Flesh fly myiasis (Diptera :Sarcophagidae) *Sarcophaga sp.* Record from fish *Pagrus pagrus* Linnaeus,1758 (Osteichtheyes: Sparidae)

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Abstract: Sarcophagidae from a diverse family in which all species are obligate parasites of domestic animals and accidentally humans causing myiasis, first-instar larva of flesh fly *Sarcophagus* sp. obtained from the gills, esophagus, stomach and intestine of fish *Pagrus pagrus*, were studied macroscopically by light and scanning electron microscope with particular reference to the anterior and posterior regions. Ultrastructural study reveals newly morphological features that not observed before and that these fine criteria may be used to differentiate it from other larvae. In addition, more detailed description was added to the cephalic and caudal regions which are of important taxonomic value. Anal division has many structures of taxonomic value and some of them can be differentiated macroscopically, posterior spiracles, papillae, sensella, and anal pseudolegs (prologs). A certain definite pattern of 2 microspines is visible on two laterals of the fleshy projection on caudal surface.

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Key words: forensic entomology, first instar larva, sparidae, myiasis, Sensilla, sarcophagidae, SEM.

1. Introduction

Sarcophagidae includes extremely varied flies in size, shape and breeding habitats. The larval stages of many species are necrophagous and the instars of many species of this family are little known and even more complicated on those groups of which taxonomy is unstable in adult stage, so few taxonomical studies (Lopes, 1943, Jirón & Bolaños, 1986, Leite & Lopes, 1989, Sukontason *et al*., 2003, Pérez- Moreno *et al*., 2006, Szpila & Pape, 2008). Interest in the study of sarcophagid larvae has increased with the step forward in forensic entomology, where they are considered potential indicators of the time of death Buenaventura *et al*., (2009). Sarcophagid female's larviposit (lay larvae instead of eggs) on the corpse and they first feed in natural orifices and injuries. It is interesting to mention that according to Zumpt (1965) female *S. carnaria* can drop its larvae from a height of about 26 inch. through wire-gauze covers put over meat. Larvae development occurs on the corpse; then, they move to less humid areas to pupate. Short time after, adults emerge and complete the life cycle. The development time of the species is the reference point for dating the death (Ames & Turner, 2003).

Lewis (1955) mentioned that most species of the subfamily Sarcophaginae breed in decaying material and some can parasitize insects. *Sarcophaga ruficornis* breeds in both faeces and carrion, and has been caught at baits of decayed carrion, rabbit, fish, liver and chicken Hanan (2010). It has been reported to cause myiasis in dogs, horses, mules and leprosy patients Amoudi *et al*. (1994). It is also a documented parasite of the toad, *Bufo melanostictus* (Roy and Dasgupta, 1977).

In Thailand, *Sarcophaga dux* is synanthropic and breeds in both faeces and carrion. This species is regarded as forensically important in Japan and Thailand Sukontason *et al*. (*2003*) and has been observed to readily enter human dwellings and larviposit on carrion and garbage.

The sea bream *Pagrus pagrus* (Linnaeus, 1758) fish is a very common in Marsy Matrouh city and has a significant commercial importance**.** Fish is one of the cheapest animal protein sources consumed over millennia and it is being used increasingly to correct protein deficiency in diets in the tropics Akinwumi (2011), *Sarcophaga* sp. caused external myiases on three cyprinid fish species caught from Atatürk Dam Lake, Turkey. A total of 130 live dipteran larvae were collected from the body surfaces and there was 100% infestation of all three species of fish, the myiases were probably accidental infections on stressed and immobilized fishes. (Oktener and Alas, 2009). Fao (2002) reported that cured fish can suffer considerable loss of weight due to feeding damage caused by insect pest. In Bangladesh, Majumder *et al*. (2012) record three important insect pests of sun-dried marine fish, viz. *Boettcherisca peregrina*, *Chrysomya megacephala* and *Lucilia cuprina.* The larvae of this species take nourishment from exposed fish and meat, and contaminate the food material by reducing the nutritional value of the food materials. *L. cuprina and B. peregrina* is the key pest of the fish drying industries of the coastal and off-shore islands in the Bay of Bengal. Together with the key pest they harm a lot of existing fish industries and have great significance for human hygiene and also in the economy of the livestock myiasis, fish and other animal food processing factories.

Dermestes maculatus is cosmopolitan and the most preponderant insect pest of dried fish in sub-Saharan Africa (Johnson and Esser, 2000), pest damage can cause fragmentation of cured fish; and this can lead to quantitative loss of the smaller fragments and loss of value due to quality reduction, visual quality loss due to contamination by live or dead pests, or by their cast skins and frass. Lale, *et al. (*2000) estimated 20 - 50% deterioration of smoked fish products during storage arising from microbial and insects' pest infestation in the tropics. More so, Esser *et al.* (1989) put physical and financial losses in Indonesia at between 10 and 50% and 25 and 90%, respectively, for the fish species they investigated. Huss, (1988) stated that reduction in post harvest losses could add another 20-30 million tonnes in the cured fish sector. Osuji (1974) screened 30 samples for the presence of insect pests in four dried fish and found that the infestation load correlated to the lipid content of the fishes. It is known that *Dermestes maculatus* develops more effectively on fish with higher fat contents. From processing throughout storage shelf life, fish is vulnerable to insect pests belonging to 3 families namely, Calliphoridea, Sarcophagidae (blowflies) and Dermestidea (beetle) (Sastawa and Lale, 1998).

