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Morphological characteristics of *Phthorimaea operculella* (Lepidoptera: Gelechiidae)
developmental stages by scanning electron microscope

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Abstract

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), or tobacco split, is considered a major pest of potato plants, widely grown in tropical and subtropical regions. This study aimed to distinguish the immature stages (last larval and pupal stages) as well as between the sexes of this insect pest by scanning electron microscope (SEM). Based on our results, scanning electron microscopy (SEM) analysis can assist in the identification of novel morphological details at the larval stage (General description of larvae, head capsule, mouthparts, antenna, stemmata, lateral ocelli, thorax and abdomen), pupae (Distinguish between male and female pupae) and adult potato tuber moths (General description, head, thorax, legs, female genitalia, and male genitalia). To this end, we reared insects of both sexes at different stages and examined them by SEM. Based on the small morphological features noted by SEM, our results can support accurate discrimination between the larval and adult stages as well as between the sexes of this insect problem. This work could facilitate the identification of this pest for effective integrated pest management programs.

Introduction

Potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a global pest of Solanaceae crops, especially potatoes, mainly grown in tropical and subtropical regions (Das and Raman, 1994; Kroschel *et al.*, 2013; Rondon, 2010 and Rondon and Xue, 2010). *P. operculella* larvae mainly bore the leaves, stems and petioles of *Solanum tuberosum* L. (Rondon, 2010). The *P. operculella* moths lay eggs on the surface of

potatoes during storage, focusing on the cracks, fissures and skins of peeled potato tubers (Al-Ali *et al.*, 1975). The newly hatched larvae then dig and burrow into the tubers (Finbarrg *et al.*, 2010 and Rondon, 2010). According to recent studies, these pests also wreak havoc on tomato and tobacco crops (Aryal and Jung, 2019 and Vaneva-Gancheva *et al.*, 2016).

Adult, egg, larva and pupa are the four stages in the life cycle of *P. operculella*. The growth, survival, and reproduction rates of *P. operculella* females and males vary greatly

depending on the quality and availability of the host (Raman 1980; Chauhan and Verma, 1991; Rondon *et al.*, 2007; Rondon and Xue, 2010) and Rondon (2010) detailed the morphology and general characteristics of females and males of *P. operculella*. Adults can live for 1-2 weeks, and copulation can occur 16-20 hrs. after the adult emergence (Gill *et al.*, 2014; Chauhan and Verma, 1985 and Makee and Saour, 2001). Oviposition takes place at night (Traynier, 1975 and 1983), in leaves or directly in tubers (Varela and Bernays, 1988). Traynier (1975), Rondon (2010), Gill *et al.* (2014) and Rondon and Gao (2018) described *P. operculella* larvae. In general, *P. operculella* can produce several generations per year, from 2 to 12 to 13 generations per year under extreme conditions (Al-Ali *et al.*, 1975). Temperature is one of the most important abiotic factors affecting insect biology, including instar length, and influences geographical distribution, population dynamics and management (Régnière *et al.*, 2012). This study aims to find good morphological features that help to accurately identify this pest to develop successful integrated management programs for this pest.

Our study is the first descriptive study using scanning electron microscopy (SEM) to observe the morphological features of different life stages of PTM, including antennae, mouthparts, legs, and segments. end of the abdomen. In addition, specific morphological structures were measured in male and female PTM pupae and adults to compare them.

Materials and methods

1. Rearing of *Phthorimaea operculella* different stages:

P. operculella were obtained from the Plant Protection Research Institute, Dokki, Giza (Ministry of Agriculture, Egypt) and reared under laboratory conditions at 26°C ± 2°C, 60% ± 5% RH., and a photoperiod of 14:10 (Light: dark). Eggs were placed in plastic containers with potato tubers free of pesticide residues, previously punctured to facilitate the entry of newly hatched larvae.

