

Two New Species of Gastropoda (Bradybaenidae and Pleuroceridae) and a New Record (Viviparidae) in Korea

Park, Gab-Man¹⁾

Catholic Kwandong University College of Medicine

ABSTRACT

An terrestrial pulmonate snail and a pleurocerids gastropod collected from Gwangneung in Gyeonggi-do, and Mungyeong in Gyeongsangbuk-do, Korea is described as a new species, respectively. A species of land snail, *Karftohelix arboretum* sp. nov., is described and the species resembles *Karftohelix kurodana* (Pilsbry, 1910), *K. koreana* (Pfeiffer, 1846) and *K. adamsi* (Kuroda & Hukuda, 1944) conchologically. but molecular analyses group it either with the polyphyletic assemblage currently considered *Karftohelix* or as a separate lineage in Bradybaenidae. This species also possesses a unique radula, but differ in without dull angulated periphery of body whorl and with the clear color band. *Semisulcospira tegulata consobrinus* is morphologically most similar to *S. tegulata* with the shape of the shell. This species also possesses a unique radula, overall sharp shape and molecular analysis revealed a different group. Preliminary results of mitochondrial 16S rDNA sequencing and phylogenetic analyses indicated that these species belonged to the Korean clade. Also, one new record belonging to the genus *Bellamyia* are described.

Key words: Gastropods, *Karftohelix arboretum*, *Semisulcospira tegulata consobrinus*,

1) PARK, Gab-Man, Department of Environmental Medical Biology, Catholic Kwandong University College of Medicine, E mail: gmpark@cku.ac.kr

Bellamyia purificata. sp. nov., molecular analyses

I. INTRODUCTION

Until recently, 24 species in the 10 genera (*Aegista*, *Lepidopisum*, *Trishoplita*, *Bradybaena*, *Chosenelix*, *Koreanohadra*, *Karaftohelix*, *Acusta*, *Euhadra* and *Nesiohelix*) from Bradybaenidae were known in Korea. Also, 8 species in the 3 genera (*Semisulcospira*, *Koreanomelania* and *Koreoleptixis*) from Pleuroceridae, and three species in the two genera (*Cipangopaludina* and *Sinotaia*) from Viviparidae (Kwan et al., 1993; Kwon et al., 2000). For many years Korean molluscs have been studied and described (Choi & Park, 2020), but priority was always given to those on the shell shape. They are now better known but many of them are still awaiting study. The mitochondrial genome (mitogenome) can provide information for phylogenetic analyses and evolutionary biology (Sengupta et al., 2009; Deng et al., 2014; Kim and Lee, 2018). This paper described a new species and a new record collected from Gwangneung, Gyeonggi-do, Mungyeong and Yecheon, Gyeongsangbuk-do, Korea. All type material has been lodged with the Laboratory of Environmental Medical Biology (LEMB), Catholic Kwandong University, Gangneung, Gangwon-do.

II. MATERIALS AND METHODS

Specimen are collected with hands and transferred to the laboratory for their morphological determination and dissection. The soft body were removed for anatomy. The genital structures were dissected with tweezers, fixed in 70% alcohol for 10-15 minutes, and examined under a Nikon XMZ 800N stereo microscope (Nikon, Japan). The experimental methods of radula and molecular analysis followed Park's (2021) and Deng et al., (2014) research method. The terminology used in the description of radula follows Kool (1987). Voucher specimens are deposited in the Department of Environmental

Medical Biology, Catholic Kwandong University, Gangwon-do, Republic of Korea.

Systematic Accounts

Phylum Mollusca Linnaeus, 1758

Class Gastropoda Cuvier, 1797

Order Stylommatophora Schmidt, 1855

Family Bradybaenidae Pilsbry, 1934

Genus *Karaftohelix* Pilsbry, 1927

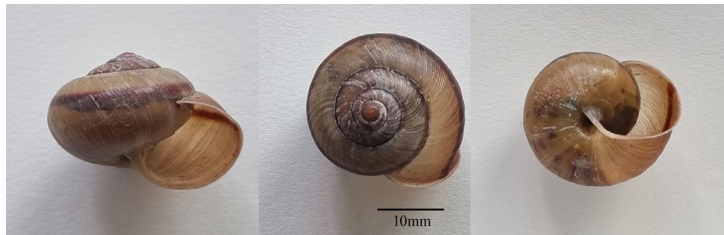
1. *Karaftohelix arboretum* n. sp. 광릉달팽이 (신칭) (Figs. 1,2)

Material examined: South Korea: Gyeonggi-do, Pocheon-si, Soheul-eup, Gwangneung

Diagnosis: Shell coiling is right-handed or “dextral”. Body whorl height and width are

