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Morphology and phylogenetic relationships of two new Antarctic species in phylogroup *Chloromonadinia* (Volvocales, Chlorophyceae)

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ABSTRACT

The phylogroup *Chloromonadinia* (Volvocales, Chlorophyceae) comprises green microalgae that inhabit a variety of environments, including freshwater, soil, and snow. Two strains were isolated from two sites in the South Shetland Islands in maritime Antarctica, and their morphological and molecular characteristics were studied. Light microscopy of the strain KSF0090 revealed ellipsoidal or broad ellipsoidal, sometimes almost spherical cells with a chloroplast without a pyrenoid, a prominent eyespot, and a hemispherical papilla. The vegetative cells of KSF0208 were ellipsoidal to ovoid cells with a chloroplast with a central pyrenoid, a linear eyespot, and a papilla. The two strains differed from other closely related species based on size and the aforementioned morphological characteristics. Nuclear small subunit rDNA sequence data indicated that each strain formed a distinct well-supported lineage within the phylogroup *Chloromonadinia*. In addition, comparative analyses of the secondary structures of internal transcribed spacer 2 and compensatory base changes were used to identify and characterize the two strains. Based on their morphological and molecular characteristics, we propose KSF0090 and KSF0208 as two new species, *Chloromonas deceptionensis* sp. nov. and *Ostravamonas greenwichensis* sp. nov., respectively.

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INTRODUCTION

Antarctica has a harsh environment due to low temperatures and the presence of minimal nutrients and excessive irradiation, but is less affected by anthropogenic activity. However, many microalgae have adapted to such extreme environmental conditions and can flourish within melting snow during spring and summer. In particular, members of the genus *Chloromonas* Gobi (Volvocales, Chlorophyceae) are generally abundant in greenish snow (Hoham 1980; Hoham & Duval 2001).

Chloromonas is a genus comprising unicellular green algae with two flagella. It has been distinguished from *Chlamydomonas* Ehrenberg by the absence of pyrenoids in the chloroplasts (Ettl 1983). However, the classification of *Chloromonas* was revised based on a phylogenetic analysis that showed that the absence of pyrenoids is not diagnostic (Pröschold *et al.* 2001). *Chloromonas* was proposed as monophyletic with *C. reticulata* (Gorozhankin) Gobi as the type species. A comprehensive phylogenetic analysis based on 18S rDNA sequences of Volvocales confirmed that the genus *Chloromonas* belongs to the phylogroup *Chloromonadinia* (Nakada *et al.* 2008). Recent studies have shown that some additional genera, including *Chlainomonas* Christen (Novis *et al.* 2008), *Gloeomonas* G.A. Klebs (Nozaki *et al.* 2010; Nakada *et al.* 2015), *Ixipapillifera* Nakada (Nakada *et al.* 2008), *Ostravamonas* Barcytè & Hodač (Barcytè *et al.* 2019), and additional species of

Chloromonas (Hoham *et al.* 2006; Matsuzaki *et al.* 2010, 2012, 2013, 2014, 2018; Muramoto *et al.* 2010; Remias *et al.* 2013; Barcytè *et al.* 2018a, b) are included in the phylogroup *Chloromonadinia*.

The genus *Paludistella* was recently established with four species of the phylogroup *Chloromonadinia* (Susanti *et al.* 2020). However, this generic name was found to be superfluous and therefore illegitimate because it originally included the type species of *Ostravamonas*, *O. chlorostellata* (E.A. Flint & H. Ettl) Barcytè & Hodač (Nakada & Susanti 2020). According to Nakada & Susanti (2020), the taxonomic and nomenclatural status of these genera were examined based on morphological, phylogenetic and internal transcribed spacer 2 (ITS2) secondary structure analyses, and two new combinations for *P. asymmetrica* H. Susanti, Masaki Yoshida, Takeshi Nakayama, Nakada & M.M. Watanabe and *P. trianguloculus* H. Susanti, Masaki Yoshida, Takeshi Nakayama, Nakada & M. M. Watanabe in *Ostravamonas* were proposed.

We isolated two *Chlamydomonas*-like strains (KSF0090 and KSF0208) from freshwater sites in the South Shetland Islands, Antarctica. The South Shetland Islands are located 120 km north of the Antarctic Peninsula, and consist of several islands with diverse environments and valuable biological resources. Although several studies have reported that microalgae live in freshwater, soil, snow, and symbiotic association with lichens, the taxonomic information on Antarctic microalgae remains

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limited (Llames & Vinocur 2007; Zidarova 2008; Chae *et al.* 2019; Kim *et al.* 2020). In this study, we performed a taxonomic analysis of the strains using light microscopy, molecular phylogenetic analysis and secondary structure analysis of the ITS2.

MATERIAL AND METHODS

Strain cultivation and microscopy

Freshwater samples were collected using a 20- μm -mesh plankton net from snowmelt streams at two sites in the South Shetland Islands, Antarctica, in January 2014; the Deception Island (62°58.86'S, 60°39.96'W; KSF0090) and Greenwich Island (62°28.72'S, 59°39.60'W; KSF0208). Aliquots (1 ml) of the samples were inoculated into 5 ml of Bold Modified Basal Freshwater nutrient solution B5282 (Sigma-Aldrich, Saint Louis, Missouri, USA). The samples were transported in refrigerated containers (2–4°C). Strains were established by isolating individual vegetative cells, using a sterile Pasteur capillary pipette to transfer single cells into a 96-well plate. The strains were cultivated at approximately 2°C at 16:8 h (light:dark) using cool-white fluorescent lamps at an intensity of 20–30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Morphological investigations of the strains were carried out using an Axio Imager A2 (Carl Zeiss, Oberkochen, Germany) equipped with differential interference contrast optics. Vegetative cells from one to three-week-old cultures were observed, and images were taken using an AxioCam HRC camera (Carl Zeiss).

Sequencing and phylogenetic analyses

Genomic DNA was extracted from the two strains using the i-genomic Plant DNA Extraction Kit (iNtRON Biotechnology, Seoul, South Korea) according to the manufacturer's instructions. We performed polymerase chain reaction (PCR) to amplify the nuclear small subunit (SSU) rDNA and ITS2, using the primer pairs NS1/NS4, NS5/NS8, and ITS1/ITS4 (White *et al.* 1990). The PCR products were purified using the MG PCR Product Purification Kit (MacroGen, Seoul, Korea) according to the manufacturer's instructions, and were then sequenced by MacroGen (Seoul, South Korea).

