GROSS ANATOMY AND HISTOLOGY OF ALIMENTARY SYSTEM OF STICK INSECT, *Pylaemenes mitratus* (PHASMID: BASILLIDAE)

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ABSTRACT

The gross anatomy and histology of the alimentary tract of the stick insect, *Pylaemenes mitratus* were described in this study. Three distinct part of its alimentary system were investigated; the foregut, midgut and hindgut were observed. The foregut consists of oesophagus, crop and proventriculus. The inner most layer of foregut and hindgut show an intima which has a chitinous layers and folded along the epithelium cell at the hindgut. The external of the gut surrounded by muscularis made up of circular muscle and longitudinal muscle. The midgut is made up of gastric caecum whereas ventriculus. The histology structure consists of epithelium layer of endoderm cells that secrete digestive enzyme and site for absorption of food nutrient. The hindgut starts at Malpighian tubule to the ileum, rectum and ends at the opening of anus as a place for faecal excretion.

Keywords: Alimentary tract, stick insect, *Pylaemenes mitratus,* morphology, histology cell

ABSTRAK

Struktur morfologi dan sel histologi salur penghadaman serangga ranting, *Pylaemenes mitratus* telah dhuraikan dalam kajian ini. Tiga bahagian utama salur penghadaman iaitu usus depan, usus tengah dan usus belakang telah dikaji. Usus depan terdiri daripada esofagus, tembolok dan proventrikulus. Bahagian dalam usus depan dan usus belakang diselaputi dengan lapisan intima yang diperbuat daripada lapisan berkitin dan tersusun secara berlipat di antara sel epitelium usus belakang. Bahagian luar usus pula diselaputi dengan lapisan otot yang terdiri daripada otot memanjang dan otot membulat. Usus tengah terdiri daripada sekum gastrik dan ventriculus. Kajian histologi menunjukkan terdapat lapisan sel epitelium yang memainkan peranan dalam rembesan enzim pencernaan dan juga bertujuan untuk proses penyerapan nutiren yang telah dicerna. Bahagian usus belakang bermula daripada struktur tubul Malfigi, ileum, rektum dan berakhir di bukaan anus sebagai lokasi perkumuhan sisa tinja.

Kata kunci: Saluran penghadaman, serangga ranting, *Pylaemenes mitratus*, morfologi, histologi

INTRODUCTION

Stick insect or Phasmid have a large diversity of species in insecta with more than 3000 individual species have been identified mostly in tropics but were poorly study especially on their morphology and histology (Blüthgen et al. 2006). Phasmid is distinguished into two forms of morphological appearance, the stick insect with long slender bodies and leaf insect with flattened and lateral dilated bodies (Bragg 2001). *Pylaemenes mitratus* is a small dark brown species, sometimes with orange and white colours. It is classified under suborder Areolatae in the family of Bacillidae and subfamily Heteropteryginae. The head crest of this species is supported by anterior pointing projection for both female and male, while spinous structure can also be observed on the thorax and the 5th abdominal segment of the male body.

The alimentary system of insect started with the opening of mouth and end at the anus which functions in secretion of undigested food. The alimentary system of insect can be divided into three part: the foregut, midgut and hindgut (Atkins 1978). The foregut is the place for food storage and grinding process while the digestion and absorption of nutrient occurred at the midgut (Tsai & Perrier 1996) and the undigested food will pass through the hindgut for reabsorption of water and important ion (Izzetoglu & Ober 2011; Nation 2008) and also the last pathway until the faecal is secreted out of the body.

Food taken by this insect is digested and moved along the alimentary tract. The gut length of insects is generally correlated with their diet (Sarwade & Bhawane 2013) but for stick insects, the gut length is the same length with their body (Bragg 2001). *Pylaemenes mitratus* is a forbivorous feeding insect, thus it may have a different morphology of the gut even to their close related family, Orthoptera that is a graminivorous consumer (Smith & Capinera 2005).

