

# The Pallial Organs of *Haliotis Asinina* Linnaeus (Gastropoda : Haliotidae)

Yaowaluk P Chitramvong<sup>a</sup>, Maleeya Kruatrachue<sup>a</sup>, E Suchart Upatham<sup>b</sup>, Sombat Singhakaew<sup>a</sup> and Krisana Parkpoomkamol<sup>a</sup>

- <sup>a</sup> Department of Biology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand.
- <sup>b</sup> Department of Biology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400 and Faculty of Science, Burapha University, Chonburi 20131, Thailand.
- \* Corresponding author, E-mail: scyct@mahidol.ac.th

Received 29 May 2000 Accepted 2 Aug 2001

Abstract The pallial organs of *Haliotis asinina* are composed of gills, osphradium, hypobranchial gland, kidney and heart. The bipectinate paired gills are on the left and right sides of the mantle cavity. The gill epithelium is composed of ciliated columnar cells and occasional mucous cells. The paired osphradia are long, slender and lie anteriorly on each side of the mantle near the gills. The ciliated sensory epithelial cells are very tall and closely packed. The paired hypobranchial glands are fused and lie along almost the entire length of the mantle. The left hypobranchial gland is more prominent than the right one. They are composed of mucous gland cells. The paired kidneys are at the postero-dorsal part of the mantle and lie anteriorly to the heart. The right kidney contains tubulo-asini with light squamous or cuboidal epithelial cells. The left kidney contains densely packed irregular cuboidal epithelial cells. The heart is at the posterior end of the mantle connecting to the gills and is composed of one ventricle and two auricles. The wall of the auricles contains strands of muscle and fibrous tissue whereas the ventricle has thick muscular walls.

KEYWORDS: Haliotis asinina, pallial organs, gills, osphradium, hypobranchial gland, kidney, heart.

#### Introduction

There are only a few studies on the anatomy and histology of the pallial organs of abalone, e.g., Haliotis tuberculata Linnaeus<sup>1</sup>, H. rufescens (Swainson)<sup>2</sup>, H. ruber Leach<sup>3</sup>, H. aurantium Ricardo and Simone.<sup>4</sup> Crofts<sup>1</sup> and Ricardo and Simone<sup>4</sup> found that the left gill was larger than the right one. The hypobranchial glands are attached to the rectum. Crofts<sup>1</sup> indicated that the mucous epithelium of the hypobranchial gland had two types of secretory cells: the pointed type and the goblet cell type. Bevelander<sup>2</sup> showed that the hypobranchial gland of H. rufescens was made up of three kinds of cells: mucous cells with rod-like elements, mucous cells with granular cytoplasm, and slender dark-staining supporting cells that, according to Hunt<sup>5</sup>, have neural connections. The paired osphradia lay on each side of the mantle near the gills. The epithelium of osphradium was composed of very tall ciliated cells which were closely packed.1 According to Bevelander2, the osphradium of *H. rufescens* was lined by two types of epithelium: a single glandular layer and a stratified sensory layer.

The heart is at the posterior end of the mantle connecting to the gills. It is composed of one ventricle and two auricles. In H. tuberculata, the wall of the auricles contained very delicate lace-like strands of muscle and fibrous tissue.1 The ventricle had thick opaque muscle walls.1 According to Bevelander<sup>2</sup>, in *H. rufescens*, the musculature ventricle was made up of a complex network of branching and anastomosing fibers2, while the walls of the auricles were extremely thin and consisted of delicate strands of muscles, connective tissue and epithelium.<sup>2</sup> Ricardo and Simone<sup>4</sup> reported that the ventricle of the heart of *H. aurantium* was large and surrounded the intestine. The left auricle was anterior to the ventricle and the right auricle was ventral to it. Russelll and Evans<sup>3</sup> mentioned that the heart of *H*. ruber lay obliquely within a fluid-filled pericardial chamber. The auricles had thin walls while the ventricle had a thicker wall.

