



# New record of two marine ulvlean species (Chlorophyta) in Korea

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## Abstract

Two marine ulvlean species (Chlorophyta) were collected from the southern and eastern coasts of Korea. One is morphologically characterized by usually unbranched or little branched ribbon-shaped thalli, fronds with spirally twisted basal portions and usually undulate margins and 1 (-2) pyrenoids per cell. The other has irregularly shaped thalli, undulate and dentate margins in the fronds, small macroscopic denticulations along the margin and (1-) 2-3 pyrenoids per cell. In phylogenetic tree based on molecular data, the two species nest in the same clade with *Ulva flexuosa* and *U. rigida*, respectively. These two Korean entities are identified as *U. flexuosa* and *U. rigida*, respectively, based on morphological and molecular analyses. This is the first record of *Ulva flexuosa* and *U. rigida* in Korea.

**Key words:** Chlorophyta, Korea, molecular analyses, morphology, new record, *Ulva flexuosa*, *Ulva rigida*

## INTRODUCTION

*Ulva* Linnaeus, which is a large genus within Ulvaceae J.V. Lamouroux ex Dumortier, includes about 100 species worldwide (Guiry and Guiry 2014). This genus is found in all aquatic habitats from freshwater to seawater environments (Loughnane et al. 2008). In the past, the taxonomy of *Ulva* had been based mainly on morphology (Bliding 1963, 1968, Koeman and van den Hoek 1981, Hoeksema and van den Hoek 1983). However, this makes it difficult to identify *Ulva* species, because of the simple thallus structure (Heesch et al. 2009). Recently, molecular analysis, which is a useful tool for differentiating taxa, has been adopted in the identification, taxonomy and phylogeny of species within this genus (Malta et al. 1999, Tan et al. 1999, Hayden et al. 2003, Loughnane et al. 2008, Hofmann et al. 2010). Many synonyms reported in *Ulva* have been the result of previous molecular analyses (Hayden et al. 2003, Brodie et al. 2007, Sfriso 2010, Alongi et al. 2014, Guiry and Guiry 2014).

A total of 15 species are currently recorded in marine algal flora of Korea (Lee and Kang 1986, 2002, Lee 2008, Bae 2010, Kim et al. 2013). During survey of indigenous species, two marine ulvlean species (Chlorophyta) were collected from the southern and eastern coasts of Korea. These two Korean entities were identified based on morphological and molecular analyses and are newly recorded in marine algal flora of Korea in the present study.

## MATERIALS AND METHODS

Samples for the present study were collected from Busan and Pohang, Korea, in 2014. All specimens were preserved in 5-10% formalin seawater, and pressed on herbarium sheets. A portion of the material was dried and preserved in silica gel for molecular analysis. Species identification was based on thallus morphology following