Lopes (1973) found larvae of *Sarcodexia lambens* on dead arthropods and *Peckia chrysostoma* on dead fish viscera in the Neotropical Region. Sarcophagidae are attracted to many types of dead vertebrate remains, including humans (Nishida 1984) some species are parasitoids, attacking and developing within other arthropods Coppel (1959), while most have been found on varying types of carrion: pig carcasses, human cadavers, and/or wild life Goff (1991).

Most species of Sarcophagidae are breed in small to medium sized vertebrate or invertebrate carrion. They can be found from deserts to rain forests, but only very few species are associated with freshwater habitats and most of these associations are very peripheral. In the large genus *Sarcophaga*, some species are facultative parasites of snails living in marshy habitats Parashar and Rao (1989), while others may at least occasionally cause myiasis, i.e., invasion of fly larvae, in adult amphibians Roy and Dasgupta (1977). Yassin and Mohamed, 2015 found that sarcophagus spread almost all over the Sudan country. Also *Wohlfahrtianuba nuba* has been bred from the dead bodies of fowl, goat guinea-pig, rat and snake. Obtained maggots from human wounds from various parts of country and from camels.

Myiases are serious health problems not only in animals but also in man, defined as "the infestation of live human and vertebrate animals with dipterous larvae, which, at least for a certain period, feed on the host's dead or living tissue, liquid body substances, or ingested food", may be classified as specific or obligatory, facultative or accidental (Zumpt, 1965).The larvae are deposited either directly on the skin or mucous membranes (e.g. *Oestrus* spp. And *Rhinoestrus* spp.), penetrate normal skin (e.g. *Gasterophilus* spp. and *Hypoderma* spp.), or become superimposed on pre-existing wounds (e.g. *Chrysomya* spp.).Facultative parasites normally free living, with larvae developing in decayingorganic matter, but which may occasionally contaminate living tissue, such as preexistingwounds, ulcers and cavities (e.g. the genera *Musca* and *Calliphora*).Accidental myiasis or pseudomyiasis it occurs when the larvae of a normally free living species are swallowed with contaminated food, passing through the alimentary canal where they may cause pathological reactions (Hall, 1995).

Myiasis is a worldwide phenomenon that is related to the latitude and the life cycle of certain species of flies it occurs mainly tropical and subtropical area and also this parasitic infection is seen in regions with a warm and humid climate (Frikh *et al*., 2009). The flies responsible for the condition prefer a warm and humid environment; therefore myiasis is restricted to the summer months in temperate zones while it is all year round in tropics (Schneider *et al*., 2007).

The flies that caused myiasis belonging to the families Calliphoridae, Sarcophagidae, Hypodermatidae, Oestridae and Gasterophilidae, especially. However, some species belonging to the other families such as Muscidae, Psychodidae etc. may cause myiasis rarely.The flies belonging to the Hypodermatidae, Oestridae and Gasterophilidae cause obligator myiasis in animals such as cattle, sheep and goat, and equids, respectively. However, these flies may cause accidental myiasis in human beings and the other animals Garcia and Bruckner, (1997).

In Egypt, many cases of human and animal myiasis have been reported, 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 *Sacophaga 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. Ahmad *et al.* (2011) record case of humanGastrointestinal Myiasis by Larvae of Sarcophaga sp and Oestrus sp.

Khalifa *et al.*(2005) discovered newly sensory array of the mouth hooks of *Gasterophilus intestinalis*and *Gasterophilus nasalis*obtained from the stomach of Egyptian equinesand was found to be different mainly in the shape of the sensilla and the number of the angled plates and that these fine criteria may be used to differentiate other larvae. Leite & Lopes (1989) 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.*

The use of scanning electron microscopy to observe and photograph the morphology of dipterous larvae has enabled observation of a suite of intra-specific differences, and to characterise some aspects (pseudocephalon, spinules, anterior and posterior spiracles, spiracular setae, rim of spiracular atrium tubercles and sensilia) not easily resolved with light microscopy (Aspoas, 1991), For this reason Cantrell (1981), indicates that it is important to describe the number of papillae forming the anterior spiracles, the shape of the peritreme of the posterior spiracles and the cephalopharingeal skeleton. The size of the circumspiracular tubercles at the caudal segment of Sarcophagid larva is an important morphological character.

