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Comparative Anatomy of the Family Bithyniidae (Prosobranchia: Mesogastropoda)

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ABSTRACT

Eight species of bithyniids, *Bithynia leachi, B. tentaculata, B. siamensis, B. misella, B. kiusiuensis, B. striatula, Bithynia* sp. from Nepal and *Gabbia australis* were collected from 1957 to 1988. The samples were relaxed with pentobarbital and fixed with 70% ethyl alcohol.

There was no significant difference in internal structure of soft body, except the male reproductive system. The alimentary canal of the bithyniids consisted of mouth, buccal mass, esophagus, stomach, intestine, rectum and anus. The heart consisted of an auricle and a ventricle, located at left side of the stomach in the visceral hump. The ctenidium comprised 40-70 subtriangular lamellae. Eight ganglia were located around upper esophagus. The female reproductive system comprised ovary, oviduct, spermatheca, and albumin gland. The male reproductive system was composed of testis, seminal vesicle, prostate gland, vas deferens, penial duct, and penis in order. Male was distinguished externally from the female by a bifid penis.

Keywords: *Bithynia leachi, Bithynia tentaculata, Bithynia siamensis, Bithynia misella, Bithynia kiusiuensis, Bithynia striatula, Bithynia, Gabbia australis,* Anatomy, Structure, Bithyniidae.

INTRODUCTION

Snails of the family Bithyniidae (Mollusca: Gastropoda: Prosobranchia: Mesogastropoda) inhabit in freshwater, and some species of them are known as intermediate hosts of human flukes, such as Chinese liver fluke and *Clonorchis sinensis* (Kobayashi, 1923; Abbott, 1948).

The bithyniid snails are widely distributed in the world except Mid and South America (Chung, 1984). Features of radula, alimentary canal, nerve and reproductive system of *Bithynia tentaculata* and some other gastropods were described briefly by Krull (1935), and Lilly (1953) gave us useful information on the anatomy of reproductive system of *B. tentaculata.* Itagaki (1965) showed outline of the external and internal morphology of *Bithynia striatula* (syn. *Parafossarulus manchouricus*). After these early efforts on morphology of the bithyniids, Mandahl-Barth (1968) described the radulae and copulatory organs of the African Bithyniidae, and Meier-Brook and Kim (1977) observed median plan of the pallial cavity of a female *B. striatula.* Fretter and Graham (1994) dealt only with *B. tentaculata* in their review of functional anatomy of British prosobranchs. Kim *et al.* (1988) compared radulae morphology of six species of bithyniids with scanning electron microscopy. Although Kim (1994) used some anatomical characteristics of bithyniid snails for his report on numerical taxonomy, their detailed feature has not been described.

MATERIALS AND METHODS

Eight species of bithyniid snails, *Bithynia leachi* Sheppard, 1823, *B. tentaculata* Linnaeus, 1758, *B. siamensis* Lea, 1856, *B. misella* Gredler, 1884, *B. kiusiuensis* Hirase, 1927, *B. striatula* Leach, 1818,

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Table 1. List of bithyniid snails for the present study.

Species/population	Locality collected	Date of collection	Collector	Habitat
Bithynia striatula				
Yoosong	Daejeon, Korea	Oct. 3, '84	Kim. C.H.	N.R.
Kimhae	Kimhae, Korea	Sep. 23, '86	Kim, J.J.	I.C.
Chongpyong	Chongpyong, Korea	Jul. 5, '70	Meier-Brook	fish pond
Haman	Haman, Korea	Nov. 3, '73	Kim. C.H.	I.C.
Yangsoori	Yangpyong, Korea	Sep. 27, '87	Kim, J.J.	pond
Bithynia tentaculata				
Schreckensee	Altshausen, Germany	N.R.	Meier-Brook	lake
Bodensee	Üeberlingen, Germany	Sep. 2, '82	Meier-Brook	lake
Warmbad	Villach, Austria	May 27, '85	Meier-Brook	lake
Allgäu	Allgäu, Germany	Apr. 21, '70	Meier-Brook	lake
Ukleisee	Malente, Germany	Sep. 27, '79	Meier-Brook	lake
Bithynia siamensis				
Khon Kaen	Khon Kaen, Thailand	Sep. 7, '86	Haas	N.R.
Bithynia sp.				
Kirthipur	Katmandu, Nepal	Apr. 22, '81	Fritsch	N.R.
Bithynia leachi				
Längsee	Kiel, Germany	Dec. 28, '60	Meier-Brook	lake
Ukleisee	Malente, Germany	May 26, '78	Meier-Brook	lake
Bithynia kiusiuensis				
Saga	Saga-Ken, Japan	May 27, '87	Terasaki	N.R.
Bithynia misella				
Haman	Haman, Korea	Nov. 3, '73	Kim C.H.	N.R.
Kaejong	Gunsan, Korea	Sep. 9, '70	Meier-Brook	I.C.
Gabbia australis				
Walcha	Walcha, Australia	Feb. 4, '72	Witten	N.R.

Remarks;
*: The nearest city in Britannic Atlas from the collection site.
N.R.: not recorded, I.C.: irrigation canal

Bithynia sp. from Nepal and *Gabbia australis* Tryon, 1865, were collected from various regions (Table 1) from 1957 to 1988. The snails were fixed with 70% ethyl alcohol immediately after collection or fixed after relaxing the soft body by the method of Meier-Brook (1976a). The procedures are briefly described as follows; 1) removed chlorine from 20 ml of tap water with sodium thiosulfate then put the snail in it, 2) added about 10 mg of pentobarbital and left it for 6-12 hours to dissolve the anesthetic slowly and to relax soft body, 3) treated with 60℃, 5% formalin for 3 min., 4) fixed with 70% alcohol with 1% glycerin.

The isolated soft bodies were dissected by micro-forceps under stereoscope, and the organs were drawn and measured with drawing tube attached to the microscope (Meier-Brook, 1975). More than 10 snails of each sex from the local collection were dissected. The lengths of penis (male verge) and flagellum of fully relaxed samples were measured, and the penial and flagellar lengths from the midpoint between them to their tips were measured.

Accessory prostate gland and vas deferens were carefully isolated from the body. They were put into absolute alcohol after being fully strengthened in 70% alcohol. The lengths of these dehydrated organs were measured.

