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# New records of two ulvophycean freshwater species, *Lithotrichon pulchrum* (Ulvales, Ulvophyceae) and *Tupiella speciosa* (Ulotrichales, Ulvophyceae)

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The Ulvophyceae are a diverse group and most species are distributed in marine environments, however, a smaller diversity occurs in freshwater and terrestrial habitats. Also, the diversity of species belonging to Ulvophyceae has been superficially investigated in Korea. Here, we identify two freshwater ulvophycean species based on the morphology, ultrastructural observation, and phylogenetic analysis. As a result, their taxonomic classifications are as follows: order Ulvales Blackman and Tansley, 1902 - family Kornmanniaceae Golden and Cole, 1986 - *Lithotrichon pulchrum* Darienko and Pröschold 2017; order Ulotrichales Borzì, 1895 - family Tupiellaceae Škaloud and Leliaert, 2018 - *Tupiella speciosa* Darienko and Pröschold 2017. The family Tupiellaceae and the two genera *Lithotrichon* and *Tupiella* are reported for the first time in Korea.

Keywords: *Lithotrichon*, phylogeny, *Tupiella*, ultrastructure, Ulvophyceae

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## Introduction

Ulvophyceae is one of the chlorophyte classes known as green seaweed (Guillou *et al*., 2004; Darienko and Pröschold, 2017) and the second most species-rich class in Chlorophyta which contain more than 1,937 species. Although the most significant diversity of Ulvophyceae is found in marine habitats, they are found over a broad salinity range, such as brackish to freshwater and semi-terrestrial habitats (Škaloud *et al*., 2018). Some of them grow by attaching to various substrates, including wood, rock, and even plastic (Nielsen *et al*., 2013; Gunnarsson and Nielsen, 2016; Škaloud *et al*., 2018).

Many filamentous algae had been assigned to Chlorophyceae based on the traditional classifications (Borzi, 1883; Nichols and Bold, 1965; Biebel, 1968; Tupa, 1974). Recently, molecular phylogenetic analyses were performed to investigate the species diversity and revealed that filamentous algal species belonged to Ulvophyceae (Darienko and Pröschold, 2017; Liu *et al*., 2019).

The phylogenetic analysis of non-marine ulvophycean species was performed based on a data set of SSU and ITS rDNA sequences by Darienka and Pröschold (2017). As a result, non-marine species belonged to two orders (Ulvales and Ulotrichales) and 12 lineages (five in Ulvales and seven in Ulotrichales). Among them, the independent lineages, showed no affiliation with the other lineages in polyphyletic genera *Dilabifilum* and *Pseudendoclonium*, were described as new genera *Lithotrichon* and *Tupiella*, respectively, and consequently, three new species have been known: *L. pulchrum*, *T. speciosa*, and *T. akineta*. After that, Liu *et al*. (2019) reported an additional new *Lithotrichon* species in China, *L. fluminense*, based on both morphological observation and phylogenetic analysis.

We report the taxonomic information on unrecorded two ulvophycean genera and species, *Lithotrichon pulchrum* and *Tupiella speciosa*, with morphology, ultrastructure, and phylogenetic data.

## Materials and Methods

### 1. Sampling and clonal culture of two ulvophycean species

The cultures of *Lithotrichon pulchrum* and *Tupiella speciosa* were single-cells isolated from natural assemblages of Samcheok Harbor (37°26′14.2″N, 129°11′34.0″E) in

**Table 1.** List of strains of the Ulvales species sequenced in this study.

| Taxon | Strain | Origin | Accession number |
|---|---|---|---|
| *Ctenocladus circinnatus* | CCMP 2158 | Ruins, Pompei, Italy | MF034603 |
| *C. circinnatus* | ULVO-16 | Solonchak, Azov-Syvash National Park, Kherson Oblast, Ukraine | MF034604 |
| *C. circinnatus* | ULVO-17 | Solonchak, Azov-Syvash National Park, Kherson Oblast, Ukraine | MF034605 |
| *C. circinnatus* | ULVO-18 | Fort Bourbon, Petruss, Casemate, Luxembourg | MF034606 |
| *C. circinnatus* | ULVO-24 | Quartz, Snake Island, Odesa Oblast, Ukraine | MF034607 |
| *C. circinnatus* | ULVO-25 | Solod, Kuyalnyk, Odesa Oblast, Ukraine | MF034608 |
| *C. printzii* | SAG 467-1 | Bog water, Rosenau/Basel, Switzerland | MF034613 |
| *Desmochloris halophila* | CCAP 6006/1 | Unknown | FM882216 |
| *D. mollenhaueri* | CCAP 6006/2 | Unknown | FM882217 |
| *D. mollenhaueri* | CCAP 6006/3 | Unknown | FM882218 |
| *Halofilum helgolandicum* | SAG 2.95 | From an enrichment culture of *Rhizoclonium riparium*, Helgoland, Schleswig-Holstein, Germany | MF034635 |
| *H. ramosum* | SAG 2050 | Isolated from lichen Wahlenbergiella striatula, In stonecracks of coastal rocks, Porth Trecastel, Anglesey, Wales, United Kingdom | MF034615 |
| *H. ramosum* | SAG 2235 | Photobiont of *Verrucaria maura*, Brittany, Roscoff, North Atlantic, France | MF034617 |
| *H. ramosum* | ULVO-19 | Green biofilm on the wall, Archeological remains, Carthage, Tunesia | MF034621 |
| *H. ramosum* | ULVO-28 | Photobiont of *Verrucaria maura*, Brittany, Roscoff, North Atlantic, France | MF034624 |
| *Lithotrichon pulchrum* | SAG 2038 | Submers from lichen *Verrucaria rheitrophila*, Near Dillenburg and Wetzlar, Gladenbacher Bergland, Germany | MF034614 |
| ***L. pulchrum*** | **Samcheokhang 190409G** | **Samcheok harbor, Samcheok-si, Republic of Korea** | **OM022027** |
| *Paulbroadya petersii* | SAG 2240 | Photobiont of *Verrucaria mucosa*, Brittany, Roscoff, North Atlantic, France | MF034620 |
| *P. petersii* | ULVO-34 | Photobiont of *Verrucaria mucosa*, Oban, North Atlantic, Scotland | MF034625 |
| *P. prostrata* | CCAP 415/4 | Green epilithic crusts on rocks, Cape Royds, Ross Island, Antarctica | MF034612 |
| *P. prostrata* | SAG 23.92 | Green epilithic crusts on rocks, Cape Royds, Ross Island, Antarctica | MF034611 |
| *Pseudendoclonium arthopyreniae* | SAG 467-2 | Photobiont of lichen *Arthopyrenia kelpii* on snail shell of *Littorina littorea*, Wangerooge, Germany | MF034609 |
| *P. incrustans* | CCAP 415/1 | Photobiont of lichen *Verrucaria aquatilis*, Austria | MF034610 |
| *P. submarinum* | SAG 2237 | Photobiont of *Verrucaria mucosa*, Oban, North Atlantic, Scotland | MF034619 |
| *P. submarinum* | ULVO-26 | Photobiont of *Verrucaria maura*, Brittany, Roscoff, North Atlantic, France | MF034623 |
| *Pseudendoclonium* sp. | SAG 2051 | On coastal rocks (Pelvetia-zone), Aberfraw, Anglesey, Wales, United Kingdom | MF034616 |
| *Pseudendoclonium* sp. | SAG 2236 | Photobiont of *Verrucaria maura*, Öland, North Atlantic, France | MF034618 |
| *Pseudendoclonium* sp. | ULVO-21 | Quartz, Snake Island, Odesa Oblast, Ukraine | MF034622 |
| *Pseudendoclonium* sp. | ULVO-62 | From rock of the tidal zone, Oakland Beach, RI, USA | MF034626 |

