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New record of *Ulva sublittoralis* (Ulvales, Chlorophyta) in Korea

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Abstract: A marine ulvalean species (Chlorophyta) was collected from the eastern coast of Korea. This species is morphologically characterized by a distromatic, dark to medium green and mostly irregularly orbicular or irregularly expanded thallus with entire or undulate margin without serrations. Vegetative cells are irregularly polygonal with distinctly rounded corners in shape, and have chloroplast completely covering the outer cell wall and one to two pyrenoids per cell. In a phylogenetic tree based on ITS (Internal Transcribed Spacer) sequences, this Korean alga nests in the same clade with *Ulva sublittoralis*, as a sister clade of *U. californica*, *U. flexuosa* and *U. tanneri*, which share the irregularly orbicular or expanded thallus normally without teeth cells. The genetic divergence between them is intraspecific within *Ulva*. Accordingly, it is identified as *U. sublittoralis* based on the morphological and molecular data. This is the first record of *Ulva sublittoralis* in the Korean marine algal flora.

Keywords: green alga, molecular analyses, morphology, first report

INTRODUCTION

Ulva, which is frequently seen in blooms called green tides caused by a huge proliferation of biomass (Blomster *et al.* 2002), is distributed globally in coastal areas (Hayden *et al.* 2003; Guiry and Guiry 2019). This genus shows highly morphological variations with habitat (van den Hoek *et al.* 1995).

Nevertheless, *Ulva* species have been distinguished by gross morphology, cell shape and size, plastid orientation, pyrenoid number per cell, and presence or absence of marginal denticulations (Koeman and van den Hoek 1981; Maggs *et al.* 2007; Loughnane *et al.* 2008). However, because of their simple and plastic morphology, it is often difficult to identify them by the features (Loughnane *et al.* 2008; Matsumoto and Shimada 2015). Recently, analyses of the internal transcribed spacer (ITS) region have provided good phylogenetic resolution at the species level in *Ulva* (Coat *et al.* 1998; Loughnane *et al.* 2008; Heesch *et al.* 2009; Duan *et al.* 2012).

More than 120 species around the world are currently accepted taxonomically (Guiry and Guiry 2019). Of these, 16 species have been recorded in Korea (Lee and Kang 1986, 2002; Lee 2008; Bae 2010; Kim *et al.* 2013; Lee *et al.* 2014; An and Nam 2017). During a survey of marine algal flora, a species of *Ulva* was collected from Uljin in Korea. Based on morphological and molecular analyses, it was identified as *U. sublittoralis*, which is newly recorded in the marine algal flora of Korea in the present study.

MATERIALS AND METHODS

Samples were collected from Uljin located on the eastern coast of Korea. They were preserved in 5% formalin seawater with herbarium specimens. A portion of the material was dried and preserved in silica gel for molecular analysis. Microscopic sections were mounted in 30% 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 were assessed by using gel electrophoresis on a 1% agarose gel and used for amplification of the internal transcribed spacer (ITS) region using published primers (Ogawa *et al.* 2013) and primer sequences are represented as follows: ITS primers (F: 5′ TCTTTGAAACCGTATCGTGA 3′ R: 5′ GGTGAACCTGCGGAGGGAT 3′). PCR amplifications were performed in a TaKaRa PCR Thermal Cycler Dice (TaKaRa Bio Inc., Otsu, Japan) with an initial denaturation step at 94°C for 1 min, 35 cycles at 94°C for 30 s, 55°C for 1 min and 68°C for 2 min and a final extension at 72°C for 5 min. The reaction volume was 30 μL, consisting of 20 ng of genomic DNA, 2 μL of 10× PCR buffer, 2 μL of 200 μM dNTP, 2 μL of each forward and reverse primer, and 0.5 units of Taq polymerase (TaKaRa Bio Inc.). Amplifications were examined using gel electrophoresis in a 1% agarose gel and amplified ITS region products were purified using a QIAquick Gel Extraction Kit (Qiagen). 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 analyses were performed using the maximum-likelihood (ML) methods. Bootstrap values were calculated with 1,000 replications. ITS sequences of other species were obtained from GenBank. *Umbraulva japonica* was used as an outgroup.

