

SCANNING ELECTRON MICROSCOPE STUDY OF WOHLFAHRTIA MAGNIFICA (SCHINER, 1862) (DIPTERA: SARCOPHAGIDAE)

I. STRUCTURES WITH PARASITIC AND POSSIBLE TAXONOMIC MEANING.

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Abstract

The larval development of Wohlfahrtia magnifica (the most important dipteran causing sheep myiasis in the Palearctic region) has been studied by means of scanning electron microscopy. The ultrastructure of mouth-hooks, oral ridges, labial lobes, body spines and anterior and posterior peritremes is described for the first time. Their possible adaptations to a parasitic lifeway are also discussed. Thus, the use of new structures in the Sarcophagidae taxonomy is proposed from the point of view of their ultrastructure and adaptative morphology.

Introduction

The application of scanning electron microscopy (SEM) techniques to the study of insect ultrastructure has become very useful (Stoffolano, 1970; Greenberg & Ash, 1972; Baccetti, 1975; Meek, 1976; Bay & Pitts, 1976; Kontermann, 1980). This technique is also useful to observe structural details of larval instars of the parasite Diptera. Nevertheless the papers on this subject are scarce (Adjidagba et al., 1985; Colwell, 1986; Principato, 1986; Sandeman et al., 1987; Principato & Tosti, 1988) due to the softness and delicateness of the larval structures. For this reason several methods for the preparation of this material have been evaluated (Grodowitz et al., 1982; Colwell & Kokko, 1986; Ruiz-Martínez et al., 1989a).

Because optical microscopic studies are insufficient to explain adaptations to parasitism (Barnard, 1977; Erzinclioglu, 1985; Lehrer & Fromuda, 1986), SEM has been used (Sandeman et al., 1987; Principato & Tosti, 1988) to determine the ultrastructural effects suffered by the hosts.

For these reasons Wohlfahrtia magnifica, which is the most important obligate parasite on sheep in the Palearctic region (Zumpt, 1965) and particularly, in the Mediterranean basin (Ruiz-Martínez et al., 1987), was studied by SEM to elucidate its taxonomic structures, their parasitic function and the changes that occurred during larval development.

Materials and Methods

One hundred and fifty specimens of Wohlfahrtia magnifica from 30 sheep were studied (40 corresponding to each instar and 15 belonging to each interinstar). This material was treated according to the method described in Ruiz-Martínez et al. (1989a). Structures from complete larvae as well as structures isolated by means of bistouri adapted to micro-handling were observed.

Larvae belonging to the three developmental instars were placed in vivo into filtered 10% formaldehyde. We removed particles adhering to the larval cuticle by a manual cleaning, using a fine brush, with alternate 10 min washes in 25 ml bidistilled water and 0.9% saline solution.

KEY WORDS: Scanning Electron Microscopy, Diptera, Wohlfahrtia magnifica, Myiasis, Taxonomy, Adaptive morphology, Larval development, Ultrastructure.

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Dehydration was carried out by lyophilization at -40°C and 125 bars, and critical point drying (CDP) with CO₂ as the transition fluid (at 31°C and 90 bars).² Samples were mounted on SEM stubs with double sided adhesive tape and silver conductive paint. Sputtering for different time periods (20 sec - 4 min) was also performed in order to determine the optimum time for sputter-coating with gold in the presence of argon gas.

Results

Buccal structures

The parts forming the buccal periphery are arranged frontally on the cephalic segment. These include the mouth-hooks (or mandibles), labial lobes and oral ridges.

The lower third of the mouth-hooks, which often projected to the outside, are covered by a cuticular expansions from the cephalic lobe. These structures exhibit remarkable ultra-structural and morphological variations during the larval ontogeny.

The first larval instar has 3 mouth-hooks, 2 lateral that are strongly curved and 1 central. Thus 3 cuticular expansions cover their proximal parts (Fig. 1). The hooks are closely disposed in the first phase (1-2 hours after laying)(Fig. 2); however, the lateral ones quickly separate to result in a 'trident'-shaped structure which is clearly evident in the interinstar I-II. At this time, the two cuticular protuberances, belonging to the new hooks of instar II, can also be observed. These occur above the central hook of instar I (in interinstar I-II the cuticle appears wrinkler and starts to shed)(Fig. 3). In the instar I a large number of canalicules are associated with the tips of the hooks (Fig. 2).

