MOUTHPART AND DIGESTIVE TRACT MORPHOLOGY OF THE SYNCHRONIZED FIREFLY, *Pteroptyx tener* (COLEOPTERA: LAMPYRIDAE)

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ABSTRACT

The firefly, *Pteroptyx tener* (Coleoptera: Lampyridae) lives in the mangrove area and is economically valuable for ecotourism because of its unique synchronous flashing behavior. It has been observed that the adult species does not have the appearance of sucking type mouthpart morphology though it is believed that its diet depends on the plant sap. This study focused on the morphology of the mouthpart and, anatomy and histology of the digestive tract of *P. tener*. From gross morphology observation, *P. tener* has hypognathus mouthpart with a crescent-like narrow apicle mandible. Scanning Transmission Electron Microscope (STEM) image showed that *P. tener* has a special food canal inside its mandible that runs from the tip to the end part of the mandible. The tip of the mandible could be used as piercing equipment for *P. tener* to suck plant sap for feeding purposes. Morphological investigations of the digestive tract showed that this species have a small crop, large ventriculus and long intestinal tract that is suitable for a liquid feeder insect. Histological sections show that the digestive tract consist of thick muscle layer especially on the hindgut and thin epithelial layer on all parts of the gut.

Keywords: Fireflies, mouthpart, digestive tract, morphology, histology, *Pteroptyx tener*.

ABSTRAK

Kelip-kelip, *Pteropyx tener* (Coleoptera: Lampyridae) hidup di paya bakau dan mempunyai nilai ekonomi dalam ekopelancongan kerana keunikan kelakuannya berkelip secara bersinkroni. Spesies dewasanya telah diperhatikan tidak menunjukkan morfologi alat mulut jenis menghisap walaupun dipercayai bahawa spesies ini bergantung kepada sap tumbuhan. Kajian ini memfokuskan terhadap morfologi alat mulut, anatomi dan histologi saluran penghadamannya. Berdasarkan pemerhatian morfologinya, alat mulut *P. tener* merupakan jenis hipognatus dengan mandibel berhujung tunggal seperti bulan sabit. Imej dari Mikroskop Transmisi Pengimbas Elektron (STEM) menunjukkan bahawa *P. tener* mempunyai saluran pemakanan khas di dalam mandibelnya bermula dari hujung tajam mandibel hingga ke hujung lebar. Hujung tajam mandibel ini dipercayai digunakan untuk mencucuk ke dalam tumbuhan bagi menghisap sap untuk mendapatkan makanan. Kajian morfologi terhadap saluran penghadamannya menyokong hipotesis bahawa *P. tener* merupakan pemakan cairan walaupun mempunyai alat mulut jenis mengunyah dengan memiliki tembolok yang kecil, ventrikulus yang besar dan saluran usus yang panjang. Histologi sel menunjukkan saluran

penghadamannya terdiri daripada lapisan otot tebal terutama di bahagian usus belakang dan lapitan epitelium nipis di keseluruhan sistem penghadaman.

Kata kunci: Kelip-kelip, alat mulut, saluran penghadaman, morfologi, histologi, *Pteroptyx tener*

INTRODUCTION

Pteroptyx tener (Coleoptera: Lampyridae) is one of the firefly species that can be found in Kg. Kuantan, Kuala Selangor, Malaysia. They prefer damp soils (usually mangrove) as its main habitat and can be found along Sungai Selangor's riverbank which is vegetated by Berembang tree (*Sonneratia caseolaris*), sagu (*Metroxylon sagu*) and others wild mangrove plants (Kazama et al. 2007; Khoo et al. 2009; Norzeana & Norela 2011; Wan Faridah et al. 2010; Wan Juliana et al. 2012).

Previously, some researchers believed that Berembang tree is the food sources for *P. tener* (Mokhtar et al. 2009, 2010; Wan Juliana et al. 2012) as it contains 20% sucrose. It also has been speculated that this beetle sucks the nectar of Berembang tree (Buck 1988; Nallakumar 2003). Recently in 2017, Cheng et al. has confirmed that there is no *S. caseolaris* cDNA detected in the gut of adult *P. tener* though it spends most of its adult life on this host plant. They obtained single plant DNA that is identical to the rbcL sequence of *Heritiera littoralis* (Malvaceae) that found along riverian area, which they speculate that this species travels further for its feeding session.