New sequence is indicated in bold type.

November 2016 and Songchonji Pond (36°22′11.5″N, 128°14′42.3″E) in January 2017, respectively. The cultures were grown in BG-11 medium at 20°C under a 14 : 10 light : dark cycle using cool white fluorescence lamps with illuminations of 150 μE $m^{-2}$ $s^{-1}$.

## 2. Light microscopy

Living *L. pulchrum* and *T. speciosa* were studied using a Nikon ECLIPSE Ni-U (Nikon, Japan) equipped with differential interference contrast optics. Images were captured using a Digital Camera (DS-Ri2, Nikon). Measuring the length and width are used NIS-Elements BR 4.50.00 program.

## 3. Transmission electron microscopy

For transmission electron microscopy, each culture of *L. pulchrum* and *T. speciosa* was prefixed in a 1 : 1 mixture of 5% (V/V) glutaraldehyde and BG-11 culture media for 1 h at 4°C. The glutaraldehyde-fixed cells were washed 3 times in BG-11 culture media and post-fixed in

**Table 2.** List of strains of the Ulotrichales species sequenced in this study.

| Taxon | Strain | Origin | Accession number |
|---|---|---|---|
| *Acrosiphonia* sp. | SAG 127.80 | Unknown | FN562430 |
| *Chamaetrichon basiliense* | CCALA 986 | Shallow lake, Andreassen Point, Ross Island, Antarctica | HF570951 HF570954 |
| *C. basiliense* | SAG 1.87 | Pool at Couchville Pike, Nashville, TE, USA | MF034630 |
| *C. basiliense* | SAG 18.88 | Queda de água (waterfall)Arganil, Portugal | MF034602 |
| *C. basiliense* | SAG 23.88 | Pond near Arazede, Amieiro, Portugal | MF034627 |
| *C. basiliense* | SAG 2396 | Deinschwanger Bach, Franconian Alb Germany | MF034631 |
| *C. basiliense* | SAG 466-1 | Garden pool, Basel, Switzerland | MF034629 |
| *C. basiliense* | ULVO-15 | Pitsford Water, Northamptonshire, UK | MF034633 |
| *C. basiliense* | UTEX 1918 | Double Lake, Pallavincinia lyelii, Sam Houston National Forest, Texas, USA | MF034600 |
| *Planophila bipyrenoidosa* | ULVO-1 | Unknown | MF034636 |
| *P. bipyrenoidosa* | ULVO-55 | Sandstone, Trakhtemyriv, Regional Landscape Park, Cherkassy Oblast, Ukraine | MF034637 |
| *P. laetevirens* | GSM-5A-LK1 | Soil sample, Great Smoky Mountains National Park, North Carolina, USA | MF034641 |
| *P. laetevirens* | SAG 2008 | Dolomites, South Tyrol, Italy | MF034638 |
| *P. laetevirens* | SAG 32.98 | Soil, Cultivo trucha, Ojo de Agua, Costa Rica | MF034639 |
| *P. laetevirens* | SAG 465-1 | Pond, Basel, Switzerland | MF034642 |
| *P. laetevirens* | ULVO-56 | Sandstone, Trakhtemyriv, Regional Landscape Park, Cherkassy Oblast, Ukraine | MF034640 |
| *Rhexinema paucicellularis* | SAG 29.93 | Soil from the shore at a lake near Suchdol, Trebon, Czech Republic | Z28970 |
| *R. paucicellularis* | SAG 463-1 | Small basin Victoria green house, Botanical garden, Basel, Switzerland | Z47997 |
| *R. paucicellularis* | SAG 466-2 | Bog water, Botanical garden, Basel, Switzerland | MF034645 |
| *R. paucicellularis* | SAG 8.90 | Sandy soil at an anthropogenic lake near Suchdol, Near Trebon, Czech Republic | MF034601 |
| *R. planctonica* | UTEX 1570 | Flooded ore pit, Mount Holy Springs, Pennsylvania, USA | MF034632 |
| *R. sancta-tomea* | ACOI 592 | São Tomé and Principe | MF034628 |
| *R. sarcinoidea* | UTEX 1710 | Soil, Bastrop National Park, Bastrop, Texas, USA | Z47998 |
| *Sarcinofilum mucosum* | SAG 24.93 | Shallow temporary pool in granite rock, Llano Co., Texas, USA | MF034649 |
| *S. mucosum* | SAG 26.88 | Porto, Portugal | MF034650 |
| *S. mucosum* | SAG 26.94 | From roots of *Lemna minor*, Garden pool, Biebergemünd/Spessart, Hessen, Germany | MF034651 |
| *S. mucosum* | SAG 29.94 | Spring Run Preserve, Dickinson Co., IA, USA | MF034652 |
| *S. mucosum* | SAG 4.90 | Vestfold Hills, Prinzess Elizabeth Land, Antarctica | MF034648 |
| *Tupiella speciosa* | CCALA 423 | Lednice n.M, Czech Republic | MF034643 |
| *T. speciosa* | ULVO-11 | Unknown | MF034644 |
| ***T. speciosa*** | **Songchonmot 180226S** | **Songchonji pond, Iksan-si, Republic of Korea** | **OM022028** |

New sequence is indicated in bold type.