2. Material and methods

This study was carried out during September to November2011. From 25 *Pagrus pagru*s fishes only 16 were found to be naturally infected with sarcophagidae larvae (64%). Specimens were collected from the fish markets of Marsy Matrouh city in Egypt, and were dissected the body cavity, gills and intestine were examined at first by naked eye for the presence or absence of any parasites. Furthermore. The affected fish had frayed and pale gills, increased mucous production and also hemorrhages on the gills caused by the feeding activity of the parasite.

Fishes were dissected and larva collected from their gills, stomach and intestines were washed several times in isotonic saline solution to get rid of the mucous that may be adherent to the body of larvae. No larvae were found in other parts of the body.

Specimens were stained in Acetic-Carmine, dehydrated and mounted in Canada balsam. Drawings were made with the aid of a Camera Lucida.

Furthermore, larvae were prepared for scanning electron microscopy (SEM), fixed overnight in 2.5% gluteraldehyde in 0.7M phosphate buffer (PH 7,4) at 4 C. they were then washed three times in phosphate buffer and post – fixed in 1% Osmium tetraoxide in the same buffer and dehydrated through a graded series of ethanol, coated with gold, using Spi – module Vac/Sputter coater. The specimens were then examined with a high resolution (Joel, 1200Ex II).

Species were identified depending on the morphological characters of the anterior spiracles, cephalo-pharyngeal skeleton and slits of the posterior spiracles in the peritrem. The collected larvae were identified as first instar Sarcophagid (Diptera: Sarcophagidae). The nomenclature of all types of sensilla in this study is based on the studies of Zacharuck & Shields (1991).

The present study aimed to study the ultrastructure of first instar *Sarcophaga sp.* by scanning electron microscope, in a trial to find some fine structures that might be useful to differentiate between different larvae (Sarcophagidae) species.

3. Result. (Figs. 1-22).

Dipterous larvae are generally with no clear distinction between thorax and abdomen often the mouthparts, with two pairs of spiracles (anterior and posterior). These are the only, practical means of identifying larvae, the present first instar larvae identified was carried out according to specific keys Greenberg (1971). Present larvae possess the general habitus characteristic for most sarcophagidae (*Sarcophaga* Meigen, 1826), being divided into pseudocephalon (pc), three thoracic segments (t1–t3), seven abdominal segments (a1–a7) and an anal division (ad), which carries the posterior spiracles (Figs.1,7,9). The integument is not sclerotized and appears as a white, tough and wrinkled skin, with some areas of small spines that give a roughened appearance, larva possessed a pair of mouth hook and associated sclerites for the attachment of muscles, collectively known as the cephalopharyngeal skeleton (cps), bands of small microspines, the tracheal and digestive systems of the larva were visible under the light microscope through transparent body cuticle. Length 2.95-0.96 mm, maximum width 0.63-0.41mm.

The prothorax **orPseudocephalon (pc)**, It has a small pair of dorsal reduced antennae, located in a depression and are formed by two segments, the apical (dome) sharpened at its end, and a pair of maxillary palpus in ventral position, each palpus shows two sensillae, retractible in the thoracic region. It is surrounded by a band of one and two sharp pointed spinules, grouped in a varying number and usually more densely arranged in the ventral part.

In the cephalic segment is pair of strong mouth hooks (mh) (Fig.12), sometimes called mandibles, parallel, retractible, decurved, heavily sclerotized, and deeply pigmented, pointed in the apex and basally square. In operation they are sickle-like in that movement is in a verticle plane. Mouth hooks are the most anterior, and articulate with the hypopharyngeal sclerite which receives the opening of the salivary duct. A small dentate sclerite unites the bases of the 2 mandibular sclerites(ms).

The anterior spiracles are located on each latero-posterior edge of the prothorax, there are number of papillae on each anterior spiracle.

Cephalopharyngeal skeleton. (cps) (Figs.2, 7).Uniformly pigmented, mandibles and maxillae are fused in a mouthhook structure with hook-like apex (Fig.2). Dental sclerite completely incorporated to the base of mouthhook structure. Subhypostomal sclerite (ss) middle moon-shaped from above. Intermediate sclerite (hypopharyngeal sclerite, hs) short, wide with rounded anterior and posterior projections, H-shaped from above and not fused to the basal sclerite. Pharyngeal sclerite (ps) relatively bulky and heavily pigmented at middle, with a parastomal bar (pb), slightly incurvated apically upwards. Dorsal cornu (dc) wider and longer than ventral cornu (vc). Window reduced to a small split dorso-apically. The ventral edge of pharyngeal sclerite more concave, with ends curved ventrally. The ventral cornu is more pigmented towards the ventral region and truncated posteriorly, approximately twice longer than wide. Clypeal arch (ca) elongated, but it does not reach the parastomal bar. The dorsal cornu split into two branches.

Anterior spinose band on **first thoracic segments** t1 broad, withspines arranged in 4–8 rows,elongated and slightly curved, size of spines decreasing gradually towards the posterior end of body; anterior spinose bands of t1 - t3 with homogeneous, strongly sclerotised, directed anteriorly, forming abroad collar band (cb). Spines on ventro-lateral surfaces of each segment larger than other spines, each anterior spinose band ventrally with a transverse lenticular gap without spines (Figs.7, 10).

