

## *Monostroma alittorale*, a marine green algal species newly recorded in Korea

Jae Woo An, Pil Joon Kang and Ki Wan Nam\*

Department of Marine Biology, Pukyong National University, Busan 48513, Republic of Korea

### \*Corresponding author

Ki Wan Nam

Tel. 051-629-5922

E-mail. [kwnam@pknu.ac.kr](mailto:kwnam@pknu.ac.kr)

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**Abstract:** A marine green algal species (Chlorophyta) was collected from the eastern coast of Korea. It is morphologically characterized by monostromatic thallus, usually undulate and entire margins, cap-like chloroplast and several pyrenoids per cell. In a phylogenetic tree based on molecular data, the Korean alga nests in the same clade as *Monostroma alittorale* originally described from Japan, as a sister clade of *M. grevillei* from France. The genetic distance for ITS (Internal Transcribed Spacer) sequences among *Monostroma* species ranges from 2.3% to 38.2%. The value between the Korean entity and *M. alittorale* was calculated as 0.01%, considered to be intraspecific divergence. This Korean entity is identified as *Monostroma alittorale* based on morphological and molecular analyses. This is the first record of *M. alittorale* in Korea.

**Keywords:** Chlorophyta, molecular analyses, morphology, first record, *Monostroma alittorale*

## INTRODUCTION

*Monostroma* Thuret (Monostromataceae), which is distributed in all aquatic habitats from freshwater to seawater environments (Loughnane *et al.* 2008), is a green alga with monostromatic leafy blades of 2–30 cm (or more) in size. This genus was lectotypified with *Monostroma bullosum* (Roth) Thuret (Pfeiffer 1874), and is delimited by some vegetative and reproductive features (Guiry and Guiry 2019).

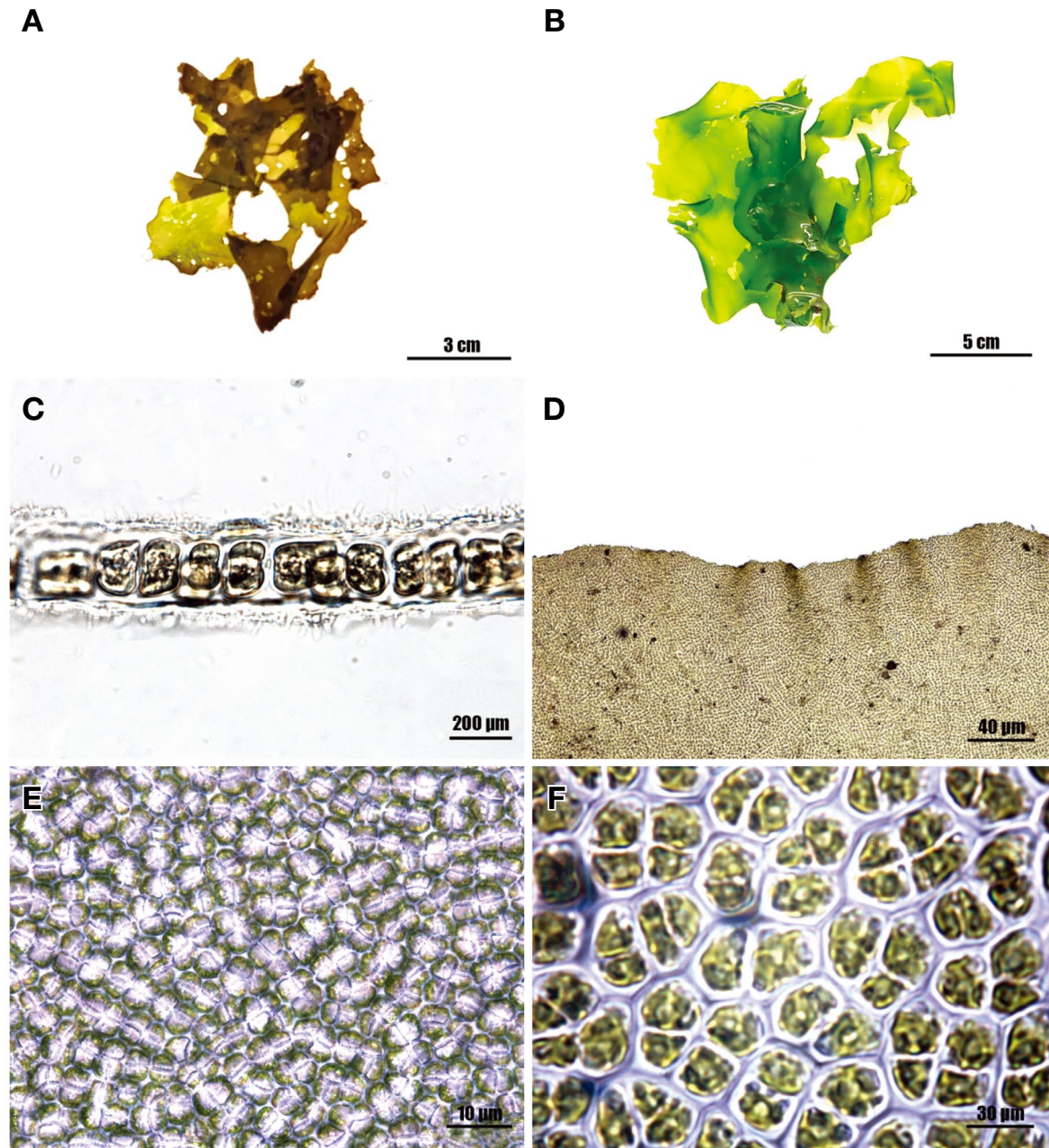
Species belonging to the genus *Monostroma* are classically defined on the basis of morphological characteristics such as size and shape of cells and thickness of thalli (Wittrock 1866; Bast *et al.* 2015). However, this makes it difficult to identify *Monostroma* species, because of the simple thallus structure (Heesch *et al.* 2009). Culture studies have been conducted in some of the taxa, and have resulted in taxonomical revisions (Gayral 1964; Bliding 1968; Bast

