**Chloromonas arctica** sp. nov., a psychrotolerant alga from snow in the High Arctic (Chlamydomonadales, Chlorophyta)

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Abstract

With the advent of molecular phylogenetic methods, it has become possible to assess the biodiversity of snow algae more accurately. In this study, we focused on a morphological, ultrastructural and taxonomic description of a new *Chloromonas*-like alga isolated from snow in the High Arctic (Svalbard). Light and transmission electron microscopy revealed broad ellipsoidal or ellipsoidal–cylindrical, occasionally spherical cells with a chloroplast without a pyrenoid, an inconspicuous eyespot and a papilla. The size difference and the aforementioned morphological traits clearly distinguished the alga from its closest counterparts within the genus *Chloromonas*. Moreover, we were able to cultivate the alga at both 5 and 20°C, revealing the psychrotolerant nature of the strain. Phylogenetic analyses of the plastid *rbcL* and nuclear 18S rRNA gene showed that the alga is nested within a clade containing a number of psychrotolerant strains within the *Chloromonadinia* phylogroup (Chlorophyceae). In the *rbcL* phylogeny, the alga formed an independent lineage, sister to the freshwater species *Chloromonas paraserbinowii*. Comparisons of secondary structure models of a highly variable ITS2 rDNA marker showed support for a distinct species identity for the new strain. The ITS2 secondary structure of the new isolate differed from the closest matches *Chlamydomonas gelatinosa* and *Chloromonas reticulata* by three and five compensatory base changes, respectively. Considering the morphological and molecular differences from its closest relatives, a new psychrotolerant species from the Arctic, *Chloromonas arctica* sp. nov., is proposed.

**INTRODUCTION**

Knowledge of Arctic snow microflora

Only a handful of psychrotolerant and psychrophilic strains of *Chlamydomonas*-like algae have been isolated from snow in polar and alpine regions compared to algae from other habitats. Consequently, there is no comprehensive modern taxonomy-based list of snow-thriving species, especially for polar regions. Although the number of Arctic isolates greatly exceeds that of Antarctic isolates (e.g. Culture Collection of Cryophilic Algae – CCCryo), detailed phylogenetic studies have mainly focused on the latter due to their unique cold adaptations [1, 2]. However, the referred Antarctic organisms were isolated from cold habitats other than snow. Therefore, the snow flora of both poles remains poorly investigated and understood. For example, no Arctic-specific *Chlamydomonas*-related taxa were recently circumscribed from the High Arctic, implying a cosmopolitan snow algae distribution in the far North [3]. However, because restricted geographical distribution is a known phenomenon in snow algae [4, 5], separate lineages might be expected in the High Arctic as well.

**Chloromonads from cold habitats**

The majority of psychrotolerant and psychrophilic strains are members of the *Chloromonadinia* clade within the Chlamydomonadales (or Volvocales) superclade of the class Chlorophyceae [6], whereas some of the best studied psychrophiles fall within the *Moeusinia* or *Monadinia* clades [1, 2]. Hoham et al. [7] studied cold-tolerant *Chloromonas* (*Cr.*) species and demonstrated that snow species are spread within at least two subclades of the...

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**Keywords:** *Chloromonas arctica*; morphology; phylogeny; snow algae; taxonomy.

**Abbreviations:** AIC, Akaike information criterion; BI, Bayesian inference; CCALA, Culture Collection of Autotrophic Organisms; CCAP, Culture Collection of Algae and Protozoa; CCCryo, Culture Collection of Cryophilic Algae; ITS, internal transcribed spacer; MCMC, Markov chain Monte Carlo; ML, maximum-likelihood; PRSF, potential scale reduction factor; rbcL, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit; SAG, Culture Collection of Algae at Göteborg University; TEM, transmission electron microscopy; UTEX, Culture Collection of Algae at the University of Texas at Austin.

