

# Newly recorded chlorophytes, *Monoraphidium subclavatum*, *Deuterostichococcus epilithicus*, and *Pseudostichococcus monallantoides* in Korea

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*Monoraphidium subclavatum* (FBCC-A409) of this study clustered in the same clade as *Messastrum gracile* strains in the 18S rDNA phylogeny. Compared to *Messastrum gracile*, *Monoraphidium subclavatum* did not form a colony, and the curvature of the cell was slightly curved or slightly crescent-shaped. This result means that the genus *Monoraphidium* is still based on the morphospecies concept, and was not monophyletic and not distinguishable as a separate genus. Two *Stichococcus*-like strains of this study (NIBRCL0000114567, NIBRCL0000114571) belong to *Deuterostichococcus epilithicus* and *Pseudostichococcus monallantoides* respectively in phylogenetic analysis using 18S rDNA sequences. These two species are consistent with recent research in the morphology and the genetic analyses using 18S and ITS rDNA sequences. We reported *M. subclavatum*, *D. epilithicus*, and *P. monallantoides* as newly recorded species in Korea.

Keywords: Aerophytic algae, *Deuterostichococcus*, *Monoraphidium*, *Pseudostichococcus*

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## INTRODUCTION

Green algae commonly inhabit terrestrial habitats, marine shorelines, streams, ponds, and lakes. Green algae also participate in wide variety of important biotic associations with a diverse range of cell organization, reproductive processes, and morphology compared with other algae (Bold and Wynne, 1978). They also contain chlorophyll tissues associated with terrestrial plants, such as pyrenoids that store photosynthetic products, and photosynthetic pigments (Happy-Wood, 1988).

*Monoraphidium* taxa of the class Chlorophyceae are solitary and mainly planktonic organisms. *Monoraphidium* cells are more or less spindle-shaped, straight, curved or spirally twisted, gradually or abruptly narrowing to acute apices. They are thin-walled and without a mucilaginous envelope, with a single chloroplast located in cell periphery and a naked pyrenoid when examined under the light microscope (Komárek and Fott, 1983; Da Silva *et al.*, 2017). Komárková-Legnerová (1969) separated this genus from *Ankistrodesmus* and transferred it to *Monoraphidium*, those single-celled species of *Ankistrodesmus* carrying serially arranged autospores and without a visi-

ble pyrenoid under the light microscope (Hindák, 1970). Species are largely recognized by cell shape, size, and characteristics associated with cell curvature. Therefore, intermediate forms may be encountered, which are impossible to classify under one or other species (John *et al.*, 2011). The genus *Monoraphidium* is not monophyletic based on *rbcL* and 18S rDNA sequences (Da Silva *et al.*, 2017).

*Stichococcus*-like algae are very common and could be found in almost all types of habitats from freshwater, brackish, marine to soil and snow (Butcher, 1952; Kol, 1968; Ettl and Gärtner, 2014). They are characterized by a simple morphology. *Stichococcus*-like algae has mostly short cylindrical and elongate or spherical to slightly oval shaped cells containing chloroplast without pyrenoid except in two species, *Deuterostichococcus marinus* (Deason) Pröschold and Darienko and *Tetrastichococcus jenerensis* (Neustupa, Eliás and Sejnohová) Pröschold and Darienko (Neustupa *et al.*, 2007; Pröschold and Darienko, 2020; Pröschold *et al.*, 2020). They reproduce vegetatively, but only *Pseudostichococcus monallantoides* was reported to have zoospore formation (Moewus, 1951). Recent studies based on molecular data have shown a high biodiversity among these organisms and put into question

the traditional generic and species concept (Nestupa *et al.*, 2007; Hodac *et al.*, 2016; Pröschold and Darienko, 2020). Pröschold and Darienko (2020) studied 34 strains assigned as *Stichococcus*, *Pseudostichococcus*, *Diplosphaera* and *Desmococcus*, and revised these genera using 18S and ITS rDNA sequences. They also proposed four new genera and four new species. Many described species of *Stichococcus*-like algae were transferred to new genera and synonymized using polyphasic studies.

The generic name *Deuterostichococcus* Pröschold and Darienko was introduced by Pröschold and Darienko (2020) for four species originally included in *Stichococcus* Nägeli. *Stichococcus deasonii* Neustupa, Eliás and Sejnohová and *S. allas* Reisingl required further nomenclatural clarification (Pröschold *et al.*, 2020). *Deuterostichococcus deasonii* (Deason) Pröschold and Darienko transferred from *Stichococcus deasonii* Neustupa, Eliás and Sejnohová needed to be changed to *D. marinus* (Deason) Pröschold and Darienko because the basionym of this is *Hormidium marinum* Deason (1969). *Deuterostichococcus allas* (Reisingl) Pröschold and Darienko was proposed to be correct in *D. tetrallantoideus* (Kol) Pröschold and Darienko because *Stichococcus allas* Reisingl (1964) was a heterotypic synonym (Pröschold *et al.*, 2020).

A total of 51 *Stichococcus* species names were in the database, but currently only eight species are accepted taxonomically (Guiry and Guiry, 2020). *Stichococcus*-like algae were reorganized into eight genera including *Stichococcus* based primarily on genetic data because these taxa are morphologically variable and are not easily distinguished for each other (Pröschold and Darienko, 2020).

In this study, chlorophytes were isolated from the microbial samples of soil surfaces in Suwon-si, Gyeonggi-do and planktonic samples from the Joochun River, Hoengseong-gun, Gangwon-do. We investigated the morphology and sequenced partial 18S rDNA gene of the three species. Our results indicate that these organisms represent three unrecorded species in Korea.

## MATERIALS AND METHODS

### Sampling, culture, and morphological analysis

The specimens were collected from Suwon-si, Gyeonggi-do in May 2019 and Joochun River, Hoengseong-gun, Gangwon-do in December 2018.

Samples were scraped from soil surface using a soft brush or sterile spatula in Suwon-si (Kiel and Gaylarde, 2006). The planktonic algae were netted vertically and horizontally using a plankton net of 25  $\mu\text{m}$  mesh and 30 cm diameter from stagnant or flowing freshwater areas of the Joochun River (Sournia, 1978).