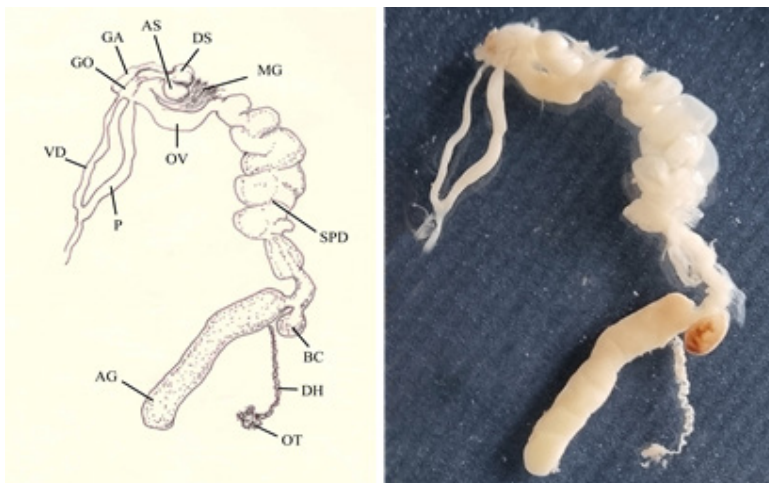


〈Fig. 1〉 Living specimen of *Karaftohelix arboretum* sp. nov. collected from Gwangneung forest.



〈Fig. 2〉 *Karaftohelix arboretum*.

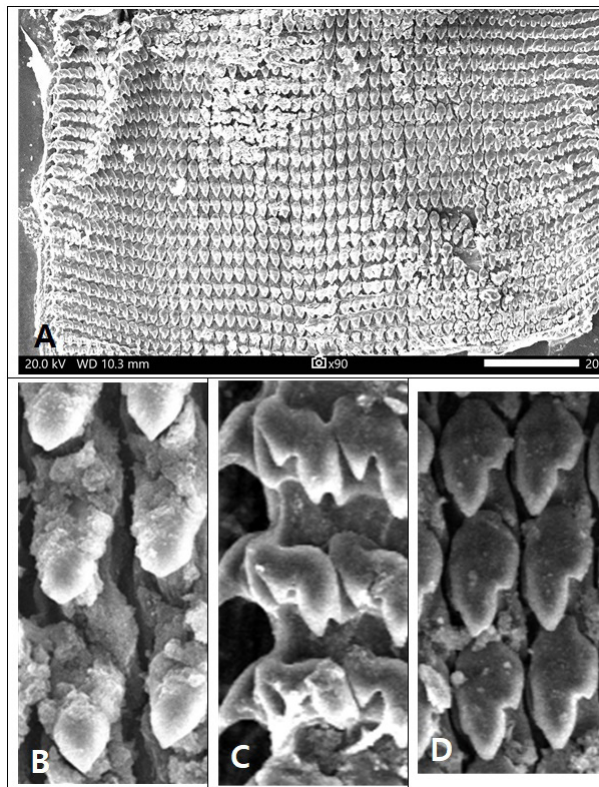
20 mm and 23 mm. Shell is globose, spire conic and rather depressed; fragile; as a whole yellowish brown in color, from the penultimate whorl to the apex light brown color, fading to a tincture of reddish brown just below the suture and at the umbilicus (Fig. 1,2). No operculum. Shell surface smooth, with fine spiral growth line, under side with fine growth line, flattened. Colored band is 0200 type. Depending on the specimens, the width of the band is slightly different, and the reddish brown bands in the body layer starring are greater. Whorls 5 1/2, each whorl moderately convex, shell with regularly increasing whorls. Suture is not deep. Umbilicus is rimate shell (with umbilical opening partially closed by apertural lip). Shell lip reflected. Protoconch is 1/2 whorl, glossy and smooth.



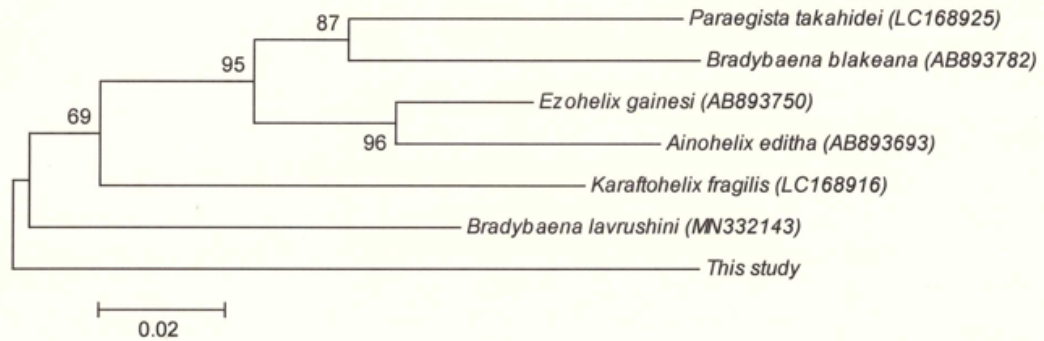
〈Fig. 3〉 Genital morphology of *Karaftohelix arboretum*. AG: albumin gland; AS: accessory-sac; BC: bursa copulatrix; DH: duct hermaphroditicus; DS: dart sac; GA: genital atrium; GO: genital opening; MG: mucous gland; OV: oviduct; OT: ovotestis; P: penis; SPD: spermoviduct; VD: vas deferens.