The GenBank/EMBL/DBJ accession numbers for the *rbcL* gene sequence and 18S-ITS1-5.8S-ITS2 rDNA region sequence of strain CCALA 10278 are MG189706 and MG189707, respectively. One supplementary figure is available with the online version of this article.
Chloromonas clade sensu Pröschold et al. [8] or Chloromonadina clade sensu Nakada et al. [6]. Further taxonomic investigations of the genus Chloromonas not only confirmed the existence of two main clades [9] encompassing psychrotolerant species, such as Cr. reticulata (Goroschanin) Gobi or Cr. augustae (Skuja) Pröschold, Marin, Schlösser et Melkonian [10], and psychophilic species, such as the assumed cosmopolitan Cr. nivalis (Chodat) Hoham and Mullet and Cr. brevispina (Fritsch) Hoham, Roemer et Mullet, but also revealed a number of novel snow lineages. Recently described Chloromonas snow species include Cr. chenangoensis and Cr. tughillensis Hoham, Berman, Rogers, Felio, Ryba et Miller found in the USA [11], Cr. miwae (Fukushima) Muramoto, Nakada, Shihtara, Hara et Nozaki described from green snow in Japan [12], Cr. fukushimeae along with Cr. tenuis Matsuzaki and Nozaki recognized, respectively, from snow in Japan and USA [13], and Cr. krienitzii Matsuzaki and Nozaki, also from Japan [4]. The increasing Chloromonas diversity shows evidence that molecular variability within the genus has not been fully assessed and that extended sampling could change the way we understand this genus, especially for snow-dwelling lineages. For example, Cr. nivalis and Cr. brevispina may in fact veil multiple cryptic species as they both were identified using just aplanozygote (resting spore) morphology [4].

**Aim of the study**

Because taxonomic studies of the genus Chloromonas were traditionally based solely on light microscopic observations [14, 15], the combination of electron microscopy and molecular phylogenies have enabled re-examination and elucidation of many cryptic taxa within the genus with new taxonomic proposals, as discussed above [4, 11–13]. Due to difficulties in cultivating snow algae leading to a limited number of existing cultures, strain-based studies are highly valuable. The aim of this study was to identify and ascertain the phylogenetic position of a new Chloromonas strain isolated from snow in the High Arctic.

**METHODS**

**Sampling and culturing**

The snow sample was taken from the surface of the Svenbreen glacier (78° 43’ 645” N 16° 17’ 037” E), Svalbard, High Arctic, in mid-August 2016. No prominent algal bloom was noticed on the glacier. The snow was dug (~5 cm deep) with a pickaxe up to the ice surface and placed in a 500 ml sterile plastic bag. An aliquot of the sample was transported to Prague, Czech Republic, in a liquid state in a 500 ml sterile plastic bag. An aliquot of the sample was transported to Prague, Czech Republic, in a liquid state in a 500 ml sterile plastic bag. An aliquot of the sample was transported to Prague, Czech Republic, in a liquid state in a 500 ml sterile plastic bag. A small subunit (18S) ribosomal RNA gene was amplified using universal eukaryotic primers NS1 [17] and 18L [18] under the following programme: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 3 min and final extension at 72 °C for 10 min. The region was sequenced with primers 34F, 370R, 1122F [19], 895R, 1422F [20] and 891F, 1122R and 1422R (T. Friedl, unpublished). The entire internal transcribed spacer (ITS) amplicon was sequenced using an ITS1 and ITS4 primer combination [17] using the following cycle parameters: initial denaturation at 95 °C for 10 min with a subsequent 30 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 10 min. The segment was sequenced with forward primers 1800F [21] and 5.8SbF [22] and reverse primers ITS2 and ITS4 [17]. A part of the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene was amplified and sequenced with primers rbcL1F and rbcL23R with given PCR cycle parameters [7]. The PCR products were purified using ethanol and sent to Macrogen for sequencing. New sequences are available in GenBank under accession numbers MG189706 and MG189707.

**Light and transmission electron microscopy**

Isolate CCALA 10278 was studied under Olympus BX43 and Nikon Eclipse E400 light microscopes. Photomicrographs were taken using Olympus DP27 and DP71 digital cameras and processed using the Quick Photo Camera 2.3 software (Promicra). For transmission electron microscopy (TEM) the sample was fixed for 24 h in 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and postfixed in 2 % OsO4 in the same buffer. Fixed cells were dehydrated in a graded ethanol series (35, 50, 70, 80, 96, 100 % for 15 min), transferred to acetone (3 × 100 % for 15 min) and finally embedded in Araldite–PolyBed 812 mixture (Polysciences). Ultrathin sections were cut on a Reichert-Jung Ultrucut E ultramicrotome and stained using uranyl acetate and lead citrate. Sections were examined using a JEOL JEM-1011 electron microscope. Photomicrographs were obtained using a Veleta CCD camera (EMSYS) equipped with the image analysis software Olympus Soft Imaging Solution. Pictures were postprocessed with Inkscape 0.91 (Free Software Foundation).