2. Preparation for Scanning Electron Microscopy:

SEM allowed us to observe in detail the different life stages of *P. operculella*. The adult wings, legs, and mouth were cut off from the body, and the long setae were partially removed from the abdomen, so that I could examine the final abdominal segment. They were placed in 2.5% glutaraldehyde for 24 hrs. Twenty live specimens were seeded on a pallet affixed to stubs using double-sided sticky tape, and sputter coated with gold-palladium, before being scanned with a JEOL GM 5200 microscope. SEM analysis was performed at the Center of Entomo-nematodes in the Experimental Research Station at the Faculty of Agriculture, Cairo University, Giza, Egypt.

Results and discussion

1. General larval description:

PTM larvae are typically 12–15 mm long, white, or yellow colored with a brown head and prothorax. Its color changes from white/yellow to pink/green as it matures. The prothoracic plate is dark brown or black, with a pale brown anterior margin and white medial sulcus. The thorax contains small black spots and bristles on each segment, and the larvae usually feed on its host plant for up to 2 weeks before pupation.

1.1. Head capsule:

1.1.1. Mouthparts:

The mouthparts are semi prognathous, the epicranium is slightly dorsoventrally flattened, the frontal sclerites are widened distally to form a broad round ecdysial line, the ecdysial suture is short and divides the adfrontal sclerites disto-medially, and the epicranial notch is deep to form two large hemispheres. Larval mouthparts consist of labrum (Lr), mandible (M), maxilla (MX), and labium (Lb). The chewing mandible is highly sclerotized, with a sharp incisor incurved apically; the mandibles cross each other apically (Figure 1A).

1.1.2. Antenna:

A pair of antennae, each of them consisting of three segments, large scape, small pedicel, short flagellum, and one sensillum chaeticum in a lateral position were observed;

in addition, the dense projections were observed at the basal segment.

1.1.3. Stemmata (Lateral ocelli):

Larval eyes, on both sides of the head, persist during the whole larval period. On the top, there are six ocelli on each side.

2. Thorax (Figure 1B):

The three thorax segments (Prothorax, meso-, and metathorax) have three small legs each, the thoracic legs are known as true legs and consist of four segments, each consisting of a coxa, a femur, a tibia, and a tarsus, always

following the same order. Each leg ended within a single claw.

3. Abdomen (Figure 1 D, E, and F):

There are 10 segments in the abdomen; segments 4–7 and 10 carry a pair of pseudo-abdominal legs called prolegs. The prolegs contain several small hooks on the tip, known as crochets. Abdominal spiracles are located on each side of the body on the first eight abdominal segments. There are two types of hair-like setae, long and short tactile setae were observed on the whole larval body. The anal opening is located at the end of the body.

