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Emended description of the genus *Eremochloris* (Trebouxiophyceae, Chlorophyta), with *Eremochloris kamchatica sp. nov.* from Kamchatka, Russia

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ABSTRACT

Three strains of a coccoid green alga were isolated from soil samples collected at the Shiveluch Volcano on the Kamchatka Peninsula. SSU rRNA gene sequence comparisons resolved the strains as members of the genus *Eremochloris* in the class Trebouxiophyceae. Analyses based on the internal transcribed spacer (ITS) rRNA secondary structure revealed that our isolates were genetically distinct from the type species of this genus. These isolates were characterized by spherical, elliptical or pyriform cells that were solitary with a single excentric nucleus. The cells harboured a single-pyrenoid parietal chloroplast, which was either simple or dissected into clear, curved or undulated lobes. Studies on the life cycle of this alga revealed that asexual reproduction takes place via autospores or biflagellated zoospores. Sexual reproduction is very scarce and is isogamous. All these features suggest a new species, which we name *Eremochloris kamchatica*. The description of the genus *Eremochloris* is emended to accommodate the features of the new species.

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KEYWORDS
18S–ITS rDNA; Green algae; Secondary structures; Shiveluch Volcano

INTRODUCTION

The phylum Chlorophyta is one of the most successful groups of algae that inhabit the most contrasting ecosystems on Earth (Friedl & Rybalka 2012). Three classes of green algae, the Chlorophyceae, Ulvophyceae and Trebouxiophyceae (UTC), make up the core of the phylum, forming the so-called UTC clade (Leliaert *et al.* 2012). The class Trebouxiophyceae accounts for approximately 900 species (Guiry & Guiry 2021) that mainly live in non-aquatic habitats, with a smaller number of representatives found in freshwater and marine habitats. Many trebouxiophycean genera are involved in symbiotic relationships with lichens, ciliates, cnidarians and even vascular plants (Friedl & Rybalka 2012; Leliaert *et al.* 2012). The class Trebouxiophyceae includes unicellular, mostly coccoid algae in addition to more complex colonial and filamentous forms, which reproduce mainly asexually via the formation of immotile autospores or motile zoospores (Leliaert *et al.* 2012). Sexual reproduction is rarely observed in this group (Fučíková *et al.* 2015). Many representatives of this class occur in extreme habitats, such as desert soils (Fučíková *et al.* 2014), caves (Abdullin 2009; Pfendler *et al.* 2018), acidic volcano soils (Darienko & Pröschold 2015) and cold environments, among others (Barcyté *et al.* 2021) and are capable of synthesizing and accumulating biologically active substances such as carotenoids (Malavasi *et al.* 2020), lipids (Kugler *et al.* 2020), flavonoids (Goiris *et al.* 2014) and polyols (Gustavs *et al.* 2011), which provide them with resistance to these types of conditions. Some of these compounds are already widely used in biotechnology (Matos 2017). In this regard, new data on the biodiversity of trebouxiophyceans is of a great theoretical and practical importance.

During a study of algal biodiversity in soils of the Shiveluch Volcano (Kamchatka Peninsula), we isolated three strains of a coccoid green alga and studied them using a polyphasic approach. SSU rRNA gene sequence comparisons placed the strains within the genus *Eremochloris* Fucíková, P.O. Lewis & L.A. Lewis (Trebouxiophyceae) clade, known from several isolates of a single species, *E. sphaerica* Fucíková, P.O. Lewis & L.A. Lewis *emend.* Mikhailyuk & Demchenko. Our strains were similar to *E. sphaerica* in morphology but differed in the presence of hemi-compensatory base changes in conservative elements of the internal transcribed spacer (ITS) rRNA secondary structure. A detailed study on the life cycle of the strains revealed additional new features and led us to describe the alga from Kamchatka as a new species, *Eremochloris kamchatica sp. nov.*

MATERIAL AND METHODS

Isolation and microscopy

Three strains of a uninucleate coccoid alga were isolated from three samples taken on 16 August 2018 from the Shiveluch Volcano, which is located on the Eastern ridge of the Kamchatka Peninsula (Kamchatka Territory, Russia) and is the northernmost of Kamchatka's active volcanoes. One sample (Kk5) was taken from volcanic soil under the dead stone birches (*Betula ermanii* Chamisso), but with grass vegetation (56°33.98′N, 161°8.41′E). Two samples, Kk8 (56°33.763′N, 161°8.34′E) and Kk10 (56°33.665′N, 161°8.615′E), were taken from pyroclastic flow deposits without grass vegetation. Sampling was carried out using standard methods (Kuzyakhmetov & Dubovik 2001). The strains were isolated

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using the micropipette method (Andersen 2005) and cultured in liquid nutrient medium Waris-H (McFadden & Melkonian 1986), recommended for green algae, at 20–22°C and an irradiance of 17.9–21.4 μmol photons m^{-2} s^{-1} with a 16:8 h (light:dark) photoperiod. The strains were maintained in the culture collection of the Laboratory of Botany in the Federal Scientific Center of East Asian Terrestrial Biodiversity, Russian Federation, with strain numbers VCA-40 (Kk5), VCA-41 (Kk8) and VCA-42 (Kk10), and their dried biomass was deposited at the Herbarium of the Federal Scientific Center of East Asian Terrestrial Biodiversity, Russia (exsiccatum numbers VLA-CA-0983, VLA-CA-1139, VLA-CA-0982).

