

Ultrastructure comparison of the sensory morphology of the first- and third-instar larvae of *Parasarcophaga argyrostoma* (Robineau-Desvoidy) (Diptera: Sarcophagidae)

Azza Awad^{1*}, Salah Abdel-Salam¹, Refat Abou El-Ela², Abdel-Aal Abdel-Aal¹ & Doaa Mohamed¹

1. Zoology Department, Faculty of Science, Assiut University
2. Entomology Department, Faculty of Science, Cairo University

ABSTRACT

Because of its considerable medical and veterinary importance, first and third larval instars of *Parasarcophaga argyrostoma* were examined by scanning electron microscope. In the cephalic region, the antennal-maxillary sensory complex, multiple types of sensilla (styloconic and basiconic) are described and compared with respect to differences in the sensory requirements of both instars. This study also deals with the ultrastructure of different sensilla (basiconic, trichoid, and coeloconic) located on the thoracic and abdominal body segments of both larval instars. Sensilla numbers, types, sizes and locations of the antennal-maxillary sensory complex were different in the two instars. The anterior and posterior spiracles of the third instar larvae are also described.

KEYWORDS: Diptera, *Parasarcophaga argyrostoma*, morphology, immature stage.

INTRODUCTION

The sarcophagid fly *Parasarcophaga argyrostoma* (= *Liopygia argyrostoma*) is commonly called "flesh flies". The species, which was originally described in 1830, occurs in association with human activity. The flies of this species belong to the genus *Sarcophaga* Meigen and to the subgenus *Liopygia* Enderlein (Martin & Christian 2002). The adult flies visit decaying substances, faeces and also feed on flowers. Larvae normally develop in decaying meat but are also known as parasitoids of various animals (Povolny & Verves 1997). Flies belonging to the Sarcophagidae have received much attention due to their myiasis potential and vector for pathogens (Greenberg 1971). *P. argyrostoma* has been reported several times in recent years as an agent of human cutaneous wounds and genito-urinal (vaginal) myiasis (Burgess 1966; Aspöck & Leodolter 1970; Zohdy & Morsy 1982; and Burgess & Spraggs 1992). Recently, attention has been focused on the Sarcophagidae because of their use in medico-criminal entomology (Wells *et al.* 2001; Povolny & Verves 1997; Byrd & Bulter 1998; Introna *et al.* 1998; Benecke 1998).

Zohdy & Morsy (1982) studied larval and pupal development of *P. argyrostoma* at various temperatures in Egypt. Hafez (1940) illustrated the morphology of the adult of *Sarcophaga faculata* Pandelle. He studied head capsule, proboscis, thorax, wings legs, abdomen, sex ratio, and sexual maturity. El-Beih (1971) compared the morphological structure of the three larval instars of *Sarcophaga misera*, which were quite similar except in size and degree of sclerotization. Abou El-Ela & El Gindi (1999) studied the external and internal morphology of the head capsule and mouthparts of *P. argyrostoma*. Martin & Christian (2002) added that, due to its high abundance in many parts of the world and the frequency with which *P. argyrostoma* appears in cases of myiasis and death, details developmental and morphological data are essential to estimate time since infestation or death. However, as far as we know, no ultrastructural details are available for this species.

Because of its medical, forensic, and legal importance, and from the entomological point of view, the present study explores morphological and ultrastructural details of *P. argyrostoma*.

* Address for Correspondence

MATERIALS AND METHODS

A colony of *P. argyrostoma* was reared under laboratory conditions ($28 \pm 1^\circ\text{C}$, 65% relative humidity and 16L:8D photoperiod). The adults were kept in glass jars (35 x 35 x 50cm) supplied with sugar solution as food and meat as larviposition medium. The meat was changed daily and the deposited larvae transferred to smaller jars for rearing under the same conditions. Larvae were fixed in glutaraldehyde, dehydrated in ethanol series, followed by critical-point drying, mounted on aluminum stubs, and sputter-coated with gold palladium. The specimens were examined in Jeol JSM T220 SEM operated at 10 – 15 Kv. The nomenclature of all types of sensilla in this study is based on the studies of Zacharuck & Shields (1991).

RESULTS

SEM of the first- and third-instar larvae reveals morphological features not observed already in *P. argyrostoma* from light microscope study. The cephalic sensory complex and spines on the thoracic and abdominal body segments of the first and the third instar larvae of *P. argyrostoma* were the main structures we studied. The antennal-sensory complex of the first-instar larvae is represented by one huge styloconic sensillum (type 1) (Plate 1). This sensillum has a large base, with a cone-like peg on its tip. On both sides of the cephalic regions of the third-instar larva, the same structure (the huge sensillum) is present with one pore in the middle on the distal margin of its base (Plate 2).

