Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(5): 147-152

Received: 03-08-2015
Accepted: 06-09-2015

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Assessment of Diptera: Stratiomyidae, genus *Hermetia illucens* (L., 1758) using electron microscopy

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Abstract
Black Soldier Flies (*Hermetia illucens*) are common in the western hemisphere as inhabitants of nutrient rich ecosystems such as manur piles and compost heaps. They pass through a five stage lifecycle i.e. larva, prepupae, pupa, and adult. The adults, 15 to 20 mm in length) have no mouth parts and therefore do not bite nor do they sting and are not known to transmit any diseases. The larva, are voraceous consumers and have been shown to be an effective maneur management tool. In this study a Scanning Electron Microscope is used to analyze and document the anatomy of both the larval and adult for of this insect. Changes in each step of their life cycle were noted as the insect grew and matures.

Keywords: Diptera, Stratiomyidae, *Hermetia illucens*, black soldier fly, biology, Scanning Electron Microscope

1. Introduction
Black soldier flies (Musca leucopa) are found throughout the Western Hemisphere in habiting nutrient rich ecossystmes such as manure piles and compost heaps.
Order Diptera, family Stratiomyidae, *genus Hermetia Illucens* (Linnaeus), most known as Black soldier fly [1], had their name changed over the time as showed below**:**
Musca illucens Linnaeus, 1758. ST? [NRS]: "South America".
Musca leucopa Linnaeus, 1767. ST 1? [LSL] "America".
Hermetia rufiventris Fabricius, 1805. ST 2 ♀[UZMC] "America meridionali".
Hermetia pellucens Macquart, 1834: explication des planches 4: Planche V, Fig. 11. Incorrect subsequent spelling.
Hermetia nigrifacies Bigot, 1879. LT♂ [BMNH, des McFadden 1972c:257]: Mexico.
Hermetia mucens Riley & Howard, 1889. Incorrect subsequent spelling.
Hermetia illucens var. *nigritibia* Enderlein, 1914c: 9. HT? [PAN]: Brazil, Santa Catarina.
Hermetia illucens: Copello, 1926. Incorrect subsequent spelling.
They are common to North and South America and the Australian region from Samoa to Hawaii as showed in Figure 1. In the continental United States, *Hermetia illucens* commonly breeds in outdoor toilets, poorly managed compost and in poultry manure. Larvae occur in greatest densities in moist rather than wet or dry media. They are scavengers and thrive on many kinds of decaying organic matter. Though they may be a nuisance, soldier flies do not have functional mouthparts, so they do not bite and are not known to transmit any diseases. In fact, this species has the beneficial effect of rendering the breeding media less suitable for the production of houseflies [2].

