www.thaiagj.org Thai Journal of Agricultural Science 2011, 44(1): 1-10

Histological Structure of the Digestive Tract of the Freshwater Pearl Mussel *Hyriopsis* (*Hyriopsis*) *bialatus* (Bivalvia: Unionidae)

S. Tantiwisawaruji[1,2], K. Chatchavalvanich[1,]*, U. Kovitvadhi[1]
A. Thongpan[3] and S. Kovitvadhi[4]

[1]*Department of Zoology, Faculty of Science, Kasetsart University*
Bangkok 10900, Thailand
[2]*King Mongkut's University of Technology Thonburi*
Bangmod, Bangkok 10140, Thailand
[3]*Department of Genetics, Faculty of Science, Kasetsart University*
Bangkok 10900, Thailand
[4]*Department of Agriculture, Faculty of Science and Technology,*
Bansomdejchaopraya Rajabhat University, Bangkok 10600, Thailand

**Corresponding author. Email: fsciknc@ku.ac.th*

Abstract

The microscopic structure of the digestive tract of *Hyriopsis (Hyriopsis) bialatus* is described. The mouth and oesophagus are lined by a simple ciliated columnar epithelium, while the stomach is divided into three regions: the gastric shield, the sorting area and the style sac. The long crystalline style (4.04 ± 0.21cm in length) in the lumen of the style sac stains positively for neutral mucin. Epithelial mucous cells containing acid and/or neutral mucins are numerous, and secretory digestive cells are also abundant. The digestive diverticula consist of blind-ending tubules composed of two cell types, digestive and basophilic cells. Mitotic figures were also observed. The anterior region of the midgut is lined by a simple ciliated columnar epithelium with no typhlosole. The internal wall of the posterior region of the midgut and rectum, however, has a typhlosole extending into the lumen. The long crystalline style in this freshwater pearl mussel is thought to assist in the digestion of the diverse phytoplankton readily available in their natural habitat.

Keywords: microscopic structure, crystalline style, tropical bivalve, mucous lining, epithelium

Introduction

Hyriopsis (*Hyriopsis*) *bialatus* Simpson 1900 is an economically important freshwater pearl mussel in Thailand. Its nacreous shell is used for inlaying furniture and making ornaments, kitchen utensils and souvenirs, while the mussels themselves produce freshwater pearls and are consumed by humans and animals (Yeemin, 1997; Kovitvadhi and Kovitvadhi, 2003). The mussels are suspension feeders, and their filtering activities help to remove particulates from the water column (Dudgeon and Morton, 1984; Pennak, 1989; Soto and Mena, 1999). They can live in different habitats as seen from their wide distribution in several provinces of Thailand (Kovitvadhi and Kovitvadhi, 2003). A remarkable difference of this mussel from other unionoideans lies in its fast growth, reaching sexual maturity at only 6 months (unpublished data). They were also found to reproduce year-round (Chatchavalvanich, et al. 2006). Another advantage of this mussel is the diversity of phytoplankton they consume, as seen from the specimen and enzyme activities found in the gastrointestinal tracts of adult mussels (Kovitvadhi et al., 2000; Areekijseree et al., 2006). At present, however, their numbers are decreasing, although attempts have been made to maintain populations in their natural habitats and through culturing (Kovitvadhi et al., 2006). A basic knowledge of the reproduction and feeding behaviour of *H.* (*H.*) *bialatus* is required for successful culturing and conservation. However, histological studies of *H.* (*H.*) *bialatus* have been limited to the structure and development of the gonads (Chatchavalvanich et al., 2006). The digestive system has not been reported upon. The aim of the present study was to elucidate the histological structure of the digestive tract of the freshwater pearl mussel *H.* (*H.*) *bialatus* in response to food processing and food digestion, and then compare the results with other bivalves.

Materials and Methods

Ten sexually mature *Hyriopsis* (*Hyriopsis*) *bialatus* were collected from the Moon River basin, Srisaket province, northeastern Thailand. Under cold-induced anesthesia, the valves were opened and the digestive organs (i.e., mouth, oesophagus, stomach, digestive diverticula, intestine, rectum and anus) removed and fixed in Bouin's fluid for 24 h. After standard histological procedures, sections 5-6 µm thick were cut and stained with haematoxylin-eosin for light microscopy. Histochemical observations for the presence of neutral mucins were carried out using periodic acid Schiff (PAS)-haematoxylin and diastase-PAS (McManus, 1946). To detect for acid mucins, alcian blue (AB) staining at pH 2.5 was used (Cook, 1982) but the pH was also lowered to 1.0 to identify acid mucins with sulphated groups (Lev and Spicer, 1964). The coexistence of acid and neutral mucins was observed by combined staining of AB and PAS (Mowry, 1956). Masson's trichrome was also used to identify collagen fibres.

Results

The digestive tract of *Hyriopsis* (*Hyriopsis*) *bialatus* consists of a mouth, a short oesophagus, and a large, thin-walled, stomach. A pair of digestive diverticula encompass the stomach, and a long twisted intestine, divided into anterior and posterior regions, joins up with the rectum and anus.

The mouth is lined by a simple ciliated columnar epithelium. Three types of mucous cells are interspersed among these columnar epithelial cells. Type A cells stain positively with PAS, type B cells with alcian blue, and type C cells with AB-PAS (Table 1). Beneath the epithelium is loose connective tissue interspersed with some smooth muscle cells (Figure 1a).

Table 1 Histochemical analysis of mucosubstances in the digestive tract of *Hyriopsis* (*Hyriopsis*) *bialatus*.