Nuclear SSU rDNA sequences of two strains and 74 other closely related phylogroup *Chloromonadinia* strains and out-group taxa were used for phylogenetic analyses (see supplemental information). The dataset containing 1676 characters was manually aligned as per Susanti *et al.* (2020; supplemental file), and sequences were assembled and edited with BioEdit 7.0.5.3 (Hall 1999). An appropriate evolutionary model was assessed using jModelTest 2 (Darriba *et al.* 2012) under the Akaike Information Criterion and the GTR + I + G nucleotide substitution model were selected as the best fit. Maximum likelihood analysis was performed using PhyML v3.0 (Guindon *et al.* 2010), applying the chosen evolutionary model with bootstrap analyses using 1000 replicates. Bayesian inference was calculated using MrBayes 3.2.6 (Ronquist *et al.* 2012) by performing two simultaneous runs (nruns = 2) and four Metropolis-coupled Markov chain

Monte Carlo (MC³) algorithms for 10×10^6 generations. The first 25% of the trees were discarded as burn-in. The phylogenetic tree data were visualized using FigTree v1.4.2 (available at <http://tree.bio.ed.ac.uk/software/figtree/>).

ITS2 secondary structure

Secondary structure models of the annotated ITS2 regions were folded based on the minimum energy criterion calculated using Mfold (Zuker 2003). The structures were compared with published ITS2 structures of *Chloromonadinia* (Barcytè *et al.* 2018a, b, 2019; Susanti *et al.* 2020), and the common secondary structures were drawn using PseudoViewer3 (<http://pseudoviewer.inha.ac.kr/>). The nuclear ITS2 rDNA secondary structures of each new strain were compared with those of closely related species to determine the occurrence of compensatory base changes (CBCs) and hemi-CBCs according to Coleman (2003). To locate CBCs and hemi-CBCs, the ITS2 secondary structures were calculated using 4SALE (Seibel *et al.* 2006, 2008).

RESULTS

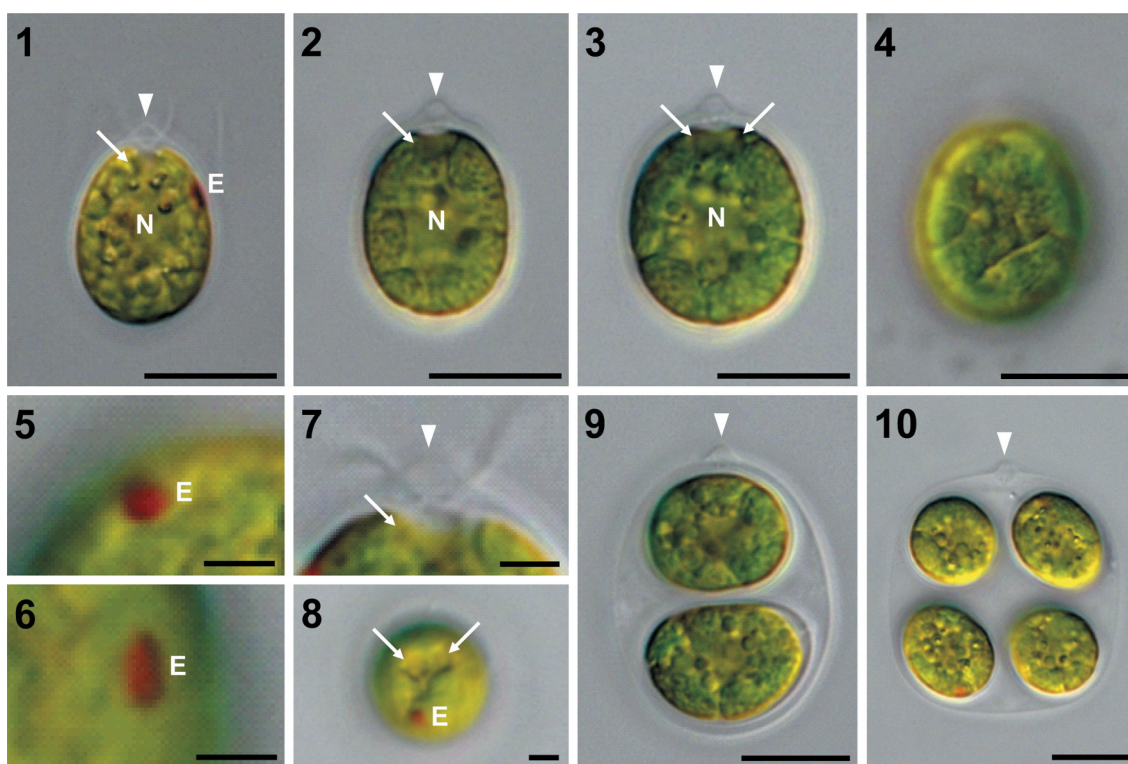
Morphological observations

The vegetative cells of strain KSF0090 were found to be ellipsoidal, broadly ellipsoidal, or often almost spherical (Figs 1–3), 11–27 μm long and 10–26 μm wide. The cell wall was thick and contained a prominent anterior hemispherical papilla (Figs 1–3, 7). Two flagella of equal length, both about as long as the cells, emerged from under the papilla (Figs 1, 7). The cells possessed cup- or urn-shaped single chloroplasts containing irregular surface slits (Fig. 4). No pyrenoids were observed. Two contractile vacuoles were located near the flagellar bases (Figs 2, 3, 8). The eyespot was oblong or round, and located in the anterior third of the cell (Figs 1, 5, 6, 8). The nucleus was in the middle part of the cell (Figs 1–3). Asexual reproduction occurred by the formation of two or four zoospores (Figs 9, 10). Sexual reproduction and cell aggregates were not observed.

