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Short communication

First Record of *Deshayesiella curvata* (Polyplacophora: Protochitonidae) from Korea

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ABSTRACT

Protochitonidae Ashby, 1925 is a family of small to medium sized chitons that includes a single fossil genus and two extant genera. Of the two extant genera, *Deshayesiella* Carpenter in Dall, 1879 contains 5 described species. Although most *Deshayesiella* species are known to be found in deep sea habitats (over 100 m), *D. curvata* (Carpenter in Pilsbry, 1892) is found from shallow waters (1−20 m). In this study, we provide details of microstructure of shell and radula characters using scanning electron microscopy and morphological features of *D. curvata*, and its partial sequence of mitochondrial DNA *cox1* gene as DNA barcode sequence. In addition, we compare morphological differences of *D. curvata* from other congeneric species.

Keywords: *Deshayesiella*, Protochitonidae, radula, SEM, mtDNA *cox1*, Korea

INTRODUCTION

The family Protochitonidae Ashby, 1925 is characterized by coarse granules on all valves' surface, the absence of an insertion plate on the anterior side of the head valve and posterior side of the tail valve, and the absence of a slit on the intermediated valves (Ashby, 1925). Originally Protochitonidae was first described from fossils species. However, this family includes two extant genera (*Deshayesiella* Carpenter in Dall, 1879 and *Oldroydia* Dall, 1894) and one fossil genus (*Protochiton* Ashby, 1925) (see Sirenko, 2006). *Deshayesiella* was previously included in the family Lepidochitonidae until Sirenko (2006) placed it into Protochitonidae. The genus *Deshayesiella* has the insertion-plate and valve-slit characteristics, typical of Protochitonidae, and concentric growth lines appearing in the form of granules. This genus contains only 5 described species (Sirenko and Clark, 2008), all of them were reported from the eastern and northwestern Pacific: *D. bidentata* (Is. Taki, 1938), *D. curvata* (Carpenter in Pilsbry, 1892), *D. sinica* (Xu, 1990), *D. sirenkoi* Saito, Fujikura & Tsuchida, 2008, and *D. spicata* (Berry, 1919). Most species inhabit hydrothermal vents or deep sea environments (Kaas and Van Belle, 1985; Saito et al., 2008; Sirenko and Clark, 2008; Saito, 2012), but *D. curvata* is found in relatively shallow seas of 1−20 m (Kaas and Van Belle, 1985).

In this study, we describe the morphological characters of *Deshayesiella curvata* from scanning electron microscope (SEM) images of the valves, girdle, and radula. In addition, we provide mitochondrial DNA (mtDNA) *cox1* sequence as DNA barcode sequence.

Specimens were collected from subtidal zone in water depths of 1−2 m (via snorkeling). The collected specimens were fixed in 95% ethanol. Specimens were identified using morphological characters of the valves and girdle under a stereoscopic microscope (Leica M205C, Wetzlar, Germany). To observe the microstructure of the valves, radula and girdle, the SEM was used. To prepare SEM samples, a specimen was immersed in 7% KOH solution and heated in a 60°C water bath for 10−15 min. After washing the sample, the valves, radula and girdle were dissected and cleaned using an ultrasonic cleaner (Shinhan 200H3L; Shinhan-Sonic, Korea). The surface of the prepared samples was coated with gold-palladium ions. Microstructures of the specimen were observed and described from SEM images (Ultra Plus, Zeiss, Germany). The specimens examined in this study were deposited in the Marine Mollusk Resource Bank of Korea (MMRBK) in Seoul, Korea (MMRBK No. 00006405, 00006406) and the National Institute of Biological Resources (NIBR) in Incheon,

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Korea (NIBR No. NIBRIV0000812929).

For sequencing mtDNA *cox1* gene fragment as DNA barcode sequence deposit, genomic DNA was extracted from the mantle tissue (hyponotum) of three individuals using an E.Z.N.A. Mollusc DNA Kit (OMEGA Bio-tek, Norcross, GA, USA). To amplify the partial sequence of the mitochondrial DNA *cox1* gene for each individual, we performed polymerase chain reaction (PCR) using TaKaRa Ex Taq (Takara Bio, Shiga, Japan) with universal primers (LCO1490, HCO2198) (Folmer et al., 1994), in 50 μL total volume reactions containing 37.25 μL of distilled water, 5 μL of 10 × Ex taq buffer, 4 μL of dNTP Mixture (2.5 mM each), 1 μL of each universal primer (LCO1490, HCO2198), 0.25 μL of TaKaRa Ex Taq and 1.5 μL of gDNA. PCR conditions were as follows: initial denaturation at 95°C for 1 min, 40 denaturation cycles 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. To purify the amplified PCR products, we used a QIAquick gel extraction kit (Qiagen, Valencia, CA, USA). The partial mtDNA *cox1* gene was sequenced in both directions by an ABI PRISM 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Fig. 1. *Deshayesiella curvata*. A, Dorsal view; B, Ventral view. Scale bar = 10 mm.