1% (W/V) osmium tetroxide for 1 h at 4°C. The fixed cells were rinsed three times with distilled water. Dehydration was carried out at 4°C using a graded ethanol series of 50, 60, 70, 80, and 90% for 10 min each and three times for 10 min changes of pure ethanol. Pellets were then brought to room temperature and transferred through propylene oxide two times for 20 min each, 50%, and 75% Spurr's embedding resin (Spurr, 1969) in propylene oxide for 1 h each, and 100% overnight. The following day, pellets were moved to new pure resin and polymerized at 70°C. Blocks were thin-sectioned on a PT-X ultramicrotome (RMC Products, Boeckeler Instruments, Tucson, AZ). Sections of 70 nm thickness were collected on slot copper grids, stained with the UranyLess solution (Electron microscopy sciences, Philadelphia, USA) and Reynold's lead citrate (Reynolds, 1963), and observed and photographed using a JEM-2100F transmission electron microscope operated at 120 kV (JEOL, Tokyo, Japan) equipped at KBSI.

## 4. DNA extraction, amplification, and sequencing

Genomic DNA was extracted using the InstaGene™ Matrix (BIO-RAD, CA, USA). SSU and ITS rDNA were amplified by PCR using the *EF-Taq* (SolGent, Daejeon, Korea) with primers EAF3 and ITS055R (Marin *et al*., 2003). SSU and ITS rDNA were amplified using a DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD, CA,

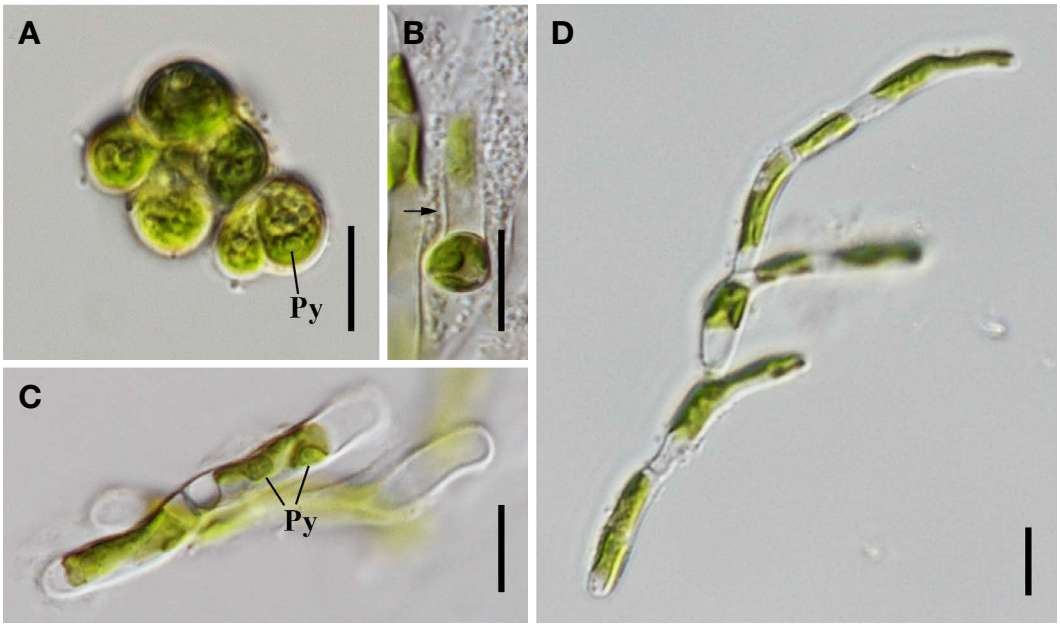



**Fig. 1.** Morphology of *Lithotrichon pulchrum* Samcheokhang190409G. A. Vegetative cells; B. The erect system formed by a short filament cell (arrow); C. Filament cells; D. Bilaterally branched from. Py, pyrenoid. Scale bars: 10 μm.

USA) with the following conditions: initial denaturation at 95°C for 2 min; 35 cycles each of 95°C for 2 min, 55°C for 1 min, and 72°C for 1 min; final extension at 72°C for 10 min and holding at 4°C. According to the manufacturer's protocol, the PCR products were purified using the Multiscreen filter plate (Millipore Corp., MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle Sequencing Kit and analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA).

### 5. Alignment and phylogenetic analyses

The sequence alignment was edited using the Genetic Data Environment program (Smith *et al*., 1994), and the aligned sequences for phylogenetic analysis were listed in Tables 1 and 2. The SSU rDNA sequences of all the strains were aligned according to their secondary structures using the structure of *Ulothrix zonata* (accession number MF034653) as a template (Darienko and Pröschold, 2017). The alignments were separated into two datasets Ulvales and Ulotrichales. The conserved regions of ribosomal DNA sequences were used for phylogenetic analysis and variation sites were excluded. Primers and ambiguously aligned regions were not used in phylogenetic analyses. The datasets were analyzed using Bayesian inference, maximum likelihood (ML), maximum parsimony (MP), and neighbor-joining (NJ). Prior to ML analysis, the best-fit model for individual and concatenated data sets was traced under Akaike Information Criterion (Akaike, 1974) using MODELTEST 3.7 (Posada and Cradall, 1998). ML analysis was carried out by RAxML v8.2.4 (Stamatakis, 2014) using the GTR + I + G model with the random sequence addition for 1,000 times, followed by the heuristic search using the tree-bisection reconnection (TRB) branch swapping. Bayesian analysis was performed using MrBayes v3.2 (Ronquist *et al*., 2012) to construct random inference trees with the GTR + G + I model for 2 million generations. The phylogenetic tree was sampled every 1,000 generations, and the burn-in point was graphically identified based on the likelihood score in the phylogenetic tree (Tracer v1.7.1; https://tree.bio.ed.ac.uk/software/tracer/). MP and NJ analyses were constructed for each data set with PAUP* v.4.0b.10 (Swofford, 2002) using a heuristic search algorithm.

