

## Note

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# *Umbraulva yunseulla* sp. nov. (Ulvaceae, Chlorophyta) from a subtidal habitat of Jeju Island, Korea

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Specimens of *Umbraulva* with greenish iridescent were collected in the subtidal zone of Jeju Island, Korea. To investigate these collections, plastid *rbcL* and *tufA* sequencing of six greenish iridescent specimens, including four *Umbraulva japonica*, were analyzed. Phylogenetic analysis of a concatenated multigene alignment found that the greenish iridescent specimens belonged to a yet undescribed taxon in the genus *Umbraulva*. We herein propose the name *Um. yunseulla* sp. nov. for this specimens. Juveniles of *Um. yunseulla* sp. nov. resemble the generitype *Um. japonica* in appearance, showing globular to subglobular and funnel-shaped habits, but the blades of this new species are not split longitudinally like those of *Um. japonica*. Although the multigene phylogenetic tree showed the polyphyletic clade of *Umbraulva* with respect to the genus *Ryuguphycus*, *Um. yunseulla* sp. nov. formed a clade with *Um. japonica* and *Um. amamiensis* by weak bootstrap support. These findings, *Um. yunseulla* sp. nov., highlight the importance of studying the biodiversity of subtidal habitats from Jeju Island, Korea and further emphasize the need for investigations of macroalgae in the mesophotic zone around the Korean peninsula.

**Key Words:** biodiversity; greenish iridescent; phylogeny; *rbcL*; taxonomy; *tufA*; *Umbraulva yunseulla* sp. nov.

## INTRODUCTION

The genus *Umbraulva* was segregated from the genus *Ulva* by Bae and Lee (2001) and includes three species previously described as *U. amamiensis* Tanaka (type locality: Amami-Oshima, Japan), *U. japonica* (Holmes) Papenfuss (type locality: Enoshima, Japan), and *U. olivascens* Dangeard (type locality: Roscoff, France). *Umbraulva* is distinguished from *Ulva* Linnaeus by its distinct olive-green blade, subtidal habitat, and the presence of the pigment siphonaxanthin (Bae and Lee 2001), which pigment can grow in deeper waters because they absorb green light in the 540-nm range (Yokohama 1981). *Umbraulva kuaweuweu* H. L. Spalding & A. R. Sherwood

and *Um. kaloakulau* H. L. Spalding & A. R. Sherwood were described as new species from specimens collected at depths of 80-125 m in mesophotic ecosystems of the Hawaiian Archipelago based on vegetative morphology and molecular phylogenetic analyses of the internal transcribed spacer (ITS) region, ribulose-1,5-biphosphate carboxylase large subunit (*rbcL*), and elongation factor Tu (*tufA*) genes (Spalding et al. 2016). Although it was known that *Umbraulva* includes five species taxonomically (Guiry and Guiry 2020), the new genus *Ryuguphycus* H. Kawai, T. Hanyuda & T. Kitayama, which includes *R. kuaweuweu* (formerly *Um. kuaweuweu*) as the generi-



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type, was recently separated from the genus *Umbraulva* based on its distinctive morphology, life history, carotenoid composition, and molecular phylogeny (Kawai et al. 2020).

Two *Umbraulva* species, *Um. amamiensis* and *Um. japonica*, have been reported from subtidal habitats in Korea (National Institute of Biological Resources 2019); however, it is very difficult to identify species correctly based on a few traditional morpho-anatomical characteristics such as blade shape and thickness, presence / absence of microscopic marginal denticulation, and rhizoidal filament type (Spalding et al. 2016, Kawai et al. 2020). The plastid markers *rbcL* and *tufA* have been used to delimit species boundaries in *Umbraulva*, including several new undescribed specimens (Heesch et al. 2009, Kirkendale et al. 2013). Therefore, it is necessary to study the systematics of this genus using molecular phylogenetic information and/or monitor the occurrence of invasive species in subtidal areas. During subtidal zone surveys around Jeju Island, Korea, we discovered a novel *Umbraulva* species; this finding is supported by molecular analyses and morphological observations. The objectives of this study were to determine the taxonomic position of this new species, assess its genetic differences from related taxa, and discuss the phylogenetic relationships of *Umbraulva* based on the plastid *rbcL* and *tufA* genes.

## MATERIALS AND METHODS

Using scuba gear, we collected six specimens of a new *Umbraulva* candidate and four of *Um. japonica* from

depths of 8-15 m in subtidal areas around four annexed islets (Hyeongjeseom, Munseom, Seopseom, and Udo) of Jeju Island, Korea. The fresh samples were photographed using an Olympus TG-4 waterproof digital camera (Olympus, Tokyo, Japan). Thallus fragments of each specimen were dried for molecular analyses using silica gel. Prior to describing morphological characters, the samples were preserved in 5% formalin in seawater and sectioned using a bench-top freezing microtome (NK-101-II; Nippon Optical Works Co., Ltd., Tokyo, Japan). Sectioned materials were stained with 1% aniline blue acidified with 1% HCl after bleaching under sunlight. Sections were mounted in 35% corn syrup and photographed under a microscope (BX43; Olympus) using an EOS 600D digital camera (Canon, Tokyo, Japan). Digitized images were edited for clarity using Adobe Photoshop software (ver. 6.1; Adobe Systems Inc., San Jose, CA, USA). Pressed herbarium specimens were deposited as voucher specimens in the herbaria of Jeju National University (JNUB) and the National Institute of Biological Resources (KB), Incheon, Korea.