Samples were separated into solid medium by addition of 1.5% agar to Bold's Basal Medium as modified by Bi-

schoff and Bold (1963) using a Pasteur pipette. The colonies in the solid medium were transferred to liquid Bold's basal medium (Bold, 1949). The algal strains were cultivated under a light/dark cycle of 16:8 with 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light at 25°C (Stein, 1973; Bold and Wynne, 1978).

One strain of this study was deposited in the Nakdong-gang National Institute of Biological Resources (specimen no. FBCC-A409) and two strains in the National Institute of Biological Resources (specimen nos. NIBRCL0000114567 and NIBRCL0000114571).

Samples were observed at 100 to 1000 $\times$  magnification using an optical microscope (Axio Imager A2, Carl Zeiss, Germany; Olympus BX53, Olympus, Japan), and images were taken using AxioCam HRC camera (Carl Zeiss, Germany) and Olympus UC-90 (Olympus, Japan) at 1000 $\times$  magnification.

We followed the taxonomic classification from AlgaeBase (Guiry and Guiry, 2020), and carried out the morphological identification of the taxa as described by Chodat (1913), Skuja (1948), Reisingl (1964), Hirose *et al.* (1977), Komárek and Fott (1983), John *et al.* (2011), Hodac *et al.* (2016), Beck *et al.* (2019), Pröschold and Darienko (2020), and Pröschold *et al.* (2020).

### DNA extraction

Cells in the exponential phase were harvested via centrifugation (3000 rpm), reconstituted with 1 $\times$  TE buffer, and stored at  $-20^{\circ}\text{C}$  until further analysis. Total genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method described by Richards *et al.* (2001).

### DNA amplification and sequencing

Nuclear 18S rDNA sequences were amplified using a long PCR technique with primers 18SF (5'-AAC CTG GTT GAT CCT GCC AGT-3') and 18SR (5'-TGA TCC TTC TGC AGG TTC ACC TAC G-3') (Katana *et al.*, 2001). PCR was carried out in 20  $\mu\text{L}$  reaction mixtures containing 17  $\mu\text{L}$  of sterile distilled water, 1  $\mu\text{L}$  of each primer (10 pmoles), and 1  $\mu\text{L}$  extracted DNA. PCR cycling was performed on a Mastercycler<sup>®</sup>Nexus gradient 6331 Model (Eppendorf, Germany) using the following program: 94°C for 3 min; followed by 30 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 60 sec; with a final extension at 72°C for 5 min (Katana *et al.*, 2001). The resulting PCR products were subjected to electrophoresis in 1.0% agarose gel (Promega, WI), stained with ethidium bromide, and visualized under ultraviolet light on a transilluminator.

The PCR products were purified using the MEGA quick-spin<sup>™</sup> Plus DNA Purification Kit (iNtRON, Korea) and sequenced (ABI 3730XL) with the same primers used for amplification.

The SSU rDNA sequence were aligned and compared with previously published data in BioEdit (Hall, 1999). All the sequences acquired were deposited in the NCBI GenBank database.

### Phylogenetic analyses

For the phylogenetic analyses, we constructed a data set of the 18S rRNA sequences, comprised of our species and other relatives obtained from the NCBI database. These sequences were aligned in BioEdit (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) and both ends trimmed to make all sequences the same length. The genetic distance was calculated using the Tamura-Nei model in MEGA 6.0 (<http://www.megasoftware.net>).

For the phylogenetic tree of the Selenastraceae, one isolate collected from this study and 39 species obtained from GenBank were used (Fig. 4). In addition, two isolates collected in this study and 24 species obtained from GenBank were used for the phylogenetic tree of the Stichococcaceae (Fig. 5). Maximum Likelihood (ML) and Neighbor Joining (NJ) analyses were both performed for respectively. For the trees, the reliability of the tree was evaluated by using 1000 bootstrap replicates, and the analyzed trees were marked with a value of 50% or higher.

## RESULTS

We identified three species of chlorophytes including *Monoraphidium subclavatum* Nygaard, *Deuterostichococcus epilithicus* Pröschold and Darienko, and *Pseudostichococcus monallantoides* L.Moewus as Korean unrecorded species using morphology and 18S rDNA sequences.

### Phylogenetic analysis

The SSU rDNA sequences in this study have been deposited in the NCBI GenBank database under the following accession numbers: MT169963 (*Monoraphidium subclavatum*), MT169962 (*Deuterostichococcus epilithicus*), and MT169960 (*Pseudostichococcus monallantoides*).

ML and NJ trees were inferred from a dataset of partial 18S rDNA sequence to understand the phylogenetic relationships between some members of Selenastraceae (1645 bp) (Fig. 4) and Stichococcaceae (1113 bp) (Fig. 5). The ML tree shows that the genera of the family Selenastraceae are not monophyletic. The genus *Monoraphidium* is distributed in several clades in the phylogenetic tree. *M. komarkovae* formed an independent clade, while *M. dybowskii* and *M. convolutum* formed a clade with *Raphidocelis subcapitata*. Also, *M. neglectum* was a member of the *Chlorobion* clade with *Chlorobion*

*braunii*. The *Monoraphidium subclavatum* strain (FBCC-A409) of this study grouped into the same clade as *Messastrum gracile* strains, and the 18S rDNA similarity was very similar (98.6-100%) between seven strains of *Messastrum gracile* and *Monoraphidium subclavatum* (FBCC-A409).

The genus *Messastrum* differs from other genera in Selenastraceae based on differences in 18S rDNA and *rbcL* gene sequences. *Messastrum gracile* (Reinsch) T.S. Garcia was transferred from *Selenastrum gracile* Reinsch (Syn. *Ankistrodesmus gracilis* (Reinsch) Korshikov) (Da Silva et al., 2017).

The morphological criteria distinguishing *Messastrum gracile* is the fusiform to semilunate cell shape. Cells frequently clustered in big colonies with an arcuated chain of cells, irregularly arranged in this case, where colonies up to 11 cells were observed (Da Silva et al., 2017). On the other hand, *Monoraphidium subclavatum* always presented solitary and pyrenoid-less cells. Cells are spindle-shaped, slightly or moderate crescent-shaped, rarely sigmoid, with both ends gradually tapering to a point, but not in long tips. The morphological differences between the two species are that *Monoraphidium subclavatum* did not form a colony, and the curvature of cell was slightly curved or slightly crescent-shaped compared to the *Messastrum gracile*.