Some short lived firefly such as *Photinus ignitus* Fall do not feed in the adult stage but some long-life adult firefly such as *Ellychnia corrusca* Linneus which last for 10 months fed during its adulthood (Lewis & Cratsley 2008). The adult of *P. tener* is reported to live for 3 to 4 weeks (Nada et. al 2012). The larva and adult firefly has been suggested to adapt for fluid feeding. The larvae of firefly are being reported to suck the liquefy food through its canal on the mandible with the toxins from the digestive enzyme (Fu & Ballantyne 2008; Labandeira 1997). However, no one ever reported on adult *P. tener* mouthpart to confirm whether these canals retains on the adults' firefly and supported fluid feeding. Therefore, mouthpart and digestive tract morphology study Fare crucial to confirm the feeding type of adult *P. tener*.

The information about *P. tener* digestive morphology is vital for its conservation efforts to ensure the survival of *P. tener* in Kg. Kuantan, Kuala Selangor that is now threaten by human activity (Norzeana & Norela 2011; Mokhtar et al. 2009). According to Enger and Smith (2004), survival rate of a species depends on several factors; some of them are competition among species for food and habitat. Hence, information on type of food taken by *P. tener* based on its digestive morphology could confirm its food preferences and this additional information could be used in its conservation in future.

MATERIALS AND METHOD

Sampling

Adult fireflies, *P. tener* (Coleoptera, Lampyridae) were collected at night by using sweep net at Kg. Kuantan, Kuala Selangor (3°21'38.78"N 101°18'4.67"E) in October 2012 and were reared in plastic containers (175 x120 x 70 mm). Few pieces of berembang's leaves were placed in the plastic containers provided with wet tissues to retain moisture and few drops of pure honey were dropped on the wet tissue.

Mouthpart Morphology
Pteroptyx tener head was separated from its body by using a small scissor and forceps under Leica stereomicroscope. External Mouthparts of *P. tener* were observed under Tabletop Scanning Electron Microscope (TM-1000 Hitachi). For observation under Scanning Transmission Electron Microscope (STEM Jeol JSM-6400), the mouthpart was embedded in resin before the block being sectioned (1 µm) using ultra-microtome to observe the food canal in the mandible.

Digestive Tract Morphology
Pteroptyx tener was placed in Phosphate Buffer Solution (PBS: 136.9 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KH_2PO_4), with pH 7.1. Dissection was done by using micro dissecting kit under Leica stereomicroscope. The internal organ was first stained with Methylene blue solution. The cuticle and fat body was separated to observe the digestive tract. Gross morphology image of digestive tract was taken by using Carl Zeiss Image Analyzer Microscope with Axiocam MRc software.

Digestive Tract Histology
Digestive tract was fixed in formalin fixative overnight. Then, the samples were put through a series of ethanol (70%, 80%, 90% and 100 %.) to dehydrate then followed by xylene immersion. Tissues were embedded in paraffin wax. Blocks of waxed tissue were then sectioned (4 µm) by Leica RM2245 microtome. Four to five sections were placed on a slide and dried in oven overcnight. Sectioned tissues were then stained according to hematoxylin & eosin (H&E) method (Behmer et al. 1976; Junqueira & Junqueira 1983). Microphotography was taken using Zeiss Axio Scope with iSolutionLite software on 20x magnification after the tissues were mounted.

RESULTS AND DISCUSSION

***Pteroptyx tener* Mouthpart Morphology**
The mouthpart of *P. tener* consists of maxillary palps, labial palps, mandible, labrum and labium. From the frontal view, the whole mouthparts were furnished with hairy structure called seta (Figure 1). These hairs would act as a sense organ that used to detect the suitable food type and to manipulate the food. These data collection supports Harald et al. (2005) who state that hair of maxillae assist an insect to absorb and push the food into its mouth. Figure 1B shows the mandible morphology that is crescent in shape with narrow apical. Mandibles usually move laterally in order to bite and chew food (Mohamad Salleh 1983). However, in this case, *P. tener* mandible has a pointed tip that is suitable for piercing.