The maxillary sensory complex of the first-instar larvae has no distinguishable sensilla, and is similar in the second- and third-instar larvae (Plate 3). The integument of this region is strongly circled, forming several cuticular rima or rings (almost a rosette-like structure). A central pore (which could be the site of the coeloconic sensillum) is located in the middle of this cuticular structure or rosette. No types of sensilla were noticed in this central region. In contrast, in the third-instar larva, the sensilla of the maxillary complex are clumped together in the central region (Plate 2). This central cluster is composed of three small styloconic sensilla (type 2), and three small basiconic sensilla (type 1). This central cluster is surrounded by several cuticular ridges (well-formed rosette like-structure). Two lateral small styloconic sensilla (type 2), which are located between the central cluster and the base of the antenna, were also found on the lateral area of these ridges. On the other side of the central cluster, one pit (possibly a coeloconic sensillum) was found (Plate 2). An array of cuticular ridges were found associated between the mouth (V-shape dorsal grooves) and the prominent hooks (Plate 4).

In the first-instar larva, a collar band of sensory spines (trichoid sensilla of types 1 and 2) are located backwardly on the anterior edges of the dorsal and ventral surface of the first thoracic segments, and on the first, second and third abdominal segments (Plate 5). No sensilla were noticed on the second and third thoracic segment. The bands were found only on the ventral surface of the first, and fifth abdominal segments. The collar bands were also found on the second, third and fourth abdominal segments (Plates 6-7). The sensory spines of the collar bands were common and numerous on the ventral surface of all the abdominal segments, while they were condensed on the dorsal surface of the first thoracic segments.

In the third-instar larvae of *P. argyrostoma*, the surface of the first thoracic segment is covered with backwardly directed spines, which could be trichoid sensilla of type 3 (Plate 8). The second and third thoracic segments are ringed by two to three rows of flattened backwardly directed basiconic sensilla (Plate 9). Small spines cover the dorsal surfaces of the sixth, seventh, and eighth abdominal segments, which could be small basiconic sensilla

(Plate 10). In the spine-free areas of the first to the fifth abdominal segments, one or two pit sensilla (coeloconic) were noticed (Plate 11).

In general in the first- and third-instar larva, in regions with lower spine densities almost all the spines tend to be broader than in other regions with higher densities, although all of them are caudally oriented. Based on fine structure, spines described in both instars (mainly trichoid and basiconic sensilla) are different in size and also in shape. Spines with double tips were also seen in third instars, particularly on the first thoracic segments (Plate 8).

In the third-instar larva, the prothoracic spiracles are located at the posterior lateral edges of the first thoracic segments. On either side, the prothoracic spiracles appear with the typical digit-like protrusions with a slit-like opening. Each spiracle has ten to eleven of these protrusions (Plate 12). This structure was not noticed in the first-instar larva.

The posterior spiracles are enclosed within the deep stigmal cavity. A collar of 10-11 cuticular papillae adorn the edge of the cavity. The ecdysial and the spiracular openings are seen in Plate 13. The spiracular openings, pores, perispiracular glands, rima and rays are also visible (Plate 14).



Plate 1: Scanning electron micrograph displaying the antennal sensory complex of the first-instar larvae of *Parasarcophaga argyrostoma*. (ST₁) styloconic sensillum of type 1- (H) hook

Plate 2: Scanning electron micrograph displaying the antennal sensory complex of the third-instar larvae of *Parasarcophaga argyrostoma*. (ST₁) styloconic sensillum of type 1-(ST₂) styloconic sensilla of type 2- (r) rima- (B₁) Basiconic sensilla

Plate 3: Scanning electron micrograph displaying the maxillary sensory complex of the first-instar larvae of *Parasarcophaga argyrostoma*. (R) Rays - (MC) maxillary complex.

Plate 4: Scanning electron micrograph displaying the array of the cuticular ridges (CR) around the mouth (M)- (R) Rays.

Plate 5: Scanning electron micrograph displaying the free sensillar areas and the absence of the collar bands in the 2nd (Th₂) and 3rd (Th₃) thoracic segments of the first-instar larvae.

