

⟨Original article⟩

## New Record of Two *Derbesia* Species (Chlorophyta) in Korea

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**Abstract** - Two siphonous green algae were collected from the eastern coast of Korea. These species share the typical features of *Derbesia* sporophytes, such as erect and prostrate siphonous filaments and the presence of basal septum in lateral branches. One is characterized by the combined features of a relatively small tufted sporophytic thalli arising from a rhizoidal base, subdichotomously branched filaments with a basal septum and one pyrenoid per subspherical to lenticular chloroplast. The other shows a larger sporophytic thallus, sparsely subdichotomously branched filaments with a basal septum and one pyrenoid per spherical chloroplast. In a phylogenetic tree based on *rbcL* sequence, the two above-mentioned Korean algae nest in the same clades as *Derbesia minima* and *D. indica*, respectively. The genetic distance between the sequences within the clades was 0.5–0.8%, which is considered to be included in the intra-specific range for the genus. These two siphonous Korean algae are identified as *D. minima* and *D. indica*, respectively, based on the morphological and molecular analyses. These species are newly recorded in the Korean marine algal flora herein.

**Keywords** : *Derbesia minima*, *D. indica*, green algae, first record, Korea

### INTRODUCTION

*Derbesia* Solier, which belongs to Bryopsidales J.H. Schaffner (Lam and Zechmann 2006; Verbruggen *et al.* 2009), is a siphonous green algal genus with a thallus composed of erect and prostrate filaments and multiflagellate zoospores. This genus was established based on *D. marina* (Lyngbye) Solier (1846), which was originally described from Quivig, Faerøes, Denmark (Lyngbye 1819). At that time, only the general features of filaments and sporangia with gross morphology were given to the type species (Lyngbye 1819). However, many studies on life history, with great interest, increased our knowledge of *Derbesia* (Kornmann 1938; Feldmann 1950; Ziegler and Kingsbury 1964; Page 1970; MacRaid and Womersley 1974; Mayhoub 1976; Kobara and Chihara 1981, 1984). It was first clarified by Kornmann (1938) that

*Derbesia marina* and *Halicystis ovalis* (Lyngbye) Areschoug are sporophytic and gametophytic phases of the one species. This suggested that *Derbesia* shows an alternation of heteromorphic generations with a *Halicystis* phase, which had been regarded as an independent genus characterized as having a coenocytic thallus consisting of an erect vesicular part and a rhizoidal part, although some authors (Kornmann 1966, 1970; Sears and Wilce 1970) have shown that direct reproduction of the sporophyte of *D. marina* can occur, as in *Pedobesia* established based on *D. clavaeformis* from Australia (MacRaid and Womersley 1974; Silva *et al.* 1996).

*Derbesia* is distinguished from the most similar genus *Pedobesia* MacRaid & Womersley in having basal septum at the portion of branching and heteromorphic life history with a gametophytic *Halicystis* stage and in lacking calcium carbonate deposited inside the cell wall (MacRaid and Womersley 1974; Woolcott *et al.* 2000, Table 2). This genus is globally distributed from the tropics to Arctic waters, and includes about 20 species worldwide (Guiry and Guiry 2018).

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Of these, three species have been recorded in Korea (Lee and Kang 1986, 2002; Kim *et al.* 2013). In the course of a floristic study of Korean marine algae, two siphonous green algae were collected from the eastern coast of Korea. This study reports unrecorded two *Derbesia* species based on morphological and molecular analyses.

## MATERIALS AND METHODS

Samples for the present study were collected from the eastern coast of Korea. Some specimens were preserved in 5–10% formalin seawater, and pressed on herbarium sheets. A portion of the material was dried and preserved in silica gel for molecular analysis. Sections of the thallus were mounted in 20% corn syrup for permanent slides.

Total genomic DNA was extracted from a silica-gel-preserved sample using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Before extraction, dried material was crushed with liquid nitrogen using a mortar and pestle. Concentrations of extracted DNA were assessed using gel electrophoresis on a 1% agarose gel. Extracted DNA was used for amplification of *rbcL* regions using following primers (forward: 5'-TCCAA AACTGAAACTAAAGCAGG-3', reverse: 5'-AATTTCT TTCCAAACTTCACAAGC-3') (Lam and Zechman 2006).