Phylogenetic analyses using 18S rDNA sequences revealed that two *Stichococcus*-like strains of this study belong to *Deuterostichococcus* clade and *Pseudostichococcus* clade within the Stichococcaceae of the Trebouxiophyceae (Fig. 5).

The *Stichococcus*-like strain (NIBRCL0000114567) collected from a rocky wall clustered with *Deuterostichococcus epilithicus* SAG 2482 (MT078170), and the similarity between *Stichococcus*-like strain and *Deuterostichococcus* clade was 95.2 to 97.8%. Therefore, the *Stichococcus*-like strain (NIBRCL0000114567) was identified to *D. epilithicus* based on the phylogeny of 18S rDNA.

The *Stichococcus*-like strain (NIBRCL0000114571) collected from the soil surface clustered with *Pseudostichococcus monallantoides* SAG 380-1 (KM020066) and Ru-s-4-2 (MH703749), and the similarity among them was 97.9 to 99.8%. Thus, we identified the *Stichococcus*-like strain (NIBRCL0000114571) as *Pseudostichococcus monallantoides* based on phylogenetic tree of 18S rDNA.

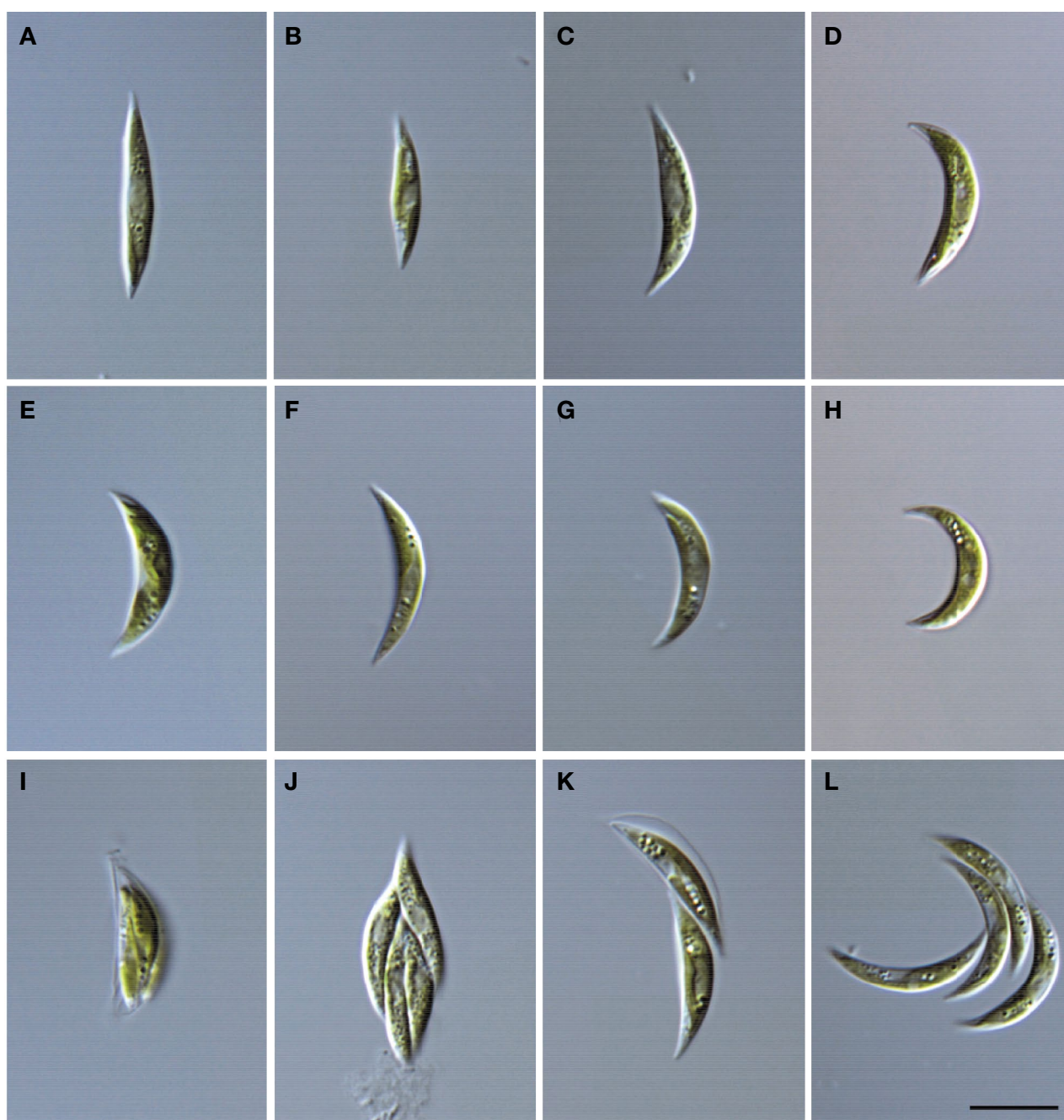
### Morphology and description

Class Chlorophyceae Wille 1884

Order Sphaeropleales Luerssen 1877

Family Selenastraceae Blackman and Tansley 1903

Genus *Monoraphidium* Komárková-Legnerová 1969



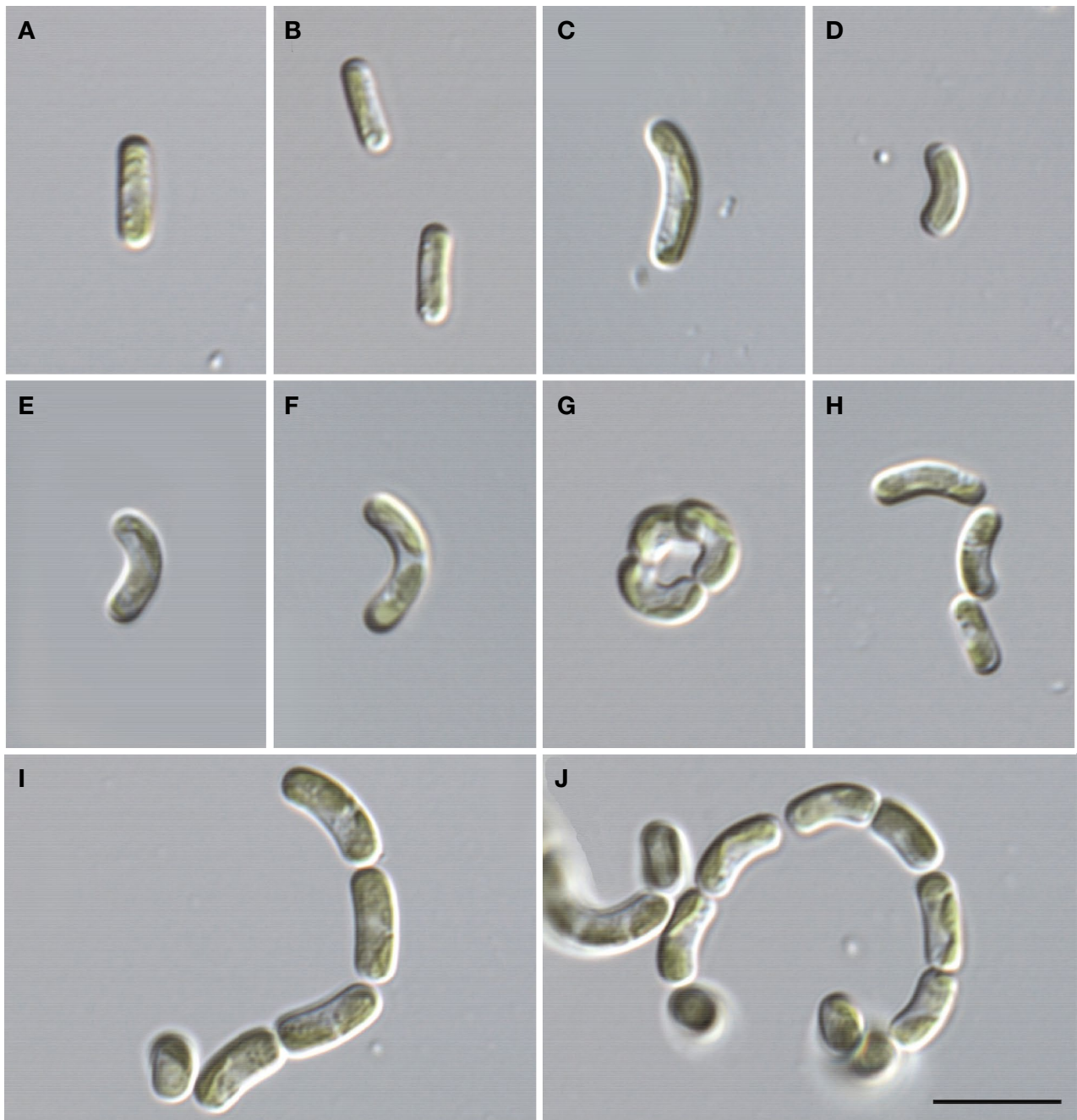
**Fig. 1.** Microscopic photographs of *Monoraphidium subclavatum* Nygaard FBCC-A409. (A, B) spindle-shapes, (C-H) slightly and moderate crescent-shapes, (I-L) autospores in the mother cell. Scale bar represents 10  $\mu\text{m}$ .

***Monoraphidium subclavatum* Nygaard 1977 (Fig. 1)**

Cells are solitary, spindle-shaped, slightly or moderate crescent-shaped, rarely sigmoid, with both ends gradually tapering to a point, but not in long tips. Chloroplast covers almost the entire cell wall, and is not clearly confined to the center, without a pyrenoid. Propagation is mostly achieved by four autospores, which are slightly

crescent-shaped in young cells, but increasingly crescent eventually. The cell length is 13.7–22.2  $\mu\text{m}$  and width is 2.4–5.3  $\mu\text{m}$ .

