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Description of Microscopic Morphology of *Leptochiton hakodatensis* (Mollusca: Polyplacophora)

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ABSTRACT

Leptochiton Gray, 1847 is one of the most ancient chiton groups which includes more than 130 species that occur in cold and deep waters worldwide. Due to their small-sized body, they are often confused as juveniles of other chiton species. Moreover, lack of morphological information makes species identification of this group very challenging. To date, only two *Leptochiton* species (*L. fuliginatus* and *L. rugatus*) have been reported from Korean waters. In this study, we found *L. hakodatensis* (Thiele, 1909) for the first time in Korea and described microscopic morphological characters of valves (tegmentum sculpture), girdle scale, and radula using a scanning electron microscopy (SEM). *Leptochiton hakodatensis* is morphologically similar to *L. fuliginatus* and *L. rugatus*, but differently characterized by having dorso-ventrally rounded (not carinated) intermediate valves, girdle (perinotum) scales sculptured with 4–7 longitudinal ribs, and bicuspid major lateral teeth of radula. In addition to morphological examination, we determined the partial mitochondrial cytochrome *c* oxidase subunit I (*cox1*) as a DNA barcode sequence information. This is the first report that describes microscopic characters (tegmentum of valves, girdle structure, and radula) of *L. hakodatensis* using a SEM. This study provides a morphological basis for describing *Leptochiton* species and discovery of a "hidden" species of this genus.

Keywords: *Leptochiton hakodatensis*, chiton, microscopic characters, SEM, mtDNA *cox1*, Korea

INTRODUCTION

The genus *Leptochiton* Gray, 1847 is one of the most diverse, and ancient chiton groups (Sirenko, 2013, 2015a) that includes more than 130 species (WoRMS, July 2021). They are found in cold waters and deep seas worldwide, including Antarctica (Sirenko, 2015b). This genus is characterized by having a small-sized body, thin valves, hardly elevated lateral areas, and finely granulose tegmentum (Kaas and Van Belle, 1985; Kiel and Little, 2006; Sigwart, 2008; Sirenko, 2015a). Previous taxonomic studies of this group have been based largely on external morphology which is generally insufficient to separate close related species, resulting in "lumping" of many species names under a single species name (Sirenko and Sigwart, 2021). Lack of morphological information makes species identification of this group challenging. Recently, morphological analysis of microscopic characters using a scanning electron microscopy (SEM) has been very useful for the description of new species and discovery of a "hidden" species (Sirenko, 2015b; Sirenko and Sigwart, 2021).

To date, two *Leptochiton* species have been reported from Korean waters: *L. fuliginatus* (Reeve, 1847), *L. rugatus* (Carpenter in Pilsbry, 1892). In this study, we found *L. hakodatensis* (Thiele, 1909) for the first time in Korea, described microscopic characters of valves (tegmentum sculpture), girdle (perinotum and hyponotum) scale, and radula using a SEM, and compared them with other congeneric species previously reported. In addition to morphological information, we determined partial sequence of mitochondrial cytochrome *c* oxidase subunit I (*cox1*) as barcode sequence information of *L. hakodatensis*.

MATERIALS AND METHODS

The specimen was collected from bivalve shell surface at a depth of 20 m and preserved in 95% ethyl alcohol (detailed collection site information is provided in the material exam-

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ined). For species identification and morphological description of the specimen, morphological characters of valves and girdle were examined using a stereoscopic microscope (Leica M205C, Wetzlar, Germany) and microstructures of valves (tegmentum surface), girdle scale (perinotum and hyponotum), and radula were examined using a SEM. For the preparation of SEM examination, the specimen was incubated in 7% KOH solution for 2–5 min, then washed with distilled water. The valves, girdle, and radula were isolated from dissected tissues, cleaned to remove residual tissue using an ultrasonic cleaner, coated with platinum ion after drying and photographed using a SEM (Ultra Plus; Zeiss, Germany). Total genomic DNA was extracted using an E.Z.N.A. mollusk DNA extraction kit (Omega Bio-tek, Norcross, USA) following the manufacturer's instructions. Partial sequence of mitochondrial *cox1* gene was PCR-amplified using universal primers (LCO1490, HCO2198) (Folmer et al., 1994) and TaKaRa Ex Taq (Takara Bio, Shiga, Japan) in a total 50 μL of mixture containing 33.75–37.25 μL of distilled water, 5 μL of 10× Ex Taq buffer, 4 μL of dNTP Mixture (2.5 mM each), 1–2 μL of each primer, 0.25 μL of TaKaRa Ex Taq and 1–3 μL of genomic DNA. PCR was performed following conditions; initial denaturation at 95°C for 1 min, 40 cycles of denaturation at 94°C for 30 s, annealing at 46°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 10 min. The amplified partial mt *cox1* gene fragment was isolated on 1% agarose gel and purified using QIAquick gel extraction kit (Qiagen, Valencia, CA, USA) following standard protocols and sequenced directly using an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The *cox1* sequence of *L. hakodatensis* was deposited in GenBank. The sequence was aligned with previously available sequences of congeneric species using Clustal X (Thompson et al., 1997) plugin in Geneious v11.0.5 (Kearse et al., 2012) and compared with other congeneric species.