Digestive structure and cell type	Histochemical reaction				
	PAS	D-PAS	AB pH 2.5	AB pH 1.0	AB-PAS
Mouth					
-mucous cell A	2 M	2 M	0	0	0
-mucous cell B	0	0	2 B	1 B	0
-mucous cell C	2 M	2 M	2 B	1 B	2 BM
Oesophagus					
-mucous cell A	2 M	2 M	0	0	0
-mucous cell B	0	0	2 B	1 B	0
-mucous cell C	2 M	2 M	2 B	1 B	2 BM
Stomach					
-gastric shield	2 M	2 M	0	0	0
-B cell of style sac	2 M	2 M	0	0	0
-crystalline style	2 M	2 M	0	0	0
Intestine					
-mucous cell A	2 M	2 M	0	0	0
-mucous cell B	0	0	2 B	0	0
-mucous cell C	2 M	2 M	2 B	0	2 BM
Rectum and anus					
- mucous cell	2 M	2 M	0	0	0

PAS = Periodic acid Schiff; D-PAS = Diastase-periodic acid Schiff; AB = Alcian blue; B = Blue; M = Magenta.
0 = Negative reaction; 1-2 = Indicates intensity of staining reaction.

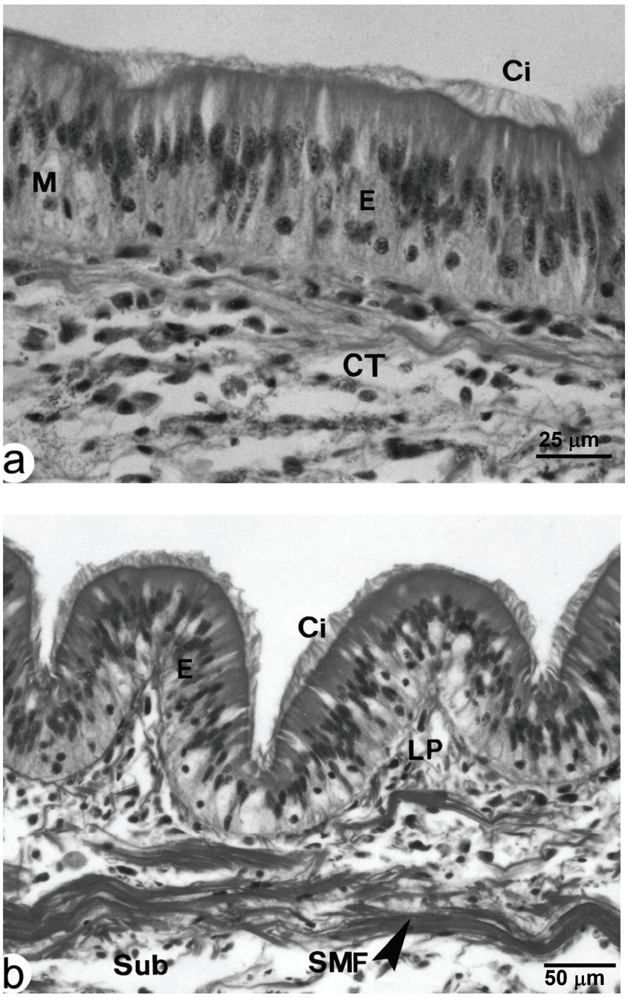

Figure 1 Mouth and oesophagus. (a) Structure of the mouth showing a simple ciliated columnar epithelium. (b) Structure of the oesophagus. Ci, cilia; CT connective tissue; E, epithelium; LP, lamina propria; M, mucous cell; SMF, smooth muscle fiber; Sub, submucosa.

The short oesophagus consists of two layers: mucosa and submucosa. The mucosa is folded and also lined by a simple ciliated columnar epithelium (Figure1b). There are three types of mucous cells interspersed among the columnar cells, similar to those in the mouth epithelium (Table 1). The lamina propria is composed of loose connective tissue; the muscularis mucosa contains a thin circular layer of smooth muscle; and the submucosa consists of loose connective tissue with some smooth muscle cells.

The stomach of *Hyriopsis* (*H.*) *bialatus* is divided into three regions: the gastric shield region, the sorting area, and the style sac region. The gastric mucosa is composed of an epithelium and lamina propria. The lining of the gastric shield is a pseudostratified columnar epithelium with a chitin-covered surface (Figure 2a). This chitinous lining is PAS-positive (Table 1). Tall columnar epithelial cells have oval nuclei. The lamina propria contains connective tissue and blood sinuses. The sorting area is characterized by ridges and grooves, and its epithelium comprises ciliated columnar cells of varying heights (Figure 2b). The style sac epithelium is composed of four cell types (Figure 2c). Type A cells are columnar (47.54 ± 6.11 µm in height) with long cilia. This cell type occupies most of the lining of the style sac. The basal cytoplasm of these cells stains intensely with eosin. The oval basophilic nuclei are found at the base of the cells (Figure 3a). Type B cells are tall and slender (115.21 ± 20.83 µm in height) with elongated nuclei. Their apical cytoplasm contains numerous granules which stain positively with PAS. Type B cells are adjacent to the crystalline style (Figure 3b). The third cell types, C cells, are 77.08 ± 14.10 µm in height. Their apical surfaces are covered with cilia which are shorter and less densely packed than those covering type A cells. Type D cells are 51.25 ± 11.30 µm in height, and are located at the junction between the style sac and intestine. Mucous cells also occur interspersed among type D cells. These mucous cells stain blue with AB-PAS (Figure 3c). The crystalline style in the lumen of the style sac stains magenta with PAS and blue with Masson's trichrome. The style is a thin translucent rod 4.04 ± 0.21 cm long, projecting into the stomach from its sac. It is apparent from the annular structure of the style, as seen in the transverse section, that its secretion is intermittent. The compaction of style material is not homogeneous along the length of the crystalline style. The material is loosely packed at the anterior (stomach) end and consists of a laminar and spiral ribbon-like matrix as seen in the longitudinal section (Figure 3d), but is more homogeneous and densely packed within the style sac (Figures 2c and 3e).