From STEM image, it is clearly observed that *P. tener* mandible has a special canal running from the tips of the mandible towards the end of the mandible (Figure 2B). According to Ballantyne and Menayah (2000; 2002), *P. tener* has chewing type mouthparts. The function of haustellate type mouthpart has been replaced by the canal in the mandible. Cicero (1994) reported for some beetles' species, especially lampyridae, the mandibles contain food canal which function to absorb nectar or liquid (Figure 3A). This statement was proven in this study by using STEM technique that portrays a similar food canal in the mandible of *P. tener*.

***Pteroptyx tener* Digestive Tract Gross Morphology**
Observation on the digestive tract of *P. tener* shows that it has a simple digestive tract with three distinct areas, foregut, midgut and hindgut. The alimentary canal consists of a small

crop that is the foregut area, a large midgut and a long hindgut that opens outside through anus (Figure 4). A few strands of Malphigian tubules are found to be the initial part of a hindgut that clearly separates these two regions. *P. tener* has a small crops compared to the ventriculus at the midgut area. Crops functions mainly as a food-storage organ, before being digested in the midgut. The foregut does not contain any proventriculus. This finding supports the hypothesis that *P. tener* do not fed on solid food. Nevertheless, the midgut is having a large ventriculus while the hindgut is quite long and narrow consists of ileum and rectum. Length of the digestive gut depends on the feeding habit of the insect (Sarwade & Bhawarne 2013). For a liquid feeder, it is expected that hindgut structure longer than other features to support the filtration process of large amount of liquid that allows maximal contact with liquid food (Chapman 1998). Hence, this proves that adult *P. tener* fed on easily digested materials such as liquid or nectars. According to Mohamed Salleh (1983), feeding type affect the digestive tract morphology. For example, solid feeders usually have proventriculus that function to grind the solid food. For *P. tener*, proventiculus is absent at the foregut area. This probably due to the feeding habit of this species that does not need this structure to grind the food that supports our hypothesis that this species is a liquid feeder by having a longer hindgut compared to the midgut and foregut which is needed in the fast filtration process of liquid type of food. The small size of *P. tener* foregut indicates that the food does not need to be stored long before it being digest in the large area of midgut. This also have proved that the adult does not stored solid food in the foregut area like other insect that fed on solid food such as crickets. Previously researchers reported that adult firefly does not fed (Buck 1988; Nallakumar 2003). It is also being speculated before that firefly probably fed on plant sap or nectar. The speculation has ended when a rearing experiment by Faust (2008) proved that individual adult firefly, *Photunis carolinus* green fed on a fruit that has increased its lifespan. Cheng et al. (2017) also had investigated the gut contain of *P.tener* confirmed few DNA food plants, that has strengthen our hypothesis that *P. tener* is a liquid feeder and fed during its adult stage.

Histological Study on Digestive Tract of *P. tener*

Generally, histological sections of *P. tener* show a single epithelial cell layer for all parts of the gut. The foregut, midgut and hindgut possess epithelium layers of cell which have different type from each other. The foregut and hindgut are ectodermal origin whereas the midgut is endodermic (Chapman 1998). Intima layers can be seen at foregut and hindgut (Figure 5 A, B, D & E). Overall, muscle layers could be seen as one of the important layers in the digestive organ. The activity of these muscles was basically myogenic in nature that involves in peristaltic movement (Lee & Lin 2003).

Foregut

Longitudinal section of foregut in *P. tener* shows that it consists of chitinous intima, simple squamous epithelium, and lumen (Figure 5A). Cross section of the foregut shows that foregut consists of circular muscle layer (Figure 5B). The squamous epithelium is flattened throughout the area. The innermost layer is the non-cellular chitinous intima that is thick and showing longitudinal folds along with the squamous epithelial layer. The thin epithelial layers showed that this area do not secretes any digestive material. Histology of foregut of adult *P. tener* is comparable with the histology of other coleopteran studied, as it possesses the well-developed muscularis, basement membrane, epithelium cell and internal intima (Berberet & Helms 1972). Smith (1968) reported that there may be a little absorption of nutrients into haemolymph occurs in the foregut of insects such as in grasshopper, *Shistocerca gregaria* that having an absorption of cholesterol in its foregut area (Joshi & Agarwal 1977).