The aperture is oblique, broadly lunate. Genital system (Fig. 3): Dart sac large, mucous glands long and tubular form. Vagina is thick and short, at terminal region divided into oviduct and stalk of receptaculum. Oviduct connected to large hermaphrodite duct, hermaphroditicus are long and slender and connected ovotestis. Penis sheath are thickened. Vas deferens divided from flagellum is contacted to vagina and then join to prostate gland. Radula (Fig. 4): 8 mm long and 2.9 mm wide. Formula 24:20:12. The central teeth are sharp and there are 20 identical shapes. Marginal and lateral teeth are pointed cusp. Preliminary results of mitochondrial 16S rDNA sequencing and phylogenetic analyses indicated that these species belonged to the Korean clade (Fig. 5).

Remarks: This new species is endemic species in Korea and type locality is



〈Fig. 4〉 Scanning electron microscope photographs of radula teeth of *Karafohelix arboretum* sp. nov. (A) Image of total part of radula teeth. (B) Central teeth. (C) Lateral teeth. (D) Marginal teeth. Scale bar = 200 μ m.



〈Fig. 5〉 Assignment of the partial 16S gene sequences (274bp) of Korean specimens of *Karaftohelix arboretum* with other Bradybaenidae species from Genebank using a neighbor-joining tree based on Kimura-2-parameter distances.

Gwangneung forest in Gyeonggi-do. This species is very rare and lives especially on trees with moss. *Karaftohelix kurodana* (Pilsbry, 1910), *K. koreana* (Pfeiffer, 1846) and *K. adamsi* (Kuroda & Hukuda, 1944) in Korea appears to be a related species (Kuroda, 1926; Pilsbry, 1926), but differ in without dull angulated periphery of body whorl, more conical form, and with the clear color band. The height of the spire is very low compared to the other species. Also, This species, unlike other species, lives on trees with moss in the forest. To elucidate the phylogeny of this species, sequenced an approximately 274bp fragment of the mitochondrial 16S gene from five individuals, and performed maximum parsimony analysis. This showed that *Karaftohelix fragilis*, *Bradybaena lavrushini* and this species are monophyletic. On the other side, *Ezohelix gainesi* an *Ainohelix editha* species belongs to the monophyletic group. In the future, it is necessary to collect more Korean species and find new species by comparing them.

Habitat: Mt. Forest. It lives on a tree with moss on it.

Order Unassigned

Family Pleuroceridae Fischer, 1885

Genus *Semisulcospira* Boettger, 1886

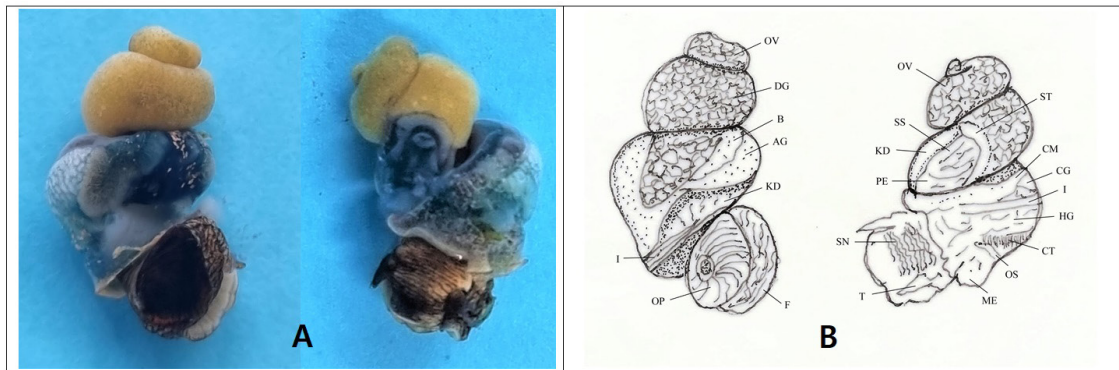
2. *Semisulcospira tegulata consobrinus* n. sp. 모산다슬기 (신칭)(Fig. 6)

Material examined: South Korea: Gyeongsangbuk-do, Mungyeong-si, Gaeun-eup, Seongjeo.

Diagnosis: Body size 14.3mm in shell height, 5.6 mm in shell width. Whorl is 7-8 (Fig. 6). The shell is very hard. The overall shape is thin, long, and pointed. Adult apex is eroded. Body colour is yellow-green or yellowish-brown. The longitudinal ribs are weakly developed with 14 rows. The overall shape is sharp as it narrows sharply from the body layer. The sutures are very deep. The longitudinal ribs of the penultimate layer are weak or not developed. The longitudinal ribs on the body layer and the penultimate layer are developed, but this species has weak or no longitudinal ribs on the body layer. Operculum is small, capped, oval in shape. Hermaphrodite. Oviparous. Operculum size is 4.5×2.1 mm, ovate, corneous, dark reddish brown in color, with 3.5 whorls. In anatomical observation, the internal organs are limited to the penultimate layer out of a total of eight layer (Fig. 7). Head-foot dark gray to black in color; in females, ovipositor groove pale in color. Foot broad with wide propodium and long anterior pedal gland along anterior margin. Ciliated egg groove forming longitudinally grooved tract



