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SHORT COMMUNICATION

Morphological Characters and Histology of *Pheretima darnleiensis*

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***Pheretima darnleiensis* is a native earthworm of Southeast Asia, India, and Japan. Although it is commonly found in Indonesia, the earthworm has never been studied well. This study was aimed to examine the morphological characters and structure of its several organs for an identification purpose, which is important for the earthworm culture. Earthworms were collected in a plot of 55-150 x 55-150 cm width and 20 cm depth at Bogor Agricultural University in Darmaga and Baranangsiang Campuses by hand sorting method. Examinations were carried out on its external as well as internal characters. The histology of the organs was studied using paraffin method. The observed characters on *P. darnleiensis* were the presence of prostate gland, one pair of male pores on segment XVIII, a cylindrical body with perichaetine setae, caeca on segment XXVII, copulatory pouches without diverticula and stalked glands, bithecal spermatheca with nephridia, and the first spermathecal pore on segment 4/5. In addition, other characters found on *P. darnleiensis* were the presence of an annular clitellum on segment XIV-XVI, an epilobus prostomium with open base, approximately 40 single pointed setae on segment XIII, one midventral female pore on segment XIV, one pair of lateroventral male pores on segment XVIII, four pairs of lateroventral spermathecal pores on segment 4/5, 5/6, 6/7, 7/8, and the first middorsal dorsal pore on segment 12/13. The histology of *P. darnleiensis* showed basic structure as found in other earthworms.**

Key words: earthworm, Megascolecidae, identification, internal organ, clitellum, seta

INTRODUCTION

Earthworm plays many important roles, such as a soil biomanipulator (Edwards & Lofty 1972) and a decomposer (Ndegwa & Thompson 2000; Arancon *et al*. 2003; Aira *et al*. 2007). They increase water infiltration into the soil (Katsvairo *et al*. 2007) and the earthworm cast increases organic compound, cytokinin and auxin concentration in the soil (Krishnamoorthy & Vajranabhaiah 1986). Earthworm is also used in traditional medical system (Costa-Neto 2005). The mucous and earthworm paste of *Lampito maurtii* contain anti-ulceral, anti-oxidative (Prakash *et al*. 2007), and anti-inflammation properties (Balamurugan *et al*. 2007). Earthworm fibrinolytic enzyme in *Eisenia foetida* demonstrates anti-tumor activity on hepatoma cell (Chen *et al*. 2007). Meanwhile, its extract decelerates the formation of clot (Popovic *et al*. 2001).

Pheretima is found as an endemic earthworm in South East Asia, Eastern India, and Japan (Stephenson 1930). Ishizuka (1999) characterized *Pheretima* in Japan, having cylindrical body with numerous setae in each segment. An annular clitellum located on segment XIV-XVI. Male pores were paired or single, opening on the surface of segment XVIII. A female pore was on segment XIV. Spermathecal pores were usually bithecal in 4/5-8/9. Internally, spermatheca was paired on segment V-IX (with or without diverticulum), copulatory pouches rarely present, and with a racemose prostate gland. A gizzard was found on segment 7/8 and 9/10 and intestinal caeca was on segment XXVII (rarely XXVI). A dorsal pore was started from segment 12/13, occasionally from segment 11/12 or 13/14.

Our present study was being focused on *P. darnleiensis* collected for the first time by Sims and Easton (1972) from Borneo. It was classified under the order Oligochaeta, suborder Neooligochaeta, family Megascolecidae, and subfamily Megascolecinae (Stephenson 1930). The earthworm is commonly found in Indonesia (personal observations), however no description of its characters was found in Ishizuka database (1999). This research, therefore, was aimed to study the structure, anatomy and histology of the worm, and the obtained result provide as basic information for its identification. Additional information from this research will hopefully can be used for an accurate assessment in culturing the earthworm.

MATERIALS AND METHODS

Study Site and Identification of *P. darnleiensis*. Earthworms were collected in a plot of 55-150 x 55-150 cm width and 20 cm depth at Bogor Agricultural University in Darmaga (S 06°33'22.5", E 106°43'29.5") and

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Baranangsiang Campuses (S 06°33’22.5", E 106°43’29.5"). All earthworms were collected by hand sorting method and were preserved in alcohol 70%. Juvenile earthworms were excluded from this study due to lacked of sexual organs.

