SCANNING ELECTRON MICROSCOPY OF *OESTRUS OVIS* (DIPTER: OSTRIDAE) LARVAE RECOVERED FROM SHEEP INFESTED WITH CUTANEOUS MYIASIS IN SAUDI ARABIA

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INTRODUCTION

Myiasis with dipterous larva is well know in human and vertebrate animals at least for certain period feeding on host's dead or living tissue, liquid body substances or ingested food (Zumpt, 1965). Some cases were recorded on animal myiasis (El-Azazy *et al.*, 1994; Cameron *et al.*, 1991; Omar *et al.*, 1988) found that *O. ovis* infested sheep nose in Kingdom of Saudi Arabia .

Zohdy and Morsy (1982); Grassberger and Reiter (2002) recorded the presence of screw worm at 30°C, while it needs longer developmental duration for larval and pupal stages at 20°C. (Hussein *et al.*, 1983; Banaja and Madbouly, 1981) described the 3rd larval instar of *Cephalopina titallator* in camel found in the western region of Saudi Arabia .The all foregoing investigators used the light microcopy for their researches. Giannetto *et al.*, (1999) described the larval instars of *O. ovis* by using SEM. The present study is the first for some ultrastructure morphological details on *O. ovis* 3rd larval instar by SEM.

MATERIAL AND METHOD

Larvae of *Oestrus ovis* were collected from the sheep cadavers of an abattoir in Dammam (eastern area in Saudi Arabia) through the year of 2004.

The larval specimens were removed manually from the lesions, and then washed several times in normal saline solution to remove any foreign debris that might obstruct the view of structures during electron microscopy. The larva was examined under binocular microscope and identified by the morphological characters given by Smith (1973). For SEM examination, the specimens were fixed in formaldehyde 10% for 24 hours then dehydrated in ascending series of acetone and the critical point of dryness was attained with liquid carbon dioxide. The

specimens were then coated with gold and examined by Jeol SEM (T330A). The third instars which were quite large were cut into three portions (head, body and caudal portion) before initial chemical treatment. Due to their large size, this additional procedure was necessary for an easy process.

RESULTS AND DISCUSSION

Body of the third instar larvae of *O. ovis* is cylindrical, approximately 2 - 2.5 cm in length and 9 mm in width; the larval body consists of 3 parts: Head, body and posterior end.

Cuticle:

The front part of cuticle (Figure 5A) showed small irregular hexagonal structure while this structure was too large on the posterior part of the body (Figure 5B) and it was very small around spiracles (Figure 5C). This result agrees with that mentioned by Giannetto *et al.*, (1999) with some variation in shape of cuticle.

Head:

Large hooks were observed consisting of two parts, protruded from the head, they are dark black in color and representing the remaining mandible as the main mouth parts (Figure 1 A, B). Under SEM, they look like large hooks having a smooth surface, (Figure 5 D).

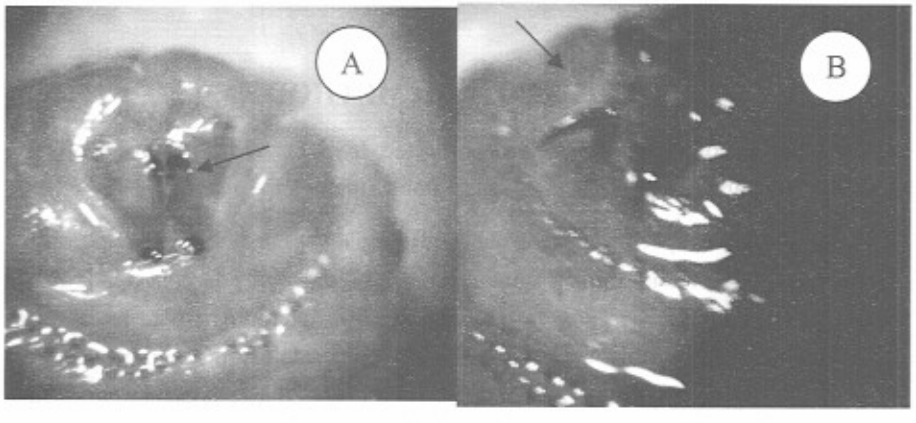

Figure1 Light Microscope for anterior part of *O. ovis* 3rd larval instar head showing the hooks. A: (front view) (40X), B: (lateral view) (40X).