This study was conducted to provide a fundamental knowledge about this stick insect and this species will contribute to a collective data between families of phasmid for a comparative study between other species of insect and for future work. This species was first described by Retenbancher at West Java, Indonesia in 1906 and the taxonomy of the insect have been revised by Brock and Okada (2005) and Seow-Choen (2005). *Pylaemenes mitratus* have been recently recorded in Gunung Ledang Johor National Park with other 46 species from 22 genus by Rabibah et. al. (2016) and Zeti & Nurul Wahida (2016).

MATERIALS AND METHODS

Sample Collection

A total of 25 individuals were collected from Mount Ledang, located in Johor State of Peninsular Malaysia (02°24'U, 102° 37'T) by active sampling during night and all the insect was kept alive in net cage with continuous supply of water and plants from the place they were found to be dissected in laboratory.

Gross Morphology of Alimentary Tract

Fresh samples were used in this study. The alimentary tract of *Pylaemenes mitratus* was dissected, the in-situ and ex-situ morphology observation of the alimentary canal were captured by image analyser microscope. The proventriculus of foregut was observed under Scanning Electron Microscope (SEM) (Table Top SEM Hitachi TM-1000).

Histology of Alimentary tract

a. Paraffin wax embedded slide preparation

The alimentary tract was removed, and the gut was cut into three parts: foregut, midgut and hindgut. Each of the section was fixed in 10% formalin buffer solution for 24 hours. They were dehydrated in 70%, 80%, 90% and absolute ethanol, 100% for a period of 1 hour each. The samples were cleared in xylene for 30 minutes. The sample were embedded in paraffin wax and allowed to solidify. The paraffin block was cut into a thin layer of paraffin ribbon (3-4 µm) and paced on the glass slide. The guts were stained with Pacific Acid Schiff (PAS), Alcian blue, Harris Haemotaoxylin and Eosin (H&E). The glass was then mounted with DPX mounting. Photomicrographs of the gut sections were taken by using Zeiss Axio Scope light microscope with iSolutionLite software version 5.0.

b. Transmission Electron Microscope (TEM)

Three parts of alimentary tract: foregut, midgut and hindgut were transferred to 4% glutaraldehyde for 12 to 24 hours. Samples were post fixed in 2% osmium tetroxide for 2 hours. The sample was then washed with phosphate buffer saline (PBS) for three times for a period of 10 minutes each. The samples were dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90% and absolute ethanol, 100%) and embedded with resin. Ultrathin sections (60-100nm) were stained with 1% aqueous uranyl acetate and lead citrate and were analysed with Philip CM12 TEM.

RESULT & DISCUSSION

The alimentary system of the stick insect, *P. mitratus* consists of three different part: the foregut, midgut and hindgut (Figure 1). The alimentary system equals the total length of the stick insect. The guts were surrounded with vast trachiol system that supplies oxygen to the muscle. The foregut consists of oesophagus, crop and proventriculus. The total length of the foregut is the longest between midgut and hindgut. The oesophagus has a very narrow opening at the anterior of the gut and it was connected to a big crop where the food is stored before the grinding process took place in the proventriculus.