Earthworms were identified at family level based on Blakemore (2002) using prostate gland and male pore characters. At genus level, Megascolecids were identified based on setae arrangement, caeca, copulatory pouches, and the presence or absence of nephridia on spermatheca. Subsequently, the characters were used to identify megascolecids at species level were the presence or absence of diverticula and stalked glands on copulatory pouches and spermathecal position and pore. Both genus and species identification were based on Sims and Easton (1972).

Observation of *P. darnleiensis*: the External and Internal Characters. Observation of the external characters was performed by using a stereo microscope. The characters observed were clitellum, prostomium, seta, female pore, spermathecal pores, and dorsal pore. The internal characters were studied through dissection and the histology of internal organs. *Pheretima darnleiensis* was dissected dorsally from anterior to posterior passing through segment XXVII (Figure 1). Internal character observed was prostate gland.

Histology Study. The organs studied histologically included body wall (including clitellum), digestive organs (pharynx, gizzard, and intestine), circulatory organs (dorsal and ventral blood vessel), nerve organs (cerebral ganglion and nerve cord), and reproductive organs (spermatheca, seminal vesicle, and prostate gland). We used FAAC as the fixative solution (formaldehyde 37% 100 ml, glacial acetic acid 50 ml, distilled water 850 ml, and calcium chloride 13 g) (6 days). Clearing was performed by soaking the organs in alcohol: xylene 1:1 and xylene I (60 min in each), and xylene II (10 min) in oven (60 °C). The organs were embedded in paraffin (3 x 45 min) prior to sectioning transversely (6 µm). The tissue was soaked in xylene and performed the dealcoholization, subsequently they were stained in Haematoxilin and Eosin (HE) for 1 and 10 minutes, respectively (Gray 1952).

RESULTS

Identification of *P. darnleiensis*. *Pheretima darnleiensis* as Megascolecids earthworm had prostate gland (Figure 1) and one pair of lateroventral male pores on segment XVIII (Figure 2a,b). The characters observed on the worm up to the genus level were a cylindrical body with perichaetine arrangement setae (evenly distributed) (Figure 2c), caeca on segment XXVII (Figure 2d), the presence of copulatory pouches (Figure 2e), and spermatheca with nephridia (Figure 2f,g). The earthworm were classified as *Pheretima darnleiensis* due to the absence of of diverticula and stalked gland in the copulatory pouches, bithecal spermatheca, and the first spermathecal pore on segment 4/5 (Figure 2h,i).

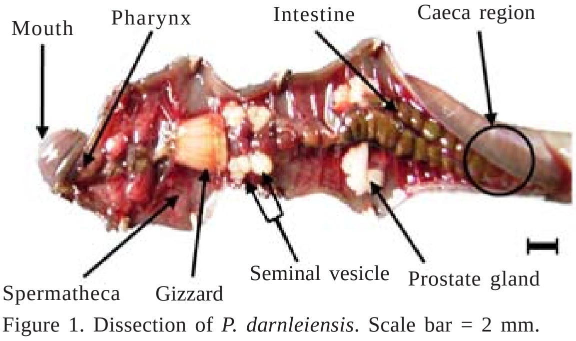


Figure 1. Dissection of *P. darnleiensis*. Scale bar = 2 mm.

We also described other characters of *P. darnleiensis* such as annular clitellum on segment XIV-XVI (Figure 2a), epilobus prostomium with open base (Figure 2j,k), approximately 40 setae single pointed (Figure 2l). One female pore located midventral on segment XIV (Figure 2a), and four pairs of spermathecal pores situated lateroventral on segment 4/5, 5/6, 6/7, 7/8 (Figure 2i). A dorsal pore was middorsal and the first dorsal pore was on segment 12/13 (Figure 2m). Prostate gland of *P. darnleiensis* was racemose in shape (Figure 2e).

Histology of *P. darnleiensis* Body wall. The body wall consisted of a cuticle layer, an epidermis, a circular muscle layer, a longitudinal muscle layer, and a peritoneum, from outer to inner layer (Figure 3). Based on a transverse section, it showed that the clitellum consisted of mucous, cocoon secreting, and albumin secreting glands (Figure 3).