## Results and Discussion

### 1. Taxonomic Summary

Class Ulvophyceae Mattox and Stewart, 1984
Order Ulvales Blackman and Tansley, 1902
Family Kornmanniaceae Golden and Cole, 1986
Genus *Lithotrichon* Darienko and Pröschold, 2017

**1) *Lithotrichon pulchrum***
**Darienko and Pröschold, 2017**

**Material examined.** Sample was collected from the Sam-

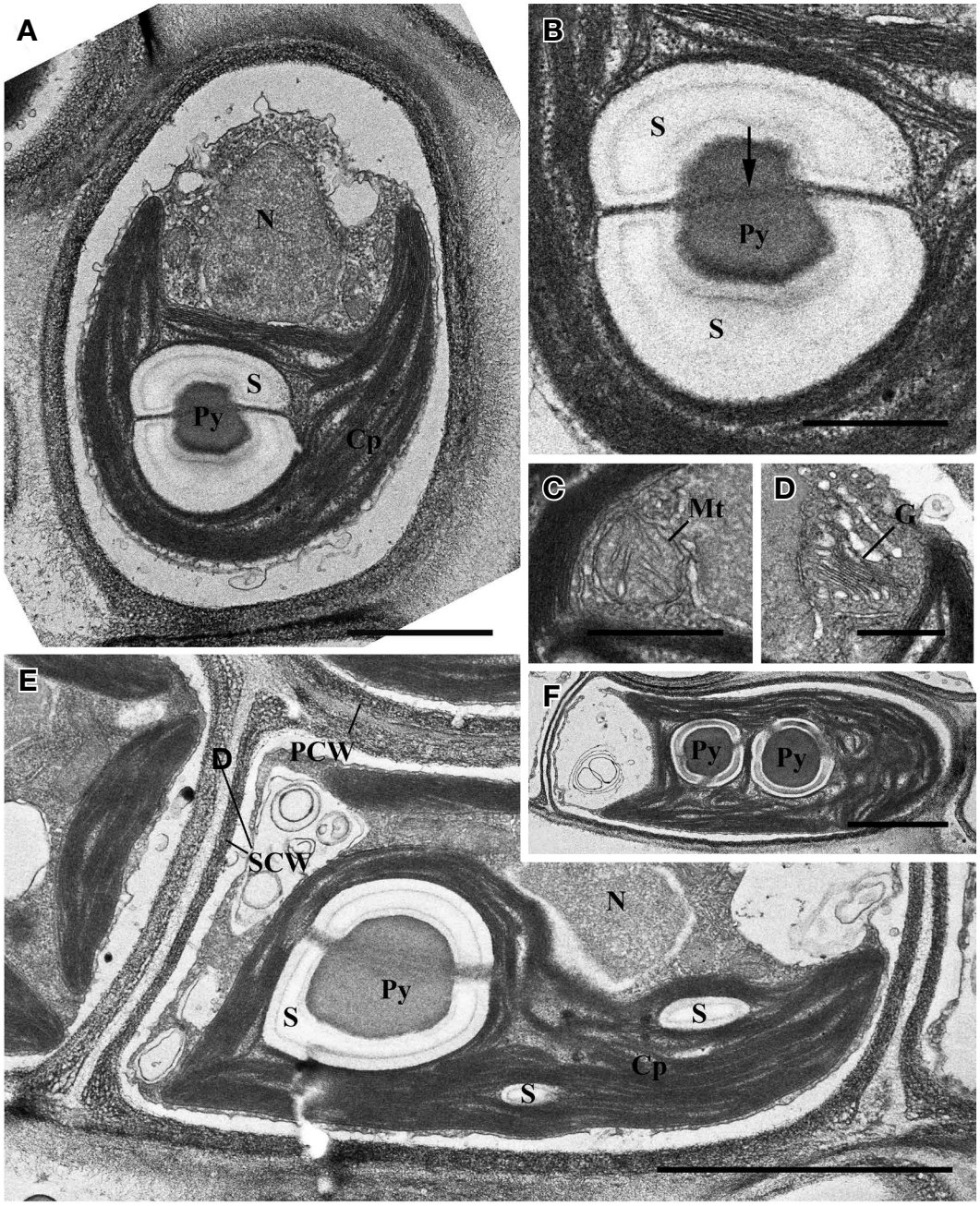



**Fig. 2.** Ultrastructure of *Lithotrichon pulchrum*. A. Vegetative cell; B. Pyrenoid penetrated by thylakoid membrane (arrow); C. Mitochondrion; D. Golgi body; E. Flament cell with one pyrenoid; F. Flament cell with two pyrenoids. Cp, chloroplast; PCW, primary cell wall; G, Golgi body; Mt, mitochondrion; N, nucleus; Py, pyrenoid; S, starch; SCW, secondary cell wall. Scale bars: A = 1 μm, B–D = 0.5 μm, E, F = 2 μm.

cheok Harbor, Jeongha-dong, Samcheok-si, Gangwon-do, Republic of Korea (37°26′14.2″N, 129°11′34.0″E) on 09 April 2019.