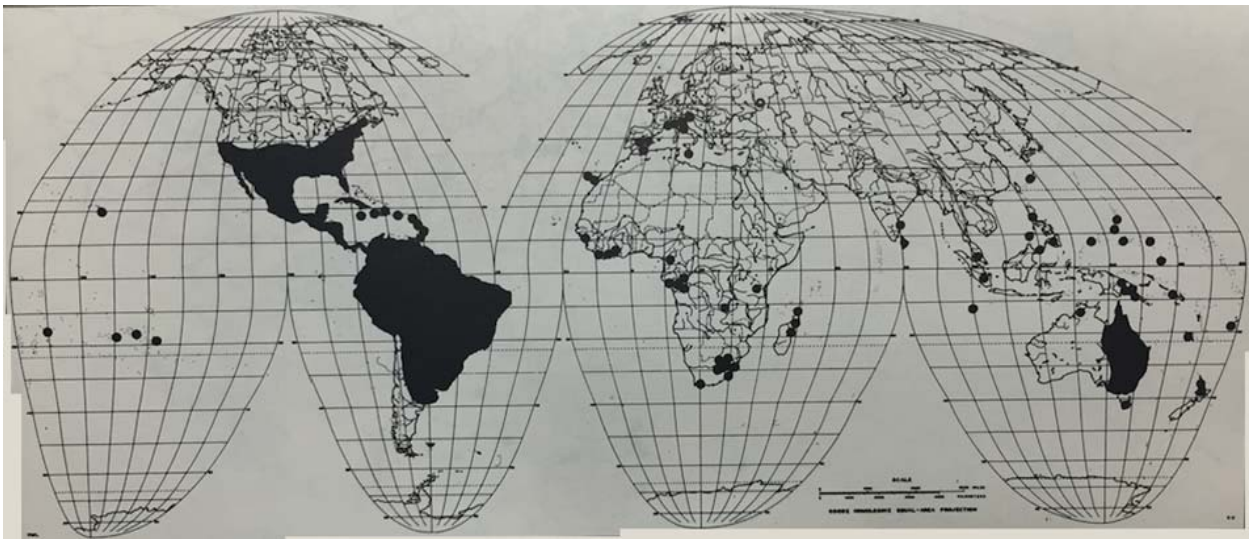


Fig 1: World distribution of *Hermetia illucens*. (Roskosny, 1983).

Hermetia illucens passes five stages of the life cycle: egg, larva, prepupae, pupa and adult [3]. The life cycle scheme is showed in Figure 2. Depending on temperature and other environmental conditions, pupation occurs in spring and lasts from 9 days to 5 months. Therefore, some adults appear as early as April but many do not emerge until late summer. The larvae seek sheltered, dry locations to pupate. Newly emerged soldier flies mate in flight. Soon afterwards females begin to deposit egg masses near edges of decaying organic matter. Eggs incubate 4 days to 3 weeks before hatching. Larvae hatch from eggs and develop through several stages before pupating inside of the last larval skin [3, 4].

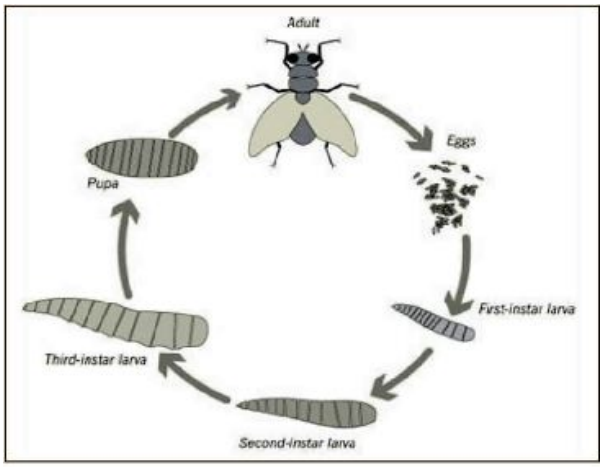


Fig 2: Lifecycle of *Hermetia illucens*. (ecosolutions.com)

In their first instar, after hatching, they are a dull, whitish in color, with a small projecting head containing chewing mouthparts. Larvae pass through three instars and require approximately 30 days to complete development [5]. During larval development, they are insatiable feeders. As adults they do not need to feed and rely on the fats stored from the larval stage [4, 5].

The pupa is formed inside the last larval skin, a puparium. The coronet of hairs on the last segment can be retracted. *Hermetiinae* is distinguished by the elongate and thickened eighth antennal flagellomere and absence of scutellar spines [1].

Other Stratiomyidae vary in color from black to metallic blue, green and purple or black and yellow patterns. Larvae are generally aquatic or semi-aquatic, feeding on algae, decomposing organic matter or on aquatic organisms. A few species are found in dung or in decaying fruit, vegetables or under the bark of rotting wood. Larvae of some species bear a rosette of hairs around the back end of their bodies used to float on the water surface and obtain air [6].

Adult flies are robust, ranging from 15 to 20 mm in length, black with smoky black wings of the plesiotypic type. Wing microtrichia very dense and cover entire wing membrane. Wings are held over the back when at rest [6, 7, 8]. The adult black soldier fly is not usually considered a pest [4] and because the larvae have been shown to be effective manure recyclers, a "Black Soldier Fly Manure Management System" has been proposed to not only reduce livestock waste, but also generate a food source for fish and other animals. In a program outlined in Newton *et al*. (2005) swine manure was fed to black soldier fly larvae, which greatly reduced the waste material. The manure was transferred into a basin containing black soldier fly larvae. As the larvae developed they reduced the manure by 50%. Approximately 45,000 larvae will consume 24 kg of swine manure in 14 days [4]. Larval and bacterial activities, not only reduce the dry matter, but also other components such as nitrogen or phosphorus. Experiments with cow manure showed a reduction of 43% nitrogen and 67% phosphorus [9]. The combination of waste treatment capacity along the generation of a product of economic value makes *Hermetia illucens* a promising tool for organic waste management.