RESULTS AND DISCUSSION

***Ulva sublittoralis* Segawa 1938: 132**

Korean name: Dong-hae-gal-pa-rae nom. nov.
(신칭: 동해갈파래)

Specimens examined: MGARB012870, MGARB012871, MGARB012872 (Mangyang-ri, Uljin, 28 July 2016), MGARB012878–012880 (Mangyang-ri, Uljin, 26 Sep. 2019)
Type locality: Kozu-sima, Japan
Habitat: Epilithic near the intertidal.
Morphology: Thalli 5–15 cm broad, mostly irregularly orbicular or expanded, dark to medium green in color (Fig. 1a, b), distromatic (Fig. 1c); margin entire, without serrations, undulate or strongly ruffled to plane (Fig. 1d); vegetative cells rectangular to polygonal in the basal region of the thallus (Fig. 1e), but irregularly polygonal with 3–6 distinctly rounded corners in the upper basal region; chloroplasts completely covering the outer cell wall, often containing many large starch grains, variously oriented to side of the outer cell wall; pyrenoids, one to two per cell, 3–8 μm in diam.; numerous rhizoidal cells present in the basal region of the thallus, bearing tubular extensions on the outside of the cell layer (Fig. 1f).

Ulva sublittoralis, which is distributed throughout temperate regions (Guiry and Guiry 2019), was originally described from Japan (Silva *et al.* 1996). According to previous reports (Segawa 1938; Bliding 1963; Brodie *et al.* 2007), it appears to be generally characterized by an irregularly expanded thallus normally with entire margin. Our specimens collected from Korea basically share this morphological feature. Even though some dissimilarities between them are found, such as in gross morphology and chloroplast shape (Segawa 1938; Ogawa *et al.* 2013; Phillips *et al.* 2016; the present study), the Korean alga is considered to be *Ulva sublittoralis*. This is also supported by molecular analysis (Fig. 2).

In morphology, this species is very similar to *U. pertusa*, which has been reduced to a synonym of *U. australis* Areschoug from South Australia. However, *U. sublittoralis* genetically differs from *U. australis* based on ITS sequence (Kirkendale *et al.* 2013; Ogawa *et al.* 2013; the present study). According to Ichihara *et al.* (2009), rhizoidal cells in most species of *Ulva* bear tubular extensions in the inside of the cell layer in longitudinal sections of the lower thallus (Bliding 1968; Koeman and van den Hoek 1981, 1982; Phillips 1988; Hiraoka *et al.* 2004). However, *Ulva sublittoralis* Segawa and *U. limnetica* Ichihara et Shimada described from Japan produce tubular extensions on the outside of the cell layer in the stipe (Ichihara *et al.* 2009). It is also similar to *Ulva limnetica* in sharing this feature (Ichihara *et al.* 2009). However, it was confirmed that both species are readily distinguished from each other by interspecific genetic distance level of 16.3% in the present study. They are currently accepted (Guiry and Guiry 2019).

In general, ITS regions have been used to analyze molecular phylogeny in *Ulva* (Malta *et al.* 1999; Hayden *et al.* 2003; Hayden and Waaland 2004; Hofmann *et al.* 2010; O'Kelly *et al.* 2010). In a phylogenetic tree based on the molecular data (Fig. 2), our specimens nest in the same clade with *U. sublittoralis* from Japan, as a sister clade of some species groups (*U. californica* and *U. tanneri* from USA and *U. flexuosa* from Italy), which share the irregularly orbicular or expanded thallus normally without teeth

