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Histological and Histochemical Description of Mesentero-Proctodeal Regions in the Striped Blister Beetle, *Epicauta waterhousei* (Haag-Rutenberg, 1880) (Coleoptera: Meloidae)

Sinlapachai SENARAT[1,*], Jes KETTRATAD[1], Pisit POOLPRASERT[2], Ezra MONGKOLCHAICHANA[2], Watiporn YENCHUM[3] and Waristha ANGSIRIJINDA[4]

[1]*Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[2]*Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand*
[3]*Bio-Analysis Laboratory, Department of Chemical Metrology and Biometry, National Institute of Metrology (Thailand), Pathum Thani 10120, Thailand*
[4]*Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok 10530, Thailand*

([*]Corresponding author; e-mail: Senarat.S@hotmail.com)

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Abstract

The present study provides a description of the histological organization of the mesentero-proctodeal regions (midgut and hindgut) of adult *Epicauta waterhousei* (Haag-Rutenberg, 1880). The histological wall of the mesentero-proctodeal regions consists of 4 layers; mucosa, submucosa, muscularis and serosa layers, respectively. Midgut was classified into 3 subparts based partially on the size and characterization of the longitudinal fold; pro-midgut, meso-midgut and hind-midgut. Among these subparts, the longitudinal fold of the hind-midgut becomes progressively higher than other subparts. The mucosal epithelial lining of the midgut is a single layer of simple cuboidal epithelium. Hindgut is classified into 2 subparts; illium and rectum. These parts are lined by simple cuboidal epithelium with a surrounding thick layer of muscularis (inner circular and outer longitudinal muscular layers). However, the muscular layer of the rectum is thicker than the ilium. The present study provides baseline information about structural histology, but further fine descriptive investigation may be needed.

Keywords: Coleopteran, *Epicauta waterhousei*, Histology, Mesentero-proctodeal regions, Thailand

Introduction

The striped blister beetle, *Epicauta waterhousei* (Haag-Rutenberg, 1880) (Coleoptera: Meloidae), is found generally in Southeast Asia, especially Thailand (Central and Northeast provinces) [1,2]. This beetle species can be distinguished from other blister beetles by its red-orange head, but other parts are somewhat brackish. Each of the Elytra (forewings) bears a broad longitudinal stripe of pubescence yellowish, spreading from the humeral angle towards the tip, narrowly along the suture, apical and lateral margins. Recently, *E. waterhousei* has mainly been considered as a pest for some economically important plants, particularly the soybean group [3,4]. However, the biology of this beetle has still been rarely seen in the available literature until now.

The digestive tracts of several coleopterans have been exclusively described using histological analysis [5-7]. With regard to *Epicauta cinerea*, Everly (1936) [6] reported that the histology of the alimentary tract distinctly consisted of midgut and hindgut, based on the differential structures and cell types. It is similar to species including *E. pennsylvania* [7]. All researches provided the acquisition of basic knowledge of their biology and the application for pest control and insect aquaculture. Therefore,

the present study is primarily focused on the detailed microstructure of the mesentero-proctodeal zones of *E. waterhousei* using histological and histochemical techniques.

Materials and methods

Adult samples of the present experimental blister beetle *E. waterhousei* (n = 30) were collected from agricultural area at Chiang Mai and Phitsanulok provinces, Thailand, from October 2012 to March 2013. All specimens were rapidly fixed in Davidson's fixative (about 24 - 36 h.) at room temperature. After then, they were processed using standard histological techniques [8]. Paraffin blocks were sectioned at 5 - 6 µm thickness and stained with Harris's haematoxylin and eosin (H&E) for histology. Other sections of each set were specifically stained with Periodic Acid-Schiff (PAS) for histochemistry. After staining, investigations were analyzed in detail under a light microscope (LM) (20× and 40×).

Results and discussion

A digestive tract of the *E. waterhousei* beetle was distinctly divided into 3 parts by considering the structure of the tissues, including foregut, midgut and hindgut. which were similar to other coleopterans such as *Rhynchophorus phoenicis* [6,9,10-12]. The histological structure of the digestive tract of the coleopterans showed some variations, due to the variation in the consumption of food materials [13]. In the current study, only the mesentero-proctodeal regions (midgut and hindgut) were characterized. The histological organization of the mesentero-proctodeal walls consisted of 4 layers; mucosa, submucosa, muscularis and serosa, respectively, as described in several species of coleopterian insects [14-16].

