*Phytotaxa* 295 (1): 060–070
http://www.mapress.com/j/pt/


**Article**

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.295.1.5

# *Asterochloris sejongensis* sp. nov. (Trebouxiophyceae, Chlorophyta) from King George Island, Antarctica

JONG IM KIM[1], SEUNG WON NAM[2], JAE EUN SO[3,4], SOON GYU HONG[3], HAN-GU CHOI[3,4] & WOONGGHI SHIN[1*]

[1] *Department of Biology, Chungnam National University, Daejeon 34134, Korea*
[2] *Nakdonggang National Institute of Biological Resources, Sangju 37242, Korea.*
[3] *Division of Polar Life Sciences, Korea Polar Research Institute, Incheon, 21990, Korea*
[4] *Department of Polar Sciences, University of Science and Technology, Incheon 406-840, Korea*
** Corresponding: Woongghi Shin (shinw@cnu.ac.kr)*

## Abstract

The new species *Asterochloris sejongensis* sp. nov. has been collected from four localities of King George Island, Antarctica, and is described as a phycobiont of the lichen species *Cladonia pyxidata* and *Sphaerophorus globosus*. This discovery is based on morphological and molecular data obtained using light microscopy, confocal laser microscopy, transmission electron microscopy, and two molecular markers; nuclear internal transcribed spacer (ITS) rDNA and actin genes. This species is characterized by five unique hemi-CBCs in the nuclear ITS transcripts, deeply lobed and echinulate chloroplasts (depending on the life stage), and two rows of pyrenoglobuli associated with two thylakoid envelopes. A molecular phylogenetic analysis of the ITS and actin gene sequences indicated that this new species is closely related to *A. woessiae* and forms a distinct well-supported lineage with the genus.

**Key words:** Antarctica, *Asterochloris*, phycobiont, phylogeny, ultrastructure

## Introduction

Lichen is a symbiotic organism mainly comprised of a fungus (mycobiont) and green algae and/or cyanobacteria (photobionts). Recently several studies reported that some of the lichen species contained multiple algal genotypes in a single lichen thallus (Casano *et al.* 2011, Grube and Muggia 2010, Guzow-Krzeminska 2006, Ohmura *et al.* 2006, Park *et al.* 2015, Piercey-Normore 2006). Among more than 40 photobiont genera, the genus *Asterochloris* Tschermak-Woess (1980a) is one of the most common lichen photobionts, and it is included in the green algal class Trebouxiophyceae. These photobionts are distributed in terrestrial environments from trophic to bipolar regions, and they occur in the thalli of more than 20 lichen genera (Piercey-Normore & DePriest 2001, Yahr *et al.* 2004, 2006, Cordeiro *et al.* 2005, Nelsen & Gargas 2006, 2008, Beiggi & Piercey-Normore 2007, Bačkor *et al.* 2010, Škaloud & Peksa 2010, Peksa & Škaloud 2011).

Antarctica is the coldest, windiest, and driest continent, and only 0.32% of its surface is ice free (Chown & Convey 2007). Because of its harsh environment and the limited impact of humans, Antarctica has attracted considerable interest from biologists. However, the difficulty of accessing Antarctica hinders the performance of biological specimen sampling and other fieldwork activities and is an obstacle to collecting data on its biological diversity. Recent studies have demonstrated the genetic diversity of lichenized photobionts and mycobionts; however, the taxonomy has not been well studied (Domaschke *et al.* 2012, Park *et al.* 2015, Pérez-Orega *et al.* 2012, Romeike *et al.* 2002).

*Asterochloris* is closely related to the genus *Trebouxia* Puymaly (1924) in vegetative morphology; however, they are differentiated by their chloroplast morphology (Tschermak-Woess 1980a). Recent molecular data have shown the paraphyly of the genus *Trebouxia* (Friedl & Zeltner 1994, Friedl & Rokitta 1997) and the close affiliation of several *Trebouxia* species with the genus *Asterochloris* (Helms *et al.* 2001, Piercey-Normore & DePriest 2001, Škaloud & Peksa 2008). Škaloud & Peksa (2010) transferred six *Trebouxia* species to the genus *Asterochloris* and re-described the genus diagnosis. In addition, they presented 16 distinct lineages belonging to *Asterochloris*, which suggests

the high cryptic diversity within the genus *Asterochloris*. In the present study, we describe a new species based on morphological and molecular data from Antarctic culture strains isolated from the two lichen species *Cladonia pyxidata* and *Sphaerophorus globosus*.

## Materials and Methods

**Taxa sampling**. Specimens of *Cladonia pyxidata* and *Sphaerophorus globosus* were collected from four different locations of King George Island, Antarctica in Febrary, 2015 (first, 62, 13.007' S, 58, 45.680' W; second, 62, 13.149' S, 58, 45.955' W; third, 62, 13.223' S, 58, 46.310' W; fourth, 62, 13.228' S, 58, 46.439' W). Identification of lichen specimens was determined by morphological characteristics and chemical substances according to the species descriptions and identification keys by Øvstedal and Smith (2001) and Olech (2004). Chemical substances were analyzed according to the standard methods (Culberson 1972, Orange *et al.* 2001).

**Isolation and cultivation of phycobionts**. The algal symbionts were isolated by the micropipette method (Ahmadjian 1967). Briefly, small pieces of rehydrated lichen thalli were fragmented by grinding between two glass surfaces, which resulted in free-lying algal cells and broken fungal hyphae. A single free-lying cell was diluted five times with 3N Bold's Basal Medium as modified by Thomas & Montes (1978) and then transferred into each well of a 96-well plate containing the same medium. The plate with isolated cells was incubated at 17 °C under a 14:10 light: dark cycle with 30 μmol photons·$m^{-2}$·$s^{-1}$ from cool white fluorescent tubes.

**Light microscopy (LM)**. The culture strains were observed and identified under an Axio Imager A2 (Carl Zeiss Inc., Hallbergmoos, Germany) equipped with differential interference contrast (DIC) optics. Images were captured with an AxioCam HRc (Carl Zeiss Inc., Hallbergmoos, Germany) photomicrographic system. The cellular dimensions were determined by measuring 25-30 cells of each taxon from photographic images.

**Confocal microscopy (CM)**. CM was performed with an LSM5 laser scanning confocal microscope equipped with an argon-krypton laser (Carl Zeiss Inc., Germany). We applied a 488 nm excitation line and an AOBS filter-free system to collect emitted light between 498 and 700 nm. The autofluorescence of chlorophyll was exploited to visualize the chloroplast structure. A series of optical sections through chloroplasts was captured and used in a three-dimensional reconstruction of their morphology. The chloroplast reconstructions were produced by the LSM 5 software version 3.5 (Carl Zeiss Inc., Germany).