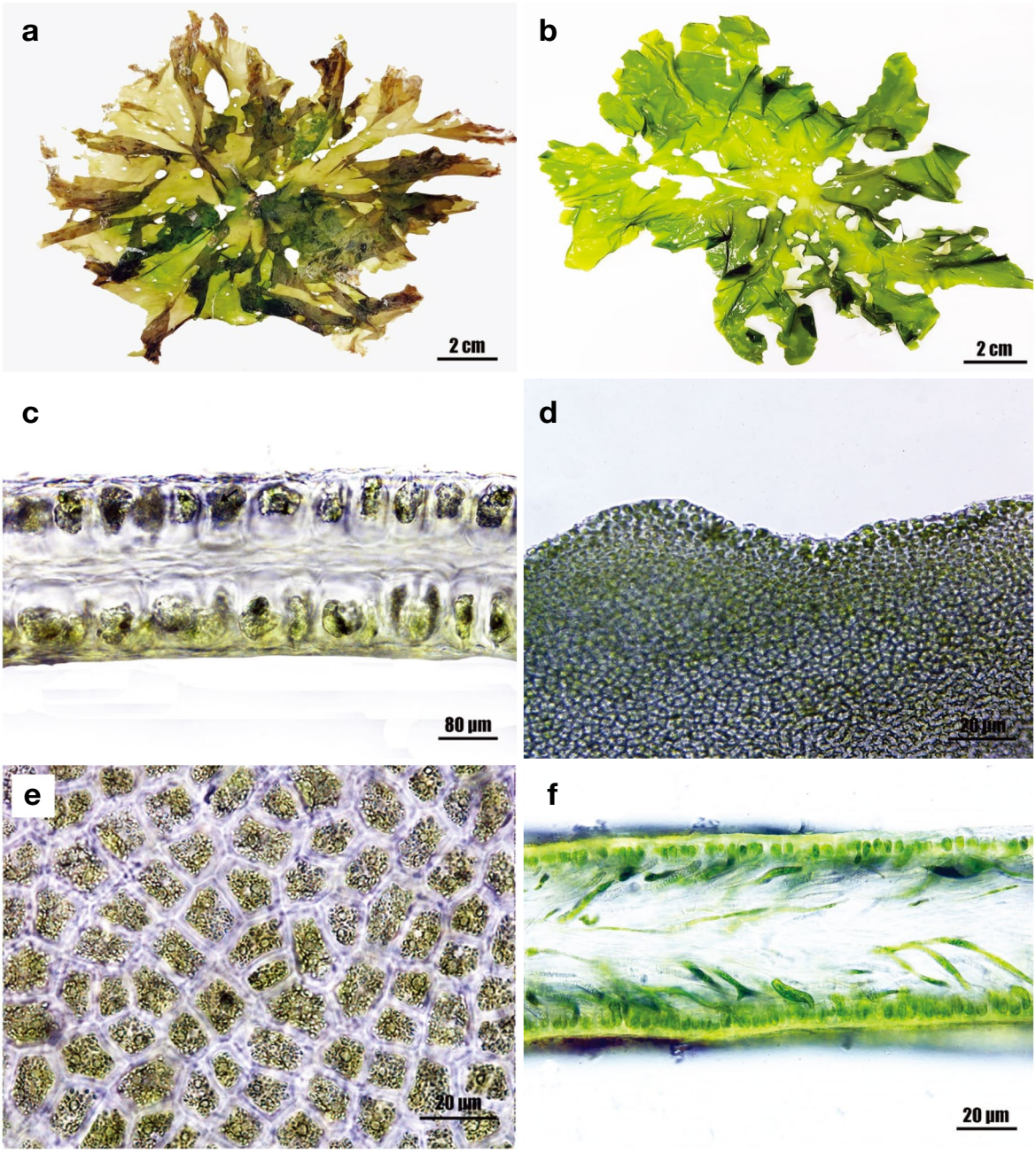

Fig. 1. *Ulva sublittoralis* Segawa. a. Herbarium specimen (MGARB012870). b. Habit of the vegetative plant. c. Transverse section view of the thallus with two cell layers. d. Entire margin of the undulate or strongly ruffled plane blade in the lower part. e. Surface view of the vegetative cells with rectangular to polygonal shapes. f. Longitudinal section view of the basal part of the thallus showing numerous rhizoidal cells bearing tubular extensions on the outside of the cell layer.

cells. The genetic distance between them within the clade was 0.01%.

According to Kirkendale *et al.* (2013), the interspecific genetic distance is 0.9–5.56% in *Ulva*. The present analysis shows a divergence range of 1.2%–21.9%. This suggests that the distance between sequences of the Korean specimens and *U. sublittoralis* is intraspecific. Based on these morphology and molecular data, our Korean specimens are identified as *U. sublittoralis*, which is newly recorded in the Korean marine algal flora.

Fig. 2. Phylogenetic tree of selected taxa obtained from maximum-likelihood analysis based on ITS sequences. Bootstrap percentages (1,000 replicates samples) are shown above the branches. Scale bar = 0.02 substitutions/site.

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