**DNA extraction, PCR and sequencing**

Total genomic DNA was extracted with the DNeasy Plant Mini Kit (Qiagen). PCRs were done using PPP Master Mix (Top-Bio) in a total volume of 25 μl. A small subunit (18S) ribosomal RNA gene was amplified using universal eukaryotic primers NS1 [17] and 18L [18] under the following programme: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 3 min and final extension at 72 °C for 10 min. The region was sequenced with primers 34F, 370R, 1122F [19], 895R, 1422F [20] and 891F, 1122R and 1422R (T. Friedl, unpublished). The entire internal transcribed spacer (ITS) region was amplified using an ITS1 and ITS4 primer combination [17] using the following cycle parameters: initial denaturation at 95 °C for 10 min with a subsequent 30 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 10 min. The segment was sequenced with forward primers 1800F [21] and 5.8SbF [22] and reverse primers ITS2 and ITS4 [17]. A part of the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene was amplified and sequenced with primers rbcL1F and rbcL23R with given PCR cycle parameters [7]. The PCR products were purified using ethanol and sent to Macrogen for sequencing. New sequences are available in GenBank under accession numbers MG189706 and MG189707.

**Phylogenetic analyses**

The closest related rbcL, 18S rRNA and ITS sequences to strain CCALA 10278 and other representatives of the Chlamydomonadales were acquired from GenBank by using the BLAST algorithm [23]. The sequence alignments were computed using MAFFTv6 [24]. The aligned sequences were
checked for possible misaligned positions in BioEdit 7.0.9.0 [25]. The rbcL alignment comprised 41 sequences/966 positions (362 variable, 294 parsimony-informative). Based on Akaike’s information criterion (AIC) in jModelTest 0.1.1 [26], the GTR+Γ+I nucleotide substitution model was selected as the best fitting for the dataset. A maximum-likelihood phylogeny was computed in RAxML 7.0.4 [27] under the proposed model and statistical support values were derived from rapid bootstrapping (1000 replicates) in the same program. For additional statistical support, Bayesian posterior probabilities were computed in MrBayes 3.2.1 [28] with sequence dataset partitioned by codon positions. We carried out two Markov chain Monte Carlo (MCMC) runs for one million generations each with one cold and three heated chains under the GTR+Γ+I evolutionary model (parameters were estimated from the data) and trees were sampled every 100 generations. After 10^6 generations the average standard deviation of split frequencies dropped below 0.008 and the potential scale reduction factor (PSRF) approached 1.000–1.001 for convergence diagnostic parameters. The 18S rRNA alignment comprised 83 sequences/1705 positions (362 variable, 262 parsimony-informative). Computation of the best substitution model and the maximum-likelihood tree were conducted in the same way as described above. Bayesian posterior probabilities were computed based on the non-partitioned dataset and after 10^6 generations the average standard deviation of split frequencies dropped below 0.006 while the PSRF approached 1.000–1.001 for convergence diagnostic parameters. The final trees were displayed using FigTree [29].

**ITS2 rDNA secondary structure analysis**

Annotation of ITS2 including the 5.8 and 28S flanking regions was accomplished by the ITS2 online database [30–34]. A minimum energy secondary structure model of ITS2 was computed with RNAstructure 5.3 [35] and displayed by Varna 3.8 [36]. A sequence and structure alignment including four sequences that were the most similar to strain CCALA 10278 (‘Chlamydomonas’ gerloffii CCAP 11/72 (FR865610), Chloromonas sp. CCAP 11/110 (FR865527), Cr. reticulata CCCryo 213–05 (HQ404885), Cr. reticulata CCCryo 338–08 (HQ404900)) was built employing the ClustalW algorithm implemented in 4SALE 1.7. [37, 38]. The same software computed compensatory base changes (CBCs) [39] among the sequences.

