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External Morphology of *Hermetia illucens* Stratiomyidae: Diptera (L.1758) Based on Electron Microscopy

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Authors' contributions

This work was carried out in collaboration between all authors. Author FRO designed the study, performed the analysis, wrote the protocol and wrote the first draft of the manuscript. Author KD supervised and managed the study and wrote the final draft. Author RPS supervised the Electron microscope study. All authors contributed at the same way, read and approved the final manuscript.

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ABSTRACT

This work aimed to study the external morphology of black soldier fly (BSF) larvae *Hermetia illucens* (L. 1758) *Hermetia illucens* (Stratiomyidae: Diptera) bred under ideal conditions with a diet based on cow manure. Observations and illustrations were made using scanning electron microscopy (SEM) at the State University of New York (SUNY), College of Environmental Science and Forestry (ESF), Department of Paper and Bioprocess Engineering (PBE) and N.C. Brown Center for Ultrastructure Studies between June 2015 and October 2015.
The study monitored an overall of 30 larvae and the transition of their instars at an temperature of 28-30°C and an moisture content of 70-80%.

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A detailed description of the anatomy of the larval and adult stage is here shown for the first time. The morphological differences between male and female flies illustrate a distinct sexual dimorphism of notable taxonomic interest.

Keywords: Hermetia illucens; black soldier larva; scanning electron microscopy; external morphology.

1. INTRODUCTION

Hermetia illucens commonly known as the black soldier fly (BSF) larva [1] has four stages in its life cycle: egg, larva, pupa and adult. Larvae hatch from eggs and develops through several stages before pupating inside the last larval skin [2]. It may take approximately 30 days to complete development [3]. During larval development, they are insatiable feeders, and in their first instar, they are white, with a small projecting head containing chewing mouthparts [4]. As adults, they do not feed and rely on the fats stored from the larval stage [5,3].

BSF larvae are not considered a pest [5] and have been shown to be effective in manure management, and a food source for fish and other animals [5] showed that swine manure fed to BSF larvae, reduced their waste material by 56% with no objectionable odor. BSF larvae management of manure offers many advantages, including a reduction up to 56% waste annually, a high quality feedstuff will be produced, and house fly populations will be controlled [6] According to [7] BSF larvae successfully reduces human feces and convert it to pre-pupal biomass, and odor was significantly reduced. Larvae fed with chicken manure reduced the waste by 50%, eliminated house fly breeding and possible disease vectors [8].

Larval and bacterial activities can reduce the dry matter, but also other components such as nitrogen or phosphorus. Experiments with cow manure showed reductions of 43% in nitrogen and of 67% in phosphorus [9]. Larvae fed on the chicken manure can convert it to 42% protein and 35% fat [8]. In addition to being a good source of oil and protein for animal feed, BSF larvae have the potential of converting organic waste into a rich fertilizer [2]. The combination of its waste treatment capacity along with the generation of a product of economic value makes *Hermetia illucens* a promising tool for organic waste management.

This study documents the external morphology of *Hermetia illucens* throughout their lifecycle and compares the differences among the three instars and adult fly using scanning electron microscopy.

2. MATERIALS AND METHODS

2.1 Breeding BSF Larvae

Thirty BSF larvae were reared in a period of two months with temperature and moisture monitored. The transition of instars, pupae and the emerging as adult flies were observed. Single BSF larvae were placed in containers and fed with cow manure. The conditions for breeding the BSF larvae were a temperature between 28-30°C and 70-80% moisture [2].

2.2 Sample Preparation for Scanning Electron Microscopy

Scanning electron microscopy was used to illustrate the external morphology of the BSF larvae. Specimens were fixed with 2.5% glutaraldehyde Phosphate Buffered Saline (PBS) buffer for one hour followed by a second fix in a 1% aqueous solution of osmium tetroxide. The samples were dehydrated in a series of alcohol solutions (30%, 50%, 70%, 95%) for 10 minutes each and finally 100% 5 minutes, 3 times. They were critical point dried and sputter coated with a gold-palladium alloy [10].

2.3 Scanning Electron Microscopy

JEOL JSM-5800 LV low vacuum scanning electron microscope. The JSM 5800 LV has superior resolution in high vacuum mode, and it was thus used for surface evaluation of the BSF larvae.

