Article available at http://www.parasite-journal.org or http://dx.doi.org/10.1051/parasite/1999061073

SCANNING ELECTRON MICROSCOPY OF *OESTRUS OVIS* LARVAE (DIPTERA: OESTRIDAE): SKIN ARMOUR AND POSTERIOR SPIRACLES

GIANNETTO S.*, SANTORO V.* & PAMPIGLIONE S.**

Summary :

The skin armour and posterior spiracles of L_1, L_2 and L_3 of *Oestrus ovis* (Diptera: Oestridae) are described and documented photographically by a scanning electron microscope. The complex and variable morphology of the attack organs of larvae of different stages makes it easier to understand better their exceeding adaptability to the host. The SEM survey has also allowed an apparently unreported anatomic particular with a probable sensory function to be detected in L_1.

KEY WORDS : *Oestrus ovis*, larvae, armour, spiracles, SEM.

Résumé : ÉTUDE AU MICROSCOPE ÉLECTRONIQUE À BALAYAGE DE L'ARMATURE DERMIQUE ET DES PLAQUES STIGMATIQUES CHEZ LES LARVES D'*OESTRUS OVIS* (DIPTERA: OESTRIDAE)

*L'armature dermique et les plaques stigmatiques chez les L_1, L_2 et L_3 d'*Oestrus ovis *sont décrites et illustrées par des photos au microscope électronique à balayage (MEB).*

La morphologie complexe et variable des organes de fixation des larves dans les différentes phases de leur développement fait mieux comprendre leur extraordinaire adaptabilité à l'hôte. La recherche au MEB a permis de mettre en évidence dans les larves L_1 un détail anatomique de fonction sensorielle probable qui ne semble pas signalé par d'autres chercheurs

MOTS CLÉS : Oestrus ovis, *larves, armature, plaques stigmatiques, SEM.*

The larvae of *Oestrus ovis* (Diptera: Oestridae) are host-specific parasites of the naso-frontal cavities of sheep, but they do sometimes attack such occasional hosts as man, dog, swine and goat. Shepherds are the human category most at risk; they may experience ocular, nasal, pharyngeal and laryngeal myiasis due to L_1 (Pampiglione, 1958*a*, *b*) and sometimes L_2 (Pampiglione & Canestri Trotti, 1991). The purpose of this paper is to provide a scanning electron micrographic (SEM) documentation of the skin armour and posterior spiracles of L_1, L_2 and L_3 of *Oestrus ovis* collected during surveys on the diffusion of the parasite in Sicily. SEM observations of the parasite larvae were made by Guitton & Dorchies (1993) who described their hooks and spines and by Giannetto *et al.* (1992) who reported the presence of sensory bristles on the ventral face of the first three larval segments.

*Cattedra di Parassitologia Veterinaria, Università di Messina.
**Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale, Università di Bologna.
Correspondence: Prof. S. Giannetto, Cattedra di Parassitologia Veterinaria, Via S. Cecilia, 30, 98100 Messina (Sicily), Italy.
Tel.: 090-2935133 – Fax: 090-2935850.

MATERIALS AND METHODS

Twenty-one larvae (ten of L_1, six of L_2 and five of L_3) were examined from sheep slaughtered at the municipal abattoir of Messina and coming from adjoining areas. After fixation in 2.5 % glutaraldehyde, the larvae were washed in a 50 % mixture of ethyl alcohol and glycerol, dehydrated, mounted on slide stubs, gold-metalised and observed by scanning electron microscope Cambridge Stereoscan 240.

RESULTS

FIRST STAGE LARVA (L_1)

The larva is spindle-shaped, about 1 mm long and 0.36 mm wide, slightly concave ventrally and consisting of 12 segments (Fig. 1).

The first segment bears two large divergent chitinous hooks with pointed, "bull's horn"-shaped ends. At their sides, there are four small papillate protrusions (Fig. 2). The ventral face of the first segment shows seven-eight rows of spines with single- and three-pointed ends (Fig. 2). The dorsal one bears four-five rows of straight or slightly bent spines with thinner ends.

The first segment is followed by three thoracic rings with two to four regular rows of triangular mainly three-pointed spines projected caudally (Fig. 3). On the sides, the spines appear as "tufts" (Fig. 2). In addition, the ventral portion of the three thoracic rings bears two symmetrical groups of three small divergent bristles 20 to 50 µm long (Fig. 2). These bristles are also present in L_2 and L_3. Dorsally, the first thoracic ring is nude, while the second and third rings show two complete and one incomplete rows of single-pointed spines, respectively (Fig. 4).