**Description.** *Lithotrichon pulchrum* is a microscopic and filamentous alga. Vegetative cells were round to laterally compressed oval-shaped and measured 5.9–8.8 μm in diameter with a thick cell wall (Figs. 1A, 2A). Cells possess a nucleus, Golgi body, mitochondria with plate-like cristae, and a single parietal chloroplast with a pyrenoid encased by starches (Figs. 1A, B, 2A–D). The pyrenoid matrix was penetrated by the thylakoid membrane(s) (Fig. 2B). The erect system is formed by short filaments, 12.0–23.5 μm long and 3.5–5.5 μm wide, generally 2–4 times longer than broad (Fig. 1B, C). Branching is mostly bilateral, and younger cells are L-shaped bent to the side (Fig. 1D). In filament form, the cell had one or two

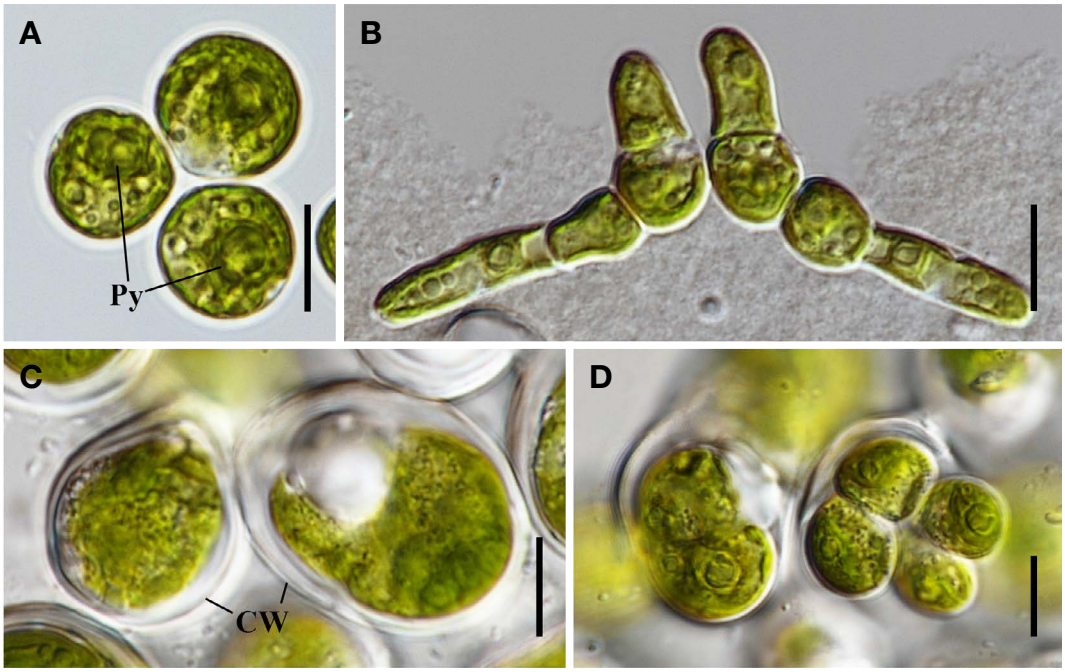



**Fig. 3.** Morphology of *Tupiella speciosa* Songchonmot180226S. A. Vegetative cell; B. Young filament; C, D. Thick cell wall-enveloped akinetes including 1−4 cells. CW, cell wall; Py, pyrenoid. Scale bars: 10 μm.

pyrenoids and two types of cell walls (Fig. 2E, F).
**Distribution.** Germany (Darienko and Pröschold, 2017) and Republic of Korea.
**Voucher slides.** Two slides of gelatin-embedded specimens (NNIBRCL16651 and NNIBRCL16654) were deposited at Nakdonggang National Institute of Biological Resources, Korea.
**Remarks.** *Lithotrichon pulchrum* was known as photobiont of lichen *Hydropunctaria rheitrophila* (Darienko and Pröschold, 2017; Sanders and Masumoto, 2021), however, Korean strain was isolated in planktonic seawater samples and cultured BG11 freshwater medium. This means *L. pulchrum* might have diverse habitats and salinity tolerance. Zoospores of German strain were contained in oval-shaped zoosporangia, but any zoospores with anterior stigma for asexual reproduction were not observed in the Korean strain (Darienko and Pröschold, 2017).

Class Ulvophyceae Mattox and Stewart, 1984
Order Ulotrichales Borzì, 1895
Family *Tupiella*ceae Škaloud and Leliaert, 2018
Genus *Tupiella* Darienko and Pröschold, 2017

**2) *Tupiella speciosa* Darienko and Pröschold, 2017**

**Material examined.** Sample was collected from the Songchonji Pond Sangchon-ri, Nakdong-myeon, Sangju-si, Gyeongsangbuk-do, Republic of Korea (36°22′11.5″N, 128°14′42.3″E) on 26 February 2018.
**Description.** *Tupiella speciosa* is a microscopic and filamentous alga. Vegetative cells were round-shaped (Fig. 3A). Cells possess a nucleus, Golgi body, mitochondria with plate-like cristae, and a single parietal chloroplast with a pyrenoid penetrated by a thylakoid membrane (Fig. 3A, 4A−D). The pyrenoid matrix was penetrated by a single thylakoid membrane (Fig. 4B). Young filament cells had a chloroplast with 1−4 pyrenoids encased by starches (Fig. 4F, G). The terminal cells are slightly pointed (Fig. 3B). Akinetes are oval-shaped and enveloped by the thick cell wall (Fig. 3C). Akinete cell divided into four (Fig. 3D). In the akinetes cell, many starches were developed between thylakoid membranes (Fig. 4E).
**Distribution.** Czech Republic (Darienko and Pröschold, 2017) and Republic of Korea.
**Voucher slides.** Two slides of gelatin-embedded specimens (NNIBRCL16652 and NNIBRCL16653) were deposited at Nakdonggang National Institute of Biological Resources, Korea.
**Remarks.** *Tupiella speciosa* cells were unicellular form in liquid medium. However, a young filament form was observed on an agar plate culture (Darienko and Pröschold, 2017). Also, mature cultures produce many swollen cells, which is growing thin filaments that become branched or produce 2−4-cell packages, but matured plant form (mature thallus) is not observed in this study (Darienko and Pröschold, 2017).