The paired kidneys are glandular in structure. In *H. tuberculata* it had cuboidal secretory epithelium.<sup>1</sup> The lumen of the kidney was folded into branched tubules.<sup>1</sup> Ricardo and Simone<sup>4</sup> indicated that in *H. aurantium* the left kidney was short and broad, while the right kidney was long and thin. Bevelander<sup>2</sup>

reported that in *H. rufescens*, the right kidney was arranged in branching tubules lined by a secretory epithelium that consisted of squamous or cuboidal cells. The left kidney epithelium was composed of irregular cuboidal cells with prominent nucleus and numerous granular inclusions.<sup>2</sup> To the best of our knowledge, there is virtually no information on the pallial organs of *Haliotis asinina* Linnaeus. Hence, the present study reports on the anatomy and histology of the pallial organs of *H. asinina*.

### MATERIALS AND METHODS

Ten mature H. asinina, five males and five females, with average shell length =  $66.6 \pm 7.5$  mm, average shell width =  $31.6 \pm 5.4$  mm, average body weight =  $97.9 \pm 12.1$  g, were collected from Samed Island, Rayong Province during May to June, 1999 to study the anatomy and histology of the pallial organs. The abalone were relaxed with 5% MgCl<sub>2</sub> and 3-4 menthol crystals for 3-4 hours. Dissections were done at the magnification of x 7.5 under an Olympus stereoscopic binocular microscope with a fiberoptic dissecting light (SAN-EI BUILDING, 22-2, NISHI-SHINJUKU 1-CHOME, SHINJUKU-KU TOKYO, JAPAN). Drawings were done with a camera lucida under the stereoscopic binocular microscope at the magnification of x 6.4 to x 16.

Pallial organs (gills, osphradia, hypobranchial glands, heart, and kidney) were dissected out and fixed in Bouin's fluid for 5 hours. The tissues were washed several times with 70 % alcohol, dehydrated through a graded series of ethanol, infiltrated with dioxane, and embedded in paraffin. Sections were cut on a rotary microtome at 5 - 6  $\mu m$  thickness and stained with hematoxylin and eosin. Sections were observed and photographed with an Olympus Vanox light microscope.

## **R**ESULTS

Pallial organs are the organs of mantle which consist of gills, osphradia, kidney, heart, and hypobranchial glands. The mantle is splitted in the middle about two thirds of the entire length of the mantle (Fig 1). The splitting part runs longitudinally from the anterior mantle margin. Therefore, the right part contains the right gill and right osphradium (Fig 1). The left part contains the left gill, left osphradium, left hypobranchial gland and posterior end of the rectum. The heart, the paired kidneys, the right hypobranchial gland and the rectum are at the fused part and posterior end of the mantle.

Gills. The bipectinate paired gills are bright yellow in color. They are located along the right and left walls of the mantle cavity. They extend from the anterior to the posterior margin of the mantle by having the latero-ventral parts pointing to each other. The gills are long and broad (Figs 2, 3). The left gill is much broader and longer than the right one. It lies ventrally to the left hypobranchial gland (Figs 1-3). The posterior parts of the gills are connected to the heart (Fig 4). The average numbers of the right and left gill filaments are  $702 \pm 5.7$  and  $716 \pm 6.6$ , respectively.

Gills are the special organs of respiration. They are composed of long gill filaments or lamellae (Fig 5A). The surfaces of the filament are bridged by very sparse connective tissue. At the base of the filament, there are V-shaped skeletal rods which form

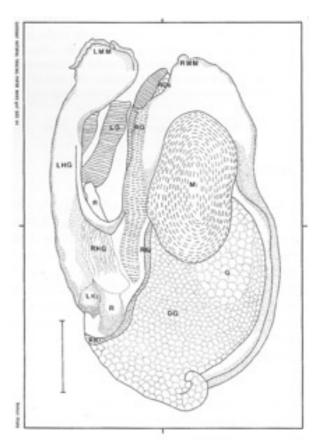


Fig 1. Dorsal view of splitted mantle of *H. asinina* showing the right gill (RG), left gill (LG) which is underneath the left hypobranchial gland (LHG), fused left and right hypobranchial glands (RHG), left (LKi) and right kidneys (RKi) and rectum (R). DG, digestive gland; G, gonad; LMM, left mantle margin; M, muscle; RMM, right mantle margin; ROs, right osphradium. Scale line = 1 cm.