Digestive System. The pharynx showed approximately 2 mm in diameter and located posterior to buccal cavity. From a transverse section, it was obvious that the pharynx consisted of columnar ciliated epitheliums (Figure 4a). The gizzard was located on segment 7/8 to 9/10. From a transverse section, we found a thick cuticle layer (Figure 4b) and a straight typhlosole infolded to lumen on dorsal part of the intestine (Figure 4c).

Circulatory System. The ventral blood vessel (Figure 5a) was smaller in diameter than the dorsal one (Figure 5b). The muscle layers of the dorsal vessel were thicker than those of the ventral vessel.

Nerve System. Nerve cells and nerve fibers were shown on the transverse section of cerebral ganglion (Figure 6a). A nerve cord with a giant fiber (Figure 6b) was located on the ventral intestine.

Reproductive Organs. From the transverse section of spermatheca (Figure 7a) and seminal vesicle (Figure 7b), we could see a sperm mass was stored inside them. Prostate gland with no definite differentiation among the cells was shown in its transverse section (Figure 7c).

DISCUSSION

Identification of *P. darnleiensis*. Blakemore (2002) stated that earthworms in the family Megascolecidae possess more than one thick cell layers in clitellum, prostate gland, and male pores on segment XVIII. Only few members of family Megascolecidae have one layer of cells in clitellum, such as Moniligastridae, Haplotaxidae, and Enchytraeidae. Prostate gland was absent in