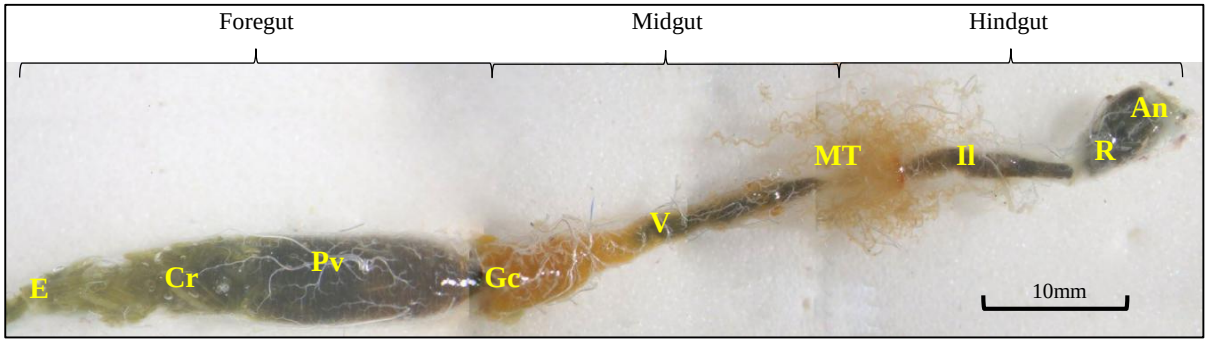

Figure 1. Ex-situ diagram of the alimentary system of *Pylaemenes mitratus*. Oesophagus (E); Crop (Te); Proventriculus (Pv); Gastric caecum (Gc); Ventriculus (V); Malpighian tubule (MT); Ileum (IL); Rectum (R); Anus (An)

Observation of the proventriculus by using SEM have shown a series of scleratin proventriculus spines teeth-like structure that is arranged longitudinally along the proventriculus separated from each other (Figure 2C). The proventriculus spines are facing

towards the midgut. The transverse section of the proventriculus of the stick have shown that it comprised of chitinous intima, cuboidal epithelium cell surrounded with thick wall of longitudinal muscles and circular muscle. The lumen is covered with a thick chitinous intima that made up the proventriculus spines (Figure 2B). There are approximately 45 folding of the thick intima that made up the teeth-like structure (Figure 2C). A single row of spinules on ridge apex were also been recorded in some phasmid species such as in *Peruphasma schultei* and *Aretaon asperrimus* (Shelomi et al, 2015). TEM view of the epitelium cell of foregut have shown a numerous number of mitochondria than supply energy for the cell to work and it is completely the same with midgut and hindgut of this species. Various types of desmosome are also have been notice in all guts cell (Figure 4C).

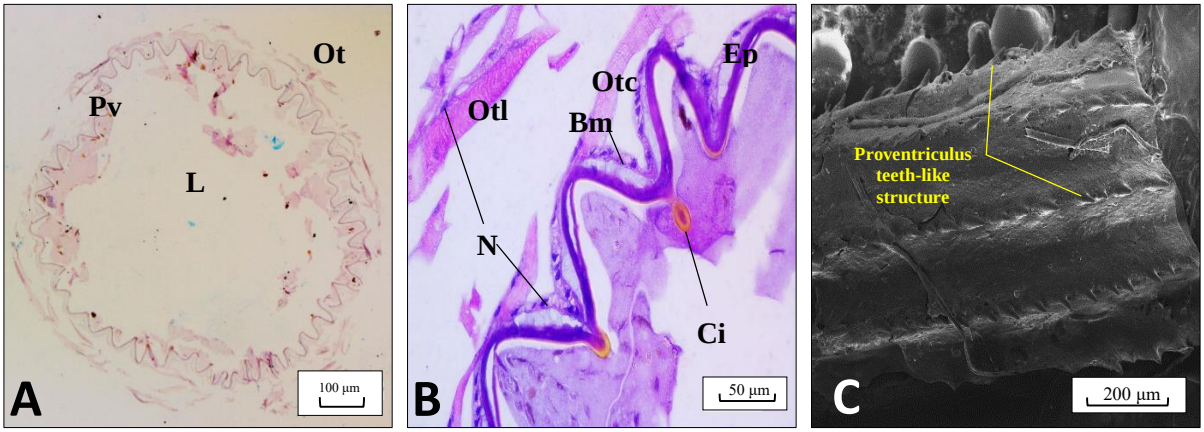

Figure 2. A) Transverse section of proventriculus (Pv). B) Close up at the histological structure of proventriculus. C) SEM view of inner proventriculus. Epithelium cell (Ep); Longitudinal muscle (Otl); Circular muscle (Otc); Chitinous intima (Ci); Lumen (L); Nucleus (N); Basement membrane (Bm)