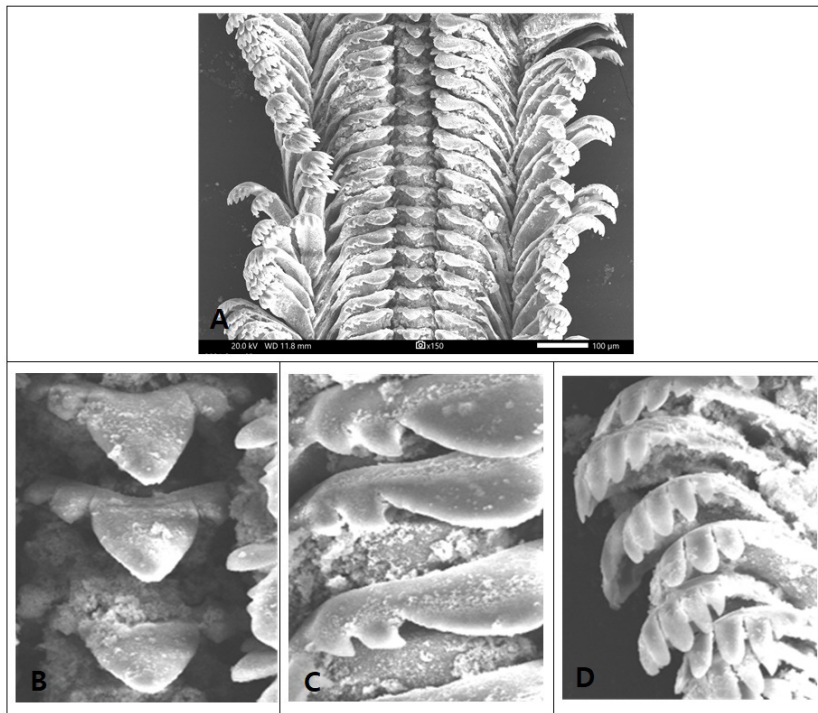
〈Fig. 6〉 Photograph of *Semisulcospira tegulata consobrinus*.



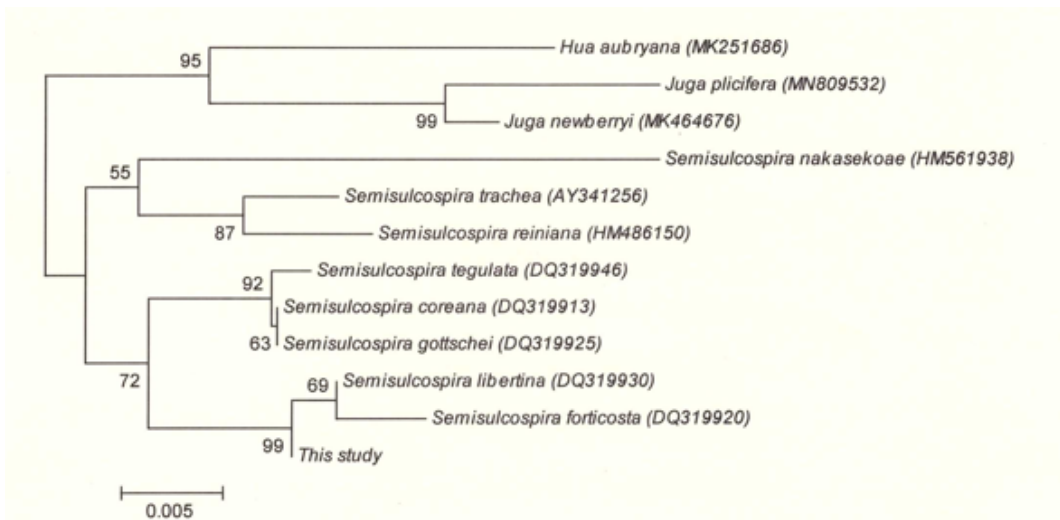
〈Fig. 7〉 External view of organs in visceral mass of *Semisulcospira tegulata consobrinus*. A) Anatomy B) AG, albumin gland; B, bladder; CG, capsule gland; CM, columellar muscle; CT, ctenidium; DG, digestive gland; F, foot; HG, hypobranchial gland; KD, main kidney chamber; I, intestine; ME, mantle edge; OP, operculum; OS, osphradium; OV, ovary; PE, paricardium; SS, style sac; ST, stomach; T, cephalic tentacle.

extending from anterior tip of pallial gonoduct and broadening continuously into shallow triangular shelf on side of neck below right cephalic tentacle. Ctenidium extending from posterior end of mantle cavity to near mantle edge, anteriorly curving toward the left. Hypobranchial gland moderately well developed with transverse ridges, especially at posterior end of mantle cavity. Radula formula is 1:1:1. Ribbon 15.1 mm long, width 1.2 mm (Fig. 8). Central tooth with rounded base, slightly broader than long, with concave upper rim and convex basal rim; cutting edge consisting of well developed central cusp; glabella with concave lateral margins, broad, extending lower margin of tooth. Lateral tooth with short lateral extension at outside; cutting edge with large main cusp. Marginal teeth saw blade shape and with broadly round cutting edges and long. Preliminary results of mitochondrial 16S rDNA sequencing and phylogenetic analyses indicated that these species belonged to this species (Fig. 9).