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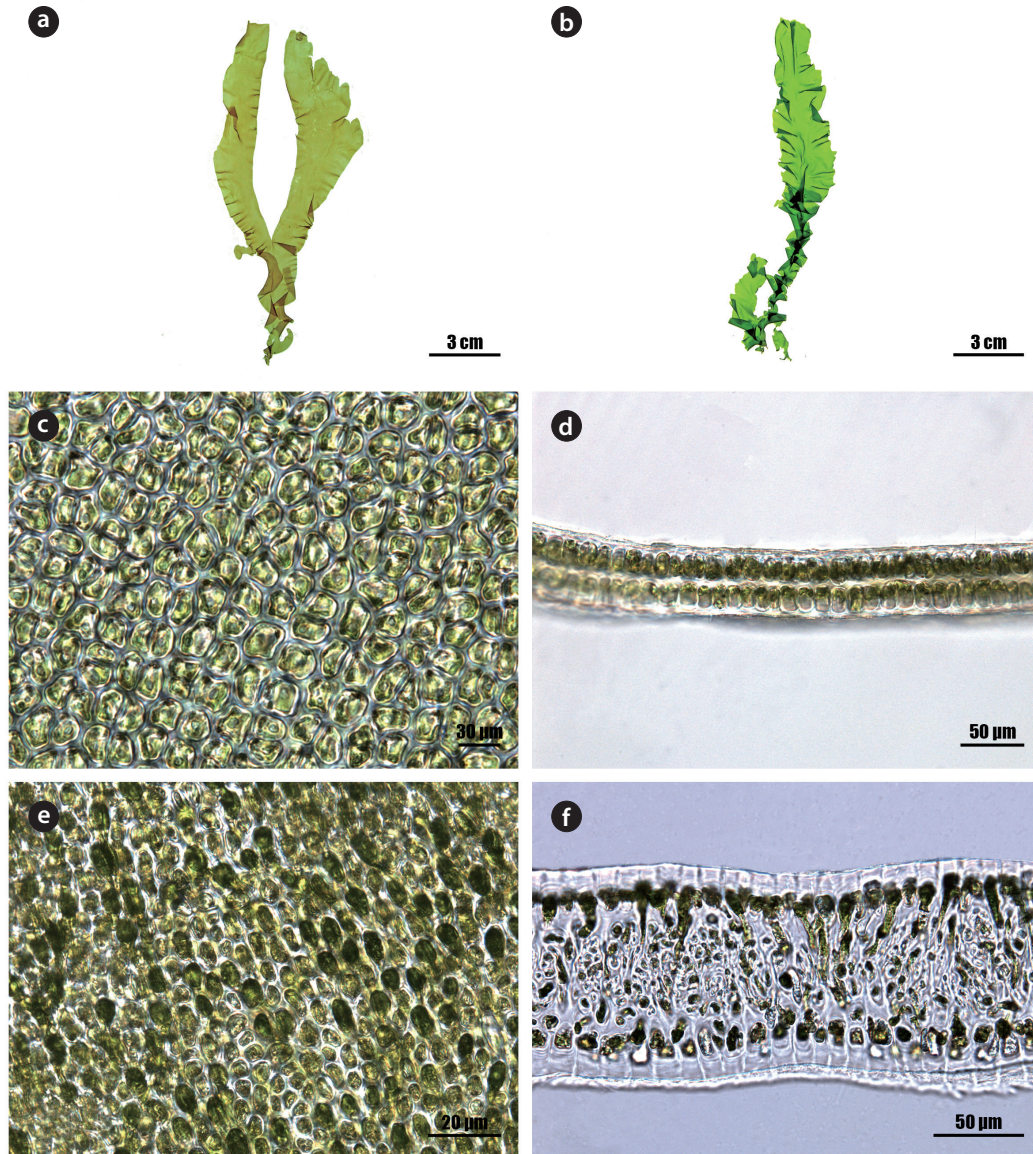