The digestive diverticula surrounding most of the stomach consist of numerous blind-ending tubules which directly connect to the stomach by a duct system. The primary duct of the digestive diverticula is lined by a pseudostratified ciliated columnar epithelium, but that of the secondary duct contains no cilia. The digestive tubules are either round or oval in cross-section. Both digestive and basophilic cells are irregularly grouped around the lumen of the tubule. However, digestive cells of variable heights and shapes–from tall columnar to irregular cuboidal–can be identified, thereby creating a star-shaped lumen (Figure 4a). The nuclei are either round or oval and are basally positioned. Their cytoplasm contains numerous granules staining light blue in Masson's trichrome and magenta with PAS. The second cell type (basophilic cells) are less numerous, and are pyramidal in shape with elongated basal nuclei. Their cytoplasm is more homogeneous and darker than the neighbouring digestive cells. Mitotic figures can also be observed in the cell, occupying one-third of the distal end.

The anterior region of the midgut found next to the style sac is lined by a simple ciliated columnar epithelium. These epithelial cells contain basally located, oval-shaped nuclei. Three types of mucous cells are interspersed among the epithelial cells (Table 1, Figure 4b). No typhlosole occur in the anterior intestinal wall. The posterior end of the midgut is convoluted in the visceral mass and has a single typhlosole extending into the lumen (Figure 5a). The upper part of the typhlosole is lined by a simple ciliated columnar epithelium (Figure 5b). The epithelium has a striated border and cilia. The supranuclear region of these cells stains intensely with eosin. There are some eosinophilic cells interspersed among the epithelial cells. The epithelium of the posterior region of the midgut (except the upper part of the typhlosole) is lined by a pseudostratified ciliated columnar epithelium (Figure 5c).

The rectum runs posteriorly through the pericardium and ends at the anus. The inner wall of the rectum has a typhlosole. The upper portion of the typhlosole is lined by a simple ciliated columnar epithelium with mucous cells, but the cells beyond the typhlosole gradually shorten, forming a low columnar epithelium. The cilia of the epithelial cells at the upper portion of the typhlosole are longer than those in other areas. The anus epithelium is the same type as that of the rectum.

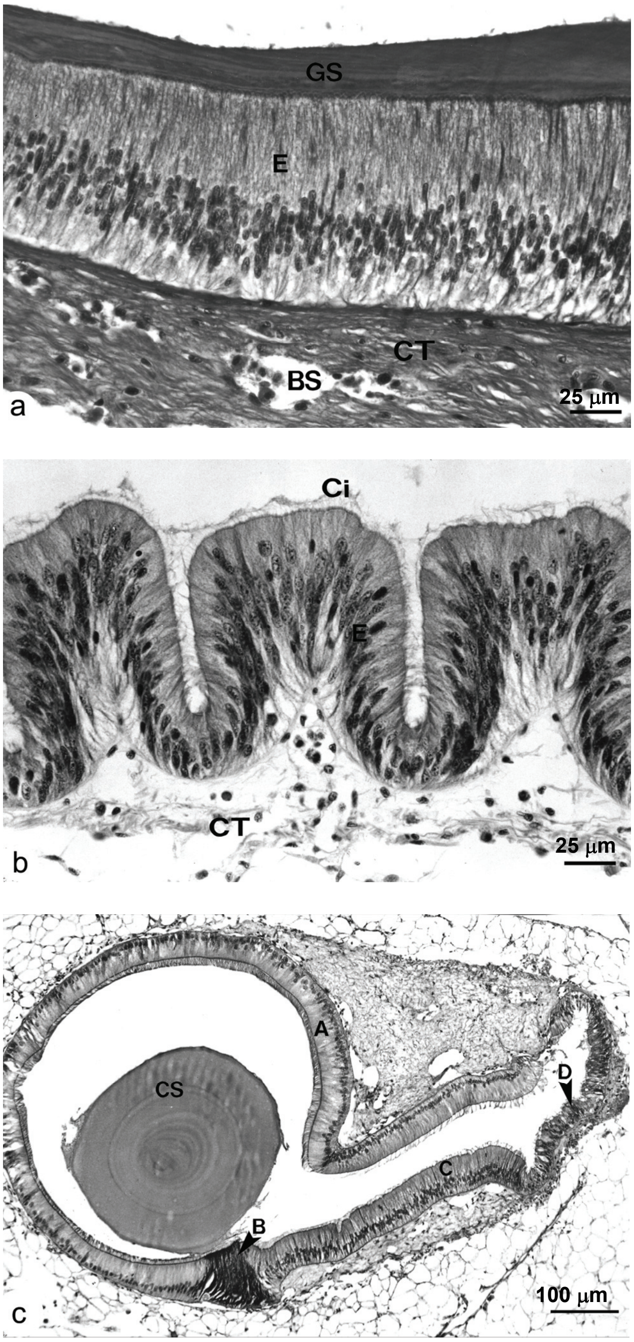

Figure 2 Stomach. (a) Gastric shield region. (b) Sorting area. (c) Style sac. A, type A cell; B, type B cell; BS, blood sinus; C, type C cell; Ci, cilia; CS, crystalline style; CT, connective tissue; D, type D cell; E, epithelium; GS, gastric shield.