Histological characterization of midgut

In this study, the histological structures of the foregut and midgut were separated by the oesophageal valve (**Figure 1A**). The midgut of *E. waterhousei* was long and coiled like in other coleopterans, such as *Cephalodesmis armiger* [17]. The midgut was exclusively divided into 3 subparts based on the characterization of the longitudinal fold in the mucosal layer. The midgut section could be described in detail as follows:

Pro-midgut

The mucosal epithelium was lined by simple cuboidal cells with no longitudinal folds. Each epithelial cell appeared with a round or oval shaped nucleus and was surrounded by basophilic cytoplasm. Based on PAS technique, the epithelial cells were positively stained. Thus, it could be concluded that this cell plays an important role in producing glycoprotein or mucous. Beneath the mucosa layer was the submucosa layer, forming a thick layer of loose connective tissues. The next layer was the muscularis which was composed of 2 muscular tissue layers (inner circular and outer longitudinal muscular layers). Externally, the serosa layer was presented, constituting of a thin layer of connective tissue (**Figure 1B**).

Meso-midgut

The histological aspect of this part was similar to the pervious part. Except for the mucosal layer, there was a higher longitudinal fold (120 - 150 µm) with numerous epithelial cells than in the pro-midgut (**Figure 1B**). Interestingly, the plant debris found in midgut of *E. waterhousei* was of the legume family (**Figure 1E**). Thus, this study strongly confirmed that this blister beetle is an herbivorous insect.

Hind-midgut

The histological structures of the hind-midgut were similar to both the pro-midgut and meso-midgut. However, the longitudinal fold of this part was the highest (200 - 220 µm) when compared with the previous parts (**Figures 1C - 1D**). The longitudinal fold of the epithelium improved the digestion and absorption efficiencies of food by increasing the cell number and surface area [13]. The characterization of this part explained that the midgut of insects is the principal part in the digestion and absorption of

food. Therefore, the epitheliums in the midgut were responsible for the production of digestive enzymes and the uptake and transfer of nutrients to the haemolymph [11,13].

Histological organization of hindgut

The hindgut is the second longest part of the digestive tract in insects [11]. In this study, the pyloric valve was observed between the midgut and hindgut. The epithelial cells of this valve were flat, elongated cells and covered by cuticles (**Figure 2F**). Structurally, the histological structures of the hindgut consisted of 2 parts based on the structure and cell types as per the following:

Ilium

In this part, the mucosa layer was raised to lumen in longitudinal folds (150 - 180 µm). The epithelium of the mucosa was identified as simple cuboidal epithelium and covered by the intima layer. Each cell contains a rounded nucleus with surrounding basophilic cytoplasm. Beneath the mucosa layer, there is a submucosa layer with a thin layer of loose connective tissue. The muscularis layer is thick and composed of 2 layers; the inner thick layer (approx. 150 µm) and outer muscular layer (approx. 100 µm) (**Figures 2A - 2B**). The outer layer of the ilium was serosa with a thin connective tissue. The residue of some food, especially plant matter, was also found in lumen in this part. However, it could not be distinguished (**Figure 2A**).

Rectum

Rectum was divided into 2 subparts; the anterior and posterior rectums (**Figures 2C - 2F**). Cross and longitudinal sections of the anterior rectum showed that the epithelial layer was covered by intima. The epithelial layer of this part was identified and lined with simple columnar cells. This cell appeared with an oval nucleus surrounded with basophilic cytoplasm. Other histological sections showed the same structure with ilium, except with a layer of muscularis of the anterior rectum (approx. 250 µm) which was thicker than ilium (approx. 150 µm). In the posterior rectum, the longitudinal fold was less than the anterior part. Histologically, the wall of the posterior rectum was similar to that described in the anterior rectum. However, the cells of the mucosal epithelium were slightly stained with PAS. Hence, it is supposed that these cells are responsible for the production of mucous substance, especially mucoglycoprotein. The functional role of ileum is the absorption of water from the feces and excretion of some materials through the anus [11].