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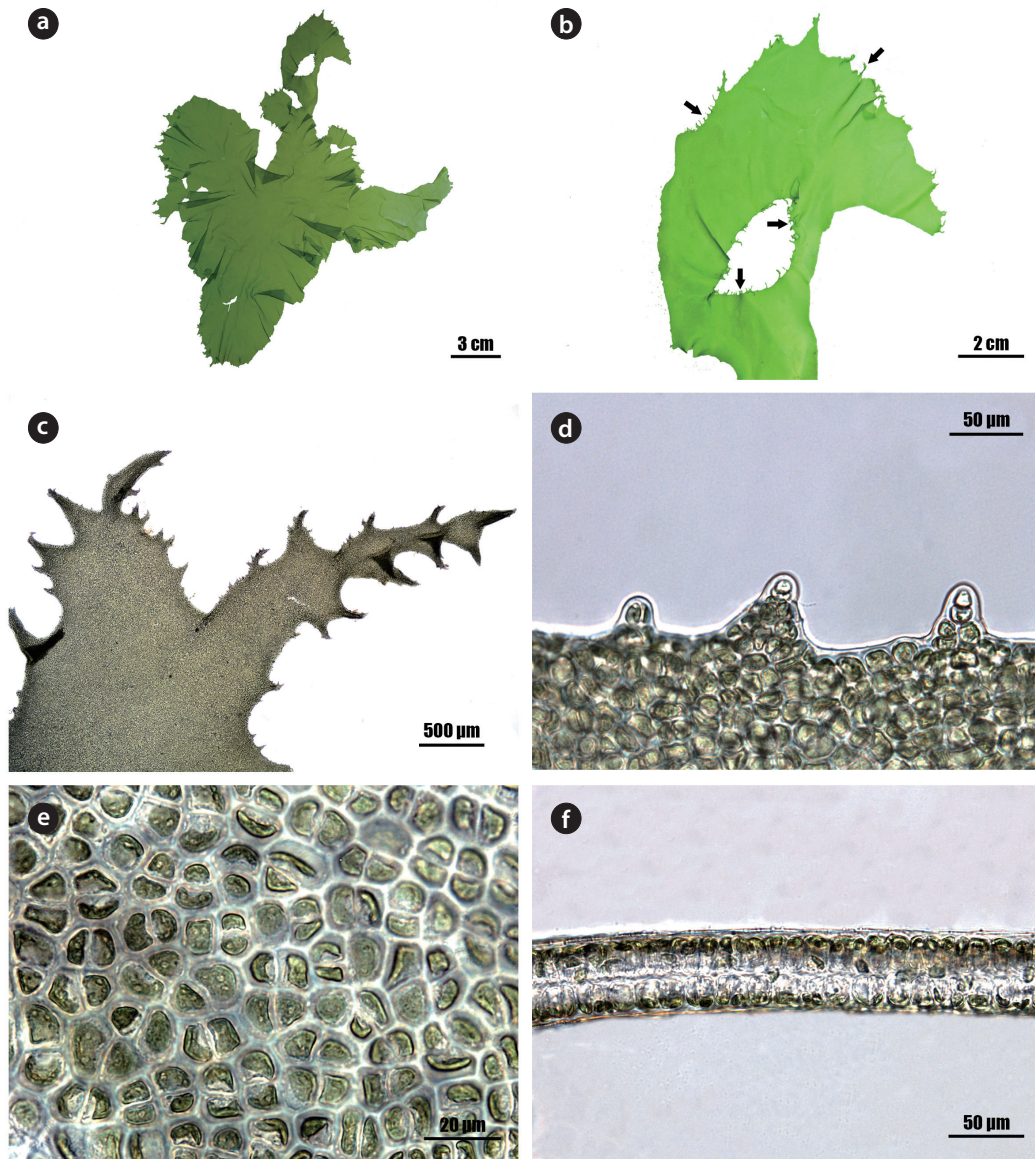