**Note.** Spindle-shaped and slightly crescent-shaped cells were mainly observed. The ends of the cells are pointed, but the ends are shorter and the blunt ends differ from those of *M. pusillum* (Printz) Komárková-Legnorová. Individuals measuring 14–16  $\mu\text{m}$  in length appeared



**Fig. 2.** Microscopic photographs of *Deuterostichococcus epilithicus* Pröschold and Darienko. NIBRCL0000114567. (A-F) slightly curved cylindrical-shapes, (G, J) colony forming circle, (H, I) weakly connected filaments. Scale bar represents 10  $\mu\text{m}$ .

frequently. Phylogenetic analyses using 18S rDNA sequences showed that the genus *Monoraphidium* is polyphyletic, and *Monoraphidium subclavatum* formed the same clade with *Messastrum gracile*. However, the two species are distinguished by cell curvature and colony formation.

**Ecology.** This is a freshwater species. They inhabit small water biotopes (Komárek and Fott, 1983). This species

was collected from planktonic samples of a eutrophic river.

**Site of collection.** Joochun river, Hoengseong-gun, Gangwon-do (37°24'44.4"N 128°09'20.0"E).

**Date of collection.** May 28, 2019.

**Specimen Locality.** FBCC-A409 in the Nakdonggang National Institute of Biological Resources.

Class Trebouxiophyceae Friedl 1995  
 Order Prasiolales Schaffner 1922  
 Family Stichococcaceae Bohlin 1901  
 Genus *Deuterostichococcus* Pröschold and Darienko  
 2020

***Deuterostichococcus epilithicus*  
 Pröschold and Darienko 2020 (Fig. 2)**

Cells are usually unicellular. Under salinity stress can form unbranched filaments out of 2–4 cells, which are easy fragmenting. On freshwater medium, cells are cylindrical with rounded ends. Cell walls thin, without mucilaginous layer. Chloroplasts are located in the cell periphery, not lobed with smooth margin, without pyrenoid. The length of the cell is 3.8–8.1 µm and the width is 2.3–3.0 µm.

**Note.** This species was newly named from *Stichococcus*-like strains based on 18S and ITS rDNA sequences by Pröschold and Darienko (2020). The SAG 2482 strain among *Deuterostichococcus epilithicus* strains is morphologically similar to the NIBRCL0000114567 strain.