SYSTEMATIC ACCOUNTS

Phylum Mollusca Linnaeus, 1758
Class Polyplacophora Gray, 1821
Order Lepidopleurida Thiele, 1909
Family Leptochitonidae Dall, 1889
Genus *Leptochiton* Gray, 1847

[1]***_Leptochiton hakodatensis_ (Thiele, 1909) (Figs. 1–4)**

Lepidopleurus hakodatensis Thiele, 1909: 10, Pl. 1, figs. 11–20; Is. Taki, 1938: 326, Pl. 14, fig. 1, Pl. 16, figs. 1–4, 6–8, 14, Pl. 17, figs. 6–8; 1962: 31; Jakovleva, 1952: 54, fig. 15, Pl. 1, fig. 1; Iw. Taki, 1964: 408; Sirenko, 1976: 88.
Chiton (*Leptochiton*) *concinnus* Gould, 1859: 164; 1862: 117; Carpenter, 1864: 586.
Lepitochiton concinnus: Dall, 1879: 316, 318.
Lepidopleurus concinnus: Pilsbry, 1892: 11; Thiele, 1909: 10; Is. Taki and Iw. Taki, 1929: 161, 163.
Leptochiton hakodatensis: Kaas and Van Belle, 1985: 98, fig. 43, Map 33; Okutani, 2000: 5, Pl. 2, fig. 5.

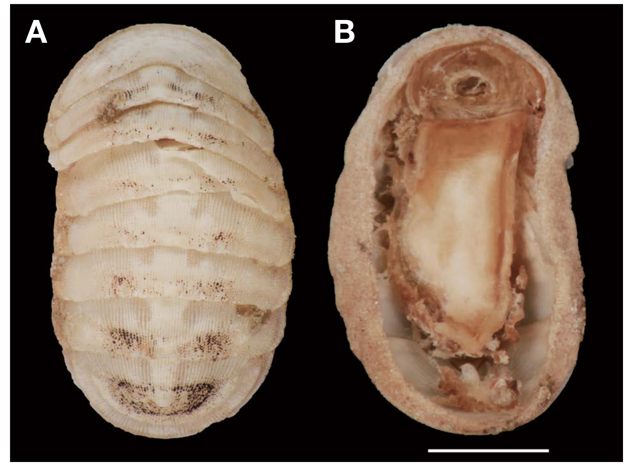

Fig. 1. *Leptochiton hakodatensis* (Thiele, 1909). A, Dorsal view; B, Ventral view. Scale bar: A, B = 2 mm.

Type locality. Hakodate, Hokkaido (Japan).
Material examined. Korea: 1 individual: Gangwon-do: Goseong-gun, Geojin-eup, Geojin-ri, 38°27′09.01″N, 128°28′03.01″E, 14 May 2020. The voucher specimen was deposited in the National Marine Biodiversity Institute of Korea (MABIK MO00178362).
Description. Body elongate-oval, small in size (body length [BL] 7 mm, body width [BW] 4 mm); valves very thin, fragile, and yellowish white in color; Girdle somewhat narrow, yellowish white in color (Fig. 1A). Gills arrangement adanal and merobranchial on both sides (Fig. 1B).