As the larvae mature they crawl out of the basin, thereby self-harvesting themselves, and are subsequently available as livestock feed. In addition to being a good source of oil and protein for animal feed, black soldier fly larvae have the potential of improving organic waste into a rich fertilizer [10]. Larvae feed on the chicken manure and can convert it to 42% protein and 35% fat [11]. Black soldier flies management of manure offers many advantages as a reduction of 25% waste annually, a high quality feedstuff will be produced, and house fly populations will be controlled [11].

In this study a Scanning Electron Microscope is used to analyze and document the external anatomy of both the larval and adult stages of this black soldier larva. Changes in each step of their life cycle are noted as the insect grew and matures.

2. Materials and Methods

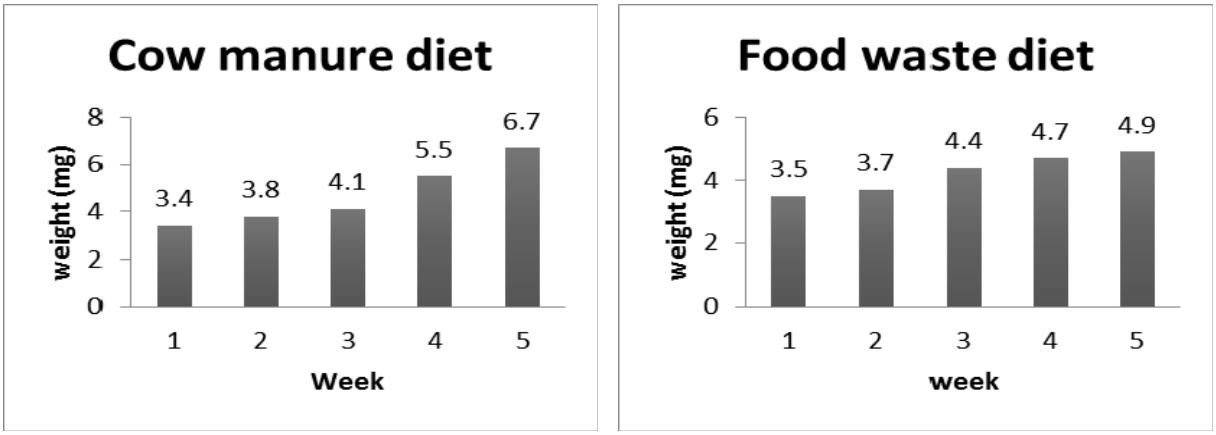
Black soldier larvae hatched after 72 hours were acquired from a local company and investigated in a period of two months with temperature and moisture monitored. Larvae were reared in ideal conditions and the transition of instars, pupating and emerging as adult flies were observed. An overall of 30 larvae were used for this experiment. They were placed in groups of 5 in containers of 6 cm x 5 cm containing 10 g of cow manure in three containers and 10 g of food waste in the other three.

The conditions for breeding the ideal life cycle were temperature between 28-30 degrees Celsius and 70 % moisture. Larvae were weighted every week.

The scanning electron microscope (SEM) made possible to see the details of the larva of this genus. Specimens were fixed with 2.5% glutaraldehyde in Phosphate Buffered Saline (PBS) buffer followed by osmium tetroxide 1% (in water). 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 dried in a desiccator and sputter coated with Au-Pd. A JEOL JSM-5800 LV low vacuum scanning electron microscope equipped with an EDAX energy dispersive x-ray spectrometer was used to collect image.

3. Results and Discussion

Throughout the three instars, *Hermetia illucens* were measured and weighted (Graph 1) every week and the color and size were taken into consideration to judge the transition of instar. Changes in size and color regarding the time, temperature and moisture remained constant for both groups fed with food waste or cow manure as it can be seen in the Table 1.



Graph 1: Weigh during a 5-week period.

First instar lasted 15 days and the larvae had an average of weight of 3.6 mg with a cow manure diet, same value acquired having food waste diet. In the transition to the second instar and the next 15 days, the larvae fed with cow manure gained more average of 1.2 mg while larvae fed with food waste acquired an average of 1mg weight. Third instar, which lasted 8 days, larvae fed with cow manure had an overall weight of 6.7 mg and the larvae fed with food waste an overall of 4.9 mg.