3. RESULTS AND DISCUSSION

3.1 External Morphology of Larvae

The head capsule on the dorsal side is long, narrow and smaller than the body, heavily sclerotic and can be retracted into the thorax. The distance between eyes and the size of the eyes were measured throughout the three instars as shown in Fig. 2. Dorsal

segments are densely hairy with several rows of small cilia (se) well developed in the anterior segments II and III (Fig. 1). The first segment is characterized by the prominent anterior spiracles (ea) that are arranged laterally.

The size of the head, as well the distance between eyes and antenna can be used as an indicator of the larvae age. Once the larval age increases the size of the head capsule increases. These structures not only help to age the larvae but also determine the instars.

The prostigma (also called anterior spiracle) is the anterior of the two pairs of spiracles opening on the pleura (Fig. 3) and is located in the first segment. Each anterior spiracle is formed by a sclerotic plaque with a heart shape stigmatic area in the center. It has two V-shaped grooves and a stigmatic scar at its base. The function of the anterior spiracle is to provide an airway into the insect's musculartho rax to aerate cells.

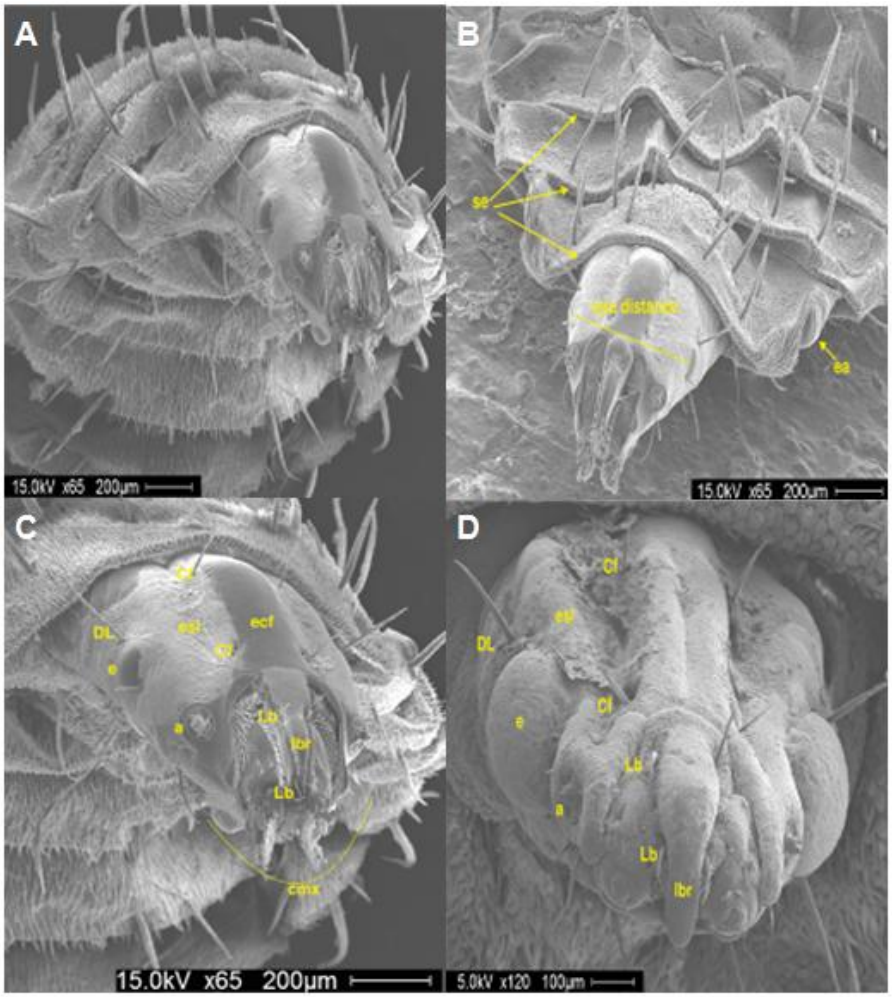


Fig. 1. Head capsule and first thoracic segment of the larvae in the first and third instars. (A). General view. (B). Distance between eyes, detail of cilia (se), anterior spiracles (ea). (C). First instar. Detail of seta frontoclypeal (Cf), lateral sclerite (esl), frontoclypeal sclerite (ecf), seta dorsolateral (DL), compound eye (e), antenna (a), seta labrum (Lb), labrum (lbr), maxilla and mandible complex (cmx). (D). Detail of head in the third instar. Detail of seta frontoclypeal (Cf), lateral sclerite (esl), frontoclypeal sclerite (ecf), seta dorsolateral (DL), compound eye (e), antenna (a), seta labrum (Lb), labrum (lbr), maxilla and mandible complex (cmx)

The thorax is composed of three segments (Fig. 3). Dorsal segments are densely hairy with several rows of small cilia well developed in the anterior segments II and III. There are three pairs of dorsal seta and one pair of dorsolateral seta in the three segments. Segment one has 2 extra pairs of anterodorsal seta.

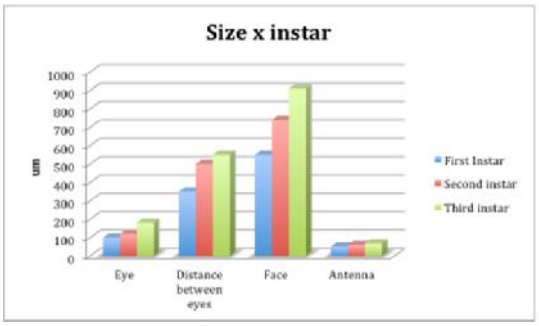