PCR amplifications were performed in a TaKaRa PCR Thermal Cycler Dice with an initial denaturation step at 94°C for 5 min followed by 35 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min and a final extension at 72°C for 7 min. The reaction volume was 20 µL, consisting of 20 ng of genomic DNA, 2 µL of 10x PCR buffer, 2 µL of 200 µM dNTP, 1 µL each of forward and reverse primer, and 0.5 units of Taq polymerase (Takara Korea, Korea). Amplifications were examined using gel electrophoresis in a 1% agarose gel and amplified *rbcL* region products were purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The PCR products were moved to Macrogen Sequencing Service for sequencing (Macrogen, Seoul, Korea).

Sequences for the *rbcL* region were aligned using BioEdit (Hall 1999). Phylogenetic analyses were performed using the maximum-likelihood (ML), neighbor-joining (NJ) and unweighted pair group method using arithmetic averages (UPGMA) methods. Bootstrap values were calculated with

1,000 replications. *RbcL* sequences of other species were obtained from GenBank. *Bryopsis pennata* J.V. Lamouroux was used as an outgroup.

## RESULTS AND DISCUSSION

### *Derbesia minima* Weber-van Bosse 1913: 95.

Type locality: Kalimantan Selatan, Selat Makassar, Lumu-Lumu, Borneo bank, Indonesia (Huisman 2015).

Korean name: Jag-eun-eong-kin-sil nom. nov. (신칭: 작은 영킨실).

Specimens examined: NIBRCL0000112655, MGARB012361, MGARB012362, MGARB012363, MGARB012364 (Gisung-ri: 5.vi.2016).

Habitat: Epiphytic near the lower intertidal.

Morphology: Sporophytic thalli up to 1 cm high, usually epiphytic on other algae, tufted, light green in color (Fig. 1A, B), with erect and prostrate systems; many erect filaments arising from a prostrate system with rhizoidal base, siphonous, cylindrical, 30–60 µm diam., subdichotomously branched several times (Fig. 1C); filamentous lateral branches with a basal septum (Fig. 1C); chloroplasts numerous, subspherical to lenticular, relatively small, measuring 3–10 µm long and 2–5 µm wide, each with a pyrenoid (Fig. 1D). Sporangia and gametophytes were not observed during the present study.

### *Derbesia indica* (R. Patel & Isabella) P.C. Silva 1996: 810.

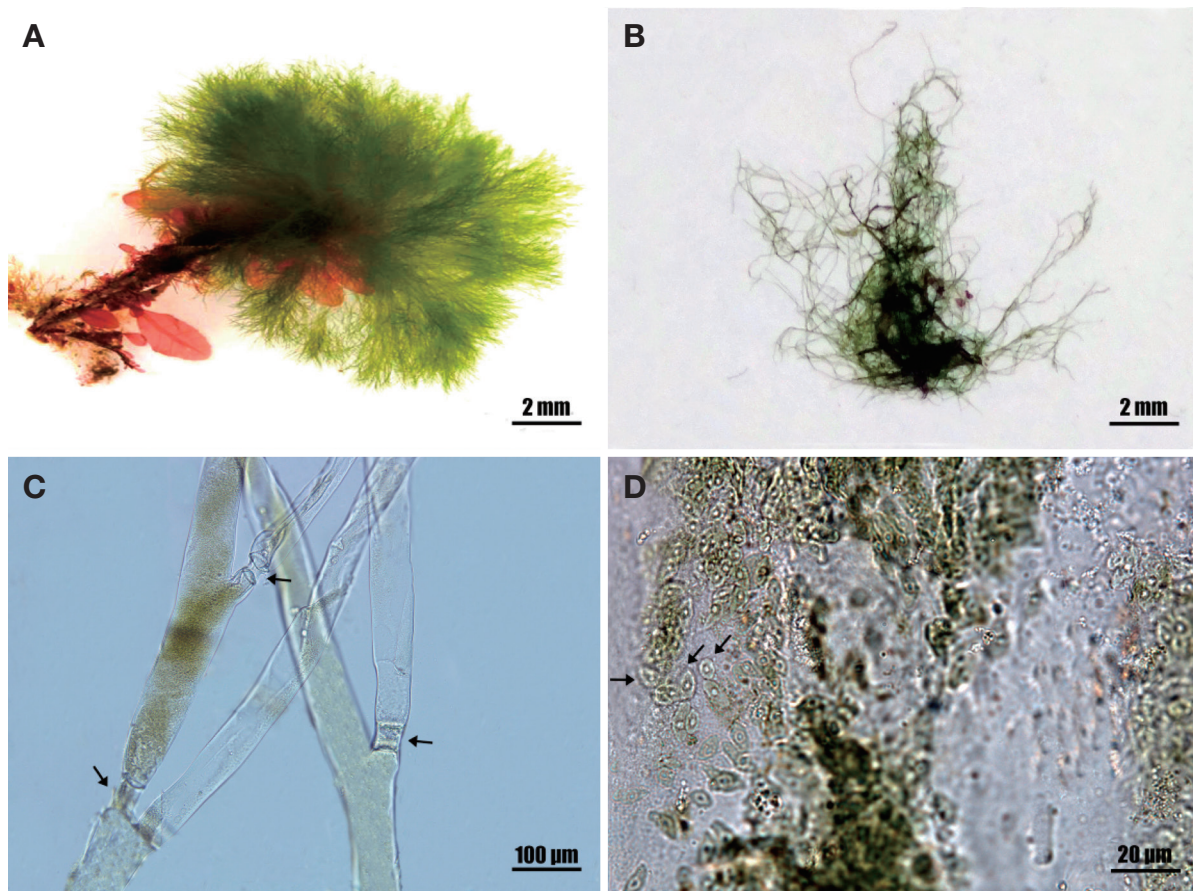
Type locality: Mithapur near Port Okha, Gujarat, India (Silva *et al.* 1996).