Midgut

Figure 5C shows the longitudinal section of midgut *P. tener*. As midgut is endodermal origin, it shows the absence of chitinous lining. Columnar cells, light striated muscle and connective tissue could be detected in this area. The ventriculus of *P. tener* is larger than the foregut and this suggests that the liquid food is being digested and absorb rapidly in this area before being transferred to the hindgut. Insect midgut is the region for both digestion of food particle as well as absorption of nutrients, and is a very metabolically active tissue. It may be the longest part of alimentary canal and its diameter decreases as it tapers towards hindgut showing anterior and posterior midgut morphologically (Sarwade & Bhawane 2013) but not in the case of *P. tener* as it has shorter midgut compared to the hindgut. Some authors (Bution et al. 2006; Rost-Roszkowska 2008; Wanderley-Texiera et al. 2006) reported the midgut epithelium arranged as a single layer and composed of columnar and regenerative cell, thus confirmed the results obtained in this research, which is found that the epithelium as a columnar epithelium type. The columnar epithelium cell in insect usually covered with mikrovilli at the lumen area, as they are known to be responsible for both secretions of digestive enzymes and absorption transfer of nutrients into the haemolymph.

Hindgut

The histology of adult *P. tener* hindgut only consists of ileum (anterior hindgut) and rectum (posterior hindgut) in this study. This part is the longest and narrowed compared to foregut and midgut.

I. Ileum

Histologically the muscularis include the single thick layer of outer circular muscle through the longitudinal and cross section image (Figure 5D and 5E). The epithelium consists of cuboidal cells supported by basement membrane. The nuclei of epithelium cell are round and small. The epithelium shows longitudinal folds of cuboidal cells with prominent nuclei. The epithelium is lined internally by thick muscularis similar with reported in other studies (Gressitt 1953; Jones 1940; Snodgrass 2018; Swingle 1930). The presence of single layer of circular muscle in the ileum of adult *P. tener* investigated here different in some beetle species of *Dendroctonus* such as, in which the external muscle has two layers in which the cryptonephredial systems is located (Silva-Olivares et al. 2003). The presence of Malphigian tubules in the initial part of the hindgut shows that the function of primary urine production in this species has been taken care of. Nevertheless, the presence of single layer of circular muscle has also been reported in ants (Villaro et al. 1999).

II. Rectum

The epithelium of rectums bears double broad folds of muscularis in which the inner layer consists of large flattened cuboidal epithellium cells with large round nuclei (Figure 5F). The epithelium is supported by distinct basal membrane. The epithelium structure is much less similar like epithelium structure in the ileum but the nuclei in the epithelium of rectum are larger.

CONCLUSION

From this study, we would like to suggest that *P. tener* is a liquid feeder that probably fed on plant saps by piercing through its mandible that has a special canal which then being directly forward to its digestive gut. Digestive tract morphological study supports the hypothesis on liquid-sucking type feeding in *P. tener* by having large ventriculus in the midgut and a long hindgut with the absence of important organ for grinding solid food that is proventiculus.