They may be used for feeding and cutting the host tissue. Modifications of mouth hooks especially in the third larval instar are helpful in explaining the ferocious feeding ability of older maggots. This result agree with Giannetto *et al.*,

(1999) who's described *O. ovis* with contradiction in size of hooks to small size than the present finding, even the front of head have a surface differs in integumental surface shape than that observed by SEM as an irregular a hexagonal surface structure which provide hardness; this shows distinction from description of Giannetto *et al.*, (1999).

Body:

The body has 12 equal segments; on dorsal surface the cuticle was so strong with dark brown lines. From the ventral side of the body, there were rows of spines (Figure 2A) on all segments, by high magnification there were 3-4 alternate rows (Figure 2B) they were all of the same size and shape. This finding agrees with that described by (Omar *et al.*, 1988; Cameron *et al.*, 1991; Grammer *et al.*, 1995; Nacapunchai *et al.*, 1995; Lucientes *et al.*, 1996; Wolfel-schneider and Wiedemann, 1996; Gregory *et al.*, 1998; Brisou and Menard, 2000; Suzzoni-Blatger *et al.*, 2000; Health and Johnston, 2001; Suarez *et al.*, 2001; Masoodi and Hosseini, 200; Hakimi and Ismail, 2004) they observed the spines in the same size and shape nearly as observed by the Light microscope. The spines cleared by SEM some like squares shaped with sharp pointed end and some other of spines were like pyramidical spines with curved tapering pointes at ends (Figure 3,4 A,B).

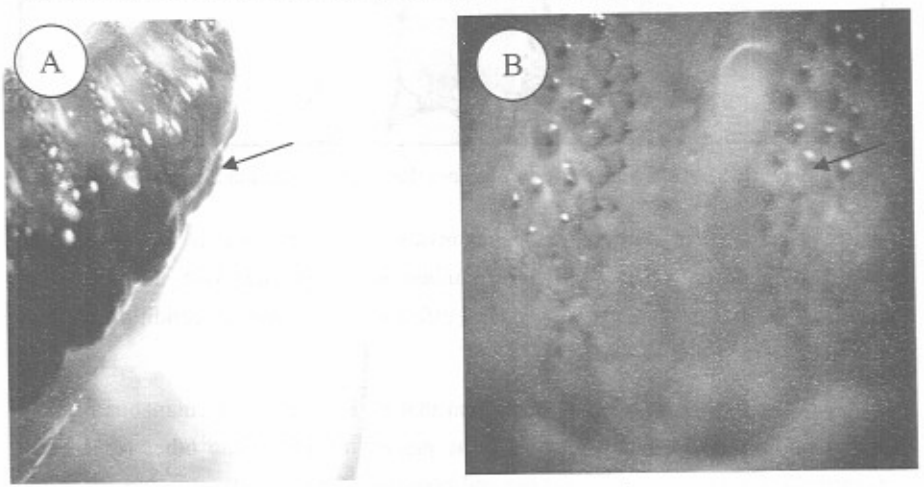

Figure 2: Light Microscope for body of the third larval instar:
A: segments of the body form ventral view showing the rows of spines. (Arrow)(20X)
B: High magnification showing the body segments from ventral view with 3-4 alternate rows of spines (40X).

Posterior end:

The larval posterior end observed like square, with two posterior spiracles, this structure is a characterization of Dipteran larvae. Each spiracle had a circle or

half-ring opening with varied integument structure around it. The spiracle consists of plate with sclerotized central portion and peripheral circle of papillae (Figure 6 A, B). In the middle, there is slit opening surrounded by a radial line, and the surface of spiracle have a smooth surface like sponge inside of pores. By high magnification, it looks like papillae on the surface (Figure 7 A, B). The spiracle has radial line around the central opening of (Figure 7 C), while spiracle surface have a node wide spear through a smooth area (Figure 7D).