In the second larval instar, the mandibular system consists of two lateral strongly curved hooks that have their lower thirds covered by cuticle (Fig. 4). No ultrastructural differences exist between this instar and the first one. In interinstar II-III, changes similar to those in interinstar can be observed.

Alternatively, in instar III the hook's surface has a random reticulate appearance (Fig. 6). The curvature of the hook is smaller than in those shown in the preceding instars. Moreover, the hooks bear a cutting edge in their inferior flanks as shown in Figures 5 and 6. In the first phases of instars II and III, a set of bands with transverse orientation respective to the hook's direction can be clearly observed in the hook's surface (Fig. 7).

Several structures appear under the hook system in instar III: 2 lateral labial lobes, an occlusive muscle of the buccal cavity and a central labial lobe (Fig. 7), which is located between the mandibles. Its formation starts during interinstar I-II. In instar III, the oral cavity is surrounded by three lips, two lateral and one central, strongly thickened and beak-shaped that are located upon the mandibles (Fig. 5).

The oral ridges, which are present during larval development of *Wohlfahrtia magnifica*, show significant structural and dispositional changes: in instar I, they are a simple cuticular fold

Figure 1. Instar I. Mouth hooks. Late developmental phase of instar I. The maximum divergence between the lateral hooks with respect to the central one can be observed. A: median hook.

Figure 2. First phases of instar I showing no great divergence amongst the lateral hooks and the median one.

Figure 3. Interinstar I-II. Frontal view of the mouth hooks. The degeneration of the integument can be observed. B: mouth hooks belonging to instar II, disposed on a trident-like structure.

Figure 4. Instar II. Frontal view of the oral region showing disposition of the mouth hook with respect to the oral papilla, oral ridges and mouth modified spines. 1: oral papilla, 2: mouth modified spines.

Figure 5. Lateral view of the cephalic and first thoracic segments belonging to larva III. C: anterior peritremes, D: median lobe, E: inferior cutting margin of the mouth hook.

Figure 6. Detail of the ultrastructure of the mouth hooks belonging to the larva III. Showing the very thin and disorderly reticulate, and expansion of the labial lobe, covering its proximal and its cutting inferior margins (F).

directed to the oral cavity, in instar II they become modified and reach their greatest complexity in instar III (Figs. 3, 4 and 7).

