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First Record of the Bathyal-microgastropod *Punctulum flavum* (Okutani, 1964) in the East Sea Korea

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ABSTRACT

During a deep-sea marine biodiversity survey, *Punctulum flavum* (Okutani, 1964) was discovered at depths ranging from 900 to 1,000 m in the East Sea, Korea. The genus *Punctulum* Jeffreys, 1884 is a minute rissoid group comprising six valid species, most of which inhabit the bathyal zone of the northwestern Pacific. Among them, *Punctulum flavum* has been reported from Japan and Russia and is known to exhibit considerable morphological variations in shell shape, sculpture, and protoconch characteristics. A detailed examination of shell morphology, including high-resolution SEM observations of the protoconch and teleoconch, indicated that the Korean specimens correspond to a "deep-water form" (*sensu* Hasegawa, 2014). The shells exhibit an inflated ovate-conical form and a thin periostracum; the protoconch bears closely spaced flat spiral cords, and the teleoconch is sculptured with weak but distinct axial riblets and fine spiral cords. The discovery of *P. flavum* in Korea extends the southwestern limit of its known distribution, providing valuable insights into the bathyal rissoid fauna of the northwestern Pacific and the genus *Punctulum*.

Keywords: Mollusca, Rissoidae, *Punctulum flavum*, SEM, deep-sea, Korea

INTRODUCTION

Taxonomic research on the deep-sea molluscan fauna of Korean waters has been limited because most deep-sea specimens were collected opportunistically from fishing gear such as gillnets and traps. However, systematic deep-sea sampling has been performed recently during collaborative research surveys conducted from 2023 to 2025 by the National Institute of Fisheries Science (NIFS) and the National Marine Biodiversity Institute of Korea (MABIK). Through this effort previously unrecorded species from the bathyal zone (at depths of 300 to 1,000 m) have been newly identified (Lee *et al.*, 2023 [Bivalvia]; Lee *et al.*, 2024 [Cephalopoda]). These findings underscore the critical need for continued taxonomic research on deep-sea biodiversity of Korea, which remains insufficiently understood.

The family Rissoidae Gray, 1847, is one of the most diverse groups of marine microgastropods and comprises 38 genera and approximately 900 valid species worldwide (MolluscaBase eds., 2025). Members of this family inhabit a wide range of environments, from shallow intertidal zones to deep-sea sediments in the bathyal and abyssal zones (Hasegawa, 2014). They are characterized by a minute size and conical shells, but shell shapes exhibit considerable intraspecific variability, and often detailed microstructural examination is required for accurate identification (Ponder, 1985). In Korean waters, eight species belonging to four genera (*Alvania* Risso, 1826, *Cingula* J. Fleming, 1818, *Onoba* H. Adams & A. Adams, 1852, and *Pusillina* Monterosato, 1884)

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have been recorded so far (National Marine Biodiversity Institute of Korea, 2025); all occur from the intertidal to shallow subtidal zones at depths of less than 100 m.

The genus *Punctulum* Jeffreys, 1884 is a minute rissoid genus that currently includes only six species (MolluscaBase eds., 2025): *P. flavum* (Okutani, 1964), *P. reticulatum* A. N. Golikov, 1986, *P. sanrikuense* Hasegawa, 2014, *P. soyomaruae* Hasegawa, 2014, *P. tanshumaruae* Hasegawa, 2014, and *P. wyvillethomsoni* (Friele, 1877). Except for the type species *P. wyvillethomsoni*, which areas at a depth of approximately 1,000 m in the North Atlantic Ocean, the others inhabit the bathyal zone of the northwestern Pacific, particularly around Japan and Russia (Hasegawa, 2014).

In this study, we report for the first time the occurrence of the genus *Punctulum* and *P. flavum* (Okutani, 1964) in Korean waters, based on specimens collected from bathyal depths in the East Sea. The morphological characteristics of the specimens are described in detail and supported by high-resolution images. In addition, their taxonomic implications and distributions are discussed.