Cells of the strain KSF0208 were ellipsoidal to ovoid (Figs 11–13), 13–20 μm long and 8–16 μm wide. Two equal flagella emerged from a prominent papilla and were approximately the same length as the cells (Fig. 15). The papilla was hemispherical to conical (Figs 11–13). The cells possessed cup-shaped chloroplasts with radial lobes and containing irregular surface slits (Figs 11–14). A large spherical pyrenoid was located centrally in the cell and covered with starch plates (Figs 11–13, 18, 19). The nucleus was located anterior to the pyrenoid (Figs 11–13). Two contractile vacuoles were located near the flagellar bases (Figs 12, 13, 17). The eyespot was elongated, linear or narrowly ellipsoidal (Fig. 16), positioned in the anterior third of the cell (Fig. 12). Asexual reproduction occurred by formation of two or four zoospores (Figs 20, 21). Sexual reproduction was not observed.

Molecular phylogenetic analyses

Phylogenetic analysis of 78 SSU rDNA sequences revealed that the two strains were clustered in two main phylogenetic lineages within the phylogroup *Chloromonadinia* (Fig. 22). In the SSU



Figs 1–10. Light microscopy of *Chloromonas deceptionensis* sp. nov. strain KSF0090. Arrowheads indicate the papillae and arrows indicate contractile vacuoles. E, eyespot; N, nucleus.

- Fig. 1.** Optical section of young cell with two flagella. Scale bar = 10 μ m.
Fig. 2. Optical section of mature cell with central nucleus. Scale bar = 10 μ m.
Fig. 3. Optical section of mature cell with two contractile vacuoles. Scale bar = 10 μ m.
Fig. 4. Surface view of cell with irregular slits in the chloroplast. Scale bar = 10 μ m.
Fig. 5. Circular eyespot. Scale bar = 2 μ m.
Fig. 6. Oblong-elliptical eyespot. Scale bar = 2 μ m.
Fig. 7. Contractile vacuole located near the base of the flagella. Scale bar = 2 μ m.
Fig. 8. Top view with two contractile vacuoles. Scale bar = 2 μ m.
Fig. 9. Zoosporangium with two daughter cells. Scale bar = 10 μ m.
Fig. 10. Zoosporangium with four daughter cells. Scale bar = 10 μ m.

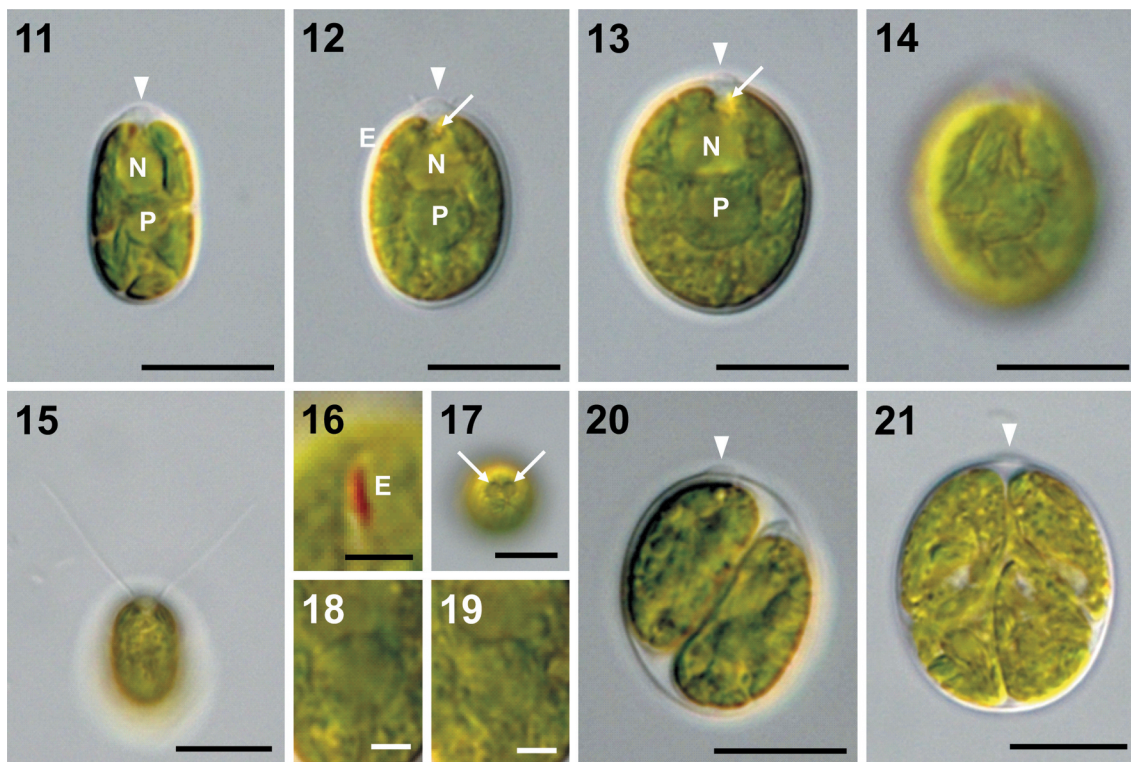
rDNA phylogenetic tree, the two strains were clustered within a diverse clade 1 (*sensu* Hoham *et al.* 2002), which was unlikely to be monophyletic. Clade 1 was divided into three subclades, and the two strains were within the first and second subclade, respectively. The first subclade (core *Chloromonas* clade; Barcytè *et al.* 2018a, 2019), containing the *Chloromonas* type species, *C. reticulata*, was moderately supported, but the relationships among strains were not supported statistically. The strain KSF0090 was placed within the core *Chloromonas* clade and was a sister group to the other strains. The second subclade consisted of *Ostravamonas* species and *C. pseudoplatyrhyncha* (Pascher) P.C. Silva. Assignment of the whole *Ostravamonas* clade was strongly supported, and strain KSF0208 was placed within the *Ostravamonas* clade. *Ostravamonas meslinii* (Bourrelly) Barcytè & Hodač SAG 75.81 was identified as the strain most similar to KSF0208, from which it differed by nine nucleotide substitutions.

