Ulva grossa sp. nov. (Ulvales, Chlorophyta) from Korea based on molecular and morphological analyses

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Abstract

A marine green algal species was collected from the eastern coast of Korea. This species shares the generic features of *Ulva*, and is characterized by irregularly shaped thalli, relatively small and thick thallus, entire undulate margins without serrations and one or two pyrenoids per cell. In a phylogenetic tree based on sequences of the nuclear-encoded internal transcribed spacer region (ITS) of ribosomal (r)DNA, it nests as a sister clade to some species including *U. ohnoi* with a relatively large thallus. This Korean alga differs from those species forming the same or subclades, such as *U. ohnoi*, *U. fasciata*, *U. reticulata*, and *U. gigantean*, in having a relatively small (3–8 cm) and thick (60–100 µm) thallus. Of these species, *U. ohnoi* originally described from Japan is similar to the Korean alga in having a more or less thick thallus of 30–90 µm, but is distinguished from the Korean species in often having microscopic serrations in the thallus margin. The genetic distance between this Korean entity and those species was calculated as 1.8–4.8%, which is considered to be an inter-specific divergence level with the genus *Ulva*. *Ulva grossa* sp. nov. (Ulinales, Chlorophyta) is described from Korea based on the morphological and molecular analyses herein.

Introduction

Ulva Linnaeus, commonly referred to as “sea lettuce”, is the most species-rich genus in the family Ulvaceae (Kazi et al. 2016), and 129 species are currently accepted in this genus (Guiry and Guiry 2019). Of these, several species are associated with notorious blooms called “green tides” (Fletcher 1996; Blomster et al. 2002; Zhao et al. 2010; Kazi et al. 2016).

Ulva exhibits various morphology in thallus, such as foliose, lanceolate, linear, ovate, cuneate or tubulose. These features are useful in part for the genus taxonomy. Other characteristics including cell size, shape and arrangement, thallus thickness, number of pyrenoids per cell and morphology of holdfast and basal region have also been adopted for the identification of *Ulva* species (Bliding 1968; Koeman and van den Hoek 1981). However, intraspecific variations were observed for these characteristics in different environments and growth phases (Blomster et al. 1998; Kazi et al. 2016). The morphological plasticity is a cause of uncertainty about the taxonomic status of most taxa in this genus, resulting in the recognition of large numbers of varieties, forms and ecotypes. This plasticity was also found at a higher taxonomic level, namely in a generic characteristic previously distinguishing *Ulva* and *Enteromorpha* Link (i.e., distromatic thalli in *Ulva* and tubular-monostromatic thalli in *Enteromorpha*) (Hayden et al. 2003; Shimada et al. 2003; Kazi et al. 2016).

Recently, molecular studies of *Ulva* have contributed significantly to its taxonomy, such as the merging of *Ulva* and *Enteromorpha* (Hayden et al. 2003), synonymization of *U. lactuca* Linnaeus and *U. fasciata* Delile (O’Kelly et al. 2010) and identification of green tide-forming taxa (Liu et al. 2010; Zhao et al. 2010; Guidone et al. 2013; Guoying et al. 2014; Kazi et al. 2016). The molecular data also helped in understanding the biogeographic history, cryptic diversity and introduction of species of *Ulva* in different regions (Heesch et al. 2009; Hofmann et al. 2010; Kraft et al. 2010; Wolf et al. 2012; Kirkendale et al.

2013; Kazi et al. 2016). These suggest that an approach to the taxonomy of *Ulva* based on both morphological and molecular data is required (Loughnane et al. 2008; Hofmann et al. 2010; Matsumoto and Shimada 2015).

A total of 16 species are currently recorded in the marine algal flora of Korea (Lee and Kang 1986, 2002, Lee 2008, Bae 2010, Kim et al. 2013, An et al. 2017). During a survey of algal flora, a marine *Ulva* species (Chlorophyta) was collected from the eastern coast of Korea. This Korean entity was newly described based on morphological and molecular analyses.

Materials And Methods

Samples for the present study were collected from Uljin and Yeongdeok, which are located on the eastern coast of Korea. All specimens were preserved in 5–10% formalin seawater, and pressed on herbarium sheets. Sections of the thallus were mounted in 20% corn syrup for permanent preparation. Measurements are given as width × length. Species identification was based on thallus morphology following the criteria of Bliding (1963, 1968) and Koeman and van den Hoek (1981). A portion of the material was dried and preserved in silica gel for molecular analysis. Total genomic DNA was extracted from a sample preserved in silica-gel 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. Concentrations of extracted DNA were assessed using gel electrophoresis on a 1% agarose gel. Extracted DNA was used for amplification of the internal transcribed spacer (ITS) regions using published primers (Blomster et al. 1998). ITS regions were PCR amplified as a single fragment with the primers ITSP1 (5' GGAAGGAGAAGTCGTAACAAGG 3') and G4 (5' CTTTTCCTCCGCTTATTGATATG 3') (Harper and Saunders 2001) or as two overlapping fragments with the primers ITSP1 and ITS1 (5' TTCAAAGAT TCGATGATTAC 3') and P5 (5' GCATCGATGAAGAACGCAG 3') and G4 (Harper and Saunders 2001). 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 µL, consisting of 20 ng of genomic DNA, 2 µL of 10x PCR buffer, 2 µL of 200 µM dNTP, 1 µL of each forward and reverse primer, and 0.5 units of Taq polymerase (Takara Korea, Korea). 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, Hilden, Germany). 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 neighbor-joining (NJ) and maximum-likelihood (ML) methods. Bootstrap values were calculated with 1,000 replications. The ITS sequences of other species were obtained from GenBank. *Umbraulva japonica* (Holmes) Bae et I.K.Lee was used as an outgroup.