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APPENDICES

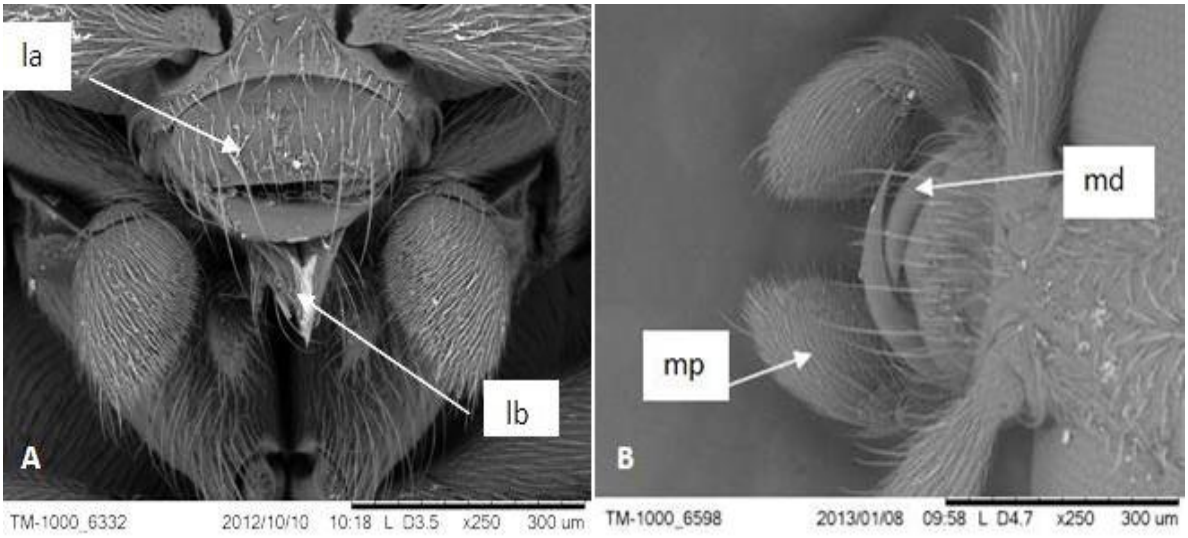

Figure 1 A: Frontal view of *P. tener* mouthpart; la labium, lb labrum; B: Dorsal view of *P. tener* mouthpart; mp maxilla palp, md mandible

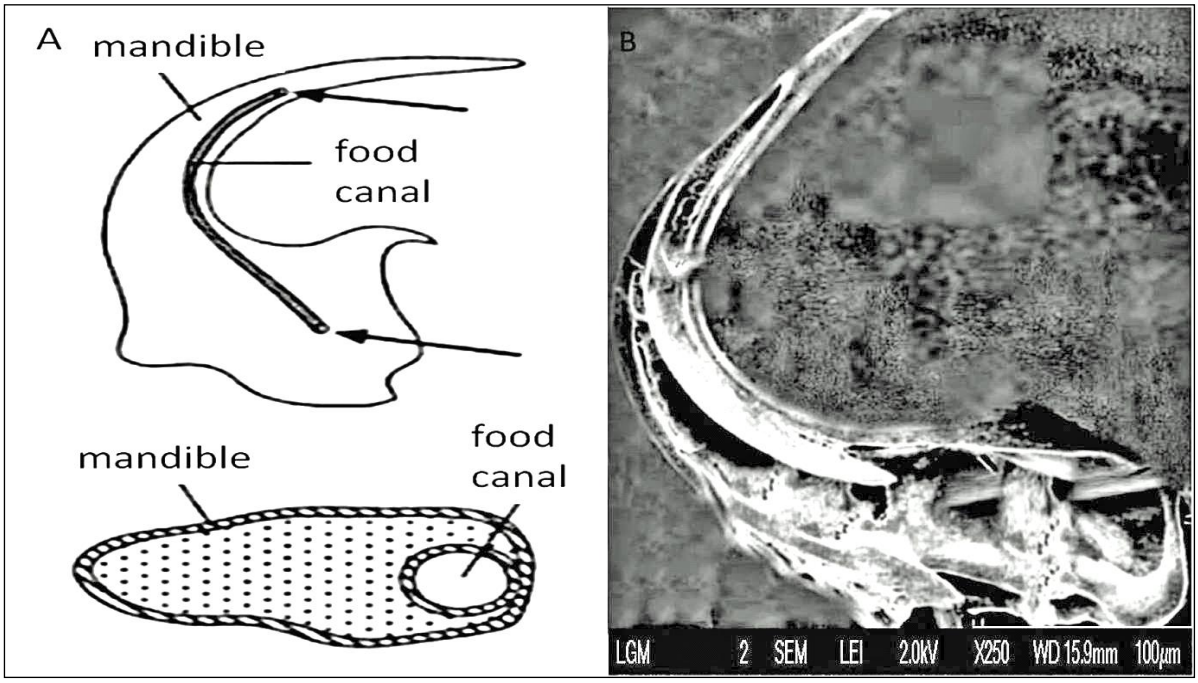

Figure 3 General food canal in insects by Cicero (1994); B: Food canal at the mandible of *P.tener*