ITS2 secondary structures

The two strains contained conserved motifs (Mai & Coleman 1997) that included a UU mismatch in helix II (Figs 23, 24). The predicted secondary structure of nuclear rDNA ITS2 of KSF0090 was compared with models of other species of the

core *Chloromonas* clade: *C. reticulata*, *C. chlorococcoides* (H. Ettl & K. Schwarz) Matsuzaki, Y. Hara & Nozaki, *C. gracillima* (H. Ettl) Barcytè & Hodač and *C. svalbardensis* Barcytè & Hodač (Fig. 23). The helix III of five strains among the core *Chloromonas* clade consisted of two sub-helices. Based on the strain KSF0090, we compared the structures of helix III, and only the first sub-helix was similar (see supplemental information). The strain KSF0090 differed from *C. reticulata* CAUP G 1302, the type species of the genus, by eight CBCs and nine hemi-CBCs in helices I, II and III. In the comparative region of ITS2, we observed 2–8 CBCs and 6–9 hemi-CBCs among the strains examined in the study (Fig. 25). Most helices of the whole *Ostravamonas* clade were similar, excluding helix III of KSF0208 and *O. meslinii* SAG 75.81, which consisted of three sub-helices, and only the first sub-helix was similar to those of other strains (Fig. 24). The base pair differences of the conserved region of ITS2 among the *Ostravamonas* species are summarized in Fig. 26. In total, one to seven CBCs and one to three hemi-CBCs were discovered.

Chloromonas deceptionensis H. Chae, H.G. Choi & Ji Hee Kim sp. nov.
 Figs 1–10



Figs 11–21. Light microscopy of *Ostravamonas greenwichensis* sp. nov. strain KSF0208. Arrowheads indicate the papillae and arrows indicate contractile vacuoles. E, eyespot; N, nucleus; P, pyrenoid.

Fig. 11. Optical section of young cell. Scale bar = 10 μ m.

Fig. 12. Optical section of mature cell with an eyespot. Scale bar = 10 μ m.

Fig. 13. Optical section of mature cell with cup-shaped chloroplast containing a pyrenoid. Scale bar = 10 μ m.

Fig. 14. Surface view of cell with irregular slits in the chloroplast. Scale bar = 10 μ m.

Fig. 15. Two equal flagella. Scale bar = 10 μ m.

Fig. 16. Linear eyespot. Scale bar = 2 μ m.

Fig. 17. Top view with two contractile vacuoles. Scale bar = 2 μ m.

Fig. 18. Spherical pyrenoid. Scale bar = 2 μ m.

Fig. 19. Surface view of starch plates covering the pyrenoid. Scale bar = 2 μ m.

Fig. 20. Zoosporangium with two daughter cells. Scale bar = 10 μ m.

Fig. 21. Zoosporangium with four daughter cells. Scale bar = 10 μ m.

DESCRIPTION: Vegetative cells unicellular, biflagellate, ellipsoid to wide ellipsoid or spherical, 11–27 μ m long and 10–26 μ m wide. Two equal flagella, about one cell length. Cell walls thick, with anterior papilla prominent and hemispherical. Chloroplast cup- or urn-shaped with irregular slits. Eyespot oblong or round, positioned in anterior third of cell. Nucleus central and two apical contractile vacuoles. Asexual reproduction by formation of two or four zoospores. Sexual reproduction unknown and cell aggregates not observed.

HOLOTYPE: Strain KSF0090, cryopreserved and deposited at Korea Polar Research Institute, Incheon, Korea.

TYPE LOCALITY: Deception Island (62°58.86'S, 60°39.96'W), South Shetland Islands, Antarctica. Collection date: 24 January 2014.

ETYMOLOGY: The specific epithet refers to Deception Island, where the new species was collected.

GENBANK ACCESSION NUMBER: MW183930 (Nuclear SSU rDNA sequence) and MW183922 (ITS rDNA sequence) of strain KSF0090.

***Ostravamonas greenwichensis* H. Chae, H.G. Choi & Ji Hee Kim sp. nov.**
Figs 11–21

DESCRIPTION: Vegetative cells unicellular, biflagellate, ellipsoid to ovoid, 13–20 μ m long and 8–16 μ m wide. Two equal flagella about one cell length. Anterior papilla prominent and hemispherical to conical shaped. Chloroplast cup-shaped with radial lobes and containing irregular surface slits. Central pyrenoid spherical, covered with starch plates. Eyespot long, linear or narrowly ellipsoid, positioned in the anterior third of the cell. Nucleus anterior to the pyrenoid. Two apical contractile vacuoles. Asexual reproduction by formation two or four zoospores. Sexual reproduction unknown.

HOLOTYPE: Strain KSF0208, cryopreserved and deposited at Korea Polar Research Institute, Incheon, Korea.

TYPE LOCALITY: Greenwich Island (62°28.72'S, 59°39.60'W), South Shetland Islands, Antarctica. Collection date: 28 January 2014.

ETYMOLOGY: The specific epithet refers to Greenwich Island, where the new species was collected.

GENBANK ACCESSION NUMBER: MW183926 (Nuclear SSU rDNA sequence) and MW183925 (ITS rDNA sequence) of strain KSF0208.