Fig. 2. Size of eyes, distance between eyes, face and antenna throughout lifecycle of BSF larvae

The abdomen consists of 8 segments that are formed by plates, roughly rectangular covered by numerous small seta, although they often grow longer and thicker caudally. There are 3 pairs of dorsal setae arranged as in the thoracic segments, plus 1 dorsolateral pair, two lateral pairs, differentiating these segments. One pair ventrolateral and three pairs of ventral setae appears. Segments 1 to 7 are characterized by spiracles on both sides (Fig. 4). The eighth abdominal segment is the last or anal segment with a rounded shape. At its dorsal end there is an aperture that is surrounded by small spiracles leading to the chamber, inside of which are in a pair of dorsal posterior spiracles. These blowholes are formed by numerous openings arranged radially. Anus appears as a longitudinal slit in the ventral half of the anal ventral segment and its edges are conical spines. The last abdominal segment, the anal segment has a rounded shape. In addition, there is an aperture surrounded by small cilia that leads to a spiracular chamber where a pair of posterior spiracles in a dorsal position is found. These spiracles are formed by several openings arranged radially on ecdysial. The anal structure appears as a longitudinal slit in the ventral half of the anal ventral segment and the edges appear scalloped with short conical spines.

Fig. 5 shows another interesting abdominal structure, called the sternal patch presented in the middle area of the segment. It is an area without trichoids but with the presence of remarkably small cuticular facets, a cuticular area with specialized glands, and with a different color from the rest of the segment.

3.2 External Morphology of Adult Fly

The head of adult flies is small and narrower (Fig. 6) than the body. Their eyes are broadly separated in both sexes. Black soldier fly do not have mouthparts to chew their food, they have a sponge-like mouthpart (Fig. 7) that allows them to lap up liquid. If the food is already in liquid form, then the insect simply places the sponge on the liquid. If the food is in solid form, the insect may first regurgitate salivary secretions onto the solid food, allowing it to liquefy before the insect feeds similar to other Musca.