Total genomic DNA of two *Umbraulva* species (Table 1) was extracted following the protocol of the LaboPass Tissue Genomic DNA Isolation Kit Mini (Cosmo Genetech, Seoul, Korea). All polymerase chain reaction (PCR) processes were performed using AccuPower PCR Premix (Bioneer, Daejeon, Korea), following the manufacturer's protocol. We amplified and sequenced the plastid genes *rbcL* and *tufA* and analyzed nuclear 18S rRNA from an unidentified *Umbraulva* species. In the present study, the primer combinations for *rbcL*, *tufA*, and 18S rRNA-5P were *GrbcL*Fi (Saunders and Kucera 2010) or RH1 (Manhart 1994) / 1385R (Manhart 1994), *tufGF*4 / *tufGR*

**Table 1.** Sequence information of *Umbraulva yunseulla* sp. nov. and *Umbraulva japonica* analyzed newly in this study

| Species<br>Voucher No.             | Date         | Site                  | GenBank accession No. |             |          |
|------------------------------------|--------------|-----------------------|-----------------------|-------------|----------|
|                                    |              |                       | <i>rbcL</i>           | <i>tufA</i> | 18S rRNA |
| <i>Umbraulva yunseulla</i>         |              |                       |                       |             |          |
| H. W. Lee, E. H. Bae & M. S. Kim   |              |                       |                       |             |          |
| MSK-GA00064                        | Jan 30, 2013 | Munseom, Jeju, Korea  | -                     | MT978114    | MT978101 |
| MSK-GA00068                        | Jan 30, 2013 | Munseom, Jeju, Korea  | -                     | MT978115    | -        |
| MSK-GA00069                        | Jan 30, 2013 | Munseom, Jeju, Korea  | MT978109              | MT978116    | -        |
| MSK-GA00070                        | Jan 30, 2013 | Munseom, Jeju, Korea  | -                     | MT978117    | -        |
| MSK-GA00071                        | Jan 30, 2013 | Munseom, Jeju, Korea  | MT978110              | MT978118    | -        |
| MSK-GA00072                        | Jan 30, 2013 | Munseom, Jeju, Korea  | -                     | MT978119    | -        |
| <i>Umbraulva japonica</i> (Holmes) |              |                       |                       |             |          |
| Bae & I. K. Lee                    |              |                       |                       |             |          |
| MSK-JDWT24                         | Dec 27, 2017 | Jongdal, Jeju, Korea  | MT978123              | MT978107    | -        |
| MSK-JDWT25                         | Dec 27, 2017 | Jongdal, Jeju, Korea  | MT978124              | MT978108    | -        |
| MSK-SYWT98                         | Jan 2, 2018  | Shinyang, Jeju, Korea | MT978125              | MT978105    | -        |
| MSK-SYWT99                         | Jan 2, 2018  | Shinyang, Jeju, Korea | MT978126              | MT978106    | -        |

(Saunders and Kucera 2010), and SSU-A (Medlin et al. 1988) / SSU\_inR (Manhart 1994), respectively. The PCR amplification procedure followed that of Saunders and Kucera (2010). All successfully amplified PCR products were purified using an AccuPrep PCR Purification Kit (Bio-ener) and sequenced by Macrogen (Seoul, Korea) using forward and reverse primers. Additional *rbcL* and *tufA* sequences for the phylogenetic analysis were selected from GenBank (Supplementary Table S1). The *rbcL* and *tufA* datasets were aligned visually using the BioEdit software (Hall 1999) after editing the *Umbraulva* sequences obtained in this study using Chromas ver. 1.45 software (Technelysium Pty Ltd., South Brisbane, Australia). Variations in the *rbcL* and *tufA* sequences were assessed based on uncorrected pairwise genetic distances (p-distance) using MEGA 5.1 software (Tamura et al. 2011) and a neighbor-joining algorithm dependent on the Kimura two-parameter distance method. To determine the taxonomic positions of our *Umbraulva* specimens (Table 1), each *rbcL* and *tufA* phylogeny was run using a maximum-likelihood (ML) algorithm with RAxML software (Stamatakis 2006) and the GTR +  $\Gamma$  + I model with 1,000 bootstrap (BS) replicates from each sequence dataset for our *Umbraulva* specimens (Table 1) and related ulvacean taxa (Supplementary Table S1), including *Ulvaria obscura* (*rbcL*, HQ603436 and HQ603651; *tufA*, HQ610415) as an outgroup. We constructed a combined phylogenetic tree inferred from the *rbcL*, *tufA*, 18S rRNA, 28S rRNA, and ITS sequence datasets (Supplementary Table S1) to delimit the generic boundaries within Ulvaceae. This concatenated phylogenetic tree was constructed using the ML method in RAxML software and expressed using FigTree ver. 1.4.0 software (Rambaut 2012).

## RESULTS

We analyzed a set of 53 *rbcL* gene sequences including two new *Umbraulva* sp. specimens (MT978109-MT978110; 741-745 bp) and four *Um. japonica* ones (MT978123-MT978126; 1,263-1,273 bp). Among all sites *rbcL*, 185 sites (14.5%) were variable and 137 sites (10.8%) were parsimoniously informative. In *tufA* analysis, a set of 40 *tufA* gene sequences were analyzed including six new *Umbraulva* sp. specimens (MT978114-MT978119; 804-859 bp) and four *Um. japonica* ones (MT978107-MT978106; 848-866 bp). Variable and parsimoniously informative sites were 327 sites (37.8%) and 169 (19.7%), respectively. In addition, a 18S rRNA-5P sequence were obtained from a new *Umbraulva* sp. specimen

(MT978101; 530 bp). Phylogenetic analysis of the plastid *rbcL* and *tufA* sequences showed that the six new *Umbraulva* sp. specimens from Jeju Island were separated from other species within *Umbraulva* clade in a fully supported clade (BS, 100), with sister to *Um. kaloakulau* from Hawaii, USA (Figs 1 & 2). The *rbcL* phylogeny showed that the novel *Umbraulva* sp. had diverged from the *Um. japonica* clade from Korea (MT978123, MT978124, MT978125, and MT978126) including the generitype from Japan (AB097612 and LC507134), as well as *Um. amamiensis* (AB097614 and LC 507131), with an interspecific variation of 2.6-3.3% and 2.6%, respectively (Fig. 1). Higher interspecific divergence among *Umbraulva* species was observed in the *tufA* phylogenetic analysis compared to the *rbcL* results. The *tufA* interspecific variation values for the novel *Umbraulva* sp. were 1.6% vs. *Um. kaloakulau*, 6.6-7.0% vs. *Um. japonica*, and 4.2% vs. *Um. amamiensis*.