RESULT

1. External morphology

When the snail is moving actively, foot, tentacles, skin flap, snout, and mantle skirt could be seen outside the shell. The snout was dark gray colored, and the mouth part was furrowed. The snail had two

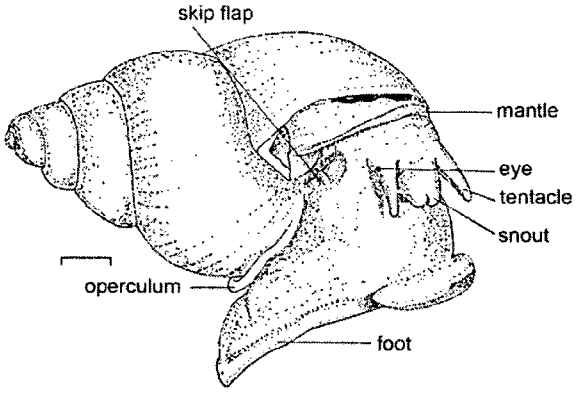

Fig. 1. External view of *Bithynia tentaculata*, Längsee population. The relaxed body was fixed with ethyl alcohol. Scale bar = 1 mm.
fo: foot, op: operculum, sn: snot, sp: skin flap, te: tentacle.

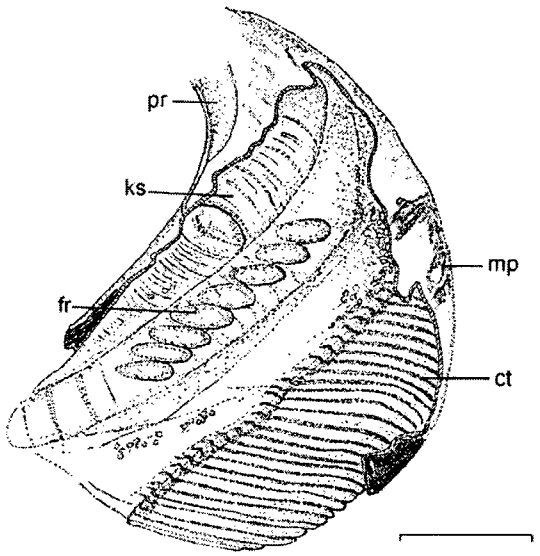

Fig. 2. Dorsal view of the anterior part of *Bithynia striatula*, Haman population. Specimens was removed the mantle edge and pigment. Scale bar = 1 mm.
ct: ctenidium, fr: feces in rectum, ks: kidney sac, mp: mantle pigment, pr: prostate gland

elongated cylindrical shaped tentacles behind the snout. Dark eye was lay at the outer side of the base, somewhat swollen externally, of the tentacles. Beneath the mantle, skin flap was on the right dorsal surface of the foot. The foot was confluent anteriorly with the

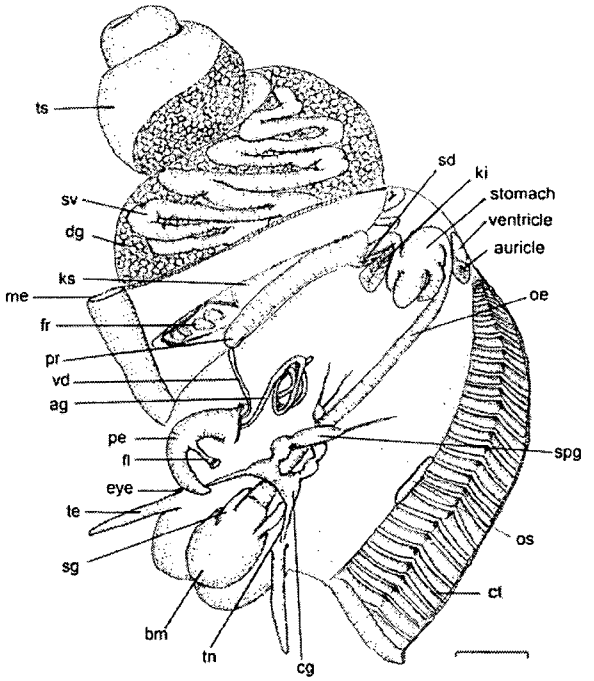

Fig. 3. Gross anatomical view of male bithyniid.
ag: accessory prostate gland, au: auricle, bm: buccal mass, cg: cerebral ganglion, ct: ctenidium, ki: kidney, ks: kidney sac, me: mantle edge, oe: esophagus, os: osphradium, pe: penis, pr: prostate gland, sd: sperm duct, sg: salivary gland, spg: supraesophageal ganglion, st: stomach, sv: seminal vesicle, te: tentacle, tn: tentacular nerve, ts: testis, ve: ventricle, vd: vas deferens

head, and had rounded projecting in the front end and somewhat sharp pointed posterior end. The operculum is attached to the postero-dorsal part of the foot (Fig. 1).

When the snails were alive, spots which had yellow color in *Bithynia striatula* and light orange color in *Bithynia misella* and *B. kiusiuensis* were scattered on the snout. These spots were concentrated near the eye, and distribution of the spots was extended to mantle edge in *B. misella* and *B. kiusiuensis.*

The pattern of mantle pigment was identical among the snails collected from a locality. All species observed, except *Bithynia leachi,* had a gray colored band on the mantle edge.

The outermost epithelial layer of the mantle of alcohol preserved snails was thin and could easily be

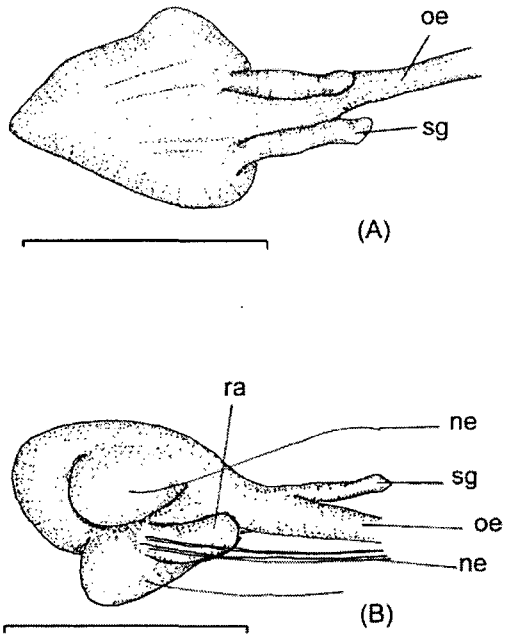

Fig. 4. Buccal mass of *Bithynia striatula,* Yoosong population. **A**: Dorsal view; **B**: Ventro-lateral view. Scale bar = 1 mm.
ne: nerve, oe: esophagus, sg: salivary gland, ra: radula sac.

