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Ulva grossa sp. nov. (Ulvales, Chlorophyta) from Korea based on Molecular and Morphological Analyses

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Abstract A green alga specimen was collected from the eastern coast of Korea. This species shared the typical features of genus *Ulva* and was 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, it nests as a sister clade to a few species including *Ulva ohnoi,* which has a relatively large thallus. This Korean algal specimen differs from the species forming the same subclades, including *U. ohnoi*, *Ulva fasciata, Ulva reticulata*, and *Ulva gigantean,* and has 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 as it had a thick thallus of 30-90 μm, but it has microscopic serrations on the thallus margin, unlike the Korean alga. The genetic distance between the Korean alga species and the aforementioned species was determined to be 1.8%-4.8%, indicating an inter-specific divergence level at the genus *Ulva*. Herein, *Ulva grossa* sp. nov. (Ulvales, Chlorophyta) from Korea is described based on the morphological and molecular analyses.

Keywords : Marine green alga, ITS sequence, Morphology, New species

Introduction

Ulva Linnaeus is the most species-rich genus in the family Ulvaceae [1], and 129 species are currently accepted in this genus [2]. Of these, several species are associated with notorious blooms called “green tides” [1, 3, 4, 5].

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 [6, 7]. However, intraspecific variations were observed for these characteristics in different environments and growth phases [1, 8]. 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 in a generic characteristic previously distinguishing *Ulva* and *Enteromorpha* Link [1, 9, 10].

Therefore, many molecular studies of *Ulva* have contributed significantly to its taxonomy [1, 5, 9,

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11, 12, 13, 14]. The molecular data also helped in understanding the biogeographic history, cryptic diversity and introduction of species of *Ulva* in different regions [1, 15, 16, 17, 18, 19]. These suggest that taxonomic approach for *Ulva* based on both morphological and molecular data is required [16, 20, 21].

A total of 16 species are currently recorded in the marine algal flora of Korea [22, 23, 24, 25, 26, 27]. 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) [6, 7, 28]. 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 [8]. ITS regions were PCR amplified as a single fragment with the primers ITSP1 (5′ GGAAGGAGAAGTCGTAA CAAGG 3′) and G4 (5′ CTTTTCCTCCGCTTATTGATATG 3′) [29] or as two overlapping fragments with the primers ITSP1 and ITSR1 (5′ TTCAAAGAT TCGATGATTCAC 3′) and P5 (5′ GCATCGATGAAGAACGCAG 3′) and G4 [29]. PCR amplifications were performed in a TaKaRa PCR Thermal Cycler Dice with an initial denaturation step at 94 ℃ for 5 min followed by 35 cycles at 94 ℃ for 1 min, 56 ℃ for 1 min, and 72 ℃ for 2 min and a final extension at 72 ℃ 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 [30]. 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: MGARB001548–MGARB001550 (Geoil-ri, Uljin: 21.vi.2018), MGARB001551 (Changpo-ri, Yeongdeok: 22.vi. 2018), MGARB001552–MGARB001553 (Daejin-ri, Yeongdeok: 21.vi. 2018).

Holotype: MGARB001548 (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 nom. nov. (신칭: 도톰갈파래)

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 [31]. 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%. 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%.