Table 1: Stages of *Hermetia illucens*

State	Size (mm)	Color	Picture	Duration (days)
1 instar	5	White/ yellow		15
2 instar	12	Light brown		15
3 instar	19	Dark brown		8
Pupa	19	Dark brown		10
Fly	20	Black		7

After hatching the larvae had a white/yellowish color and 5mm +- 0.5. As they transited to the second instar in a period of 15 days, they doubled size and became light brown. Third instar was reached after 30 days and the larvae grew, changed its color to dark brown and became rigid. Pupating lasted 10 days and a fly emerged from chrysalis with sole purpose of mating and lay eggs. Appearance of black soldier larva in Figure 3 shows the division of thoracic and abdominal segments as well the details of the cuticle, hair and body. The head capsule on the dorsal, long, narrow and small than the body, is heavily sclerotic and can be retracted into the chest.

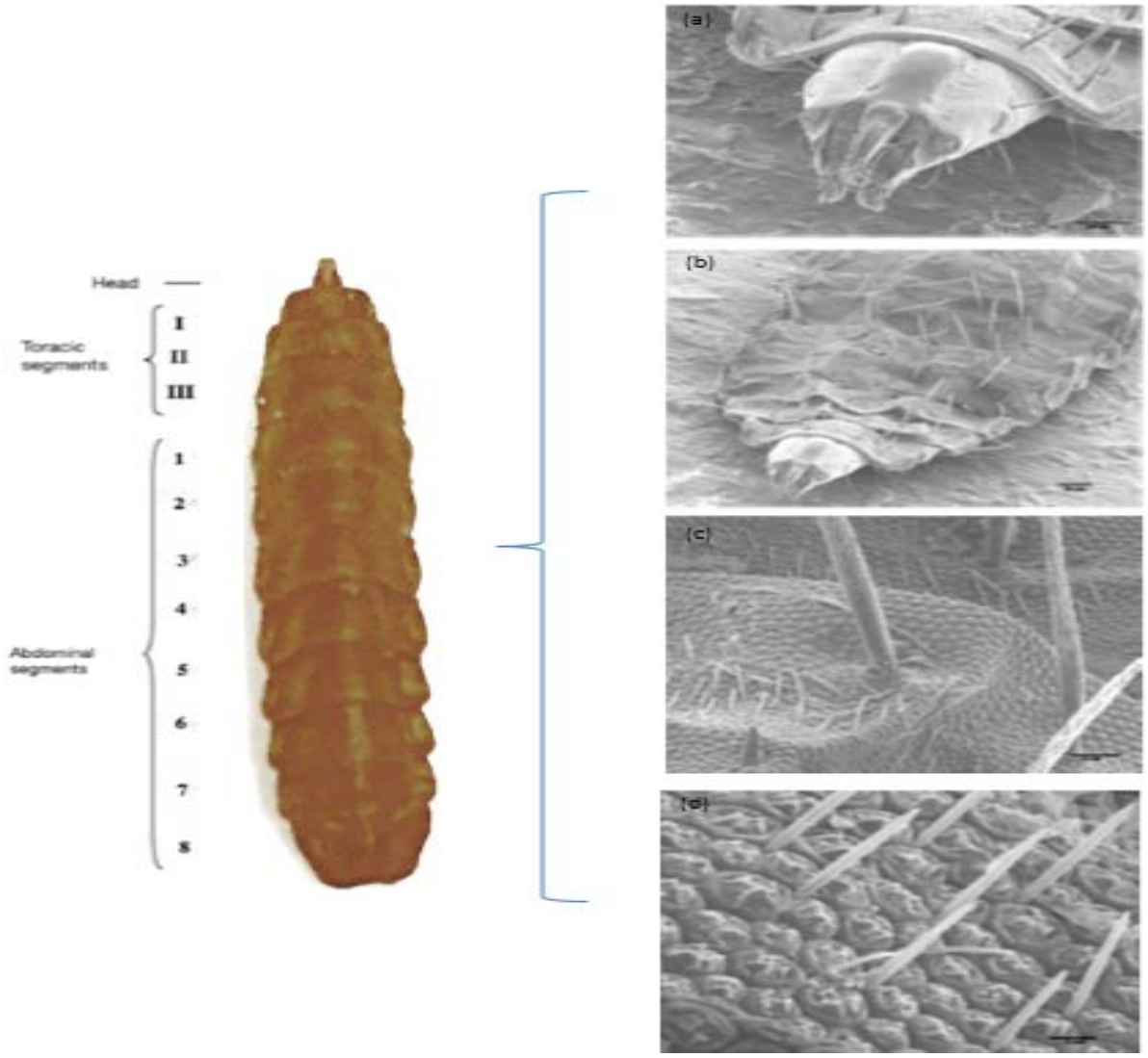


Fig 3: Division of thoracic, abdominal and head of the dorsal. Details of body and hairs.