**Transmission electron microscopy (TEM)**. For the TEM of *Asterochloris sejongensis*, aliquots of the culture were centrifuged for 20 min at 3,000 rpm in an Eppendorf centrifuge 5415D (Eppendorf, Hamburg, Germany) to form cell pellets. After removing the supernatant, the pelleted cells were mixed 1:1 with cold 5% glutaraldehyde in 3N BBM culture medium and fixed for 1 h at 4 °C. Glutaraldehyde-fixed cell pellets were washed 3 times in the same medium and post-fixed in 1% $OsO_4$ for 1 h at 4 °C. Before dehydration, the cells were rinsed 3 times in the culture medium. Dehydration was conducted at 4 °C using a graded ethanol series of 50%, 60%, 70%, 80%, 90% (10 min each) and absolute ethanol (three 10 min exchanges). The pellets were brought to room temperature, rinsed in propylene oxide two times for 20 min each, and then infiltrated with 50% Spurr's embedding resin (Spurr 1969) in propylene oxide for 1 h, 75% for 1 h, and 100% overnight. The following day, the pellets were transferred to new pure resin and polymerized at 70 °C. Blocks were thin-sectioned on a PT-X ultramicrotome (RMC Products, Boeckeler Instruments, Tucson, AZ, USA). Sections with a thickness of 70 nm were collected on slot copper grids, stained with 3% uranyl acetate and Reynold's lead citrate (Reynolds 1963), and then observed and photographed using a JEM-1010 transmission electron microscope (JEOL, Tokyo, Japan) operated at 80 kV. Images of the sections were recorded on Kodak EM Film 4489 (Eastman Kodak Co., Rochester, NY) and scanned to digital format using an Epson Perfection V700 Photo Scanner (Epson Korea Co., Ltd, Seoul, Korea).

**DNA isolation, amplification and sequencing**. All of the cultured cells were harvested by centrifugation during the exponential growth phase (Eppendorf 5415D, Hamburg, Germany). Total genomic DNA was isolated from the cultures with the DNeasy Blood & Tissue Kit (Cat. no. 69504; Qiagen Co., Valencia, CA, USA) using the animal tissue protocol provided by the manufacturer. The nuclear ITS rDNA and actin type I genes were amplified using different combinations of forward and reverse primers in a T100™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). The amplifications of ITS rDNA and actin type I gene were performed using the following primers: nrSSU1780 (5′-CTG CGG AAG GAT CAT TGA TTC-3′; Piercey-Normore & DePriest 2001) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′; White *et al.* 1990); and ActinF2 Astero (5′-AGC GCG GGT ACA GCT TCA C-3′) and ActinR2 Astero (5′-CAG CAC TTC AGG GCA GCG GAA-3′; Škaloud & Peksa 2010), respectively. All of the genes were

amplified using a total 25 µL reaction mix contained 2 µL DNA (20 ng), 16.35 µL distilled water, 2.5 µL 10X Ex Taq™ buffer, 2.0 µL each 2.5 mM dNTPs, 1.0 µL each 10 pmol primer and 0.15 µL (5 U µL $^{-1}$) TaKaRa Ex Taq™ DNA polymerase (TaKaRa Bio Inc, Shiga, Japan). The amplifications were performed using the following program: 5 min of denaturation at 94 °C; 30 cycles of 94 °C for 30 sec, 45-52 °C for 1 min, and 72 °C for 2 min; a final extension at 72 °C for 11 min, and an unlimited hold at 4 °C. The PCR products were sized on agarose gels and then purified using the MinElute Gel Extraction Kit (Qiagen Co., Valencia, CA, USA) according to the manufacturer's instructions. The purified amplification products were sequenced using the PCR primers in an Applied Biosystems (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) automated sequencer (ABI 3730XL) at Macrogen Corp. (Seoul, Korea). A total of 8 new sequences were generated, including 4 sequences of the nuclear ITS rDNA and 4 sequences of the actin type I gene. These sequences were aligned by eye using the Genetic Data Environment (GDE 2.5) program (Smith *et al.* 1994) and the secondary structure of the nuclear ITS1 and ITS2 rDNA molecules of *Asterochloris* (Škaloud & Peksa 2010) as a guide. The conserved regions of the two genes were readily aligned across taxa and used for the phylogenetic analyses. The nucleotides that could not be aligned were excluded from the phylogenetic analyses.

**Phylogenetic analyses.** A combined dataset of 98 taxa consisting of previously published sequences as well as new sequences representing 98 nuclear ITS rDNA sequences and 88 actin gene sequences was generated for the phylogenetic analyses, and these new sequences represented 100% and 89.8% of the sequence dataset of each gene. The dataset consisted of 994 characters (nuclear ITS=508, and actin=486) for the phylogenetic analyses.

Maximum likelihood (ML) analyses were performed using RAxML version 8.0.0 (Stamatakis 2014) with a single general time-reversible plus gamma (GTR+GAMMA) model obtained automatically by the program [rate matrix (1.1199, 2.4396, 1.0802, 0.5968, 2.9211, 1.0); state frequency (0.2034, 0.2759, 0.2929, 0.2278); with gamma (0.3414)]. We used 1000 independent tree inferences using the -# option of the program to identify the best tree. Bootstrap values (MLBS) were calculated using 1000 replicates with the same substitution model.

Bayesian analyses were run using MrBayes 3.2.5 (Ronquist *et al.* 2012) with a random starting tree, two simultaneous runs (nruns=2) and four Metropolis-coupled Markov chain Monte Carlo ($MC^3$) algorithms for 10 X $10^6$ generations, with one tree retained every 1,000 generations. The molecular data were analyzed with a single TrNef+I+Γ model obtained automatically by the program [Prset revmatpr=dirichlet (1.2578, 2.8112, 1.1722, 0.5713, 2.8112, 1.0); statefreqpr=dirichlet (0.1921, 0.2888, 0.2836, 0.2355); shapepr=exponential (1.0874); pinvarpr=fixed (0.4284)]. The burn-in point was identified graphically by tracking the likelihoods (Tracer v.1.6; http://tree.bio.ed.ac.uk/software/tracer/). The first 1,250 trees were discarded, and the remaining 8,751 trees were used to calculate the posterior probabilities (PP) of each clade. Additionally, the sump command in MrBayes was used to confirm the convergence. This analysis was repeated twice independently, and both analyses resulted in the same tree. The trees were visualized using the FigTree v.1.4.2 program, available at http://tree.bio.ed.ac.uk/software/figtree/.