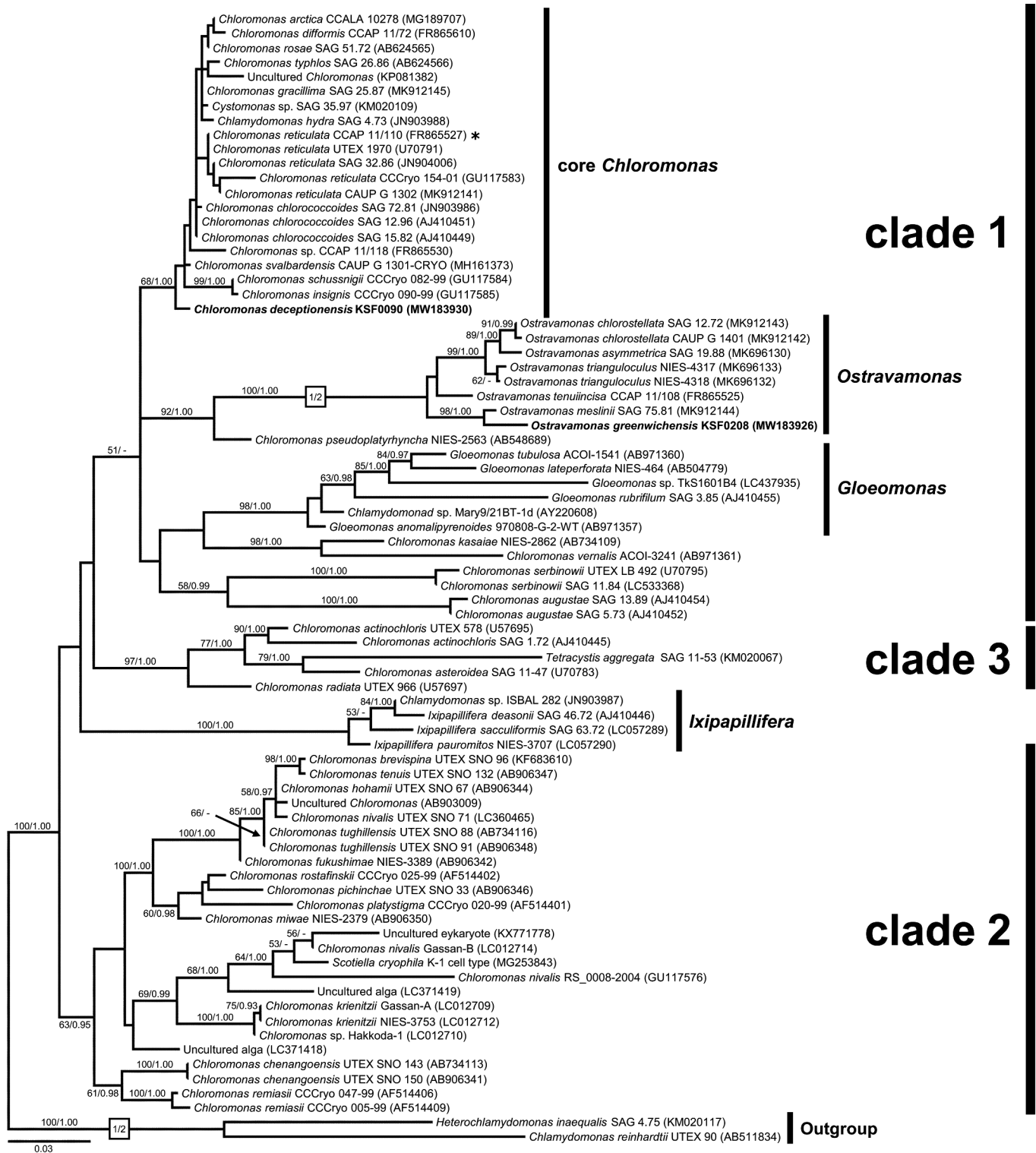


Fig. 22. Maximum likelihood tree of the phylogroup *Chloromonadina* constructed from SSU rDNA sequences. Numbers at each node are the Maximum Likelihood (> 50%, left) and Bayesian probability (> 0.95, right). *Chloromonas* clades 1, 2 and 3 were delimited according to Hoham *et al.* (2002). New sequences are in bold; the asterisk marks the authentic strain of *C. reticulata*, the type species of *Chloromonas*.

DISCUSSION

The molecular phylogenetic tree based on SSU rDNA sequences showed that the two strains of the phylogroup *Chloromonadina*, KSF0090 and KSF0208, were closely related to the genera *Chloromonas* and *Ostravamonas*,

respectively (Fig. 22). Members of the core *Chloromonas* usually possess a cup- or urn-shaped chloroplast containing parietal lobes, and lack pyrenoids (Ettl 1970, 1976; Matsuzaki *et al.* 2012; Barcytė *et al.* 2018a, b, 2019; Makino *et al.* 2019). The newly isolated *Chloromonas deceptionensis* sp. nov. KSF0090 showed several morphological differences from