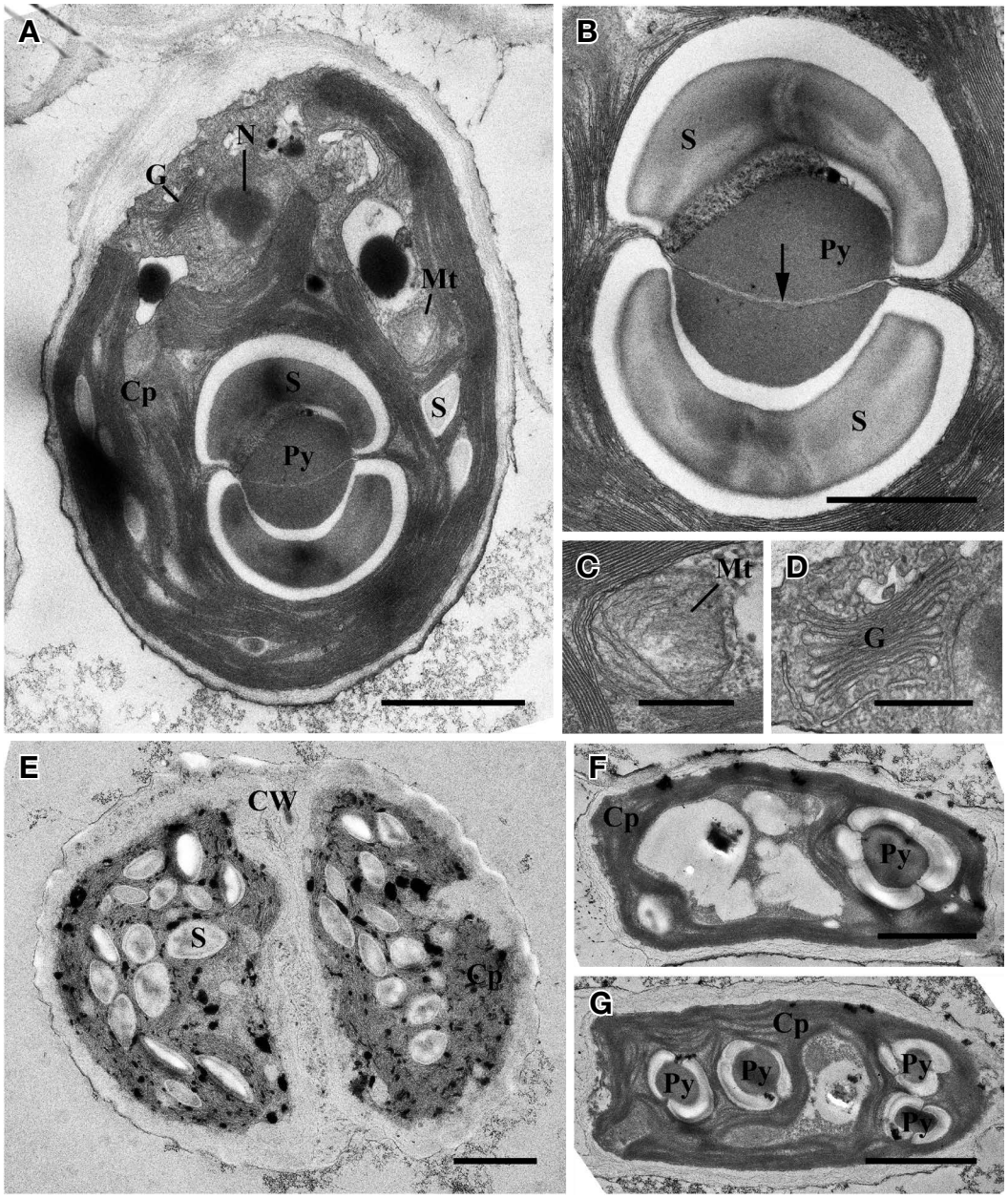



**Fig. 4.** Ultrastructure of *Tupiella speciosa*. A. Vegetative cell; B. Pyrenoid penetrated by thylakoid membrane (arrow); C. Mitochondrion; D. Golgi body; E. Akinete including two cells; F. Flament cell with one pyrenoid; G. Flament cell with four pyrenoids. Cp, chloroplast; CW, cell wall; G, Golgi body; Mt, mitochondrion; N, nucleus; Py, pyrenoid; S, starch. Scale bars: A, E–G = 2 μm, B = 1 μm, C, D = 0.5 μm.

## 2. Phylogeny

Phylogenetic analyses were separated into two datasets: concatenated dataset of 29 SSU and ITS rDNA sequences (2,554 bp) of Ulvales (Fig. 5), concatenated dataset of 31 SSU and ITS rDNA sequences (2,355 bp) of Ulotrichales (Fig. 6).

### 1) Ulvales (Fig. 5)

The phylogenetic tree based on the ML analysis was rooted with three strains of *Desmochloris* serving as outgroups (pp = 1.00 and ML, MP, and NJ = 100%). The Bayesian, ML, MP, and NJ analyses recovered trees with almost identical topology. Ulvales phylogenetic tree was comprised of members of the genera *Ctenocladus*, *Desmochloris*, *Halofilum*, *Lithotrichon*, *Paulbroadya*, and *Pseudendoclonium*. All genera were monophyletic with strongly support values. Newly recorded species *L. pulchrum* formed a monophyletic clade with type strain, *L. pulchrum*