a) Head of larva. 15.0 Kv, x30, spot size 8. b) body of the larva (plates). 10.0 Kv, x33, spot size 8. c) body of the larva (hairs). 15.0 Kv, x270, spot size8. d) body of the larva (hairs). 15.0 Kv, x1000, spot size 9.

The thorax is composed of three segments (Fig.4). Dorsal segments are densely hairy with several rows of small cilia well developed in the anterior segments II and III.

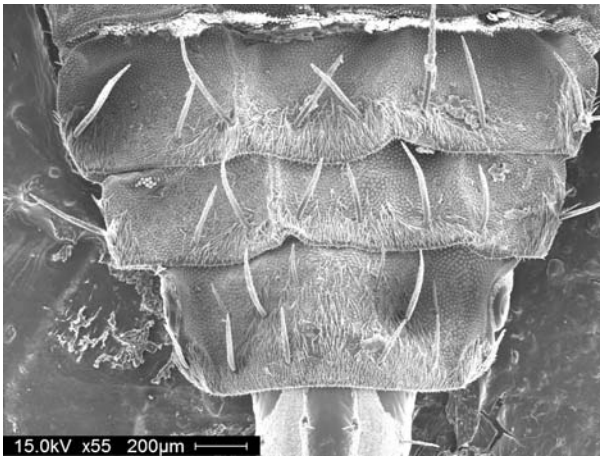
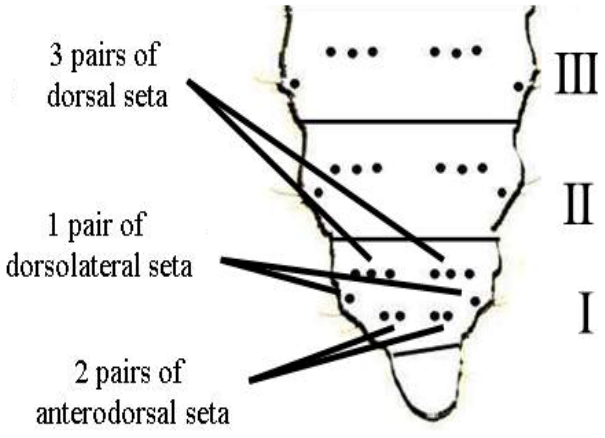


Fig 4: Division of thoracic, abdominal segments of the dorsal.



Source: Adapted from Gobbi, 2012.

The abdomen consists of 8 segments that are formed by plates, roughly rectangular covered by numerous small setas, although they often grow longer and stronger caudally. There are 3 pairs of dorsal setae arranged as in the thoracic segments, plus 1 dorsolateral pair, two lateral pairs, differentiating these segments abs. Ventral one pair ventrolateral and three pairs of ventral setae appears. The segments 1 to 7 are characterized by both sides spiracles. The eight abdominal segment is the last or anal segment with a rounded shape. At its end there is an aperture surrounded by small spiracles leading to the chamber, inside which are in a pair of dorsal posterior spiracles. These blowholes are formed by numerous openings arranged radially on scar. Anus appears as a longitudinal slit in the ventral half of the anal ventral segment and its edges are conical spines.

The adult flies' abdomen is slender consisting of 5 visible segments (Fig. 5). The first abdominal segment has clear areas. Soldier flies also have two translucent "windows" located on the first abdominal segment.

The head of adult flies is small and narrower (Fig. 6) than the body. Their eyes are broadly separated in both sexes. The antennae have a long terminal segment characterized by a elongated, flagellum. It is twice as long as the head.

