INTRODUCTION

Coralline algae are the most distinctive and recognizable benthic marine plants and commonly constitute a dominant component of intertidal and subtidal communities (Woelkerling 1988). Despite their ubiquity, however, they are not easily recognised and have been a relatively poorly studied group of marine organisms (Maneveldt et al. 2008). In Korea, corallines are common both in intertidal and subtidal habitats, but they are poorly documented in the flora of Korea.

Lithothamnion Heydrich (1897) was proposed as conservation of the latter homonym for Lithothamnium Philippi (1837) (Woelkerling 1985). It was based on L. muelleri Lenormand ex Rosanoff collected from Western port bay, Victoria, Australia (Wilks and Woelkerling 1995: 555). Currently, 83 species of Lithothamnion have been recognized mostly based on morphological analyses (Guiry and Guiry 2018). Of them, Lithothamnion japonicum Foslie is recorded as endemic species in the North-west Pacific. Lithothamnion japonicum was originally reported with simple description from Murotan, Iburi Subprefecture, Hokkaido in Japan (Foslie 1900). It was characterized by a knotty thallus with subdichotomous branched or unbranched short and cylindrical protuberances, conical shaped spermatangial conceptacles, branched (dendroid) spermatangial systems formed on floor, walls, and roof ofconceptacle chamber, cylindrical shaped spermatangial conceptacle canal, 9–10 cell layered spermatangial conceptacle roof, raised tetra/bisporangial conceptacles without rims, flattened tetra/bisporangial conceptacle pore plate, 16–50 pores on each pore plate, 6–8 rosette cells surrounded by each pore, pore canal lining filaments composed of tetra/bisporangial conceptacle roof, and buried senescent tetra/bisporangial conceptacles completely infilled with relatively large and irregularly arranged calcified sterile cells. In this study, we report a new record of Lithothamnion japonicum from Korean coasts.

Keywords : Hapalidiaceae, Korea, Lithothamnion japonicum, morphology, taxonomy

Morphology of Lithothamnion japonicum (Hapalidiaceae, Rhodophyta):
A New Record of Coralline Species from Korea

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Abstract - The morphology, anatomy and reproduction (tetra/bisporic and male) of Lithothamnion japonicum Foslie was studied, using holotype material and materials collected from Korea. Lithothamnion japonicum is characterized based on the presence of encrusting, warty to fruticose thallus, branched or unbranched short and cylindrical protuberances, conical shaped spermatangial conceptacles, branched (dendroid) spermatangial systems formed on floor, walls, and roof of conceptacle chamber, cylindrical shaped spermatangial conceptacle canal, 9–10 cell layered spermatangial conceptacle roof, raised tetra/bisporangial conceptacles without rims, flattened tetra/bisporangial conceptacle pore plate, 16–50 pores on each pore plate, 6–8 rosette cells surrounded by each pore, pore canal lining filaments composed of tetra/bisporangial conceptacle roof, and buried senescent tetra/bisporangial conceptacles completely infilled with relatively large and irregularly arranged calcified sterile cells. In this study, we report a new record of Lithothamnion japonicum from Korean coasts.

Keywords : Hapalidiaceae, Korea, Lithothamnion japonicum, morphology, taxonomy

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observed their morphology and anatomy in detail and compared them with the holotypes of *L. japonicum* and *L. fretense* which was already synonymized with *L. japonicum*. In this study, we confirm the validity of *L. japonicum*, represent its detailed anatomical structures according in modern context, and extend its distribution to Korea.

**MATERIALS AND METHODS**

1. **Samples**

Fresh samples of *Lithothamnion japonicum* were collected from intertidal zones of Sokcho, Pohang, Oeyon-do, Heuksan-do, and Hong-do in Korea during 2014–2016 and compared with their type materials. Our samples were removed from the bedrock, small boulders, and mollusks using a chisel and hammer and were preserved in silica gel for anatomical morphology and molecular investigation. In addition, the holotype materials of *L. japonicum* and *L. fretense* were borrowed from the Herbarium, Museum of Natural History and Archaeology, Norwegian University of Science and Technology, Trondheim, Norway (TRH).

