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〈Original article〉

New Record of a Marine Algal Species, *Membranoptera alata* (Delesseriaceae) in Korea

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Abstract - A marine algal species was collected from Sacheonjin, Gangneung located on the eastern coast of Korea during a survey of marine algal flora. This alga shares the generic features of *Membranoptera* belonging to the subfamily Delesserioideae and is characterized by the presence of combined features of membranous, monostromatic thalli attached by a solid discoid holdfast, blades with a conspicuous terete stipe-like midrib and microscopic lateral veins, entire margins, irregularly alternate to dichotomous branching, and obtuse apices growing apically. In a phylogenetic tree based on *rbc*L sequences, the Korean alga nests in the same clade with *M. alata* from the eastern North Atlantic. The genetic distance between both the sequences within the clade was calculated as 0.0%. Based on the morphological and molecular analyses, this Korean species is identified as the generic type, *M. alata*. This is the first record in the list of Korean marine algal flora.

Keywords : *Membranoptera alata*, Delesseriaceae, marine alga, first record, Korea

INTRODUCTION

The Delesseriaceae is a large red algal family that includes about 100 genera inhabiting the intertidal and subtidal zone (Lin *et al*. 2001; Wynne 2001; Nam and Kang 2012; Guiry and Guiry 2018). This family has been divided into three subfamilies, Delesserioideae, Nitophylloideae and Phycodryoideae, based on molecular analyses (Lin *et al*. 2001). In the subfamily Delesserioideae procarps are located on primary axes (except in *Pseudophycodrys* Skottsberg) near the blade tip, whereas in the Nitophylloideae the procarps are scattered on the blade surface and a fusion cell is lacking and the pit connections between gonimoblasts cells broaden (Maggs and Hommersand 1993; Wynne 1996; Lin *et al*. 2001; Nam and Kang 2012). In the Phycodryoideae, the procarps are scattered and a large fusion cell is formed by the progressive incorporation of neighboring gametophytic and inner gonimoblast cells around the pit connections (Lin *et al*. 2001). The Delesserioideae consists of several tribes, including the Delesserieae (Kylin 1956; Lin *et al*. 2001; Wynne 2001).

Membranoptera Stackhouse (1809) belongs to the tribe Delesserieae within the subfamily Delesserioideae and was established based on three entities, *M. alata, M. angustifolia* and *M. costata*, all of which are representative of *Fucus alatus* Hudson (1762) (Papenfuss 1950). This genus has the following features: growth by a transversely dividing apical cell, discoid holdfast, monostromatic to polystromatic blades, branching from margin, presence of midrib, presence or absence of macroscopic or microscopic veins, absence of intercalary cell division, spermatangial sori scattered, procarps borne on both sides of blade near apex and tetrasporangia produced from surface cells, and is distinguished from other members of the tribe Delesserieae by those combined features (Wynne 1985; Maggs and Hommersand 1993; Nam and Kang 2012). It is distributed on both sides of the North Atlantic and in the northeastern North Pacific (Wynne and Saunders 2012). Eleven species are currently accepted in this

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genus (Guiry and Guiry 2018).

In Korea, only *Membranoptera robbeniensis* Tokida, originally described from Robben Island (Sea of Okhotsk), has been reported (Kang 1966; Nam and Kang 2012). During a survey of marine algal flora, a red algal species belonging to the family Delesseriaceae was collected from Sacheonjin, Gangneung on the east coast of Korea. This alga was identified based on morphological and molecular analyses and is newly recorded in Korea herein.

MATERIALS AND METHODS

Specimens for this study were collected from Sacheonjin, Gangneung on the east coast of Korea. Taxonomic data were obtained from fresh, liquid-preserved and herbarium specimens. Liquid-preserved material was stored in a 10% solution of formalin/seawater. Blades dissected from the cleared materials were hand-sectioned, transferred to a slide with distilled water, and mounted in pure glycerin. Measurements are given as width and length. For permanent slides, the glycerin was exchanged with 10−20% corn syrup.