*et al.* 2015). Recently, many entities that had been included in *Ulva* were transferred to *Monostroma* based on molecular analyses (Brodie *et al.* 2007; Sfriso 2010; Alongi *et al.* 2014; Guiry and Guiry 2019). Therefore, about 30 species are currently accepted in this genus worldwide (Guiry and Guiry 2019).

A total of nine species are currently recorded in Korea (Lee and Kang 1986, 2002; Lee 2008; Bae 2010; Kim *et al.* 2013). During a survey of marine algal flora, one monostromatic species (Chlorophyta) was collected from the eastern coast of Korea. This Korean entity was first recorded based on morphological and molecular analyses in the present study.

## MATERIALS AND METHODS

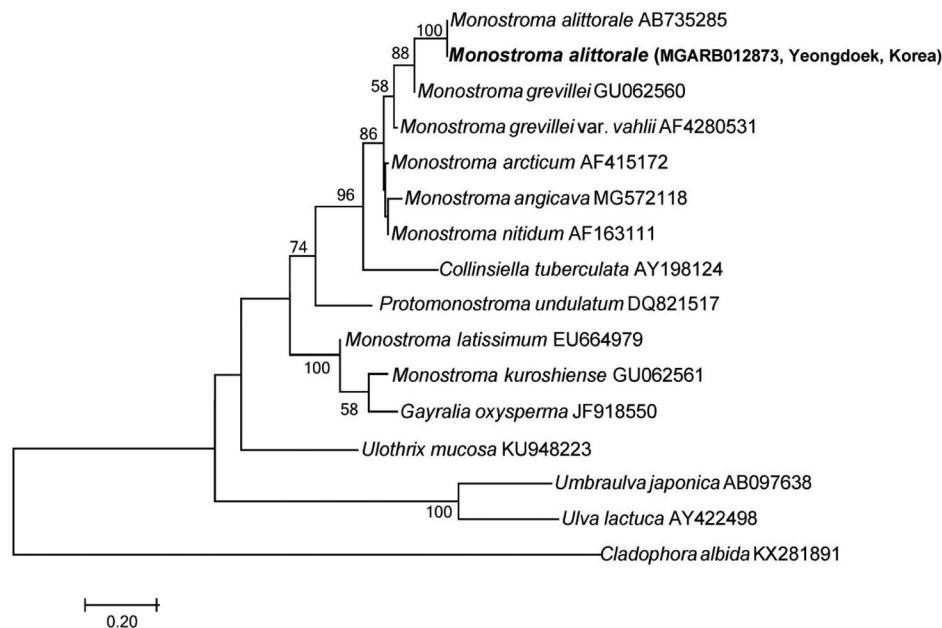
Samples for the present study were collected from Sa-



**Fig. 1.** *Monostroma alittorale* T. Tanaka & K. Nozawa. A. Herbarium specimen (MGARB012873). B. Habit of the vegetative plant. C. Monostromatic thallus with cap-like chloroplast in the transverse section. D. Undulate and entire margin of the thallus. E. Surface view of the rectangular-to-polygonal cells with round corner near the upper portion. F. Several pyrenoids in the surface view.

jin-ri, Yeongdeok located on the eastern coast of Korea. All specimens were preserved in 5–10% formalin seawater solution, 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 preparation.