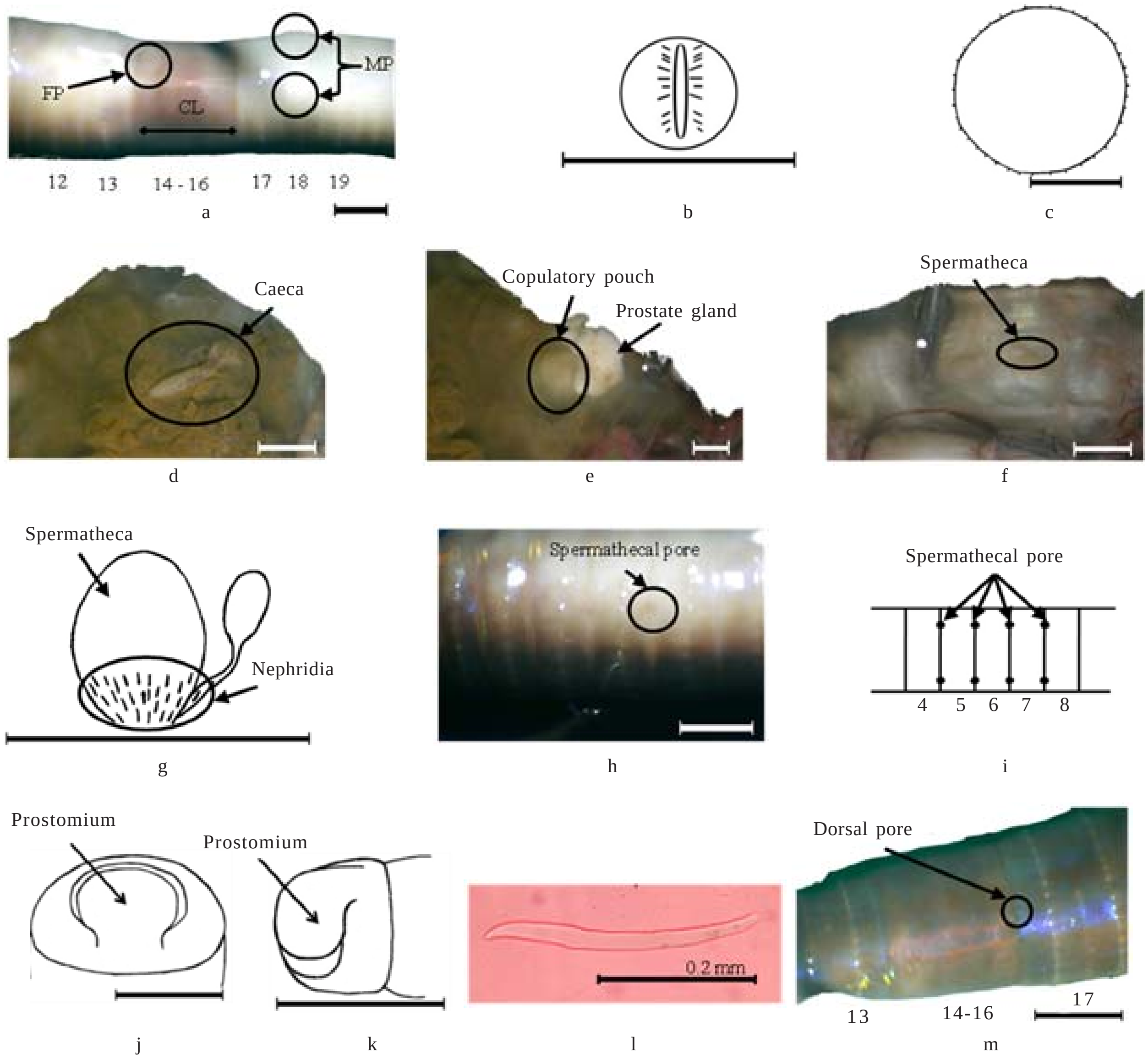


Figure 2. Characters of *P. darnleiensis*: (a) clitellum (CL) located on segment XIV–XVI, male pores (MP) on segment XVIII and female pore (FP) on segment XIV; (b) schematic of male pore; (c) schematic of seta on segment XIII; (d) caeca; (e) copulatory pouch under prostate gland; (f) spermatheca; (g) schematic of spermatheca with nephridia; (h) spermathecal pores; (i) schematic of spermathecal pores position; (j) top view schematic of prostomium; (k) lateral view schematic of prostomium; (l) seta shape; (m) dorsal pore behind clitellum region. Scale bar = 2 mm except for seta = 0.2 mm.

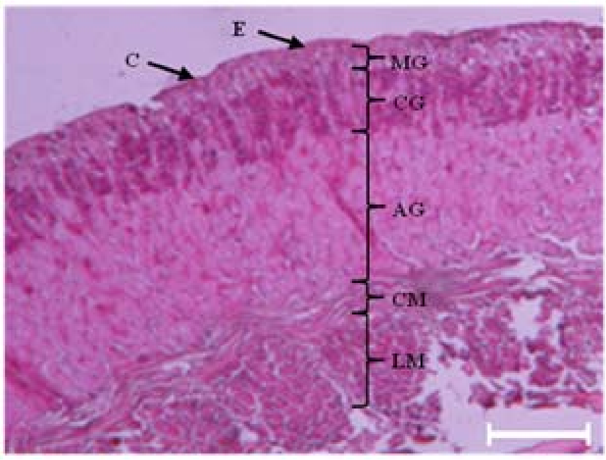
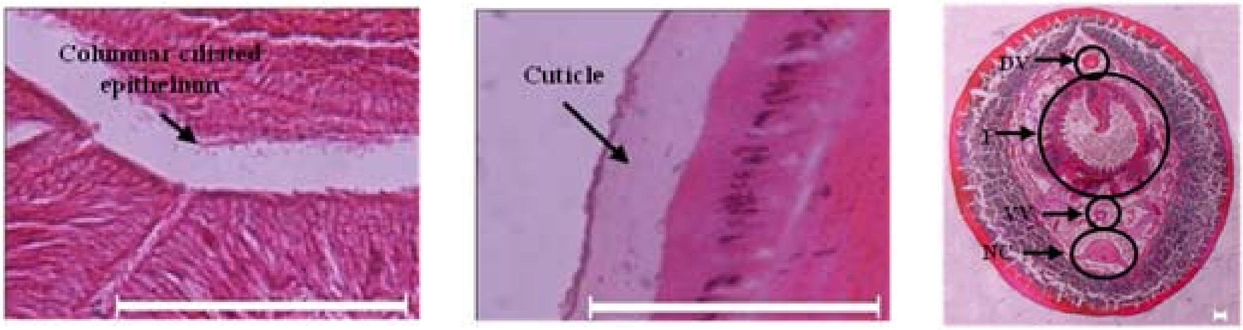


Figure 3. Photomicrograph of *P. darnleiensis* body wall with clitellum. C: cuticle, E: epidermal cells, MG: mucous secreting glands, CG: cocoon secreting glands, AG: albumin secreting glands, CM: circular muscles, LG: longitudinal muscles. Scale bar = 0.1 mm.