SYSTEMATIC ACCOUNTS

Phylum Mollusca Linnaeus, 1758
Class Polyplacophora Gray, 1821
Order Lepidopleurida Thiele, 1909
[1*]Family Protochitonidae Ashby, 1925

Type genus. *Protochiton* Ashby, 1925.
Diagnosis. Body small to medium sized; distinct granules on valves; slitless insertion plates except for both ends of the head and tail valves; clear mucro; and tegmentum expanded posteriorly beyond the callus on the tail valve.

[2*]Genus *Deshayesiella* Carpenter in Dall, 1879

Type species. *Deshayesiella* (*Leptochiton*) *curvatus* Carpenter in Dall, 1879 (nom. nud.) [= *Lepidopleurus* (*Deshayesiella*) *curvatus* (Carpenter in Pilsbry, 1892) = *Deshayesiella curvata* (Carpenter in Pilbry, 1892)], by subsequent designation (Sirenko, 2006).
Diagnosis. Body small to medium sized, elongate-oval shaped; marked concentric growth lines on valves; no insertion plates on head valve nor tail valve; no slit on insertion plates of intermediated valves; bidentate cusps on major lateral tooth.

[3*]***Deshayesiella curvata*** **(Carpenter in Pilsbry, 1892) (Figs. 1–3)**

Deshayesiella (*Leptochiton*) *curvatus* (Carpenter MS) Dall, 1879: 314, nom. nud.
Lepidopleurus (*Deshayesiella*) *curvatus* (Carpenter MS) Pilsbry, 1892: 16, pl. 4, figs. 78–81.
Oldroydia percrassa; Jakovleva, 1952: 52, fig. 19, pl. 2, fig. 1 (non *Oldroydia percrassa* Dall, 1894).
Deshayesiella extensibilis Sirenko, 1973: 660, fig. 1.
Leptochiton (*Leptochiton*) *curvatus*: Kaas and Van Belle, 1985: 96–98, fig. 42.

Material examined. Korea: 3 individuals, Gangwon-do, Goseong-gun, Toseong-myeon, Ayajin-ri, 2 Sep 2016.
Description. Body elongate oval, small in size (length 12.5–16 mm, width 5–6 mm); valves bark-like, yellowish white and brown colored; apex of valves dark brown (Fig. 1A). Girdle brown to dark brown. Gills arrangement adanal and merobranchial (Fig. 1B).

Head valve semicircle in shape, small granules on and around apex, granules of irregular size and shape on thick concentric growth lines, posterior area widely V-shaped (Fig. 2A). Articulamentum white, no insertion plate, ventral teg-