The thorax is followed by eight abdominal rings with no spines on their dorsal faces except for the first one which shows an incomplete row (Fig. 4). Ventrally, the rings are provided with a double row of prevailingly single-pointed spines which may be accompanied in the middle line by another parallel short row of eight to ten spines. Laterally, the spines appear longer and are grouped into small tufts (Fig. 1).

On the ventral face of the second abdominal ring there is an odd group of three 8 to 10 µm long bristles stemming from the base of a triangular skin prominence sited just below the spines at about 1 mm from the middle line (Fig. 5).

The last segment is more elongated and ends with the anal opening. Its ventral face bears 20 to 24 ventrally bent, "cat's claws"-shaped hooks distributed in a fan-like manner in three groups, one laterally and two centrally (Fig. 6).

As to the posterior spiracles, we were not able to evidence them by SEM. This was probably due to their position in the groove between the last and second-last segment, so that they were covered by the ridge of the latter. We were able, however, to photograph them at the optical microscope, by previous treatment of the larva with Hoyer's fluid (Fig. 7). They were slightly sclerotized, cylindrical, with minute holes thickly distributed at the surface. Their dimensions were about 30 × 20 µm.

Second stage larva (L_2)

It is 3 to 12 mm long, with a skin armour much less conspicuous than the one of the 1st-stage larva. Actually, the hooks of the first segment are not bull's horn-shaped as is typical of the L_1, but they are less robust and more curved (Fig. 8).

The segments of the dorsal face are disarmed save the second one which bears a series of small spines with occasional blunt ends.

On the ventral face, the thoracic and abdominal segments show single-ended, caudally projected spines. The posterior spiracles consist of sclerotized, almost circular plates with a medial interruption, which nearly entirely circumscribe the outlet of the respiratory canal that appears nearer to the internal than to the external border. The sclerotized plates bear several little holes arranged radially (Fig. 9).

Third stage larva (L_3)

It is longer than 20 mm (Figs. 10-11), with the first segment bearing again large hooks.

On the ventral face of the larva there are strong spines with broad supporting bases. Some of them are small and distributed along two rows on the second ring; others are larger and arranged in three rows on the second ring, and in four or even five-six rows on the subsequent ones. Their number decreases on the last rings (Fig. 10).

The dorsal face of each segment, except for the cephalic one, shows a stout sclerotized plate in the shape of a parallelogram with rounded edges (Fig. 11). The plates of the posterior spiracles appear strongly sclerotized and surround the outlet of the respiratory canal completely. The small holes are more prominent than those of the L_2 and are irregularly scattered (Fig. 12).

DISCUSSION

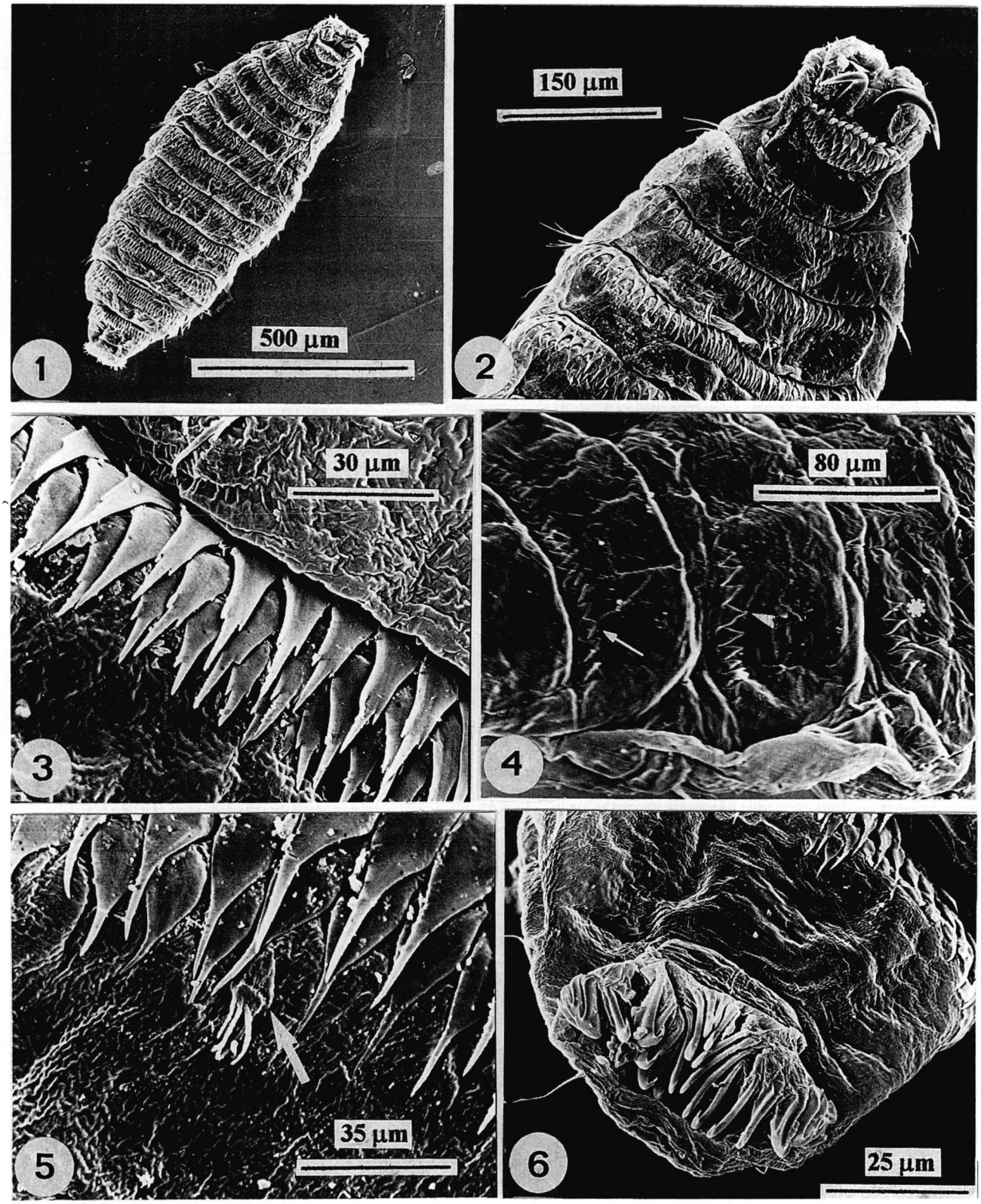
The SEM examination of *Oestrus ovis* larvae allowed us to put into clear evidence their surface morphology and to better understand their extraordinary adaptability to the host.