Glossoscolecidae and Lumbricidae and male pores were not on segment XVIII (Blakemore 2002).

The present study found that *Pheretima* had cylindrical body, perichaetine arrangement of seta, and caeca was present. The characters differentiated *Pheretima* with *Planapheretima* that had depressed body with ventrally crowded setae and with *Epithemara*, *Archipheretima*, and *Metapheretima* that had cylindrical body but caeca was absent (Sims & Easton 1972). Ishizuka (1999) also stated the same characteristics of genus *Pheretima* with these observed characters, except for copulatory pouches.

Ishizuka (1999) stated that caeca, spermathecal pores, spermathecae, and male pores were important morphology in identifying *Pheretima* in the species level. However, this study based on Sims did not include those characters. We used diverticula and stalked glands on copulatory



a b c

Figure 4. Photomicrograph of *P. darnleiensis* digestive system: (a) pharynx, (b) crop, and (c) intestine with dorsal blood vessel (DV), intestine with typhlosole (I), ventral blood vessel (VV), and nerve cord (NC). Scale bar = 0.1 mm.

pouches, position of spermatheca, and spermathecal pores to identify species *P. darnleiensis*. Base on this study, we concluded that those three characters were sufficient to differentiate *P. darnleiensis* from other species within *Pheretima*. This was shown between *P. darnleiensis* and *P. barbara*, such diverticula was absent on the former whereas present in the later. *Pheretima darnleiensis* had bithecal spermatheca and the first spermathecal pore was on segment 4/5. Those two characters distinguished *P. darnleiensis* from *P. ambonensis* that had monothecate spermatheca and from *P. racemosa* that had bithecal spermatheca but the first spermathecal pore was on segment 8/9.

Structure and Function of *P. darnleiensis*. Histology of *P. darnleiensis* showed basic structure as found in other earthworms (Stephenson 1930). *Pheretima darnleiensis* body wall consisted of a cuticle layer, an epidermis, a circular muscle layer, a longitudinal muscle layer, and a peritoneum, from outer to inner layer (Figure 3). The cuticle is a colourless noncellular layer. The epidermis was supported with single columnar epithelium layer. Coggeshall (1966) stated that epidermal epithelium of *Lumbricus terrestris* is 50 to 70 µ in height. The circular and longitudinal muscle layer provided locomotion of *P. darnleiensis*. Hanson (1957) mentioned that longitudinal muscle consists of smooth muscle fibres with the extended length may be as much as 2 or 3 mm. Setae on the posterior segment were protruded to keep the rear body fixed, whereas the anterior circular muscles contracted causing anterior segment to extend forward. The anterior longitudinal muscles contracted in turn, drawing the posterior part and causing the forward movement (Edwards & Lofty 1972).

The clitellum presents only at mature earthworms such as *Eisenia fetida* that mature after 10 weeks (Spurgeon & Hopkin 1996). Its transverse section displayed mucous, cocoon, and albumin secreting glands (Figure 3). The mucous gland secretes mucous to maintain earthworm position when copulating, while the cocoon secreting gland secretes proteinaceous sleeve enveloping the ovum. Albumin secreting gland secretes albumin for the nutrition supply for the embryo (Edwards & Lofty 1972).

The ciliated columnar epithelium (Figure 4a) on pharynx supports its function to pass the food to the

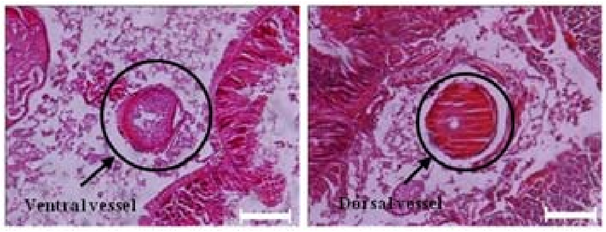


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Figure 5. Photomicrograph of *P. darnleiensis* circulatory system: (a) ventral blood vessel and (b) dorsal blood vessel. Scale bar = 0.1 mm.