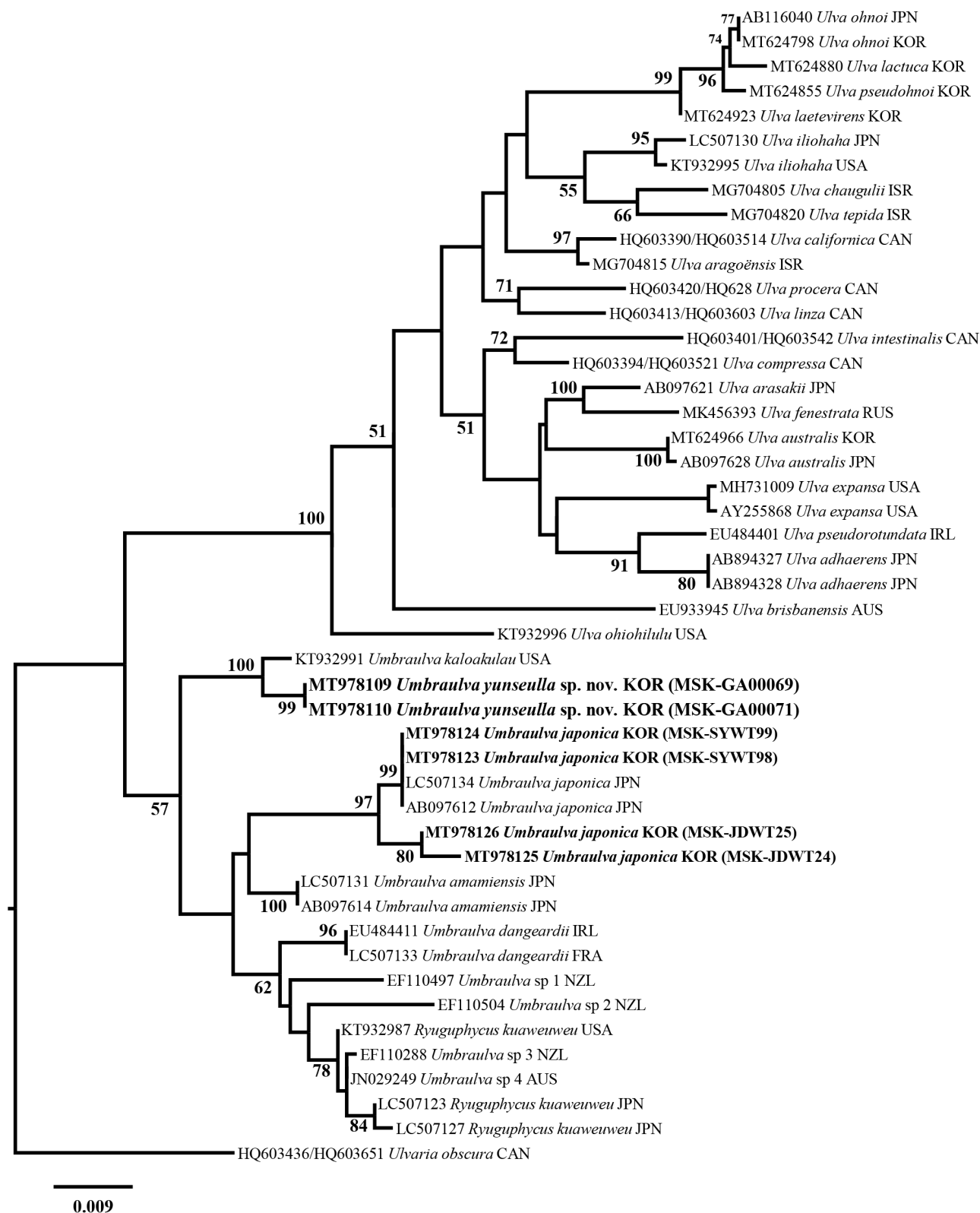
Although the genus *Ulva* is monophyletic based on the *rbcL* and *tufA* genes ML phylogeny, *Umbraulva* clade was not monophyletic because of the genus *Ryuguphycus* embedded within this clade. In addition, the concatenated ML phylogeny showed that *Umbraulva* consists of two clades, one containing *Um. japonica*, *Um. amamiensis*, *Um. kaloakulau*, and the novel *Umbraulva* sp. (Fig. 3) with weak ML support (BS, 69%), and the other consisting of *Um. dangeardii* from Ireland and France (EU484411 and LC507133, respectively), *Umbraulva* sp. specimens from New Zealand (EF110497, EF110504, and EF110288) and Australia (JN029249, *rbcL*, and JN029348) (Supplementary Table S1), and *Ryuguphycus* with no support (Fig. 3). Although the clade of *Umbraulva* is unstable on the basis of the molecular evidence, we propose a new species of *Umbraulva*, hereby named *Um. yunseulla* H. W. Lee, E. H. Bae & M. S. Kim sp. nov.