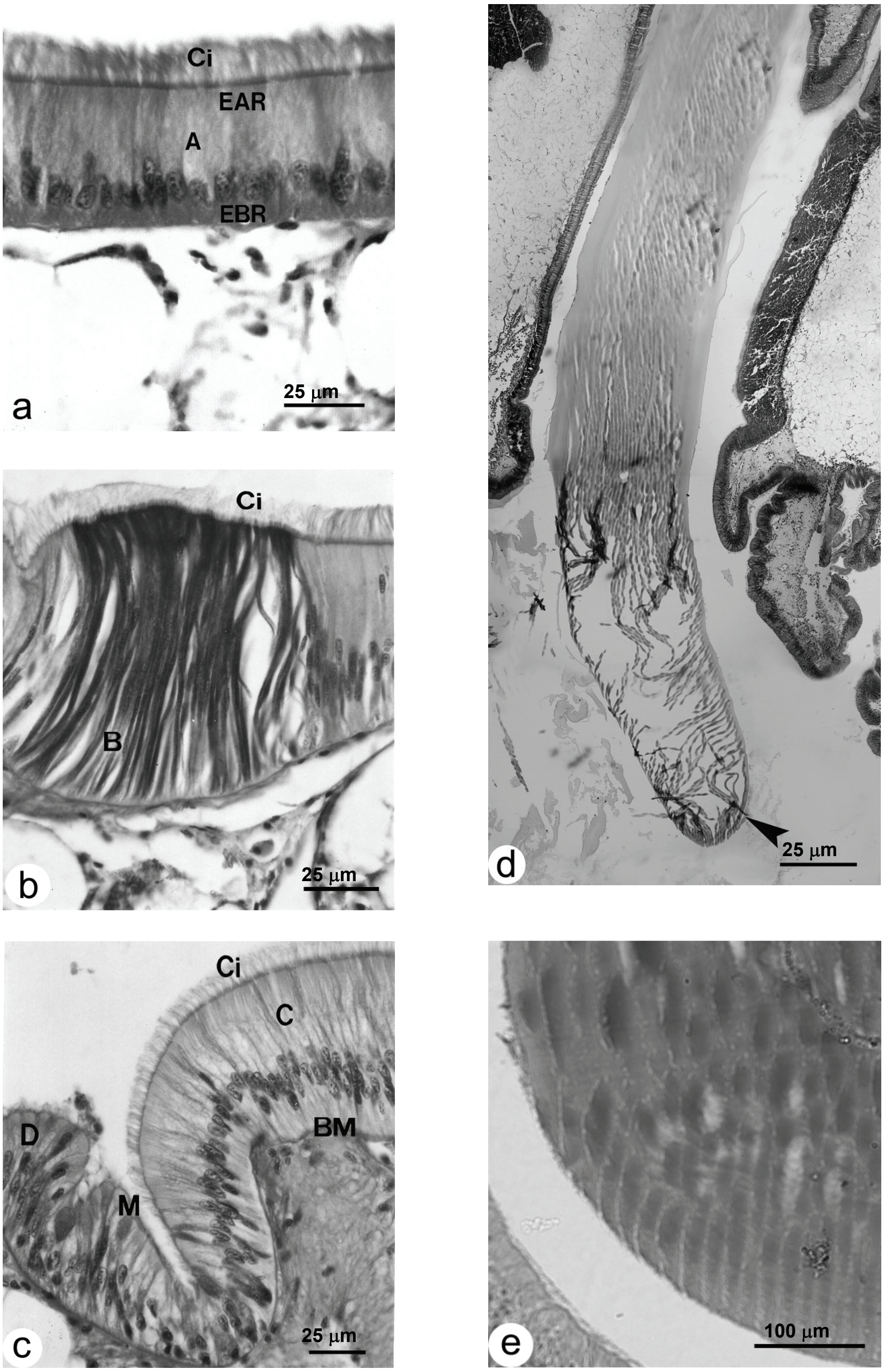

Figure 3 Style sac and crystalline style. (a) Epithelial lining of style sac revealing type A cell. (b) Type B cell. (c) Type C and D cells. (d) Longitudinal section of the crystalline style. (e) Transverse section of the crystalline style at the anterior end. A, type A cell; B, type B cell; BM, basement membrane; C, type C cell; Ci, cilia; D, type D cell; EAR, eosinophilic apical region; EBR, eosinophilic basal region; M, mucous cell; arrowhead indicated the anterior end.