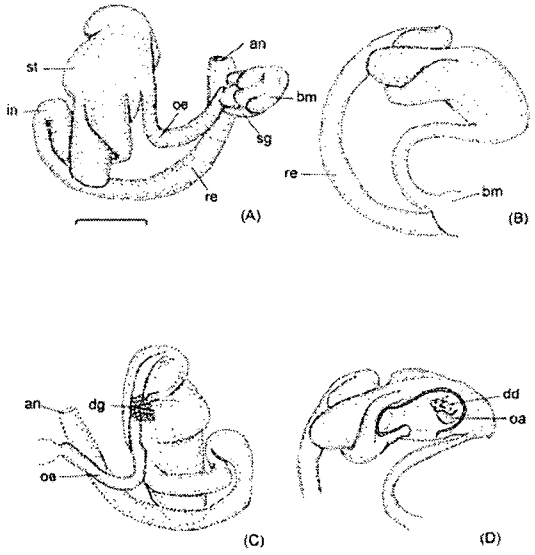

Fig. 5. Alimentary system of *Bithynia tentaculata,* Schreckensee population. **A**: dorsal view of stomach and ventral view of buccal mass; **B**: Lateral view; **C**: Ventro-lateral view; **D**: Lateral view with partially dissected stomach. Scale bar = 1 mm.
an: anus, bm: buccal mass, dd: opening of duct of digestive glands, dg: digestive glands, in: intestine, oa: esophageal aperture, oe: esophagus, re: rectum, st: stomach.

peeled off (Fig. 2).

2. Alimentary system

The alimentary canal of the bithyniids consisted of mouth, buccal mass, esophagus, stomach, intestine, rectum and anus (Fig. 3). The mouth opened into anterior end of buccal mass, and muscles were connected to inner wall of the snout and mouth part. Grape seed-shaped buccal mass had sharp anterior and blunt posterior ends, and a pair of cylindrical salivary glands (Fig 4A). Two ball-shaped projections, which had nerve fibers, could be seen on ventral side of the buccal mass, and a part of radula sac projected posteriorly along medial line (Fig. 4B).

The esophagus from buccal cavity was connected to posterior end of stomach in stomodeal section. This organ was a slender canal and passed under the cephalic ganglion, and joined to the left side of the stomach at its posterior end (Fig. 3, 5B). Stomach was located in left side of the kidney and right side of the heart, just behind the heart. Stomach had two parts; the anterior end had a large gastric shield and posterior end consisted canal like projection (Fig. 5A). Right beside where digestive gland joined anterior stomach, esophagus was attached (Fig. 5C). Duct of digestive glands and esophageal aperture were opened to inside of the stomach wall (Fig. 5D).

Intestine had blunt end at its base and ran under the posterior portion of the stomach and curved at the columellar axis (Fig. 5A).

Rectum was attached to inner wall of the mantle, and lay on the left side of prostate gland or pallial oviduct. Sausage-shaped feces filled the rectum obliquely, and pushed out to anus in mantle edge.

Digestive gland occupied largely inside of coiled whorls, and consisted of yellowish to dark green granules with tiny whitish spots.

3. Circulatory system

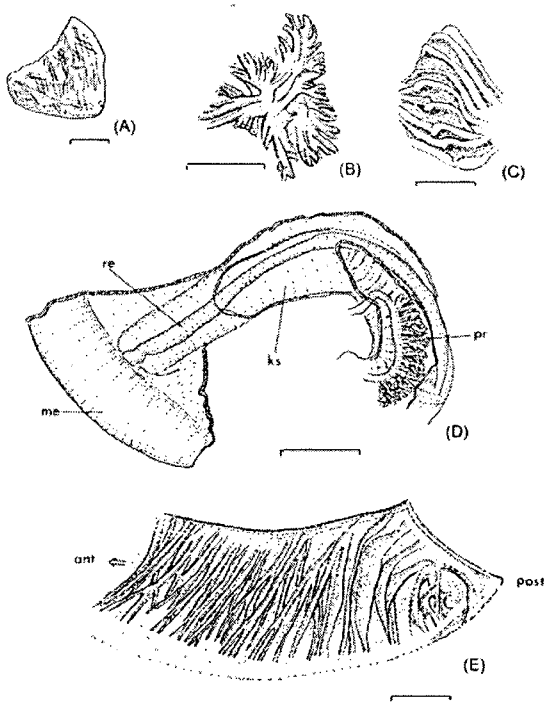

Fig. 6. Circulatory, respiratory and excretory system of the Bithyniidae. **A**: Outer view of the auricle of *Bithynia striatula,* Kimhae population; **B**: Inner view of the wall of the auricle of *Bithynia striatula,* Haman population; **C**: A part of ctenidium of *Bithynia siamensis,* Khon Kaen population; **D**: Kidney sac of *Bithynia misella,* Haman population; **E**: Inner view of the wall of kidney sac of *Bithynia striatula,* Haman population. Scale bar = 0.5 mm.
ant: anterior, ks: kidney sac, me: mantle edge, post: posterior, pr: prostate gland, re: rectum.

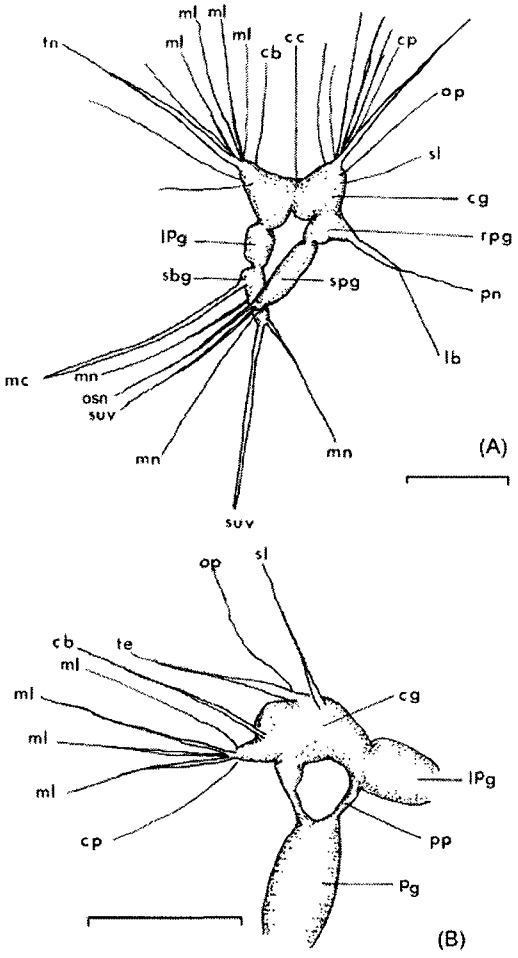

Fig. 7. Nervous system of *Bithynia striatula,* Yoosong population. **A**: Dorsal view of the central nerve system; **B**: Lateral view of the cerebral ganglion. Scale bar = 0.5 mm.
cb: cerebro-buccal connective, cc: cerebral commissure, cg: cerebral ganglion, cp: cerebro-pedal ganglion, lb: nerve to lateral body wall, lpg: left pleural ganglion, mc: mid-columellar nerve, ml: labial nerve, mn: mantle nerve, op: optic nerve, osn: osphradial nerve, pg: pedal ganglion, pn: penial nerve, pp: pleuro-pedal connective, rpg: right pleural ganglion, sbg: subesophageal ganglion, sl: supralabial nerve, spg: supraesophageal ganglion, suv: supravisceral connective, tn: tentacular nerve.