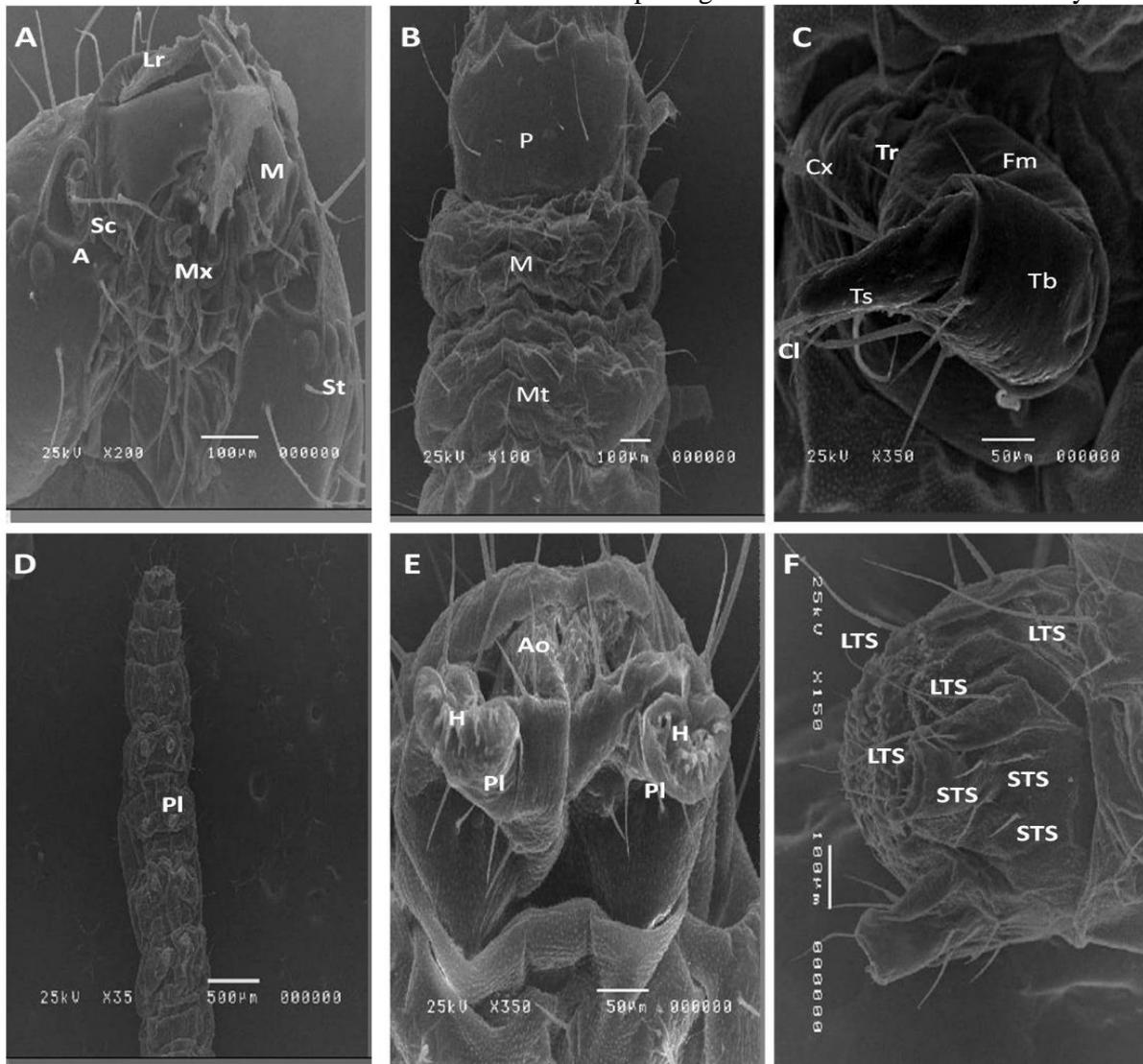


Figure (1): Electron micrographs of fourth larval instar of *Phthorimaea operculella* showing: (A) Higher magnification of antenna and mouthparts, Lr: Labrum, M: mandibles, Mx: maxilla, A: antenna, Sc: sensilla chaetica, St: stemma; (B) Three thorax segments P: prothorax, M: Mesothorax, Mt: metathorax; (C) Higher magnification of thoracic leg, Cx: coxa, Fm: femur, Tb: tibia, Ts: tarsus; Cl: claw; (D) Ventral view of larvae showing prolegs; (E) abdominal leg in the last body segment and anal opening; (F) two types of hair-like setae, Lts: long tactile setae, Sts: short tactile setae.

2. General pupal stage description:

P. operculella pupae are narrow in width and typically 1.27 centimeters in length. They are usually smooth golden yellow, with thin brown lines demarcating sclerites and need 10–30 days to develop, depending on environmental conditions. Vertex rounded; front-clypeus convergent, broadly rounded distally; labial palpi slightly visible; antennae broadly rounded encircling sclerites of maxillae, forelegs and mid legs, meeting medially slightly beyond mid length, extending distally in parallel, diverging distally slightly exposing meso thoracic legs; meso thoracic legs shorter or extending to lengths of antennae and forewings; maxillary palpi and forelegs extending to a common point anterior to mid legs; abdominal spiracles slightly raised. SEM allowed a clear differentiation between male and female pupa. Figures (2A–D) show a dorsal view of the head in male and female pupae. In Figures 2A–B, there is a pear shape between the two eyes pointing toward the tip on the dorsal surface of the male head. In the dorsal surface of the female head, in contrast, there is a bow shape between the two eyes, with two clear setae at the tip as shown in Figures (2C–D). Pupal sex can also be determined by the appearance of the genital area on the ventral side of the last abdominal segments. Furthermore, the shape of the genitalia opening at the end of the abdomen is different between sexes. Compared to the female's oviduct aperture and bursa copulatrix, the male opening is located away from the proximal side of the final abdominal segment. The genital aperture of male pupa is approximately in the middle third of the last abdominal segment, whereas the genital aperture of the female pupa is approximately in the first third of the last abdominal segment, closer to the proximal side, giving the impression that the male pupa has an additional segment before the genital aperture and the anus. To identify the unique characteristics of the pupa, the dorsal and ventral surfaces of the last abdominal segments of the male pupa are displayed in Figures (2 E and F) and the female pupa's genital region is shown