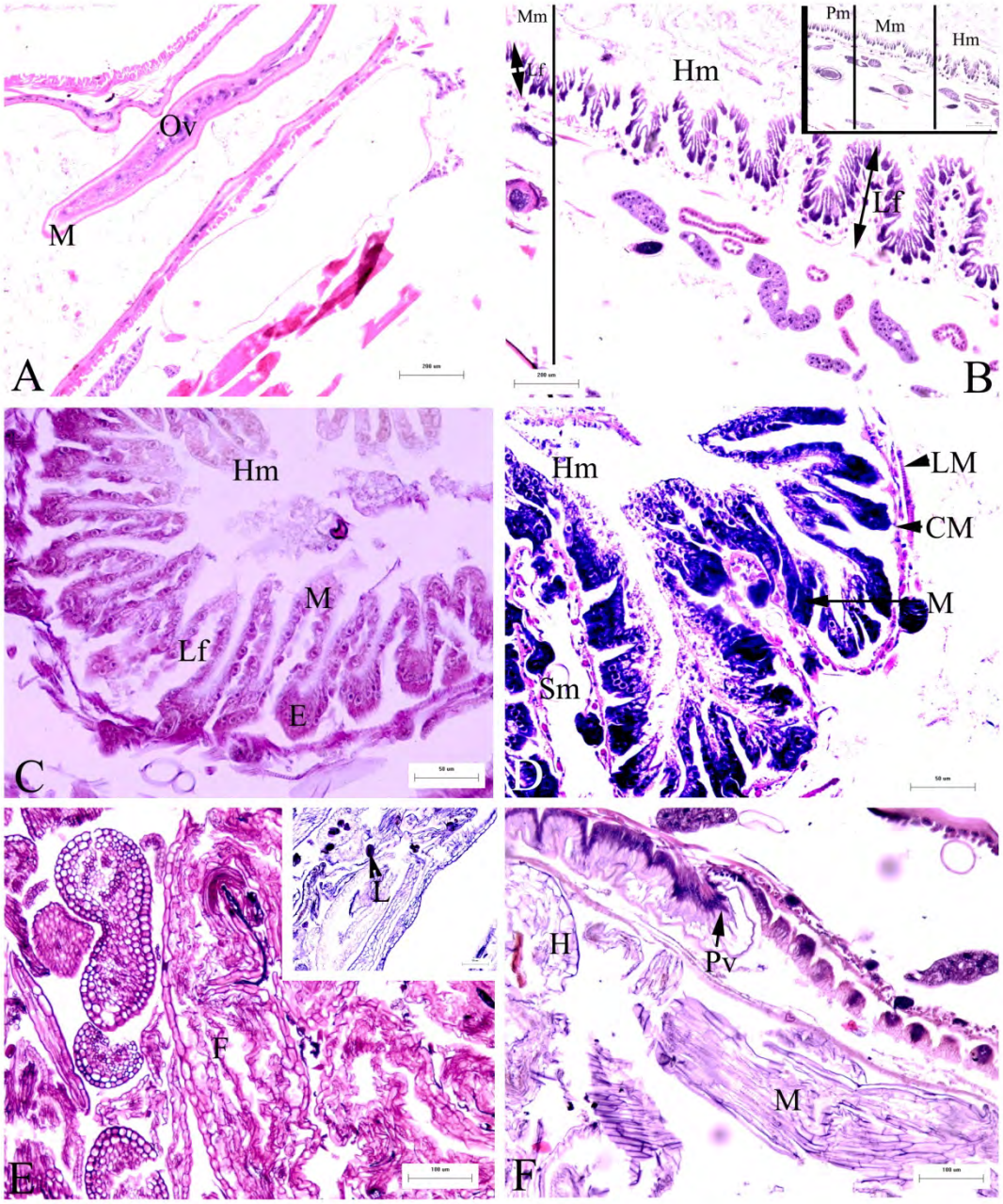

Figure 1 Micrograph of midgut of *Epicauta waterhousei; (A-F); Ov = oesophageal valve, M = midgut, Pm = promidgut, Hm = hind midgut, Hm = hind midgut, M = mucosa, Sm = submucosa, Lf = longitudinal fold, E = epithelial layer, CM = inner circular layer, LM = outer longitudinal muscular layer, F = food, L = legume, Pv = pyloric valve (A-B, D, F = H&E; C, E = PAS).*