Remarks: The freshwater snail genus *Semisulcospira* Boettger, 1886 contains 26 extant and 10 fossil species indigenous to inland waters of Japan, Korea, Taiwan, and China (Du et al. 2019; Matsuoka and Miura 2019). For a long time, because of the great diversity of morphological variations within species, there has been a question about how many



〈Fig. 8〉 Scanning electron microscope of radula of *Semisulcospira tegulata consobrinus* (A) (JSM-IT500 (Jeol, Japan). Central teeth (B), Lateral teeth (C), and Marginal teeth (D).



〈Fig. 9〉 Assignment of the partial 16S gene sequences (518bp) of Korean specimens of *Semisulcospira tegulata consobrinus* with other *Semisulcospira* species from Genebank using a neighbor-joining tree based on Kimura-2-parameter distances, 1,000 bootstrap replicates and both *Hua aubryana*, *Juga plicifera* and *Juga newberryi* as outgroups.

species of *Semisulcospira* exist in Korea. Among the two genera and 7 extant species described in Korea (Kwon et al., 2000). Shell width of *Semisulcospira tegurata* is wide then this species. This new species is characterized by its narrow width compared to its length among the *Semisulcospira* spp. Prozorova & Rasshepkina (2005) have been anatomically studied that specimens of three *Semisulcospira* species (*S. gredleri* (Boettger, 1886) from Tai Hu Lake (Jiangsu Province, China), *S. libertina* (Gould, 1859) from Chiba Lake (Chiba Prefecture, Japan), and *S. forticosta* (Martens, 1886) from a small river in the Korean Peninsula (Chungchongnam Province, South Korea). These species have pallial oviducts of similar structure. According to their study, the lateral lamina of all species consists of the brood pouch with embryos and a small lateral gland disposed in its proximal part. Some differences in the position of the seminal receptacle of studied *Semisulcospira* species were revealed. Seminal receptacles of both species *S. libertina* and *S. gredleri* are located under pallial pocket from its left side, closer to inner part of medial lamina. The seminal receptacle of *S. forticosta* is located on the right side of pallial pocket. So, sperm gutter of *S. forticosta* take place on the right side of pallial pocket. Sperm gutters of two other species are displaced from right side in the distal part of pallial pocket to its left side in proximal part. *S. gredleri* differs from *S. libertina* in more high location of seminal receptacle on the pallial pocket. In this study, some interspecific differences in the shape and position of the seminal receptacles were revealed. In this study, *Semisulcospira tegulata consobrinus* was very similar morphology with *S. tegulata* and *S. forticosta*. In rarhachidan teeth, this species have blunt teeth, while *S. tegulata* have sharp teeth.

Habitat: It lives in clear water and inhabits spring water from caves with little change in water temperature throughout the year.