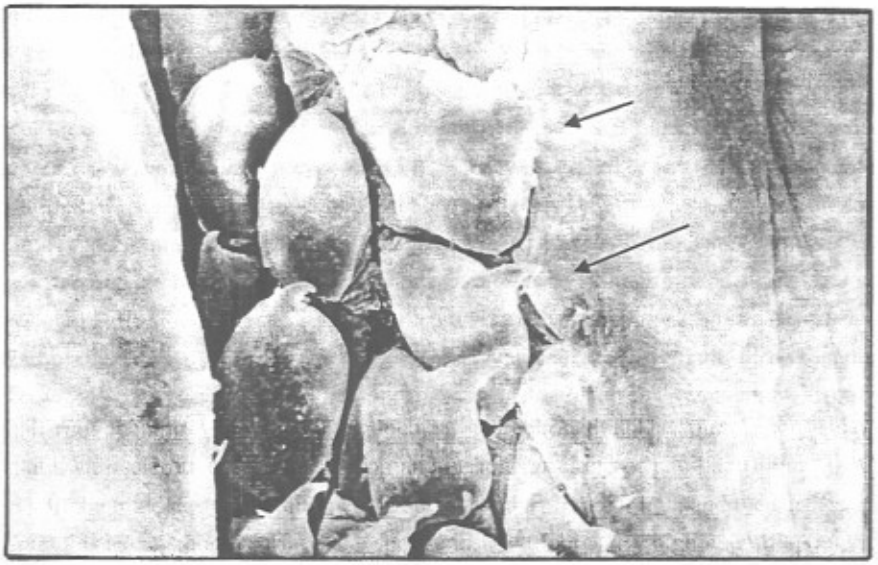

Figure3: SEM showing the rows of spines that differ in size and Shape (150X).

The present study on cutaneous myiasis caused by *O. ovis* was not studied in K.S.A or described by SEM; most studies were concerned with the infestation levels of *O. ovis* previous life cycle, and effect of environmental condition on their presence as recorded in (Table 1).

Gregory *et al.*, (2004) mentioned that *O. ovis* caused a cutaneous myiasis for goats and sheep that agreed with the present results; while other researchers recorded infestation in the head, nose and eyes for different hosts.

(Table1) cleared the present of infestation by *O. ovis* in different areas around Saudi Arabia that have same environmental conditions of temperature and humidity like Egypt (El-baky, 2001). (Table 2) recorded the SEM studies for other species of myiasis in Kingdom of Saudi Arabia and Kuwait that were observed by light microscope.