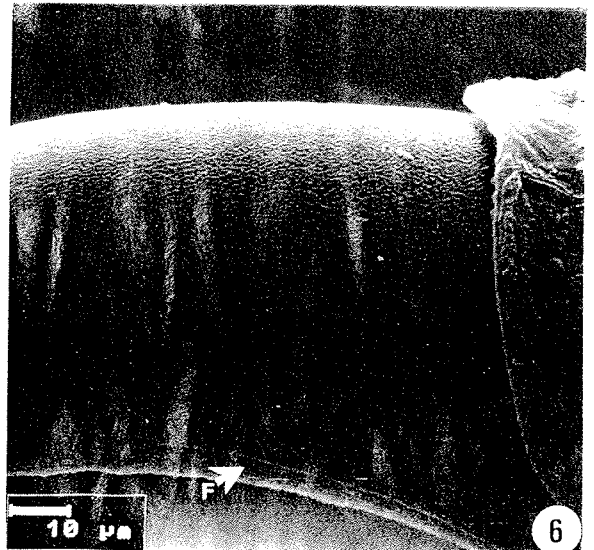
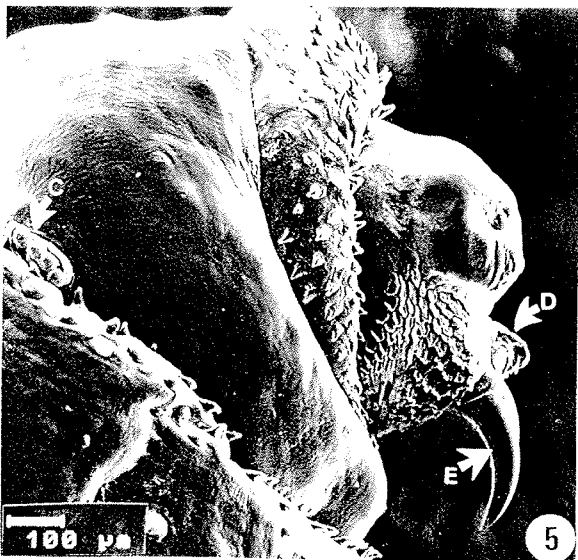
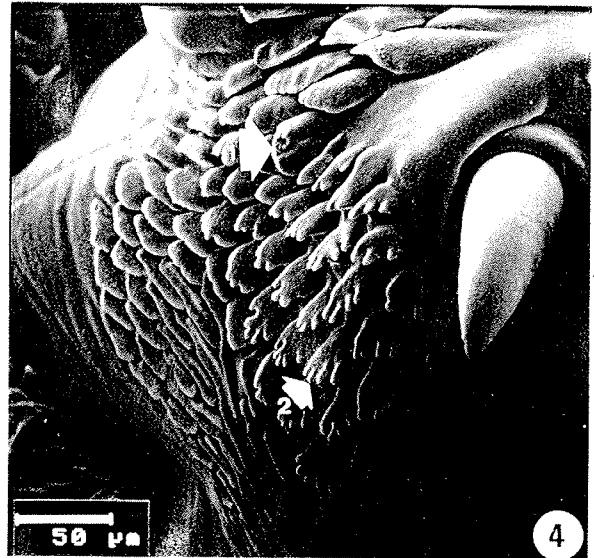
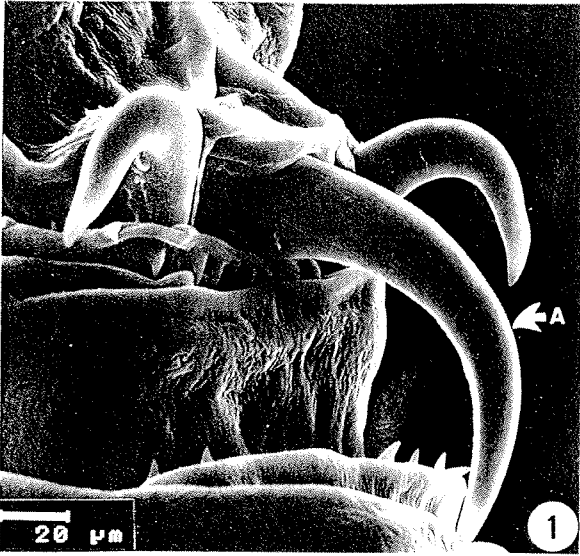
In instar II the oral ridges are organized into several bands formed by polyhedral cuticular scale-like expansions. Their anterior end is simple, but they show a greater complexity near the external edge of the hook (they show 1 to 4 lobes per scale)(Figs. 4 and 5). This system forms four canals that lead to the oral cavity. This structure is constant in instar III, with the scale-like expansions being more complex (with 3 to 8 lobes or expansions)(Fig. 7).

Body segments

The anal cavity, which is located ventrally in segment XI (Fig. 8) maintains a consistent structure during larval development. This cavity is delineated posteriorly by two rounded anal papillae, bearing a large papillar sensilla in their apices (Fig. 8). The sensillae are surrounded by a simple set of small cells in instar I. In instar III the number of these cells is larger. Near the papillar sensillae, campaniform sensillae can be observed (Fig. 8).

In instar I, the anal cavity bears 1 or 2 rows of spines in its anterior part; 3 to 4 rows occur in instar III. Between these spines and the anal papillae lies the anal opening. This opening is occluded by two muscles converging to its middle.