The morphology of vegetative and reproductive cells was examined using an Olympus BX 53 light microscope equipped with Nomarski DIC optics and an Olympus DP27 digital camera. Cultures were repeatedly examined throughout the life cycle stages, i.e. in cultures of different age since transfer.

Examination of chloroplast fluorescence in living algal cells was performed with LSM 510 META and LSM 710 LIVE confocal laser scanning microscopes (Carl Zeiss, Germany) at the Instrumental Centre of Biotechnology and Gene Engineering of FSCEATB FEB RAS. Chloroplast fluorescence was excited with an argon laser line at 488 nm and the emission was collected through a 505-nm long-pass filter. For a detailed study of the nucleus, algal cells were stained with DAPI (4′,6-diamidino-2-phenylindole, dilactate, Molecular Probes, USA) with excitation at 405 nm and emission at 410–505 nm (multitrack). The objective used was a Plan-Apochromat 63×/1.40 Oil DIC M27. Files with the 3D captured images were recorded and analysed with LSM 510 Release v.4.2 and ZEN 2011 software.

DNA extraction, amplification and sequencing

For the DNA analysis, cultures were harvested during exponential growth phase and concentrated by centrifugation. Total genomic DNA was extracted as described previously by Echt *et al.* (1992) with some modifications (Kiselev *et al.* 2015). For amplification of the SSU rRNA gene and ITS region the following primers were used: 82F (5′-GAAACTGCGAATGGCTC-3′; López-García *et al.* 2003), ITS4R (5′-TCCTCCGCTTATTGATATGC-3′; White *et al.* 1990), with sequencing primers 82F, Bd18SF1 (5′-TTTGTACACACCGCCCGTCGC-3′; Goka *et al.* 2009) and ITS4R. PCR amplification was performed using the Encyclo Plus PCR kit (Evrogen, Moscow, Russia) with a T100 Thermal Cycler (Bio-Rad Laboratories, Inc., USA) and parameters for ITS rDNA region: 95°C, 5 min, 38 × (95°C, 20s; 55°C, 20s; 72°C, 2 min 40s) and 72°C, 5 min. The PCR products were purified by ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix Inc., USA) and sequenced in both directions at the Instrumental Centre of Biotechnology and Gene Engineering of FSCEATB FEB RAS using an ABI 3500 genetic analyser (Applied Biosystems, USA) with a BigDye terminator v.3.1 sequencing kit (Applied Biosystems, Maryland, USA). Sequences were assembled with the Staden Package v.1.4 (Bonfield *et al.* 1995), aligned manually in the SeaView program (Galtier *et al.* 1996). Sequences were deposited in GenBank under accession numbers MW396942–MW396944.

Phylogenetic analyses

Maximum likelihood (ML) analysis was carried out using PAUP 4.0b10 (Swofford 2002). Bayesian inference (BI) was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). To determine the most appropriate DNA substitution model for our datasets, the Akaike information criterion (AIC; Akaike 1974) was applied with jModelTest 2.1.1 (Darriba *et al.* 2012). MEGA v.7.0.26 (Kumar *et al.* 2016) was used to estimate intraspecies and interspecific pairwise distances (*p*-distances). ML analysis was done using heuristic searches with a branch-swapping algorithm (tree bisection-reconnection). In BI, four runs of four Markov chains were carried out for 2 million generations, sampling every 100 generations for a total of 20,000 samples. Convergence of the two chains was assessed, and stationarity was determined according to the 'sump' plot with the first 5,000 samples (25%) discarded as burn-in; posterior probabilities were calculated from trees sampled during stationary phase. The robustness of the ML trees was estimated by bootstrap percentages (BP; Stamatakis *et al.* 2008) and posterior probabilities (PP) in BI. BP < 50% and PP < 0.95 were not considered. ML-based bootstrap analysis was inferred using the web service RAxML v.7.7.1 (http://embnet.vital-it.ch/raxml-bb/; Kozlov *et al.* 2019).

The Mfold web server (http://www.unafold.org/mfold/applications/rna-folding-form.php; Zuker 2003) was used with the default settings to generate the folding pattern of ITS1 and ITS2 rRNA secondary structure. An ITS2 model was constructed based on models proposed by Mikhailyuk *et al.* (2019). The model of ITS1 secondary structure was not available for any *Eremochloris* strain; therefore, it was generated based on folding patterns of Hoshina *et al.* (2018) and Darienko *et al.* (2019b) for Chlorellaceae.