The heart consisted of an auricle and a ventricle, and was located at left side of the stomach in the visceral hump (Fig. 3). An aorta rose from the slender posterior part of the ventricle, and divided into two arteries. The auricle lay at posterior to ctenidium and at anterior to stomach, and was blunt cone shaped. Irregular oblique patterns were observed in the transparent auricle in alcohol preserved samples (Fig. 6A). Branched muscles were located in the inner wall of the auricle (Fig. 6B).

4. Respiratory system

The ctenidium elongated inside of the left mantle (Fig. 3), and consisted of 40-70 gill lamellae, which was subtriangular shaped (Fig. 6C). The number of lamellae varied in species. Generally, bigger species

such as *Bithynia siamensis* had about 70 lamellae, but smaller species, *B. leachi,* had less than 40 lamellae.

5. Excretory system

Excretory system of the bithyniids was monocardian type, which had a single kidney. The kidney was located on the right side of stomach, and surrounded by intestine which curved out from stomach. The kidney consisted of two lobes which were elongated triangular shape. The kidney connected to anterior kidney sac, which was extended along the inside of mantle on the left side of pallial oviduct or prostate gland (Fig. 6D). The kidney sac was cylindrical and had many folds (Fig. 6E).

6. Nervous and sensory system

Nervous system consisted of eight ganglia (paired cerebral, pleural, and pedal ganglia, and unpaired sub-esophageal and supra-esophageal ganglia) which surround the esophagus, and many peripheral nerves from the ganglia (Fig. 7A).

Both of cerebral ganglia connected with short commissure, and joined posteriorly to pleural ganglia and ventrally to pedal ganglia (Fig. 7B). Four pairs of labial, supra-labial, tentacular, and optic nerves, and cerebro-buccal connective and cerebro-pedal connective were risen from a cerebral ganglion (Fig. 7A).

Right pleural ganglion was oval shape, but left one showed columnar shape. Penial nerve and nerves to body wall connected to the right pleural ganglion, however, the nerve from the left pleural ganglion could not be found. The pleural ganglia joined to the pedal ganglia with connective (Fig. 7B).

Sub-esophageal ganglion was under the esophagus and connected with left pleural ganglion. A mid-columellar nerve and a mantle nerve started from the right side of sub-esophageal ganglion, and nerves to mantle and viscera lay posterior to the ganglion.

Supra-esophageal ganglion was longer and larger than sub-esophageal ganglion. Mantle, visceral and osphradial nerves rose from the supra-esophageal ganglion.

Paired podal ganglia were linked to both cerebral and pleural ganglia on the ventral side. Each pedal

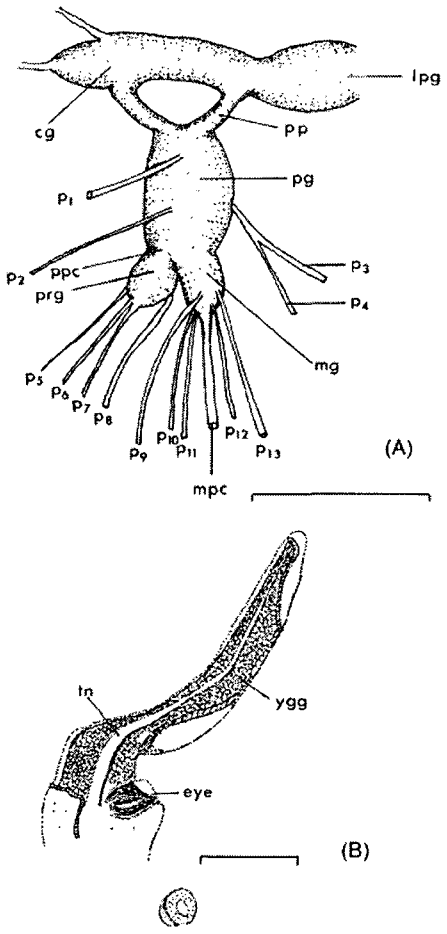

Fig. 8. Nervous and sensory system of Bithyniidae. **A**: The pedal ganglion of *Bithynia striatula,* Yangsoori population; **B**: A dissected tentacle and eye of *Bithynia siamensis,* Khon Kaen population. The tentacular nerve was embedded in the yellow and dark gray colored granules. The base of the eye was black and the lens (bottom) was transluscent and the center of the lens was white colored. Scale bar = 0.5 mm.
cg: cerebral ganglion, lpg: left pleural ganglion, mg: metapodial ganglion, mpc: metapodial connective, p1: lateral retractor nerve, p2: nerve to the anteroventral wall of the pedal haemocoel, p3: major lateral nerve of pedal ganglion, p4: minor lateral nerve of pedal ganglion, p5-8: nerves from propodial ganglion, p9-13: nerves from metapodial ganglion, pp: pleuro-pedal connective, ppc: propodial connective, prg: propodial ganglion, tn: tentacular nerve, ygg: yellow and dark gray colored granules.

ganglion was subdivided into propodial and metapodial ganglia at the ventral end. A total of 13 nerves to foot and body wall were connected to a pedal ganglion as shown in Fig 8A.

Sensory system of the snails comprised tentacles and an osphradium. Tentacular nerves from the cerebral ganglia were surrounded with yellowish and grayish granules in the tentacles. Eye, located at the proximal end of the tentacle, had a blackish eye ball which had a transparent lens and a pupil (Fig. 8B). Long cylindrical shaped osphradium lay between body wall and mantle near columellar, and was attached to the ctenidium (Fig. 3).