Valves: Head valve semicircle in shape, with roundish and flat granules arranged in fine radial lines, granules small and regular in size, posterior area widely V-shaped (Fig. 2A). Intermediate valves similar in width except for 2nd valve (Fig. 2B; shorter than other intermediate valves), oblong shaped, not beaked (Fig. 2B, C). Intermediate valves dorso-ventrally rounded, not carinated, and moderately elevated in frontal view (Fig. 2E; elevation ratio of 0.61 in the 6th valve); an-

Korean name: [1]*북방상아군부 (신칭)

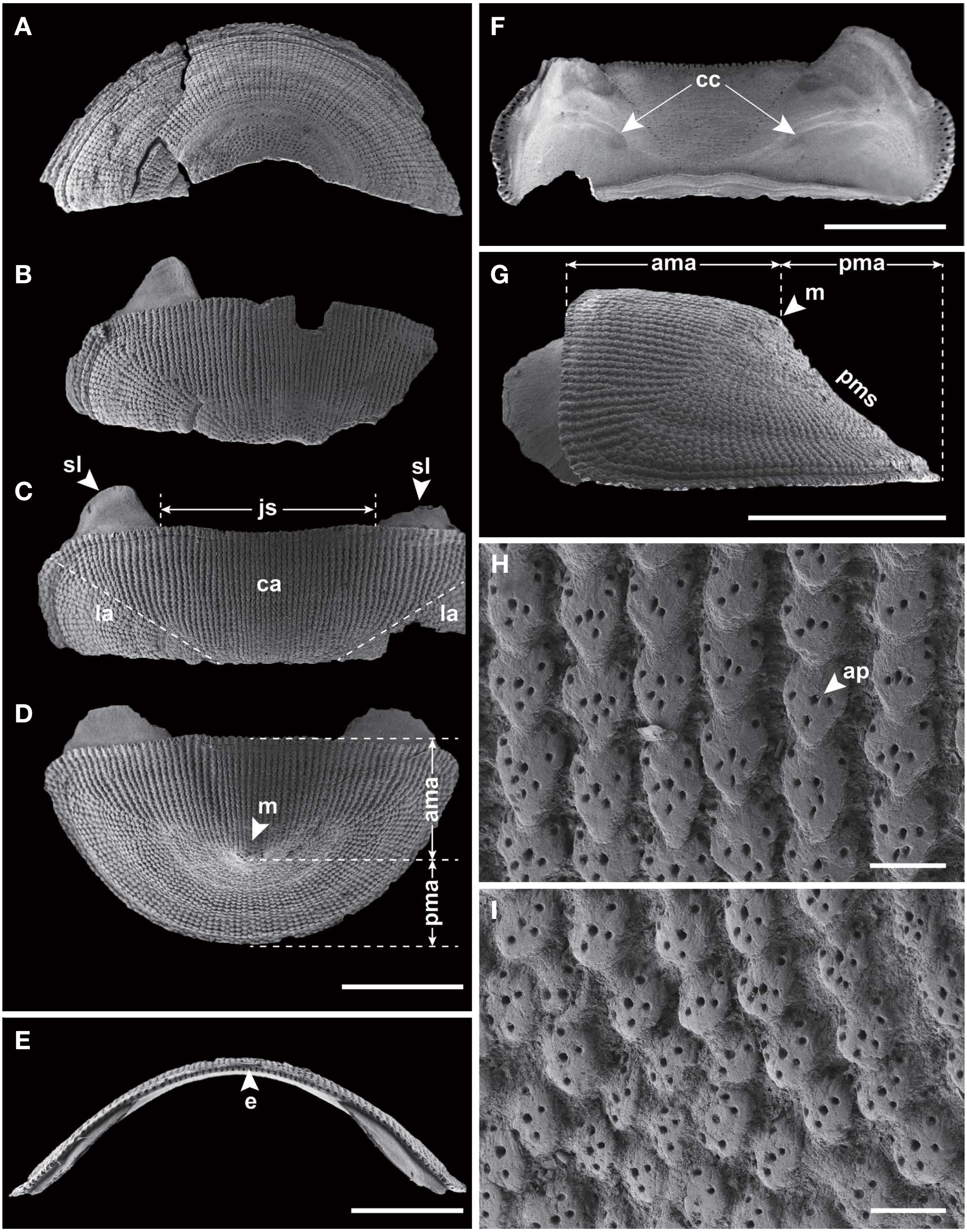

Fig. 2. Scanning electron microscope images of valves of *Leptochiton hakodatensis*. A, Head valve, dorsal view; B, 2nd valve, dorsal view; C, 6th valve, dorsal view; D, Tail valve, dorsal view; E, 6th valve, frontal view; F, 6th valve, ventral view; G, Tail valve, lateral view; H, 6th valve, detail of tegmentum surface of central area; I, 6th valve, detail of tegmentum surface of central and lateral area. ama, antemucronal area; ap, aesthete pore; ca, central area; cc, central callus; e, eave; js, jugal sinus; la, lateral area; m, mucro; pma, postmucronal area; pms, postmucronal slope; sl, sutural laminae. Scale bars: A–G = 1 mm, H, I = 500 μm.