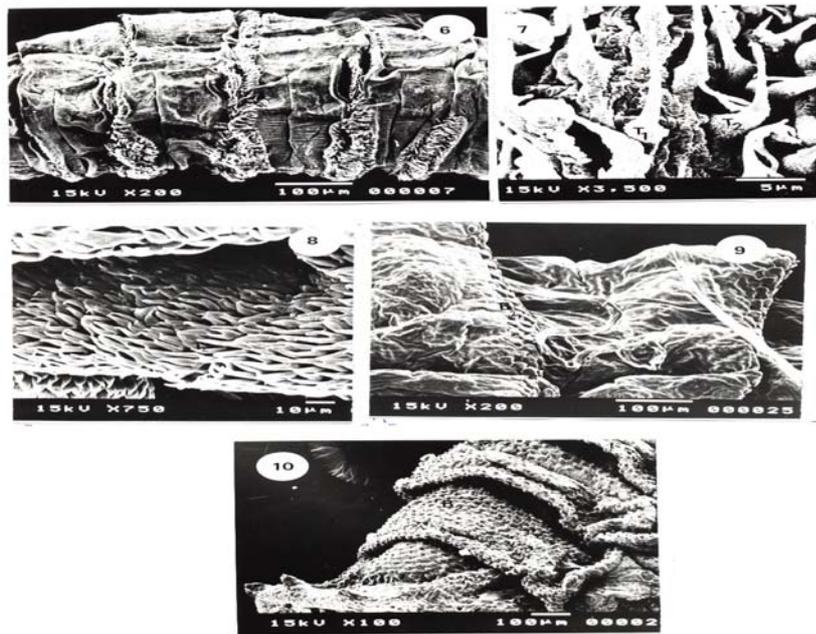


Plate 6: Scanning electron micrograph displaying the presence of the collar bands, and the backwardly directed spines over the body segment of the third-instar larva.

Plate 7: Scanning electron micrograph displaying the trichoid sensilla of type 1 and 2 (T₁- T₂) in the third-instar larva (higher magnification than the previous micrograph).

Plate 8: Scanning electron micrograph displaying the double tip trichoid sensilla in the third-instar larva.

Plate 9: Scanning electron micrograph displaying the flattened backwardly directed basiconic sensilla (B₁) in the 2nd and 3rd thoracic segments of the third-instar larva.

Plate 10: Scanning electron micrograph displaying the basiconic sensilla (B₂) on the dorsal surface of the 6th, 7th, and 8th abdominal segments of the third-instar larva.

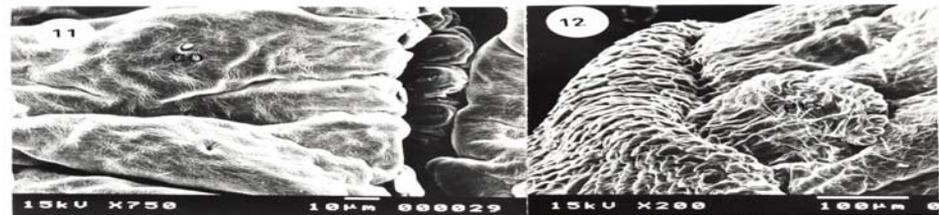


Plate 11: Scanning electron micrograph displaying the spine-free areas from the 1st to the 5th abdominal segment of the third-instar larva, and the presence of some coeloconic sensilla (CO).

Plate 12: Scanning electron micrograph displaying the prothoracic spiracles (Pt.S) with 10- 11 digit like-protrusions

Plates 13 & 14 : Scanning electron micrographs displaying the posterior spiracles (Ps. S), with the perispiracular glands (g), rima (r) and papillae (P)



DISCUSSION

The present study is concerned mainly with the ultrastructure, morphology and distribution of sensilla and spines of the first- and third-instar larvae of *P. argyrostoma*. As in some other studies on *Sarcophaginae* and *Parasarcophaginae*, the present work concentrates on the cephalic sensory sensilla (antennal-maxillary sensory complex). The differences between sensilla located on the thoracic and abdominal body segments of both instars (spinal bands) were studied. In contrast to some other work, this study tries to nominate the different types of spines (sensilla) in these regions, which are represented by two forms of styloconic, and trichoid sensilla, basiconic, and coeloconic sensilla. With respect to differences in the sensory requirements of both instars, the difference in sensillar numbers, types, sizes and locations of the antennal-maxillary sensory complex in both instars were indicated. The present study focussed also on the formation and development of the antennal-maxillary complex. Those areas in both instars were different in their rosette-like structure and in the development of the cuticular rima. The anterior and posterior spiracles of the third instar larvae were also described.