in (Figures 2 G and H) at a higher magnification.

3. General adult stage description:

Males and females are morphologically distinguishable by SEM. Adult moths are small, and nocturnal, with brown to silver scales and black spots on the forewings. The antennae are filiform, and the wings are fringed. Males are slightly darker and smaller than females. The abdomen of the female moths is wider, whereas that of males is narrower.

1. Head (Figure 3A):

The mature *P. operculella*'s antenna, seen from the front, is composed of 11 annular flagella, a scape, and a pedicel. Near the distal end of the antennae, the annuli are cylindrical and get shorter (Figure 3B).

2. Thorax and legs (Figure 3C):

Legs without sexual dimorphism. Legs with elongated and cylindrical coxa, a proximal articulation with episternum, and a distal one with trochanter, femur, tibia, and five tarsi. The hindwings had concave outer margins and pointed tips, in contrast with the more typical, narrow forewings (Figures 3D–F).

3. Female genitalia (Figure 3G):

The intersegmental membrane between abdominal segments 8 and 9/10 is visible in electron micrographs of the female *P. operculella*'s terminal 8–10 abdominal segments. The 8th abdominal segment, which was severely sclerotized, had a smooth surface where the ostium bursae (Arrow) and the papillae annales with their long setae were located. An apophysis divides the cylindrical intersegmental membrane roughly into dorsal and ventral halves.

4. Male genitalia (Figure 3H):

Phothermaea identification was based on adult morphological characters, as well as on the shape of the uncus, digitate, setosevalvae, and the well-developed vinculum and phallus of the male genitalia. Our results can facilitate accurate differentiation of adult and immature sex stages of this insect pest based on the fine morphological features detected by SEM. This work is the first to perform morphological differentiation of the stages of *P. operculella* by SEM.