**Ecology.** This is a terrestrial species. They inhabit rock and stone walls (Reisigl, 1964).

**Site of collection.** Iui-dong, Yeongtong-gu, Suwon-si, Gyeonggi-do (37°18'03.9"N 127°02'29.3"E).

**Date of collection.** December 14, 2018.

**Specimen Locality.** NIBRCL0000114567 in the National Institute of Biological Resources.

Genus *Pseudostichococcus* L.Moewus 1951

***Pseudostichococcus monallantoides*  
 L.Moewus 1951 (Fig. 3)**

Cells are solitary or formed only two-celled, rarely four-celled filaments or sometimes packages of four cells. Cells are cylindrical with rounded cell ends. Chloroplast is plate-like, without pyrenoid, covering half of cell. Usually several large oil drops are present. The length of the cell is 7.5–34.1 µm and the width is 1.6–3.2 µm.

**Note.** The strain (NIBRCL0000114571) used in this study is morphologically similar to the *Pseudostichococcus* strains described in Pröschold and Darienko (2020).

**Ecology.** This is a terrestrial species. They usually inhabit the soil surface (Skuja, 1948).

**Site of collection.** Iui-dong, Yeongtong-gu, Suwon-si, Gyeonggi-do (37°17'49.1"N 127°03'02.1"E).

**Date of collection.** December 14, 2018.

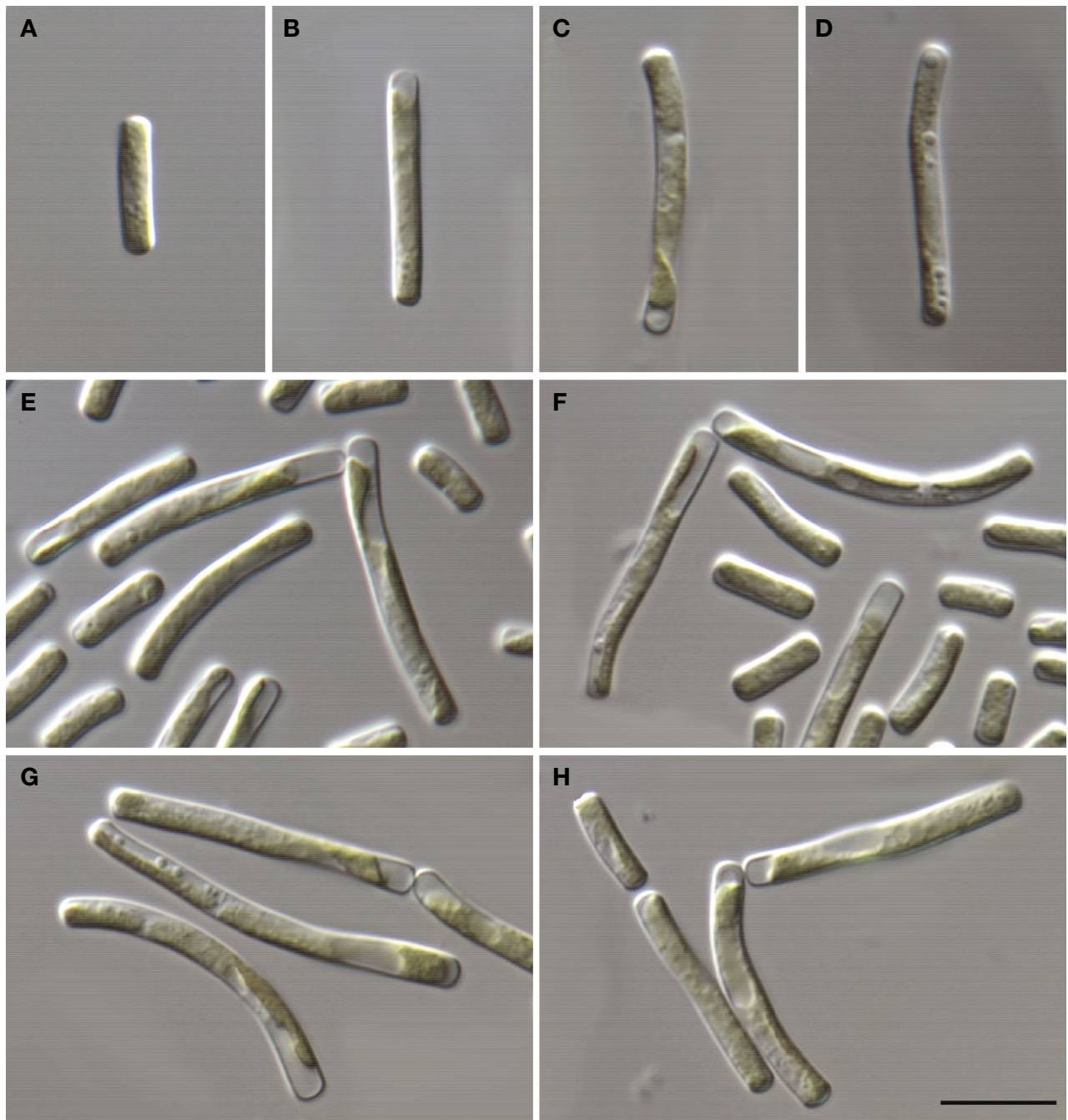
**Specimen Locality.** NIBRCL0000114571 in the National Institute of Biological Resources.

## DISCUSSION

Selenastraceae are ubiquitous, freshwater green algae with uncertain taxonomy. Although genera of Selenastraceae are delimited by apparently distinguishable morphological features, such as solitary or colonial habit, presence of mucilage pads, and overall cell-shape, these features have been shown to be ambiguous at the genus level by 18S rDNA sequence analysis (Krienitz *et al.*, 2001). The application of morphospecies concept in the Selenastraceae including *Monoraphidium* Komárková-Legnerová, *Ankistrodesmus* Corda, and *Raphidocelis* Hindák emend. Marvan *et al.* has resulted in an underestimation of species diversity of this family and probably erroneous conclusions about the distribution of species (Fawley *et al.*, 2006). In the phylogeny of the Selenastraceae using 18S rDNA, *rbcL*, and ITS-2 analyses, the genera *Monoraphidium*, *Kirchneriella*, and *Selenastrum* were not monophyletic and not distinguishable as separate genera. The polyphyly of the genus *Selenastrum* led to the description of two new genera, *Curvastrum* gen. nov. and *Messastrum* gen. nov. (Da Silva *et al.*, 2017).

