

New record of *Callophyllis mageshimensis* (Gigartinales, Kallymeniaceae) in Korea

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Abstract: A marine red algal species was collected from Pyengdae-ri, Gujwa in Jeju, Korea during a survey of marine algal flora. This alga shares the generic features of *Callophyllis* (Gigartinales, Kallymeniaceae), and is characterized by erect and membranous thalli, branches dichotomously or trichotomously divided, margin somewhat undulate or entire and tetrasporangia scattered irregularly in the cortex. In a phylogenetic tree based on *rbcL* sequences, the Korean alga nests in the same clade as *C. mageshimensis* originally described from Japan. The genetic distance between both sequences within the clade was 0.0–0.1%. Based on the morphological and molecular data, the alga was identified as *Callophyllis mageshimensis*. This is the first record of *C. mageshimensis* in Korean marine algal flora.

Keywords: *Callophyllis mageshimensis*, red alga, morphological and molecular data, Korea

INTRODUCTION

Kallymeniaceae Kylin (Gigartinales, Rhodophyta), which includes approximately 44 genera and 193 species, is characterized by a female reproductive structure with one or several 3-celled carpogonial branches on the supporting cell and sterile subsidiary cells of similar morphology to the lower two carpogonial branch cells, which are large and often prominently lobed (Womersley 1994). The auxiliary cell systems in non-procarpic genera are also distinctive, with several 1- or 2-celled chains of subsidiary cells (Womersley 1994). Many taxa in this family have refractive, darkly staining, usually branched stellate or ganglionic cells in the medulla (Womersley 1994).

Callophyllis Kützinger is the most diverse genus in the family. This genus involves approximately 64 species that occur in cold and temperate waters of both hemispheres (Guiry and Guiry 2020). The distinction among *Callophyllis* species is

based on vegetative features such as color, size, and branching pattern, as well as reproductive characteristics such as number of carpogonial branches per supporting cell, position and size of cystocarps and number of ostioles, and whether or not tetrasporangia are produced in sori (Dawson 1954; Norris 1957; Arakaki *et al.* 2011).

Eleven species are currently recorded in algal flora of Korea (Lee and Kang 2002; Kim *et al.* 2013; Lee and Kim 2014). During a survey of marine algal flora, a species belonging to *Callophyllis* was collected from Jeju in Korea. This was identified based on morphological and molecular data, and is newly recorded in Korea.

MATERIALS AND METHODS

Specimens for this study were collected from Pyengdae-ri, Gujwa in Jeju, 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 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. Extracted DNA was used for amplification of ribulose-1, 5-bisphosphate carboxylase large subunit (*rbcL*) regions. For *rbcL*, the gene was amplified in three overlapping parts with the primer pairs *FrbcL* start (*S'*-TGTGTTGTCGACATGTCTA-*ACTCTGTAGAAG-3'*) - R753 (*S'*-GCTCTTTCATACATATCTTCC-*3'*), F492 (*S'*-CGTATGGATAAATTTGGTCG-*3'*) - R1150 (*S'*-GCATTTGTCCGCAGTGAATACC-*3'*), and F993 (*S'*-GGTACTGTTGTAGG-TAAATTAGAAGG-*3'*) - *RrbcS* (*S'*-TGTGTTGCGCC-*CGCCCTTGTGTT AGTCTCAC-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 MacroGen Sequencing Service for sequencing (MacroGen, Seoul, Korea). Sequences for the *rbcL* 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. *RbcL* sequences of other species were obtained from GenBank. *Kallymenia reniformis* (Turner) J. Agardh was used as an outgroup.

RESULTS AND DISCUSSION

Callophyllis mageshimensis Tanaka 1963

Korean name: Je-ju-bul-geun-ip nom. nov.

(신칭: 제주붉은잎)

Type locality: Mageshima, Tanegashima, Japan (Tanaka 1963).

Specimens examined: NIBRAL0000152471, NIBRRD0000004777, NIBRRD0000004778 (Pyeongdae-ri, Jeju: 04.xi.2013).

Habitat: Epilithic or epiphytic on other algae near upper to lower intertidal.

Morphology: Thalli 3–6 cm high, erect, compressed, bright to dark red in color, membranous in texture, attached to substratum by rhizoidal pad; main axes issuing branches dichotomously, trichotomously or occasionally palmately and proliferations very rarely on the margin, 5–20 mm in width; branches with round apex, somewhat undulate or entire in margin; cortex of small, round to elliptical cells of 2–5 × 3–5 μm in size, one to three cell layers thick; medullary cells large, ovoid to elliptical, intermixed with filaments of small cells, 30–100 μm in diam.; tetrasporangia scattered irregularly in cortex near the apex of branch, produced from the inner cortical cell, round to elliptical, with cruciate division, 10–15 × 10–15 μm in size. Sexual plants were not observed during the present study.

Callophyllis species have been recognized based on following features: thallus size and shape, branching pattern, marginal type, size and location of cystocarps, shape and number of ostioles, and presence or absence of tetrasporangial sori (Setchell 1923; Dawson 1954; Norris 1957; Abbott and Norris 1966; Womersley and Norris 1971; Lee and Kim 2014).