The body spines show remarkable changes at several levels (shape, structure and arrangement) during larval development. In instar I, the body spines, which are sharpened and hooked, are especially abundant on the ventral side of the thoracic segments (Fig. 9). The spines are replaced in each interinstar; consequently, it was possible to observe the disposition of the spines



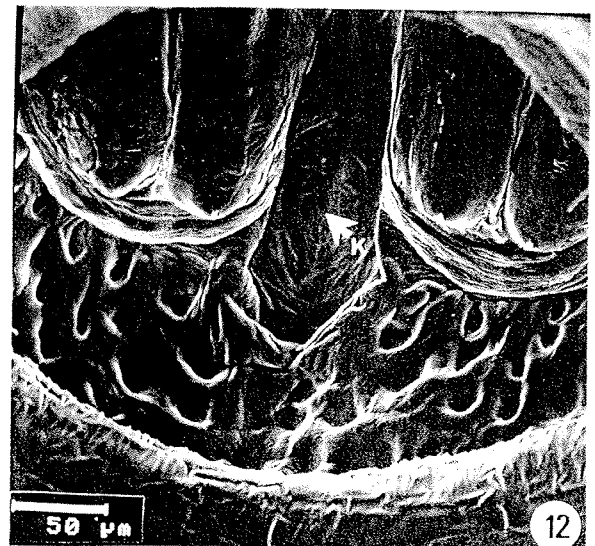
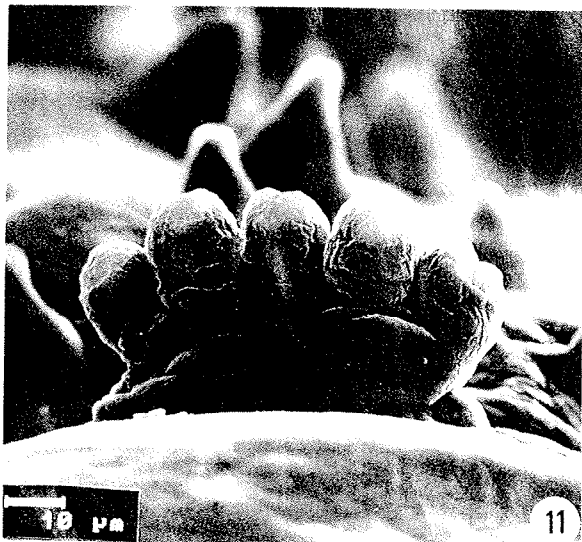
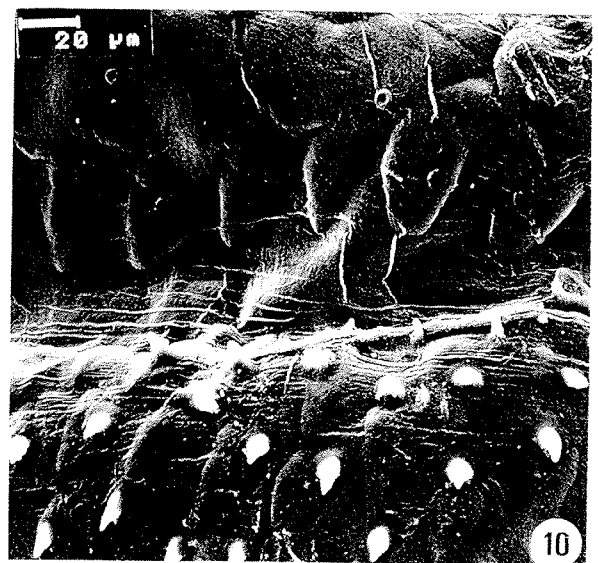
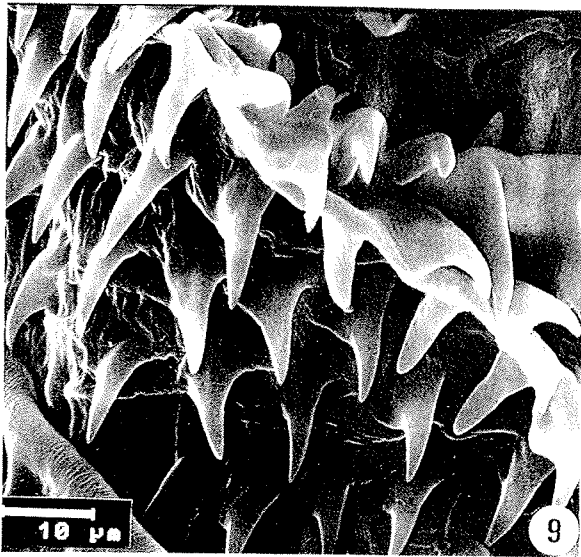
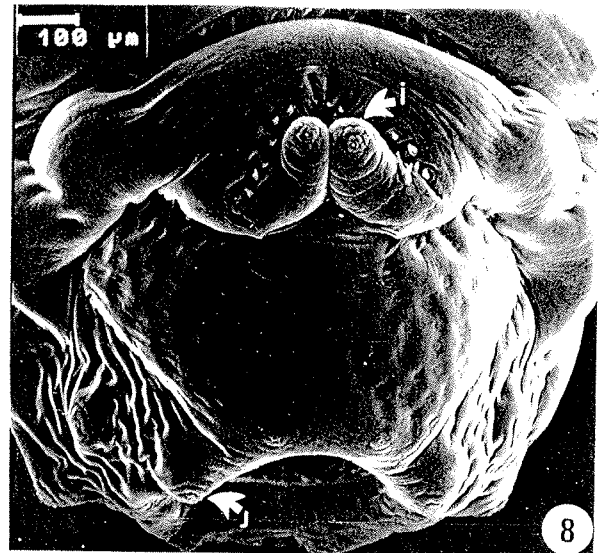