The two heavy buccal, bull's horn-shaped hooks, the long single- and three-pointed spines and those in the form of cat's claws are needed by L_1 to progress towards the frontal cavities and to anchor to the mucous surface in order not to be expelled by the strong expirations of the host or by the sneezing provoked by their irritating action. At the same time, this irritating action secures the production of an inflammatory exsudate which is prerequisite to the larva's feeding.

The L_2 bears a limited number of spines, this being due to the fact that it is situated in the host's paranasal and frontal cavities where there are no vertical air currents of the nasal cavities, and their function is limited to the production of exudate and to little movements.

Again, the L_3 is provided with large hooks, stout spines and dorsal plates which serve to favour its gradual descend in the nasal cavities to the outside environment and perhaps to its subsequent sinking into the soil for a few centimetres to change into a pupa.

Finally, the SEM confirms the presence of two symmetrical groups of three small divergent bristles 20 to 50 µm long in the ventral portion of the three thoracic rings L_1, L_2 and L_3. These bristles, with probable sensitive functions, where described in L_2 by optical microscope (Pampiglione & Canestri, 1991) and in L_2 and



Figs 1-6. – SEM *Oestrus ovis* L_1. 1: Ventral view. 2: Detail of the anterior region. Note the two symmetrical group of three small divergent bristles (arrows) located on the three thoracic segments. 3: Ventral face of the second and third toracic segment. The spines are prevailingly three-pointed. 4: Dorsal face with the double complete row of spines on the second thoracic segment (arrow) and the single incomplete row on the third thoracic (arrowhead) and first abdominal segments (asterisk). 5: Ventral face of the second abdominal segment. Skin prominence with bristles of probable sensory origin (arrow). 6: Ventral face of the last segment showing the "cat's claws" hooks.