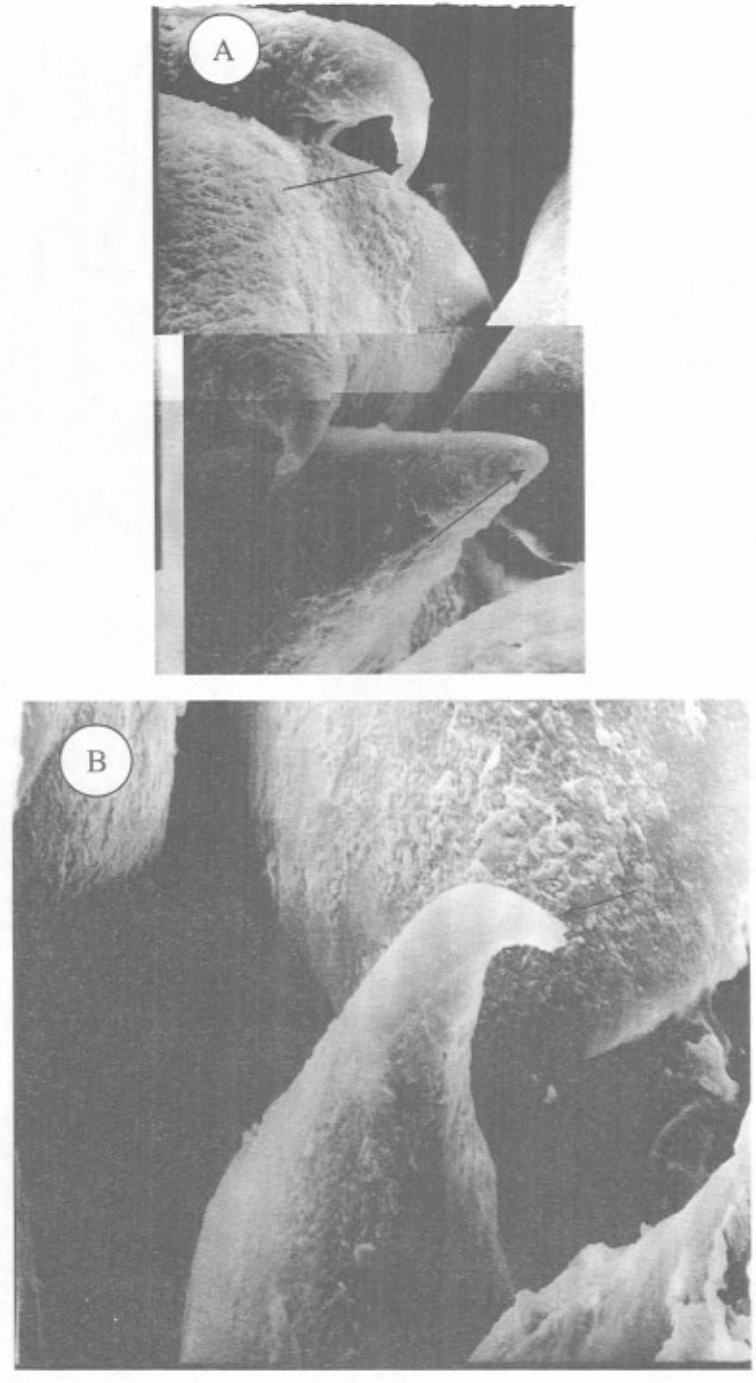

Figure 4: A: High magnification of spines, not equal in shape with curved pointed end (1500X). B: Sharp curved tip of spine (1500X). (Arrows)