Total genomic DNA was extracted from silica-gel-preserved samples 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. Extracted DNA was used for the amplification of ribulose-1, 5-bisphosphate carboxylase large subunit (*rbc*L) regions. For *rbc*L, the gene was amplified in three overlapping parts with the primer pairs F*rbc*L start (5′-TGTGTTGTCGACATGTCTAACTCTGTAGAAG-3′) - R753 (5′-GCTCTTTCATACATATCTTCC-3′), F492 (5′-CG TATGGATAAATTTGGTCG-3′) - R1150 (5′-GCATTTGTC CGCAGTGAATACC-3′), and F993 (5′-GGTACTGTTGTA GGTAAATTAGAAGG-3′) - R*rbc*S (5′-TGTGTTGCGGCC GCCCTTGTGTTAGTCTCAC-3′) (Freshwater and Rueness 1994). PCR amplifications were performed in a TaKaRa PCR Thermal Cycler Dice (TaKaRa Bio Inc., Otsu, Japan). PCR was performed with an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C, with a final 7-min extension at 72°C. The PCR products were moved to the Macrogen Sequencing Service for sequencing (Macrogen, Seoul, Korea). Sequences for the *rbc*L region were aligned using BioEdit (Hall 1999). Phylogenetic analyses were performed using neighbor joining and maximum-likelihood methods. Bootstrap values were calculated with 1,000 replications. *Rbc*L sequences of other species were obtained from GenBank. *Ceramium virgatum* Roth was used as an outgroup.

RESULTS AND DISCUSSION

***Membranoptera alata* (Hudson) Stackhouse 1809**

Korean name: Nal-gae-geu-mur-ip nom. nov. (신칭: 날개그물잎).

Type locality: ?

Specimens examined: NIBR00002113415 (Sacheonjin, Gangneung, Korea: 03.vii.2017), MGARBb000752 (Sacheonjin, Gangneung, Korea: 03.vii.2017).

Habitat: Epilithic in upper to lower intertidal.

Morphology: Thalli up to 1−5 cm high, membranous, monostromatic except for midrib, irregularly alternately to dichotomously branched in one plane, bright to dark red in color, attached by a solid discoid holdfast (Fig. 1A); blades 0.5−2.0 mm wide, without intercalary cell division, with a conspicuous midrib 0.1−0.5 mm in width, with microscopic lateral veins and dentate margins, with 1-2 cell-layered cortex around midrib in middle and lower portion (Fig. 1B−E); apical cell obtuse, hemispherical, transversely dividing, 10−15 μm in diameter (Fig. 1F). Sexual and tetrasporangial plants were not found during the present study.

Membranoptera was lectotypified with *M. alata* (Papenfuss 1950). Previously, the type species had been recognized on both sides of the North Atlantic (Rosenvinge 1923, 1924; Taylor 1962; Bird and McLachlan 1992; Maggs and Hommersand 1993; Sears 1998; Loiseaux-de Goer and Noailles 2008; Wynne 2013). However, Wynne and Saunder (2012) reported that the type species is restricted to Europe, as suggested by Hommersand and Lin (2009). They clarified that *M. alata* from the North American Atlantic coast is genetically distinct from the European entity and is conspecific with *Pantoneura fabriciana* (Lyngbye) M.J. Wynne, one of two species of *Pantoneura* Kylin which was usually recognized in that region, but it was difficult to separate it from *Membranoptera*. Thus, it has been known with the combined name *M. fabriciana* (Lyngbye) Wynne et Saunder (Guiry and