According to the original description (Tanaka 1963), *Callophyllis mageshimensis* is characterized by thinly membranaceous thalli and di- or trichotomously or somewhat palmately divided branches, minutely undulate or entire margin and cystocarps without horn-like protuberances. Even though cystocarpic plants were not collected in the present study, the vegetative features were confirmed in the Korean alga collected from Jeju (Fig. 1).

It appears that the presence or absence of tetrasporangia sori is an important diagnostic characteristic in *Callophyllis* except for Chilean species (Arakaki *et al.* 2011). Unlike in *Callophyllis linearis* (Abbott and Hollenberg 1976), tetrasporangia in *Callophyllis mageshimensis* do not form sori, but are scattered in the cortex (Fig. 1F).

Callophyllis mageshimensis is similar to *C. repens* in having a creeping habit. However, while the latter species has adherent branches attached to the substratum by discoid and minute radical discs, the former has no such adherent branches. *C. mageshimensis* is also distinguished from the latter in having a round rather than acute apex in branches.

The Korean alga collected from Jeju is referred to *C. mageshimensis* based on the morphological features. This is also supported by molecular analysis (Fig. 2).

In the phylogenetic tree based on *rbcL* sequence, the sequence of the Korean alga nests in the same clade as that

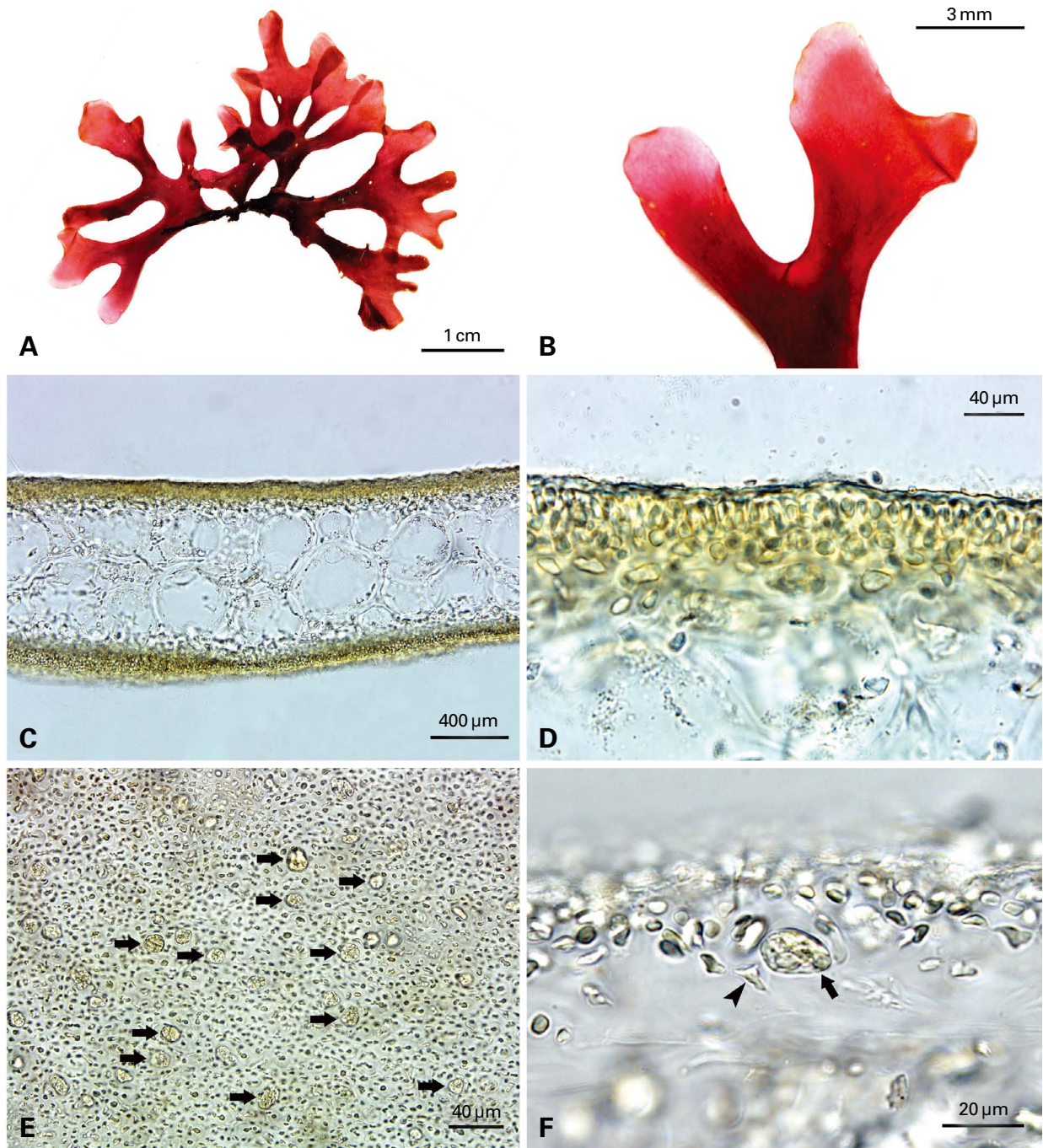


Fig. 1. *Callophyllis mageshimensis* Tanaka. A. Habit of vegetative plant. B. Details of branch with round apex. C. Transverse section of branch with round medullary cells. D. Cortical cell layers. E. Tetrasporangia (arrows) scattered irregularly in cortex in surface view. F. Developing tetrasporangium (arrow) from cortical cell (arrowhead).