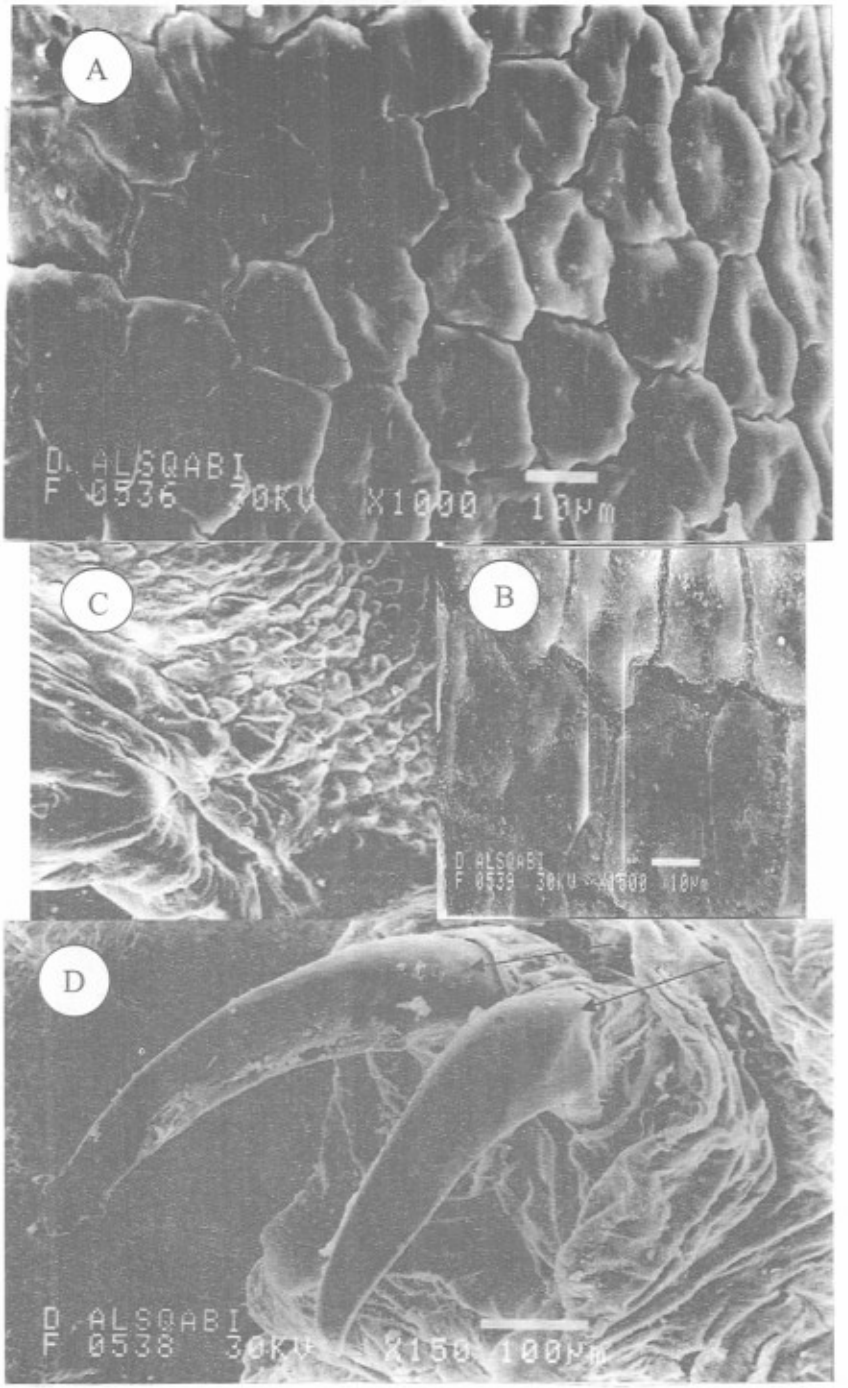

Figure 5: Body wall and hooks. Body wall integument with hexagonal structure; A (1500X); B (1500X) (in front) and C (1500X) (posterior part); D: hooks protruded form front head and mouth part (150X) (arrows).