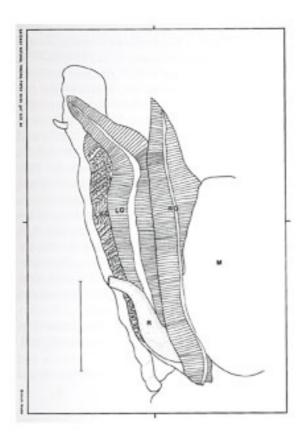


Fig 2. *H. asinina*, showing the left (LG) and right gills (RG), left hypobranchial gland (LHG) and rectum (R) after the right and the mid-mantle were dissected out. M, Muscle, Scale line = 1 cm.

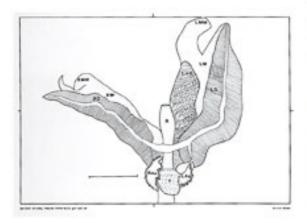


Fig 3. *H. asinina*, ventral view showing the left (LG) and right (RG) gills, left hypobranchial gland (LHG), heart and rectum (R) after the whole mantle is dissected out. LAu, left auricle; LM = left mantle; LMM, left mantle margin; RAu, right auricle; RM, right mantle; RMM, right mantle margin; V, ventricle. Scale line = 1 cm.

a basal support and keep the filaments erect (Figs 5A, 5B).

The gill epithelium is composed of ciliated columnar cells mixed with occasional mucous goblet cells. In the central region of each filament, the cells are short with short cilia. Towards the skeletal rods, the cells are taller with long cilia (Fig 5C). At the tips of the filaments, there are clusters of mucous goblet cells. The convoluted region of the gills is lined by squamous epithelium.

Osphradium. The paired osphradia are long, slender and opaque white in color. Each has a long ridge with a central groove. Its anterior part has a thick ridge. It lies at the anterior margin and on each side of the mantle near the gills (Fig 1).

The epithelium of the osphradium is composed of tall ciliated sensory cells and some mucous goblet cells (Fig 6). The interior of the osphradium is composed of connective tissue and osphradial nerve with glial cells (Fig 6B).

Hypobranchial gland. The left hypobranchial gland is very well developed. It is creamy-white in color. It is long, broad, full of folded-branched ridges, and very glandular (Figs 2, 3). It lies dorsal

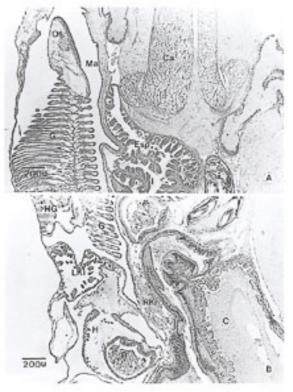


Fig 4. A low-power micrograph of the mantle cavity and associated structures. A. Gills (G), osphradium (Os), esophagus (Eso) and cartilage (Ca). B. Left (LKi) and right kidneys (RKi), heart (H), crop (C) and rectum (R).

to the left gill almost along the entire length of the mantle (Figs 1-3). The right anterior part is attached to the rectum (Fig 1). The posterior end of the gland is attached to the left kidney (Fig 1). The right hypobranchial gland lies laterally at the right side of the left one and it is much smaller and fused to the left one (Fig 1).

Figure 7A shows the histology of hypobranchial gland. It is composed of three types of cells (Fig 7B): mucous cells with fibrous elements in the cytoplasm, mucous cells with granular cytoplasm and slender supporting ciliated cells.

*Kidney.* The kidneys are paired structures. The anterior lobe of the kidney lies along the transverse part of the digestive gland. The left kidney is very small and attached to the posterior part of the left hypobranchial gland (Fig 1). It is reddish-brown in color.

The right and left kidneys have different histological organization: tubulo-acinus in the right, and branching folia in the left (Fig 8A). The right kidney contains light epithelial cells with squamous or cuboidal in shape (Fig 8B). The nuclei are located basally or centrally. These cells enclose a definite

lumen. The epithelium of the left kidney contains densely packed epithelial cells sitting on a connective tissue core (Fig 8C). These cells are irregular cuboidal in shape and have prominent nuclei (Fig 8C). In section, the cells appear to have irregular borders.