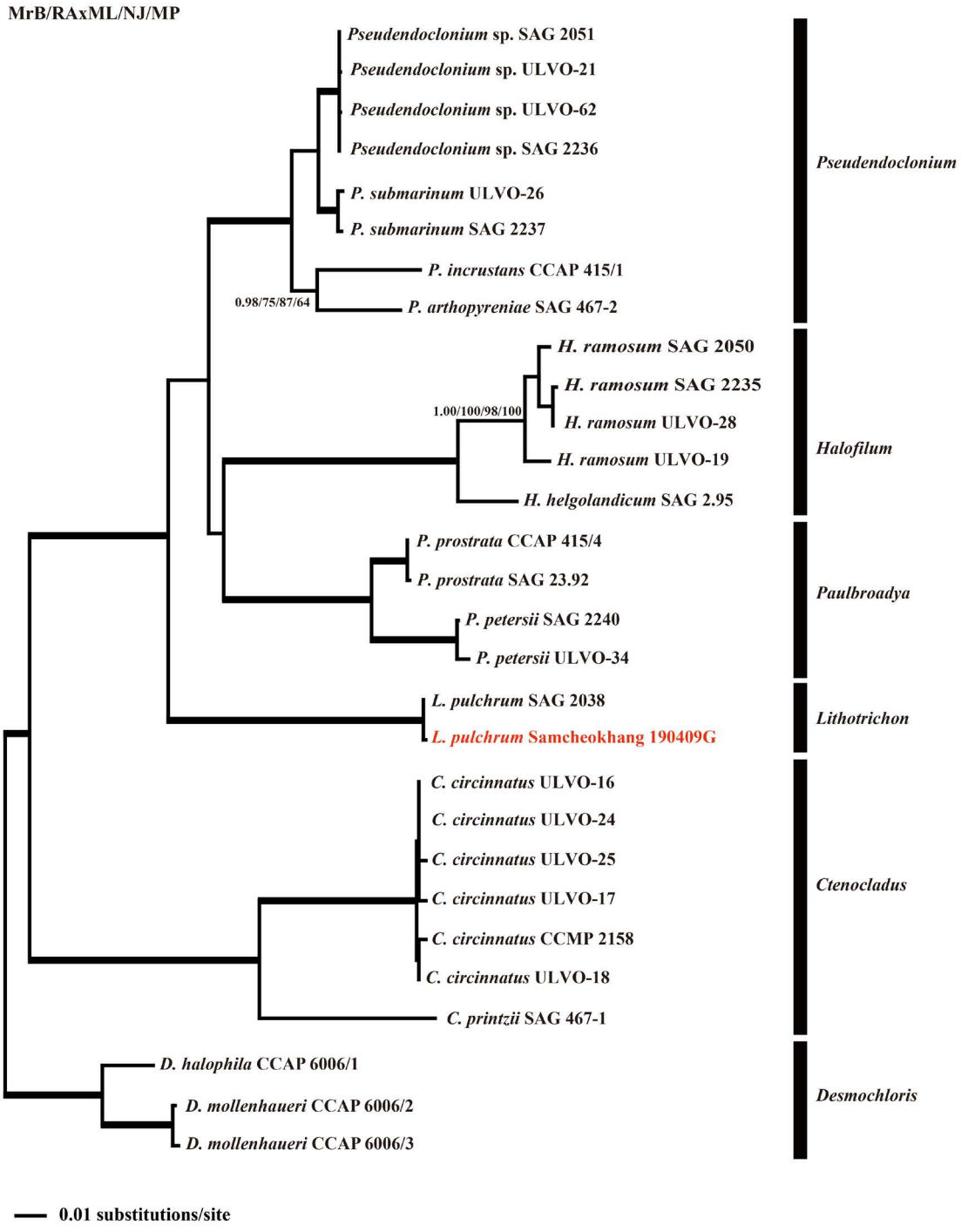



**Fig. 5.** Molecular phylogeny of Ulvales. The phylogenetic trees shown were inferred using the maximum likelihood method based on the SSU and ITS rDNA data sets (2554 aligned positions of 29 taxa) using RAxML. The Bayesian posterior probability (pp), maximum likelihood (ML) bootstrap, maximum parsimony (MP), and distance values (NJ) are shown above or below the branches. The bold branches indicate strongly supported values (pp = 1.00 and ML, MP, and NJ = 100%). Scale bar indicates the number of substitutions/sites.

SAG 2038 (pp = 1.00 and ML, MP and NJ = 100%).

**2) Ulotrichales (Fig. 6)**

The phylogenetic tree based on the ML analysis was rooted with a single strain of *Acrosiphonia* serving as outgroups (pp = 1.00 and ML, MP, and NJ = 100%). The Bayesian, ML, MP, and NJ analyses recovered trees with almost identical topology. Ulotrichales phylogenetic tree