**Phycobiont ITS secondary structure**. The secondary structures of the nuclear ITS sequences were constructed using the mfold computer program (version 2.3; Walter *et al.* 1994; Zuker 2003), and the folding temperature were set to 25 °C. The structures were compared with the published ITS secondary structure of *Asterochloris* photobionts (Beiggi & Piercey-Normore 2007), and the common secondary structures were created with PseudoViewer3 (http://pseudoviewer.inha.ac.kr/) and used to identify the compensatory base changes (CBCs) and hemi-CBCs.

## Results

### *Morphology*

#### 1) LM and CM

The vegetative cells of *Asterochloris sejongensis* ranged from 5 to 28 µm in diameter. The cells were generally spherical but occasionally oval or pyriform. The cell wall was thick, and localized thickening of the wall was detected in mature cells. The majority of the cell volume was occupied by the chloroplast, which was parietal and deeply lobed in young cells (Fig. 1A, B) and moved to a central position and developed deeply lobed or echinate chloroplasts in mature cells (Fig. 1E, F). The chloroplast lobes were simply terminated, elongated, or pointed at their ends (Fig. 1G, H). One to two pyrenoids were positioned at the center of the chloroplast (Fig. 1A, C). A single nucleus with a distinct nucleolus was situated parietally in the broad chloroplast infolding (Fig. 1 E, G). Asexual reproduction by 64-128 aplanospores or zoospores produced spherical or ellipsoidal sporangia (Fig. 1I, J).

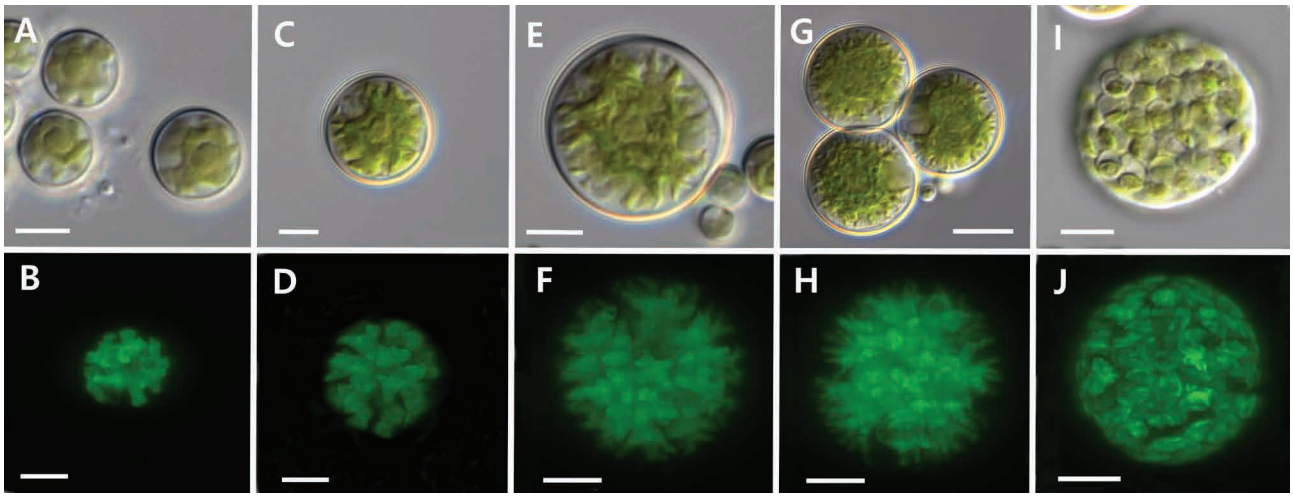


**FIGURE 1.** Light micrographs and confocal reconstructions of the chloroplast structures in *Asterochloris sejongensis* sp. nov. (A, B) Parietal lobed chloroplast in young cell. (C, D) Deeply lobed chloroplast; (E, F) crenulated chloroplast; and (G, H) deeply lobed chloroplast with flat lobe ends in the mature cell. (I, J) Aplanospores. Scale bar=5 µm.

**2) TEM**

The mature vegetative cells and young cells of an *Asterochloris sejongensis* 2015KGS-007A strain were spherical or ovoid in shape (Fig. 2A, B), and most of the cytoplasm was occupied by a chloroplast (Fig. 2A). However, in the young cells (Fig. 2B), the chloroplast was surrounded by two chloroplast membranes (arrows in Fig. 2C) and thylakoid membranes stacked in 2 to 4 layers (arrowheads in Fig. 2C). Several small mitochondria in the vegetative cells had plate-like cristae (Fig. 2A). In the young vegetative cells, the pyrenoid could not be recognized (Fig. 2B) and the arrangement of pyrenoid tubules was poorly developed. In the mature vegetative cells, the pyrenoid was of the irregularis-type as defined by Friedl (1989). Several thin and curved thylakoid membranes were invaginated into the matrix and surrounded by numerous small pyrenoglobuli (Fig. 2D).

***Phylogenetic analyses***

The concatenated Bayesian analysis of the new phycobiont nuclear ITS rDNA and actin dataset revealed more than 15 well-resolved lineages within the genus *Asterochloris* (Fig. 3). The 14 previously described species (*A. erici, A. excentrica, A. glomerata, A. irregularis, A. italiana, A. magna, A. phycobiontica, A. echinata, A. friedlii, A. gaertneri, A. leprarii, A. lobophora, A. woessiae,* and *A. mediterranea*) formed well-recognized and distinct lineages. The relationships among the lineages grouped in three major clade corresponded well with the previously presented phylogeny (Škaloud & Peksa 2010, Moya *et al.* 2015, Škaloud *et al.* 2015). The first clade was located at the top of the tree and formed a monophyletic group with the four species *A. magna, A. erici, A. glomerata*, and *A. irregularis* (pp=1.00, ML=99). The second clade was grouped with the three recently reported species *A. leprarii* and *A. gaertneri* and *A. excentrica* as a long branch attraction (pp=0.97, ML=67). The third clade was grouped together with six described species (*A. wossiae, A. friedlli, A. italiana, A. phycobiontica*, *A. echinata, A. lobophora* and *A. mediterranea*) and the new species from Antarctica (pp=1.00 ML=97). The new species *A. sejongensis* formed sister relationships with *A. wossiae* (pp=1.00, ML=100). The relationship among the lineages belonging to the third clade remains unresolved, although two exceptions of close significant relationships were observed between [*A. phycobiontica* and *A. lobophora*] and [*A. wossiae* and *A. sejongensis*].