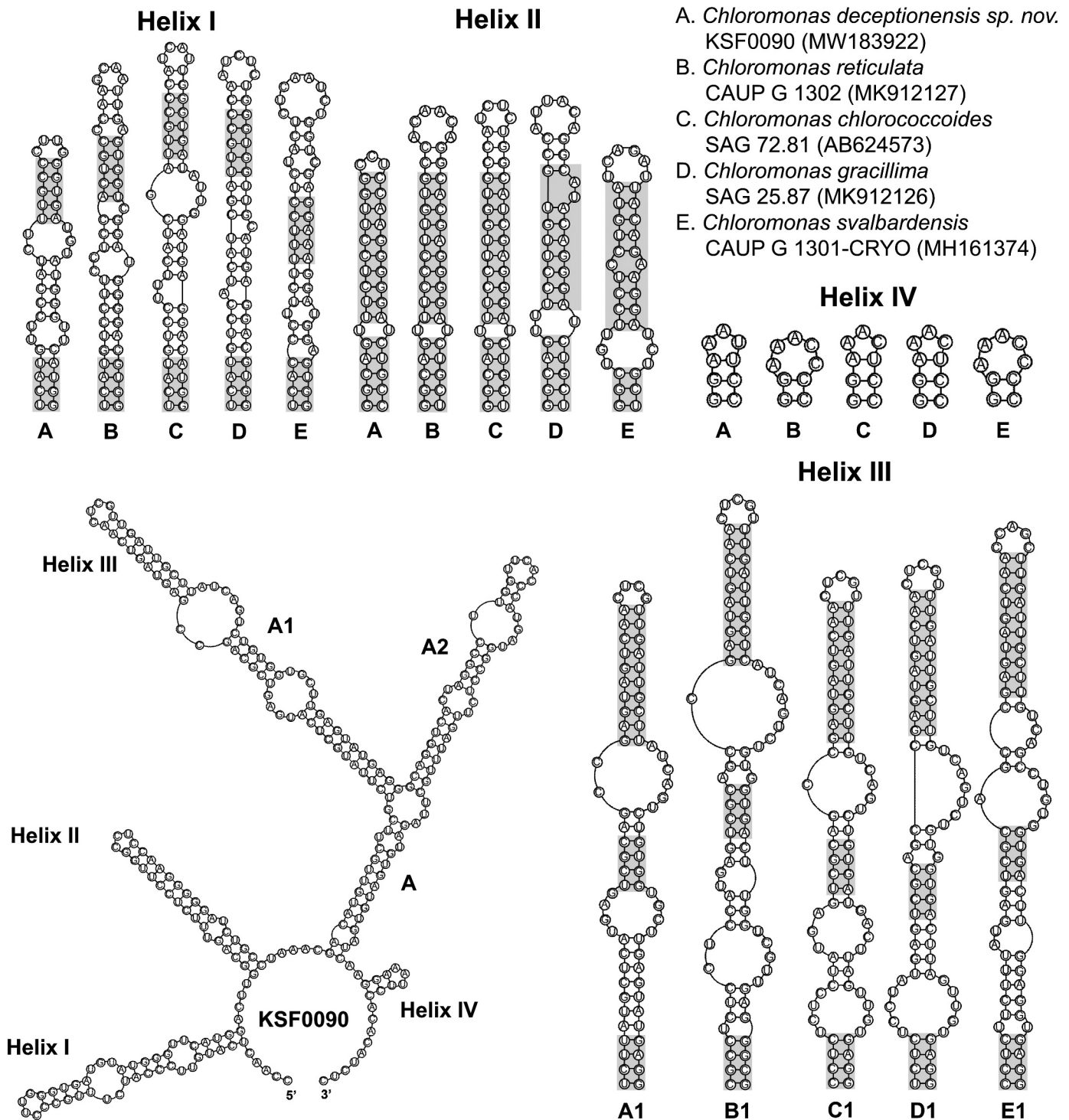
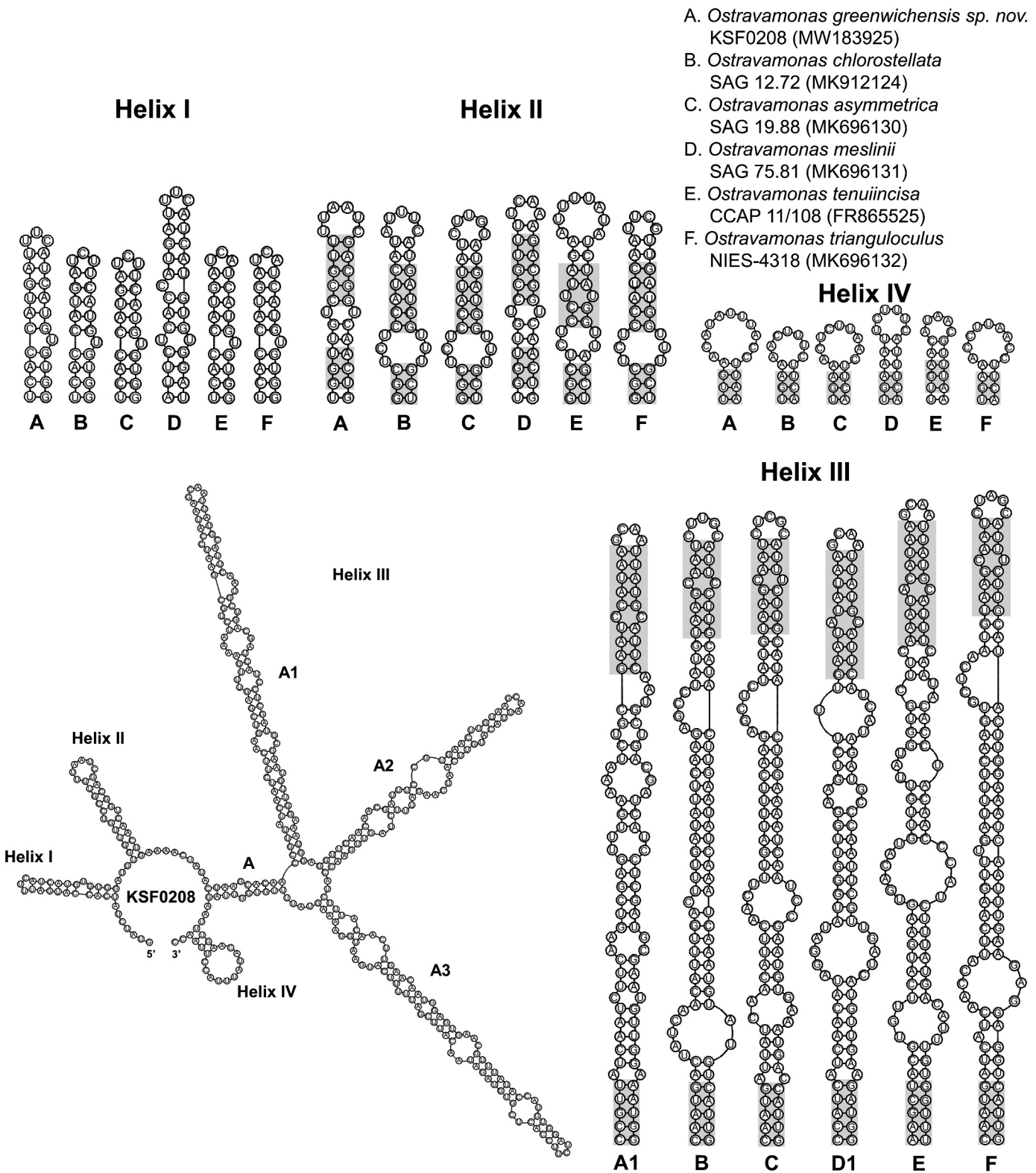


Fig. 23. Secondary structure of ITS2 rDNA sequences among the species of the core group of *Chloromonas*. The helix III of five strains consists of two sub-helices and the comparable positions were found in sub-helix 1. The compensatory base changes (CBCs) and hemi-CBCs are marked by grey boxes.

other species of the core *Chloromonas* (Table 1). *Chloromonas deceptionensis* showed morphological characteristics similar to those of *Chloromonas* type species, *C. reticulata*; however, a round eyespot is not observed in *C. reticulata* (Matsuzaki *et al.* 2012). Features of a closely related species, *C. svalbardensis*, were similar to the description of *C. deceptionensis*; however, *C. deceptionensis* often showed spherical cells and was larger than *C. svalbardensis*. Another close relative, *C. insignis* (Anakhin) Gerloff &

H. Ettl, has broadly ovoid to spherical cells with a low hemispherical papilla and elliptical eyespot. However, *C. deceptionensis* often showed a round eyespot and was larger than *C. insignis*. In addition, *C. deceptionensis* differed from yet another close relative, *C. schussnigii* H. Ettl, in the position of the eyespot, which in *C. schussnigii* is in the middle part of the cell.