Heart. The heart has one ventricle and two auricles (Fig 3). The ventricle is a strong muscular sac appearing opaque pale yellow in color. It is connected and lies posterior to the gills and surrounds the anterior rectum (Fig 3). The auricles are on the left and right side of the ventricle. They are pale yellow thin sacs with serrated outline and have a leaf-liked shape (Fig 3). The right auricle is a little longer and narrower than the left one. It lies more ventrally to the ventricle. One end of the right auricle is attached to the rectum (Fig 3). The left auricle is broader and shorter than the right auricle. It lies more dorsally to the ventricle. One end of the left auricle is attached to the left gill. There is a thin sac underneath the left auricle (Fig 3).

The ventricle has a very thick musculature (Figs 9A, 9B). The walls of the auricle are extremely thin, and consist of delicate strands of muscle and endothelium(Fig 9C). The lining of the auricle and

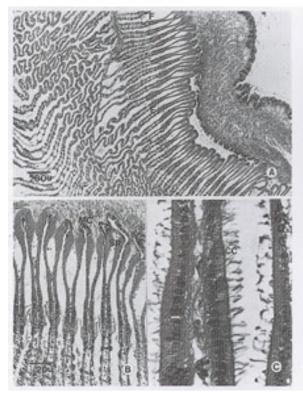


Fig 5. A. A low-power micrograph showing gills which are composed of numerous gill filaments (F). B. The basal part of the gill contains skeletal rods (SR) and clusters of mucous cells (MC). C. The middle part of gill filament showing numerous ciliated cells (CC).

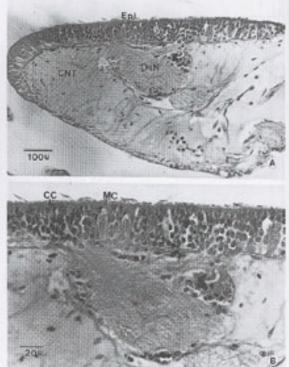


Fig 6. A. A low-power micrograph of osphradium whose interior is filled with connective tissue (CNT). Epi, epithelium; OsN, osphradial nerve; GC, glial cell. B. High magnification of osphradial epithelium which is composed of ciliated sensory cells (CC) and some mucous goblet cells (MC).

ventricle is composed of low cuboidal cells resting on a fibrous basement membrane (Figs 9B, 9C).

## DISCUSSION

The sizes of paired pallial organs of *H. asinina* are uneven. The left pallial organs are always decidedly larger or more obvious than the right ones except the right kidney which is more prominent than the left one. These have been used as evidence that the organs on the right side show a tendency towards disappearance.

The paired gills of *H. asinina* are asymmetric similar to those of *H. tuberculata*<sup>1</sup>, *H. rufescens*<sup>2</sup> and *H. ruber*. The number of gill filaments of *H. asinina* is not equal on both sides; unlike that of *H. tuberculata*, whose gills contained equal number of filaments on both sides. Epithelial cells lining the gills of *H. asinina* are similar to those of *H. tuberculata*<sup>1</sup> and *H. rufescens*. Crofts¹ also found that the sensory cells were scattered throughout the epithelium of the gills. The gills carry out several functions, such as the

The glis carry out several functions, such as the

Fig 7. A. A low-power micrograph of hypobranchial gland (HG). The cells are arranged in a single layer resting on a basal lamina (BL). B. High magnification of hypobranhcial gland showing three types of cells: mucous cells with rod-like elements (MC<sub>1</sub>), mucous cells with granular cytoplasm (MC<sub>2</sub>), and supporting ciliated cells (CC).

transport of blood, gaseous exchange, and water movement.