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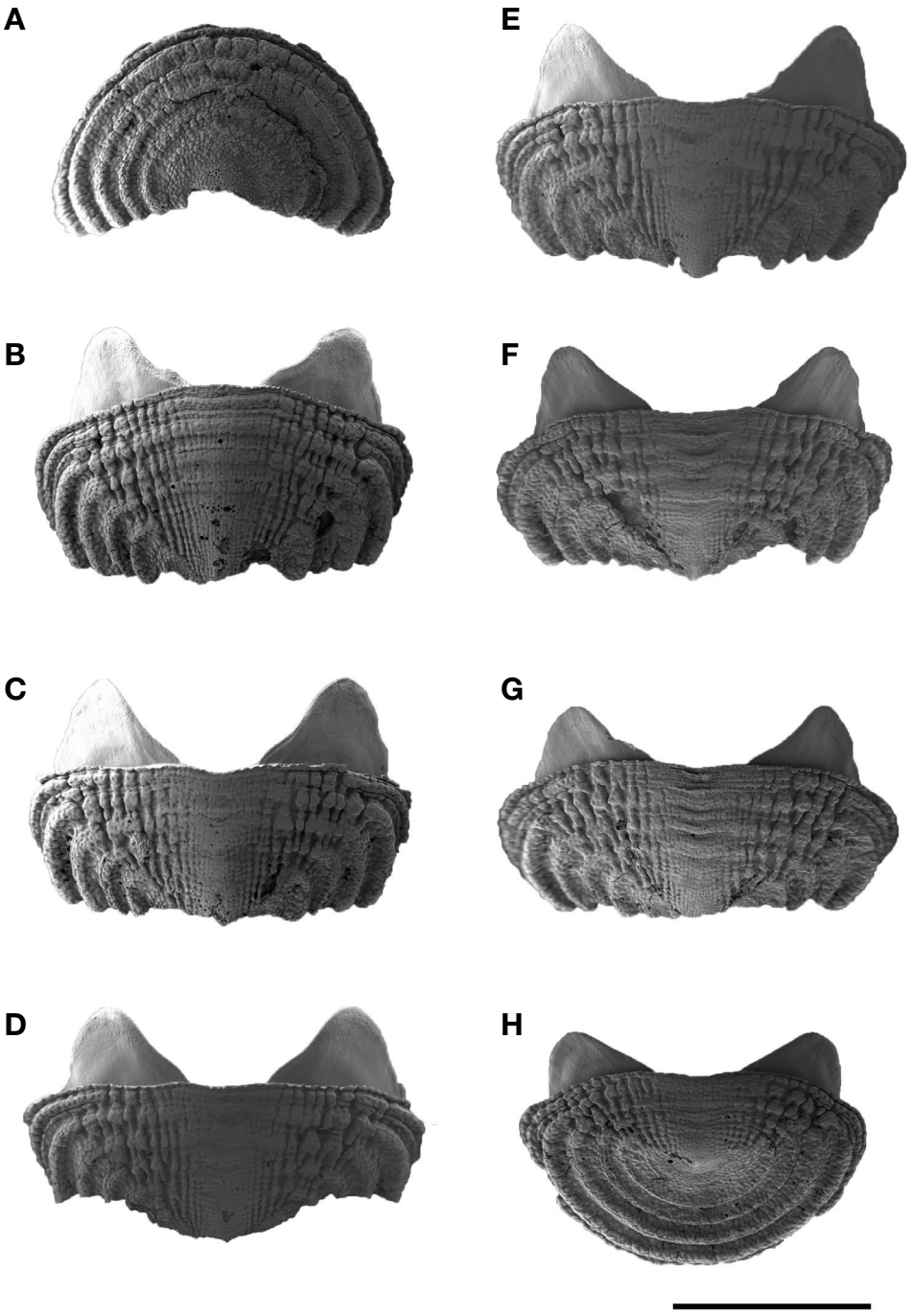

Fig. 2. *Deshayesiella curvata*. A, Head valve; B, 2nd valve; C, 3rd valve; D, 4th valve; E, 5th valve; F, 6th valve; G, 7th valve; H, Tail valve. Scale bar = 3 mm.

mental callus thin.

Intermediate valves rounded-rectangle shaped, growth lines present, anterior and side margins roundish, posterior margin somewhat protruding to form a central apex, both sides roughened by the growth lines, central area with distinguished jugal and pleural areas, jugal area wedge shaped and with small granules on longitudinal ridges, pleural area granules bigger than jugal area granules, lateral area granules on thick growth lines (Fig. 2B–G). Articulamentum white, apophysis rather thin and roundish-triangular, insertion plates reduced, without slit.

Tail valve semicircular, mucro antemedian, antemucronal

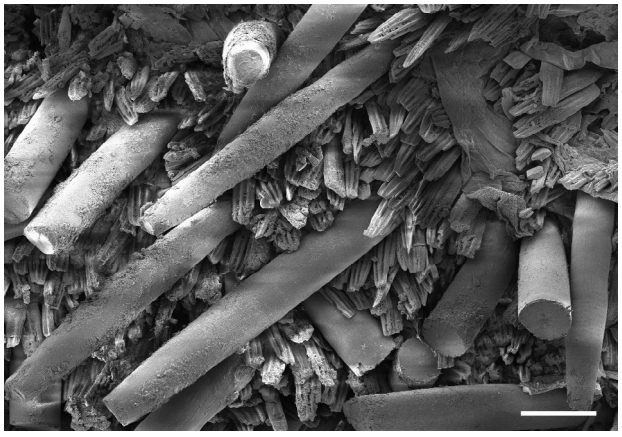

Fig. 3. Microstructure of spicules on girdle of *Deshayesiella curvata*. Scale bar = 50 μm.