7. Female reproductive system

There was no significant difference in shape of female reproductive system among the eight bithyniids, but they varied in size. The female reproductive system comprised ovary, oviduct, spermatheca, and albumin gland. Ovary occupied 1.5 whorls, covered digestive gland, and could be seen through the mantle (Fig. 9A). The size of the ovary was smaller than the testis in a species. Oval and tiny eggs in the branched ovary could be seen in the alcohol preserved specimens (Fig. 9C). The branched tubules of the ovary converged to join an oviduct (Fig. 9B). Oviduct ran along the columellar axis, and was surrounded by the digestive gland. Oviduct was shorter and thinner than seminal vesicle of the male. The duct was connected to the bursa copulatrix and pallial oviduct, which was larger than prostate gland of male, and lay on the right side of the body. The color of pallial oviduct was whitish or yellowish. Albumin gland, in which eggs could be found, was in the middle portion of the pallial oviduct.

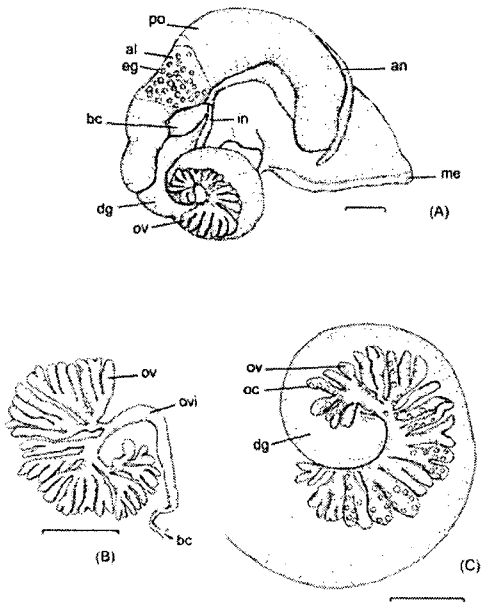

Fig. 9. Female reproductive system. **A**: Female reproductive system of *Bithynia striatula,* Yangsoori population; **B**: Ovary and oviduct of *Bithynia striatula,* Yangsoori population, fresh sample; **C**: Apical part of female of *Bithynia striatula,* Yangsoori population. White colored oocytes could be seen. Scale bar = 1 mm
al: albumin gland, an: anus, bc: bursa copulatrix, dg: digestive gland, eg: egg, in: intestine, me: mantle edge, oc: oocyte, ov: ovary, ovi: oviduct, po: pallial oviduct.

8. Male reproductive system

Male reproductive system of the eight bithyniids

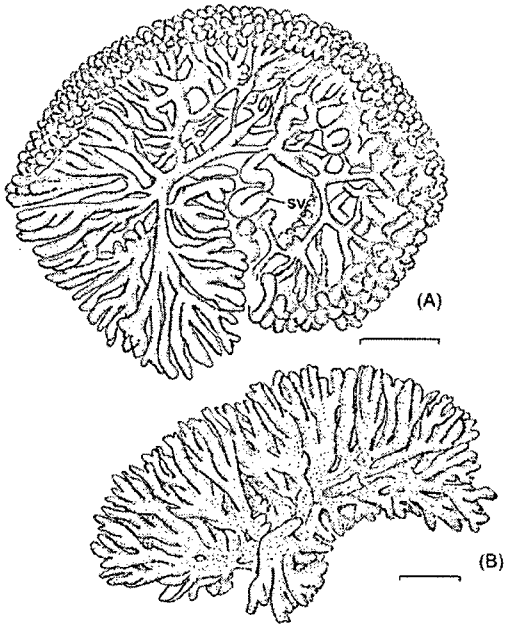

Fig. 10. Testis of *Bithynia striatula.* A: Whole testis, Kimhae population; B: A part of the testis, Chongpyong population. Scale bar = 0.5 mm.
sv: seminal vesicle.

observed in this study had morphological differences as well as size variations of reproductive organs. The male reproductive system comprised testis, seminal vesicle, prostate gland, vas deferens, penial duct, and penis in order. A flagellum was attached to distal half of the penis, and blind ended accessory gland ran through flagellum and opened to tip of the flagellum (Fig. 3).

1) Testis

The testis occupied most of 2-2.5 apical whorls, and spread over the surface of the digestive gland. The testis was multibranched and bigger than the ovary of the same species (Fig. 10A). The tubules of multibranched testis converged to join seminal vesicle (Fig. 10B). Before merging to seminal vesicle, tubules were subdivided into two portions in *Bithynia striatula, B. tentaculata, B. siamensis* and *Bithynia* sp. (Fig. 10A), but tubules were located in only a single side in *B. misella* and *B. leachi* (Fig. 11A, B).

2) Seminal vesicle

Seminal vesicle left from testis and ran zigzag through the digestive gland to the prostate gland (Fig. 12). Thickness of the seminal vesicle varied in the portion and the species. Thicker portion of the seminal vesicles of *Bithynia striatula, B. tentaculata* and *B. siamensis* was 0.5-0.55 mm in diameter, but the portion of merging out testis was generally slender, around 0.1 mm in thickness. The most swollen portion had less than 0.25 mm in thickness in *B. leachi, Bithynia* sp. and *B. misella.* The length of the seminal vesicle showed difference depending on the shell size. The seminal vesicle had creamy color and was covered with gray-whitish color sporadically.

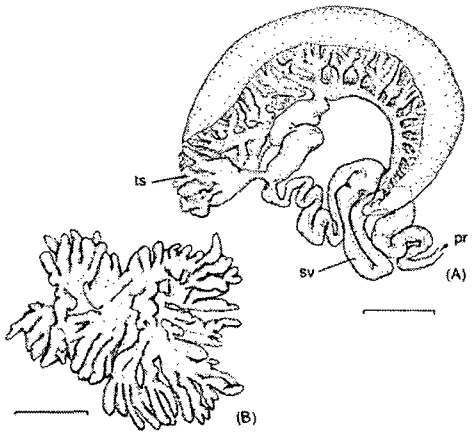

Fig. 11. Male reproductive system. **A**: Testis and seminal vesicle of *Bithynia misella*, Kaejong population. Multibranched and glandular shaped tubules were distributed in a single side of the seminal vesicle; **B**: A part of the testis of *Bithynia* sp., Kirthipur population. The tubules were distributed in the both sides of seminal vesicle. Scale bar = 0.5 mm.
pr: prostate gland, sv: seminal vesicle, ts: testis