The paired osphradia are located in the region where the gill axis joins the mantle skirt on the anterior border of the gill supports. Hence, they are in the advantageous position to test the quality of the water before it flows through the gills for oxygenation of the blood. The osphradia of *H. asinina* are opaque white in color, whereas those of *H. tuberculata* are yellow green in color. Each has a long ridge with a central groove. In *H. tuberculata*, the osphradia passed along the gills and supported the inside of the gill chamber. The osphradial epithelium of *H. asinina* contains numerous ciliated sensory cells and a few mucous goblet cells, which are similar to those of *H. tuberculata*<sup>1</sup> and *H. rufescens*.<sup>2</sup>

The osphradium occurs in the cavity either on or near the gill.<sup>6-9</sup> It is generally accepted to be a sense organ with two functions: chemoreceptor, involved in food location or detection of attractive substances<sup>10-15</sup>, and a tactile organ concerned with estimating the amount of sediment carried into the

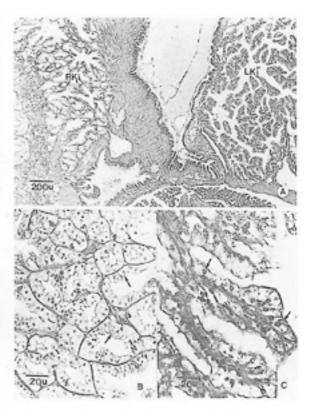


Fig 8. A. A low-power micrograph of left (LKI) and right kidneys (RKi) showing numerous tubules (T). B. High magnification of the right kidney showing secretory epithelium which is composed of cuboidal cells (arrows) with basal nuclei and granular cytoplasm. C. High magnification of the left kidney showing secretory epithelium which is composed of irregular-shaped cuboidal cells (arrows).

mantle cavity, or assists the regulation of resperation by detecting changes in the pH of sea water. 16,17

The paired hypobranchial glands of H. asinina are located more toward the left of the rectum. This is the same as those of H. tuberculata and H. aurantium which have fused hypobranchial glands and the left gland is more extensive than the right one. 1,3 Crofts1 indicated that the mucous cells of H. tuberculata were of two types based on their secretion: the cell containing pointed spindles and the mucous goblet cell. The hypobranchial gland of H. asinina is composed of three types of cells similar to those in H. rufescens described by Bevelander.2 They are mucous cells with fibrous elements, mucous cells with granular cytoplasm and slender supporting cells. Bevelander<sup>2</sup> indicated that the first type of mucous cells (with fibrous elements) produced acid mucopolysaccharide and the second type (with granular content) produced glycoprotein. The function of hypobranchial gland is secretion of mucus.<sup>13</sup> The glandular epithelium is folded into large pleats to increase the secreting area. The quantity of mucous discharging into the

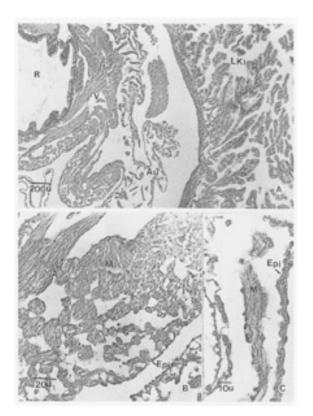


Fig 9. A. A low-power micrograph of ventricle (V) and auricle (Au) of the heart. LKi, left kidney; R, rectum. B. High magnification of ventricle with very thick musculature (M). Epi, epithelium. C, High magnification of auricle with thin walls consisting of delicate strands of muscle (M) and epithelium (Epi).

respiratory chamber increases suddenly if the animal is irritated.<sup>1</sup> It is produced for protection and for surrounding and clearing away debris from the anus and renal organs, so keeping the gills clean.<sup>13</sup>

The paired kidneys are found in *H. tuberculata* and *H. aurantium*. <sup>1,4</sup> Similar structures were found also in *H. asinina*. In *H. aurantium*, the left kidney is short and broad with a short papillated nephrostome in the ventral base of rectum. The right kidney of *H. asinina* is very extended. Its anterior lobe runs transversely along the digestive glands. In comparison, the left kidney of *H. asinina* is very small, and its shape is short and broad similar to that of *H. aurantium*.

The observation of both left and right kidneys of H. asinina revealed the similar histology to that described by Bevelander<sup>2</sup> in H. rufescens. In addition, Bevelander<sup>2</sup> also indicated that the left kidney had a much greater resorption capacity than that of the right one.