MATERIALS AND METHODS

1. Sample collection and processing

Specimens were collected from survey areas 76 and 77 at depths of 900 and 1,000 m in the East Sea during a deep-sea research survey conducted by the National Institute of Fisheries Science (NIFS) and the National Marine Biodiversity Institute of Korea (MABIK) from 2023 to 2024 (Fig. 1). Sampling was conducted in the offshore waters of Gyeongsangbuk-do by the research vessel *Tamgu 22-ho* (1,458 G/T). Bottom-trawl hauls lasted around 30 minutes at an average ground speed of 3.2 knots. The start of each haul was defined as the moment the trawl net reached the seabed, and the end as the time the winches began hauling back the net (bottom time). Door spread, wing spread, and net opening height were monitored and recorded during each haul using onboard net sensors.

After retrieval, sediments were sieved onboard to separate specimens from mud and immediately fixed in 99% ethanol. In the laboratory, specimens were examined and identified under a stereomicroscope and placed in 100% ethanol for long-term storage and potential molecular analyses. The voucher specimens were deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seocheon, Republic of Korea.

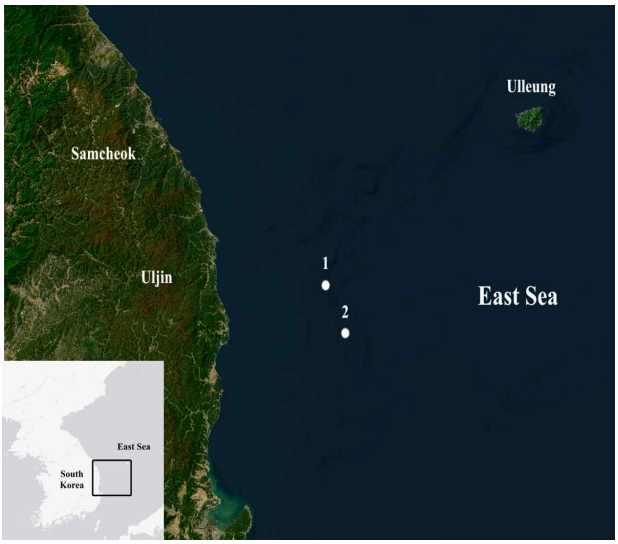

Fig. 1. Map showing the sampling sites in this study. 1, Station 1 at 900 m depth in survey area 76; 2, Station 2 at 1,000 m depth in survey area 77 of the East Sea, Korea.

2. Species identification and morphological examination

Morphological features were initially examined under a stereomicroscope (Leica MZ205C, Germany). Photographs of a selected representative specimen and its shell were taken using a DSLR camera (Nikon D800, Japan). Shell length (SL) and width (SW) were measured on selected specimens to the nearest 0.01 mm using digital calipers. Shells with the least-damaged protoconch were selected for scanning electron microscope (SEM) studies. Samples were washed with distilled water, without any chemical treatment to preserve the periostracum, and mounted on aluminum stubs. After drying, shells were coated with gold using an ion sputter coater (MC1000; Hitachi, Tokyo) and examined using a high-resolution SEM (Zeiss Sigma 500 VP FE-SEM, Carl Zeiss,

Germany).

3. DNA extraction, PCR-amplification, and Sequence analysis

Total genomic DNA was extracted from the whole body of each specimen, excluding visceral tissues to prevent contamination, using the QIAamp DNA Micro Kit (Qiagen, California, USA), following the manufacturer's protocols. Partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (COI; 658 bp) and 16S rDNA (16S; 548 bp) genes were amplified by polymerase chain reaction (PCR) using AccuPower HotStart PCR PreMix (Bioneer, Daajeon, Korea) with universal primer sets (COI: LepF1/LepR1,16S: 16sar-L/16sbr-H) (Hebert *et al.*, 2003; Palumbi *et al.*, 1991). Each PCR reaction was conducted in a total volume of 20 μL, containing 2 μL of each primer, 3 μL of genomic DNA template, and distilled water added to reach the final volume. The PCR conditions were as follows: an initial denaturation at 95°C for 1 min; 40 cycles of denaturation at 94°C for 30 s; annealing at 50°C for 30 s, and extension at 72°C for 30 s; followed by a final extension at 72°C for 7 min. PCR products were sequenced bidirectionally using an ABI 3730xl DNA analyzer (PE Applied Biosystems, Foster City, CA, USA). The sequences obtained in this study were deposited in GenBank (accession numbers PX736146 and PX736147).