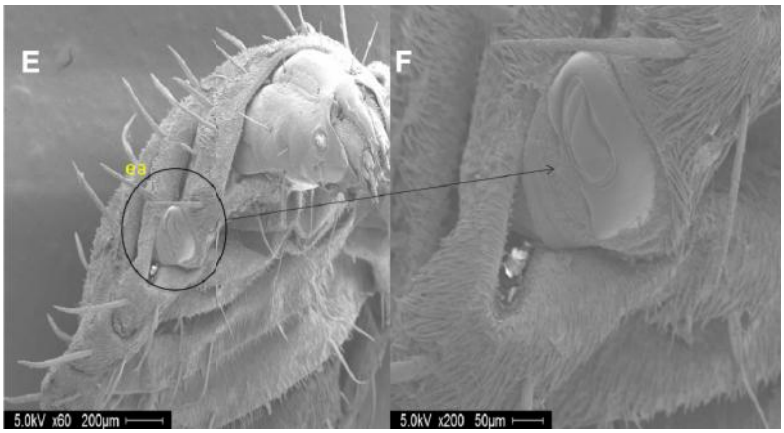


Fig. 3. Details of anterior spiracle (ea) in the first thoracic segment in the first instar. (E). Anterior spiracle x60. (F). Anterior spiracle

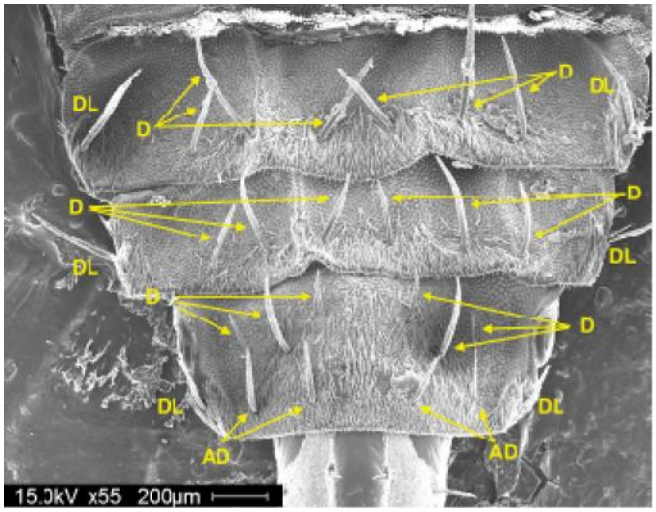


Fig. 4. Details of the seta in the thoracic segments. Dorsal seta (D), anterodorsal seta (AD), dorsolateral (DL)

Black soldier fly is a nonbiting fly, so the mandibles are absent and other structures are reduced. Maxillary palps are present, although short.

A detail of the antenna is showed in Fig. 8. It is morphologically and functionally differentiated into three parts: the scape that is the proxymal antennomere, the pedicel (second antennomere) and the flagellum, (a long terminal segment characterized by flagellomeres). It is twice as long as the BSLF head in lateral view with short bristles. The length of the antenna is important for sensory perception and some of the sensory uses of antenna include motion and orientation, odor, sound, humidity, and a variety of chemical cues.

The order Diptera comprises primarily aerial insects and the thorax can be considered the main part of their body, which bears the only pair of wings with internal flight muscles. The thorax is divided into three separate and visible sections called from the front: the prothorax, mesothorax and the metathorax. The adult flies' abdomen is slender consisting of five visible segments. Soldier flies also have two hairless translucent windows located on the first abdominal segment that may protect the insect's internal anatomy from the outside environment, as a skeleton to provide support and allow for movement or possible bioluminescent signalling (Fig. 9).

The female is usually larger than the male, although there is no obvious external sexual dimorphism. Genital structure (Fig. 10) represents the sexual dimorphism of this species. Male genitalia are short with two pairs of posterior side lobes, a pair of rims and a pair of very small gonostilos. The edeagal complex is very thin and is dilated in its basal part. Terminalia of a female consists of a long pair formed by two segments; subgenital has a long plate in its distal portion with a pointed shape and genital subtriangular furca.

The legs have precisely arranged bristles that also function in chaetotaxy. The femur and tibia may bear combinations of dorsal, anterodorsal, posterodorsal, ventral, anteroventral and posteroventral bristles. The leg flexes (tibia on femur) in the dorsal ventral plane. Typical insect leg as shown in Fig. 11 consists of coxa, this is the most basal aspect of the insect leg and articulates with the sternites, the trochanter that is usually small and serves as a joint between the coxa and the femur. The femur is long and contains the main skeletal muscles used in running, jumping and digging. The tibia is a long serving to increase the length of the leg, as well as adding an extra joint and flexibility. The tarsus is the foot of the insect leg and can consist of between one and five segments. The claws are at that the end of the tarsus serve to assist the insect in holding onto the substrate.