The heart of H. asinina lies posteriorly to the paired gills. It is composed of one large, thick-walled ventricle and two small, thin-walled auricles. These characters are the same as those of H. tuberculata<sup>1</sup>, H. rufescens<sup>2</sup>, H. ruber<sup>3</sup> and H. aurantium.<sup>3,4</sup> The right auricle of H. asinina is a little larger than the left one. This is similar to those of H. tuberculata<sup>1</sup>, H. rufescens<sup>2</sup> and H. aurantium.<sup>4</sup> Crofts<sup>1</sup> mentioned that the auricles received oxygenated blood from the two ctenidia. He also mentioned that the posterior twothirds of the cavity of the ventricle had a spongy character, because the muscular strands suspended the ventricle wall to the wall of the intestine. The histology of the ventricle and auricle of the heart of H. asinina is also similar to those described in H. tuberculata<sup>1</sup>, H. rufescens<sup>2</sup> and H. aurantium.<sup>4</sup>

### **A**CKNOWLEDGEMENTS

This study received a financial support from the Thailand Research Fund BRG/04/2543.

## REFERENCES

- Crofts DR (1929) Haliotis. Liverpool Mar Biol. Committee Memoirs Typical Br Mar Plants Animals 29, 1-174.
- Bevelander G (1988) Abalone Gross and Fine Structure. The Boxwood Press, Pacific Grove, California.
- Russell CW and Evans BK (1989) Cardiovascular anatomy and physiology of the black-lip abalone, Haliotis ruber. J Exp Zool 252, 105-17.
- Ricardo L and Simone L (1998) Morphology of the Western Atlantic Haliotidae (Gastropoda, Vetigastropoda) with description of a new species from Brazil. Malacologia 39(1-2), 59-75.

5. Hunt B S (1973) Fine structure of the secretory epithelium in the hypobranchial gland of the prosobranch gastropod mollusc *Buccinium undatum*, L. *J Mar Biol Assoc UK* **53**, 59-71.

- 6. Newell P F and Brown A C (1977) The fine structure of the osphradium of *Bullia digitalis* Meuschen (Gastropoda, Prosobranchia). *Malacologia* **16**(1), 197-205.
- 7. Brown A C and Noble R G (1960) Function of the osphradium in *Bullia* (Gastropoda). *Nature* 188 (4755), 1045.
- 8. Garton D W, Roller R A and Caprio J (1984) Fine structure and vital straining of osphradium of the southern oyster drill, *Thais haemastoma canaliculata. Biol Bull* 167, 310-21.
- Haszprunar G (1985) The fine morphology of the osphradial sense organs of the mollusc. I Gastropoda, Prosobranchia. Phil Trans R Soc Lond B 307, 457-96.
- 10. Michelson E H (1960) Chemoreception in the snail Australorbis glabratus. Amer J Trop Med 9, 480-487.
- 11. Bailey D F and Laverack M S (1963) Central nervous responses to chemical stimulation of a gastropod osphradium. *Nature* **200**, 1122-3.
- Bailey D F and Laverack (1966) Aspects of the neurophysiology of Buccinum undatum L (Gastropoda). J Exp Biol 44, 131-48.
- Hyman L H (1967) The invertebrates, Mollusca. Volume 6. McGraw-Hill, New York.
- Townsend C R (1973) The role of the osphradium in chemoreception by the snail *Biomphalaria glabrata* (Say). *Anim Behav* 21, 549-56.
- 15. Taylor J D and Miller J A (1989) The morphology of the osphradium in relation to feeding habits in Meso-and Neogastropods. *J Moll Stud* 55, 227-37.
- Parwar B J, Smith M and Baumgarten RV (1969) Activation of neurosecretory cells in *Aplysia* by osphradial stimulation. *Amer J of Physiol* 216(5), 1246-57.
- Stinnakre J and Tauc L (1969) Central neuronal response to the activation of osmoreceptors in the osphradium of *Aplysia*. J Exp Biol 51, 347-61.