The ML tree of 18S rDNA sequences showed that the Selenastraceae family was not clearly separated into each genus by classification level (Fig. 4). The strains of genus *Monoraphidium* were distributed in several clades in the 18S rDNA phylogenetic tree. The *Monoraphidium subclavatum* (FBCC-A409) of this study were in the same clade as *Messastrum gracile* strains. The 18S rDNA similarity was very similar to 98.6–100% between seven strains of *Messastrum gracile* and *Monoraphidium subclavatum* (FBCC-A409). Compared to *Messastrum gracile*, cells of *Monoraphidium subclavatum* do not form a colony, and the shape of cell was slightly curved or slightly crescent-shaped. This result means that the genus *Monoraphidium* is still based on the morphospecies concept, and was not monophyletic and not distinguishable as a separate genus.

Originally the genus *Stichococcus*, with its type species *S. bacillaris*, was described by Nägeli (1849). Since its first description, more than 100 species names could be found in the literature, but very little is known about their morphology (Guiry and Guiry, 2020). *Stichococcus*-like algae has been identified using simple morphological features. The main features were shape of cylindrical cells, presence or absence of pyrenoids, and presence or absence of filamentous formation. *Stichococcus*-like algae are polyphyletic, and the generic and species concept remained unresolved in the studies Neustupa *et al.* (2007) and Hodac *et al.* (2016). Pröschold and Darienko (2020) studied the order Prasiolales, including *Stichococcus*-like organisms, using a polyphasic approach and proposed a new generic and species concept for this group. *Stichoc-*

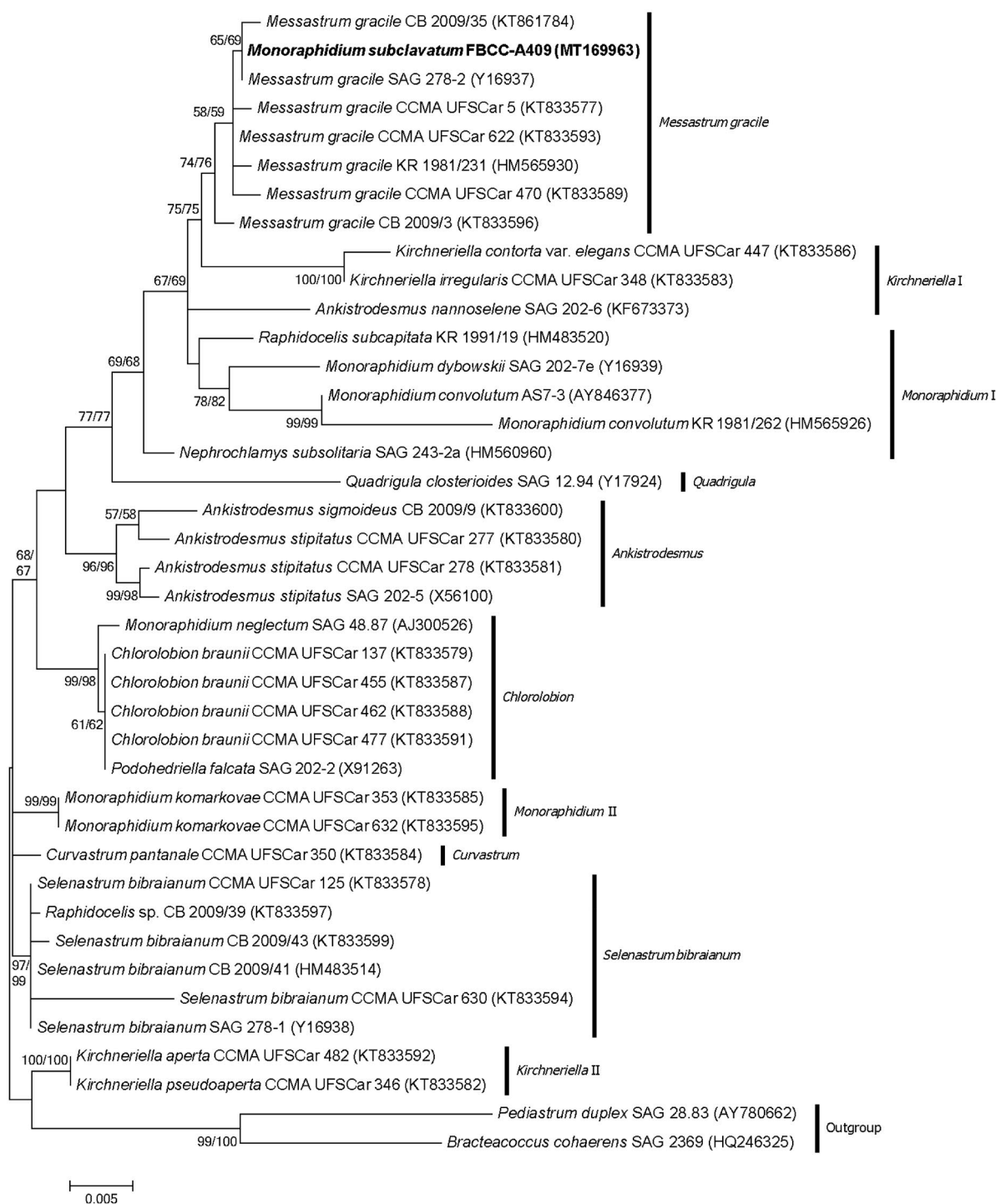


**Fig. 3.** Microscopic photographs of *Pseudostichococcus monallantoides* L.Moewus. NIBRCL0000114571. (A–D) long and cylindrical shapes, (E–H) mostly grows in a solitary state during culture. Scale bar represents 10  $\mu\text{m}$ .

*occus*-like algae belong to eight independent lineages within the *Prasiola* clade of the Trebouxiophyceae in the phylogenetic analyses using a multigene approach.