### *Umbraulva yunseulla* H. W. Lee, E. H. Bae & M. S. Kim sp. nov. (Fig. 4A-L)

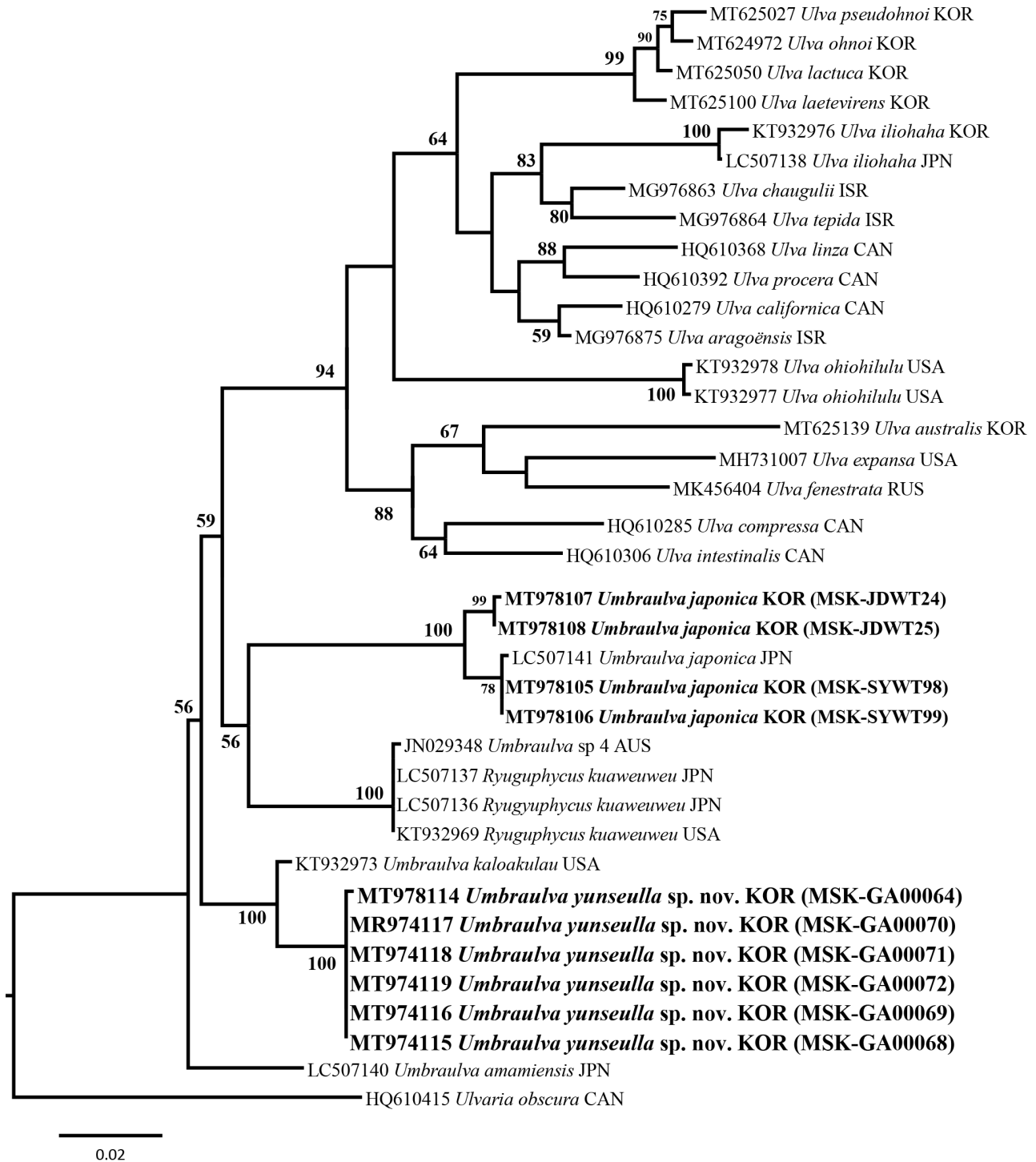
**Holotype.** MSK-GA00069 (Fig. 4B), vegetative, Munseom (annexed islet), Jeju Island, Korea, Jan 30, 2013, deposited in the JNUB herbarium: GenBank accession No. MT978109, *rbcL*; MT978116, *tufA*.

**Isotypes.** MSK-GA00068, MSK-GA00071, vegetative, Munseom (annexed islet), Jeju Island, Korea, Jan 30, 2013 (deposited in JNUB). MSK-GA00064, MSK-GA00070 (Fig. 4C), MSK-GA00072, vegetative, Munseom, Jeju Island, Korea, Jan 30, 2013 (deposited in KB).

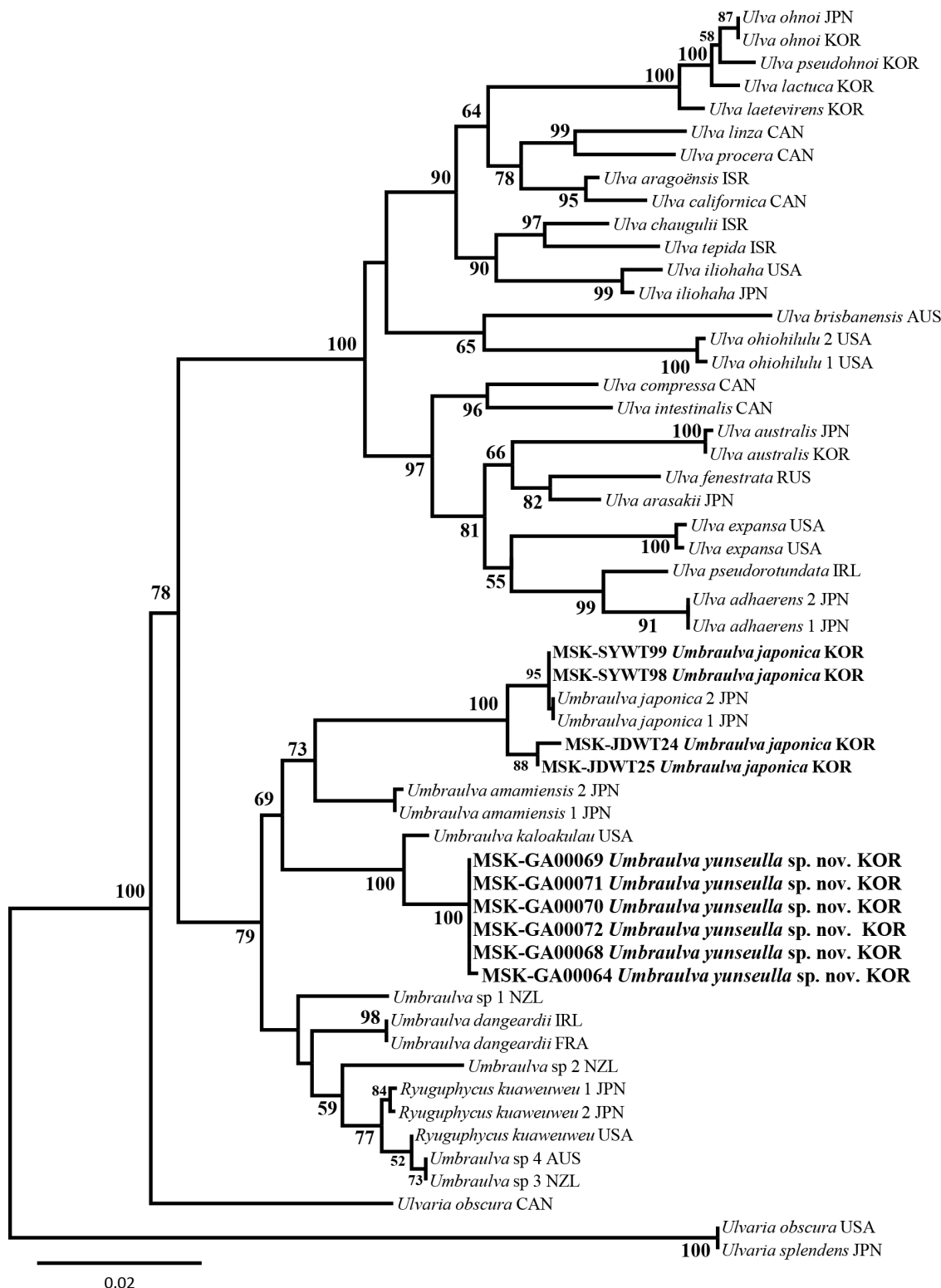
**Type locality.** Munseom, Jeju Island, Korea (33°13'39" N, 126°33'57" E).