***ITS secondary structure***

A common overall organization of the ITS1 and ITS2 secondary structures could be identified in strains of *Asterochloris sejongensis* (Fig. 4). The ITS secondary transcripts were compared with the closely related species *A. woessiae* to verify the occurrence of CBCs (nucleotide changes at both sides of a paired base) and hemi-CBCs (change at only one side of the nucleotide pair with the pairing preserved) according to Coleman (2000, 2003) and Ma *et al.* (2015). The ITS1 secondary structure consists of four paired regions (helices I–IV), with helix I the most divergent in sequences (Fig. 4A). The base was changed from GUUCC or GUCUC – GUCCC in helix I; and from U:G or U:A – C:G and G:C – G:U in helix II of ITS1 (Fig. 4A). The ITS2 secondary structure possessed conserved motifs among green algae (Mai & Coleman 1997), including a four-fingered hand (helices I–IV), a UU mismatch in helix II, and a conserved sequence

of UGGU on the 5' side of helix III (Fig. 4B). The base was changed from U:G – C:G and C:G – U:G in helix I and one base deletion AGU-AU between helix II and helix III of ITS2 (Fig. 4B). Five hemi-CBC sites were revealed in helices I and II of ITS1 and in helices I and III of ITS2in the ITS secondary structure transcripts. In all cases, the base change C:G or U:G – U:G or C:G was detected. The ITS secondary structure of *A. sejongensis* was also compared with the previously published structures of all *Asterochloris* lineages. Interestingly, two base changes in the ITS2 secondary structures of *A. sejongensis* have been newly identified as diagnostic characters; one hemi-CBC at position 32 and one deletion at position 90 (Fig. 4B. star marked). These changes were not previously identified (Škaloud & Peksa 2010, Škaloud *et al.* 2015).

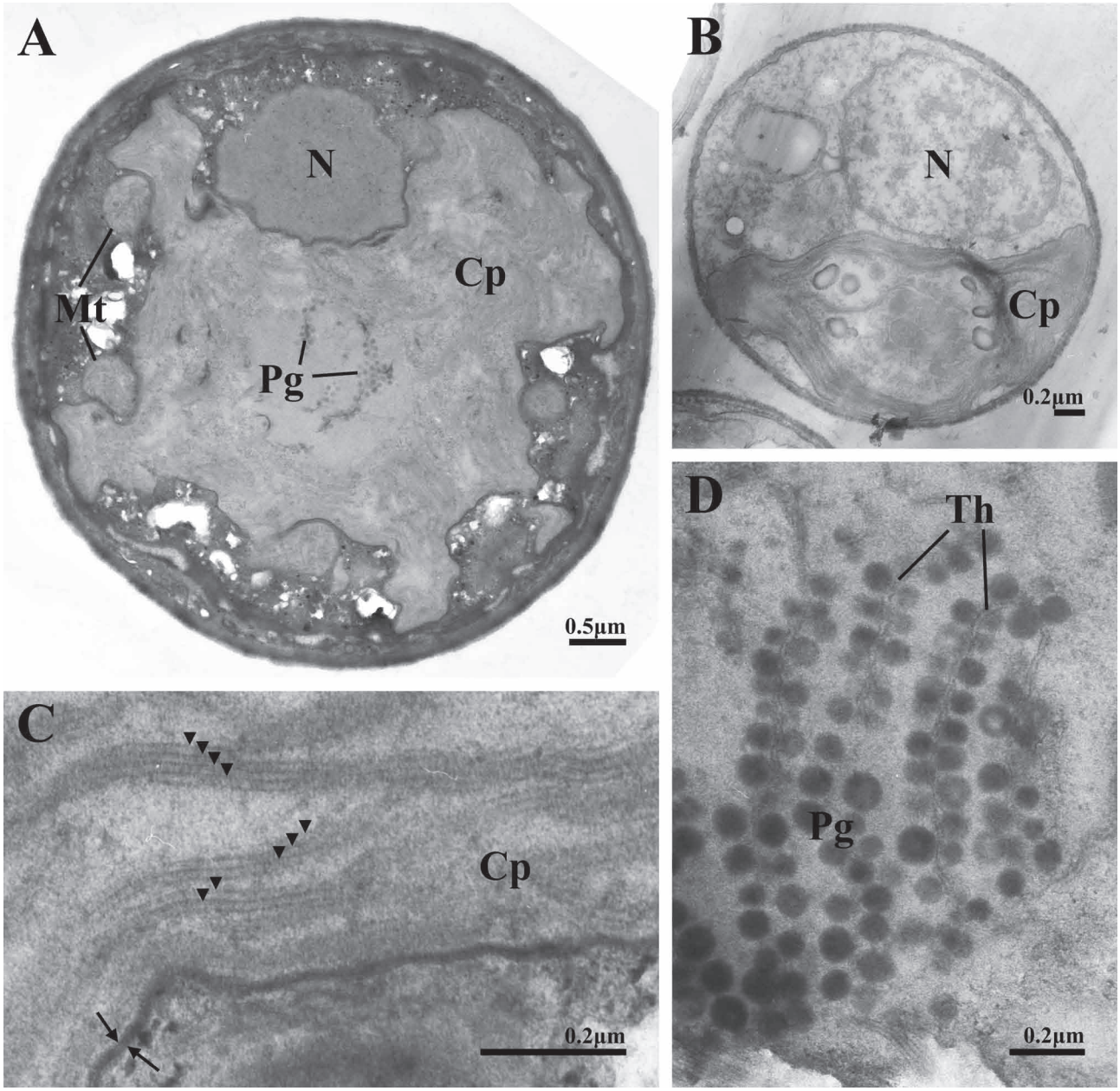


**FIGURE 2.** Transmission electron micrographs of 2015KGS-007A. (A) Vegetative cell of 2015KGS-007A. (B) Spore cell of 2015KGS-007A. (C) Magnified image of thylakoid and chloroplast membrane. (D) Pyrenoglobuli in the chloroplast. Cp, chloroplast; Mt, mitochondria; N, nucleus; Pg, pyrenoglobuli; Th, thylakoid.