2. **Morphology**

   For light microscopy (LM), the dried samples in silica gel were completely decalcified in 0.6 M nitric acid for a minimum of 30 min until gas bubbles cease forming, rinsed with distilled water, and then stained using a 3:7 mixture of 1% aqueous aniline blue and glycerin solution (1:9 glycerol:80% ethanol) for more than 30 min. Sections of thallus (5–20 μm thickness) were prepared using an embedding matrix (O.C.T.; CellPath, Ltd., Newtown, Wales, UK) and a freezing microtome (Shandon Cryotome FSE; Thermo Shandon, Ltd., Loughborough, UK) and the sections were stained using a 3:7 mixture of 1% aqueous aniline blue and glycerin solution and then treated with Mayer’s hematoxylin (Electron Microscopy Sciences, PA, USA). Photomicrographs were taken using an Olympus microscope (BX51TRF; Olympus, Tokyo, Japan) and an Olympus DP71 camera.

   For scanning electron microscopy (SEM), the dried samples were fractured by hammer and chisel, mounted on aluminum stubs using double-sided adhesive carbon tape (Nisshin EM Co., Ltd., Tokyo, Japan), and coated with gold for 10 min using digital ion coater (SPR-20, COXEM Co., Ltd., Daejeon, Korea). The samples were examined using a COXEM EM-30 PLUS + scanning electron microscope (Mini SEM; COXEM Co., Ltd, Korea) with an accelerating voltage of 15 kV.

   Thallus anatomical terminology follows Chamberlain (1990). Morphological (growth forms) terminology follows Woelkerling et al. (1993). In cell measurement, length denotes the distance between primary pit connections, and diameter the maximum width of the cell lumen at right angles to this. Conceptacle measurements follow Adey and Adey (1973).

**RESULTS**

*Lithothamnion japonicum* Foslie, 1900  돌기ânlag (신칭) (Figs. 1–5)

**Heterotypic synonym:** *Lithothamnion fretense* Foslie, 1907.

**Holotype:** TRH C16-3267; Miyabe No. 7; slides 391, 1158 (Woelkerling et al. 2005: 456).

**Type locality:** Muroran, Iburi Subprefecture, Pacific Coast, Hokkaido, Japan (Yoshida 1998; Woelkerling et al. 2005: 456).

**Material examined:** Holotype of *Lithothamnion japonicum*, TRH C16-3267 (Muroran, Iburi Subprefecture, Hokkaido, Japan, collected by Miyabe, 21.iii.1897, attached on the holdfasts of *Laminaria*, tetrasporophyte); holotype of *L. fretense*, TRH C15-3228 (Kaifuura, Echigo Province, Japan, collected by Yendo, no date, 1899, no habitat data, tetrasporophyte); CUK12890 (Daepo harbor, Daepo-dong, Sokcho-si, Gangwon-do, Korea, collected by S.Y.J and T.O.C, 31.vii.2014, epilithic on rock in intertidal zone, tetrasporophyte); CUK13407 (Oeyondo-ri, Ocheon-myeon, Boryeong-si, Chungcheongnam-do, Korea, collected by S.Y.J and T.O.C, 20.i.2015, epizoic on limpet in intertidal zone, tetrasporophyte); CUK13415, CUK13417, CUK13428 (Gorageum, Oeyondo-ri, Ocheon-myeon, Boryeong-si, Chungcheongnam-do, Korea, collected by S.Y.J and T.O.C, 19.i.2015, epilithic on rocks in intertidal zone, male and tetrasporophyte); CUK15495 (Seokbong 2-ri, Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do, Korea, collected by S.Y.J and T.O.C, 26.xii.2015, epizoic on gastropods in intertidal zone, tetra-
Morphology of Lithothamnion japonicum