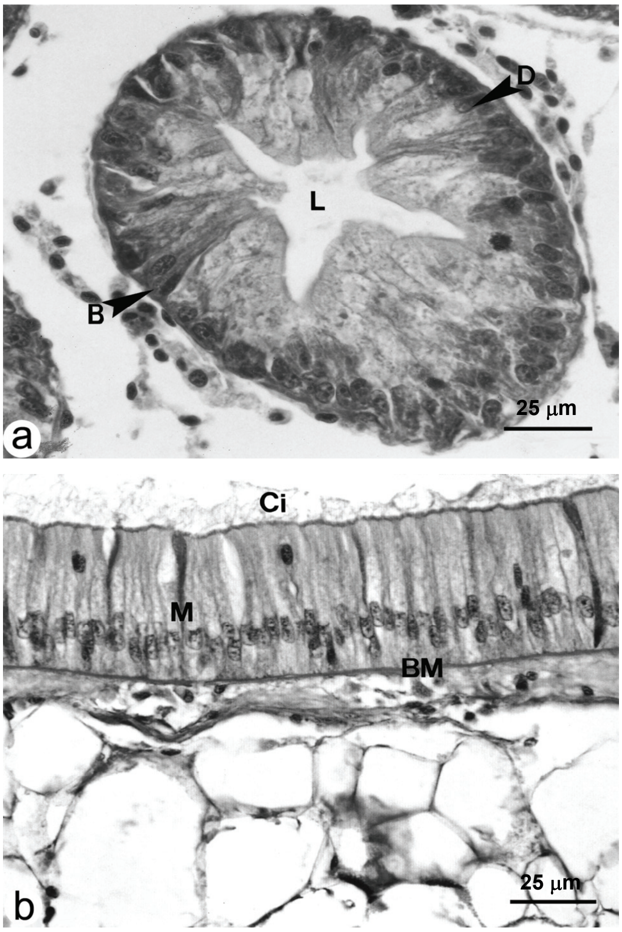

Figure 4 Digestive diverticula and anterior intestine. (a) Digestive tubule consisting of digestive and basophilic cells. (b) Simple ciliated columnar epithelium of anterior intestine. B, basophilic cell; BM, basement membrane; Ci, cilia; D, digestive cell; L, lumen; M, mucous cell.

Discussions

The most significant finding with respect to the digestive tract of *Hyriopsis* (*Hyriopsis*) *bialatus* is the long crystalline style (4.04 ± 0.21 cm, or 35.3% of the shell length). This is noteworthy, and contrasts with another suspension feeding bivalve, *Lyonsia hyalina,* from which a thin crystalline style ~1 mm long (or 5% of the shell length) protrudes from the style sac into the stomach (Thomas, 1993). The crystalline style is reported to be a source of enzymes facilitating extracellular digestion in the stomach (Kato and Kubomura, 1954; Owen, 1955; Hylleberg Kristensen, 1972; Judd, 1979).The role of the crystalline style in the secretion of amylases and other carbohydrases is well-documented (Owen, 1974). Since *H.* (*H.*) *bialatus* live in different habitats in the tropics where diverse phytoplankton are abundant year-round, having a long crystalline style would be greatly beneficial for food digestion. Hummel et al. (1988) also reported that the maximum lengths of the crystalline styles of *Macoma balthica* were recorded in the season of high food uptake. From our observations, the crystalline style of the freshwater bivalve *Anodonta cygnea* (a unionid from Lagoon Lake, Portugal) is 8.39 ± 0.69 cm long (or 64.4% of the shell length); that of other Thai freshwater mussels, e.g. *H.* (*Lymnoscapha*) *desowitzi* is 3.0 ± 0.2 cm (36.01% of the shell length); and that of *Chamberlainia hainesiana* is 6.5 ± 0.3cm (49.62% of the shell length). Therefore, having a long crystalline style seems to be rather typical of all unionids. The advantage of food digestion and absorption can be seen from the rapid growth of these mussels, which reach sexual maturity at the early age of 6-8 months (unpublished data), while those of other freshwater bivalves (e.g. *Margaritifera margaritifera*, *M. hembeli* and *Lampsilis rafinesqueana*) require a longer period of time (2-12 years) to reach this stage (Bauer, 1987; Smith, 1979, 1988; Shiver, 2002).

Numerous PAS-positive granules in the apical cytoplasm of the type B cells of the style sac strongly indicate that they are the secretion site for the crystalline style in *Hyriopsis* (*H.*) *bialatus*. Our results agree with that of Judd (1979) who showed B cells to be the most active secretory cells, as seen from the positively carbohydrate-stained material extruded from their apical surfaces. In *Dreissena polymorpha*, the ground substance is secreted by either the type D epithelium or the typhlosole of the style sac (Morton, 1969, 1973). Kato and Kubomura (1954) suggested that gelatinous tissue underlying the epithelia of the style sac possesses mesenchymal secretory cells which pass secretory material into the lumen of the style sac. The loosely packed anterior end of the style is probably due to its dissolution. This dissolution releases enzymes into the stomach, breaking down mucoid-bound food strings and effecting primary extracellular digestion (Morton et al., 1998). The crystalline style of several bivalves rotates clockwise to grind