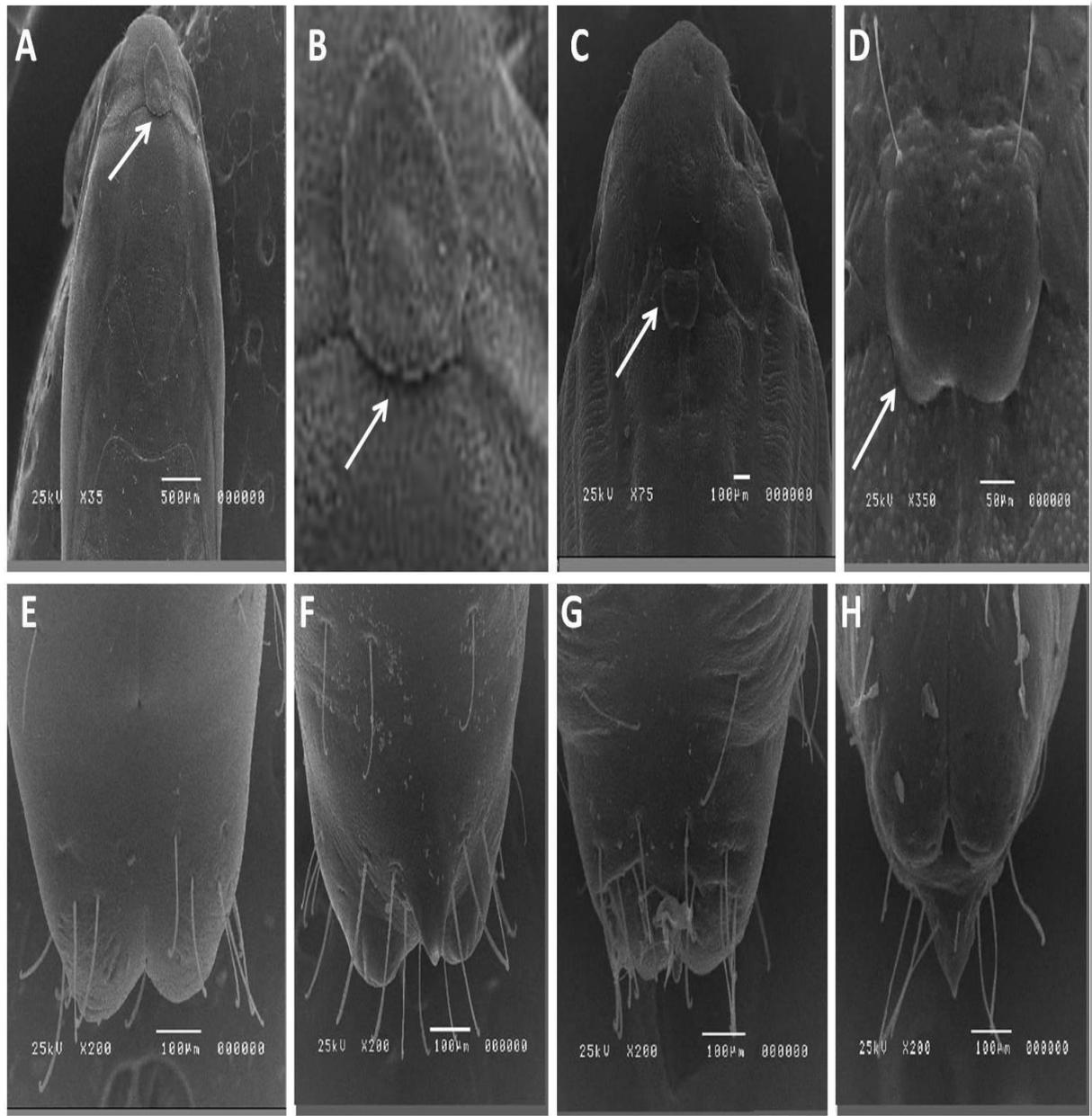


Figure (2) : Electron micrographs of mature *Phthorimaea operculella* pupa showing: (A and B) dorsal surface of the male head. The arrow points to a pear shape between the two eyes; (C and D) dorsal surface of the female head. The arrow points to an enlargement of a unique plate bow shape with two setae between the two eyes, the dorsal and ventral surfaces of the last abdominal segments of the male pupa (E and F), and the genital region of the female pupa (G and H) at higher magnification on the dorsal and ventral surfaces of the last abdominal segments (E and F, G and H).