**RESULTS**

**Chloromonas arctica Barcytè and Hodač, sp. nov.**

Diagnosis: solitary vegetative cells, 10–20µm long and 6–16µm wide, broad ellipsoidal or ellipsoidal–cylindrical in shape; or spherical. Two flagella of equal length; 1.0× cell length or longer. Chloroplast single, parietal, cup- or urn-shaped and lobed with number of lobes ranging from two to eight. Lobes are never disconnected. Eyespot pale red, small and elliptical in the lateral anterior part of the cell; in older cells usually not visible. Two apical contractile vacuoles. No pyrenoid. Papilla non-distinct, hemispherical. Nucleus central. Old cells globular with cytoplasmic oil droplets occupying most of the cell volume. Asexual reproduction via production of two, four or eight zoospores. Zoospores may lose their flagella, become spherical and act as aplanospores. Formation of cell aggregates is a common phenomenon in culture. Sexual reproduction unclear. The species differs from other species of the genus in the nuclear 18S rDNA, ITS rDNA and plastid rbcL gene sequences.

Holotype: the alga is preserved permanently at the Herbarium of University of South Bohemia in České Budějovice (CBFS) under number A-90–1. The authentic strain has also been deposited at the Culture Collection of Autotrophic Organisms (CCALA, http://ccala.butbn.cas.cz/) in Treboň, Czech Republic, as an active culture under strain number 10278. Figs 1, 2 and 3 show the morphology of the holotype.

Type locality: snow on the Svenbreen glacier, Svalbard, Norway (78° 43′ 645′′ N 16° 17′ 037′′ E; altitude 391 m above sea level).

Etymology: arc’ti.ca. L. fem. adj. arctica northern, from the Arctic.

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**Fig. 1.** Morphology of Cr. arctica CCALA 10278: (a) vegetative cells; (b) zoospore with two equal flagella; (c) old cells full of lipid droplets. White arrow points to the eyespot, grey arrows to the mother cell walls and black arrow to the papilla. Bars, 10µm (a); 20µm (b and c).
Morphology and ultrastructure

The alga investigated in this study grew at both 5 and 20°C, revealing the psychrotrophic nature of the organism rather than it being an obligate psychrophile. The isolate had general morphological and ultrastructural characteristics common to Chloromonas-like microalgae. It was unicellular, with broad ellipsoidal or elliptoidal-cylindrical cells (Fig. 1a, b). Spherical cells were observed in culture that was more than 1 month old (Fig. 1c). Single cup- or urn-shaped multilobed parietal plastid occupied most of the cell volume and surrounded the central nucleus; no pyrenoid was observed (Figs 1 and 2). Small starch grains were spread in the interthylakoidal spaces over the entire chloroplast (Fig. 2a). It reproduced asexually by two to eight zoospores which soon became immotile. Motile cells (Fig. 1b) were only observed when aliquots of the culture were transferred to fresh medium, in which case motility lasted for a very short time (noticed only at 20°C). Zoospores then quickly lost their flagella and became aplanospores. These cells were always surrounded by a parental cell wall (Fig. 1a). The young cells had eyespots and inconspicuous papillae (Figs 1a, b and 2a), while in mature older cells stigmata and papillae were not visible. Repeated divisions of daughter cells in parental cell walls resulted in cell aggregates covered by mother cell walls (Fig. 1a), especially under lower temperature conditions (5°C compared to 20°C). Sexual reproduction was not observed. Large oil droplets were noticed in the senescent cells (Figs 1c and 2c).