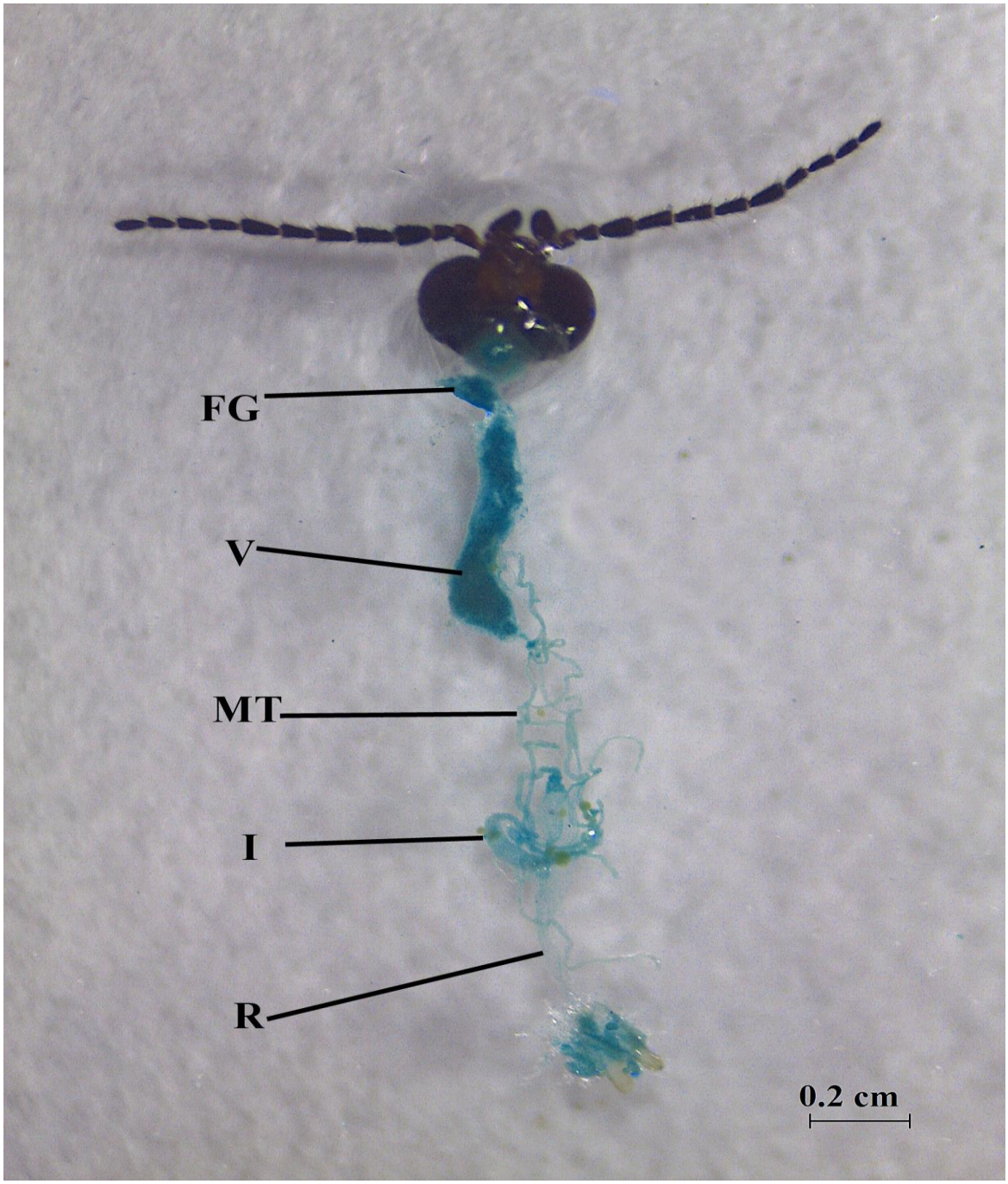

Figure 4 Gross morphology of *P. tener* digestive tract. Fg: Foregut; V: Ventriculus, MT: Malphigian tubules; I: Ileum; R: Rectum