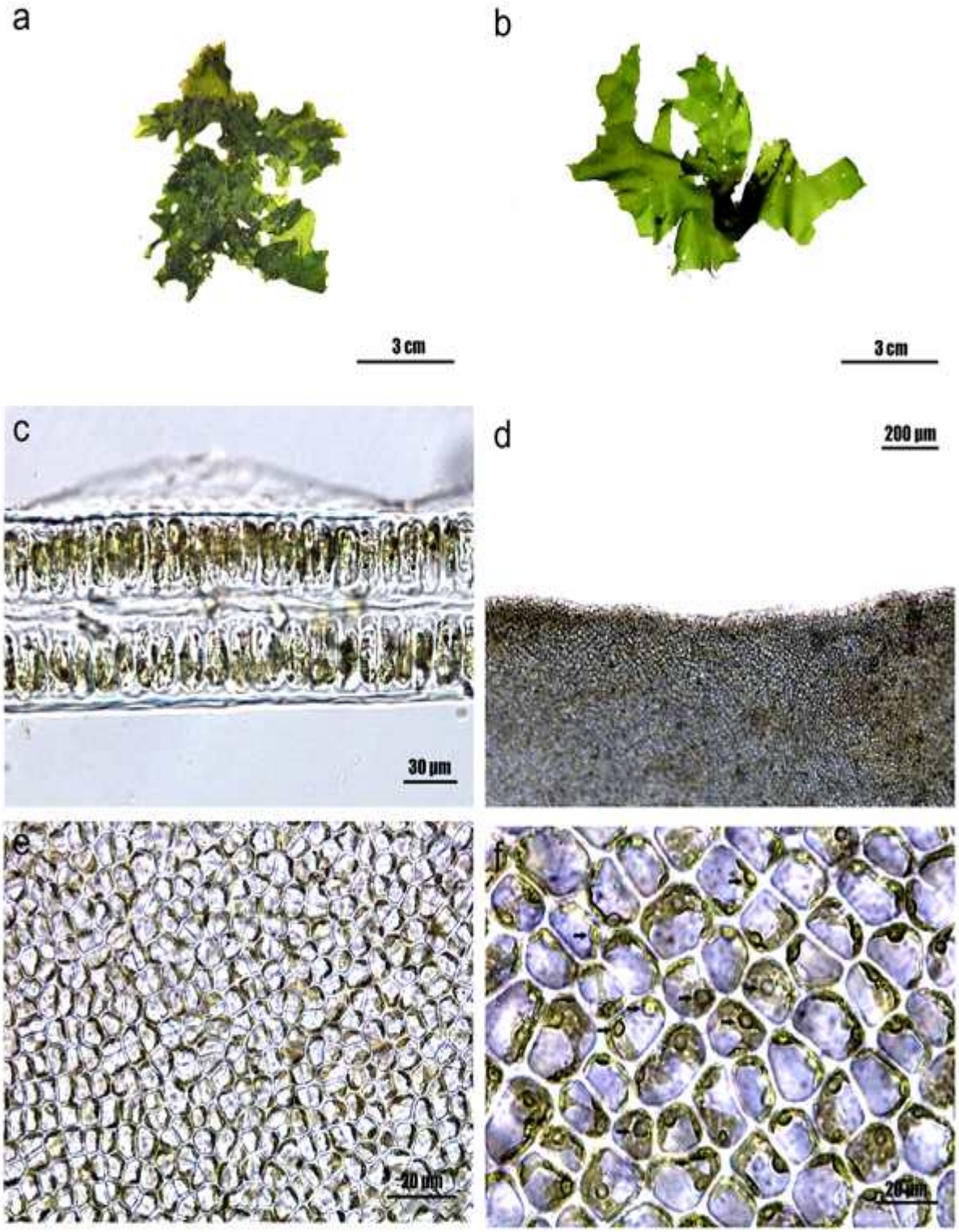

Fig. 1 *Ulva grossa* sp. nov. **a** Holotype specimen (MGARB001548). **b** Habit of vegetative plant from liquid preserved specimen. **c** Transverse section of thallus with two cell layers. **d** Entire margin of thallus. **e** Rectangular to polygonal cells with round corner near upper portion of thallus. **f** Cap-like parietal chloroplasts with one or two pyrenoids (arrows) in surface view.