The midgut is made up of gastric caecum and ventriculus. It is made up of short tube started from first abdomen until the fourth abdomen. The midgut length may be influenced by the diet of this insect, since it consume a forbs type of plant it has a shorter midgut compare to most of sucking insect that consume fluid food (Lopez-Guerrero 2002). At the anterior of the midgut it is covered with finger like projection, so called gastric caecum. In this species, there are seven projection of gastric caecum (Figure 3A). The outer surface of ventriculus is covered densely with a smaller projection than gastric caecum known as the crypts papillae. While the transverse section of gastric caecum of midgut shows that the gastric caecum is made up of pseudostratified columnar epithelium cell (Figure 3C). It is surrounded by layers of longitudinal muscle. The lumen of ventriculus of this insect lacks chitinous intima because it is mesodermal origin unlike the foregut and hindgut which is a ectodermal origin (Wan Nurul Ain, & Nurul Wahida 2015). The lumen is supported by a single layer of columnar epithelium cell that has formed a folded formation in the lumen.

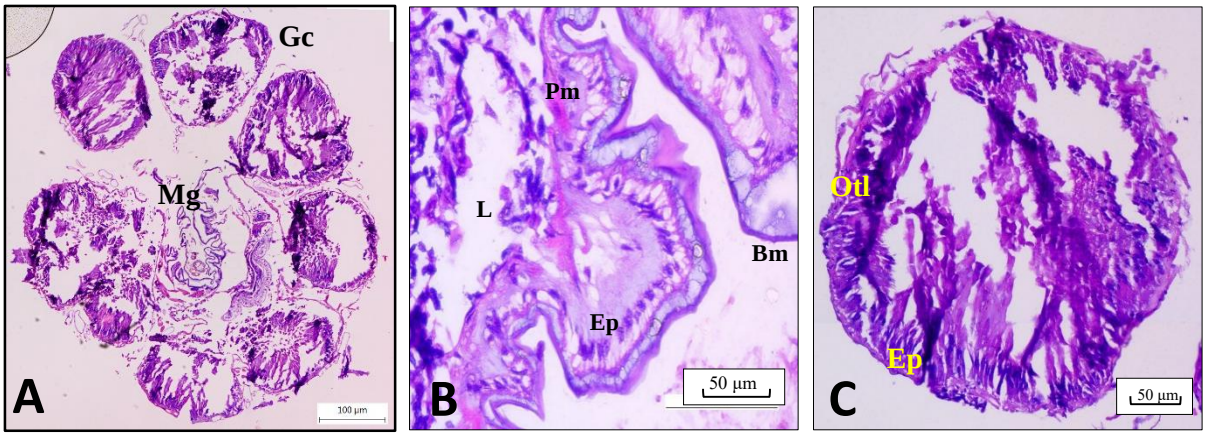

Figure 3. A) Transverse section of midgut (Mg) with 7 structure of gastric caecum (Gc). B) Peritrophic membrane (Pm) of the midgut of *Pylaemenes mitratus*. C) Gastric caecum transverse section. Epithelium cell (Ep); Longitudinal muscle (Otl); Circular muscle (Otc); Lumen (L); Basement membrane (Bm)

The epithelium has been proposed to play the role in secretion of digestive enzyme and absorbing nutrient in the midgut (Junquiera et al. 1989; Omotoso & Adedire 2010). The internal surface of ventriculus is bounded by a peritrophic membrane secreted by the epithelial cell (Figure 3B). The peritrophic membrane of the midgut have several function: prevent friction between food molecule to the epithelium cell and permeable to various kind of food molecule that increase the efficiency of absorbing nutrient (Lopez-Guerrero 2002). For insects that does not have peritrophic membrane for example Hemipteran species, *Brontocoris tabidus* and *Oncopeltus fasciatus* (Fialho et al. 2009; Hood 1937), there is another layer that is secreted by the epithelium cell that have the same function as peritrophic membrane known as perimicrovillar membrane (Silva et al. 2007). The folding formation of the membrane increases the surface area for absorption of nutrient. The midgut is also surrounded by circular muscle and longitudinal muscle that is well developed. The longitudinal muscle is thicker inside the circular muscle.