Korean name: Na-gog-eong-kin-sil nom. nov. (신칭: 나곡 영킨실).

Specimens examined: NIBRCL0000112654, MGARB012365, MGARB012366, MGARB012367, MGARB012368 (Nagok-ri: 24.vii.2015).

Habitat: Epilithic or epiphytic near the intertidal.

Morphology: Sporophytic thalli up to 2 cm high, epilithic or epiphytic on other algae, tufted, light green in color (Fig. 2A, B), composed of erect and prostrate systems; many erect filaments arising from rhizoidal base of a prostrate system, siphonous, cylindrical, 50–80 µm diam., sparsely subdichotomously branched (Fig. 2C); filamentous lateral branches

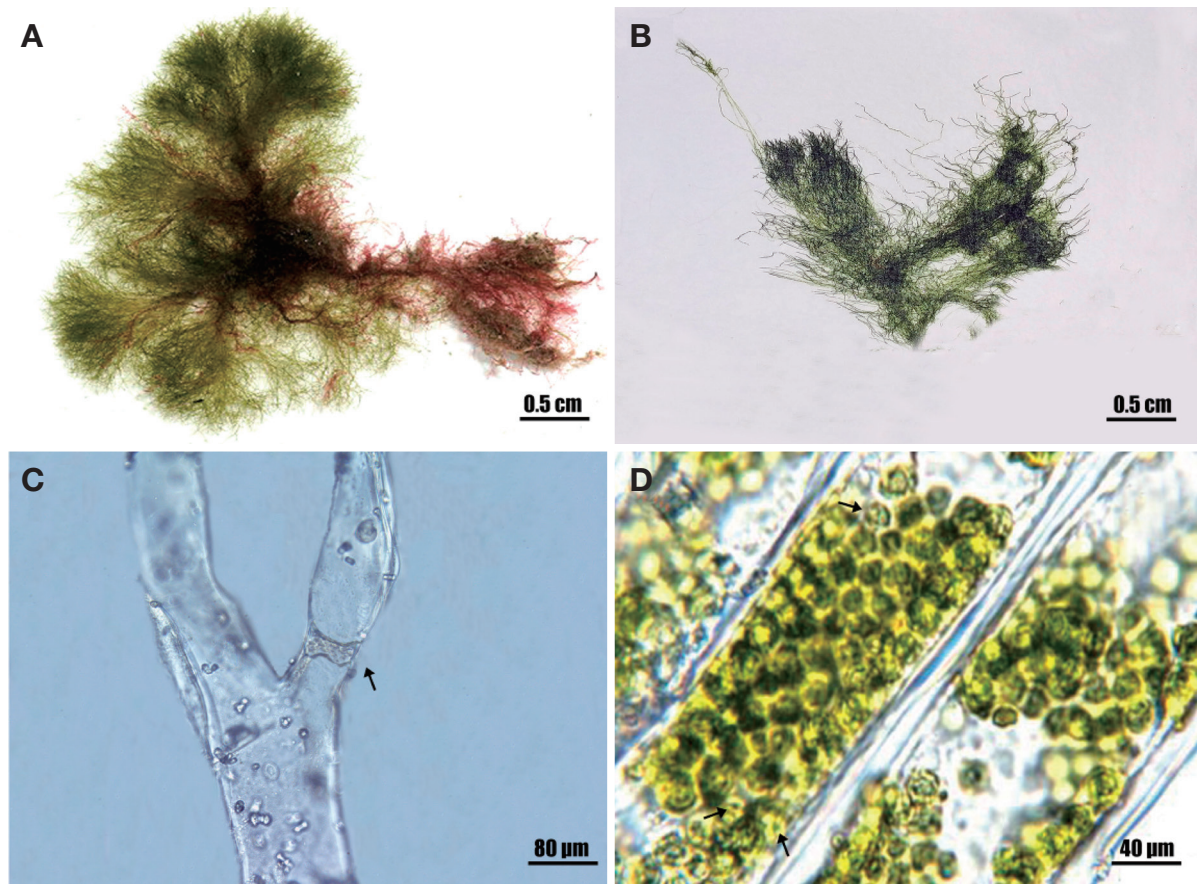


**Fig. 1.** *Derbesia minima* Weber-van Bosse. A: Sporophytic plant being epiphytic on other algal species; B: Herbarium specimen of sporophytes; C: Subdichotomously branched filaments with a basal septum (arrows); D: Subspherical to lenticular chloroplasts (arrows) with a pyrenoid in thalli.

with a basal septum (Fig. 2C); chloroplasts numerous, spherical, measuring 5–15 µm diam., each with a pyrenoid (Fig. 2D). Sporangia and gametophytes were not observed during the present study.

*Derbesia minima*, which occurs in various localities including Japan, Australia, New Zealand and Micronesia (Guiry and Guiry 2018), was originally described from Indonesia (Huisman 2015). This species is characterized by the combined features of relatively small tufted sporophytic thalli arising from an irregularly lobed rhizoidal base, cylindrical erect branches with transverse and basal septum, ovoid or pyriform sporangia with a short pedicel and double basal septa, and each subspherical chloroplast with one pyrenoid (Huisman 2015; the present study). Of these features, the basal septum at point of branching is one of the important

generic features of *Derbesia* (MacRaid and Womersley 1974; Woolcott *et al.* 2000). According to Huisman (2015), apart from this basal septum, transverse septa in vegetative siphons are also occasionally observed in this species from Australia. *D. minima* is distinguished from similar species with a small thallus (lower than 5 mm in height), such as *D. attenuate* E.Y. Dawson, *D. furcate* R.W. Ricker and *D. padinae* Trono, by the occasional presence of the septum (Dawson 1954; Trono 1971; Ricker 1987; Huisman 2015). *Derbesia minima* appears to be distinct in having a transverse septum, which is not a typical feature of the genus. These transverse septa were not found in Korean specimens. The Korean specimens also differ from the species from Australia and Japan in branching of erect filaments. It shows subdichotomous branching several times, while Australian and Japanese species branches once or not at all (Yoshida 1998; Huisman