**Fig. 1.** *Ulva flexuosa* Wulfen. (a, b) Habit of vegetative plant. (c, d) Surface view (c) and transverse section (d) of the rectangular to polygonal cells with round corner near upper portion. (e, f) Surface view (e) and transverse section (f) of the oval to rectangular cells near basal portion. Scale bars are presented on the figures.

the criteria of Bliding (1963, 1968) and Koeman and van den Hoek (1981). Sections of the thallus were mounted in 20% 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. Before extraction, dried material was crushed with liquid nitrogen using a mortar and pestle. Concentrations of extracted DNA were assessed by 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). 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 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 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



**Fig. 2.** *Ulva rigida* C. Agardh. (a) Habit of vegetative plant. (b) Details of frond with many denticulations (arrows) along margin. (c) Denticulations with branching. (d) Dentate margin of frond. (e, f) Surface view (e) and transverse section (f) of rectangular to polygonal cells with round corner near basal portion. Scale bars are presented on the figures.

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. ITS sequences of other species (excluding *U. flexuosa* and *U. rigida* collected from Korea during the present study) were obtained from GenBank. *Umbraulva japonica* was used as an outgroup.

## RESULTS AND DISCUSSION

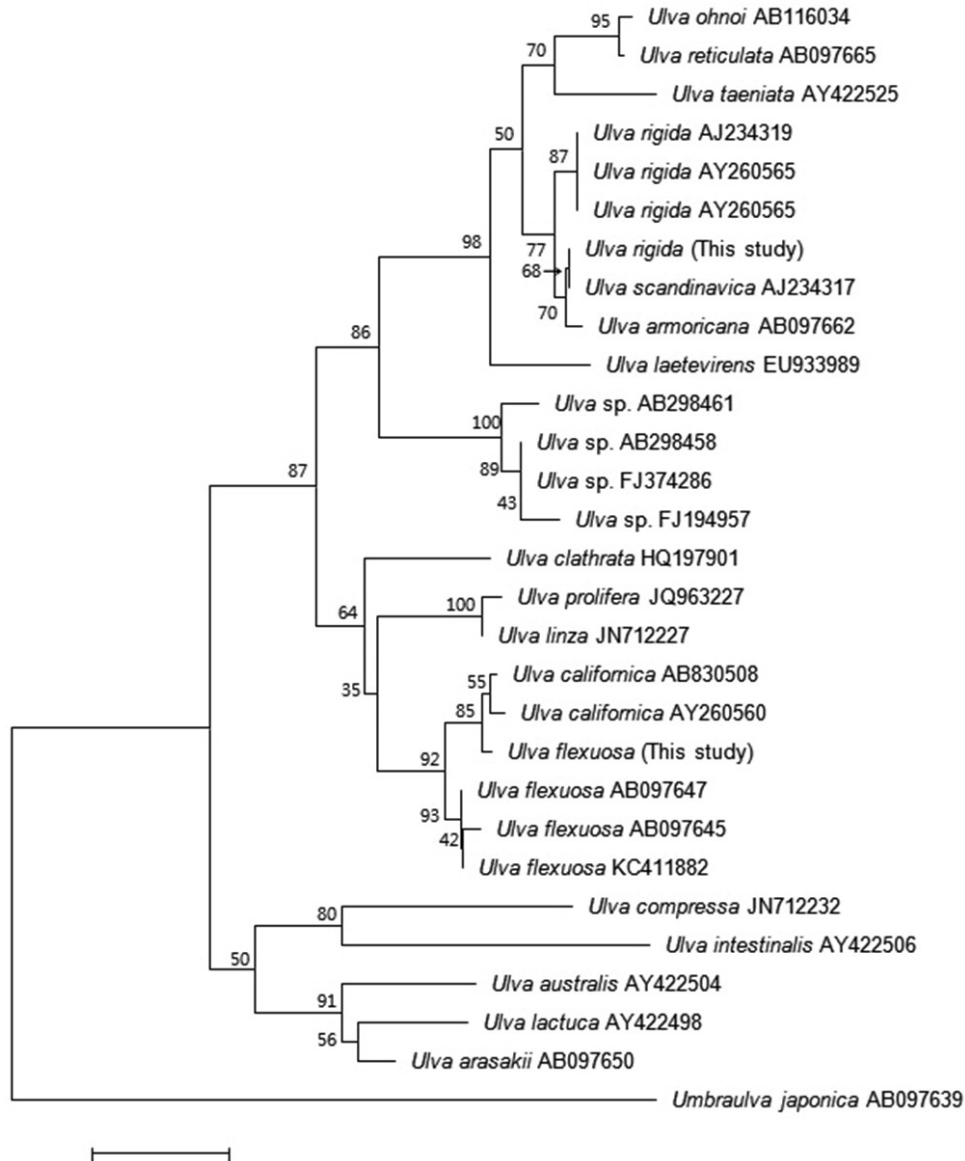
### *Ulva flexuosa* Wulfen 1803: xxii

Korean name: Yeon-gal-pa-rae nom. nov. (신칭: 연갈파래).

Specimens examined: NIBRAL0000143269, PKNU0000134878 (Cheongsapo: 17 May 2014).

Habitat: Epilithic near the lower intertidal.

Morphology: Thalli 15-25 cm high (Fig. 1a and 1b), erect, membranous, distromatic, usually unbranched or little branched conical to ligulate shape, light to dark green in color, soft in texture, attached by a small holdfast (<5 mm)



**Fig. 3.** Phylogenetic tree of selected taxa obtained from neighbor-joining analysis based on ITS sequences. Bootstrap percentages (1000 replicates samples) are shown above branches. Scale bar = 0.02 substitutions/site.

on rocks near the lower intertidal; frond ribbon-shaped, with a spirally twisted basal portion, usually undulate at the margin, 40-60  $\mu\text{m}$  thick in the upper portion (Fig. 1d), 80-120  $\mu\text{m}$  thick in the basal portion (Fig. 1f); cells usually arranged in pairs, rectangular to polygonal near the middle to upper portion (Fig. 1c), oval to rectangular with round corners near the basal portion in the surface view (Fig. 1e), transformed into rhizoidal cells near the base, 15-25  $\mu\text{m} \times$  10-15  $\mu\text{m}$ , with a length to width ratio of 1.6-1.8 in the transverse section; chloroplasts cap-like, parietal, with 1 (-2) pyrenoids (5-6  $\mu\text{m}$ ).