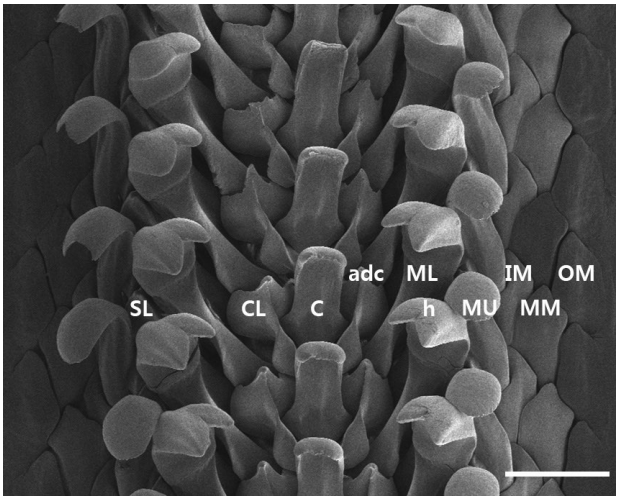

Fig. 4. Radula of *Deshayesiella curvata* from scanning electron microscope image. adc, antero-dorsal corner of centro-lateral tooth; C, central tooth; CL, centro-lateral tooth; h, head of major lateral tooth; IM, inner marginal tooth; ML, major lateral tooth; MM, middle marginal tooth; MU, major uncinus tooth; OM, outer marginal tooth; SL, small lateral tooth. Scale bar = 100 μm.

area short, granules longitudinal in arrangement and bigger on both sides, postmucronal area with thick growth lines, slope concave (Fig. 2H). Articulamentum white, apophysis similar to that of intermediate valves, no insertion plate on posterior region.

Girdle slightly narrow, disorderly covered with spicules and scales (Fig. 3). Spicules large, about 300 μm in length, white or opaque, with smooth surface and blunt tip. Scales small, 60–70 μm in length, dark brown, flat, longitudinally ribbed on surface.

Radula symmetrical rows (Fig. 4). Central tooth oblong, basal part wide. Centro-lateral tooth rather thin, antero-dorsal corner slightly protruding. Major lateral tooth with blunted bidentate cusps. Major uncinus tooth ladle shaped, with longitudinal groove on both sides. Marginal tooth flattened.

Habitat. Subtidal zone in rocky shores.

Distribution. Korea, Japan, and Russia.

Remarks. Four *Deshayesiella* species were previously classified into 3 different genera: *Hanleya* Gray, 1857 (*H. sinica, H. spicata*), *Leptochiton* Gray, 1847 (*L. curvata*), and *Oldroydia* Dall, 1894 (*O. bidentata*). These genera are distinguished by a number of morphological characters. *Deshayeisella* lacks an insertion plate on both terminal valves (Fig. 2A, H), while *Hanleya* has an insertion plate on the head valve (Kaas and Van Belle, 1985). In *Deshayesiella*, the jugal and pleural areas on the intermediate valve are separated (Fig. 2B–G) but their areal distinction is not clear in *Leptochiton* (Saito et al., 2008). *Deshayesiella* has a slightly reduced or unreduced pleural area (Fig. 2B–G), while *Oldroydia* has a pleural area which is reduced on the anterior part of the intermediate valves (Sirenko and Clark, 2008).

Ecologically, *D. curvata* differs from other congeneric species in that it inhabits shallow water. Morphologically, *D. curvata* and *D. sinica* have unreduced pleural areas on the intermediate valves (Fig. 2B–G) (Saito, 2012), but the other three congeneric species have slightly reduced pleural areas (Kaas and Van Belle, 1985; Saito et al., 2008; Sirenko and Clark, 2008). In addition, *D. curvata* can be easily distinguished from other congeneric species including *D. sinica* by having large, thick granules on valves (Figs. 1, 2) (Saito, 2012).

The partial mtDNA *cox1* sequences from 3 individuals were determined as DNA barcode (GenBank accession Nos. MH445300, MH445301, MH445302) and compared with the *D. curvata* sequence deposited on GenBank (accession No. HQ907843), differing less than 2 bp from each other.

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