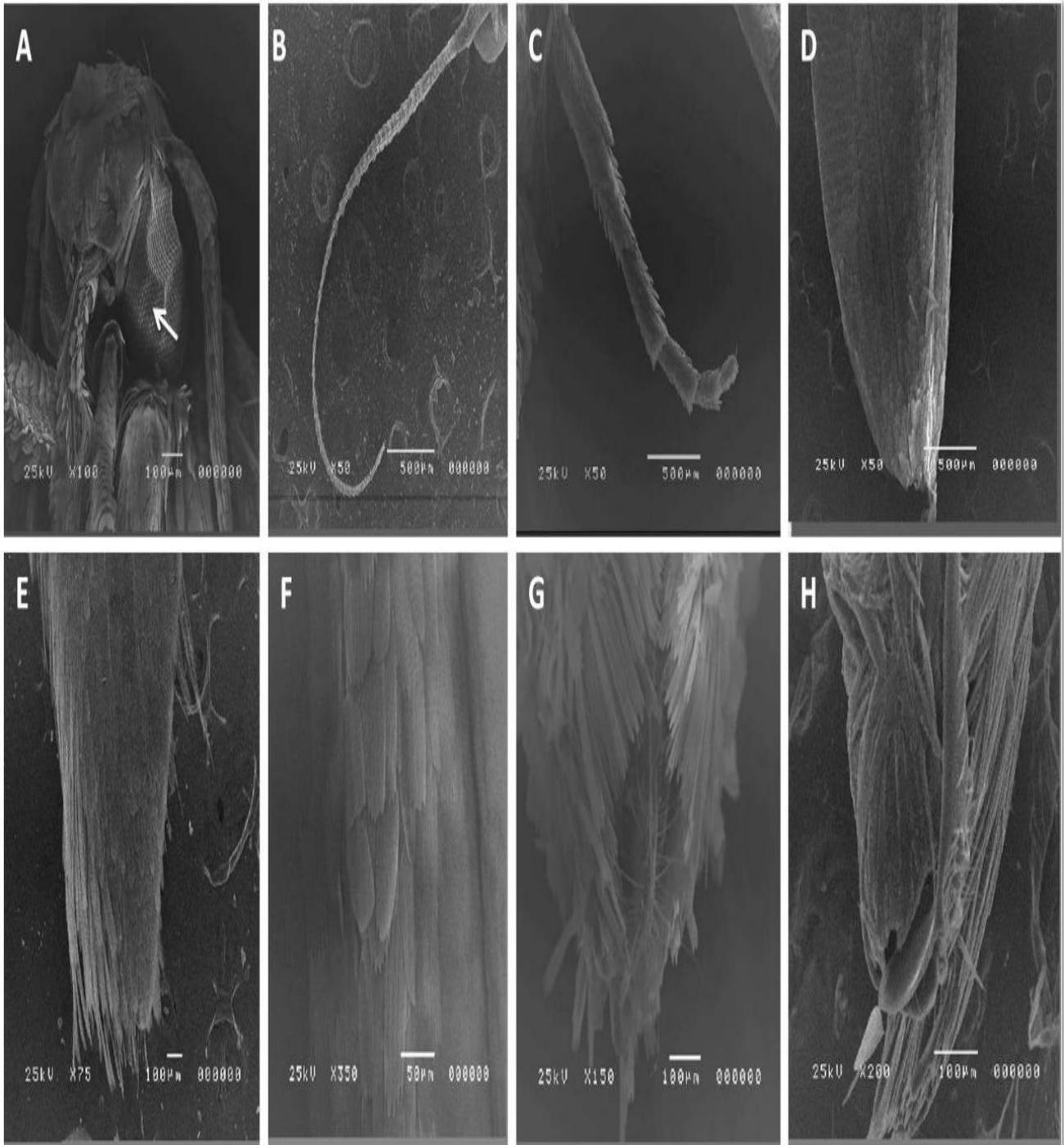


Figure (3) : Electron micrographs of *Phthorimaea operculella* adults showing: (A) Dorsal view of the head; (B) Higher magnification image of the filiform antenna; (C) Legs consisting of coxa, trochanter, femur, tibia, tarsus, and claw; (D) Fore wings; (E) Hind wings; (F) Scales on the wings; (G) Ventral view of the last abdominal segments of an adult female and (H) Abdominal end of a male showing the genital area.

However, key biological and morphological aspects of *P. operculella* are important in the selection of management measures to control it (Rondon and Xue, 2010 and Rondon and Hervé, 2017). Although the external morphology of *P. operculella* has been described (Moregan and Crumb, 1914 and Trivedi and Rajagopal, 1992; Rondon, 2010; Gill *et al.*, 2014; Rondon and Gao, 2018 and Salem *et al.*, 2021), we have here performed a more detailed examination of the external morphology using SEM.

At the beginning, we illustrate the shape of the antenna which consists of a large scape, small pedicel, and short flagellum, as well as the mouth parts of the larvae was also shown from a ventral view. On each side of the head, there are usually six stemmata just above the mandibles. These stemmata are arranged in a semicircle. Below the stemmata, there is a small pair of antennae, one on each side (Seema and Subbarayalu, 2017).

Our morphological study agrees with previous reports (Hannes, 1986; Lu *et al.*, 2014; Sabry, 2018; Seema and Subbarayalu, 2017 and David *et al.*, 2020). They described the location and number of lateral ocelli on both sides of the head, a sensillum chaeticum on the antennae, and two types of hair-like setae over the larva's body. Furthermore, our results show that the thorax has three pairs of legs, one for each segment.