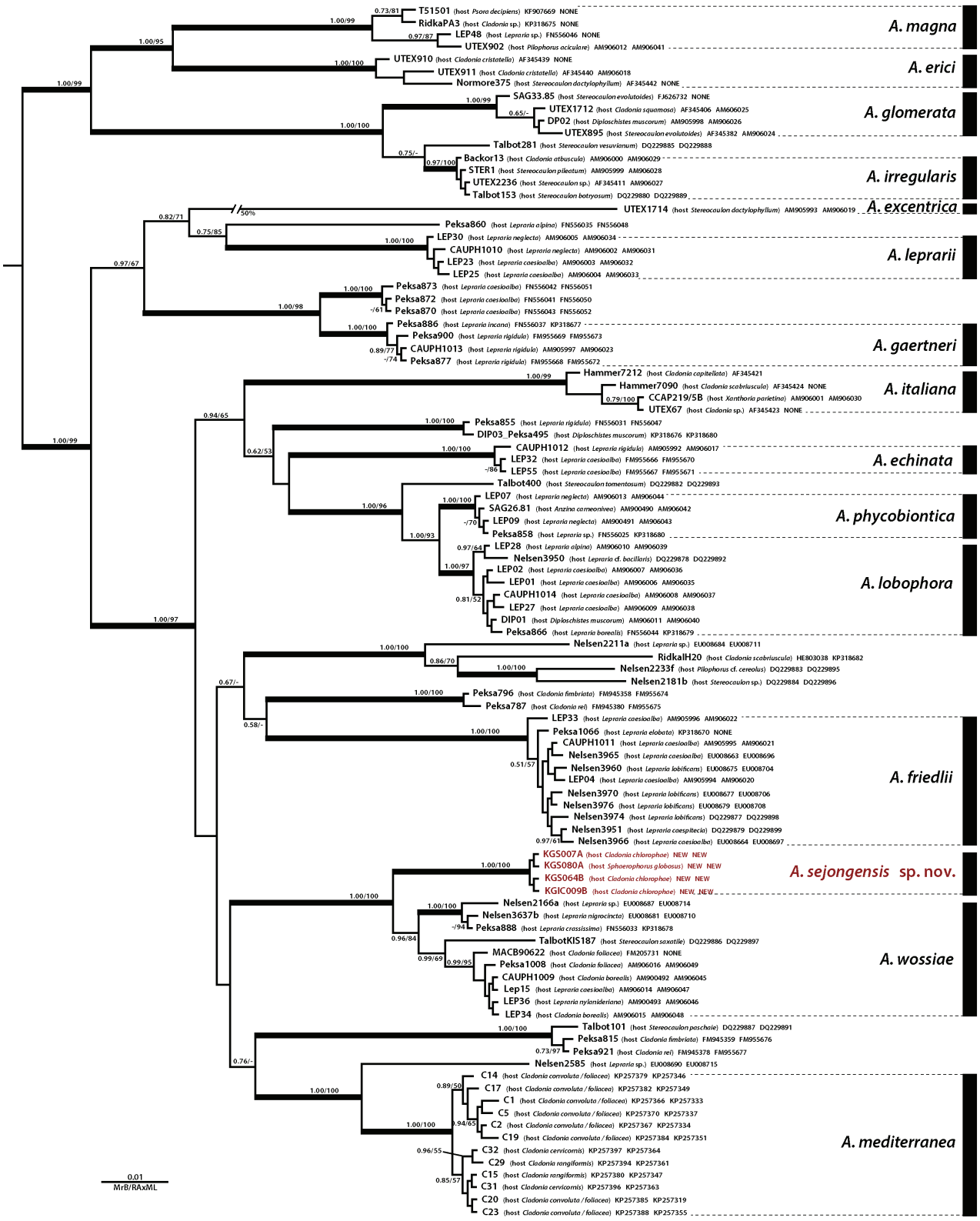


**FIGURE 3.** Bayesian majority role tree based on concatenated ITS rDNA and actin gene sequences. The numbers on each node represent posterior probabilities (left) and bootstrapping values (right). The bold branches indicate strongly supported values (pp=1.00 and ML=100%).

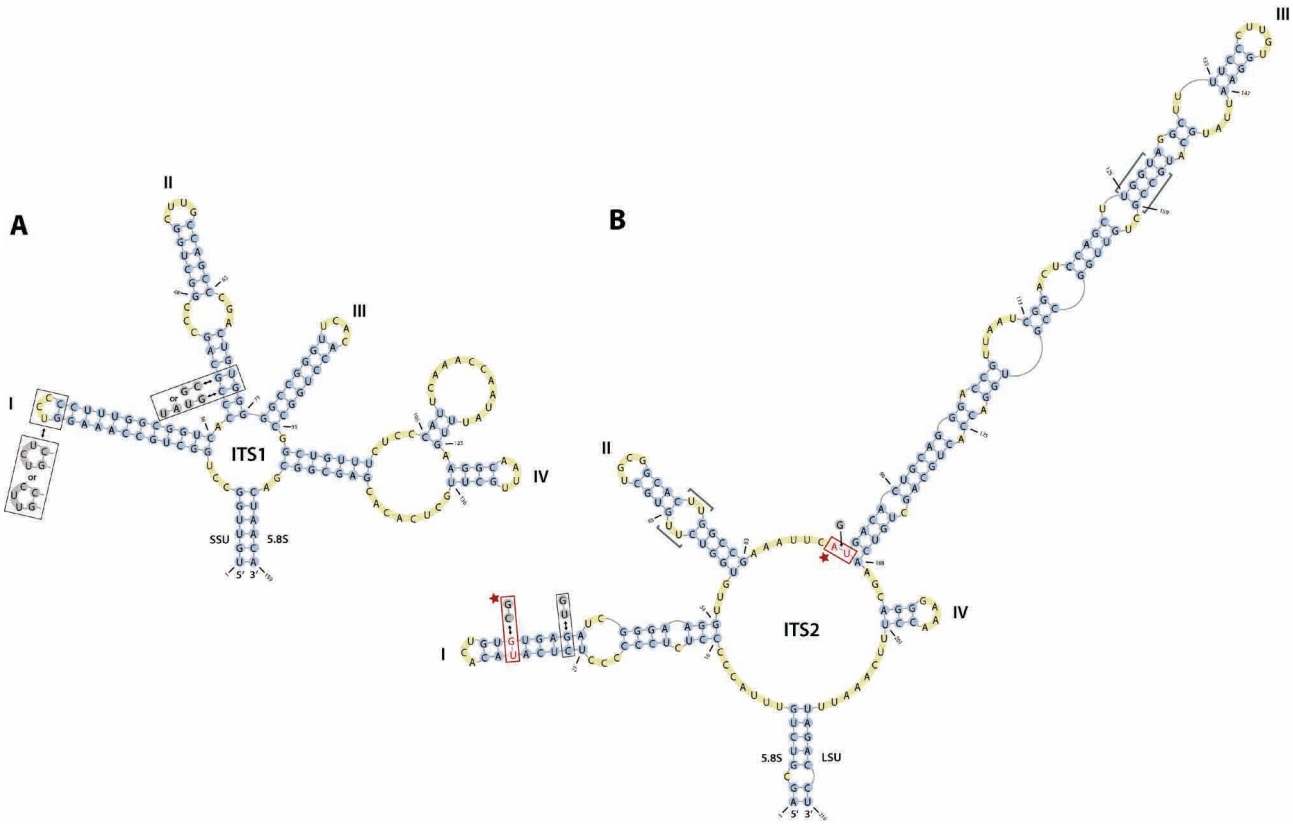


**FIGURE 4.** Predicted secondary structures of the ITS1 (A) and ITS2 (B) transcripts of *Asterochloris sejongensis* sp. nov. (2015KGS-007A) derived by a comparison of the closely related species *A. woessiae*. Base changes between two *Asterochloris* genotypes are indicated: the boxed base pair indicates hemi-compensatory base changes (hemi-CBCs); the grey circles indicate single base changes; and the large boxes indicate changes of the ends in the helices. ITS2 transcripts, highly conserved U-U mismatches and UGGU motifs are marked with [ ]. The two star markers in the ITS2 transcript structure indicate specific base changes in the *Asterochloris sejongensis* sp. nov. (2015KGS-007A) clades.

