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Leptoplax doederleini (Polyplacophora: Acanthochitonidae) from South Korea

Youngheon Shin[1], Jina Park[2], Yucheol Lee[1], Taeho Kim[2], Joong-Ki Park[2,*]

[1]Department of Biological Sciences, Sungkyunkwan University, Suwon 16419, Korea
[2]Division of EcoScience, Ewha Womans University, Seoul 03760, Korea

ABSTRACT

Acanthochitonidae Pilsbry, 1893 is one of the chiton families characterized by 9 pairs of sutural tufts on a well-expanded girdle that overgrows plates. It is found in intertidal and subtidal zones worldwide. Of its eight genera, only two have been previously reported in South Korea: *Acanthochitona* Gray, 1821 and *Notoplax* H. Adams, 1862. Here we report *Leptoplax doederleini* (Thiele, 1909) as the first record of the genus *Leptoplax* in South Korea and describe and compare its morphological features with *N. kaasi* Hong, Dell'Angelo and Van Belle, 1990. In addition, we provide the partial sequence of the mitochondrial DNA cytochrome *c* oxidase subunit I gene as a DNA barcode sequence record.

Keywords: *Leptoplax*, Acanthochitonidae, shell eyes, radula, Korea

INTRODUCTION

The family Acanthochitonidae Pilsbry, 1893 is characterized by 9 pairs of sutural tufts on the girdle and 5 slits on the head valve. This family is found throughout the world in intertidal and subtidal zones. Of its eight genera, only two have been recorded previously in South Korea: *Acanthochitona* Gray, 1821 (6 species) and *Notoplax* H. Adams, 1862 (1 species) (Hong et al., 1990). Here we report the first record of a third acanthochitonid genus, *Leptoplax*, from Korea. The genus *Leptoplax* Dall, 1882 was a synonym of *Notoplax* until it was depicted as a valid genus by Gowlett-Holmes (2001). Members of this genus are found from intertidal zones to shallow water habitats in the Indo-Pacific Ocean (Schwabe, 2007). *Leptoplax* and *Notoplax* are morphologically similar, both having a reduced tegmentum and well developed insertion plates with slits (formula 5/1/3-10). However, they differ in the presence or absence of diagonal ridges on their valves, the postmucronal slope and apophysis of the tail valve, and in their radula arrangement (Sirenko and Saito, 2017).

In this study, we describe morphological details of the valves, girdle and radula of *L. doederleini* (Thiele, 1909) using scanning electron microscope (SEM) images, and provide mitochondrial DNA cytochrome *c* oxidase subunit I (*cox1*) sequences. In addition, we describe the morphological differences between *Leptoplax* and *Notoplax* through comparison with *N. kaasi* Hong, Dell'Angelo and Van Belle, 1990, which is also previously reported from Korea.

MATERIALS AND METHODS

Specimens were collected from the rocky intertidal zone in Jindo and fixed in 95% ethanol. The specimens were identified by their morphological features observed under a stereoscopic microscope (Leica M205C, Wetzlar, Germany). SEM images were prepared in order to examine the microstructure of the valves, girdle and radula. One specimen was boiled in 7% KOH solution for 10−15 min. After the internal tissues had dissolved, the specimen was rinsed with tap water and the valves, girdle and radula were dissected. The dissected parts were cleaned to remove residual tissue using an ultrasonic cleaner (Shinhan 200H3L; Shinhan-Sonic, Korea), coated with gold-palladium, and photographed using an SEM (Ultra Plus; Zeiss, Germany). The specimens used in this study were deposited in the Marine Mollusk Resource Bank of Korea (MMRBK) in Seoul, Korea (MMRBK Nos. 00006403, 00006404, and 00006407) and the National Institute of Bio-

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***To whom correspondence should be addressed**
Tel: 82-2-3277-5948, Fax: 82-2-3277-2385
E-mail: jkpark@ewha.ac.kr

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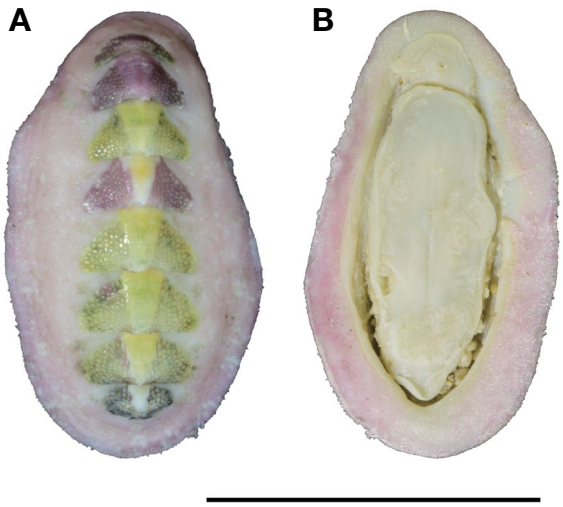

Fig. 1. *Leptoplax doederleini*. A, Dorsal view; B, Ventral view. Scale bar: A, B = 10 mm.

logical Resources (NIBR) in Incheon, Korea (NIBR No. NIBRIV0000812928).