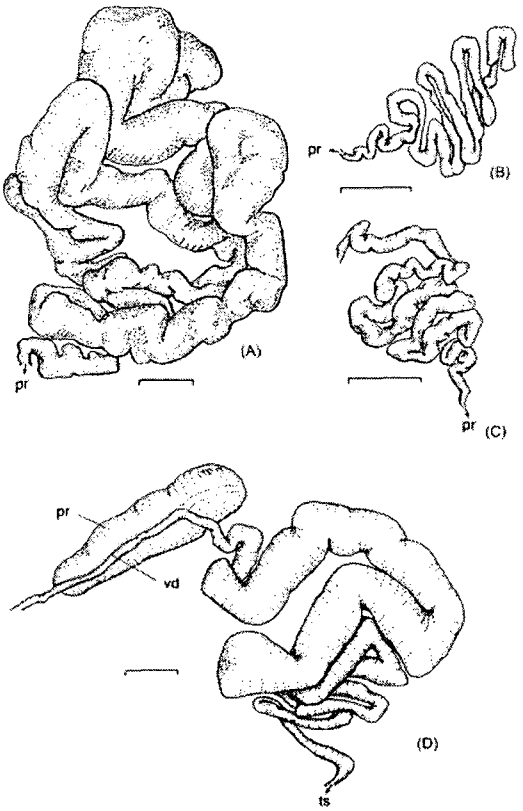

Fig. 12. Seminal vesicles of Bithyniidae. **A**: *Bithynia striatula*, Chongpyong population; **B**: *Bithynia* sp., Kirthipur population; **C**: *Bithynia leachi*, Längsee population; **D**: *Bithynia tentaculata*, Bodensee population. Scale bar = 0.5 mm
pr: prostate gland, vd: vas deferens, ts: testis

3) Prostate gland

The prostate gland was superficial to the columellar axis and located on the right side of the rectum (Fig.

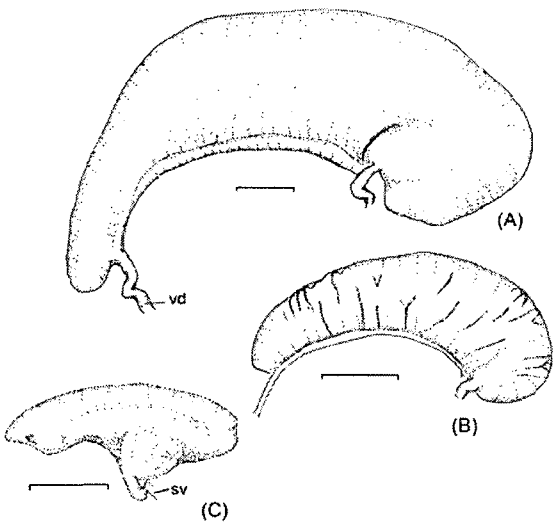

Fig. 13. Prostate glands of Bithyniidae. **A**: *Bithynia striatula,* Chongpyong population; **B**: *Bithynia* sp., Kirthipur population, **C**: *Bithynia leachi,* Längsee population. Scale bar = 0.5 mm.
sv: seminal vesicle, vd: vas deferens.

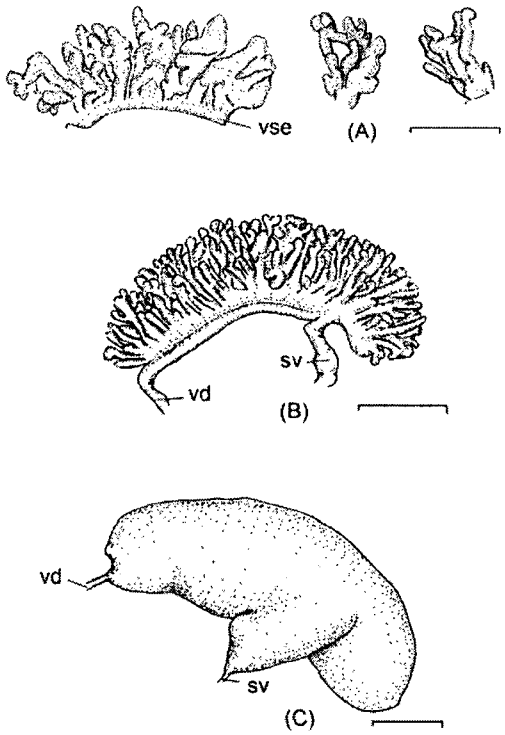

Fig. 14. Prostate glands of Bithyniidae. **A**: Prostate gland of *Bithynia tentaculata,* Warmbad population. Multibranched tubules were connected to the vas efferens (left). A gland with branched tubules (right); **B**: Prostate gland of *Bithynia misella,* Kaejong population. Glandular shaped prostate gland was coated with the presumable calcium carbonate granules as like of Fig. 13; **C**: Prostate gland of *Gabbia australis,* Walcha population. Scale bar = 0.5 mm.
sv: seminal vesicle, vd: vas deferens, vse: vas efferens.

3). The portion which join to seminal vesicle was broader, but the anterior part was narrow (Fig. 13). The emerging portion of seminal vesicle to prostate differed from species to species; in *Bithynia striatula, B. tentaculata, B. siamensis* and Bithynia sp., seminal vesicle emerged to posterior prostate (Fig. 13A, B); in *B. leachi* (Fig. 13C), *B. kiusiuensis, B. misella* and *G. australis* (Fig. 14C), they joined in the mid-posterior portion. The narrow vas deferens left it anteriorly, and ran over the head to the base of the penis and became the penial duct.

The prostate looked like a lump as shown in Fig. 13 in fresh samples. But when the granules covering prostate were peeled off, branching tubules merged to the vas efferens at their base could be seen in alcohol preserved materials or even in fresh samples (Fig. 14A, B).

4) Penis

The penis was located slightly posterior to the right tentacle, and these features help to distinguish the sex of living organism (Fig. 3). The shape and size of the penis and its accessory organs were apparently distinctive. Generally the distal half of the penis was bifid and had a cylindrical flagellum.

Penis was filled with blackish or dark gray granular tissue and had a penial and a flagellar duct which was separated and occasionally visible in alcohol preserved samples (Fig. 15B). Penis was generally curved in ark-shape (Fig. 15A, C), but curved in S-shape in bigger species, *Bithynia siamensis* (Fig. 15C). Length of the penis was 2 mm in most species, except *B. siamensis* (longer than 4 mm) and *B. leachi,* and *B. misella* and *G. australis* had relatively larger penis compared to their body size (Table 2, Fig. 16D, E).