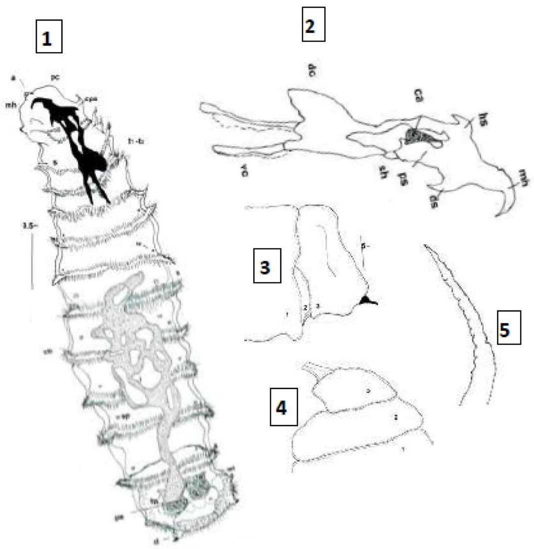
The abdomen is cylindrical in shape and consists of 7 segments, each with bands of microspines. The 4^{th} - 7^{th} segments are the largest. Abdominal segments present sharp pointed spinules directed both anteriorly and posteriorly. Although all spinules are sharp pointed, the ones located in segments 2-4 and around the spiracular atrium are slightly more slender. In lateral view the bands of microspines are wider on the ventral surface, probably to aid in locomotion, with anterior spinule bands one pointed and anteriorly projected. One of these bands narrower than the others and with the spinules posteriorly projected. Inter-band areas are devoid of spinules.

Anal segment (Figs.8,19-21**)** or anal pads rounded, small and slightly protruding, anal tuft with several spines dorsally and ventrally, readily apparent in light microscope; hair-like spines around spiracular cavity present but sparse, strong trabeculae are visible through the spiracular slits. The anal tubercles have small plate and the anus is anteriorly and posteriorly bordered by thorn-like spines. Posterior spiracles concealed in deep cavity on posterior ‘face’ of last segment, with a distinct cavity are set within a deep spiracular atrium. There are 8 tubercles (t) located on the rim of the spiracular atrium each one with an apical setae. The posterior spiracle is D-shaped with an incomplete peritreme, posterodorsal in the atrium, on a spiracular plate. Spiracles consisting of three elongate slits (s) oriented vertically, with the openings disposed radially, each with an incomplete sclerotized peritreme (p). The inner most slits are longest and tilt centrally while the outermost slits are shortest. Posterior spiracles are located in a conical depression. The outer ring to spiracular atrium is covered with sharp pointed spinules. Anal division has many structures of taxonomic value.There are six dorsal papillae, sensella, and two pairs of prolegs (pseudolegs).

Scanning electron microscope study reveals morphological features not observed already inSarcophagidae from light microscope study, the cephalic sensory complex and spines on the thoracic and abdominal body segments. Palpi bearing numerous papillae and delicate furrows to mouth on pseudocephalon. Maxillary palpus encircled by several cuticular folds, central cluster of sensilla with three sensilla coeloconica and three sensilla basiconica (sb) (Figs.11,13).The antennal-sensory complex is represented by one huge styloconic sensillum. this sensillum has a large base, with a cone-like peg on its tip.At both sides of the mouth appear bands of little deep subparallel oral ridges (or). The base of the atrial cavity (ac) shows a sensilla and papillae. The integument of the maxillary region is strongly circled, forming several cuticular rima or rings (almost a rosette-like structure). Arrays of cuticular ridges were found associated between the mouth (V-shape dorsal grooves) and the prominent hooks. Anterior spiracles fan shaped and are located at the posterior lateral edges of the prothorax, appear with the typical digit-like protrusions (4-6 lobes) with a slit-like opening. The entire surface of the abdominal segments present filamentous spines directed backwards and forwards such as hair junction of haed, provide with sensory buds; on the lateral surface of each segment there is a sensorial papilla (sp). All the spines tend to be broader than in other regions with higher densities. Based on fine structure, spines described (mainly trichoid and basiconic sensilla) are different in size and also in shape. Spines with double tips were also seen, particularly on the first thoracic segments. A collar band of sensory spines (trichoid sensilla) 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.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 thoracic segments. The surface of the first thoracic segment is covered with anterior directed spines, which could be trichoid sensilla (Figs.14- 16).