RESULTS

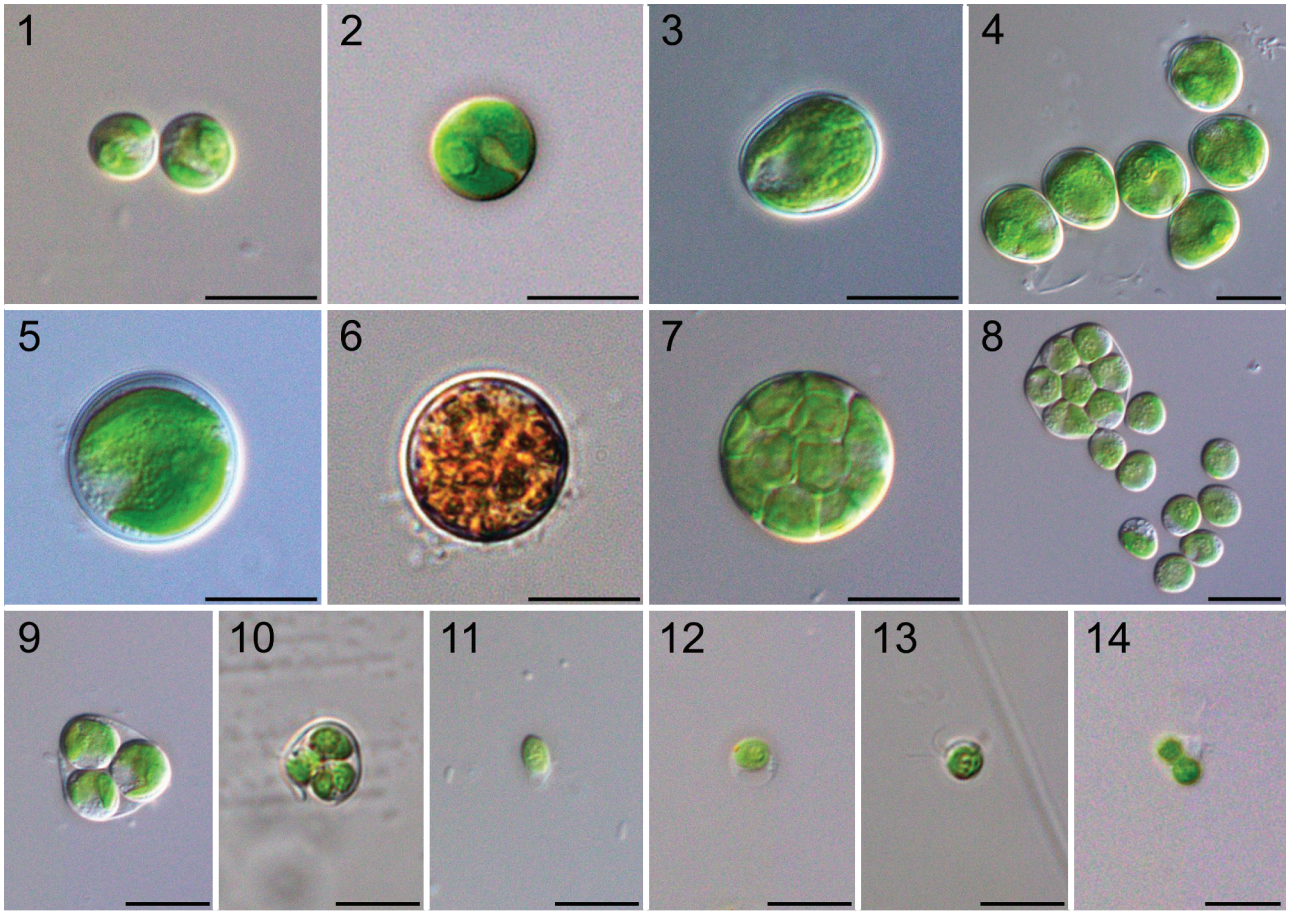
The strains isolated from soil samples collected at the Shiveluch Volcano (Kamchatka Territory, Russia) were genetically distinct from *E. sphaerica* but were located within the *Eremochloris* clade. In addition, we documented some morphological characteristics not previously observed in the type species *E. sphaerica* (e.g. pyriform cells), including life cycle features and sexual reproduction in these strains. We hereafter refer to these strains by the name of *E. kamchatica sp. nov.* and emend the description of the genus *Eremochloris.*

Eremochloris Fučíková, P.O. Lewis & L.A. Lewis *emend.* Abdullin & A. Gontcharov

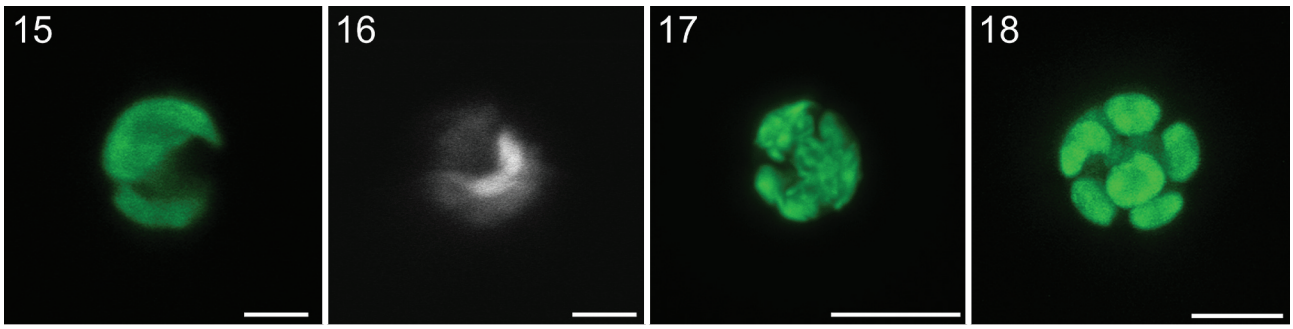
PREVIOUS DESCRIPTIONS: Fučíková *et al.* (2014, p. 304, fig. f–1i); generic description emended by Mikhailyuk & Demchenko in Mikhailyuk *et al.* (2019, p. 284, figs 53–55, 62, 63).