### *Ulva rigida* C. Agardh 1823: 410

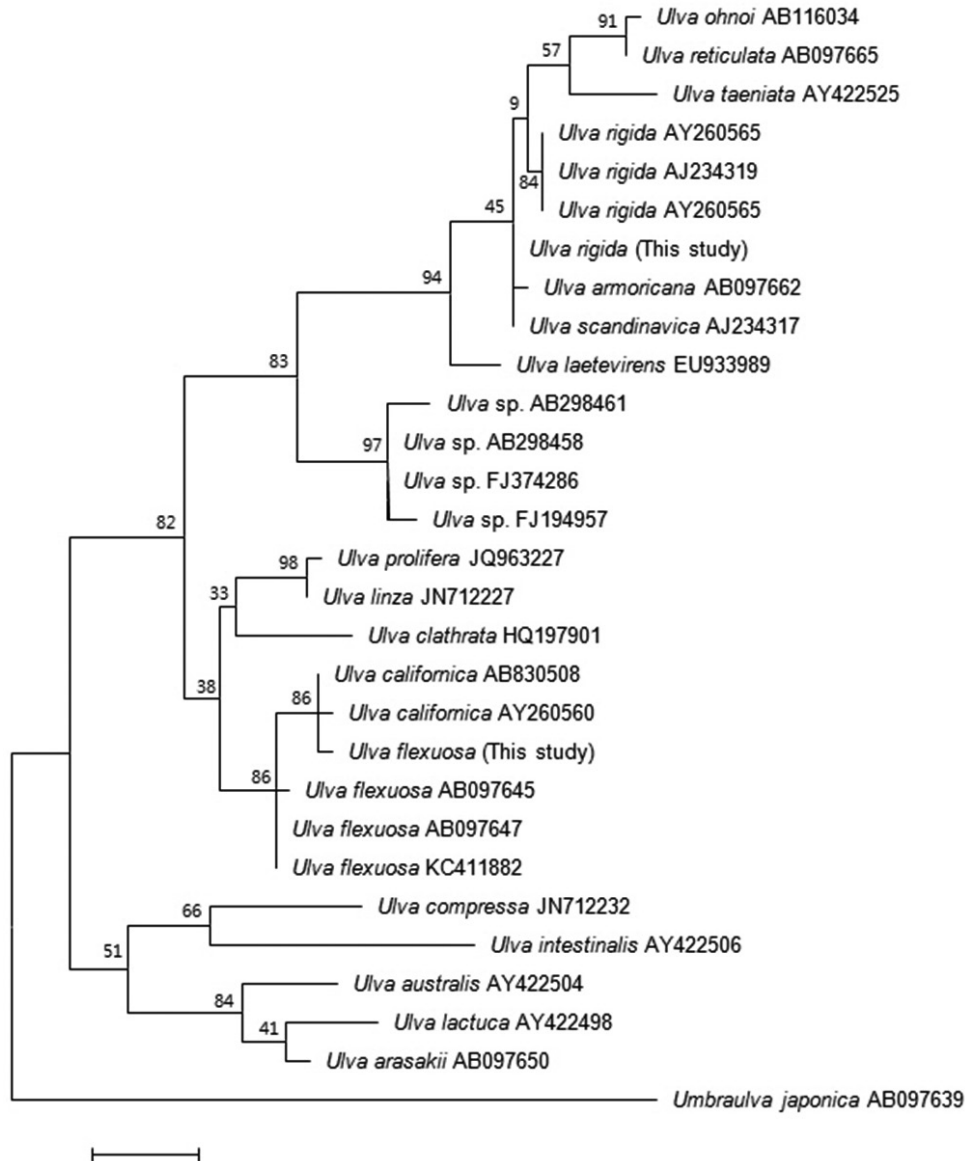
Korean name: Gyeong-gal-pa-rae nom. nov.

(신청: 경갈파래).

Specimens examined: NIBRAL0000143268, PKNU 0000 134875, PKNU 0000134879 (Duhodong: 3 July 2014).

Habitat: Epilithic near the subtidal.

Morphology: Thalli 20-30 cm high (Fig. 2a), membranous, distromatic, irregular in shape, light to dark green in color, stiff in texture, attached by small holdfast on rocks near subtidal; frond irregular in shape, usually undulate and dentate at the margin (Fig. 2d), with small macro-



**Fig. 4.** Phylogenetic tree of selected taxa obtained from maximum-likelihood analysis based on ITS sequences. Bootstrap percentages (1000 replicates samples) are shown above branches. Scale bar = 0.02 substitutions/site.

scopic denticulations along the margin (Fig. 2b), 40-60  $\mu\text{m}$  thick (Fig. 2f); denticulations usually branched (Fig. 2c); cells usually arranged in pairs, rectangular to polygonal with round corners in the surface view (Fig. 2e), 5-15  $\mu\text{m} \times$  5-10  $\mu\text{m}$ , with length to width ratio of 1.2-2.0 in the transverse section; chloroplasts cap-like, parietal, with (1-) 2-3 pyrenoids (2-3  $\mu\text{m}$ ).