The second and third thoracic segments are ringed by two to three rows of basiconic sensilla (filamentous spines) with apices, all directed anteriorly. Ultra structural study reveals that the outer surface of the body have cavities Walt possible help a function directorial, small spine cover the dorsal surfaces of the abdominal segments, which could be small basiconic sensilla. Also found two sensorial pits with triangular loped process and two additional papillae are present on each of the abdominal segment, secretary lope inside sensory pit. Scattered sessile papillae (arrow head) are found in the center of the abdominal segments and long setae (arrow) dispersed in different directions. The caudal segment is composed of deep stigmal cavity and 8 pairs of moderate circumspiracular tubercles (Figs.8, 9, 14-18). The posterior spiracles are enclosed within the deep stigmal cavity, each spiracular disc bears spiracular openings (slits) enclosed by an incomplete peritreme (Figs.8,19-21) Spiracles consisting of 4-6 elongate slits oriented vertically, with the openings disposed radially, each surrounded by an incomplete sclerotized peritreme (p). Tubercles (t) on the atrium rim, the fleshy projections (fp) on the anal segment, analconic sensilla and postconic sensilla and small papillae.The circumspiracular cavity shows long hair-shaped spins.

The spiracular opening is wrinkled. Near the rim of slits are the heavy spiracular hairs,perispiracular glands, rima and rays are also visible.The circumspiracular cavity shows long hair-shaped spines and the outer dorsal tubercles large, inner and middle dorsal ones small; outer ventral a little more developed than the others which are reduced. Each peritreme has **4- 6** peritremal slites (caves) the peritremal cavity appears as a deep depression. Along the margin of this depression a group of peritremal tubercles is symmetrically arranged. A group of modified spines, like (fine hairs) occurs both dorsally and ventrally around the inner margin of the peritremal cavity. They are short, thick, and rigid and curved, grouped into clusters bearing 4, 5 or 6 hairs. Two pairs anal protuberances pseudolegs (prolegs) one pair claw leg and the other walking leg occurs near ventral of the spiracular cavity. A certain definite pattern of 2 microspines is visible on two laterals of the fleshy projection analconic sensilla (as) and on caudal surface postconic sensilla (ps) (Figs.14-18.).



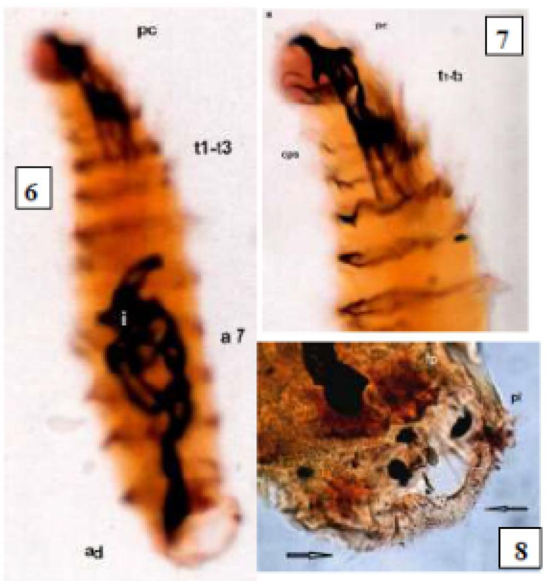
Camera lucida drawings:

Fig.1. Larvae elongated, truncated posteriorly and tapering toward the anterior extreme curved downward, they are cylindrical, segmented. The body isdivided into pseudocephalon (pc), three thoracic segments (t1–t3), seven abdominal segments (a1–a7) and an anal division (ad), which carries the posterior spiracle (ps) intestine (i). **Fig.2**. The cephalopharyngeal skeleton(cps) apparent anterodorsal process mouth hooks large (mh), intermediate sclerite H shaped in ventral view (is),parastomal bars (Pb) broad and straight in lateral view ; vertical plate very wide; dorsal cornua(dc) slightly longer than ventral cornua (vc). **Figs. 3, 4**. Claw and walking (protolegs) pseudolegs.**Fig.5**.High magnification of filamentous spines, the base of each spine is wrinkle, the tip is a sharp triangular shape and provided by sensory buds.

4. Discussion

Comparing the present first instar larvae with other sarcophagidae species, although they are morphological similar in appearance and difficulty in identification, our preliminary study using LM revealed that the anterior spiracle located, at the lateral side of the prothorax, shows a single row of 5–7 papillae marginally, the number and arrangement of papillae was morphological different; 10–15 papillae arrange in a single row of *L. ruficornis*; 21–27 papillae arrange in two rows in *B. nathani*; 24–26 papillae arrange in one or two rows in *B. peregrina*; 20–28 papillae.

The first thoracic to the end somatic segment, is densely covered with spines of a characteristic distribution, could facilitate anchoring to the host.thus, they are adapted to a parasitic way of life, as their density and size suggest.the spines are generally sharp and hooked, the ventral spines are usually large than the dorsal ones.ventrally. A group of small trified spines arranged transversely exist along with epidermal pores, these are; mainly used in locomotion since no pseudopods are present. The presence of these spines makes removal of the larva from its host difficult (Ramli and Rahman, 2002). Also, larvae have claw/s on the ends of their prolegs (pseudolegs) to help them stick to surfaces.