The prothorax (T1) has a functional spiracle derived from the mesothorax (T2) while the metathorax has a reduced spiracle, not externally open and lying beneath the cuticle (Scoble, 1995). On the other hand, the thoracic legs consist of the coxa, trochanter, femur, tarsus, and claw and are constant in order. However, they are reduced in certain leaf-miners and elongated in certain Notodontidae. In Micropterigidae, the legs are three-segmented, as the coxa,

trochanter, and femur are fused (Scoble, 1995).

Our results are consistent with Triplehorn *et al.* (2005) who described those abdominal segments 3–6 and 10 may each bear a pair of more fleshy legs. The thoracic legs are known as true legs and the abdominal legs are called prolegs (Wagner, 2005).

Lepidoptera families have different proleg numbers and positions. Some larvae such as inchworms (Geometridae) and loopers (Plusiinae) have less than or equal to five pairs of prolegs, while others like Lycaenidae and slug caterpillars (Limacodidae) lack prolegs (Triplehorn *et al.*, 2005 and Wagner, 2005). We found three pairs of true legs on the thorax, ending with large claws, and five pairs of pseudo legs, four abdominal and one anal. The abdominal spiracles are located on each side of the body on the first eight abdominal segments (Wagner, 2005).

The pupation occurs in a cocoon among dead potato leaves, on the soil, or on the stored potato tuber (Hill, 1975, Foot, 1998 and Alvarez *et al.*, 2005). Pupation takes about 6 to 26 days depending on temperature. The pupa is about 8.2 mm long and spindle-shaped. It is usually found in a white and uniform brown cocoon (Sorensen, 1994). Gamboa and Notz (1990) measured the length of the pupa to be 6.2 ± 0.32 mm, whereas we found it to be yellowish or reddish to dark brown and about 6 mm long, as reported in other reports. other (Broodryk, 1977 and David, 1984).

In this study, we found two sex differences. First, a bow shape between the eyes of the female pupa, was seen for the first time in SEM. Second, observe the shape of the genital opening at the end of the abdomen. This point has also been recently applied by Salem *et al.* (2021), who used the morphological characteristics of male and female larvae (L.) and pupae (p.) of *P. operculella*. On the other hand, the

genital opening of the male pupa is located about the middle 1/3 of the last abdominal segment, while the genital opening of the female pupa is located near the proximal side of the segment, providing a further point of differentiation.

Our morphological study shows that adults are small, and females are slightly longer than males. The slender forewings have a fringe of hair near the base (SimSar, 2002) and are gray to grayish brown with darker brown spots or dots in males and forming an "X" in females (Rondon, 2010; Chauhan and Verma, 1991; Rondon *et al.*, 2007; Raman, 1980 and Rondon and Xue, 2010). These results are consistent with previous work (Sorensen, 1994; Alvarez *et al.*, 2005 and Hypp on line 2005). Sorensen (1994) considers size to be one of the main distinguishing features between adult males and females, alongside the structure and shape of the anal part. As a result of the present study, the yellowish-brown hind wings are shorter and contain fringed hair along the entire rear margin, (Sorensen, 1994; Alvarez *et al.*, 2005, and Hypp online, 2005). Shashank *et al.* (2018) described the male and female genitalia of *Tuta absoluta* as well as the genital opening in the pupae as useful features for sex discrimination of the moth.

This study aimed to distinguish between the immature stages (Final larval and pupal stages) as well as between the two sexes of this insect pest by using scanning electron microscopy. (SEM). The general description of the larval body, the mouthpart as well as abdominal end are observed in detail. SEM allows a clear distinction between male and female pupae. Adult males and females were distinguished morphologically by SEM.

The current study is the first to use SEM to describe the different stages of *P. operculella*. The findings are encouraging, and SEM analysis may help in the discovery

of unique morphological characteristics in potato tuber moth larvae, pupae, and adults. This study can help identify this pest so that integrated pest management programs can be used to control it.

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