In addition to the morphological evidence, molecular data have also provided support for identification of *C.*



deceptionensis. The species was distinguished from other members of the core *Chloromonas* clade by the SSU rDNA and ITS2 sequences. The topology of the phylogenetic trees along with the ITS2 CBC species concept (Wolf *et al.* 2013) suggested the recognition of *C. deceptionensis* as a new

species. Our phylogenetic tree, along with a number of previous studies (Remias *et al.* 2013; Nakada *et al.* 2015, 2016; Barcytė *et al.* 2018a, b), demonstrated that *Chloromonas* is paraphyletic, and that the genus can be divided into several smaller entities.

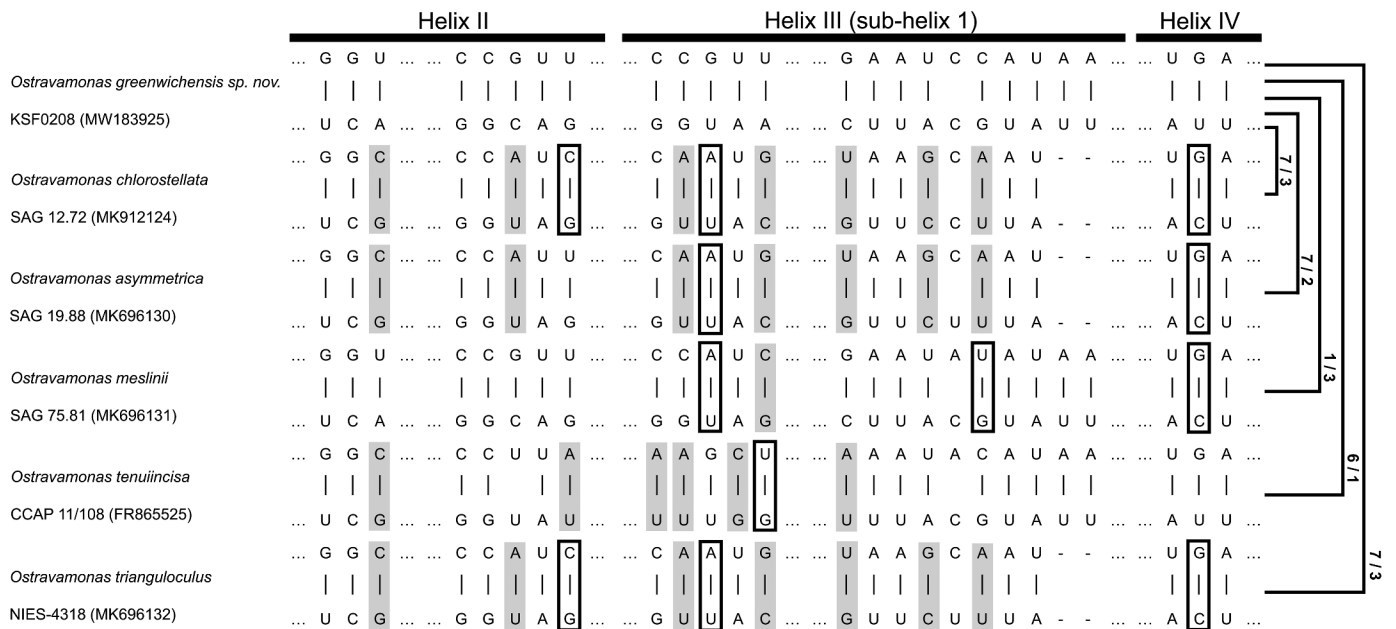


Fig. 26. Comparison of ITS2 secondary structure of *Ostravamonas greenwichensis* sp. nov. with other *Ostravamonas* clade species. Hyphens (-) and dots (.) indicate gaps and omitted regions, respectively. The positions marked by grey boxes and empty boxes respectively indicate the presence of compensatory base changes (CBCs) and hemi-CBCs compared to the *O. greenwichensis*. Numbers of CBCs and hemi-CBCs are indicated on the right.

Table 1. Morphological comparisons between core *Chloromonas* clade species.

Species	Cell shape	Papilla shape	Eyespot	Pyrenoid	Cell length × cell width (μm)	Reference
<i>Chloromonas deceptionensis</i> sp. nov. KSF0090	Ellipsoid to broad ellipsoid or spherical	Hemispherical	Oblong or round, in anterior 1/3 of cell	Absent	11–27 × 10–26	This study
<i>Chloromonas reticulata</i>	Ellipsoid or ovoid	Hemispherical, with or without flattened anterior face	D- or rod-shaped, in anterior 1/2–1/3 of cell	Absent	11–20 × 5–15	Matsuzaki <i>et al.</i> (2012)
<i>Chloromonas arctica</i> Barcytė & Hodač	Broad ellipsoid or ellipsoid-cylindrical or spherical	Hemispherical	Elliptical, in lateral anterior part of cell	Absent	10–20 × 6–16	Barcytė <i>et al.</i> (2018b)
<i>Chloromonas chlorococcoides</i>	Cylindrical or elongate-ovoid	Hemispherical	Ellipsoid, in anterior 1/3 of cell	Present	10–18 × 5–10	Matsuzaki <i>et al.</i> (2012)
<i>Chloromonas difformis</i> Tomo, Makino, Matsuzaki & Nozaki	Broadly ellipsoid	Hemispherical, often with a flat top face	Short rod or ellipsoid, in anterior 1/3 to 2/5 of cell	Present	10–18 × 8–15	Makino <i>et al.</i> (2019)
<i>Chloromonas gracillima</i>	Broadly ellipsoid or cylindrical or spherical	Hemispherical	Ellipsoid or slightly roundish, in anterior 1/3 of cell	Present	12–22 × 7–18	Barcytė <i>et al.</i> (2019)
<i>Chloromonas insignis</i>	Broadly ovoid to spherical	Low, hemispherical	Elliptical, in anterior half of cell	Absent	18–23	Ettl (1970)
<i>Chloromonas rosae</i> (H. & O. Ettl) H. Ettl	Cylindrical or elongate-ellipsoidal	Obtuse, cone-shaped	Narrow and oblong, in anterior 1/3 of cell	Absent	10–22 × 6–14	Matsuzaki <i>et al.</i> (2012)
<i>Chloromonas schussnigii</i>	Spherical	Low, hemispherical	Large and spot-shaped, in middle part of cell	Absent	7–10	Ettl (1970)
<i>Chloromonas svalbardensis</i>	Ellipsoid or wide ellipsoid	Hemispherical	Oblong or round, in anterior 1/3 of cell	Absent	12–20 × 7–16	Barcytė <i>et al.</i> (2018a)
<i>Chloromonas typhlos</i> (Gerloff) Matsuzaki, Y. Hara & Nozaki	Ellipsoid or ovoid	Hemispherical	Absent	Present	8–17 × 6–13	Matsuzaki <i>et al.</i> (2012)
<i>Chlamydomonas hydra</i> H. Ettl	Ellipsoid or ovoid	Low, keel-shaped	Large and elliptical, in anterior half of cell	Present	9.5–14 × 6–12	Ettl (1976)