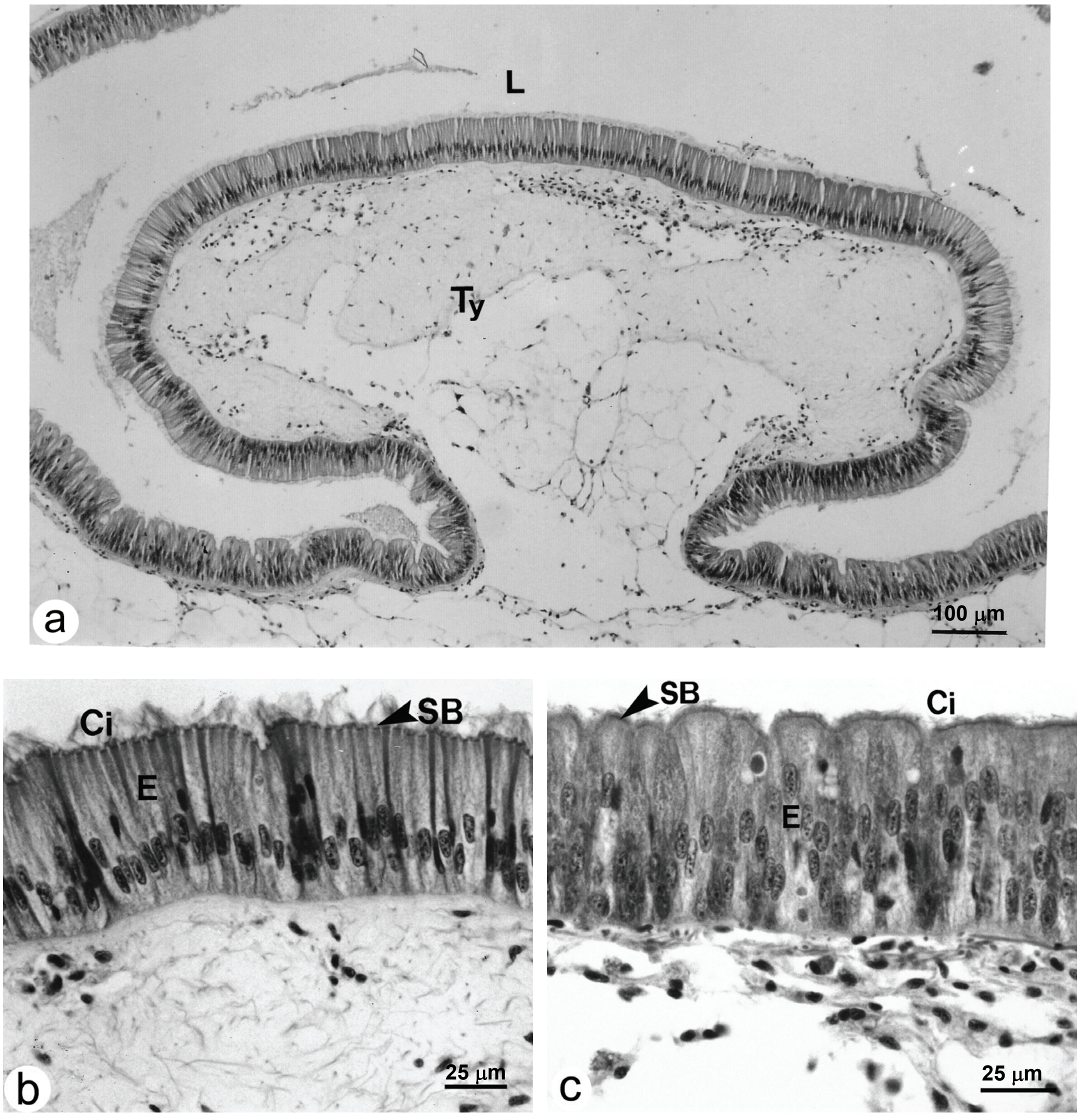

Figure 5 Posterior intestine. (a) Structure of a typhlosole in posterior intestine. (b) Epithelium of the upper part of typhlosole lined by a simple columnar epithelium. (c) Other area of posterior intestine lined by a pseudostratified ciliated columnar epithelium. Ci, cilia; E, epithelium; L, lumen; SB, striated border; Ty, typhlosole.

the food string against the gastric shield (Morton, 1969; Thomas, 1993). In *H.* (*H.*) *bialatus* the same mechanism is anticipated, since the ribbon-like matrix that constitutes the style at the anterior end is spiral in appearance, reflecting its rotational action.

The histological structure of the different parts of the digestive tract of *Hyriopsis* (*H.*) *bialatus* correlates well with their functions. The mucous cells are known to produce mucoids to lubricate and coalesce ingested food, as well as to coat the luminal surface of the digestive tube (Roldan and Garcia-Corrales, 1988; Thomas, 1993). Mucous cells containing sulfated acid mucin found only in the mouth and oesophageal epithelium serve as food lubricants to move ingested food. Subsequently, the food-bearing strands of mucus which proceed into the stomach are caught up and wound into a spiral mass by the rotation of the style. They are simultaneously mixed with digestive secretions, so that the extracellular digestion of carbohydrates is initiated (Barrington, 1979). Acid and neutral mucins in the intestine and rectum provide the mucus covering of the faecal pellets and help in compressing the faecal material (Roldan and Garcia-Corrales, 1988), thereby completing the digestion and absorption processes.

