.

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MYCOTOXINS RESIDUES IN SOME EGYPTIAN POULTRY MEAT PRODUCTS

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SUMMARY

The objective of the present study is to determine the aflatoxin B_1 (AFB_1) and ochratoxin A (OCHA) level in some chicken meat products. AFB_1 and OCHA were evaluated in 68 samples of chicken meat products collected from different companies in Giza city. The tested processed chicken meat products were chicken burger, grilled shish, pre-fried pate, chicken kebbeh balls, chicken nuggets, sesam fillet chicken, chicken luncheon and smoked chicken luncheon. Highest incidence of AFB_1 was detected in chicken kebbeh balls (70%), while lowest incidence was reported in chicken nuggets (8.34%). No AFB_1 was detected in grilled shish, chicken luncheon and smoked chicken luncheon. All positive samples contained more than 5 ppb AFB_1 except chicken nuggets which contained 3.1 ppb. Frying decreased the level of AFB_1 and roasting was more effective in this respect. Highest incidence of OCHA was detected in grilled shish (71.43%) , while lowest incidence was reported in chicken nuggets (25%). No OCHA was detected in chicken luncheon and smoked chicken luncheon. All positive samples contained more than 10 ppb OCHA. Frying and roasting decreased the level of OCHA but its level is still above the permissible limits (10 ppb).

INTRODUCTION

Mycotoxins are toxic metabolites produced by certain toxigenic microscopic fungi in or on foods. Some mycotoxins are carcinogenic and others cause pathological effects on the body. Mycotoxin containing foods have been found all over the world: Africa, Asia, North and South America, Australia and Europe (Ostr, 1999). Natural contamination of foods and feeds by