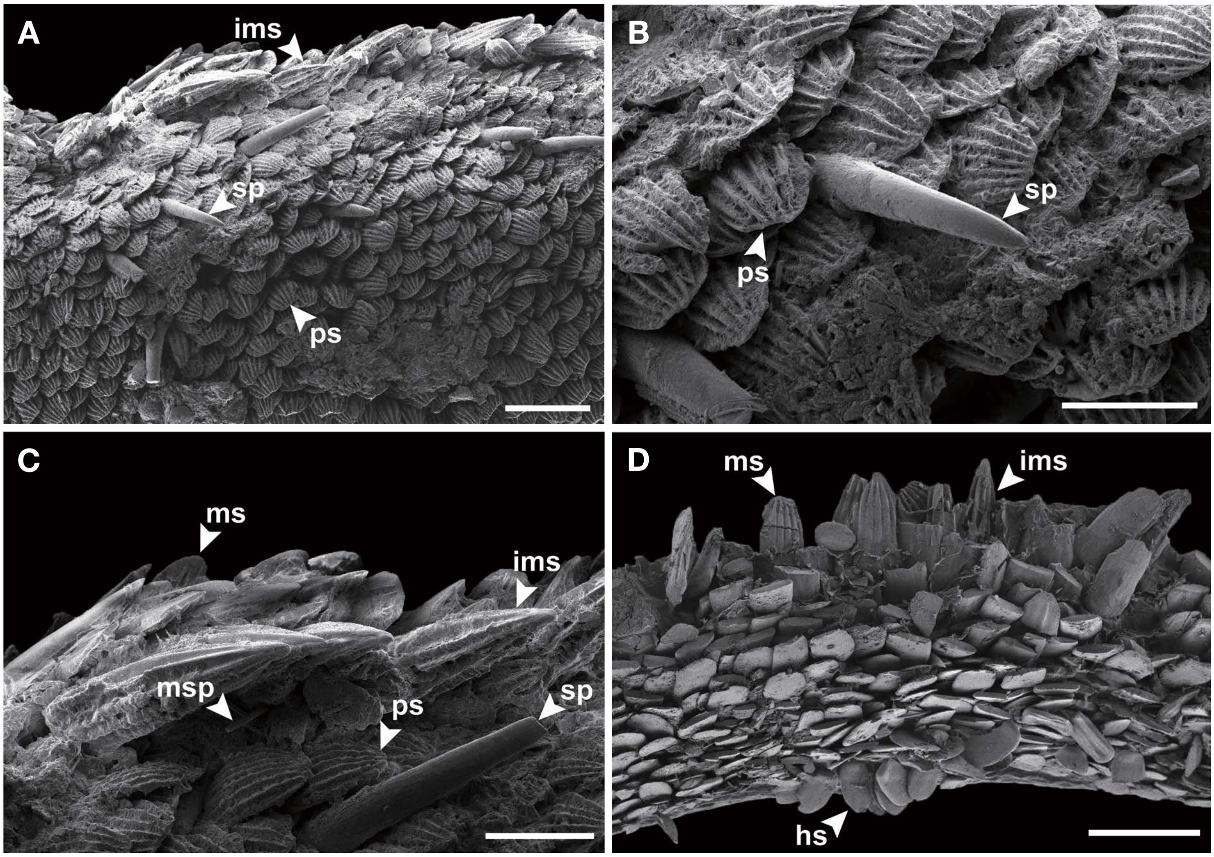

Fig. 3. Scanning electron microscope images of microstructure of girdle in *Leptochiton hakodatensis*. A, B, Perinotum, marginal scales and spines; C, Marginal scales and spicules; D, Hyponotum and marginal scales. hs, hyponotum scale; ims, infra-marginal scale; ms, marginal scale; msp, marginal spicule; ps, perinotum scale; sp, perinotum spine. Scale bars: A, D=100 μm, B, C=50 μm.

terior and posterior margins of intermediate valves nearly straight, lateral margins rounded; tegmentum of central area (ca) sculptured with rounded granules (each having 5–7 aesthete pores, ap) densely arranged in longitudinal rows (Fig. 2H); lateral areas (la) not raised, but separated from central area by interstice of granules arranged on radial lines (Fig. 2B, C, I); sutural laminae (sl) triangular in shape, small, hyaline; jugal sinus (js) wide, flat; eaves (e) narrow with equal pore size on granules of tegmentum surface; central callus (cc) thin (Fig. 2F). Tail valve semicicular, almost as wide as head valve; mucro (m) slightly antemedian, distinct, not pointed; sculpture of antemucronal area (ama) same as central area of intermediate valves; postmucronal area (pma) similar to head valve (Fig. 2D); anterior margin straight; postmucronal slope (pms) concave behind the mucro (Fig. 2G); sutural laminae and jugal sinus similar to intermediate valves. Articulamentum white, no insertion plate.