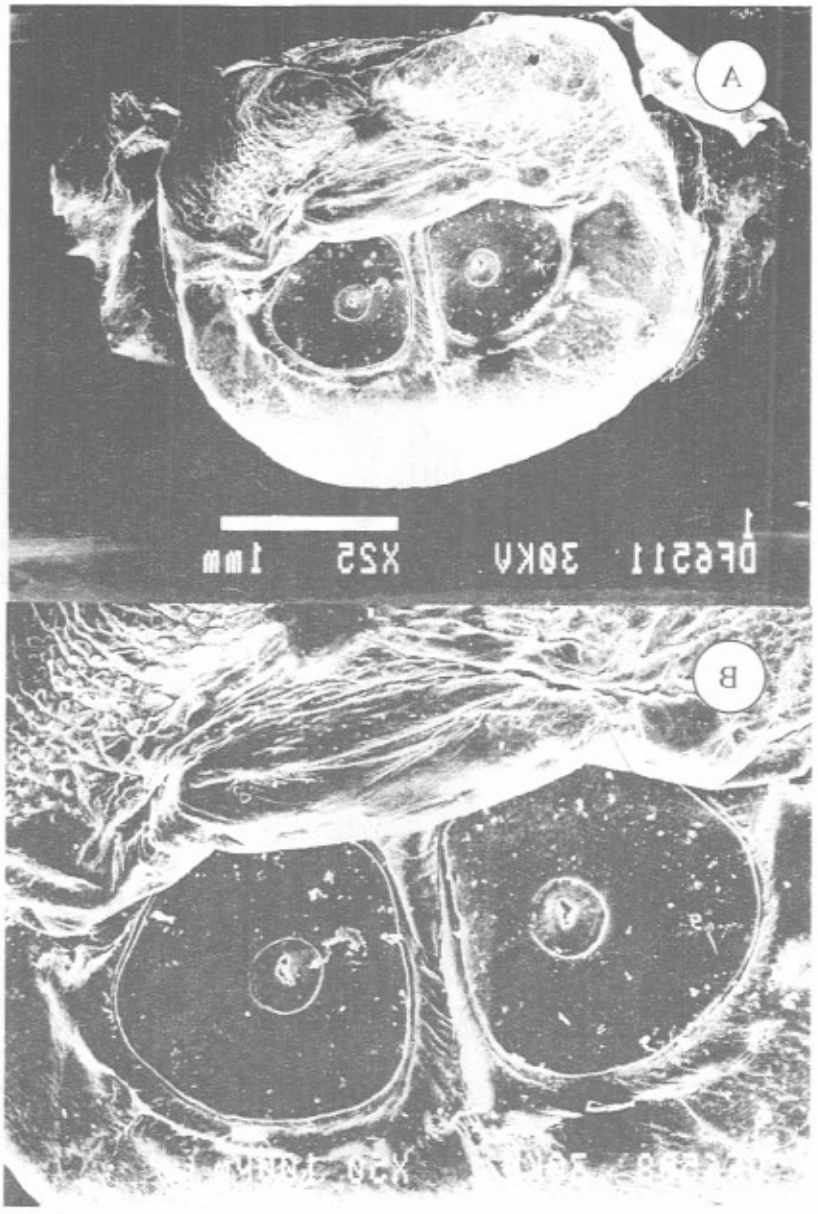

Figure 6: Spiracles. A: Spiracle with round plate and small central pore (25X). B: High magnification of spiracles showing the surface of spiracle having many small pores as spongy surface (50X)(arrows).