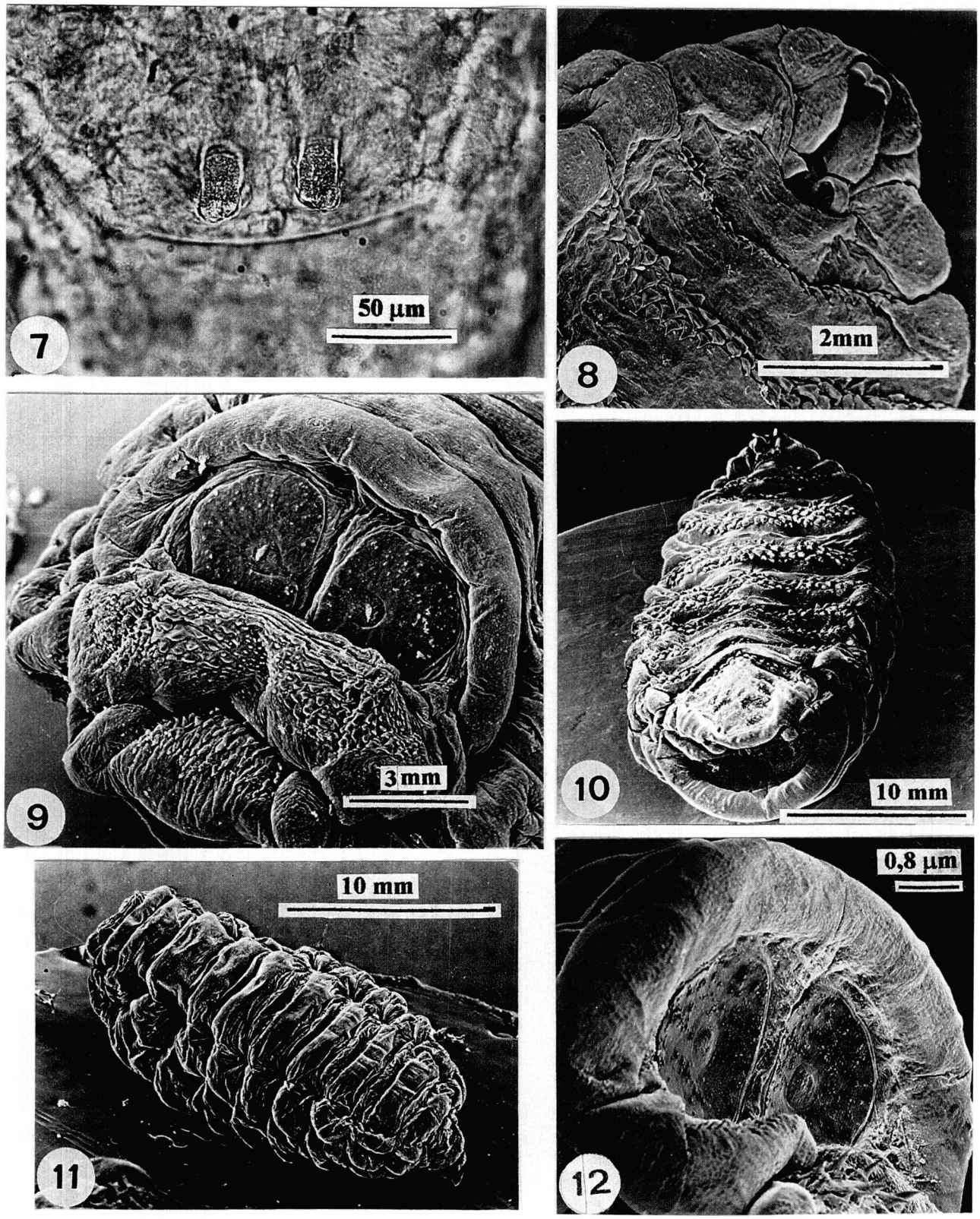


Fig. 7. – Posterior spiracles of *Oestrus ovis* L_1 (Light Microscopical).
Figs 8-12. – SEM *Oestrus ovis* larvae L_2, L_3. 8: Ventral view of the anterior part of L_2. 9: Posterior spiracles of L_2. 10: Ventral view of L_3. 11: Dorsal view of L_3. 12: Posterior spiracles of L_3.

L_3 by SEM (Giannetto *et al.*, 1992). We can add their presence now in L_1 also. This anatomic particular consists of the presence of an odd group of three 8 to 10 µm long bristles stemming from the base of a triangular skin prominence sited on the ventral face of the second abdominal ring. Histological investigations are already being carried out which may possibly ascertain whether they are sensory receptors which are not rare in insect larvae (Davies, 1991).

REFERENCES

Davies R.G. *Lineamenti di entomologia.* Ed. Zanichelli, Bologna, 1991, 42-44.

Giannetto S., Pampiglione S. & Canestri Trotti G. Su alcuni dettagli morfologici delle larve di II e III stadio di *Oestrus ovis*. *Parassitologia*, 1992, *34*, 207-208.

Guitton C. & Dorchies P.H. Étude des Larves d'*Oestrus ovis* (Linné, 1761) en microscopie électronique à balayage. *Revue de Médecine Vétérinaire*, 1993, *144*, 687-692.

Pampiglione S. Indagini epidemiologiche sulla miasi congiuntivale umana da *Oestrus ovis* in Italia. Nota I. *Nuovi Annali di Igiene e Microbiologia*, 1958*a*, *9*, 242-263.

Pampiglione S. Indagine epidemiologica sulla miasi umana da *Oestrus ovis*. Nota II. *Nuovi annali di Igiene e Microbiologia*, 1958*b*, *9*, 494-517.

Pampiglione S. & Canestri Trotti G. Miasi umana nasofaringea con reperto di larve di *Oestrus ovis* L. di II stadio. *Biologia Oggi*, 1991, *6*, 167-170.

Reçu le 7 octobre 1998
Accepté le 29 octobre 1998