Adult flies have only one pair of wings, on the second thoracic segment. The halteres vibrate up and down in time with the wings and act as a gyroscope sensory organ that is essential for flight. If the fly yaws, rolls, or pitches during flight, the halteres, maintaining their original

plane of movement, twist at their bases, where special nerve cells detect the twist and cause the fly to correct its flight attitude [11]. Veins in the wing (Fig. 12) provide support for the thin, delicate membranous cuticle that forms the wing itself and blood supply. Veins have tracheae and provide a passage for haemolymph which is essential for the functioning of the numerous sense organs found on the wings, including wind sensitive hairs. Wing venation is also an important morphological characteristic in taxonomy and can be used to identify many insect groups.

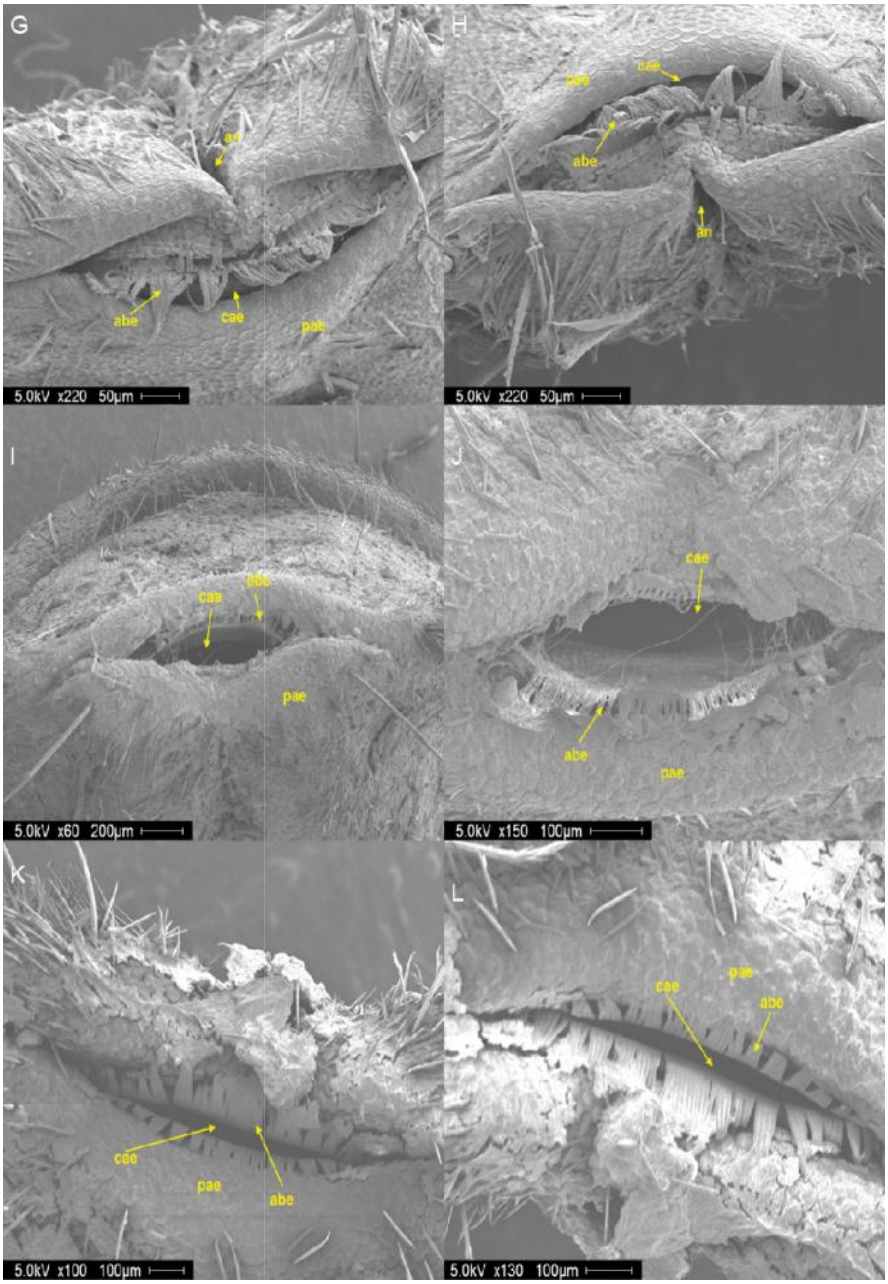


Fig. 5. Dorsal and ventral view of the last segment of *Hermetia illucens*. First instar: Dorsal view (G). Ventral view (H). Second instar: Dorsal view (I). Ventral view (J). Third instar: Dorsal view (K). Ventral view (L). Anal segment (An), spiracle camara (cae), sternal patch (pae), spiracle opening (abe)

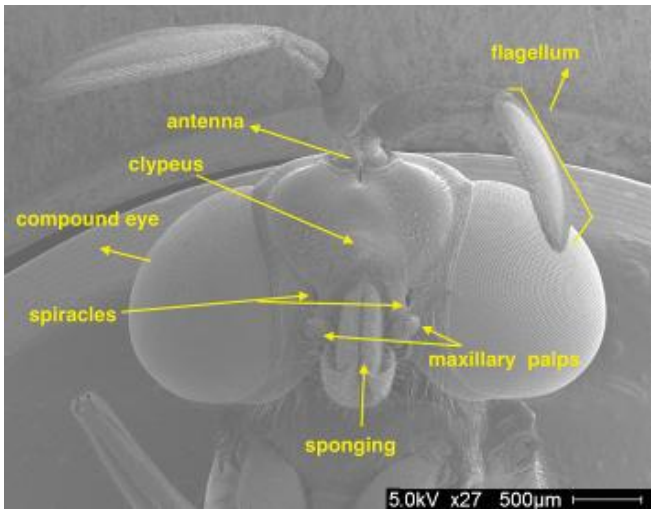


Fig. 6. Frontal view of the head of a female Black soldier fly. In the detail the antenna, spiracles, maxillary palps, sponging, clypeus, flagellum and compound eyes

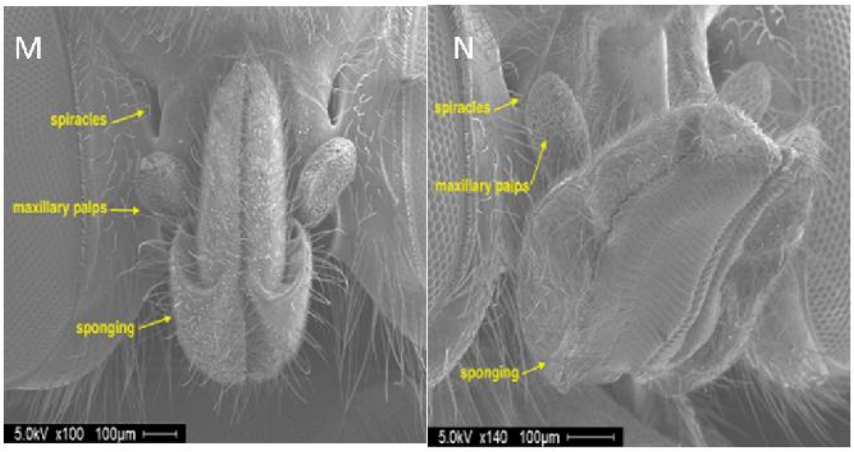


Fig. 7. Frontal view of the sponging, spiracles and maxillary palps of Black soldier fly. (M) and (N) during third instar