Order Architaenioglossa Haller, 1890

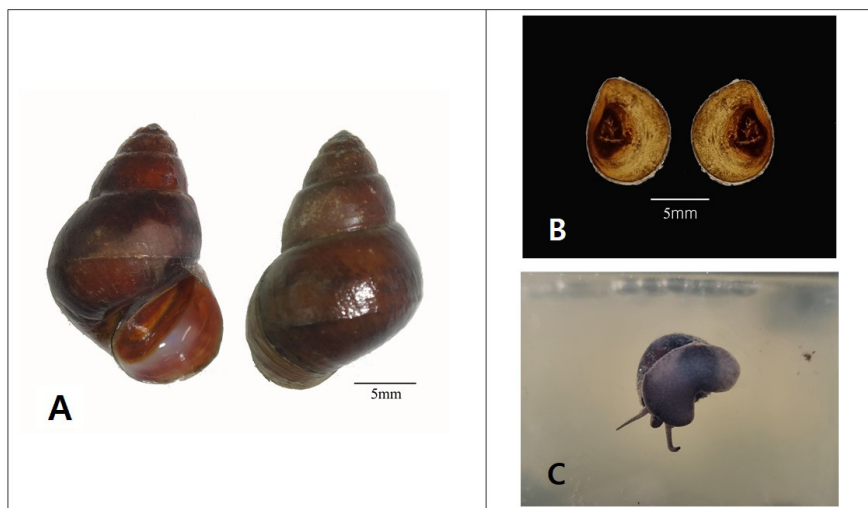
Family Viviparidae Gray, 1847

Genus *Bellamyia* Jousseaume, 1886

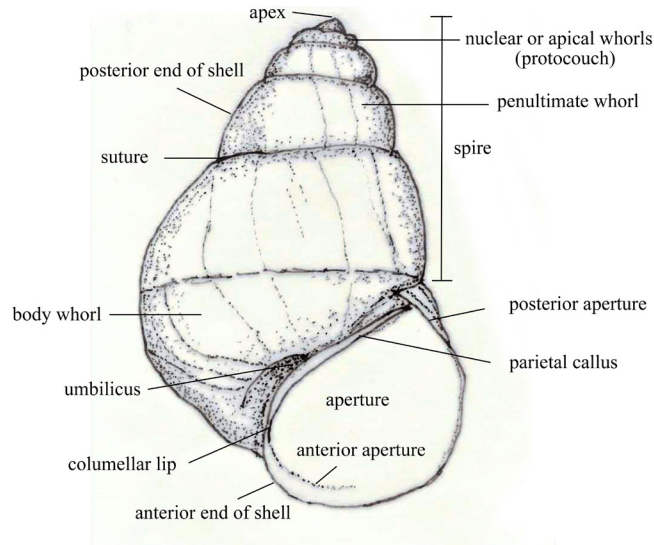
3. *Bellamya purificata* (Heude, 1890) 정화우렁이 (신칭) (Fig. 10)

Material examined: Korea: Gyeongsangbuk-do, Yecheon-gun, Pungyang-myeon, Deogam-ro. Gaeun-eup, Seongjeo.

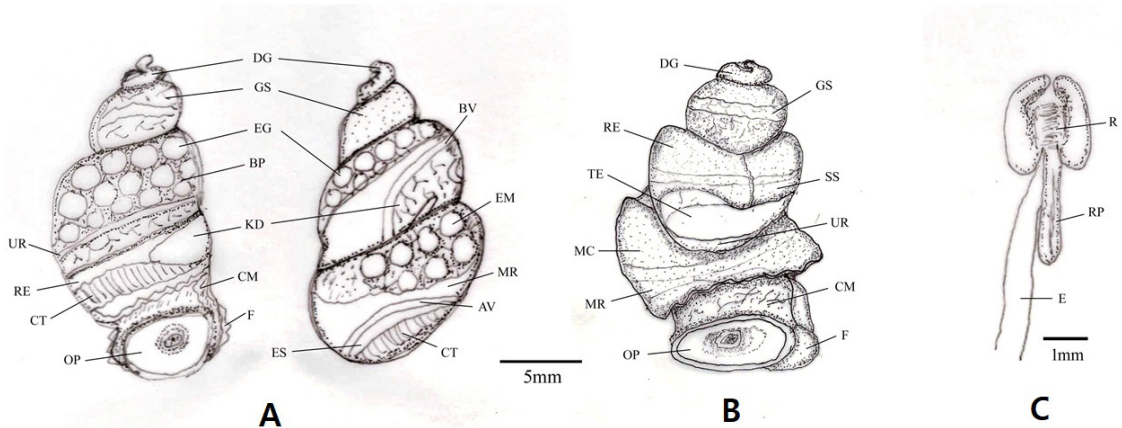
Diagnosis: Thick, elongated and conical shell, In females, height \times width 24.3×16.2 , and in males, 21.4×13 , the size of females is slightly larger. The spire is high and conical (Fig. 10). The sutures are distinct and the bare layers are 4 layers, and each part gradually erodes after growth. The shell is rough and black-brown and outer margin of aperture blackish. Aperture pear-circular (Fig. 11), blackish axial growth lines usually present on first whorls, low spiral carina. Five whorls slightly convex, separated by deep sutures, with a thin brownish band below suture on spire. Body layer accounts for 60% of height. Circular, horny operculum, smaller than the aperture, with concentric growth lines, occupying dorsal foot surface. The body layer has projections along the periphery with hairs, and is slightly angled. This species is fertilized in vivo, ovoviviparous, and females have an average of 24 larvae. The uterus varies from immature eggs to full-grown larvae. They have a sense of touch and have eyes outside the lower part of the sense of touch. In males, the right tentacle is curled up and serves as a mating stage (Fig. 12). Radula: size 4.5 mm in length and 0.5 mm in width. Rachidian tooth with rectangular



〈Fig. 10〉 Photograph of *Bellamya purificata* (A). Operculum (B), Curved right tentacle (C).