TYPE SPECIES: *Eremochloris sphaerica* Fucíková, P.O. Lewis & L.A. Lewis.

EMENDED DESCRIPTION: Cells spherical, elliptical or pyriform, with a single excentric nucleus. Chloroplast single, parietal, dissected into lobes, cup-shaped in young cells. Pyrenoid single, prominent, surrounded by several starch grains. Asexual reproduction via autospores and zoospores. Zoospores biflagellate with a lateral stigma, wall-less. Sexual reproduction rarely observed, by isogamous biflagellate gametes fusing by the cell sides without losing flagella. The gametes differ morphologically from zoospores only in the absence of stigma.



Figs 1–14. Light micrographs of general morphology in *Eremochloris kamchatica*. Scale bars = 10 µm.
Fig. 1. Young cells.
Fig. 2. Adult spherical cell.
Figs 3, **4**. Adult elliptical and pyriform cells.
Fig. 5. Old vegetative cell.
Fig. 6. Tan and very granulated old vegetative cell.
Figs 7, 9. Autosporangia.
Fig. 8. Autospores.
Fig. 10. Zoosporangium.
Figs 11–13. Zoospores.
Fig. 14. Fusion of gametes.



Figs 15–18. Confocal reconstructions of chloroplasts and nucleus in *Eremochloris kamchatica*. Scale bars = 2 µm.
Fig. 15. Chloroplast in young cell.
Fig. 16. Nucleus.
Fig. 17. Chloroplast in old vegetative cell.
Fig. 18. Autosporangia.

Eremochloris kamchatica Abdullin & A. Gontcharov *sp. nov.*

Figs 1–18

DESCRIPTION: Cells spherical (7.0–15.3 μm in diameter), elliptical or pyriform (9.8–13.2 μm long and 8.2–11.4 μm wide; Figs 1–6), with a single, parietal chloroplast dissected into 2–4(–8) lobes (Fig. 17), cup-shaped in young cells, with single excentric nucleus (Fig. 16). Pyrenoid single, prominent, surrounded by several starch grains (Fig. 2). Young cells spherical, 4.9–6.9 μm in diameter (Figs 1, 15); old cells 9.6–19.2 μm in diameter (Figs 5, 6), yellowish-brown and granulated (Fig. 6). Autosporangia 8.5–14.0 μm in diameter, with (4–)8(–16) autospores (Figs 7–9, 18). Autospores spherical, 3.3–5.4 μm in diameter (Fig. 8). Zoosporangia spherical, 8.8–9.6 μm in diameter (Fig. 10), producing 4–8 biflagellate zoospores; flagella 2.0–5.4 μm long. Zoospores elliptical while moving (4.8–7.5 μm long and 2.6–4.1 μm wide), spherical when stopping (3.8–5.8 μm in diameter), with a lateral stigma (0.7–1.0 μm long and 0.4–0.5 μm wide) and granules (Figs 11–13). Gametes 2.8–3.2 μm in diameter (Fig. 14).

HOLOTYPE: Exsiccatum number VLA-CA-0983, a dried, metabolically inactive biomass of the reference strain deposited in the Herbarium, Federal Scientific Center of East Asian Terrestrial Biodiversity, Russia (VLA).

AUTHENTIC STRAIN: *E. kamchatica* strain VCA-40, isolated from a Shiveluch Volcano soil sample (Kamchatka Territory, Russia) and deposited in the Culture Collection of the Laboratory of Botany, Federal Scientific Center of East Asian Terrestrial Biodiversity, Russia.

TYPE LOCALITY: 56°33.98′N, 161°8.41′E, the Shiveluch Volcano, Kamchatka Peninsula (Kamchatka Territory, Russia).

ETYMOLOGY: The species is named after the Kamchatka Peninsula.

MOLECULAR VOUCHERS: MW396942 (18S rDNA, ITS1, 5.8S rDNA, ITS2), MW396943 (18S rDNA, ITS1, 5.8S rDNA, ITS2), MW396944 (18S rDNA, ITS1, 5.8S rDNA, ITS2).

HABITAT: volcanic soil and pyroclastic flow deposits.

Life cycle

The life cycle and reproductive processes of *E. kamchatica* are shown schematically in Fig. 19. Asexual reproduction involves the formation of autospores and zoospores. Autospores were produced in relatively small numbers (mostly eight or 16) and liberated by the mother cell wall rupturing without producing any special openings. Typically, eight zoospores are formed. The zoospores were all liberated simultaneously with the rupturing of the mother cell wall. The zoospores had two equal anterior flagella, a parietal chloroplast, apparent granules in the cytoplasm, and a lateral stigma. Zoospores were round just after release and elongated while moving, which suggests a lack of cell wall. Zoospores with this general morphology are not typical for the Trebouxiophyceae but have been recorded in the class (Gaysina *et al.* 2013; Škaloud *et al.* 2016; Baudelet *et al.* 2017; Darienko *et al.* 2019a). Before stopping, each zoospore moved around its axis, gradually rounding up and finally losing the flagella. The release of zoospores was observed in 20–22-day-old liquid cultures. Sexual reproduction was observed only once, in strain VCA-40 (Kk5). Biflagellate isogamous gametes differed morphologically from zoospores only in lacking a stigma and in having a somewhat smaller size. The gametes fused by their sides without losing flagella. Gametangium, release of gametes, and zygote formation and germination were not observed.