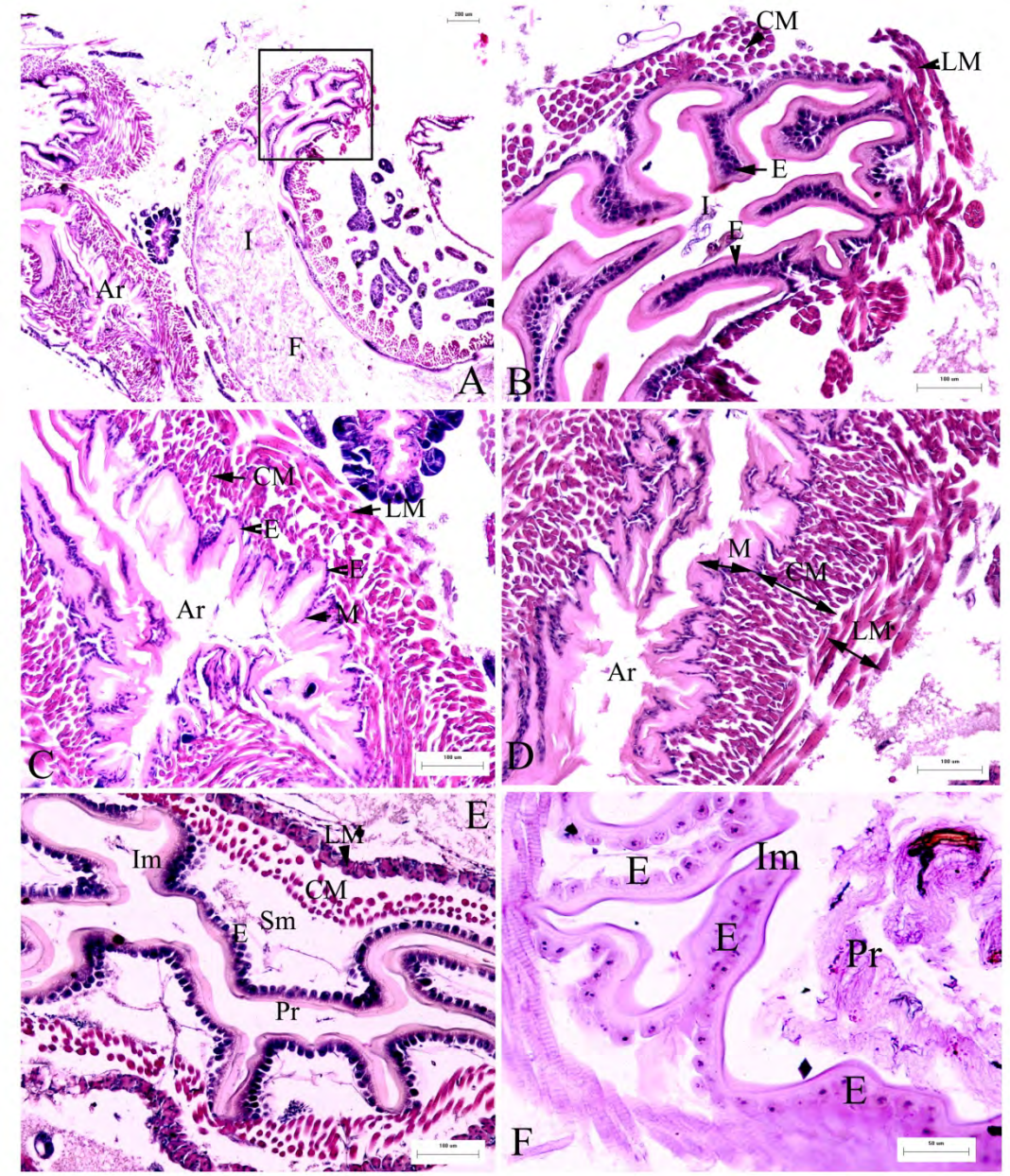

Figure 2 Micrograph of hindgut of *Epicauta waterhousei; (A-F); I = ilium, E = epithelial cell, CM = inner circular layer, LM = outer longitudinal muscular layer, F = food, Ar = anterior rectum, Pr = posterior rectum, I = intima, M = mucosa, Sm = submucosa (A-E = H&E, F = PAS).*

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