The morphological characteristics observed in *Ostravamonas greenwichensis* sp. nov. strain KSF0208 were similar to other *Ostravamonas* species, which formed a well-supported clade in

SSU rDNA phylogeny (Fig. 22). Molecular phylogenetic analyses identified *O. meslinii* as the closest relative of *O. greenwichensis*, and the two species differ from other *Ostravamonas* species in that

Table 2. Morphological comparisons between *Ostravamonas* species.

Species	Cell shape	Papilla shape	Eyespot	Akinete	Cell length × cell width (µm)	Reference
<i>O. greenwichensis</i> sp. nov. KSF0208	Ellipsoid to ovoid	Hemispherical to conical	Long, thin, linear, in anterior 1/3 of cell	Unknown	13–20 × 8–16	This study
<i>O. chlorostellata</i>	Cylindrical to broad ellipsoid or almost spherical	Hemispherical to conical	Oblong to elliptical, in anterior 1/2–1/3 of cell	Unknown	(12–) 15–27 × (6–) 9–22	Barcytė et al. (2019) Susanti et al. (2020)
<i>O. asymmetrica</i>	Cylindrical to broad ellipsoid, frequently asymmetrical in young cell	Hemispherical to conical	Oblong to small elliptical, in anterior 1/3 of cell	Present	13–20 × 8–18	Susanti et al. (2020)
<i>O. meslinii</i>	Cylindrical to ellipsoid	Hemispherical to conical	Long, thin, linear, in anterior 1/3 of cell	Unknown	(13–) 16–27 × (6–) 7–20	Barcytė et al. (2019) Susanti et al. (2020)
<i>O. tenuincisa</i>	Broadly ellipsoid to ovoid	Low keel-shaped	Small, thin, linear or narrowly ellipsoid, in anterior 1/2–1/3 of cell	Unknown	14–25 × 7–21	Barcytė et al. (2019)
<i>O. trianguloculus</i>	Cylindrical to ellipsoid	Hemispherical	Triangular to small elliptical, in anterior 1/3 of cell	Unknown	10–19 × 5–15	Susanti et al. (2020)

three sub-helices appear in helix III in the ITS2 secondary structure. However, the two species show morphological differences, such as shape and size of cells, and sometimes *O. meslinii* revealed two pyrenoids. The type species, *O. chlorostellata*, is larger than *O. greenwichensis* and has an eyespot of different shape. *Ostravamonas greenwichensis* differs from *O. asymmetrica* (H. Susanti, Masaki Yoshida, Takeshi Nakayama, Nakada & M.M. Watanabe) Nakada & H. Susanti and *O. trianguloculus* (H. Susanti, Masaki Yoshida, Takeshi Nakayama, Nakada & M.M. Watanabe) Nakada & H. Susanti in cell and eyespot shape, and from *O. tenuincisa* Barcytė & Hodač in cell size, and papillar and eyespot shape. The morphological characteristics mentioned above are summarized in Table 2. *Chloromonas pseudoplatyrrhyncha*, which forms a sister lineage to *Ostravamonas*, has ovoid to spherical cells containing cup-shaped chloroplasts, and multiple atypical pyrenoids, including a large D- or rod-shaped eyespot positioned in the lateral-central part of the cell (Matsuzaki et al. 2010). In addition, the results of the ITS2 rDNA secondary structure analyses strongly supported the morphological classification of *O. greenwichensis* and other members of *Ostravamonas* (Figs 24, 26).

Chloroplast morphology and ecological plasticity can probably explain the division and revision of the phylogroup *Chloromonadinia* (Barcytė et al. 2018b). A molecular phylogenetic study based on SSU rDNA supports the division of the phylogroup *Chloromonadinia* into three clades. Clade 1 (containing core *Chloromonas*) members have a parietal cup- or urn-shaped chloroplast with a number of continuously connected lobes, and comprises mesophilic and psychrotolerant organisms (Barcytė et al. 2018b). Clade 2 members bear chloroplasts mostly composed of parietal bands, platelets or disks, which may or may not be interconnected (Hoham et al. 2006; Matsuzaki et al. 2014, 2018), and comprise psychrophilic organisms that form blooms. Clade 3 members exhibit asteroid chloroplast morphology (Pröschold et al. 2001) and only include mesophilic algae. We observed that *C. deceptionensis* and *O. greenwichensis* contained cup-shaped chloroplasts and could grow in the cold environment of Antarctica, which are the same characteristics as other species in clade 1. In particular, *O. greenwichensis* was isolated from a snowmelt stream, which resembles the habitat where other species of *Ostravamonas* were found.

The limited taxonomic studies of Antarctic species and strains are probably due to the inaccessibility of Antarctica. Further taxonomic studies of Antarctic microalgae could improve understanding of ecological speciation of the phylogroup *Chloromonadinia* and its evolution in various environments.

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