Vegetative morphology: Plants are dark red in color, encrusting, firmly attached to substrate (sometimes easy to detach) with or without conspicuous white edges, matt textured, warty to knobby (Figs. 1A–C, 5A). Protuberances are short, cylindrical, branched or unbranched (simple), 5–7 mm long and 6–60 mm wide at the distal end. Surface cells have thickened calcareous ridges that surround central concavities (Fig. 2A). Thalli are pseudoparenchymatous, monomeroius, and dorsiventrally organized (Fig. 2B). In protuberances, they are composed of a central core of filaments and a peripheral region where distal portions of core filaments or their derivatives curve outwards towards the thallus surface (Fig. 5B). Haustoria are absent. The crustose portion of the thallus is 147–716 μm thick and composed of medulla and cortex. The medulla is plumose (non-coaxial), 21–91 μm thick, and comprised of approximately 5–12 cell layers, which is usually less than 8–39% of the mature thallus thickness. Cells of the medullary filament are rectangular to elongated with rounded corners, 2–3 times as long as wide, 6–27 μm in length, and 3–7 μm in diameter (Fig. 2C). The ventrally situated medullary filaments curve downwards toward the substratum and terminate into more- or less triangular-shaped cells (Fig. 2C). The medullary cells are composed of filaments running more or less parallel to the thallus surface (Fig. 2D). Medullary filaments curve toward the thallus surface to form a zoned cortex (cortical region). The cortex is 70–624 μm and comprising 61–92% of the thallus thickness. Cells of the cortical filaments are square to oblong, 2–13 μm in length, and 4–6 μm in diameter (Figs. 2E and 5C). Epithallial cells are arranged in a single layer, flattened and flared, 1–3 μm

Fig. 1. Diversity growth forms of Lithothamnion japonicum. A. Holotype specimen of Lithothamnion japonicum (TRH C16-3267); B. Epilithic specimens of L. japonicum (CUK13415) on the rock; C. Epizoic specimens of L. japonicum (CUK13407) on shell. Scale bars: A–C = 1 cm.
in length, and 4–5 μm in diameter (Figs. 2F and 5D). Subepithallial initials are rectangular with rounded corner, as long as or longer than the cells immediately subtending them, 2–9 μm in length, and 3–7 μm in diameter (Figs. 2F and 5D). Fusions between the cells of contiguous medullary and cortical filaments are abundant and frequently occupy most of

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**Fig. 2.** Vegetative structures of *Lithothamnion japonicum* (TRH C16-3267, CUK13407, CUK13415, and CUK13428). A. Surface view of thickened calcareous ridges surrounding the central concavities; B. Vertical section view of thallus; C. Vertical section view of ventrally situated medullary filaments showing their termination in cells with angular end walls (arrows); D. Vertical section view of medullary systems showing monomorous construction; E. Vertical section view of cortical filaments; F. Vertical section view of outer cortical filaments showing flared epithallial cells (e) and subepithallial initials (i) that are as long as or longer than the subtending them; G. Vertical section view of cortical region showing cell fusion (f) connecting the adjacent filament. Scale bars: A, F = 5 μm; B, D, E = 20 μm; C = 10 μm; G = 2 μm.
the walls of adjoining cells (Fig. 2G). Secondary pit connections and trichocytes have not been observed.