Genomic DNA was extracted from mantle tissue of four individuals using an E.Z.N.A. Mollusc DNA Kit (OMEGA Bio-tek, Norcross, GA, USA). The partial sequence of the mtDNA *cox1* gene was amplified by polymerase chain reaction (PCR) using TaKaRa Ex Taq (Takara Bio, Shiga, Japan); the mixture consisted of 37.25 μL distilled water, 5 μL of 10 × Ex taq buffer, 4 μL dNTP Mixture (2.5 mM each), 1 μL of each universal primer (LCO1490, HCO2198) (Folmer et al., 1994), 0.25 μL of TaKaRa Ex Taq, and 1.5 μL of genomic DNA. PCR was performed with an initial denaturation at 95°C for 1 min, 40 consecutive cycles of denaturation at 94°C for 30 sec, annealing at 46°C for 30 sec, elongation at 72°C for 30 sec, and a final elongation at 72°C for 10 min. The PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA). Sequencing of the PCR products was performed using an ABI PRISM 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA).

SYSTEMATIC ACCOUNTS

Phylum Mollusca Linnaeus, 1758
Class Polyplacophora Gray, 1821
Order Chitonida Thiele, 1909
Family Acanthochitonidae Pilsbry, 1893
[1*]Genus *Leptoplax* Dall, 1882

Type species. *Chiton coarctatus* G. B. Sowerby II, 1841 [= *Leptoplax coarctatus* (G. B. Sowerby II, 1841)], by subsequent designation (Gowlett-Holmes, 2001).

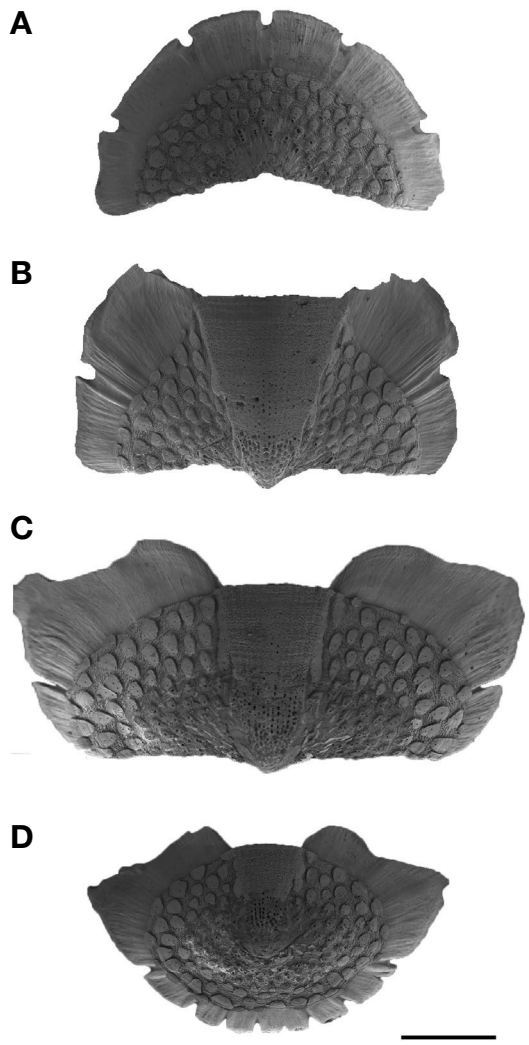

Fig. 2. *Leptoplax doederleini*. A, Head valve; B, 2nd valve; C, 7th valve; D, Tail valve. Scale bar: A–D = 1 mm.

Diagnosis. Body small to medium sized, reduced tegmentum, developed apophysis and insertion plates, lateral and pleural areas of intermediate valves not separated, postmucronal slope of tail valve concave, 9 pairs of low density sutural tufts, radula symmetrical arrangement.

[2*]***Leptoplax doederleini*** **(Thiele, 1909)**

Notoplax döderleini Thiele, 1909: 39, Pl. 5, figs. 32–38; Kaas and Van Belle, 1980: 40; 1998: 64.

Notoplax (*Notoplax*) *doederleini*: Van Belle, 1980: 478–480, fig. 4.