Results

Ulva grossa sp. nov.

Description: Thalli 5–10 cm high (Fig. 1a), erect, membranous, distromatic (Fig 1c), usually irregularly shaped and unbranched or little branched conical to ligulate shape (Fig. 1b), relatively small and thick thallus, light to dark green in color, soft in texture, attached by a small holdfast on rocks near the lower intertidal; frond irregular shaped, with a spirally twisted basal portion, with usually entire, undulate margin without serrations (Fig. 1d), 50–60 µm thick in the upper portion, 100–130 µm thick in the basal portion; the 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), transformed into rhizoidal cells near the base, 10–20 µm × 10–18 µm, with a length-to-width ratio of 1.5–2.0 in the transverse section; chloroplasts cap-like, parietal, with one or two pyrenoids (Fig. 1f).

Habitat: Epilithic near the lower intertidal.

Specimens examined: MGARB00001548–MGARB00001550 (Geoil-ri, Uljin: 21.vi.2018), MGARB00001551 (Changpo-ri, Yeongdeok: 22.vi. 2018), MGARB00001552–MGARB00001553 (Daejin-ri, Yeongdeok: 21.vi. 2018).

Holotype: MGARB00001548 (Fig. 1a).

Type locality: Geoil-ri (N 36° 41' 49.8" E 129° 28'22.5"), Hupo-myeon, Uljin-gun, Gyeongsangbuk-do, Korea.

Etymology: The specific epithet is derived from the relatively small, thick and coarse thallus.

Korean name: Do-tom-gal-pa-rae

Phylogenetic analyses

Thirty-three species were contained in the dataset alignment based on ITS sequences. Thirty-five sequences were obtained from 32 samples of *Ulva* in GenBank and three samples of *U. grossa* collected from Korea in the present study. The phylogenetic tree was inferred by using the NJ and ML method based on the Tamura 3-parameter model (Tamura 1992). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There was a total of 173 positions in the final dataset.

U. grossa formed a sister clade to some species including *U. ohnoi* (Fig. 2). The genetic distance between sequences of *U. grossa* collected from the three localities in Korea was ranged from 0.0% to 0.1%. However, the values between *U. grossa* and other *Ulva* species were calculated as 1.8–6.7% in the present study. In particular, the genetic divergence within the sister clade to *U. grossa* was 1.8%–4.8%.

Discussion

Ulva was established based on *U. lactuca* originally described from the Atlantic Ocean (Linnaeus 1753). In basic thallus morphology, *Ulva* is a flattened distromatic or tubular monostromatic (Hayden et al. 2003), and its blades are broadly expanded foliose, irregularly lobed, cuneate, linear, ovate, lanceolate, oblanceolate, and deeply divided into linear lacinae or tubulose (Guiry and Guiry 2019). Even though intra- or inter-specific variation and overlap are found, cell size, shape and arrangement, thallus thickness, chloroplast disposition and number of pyrenoids per cell, and morphology of holdfast and basal region are useful as taxonomic characteristics for this genus (Bliding 1968; Steffensen 1976; Mshigeni and Kajumulo 1979; Koeman and van den Hoek 1981; Tanner 1986; Phillips 1988; Malta et al. 1999).

The type species *U. lactuca* is commonly distributed along the coasts of Korea (Lee and Kang 2002). However, it is occasionally confused with *U. ohnoi*, which was originally described from Japan, in gross morphology with ovate or fan-shaped thallus in Korea (Hiraoka et al. 2003; the present study). However, *U. lactuca* is separable from the latter species in having relatively small and thick thallus (Womersley 1984; Bae 2010; Dawes and Mathieson 2008), even though the thallus size of *U. ohnoi* varies greatly with habitat (Hiraoka et al. 2003; O'Kelly et al. 2010; the present study). *U. lactuca* shows broadly expanded, lanceolate, ribbon-like and more or less deeply divided thallus. In addition, *U. lactuca* appears to lack the features of mostly orbicular-shaped thallus of two layers separated easily, which are found in *U. ohnoi* (Hiraoka et al. 2003). The specimens collected from the eastern coast of Korea share the generic features found in *Ulva lactuca*, and are characterized by the following combination of characteristics: irregularly shaped thalli, relatively small and thick thallus, entire undulate margins without serrations and one or two pyrenoids per cell.