Figure 7. Instar III. Inferior view of the cephalic segment. Showing type of growth in the mouth hooks (G), the arrangement of the oral complex and the occlusive muscle of the buccal cavity (H).

Figure 8. Ventral view of the last abdominal segment showing the anal lobes (i) and the peritremal tubercles surrounding the peritremal cavity (J).

Figure 9. Instar I. Body spines in the first thoracic segment.

Figure 10. Interinstar I-II. Body spines. This figure shows the variation in size and disposition of the spines.

Figure 11. Anterior peritremes of Wohlfahrtia magnifica.

Figure 12. Instar II. Posterior peritremes. In the bottom of the peritremal cavity the wall connecting both peritremes can be observed (K).

on the new larval integument progressively degenerated during the following instar (Fig. 10). In instars II and III the spines are thicker, have a conical base and bear several grooves perpendicularly oriented in relation to the direction of the spine.

Spiracles

The anterior peritremes are laterally disposed in a small cuticular hollow of the base of the first thoracic segment (Figs. 5 and 11). Generally, they project towards the outside.

They consist of chitinous tubes each bearing 4 or 5 peritremal splits in instar II and 5 or 6 splits in instar III. On their apex, a cuticular expansion (which is able to close) is disposed. Each peritremal split is supported by 'Y'-shaped stigmatic sclerites (Lehrer & Fromuda, 1986; Principato, 1986; Ruíz-Martínez et al., 1989b).

The posterior peritremes are located in a deep cavity (typical in Sarcophagidae), where the peritremal splits are situated. They are oval, never closed and numbered 1, 2 or 3 in instar I, II or III, respectively. The ecdysial scar is always present at a lower level. The peritremes are placed in the inner wall of the XIth segment. Between these structures their joint suture can be observed (Fig. 12).

Discussion

Earlier morphological studies on Wohlfahrtia magnifica (Yasuda, 1940; Valentyuk, 1971; Schumann et al., 1976; Lehrer & Fromuda, 1986), failed to reveal the possible function of structures due to the limitations of the optical microscope. However, in our opinion, results from scanning electron microscopy can resolve several questions.

The mouth-hooks system shows a remarkable adaptation to the parasitic nature of Wohlfahrtia magnifica. A study of Lucilia cuprina recently determined if the onset of myiasis depended on

the larval development or on other infections or predisposing factors (Monzu, 1983, Sandeman et al., 1987). We have observed the developmental changes shown by the hooks in instar I during its first hours: from a folded disposition (this "folded" disposition would facilitate the exit of larvae across the uterus) to the lateral hooks diverging more with regard to the central one. Thus, an 'effective' anchor in a plane is acquired. Then, larva I has the capability to produce primary myiasis. The authors came to this conclusion in an earlier paper (Ruíz-Martínez et al., 1987; Ruíz-Martínez et al., 1989b).