As for food movement and the enzymatic process, cilia are found throughout the digestive tract and are particularly dense in the style sac. The ciliated cells in the mouth are associated with particle transport, whereas the ciliary activities of the oesophageal cells move food particles into the gut (Thomas, 1993). The ciliated cells of the style sac presumably serve to rotate the crystalline style against the gastric shield, though they may also be responsible for the secretion of some enzymes (Morton, 1973). The digestive enzymes found in the gastrointestinal tract of adult *Hyriopsis* (*H.*) *bialatus* are amylase, proteinase, lipase and cellulose (Areekijseree et al., 2004). These enzymes correspond to its main food phytoplankton (Paterson, 1986; Kovitvadhi et al., 2000). The gastric shield protects the stomach wall from the abrasive effect of the rotating crystalline style, although it may also assist in the trituration of stomach contents (Morton, 1973). Moreover, it is also enzymatically active (Halton and Owen, 1968; McQuiston, 1970). The chitinous lining on the internal wall of the stomach is presumably secreted from the columnar epithelial cells (Thomas, 1993). However, McQuiston (1970) suggested that the chitin of the gastric shield matrix in the bivalve *Lasaea rubra* is formed by the conversion of glycogen reserves in the underlying cells. The sorting areas of the stomach in the Lamellibranchia remove large or indigestible particles to the intestine, leaving a suspension of fine particles for primary extracellular digestion by enzymes liberated by the style. These are subsequently transmitted to the digestive diverticula for intracellular digestion (Purchon, 1960, 1987; Morton, 1973). Eosinophilic regions in type A cells of the style sac probably result from the numerous mitochondria present in them, as reported for other bivalves (Kato and Kubomura, 1954). These mitochondria function to provide energy for the cilia (Thomas, 1993). Striated borders at the apical surface of the epithelial cells of the intestine have been described as being related to absorption and the transport of relatively large volumes of water, salts and protein over a short period of time (Palay and Karlin, 1959). The typhlosole found in the intestinal wall may increase the surface area and help to move food particles (Roldan and Garcia-Corrales, 1988). The simple ciliated columnar epithelium of the upper part of the typhlosole may function in absorption, while the pseudostratified ciliated columnar epithelium of the other areas of the posterior midgut is responsible for transportation (Sheedlo, 2005). The digestive cells of the digestive tubule ingest particles by endocytosis and digest them intracellularly (Owen, 1970, 1973; Pal, 1972). In this process, nutrients are absorbed in the basal area and passed into the haemocoel; whereas waste products are aggregated at the apex of the cell, released as waste spherules into the lumen of the tubule, and transported by ciliary currents out of the digestive diverticula into the stomach (Thomas, 1993). The basophilic cells, however, may serve in digestive cell replacement due to the presence of mitotic figures (Palmer, 1979).

In conclusion, *Hyriopsis* (*H.*) *bialatus* has all the typical features of the digestive tract of tropical freshwater bivalves. The striking features specifically found in this freshwater pearl mussel are: a thin, long, crystalline style; the site of secretion of material which constitutes the style; and an epithelium with numerous mucous cells and cilia lining entire the digestive tract. The information presented here offers a baseline for in-depth analysis of the digestive tract composition and function of *H.* (*H.*) *bialatus* at the ultrastructural level.

Acknowledgments

This work was partially supported by the Graduate School, Kasetsart University and the Development and Promotion of Science and Technology Talent Project. We would also like to thank the Department of Aquaculture, Faculty of Fisheries, Kasetsart University for providing a culturing pond and special thanks to Prof. Jorge Machado and Paula Lima (Porto University, Portugal) for providing the freshwater bivalve *Anodonta cygnea*.

References

Areekijseree, M., A. Engkagul, U. Kovitvadhi, A. Thongpan, M. Mingmuang, P. Pakkong and K. Rungruangsak-Torrissen. 2004. Temperature and pH characteristics of amylase and proteinase of adult freshwater pearl mussel, *Hyriopsis* (*Hyriopsis*) *bialatus* Simpson, 1900. Aquaculture 234: 575-587.

Areekijseree, M., A. Engkagul, S. Kovitvadhi, U. Kovitvadhi, A. Thongpan and K. Rungruangsak-Torrissen. 2006. Development of digestive enzymes and *in vitro* digestibility of different species of phytoplankton for culture of early juveniles of the freshwater pearl mussel, *Hyriopsis* (*Hyriopsis*) *bialatus* Simpson, 1900. Invert. Reprod. Develop. 49: 255-262.

Barrington, E.J.W. 1979. Invertebrate Structure and Function, 2nd ed. John Wiley and Sons, Inc., New York.

Bauer, G. 1987. Reproductive strategy of the freshwater pearl mussel *Margaritifera Margaritifera*. J. Anim. Ecol. 56: 691-704.

Chatchavalvanich, K., P. Jindamongkon, U. Kovitvadhi, A. Thongpan and S. Kovitvadhi. 2006. Histological structure of gonads in the freshwater pearl mussel, *Hyriopsis* (*Hyriopsis*) *bialatus* Simpson, 1900. Invert. Reprod. Develop. 49: 245-253.

Cook, H.C. 1982. Neutral mucin content of gastric carcinomas as a diagnostic aid in the identification of secondary deposits. Histopathology 6: 591-599.

Dudgeon, D. and B. Morton. 1984. Site selection and attachment duration of *Anodonta woodiana* (Lea, 1834) (Bivalvia: Unionacea) glochidia on fish hosts. J. Zool. 204: 355-362.

Halton, D.W. and G. Owen. 1968. The fine structure and histochemistry of the gastric cuticle of the Protobranchiate bivalve, *Nucula sulcata* Bronn. Proc. Malacol. Soc. Lond. 38: 71-81.

Hummel, H., W. De Bruin, G. Nieuwland and I.F. Hummel-Poel. 1988. Seasonal and tidal changes in the length of the crystalline style intertidally living *Macoma balthica* (Mollusca, Bivalvia). Mar. Biol. 98: 529-534.

Hylleberg Kristensen, J. 1972. Structure and function of crystalline styles of bivalves. Ophelia 16: 91-108.

Judd, W. 1979. The secretions and fine structure of bivalve style sacs. Ophelia 18: 205-233.

Kato, K. and K. Kubomura. 1954. On the origin of the crystalline style of lamellibranches. Sci. Repts. Saitama Univ. B3: 135-152.

Kovitvadhi, U. and S. Kovitvadhi. 2003. Development of freshwater pearl culture in Thailand. Report submitted to the National Research Council of Thailand. Bangkok, Thailand. (In Thai).