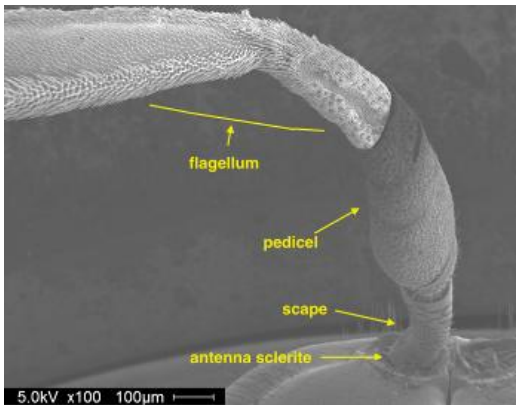
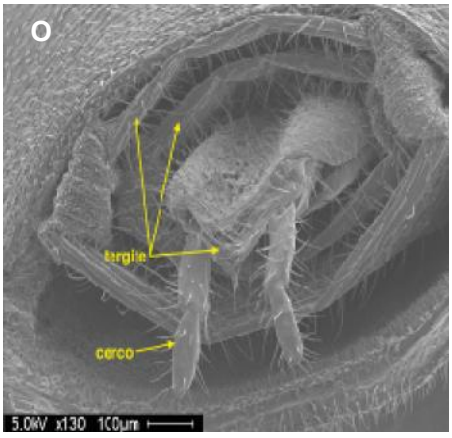


Fig. 8. Detail of the antenna of Black soldier fly

Costa (C) the leading edge of the wing. Subcosta (Sc) second longitudinal vein (behind the costa), typically unbranched. Radius (R) third longitudinal vein, one to five branches reach the wing margin. Media (M) fourth longitudinal vein, one to four branches reach the wing margin. Radio-medial (r-m) connects the posterior branch of the radius and the anterior branch of the media. Cubitus (Cu) fifth longitudinal vein, one to three branches reach the wing margin. Anal veins (A1, A2, A3) unbranched veins behind the cubitus. Medio cubital (m-cu) connects the posterior branch of the medius and the anterior branch of the cubitus. Cubito-anal (cu-A) connects the posterior branch of the cubitus and the first anal vein.

Fig. 9. The transparent window in the abdomen



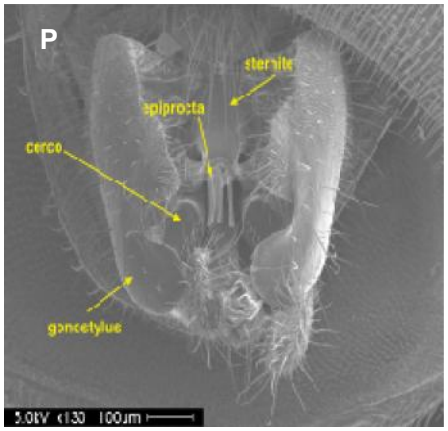
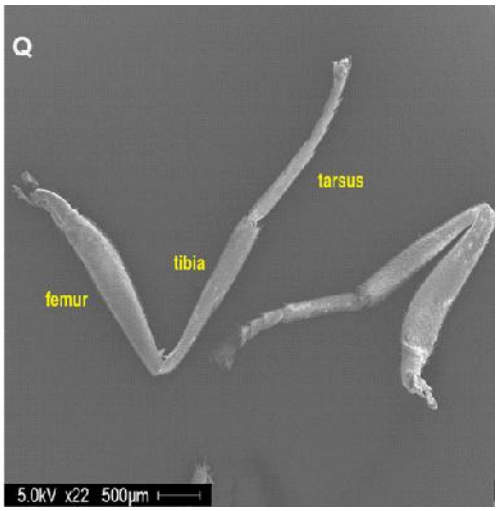
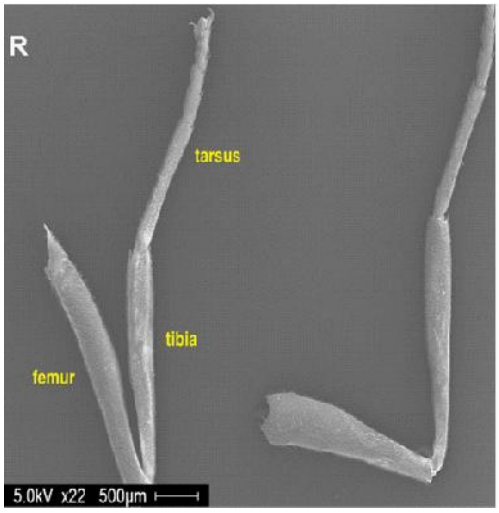


Fig. 10. Genital structure of Black soldier fly. (O). Terminalia of a female. (P). Male genitalia