The mouth-hooks of Wohlfahrtia magnifica are always projected according to their length and curvature, because their dimensions limit the retraction into the cephalic segment. This does not happen in Cuterebra fontinella (Colwell, 1986) and Lucilia cuprina (Sandeman et al., 1987).

The central hook is identical to the lateral ones, morphologically and ultrastructurally. Thus, we consider the "central hook" as the appropriate denomination for this structure, instead of "central sclerite". The presence of this central hook is possibly consistent with the parasitic nature of W. magnifica. This feature probably must be shown by W. vigil (Walker, 1937) and W. opaca (James & Gassner, 1947) which are the Nearctic species equivalent to W. magnifica. Nevertheless, these authors used "central sclerite" to designate this structure. Perhaps, this term may be adequate for similar occasional parasites such as W. pattoni (Hilton, 1973), W. bella (Gil Collado et al., 1974), W. meigeni and W. balassogloi (Valentyuk, 1971).

At the ultrastructural level, the presence of canalicules converging to the hook's apex in instar I and II may favour the penetration of the hook into the host's tissues. This fact is not observed in instar III; however the hook's thickness, its associated musculature and the presence of a cutting inferior margin in the hook may assist its penetration into the host tissues (Figs. 5-E and 6-F). Moreover, in this instar the most effective and prolonged penetration in development of this parasite is observed (its injuries are bigger and average life in myiasis is between 3-5 days). In this case, the mandibles should reveal the parasitic nature of this species; the maxillae are characteristic of other species such as Gasterophilus sp. (Principato & Tosti, 1988).

The set of transversal bands observed in the hooks during the first stages of each instar denotes their type of growth during the precedent interinstar (Ruíz-Martínez et al., 1989b) and shows the distinct chitinous coats deposited during the hook's formation. This process is similar in all instars (II, III). Alternatively, the structure and disposition of the labial lobes suggest that they control extension-retraction of the mouth-hooks during the larval locomotion in the myiasis.

The simplicity of the oral ridges in instar I is sufficient to direct the food towards the oral cavity, taking into account the anchorage system and the short length of this instar (6 to 8 hours). The posterior specialization and complexity of the oral ridges during instars II

and III is paralleled by the increase in size, invading power and food requirements. In this way, the "modified spines" not only serve as an accessory support to the hook during the larval locomotion (since these spines elevate themselves at a certain angle during the projection of the hook) but also produce an abrasive effect (mechanic effect of rubbing) and direct the food towards the oral ridges, located immediately below these structures. The presence of these oral ridges in *W. magnifica* suggests the way in which this species feeds itself. One may assume that the absence of this structure indicates a higher degree of parasitism, as was argued for *Cuterebra fontinella* and *Hypoderma* sp. (Colwell, 1986). The weak oral ridges in instar I (bearing no modified spines) seem to be compensated in some manner by the presence of abundant spines on the ventral side of the first thoracic segment. Thus, these spines should functionally substitute for those appearing in the oral ridges in instars II and III.

The abundant body spinulation shown by *W. magnifica* might serve as a complementary anchorage system, because these spines present an antero-posterior orientation (in addition, this fact is reinforced by the musculature of the body wall). Alternatively, the grooves of the body spine apex are more obvious in instar III (which is longer and in it, the friction of the larvae is largest). The function of these grooves, which have a constant structure, must be determined. Perhaps these grooves provide for chitine transport to compensate for the erosion suffered by the spine.

In *L. cuprina* (Sandeman et al., 1987) the anterior spiracles are associated with a cuticle which can close over them. This structure is also observed in the posterior spiracles, that are located in a deep peritremal cavity which can be closed by the larva. The function of these structures might be related to gas regulation preventing the entry of waste substances across the respiratory system.

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