Two *Stichococcus*-like strains of this study belong to *Deuterostichococcus* clade and *Pseudostichococcus* clade, respectively, within the Stichococaceae of the Trebouxiophyceae in phylogenetic analysis using 18S rDNA

data (Fig. 5). The *Stichococcus*-like strain (NIBRCL0000114567) formed a cluster with *Deuterostichococcus epilithicus*. The strain NIBRCL0000114567 was similar to SAG 2482, one of the strains identified as *Deuterostichococcus epilithicus* based on morphological features. They shared the features of 2–5 cell filaments and severely curved cells. The *Stichococcus*-like strain (NIBRCL0000114571)

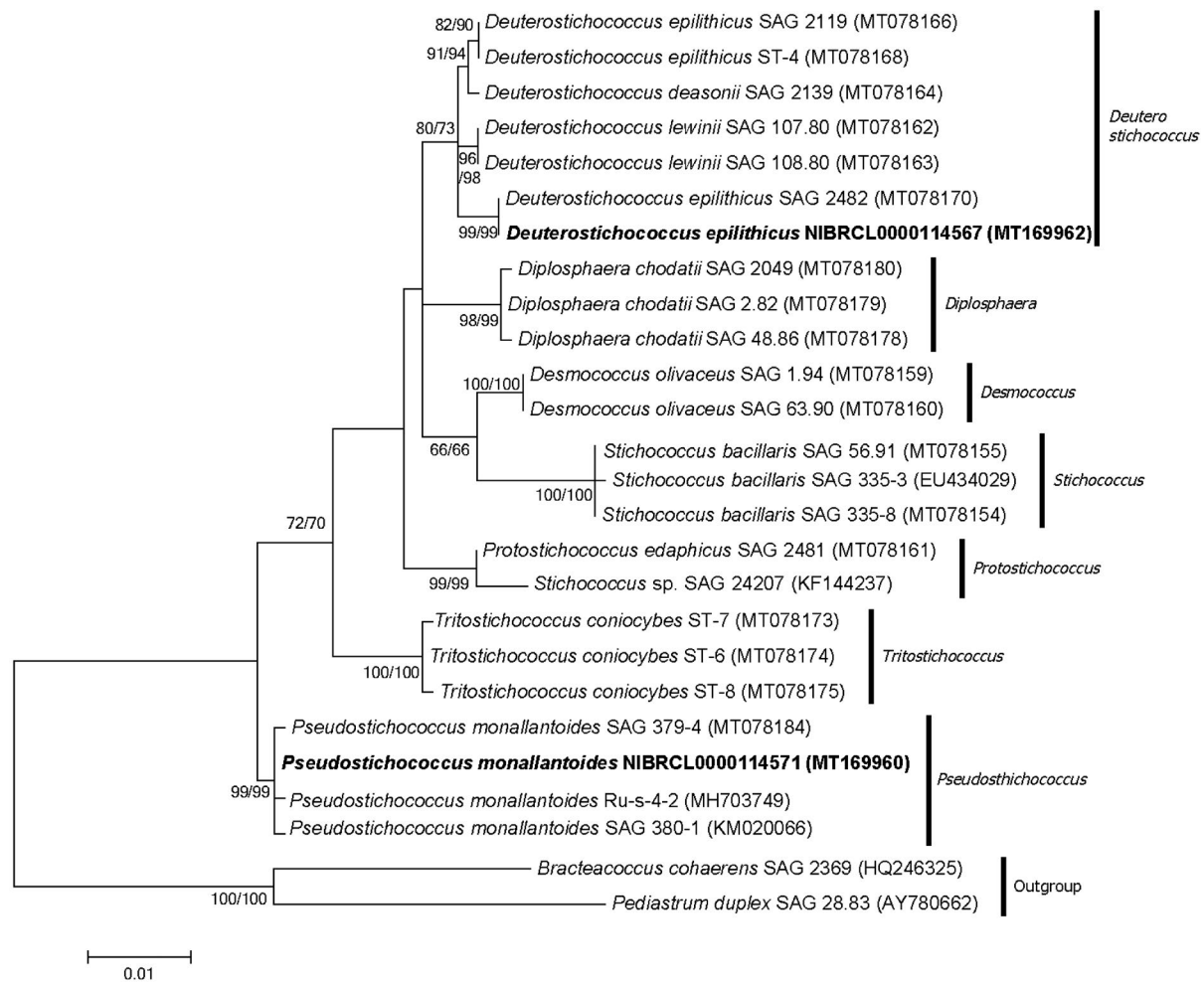


**Fig. 4.** Phylogenetic relationships of chlorophytes within the family Selenastraceae inferred from nearly complete 18S rDNA sequences with the maximum-likelihood (ML) algorithm. 18S sequences of genera (*Bracteacoccus cohaerens*, Accession No. HQ246325/ *Pediastrum duplex*, Accession No. AY780662) were included as the outgroup. Additionally, the probability of NJ analysis was incorporated into the ML tree to support the strength of each branch. The first and second numbers at the nodes display the bootstrap proportions (BP) (> 50%) in ML and NJ, respectively. The branch lengths are proportional to the scale given.

was identified to be *Pseudostichococcus monallantoides* in the phylogenetic tree of 18S rDNA. The morphology

of strain NIBRCL0000114571 was similar to *Pseudostichococcus monallantoides*, SAG 380-1. Their cells were





**Fig. 5.** Phylogenetic relationships of chlorophyte species within the family Stichococcaceae inferred from nearly complete 18S rDNA sequences with the maximum-likelihood (ML) algorithm. 18S sequences of genera (*Bracteacoccus cohaerens*, Accession No. HQ246325/ *Pediatrum duplex*, Accession No. AY780662) were included as the outgroup. Additionally, the probability of NJ analysis was incorporated into the ML tree to support the strength of each branch. The first and second numbers at the nodes display the bootstrap proportions (BP) (> 50%) in ML and NJ, respectively. The branch lengths are proportional to the scale given.

solitary or formed only two-celled rarely four-celled filaments, or sometimes packages of four cells. Through the above results, the generic and species concept of *Stichococcus*-like algae seems to be well established in Pröschold and Darienko (2020).