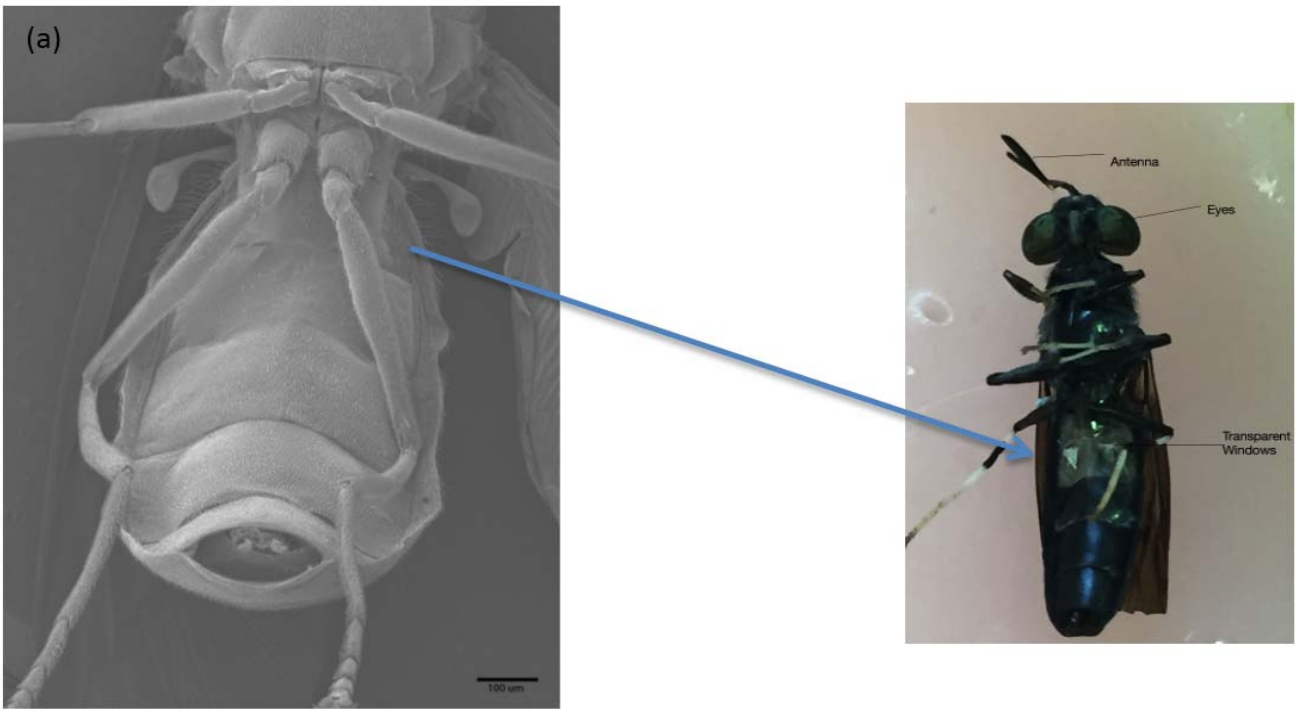


Fig 5: Black soldier fly. a) Abdomen in ventral view. 5.0 Kv, x22, spot size 11. b) Picture showing the transparent window in the abdomen.

The head of adult flies is small and narrower (Fig. 6) than the body. Their eyes are broadly separated in both sexes. The antennae have a long terminal segment characterized by an elongated, flagellum. It is twice as long as the head.

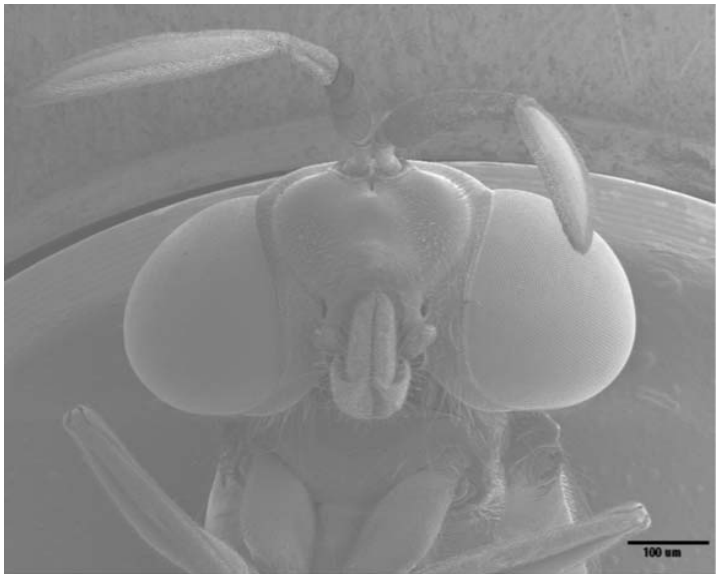


Fig 6: Frontal view of the head of Black soldier fly. 5.0 Kv, x27, spot size 11.