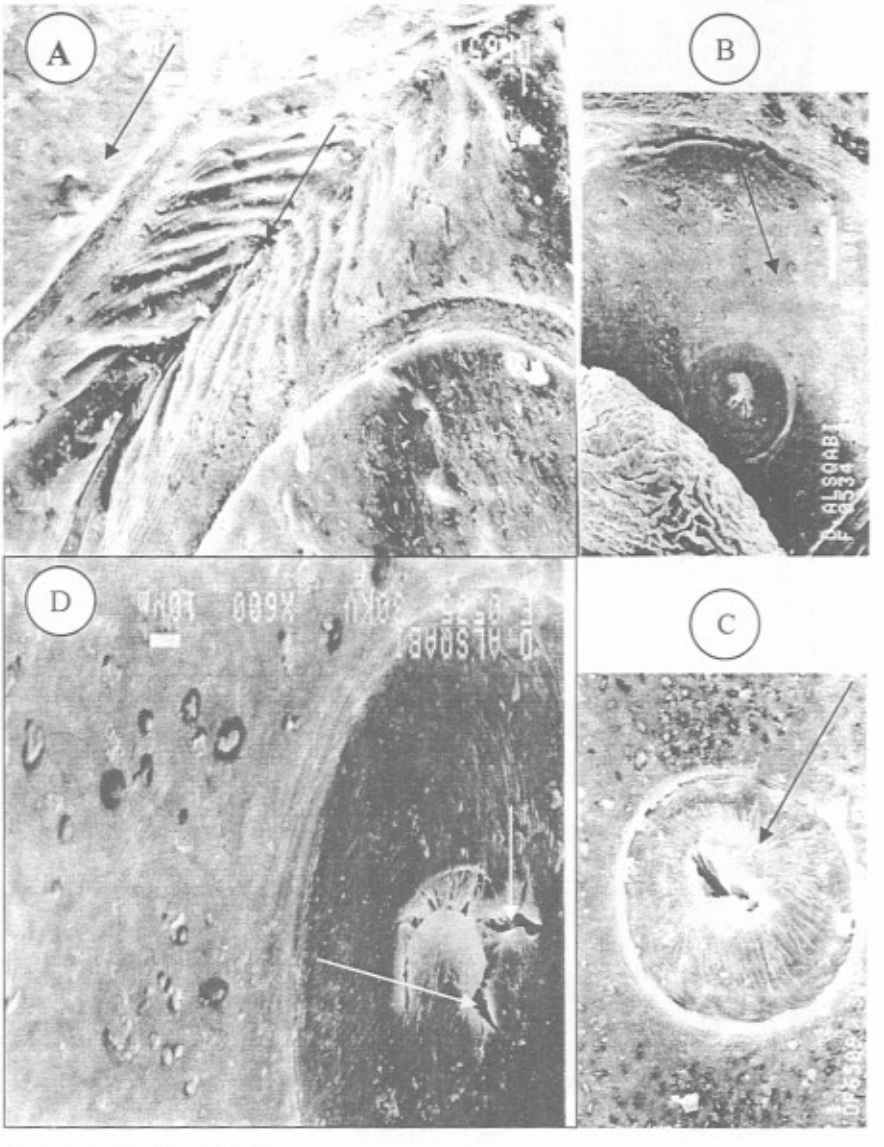

Figure7: Structure of spiracles.

A: High magnification of spiracle showing the space between the spiracles with the horizontal radial line adherent from the middle and the surface have a node wide speared on the spiracle (800X) (Arrows)..

B: Higher magnification of spiracle surface has papillae on it (600X) (Arrows).

C: Higher magnification of spiracle showing the radial line around the central opening of spiracle (1500X) (Arrows).

D: Higher magnification for spiracle surface has a node wide spear through a smooth area (100X).(Arrows).

In Italy *O. ovis* was studied by Giannetto *et al.*, (1999) which cleared differentiation in the shape and size of hooks where they were smaller and differ in shape. The present study shed light on the importance of studies by SEM for fine structures of *O. ovis* that could be useful for future identification of Osteridae larval species that exists in Saudi Arabia.

TABLE (I)

The most important studies on myiasis, *Osetrus ovis*.

Region	**Site Of infection**	**Author & year**	**Host**
Saudi Arabia	nose	Omar *et al.*, (1988)	Sheep
South west Germany	Right eye	Grammer *et al.*,(1995)	Man 54 year old
German	Nasal cavities Eye	Wolfel- schneider & Wiedemann (1996)	Human Sheep goat
Spin	Nasal eye external auditory canal of human	Lucientes *et al.*, (1996)	Human Sheep
Oman	Nasal left eye	Victor &Bhargra (1998)	Human 21 year old
Iraq	Skin eye nose	Gregory *et al.*, (2004)	Sheep human
Italy	Skin	Giannetto *et al.*, (1999)	Sheep
Beach in var (France)	eye	Brisou &Menard (2000)	Young women
France (Toulouse)	nose	Suzzoni-Blatger *et al.*, (2000)	Child
U K (London)	Nose	Stevens *et al.*, (2000)	Sheep
German	nose	Weinand & Bauer (2001)	Man 28 year old
Egypt (south eastern desert)	bodies	El-baky (2001)	Sheep goats
Argentina western pampas	head	Suarez *et al.*, (2001)	Sheep

TABLE (II)
Important microscopic studies on myiasis.

Region	Genus	Author & year	Type of microscopy
ITALY	*Osetrus ovis*	Giannetto *et al.*, (1999)	SEM
U K (London)	*Osetrus ovis*	Stevens *et al.*, (2000)	SEM
Canada	*Sarcophagid*	Colwell & Connor (2000)	SEM
Thailand	*Chrysomya megacephala*	Sukontason *et al.*, (2002a)	SEM
Thailand	*Megaselia scalaris*	Sukontason *et al.*, (2002b)	SEM
Iran	*Osetrus ovis*	Masoodi & Hosseini (2003)	light
Thailand	*Sarcophagid*	Sukontason *et al.*, (2003)	SEM
Thailand	*Megaselia scalaris (Dipteraphordae)*	Boonchu *et al.*, (2004)	SEM
Kuwait	*Lucilia sericata*	Hira *et al.*, (2004)	Light
Saudi Arabia	*Chrysommya bezziana*	El-azazy & El- Metenawy (2004)	Light
USA	*Gastrophilus*	Cogley (2004)	Light

SUMMARY

Oestrus ovis were recovered from the skin bodies of sheep's in Dammam (Saudi Arabia). Morphological ultrastructure studies of the third larva instar was observed by SEM. The cephalothoracic and terminal abdominal sensors, spiracle structure were observed. Spines of the various body regions were described.

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