Korean name: [1*]얇은판가시군부속(신칭), [2*]옅은분홍군부(신칭)

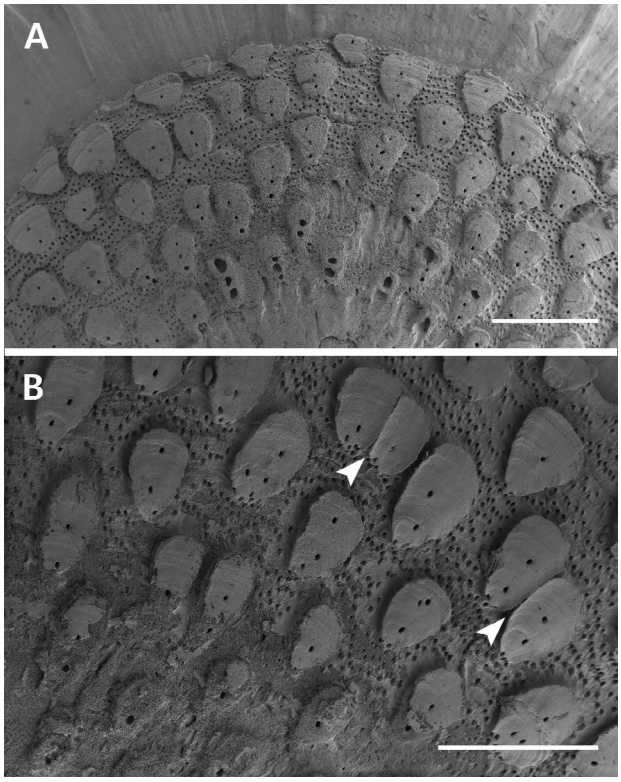

Fig. 3. *Leptoplax doederleini*, microstructure of granules. A, Surface of head valve; B, Surface of 7th valve, with granules indicated by white arrowheads. Scale bars: A, B = 300 μm.

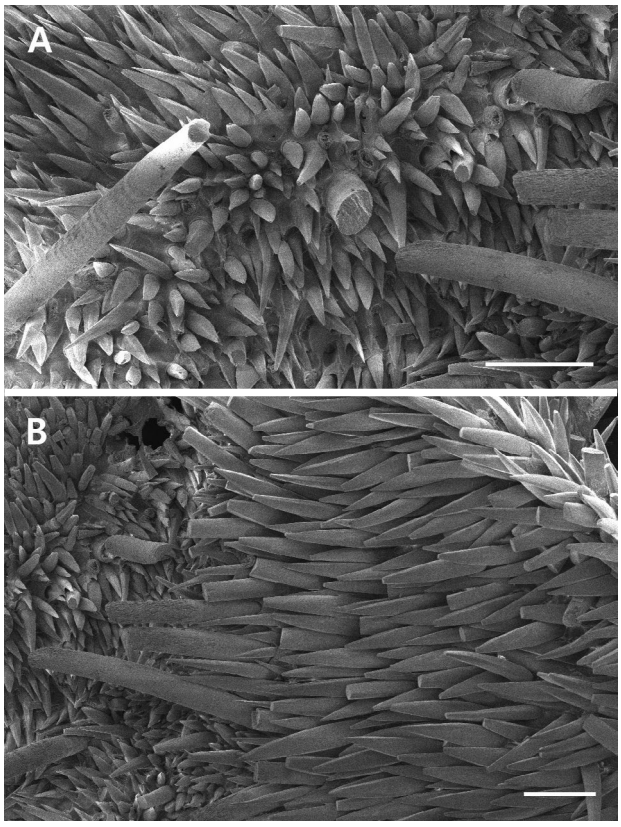

Fig. 4. *Leptoplax doederleini*, microstructure of spicules on girdle. A, Perinotum; B, Hyponotum. Scale bars: A, B = 50 μm.

"*Notoplax*" *doederleini*: Saito, 1998: 160, fig. 2N; 2000: 102.
Leptoplax doederleini: Saito, 2001: 200, fig. 14; 2006: 217; Sirenko and Saito, 2017: 460–463, figs. 9–12, 43D.

Material examined. Korea: 3 individuals, Gyeonsangnam-do: Jindo-gun, Gogun-myeon, Geumgye-ri, Gagye seawall, 6 Jul 2016; 1 individual, Jindo-gun, Gogun-myeon, Geumgye-ri, Gagye seawall, 14 Dec 2016.

Description. Body elongate oval shaped, small in size (length 12–14 mm, width 6–8 mm). Head valve, 2nd valve, and 4th valve pinkish red; the other valves yellowish white and maculated with olive green or pink; jugal area of intermediate valves greenish white except for 2nd valve (Fig. 1A). Girdle pink with pale pink bands. Gills arrangement abanal and merobranchial (Fig. 1B).