## Taxonomic revision

### *Taxon description*

*Asterochloris sejongensis* J.I. Kim *et* W. Shin sp. nov. (Fig. 1)

Vegetative cells that are usually spherical but occasionally oval and pyriform and have diameters of 5-7.5 to 24-28 µm (Fig. 1). Thick cell walls (Fig. 1) with a flat local thickening can be distinguished. Rarely, the cell wall is slightly thickened along its entire surface. Parietal, deeply lobed chloroplasts in young cells, and central deeply lobed or echinulated chloroplasts in mature cell. The chloroplast lobes are simply terminated (Fig. 1A, B), elongated (Fig. 1C, D), or pointed at their ends (Fig. 1E-H). One to two pyrenoids are positioned at the center of the chloroplast. Asexual reproduction occurs by 64– 128 aplanospores or zoospores produced in spherical or ellipsoidal sporangia (Fig. 1I, J).

**Molecular signatures**: Hemi-CBCs in helix I (C:G – U:G) and one base deletion between helix II and helix III (AGU-AU) of the ITS2; compared with the ITS2 secondary transcripts of all *Asterochloris* species.

**Holotype**: Designated here at Fig. 1G. Holotype is stored at the collection of specimens on TEM block CNU071737, deposited in the Herbarium of Chungnam National University, Daejeon, Korea (CNUK).

**Type strain**: Deposited at the culture collection of Chungnam National University (strain number; 2015KGS-007A).

**Type locality**: Phycobiont of *Cladonia pyxidata* and *Sphaerophorus globosus* collected on rocks and soil crusts in the vicinity of the King Sejong Station, King George Island, Antarctica in February 2015. The lichen specimen has been deposited in the herbarium of the Korea Polar Research Institute, (2015KGIC-009, 2015KGS-007, 2015KGS-064, 2015KGS-080).

**Etymology**: The name "*sejongensis*" is derived from the name of the King Sejong Station (Korea Antarctic Research Station), the location where the lichens were collected.

**Distribution**: Thus far, the only known distribution is in the vicinity of Barton Peninsula, Antarctica.

**Ecology**: *Cladonia pyxidata* is a cosmopolitan species and grows usually on gravelly soil and mosses, and frequently on mosses in Barton Peninsula, King George Island. *Sphaerophorus globosus* is a bipolar species inhabiting on mosses, especially on *Chorisodontium aciphyllum* and *Polytrichcum strictum* turf bank in Barton Peninsula.

**Specificity**: Found in the thalli of the lichen species *Cladonia pyxidata* and *Sphaerophorus globosus*.

## Discussion

Limited taxonomic studies of lichenized photobionts have been performed despite the high diversity of lichen species, and most of the available studies have primarily focused on lichen-forming mycobionts. Most eukaryotic photobionts are members of trebouxiophycean genera, especially *Trebouxia* de Puymaly (1924: 109), *Asterochloris* Tschermak-Woess (1980a: 291), *Myrmecia* Printz (1921: 13), *Coccomyxa* Schmidle (1901: 23), *Dictyochloropsis* Geitler (1966: 155) and *Elliptochloris* Tschermak-Woess (1980b: 72). Among these genera, *Trebouxia* and *Asterochloris* species are among the most common and represent important partners with lichenized mycobionts (Friedl & Büdel 2008, Škaloud *et al.* 2015). Recently, Vančurová *et al.* (2015) described a new lichenized photobiont genus *Vulcanochloris* Vančurová, Peksa, Němcová et Škaloud isolated from the thalli of *Stereocaulon vesuvianum* growing on the slopes of volcanoes and lava fields of La Palma, Canary Islands, Spain. *Asterochloris* and *Vulcanochloris* species are morphologically similar to each other; however, they are differentiated from *Trebouxia* species by their axial, deeply lobed chloroplasts, chloroplast transformation prior to sporogenesis, and production of many daughter cells (Vančurová *et al.* 2015). According to Vančurová *et al.* (2015), *Vulcanochloris* is differentiated morphologically from *Asterochloris* species by its unique formation of spherical incisions with the pyrenoid. In this study, we found a new *Asterochloris* species that was isolated from the thalli of the Antarctic lichen species *Cladonia pyxidata* and *Sphaerophorus globosus*, and this new species was differentiated from other species using morphological and molecular evidence.

The chloroplast is an important characteristic for species delimitation within the genus *Asterochloris* (Škaloud *et al.* 2015). *Asterochloris sejongensis* has a typical chloroplast of the genus and shares chloroplast features with other *Asterochloris* species. The newly described species, however, has a characteristic type of chloroplast: in young vegetative cells, the chloroplast is axial and deeply incised into a centrally positioned pyrenoid, whereas in mature cells, the chloroplast margin of the vegetative cell is more deeply incised and spiny-shaped (Fig. 1G, H). This chloroplast type is most comparable to those of *A. echinata* Škaloud et Peksa, *A. gaertneri* Škaloud et Peksa, and *Vulcanochloris symbiotica* Vančurová, Peksa, Němcová et Škaloud. However, the chloroplast margin is fine and becomes a spiny form at the tip of the margin. In addition, the pyrenoid ultrastructure shows that *A. sejongensis* has an irregularis type (Friedl 1989) because of the invagination of a small number of curved thylakoid membranes into the matrix and numerous pyrenoglobuli associated with the membranes. The irregularis-type pyrenoid is found in *A. excentrica*, *A. glomerata*, *A. irregularis*, *A. italiana*, and *A. mediterranea* Barreno, Chiva, Moya et Škaloud (2015: 1838).