of *C. mageshimensis* with a genetic distance of 0.0–0.1% (Fig. 2). These morphological and molecular data suggest that this Korean species should be identified as *C. mageshi-*

mensis. This is the first record of *Callophyllis mageshimensis* in Korea.

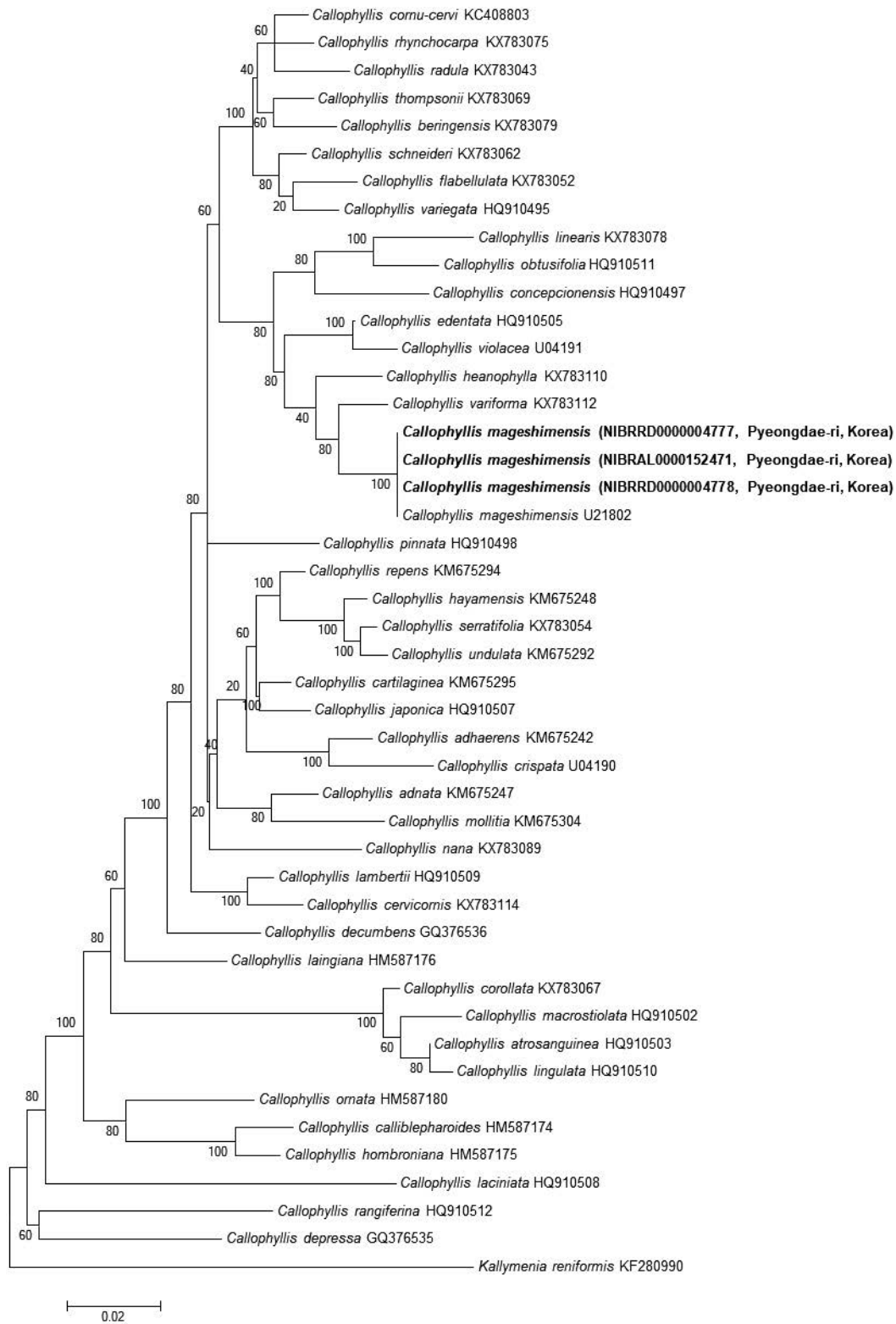


Fig. 2. Phylogenetic tree of *Callophyllis* species obtained from maximum-likelihood method based on *rbcL* sequences. Bootstrap proportion values (1,000 replicates samples) are shown above branches. Scale bar = 0.02 substitutions/site.

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