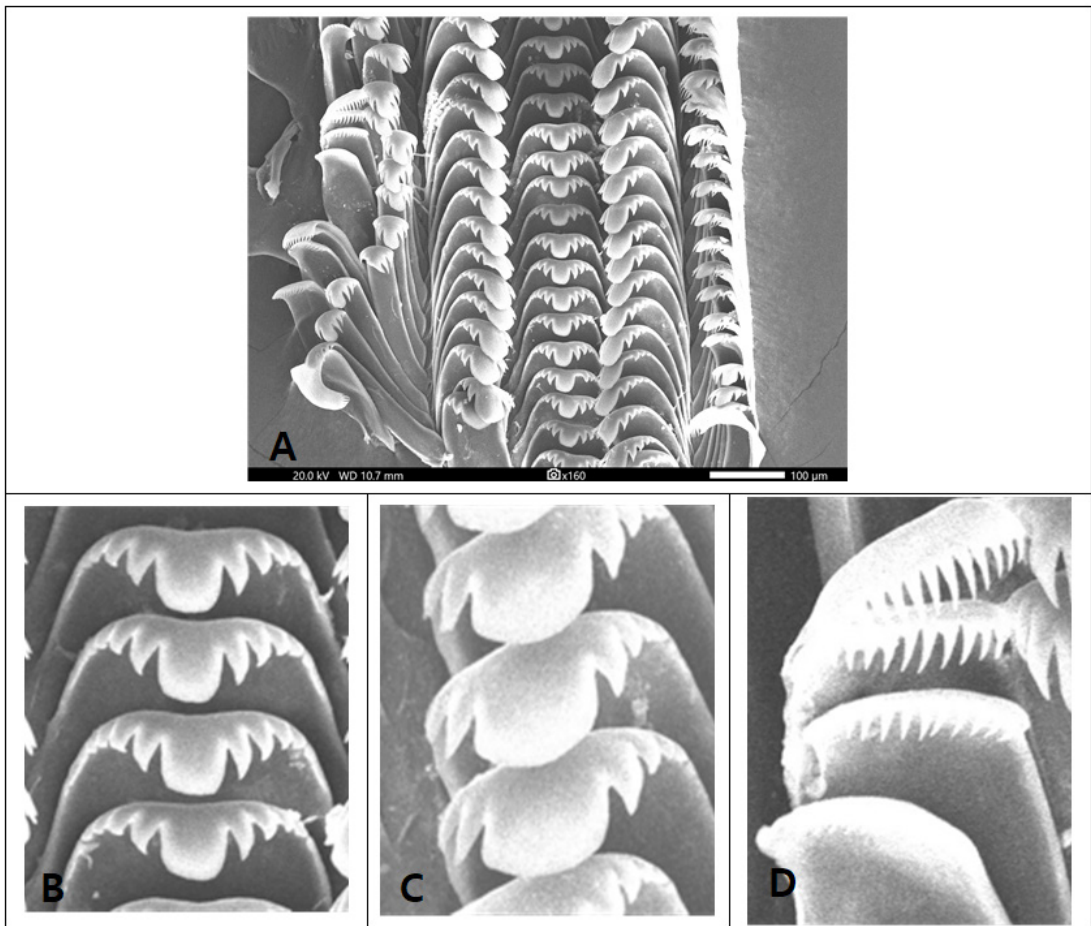
〈Fig. 11〉 Shell terminology.



〈Fig. 12〉 Body morphology and anatomy of *Bellamyia purificata*. A) Female in apertural and adapertural view; B) Male in apertural view; C) Buccal cavity. Abbreviations: AV, afferent branchial vessel; BP, brood pouch; BV, blood vessel; CM, columellar muscle; CT, ctenidium; DG, digestive gland; E, esophagus; EG, egg; EM, embryo; ES, endostyle; F, foot; GS, gastric chamber; KD, kidney; MC, mental collar; MR, mental roof; OP, operculum; R, radula; RE, rectum; RP, radula pouch; SS, style sac; TE, testis; UR, ureter.

central cusp followed by 4–6 triangular cusps on each side. Two lateral teeth per row, curved and multicuspid, similar to the central teeth. Body with width cylindrical snout

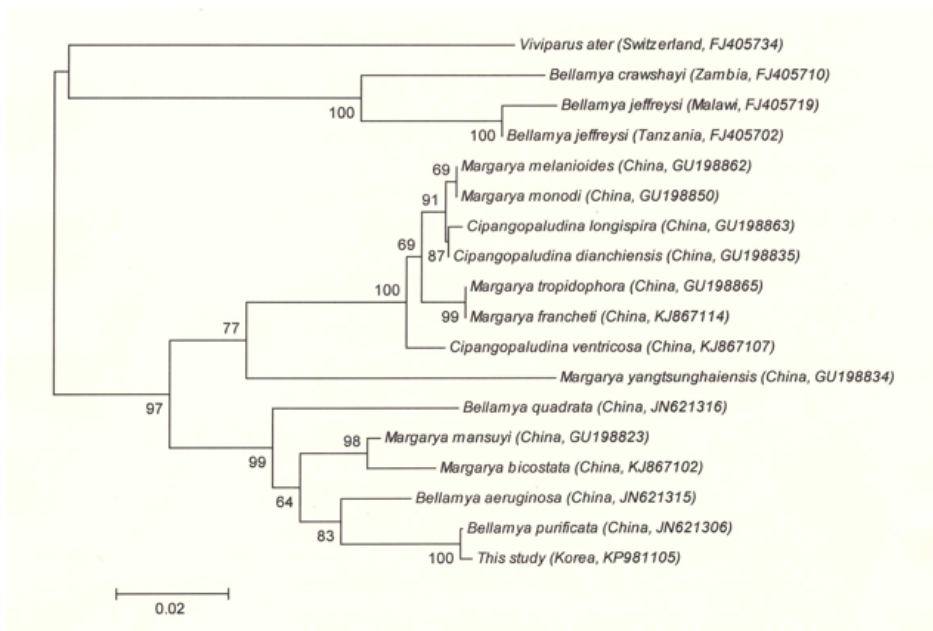
with an aperture in the anterior margin. Males with modified right tentacle that serves as a copulatory organ. Males and female specimens differing in the right margin of the mantle cavity, which is filled by the oviduct in females. This oviduct works as a brood pouch filled by capsules, covered by a thin membrane, with different stages of developing oocytes, embryos and juvenile snails. Radula (Fig. 13): Radula formula is 1:2:1. Ribbon 12 mm long and 1.5 mm wide. Central tooth with rounded base, slightly broader than long, with concave upper rim and convex basal rim; cutting edge consisting of well developed central cusp and three accessory cusps on each side that taper in size; glabella with concave lateral margins, broad, not extending lower margin of tooth. Lateral tooth with