Phylogenetic analyses

Results of BLAST searches showed that rRNA gene sequences (partial 18S rDNA, ITS1, 5.8S rDNA, ITS2; 2409 bp) in our strains is highly similar to those of *E. sphaerica* (>97.8%). Phylogenetic analyses based on comparisons of 18S rRNA gene sequence of 84 representatives of major groups of the Trebouxiophyceae placed the new species as a member of the robust and relatively long-branched *Eremochloris* generic clade (100/1.00; Fig. 20), which occupied an unresolved position among other Trebouxiophyceae. The 18S rRNA gene sequence divergence did not exceed 1% in the *Eremochloris* clade (results not shown), but it was sufficiently higher in the ITS1–5.8S–ITS2 region. *P*-distances between available ITS sequences MH703778, MH703757 and MW396942–MW396944 (622 aligned positions) ranged from 0% (three *E. kamchatica* strains) to 7% between *E. sphaerica* and *E. kamchatica*. Divergence between *E. sphaerica* accessions was 2.28%.

ITS1 and ITS2 secondary structure

To access relationships between the new species and *E. sphaerica* we compared their ITS secondary structures. It is widely accepted that the presence of compensatory and hemi-compensatory base changes (CBCs and hCBCs) in the conserved helices (II and III) of ITS2 region is an indication of sexual incompatibility that marks biological species (Müller *et al.* 2007; Ruhl *et al.* 2010; Schill *et al.* 2010; Wolf *et al.* 2013; Zhan *et al.* 2019). Figure 21 illustrates the proposed base pairing in ITS1 and ITS2 of *E. kamchatica*. According to our predictions, both spacers were characterized by the presence of four helices and five single-stranded domains. ITS1 and ITS2 had similar lengths (237 and 227 base pairs respectively), but in ITS1 all helices were shorter. Approximately 58% of nucleotides were involved in formation of the hairpin loops in ITS1, while in ITS2 this value was *c.* 90%. Helical domains I and III were the most conservative in ITS1, while in ITS2 the most conservative were II and III. We found only hemi-compensatory base changes, rather than CBCs in almost every helical domain (except helix II in ITS2). *Eremochloris kamchatica sp. nov.* differed from *E. sphaerica* in 29 and 22 substitutions (of which five and six were hCBCs) and three (one insertion and two deletions) and seven (two insertions and five deletions) indels in ITS1 and ITS2, respectively. These substitutions and indels were mostly located in the single stranded domains.

DISCUSSION

The new species found in soils collected from the Shiveluch Volcano (Kamchatka Peninsula, Russia) was confidently resolved as a member of the *Eremochloris* clade based on SSU rDNA data (Fig. 20). The genus is characterized by a relatively long branch reflecting a large number of autapomorphic characters that likely contribute to its unresolved