SYSTEMATIC ACCOUNTS

Phylum Mollusca Linnaeus, 1758
Class Gastropoda Cuvier, 1795
Order Littorinimorpha A. N. Golikov & Starobogatov, 1975
Family Rissoidae J. E. Gray, 1847
Genus *Punctulum* Jeffreys, 1884 배꼽꼬마고둥속(신칭)

Type species: *Rissoa wyvillethomsoni* Friele, 1877, by subsequent designation (Ponder, 1985)

Diagnosis. Shell minute (typically < 5 mm), ovate to depressed conical, with approximately five whorls; body whorl large, inflated. Protoconch conical, comprising approximately 2.5 whorls, smooth or bearing very fine spiral lines and faint axial striae. Teleoconch weakly clathrate to finely spirally sculptured, with axial ribs faint or becoming coarse on lower part of body whorl. Umbilicus small but distinct. Aperture ovate to rounded; outer lip thin; varix absent. Periostracum thin, translucent, ranging in color from whitish to yellowish. Operculum corneous, thin, ovate, paucispiral; nucleus subcentral. Radula taenioglossate; central teeth broad, low, with 2-4 cusps; lateral teeth with well-developed cusps; marginal teeth slender, simple, inner cusps absent.

Distribution. Bathyal and cold-water regions of the North Atlantic and North Pacific.

Remarks. The genus is distinguished from *Alvania* Risso, 1826 and *Frigidoalvania* Warén, 1974 by its smooth conical protoconch, thin unthickened outer lip, distinct umbilicus, and lack of pigmented eyes (Hasegawa, 2014). Significant morphological variation is observed between representatives from the northeastern Atlantic and the northwestern Pacific (NWP). The Atlantic species (*P. wyvillethomsoni*) possesses a smooth protoconch, a weakly clathrate teleoconch, and a relatively narrow or closed umbilicus, as described by Ponder (1985). In contrast, NWP species (*P. flavum*, *P. reticulatum*, *P. sanrikuense*, *P. soyomaruae*, and *P. tanshumaruae*) described by Hasegawa (2014) generally exhibit a more depressed shell shape, a wider and more distinct umbilicus, and a coarsely sculptured protoconch characterized by distinct spiral striae. These characteristics collectively support the broader morphological concept of *Punctulum* sensu Hasegawa (2014), which includes species from the Atlantic and Pacific regions.

Punctulum flavum (Okutani, 1964) 노랑배꼽꼬마고둥 (신칭) (Figs. 2, 3)

Microstelma flava Okutani, 1964: 389, 390, pl. 6, fig. 5.
Punctulum delicatum Golikov and Sirenko, 1998: 102, figs. 3D, 15E, L; Kantor and Sysoev, 2006: 75, pl. 36, fig. H.
Punctulum ochotense Golikov and Sirenko, 1998: 102, figs. 3E, 15D, K; Kantor and Sysoev, 2006: 75, pl. 36, figs. F, G.
Alvania (*Alvania*) *flava*: Hasegawa, 2000: 148, 149, pl.