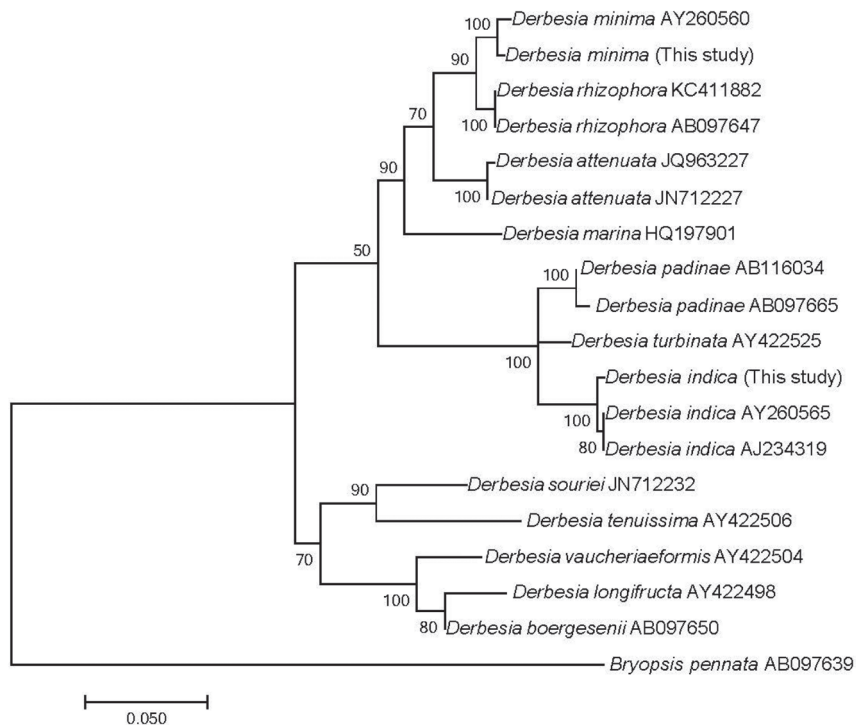
**Fig. 2.** *Derbesia indica* (R. Patel & Isabella) P.C. Silva. A: Habit of sporophytic plant; B: Herbarium specimen of sporophytes; C: Sparsely subdichotomously branched filaments with a basal septum (arrow); D: Numerous spherical chloroplasts (arrows) possessing a pyrenoid.

2015). However, other vegetative features are shared with *D. minima*. Although sporangia and gametophytes were not observed, the Korean specimens appear to be referred to *D. minima* based on vegetative morphology. This is also supported by molecular data.

*Derbesia indica* was originally described from India based on gametophytes (Patel and Isabella 1978). So, at that time, it was established as *Halicystis indica*, but later transferred to *Derbesia* (Silva *et al.* 1996). This species is characterized by the combined features of tufted sporophytic thalli arising from rhizoidal base, sparsely subdichotomously branched filaments with a basal septum and each spherical chloroplast with one pyrenoid (the present study). *D. indica* is similar to *D. minima*, *D. attenuata*, *D. tenuissima* (Moris & De Notaris) P. Crouan & H. Crouan, and *D. boergesenii* (M.O.P. Iyengar & Ramanathan) Mayhoub in having one pyrenoid per chloroplast. However, *D. indica* is readily distinguished from *D.*

*minima* and *D. attenuata* by thallus size. *D. indica* shows a relatively large thallus (about 2 cm), while the latter two species have thallus height of about 2 mm and 5 mm, respectively. *D. tenuissima* differs from *D. indica* in lacking a cross septum (Wormersley 1984; Kim and Klochkova 2010) or in occasionally having a basal septum in lateral branches (Abbott and Huisman 2004; Huisman 2015). *D. boergesenii* is also distinguished from *D. indica* in branching pattern and size of lateral filaments. The former species shows irregular branching and a relatively small size of 15–36 μm diameter (Mayhoub 1976) rather than sparsely subdichotomously branching and large size of 50–80 μm diameter. More importantly, it is distinguished from *D. indica* in being fusiform rather than spherical chloroplast (Mayhoub 1976).

In a phylogenetic tree based on *rbcL* sequence, the Korean algae nest in the same clades as *D. minima* and *D. indica*, respectively (Fig. 3). According to Woolcott *et al.* (2000,



**Fig. 3.** Phylogenetic tree of selected taxa obtained from maximum-likelihood analysis based on *rbcL* sequences. Bootstrap percentages (1000 replicates samples) are shown above the branches. Scale bar = 0.05 substitutions/site.

Table 4), the genetic distance for *rbcL* sequence between *Derbesia* and *Pedobesia* is 13.9%. In the present study, the interspecific genetic distance in *Derbesia* was calculated as 1.4–11.9%. Based on these data, the genetic divergence between the sequences within the clades, which was 0.5–0.8%, is considered to be included in the intra-specific range for the *Derbesia*.

These morphological and molecular analyses indicate that the two siphonous Korean algae are identified as *Derbesia minima* and *D. indica* originally described from Indonesia and India, respectively. This study reports the first occurrence of the species in Korea.

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