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## REFERENCES

- Beck, A., J. Bechteler, A. Casanova-Katny and I. Dzhilyanova. 2019. The pioneer lichen *Placopsis* in maritime Antarctica: Genetic diversity of their mycobionts and green algal symbionts, and their correlation with deglaciation time. *Symbiosis* 79(1):1-24.
- Bischoff, H.W. and H.C. Bold. 1963. Phycological studies. IV. Some algae from enchanted rock and related algae species. University of Texas Publishers, Texas.
- Bold, H.C. 1949. The morphology of *Chlamydomonas chlamydogama* sp. nov. *Bulletin of the Torrey Bot Club* 76(2):101-108.
- Bold, H.C. and M.J. Wynne. 1978. Introduction to the algae. Prentice-Hall, New Jersey.
- Butcher, R.W. 1952. Contributions to our knowledge of the

- smaller marine algae. *Journal of the Marine Biological Association of the United Kingdom* 31(1):175-191.
- Chodat, R. 1913. *Monographies d'algues en culture pure. Matériaux pour la flore cryptogamique Suisse*. Berne 5(2):1-266.
- Da Silva, T.G., C. Bock, C.L. Sant'Anna, I.L. Bagatini, S. Wodniok and A.A.H. Vieira. 2017. Selenastraceae (Sphaeropleales, Chlorophyceae): *rbcL*, 18S rDNA and ITS-2 secondary structure enlightens traditional taxonomy, with description of two new genera, *Messastrum* gen. nov. and *Curvastrum* gen. nov.. *Fottea* 17(1):1-19.
- Ettl, H. and G. Gärtner. 2014. *Syllabus der Boden-, Luft- und Flechtenalgen*. Berlin and Heidelberg, Springer.
- Fawley, M.W., M.L. Dean, S.K. Dimmer and K.P. Fawley. 2006. Evaluating the morphospecies concept in the Selenastraceae (Chlorophyceae, Chlorophyta). *Journal of Phycology* 42(1):142-154.
- Guiry, M.D. and G.M. Guiry. 2020. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway [Available from: <http://www.algaebase.org/>, accessed 12 June 2020].
- Hall, T.A. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* 41(1):95-98.
- Happy-Wood, C.M. 1988. Ecology of freshwater planktonic green algae. In: C.D. Sandgen (ed.), *Growth and Reproduction Strategies of Freshwater Phytoplankton*, Cambridge University Press, Cambridge. pp. 175-226.
- Hindák, F. 1970. A contribution to the systematics of the family Ankistrodesmaceae (Chlorophyceae). *Algological studies* 1:7-32
- Hirose, H., M. Akiyama, M. Hirano, K. Imahori, T. Ioriya, H. Kasaki, H. Kobayasi, S. Kumano, E. Takahashi, K. Tsumura and T. Yamagishi. 1977. *Illustrations of the Japanese freshwater algae*. Uchida Rokakuho, Tokyo.
- Hodac, L., C. Hallmann, K. Spitzer, J. Elster, F. Faßhauer, N. Brinkmann, D. Lepka, V. Diwan and T. Friedl. 2016. Widespread green algae *Chlorella* and *Stichococcus* exhibit polar-temperate and tropical-temperate biogeography. *FEMS Microbiology Ecology* 92(8):1-16.
- John, D.M., B.A. Whitton and A.J. Brook. 2011. *The freshwater algal flora of the British Isles*. Second edition. Cambridge University Press, Cambridge.
- Katana, A., J. Kwiatowski, K. Spalik, B. Zakryś, E. Szalacha and H. Szymańska. 2001. Phylogenetic position of *Koliella* (Chlorophyta) as inferred from nuclear and chloroplast small subunit rDNA. *Journal of Phycology* 37(3):443-451.
- Kiel, G. and C.C. Gaylarde. 2006. Bacterial diversity in biofilms on external surfaces of historic buildings in Porto Alegre. *World Journal of Microbiology and Biotechnology* 22(3):293-297.
- Kol, E. 1968. *Kryobiologie. Biologie und limnologie des schnees und eises. I. Kryovegetation. Die Binnengewässer* 24(1):1-216.
- Komárek, J. and B. Fott. 1983. *Das Phytoplankton des Süßwassers*. Schweiz. Verlag, Stuttgart.
- Komárková-Legnerová, J. 1969. The systematics and ontogenesis of the genera *Ankistrodesmus* Corda and *Monoraphidium* gen. nov.. In: B. Fott (ed.), *Studies in phycology*, Academia publishing house of the Czechoslovak academy of sciences, Praha. pp. 75-122.
- Krienitz, L., I. Ustinova, T. Friedl and V.A. Huss. 2001. Traditional generic concepts versus 18S rRNA gene phylogeny in the green algal family Selenastraceae (Chlorophyceae, Chlorophyta). *Journal of Phycology* 37(5):852-865.
- Moewus, L. 1951. Systematische bestimmung einzelliger grüner algen auf grund von kulturversuchen. *Botaniska Notiser* 4(1):287-318.
- Nägeli, C. 1849. *Gattungen einzelliger algen. Physiologisch und systematisch bearbeitet*. Friedrich Schulthess.
- Neustupa, J., M. Eliáš and L. Šejnohová. 2007. A taxonomic study of two *Stichococcus* species (Trebouxiophyceae, Chlorophyta) with a starch-enveloped pyrenoid. *Nova Hedwigia* 84(1-2):51-63.
- Pröschold, T. and T. Darienko. 2020. The green puzzle *Stichococcus* (Trebouxiophyceae, Chlorophyta): New generic and species concept among this widely distributed genus. *Phytotaxa* 441(2):113-142.
- Pröschold, T., T. Darienko and M.D. Guiry. 2020. Nomenclatural corrections in the green algal genus *Deuterostichococcus* Pröschold and Darienko (Trebouxiophyceae). *Notulae algarum* 137(1):1-2.
- Reisigl, H. 1964. Zur systematik und Ökologie alpiner Bodeenalgen. *Österreich Botanische Zeitschrift* 111(4):402-499.
- Richards, E., M. Reichardt and S. Rogers. 2001. Preparation of genomic DNA from plant tissue. In: F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl (eds), *Current protocols in molecular biology*, John Wiley and Sons, Inc., New York. pp. 2.3.1-2.3.7.
- Skuja, H. 1948. Taxonomie des Phytoplanktons einiger seen in uppland, Schweden. *Symbolae Botanicae Upsalienses* 9(3):1-399.
- Sourmia, A. 1978. *Phytoplankton manual*. UNESCO, Paris.
- Stein, J.R. 1973. *Handbook of phycological methods, culture methods and growth measurements*. Cambridge University Press, Cambridge.

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