Head valve semicircular, anterior margin roundish, posterior margin widely V-shaped, irregularly sized circular granules on surface (Fig. 2A). Articulamentum white, insertion plate symmetric and wide with 5 slits; shallow channels and wrinkles.

Intermediate valves trapezoid, jugal area wedge shaped and smooth, pleural and lateral areas not distinguished; with oval granules on surface (Fig. 2B, C). Articulamentum white, central callus rather thick, apophysis thin and slightly protruded; separated without jugal laminae, 1 slit on each side of insertion plate; deep channels and strong wrinkles near channels, ventral tegmental callus thin and slightly wide.

Tail valve oval, central mucro (Fig. 2D). Antemucronal area with smooth central surface and with oval granules on both sides. Postmucronal area similar to head valve, postmucronal slope concave. Articulamentum white, apophysis rather protruding without jugal laminae and anterolateral side concave, insertion plate asymmetric and with differently sized teeth with 9 slits.

Granules usually in quincuncial arrangement (Fig. 3A); sometimes in irregular arrangement and attached closely side by side (Fig. 3B; white arrowheads). Usually 2–3 shell eyes on each granule, sometimes only one shell eye. Located on the surface except for granules with dense shell eyes.

Girdle rather wide. Perinotum with 9 pairs of not noticeable sutural tufts; seven pairs of sutural tufts between each valve and two pairs of sutural tufts at the ends of both the head and tail valves, densely covered with very small spines,

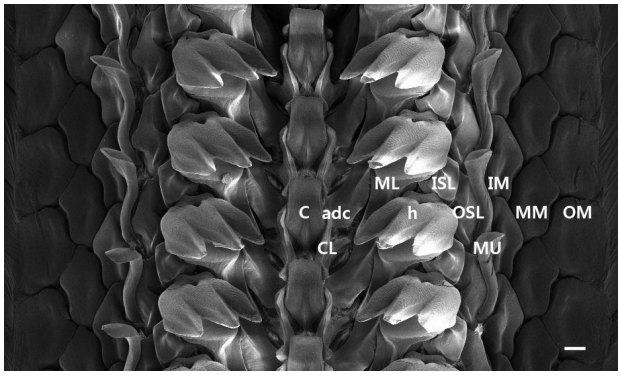

Fig. 5. *Leptoplax doederleini* radula, scanning electron microscope image. adc, antero-dorsal corner of centro-lateral tooth; C, central tooth; CL, centro-lateral tooth; h, head of major lateral tooth; IM, inner marginal tooth; ISL, inner small lateral tooth; ML, major lateral tooth; MM, middle marginal tooth; MU, major uncinus tooth; OM, outer marginal tooth; OSL, outer small lateral tooth. Scale bar = 20 μm.

rarely large spines (Fig. 4A). Small spines sharply pointed, sometimes with narrow longitudinal grooves on the tip. Large spines long and smooth. Hyponotum densely covered with flattened spines directed outward (Fig. 4B).

Radula symmetric rows (Fig. 5). Central tooth oblong, basal part narrow. Centro-lateral tooth rather thin, antero-dorsal corner hardly protruding. Head of major lateral tooth tricuspid, three cusps of similar length, middle cusp slightly longer than the others, long groove along lateral side of shaft. Small lateral tooth rather thick. Major uncinus tooth S-curved; spoon-like in shape on the upper side. Marginal tooth flattened, slightly dented on the central part.

Habitat. Intertidal zones of rocky shores.

Distribution. Hong Kong, Japan, Korea, and Vietnam.

Remarks. *Leptoplax* and *Notoplax* are morphologically similar to each other: both have a reduced tegmentum, well developed articulamentum, slit formula of 5/1/3-10, wide and thick girdle with dense spicules, and inconspicuous sutural tufts. But these two genera are distinguished by several morphological characters: diagonal lines, postmucronal slope of the tail valve, and radula arrangement. In *N. kaasi*, the head valve has five diagonal ridges with enlarged granules compared to the granules that are not on the ridges; the pleural and lateral areas of the intermediate valves are distinguished by a diagonal line; the postmucronal slope is convex and precipitous; and the radula are arranged obliquely (Hong et al., 1990). On the other hand, *L. doederleini* lacks diagonal lines, and so the pleural and lateral areas are not distinguished (Fig. 2B, C); the postmucronal slope of the tail valve is concave (Fig. 2D); and the radula is arranged symmetrically (Fig. 5).

A 658-bp partial *cox1* sequence was obtained from 4 individuals, each differing by 3–9 bp. The sequences were deposited in GenBank (accession Nos. MH445296, MH445297, MH445298 and MH445299).

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