Phylogenetic analyses

Cr. arctica CCALA 10278 was placed within the Chloromonadina phylogroup [6] of the class Chlorophyceae. In the rbcL phylogeny, Cr. arctica CCALA 10278 clustered within clade 1 (Fig. 3) as a part of a supported subclade [maximum-likelihood support (ML)/Bayesian inference (BI): 90/1.00] containing the Chloromonas type species Cr. reticulata with the epitype strain UTEX 1970 (=SAG 29.83) [8]. The most similar rbcL gene sequence in GenBank was Cr. paraserbinowii SAG 71.72, which differed by 28 nucleotides from Cr. arctica CCALA 10278. 18S rRNA gene sequence analysis supported the same phylogenetic placement (Fig. 4). Cr. arctica CCALA 10278 differed by two nucleotides from ‘Chlamydomonas’ gerloffii CCAP 11/72, its closest relative available in GenBank. Both sequences were nested within a moderately supported (ML/BI: 65/0.99) subclade of clade 1 (upper part of Fig. 4) along with Cr. polyptera gerloffii CCAP 11/72, although both sequences still differed by three compensatory base changes within helix III (Fig. 5). Three other strains of Cr. reticulata with similar ITS2 sequences (CCAP 11/110, CCCryo 213-05, CCCryo 338-08) differed by five compensatory base changes from Cr. arctica CCALA 10278.

DISCUSSION

Biogeography of snow algae

Limited research has been undertaken to explore the biodiversity of eukaryotic snow microalgae using modern methods, particularly in the polar regions [3]. On the other hand, polyphasic taxonomic approaches have revealed a number of new green algal taxa from snowfields of the USA and Japan [4, 11–13]. Interestingly, the newly described species appeared to have a local rather than cosmopolitan distribution, implying the existence of both large- and small-scale biogeographical patterns [4]. Thus, the discovery here of the novel species Cr. arctica in the High Arctic is not surprising. In contrast, Lutz et al. [3] revealed a distribution of cosmopolitan snow algae throughout the Arctic. For example, Cr. polyptera (Fritsch) Hoham, Mullet et Roemer, known only from Antarctica, was shown to be the second most abundant snow alga in the Arctic [3]. However, such results should be interpreted with great care because the authors used the evolutionary highly conserved 18S rRNA gene which has been shown to be poorly discriminating.
between *Chloromonas* species. In addition, *Cr. polyptera* is found only in ecosystems with high animal nutrient input [9]. Our study also showed that the 18S rRNA gene would not have been adequate in defining *Cr. arctica*. Therefore, the conclusion that distinct snow algal species are cosmopolitan based on this single marker [3] should be reconsidered because only the application of a multigene approach along with modern microscopy can accurately distinguish taxa of the genus *Chloromonas* [40]. The study by Lutz et al. [3] serves as a good example of how different methods and approaches can affect the assessment of Arctic (and not only) snow algae distribution.

**Observations at the type locality**

The best-investigated site for Arctic snow algae is probably north-western Spitsbergen of the Svalbard archipelago, where many psychrotolerant and psychrophilic strains were isolated from persistent snowfields and glaciers at the coast.

Fig. 3. Phylogenetic position of *Cr. arctica* CCALA 10278 within the *Chloromonadinia* phylogroup (Chlorophyceae) based on maximum-likelihood tree of *rbcL* gene sequences. *Reinhardtinia* lineages were used as an outgroup. Numbers next to branches indicate statistical support values (maximum-likelihood bootstraps/Bayesian posterior probabilities). Thick lines indicate branches with high statistical support. *Chloromonas* clades 1, 2 and 3 were delimited according to Hoham et al. [7]. Snow strains in clade 1 are marked in bold. Asterisk shows the type species of the genus *Chloromonas*. Bar, 0.1 changes per nucleotide position.
Species of the genera Chloromonas and Chlamydomonas were the most frequently occurring representatives of the High Arctic cryoflora [41]. The Svenbreen glacier where Cr. arctica CCALA 10278 was found is located in Petuniabukta, Central Svalbard, where no comprehensive study of the cryoflora has been performed. The first attempt to briefly describe the community of snow algae there was done by Kviderová [42]. However, that study encompassed the cryoflora of temporary snowfields only and was based just on microscopic observations. The samples were dominated by coloured cysts of Chlamydomonas cf. nivalis [42]. Cr. arctica CCALA 10278 was isolated from a permanent supraglacial habitat exposed to the sun and meltwater during the boreal summer. The alga was found along with the common ice species zygnematophytes Ancylonema nor-denskioeldii Berggren and Cylindrocystis brebissonii Ralfs (De Bary) and spherical red spores resembling Chlamydomonas cf. nivalis (see Fig. S1, available in the online version of this article). The ice species probably appeared due to snow and ice mixing in the sample taken. The reason why Cr. arctica was not previously discovered may not be only the lack of sampling of snow habitats but also the possibility that the alga is not a dominant or bloom-forming species of snow algal communities, such as Cr. nivalis or Cr. brevispina [14]. Therefore, it could have been overlooked among other conspicuous and abundant snow species and their different life stages. For example, strain CCALA 970 was isolated from a red snow (it belongs to the species Cr. reticulata; pers.
commun. with Nedbalová) but it did not form the initial algal bloom. In addition, the distribution of _Cr. arctica_ could also be influenced by habitat type and its physico-chemical conditions [11]. On the other hand, the alga may be an endemic species to the Arctic where it commonly occurs on the permanent snowfields. For example, _Cr. polyptera_ is acknowledged to be an endemic species to Antarctica [9], or the recently described _Cr. nivalis_ subsp. _tatrae_ Procházková, Remias, Řezenka et Nedbalová was revealed to be a likely endemic taxon to the High Tatra Mountains (Slovakia) [5].