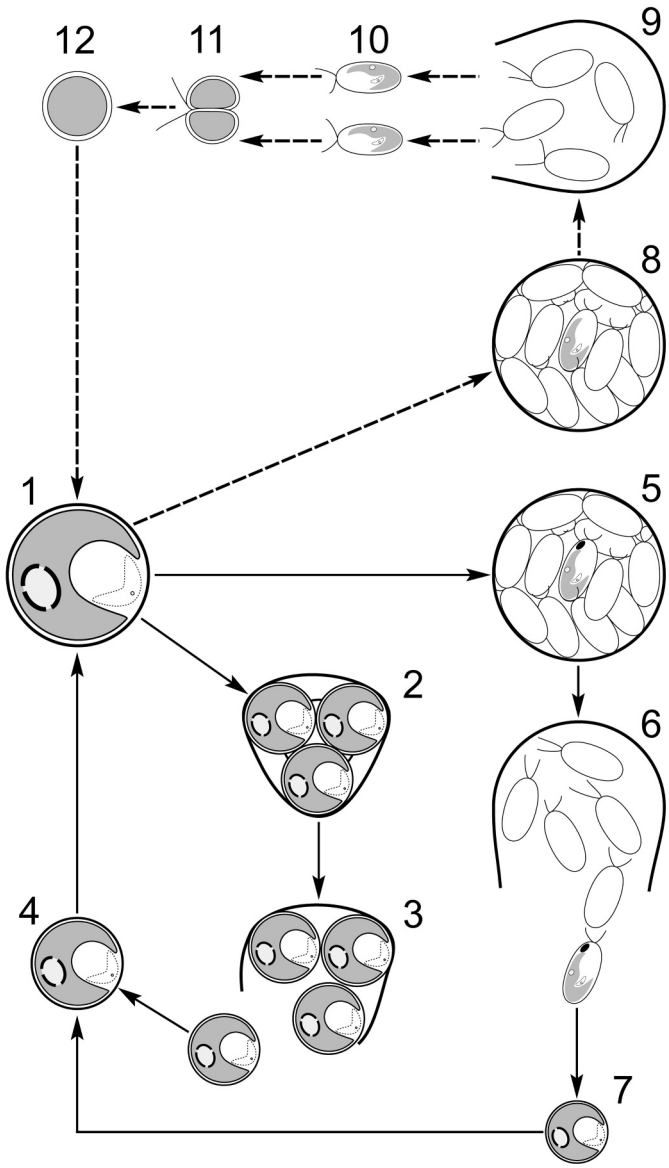


Fig. 19. Schematic representation of the life cycle in *Eremochloris kamchatica*. 1, vegetative cell; 2, 3, autosporogenesis: 2, autosporangium, 3, release of autospores; 4, young cell; 5–7, zoosporogenesis: 5, zoosporangium, 6, release of zoospores, 7, immobile zoospore without flagella; 8–12, sexual reproduction: 8, gametangium, 9, release of gametes, 10, gametes, 11, fusion of gametes, 12, zygote. The dotted line shows parts of the sexual reproduction cycle that were not fully observed.

affiliation among the Trebouxiophyceae (Fučíková *et al.* 2014). *Eremochloris* lacks pronounced phenotypic characters that would permit its unambiguous identification; therefore, the genus could be differentiated from other class members based on molecular data only. We extended the genus description by adding data on its life cycle. Asexual reproduction by autospores is typical for the members of the class, including *Eremochloris* (Fučíková *et al.* 2014). Zoosporogenesis is less frequent in Trebouxiophyceae and reproduction by zoospores is not clade or genus-specific (Fučíková *et al.* 2015). The present study described *Protosiphon*-type (Starr 1955) zoospore formation in the genus *Eremochloris* and thus extended the list of trebouxiophycean lineages with flagellated life cycle stages. Moreover, we observed a fusion of flagellated cells that we regarded as gametes. These cells were distinct from zoospores in the lack of stigma and smaller dimensions (Table 1). Occurrence of the sexual reproduction in *Eremochloris* was not unexpected and has been hypothesized based on the partially sequenced nuclear genome of *E. sphaerica* (Fučíková *et al.* 2015). Data provided by these authors suggested that sexual reproduction in Trebouxiophyceae could be widespread but may require rarely observed specific conditions. Only minimal information concerning morphological differences between zygospores and gametes in the class can be found. Unlike *Eremochloris*, in most cases they are indistinguishable in shape, size and presence or absence of stigma (Ahmadjian 1960; Ettl & Gärtner 1995; Škaloud *et al.* 2015).

To date, only a few *Eremochloris* isolates and sequences are known; therefore, it is difficult to access the genus genetic and phenotypic diversity. Previous records of *Eremochloris* were based mostly on molecular data with scarce information on morphological characteristics and life cycle features. Our study partially filled in these gaps by characterizing asexual reproduction and providing evidence for sexual reproduction by isogamy in *E. kamchatica*. Divergence of SSU rDNA sequences between isolates/species in the genus is low (<1%) and cannot be used to differentiate species. In contrast, ITS rDNA readily delineated the new species from *E. sphaerica* (see Results). Although no CBCs in ITS2 were observed between the species, this spacer carried six hCBCs, two deletions and five indels. Mikhailyuk *et al.* (2019) regarded 2 hCBCs found in ITS2 between authentic strain of *E. sphaerica* and their isolate from the Baltic Sea coast as intraspecific variation but concluded that 1–3 hCBCs and additional substitutions in unpaired bases between their *Tetradesmus* isolate and related taxa justified the description of a new species. Moreover, there is evidence that some lineages up to the order level may lack any CBCs in the conserved regions of the ITS2 (Caisová *et al.* 2011, 2013; Samanta *et al.* 2018).

Morphological traits of the newly described *E. kamchatica* (small, spherical vegetative cells with a single parietal chloroplast with pyrenoid) were generally in line with the characteristics described for the type species *E. sphaerica* (Fučíková *et al.* 2014; Mikhailyuk *et al.* 2019; Table 1). In addition to the finding of spherical vegetative cells in the liquid cultures of *E. kamchatica*, as is common in trebouxiophyceans, we sometimes observed pyriform cells, which had not been demonstrated in *Eremochloris* previously; this feature could be used to distinguish the new species. In the class Trebouxiophyceae, similar cell morphology is typical for some genera (*Chloroidium* Nadson, *Chloropyrula* Gaysina, M. Eliáš, Němcová & Škaloud, *Coleochlamys* Korshikov, *Myrmecia* Printz, *Xylochloris* Neustupa, M. Eliáš & Škaloud) as well as for some species of *Asterochloris* Tschermak-Woess, *Parietochloris* Shin Watanabe & G.L. Floyd and *Trebouxia* Puymaly. Pyriform cell morphology is probably a homoplastic trait that arose independently in unrelated members of the class.