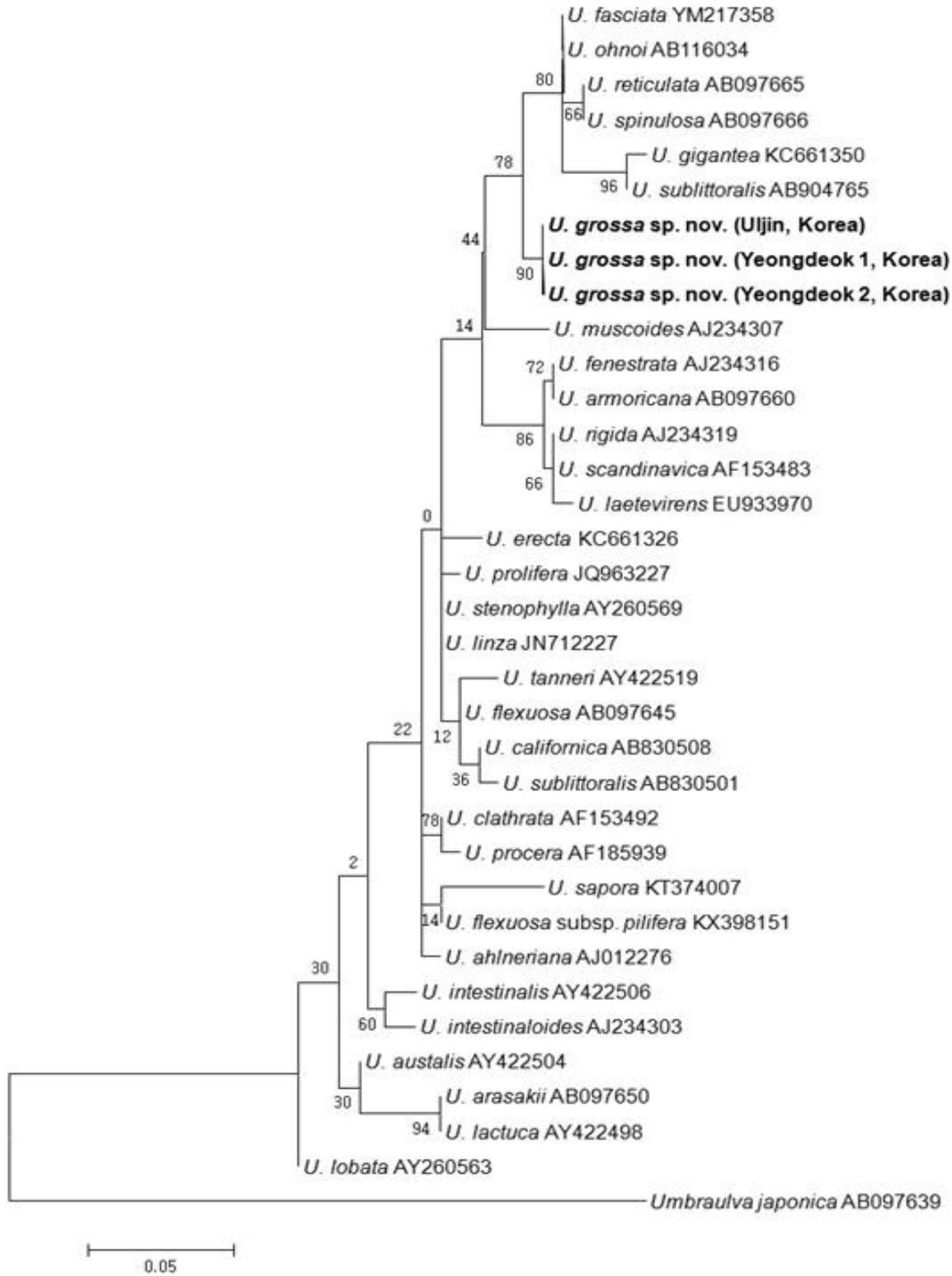

Fig. 2 Phylogenetic tree of selected taxa obtained from ML analysis based on ITS sequences. Bootstrap percentages (1000 replicates samples) are shown above branches. Scale bar = 0.05 substitutions/site.

Discussion

Ulva was established based on *U. lactuca* originally described from the Atlantic Ocean [32]. In basic thallus morphology, *Ulva* is a flattened distromatic or tubular monostromatic [9], and its blades are broadly expanded foliose, irregularly lobed, cuneate, linear, ovate, lanceolate, oblanceolate, and deeply divided into linear lacinae or tubulose [2]. 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 [6, 7, 33, 34, 35, 36, 37].

The type species *U. lactuca* is commonly distributed along the coasts of Korea [23]. 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 [38, the present study]. However, *U. lactuca* is separable from the latter species in having relatively small and thick thallus [25. 39, 40], even though the thallus size of *U. ohnoi* varies greatly with habitat [38, 11, 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* [38]. 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 [38], 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 [41] and *U. gigantean* 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. gigantean* is irregularly or palmately to

linear, deeply and irregularly lobed, or deeply laciniate (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 [10, 42, 43]. 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.

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

Features	*U. grossa* sp. nov.	*U. lactuca* (type)	*U. ohnoi*	*U. fasciata*	*U. reticulata*	*U. gigantea*
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 laciniate 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 (μm)	5–20 × 20–35	15 × 20	14–20 × 7–15 (upper) 14–30 × 12–20 (basal)	8–20 × 14–40	-	15–22 × 12–15
Thickness (μm)	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	[39, 25, 40]	[38]	[39, 40, 44]	[44]	[45]

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