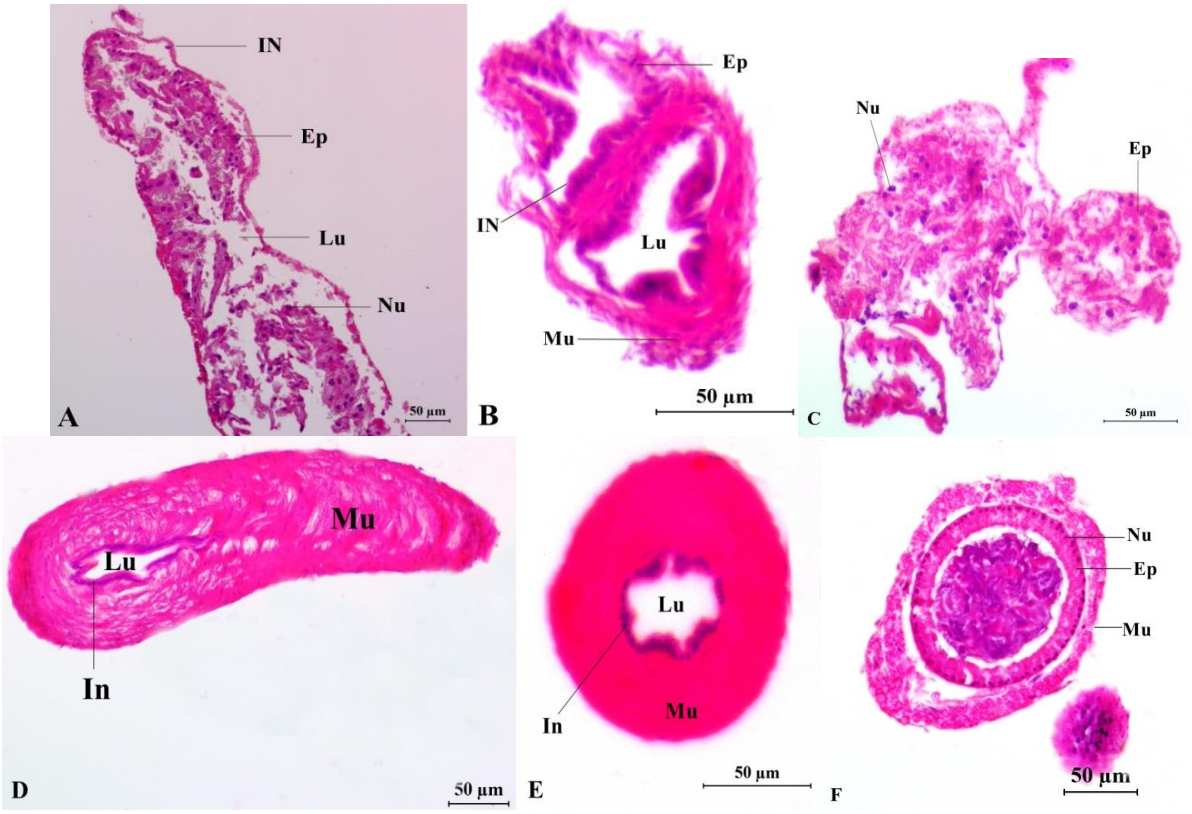

Figure 5 A: Longitudinal section of foregut; B: Cross-section of foregut; C: Longitudinal section of midgut; D: Ileum-anterior; E: Ileum-Posterior; F: Rectum IN: Intima; Ep: Epitelium; Lu: Lumen; Nu: Nucleus; Mu: Muscle