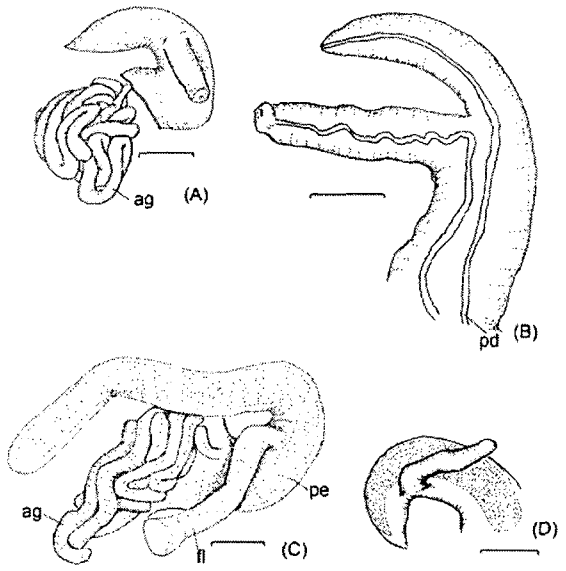

Fig. 15. Male verges. **A**: *Bithynia striatula,* Chongpyong population, with accessory prostate gland; **B**: *Bithynia tentaculata,* Allgäu population. Penial duct and flagellar duct were embedded; **C**: *Bithynia siamensis,* Khon Kaen population, with accessory prostate gland, **D**: *Bithynia* sp., Kirthipur population. Scale bar = 0.5 mm
ag: accessory prostate gland, fl: flagellum, pd: penial duct. pe: penis.

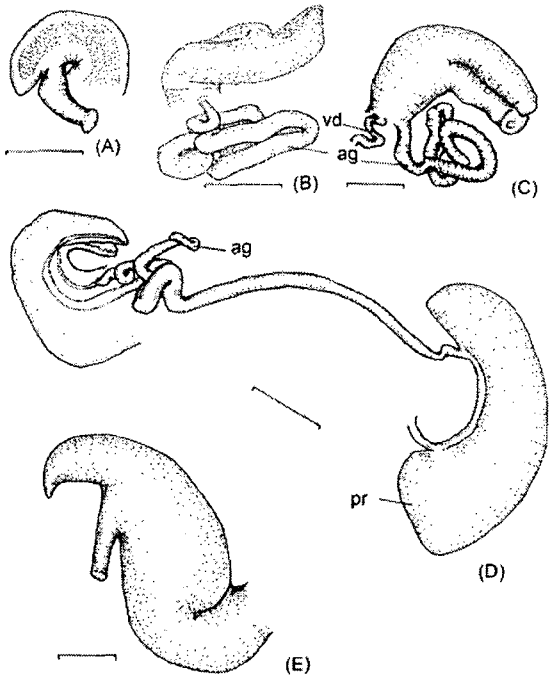

Fig. 16. Male verges. **A**: *Bithynia leachi,* Längsee population; **B**: *Bithynia leachi,* Ukleisee population, with accessory prostate gland; **C**: *Gabbia australis,* Walcha population, with accessory prostate gland; **D**: *Bithynia misella,* Haman population. Vas deferens was coiled with the accessory prostate gland before merged into the penis; **E**: *Bithynia kiusiuensis,* Saga population. Scale bar = 0.5 mm.
ag: accessory prostate gland, pr: prostate gland, vd: vas deferens.

The length from penis tip to bifid point (PTL), full length of penis (P) and length of flagellum (F) were measured as shown in Fig. 17 and their ratios were calculated; F/PTL and P/PTL (Table 2). These ratios were relatively constant among snails in a species. F/PTL ratios of *Bithynia striatula, B. tentaculata, B. misella, B. kiusiuensis* and *Gabbia australis* were *c.a.* 1.0, and the ratios of *B. siamensis* and *B. leachi* was 0.4 and 2.1, respectively. These ratios mean that flagellum of *B. siamensis* forked near base of penis, but that of *B. leachi* separated near distal end of penis.

Average length of flagellum in fixed samples ranged from 514 to 1,302 μm in the species. Tip of the flagellum was blunt and had an opening of flagellar duct (Fig. 15B). Accessory gland had blind end, lumped in disarray, and connected to flagellar duct at proximal end of the penis. This structure could often be seen outside through transparent soft body in alcohol preserved samples.

The accessory gland of *Bithynia siamensis* was as long as 17.66 mm, and the lengths of *B. striatula* and *Bithynia* sp. were 11.0 and 9.8 mm, respectively, and the lengths of the other species observed were around 5 mm (Table 3).

Vas deferens from anterior prostate ran across columellar muscle to central visceral mass and directly connected with penial duct at the base of the penis (Fig. 3). However, the vas deferens of *Bithynia kiusiuensis* and *Gabbia australis* were tangled with accessory prostate gland before connecting to penial duct (Fig. 16D). Vas deferens of these two species coiled and tangled with accessory gland in the body

Table 2. Dimension of male verges of eight bithyniid snails.

Species	No. examined	Penis length (μm)	Flagellum length (μm)	P/PTL	F/PTL
Bithynia striatula	24	2043 ± 95.6*	751 ± 45.1	2.7 ± 0.14	1.0 ± 0.07
Bithynia tentaculata	23	2181 ± 181.4	928 ± 134.4	2.7 ± 0.18	1.0 ± 0.07
Bithynia siamensis	10	4220 ± 179.5	1302 ± 106.3	1.4 ± 0.04	0.4 ± 0.03
Bithynia sp.	14	2254 ± 216.7	813 ± 84.6	4.1 ± 0.27	1.5 ± 0.14
Bithynia leachi	13	979 ± 78.4	541 ± 48.3	4.0 ± 0.46	2.1 ± 0.26
Bithynia kiusiuensis	11	1872 ± 96.5	587 ± 62.3	3.0 ± 0.44	0.9 ± 0.11
Bithynia misella	16	1919 ± 116.6	610 ± 68.7	3.1 ± 0.31	0.9 ± 0.09
Gabbia australis	12	2034 ± 125.5	709 ± 79.9	2.9 ± 0.28	1.0 ± 0.15

* Mean ± S.E.

Table 3. Dimensions of the accessory prostate glands of eight bithyniid snails.