**Fig. 1.** Maximum likelihood phylogenetic tree inferred from the plastid *rbcL* of Ulvaceae species with *Umbraulva yunseulla* sp. nov. Numbers on the branches indicate the bootstrap values with only  $\geq 50\%$ . Sequences produced in this study are marked in bold font. Branch lengths are proportional to substitution rate.



**Fig. 2.** Phylogenetic tree inferred from the plastid *tufA* of *Umbraulva yunseulla* sp. nov. constructed by maximum likelihood analysis. Numbers on the branches indicate the bootstrap values with only  $\geq 50\%$ . Sequences produced in this study are marked in bold font. Branch lengths are proportional to substitution rate.



**Fig. 3.** Maximum likelihood phylogeny of Ulvaceae inferred from five gene regions, *rbcl*, *tufA*, 18S rRNA, 28S rRNA, and ITS (total 5,634 bp). Numbers on the branches indicate the bootstrap values with only  $\geq 50\%$ . The GenBank accession numbers of each sequence data set are shown in Supplementary Table S1. Specimens analyzed in this study are marked in bold font. Branch lengths are proportional to substitution rate.

**Etymology.** The specific epithet, *yunseulla*, is derived from the Korean traditional noun *yunseul*, meaning “calm wave glittering with sunshine or moonlight” in reference to the greenish iridescence observed on the underwater thallus surface.

**Korean name.** 윤슬초록갈파래 (국명신칭).

**Habitat.** Attached to hard substrates such as rocks, shells, or nongeniculate corallines in the subtidal zone (generally 8-15 m deep).

**Distribution.** Jeju Island, Korea.

**Specimens examined.** MSK-GA00064, MSK-GA00068, MSK-GA00069, MSK-GA00070, MSK-GA00071, MSK-GA00072, Jan 30, 2013, Munseom, Jeju Island, Korea; MSK150802-06, Aug 2, 2015, Sagye, Jeju Island, Korea; MSKL160414-14, MSKL160414-15, MSKL160414-16, MSKL160414-17, Apr 14, 2016, Munseom, Jeju Island, Korea; MSKL160419-03, MSKL160419-12, MSKL160419-13, MSKL160419-14, Apr 16, 2016, Munseom, Jeju Island, Korea; MSKL160419-19, MSKL160419-22, Apr 19, 2016, Seopseom (annexed islet), Jeju Island, Korea; MSKL160520-10, May 20, 2016, Munseom, Jeju Island, Korea; MSKL160528-16, MSK160528-17, May 28, 2016, U-do (annexed islet), Jeju Island, Korea (deposited in JNUB).

**DNA sequence data.** *rbcL*: MSK-GA00069 (MT978109), MSK-GA00071 (MT978110). *tufA*: MSK-GA00064 (MT978114), MSK-GA00068 (MT978115), MSK-GA00069 (MT978116), MSK-GA00070 (MT978117), MSK-GA00071 (MT978118), MSK-GA00072 (MT978119). 18S rRNA: MSK-GA00064 (MT978101).