Our tree constructed according to the nuclear ITS and actin-coding gene sequences was divided largely into three main clades, and this result is very similar to the tree previously published by Škaloud *et al.* (2015) and Moya *et al.* (2015). The phylogenetic tree revealed a new strongly supported monophyletic clade (pp=1.00, ML=100) and showed that the new species is closely related to *A. wossiae* with strong support values (pp=1.00, ML=100). The putative ITS secondary structure model of *A. sejongensis* sp. nov. was compared with those of other *Asterochloris* species, and the results showed that it has a unique hemi-CBC in helix I and one nucleotide deletion between helix II and III of ITS2. In summary, the newly described species was isolated from Antarctic *Cladonia pyxidata* and *Sphaerophorus globosus* and included in the genus *Asterochloris*. The morphological and molecular data showed that all of the strains have unique characteristic features, such as irregularis-type pyrenoids, spiny tips at the chloroplast margin, and unique hemi-CBC in helix I of ITS2. In addition, the phylogenetic tree showed that all of the isolates were of a monophyletic lineage with strong support.

## ACKNOWLEDGEMENTS

This research was supported by the Korea Polar Research Institute (grant PE15020) to W. Shin.

## References


Ahmadjian, V. (1967) A guide to the algae occurring as lichen symbionts: isolation, culture, cultural physiology, and identification. *Phycologia* 6: 127–60.
https://doi.org/10.2216/i0031-8884-6-2-127.1

Bačkor, M., Peksa, O., Škaloud, P. & Bačkorová, M. (2010) Photobiont diversity in lichens from metal-rich substrata based on ITS rDNA sequences. *Ecotoxicology and Environmental Safety* 73: 603–612.
https://doi.org/10.1016/j.ecoenv.2009.11.002

Beiggi, S. & Piercey-Normore, M.D. (2007) Evolution of ITS ribosomal RNA secondary structures in fungal and algal symbionts of selected species of *Cladonia* sect. *Cladonia* (Cladoniaceae, Ascomycotina). *Journal of Molecular Evolution* 64: 528–542.
https://doi.org/10.1007/s00239-006-0115-x

Casano, L.M., del Campo, E.M., García-Breijo, F.J., Reig-Armiñana, J., Gasulla, F., del Hoyo, A., Guéra, A. & Barreno, E. (2011) Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? *Environmental Microbiology* 13: 806–818.
https://doi.org/10.1111/j.1462-2920.2010.02386.x

Chown, S.L. & Convey, P. (2007) Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 362: 2307–2331.
https://doi.org/10.1098/rstb.2006.1949

Coleman, A.W. (2000) The significance of a coincidence between evolutionary landmarks found in mating affinity and a DNA sequence. *Protist* 151: 1–9.
https://doi.org/10.1078/1434-4610-00002

Coleman, A.W. (2003) ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends in Genetics* 19: 370–375.
https://doi.org/10.1016/S0168-9525(03)00118-5

Cordeiro, L. M., Reis, R. A., Cruz, L. M., Stocker-Wörgötter, E., Grube, M. & Iacomini, M. (2005) Molecular studies of photobionts of selected lichens from the coastal vegetation of Brazil. *FEMS Microbiology Ecology* 54: 381–390.
https://doi.org/10.1016/j.femsec.2005.05.003

Culberson, C.F. (1972) Improved conditions and new data for the identification of lichen products by a standardized thin−layer chromatographic method. *Journal of Chromatography* 72: 113–125.

Domaschke, S., Fernández-Mendoza, F., García, M.A., Martín, M.P. & Printzen, C. (2012) Low genetic diversity in Antarctic populations of the lichen-forming ascomycete *Cetraria aculeata* and its photobiont. *Polar Research* 31: 1–13.

Friedl, T. (1989) Comparative ultrastructure of pyrenoids in *Trebouxia* (Microthamniales, Chlorophyta). *Plant Systematics and Evolution* 164: 145–159.
https://doi.org/10.1007/BF00940435

Friedl, T. & Büdel, B. (2008) Photobionts. *In*: Nash III, T.H. (Ed.) *Lichen biology*, Cambridge University Press, New York, pp. 9–26.

Friedl, T. & Rokitta, C. (1997) Species relationships in the lichen alga *Trebouxia* (Chlorophyta, Trebouxiophyceae): molecular phylogenetic analyses of nuclear-encoded large subunit rRNA gene sequences. *Symbiosis* 23: 125–148.

Friedl, T. & Zeltner, C. (1994) Assessing the relationships of some coccoid green lichen algae and the Microthamniales (Chlorophyta) with 18S ribosomal RNA gene sequence comparisons. *Journal of Phycology* 30: 500–506.
https://doi.org/10.1111/j.0022-3646.1994.00500.x

Geitler, L. (1966) Die Chlorococcalen *Dictyochloris* und *Dictyochloropsis* nov. gen. *Österreichische Botanische Zeitschrift* 133: 155–164.

Grube, M. & Muggia, L. (2010) Identifying algal symbionts in lichen symbioses. *In:* Limis, P.L. & Lebbe, R.V. (Ed.) Tools for Identifying Biodiversity: Progress and Problems. *Proceedings of the International Congress, Paris, September 20–22, 2010.* EUT Edizioni Universit_a di Trieste, Trieste, pp. 295–299.

Guzow-Krzeminska, B. (2006) Photobiont flexibility in the lichen *Protoparmeliopsis muralis* as revealed by ITS rDNA analyses. *Lichenologist* 38: 469–476.

Helms, G., Friedl, T., Rambold, G. & Mayrhofer, H. (2001) Identification of photobionts from the lichen family Physciaceae using algal-specific ITS rDNA sequencing. *Lichenologist* 33: 73–86.
https://doi.org/10.1006/lich.2000.0298

Ma, S., Han, B., Huss, V.A.R., Hu, X., Sun, X. & Zhang, J. (2015) *Chlorella thermophila* (Trebouxiophyceae, Chlorophyta), a novel thermo-tolerant *Chlorella* species isolated from an occupied rooftop incubator. *Hydrobiologia* 760: 81–89.

Mai, J.C. & Coleman, A.W. (1997) The internal transcribed spacer 2 exhibits a common secondary structure in green algae and flowering plants. *Journal of Molecular Evolution* 44: 258–271.

https://doi.org/10.1007/PL00006143

Moya, P., Škaloud, P., Chiva, S., García-Breijo, F. J., Reig-Armiñana, J., Vančurová, L. & Barreno, E. (2015) Molecular phylogeny and ultrastructure of the lichen microalga *Asterochloris mediterranea* sp. nov. from Mediterranean and Canary Islands ecosystems. *International Journal of Systematic and Evolutionary Microbiology* 65: 1838–1854.
https://doi.org/10.1099/ijs.0.000185

Nelsen, M.P. & Gargas, A. (2006) Actin type I introns offer potential for increasing phylogenetic resolution in *Asterochloris* (Chlorophyta: Trebouxiophyceae). *The Lichenologist* 38: 435–440.
https://doi.org/10.1017/S0024282906005779

Nelsen, M.P. & Gargas, A. (2008) Dissociation and horizontal transmission of codispersinglichen symbionts in the genus *Lepraria* (Lecanorales: Stereocaulaceae). *New Phytologist* 177: 264–275.
https://doi.org/10.1111/j.1469-8137.2007.02241.x

Orange, A., James, P.W. & White, F.J. (2001) *Microchemical methods for identification of lichens.* British Lichen Society, London.