Total genomic DNA was extracted from silica-gel-preserved sample using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Extracted DNA was used for amplification of ITS regions using following primers (Ent18SA: 5' GAGGCA ATAACAGGTCTGTGATGC 3', ITS2: 5' GCTGCGTT



**Fig. 2.** Phylogenetic tree of *Monostroma* species obtained from ML (Maximum-likelihood) analysis based on ITS (Internal Transcribed Spacer) sequences. Bootstrap percentages (1,000 replicates samples) are shown above the branches. Scale bar = 0.20 substitutions/site.

CTTCATCGATGC 3', ITS4: 5' TCCTCCGCTTATTGATATGC 3') (White *et al.* 1990; Blomster *et al.* 1998). 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  $\mu$ L, consisting of 20 ng of genomic DNA, 2  $\mu$ L of 10 $\times$  PCR buffer, 2  $\mu$ L of 200  $\mu$ M dNTP, 1  $\mu$ L each of forward and reverse primer, and 0.5 units of Taq polymerase (Takara Korea, Korea). The PCR products were moved to MacroGen Sequencing Service for sequencing (MacroGen, Seoul, Korea). The PCR primers were also used for sequencing.

Sequences for the ITS region were aligned using BioEdit (Hall 1999). Phylogenetic analysis was performed using the maximum-likelihood (ML) methods. Bootstrap values were calculated with 1,000 replications. *Cladophora albida* (Nees) Kutzing was used as an outgroup.

## RESULTS AND DISCUSSION

### *Monostroma alittorale* T. Tanaka & K. Nozawa in Tanaka 1963: 75

Korean name: Dong-hae-hot-pa-rae nom. nov.  
(신칭: 동해홀파래)

**Specimens examined:** MGARB012873–MGARB012874 (Sajin-ri, Yeongdoek, 17 Feb. 2016), MGARB012875–012877 (Sajin-ri, Yeongdoek, 25 Sep. 2019).

**Habitat:** Epilithic near the lower intertidal.

**Type locality:** Mage Island, Tanegashima, Japan

**Morphology:** Thalli 5–12 cm high (Fig. 1A, B), erect, membranous, monostromatic (Fig. 1C), usually unbranched or little branched conical to ligulate shape, light to dark green in color, soft in texture, attached by a small cuneate holdfast (< 5 mm) on rocks near the lower intertidal, with usually undulate and entire margin (Fig. 1D), 40–60  $\mu$ m thick in the upper portion, 80–120  $\mu$ m thick in the basal portion; cells usually arranged in pairs, rectangular to polygonal near the middle to upper portion, oval to rectangular with round corners near the basal portion in the surface view (Fig. 1E, F), transformed into rhizoidal cells near the base, 15–25  $\mu$ m  $\times$  10–15  $\mu$ m, with a length to width ratio of 1.6–1.8 in the transverse section; chloroplasts cap-like, parietal, with several pyrenoids (3–4  $\mu$ m) (Fig. 1C, F).

*Monostroma alittorale* was originally described from Japan (Tanaka 1963). Since then, the occurrence of this species has only been recorded in Japan (Yoshida *et al.* 1990; Yoshida 1998; Guiry and Guiry 2019). According to Tanaka (1963), this species seems to be characterized by having undulate or entire margin without microscopic teeth, short cuneate stipe and somewhat costate thalli in the basal por-

tion, chromatophore in most of the cells not filling the interior, and several pyrenoids per cell.

Most of these features including gross morphology are also found in our Korean specimens with the exceptions of a lack of basal costate thallus and cap-like parietal position of chloroplast. However, the basal features of thallus and chloroplast position appear to be variable with habitat.

Several studies have used the ITS region to analyze the phylogenetic relationships among *Monostroma* species (Hayden *et al.* 2003; Hayden and Waaland 2004; Bast *et al.* 2014; Bast 2015). *Monostroma* appears to not be monophyletic based on the ITS sequence (Fig. 2). *M. latissimum* Wittrock and *M. kuroshiense* F. Bast form the same clade as *Gayralia* K.L. Vinogradova. Phylogenetic consideration of these species needs further investigation. In a phylogenetic tree based on the ITS sequence (Fig. 2), the Korean alga nests in the same clade as *M. alittorale* from Japan, as a sister clade of *M. grevillei* (Thuret) Wittrock from France sharing a polygonal cell shape and several pyrenoids per cell.

According to Bast *et al.* (2014, table 3), the genetic distance for ITS sequences among *Monostroma* species ranges from 0.48% to 6.55%. The values were calculated as 2.3%–38.2% (the present study). The genetic distance of 0.01% between sequences from the Korean specimens and *M. alittorale* from Japan suggests that our specimens are referred to *M. alittorale*. Based on these morphological and molecular data, the Korean alga is identified as *M. alittorale*, which is first recorded in the Korean marine algal flora.

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