Species	No. examined	Length (μm) Mean ± S.E.	Width (μm) Mean ± S.E.
Bithynia striatula	19	11009 ± 1334 (2325 - 22500)	128 ± 8.0 (70 - 198)
Bithynia tentaculata	13	5437 ± 774 (2825 - 11250)	131 ± 2.7 (120 - 149)
Bithynia siamensis	8	17656 ± 1675 (13000 - 25150)	145 ± 3.8 (125 - 155)
Bithynia sp.	10	8810 ± 924 (3175 - 13550)	84 ± 6.2 (50 - 125)
Bithynia leachi	12	4271 ± 504 (2025 - 8307)	84 ± 6.2 (55 - 125)
Bithynia kiusiuensis	8	4924 ± 578 (1835 - 8875)	97 ± 5.4 (67 - 123)
Bithynia misella	11	5908 ± 660 (1933 - 9000)	102 ± 7.1 (70 - 133)
Gabbia australis	4	4587, 7564, 3982, 5863	63, 77, 147, 83

cavity, but this feature could not be observed in the other species.

The length of the vas deferens ranged from 2 to 4.5 mm, and the diameter ranged 34-89 μm in the species (Table 4). *Bithynia siamensis* had relatively longer but thinner vas deferens, and the vas deferens of *B. kiusiuensis* and *B. misella* were relatively longer and thicker depending on their body size.

DISCUSSION

The shape and the number of radulae of bithyniids observed by optical or scanning electron microscope were reported by various authors (Sugihara and Okamoto, 1973; Nagahana *et al.,* 1973; Chun, 1964; Chung, 1984; Kim *et al.,* 1988). On the contrast, there is little information on internal morphology of bithyniids because their size is less than 15 mm.

Fully relaxed soft body are effective to dissect and observe, but the snails which have thick operculum close their apertures tightly in an unfavorable condition. Anaesthetic, sodium pentobarbital is used to relax the muscle for the non-operculated snails, but it is not effective in operculated snails due to solubility. Insolubility of pentobarbital which has no sodium salt helps relax the operculated snails, *i.e.* the snails do not sense anesthetic while it dissolves steadily. In consequence some snails are fully relaxed (Meier-Brook, 1976a). Because the level of relaxation of the soft body can affect measuring the size of organs (Meier-Brook, 1976b), the measurement was performed on fully relaxed samples in this study. Some ducts were straighten to measure the length by

Table 4. Dimensions of vas deferens of eight bithyniid snails.

Species	No. examined	Length (Mean ± S.E.; μm)	Width (Mean ± S.E.; μm)
Bithynia striatula	15	3150 ± 160	86 ± 8.7
Bithynia tentaculata	16	4699 ± 375	72 ± 6.4
Bithynia siamensis	7	4483 ± 130	42 ± 5.5
Bithynia sp.	8	1925 ± 325	58 ± 3.9
Bithynia leachi	9	N.D.	34 ± 3.8
Bithynia kiusiuensis	6	3765 ± 123	62 ± 3.8
Bithynia misella	15	4216 ± 237	89 ± 6.3
Gabbia australis	3	3120, 2743, 4144*	66, 78, 49

All specimens were relaxed with pentobarbital.
* Values of each measurement.
N.D.: Not done.

putting them into absolute alcohol, but most of the measurements were performed based on the drawings using drawing apparatus.

Some samples used in this study were fixed just before the observation, but some specimens had been preserved up to 30 years, especially *Bithynia leachi.* Sometimes this prolonged fixation was helpful in distinguishing the organs because mucus and some connective tissue were broken down.

Krull (1935) showed precise structure of the stomach of *Bithynia tentaculata.* I also observed the same structure of the organ, but it was very difficult to identify the junction between the digestive gland and the stomach. I found the opening to digestive gland near esophagus merging to anterior stomach in only two specimens.

Itagaki (1965) gave an outline of internal morphology of *Bithynia striatula* with simple drawings. He described that *B. striatula* had nine ganglia, and a visceral ganglion lay near spermatheca shown in his Fig. 24., but, I could not find visceral ganglion. Most of the ganglia excluding visceral ganglion clustered around the upper esophagus. I cannot completely exclude the possibility that I missed an isolated visceral ganglion.

Lilly (1953) gave accurate figures of the reproductive system of *Bithynia tentaculata* after Krull (1935). Even though she only described the reproductive system, her drawings of anatomy on male and female help understand other organ system. General plan shown in Fig. 3 was basically the same as that of her report. However, I could not see the kidney aperture and meandering tubules in the prostate. Instead, I found branching tubules merged to the vas efferens at their base in the prostate gland (Fig. 14B).

It is known that bifid male verge is one of the characteristics of Bithyniidae (Burch *et al.,* 1987). All samples of Australian *Gabbia australis* and Japanese *Bithynia kiusiuensis* observed had peculiar structures, accessory prostate gland tangled with vas deferens before merging to penis. But vas deferens in all the other species examined did not coil with accessory gland. Taxonomically Korean *B. misella* has belonged to the genus *Gabbia, i.e. Gabbia misella* by many authors. An allozyme analysis on *B. striatula, B. misella,* and *B. kiusiuensis* showed that *B. misella* was closer to *B. kiusiuensis* than *B. striatula* (Kim and Kim, 1990). To clarify the taxonomic position of Korean *B. misella,* Burch *et al.* (1987) suggest comparing it with Australian *Gabbia.* Giving weight to this character of accessory gland coiling for taxonomy, Japanese *B. kiusiuensis* rather than Korean *B. misella* is close to Australian *Gabbia.*

Generally the species employed can be divided into three groups by shell size, 1) large-sized group, *B. siamensis;* 2) mediate-sized group, *B. tentaculata, B. striatula,* and *Bithynia* sp.; 3) small-sized group, *B. leachi, B. kiusiuensis, B. misella,* and *Gabbia misella.*

Although this comparative anatomy pursued finding differences between species, no significant morphological divergence was observed even in female

reproductive system. But male reproductive systems slightly varied in shape and size of testis, penis, seminal vesicle and accessory gland (Fig. 11-16). Testicular tubules were subdivided into two parts in large- and mediate-sized snails, *Bithynia striatula, B. tentaculata, B. siamensis* and *Bithynia* sp. (Fig. 10A), but the tubules were located in only a single side in small-sized *B. misella* and *B. leachi* (Fig. 11A, B). The seminal vesicles of *B. striatula, B. tentaculata,* and *B. siamensis* were relatively thicker than other species.

The emerging part of seminal vesicle to prostate differed from species to species; in *Bithynia striatula, B. tentaculata, B. siamensis* and *Bithynia* sp. emerging points of seminal vesicle shift to posterior prostate; but in smaller group (*B. leachi, B. kiusiuensis, B. misella* and *G. australis)* seminal vesicles joined to mid-posterior prostate (Fig. 13-14). Flagellum of *B. siamensis* forked near the base of penis, but that of *B. leachi* separated near distal end of penis.

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