Kovitvadhi, U., A. Nagachinta and K. Aungsirirut. 2000. Species composition and abundance of plankton in the gut contents of freshwater pearl mussel, *Hyriopsis* (*Limnoscapha*) *myersiana*. J. Med. Appl. Malacol. 10: 203-209.

Kovitvadhi, S., U. Kovitvadhi, P. Sawangwong, A. Thongpan and J. Machado. 2006 Optimization of diet and culture environment for larvae and juvenile freshwater pearl mussels, *Hyriopsis* (*Limnoscapha*) *myersiana* Lea, 1856. Invert. Reprod. Develop. 49: 61-70.

Lev, R. and S.S. Spicer. 1964. Specific staining of sulphate groups with alcian blue at low pH. J. Histochem. Cytochem. 12: 309.

McManus, J.F.A. 1946. Histological demonstration of mucin after periodic acid. Nature 158: 202.

McQuiston, R.W. 1970. Fine structure of the gastric shield in the lamellibranch bivalve, *Lasaea Rubra* (Montagu). Proc. Malacol. Soc. Lond. 39: 69-75.

Morton, B. 1969. Studies on the biology of *Dreissena polymorpha* Pall. I. General anatomy and morphology. Proc. Malacol. Soc. Lond. 38: 301-321.

Morton, B. 1973. A new theory of feeding and digestion in the filter-feeding Lamellibranchia. Malacologia 14: 63-79.

Morton, B., R.S. Prezant and B. Wilson. 1998. Class Bivalvia, pp.195-234. In P.L. Beesley, G.J.B. Ross and A. Wells, eds., Mollusca: The Southern Synthesis. Fauna of Australia Vol. 5. CSIRO Publishing, Melbourne, Australia.

Mowry, R.W. 1956. Observations on the use of sulphuric ether for the sulphation of hydroxyl groups in tissue sections. J. Histochem. Cytochem. 4: 407.

Owen, G. 1955. Observations on the stomach and digestive diverticula of the Lamellibranchia. I. The Anisomyaria and Eulamellibranchia. Quart. J. Microsc. Sci. 96: 517-537.

Owen, G. 1970. The fine structure of the digestive tubules of the marine bivalve *Cardium edule*. Philos. Trans. R. Soc. Ser. B. 258: 245-260.

Owen, G. 1973. The fine structure and histochemistry of the digestive diverticula of the protobranchiate bivalve *Nucula sulcata*. Proc. R. Soc. Ser. B. 183: 249-264.

Owen, G. 1974. Feeding and digestion in the Bivalvia. pp. 1-35 *In*: O. Lowenstein, ed., Advances in Comparative Physiology and Biochemistry, Vol. 5. Academic Press, New York, USA.

Pal, S.G. 1972. The fine structure of the digestive tubules of *Mya arenaria* L. II. Digestive cell. Proc. Malacol. Soc. Lond. 40: 161-170.

Palay, S.L. and L.J. Karlin. 1959. An electron microscopic study of the intestinal villus. II.The pathway of fat absorption. J. Biophys. Biochem. Cytol. 5: 373-384.

Palmer, E.R. 1979. A histological and histochemical study of digestion in the bivalve *Arctica islandica* L. Biol. Bull. 156: 115-129.

Paterson, C.G. 1986. Particle-size selectivity. I. The freshwater bivalve *Elliptio complanata* (Lightfoot). Veliger 29: 235-237.

Pennak, R.W. 1989. Freshwater Invertebrates of the United States: Protozoa to Mollusca. 3rd ed. John Wiley and Sons, Inc., New York.

Purchon, R.D. 1960. The stomach in the Eulamellibranchia: stomach types IV and V. Proc. Zool. Soc. Lond. 135: 431-489.

Purchon, R.D. 1987. The stomach in the bivalvia. Philos. Trans. R. Soc. Ser. B. 316: 183-276.

Roldan, C. and P. Garcia-Corrales. 1988. Anatomy and histology of the alimentary tract of the snail *Theba pisana* (Gastropod: Pulmonata). Malacologia 28:119-130.

Sheedlo, H.J. 2005. USMLE Road Map Histology. International edition. The McGraw-Hill Companies, Inc. Singapore.

Shiver, M.A. 2002. Reproduction and propagation of the neosho mucket, *Lampsilis rafinesqueana*. M.S. Thesis, Southwest Missouri State University, Missouri, USA.

Smith, D.G. 1979. Sexual characteristics of *Margaritifera margaritifera* (Linnaeus) populations in central New England. Veliger 21: 381-383.

Smith, D.G. 1988. Notes on the biology and morphology of *Margaritifera hembeli* (Conrad, 1838) (Unionacea: Margaritiferidae). Nautilus 102: 159-163.

Soto, D. and G. Mena. 1999. Filter feeding by the freshwater mussel, *Diplodon chilensis*, as a biocontrol of salmon farming eutrophication. Aquaculture 171: 65-81.

Thomas, K.A. 1993. The functional morphology of the digestive system of *Lyonsia Hyalina* Conrad, 1831 (Bivalvia: Anomalodesmata: Pandoroidea). J. Moll. Stud. 59: 175-186.

Yeemin, M. 1997. Sizes and Shapes of Transplanted Mantle Pieces for Pearl Formation in Freshwater Pearl Mussels*, Hyriopsis (Hyriopsis) bialatus and Pseudodon vondembuschianus ellipticus.* M.S. thesis, Chulalongkorn University, Bangkok, Thailand.

Manuscript received 28 June 2010, accepted 21 December 2010