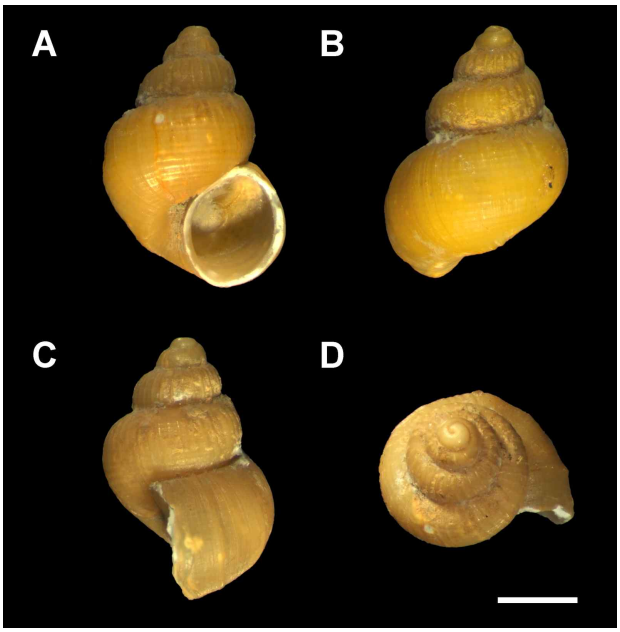

Fig. 2. Shell of *Punctulum flavum* (Okutani, 1964). **A**, ventral view; **B**, dorsal view; **C**, lateral view; **D**, apical view. Scale bar = 1 mm.

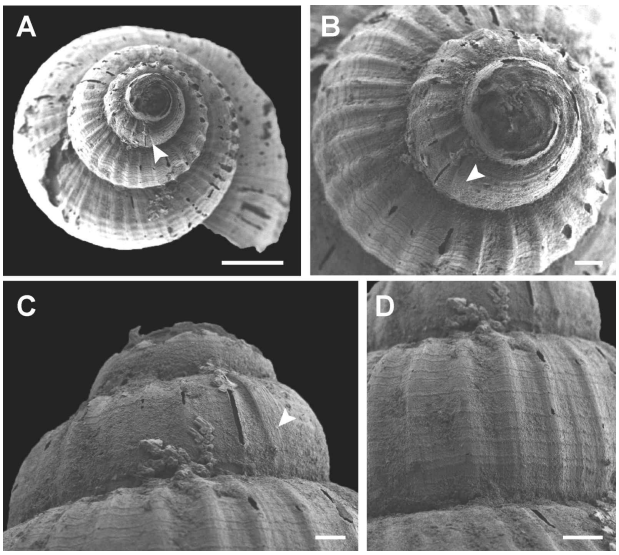

Fig. 3. Scanning electron micrographs of the shell of *Punctulum flavum* (Okutani, 1964). **A**, apical view; **B**, detail of the protoconch microstructure (apical view); **C**, detail of the protoconch microstructure (ventral view); **D**, detail of the teleoconch microstructure (apical view). Arrowheads indicate the protoconch-teleoconch boundary. Scale bars: **A** = 200 μm; **B-D** = 100 μm.

74, fig. 6.

Alvania flava: Hasegawa, 2001: 134.

Punctulum flava [sic]: Hasegawa, 2009: 255, 256, fig. 65; Hasegawa and Okutani, 2011: 113, fig. 18.

Punctulum flavum: Hasegawa, 2014: 101-112, figs. 15-25, 49C, Table 6; 2017: 116, 805, pl. 72, fig. 5.

Materials examined. Total of five individuals: Three individuals (MO00191601): 100% ethanol-preserved specimens, survey area 76, East Sea (36°53'35.90"N, 129°55'13.16"E), collected by bottom trawl at a depth of 900 m, from sieved muddy sediment, 11 October 2023, collectors: Kang, C.B., Shin, M.H. and You, C.; One individual (MO00191602; Figs. 2, 3): dry specimen, survey area 76, East Sea (36°53'35.90"N, 129°55'13.16"E), collected by bottom trawl at a depth of 900 m, from sieved muddy sediment, 11 October 2023, collectors: Kang, C.B., Shin, M.H. and You, C.; One individual (MO00191603): 100% ethanol-preserved specimen, survey area 77, East Sea (36°43'4.75"N, 130°0'37.86"E), collected by bottom trawl at depth of 1,000 m, from sieved muddy sediment, 8 September 2024, collectors: Kim, C.H., and Shin, M.H.