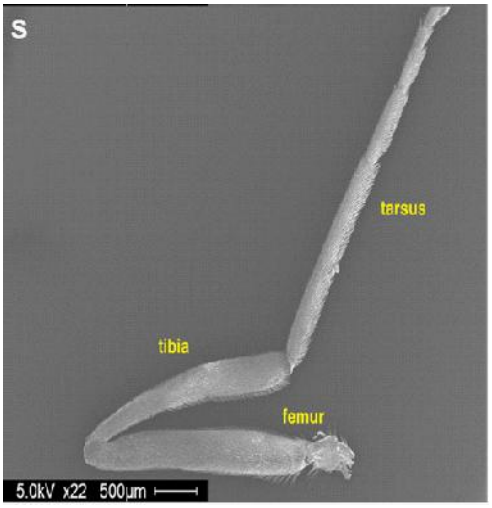


Fig. 11. Detail of the three pairs of legs of black soldier fly showing the femur, tibia and hind parts

(Q). First pair of legs, (R). Mid legs, (S). The hind leg.

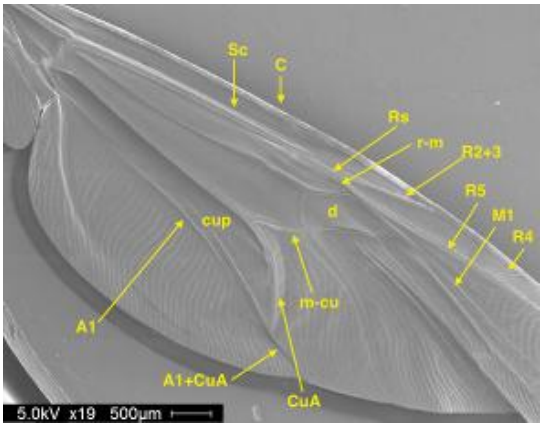


Fig. 12. Wing venation of black soldier fly

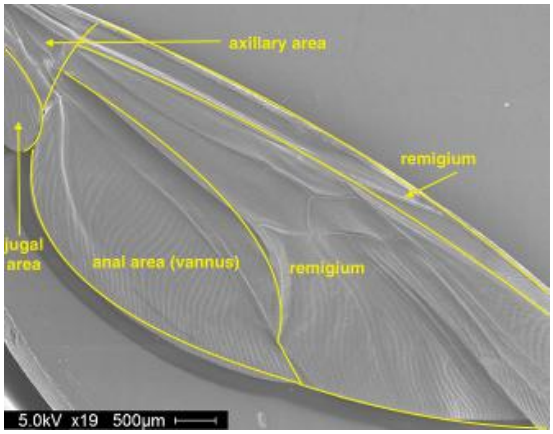


Fig. 13. Detail of four fields in the wing of black soldier fly.

There are about four different fields (Fig. 13) found on the insect wings, the remigium which is responsible for the flight, the anal area (vannus), the jugal area and the axillary area.

4. CONCLUSION

BSF larvae were reared for a detailed description of the anatomy during larval and adult stages. This study showed modifications in the structures of the BSF larvae during their lifecycle.

The BSF larvae head is small and narrower than the body possibly for burrowing. The first segment is characterized by anterior spiracles that provide the air entrance into the thorax.

The arrangement of setae in the thoracic segments help the larvae to remain on the surface of aquatic environments and get the air supply needed to survive.

The number and placement of seta is the same in all larvae. A pair of anterior spiracles is found in the first thoracic segment which allows the entrance of air in the trachea in aquatic environments.

Adult flies do not chew their food, they have a sponge-like mouthpart that allows them to lap up liquid. Because the reduced structure they do not eat and live for a couple days only. Their main purpose after emerging as an adult fly is to mate and lay eggs. The antenna is twice as long as the flies head in lateral view with short bristles and used for sensory perception.

Adult flies have a transparent window on the first abdominal segment, that may protect the insect's internal anatomy from the outside environment and as a skeleton provide support and allow for movement or bioluminescence.

Our study showed a morphologic difference between male and female flies compare the genital structure based on the size of flies.

Adult flies have one pair of wings on the second thoracic segment.

Wing venation is an important characteristic in taxonomy and can be used to identify many insect groups.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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