Information on the *Eremochloris* life cycle and characteristics of zoosporangium, zoospores and gametes is reported here for the first time; therefore, no full comparison could be made between *E. sphaerica* and *E. kamchatica*. Moreover, previous publications were not consistent regarding vegetative cell dimension ranges in

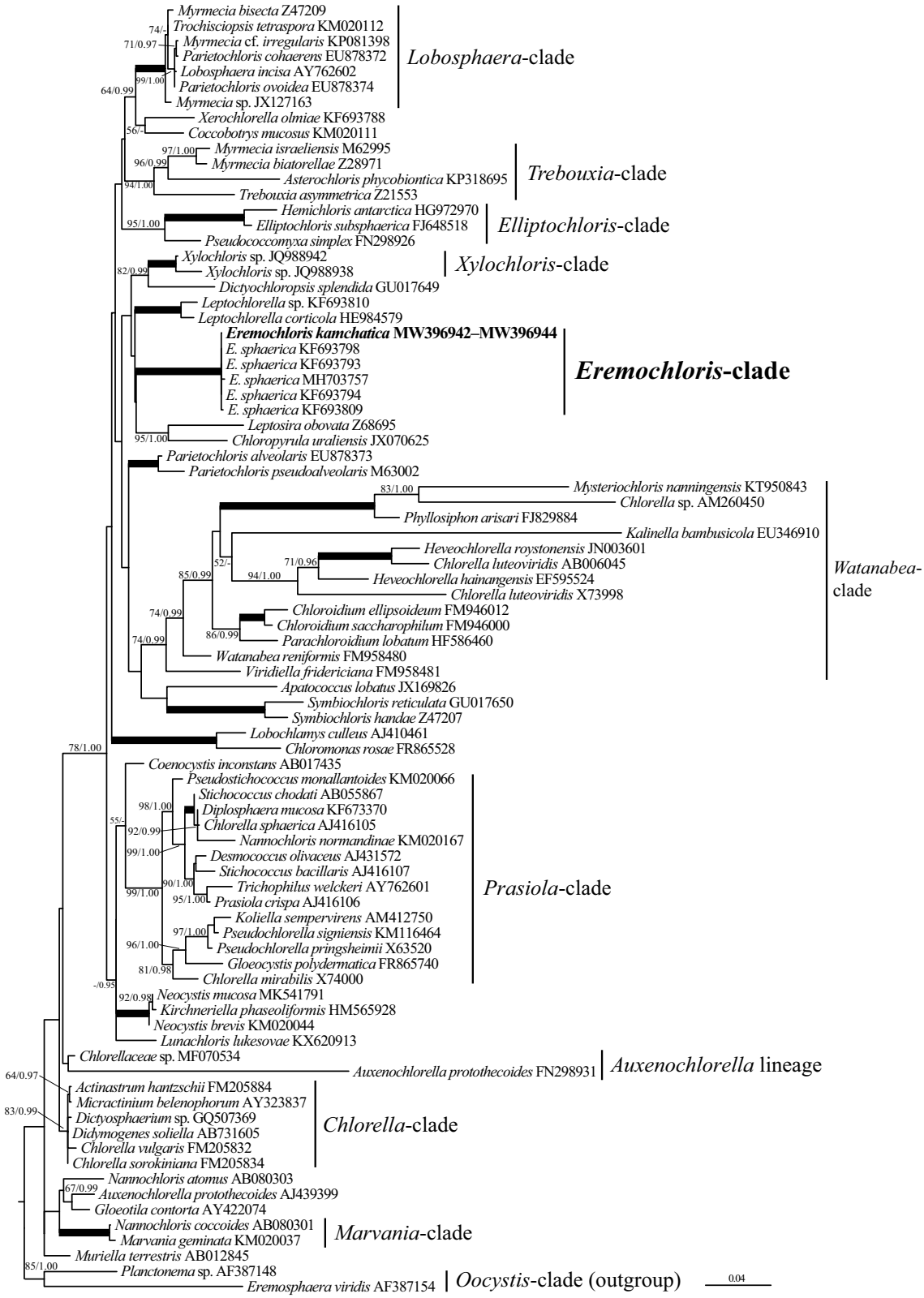


Fig. 20. ML phylogenetic tree showing position of the genus *Eremochloris* and the new species *Eeremochloris kamchatica* in the Trebouxiophyceae based on 18S rRNA gene sequence data (TIM1+I+Γ model). Supports for ML and BI (BP ≥ 50% and PP ≥ 0.95, respectively) are given above or below the branches. Branches with 100% BP, 1.00 PP and sequences obtained for this study are shown in boldface. Clade designations follow Fučíková *et al.* (2014), Hodač *et al.* (2015), Darienko *et al.* (2016) and Mikhailyuk *et al.* (2019).