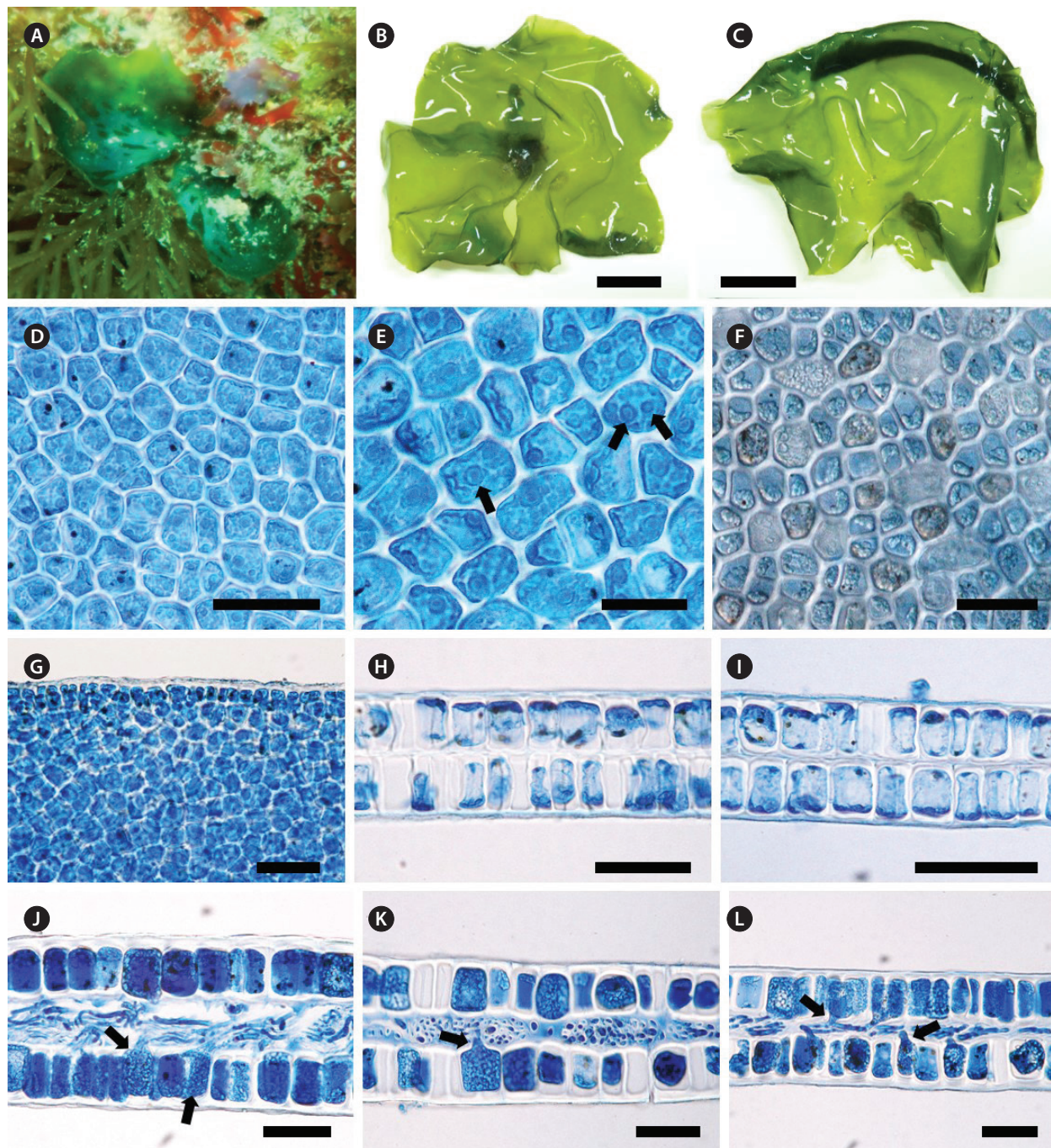
**Habit and morphology.** *Umbraulva yunseulla* sp. nov. has a foliose (Fig. 4B & C) and distromatic (Fig. 4H & I) thallus with slightly ruffled to curled margins (Fig. 4B & C). Greenish iridescence derived from the natural habitat glitters on the surface radially from the lower part or at the margin of the thallus (Fig. 4A). Thallus green to dark green (Fig. 4B & C). Thallus entirely globular to subglobular and funnel-shaped, growing dorsiventrally decumbent when young to erect when mature (Fig. 4A), 5-7 cm wide and 4-5 cm high, to a maximum of 10 cm (Fig. 4B & C). Thallus apex rounded to slightly emarginated, base cordate to lobate (Fig. 4B & C). Thallus attached by a distinct discoid holdfast without a stipe (Fig. 4B & C) and generally composed of a single or 2-3 (5) blades (Fig. 4B & C). Distromatic thallus is composed of entirely cuboidal to polygonal cells, some subspherical in the upper part of the blade in surface view, 11-26  $\mu\text{m}$  long by 9-16  $\mu\text{m}$  wide (Fig. 4D). In the center of the blade, cells are subspherical, cuboidal to polygonal, 15-35  $\mu\text{m}$  long by 9-18  $\mu\text{m}$  wide, and are larger and more numerous than in the upper blade (Fig. 4E). At the base, subspherical, cuboi-

dal and polygonal cells, 18-55  $\mu\text{m}$  long by 9-26  $\mu\text{m}$  wide, are intermixed (Fig. 4F). Pale cells are greatly expanded, and darker cells are filled with cytoplasm in a compact arrangement (Fig. 4F). Each cell contains 1-2 pyrenoids (Fig. 4E), up to 4 per cell. Cell arrangement throughout the thallus irregular (Fig. 4D-F). Thallus margin entire, plain, and rounded, without microscopic protuberances (Fig. 4G). In transverse section, cell shape is cuboidal throughout the entire blade (Fig. 4H-J). Cell size in transverse section is taller and wider toward basal part, 26-32  $\mu\text{m}$  tall by 11-13  $\mu\text{m}$  wide in upper, 23-28  $\mu\text{m}$  tall by 13-17  $\mu\text{m}$  wide in middle, 40-60  $\mu\text{m}$  tall by 23-37  $\mu\text{m}$  wide in basal, but similar throughout the thallus on both the dorsal and ventral surfaces (Fig. 4H-J). Cell thickness ranges from 50-70  $\mu\text{m}$  in the upper thallus to 100-140  $\mu\text{m}$  in the basal portion of the blade. In transverse section, the basal portion of the blade, fine rhizoidal filaments are observed budding off from cells inward filled with cytoplasmic contents compactly, and are interwoven with longitudinal arrangement and fill between distromatic cell planes densely (Fig. 4J-L). Gametophytes were not observed.

## DISCUSSION

Molecular analyses of the genus *Umbraulva* from subtidal areas of Jeju Island, Korea, have expanded our understanding of the phylogenetic affinity between *Umbraulva* and related taxa (Bae and Lee 2001). This approach has facilitated the discovery of the newly recognized species *Um. yunseulla* sp. nov., thus enhancing studies of macroalgal biodiversity. The plastid markers *rbcL* and *tufA* were previously demonstrated to resolve new species and delimit boundaries among *Umbraulva* species (Heesch et al. 2009, Kirkendale et al. 2013). In this study, the morphological delimitation, such as size and thickness of thallus, size and shape of cell, and number of pyrenoids, provide less resolution to identify species definitely because morphological characteristic range is overlapped between *Umbraulva* species (Table 2). However, the phylogenetic analyses inferred *rbcL* and *tufA* are helpful to reveal a new member of *Umbraulva*, *Um. yunseulla* sp. nov. from Jeju Island, Korea (Figs 1 & 2), which has the smallest and a globular to sub-globular and funnel-shaped thallus with greenish iridescence along dorsal surface (Table 2).