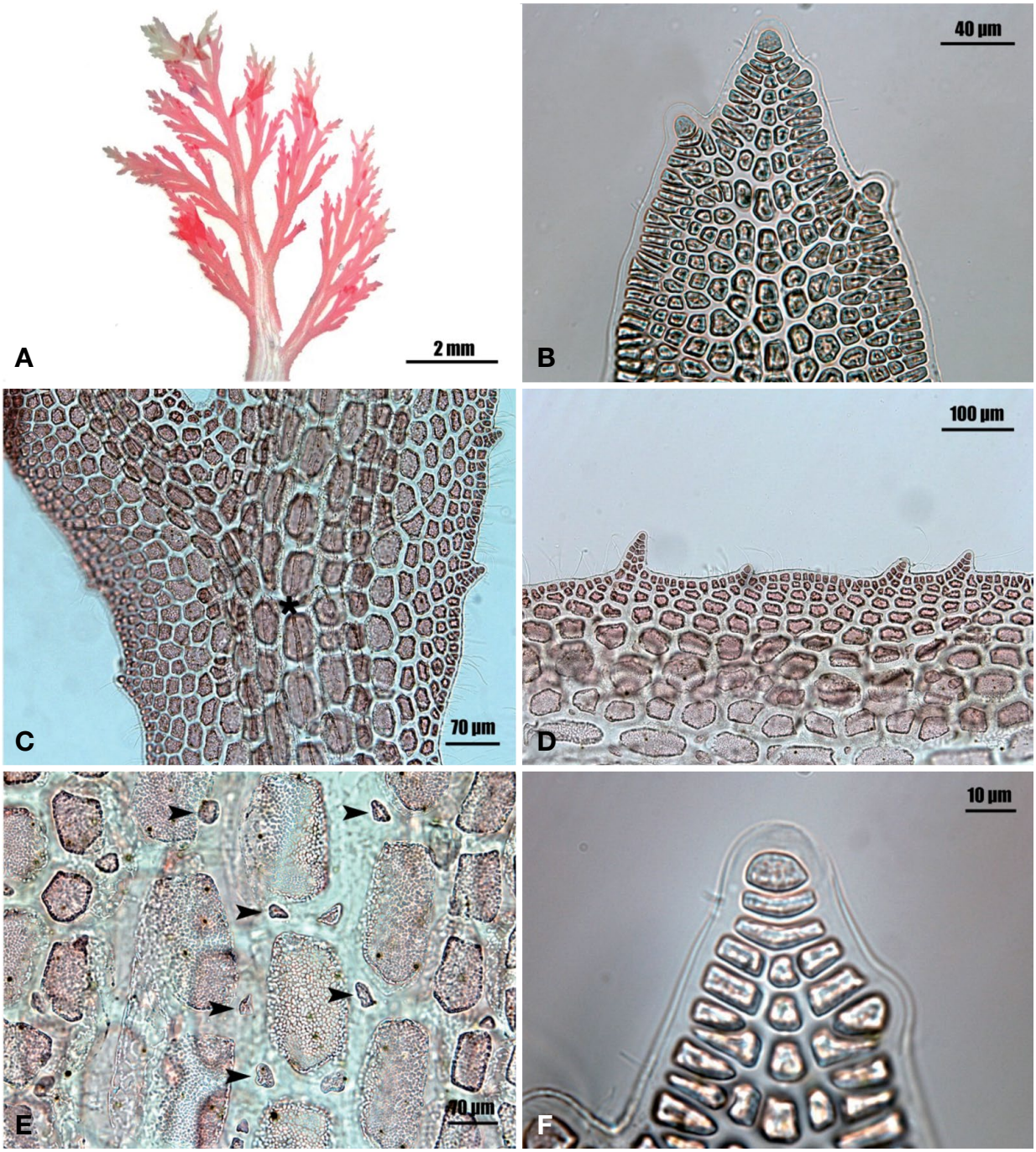

Fig. 1. *Membranoptera alata*. A. The habit of vegetative plant. B. Cell arrangement without intercalary cell division in the upper portion of the branch. C. Branch with a conspicuous midrib (asterisk). D. The dentate margin of the branch. E. Initial cell (arrowheads) of cortex around midrib in the middle portion of the blade. F. Branch apex with a distinct hemispherical apical cell dividing transversely.