However, the dispersal capabilities of snow algal species probably differ. Based on extensive sampling in the states of Colorado and Washington (USA), Brown _et al._ [43] demonstrated that _Coenochloris_ species populations from snow were strongly geographically structured, whereas _Chlamydomonas_ species were not.

**Growth and morphology of _Cr. arctica_**

The new isolate is a psychrotolerant alga because it grew at both tested temperatures (5 and 20°C). This is not...
surprising considering that only few species isolated from snow are true psychrophiles that form blooms [10] and the majority of algae are usually mesophiles that have some degree of cold tolerance [44]. With ongoing climate change, the unique polar or alpine ecosystems could soon be lost by accommodating more psychrotolerant or cosmopolitan organisms [45]. Thus, the identification of organisms and description of their distribution could not only help to better understand the biotic interactions within cold ecosystems but also allow us to predict how they could change in the future [46].

Cells of Cr. arctica were observed mostly as non-motile vegetative stages. Stibal [47] also reported the reproduction of Cr. nivalis solely by non-motile stages in the culture. It is not clear what exact growth conditions could induce the motility of the zoospores or loss of flagella. Sensitivity to changes of environmental/growth conditions is well known in snow flagellates [48]. The observed morphological features were consistent with those reported for Chloromonas-like algae [49, 50], including the standard lack of a pyrenoid. The two sister lineages of Cr. arctica, Cr. rosea Ettl and Cr. serbinowii Wille, also do not have pyrenoids. Cr. gerloffii (=Chlamydomonas gerloffii) Ettl the closest relative of Cr. arctica (revealed by 18S rRNA gene and ITS2 rDNA analyses), has stretched, egg-like cells without papillae [50]. Moreover, the alga has a large protruding eyespot. In contrast, the eyespot of Cr. arctica is inconspicuous and usually not present (or not visible) at all, as also noted, for example, in its other sister lineage Cr. variabilis (Dangeard) Wille [50]. In addition, cells of Cr. gerloffii are almost twice as narrow (4–8 µm) as those we report for Cr. arctica (6–16 µm), although the length of the cells is the same (10–16 µm for Cr. gerloffii and 10–20 µm for Cr. arctica). Another closely related taxon, Cr. paraserbinowii (Skuja) Gerloff and Ettl (revealed by rbcL analysis), has bigger cells (20–38 µm long and 13–29 µm wide) that are ovate or ellipsoid-ovate in shape. In contrast to the aforementioned Chloromonas species, Cr. paraserbinowii has an exceptionally large papillae. Like Cr. arctica, it has a large chloroplast composed of many closely connected lobes, although the eyespot is big and located in the lateral middle part of the cell [50]. The presence of the parental cell wall (=primary cell wall) is a common phenomenon in Chloromonas snow species observed both in the field and under culture conditions [9, 51].

Conclusions

We predict that Arctic Chlamydomonas/Chloromonas-like snow flora may hide a number of novel, possibly endemic, species yet to be discovered. Here we showed that the isolation and cultivation of unialgal strains is a valuable and vital tool for better understanding of actual snow microalgae biodiversity. Therefore, this study serves as a good starting point for looking more deeply into Arctic cryoflora using a combination of both culture-based and modern molecular techniques.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

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