Light microscope photographs, showing
Fig.6-lateroventral view of the whole larva with body segmentation, pseudocephalon (pc), mouth hooks (MH), cephalopharyngeal skeleton (cps), three thoracic segments (t1–t3), seven abdominal segments (a1–a7) and an anal division (ad).The surface of thoracic and abdominal segments displays bands of spinules rounded in form the inter-band areas are devoid of spinules,. (X 4). **Fig.7**-lateral view pseudocephalon, retractible in the thoracic region, two very reduced antennae, located in a depression and are formed by two segments, the apical (dome) sharpened at its end, it is surrounded by a band of acute spinules, Cephalopharyngeal skeleton(cps)heavily pigmented. (X 10). **Fig.8.** Anal segment rounded, slightly protruding, with several spines dorsally and ventrally; hair-like spines around spiracular cavity present but sparse, Strong trabeculae are visible through the spiracular slits, depression in which the posterior spiracles are found, three darkly coloured pyramidal elevations (arrows or fleshy projection) shwoing claw and walking (protolegs) (pl) pseudolegs. (X 10).

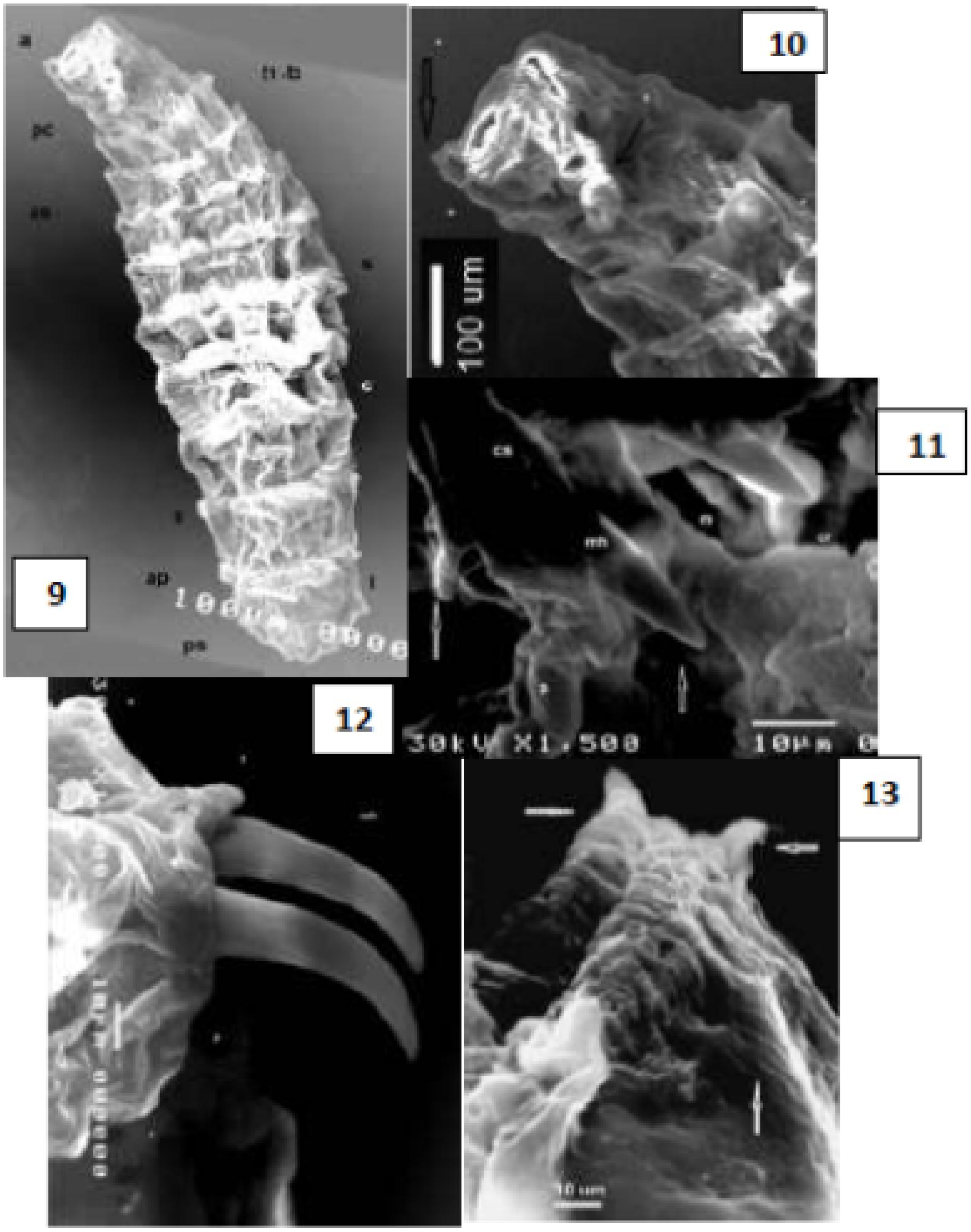
The spines not only serve as an accessory support to the hook during the larval locomotion but also produce an abrasive effect and direct the food towards the oral ridges, located immediately below these structures The abundant body spinulation shown by *W. magnifica* might serve as a complementary anchorage system, because these spines present an antero-posterior orientation (in addition, this fact is reinforced by the muscular of the body wall).alternatively, the grooves of the body function may be providing for chitin transport to compensate for the erosion suffered by the spine (Colwell, 1989).