*Umbraulva yunseulla* sp. nov. was first collected by Bae and Lee (2001) from Munseom, Jeju Island, Korea, but it was identified as *Um. amamiensis*, which is described as



**Fig. 4.** *Umbraulva yunseulla* H. W. Lee, E. H. Bae & M. S. Kim sp. nov. (A) Natural habit of *Um. yunseulla* sp. nov. from Munseom, Jeju, Apr 14, 2016, glittering with greenish iridescence. Thallus composed of two globular blades having ruffled margins. (B) MSK-GA00069, the holotype specimen, from Munseom, Jeju, Jan 30, 2013. Dorsiventral thallus habit composed a big cordate blade having rounded to slightly emarginate apex partially and another small subglobular blade. Both blades having slightly ruffled margin with curled parts. (C) MSK-GA00070, from Munseom, Jeju, Jan 30, 2013, subglobular and dorsiventral thallus having curled margins. (D) Sub-roundish or cuboidal to polygonal cells at the surface view of upper part with irregular arrangement. (E) Sub-roundish or cuboidal to polygonal cells at the surface view of middle part with irregular arrangement. Each cell containing one to two pyrenoid (arrows). (F) Sub-roundish or cuboidal cells to polygonal at the surface view of basal part with irregular arrangement. (G) Thallus margins plain without marginal denticulations or microscopic protuberances. (H) Upper part transection of blade having solid cell shape, composed of cuboidal cells. (I) Middle part transection of blade having solid cell shape, composed of cuboidal cells. (J-L) Basal part transection of blade having solid cell shape, composed of cuboidal cells with compact and interwoven rhizoids between both distromatic arrangements. Interstitial rhizoids are originated from each cell inward. Rhizoidal filaments are originated from inner part of cells filled with cytoplasmic contents compactly (arrows). Scale bars represent: B & C, 1 cm; D & F-L, 50  $\mu$ m; E, 25  $\mu$ m.



**Table 2.** A comparison of morphological characteristics among *Umbraulva yunseulla* sp. nov. and other *Umbraulva* species

|                    | <i>Umbraulva yunseulla</i><br>H. W. Lee, E. H. Bae & M. S. Kim   | <i>Umbraulva japonica</i><br>(Holmes) Bae & I. K. Lee                          | <i>Umbraulva amamiensis</i><br>(Tanaka) Bae & I. K. Lee                     | <i>Umbraulva dangardii</i> M. J. Wynne & G. Furnari                                    | <i>Umbraulva katoakulau</i><br>H. L. Spalding & A. R. Sherwood                   | <i>Ryuguphyicus kuaueuueu</i><br>(Spalding & Sherwood)<br>H. Kawai, T. Hanyuda & T. Kitayama (= <i>Um. kuaueuueu</i> ) |
|--------------------|--|--|---|--|--|--|
| Type locality      | Munseom, Jeju Island, Korea  | Enoshima, Kanagawa, Japan  | Amami Islands, Kagoshima, Japan   | Roscoff, Brittany, France  | Maui Island, Hawaii, USA   | Moloka Island, Hawaii, USA   |
| Habitat            | Subtidal from 8 to 15 m deep, attached on rocks, shells or non-geniculate coralline algae                        | Subtidal from 10 to 20 m deep, attached on the hard substrates like rocks      | Subtidal from 3-13 m deep, growing on rocky bottom                          | Subtidal up to 15 m deep, attached on rocks  | Subtidal from 85 to 125 m deep, attached on carbonate substrates                 | Subtidal from 65 to 80 m deep, attached on rhodoliths and carbonate reef   |
| Thallus shape      | Globular to sub-globular and funnel-shaped having rounded to slightly emarginate apex and cordate to lobate base | Ellipsoidal to flabellately expanded, often split radially with perforations   | Irregularly perforate, linear lanceolate to ovate lanceolate into 2-5 lobes | Slightly perforate, lanceolate to cuneate or irregularly orbicular, expanded and lobed | Irregularly perforate, elliptical to cuneate                                     | Slightly to extensively perforate, elliptical to flabellately expanded   |
| Holdfast           | Distinct discoid   | Distinct discoid   | Discoid   | Distinct discoid   | Very small, difficult to discern   | Distinct   |
| Margin             | Entirely, slightly ruffled to heavily curled, sometimes slightly torn in old                                     | Entire, slightly ruffled, lacinate with irregular microscopic protuberances    | Entire, smooth, no microscopic protuberances                                | -  | -  | Entire margin  |
| Color              | Olive green to dark green, greenish iridescent along dorsal surface of blades                                    | Dark olive green   | Green to dark green   | Olive green  | Deep green to dark olive green   | Light to dark olive green  |
| Height (cm)        | 4-5 (up to 10)   | 8-20   | 20-60 (up to 115)   | <30 (up to 100)  | 12-37  | 7-21   |
| Width (cm)         | 5-7  | 10-26  | 10-35 (up to 50)  | Up to 40   | 15-20  | 7-17   |
| Thickness (µm)     | 50-70 (100-140 at basal)   | 100-125 (up to 180-400)  | 100-170 (lower part)  | 40-95 (100-115 at basal)   | 30-94 (middle part)  | 24-98 (middle part)  |
| Structure          | Solid  | Solid  | Solid   | Solid  | Solid  | Solid or hollow  |
| Surface view       | Cuboidal to rounded polygonal 11-55 µm long by 9-26 µm wide  | Roundish to irregularly polygonal  | Polygonal with rounded corners 27-40 µm in diameter                         | Polygonal to rectangular with rounded corners 24-35 µm long by 18-24 µm wide           | Polygonal and angular, irregularly arranged 14-42 (-49) µm long by 11-26 µm wide | Polygonal and angular, irregularly arranged 17-49 (-53) µm long by 8-25 (-39) µm wide                                  |
| Transverse view    | Cuboidal 23-60 µm tall by 11-37 µm wide  | Longitudinally rectangular with rounded corners 20-45 µm tall by 15-35 µm wide | Polygonal to oblong 27-40 µm tall   | Cuboidal 13-60 µm tall by 11-37 µm wide  | Cuboidal to rectilinear 11-39 µm tall by 12-41 µm wide                           | Cuboidal to rectilinear 9-49 µm tall by 10-50 µm wide  |
| No. of pyrenoids   | 1-2 per cell (up to 4 per cell)  | 2-3 per cell (up to 4 per cell)  | 1 per cell  | 1 per cell (2-4 per cell at basal)   | 1-2 per cell   | 1-2 per cell   |
| Rhizoidal filament | Longitudinally interwoven and dense in basal   | Densely filling inwardly in basal part   | Dense between inner part of basal thallus                                   | Closely packed bundles in lower basal region   | Lacking rhizoidal filaments  | Densely and longitudinally packed rhizoidal filaments  |
| Reference          | This study   | Bae and Lee (2001), Kawai et al. (2020)  | Tanaka (1956)   | Hoeksema and van den Hoek (1983), Burrows (1991)                                       | Spalding et al. (2016)   | Spalding et al. (2016), Kawai et al. (2020)  |