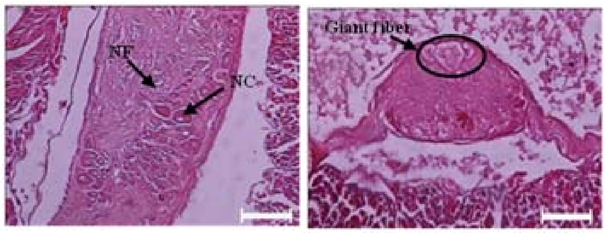

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Figure 6. Photomicrocgraph of *P. darnleiensis* nerve system: (a) cerebral ganglion with nerve fiber (NF) and nerve cell (NC), (b) nerve cord with giant fiber. Scale bar = 0.1 mm.

gizzard. The thick cuticle (Figure 4b) of gizzard was due to the function for grinding food. The typhlosole is infolding of intestine (Figure 4c) to increase the absorption area. Each type of earthworms may have different shape of typhlosole and many earthworms do not have typhlosole such as *Drawida barwelli*, *Eukerria kuekenthali*, *Nematogenia panamaensis*, and *Rhododrilus kermadecensis*. The straight shape of *P. darnleiensis* typhlosole found in this study compare to other V shape and T shape typhlosole (Blakemore 2002).

The ventral blood vessel flows the blood from anterior to posterior parts of the body as well as to the most organs, whereas the dorsal vessel flows the blood vice versa (Edwards & Lofty 1972). The ventral blood vessel (Figure 5a) was smaller in diameter than dorsal vessel (Figure 5b) as also stated by Hama (1960) in *Eisenia foetida*. The dorsal vessel *E. foetida* was larger than ventral vessel due to additional outer longitudinal muscle layer at lateral portion.

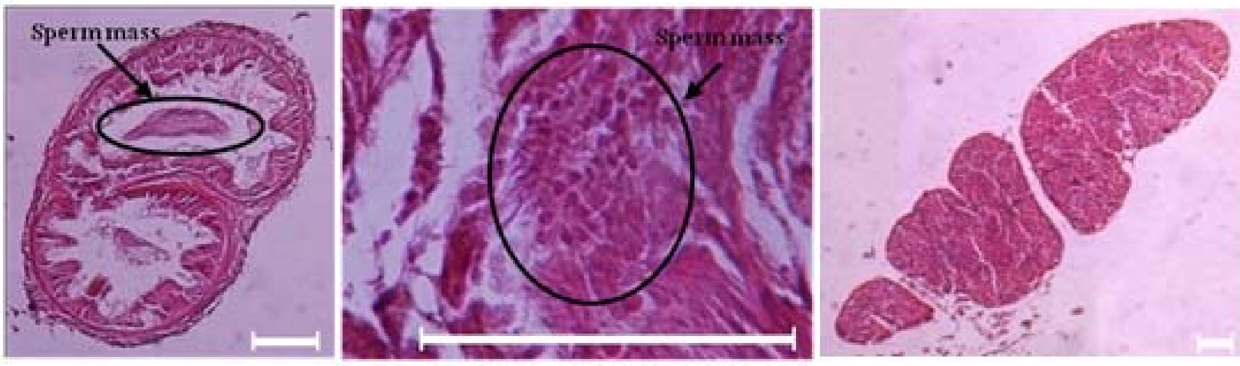

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Figure 7. Photomicrograph of *P. darnleiensis* reproductive organ: (a) spermatheca with sperm mass, (b) seminal vesicle with sperm mass, and (c) prostate gland. Scale bar = 0.1 mm.

The cerebral ganglion associated with the anterior part (prostomium), serves as a sensory organ. The ganglion was connected by a circumpharyngeal nerve to a nerve cord that laid ventrally (Edwards & Lofty 1972). The giant fiber was located ventrally of the intestine (Figure 6b) that facilitates rapid impulse condition bypassing the ganglia (Stephenson 1930). Median and lateral giant fiber sheath of *Eisenia foetida* consist of 15 to 30 and 2 to 15 layers of lamellae respectively (Hama 1959).

The spermatheca (Figure 7a) stores mass of sperms when the earthworm is copulating and keeps the sperms until fertilization. During maturation, seminal vesicle (Figure 7b) provided an area for developing sperms and prostate gland secretes semen for sperms (Stephenson 1930).

More researches are needed to examine the morphological characters and histology of other earthworms found surrounding *P. darnleiensis*, such as *Amynthas*, *Metaphire*, and *Pontoscolex* (personal observation). There might be association among *Pheretima* and the other earthworms (Edward & Lofty 1972).

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