The function of the sensory arrays may be locating the proper attachment site, sensing changes in the stomach contents or possibly operating in regulating mechanisms that prevent the attached larvae from perforating the gastrointestinal wall (Cogley and Cogley, 1999).

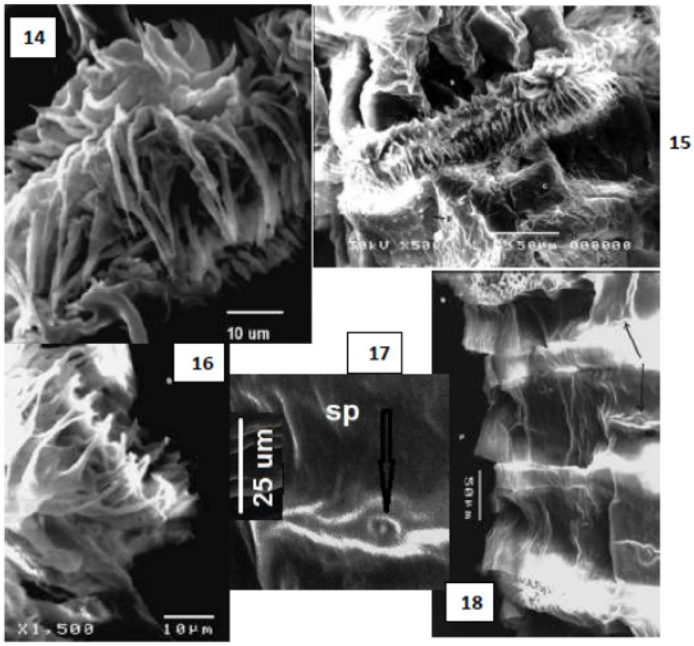
Hook system facilitates the anchorage of larva to a plane and, furthermore, could be used in penetrating the host s body in primary invasion and feeding habits. Awad *et al.* (2003) show a mouth hook with delicate pits and ridges to assist its penetration into carrion or wounds of animals (Mahmoud *et 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, larvae can clean wounds and may reduce bacterial activity and the chance of a secondary infection. They dissolve dead tissue by secreting digestive enzymes onto the wound as well as actively eat the dead tissue with “mouth hooks,” two probing appendages near their toothless mouth**.**

At the ultrastructural level, the presence of canalicules converging to the hooks apex may favour the penetration of the hook into the hosts tissues; however the hooks thickness, its associated musculature and the presence of a cutting inferior margin in the hook may assist its penetration into the host tissues. Each Antennomaxillary complex has two characteristic spheroidal flower-like sessile structures (arrows) which may be sensory in origin. The larvae use their anterior spines and mouth hooks to attach to the wall of the gastrointestinal tract and ultimately form ulcers but with rare reports of perforation (Principato and Tosti, 1988).

The cephalopharingeal skeleton is very similar in other saprophagous diptera, but there are some differences. The mouth hooks are proportionally larger and more curved. Other authors have noted a limit in the mouthhooks retraction into the cephalic segment in other species such as *Wohlfahrtia magnifica* (Ruiz-Martı´nez *et al*., 1990). This character state should be analyzed in other Sarcophaga species.

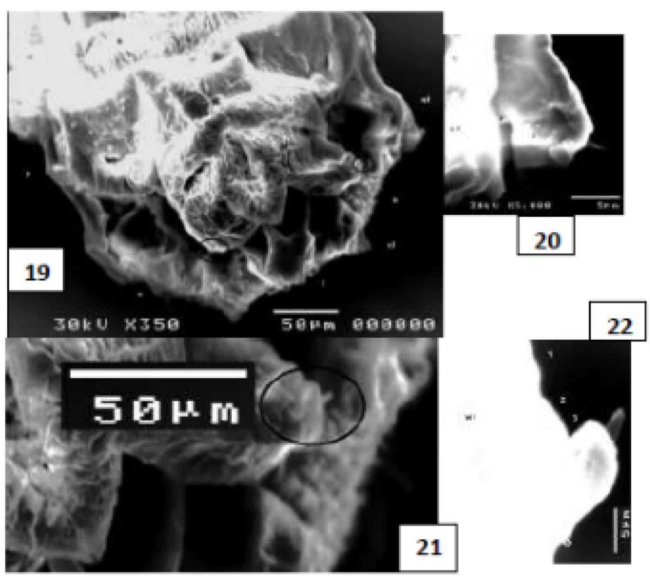


Scanning electron micrographs showing:
Fig.9. The whole body, ventral view showing pseudocephalon (PC), anterior spiracles (as) the posterior spiracles (Ps.), well developed annular bands (ab) of intersegmented spines (s) is in 4-6 rows of pinted spines, the grooves of the body are obvious. **Fig.10**. Anterior portion showing 3 thoracic segments with anterior spiracular. **Fig.11**. Higher magnification, maxillary palpus consists of several sensillae, In the frontal and inner margins,a group of small spines delimiting the atrial cavity, the array (R) of the cuticular ridges (CR) were found associated between the mouth (V-shape dorsal grooves) and the prominent hooks. (cs) Cephalo-pharyngeal skeleton. **Fig.12.** Higher magnification lateral view showing the sharp bladed mouth hook (mh), antenna is composed of a dome and a basal ring with external sensilla**.** perispiracular glands (pg), rima (r) and papillae (P). **Fig.13**. The integument of the maxillary region is strongly circled, forming several cuticular rima or rings,vacules (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. atrial cavity (ac).