having a height of 20-60 cm (to 115 cm) and width of 10-35 cm (to 55 cm), with abundant perforations (Tanaka 1956). *Um. yunseulla* sp. nov. has much smaller thallus, ranged 5-7 cm wide and 4-5 cm high up to a maximum of 10 cm, compared to *Um. amamiensis*, although these two species inhabit the similar depth range of subtidal (Table 2). The specimens of Bae and Lee (2001) were characterized by cordate or funnel-shaped thalli 5-7 cm in height with wavy margins and epifluorescent illumination at the surface of the blade in nature. These characteristics are identical to our specimens of *Um. yunseulla* sp. nov. (Table 2). On the contrary, *Um. amamiensis* is linear lanceolate to ovate-lanceolate with irregular perforation, and the epifluorescent illumination of *Um. amamiensis* specimens was undescribed (Table 2). The iridescent coloration along thallus surface is one of morphological features of *Um. yunseulla* sp. nov. distinguished from other *Umbraulva* species (Table 2). In marine macroalgae, external iridescence is caused by unique nanostructural coloration mechanisms such as intracellular iridescent bodies or multi-layered cuticle reflection (Chandler et al. 2017). Some brown and red seaweeds such as *Cystoseria*, *Dictyota*, *Chondria*, and *Cottoniella* have nanostructural coloration that produces iridescent bodies (Chandler et al. 2017). In *Um. yunseulla* sp. nov., greenish iridescence appears around the base or middle part of the thallus (Fig. 4A). In surface view, *Um. yunseulla* sp. nov. exhibits larger subspherical and expanded cells than it does cuboidal or polygonal cells; these are mainly distributed at the middle and basal parts of the thallus, and have tiny globules scattered throughout (Fig. 4E & F). Further investigation may show that these tiny globules are iridescent bodies of *Um. yunseulla* sp. nov., which would provide new insight into the mechanism by which iridescent marine macroalgal species adapt to environmental conditions such as radiation intensity and turbidity (Chandler et al. 2017).

The monophyly of *Umbraulva* was not well-supported by *rbcl* and *tufA* phylogenetic analyses (Figs 1 & 2), which is inconsistent with a previous study of the phylogenetic relationship between *Umbraulva* and *Ryuguphycus* (Kawai et al. 2020). Because the generic boundary of *Umbraulva* was delimited by only weak support and the *Ryuguphycus* clade was supported by poor BS support in our concatenated analyses inferred from 18S rRNA, 28S rRNA, ITS, *rbcl*, and *tufA* (Fig. 3), the phylogenetic topology between *Umbraulva* and *Ryuguphycus* presented in this study remains uncertain. The recently established genera *Umbraulva* and *Ryuguphycus* have been subject to fewer phylogenetic analyses than *Ulva*,

which has been examined using many molecular approaches (Kirkendale et al. 2013, Spalding et al. 2016). Future studies should attempt to discover new members of *Umbraulva* and *Ryuguphycus* to fill phylogenetic gaps and strengthen generic boundaries among these genera.

Few taxonomic studies have examined ulvacean organisms inhabiting subtidal zones of Korea, with the exception of *Umbraulva* species (Bae and Lee 2001). Recent studies have delimited species boundaries within the genus *Codium* Stackhouse, including five subtidal species, and for *Palmophyllum crassum* (Naccari) Rabenhorst, which inhabits deep marine areas around Jeju (Lee and Kim 2015, 2017). Many red algae species have been discovered in the subtidal zones of Jeju Island, including *Pseudopolyneura hyacinthia* (J. C. Kang & M. S. Kim) M. J. Wynne (as *Erythroglossum hyacinthinum*, Kang and Kim 2014) and *Pachymeniopsis volvita* M. Y. Yang & M. S. Kim (Yang and Kim 2015). Although Jeju Island has the potential to be named a seaweed genetic and species diversity hotspot (Yang et al. 2020), our knowledge of green seaweeds in subtidal zones remains poor. A few new ulvacean species have been reported in Hawaii and Japan, including *U. oiophilulu*, *Um. kaloakulau*, and *R. kuaweuweu* collected from the mesophotic zone, at depths of 30-125 m (Spalding et al. 2016, Kawai et al. 2020). Continuous surveys of green algae in the subtidal zone contribute to the expansion of ecological knowledge, which is essential for habitat conservation and species diversity enrichment. Therefore, we further emphasize the necessity of investigating green macroalgal diversity in the mesophotic zone to discover new species of the ulvacean genera *Ulva*, *Umbraulva*, and *Ryuguphycus*.

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## SUPPLEMENTARY MATERIALS

**Supplementary Table S1.** The representative sequence datasets of *Umbraulva* and *Ulva* from GenBank including outgroups (<https://e-algae.org>).

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