〈Fig. 13〉 Scanning electron microscope photographs of radula teeth of *Bellamya purificata* (A). Central teeth (B), Lateral teeth (C), and Marginal teeth (D).

short lateral extension at outer side; cutting edge with large main cusp, one accessory cusp on inner and two to three on outer side. Preliminary results of mitochondrial 16S rDNA sequencing and phylogenetic analyses indicated that these species belonged to the this species (Fig. 14) (Table 1).

Remarks: As one of the widely distributed freshwater gastropods in Asia, India and Africa, *Bellamyia* has been identified as relatively recent radiation (Sengupta et al., 2009; Schultheiss et al., 2011). This species is an ovoviviparus species (commonly referred only as “viviparus”, in which the eggs develop within the female body and are birthed live. Fertilized and unfertilized eggs and embryos in different stages of development were subspherical to slightly trapezoidal in shape. This species is almost similar to *Sinotaia quadrata* in morphological and anatomical features (Lee, 2009). This species has 5 spire, whereas *S. quadrata* has 6. The number, shape, and specialized arrangement of molluscan teeth in each transverse row is consistent on a radula, and the different



(Fig. 14) Phylogenetic relationships among the taxa analyzed, based on 16S rRNA (431bp) gene sequences with other Viviparidae species from Genbank using a neighbor-joining tree based on Kimura-2-parameter distances.

〈Table 1〉 Species used in this study with collection localities and sequence sources

Family Viviparidae	Collection locality	16S
Subfamily Viviparinae		
<i>Viviparus ater</i>	Switzerland	FJ405734
<i>Margarya bicostata</i>	China	KJ867102
<i>M. francheti</i>	China	KJ867114
<i>M. mansuyi</i>	China	GU198823
<i>M. melanioides</i>	China	GU198862
<i>M. monodi</i>	China	GU198850
<i>M. tropidophora</i>	China	GU198865
<i>M. yangtsunghaiensis</i>	China	GU198834
Subfamily Bellamyinae		
<i>Bellamya jeffreysi</i>	Malawi	FJ405719
<i>B. jeffreysi</i>	Tanzania	FJ405702
<i>B. crawshayi</i>	Zambia	FJ405710
<i>B. aeruginosa</i>	China	JN621315
<i>B. purificata</i>	China	JN621306
<i>B. quadrata</i>	China	JN621316
<i>Cipangopaludina chinensis</i>	USA	-
<i>C. dianchiensis</i>	China	GU198835
<i>C. japonica</i> *	USA	-
<i>C. longispira</i>	China	GU198863
<i>C. ventricosa</i>	China	KJ867107

**Cipangopaludina japonica* from USA has invaded from the Orient.

patterns can be used as a diagnostic characteristic to identify the species in many cases. To elucidate the phylogeny of these species, we sequenced an approximately 431bp fragment of the mitochondrial 16S gene from five individuals per species, and performed maximum parsimony analysis. Phylogenetic tree compared with this species were exactly matched with Chinese *Bellamya purificata* (JN621306). In the future, it is necessary to collect more Viviparidae other species in Korea and find new species by comparing them.

Habitat: Fresh water

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- of the freshwater snail genus *Semisulcospira* (Cerithioidea: Pleuroceridae). *Molecular Phylogenetics and Evolution*, 43: 386-397.
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October. 20, 2022

Dear Editor-in-Chief :

I have recently obtained some result of the “Two New Species of Gastropoda (Bradybaenidae and Pleuroceridae) and a New Record (Viviparidae) in Korea”. I am submitted to the online herewith of the manuscript and figures. The manuscript consists of 14 pages of text with references and figure. This paper described a new species and a new record collected from Korean peninsula based on the external morphological features, radura and genital structures and 16S rDNA.

The paper has been written according to the author’s guidelines provided by the Korean Journal of Nature Conservation. I would appreciate very much if you could review it for publication in the Korean Journal of Nature Conservation. The manuscript has never been published before elsewhere.

Sincerely yours,

Prof. Gab Man Park
Catholic Kwandong University, College of Medicine.