In a phylogenetic tree based on sequences of the nuclear-encoded internal transcribed spacer region (ITS) of ribosomal (r)DNA, it forms a sister clade to some species groups including *U. ohnoi* with a relatively large thallus. This Korean alga differs from those species forming the same or subclades, such as *U. ohnoi*, *U. fasciata*, *U. reticulata*, and *U. gigantea*, in having a relatively small (3–8 cm) and thick (60–100 µm) thallus (Table 1). Of these species, *U. ohnoi*, which was originally described from Japan (Hiraoka et al. 2003), is similar to *U. grossa* in having a more or less thick thallus of 30–90 µm (Table 1). However, it is distinguished from the Korean species in often having microscopic serrations in the thallus margin. *U. grossa* has an entire thallus margin without serrations. More importantly, both species are distinguishable from each other by thallus size and habitat. *U. grossa* is small (3–8 cm) in thallus size and always saxicolous, while those in *U. ohnoi* are respectively large (20–30 cm) and saxicolous or floating.

In addition to the characteristics of thallus size and thickness, the dividing and marginal features of thallus appear to be useful in part for distinguishing *U. grossa* from the other species nesting in the sister clade. *U. fasciata* from Egypt (Silva et al. 1996) and *U. gigantea* from France occasionally show a ruffled and macroscopic serration margin rather than entire margin in thallus, respectively (Table 1). Thallus dividing in *U. grossa* is irregular, while that in *U. fasciata*, *U. reticulata* and *U. gigantea* is irregularly or palmately to linear, deeply and irregularly lobed, or deeply lacinate (Table 1).

Molecular data also confirm the distinction of *U. grossa* from those species. The genetic distance between *U. grossa* and those species of the sister clade was calculated as 1.8–4.8%. This estimated sequence divergence for ITS rDNA sequence is well within the inter-specific range based on the previous reports (Hayden and Waaland 2002; Shimada et al. 2003; Ichihara et al. 2009). This warrants its recognition as a new species in the genus *Ulva*. Accordingly, *Ulva grossa* sp. nov. (Ulvales, Chlorophyta) is described from Korea based on the morphological and molecular analyses herein.

Declarations

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Conflicts of interest The authors declare no conflict of interest.

Ethics approval Not applicable

Consent for publication All authors consent for publication.

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Tables

Table 1 Comparison of morphological features between *Ulva grossa* sp. nov. and the relative species

Features	<i>U. grossa</i> sp. nov.	<i>U. lactuca</i> (type)	<i>U. ohnoi</i>	<i>U. fasciata</i>	<i>U. reticulata</i>	<i>U. gigantea</i>
Blade	Distromatic	Solitary or clustered	Foliose, saxicolous or floating, fragile, easily torn, orbicular, obovate or ovate	Distromatic, thin	Becoming perforated by many pores	Variable in shape
Size (cm)	3-8	20-70	20-30	10-50 (100)	36	10-40
Color	Green	Green	Light green	Bright green	-	Light green
Stalk	None	None	-	Inconspicuous or absent	-	-
Dividing	Irregular	Orbicular to irregular	Often more or less split in the upper portion	Irregularly or palmately into linear	Deeply and irregularly lobed or divided	Entire and rounded to deeply lacinate with large marginal lobes, ruffled, frilly, or flat, sometimes rosette-like
Margin	Entire without serrations	Entire	often with microscopic serrations	Entire and smooth or ruffled	-	Entire or with macroscopic teeth by no microscopic teeth
Cell size (W μ m $\times$ L μ m)	5-20 $\times$ 20-35	15 $\times$ 20	14-20 $\times$ 7-15 (upper) 14-30 $\times$ 12-20 (basal)	8-20 $\times$ 14-40	-	15-22 $\times$ 12-15
Thickness (μ m)	60-100	40-60	30-90		32-76	30-55
Pyrenoids	1-2	1-2	1-3	1-2	-	1-3
References	The present study	Womersley (1984); Bae (2010); Dawes and Mathieson (2008)	Hiraoka et al. (2003)	Womersley (1984); Abbott and Huisman (2004); Dawes and Mathieson (2008)	Abbott and Huisman (2004)	Brodie et al. (2007)

Figures

Figure 1

Ulva grossa sp. nov. **a** Holotype specimen (MGARB00001548). **b** Habit of vegetative plant from liquid preserved specimen. **c** Transverse section of thallus with two cell layers. **d** Entire margin of thallus. **e** Rectangular to polygonal cells with round corner near upper portion of thallus. **f** Cap-like parietal chloroplasts with one or two pyrenoids (arrows) in surface view

Figure 2

Phylogenetic tree of selected taxa obtained from ML analysis based on ITS sequences. Bootstrap percentages (1000 replicates samples) are shown above branches. Scale bar = 0.05 substitutions/site