**Reproductive structures**: Gametangial thalli are dioecious. In male plants, the spermatangial conceptacles are initially more or less flush with the thallus surface, shed a thin white cap (Fig. 3A), raised to conical shape, occur mainly on the tips of protuberances, and 46–61 μm in thickness above the thallus surface without rims (Fig. 3A and B). Spermatangial...
Fig. 4. Tetra/bisporangial structures of *Lithothamnion japonicum* (TRH C16-3267, CUK12890, CUK13407, CUK13415, and CUK13417). A. Surface view of tetra/bisporangial conceptacles; B. Surface view of tetra/bisporangial conceptacles showing a raised multiporate pore plate; C. Detailed view of pore plate comprised of a number of pores; D. Surface view of pores (p) bordered by a rosette of 6–7 cells (labelled 1 to 6–7); E–G. Developmental series of tetra/bisporangial conceptacle formation; H. Vertical section view of tetra/bisporangial conceptacles showing tetrasporangia (t); I. Detailed view of apical plugs (arrows) and pore canal lining cells (asterisks); J. Vertical section view of old tetra/bisporangial conceptacle showing peripheral filaments enclosing the chamber; K. Vertical section view of several buried tetra/bisporangial conceptacle chambers; L. Vertical section view of buried tetra/bisporangial conceptacle chamber infilled with calcified sterile cells. Scale bars: A = 200 μm; B, K, L = 100 μm; C, H = 50 μm; D, I = 10 μm; E–G, J = 25 μm.
conceptacles are 308–452 μm in external diameter. Conceptacle chambers are hemispherical, 83–145 μm in diameter, and 26–59 μm in height (Fig. 3C and D). Spermatangial conceptacle primordia develop from small group of subepithallial initials of the dorsal region of the thallus. Protective cells above spermatangial initials are not observed in young male conceptacles. Branched (dendroid) spermatangial systems develop across the floor, walls, and roof in mature conceptacles (Fig. 3D). During the early developmental stages of conceptacle, a group of meristematic cells differentiates into spermatangial initials. Spermatangial initials cut off into two to five spermatangial mother cells that then form a dense, deeply stained layer constituting the fertile area of the conceptacle (Fig. 3E–G). Spermatangial mother cells are elongated and divided to form dendroid branching systems and cut off the spermatangia (2 × 2 μm in size) (Fig. 3H). The roofs of conceptacles are 27–36 μm thick and formed from peripheral filaments that arch over and enclose the chamber. Pore canals of conceptacles are cylindrical, 17–20 μm in diameter, and 35–40 μm in length (Fig. 3I). The roof filaments are composed of 9–10 cell layers and their terminal initials are developed into papillae along the pore canal (Fig. 3I). Papillae project into the pore canal and are orientated more or less parallel to perpendicular to the conceptacle roof surface (Fig. 3I). Senescent male conceptacles are buried in the tissue (Fig. 3C). Female plants have not been observed.

In tetra/bisporangial plants, conceptacles are multiporate, raised to low-domed to polygonal shape, crowded, not confluent, 208–407 μm in external diameter, and 28–70 μm in thickness above the surrounding thallus surface without rims (Fig. 4A–C). Pore plates are flattened, approximately 152–225 μm in diameter, and composed of 16–50 pores (Fig. 4C). The pores are usually crowded in the central part on the pore plates (Fig. 4C), surrounded by 6–8 rosette cells, and not protruded or sunken (Fig. 4D). Conceptacle primordia develop from small group of subepithallial initials within the dorsal region of the thallus (Fig. 4E–G). Tetra/bisporangial chambers are rounded to elliptical, 114–340 μm in diameter, and
85–173 μm in height (Fig. 4H). Tetrasporangia are zonately divided, scattered across the chamber floor, 55–100 μm in height, and 12–20 μm in diameter. Bisporangia were not detected. The roofs of tetra/bisporangial conceptacles are 16–32 μm in thickness and composed of pore canals, pore canal lining filaments, and roof filaments. The pore canals of conceptacles are 4–7 μm in diameter and blocked by apical plugs (Fig. 4I). The filaments lining the pore canal are composed of 4–6 cells and are similar to roof filaments in the shape and size (Fig. 4I). Senescent tetra/bisporangial conceptacles are buried and completely infilled with relatively large and irregularly arranged calcified sterile cells (Fig. 4J and K).

**Habitat and phenology:** Plants are collected from high to low intertidal zone in sheltered to wave-exposed area of the reef. They occur in pink to rose-colored patches. Holotype material of *L. japonicum* was epiphytic on the holdfasts of *Laminaria*. Most plants grow on gastropod, limpet, or rock. Male plants are collected in January and May. Tetra/bisporangial plants are collected in January, March, May, July, and December.