Morphology of hindgut is divided into Malpighian tubule, ileum, rectum and anus. The hindgut has the same length with the midgut which is shorter than foregut. Ileum is a narrow thin walled leading to the rectum. The rectum and anus of this insect is convoluted and have a larger diameter than the ileum. Abundance of Malpighian tubule arising are observed at the beginning of ileum. The Malpighian tube is a thin tube like structure that is arranged around the ileum that have half of ileum length that help to carry out excretion process and prevent water loss from the body throughout the process (Maddrell & O'donnell 1992; Omotoso & Adedire 2010).

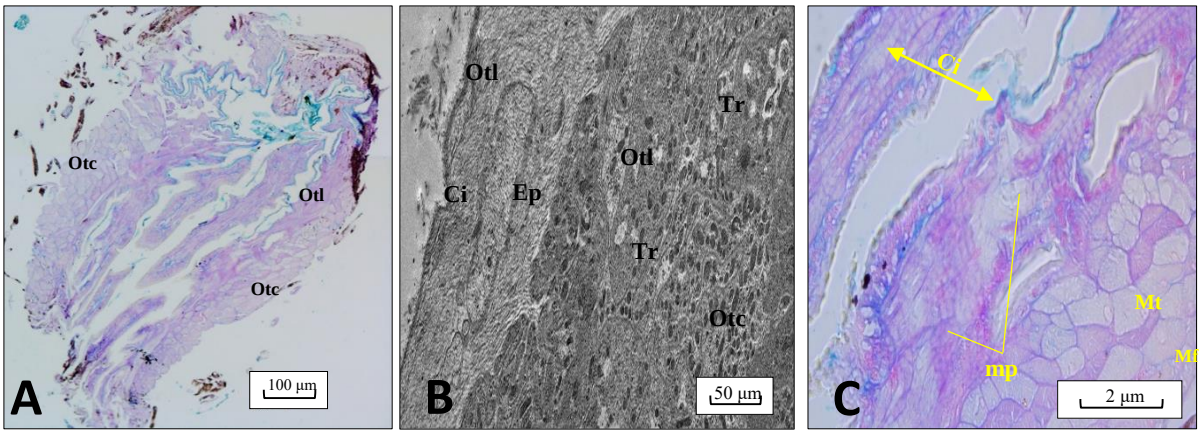

Figure 4. A) Transverse section of rectum of hindgut. B) Muscle layers of the hindgut. C) TEM view showing infolded of epithelium membrane (mp) and mitochondria (Mt). Epithelium cell (Ep); Longitudinal muscle (Otl); Circular muscle (Otc); Lumen (L); Chitinous intima (Ci); Trachiole (Tr).

Histological structure of hindgut consists of chitinous intima, cuboidal epithelium cell, inner longitudinal muscle and outer circular muscle based on the transverse section of rectum (Figure 4A). The chitinous intima of hindgut is thinner than the chitinous intima found at the foregut. It may be permeable cuticle layer to avoid loss of important substances (Sarwade & Bhawane 2013). There is a teeth structure that facing towards the anus that may help to move the undigested food towards the opening of anus. The epithelium is very much folded and made up of small cuboidal cell with round nuclei. The longitudinal muscle is surrounded by thick layer of well-developed circular muscle (Figure 4B) for the movement of faeces towards the anus. The muscle layer helps the transportation of food through the alimentary tract and prevent flow back of the undigested food (Levy et al. 2004). The layer of muscle in hindgut is much thicker than muscle layer that can be found in other gut. TEM view of hindgut shows a layer of cuticle that is connected to the membrane of epithelium cells. The membrane of the epithelium cell is highly folded inward the cytoplasm with numerous number of mitochondria lies in the cytoplasm (Figure 4C).

CONCLUSION

The alimentary system of *Pylaemenes mitratus* is a straight tube than have the exact length of the insect from thorax until the end of the abdomen. The foregut is longer than both midgut and hindgut. The structure of proventriculus is different compare to the other insect and other phytophagy. This study provides some new knowledge in understanding stick insect and will be useful for further work on stick insect.

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