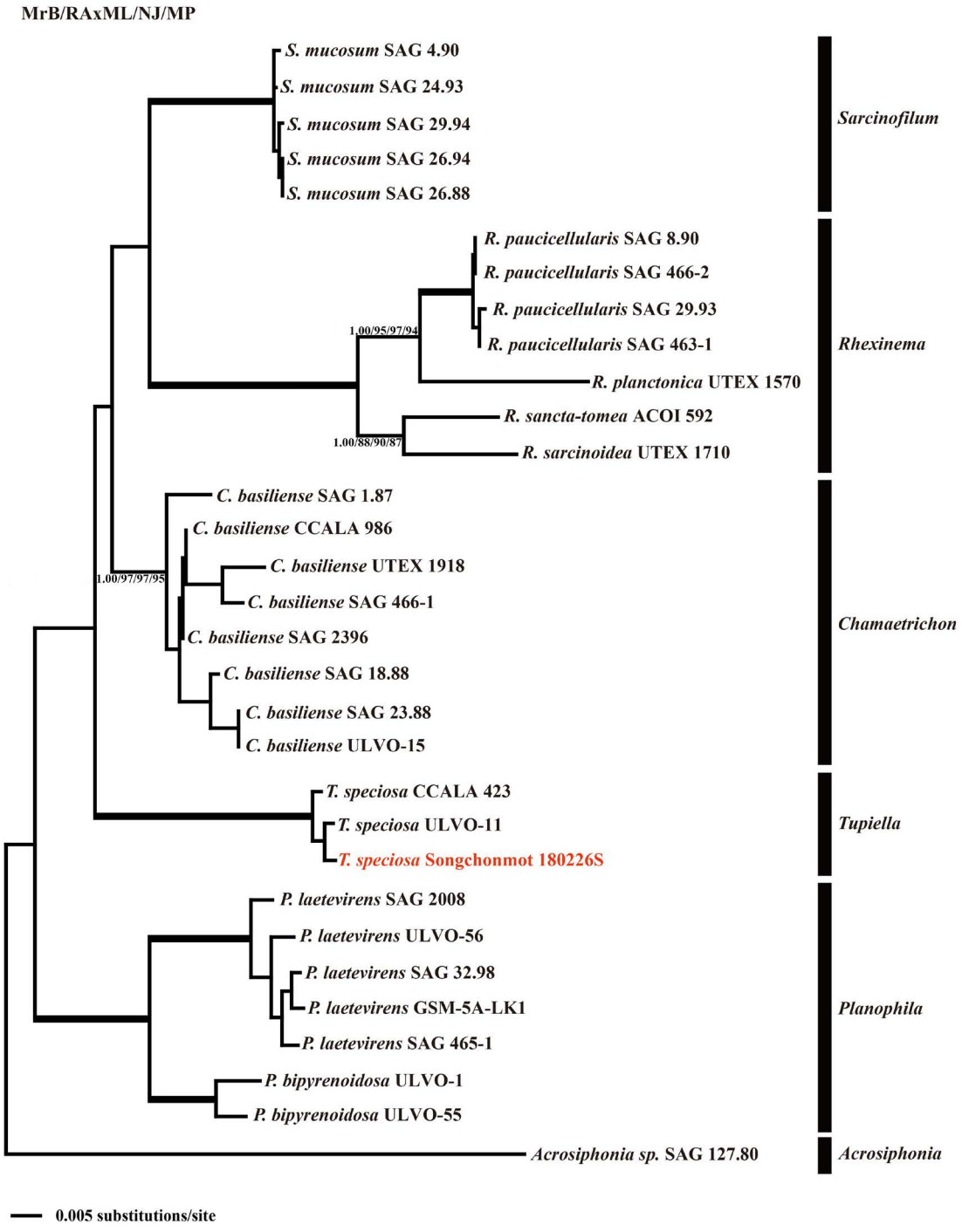



**Fig. 6.** Molecular phylogeny of Ulotrichales. The phylogenetic trees shown were inferred using the maximum likelihood method based on the SSU and ITS rDNA data sets (2355 aligned positions of 31 taxa) using RAxML. The Bayesian posterior probability (pp), maximum likelihood (ML) bootstrap, maximum parsimony (MP), and distance values (NJ) are shown above or below the branches. The bold branches indicate strongly supported values (pp = 1.00 and ML, MP, and NJ = 100%). Scale bar indicates the number of substitutions/sites.

was comprised of members of the genera *Acrosiphonia*, *Chamaetrichon*, *Planophila*, *Rhexinema*, *Sarcinofilum*, and *Tupiella*. All genera were monophyletic with strongly support values. Newly recorded species *Tupiella speciosa* formed a monophyletic clade with *T. speciosa* CCALA 423 (type strain) and UKVO-11 (pp = 1.00 and ML, MP and NJ = 100%).

## Acknowledgements

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## References

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control. 19(6):716-723.

Biebel, P. 1968. Reproduction in *Helicodictyon planctonicum* (Whitford) Whitford and Schumacher. Journal of Phycology 4(1):55-58.

Borzi, A. 1883. Saggio di ricerche Sulla biologia delle alghe. Ctenocladus, gen. nov. Studi Algologici 1:27-50.

Darienko, T. and T. Pröschold. 2017. Toward a monograph of non-marine Ulvophyceae using an integrative approach (Molecular phylogeny and systematics of terrestrial Ulvophyceae II.). Phytotaxa 324(1):001-041.

Guillou, L., W. Eikrem, M. Chretiennot-Dinet, F. Le Gall, R. Massana and K. Romari. 2004. Diversity of picoplanktonic prasinophytes assessed by direct nuclear SSU rDNA sequencing of environmental samples and novel isolates retrieved from oceanic and coastal marine ecosystems. Protist 155(2):193-214.

Gunnarsson, K. and R. Nielsen. 2016. Culture and field studies of Ulvellaceae and other microfilamentous green seaweeds in subarctic and arctic waters around Iceland. Nova Hedwigia 103 (1-2):17-46.

Liu, B., Q. Wang, S. Li, J. Fang, G. Liu and Z. Hui. 2019. Taxonomic transfer of *Gongrosira fluminensis* Fritsch (Chaetophorales, Chlorophyceae) to *Lithotrichon* Darienko et Proschold (Ulvales, Ulvophyceae) based on morphological observation and phylogenetic analyses. Fottea 19(1):25-32.

Marin, B., A. Palm, M. Klingberg and M. Melkonian. 2003. Phylogeny and taxonomic revision of plastid-containing euglenophytes based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure. Protist 154:99-145.

National Institute of Biological Resources, Ministry of Environment. 2002. National species list of Korea, 2020. National Institute of Biological Resources, Incheon [Available from: https://species.nibr.go.kr/, accessed 23 Dec, 2021].

Nichols, H.W. and H.C. Bold. 1965. *Trichosarcina polymorpha* gen. et sp. nov. Journal of Phycology 1(1):34-38.

Nielsen, R., G. Petersen, O. Seberg, N. Daugbjerg, C.J. O'Kelly and B. Wysor. 2013. Revision of the genus *Ulvella* (Ulvellaceae, Ulvophyceae) based on morphology and *tufA* gene sequences of species in culture, with *Acrochaete* and *Pringsheimiella* placed in synonymy. Phycologia 52(1):37-56.

Posada, D. and K.A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14(9):817-818.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. Journal of Cell Biology 17(1):208-212.

Ronquist, F., M. Teslenko, P.V.D. Mark, D.L. Ayres, A. Darling, S. Höhna and J.P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539-542.

Sanders, W.B. and H. Masumoto. 2021. Lichen algae: the photosynthetic partners in lichen symbioses. The Lichenologist 53(5):347-393.

Škaloud, P., F. Rindi, C. Boedeker and F. Leliaert. 2018. Freshwater Flora of Central Europe, Vol 13: Chlorophyta: Ulvophyceae (Süßwasserflora von Mitteleuropa, Bd. 13: Chlorophyta: Ulvophyceae). Springer-Verlag.

Smith, S.W., R. Overbeek, C.R. Woese, W. Gilbert and P.M. Gillevet. 1994. The genetic data environment an expandable GUI for multiple sequence analysis. Bioinformatics 10(6):671-675.

Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Phycology 26(1-2):31-43.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9):1312-1313.

Swofford, D.L. 2002. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10. Sunderland, MA, USA: Sinauer Associates.

Tupa, D.D. 1974. An investigation of certain chaetophoralean algae. Beihefte zur Nova Hedwigia 46:1-155.

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