*Ulva flexuosa*, which occurs in various localities including China and Japan (Guiry and Guiry 2014), was originally described from Duino, Adriatic Sea (Womersley 1984). This species is very similar to *U. linza* and *U. lactuca* in

habit. However, *U. flexuosa* is distinguished from the two species by thalli with a spirally twisted basal portion, undulate margin and ribbon-shaped frond. Specimens collected from Cheongsapo, Busan, during the present study share these features as described above (Fig. 1a and 1b).

*Ulva rigida* resembles *U. laetevirens* Areschoug in having macroscopic denticulations along the thallus margin (Sfriso 2010). However, the denticulations in *U. rigida* are very numerous and branched, while those in *U. laetevirens* are occasionally isolated and rarely branched (Sfriso 2010). This type of denticulation is also found in Korean specimens collected during the present study.

Many studies have used the ITS region to analyze the phylogenetic relationships among *Ulva* species (Malta et al. 1999, Hayden et al. 2003, Hayden and Waaland 2004, Hofmann et al. 2010, O'Kelly et al. 2010). In phylogenetic tree obtained from the present study based on the molecular data (Figs. 3 and 4), the former Korean entity forms the same clade with *U. californica* as a subclade of the group containing *U. flexuosa* from Greece (near type locality) and China and *U. californica* from USA (type locality) and Japan with relatively high bootstrap value of 92% in NJ tree and 86% in ML tree. In this group supported by the undulate margin of thalli, genetic distance between the Korean entity and *U. flexuosa* ranged from 1.1% to 1.3%, while that between *U. californica* and *U. flexuosa* was 0.8 - 1.3%. According to Mareš et al. (2011), the genetic distance for ITS sequences among *U. flexuosa* subspecies range from 3.8% to 5.9%. Based on this report, the Korean entity and *U. californica* are categorized into a specific variation within *U. flexuosa*. The present analyses, which show interspecific genetic distance of 2.1-13.3% in ulvlean species, also support strongly the intraspecific variation between those species. Accordingly, *Ulva californica* Wille in Collins et al. (1899), which is currently accepted (Guiry and Guiry 2014), is synonymized with *U. flexuosa*, here.

The second Korean entity nests in the same clade with *U. armoricana* and *U. scandinavica*. Recently, however, *U. armoricana* and *U. scandinavica* were reduced to synonymy with *U. rigida* based on molecular studies (Brodie et al. 2007). Although this Korean entity forms a sister clade to other *U. rigida* from Spain (type locality) and Scotland in NJ tree, genetic distance between those species is 0.5% within intraspecific variation range.

Based on these morphology and molecular data, our Korean entities are identified as *U. flexuosa* and *U. rigida*, respectively. This is the first record of these two ulvlean species in Korea.