The female is usually larger than the male, although there is no obvious sexual dimorphism. Genital structure represents the sexual dimorphism of this species. Male genitalia is short and has 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.

Terminal structure of a female (Fig. 7) 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.

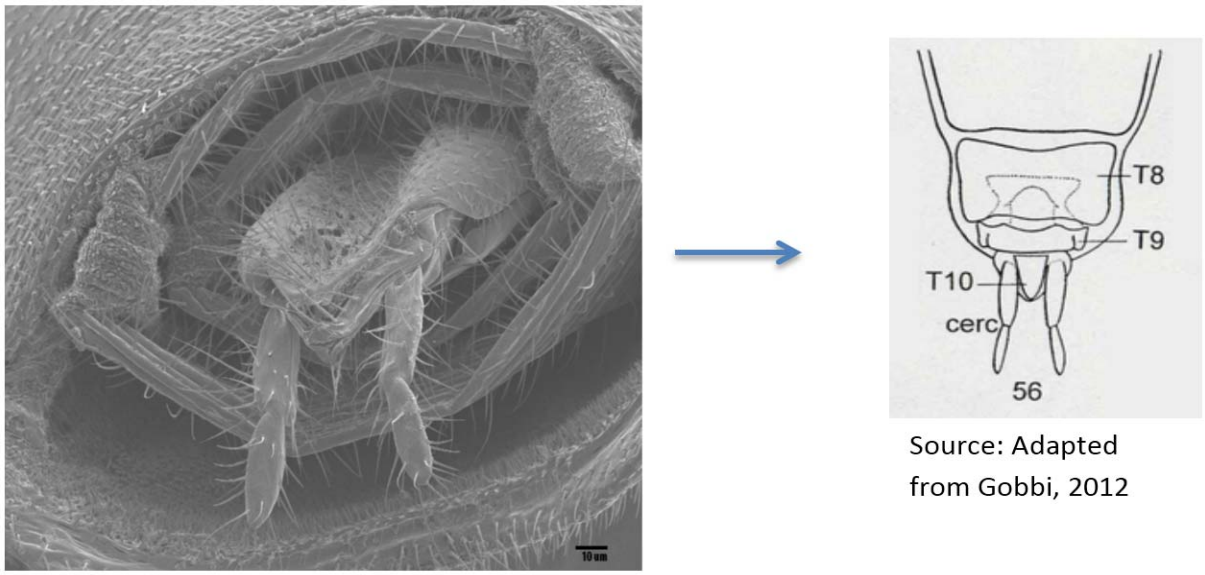


Fig 7: Genital structure of a female. 5.0 Kv, x130, spot size 11. (cerc: cerco, T: tergum).

4. Conclusions

Black soldier larva has been widely used in composting piles and manure management. This study shows the morphology of this insect and the modifications along the lifecycle.

Larvae are elongate and may be flattened, with the skin or exoskeleton firm and tough with average of 2 to 19 mm. Head is small and narrower than the body. The body bears no legs or other prominent features except body spines. Pupation occurs in the last larval skin when they stop feeding. This stage last between 1 to 5 weeks depending on the breed conditions.

Adult flies resemble wasps in appearance and behavior. Wings at rest are folded scissorlike across the abdomen. Antennae characteristic in having a long terminal segment consisting of eight flagellomeres.

The diet based on different sources as cow manure and food waste does not interfere in the instars and adult stage of *Hermetia illucens*. However, monitoring temperature and moisture conditions are crucial to the development of the larvae.

In addition, the larvae are good source of oil and protein for animal feed and have the potential of improving organic waste into a rich fertilizer.

5. Acknowledgment

Thanks to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Ministério da Educação (Capes/MEC) for funding, TRINITY Institute at ESF and N.C. Brown Center for Ultrastructure at ESF for providing the electron microscope.

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