Description. Shell minute (Fig. 2A-C; in examined specimen, shell length [SL] 3.32 mm, shell width [SW] 1.86 mm), solid, thick, ovate-conical. Shell color light yellow to yellowish brown, covered with thin periostracum. Protoconch low, dome-shaped, consisting of 1.0-2.0 whorls (Figs. 2D, 3A, 3B; in examined specimen approximately 1.75 whorls), surface nearly smooth with closely spaced flat spiral cords arranged at regular interspaces (Figs. 2, 3A-C). Protoconch-teleoconch boundary clearly demarcated by slightly thickened axial riblet (Fig. 3A-C; arrowheads). Teleoconch 2.5-3 whorls (Figs. 2D, 3A; in examined specimen approximately 2.8 whorls), sculptured with weak but distinct axial riblets and fine spiral cords (Figs. 2, 3); axial riblets gradually faint toward base of body whorl, spiral cords regularly arranged with equal interspaces. Spire short, approximately one-third of shell length, rounded at shoulder; each whorl separated by deeply impressed suture. Body whorl large, occupying approximately two-thirds of entire shell length, roundly inflated. Aperture oval (Fig. 2A); outer lip rather thick, with weak apertural varix behind lip; inner lip gently curved. Umbilicus narrow, deep, with minute perforation.

Operculum thin, chitinous, oval, paucispiral.

GenBank accession numbers. mtDNA COI: PX736146 (MO00191601), 16S rDNA: PX736147 (MO00191602).

Distribution. Bathyal region of the northwestern Pacific. **Japan**: the Rebun Island southwards along the western coast (200–2,027 m; Hasegawa, 2014) and along the Pacific coast (480–2,075 m; Hasegawa, 2014) of the Japanese archipelago, extending from Sagami Bay (type locality; ca. 550 m; Okutani, 1964) south to Tosa Bay and Suruga Bay (500–776 m; Hasegawa, 2001), extending to Hyuga–nada, east of Kyushu; **Russia**: off the Okhotsk Sea coast of Paramushir Island, Kurile Islands (ca. 500 m; Golikov and Sirenko, 1998); **Korea**: the East Sea (900–1,000 m; present study).

Remarks. According to Hasegawa (2014), *Punctulum flavum* is identified two depth–related morphological forms: a "shallow–water form" (less than 1,000 m) and a "deep–water form" (deeper than 1,000 m). The "shallow–water form" is characterized by a lower conical shell, stronger axial and spiral sculpture, and a thicker, yellowish–brown periostracum, whereas the “deep–water form” possesses a relatively inflated shell, weaker teleoconch sculpture, and a thin, translucent periostracum.

The protoconch of the Korean specimen (Figs. 2, 3A–C) exhibited a nearly smooth surface, bearing closely spaced flat spiral cords with regular interspaces, consistent with the description of both depth–related forms in Hasegawa (2014). This feature is considered one of the most stable characteristics of the species. The protoconch size (approximately 1.75 whorls in the examined specimen; Figs. 2D, 3A, 3B) also corresponds to the depth–related trend observed in Japanese specimens, where individuals dwelling at greater depths tend to have relatively larger protoconchs (Hasegawa, 2014).

Korean specimens differ significantly from the "shallow–water form" in having weaker teleoconch sculpture, more inflated shell outline, and a lighter periostracum. However, they closely match the "deep–water form" reported from the Pacific coast of northern Japan and the western coast of the Japanese archipelago. Thus, the Korean specimens exhibited intraspecies variation and lacked diagnostic characteristics indicative of more than a single species, despite the broader morphological variability documented by Hasegawa (2014).

This study represents the first record of the genus *Punctulum* and *P. flavum* in Korean waters. The discovery of this species in the bathyal sediments of the East Sea extends the southwestern limit of its known distribution and supports the interpretation that Japanese–Okhotsk–East Sea bathyal fauna forms a morphologically coherent group of rissoids that share a characteristic protoconch morphology.

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