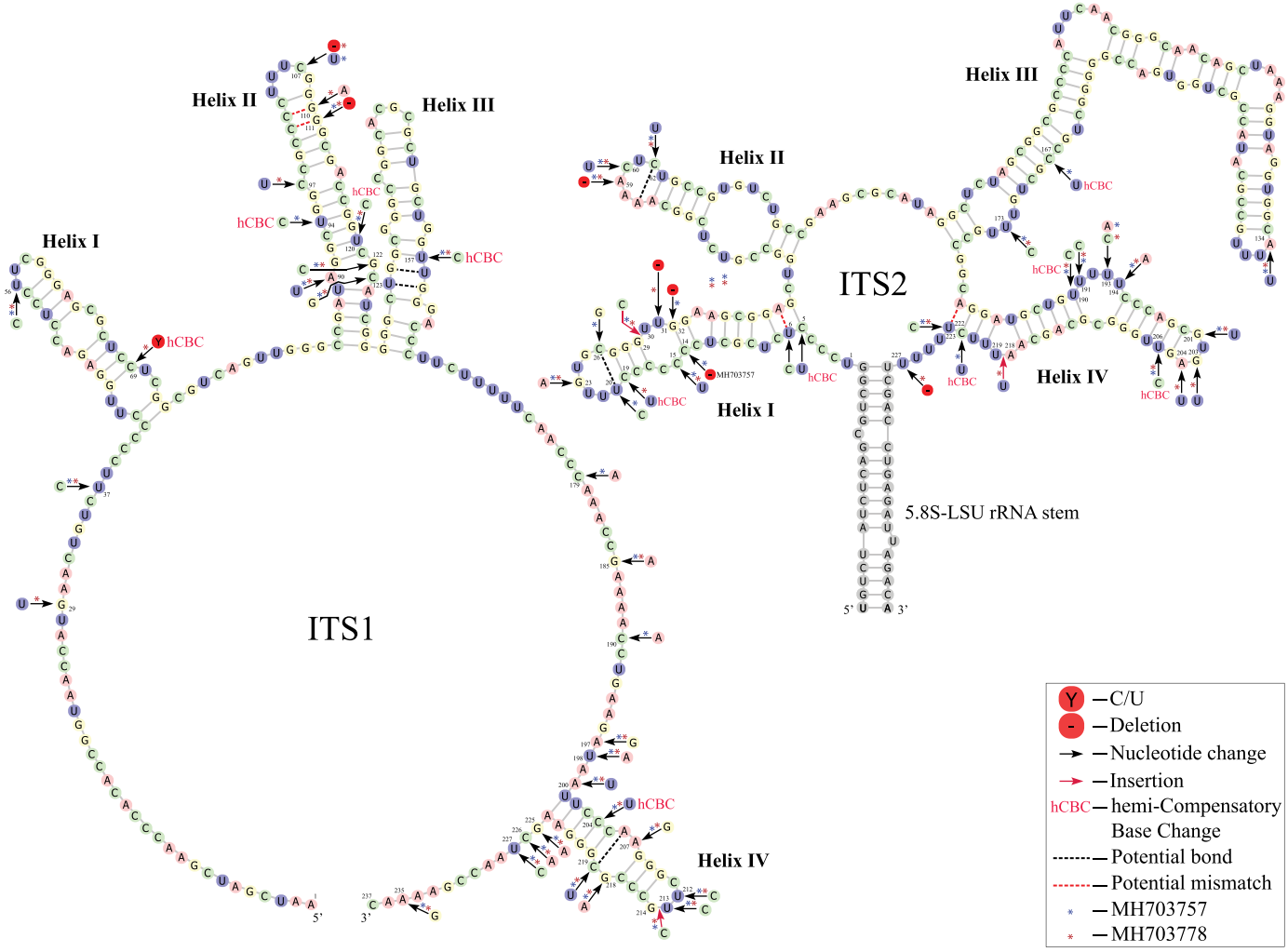


Fig. 21. Secondary structure models of ITS1 and ITS2 of investigated strains of *Eremochloris* based on Mfold predictions. The numbers indicate the numeration of nucleotides.

Table 1. Cell dimensions (μm) of *Eremochloris* species. no – not observed, nd – no data.

	E. kamchatica	*E. sphaerica*	
Species	This study	Fučíková *et al.* 2014	Mikhailyuk *et al.* 2019
Young cell diameter	5.0–7.0		
Adult spherical cell diameter	7.0–15.3	5–16	(15.0) 18.3–23.3 (29.4)
Old vegetative cell diameter	9.6–19.2		
Adult pyriform, elliptical cell length	9.8–13.2	no	no
Adult pyriform, elliptical cell width	8.2–11.4	no	no
Autosporangium diameter	8.5–14.0	nd	nd
Autospore diameter	3.3–5.4	nd	5.0–7.2
Zoosporangium diameter	8.8–9.6	no	no
Round zoospore body diameter	3.8–5.8	no	no
Elliptical zoospore body length	4.8–7.5	nd	nd
Elliptical zoospore body width	2.6–4.1	nd	nd
Zoospore flagella length	2.0–5.4	no	no
Zoospore stigma length	0.7–1.0	no	no
Zoospore stigma width	0.4–0.5	no	no
Gamete body diameter	2.8–3.2	no	no

E. sphaerica. Mikhailyuk *et al.* (2019) reported bigger cells and larger autospores than we observed in *E. kamchatica*, while vegetative cell dimensions in the original description of the former species (Fučíková *et al.* 2014) generally correspond to those recorded here (Table 1).

Apparently, this recently described genus has a wide distribution, with its representatives already reported from three continents (North America, Europe and Asia). *Eremochloris sphaerica* has been found in different climates and geographic regions but in ecologically similar habitats, including maritime sand dunes (Europe) and sandy desert soils (North America) (Fučíková *et al.* 2014; Mikhailyuk *et al.* 2019). *Eremochloris kamchatica* was isolated from the Shiveluch Volcano (Kamchatka Territory, Russia). However, its habitat conditions are similar to *E. sphaerica* (volcanic soils and pyroclastic flow deposits).

DISCLOSURE STATEMENT

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