Scanning electron micrographs showing:

Fig.**14.** Higher magnification of abdominal filamentous spines directed backforwards and forwards provide with sensory buds with 6-8 rows of pinted spines.**Fig.15.** Abdominal segments ventral view, the band of spines appears anteriorly and posteriorly directed on each segment as head junction hairs. **(Fig.16).** The dorso-anterior edge of the third thoracic segment, the spine band appears rows of anteriorly projecting, a collar band sensory spines (trichoid sensilla and basiconic sensilla), are different in size and also in shape, spines with double tips were also seen.**Fig.17.** Higher magnification of sensorial pits (sp) note secretary lope inside it. **Fig.18**. Sensorial pits with triangular loped process and two additional papillae (p) are present on each segment of the abdomen.



Scanning electron micrographs showing.

Fig. 19. Anal segment, the posterior spiracles are enclosed within the deep stigmal cavity,the spiracular openings (caves), pores, perispiracular glands and sensilla, rima and rays are also visible, the fleshy projections (fp). **Figs.20 & 22.** Two pairs anal protuberances pseudolegs (prolegs) one pair claw leg and the other walking leg.**Fig.21.** Spinules distributed around the rim and extending as far as the outer circumference of the ring formed by the tubercles. Spiracles consisting of 4-6 elongate slits oriented vertically, with the openings disposed radially, each surrounded by an incomplete sclerotized peritreme (p). Tubercles (t) on the atrium rim, the fleshy projections (fp) on the anal segment, analconic sensilla and postconic sensilla and small papillae. The circumspiracular cavity shows long hair-shaped spins.

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 insect. 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.

The spiracles are the breathing holes of fly larvae and can appear as black dots on the cuticle surface. They are very distinctive and are often the only way of identifying the species without rearing it on to adult hood.

The disposition and shape of the slits on the posterior spiracles as well as the presence of an ecdysial scar is a useful tool in the identification of the Sarcophaginae species Cantrell (1981), with these characters being repeatedly described, number of papillae in the anterior spiracles; pigmentation, arrangement and hardness of the spinules on the body segments and cephalo-pharyngeal skeleton (James and Gassner, 1947). Kano and tange (1951) add arrangement of papillae in the anterior spiracles, tubercles on upper border of anal segment, inner projections of peritreme and posterior spiracles. Ishijima (1967) completes the keys adding: distance between posterior spiracles and morphology of slits.

In the present specimens the anterior spiracles are associated with a cuticle fleshy projection (fp) which can close over them. This structure is also observed in the posterior spiracles, which 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. The anterior spiracles of the present species different from the larvae described by Cantrell (1981).

After using SEM, new characters have been incorporated into the species diagnosis. Awad *et al.* (2003) take into account new ultrastructure characters: sensillar numbers and types, sizes and locations of the antennal-maxillary sensory complex. Although the first instar larvae of the present specimens had been considered very similar (Lopes, 1983..Leite and Lopes, 1989), some differences can be observed by sem. the pseudocephalon of *S. lambens* bears antennae more elongated than that of present larvae and the circumspiracular tubercles are very large; all thoracic segments of present larvae have similar spines while *P.chrysostoma* shows strong spines on first thoracic segments and filamintus spines on the other two. The spines of the abdominal segments of *S.lambens*, except the eight, do not cover the whole surface but in p. chrysostoma the slender spines conceal the segments. The spiracular hairs are similar but those of *S. lambens* show wider points. The spiracular hair of larva in this present study markedly differed from those of *Sarcophaga cruentata* Meigen*, S. exuberans* Pandelle and *S. tibialis*.

The shape and position of the posterior spiracles indicate the highly developed system of protection of these spiracles which helps the larva to live in the gastrointestinal tract and keep them away from obstruction by gastrointestinal contents. This comes in agree-ment with Principato and Tosti (1988). The branching peculiarity of spiracular hairs, adjacent to the posterior spiracular opening, has been shown as a distinctive feature of sarcophagid larvae (Sukontason *et al.,* 2003).

As a general reflection from the preceding compilation and the new data provided in our descriptions, it can be concluded that the most useful morphological characters for diagnosis at specific level are: the structures of both, anterior and posterior spiracles, the morphology of the pseudocephalon (including oral ridges, antenna, maxillary palp, sensilla and ventral organ) and the morphology and distribution of the spinules and papilla in the body segments.

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