Leite & Lopes (1987, 1989) and Lopes & Leite (1987) said that more detailed studies of specific groups of sarcophagids would be necessary to discern any patterns and to potentially develop useful keys. Mangan & Welch (1990) added that an evaluation on the influence of host and nutritional state on spine structure also would be necessary, particularly in light of a study of spine morphological variation in the Calliphorid *Cochliomyia hominivorax*. However the immature stages of Parasarcophaginae have received little attention, although some authors have described some morphological details. Cantrell (1980) worked on the larvae of *Blaesoxipha* Loew, while Ferrar (1979) described the larvae of *Parasarcophaga knabi* (Parker). Cantrell (1981) also studied the immature stages of some Sarcophaginae, and he pointed that all species examined show great similarities in morphology, but attention should be given to the number of rays in the anterior spiracles, the shape of the posterior spiracles, and the cephalopharyngeal skeleton. In contrast to our concerns, Cantrell allowed identification without resort to examination of the cuticular spine bands in most cases of the second and third instar larvae. He added, however, that with first-instar larvae where spine bands are readily observed, examination of these is a useful aid to identification.

Some authors have published illustrations of the cephalopharyngeal skeletons of first-instar larvae of various dipteran species. Ishijima (1967) described the third-instar larvae of *Boettcherisca peregrina* (Robineau-Desvoidy). Schmidt (1993) made a comparison of the sensory morphology of the cephalic region of first- and third-instar *Arachnidomyia aldrichi* (Parker). He said that the cephalic sensory structure of *A. aldrichi* is similar in arrangement and structure to those reported for other sarcophagous and herbivorous muscomorph dipteran larvae. We disagree with his results which suggest that sensilla numbers and types are similar in both instars; our results indicate differences in different types of sensilla in both instars of *P. argyrostoma*.

Thelma De Fillips & Leite (1997) worked on the first-instar larvae of *Dermatobia hominis*. They found on the pseudocephalon basiconic and trichoid sensilla in an antennal sensory complex, and basiconic, coeloconic and campaniform sensilla in a maxillary sensory complex. They reported that these types of sensilla might have mechanical, chemical and olfactory functions or a combination of all these functions. They also suggested that the multiplicity of types of sensilla and their distributions on the integument of the first-instar larvae of *Dermatobia hominis* may have importance in establishing the parasitic phase of the life cycle of this insects. In 1998 they also studied the second- and third-instar larvae of *D. hominis* by SEM. They reported that on the pseudocephalon the

second- and third-instar carries an antenna (with coeloconic sensilla), and coeloconic and basiconic sensilla on the maxillary sensory complex. In contrast to their results, the antennae of *P. argyrostoma* are represented by a single huge styloconic sensilla.

Colwell & O'Connor (2000) studied the second instar of unidentified sarcophagid maggots recovered from the foot of a 2-month-old child. They examined its external features by SEM: these were typical for muscomorph larvae and shared features common to other sarcophagids. Their results were similar to those of Schmidt (1993) for the cephalic sensilla of third instar *Arachnidomyia aldrichii* (Parker). We differ from their description of the antennal sensory complex as large cone-shaped sensillum surrounded by a cuticular ring, since we describe the antenna of *P. argyrostoma* as a huge styloconic sensillum. Our description of the maxillary sensory complex of the second- and third-instar larva of *P. argyrostoma* almost corresponds to their description for the same region. The central cluster is composed of numerous papillae (three small styloconic, plus three other small basiconic sensilla) surrounded by concentric cuticular ridges, and with several lateral additional sensory papillae. Mahmoud *et al.* (2002) worked on *Musca domestica* larvae using SEM. They pointed to the functions of the mouth hooks as tools of penetration. They suggest that the complexity of the oral ridges satisfies the invading power and food requirements. They also add that the modified spines not only serve as accessory supports to the mouth-hooks during locomotion, but also produce an abrasive effect and direct the food towards the oral ridges located immediately below these structure. The posterior spiracles might be related to gas regulation, preventing the entry of waste substances across the respiratory system.

There was little basis for comparisons with our detailed examination of the spines since these have not been reported by other authors who have worked on *P. argyrostoma*. Therefore this study enhances the positions and nomenclature of the spines and sensilla on the body and the antennal-maxillary sensory complex of first- and third-instar larvae of *P. argyrostoma*.

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