Guiry 2018). *M. alata* appears to be distinct from *M. fabriciana* in having relatively wide blades, axillary laterals and cystocarp with non-projecting ostiole (Maggs and Hommersand 1993; Wynne 1997). It also distinguished from *M. spinulosa* (Ruprecht) Kuntze with syntype localities in the Sea of Okhotsk and Bering Sea, but recently occurring in the northeastern Atlantic (Mathieson *et al*. 2009), by branching type, axillary tufts and cystocarp shape (Wynne 1970; Maggs

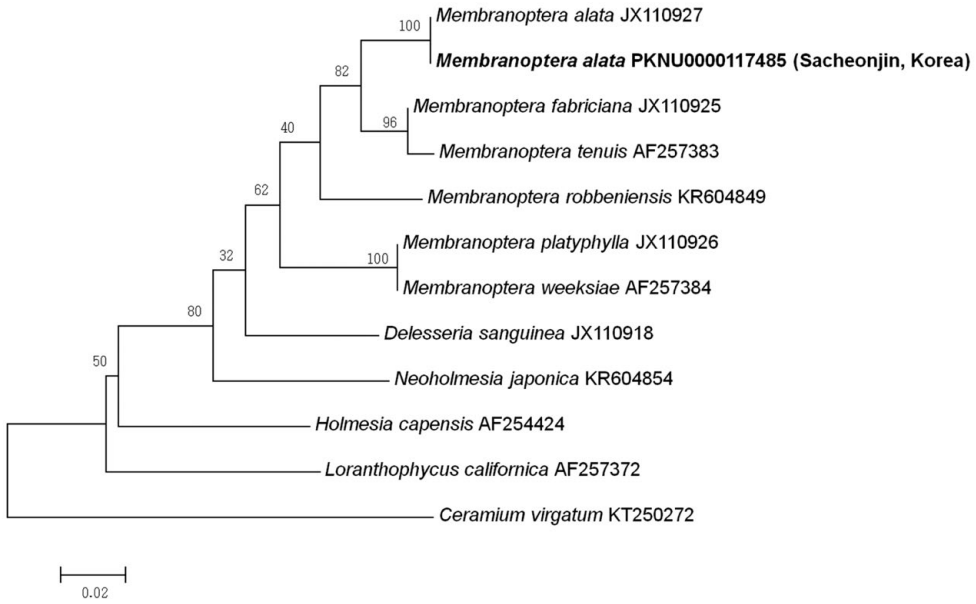

Fig. 2. Phylogenetic tree of *Membranoptera* species obtained from a maximum-likelihood method based on *rbc*L sequences. Bootstrap proportion values (1,000 replicates samples) are shown above the branches. Scale bar = 0.02 substitutions/site.

and Hommersand 1993). *Membranoptera platyphylla* (Setchell et N.L. Gardner) Kylin, which had been previously recognized as several species in *Membranoptera* populations from the northeastern North Pacific (Wynne and Saunders 2012), is distinct from *M. alata*, but it is needed to examine the characters used to distinguish it from *M. multiramosa* N.L. Gardner and *M. spinulosa* (Gabrielson *et al*. 2004, 2006).

The Korean alga collected from Sacheonjin in the present study shares the generic features of *Membranoptera* (Fig. 1) and fits the description of *M. alata* by Maggs and Hommersand (1993). This is supported by molecular data (Fig. 2).

In a phylogenetic tree based on *rbc*L sequences, the Korean alga nests in the same clade with *M. alata* from Ireland in the eastern North Atlantic as a sister clade of *M. fabriciana* and *M. tenuis* (Fig. 2). In general, the value of interspecific divergence in the family Delesseriaceae varies from 5.6% to 15.95% (Lin *et al*. 2001), and in *Membranoptera* it was calculated as 3.4−11.6% (the present study). The genetic distance between both sequences within the clade was 0.0%. Based on these morphological and molecular analyses, this Korean species is identified as the generic type, *M. alata*, the distribution of which is currently known as being restricted to Europe (Hommersand and Lin 2009; Wynne and Saunder 2012). This is the first record of *M. alata* in Korea.

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