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## LITERATURE CITED

- Agardh CA. 1823. Species Algarum Rite Cognitae, Cum Synonymis, Differentiis Specificis et Descriptionibus Succinctis. Volumen Primum Pars Posterior. Ex Officina Berlingiana, Lundae.
- Alongi G, Cormaci M, Furnari G. 2014. A nomenclatural reassessment of some of Bliding's Ulvaceae. *Webbia: J Plant Taxon Geogr* 69: 89-96.
- Bae HB. 2010. Ulotrichales, Ulvales. In: *Algal Flora of Korea*. Volume 1, Number 1. Chlorophyta: Ulvophyceae: Ulotrichales, Ulvales, Cladophorales, Bryopsidales. Marine Green Algae (Bae EH, Kim HS, Kwon CJ, Hwang IK, Kim GH, Klochkova TA, eds). National Institute of Biological Resources, Incheon, pp 7-52.
- Bliding C. 1963. A critical survey of European taxa in Ulvales. Part I. *Capsosiphon, Percursaria, Blidingia, Enteromorpha*. *Op Bot Soc Bot Lund* 8: 1-160.
- Bliding C. 1968. A critical survey of European taxa in Ulvales. II. *Ulva, Ulvaria, Monostroma, Kornmannia*. *Bot Notiser* 121: 535-629.
- Blomster J, Maggs CA, Stanhope MJ. 1998. Molecular and morphological analysis of *Enteromorpha intestinalis* and *E. compressa* (Chlorophyta) in the British Isles. *J Phycol* 34: 319-340.
- Brodie J, Maggs CA, John DM. 2007. *Green Seaweeds of Britain and Ireland*. British Phycological Society, London.
- Collins FS, Holden I, Setchell WA. 1899. *Phycotheca Boreali-Americana*. A Collection of Dried Specimens of the Algae of North America, Malden, MA.
- Guiry MD, Guiry GM. 2014. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>. Accessed 05 September 2014.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41: 95-98.
- Hayden HS, Blomster J, Maggs CA, Silva PC, Stanhope MJ, Waaland JR. 2003. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *Eur J Phycol* 38: 277-294.
- Hayden HS, Waaland JR. 2004. A molecular systematic study of *Ulva* (Ulvaceae, Ulvales) from the northeast Pacific. *Phycologia* 43: 364-382.
- Heesch S, Broom JES, Neill KF, Farr TJ, Dalen JL, Nelson WA. 2009. *Ulva, Umbraulva* and *Gemina*: genetic survey of New Zealand taxa reveals diversity and introduced species. *Eur J Phycol* 44: 143-154.
- Hoeksema BW, van den Hoek C. 1983. The taxonomy of *Ulva* (Chlorophyta) from the coastal region of Roscoff (Brit-

- tany, France). *Bot Mar* 26: 65-86.
- Hofmann LC, Nettleton JC, Neefus CD, Mathieson AC. 2010. Cryptic diversity of *Ulva* (Ulvales, Chlorophyta) in the Great Bay Estuarine System (Atlantic USA): introduced and indigenous distromatic species. *Eur J Phycol* 45: 230-239.
- Kim HS, Boo SM, Lee IK, Sohn CH. 2013. National List of Species of Korea, Marine Algae. Jeonghaengsa, Seoul. (in Korean)
- Koeman RPT, van den Hoek C. 1981. The taxonomy of *Ulva* (Chlorophyceae) in the Netherlands. *Brit Phycol J* 16: 9-53.
- Lee IK, Kang JW. 1986. A check list of marine algae in Korea. *Kor J Phycol* 1: 311-325.
- Lee YP. 2008. Marine Algae of Jeju. Academy Publishing Co, Seoul. (in Korean)
- Lee YP, Kang SY. 2002. A Catalogue of the Seaweeds in Korea. Jeju National University Press, Jeju.
- Loughnane CJ, McIvor LM, Rindi F, Stengel DB, Guiry MD. 2008. Morphology, *rbcL* phylogeny and distribution of distromatic *Ulva* (Ulvophyceae, Chlorophyta) in Ireland and southern Britain. *Phycologia* 47: 416-429.
- Malta EJ, Draisma SGA, Kamermans P. 1999. Free-floating *Ulva* in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison. *Eur J Phycol* 34: 443-454.
- Mareš J, Leskinen E, Sitkowska M, Skácelová O, Blomster J. 2011. True identity of the European freshwater *Ulva* (Chlorophyta, Ulvophyceae) revealed by a combined molecular and morphological approach. *J Phycol* 47: 1177-1192.
- O'Kelly CJ, Kurihara A, Shipley TC, Sherweed AR. 2010. Molecular assessment of *Ulva* spp. (Ulvophyceae, Chlorophyta) in the Hawaiian Islands. *J Phycol* 46: 728-735.
- Sfriso A. 2010. Coexistence of *Ulva rigida* and *Ulva laetevirens* (Ulvales, Chlorophyta) in Venice Lagoon and other Italian transitional and marine environments. *Bot Mar* 53: 9-18.
- Tan IH, Blomster J, Hansen G, Leskinen E, Maggs CA, Mann DG, Sluiman HJ, Stanhope MJ. 1999. Molecular phylogenetic evidence for a reversible morphogenetic switch controlling the gross morphology of two common genera of green seaweeds, *Ulva* and *Enteromorpha*. *Mol Biol Evol* 16: 1011-1018.
- Womersley HBS. 1984. The Marine Benthic Flora of Southern Australia. Part I. Government Printer, Adelaide.