Girdle: Perinotum densely covered with flat, ovate scales (ps: length 45.8 μm, width 40 μm) of 4–7 fine longitudinal ribs (Fig. 3A–C); perinotum spines (sp: length 111.7 μm, width 16 μm) smooth, slender, pointed sporadically interspersed (Fig. 3A–C); along outer margin, marginal scales (ms: length 95 μm, width 31.4 μm) longer, slenderer and pointed than perinotum scales (Fig. 3C); infra-marginal scales (ims: length 116.5 μm, width 30.5 μm) with well-developed longitudinal ribs (Fig. 3C); marginal spicules (msp: length 24.3 μm, width 3.8 μm) small, smooth, needle-like (Fig. 3C); hyponotum covered with overlapping, oblong scales (hs: length 45.2 μm, width 30.3 μm) with longitudinal grooves (Fig. 3D).

Radula: Radula teeth symmetrical (Fig. 4). Central tooth (c) small, oblong with a cusp bent inwardly; two centro-lateral teeth (cl) with a wing-like blade; head (h) of major lateral teeth (mlt) bicuspid, each cusp bluntly pointed; major uncinal teeth (mu) long oar shaped, bent inwardly, with a rounded tip.

Habitat. On hard substrate (e.g., shells and stones) in subtidal zone.

Distribution. Japan, Korea, and Russia.

Remarks. Two *Leptochiton* species (*L. fuliginatus* and *L. rugatus*) have been reported in Korea. The body size of *L. hakodatensis* (BL 7 mm, BW 4 mm) is similar to *L. rugatus*

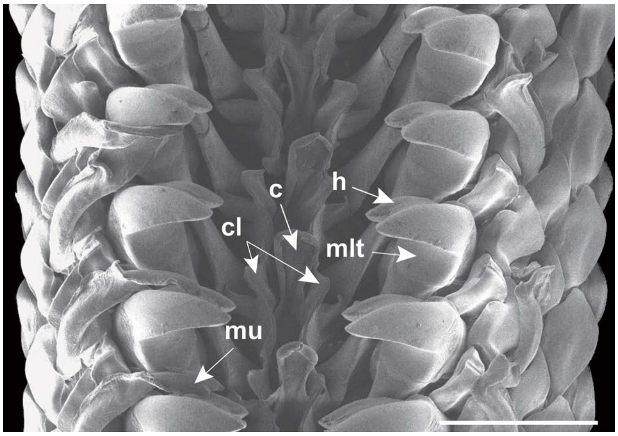

Fig. 4. Scanning electron microscope images of microstructure of radula of *Leptochiton hakodatensis*. c, central tooth; cl, centro-lateral tooth; h, head of major lateral tooth; mlt, major lateral tooth; mu, major uncinus tooth. Scale bar=100 μm.

(BL 6 mm, BW 4.58 mm), but much smaller than *L. fuliginatus* (BL 21 mm, BW 8 mm) (Kaas and Van Belle, 1985). In addition, overall morphology of intermediate valves of *L. hakodatensis* and *L. rugatus* is also similar in that they are dorso-ventally rounded, with their lateral areas not elevated, differing from *L. fuliginatus* where intermediate valves are subcarinated with slightly raised lateral area. *Leptochiton hakodatensis* differs from *L. rugatus* in microstructure of perinotum scale and radula characters: Dorsal scales of *L. hakodatensis* are flat and ovate that is sculptured with 4–7 longitudinal ribs, whereas in *L. rugatus* they are sculptured with 13–16 longitudinal ribs, and in *L. fuliginatus* they are sculptured with 4 longitudinal ribs not reaching the base (Kaas and Van Belle, 1985). Major lateral teeth of *L. hakodatensis* and *L. fuliginatus* radulae are bicuspid, but *L. rugatus* has unicupid major lateral teeth (Kaas and Van Belle, 1985).

DNA sequence information of *cox1*. GenBank accession no. MZ733400.

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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