Øvstedal, D.O. & Smith, R.I.L. (2001) *Lichens of Antarctica and South Georgia. A guide to their identification and ecology.* Cambridge University Press, Cambridge.

Olech, M. (2004) *Lichens of King George Island, Antarctica.* The Institute of Botany of the Jagiellonian University, Kraków.

Ohmura, Y., Kawachi, M., Kasai, F., Watanabe, M.M. & Takeshita, S. (2006) Genetic combinations of symbionts in a vegetatively reproducing lichen, *Parmotrema tinctorum*, based on ITS rDNA sequences. *Bryologist* 109: 43–59.

Park, C.H., Kim, K.M., Elvebakk, A., Kim, O.S., Jeong, G. & Hong, S.G. (2015) Algal and fungal diversity in Antarctic lichens. *Journal of Eukaryotic Microbiology* 62: 196–205.
https://doi.org/10.1111/jeu.12159

Peksa, O. & Škaloud, P. (2011) Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Molecular Ecology* 20: 3936–3948.
https://doi.org/10.1111/j.1365-294X.2011.05168.x

Pérez-Ortega, S., Ortiz-Álvarez, R., Green, T.G.A. & de los Ríos, A. (2012) Lichen myco- and photobiont diversity and their relationships at the edge of life (McMurdo dry valleys, Antarctica). *FEMS Microbiology Ecology* 82: 429–448.
https://doi.org/10.1111/j.1574-6941.2012.01422.x

Piercey-Normore, M.D. (2006) The lichen-forming ascomycete *Evernia mesomorpha* associates with multiple genotypes of *Trebouxia jamesii*. *New Phytologist* 169: 331–344.

Piercey-Normore, M.D. & DePriest, P.T. (2001) Algal switching among lichen symbioses. *American Journal of Botany* 88: 1490–1498.
https://doi.org/10.2307/3558457

Printz, H. (1921) Subaërial algae from South Africa. *Det Kongelige Norske Videnskabers Selskabs Skrifter* 1920: 1–41, I–XIV.
https://doi.org/10.5324/dknvss.v0i0.1271

Puymaly, A. de (1924) Le *Chlorococcum humicola* (nag.) Rabenh. *Revue Algologique* 1: 107–14.

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: 208–212.
https://doi.org/10.1083/jcb.17.1.208

Romeike, J., Friedl, T., Helms, G. & Ott, S. (2002) Genetic diversity of algal and fungal partners in four species of *Umbilicaria* (lichenized ascomycetes) along a transect of the Antarctic peninsula. *Molecular Biology and Evolution* 19: 1209–1217.
https://doi.org/10.1093/oxfordjournals.molbev.a004181

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
https://doi.org/10.1093/sysbio/sys029

Schmidle, W. (1901) Über drei Algengenera. *Berichte der Deutschen Botanischen Gessellschaft* 19: 10–24.

Škaloud, P. & Peksa, O. (2008) Comparative study of chloroplast morphology and ontogeny in *Asterochloris*. *Biologia* 63: 869–876.

Škaloud, P. & Peksa, O. (2010) Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga *Asterochloris* (Trebouxiophyceae, Chlorophyta). *Molecular Phylogenetics and Evolution* 54: 36–46.
https://doi.org/10.1016/j.ympev.2009.09.035

Škaloud, P., Steinová, J., Řídká, T., Vančurová, L. & Peksa, O. (2015) Assembling the challenging puzzle of algal biodiversity: species delimitation within the genus *Asterochloris* (Trebouxiophyceae, Chlorophyta). *Journal of Phycology* 51: 507–527.
https://doi.org/10.1111/jpy.12295

Smith, S.W., Overbeek, R., Woese, C.R., Gilbert, W. & Gillevet, P.M. (1994) The genetic data environment an expandable GUI for multiple sequence analysis. *Computer Applications in the Biosciences* 10: 671–675.
https://doi.org/10.1093/bioinformatics/10.6.671

Spurr, A.R. (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* 26: 31–43.
https://doi.org/10.1016/S0022-5320(69)90033-1

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

Thomas, D.L. & Montes, J.G. (1978) Spectrophotometrically assayed inhibitory effects of mercuric compounds on *Anabaena flos-aquae* and *Anacystis nidulans* (Cyanophyceae). *Journal of Phycology* 14: 494–499.
https://doi.org/10.1111/j.1529-8817.1978.tb02475.x

Tschermak-Woess, E. (1980a) *Asterochloris phycobiontica*, gen. et spec. nov., der Phycobiont der Flechte *Varicellaria carneonivea*. *Plant Systematics and Evolution* 135: 279–294.

Tschermak-Woess, E. (1980b) *Elliptochloris bilobata*, gen. et spec. nov., der phycobiont von *Catolechia wahlenbergii*. *Plant Systematics and Evolution* 136: 62–72.

Vančurová, L., Peksa, O., Němcová, Y. & Škaloud, P. (2015) *Vulcanochloris* (Trebouxiales, Trebouxiophyceae), a new genus of lichen photobiont from la Palma, Canary Islands, Spain. *Phytotaxa* 219: 118–132.
https://doi.org/10.11646/phytotaxa.219.2.2

Walter, A.E., Turner, D.H., Kim, J., Lyttle, M.H., Müller, P., Mathews, D.H. & Zuker, M. (1994) Coaxial stacking of helixes enhances binding of oligoribonucleotides and improves predictions of RNA folding. *Proceedings of the National Academy of Sciences of the United States of America* 91: 9218–9222.
https://doi.org/10.1073/pnas.91.20.9218

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*, Academic Press, San Diego, California, pp. 315–322.

Yahr, R., Vilgalys, R. & DePriest, P.T. (2004) Strong fungal specificity and selectivity for algal symbionts in Florida scrub *Cladonia* lichens. *Molecular Ecology* 13: 3367–3378.
https://doi.org/10.1111/j.1365-294X.2004.02350.x

Yahr, R., Vilgalys, R. & DePriest, P.T. (2006) Geographic variation in algal partners of *Cladonia subtenuis* (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. *New Phytologist* 171: 847–860.
https://doi.org/10.1111/j.1469-8137.2006.01792.x

Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* 31: 3406–3415.
https://doi.org/10.1093/nar/gkg595