**World distribution:** Korea, China, and Japan.

**Deposition:** Collected specimens are deposited in the National Institute Biological Resources (NIBR) and herbarium of Chosun University (CUK), Korea.

**Identifier:** Tae Oh Cho and So Young Jeong.

## DISCUSSION

Within the genus *Lithothamnion*, most species are poorly known, and in the absence of a world monograph, species delimitation is attended by many uncertainties. The original diagnostic characters of many coralline species have been based on only a few anatomical features of slight or doubtful taxonomic relevance (Aguirre and Braga 2005). Because of the lack of reliable definitions, available names in the literature have rarely been used by subsequent authors (Bassi *et al.* 2007). Recently, however, reassessments of the original collections and freshly material have been carried out in the context of modern taxonomic understanding (e.g. Basso and Rodondi 2006; Tâmega *et al.* 2014; Peña *et al.* 2018).

*Lithothamnion japonicum* is characterized such as encrusting, warty to fruticose thallus, branched or unbranched short and cylindrical protuberances, conical shaped spermatangial conceptacles, branched (dendroid) spermatangial systems formed on floor, walls, and roof of conceptacle chamber, cylindrical shaped spermatangial conceptacle canal, 9–10 cell layered spermatangial conceptacle roof, raised tetra/bisporangial conceptacles without rims, flattened tetra/bisporangial conceptacle pore plate, 16–50 pores on each pore plate, 6–8 rosette cells surrounded each pore, pore canal lining filaments composed at tetra/bisporangial conceptacle roof, and buried senescent tetra/bisporangial conceptacles completely infilled with relatively large and irregularly arranged calcified sterile cells (Foslie 1900; Baba 2000; Xia 2013). In this study, we observed that the morphology of our fresh materials collected from Korea correspond to those of the holotype of *Lithothamnion japonicum*. However, while the protuberances in holotype are subdichotomously to irregularly branched, protuberances in our materials collected from Korea are unbranched. Although many species have been delimited using apparent differences in the extent and nature of protuberance development, as evidenced (Woelkerling 1988), Adey (1966) provided conclusive evidence that protuberance development and form are highly variable within species and event within single plants and that this variability results from a variety of ecological conditions. Indeed, the development and form of protuberances have provided additional problems associated with species delimitation in *Lithothamnion* (Woelkerling 1988). Moreover, a number of studies have shown that considerable variation in growth-form can occur within a species (e.g. see Penrose and Woelkerling 1991; Woelkerling and Campbell 1992; Woelkerling and Harvey 1992). As the result, the differences of the shape of protuberances within *L. japonicum* did not seem to be significant enough to indicate separate species.

Gametangial conceptacles of *Lithothamnion japonicum* are very poorly known. When Foslie (1900) described *L. japonicum*, only tetra/bisporangial conceptacles were found. Masaki (1984) found both spermatangia and carpogonia within single conceptacles of *L. japonicum*. Baba (2000) illustrated with photographs of spermatangial conceptacles from *L. japonicum*. He recognized its characters in very simple way as follows: 1) conical shape of spermatangial conceptacles, 2) branched spermatangial filaments on the conceptacle chamber, and 3) senescent male conceptacles buried in to the thallus. In this study, we observed the detailed anatomical mor-
Morphology of Lithothamnion japonicum

We suggest the followed additional diagnostic characters for the delimitation of species level of genus Lithothamnion: 1) shape of spermatangial conceptacles, 2) raised or sunken degree of spermatangial conceptacles, 3) shape of spermatangial conceptacles chamber, 4) shape of spermatangial conceptacle canal, 5) number of cells of spermatangial conceptacle roof, and 6) presence or absence of buried spermatangial conceptacles.

Although Lithothamnion japonicum has been reported from Japan and China (Okamura 1936; Yoshida et al. 1990; Yoshida 1998; Liu 2008; Zeng 2009; Xia 2013), in this study, we confirm extension of its distribution to the Northeast Asia including Korea.

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